A label-free electrochemical protein sensor of perchloric acid doped polyaniline

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ABSTRACT
In this study, we developed a potentiometric based protein sensor utilizing the interactions between charged functional moieties of a target protein-protein interaction and the complexation between PANI and dopant molecules. The sensor response depended on the isoelectric point of the protein. However, this dependency may be exploited to enhance specificity of protein sensors at specific pH dependence. The conducting polymer was found to respond by changing potential in the presence of biomolecules, demonstrating a direct chemical to electronic transduction method. The influence of polymer surface and morphology of finished films was also studied. This study demonstrated a conducting polymer able to respond to proteins at physiological conditions, in other words a step towards the integration of these materials into implantable sensing system. Additionally this sensor also had better ability to recognize the specific protein-protein interaction.

Keywords: Poly aniline, Conducting polymers, Biosensors, Proteins.

INTRODUCTION
A great attention has been paid to the research and examination in the biosensors which has brought deep growth in the development of new biosensors in the past two decades (Wilson and Gifford, 2005; Kissinger, 2005; Murphy, 2006). A biosensor is a sensor that uses biological selectivity to limit its examination to specific key molecules. These types of biosensor require the expansion of novel sensing methods, which are sensitive, specific, effective and low-cost. To address this need, the ability of a polyaniline (PANI) doped with a variety of acids, was already used for detecting different biomolecules by taking advantages of its properties such as pH sensitivity, ionic strength, electrochromism and conductivity (Kim et al., 2000). Polyaniline is one of the most main conducting polymers among organic and polymer electronics (Genies et al.,1988; Saxena and Malhotra, 2003; Lee et al., 2005; Lee et al., 2006) due to its promising electrical and optical properties, as well as environmental stability (Chinn et al., 1995; Chinn et al.,1997; MacDiarmid,1997). In its emeraldine base form (see Scheme 1a), PANI behaves as an insulator. To obtain the conducting emeraldine salt forms of PANI (see Scheme 1b and 1c), the emeraldine base is chemically doped by exposure to an acid (see Figure 1). Polyaniline syntheses are carried out mostly using aniline hydrochloride solution or a mixture of an aniline monomer and a dilute hydrochloride acid (Stejskal and Gelberg, 2002). To obtain a conducting form of polyaniline, three
steps are required: (1) polymerization of aniline with hydrochloride acid, (2) treatment of the obtained product with base to produce non-conducting polyaniline, and (3) successive protonation with an appropriate acid to form a conducting polyaniline salt (Laska and Widlarz, 2003a). During the pathway, every step is a separate process and requires purification and filtration processes. Most of the current studies on polyaniline are focused on regulatory the macromolecule properties of the polymer, such as its conductivity, molecular weight and solubility in water (Salaneck and Lundstrom, 1987; Stejskal et al., 1996, 1998; Laska and Widlarz, 2003a). Among several other processes, one way to modify the polyaniline properties is by polymerization in various acids. Inorganic acids such as sulfuric acid (Neoh et al., 1990), phosphoric acid (Yoon et al., 1996), and water soluble organic acids, such as phosphonic and sulphonic acids (Laska and Widlarz, 2003 a,b), have been investigated with the probability of increasing conductivity and solubility in water. It has also been reported that the temperature at which the polymerization is conducted controls the molecular structure of the synthesized polyaniline. When polymerization of aniline is carried out at a sub-zero temperature, noticeable rises in both molecular weight and crystallinity have been observed (Stejskal et al., 1998). The opportunity of directly converting a biomolecular chemical interaction into an electrical signal makes the application of conducting polymers a promising sensor of transduction method. The interaction of molecules with the conducting polymer substrate can change the dopant charge concentration, and therefore the conducting properties of the material. This effect was demonstrated in both gaseous (Bai and Shi 2007; Prasad et al. 2005; Virji et al. 2006) and liquid phases (Janata and Josowicz 2003; Lange et al. 2008). A novel conducting polymer sensing mechanism, utilizing the interaction between charged functional moieties of a target protein, the complexion between the PANI and the dopant molecule, is presented in this work. Non-covalent interactions between the protein functional moieties, the acid and the conducting polymer itself, cause modifications in the extent of doping present in the conducting polymer materials. The control of these interactions can be easily changed by changing the pH of solution. So, in the presence of a protein, the charge delocalization of the conducting polymer chain, or the link between the dopant acid will be modified in a measurable way. As PANI films do not maintain their conducting character in non-acidic media (Naudin et.al., 1998) (i.e. the neutral surroundings necessary for most proteins to function optimally), the electro polymerisation process has to be carried out in the presence of a dopant. For this reason, we focused on the development of biosensors incorporating with the perchloric acid (PA) doped PANI. The insertion of the latter retains electrical neutrality in the oxidised form of the polymer and also leads to increments in its structural stability and conductivity at a broader range of pH values. Polyaniline synthesized with PA is shown to have the highest conductivity in a near neutral solution (pH 6.6). The characteristic of high conductivity in a neutral solution is desirable to accommodate the requirement for optimal antibody-antigen reaction (Barbourand George, 1997). Therefore, polyaniline polymerized with PA was expected to result in the best biosensor performance among the other acids and the sensitivity of the biosensor with polyaniline protonated with PA was 103 CCID/ml of Bovine viral diarrhea virus (BVDV) samples and the rest of the biosensors ranged from 104 to 105 CCID/mls (Tahir et al., 2005). PANI exhibits unprecedented electroactivity over 8 decades of pH. We attribute this unusual electrochemical stability of PANI-PA to the same specific interactions that are responsible for the enhanced conductivity.

