



## FABRICATION OF POLYPYRROLE / MWCNT NANOCOMPOSITE FILMS AS LACTATE BIOSENSOR FOR SWEAT ANALYSIS

**B. Meshram<sup>1\*</sup>, S. Kondawar<sup>1</sup>, R. Mahore<sup>2</sup>, A. Mahajan<sup>1</sup>, D. Burghate<sup>2</sup>**

<sup>1</sup>Department of Physics, R.T.M Nagpur University, India

<sup>2</sup>Department of Physics, Shri Shivaji Science College, Nagpur, India

\*Corresponding author email: [bhavna.ilamkar@gmail.com](mailto:bhavna.ilamkar@gmail.com)

### Abstract

The nanocomposite films of polypyrrole (PPy) and functionalized multiwalled carbon nanotubes (MWCNT) were electrochemically synthesized on stainless steel in 0.1 M aqueous solution of dodecylbenzene sulfonic acid. The films deposited on electrode were used for the immobilization of enzyme for determination of lactate in sweat. The enzyme, lactate oxidase (LOD) was immobilized by cross-linking via glutaraldehyde onto PPy/MWCNT film deposited on stainless steel. The immobilization was done by cross-linking 2 mg/ml LOD via 0.1% glutaraldehyde at 0.1 M phosphate buffer with 7.0 pH. The synthesized composite films were characterized using Cyclic Voltammetry (CV), Fourier Transform Infrared spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). The porous morphology of the composite films and ability to promote electron transfer reaction of MWCNT fillers leads to high enzyme loading and an increase in lifetime, stability and fast response time of the enzyme electrode. These PPy/MWCNT/LOD electrodes when tested for lactate in artificial sweat have shown a detection limit of 0.1 mM/l, a response time of about 60s and a shelf-life of about 2 weeks with a linear response range from 0.5 to 6 mM.

**Keywords:** Lactate biosensor, Enzyme immobilization, Conducting polypyrrole, Carbon nanotubes.

### 1. Introduction

A Biosensor is an analytical device, which converts a biological response into a quantifiable signal [1]. A large number of Biosensors are available in medical analysis all based on in-vivo method. Outcome through a non-invasive method is the main aim to be achieved in health care. The advantage of measurement of metabolite in media other than blood has become increasingly significant. In our research we are using sweat as a non-invasive analyte for detection of Lactate. Lactate detection is helpful for monitoring respiratory insufficiency, shocks, heart failure, metabolic disorder and Psychological disorder [2]. Nanocomposite films of carbon nanotubes with material such as conducting polymer are very attractive



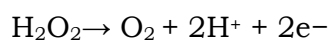
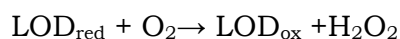
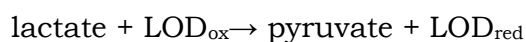
combinations for the development of electrochemical sensors and biosensors. [3]. Carbon materials have been used as components in electrochemical biosensors for over a decade. Carbon nanotubes (CNTs) are promising materials for sensing applications due to several intriguing properties [4]. In particular, their large length-to diameter aspect ratios provide for high surface-to-volume ratios. Moreover, CNTs have an outstanding ability to mediate fast electron-transfer kinetics for a wide range of electroactive species, such as hydrogen peroxide or NADH [5]. In addition, Carbon nanotubes have established themselves as a good material for immobilization of enzymes, as these tubes could retain enzyme activity, due to the desirable microenvironment [6].

Several studies in the fields of new materials have introduced the possibility to use Conducting Polymers as suitable matrices to disperse nanostructural elements, such as carbon nanotubes (CNTs). It has been shown that the introduction of CNTs into a polymer matrix can improve the electrical conductivity and the mechanical properties of the original polymer matrix [7]. Among various conducting polymers, polypyrrole (PPy) as an intelligent material plays an important role in the electrochemical biosensors [8]. The versatility of this polymer is determined by its biocompatibility, capability to transduce energy arising from the interaction of analytes and analyte recognizing sites into electrical signals that are easily monitored, capability to protect electrodes from interfering material, and easy way for electrochemical deposition on the surface of any type of electrode. PPy films can be easily formed from aqueous solutions by electrochemical routes at neutral pH [9]. A variety of urease biosensors with high sensitivity and excellent reproducibility based on polypyrrole and carbon nanotubes nanocomposites has been reported [10]. Electrochemical biosensors are based on monitoring electroactive species that are either produced or consumed by the action of the biological components (e.g., enzymes and cells) [11]. These electrochemical transducers use the powerful features of the corresponding electrochemical methods, for example, potentiometry, amperometry, cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). In the case of potentiometric sensors a local equilibrium is set up at the sensor interface, and the electrode potential is measured. For amperometric sensors, a potential is applied to drive the



electrode reaction, and the resulting current is measured. In cyclic voltammetry, the electrode potential is scanned linearly using a triangular potential waveform, and the current is measured [12, 13].

