Enzymatic Synthesis and Characterization of a Novel Water-Soluble Polyaniline: Poly(2,5-diaminobenzenesulfonate)

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ABSTRACT: A novel water-soluble polyaniline has been synthesized by horseradish peroxidase catalyzed oxidative free-radical coupling of 2,5-diaminobenzenesulfonate. The polymerization reaction is rapid, and the average molecular weight of the resulting polymer is on the order of 18 000. Unlike the sulfonated polyanilines synthesized through posttreatment of polyaniline with fuming sulfuric acid, polyaniline reported here is fully sulfonated. This electrochemically active polymer shows strong pH dependence of absorption and other physical characteristics. This class of novel electroactive polyelectrolytes can be self-assembled into multilayer structures by interleaving with a variety of polyelectrolytes.

1. Introduction

Polyaniline is one of the important classes of conjugated polymers studied due to its interesting electrochemical and optical properties and environmental stability.[1−4] Polyaniline can be doped to its conducting form by treating with protic acids without changing the number of π electrons.[5] The rich oxidation−reduction behavior and electrical conduction of the polymer has been extensively investigated.[6,7] Polyanilines, due to their remarkable electronic, optical, and unique redox properties as well as their environmental stability, processability, and mechanical properties including tensile properties, have found a number of practical applications, such as in biosensors as hosts for biomaterials, EMI shielding, and batteries and as electrochromic materials among others.[3,4,8] Significant advances have been made to improve the oxidative synthesis of polyanilines but is limited to the structure and function of the monomer. The substituted anilines react less successfully than aniline itself.[6] Therefore, the modification of the polymer after oxidative polymerization has become more attractive than the derivatization of aniline monomer per se.

Attempts are being made to solubilize the polyanilines in order to improve their processability.[2,5,9] A majority of these efforts rely on the posttreatment of the polymer, such as treating with fuming sulfuric acid in order to introduce a sulfonic acid functional group in the benzene ring.[2,5,9] Sulfonated polyaniline is of interest because of its unusual electroactive properties, improved processability, and potential industrial applications.[4,7] Sulfonated polyanilines are self-doped and may be soluble in water. Unfortunately, many of the chemically synthesized sulfonated polyanilines are water-soluble only at higher pH conditions, where the polymer is in its undoped form.[7] We have recently reported the synthesis of water-soluble polyanilines from *p*-aminobenzoic acid.[10] This polymer is soluble in neutral or alkaline conditions.

Recently, self-assembled polymer architectures created by a layer-by-layer deposition technique through electrostatic interaction of polyelectrolytes have become an attractive technology for the fabrication of thin film devices with molecular level control on the organization.[11,12] Rubner and co-workers for example have reported the use of partially sulfonated polyanilines in the fabrication of multilayers.[12] However, for applications where the electroactive polymer is an active component of the device, such as in biosensors, conjugated polymer soluble in physiological pH conditions in its doped form would be important. The need for the development of polyanilines soluble in their doped form has become more apparent. Therefore, an alternate route, where the reaction steps involved are chemically mild and allow the control over the extent of sulfonation and level of doping is desired.

With hazardous waste becoming increasingly expensive to treat, biochemical reactions are becoming more attractive as alternate synthetic routes, for synthesis of fine chemicals.[13,14] This "natural way" of producing polymers from monomers is also attracting the interests of chemical industry seeking methods to synthesize their products without generating toxic byproducts. Horseradish peroxidase (HRP) has been used for the synthesis of polyanilines and polyphenols through oxidative free-radical coupling.[14] The polymerization has been studied in conditions including aqueous, organic, solvent mixtures, micelles, reverse micelles, and emulsions and at the air−water interface to improve the processability and ordering of the polymer.[15,16]

In the presence of hydrogen peroxide, HRP catalyzes the oxidation of aromatic amines and phenols to generate their respective free radicals. These free radicals undergo coupling to produce dimers. Successive oxidation and coupling reactions result in the polymer.[10,14,17,18] This polymerization is remarkably versatile, and although the final polymer structure is somewhat ill-defined, a variety of phenolic and anilinic monomers may be employed. The polymerization of aniline and phenol in aqueous media resulted in low molecular weight oligomers due to the insoluble nature of the product. Therefore, microemulsions and solvent mixtures were utilized to polymerize phenols and alkyl derivatives of phenols to obtain polymers soluble in organic solvents with improved molecular weights.[16]