The recognition of proteins is rare, due to several technical difficulties: the relative complexities of the protein surfaces, which carry large numbers of competing binding sites; their conformational sensitivity to temperature, pH, the nature of the solvent; poor solubility in apolar solvents; biocompatibility, and relatively large molecular sizes (Sellergren, 2001; Turner et al., 2006). In most of the reports for the application of MI technique in protein sensors, elegant chemistry complementarities and polymerization procedures are essential for successful recognition (Lin et al., 2004; Turner et al., 2006). Label-free protein detection is therefore commonly achieved by employing biomolecules with high affinity for the target protein. This ensures much improved specificity, especially when dealing with a more complex sample matrix such as urine, cerebral spinal fluid, and serum, which contains high levels of serum albumin and immunoglobulins. The goal
of this work is to recognize protein–protein interaction by using PANI-PA film.

**EXPERIMENTAL METHODS**

**Materials**

Amino propyl triethoxysilane (APTES), aniline, ammonium persulfate (APS), sodium acetate, lysozyme (Lys), bovine serum albumin (BSA), and thaumatin were obtained from Sigma Aldrich Co, sodium chloride (NaCl) from Merck and perchloric acid (PA) from fluka. All the proteins and chemicals were used as received. The potentiometric measurements were made in a 25 mL beaker equipped Fluke Scopemeter 124 with a magnetic stirrer. The two-electrode system consisted of an Ag/AgCl as the reference electrode and the PANI film doped with PA as the working electrode. The potentials of the working electrode against the reference electrode were measured with a potentiometer. The electrochemical response was defined as the change of potential after the testing molecules were added into the solution compared with that before addition of the test molecules (expressed as ΔE = E - E₀, where E is the potential after adding the test molecules and E₀ is the potential before adding the test molecules). For selected experiments, a pH electrode was connected to the other channel of the potentiometer to measure the pH value of the solution.

**Fabrication of conducting polymer sensor**

PANI was template-synthesized with PA to generate PA doped PANI. PA was chosen for these experiments since it was demonstrated to be a useful polymer acid dopant which could produce materials with high conductivity (Moon and Park 1998; Catedral, et al., 2004; Tahir et al., 2005). In order to make the PANI-PA adhere to a microfabricated substrate for integration into the sensing device, APTES was formed on the glass substrate and the PANI was polymerized in the presence of the substrate, using what is termed a dip coating or in situ process. Glass slides were first cleaned in solvent prior to the silane functionalization to form the silane layer. The slides were then rinsed and toluene, acetone again and finally by methanol. The slides were then rinsed in deionized water ten times. The slides were subsequently cleaned with a Piranha solution (Sulfuric acid: hydrogen peroxide in a 2:1 ratio). Sulfuric acid was added to hydrogen peroxide in a 2:1 ratio, and the slides were soaked in this solution for 30 min. The slides were again rinsed with deionized water ten times. Next, the slides were immersed in a solution of 2 wt% APTES in acetone for 1 h. Then they were rinsed in acetone, followed by methanol, and dried with pressurized nitrogen. The slides were then heated up to 100°C for at least 12 h to ensure formation of covalent bonds between the glass and the APTES (Plueddemann 1991). The functionalized slides were stored at ambient conditions until use.