In this paper, we report electrochemical synthesis of polypyrrole-multiwalled carbon nanotube (PPy-MWCNT) nanocomposite films by direct oxidation of pyrrole in 0.1 M aqueous solution of dodecylbenzene sulfonic acid (DBSA) and containing a certain amount of MWCNT on steel electrode as redox mediator. Lactate oxidase (LOD) then immobilized by cross-linking with glutaraldehyde on top of a nanocomposite film of polypyrrole/MWCNT. Reaction Mechanism of enzyme biosensor LOD catalyzes the conversion of lactate to pyruvate and hydrogen peroxide, which can be oxidized at the electrode surface, according to the following reactions [14];



## 2. Experimental

### 2.1 Chemical and Reagents

Lactate Oxidase from *Pediococcus* lyophilized powder,  $\geq 20$  units/mg solid from Sigma, Pyrrole (with 98% purity) from Sigma-Aldrich, Multiwalled Carbon nanotubes (MWCNT) from NPL Laboratory Delhi, dodecylbenzenesulphonic acid (sodium salt) and glutaraldehyde from Merck were purchased and used as received. All the other reagents were of AR grade. All solutions were prepared using de-ionized water.

### 2.2 Fabrication of PPy/MWCNT nanocomposite films

Electrochemical synthesis of PPy/MWCNT nanocomposite film was performed in a one-compartment three-electrode glass cell at room temperature (27 °C) using a potentiostat-galvanostat under computer control. The stainless steel plate (5cm x 0.5cm) was used as a working electrode, platinum wire as a counter electrode and Ag/AgCl was used as a reference electrode. To perform the experiment, MWCNTs were functionalized by sonication using 3:1 sulfuric-acid/nitric-acid mixture for 3 hrs. Subsequently, the pretreated CNT were washed with water, then with 0.1M NaOH (to reach neutrality of pH 7.0), filtered, and dried overnight at 80°C [15]. The functionalized MWCNT (5%, 10%, and 15% by weight of pyrrole) were dispersed in 0.1 M DBSA aqueous solution and sonicated for 1 h. Pyrrole 0.1 M was dissolved in this solution under ultrasonic stirring for 15 min at



room temperature. Then, nanocomposite films were electrochemically synthesized by electrolyzing this medium on a steel electrode. PPy/MWCNT films were washed repeatedly with de-ionized water and methanol and then dried at room temperature under vacuum. Electrochemical polymerizations were performed by sweeping the potential on the working electrode, from  $-0.8$  to  $+0.8$  V versus a standard counter electrode, at room temperature with a scan rate  $50$  mV/s.

### **2.3 Immobilization of LOD on PPy/MWCNT nanocomposite films**

The enzyme LOD was immobilized by cross-linking via glutaraldehyde onto PPy/MWCNT films, thus restricting the leaching of the enzyme from the film. The stock solution of LOD was prepared in phosphate buffer ( $0.1$  M, pH  $7.2$ ).  $2$  mg/ml LOD was adsorbed onto the surface of PPy/MWCNT films. These films were subsequently dipped in  $0.1\%$  glutaraldehyde solution, left for about  $30$  min and were later washed  $2-3$  times with excess of phosphate buffer. This kind of immobilization results in a greater physical and chemical stability of the catalytic material due to the cross-linking formed with the glutaraldehyde and enzyme. In this case, the active sites of the enzyme could be more accessible for the enzymatic reaction [16]. The enzyme electrode prepared was immersed in a phosphate buffer and kept at  $4$  °C in a refrigerator overnight.

