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A Microelectrochemical Enzyme Transistor Based on an N-Alkylated Poly(Aniline) and Its Application to Determine Hydrogen Peroxide at Neutral pH

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Conducting polymers are important materials for several applications, among them, newly developed sensors that are based on changes in the conductivity of polyaniline (PANI). These sensors usually have pH limitations related to PANI characteristics (need for positive charge on the nitrogen atoms to get high conductivities). In this work, we report on a simple and fast treatment with propane sultone to dramatically improve PANI conductivity at neutral pH. As a result of this treatment, conductivity of the partially sultonated PANI (PSPANI) keeps its high conductivity at low pH almost unchanged, and as the pH approaches neutrality, PSPANI remains remarkably conductive (resistance grows less than 10 fold), while resistance in PANI increases up to 6 orders of magnitude. Electrochemical techniques and X-ray photoelectron spectroscopy are used to study the reaction. An application of this treatment to a hydrogen peroxide sensor is presented. Detection of <1 ppm at neutral pH could be achieved.

In recent years, several microelectrochemical enzyme transistors (also referred to as enzyme switches) have been described. They integrate the analyte signal and could therefore be an attractive approach for detection of low analyte concentrations using simple electronics. In an enzyme transistor, an enzyme-catalyzed reaction is used to change the oxidation state of a conducting polymer film deposited across the gap between two electrodes. This oxidation or reduction of the conducting polymer causes a change in the conductivity of the device, which is sensed by measuring the current flowing between the two electrodes. This type of detection is different from the amperometric and potentiometric approaches to electrochemical detection. In this case, no reference electrode is required to make the measurement; the circuitry is very simple; and although low analyte concentrations are detected, the changes in currents to be measured are not necessarily small.[1]

Bartlett and co-workers have demonstrated that poly(aniline) (PANI) is a very efficient surface for the direct electrochemical reduction of horseradish peroxidase (HRP). Thus, at pH 5, HRP catalyzes the reduction of hydrogen peroxide at a polyaniline-coated electrode at 0.05 V vs SCE without the need for any added mediator.[2] In a later work, the same authors presented an enzyme transistor based on horseradish peroxidase.[3] For this device, the change in resistance is due to the following set of reactions:

$$\mathrm{H_2O_2 + HRP\ (red) \rightarrow HRP\ (compound\ I) + 2H_2O}$$

$$\mathrm{HRP\ (compound\ I) + M + H^+ \rightarrow HRP\ (compound\ II) + M^+}$$

$$\mathrm{HRP\ (compound\ II) + M + H^+ \rightarrow HRP\ (red) + M^+}$$

where HRP (red) is the peroxidase in the ferric form, HRP (compound I) is the oxidized form of the enzyme, HRP (compound II) is the intermediate form of the enzyme, and M and M^+ represent the PANI in the conducting and the insulating forms, respectively.

The work described above was carried out using poly(aniline) at pH 5. Low-level detection of peroxide is an important goal, since peroxidase is used in binding assays. In addition, it would be useful for the detection of residual peroxides in devices used in hemodyalisis, which are cleaned with peroxide solutions, since traces of these compounds can be harmful for patients. In both cases, the samples are around pH 7, so it is desirable to remove the constraint mentioned above and to be able to work at neutral pH. Poly(aniline) behaves as a conductor in only the half-oxidized form (emeraldine) when it is protonated; above pH 5, the emeraldine deprotonates, becoming an insulator. For a poly(aniline) film, the deprotonation of the emeraldine form is associated with the egress of both protons and the associated anions from the film. This is only possible if the anions are small and mobile, for example chloride or bisulfate anions. If these mobile anions are replaced by long-chain polymeric counteranions, these become trapped within the poly(aniline) film, and the overall process changes. As a consequence of this change, the conductivity of poly(aniline) can be maintained at a much higher pH.[1] Bartlett et al. used this effect to deposit films of poly(aniline) with

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polymer counterions such as poly(vinylsulfonate)[4] or poly(styrenesulfonate),[5] which remain electrochemically active and conducting at neutral pH. This ingenious solution has several limitations: the film electrochemistry changes with time, and it must be returned to a pH 5 solution to reverse this process. If the film is left overnight at open circuit in a pH 7 solution, noticeable decreases in the peak currents were observed upon returning to pH 5 buffer. Furthermore, the conductivity of this composite material is 2 orders of magnitude lower than the corresponding values for poly(aniline)/bisulfate films, both at pH 5.[5] The limitation of this device is that after a period of time, proton concentration in the film equilibrates with the concentration in solution, and the positive charge on the emeraldine is lost.

