

Enhanced Loading of Glucose Oxidase on Polyaniline Films Based on Anion Exchange

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ABSTRACT: The temporal aspects of anion self-exchange in electrochemically prepared polyaniline films have been experimentally investigated. The exchange of bulkier tosylate–ferricyanide ion with Cl^- ion has been monitored by photometry and electrochemical techniques. The relative changes in porosity brought about by self-exchange have been experimentally determined to be 323 and 2125/k in tosylate-exchanged and ferricyanide-exchanged polyaniline films, respectively. Scanning electron microscopy has been used to delineate the surface morphology of polyaniline films. It is seen that the polyaniline films exhibit enhanced loading of glucose oxidase after a self-ion exchange, and, hence, they can be used for the fabrication of a third-generation glucose biosensor. © 1998 John Wiley & Sons, Inc. *J Appl Polym Sci* 70: 1447–1453, 1998

Key words: polyaniline; glucose oxidase; self-exchange; biosensor

INTRODUCTION

To date, several conducting polymers, such as polypyrrole (PPY),^{1–2} poly(*N*-methyl-pyrrole),³ polyindole,⁴ polyaniline (PANI),⁵ polycarbazole,⁶ and copolymers of *N*-substituted pyrrole⁷ have been used to immobilize desired enzymes (glucose oxidase–urease–cholesterol oxidase) using physical adsorption and physical entrapment techniques.⁸ For this purpose, PANI has been considered to be an attractive polymer since this electronic material exhibits two redox couples in the right potential range to facilitate an enzyme–polymer charge transfer. Besides this, PANI has been shown to have a variety of applications, such as in electrochemical transistors,⁹ rechargeable batteries,¹⁰ electrocatalysis,¹¹ antistatic coatings,¹² electrochromic displays,¹³ gas separation,¹⁴ and biosensors.¹⁵ However, not much information has as yet

been reported on the role of structural and mechanical behavior of PANI for its application to biosensors.

Kumaran et al.¹⁶ have recently reported the immobilization of glucose oxidase (GOD) via manipulation of pore size in PPY to improve the loading parameters of the enzyme. Keeping this in view, we have undertaken systematic studies on the spontaneous ion-exchange behavior in PANI films as it is well known that the nature of the anion species present as a counter ion plays an important role in determining the physical properties of conducting polymers. Also, the study of coupled process of ion transport and faradaic current flow in these polymer electrodes may perhaps help in improving the GOD loading.

The present article deals with the temporal aspects of the ion self-exchange process using PANI films containing paratoluene sulphonate (tosylate)–ferricyanide as dopant anions. It is known that ion exchange offers the possibility of maintaining the degree of polymer oxidation at a constant level while simultaneously varying the dop-

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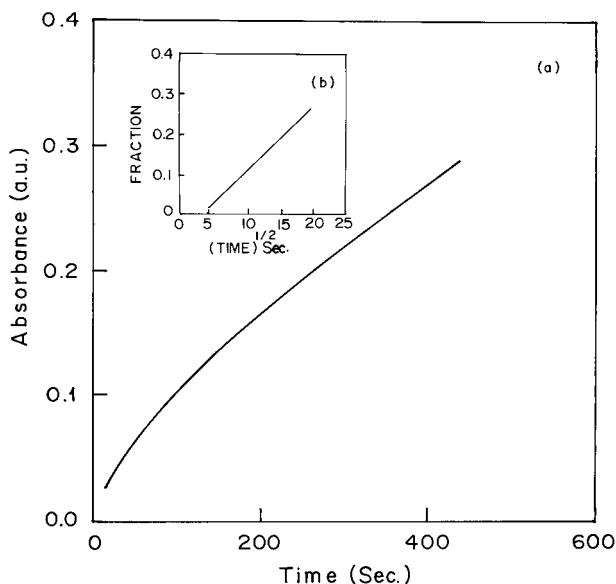


Figure 1 (a) Plot of absorbance of tosylate in solution as a function of time for the self-exchange of tosylate in polyaniline films. (b) Fraction of tosylate exchanged with Cl^- ions versus $t^{1/2}$.

ant anion. The selection of PANI-tosylate¹⁷ or PANI-ferricyanide films for such studies has been carried out due to the bulky sizes of the tosylate-ferricyanide anions that are likely to provide a significant change in porosity after their respective exchange with Cl^- . The scanning electron microscopy (SEM) technique has been used to delineate the surface morphology of PANI-tosylate and PANI-ferricyanide films containing GOD. Besides this, an attempt has been made to investigate the effect of various parameters such as pH, polymer morphology, and composition of bathing medium, to optimize the performance of the glucose biosensor thus obtained.

