Available online at www.sciencedirect.com
SCIENCE DIRECT®
Current Applied Physics 5 (2005) 174–177
Current Applied Physics
Physics, Chemistry and Materials Science
www.elsevier.com/locate/cap

# Application of polyaniline as enzyme based biosensor

Manju Gerard [a,*], B.D. Malhotra [b]

[a] Department of Chemistry, Allahabad Agricultural Institute–Deemed University, Allahabad, U.P. 211 007, India
[b] Biomolecular Electronics and Conducting Polymer Research Group, National Physical Laboratory, New Delhi 110012, India

Available online 20 July 2004

**Abstract**

The PANI films have been synthesized electrochemically and are used as matrix for immobilization of glucose oxidase (GOD) and lactate dehydrogenase (LDH) enzymes. The temporal aspects of anion self-exchange in PANI films have been 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. 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.

Lactate is determined by the photometric detection of NADH formed in the reaction catalysed by LDH. Studies have been carried out with PANI as a matrix for the immobilization of LDH and its feasibility as a biosensor. The results of the photometric and amperometric measurements conducted on such LDH/PANI electrodes show a response to pyruvate concentration upto 0.45 mM, a response time of 90 s and a shelf life of about two weeks.


*PACS:* 82.35.Cd; 82.45.Aa; 82.47.Rs; 87.80.−y
*Keywords:* Polyaniline; Electrochemical; Glucose; Lactate; Biosensor

## 1. Introduction

Recently conducting polymers have attracted much interest in the development of biosensors. The electrically conducting polymers are known to posses numerous features which allow them to act as excellent material for immobilization of biomolecules and rapid electron transfer for the fabrication of efficient biosensors. To date, several conducting polymers such as polypyrrole (PPY), poly (*N*-methyl pyrrole), polyindole, polyaniline (PANI), polycarbazole etc. have been used to immobilize desired enzymes (glucose oxidase, urease, cholesterol oxidase) etc. using physical adsorption and entrapment techniques [1,2]. For this purpose PANI is considered to be an attactive 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, rechargeable batteries, electrocatalysis, antistatic coatings, electrochromic displays, gas seperation and biosensors [3,4]. However, the role of structural and mechanical behavior of PANI for its application to biosensor is yet to be explored.

The rapid, accurate and selective assay of L-lactate and pyruvate has been considered necessary in clinical biology and in food processing chemistry. Increased lactate concentration levels in blood indicate various pathological states such as respiratory insufficiencies, heart and liver diseases etc. [5]. Pyruvate is a key intermediate in the pathway leading to acetyl co-enzyme which is the precursor of Kreb's cycle. Its determination is therefore important as a considerable number of relevant metabolites and enzyme activities can be traced back

* Corresponding author.
*E-mail address:* manjuge@rediffmail.com (M. Gerard).

1567-1739/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.cap.2004.06.016

to pyruvate into L-lactate and the reverse reaction of L-lactate oxidation into pyruvate.

The present article deals with the temporal aspect of the ion self-exchange process using PANI films containing paratoulene sulphonate (tosylate) and 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 dopant anion. 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. Studies have been carried out on immobilization of LDH physically adsorbed on electropolymerised PANI films. And the photometric and amperometric measurements have also been carried out.

## 2. Experimental

PANI-tosylate and PANI-ferricyanide films were electrochemically synthesized by potentiostatic technique. Anion exchange was performed at about $27 \pm 1$ °C by soaking PANI films in a bathing medium of 0.1 M KCl. Spectrophotometeric measurements were carried out on a Shimadzu UV-visible spectrophotometer. Cyclic voltammetric studies were undertaken on an electrochemical interface connected to a three-electrode set up. Morphological studies were carried out using a scanning electron microscope. GOD (EC 1.1.3.4) from Sigma along with buffer solution was adsorbed on the electrochemically prepared PANI-tosylate and PANI-ferricyanide films. The amperometric response to varying glucose concentration was measured by a Keithley electrometer and the activity of GOD was estimated by the *o*-dianisidine dye oxidation procedure.