A possible solution to this problem would be modifying the N atom in the backbone with an alkyl group. By doing so, the polymer will be doped with positive charge when in the oxidized form, and the only limitation will be the migration of anions.

A disadvantage of this approach is the disorder produced in the structure. Dao et al.[6] found an important decrease in the conductivity as the size of the group increased for a series of N-alkylated poly(anilines). Mikhael et al.[7] studied the effect of various alkylating and acylating agents, which were allowed to react with poly(aniline) in its emeraldine form. The replacement of N-hydrogens by acyl or alkyl groups strongly affected the conductivity of the polymer. With the acyl derivatives, they observed a decrease in the electrical conductivity attributed to the depletion of electron density on the polymer chains by the electron withdrawing effect of the acyl groups. The same effect was observed by Wrighton and co-workers.[8] Meanwhile, the alkylated polymer shows good electrical conductivity. These results are attributed to the fact that alkyl groups improve the Π electron density along the conjugated polymeric chain as a result of their donating effect.[7]

Several authors have modified poly(aniline) with propane sultone to obtain poly(aniline-*co*-*N*-propane sulfonic acid aniline) (PSPANI),[9,10] In particular, Chen et al.[10] observed a semiconductor behavior in chemically synthesized polymers.

On the basis of these results, we hypothesize that it may be possible to synthesize a polymer able to work at neutral pH by modifying PANI on the N atom with an alkyl sultone group. With this procedure, the hydrogen on the N atom could be replaced by an alkyl group covalently bound, which in turn produces a positively charged N when the polymer is oxidized. The anion at the end of the group, which will be retained in the polymer structure, will compensate the positive charge generated. In this paper, as part of the work to improve PANI properties for analytical application,[11] we present results of a partially sultonated electrochemically grown poly(aniline). This polymer shows a dramatic improvement in conductivity with respect to the starting material when immersed in a buffer solution at pH 7. It also exhibits a higher stability with respect to composite materials and a fast response when combined with horseradish peroxidase or microperoxidase to determine concentrations lower than 1 ppm of hydrogen peroxide.

EXPERIMENTAL SECTION

Materials. Horseradish peroxidase and microperoxidase (MP-11) were from Sigma; all other chemicals were obtained from Aldrich and were used as received and without further purification. Buffer solutions were freshly prepared, and pH was carefully controlled.

Equipment. Electrochemical experiments were performed in a Pine AFRDE5 bipotentiostat. Acquisition of the data was accomplished with a National Instruments acquisition card. A three-electrode electrochemical cell was used. An Ag|AgCl reference electrode (BAS) was used in all experiments (all potentials in this work are referred to Ag|AgCl unless otherwise stated). A platinum foil was used as counter electrode.

For resistance measurements and sensor applications, dual carbon microband electrodes were used.

The XPS experiments were performed in an ultrahigh vacuum (UHV) multitechnique Imaging XPS system (Thermo VG Scientific ESCALab 250) equipped with a hemispherical analyzer (of 150-mm mean radius) and a monochromatic Al Kα (1486.60 eV) X-ray source. The high sensitivity of this instrument obtained at a routine instrumental energy resolution of 0.5 eV fwhm at 20 eV pass energy allowed us to characterize spectral features in the core−shell regions. No charge compensation was found to be necessary for the samples studied in the present work. For XPS experiments, a specially designed working electrode was used, which consists of a 3-mm-diameter gold disk in a Teflon holder that allows the subsequent disassembly and extraction of the gold disk. The gold disk was attached onto a sample holder by double-sided carbon tape and transferred into the UHV system.

Dual Carbon Microband Electrodes Construction. The selected substrate for the construction of the dual microband electrodes was a polyimide (Kapton from Dupont). The film is heat resistant and has a thickness of 12.5 μm. Onto the substrate partially covered with tape (as to limit the zone to be painted), carbon paste was poured. The excess was then removed with a suitable straight element (such as a ruler), and only a thin film of carbon paste (as thin as the tape) was left. This process was repeated on the other side of the Kapton in such a way that both painted sides coincided. Once the carbon tape was dried in the oven at 80 °C, a wire was pasted on each side of the painted substrate (contacts). The carbon bands have a resistance of ~150 Ω. Finally, the whole electrode was covered with Araldite to insulate everything except the exposed edge and to make the electrode easy to handle. These electrodes can be reused by polishing the edge.