Our approach to develop water-soluble electroactive polymers based on anilines was simply to adopt a monomer design that was water-soluble and led to a

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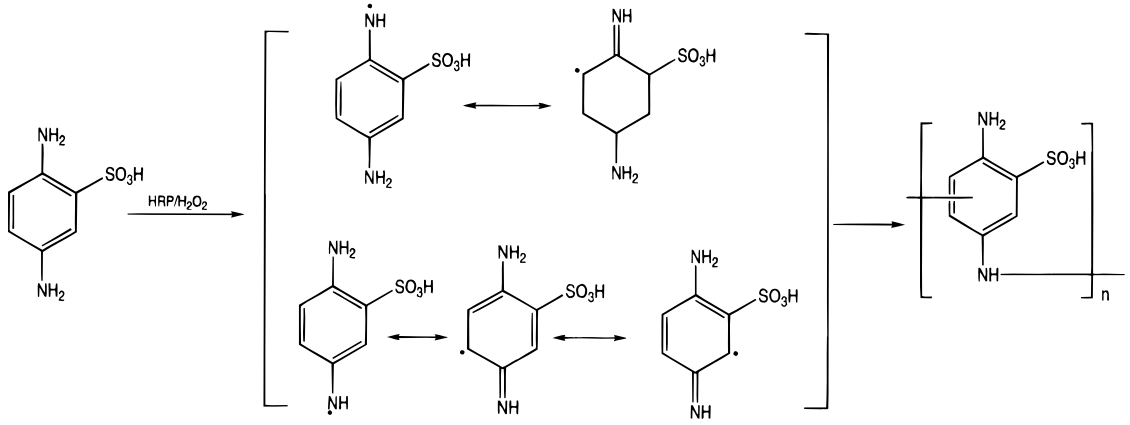
Scheme 1. Schematic of Enzyme-Catalyzed Polymerization of 2,5 Diaminobenzene Sulfonic Acid



polymer structure that was substantially soluble as well. We have recently reported the synthesis of water-soluble polyanilines from *p*-aminobenzoic acid by enzyme-catalyzed polymerization.[10] Solubility of the polymer was, however, limited to neutral or alkaline conditions. Here, we describe the synthesis and spectroscopic characteristics of a self-doped sulfonated polyaniline which is soluble at all pH conditions. 2,5-Diaminobenzenesulfonate (DABSA) has been polymerized by HRP-catalyzed oxidative free-radical coupling in the presence of hydrogen peroxide to obtain a water-soluble polymer (PDABSA). Scheme 1 gives the possible mechanism involved in the HRP-catalyzed oxidative free-radical coupling of DABSA. This is analogous to the mechanism proposed for the enzymatic polymerization of phenols which has been extended to the polymerization of anilines.[10,15,17] Though these coupling reactions can result in C−C, C−N−C, and N−N type bonds, only C−C and C−N−C bonds result in growth of the polymer chain. This approach can be extended to incorporate groups with tailored functionalities without much loss in the reactivity unlike the chemical synthetic routes reported to date.[6,19,20] These self-doped, water-soluble polyanilines show unusual pH-dependent optical properties. These electrochemically active polymers show promise as potential materials in the fabrication of thin films of biomaterials through a layer-by-layer deposition technique.[21] It has been demonstrated that the solubility of the polymer at all pH conditions can be used to fabricate thin films of polyelectrolytes with a wide range of properties.

2. Materials and Methods

2.1. Materials. Horseradish peroxidase (EC 1.11.1.7) was purchased from Sigma Chemicals Co., St. Louis, MO. 2,5-Diaminobenzenesulfonic acid was purchased from Aldrich Chemicals Co., Inc., Milwaukee, WI. All other chemicals and solvents used were of analytical grade or better and used as obtained.