EXPERIMENTAL

PANI-tosylate and PANI-ferricyanide films were electrochemically synthesized by the potentiostatic technique.¹⁸ Polymerization was carried out at a potential of 0.8 V versus Ag-AgCl on an indium-tin-oxide (ITO)-coated glass substrate (sheet resistance $\sim 22 \Omega$, Balzers). The electrolyte consisted of 0.5 M aniline in 1 M HCl/0.2 M PTS (or 0.2 M ferricyanide) and was purged with N_2 gas for about 15 min prior to polymerization. Extensive washing and soaking of such PANI

films in deionized water ($R = 18 M\Omega$; Millipore, Milli-R0 10TS) was carried out to ensure that no free tosylate or HCl solution was present on the surface of the PANI film.

Anion exchange was performed at about $27 \pm 1^\circ\text{C}$ by soaking PANI films (the geometrical area was $0.9\text{--}1.0 \text{ cm}^2$) in a bathing medium containing 0.1 M KCl. Spectrophotometric measurements were carried out on a Shimadzu ultraviolet-visible (UV-VIS) spectrophotometer (Model 160 A) at 221-nm wavelength. Aliquots (3 mL) of the bathing solution were periodically used for the analysis of the tosylate content. Uniform electrode geometry was maintained throughout all the measurements, and the conductivity of the film on the electrode was monitored by four-points probe method.

Cyclic voltammetric (CV) studies were undertaken on an electrochemical interface (SI 1286, Schlumberger), which was connected to a three-electrode setup consisting of a PANI-tosylate-ITO or PANI-ferricyanide-ITO as working electrode (1 cm^2), a platinum plate (2 cm^2) as a counter electrode, and Ag-AgCl wire as a reference electrode. The bathing medium consisted of 0.1 M KCl. Morphological studies were carried out using a JEOL (JSM-35) scanning electron microscope. Thickness measurements of various PANI films were conducted by using a Talystep (Rank Taylor Hobson).

GOD (EC 1.1.3.4) from Sigma, 12.5 mg mL^{-1} , was solvated in 0.1 M phosphate buffer, pH 6, and 50 μL aliquots were physically adsorbed on electrochemically prepared PANI-tosylate and PANI-ferricyanide films. These modified elec-

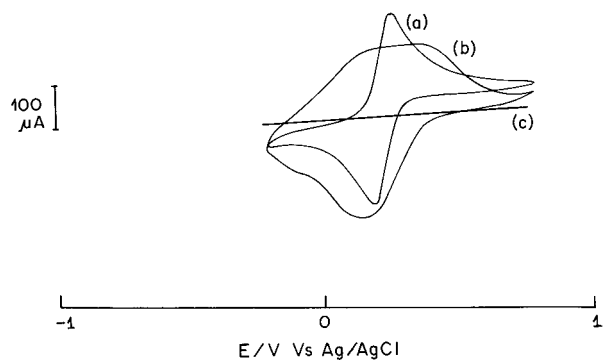
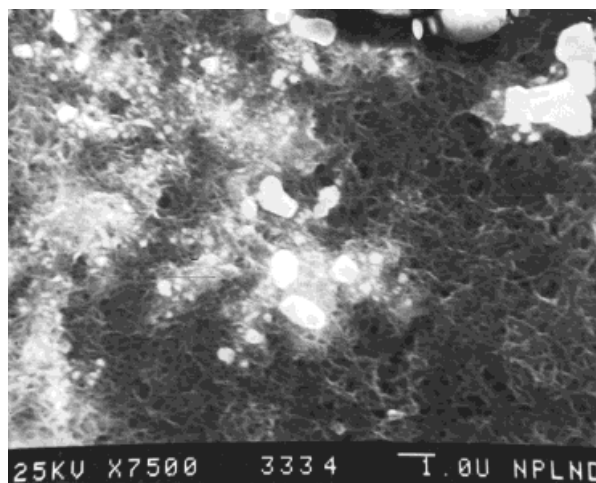
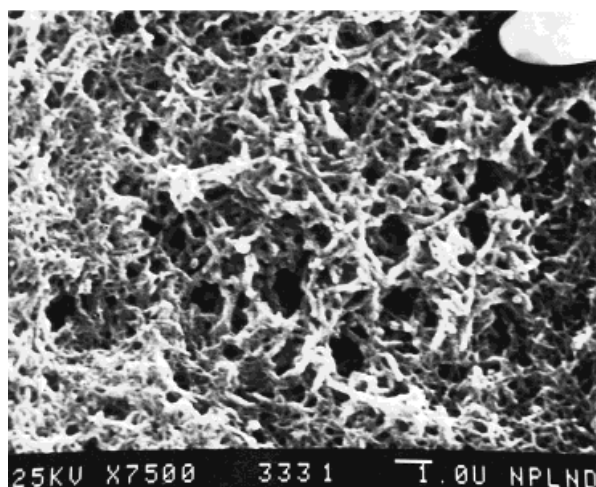