To form the PA doped PANI nano film, we prepared polymerization solutions. The standard procedure for the polymerization of aniline was followed and the substrates were immersed in the solutions during the polymerization reaction see (Figure 2). A 0.25 M aqueous solution of APS was mixed with 2 mg/mL of aniline in 1 M PA as dopant. The mixture was stirred and maintained at 4°C in an ice bath and after that, the silane-treated substrates were immersed in the chilled polymerization solution. Upon the addition of APS, the PA-aniline solution turned yellow, and then eventually brown and blue with time. Finally, the polymerization medium turned green. This final color change indicates the presence of the conducting emeraldine salts of PA doped PANI. After reacting for 24 hrs, the solution was poured off, and the substrates were rinsed for 3 days in deionized water, changing water daily. A sample of the last rinse was taken and the absorbance of the solution was analyzed with a UV-vis equipment to determine if detectable unreacted aniline or unadhered PANI continued to diffuse from the surface after rinsing.

Next, 0.05 M sodium acetate buffer was prepared, with a pH of 6.6 and 8.5. Into this solution, 150 mM sodium chloride was added to control the ionic strength. Using this buffer, solutions with 10 mg/mL Lys, 10 mg/mL BSA, and 10 mg/mL thaumatin were prepared. PA doped PANI films were exposed to the buffered protein solutions of Lys and BSA overnight.

**Principle of potentiometric detection**

Proteins in aqueous solutions are polyelectrolytes and have a net electrical charge; the magnitude of which depends on the isoelectric point of the protein and on the ionic composition of the solution. It was demonstrated that when the charged proteins are trapped into the thin insulating layer, which is deposited onto a metallic conductor, the change of the surface potential will occur, and
this change can be measured potentiometrically using a reference electrode immersed in the same solution (Janata, 1975).

**Protein-protein interaction based biomarker**

Protein-protein interaction based biomarker was based on a domain-domain interaction network flow model to identify signaling pathways from protein-protein interaction network (Figure 3) it is centered on two underlying mechanisms. In the first mechanism, the binding of the small inducer molecule induces conformational changes at the protein interacting interface and therefore weakens the interaction. The second mechanism involves two small molecules that are connected via a flexible linker. In addition, the proteins belonging to the same community are more likely to have similar functions. Such method detects the community structure in the molecular network (Wang et. al., 2007) and found some interesting hubs of network motifs in the protein-protein interaction network (Jin et.al. 2007). The modular or motif underlying biological networks can provide insight into biomarker prediction. Targeting protein-protein interactions, which are of central importance to virtually every cellular process, has enormous therapeutic potential. Many researchers focus on the identification of small molecules that specifically disrupt disease-promoting protein-protein interactions for therapeutic applications. Although considerable progress has been made in the past several years, the discovery of small molecule drugs that disrupt protein–protein interactions still faces a number of significant challenges. As an example, small and deep cavities that can serve as binding sites for small molecules are rarely found at the interface of interacting protein pairs, as the contact surfaces involved in protein–protein interactions are large and generally flat.

**RESULTS AND DISCUSSION**

**Conducting polymer sensor fabrication and role of doped sample**

The process to fabricate substrates with adherent conducting polymer films resulted in a dip coating polymer layer on the substrate surface (Figure 4). While the conducting polymer grew a negatively charged surface, the silane was a key to the formation of a stable layer. Without the silane, the films easily delaminated. In the presence of PA, the polymer film presented an emerald green color indicative of emeraldine salt. Previous studies showed undoped sample had a conductivity of $5 \times 10^{-4} \text{S/cm}$ and the highest conductivity of 109.04 S/cm was observed for the PA-doped sample (Catedral, et al., 2004). The various electron states in conducting polymers are in terms of the gap between highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) or the HOMO-LUMO energy gap (Vardeny & Wei, 1998). The experimental values of conductivity were found to exhibit roughly an inverse correlation with the computed values of the energy gap between the HOMO and LUMO; these values have been reported by other researcher groups (Atienza et al., 2004; Pascual, 2003). In this way, the smallest HOMO-LUMO gap was observed for the PA-doped sample, while the biggest energy gaps were obtained for hydrochloric acid and hydroiodide acid doped samples. The HOMO-LUMO gap is the molecular counterpart of the band gap for the macroscopic polymeric solid. The PANI-ES samples with varying dopants exhibit varying microstructures. Variation in microstructure leads to different conductivities of the samples. The addition of acid dopants alters the polymer lattice, which leads to the ionization of sites in the chains. The defects in the chain due to the dopant ions provide the mobility of the charge carriers on which conduction depends (Kroschwitz, 1988). The conductivity is also dependent on the number of charge carriers. The undoped sample displays globular but irregular morphology, where PA-doped sample has coral-like structures with elongated bodies (Catedral, et al., 2004). This current investigation of the use of polymer electronics is to find new methods of controlling its properties electrochemically and is promising routes toward harnessing the wide range of potential uses of PANI.