The infrared spectrum was recorded with a Perkin-Elmer 1760X FTIR spectrometer. The UV−vis spectra were recorded using a Perkin-Elmer Lambda-9 UV/Vis/near-infrared spectrophotometer. The emission characteristics of the polymer were studied using a SLM 8100 spectrofluorometer. The NMR spectra were recorded using a Bruker 250 MHz NMR spectrometer. The electrochemical characterization of the polymer was carried out using a potentiostat (EG&G potentiostat/galvanostat Model 263). A platinum wire was used as the working electrode. Electric potential was applied with respect to silver/silver chloride electrode using platinum mesh as the counter electrode. The reaction was carried out in 0.1 M sodium phosphate buffer, pH 6.0, under a nitrogen atmosphere. Thermal properties of the dried polymer (dried in vacuum over at 95 °C for 5 h) were studied using TGA 2950 (TA Instruments, Inc.) and DSC 2910 (TA Instruments, Inc.). The molecular weight was determined using gel permeation chromatography utilizing a Waters Model 510 pump and a Waters Model 410 refractive index detector with Jordi columns relative to polystyrene standards. Dimethylformamide containing 1% LiBr was used as the eluent.

2.2. Enzymatic Synthesis of the Polymer. The polymerization of DABSA was carried out in 0.1 M sodium phosphate buffer, pH 6.0. A total of 0.1 g of DABSA was dissolved in 50 mL of sodium phosphate buffer containing 3 units of the enzyme. The reaction was initiated with the addition of 100 μL of 30% hydrogen peroxide with stirring. The polymerization reaction starts instantaneously. The reaction was allowed to continue at room temperature for a minimum of 3 h with constant stirring. The reaction medium was dialyzed (cutoff 2000M_w) overnight against water to remove the unbound buffer, and water was evaporated off to get a dark brown solid. The polymer was then extracted with methanol, which was later evaporated off to obtain dark brown colored polymer with 80% yield.

2.3. Thin Films by a Layer-by-Layer Deposition Technique. Self-assembly of the polyaniline poly(DABSA) on a glass slide was carried out by the layer-by-layer deposition technique.[11] A glass slide treated with alkali (Chemsolv) was exposed to polycation and polyanion solutions repeatedly with in between washes to transfer monolayers of these polyelectrolytes per every exposure. A 1 mg/10 mL solution of poly(diallyldimethylammonium chloride) (PDDAC) at pH 2.5 was used as the polycation, while a 1 mg/10 mL solution of PDABSA also at pH 2.5 was used as the polyanion. The glass slide was exposed to the polyelectrolyte solution for 10 min and washed with water at pH 2.5 to remove any unbound polymer from the surface. This process was repeated to obtain the desired number of bilayers.

3. Results and Discussion

3.1. Kinetic Experiments. The polymerization reaction was followed by UV−vis spectroscopy. In this experiment, the concentration of hydrogen peroxide, DABSA, and the solution pH were chosen such that the reaction rate is low enough to be followed by UV−vis spectroscopy. Polymerization was initiated with the addition of 10 μL of 3% hydrogen peroxide to 3.0 mL of a DABSA (1 mg/100 mL) solution in sodium phosphate buffer at pH 8.0 containing about 0.1 units of the enzyme. Figure 1 gives the absorption spectra recorded before and after 20 min of the initiation of the polymerization. The spectra were recorded using DABSA and the enzyme in the buffer as the reference. The inset in Figure 1 gives the change in absorbance recorded at 420 nm, corresponding to the absorption maximum of the polymer, as a function of time. The changes in absorbance are dramatic in the initial stages of the reaction,