Figure 2 (a) Cyclic voltammogram of $\text{Fe}^{2+}/\text{Fe}^{3+}$ in 0.1 M KCl solution. Cyclic voltammogram of 10- μm -thick PANI films obtained in the region of -0.2 to 0.8 V versus Ag-AgCl at a scan rate of 20 mV/s. (b) PANI doped with ferricyanide in 0.1 M KCl medium. (c) CV of the same in 0.1 M KCl solution after reduction at -1 V for 2 h.



(a)



(b)

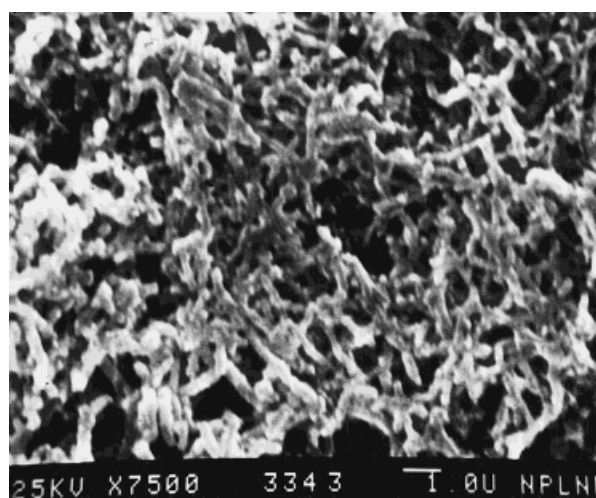
Figure 3 Electron micrographs of PANI-tosylate films (a) before exchange and (b) after exchange.

trodes were left for about 24 h at 4°C and were subsequently rinsed with phosphate buffer (pH 6) for the removal of any superficially attached GOD molecules onto the polymer matrix.

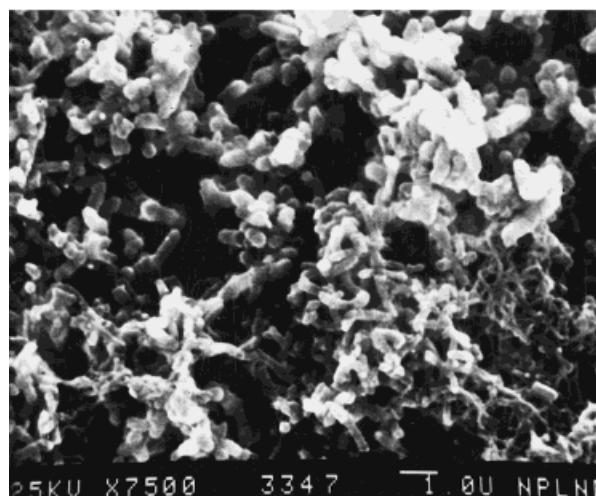
D-glucose (BDH) solution was prepared in a 0.1M phosphate buffer, pH 6, and was allowed to mutarotate at $27 \pm 1^\circ\text{C}$ for about 2 h. The amperometric response to varying glucose concentration was measured by a Keithley electrometer (Model No. EC 617) at an applied potential of 0.7 V versus Ag-AgCl on PANI-tosylate-ITO and PANI-ferricyanide-ITO working electrodes. The background current ($1 \mu\text{A}$) was first allowed to stabilize for about 1 h, after which the amperometric response measurements were systematically conducted. The activity of GOD was estimated by the *o*-dianisidine dye oxidation procedure.¹⁹