**UV-vis Spectroscopy of polyaniline**

UV-vis absorbance spectra of the PA doped PANI films were acquired with a UV-vis spectrophotometer JASCO V-530. After creating the baseline, films were inserted into the sample holder, and the absorbance of sample was recorded over the spectra from 200 nm to 1200 nm in steps of 1 nm. The PA doped PANI samples (Figure 5) exhibited two pronounced absorption bands in their spectra. The broad band between 310 nm and 450 nm were attributed to the $\pi - \pi^*$ transition of the...
benzenoid ring (Ginder and Epstein, 1990; McManus et al., 1987; Stafstrom et al., 1987). The other absorption band, at approximately 800 nm, corresponded to a polaron interband transition (Ginder and Epstein, 1990; McManus et al., 1987; Stafstrom et al., 1987). The presence of both these absorptions indicated that our material was the conducting salt form of PANI. Figure 4 presents spectrum recorded for protonated (emeraldine salt, green) PANI spot (from diluted PANI dispersion). For this polymer layer, an absorption maximum was observed at wavelength close to 770 nm and a broad minimum in the range of 480 nm and 600 nm. The spectrum was also dependent on pH of solution, where the spot was immersed; in PA solution, deprotonated PANI changed its spectrum to typical for protonated polymer. Typical spectra of protonated PANI were recorded and neither significant nor regular changes in the spectrum were observed pointing to lack of chemical reactions with the polymer. These results suggested simple ion exchange equilibrium between the polymer/dopant and solution, without noticeable change of the polymer composition.

### PANI-PA film morphology

During the polymerization of polyaniline in the presence of PA was observed a short and thick rod like structure (Tahir et al., 2005) which was similar to one observed by (Abe et al. 1989). In general, the polyaniline compounds synthesized with the selected acids were shown to have a rod shape structure. The PA-doped sample exhibited a coral-like structure, (Catedral et al., 2004). By using this method a more stable coating and desired surface can be created by adjusting the pH during preparation. This method expects the similar environment to control the polymer morphology, where the dopant anions are inserted during electrochemical polymerization method fulfilling the request of electroneutrality. Therefore, their concentrations are on the stoichiometric levels, for it is reasonable that their presence have strong influence on polyaniline morphology, conductivity, and electrochemical activity and the polymerization process itself (Arsov et al., 1998; Cordova et al., 1994; Dhaouci et al., 2008; Koziel, 1993, 1995; Lapkowski, 1990, 1993; Lippe & Holze, 1992; Okamoto & Kotaka, 1998; Pron et al., 1992; Pron & Rannou, 2002). Moreover, it was experimentally confirmed that polyaniline obtained in the presence of so called “large dopant anions”, originated from hydrochloride acid, sulfuric acid, nitric acid, p- toluensulfonic acid, and sulfosalicylic acid promoted formation of more swollen and open structured film, while the presence of “small ions” such is ClO$_4^-$ or BF$_4^-$, resulted in formation of a more compact structure (Nunziante & Pistoria, 1989; Pruneanu et al., 1998; Zotti et al., 1988). The order of the polyaniline growth was also proved to increase with the size of the dopant anion (Inzelt et al., 2000) but here we can also control the polymer morphology by adjusting the pH during PA doped PANI film preparation. Further investigation of the morphology of samples will give a better understanding of conductivity.