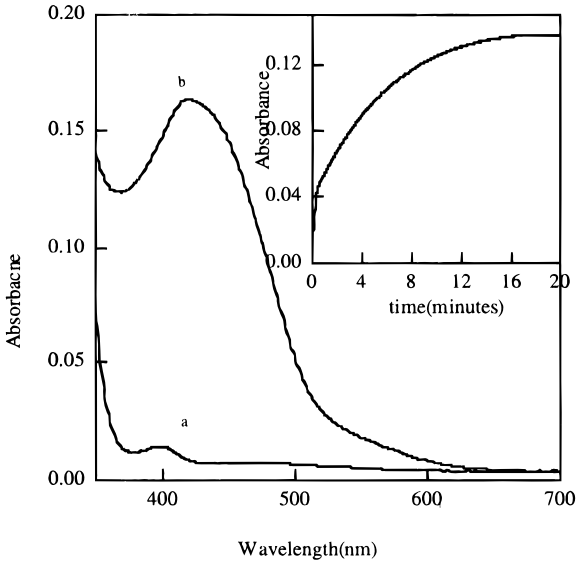


Figure 1. Absorption spectra of 2,5-diaminobenzenesulfonate (a) before addition of hydrogen peroxide and (b) 20 min after the addition of hydrogen peroxide in sodium phosphate buffer, pH 8.0. The inset gives the change in absorbance at 420 nm recorded as a function of time during the polymerization.

which reaches near completion in about 15 min. We also observed that the maximum conversion of DABSA is achieved at pH 6.0. Therefore, the bulk polymerization was carried out at pH 6.0. These spectroscopic experiments were carried out under dilute concentration conditions. In bulk polymerization, the reaction was allowed to continue for 3−4 h with intermittent addition of hydrogen peroxide to ensure completion of the reaction. The reaction mixture was then dialyzed against water and extracted to methanol. GPC analysis shows that the polymer has a molecular weight (M_w) on the order of 18 000. Elemental analysis estimates of C, N, S, O, and H adds up to less than 100%, confirming that the polymer was self-doped with the buffer salt. The ratio of C/N (2.48) agrees with the estimated C/N ratio (2.57), however, these data could not be used for any further structural elucidation.

3.2. Nuclear Magnetic Resonance Studies. The polymerization process was also followed by *in-situ* NMR spectroscopy. The polymerization reaction was carried out in D_2O (sodium phosphate buffer pH 6.0) in a NMR tube. Reaction was initiated by the addition of 2 μL of 30% hydrogen peroxide, and the NMR spectra were recorded at different reaction time intervals. Characteristic spectra recorded during the polymerization are given in Figure 2. Figure 2a is the spectrum of the monomer containing the enzyme, before the addition of hydrogen peroxide. The peak at 7.5 ppm arises from the proton at position 6. Protons at positions 3 and 4 give rise to peaks at 7.0 and 7.15 ppm, respectively, which appear to have doublet characteristics. All three peaks have identical intensities. The spectral widths are very broad possibly due to the neighboring exchangeable protons. The molar concentration of the enzyme is very low compared to DABSA; hence, its protons do not appear in the NMR spectrum. Parts b−d of Figure 2 represent the spectra recorded 4, 9, and 13 min after the addition of hydrogen peroxide, respectively. As the polymerization reaction progresses, the peak pattern changes, with the appearance of new peaks at 8.15 ppm. The peaks show multiple splitting patterns which is due to the change in the chemical environment of the neighboring protons and their long-