Immobilization of LDH (EC 1.1.1.27 Type XI extracted from rabbit Muscle) onto PANI films was carried out by physical adsorption. The activity of LDH both in solution and in immobilized PANI films was measured by a photometric assay using a UV-visible spectrophotometer. The amperometric response measurements of LDH/PANI/ITO electrodes were performed with a Keithley programmable electrometer (Model 617).

## 3. Results and discussions

Homogenous PANI-tosylate films were obtained using an electrolyte comprising of 0.5 M aniline, 0.2 M tosylate and 1 M HCl by electrochemical technique. The exchange of tosylate ions was carried out in bathing medium of 0.1 KCl solution (Fig. 1(a)). As a consequence of 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. Fig. 1(b) exhibits temporal evolution of the tosylate anion from the PANI tosylate films in 0.1 M KCl. The observed data was used to calculate the apparent diffusion coefficient (Dapp) of the tosylate exchanged PANI films using the following equation:

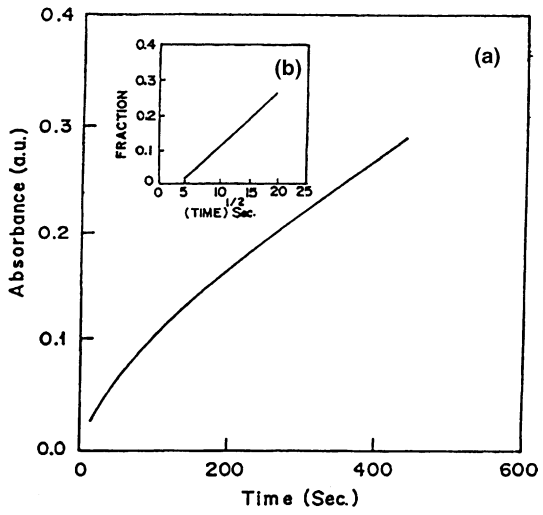


Fig. 1. (a) Plot of absorbance of tosylate in solution as a function of time for the self-exchange of tosylate in polyaniline films. (b) Fraction of tosylate exchanged with $Cl^-$ ions versus $t^{1/2}$.

$$(\mathrm{Dapp})^{1/2} = \frac{F \cdot \pi^{1/2} d}{4t^{1/2}},$$

where $F$ is the fraction of the tosylate in the solution at time $t$ to that at $\alpha$ and $d$ is the film thickness. Using PANI films thickness of $6\times10^{-3}$ cm, the value of $D$ (diffusion coefficient) has been found in the range of $10^{-10}$–$10^{-15}$ $\mathrm{cm^2 s^{-1}}$. And Fig. 2(a) and (b) shows the choronoamperogram for the PANI films (60 μm thick). In contrast to the behaviors of tosylate films, ferricyanide doped films do not undergo spontaneous self-exchange, possibly due to the bulky nature (0.4 nm). The SEM photographs obtained for the PANI-tosylate films show a fibrillar morphology with the maximum pore size of (0.57 μm) for unexchanged PANI-tosylate and (1.14 μm) for exchanged films. Similarly, PANI-ferricyanide films show a loosely packed network with pore size of (1.42 μm) for unexchanged and (2.85 μm) for exchanged films. Thus the exchange results in increase in number of pores. Similar work has been carried out by Desilvestro and Scheifele [6,7].

Following ion exchange GOD was immobilized on tosylate and ferricyanide (exchanged & unexchanged) films. The amount of GOD adsorbed onto the PANI-tosylate films before (0.0029 IU) and after exchange (0.2049 IU) and for PANI-ferricyanide films before (0.656 IU) and after exchange (1.98 IU) indicate enhanced loading of enzyme. The relative porosity for PANI- tosylate exchanged films is $323/k$ and for PANI-ferricyanide exchanged films is $2125/k$ where $k$