Electrosynthesis of PANI. Polyaniline was electrochemically synthesized from a solution of 0.52 M aniline in 1.8 M H_2SO_4 onto the dual-band electrode. The potential was cycled between −0.2 and 0.8 V. For the XPS samples, PANI was synthesized with the same technique but from a solution of 0.52 M aniline in 2 M HCl.

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Sultonation of PANI. The derivatization of PANI with propane sultone was carried out as follows: the PANI-modified electrode was held at −0.2 V for 10 min to obtain the reduced state (leucoemeraldine). It was then rinsed with deionized water and placed in a solution of NaOH to take protons out of the film and obtain the base form of the polymer. The polymer was then dried carefully with tissue paper and a drop of neat propanesultone was placed on it covering the polymer. The electrode was left overnight and then rinsed with abundant water to remove the excess of sultone.

Peroxidase Modification. The process for the electrode modification with peroxidase was similar to that used by Bartlett and co-workers.[3] Briefly, the electrode with sultonated PANI was placed in an electrochemical cell containing a solution of the enzyme (HRP or MP-11), ~120 units cm^{-3}, and 25 mM *p*-phenylenediamine. It was left at open circuit for 20 min to allow adsorption, and then 0.4 V was applied for 4 min. After that, the electrode was rinsed with abundant water and used as sensor.

Conductivity (Resistance) Measurements. Measurement of resistance of the polymers synthesized on the band electrodes was carried out modifying a technique presented by Wrighton and co-workers.[12] It consisted of fixing the potential of one of the microbands and sweeping the potential of the other some millivolts up and down. When a bias of 20 mV is applied it can be assumed that the current flowing through the polymer is the result of the electronic conduction of the film. Thus, it is inversely proportional to the resistance ($R = E/I$; R = resistance; E = potential; I = current). A source of error in the determination is the capacitive current, but it can be avoided by stopping the voltage sweep at the positive (or negative) limit until the value of current is constant. The values of resistance obtained by using this method are absolute, not specific, so the comparison between switches is only valid for polymers grown with similar geometric characteristics.

Hydrogen Peroxide Determination. The hydrogen peroxide determinations were performed as follows: the electrode with sultonated PANI and the corresponding enzyme immobilized was placed in a buffer solution of the desired pH and potentiostatized at 0 V. A potential source was connected to the microband electrodes applying a potential difference of 20 mV between them. The current flowing through the polymer was measured as a function of time. Note that no reference or counter electrode was used during this experiment. The solution was constantly stirred during the measurement. Hydrogen peroxide is added to the solution, and the current falls faster or slower depending on the concentration of the analyte, and a switching rate can be calculated. To improve the correlation, maximum switching rates are divided by the current of the switch before the addition of H_2O_2 and plotted against concentration.[3]

RESULTS AND DISCUSSION

Chemical Characteristics of the Sultonated PANI. Several authors have carried out derivatization reactions of PANI in solution, including sultonation. This method involves further purification steps. The method presented here is very simple. An electrode in which PANI was grown is exposed to neat propane sultone at room temperature for 12 h and then rinsed with water.