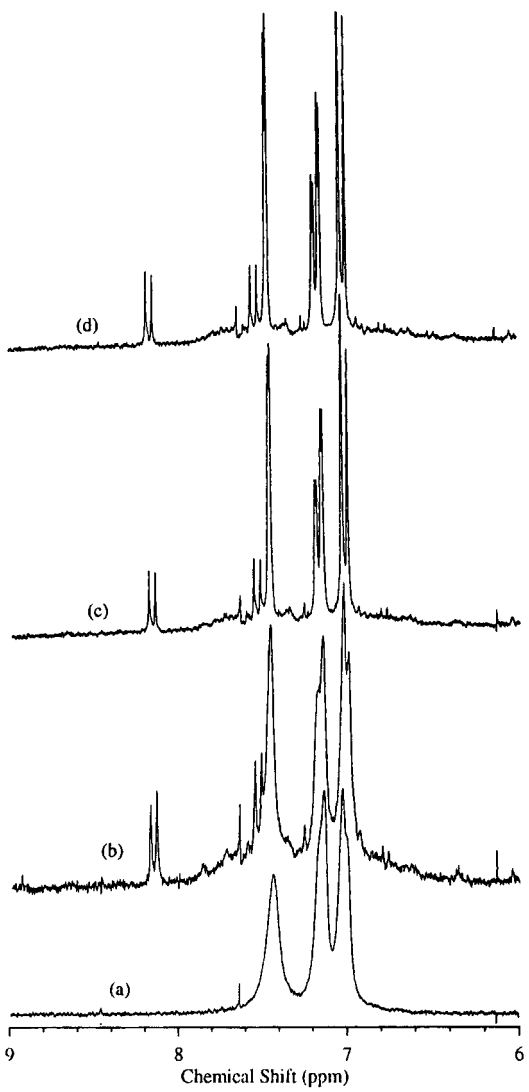


Figure 2. Proton magnetic resonance spectra during the polymerization of 2,5-diaminobenzenesulfonate in D_2O: (a) 2,5-diaminobenzenesulfonate with horseradish peroxidase, (b) 4 min after the addition of hydrogen peroxide, (c) 9 min after the addition of hydrogen peroxide, and (d) 13 min after the addition of hydrogen peroxide.

range couplings. The other striking observation is that the peaks become sharper with the progress of the polymerization reaction. The intensities of the peaks at the end of 13 min compared to the one before the initiation of the polymerization decrease to 62 ± 3%, 63 ± 3%, and 72 ± 3% for the protons at positions 6, 3, and 4, respectively, indicating that all three positions have almost identical probabilities of oxidative coupling. The peaks at 7.55 and 8.15 ppm have a J value of 9 Hz, identical to that of the peak at 7.0 ppm arising due to the coupling at position 6. The peak at 7.15 ppm is a finely split doublet with J values of 3 and 9 Hz, respectively, while the peak at 7.45 ppm is a doublet with a J value of 3 Hz. The doublet at 7.2 (J value of 6 Hz) can be considered as two singlets arising from the couplings at positions 3 and 4. This suggests that the positions 3, 4, and 6 are involved in the coupling reaction. The reaction mixture in the NMR tube consists of both the monomer and the growing oligomer chains, since the reaction was followed *in-situ* and not optimized for final products. The isolated polymer from bulk enzymatic synthesis, however, gives a broad featureless proton NMR spectrum. We could not obtain a ^{13}C spectrum; therefore, further details on the resulting

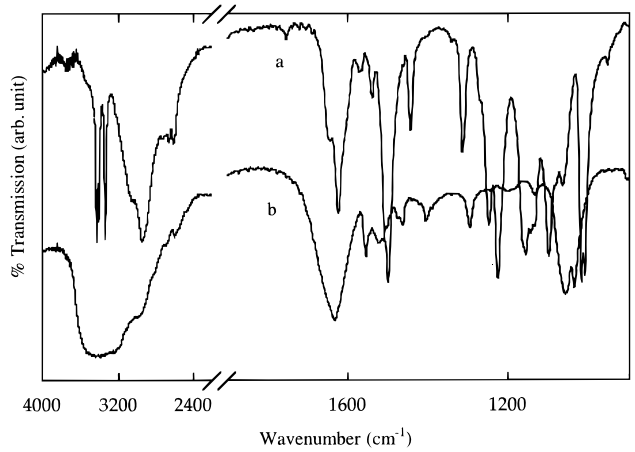


Figure 3. FT-IR spectra of (a) 2,5-diaminobenzenesulfonate and (b) poly(2,5-diaminobenzenesulfonate) in KCl.

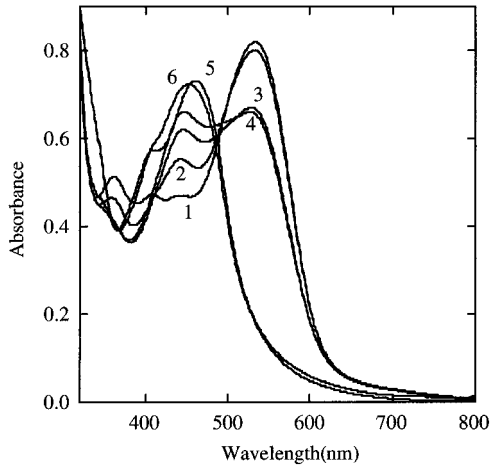


Figure 4. Absorption spectra of poly(2,5-diaminobenzenesulfonate) in solutions of different pH values: (1) 1.2, (2) 3.0, (3) 6.0, (4) 9.0, (5) 10.0, and (6) 12.8.