### Lysozyme sensor

The sensitivity and selectivity of sensor were tested and shown in Figure 6 and 7. It has been observed that the potential of the Lys sensor changed drastically with the addition of Lys before reaching a stable value at the concentration of around 100 µg/mL, after which ΔE changed slowly. On the other hand, by adding the BSA and thaumatin only very slight potential response was observed. This result indicated that the PA doped PANI electrode based Lys sensor had little or no affinity to the other protein molecules. This is consistent with the sensing mechanism where Lys was initially incorporated into the adsorbed film and extracted away to create the molecular recognition cavity. Moreover, the potential of the sensing electrode is closely related to the charge of the protein, and the net charge of the proteins is highly dependent on the pH of the solution; the pH of the testing medium is important to the response of the electrode. The isoelectric point of the protein is defined as the pH at which the net charge on the protein surface is zero. Usually, in the solution where pH is higher than the isoelectric point of the protein, it is negatively charged, while in the media with pH lower than its isoelectric point, the protein is positively charged. In our experiment, the pH of the buffer used as medium was 6.6 and 8.5. Our aim was to see if the pH was the reason that contributed to the insensitivity of the Lys sensor. Further, pH 6.6 was chosen as the pH of the buffer solution because polyaniline synthesized with PA is shown to have the highest conductivity in a near neutral solution. The characteristic of high conductivity in a neutral solution is desirable to accommodate the requirement for optimal antibody-antigen reaction (Barbourand George,
The isoelectric point of Lys, BSA and thaumatin are about 10.4, 4.6 and 12.7 respectively (see table 1). Hence, in the above experimental condition, the Lys showed to be more positively charged and had good response at both pH but in case of BSA and thaumatin the protein nature was basic and acid, respectively, but did not show noticeable response remaining still insensitive towards both proteins. This result clearly excluded the possibility that the insensitivity of the sensor to other proteins was due to the zero net charge of the target molecules or no domain-domain interactions.

**Bovine serum albumin sensor**

Similarly, the BSA sensing electrode was prepared by exposed PA doped PANI film to the buffered protein solutions of BSA overnight. Upon this condition, a big potential response was shown by the BSA while very slight or no response to Lys and thaumatin shown in Figure 8 and 9. Since in the practical application, target analyze usually coexists with a large number of interfering species, the sensor is expected to be able to identify a target molecule. It means this electrode sensor have ability to recognize a specific target even in the multi-component system. The potential of the sensor was plotted as a function of the concentration and a big potential change was observed with the addition of protein solution in the initial state, which became constant after certain concentration.

**Response time of the sensor**

The response time of the sensor was the time needed for reaching a stable reading of the potentiometer after a stepwise increase of the target molecule concentration in 2–10 min.

**Discussion about the binding/doped mechanism factors**

The mechanism of this method was based on the involvement of covalent bindings as well as the combination effect of other attractive forces between the protein molecule and PA doped PANI film surface. Moreover, the hydrogen bonding between the hydrophilic groups of the protein surface and the polymer chain as well as the specific arrangement of these interactions in shape and orientation, determined the recognition and selectivity of the sensor (Shi et al., 1999; Kaufman et al., 2007). Although the sensor was demonstrated to work well with the proteins tested so far, but still a question remains to answer: how important is geometric match between sensing molecules and sensor to function properly? The sensitivity of the sensor to the similar protein molecules indicates the geometrical complementarity is crucial to function properly. Even though the different protein molecules can have the domain-domain interactions, two possible processes may prevent them from inducing an electrochemical signal. Firstly, the protein molecule tightly adsorbed on the electrode surface may be denatured quickly because of surrounding environment and hence loses its electrochemical activity. Secondly, the loosely attached protein molecules that do not have hydrogen bonds with the electrode surface have a bigger chance to escape into the solutions instead of staying in the monolayer matrix, so that the adsorption is unstable because of kinetic reasons. Since the potential of the sensing electrode is closely related to the amount of charges on the electrode surface, the less accumulated charges due to the escaping of the approached molecules into the solution will lead to an unchanged potential of the sensing electrode. Additionally, the best potentiometric response was achieved at high protein concentration due to maximum mass of protein on the electrode surface which results maximum binding capacity of the electrode surface. When the polymer backbone of film interacted with protein, the potentiometric response not only depended on the concentration of protein being bound to the surface but also on the nature of the protein used. For example, the protein with higher molecular weight shows less change in potentiometric response. It could be because of steric hindrance as overcrowding of protein may have blocked the access of substrate to protein located closer to the electrode surface. In this work, we observed that the potentiometric response of different protein were different and it was restricted by the molecular weight of protein and to noted less change in potentiometric response to huge protein (protein having high molecular weight such as BSA and thaumatin). Moreover, the electronic state of the PA doped PANI films also changed significantly after protein exposure in the presence of BSA and Lys with different pH of buffer solution. The pH-controlled of buffer solution during the experiment provided enormous flexibility to control molecular organization, composition as well as the surface properties of PA doped PANI film. In the experiment, all the work
was done at two different pH. As we know, electrostatic and non-electrostatic interactions are related to pH phenomena. So that the biopolymers which had isoelectric point less than pH 7 acted as basic and were ruled by non-electrostatic interactions, for example in the case of BSA. On the other hand the biopolymer like Lys which had isoelectric point of 11 acted as an acid at pH 7 and was ruled by electrostatic interactions. Here, we report the pH dependence of the oxidation/dopant states of PANI. Our studies also indicated that the electroactivity of PANI was stable at pH 8.5.