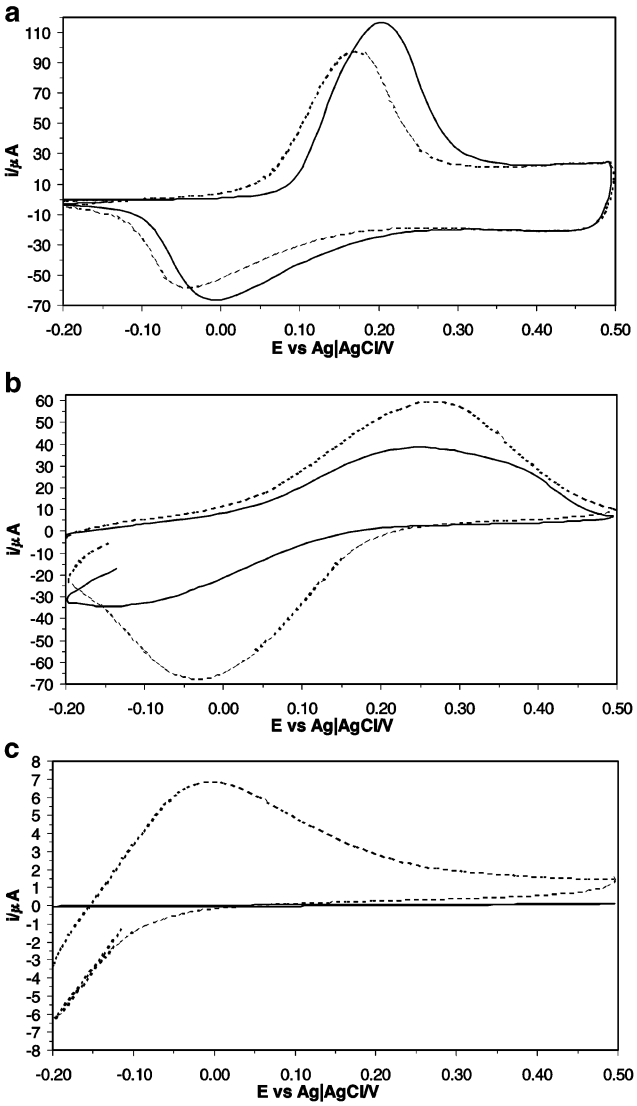

Figure 1. Cyclic voltammograms of PANI before (full line) and after (dashed line) derivatization with propane sultone in different media. (a) 1.8 M H_2SO_4; (b) 0.1 M acetate buffer pH 4.7; (c) 0.1 M HEPES, 0.1 M KNO_3 pH 7. Sweep rate: 25 mV/s

Figure 1 shows the electrochemical response of PANI-modified electrode before and after sultonation at different pHs. Figure 1a shows the electrochemical response of the modified electrode in 1.8 M sulfuric acid. It can be observed that the propane sultonated PANI (PSPANI) practically shows no changes in the amount of charge passed through the electrode and a slight shift to lower potentials for the oxidation peak. In Figure 1b, it can be seen that at pH 4.7, the PSPANI shows double the charge of PANI, and the redox couple shows a more reversible behavior in the case of PSPANI. At pH 7.0 (Figure 1c), the oxidation peak for PANI is negligible with respect to the sultonated product. These changes can be related to an improvement in the conductivity within the film as a result of the treatment. Even though there is an important enhancement in the electrochemical response, the charge observed in sulfuric acid is more than 10 times higher than that at pH 7. This is probably due to the fact that not all of the nitrogen atoms are alkylated.

The XPS spectra for sulfur and nitrogen shows that 23% of the N atoms in PANI were modified by the sultone. Figure 2 shows

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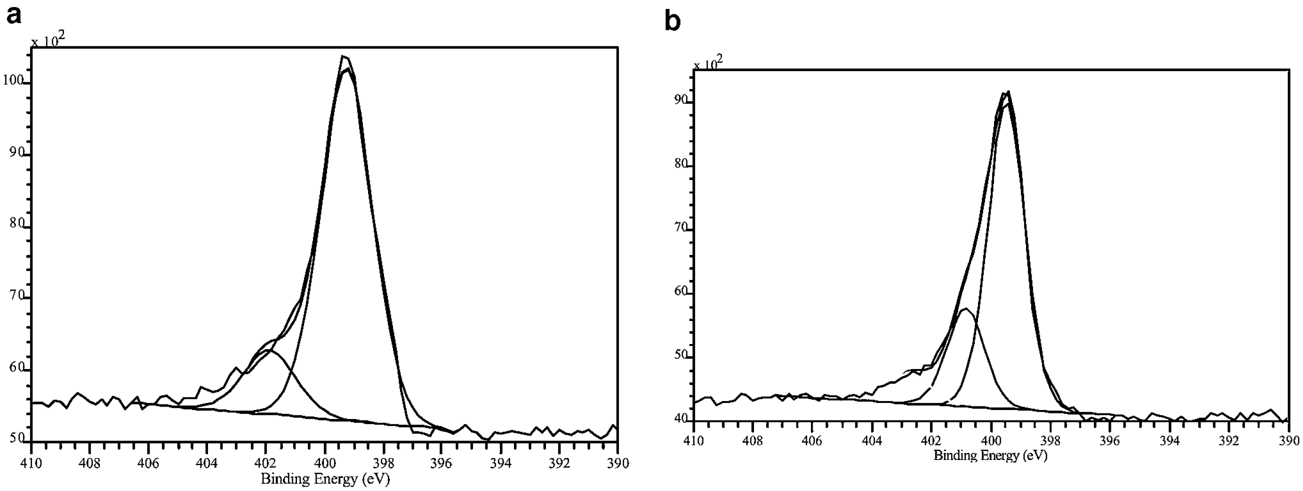

Figure 2. XPS spectra of N1s region for (a) PANI and (b)PSPANI.