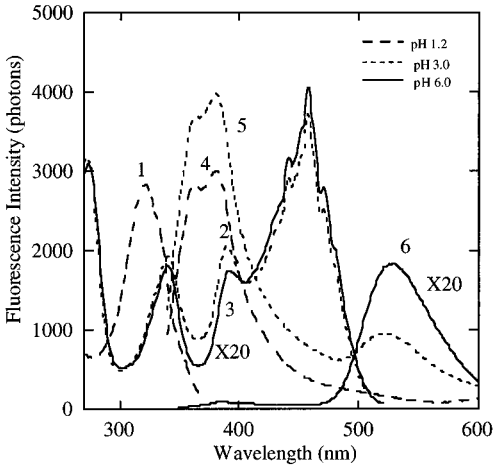


Figure 5. Excitation and emission spectra of poly(diaminobenzenesulfonate) at pH 1.2 (curves 1 and 4), pH 3.0 (curves 2 and 5), and pH 6.0 (curves 3 and 6). The intensities in curves 3 and 6 have been divided by 20 in this representation. The spectra above pH 6.0 are identical to those at pH 6.0.

polymer structure could not be obtained. In a separate study, in a more amenable system we have investigated the enzyme-catalyzed polymerization of HQS using HRP in an aqueous medium. In this case the mechanism of enzyme-catalyzed polymerization was clearly established through *in-situ* ^{1}H NMR spectroscopy and ^{13}C NMR spectroscopy.[17] We believe a similar mechanism of polymerization is in place here in the polymerization of water-soluble polyanilines.

3.3. FT-IR Spectroscopy. The FT-IR spectra of DABSA and its polymer in a KCl matrix are given in Figure 3. The monomer (curve a) shows characteristic amine vibration bands at 3430 cm^{-1} (with a doublet pattern) and a sharp peak at 3340 cm^{-1}. The −OH shows a broad peak at 2935 cm^{-1} with a shoulder at 3200 cm^{-1}. The polymer (curve b), on the other hand, shows a broad peak centered around 3450 cm^{-1} with a shoulder around 2940 cm^{-1}. The peaks at lower energy regions also become broader upon polymerization, resulting in the loss of multiplicity of the absorption bands. The number of vibrational bands in the ring hydrogen rocking region (1250−1000 cm^{-1}) is lower in the case of polymer as compared to the monomer, indicating the disappearance of the ring hydrogens upon polymerization. The broad intense absorption band around 1000 cm^{-1} is arising due to the increased ring substitution. The disappearance of the peak at 1250 cm^{-1} suggests the formation of a zwitterionic structure in the polymer.

3.4. Absorption Characteristics. Figure 4 demonstrates the effect of pH on the absorption spectrum. The stock solution of the polymer at pH 7.0 was diluted to a constant dilution in a 0.1 M KCl solution at various final pH values. It can be observed from the figure that the absorption characteristics undergo a series of changes upon increasing the solution pH from 1.2 to 12.8. The absorption at 540 nm decreases with increasing pH, while a new peak appears around 445 nm upon increasing the solution pH. The absorption band at 540 nm is assigned to the doped form of the polymer, while at 445 nm the conjugated polymer exhibits its characteristic absorption band. The chemically synthesized polyaniline in the undoped form has an absorption maximum of 563 nm. The blue-shifted absorption maximum of the polymer reported here (445 nm) can be ascribed to the branching in the polymer due to the nature of the coupling reaction and loss of conjugation. The conversion from the doped to the undoped form of the polymer is instantaneous as observed by spectral changes, indicating that the undoping kinetics is rapid. The polymer can be shuttled between its doped and undoped forms by the proper choice of solution pH. The absorbance of the polymer follows a linear relation with the concentration of the polymer up to 0.5 mM of the polymer. Thin polymer films cast from an aqueous solution of the polymer are optically clear and give an absorption maximum of 530 nm.