**Figure 1: PANI form**

![Figure 1](image1)

**Figure 2: Experiment set up for the preparation of PA doped PANI film**

![Figure 2](image2)

**Figure: 3 Inferring domain-domain interactions from protein-protein interactions**

![Figure 3](image3)
Figure 4. PA doped PANI film

Figure 5: UV spectroscopy graph of PA doped PANI film

Table: 1 Protein nature and its behavior at pH 6.6 & 8.5 with comparison of PA doped PANI

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular weight (kD)</th>
<th>Isoelectric point</th>
<th>Protein Nature at pH 6.6</th>
<th>Protein Nature at pH 8.5</th>
<th>PANI-PA film behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>14400</td>
<td>10.4</td>
<td>Acidic</td>
<td>Less Acidic</td>
<td>Better at pH 6.6 but also show good electroactivity at pH 8.5</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>67000</td>
<td>4.6</td>
<td>Neutral</td>
<td>More Neutral</td>
<td>Better at pH 6.6</td>
</tr>
<tr>
<td>Thaumatin</td>
<td>22000</td>
<td>12</td>
<td>More Acidic</td>
<td>Less Acidic</td>
<td>Better at pH 8.5 where less acidic environment</td>
</tr>
</tbody>
</table>

Figure 6: Potentiometric response of Lysozyme sensor at pH 6.6

Graph is $\Delta E$ (mV) vs Concentration (µg/mL) (y axis vs x-axis)
Figure 7: Potentiometric response of Lysozyme sensor at pH 8.5

Figure 8: Potentiometric response of Bovine serum alumina (BSA) sensor at pH 6.6

Figure 9: Potentiometric response of Bovine serum albumin (BSA) sensor at pH 8.5
CONCLUSIONS
In this study, a protein sensor was built by a novel conducting polymer sensing mechanism, utilizing the interaction between charged functional moieties of a target protein and the complexation between the PANI and the dopant molecule. This process was demonstrated to be efficient in recognizing Lys and BSA. The selective adsorption of the target protein molecules onto the sensing electrode induced a significant potential change of the electrode and this change became more gradual above a certain concentration due to saturation of the accepting sites. The sensor also had better ability to recognize the specific protein-protein interaction. The size and shape match was demonstrated to be crucial for the precise recognition.

The results obtained in this research work indicated the ability of the synthesized PA doped PANI film to act as a biomolecular sensor at physiological pH. We aim to do further studies which will be keen on assessing other polymerization approaches and other protonic acids to increase conductivity and solubility of polyaniline at neutral pH. One potential manner would be to integrate sulphonate groups onto the polymer backbone. The addition of dopant, for example polyvinyl sulphonate, was presented to enhance conductivity of polyaniline at neutral pH. The water solubility of polyaniline synthesized from the projected way so far required to be examined. Research on the influence of the polymerization temperature below 0 °C and its corresponding a macromolecular sensor performance will examined too.

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REFERENCES
[9] Dhal PK 2001 In Molecular Imprinted Sellegren B (ed.) Elsevier Amsterdam p.185


[38] Pron A.; Laska J.; Österholm J.E. & Smith P., 1993, Processable conducting polymers obtained via protonation of polyaniline with phosphoric acid esters. Polymer, Vol. 34, No. 20, 4235-4240 ISSN0032-3861


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