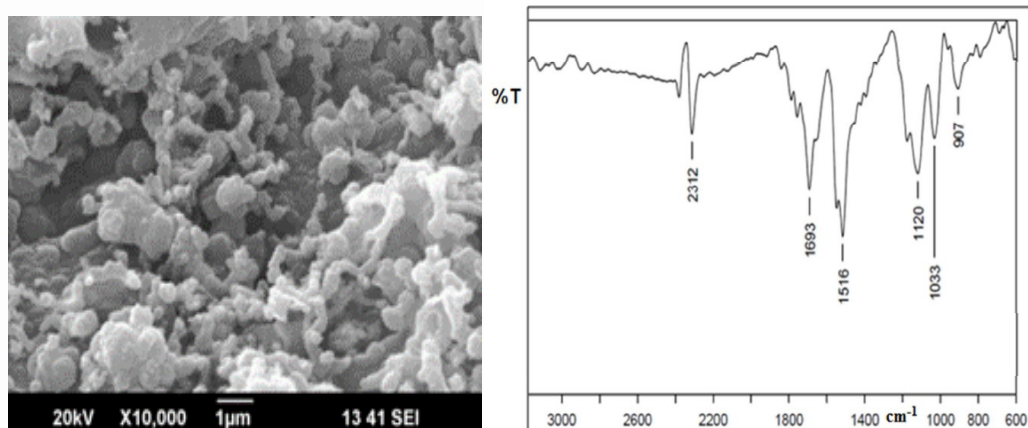
### **2.4 Characterization**

The morphology of the film was studied using scanning electron microscope (SEM, JSM-6390 model). The infrared spectrum of the film was obtained using Bruker Alpha FT-IR spectrometer. The absorption spectra of the film were obtained from a UV-Vis spectrophotometer Shimadzu-UV1800. Cyclic Voltammetry (CV) of PPy/MWCNT films was studied using CHI600D Electrochemical Analyzer. Amperometric response measurements for the detection of lactate were performed using PPy/MWCNT/LOD as working electrode and platinum (Pt) as counter electrode. The electrodes were polarized at a potential of  $0.4$  V. All measurements were carried out at room temperature on CHI600D Electrochemical Analyzer with successive addition of  $0.1$  ml of  $1$  mM Lactic acid in  $0.1$  M Phosphate Buffer Solution (pH  $7.2$ ) at potential of  $-0.5$  V.

## **3. Results and discussion**

SEM image of PPy/MWCNT nanocomposite is shown in Fig. 1. It can be seen from the image that the composite exhibits well dispersed carbon nanotubes enwrapped uniformly with PPy. This suggests that the interaction between polymer molecules and MWCNTs overcomes the Van-der Waals interaction

between MWCNTs. Such morphology is in agreement with early studies of conducting polymer/CNT composites [19, 20].

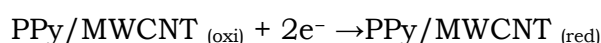
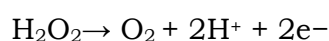
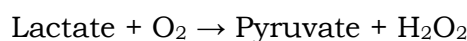


**Fig. 1: SEM image of PPy/MWCNT Fig. 2: FTIR spectra of PPy/f-CNT**

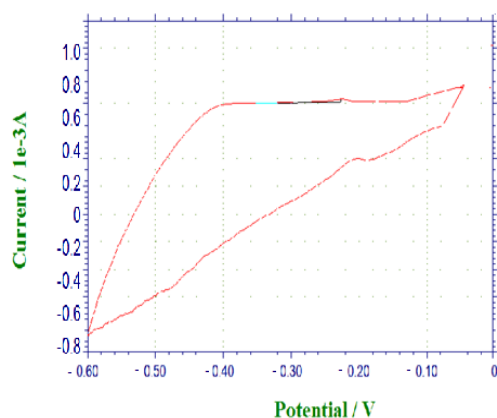
FTIR spectrum of PPy/MWCNT nanocomposites is shown in Fig. 2. The characteristic bands at 1517, 1177, 1033, 907 and 650  $\text{cm}^{-1}$  corresponds to C=C vibration, C–C–O stretching vibration, C–H bonding and ring deformation in PPy [21]. Significant differences between IR spectra of pure PPy and as-synthesized PPy/MWCNT nanocomposites appear for the bands ascribed to pyrrole ring vibrations, located around 688, 912, 1177  $\text{cm}^{-1}$ . These absorption bands are sensitive to the oxidation level and to the conjugation length of the PPy chain. The shift of these bands to lower frequencies in the nanocomposites spectra as compared with PPy suggests that an interaction between the polymer and CNT occurs [22].

The electroactivity of PPy/MWCNT nanocomposite films were studied in a monomer-free 0.1 M aqueous solution of  $\text{LiClO}_4$ . As can be seen from the Fig 3, the CV shows a couple of broad cathodic and anodic peaks. The anodic peak was found at about -0.4 V and the cathodic peak was at about -0.2 V [17]. The rise in the anodic current at -0.4 V potential range corresponds to the oxidation of the pyrrole monomer [18]. There is no significant difference between the CVs of pure PPy and PPy/MWCNT, indicating that the electrochemical activity of the composite film results from its PPy component. Fig 4 shows Amperometric response of PPy/MWCNT/LOD biosensor, with successive addition of 0.1ml of 1 mM