3.5. Emission Characteristics. Figure 5 shows the excitation and emission spectra of the polymer at various pH conditions. The emission characteristics show an interesting pH dependence. The polymer at pH 1.2 has emission (curve 4) only in the blue region. The emission maximum is 380 nm with an excitation maximum at 320 nm (curve 1). Upon increasing the pH to 3.0, a new emission band at 530 nm (curve 5) appears with an intensity comparable to that at 380 nm. The excitation spectrum (curve 2) shows multiple bands with peak maxima at 340, 380, and 460 nm unlike that at pH 1.2. Upon increasing the pH to 6.0, the emission at 530 nm (curve 6) increases and the intensity is about an order of magnitude higher than that of a low pH solution. The excitation spectrum is identical to that at pH 3.0 except for the intensity. These observations indicate that the polymer exists predominantly in the acid form at pH 1.2. At pH 3.0, the doped and the

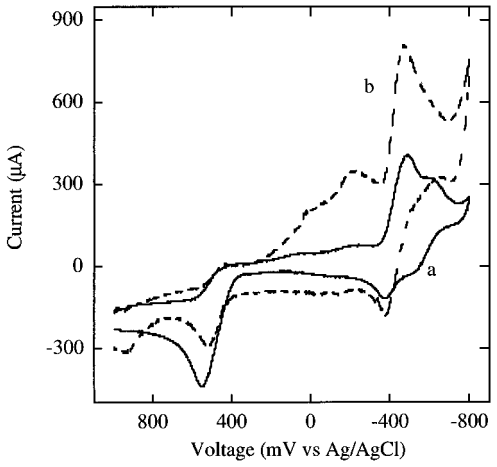


Figure 6. Cyclic voltammograms of (a) 2,5-diaminobenzenesulfonate and (b) poly(2,5-diaminobenzenesulfonate) under a nitrogen atmosphere. The potential at the platinum wire working electrode was varied at a scan rate of 20 mV/s with respect to a Ag/AgCl reference electrode. A platinum mesh electrode was used as the counter electrode.

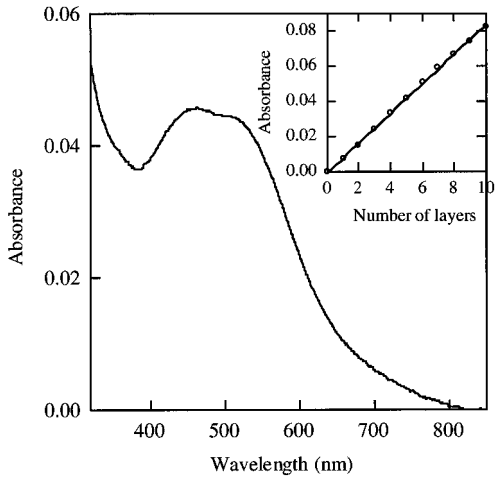


Figure 7. Absorption spectrum of six bilayers of PDABSA and PDDAC deposited by a layer-by-layer technique. The inset gives the absorption maximum at 535 nm as a function of the bilayers.

undoped forms of the polymer coexist. At pH 6.0, the undoped form of the polymer dominates in the solution. Similar spectral features were observed at pH values above 6.0. It can be observed from the absorption and excitation spectrum that the emission properties arise from the structural defects in the polymer and not from the conjugated polymer chain. The ground-state and excited-state acidities of organic molecules can differ, causing different pH dependencies of absorption and fluorescence.[22] The quantum efficiencies of the acid and its conjugate base can also be different. Moreover, in the present case, the polymer conformation, level of doping, and oxidation state of the polymer are also pH dependent.

3.6. Electrochemical Properties. Figure 6 examines the cyclic voltammograms of the monomer and the polymer at a platinum electrode at pH 6.0. Curve a is the cyclic voltammogram of the monomer recorded at a scan speed of 20 mV/s. The monomer displays a oxidation potential of 0.58 V with respect to Ag/AgCl. The reduction cycle of the monomer displays two single electron-transfer reversible redox reactions at a potential of −0.45 and −0.55 V, respectively. At higher scan speeds (50 mV/s and higher), the reduction peak appears at a single potential of −0.55 V. However, the corresponding oxidation profiles show a two-electron-transfer reaction. Curve b represents the cyclic voltammogram of the poly(2,5-diaminobenzenesulfonate) recorded at 20 mV/s. Upon polymerization, the molecule displays redox properties different from those of the monomer. A new two-electron reduction peak appears at a potential of −0.225 V which corresponds to the polyaniline, which does not appear to be completely reversible. The peak at −0.55 V observed in the monomer does not appear in the polymer even at slow scan speeds. It is also noteworthy that the oxidation potential (+0.52 V with respect to Ag/AgCl) of the polymer is shifted by 30 mV to lower potential as compared to that of the monomer. However, the oxidizable species are lower in concentration in the case of polymer, resulting in a lower peak current. The complex nature of the polymer structure, as suggested in Scheme 1, supports such a complex nature of the redox characteristics of the polymer.