Table 1. Resistance of PANI and PSPANI on Molecular Switches

medium	R of PANI/Ω	R of PSPANI/Ω
1.8 M H_2SO_4	1.7×10^3	1.7×10^3
0.1 M HAc/Ac^-, pH 4.7	4.1×10^4	1.9×10^3
0.1 M HEPES, pH 7	1.1×10^7	1.3×10^4

the XPS spectra of the completely reduced form of PANI (leucoemeraldine) and of PSPANI. For leucoemeraldine, the N1s spectrum (Figure 2a) can be deconvoluted into two peaks, with the more prominent peak at 399.3 eV corresponding to amine nitrogen and the weaker peak at 401.9 eV attributed to protonated nitrogen.[10] In contrast with leucoemeraldine (Figure 2a), the N1s spectrum for PSPANI (Figure 2b) evidently shows a shoulder. It can be deconvoluted into three peaks centered at 399.3 eV for unsubstituted amine, 400.8 eV attributed to sultone-modified N, and 403.6 eV that can be assigned to positively charged N.[10] The sultone-modified peak represents 23% of the total N area. The area of this peak is in a ratio of ~1:1 with the area of the S2p peak at 168.5 eV.

Table 1 shows the resistance of a molecular transistor constructed by growing PANI between two band electrodes before and after modification with propane sultone. At pH 7.0, a dramatic change in resistivity (reflected in a change of resistance) from 1.1×10^7 Ω for PANI to 1.3×10^4 Ω for PSPANI can be observed. The conductivity in sulfuric acid shows the mildness of the synthetic method, since the resistance in this medium is practically unchanged after the reaction. It should be noted that these molecular switches are very stable upon exposure to air or neutral solutions. The molecular switches can be left at room temperature for 2 days, and their conductivity remains almost unchanged. They can also be left in a pH 7 solution overnight and 70% of the conductivity remains the following day.

Peroxidase Modification and Hydrogen Peroxide Determination. PSPANI was modified by immersion in a solution containing HRP (or MP-11) and *p*-phenylenediamine, followed by the electropolymerization of the *p*-phenylenediamine. The peroxidase/PSPANI devices were set at 0 V in a pH 7.0 buffer solution, and then the circuit was set for the measurement of the current circulating between the bands. The device was exposed to hydrogen peroxide, and the drain current (current flowing between the two bands, through the polymer) was measured as a function of time. In these experiments, the only potential fixed from outside is the drain potential (E_{drain}), that is, the potential applied between the two bands. The potential of the whole device (E_{gate}) is free to move according to the solution; in fact, the effect of the hydrogen peroxide is that it oxidizes the polymer (via the corresponding peroxidase) and changes the oxidation state of the PSPANI. Consequently, the resistance of the film changes, and the current falls at a rate that is proportional to the H_2O_2 concentration. Figure 3 shows the response for different concen-

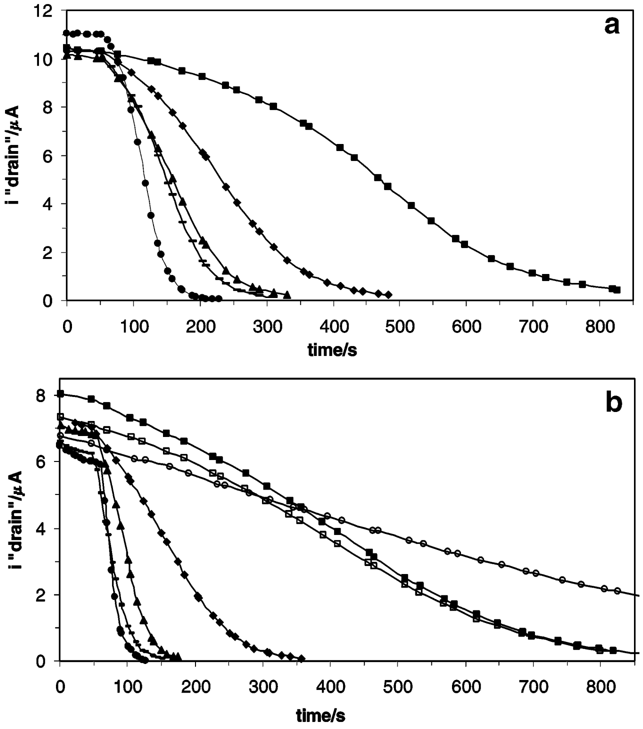

Figure 3. Switching rate (maximum slope divided by maximum current) vs time for molecular switches of PSPANI with HRP (a) and MP-11 (b) immobilized with *p*-phenylenediamine. 0.1 M HEPES pH 7, 0.1 M KNO_3. $E_{\text{drain}} = 20$ mV. Initial $E_{\text{gate}} = 0$ V vs Ag/AgCl. H_2O_2 was added at time = 50 s. Final concentrations: ●, 1; —, 0.5; ▲, 0.3; ◆, 0.2; ■, 0.1; □, 0.05; and ○, 0.025 mM. For each case (a and b), all the data correspond to one device that was reused.