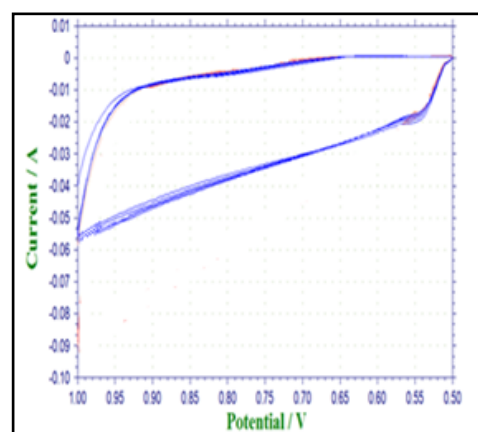
Lactic acid in 0.1 M PBS (pH 7.2) at potential of -0.5 V. As soon as the lactate was introduced into the buffer, the Lactate was converted into pyruvate and  $H_2O_2$  by LOD immobilized onto PPy/MWCNT. The electrons thus generated from  $H_2O_2$  were detected at the applied potential utilizing PPy/MWCNT/LOD as electron transferring medium. The reactions involved were as follows:



It can be seen from Fig. 4 that the response current increases linearly with increasing concentration of lactate in the range of 0.5 - 6 mM, which is due to the produced ammonium from the enzymatic reaction, then reached saturation.

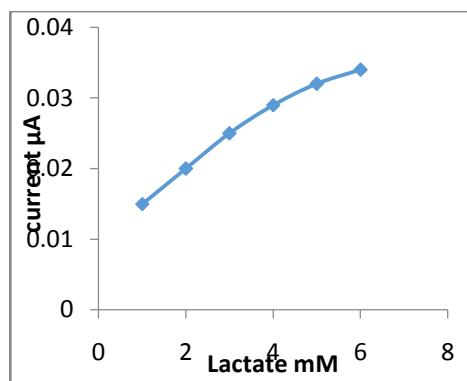


**Fig. 3: Cyclic voltammograms of PPy/MWCNT nanocomposite**

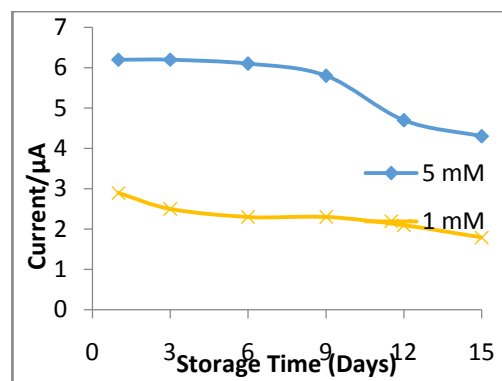


**Fig. 4: Amperometric response of PPy/MWCNT nanocomposite**

The calibration lines as shown in Fig. 5, were calculated by taking the maximum current reading for each concentration in Fig. 4 subsequently plotting current value versus lactate concentration. The detection limit and sensitivity was calculated to be 0.1 mM/L and 0.028 $\mu$ A/mM. The greater sensitivity was due to the incorporation of CNT deposited PPy film of the electrode. Fig 6 shows the response of PPy/MWCNT/LOD electrodes as a function of storage time in the presence of lactate 1 mM ( $\diamond$ ) and 5 mM ( $\times$ ) in phosphate buffer (0.1 M, pH 7.2).



**Fig. 5: The lactate calibration curve of PPy/MWCNT/LOD biosensor**



**Fig. 6: Response of PPy/MWCNT/LOD electrode as a function of storage**

PPy/ *f*-MWCNT/ LOD electrodes were tested for stability over 2 weeks. When not in use, the electrodes were stored at 4–10°C. Fig. 6 depicts the response of PPy/MWCNT/LOD electrodes for 1 and 5mM of lactate. It can be seen that there is an initial sharp decline in response followed by a gradual decrease. After about 10 days the sensor response is still significant and thus these can be used for lactate determination for about 15 days.

#### 4. Conclusion

Fabrication of sensitive redox lactate biosensor based redox species mediated and immobilization of LOD on PPy/MWCNT nanocomposite film on stainless steel electrode by electrochemical method was successfully done. PPy/MWCNT/LOD electrode when tested for lactate in artificial sweat, have shown detection limit of 0.1 mM/l, response time of about 60s, shelf-life of about 2 weeks, linear response range from 0.5 to 6 mM. The simple fabrication method of the biosensor has many advantages such as ease of fabrication, enhanced electrocatalysis, and efficiently preserving the activity of biomolecules. The biosensor can be tested to simultaneously detect several analytes in human sweat.

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