RESULTS AND DISCUSSION

Homogenous PANI-tosylate films were obtained using an electrolyte comprising of 0.5M aniline, 0.2M tosylate, and 1M HCl by the electrochemical technique with an electrical conductivity of 10^{-1}S/cm , and these films were used for the ion exchange. The exchange of tosylate ions in a bathing medium containing 0.1M KCl for the PANI-tosylate film was investigated using a UV-vis spectrophotometer [Fig. 1(a)]. As a consequence of the exchange, the tosylate ions egress out of the PANI film into the bathing medium, and the tosylate exchanged from the film was monitored with increasing time interval. Considering the exchange process across the PANI-tosylate bathing



(a)



(b)

Figure 4 Electron micrographs of PANI-ferricyanide films (a) before exchange and (b) after exchange.

Table I Swelling Effect of Polyaniline Films Before and After Exchange

Nature of the Film	Dopant Anion	Weight Before Exchange (g)	Weight After Exchange (g)	Change in Weight (g)
PANI-tosylate	Cl ⁻	0.7469	0.7784	0.0315
PANI-ferricyanide	Cl ⁻	0.6537	0.7023	0.0486

medium to be nonrate-limiting,²⁰ and the observed diffusion profiles are such that they do not effect the film boundaries, the semi-infinite planar diffusion²¹ model could be applied.

Figure 1(b) exhibits temporal evolution of the tosylate anion from the PANI-tosylate films in 0.1M KCl. The observed data was used to calculate the apparent diffusion coefficient (D_{app}) of the tosylate exchanged PANI films using the following equation²²:

$$(D_{app})^{1/2} = \frac{F \cdot \pi^{1/2} \cdot d}{4t^{1/2}} \quad (1)$$

where F is the fraction of tosylate in solution at time t to that at α , and d is the film thickness.

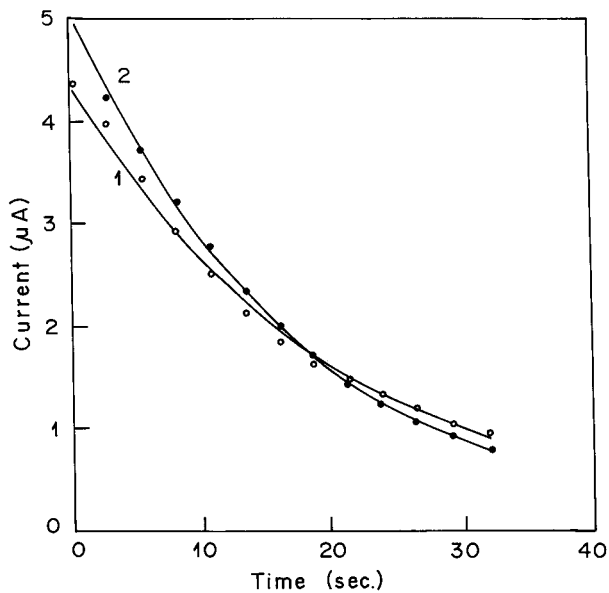
The plot of F versus $t^{1/2}$ [Fig. 1(b)] shows that the planar diffusion model is obeyed in the case of PANI-tosylate films. A similar model for the self-exchange process in polyacetylene has recently been described by Schlenoff and Chien.²³ Diffusion coefficients obtained for the PANI film (thickness = 10 μm) from the slope in Figure 1(b) yield an overall D_{app} value of $4.868 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1}$, which is in accordance with the expected dynamics of ion transport through the PANI-tosylate films.²⁴