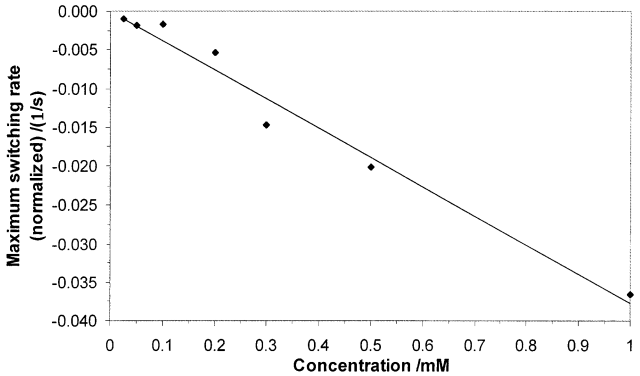

Figure 4. Calibration curve for molecular switch of PSPANI with MP-11 immobilized with *p*-phenylenediamine. 0.1 M HEPES pH 7, 0.1 M KNO_3. $E_{drain} = 20$ mV. Initial $E_{gate} = 0$ V vs Ag/AgCl. Criterion used: maximum switching rate divided by maximum current. All of the data correspond to one device that was reused. See Experimental Section for details.

Table 2. Maximum Switching Rate at Different pHs

pH	maximum slope (μA/s)
5	-8.52×10^{-2}
6	-1.00×10^{-1}
7	-1.26×10^{-1}
8	-1.65×10^{-2}

trations of hydrogen peroxide for both electrodes. Figure 3a corresponds to HRP/PSPANI. The same experiment was carried out for MP-11/PSPANI (Figure 3b). In this case, the current changes are faster than the HRP/PSPANI device, making more attractive its use for fast determinations, such as total residual peroxide.

The slope of the steepest part of the device response to hydrogen peroxide depends on the concentration of H_2O_2 and can be taken as a criterion for calibration.[13] Figure 4 depicts that the correlation between this variable and concentration closely follows a linear behavior for an extended range of concentrations for MP-11/PSPANI. The disadvantage of this method is the time consumed until the maximum slope of the current curve is obtained, mainly at concentrations bellow 0.2 mM (~7 ppm). Note that once the slope reaches its maximum, the assay can be stopped, so it is not necessary to wait until the current falls to 0. For example, for a 25 μM hydrogen peroxide solution (<1 ppm), the maximum slope is reached around 300 s.

For low concentrations, it is possible to use a different criterion. Because the change is slower, the first few seconds of the response can be taken, a straight line can be fitted (regression coefficients higher than 0.95, $n > 100$), and its slope can be calculated. By this procedure, we found that for 25 μM, the slope given by the first 100 s is three times the slope observed for the switch when it is immersed in the same buffer solution free of hydrogen peroxide.

Another aspect studied was the dependence of the time response with the pH. Table 2 shows the slope for the steepest part of the device response of MP-11/PSPANI to 0.5 mM H_2O_2 at four different pHs. The results show the fastest response at pH 7. The sharp decrease in the response at pH 8 may be attributed to the decrease in conductivity of the polymer, which in turn slows the electron-transfer process between the polymer and the microperoxidase. The decrease at lower pHs may be attributed to changes in microperoxidase reaction kinetics. Further studies must be carried out to elucidate this topic.

CONCLUSIONS

We have shown that modification of PANI with propane sultone at an extent of 20% could produce a marked improvement in the conductivity of the polymer at neutral pH without compromising its properties at lower pH. This improvement is the sum of two effects: creation of permanent positive charge on the N atoms and Donnan potential produced by immobilization of the negative charge in the polymer. Its application to hydrogen peroxide sensors is demonstrated, and its potential use in analytical devices is suggested. Work is in progress to improve the sensitivity, detection limit, and analysis time by optimizing several parameters, among them, film thickness, degree of sultonation, and adsorption time.

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