The polymer as synthesized shows a conductivity in the semiconducting region (10^{-5} S/cm) at pH 6, sufficient for many biosensor applications. This level of conductivity can be attributed to the complex structure of the polymer and the fact that at this pH the chains may not be significantly doped. It should be possible to improve the conductivity by copolymerizing with underivatized aniline with a stoichiometry such that solubility is still maintained at the appropriate pH.

3.7. Thermal Properties. Thermogravimetric analysis of the polymer shows very good thermal stability of the polymer. There is about 2% weight loss to 176 °C, where the first significant weight loss is observed, with 92% weight retention at 201 °C. There is less than 19% mass loss to 332 °C and less than 25% mass loss to 500 °C. The DSC trace is featureless to 180 °C and shows a sharp endotherm at 196 °C in the first scan, which is absent on the reverse and the second forward scans. The mass loss at this temperature in TGA and the presence of the endotherm in the first DSC scan are well correlated. The disappearance of this endotherm in subsequent scans is related to loss of bound water. As expected in a rigid backbone polymer, the presence of a significant glass transition temperature is not indicated.

3.8. Thin Films by a Layer-by-Layer Technique. Ultrathin organic films are currently gaining interest in many areas such as integrated optics, sensors, coatings, and surface orientation layers.[15,16] A recent approach to build multilayer assemblies is by consecutive alternate adsorption of anionic and cationic polyelectrolytes.[11,12,23] The Coulombic attraction between opposite charges is the driving force in the multilayer buildup, which results in 100% coverage of the substrate independent of the substrate size and surface topography.[12] Polymers with a wide range of properties such as electrical conductivity, electroluminescence, nonlinear optical, and redox properties can be assembled into thin films with controlled supramolecular architecture.

The solubility of the polyaniline described in this study at all pH conditions makes it a perfect "command layer" for the fabrication of thin films by the layer-by-layer deposition technique. This polymer can be used as polyanion with a variety of other polycations of interest. In the preliminary studies on fabrication of multilayers by this technique, we have demonstrated that one can build multilayers of this polyaniline at any pH condition with a proper choice of the polycation. Figure 7 gives the absorption spectrum of six bilayers of polyaniline with poly(diallydimethylammonium chlo-

ride) (PDAC) deposited on a glass slide at pH 2.5. Since PDAC is not absorbing in the spectral region scanned in this study, the absorbance is solely due to the polyaniline. The absorption spectrum of the multilayer assembly gives the absorption maxima at 535 and 450 nm, respectively. The inset in Figure 7 gives the absorbance recorded at 535 nm as each bilayer was deposited on the glass slide. The constant change in absorbance per bilayer indicates that one can build thin films with precise control over thickness and organization. Similar observation was also made at neutral pH conditions. This approach can be extended, for example, to the fabrication of thin films of biomolecules sandwiched between conjugated polymer layers for biosensing applications. Results from these investigations will be reported elsewhere.

4. Conclusion

In conclusion, we have synthesized a class of electrochemically active, water-soluble polyanilines through enzyme-catalyzed polymerization. The polymerization process is chemically mild and environmentally safe. The presence of sulfonic acid groups throughout the polyaniline makes it water-soluble at all pH conditions. The polymer can be organized into multilayers by the "layer-by-layer" deposition technique. Individual components of these multilayers can be engineered to obtain molecular assemblies of tailored properties. The solubility of the polymer at all pH conditions makes it a suitable material for self-assembly into organized structures with biological macromolecules such as enzymes for fabrication of biosensors. The solubility and the electronic and optical properties of the polymer can be manipulated further by copolymerization with aniline or phenolic monomers.

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