Metal complexes have been shown to electrostatically bind to highly charged polymer films.²⁵⁻²⁶ The *in situ* binding of ferricyanide-ferrocyanide ion to PPY has been reported recently.²⁷⁻²⁸ In contrast to the behavior of tosylate films, ferricyanide-doped films do not undergo spontaneous self-exchange, possibly due to the bulky nature (0.4 nm). They can thus be flushed out into the electrolyte solution by reducing the respective films. This is also demonstrated by the cyclic voltammogram (Fig. 2) of the ferricyanide-doped PANI solution containing 0.1M KCl as the electrolyte. Figure 2(a) shows the standard $\text{Fe}^{+2}/\text{Fe}^{+3}$ redox peaks on ITO electrode in 0.1M KCl solution. CV [Fig. 2(b)] shows broad peaks, which can be attributed to the overlapping of PANI and the $[\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}]$ redox couple arising due to the high pH (= 6) of the electrolyte. However,

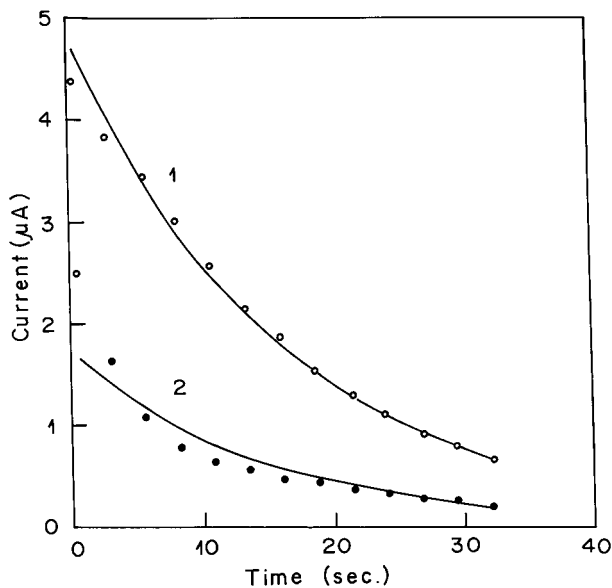
the exchange of ferricyanide may perhaps be due to the electrochemical reduction at an applied voltage of -1 V in the KCl medium following a procedure in the literature.²⁹ Such an exchange can be clearly seen in Figure 3, which shows the CV of PANI films before [Fig. 2(b)] and after the exchange [Fig. 2(c)]. The absence of any ferricyanide-ferrocyanide peak demonstrates the successful replacement of the ferricyanide by Cl⁻ ions.

Desilvestro and Scheifele³⁰ have investigated the influence of different anions on the morphology of the electrochemically prepared PANI. Figure 3 shows the SEM micrographs obtained for exchanged and unexchanged PANI films. The PANI-tosylate films after the exchange with Cl⁻ exhibit high porosity. However, unexchanged PANI-tosylate films [Fig. 3(a)] show open porous structures having fibrillar surface morphology with higher pore density and a maximum pore size of 0.57 μm , which is in agreement with the value reported.³⁰⁻³¹ After exchange [Fig. 3(b)], the morphology of the PANI film surface shows a distinct change with increased pore size (1.14 μm). On the other hand, electron micrographs of the PANI-ferricyanide unexchanged films [Fig. 4(a)] show a loosely packed network, nonuniform surface indicating granular structure (with a maximum pore size of 1.42 μm) with large cavities. It is significant to note that in the exchanged films [Fig. 4(b)], the pores appear to be interconnected with a maximum diameter of about 2.85 μm .

The increase in the number of pores is likely to result in the increase in the internal volume of the PANI film since the polymers are known to swell in the electrolyte solutions, resulting in morphological changes.³² Recently, Pisarevskaya and coworkers³³ have studied the influence of the porous structure, microkinetics, and diffusion properties on the charge-discharge behavior of conducting polymers and showed that swelled PANI and PPP films show an appreciable internal porosity and a high internal surface. With this in view, the swelling effects have been estimated in



(a)



(b)

Figure 5 (a) Chronoamperogram recorded for PANI-tosylate films in the potential range of -1.17 to +0.68 V (60 µm thick) (1) before exchange with Cl⁻ and (2) after exchange with Cl⁻. (b) Chronoamperogram recorded for PANI-ferricyanide films (1) before exchange with Cl⁻ and (2) after exchange with Cl⁻.

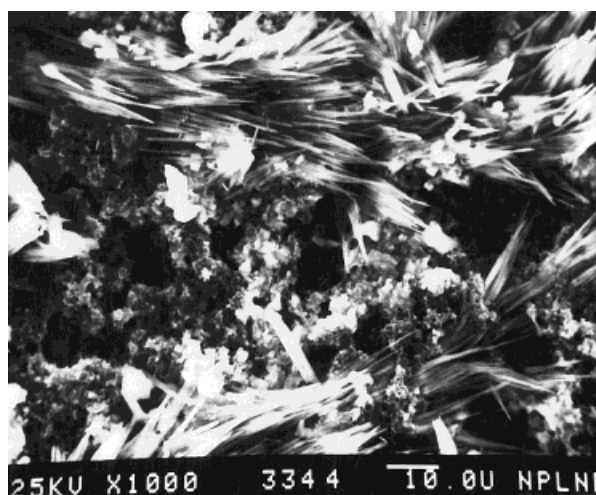
terms of total exchangeable volume, as shown in Table I. The effect of swelling after its exchange in the aqueous KCl medium could be observed by the changes in weights, which may perhaps be ascribed to the increased solvent retention after exchange. Besides this, it may be noted that the increase in volume-to-area ratio in ferricyanide-

exchanged PANI films is larger than that of tosylate-exchanged PANI films.

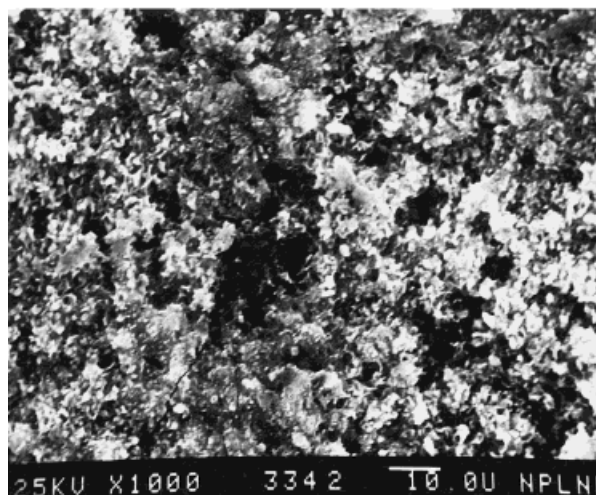
In order to delineate the porous nature of the PANI films, the chronoamperometric measurements (Fig. 5) have been conducted. Pajkossy and Nyikos³⁴ have shown that it is possible to calculate a fractal dimension D_f by fitting the chronoamperometric current transients to the following expression:

$$i = kt^{-x} \tag{2}$$

where $x = (D_f - 1)/2$ and has values ranging from 0.3 to 0.8. This equation is valid up to a temporal cutoff determined by the condition, $l > (Dt)^{1/2}$,



(a)



(b)

Figure 6 Electron micrographs of PANI-ferricyanide films (a) with glucose oxidase and (b) without glucose oxidase.

Table II GOD Activity in PANI-Tosylate and PANI-Ferricyanide Films and The Relative Changes in Porosity

Film	Exchanging Ion	GOD Activity		P
		Unexchanged Films (IU)	Exchanged Films (IU)	
PANI-tosylate	Cl ⁻	0.0029	0.2049	323/K
PANI-ferricyanide	Cl ⁻	0.656	1.98	2125/K

where l is the film thickness and D is the diffusion coefficient. Using PANI film thickness of 6×10^{-3} cm, the value of D (diffusion coefficient) has been found to be in the range of 10^{-10} – 10^{-15} cm² s⁻¹. Thus, in the time scale of 100 s, diffusion conditions may be expected to apply. Application of eq. (2) to the current transients for the PANI-tosylate electrodes thus yields a fractal dimension of 1.096 for unexchanged films and 1.116 for ion-exchanged film, indicating the increased porosity. Keeping in view the fact that differences in fractal dimensions are small, it is difficult to ascertain at this stage that this leads to a three-dimensional network in the polyaniline films. Further experiments to delineate these properties are being carried out. In the case of PANI-ferricyanide films, the values of D_f for unexchanged and exchanged films have been found to be 1.122 and 1.136, respectively, sug-

gesting increased porosity after ion exchange. The observed low value of D_f compared to the value of 2.6–2.9 in the case of PPY¹⁶ may be attributed to the disordered nature of the PANI film.

Porosity values of PANI have been calculated by Desilvestro et al.^{30,35} from the surface charge and film thickness based on an *in situ* gravimetric investigation. Presently, we have used the chronoamperometric approach to quantitate changes in porosity. Since our interest is only in the relative changes in the porosity, the following equation¹⁶ has been used:

$$\delta q/k = \delta p \quad (3)$$

where δp is the porosity change following ion exchange, δq is the difference between charge integrals for the unexchanged and the exchanged PANI films, and k is the charge integral for the nonporous PANI films having similar thickness. It is interesting to see that the relative changes in porosity calculated in tosylate-exchanged PANI film provide a porosity change of 323/ k , while the ferricyanide-exchanged PANI films show a porosity of 2125/ k . Following ion exchange, GOD was immobilized on tosylate-exchanged-unexchanged PANI films by the physical adsorption technique. Compared to the native PANI-ferricyanide films [Fig. 6(a)], SEM pictures of PANI-ferricyanide-GOD films show flakes or platelike structures, indicating the presence of GOD on the polymer surface [Fig. 6(b)].

The amounts of GOD adsorbed onto the PANI films before and after exchange indicate enhanced loading of enzyme, shown in Table II. The amperometric response of the PANI-tosylate and PANI-ferricyanide films are shown in Figure 7. It has been found that PANI-tosylate films (curves 1 and 2) compared to that of PANI-ferricyanide film (curves 3 and 4) show improved amperometric response. The higher currents obtained with ion-exchanged electrodes comprising PANI films (curves 2 and 4) clearly bring out the importance

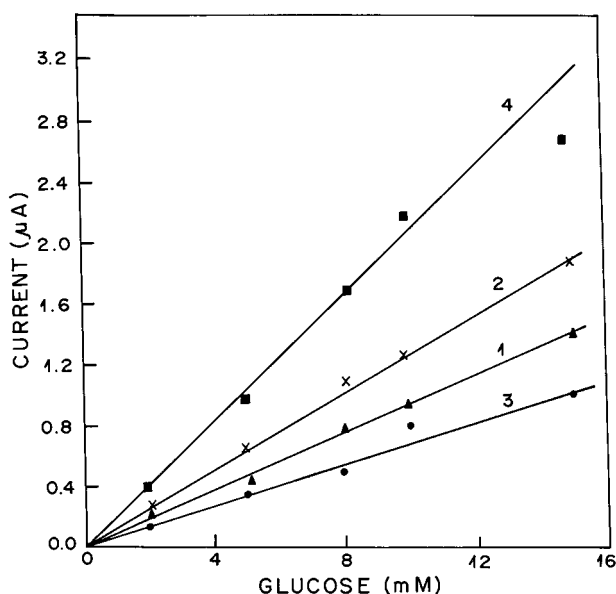


Figure 7 Amperometric response of physically adsorbed GOD to varying concentrations of β -D-glucose of PANI-tosylate films before exchange (curve 1) and after exchange (curve 2), and PANI-ferricyanide films before exchange (curve 3) and after exchange (curve 4).

of ion self-exchange as a useful technique for the development of a reagentless PANI-based glucose biosensor.

CONCLUSIONS

It has been demonstrated that self-ion-exchange in PANI-tosylate and PANI-ferricyanide films plays an important role towards the surface modification of the conducting polymers, enabling enhanced loading of enzymes. The porosity changes following self-exchange have been shown to be more pronounced in PANI-ferricyanide films compared to PANI-tosylate films. Experiments are presently in progress to delineate the nature of interaction of the immobilized GOD with conducting PANI using fluorescence microscopy³⁶ and dielectric techniques.

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