World Applied Sciences Journal 9 (Special Issue of Nanotechnology): 23-25, 2010
ISSN 1818-4952

Immobilization of Horseradish Peroxidase onto Polyaniline Nanoparticle Monolayer

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Abstract: Enzyme have been covalently bonded to a wide variety of water- insoluble polymeric supports. Recently, the class of organic polymers called ‘ conductive polymers’ has attracted considerable attention and has been used as supports for enzyme immobilization. Among these polyaniline emeraldine base (PANI-EB) occupies an important place because of its unique properties, including good environmental stability, the simplicity of doping process and its excellent redox recyclability enabling polymers with significant differences to be constructed by means of simple acidic or basic treatments. This study intends to see the effect and properties Horseradish Peroxidase (HRP) when it is deposited onto PANI-EB nanoparticle monolayer by using electrodeposition technique. During electrodeposition, HRP will be deposited on PANI-EB monolayer that act as a cathode. For U.V.- visible spectrum the PANI-EB with and without HRP shows two sharp absorption peaks at ~320nm and ~800nm. HRP helps in increasing the conductivity of PANI-EB. This support by a resistivity value obtained from four point probe measurement. Uniformity during electrodeposition can be seen from VPSEM result. As a conclusion, immobilization of enzyme onto PANI-EB can helps in increasing the conductivity properties of PANI-EB.

Key words: HRP · PANI-EB · Enzyme · Polymer · Conductivity

INTRODUCTIONS

Organic conducting polymers have attracted interest in recent years because they exhibit a wide range of novel electrochemical properties. Among the most studied is polyaniline (PANI) which has been studied extensively as an important conducting material that possesses interesting electrical, electrochemical and optical properties. Advantages of utilizing polyaniline- coated electrides in biosensors are impressive signal amplification and elimination of electrode fouling. This polymer also provides a suitable environment for immobilization of biomolecules. Immobilised enzymes can offer many advantages over their soluble forms making this a topic of active research in enzyme technology for industrial applications [1,2]. Operational advantages of immobilised enzymes include reusability, rapid termination of reactions, control of product formation and easy preparation from the reaction medium [3]. There are various methods for enzyme immobilisation as well as support materials. The methods and supports used for immobilisation of certain enzymes are chose to ensure the highest retention of activity and its stability. Thus the activity of a covalently bound enzyme can vary from zero to high values depending on the success of the immobilisation process. Polyaniline (PANI) is a environmentally stable polymer with excellent physical and chemical properties that has attracted considerable attention in recent years, especially because of electrical conductivity of this polymer family, which is used in the construction of conductimetric biosensor.

This paper reports the process of immobilised enzymes on polymeric substrate and a role of enzymes in PANI-EB conductivity. The characterization was done by using Uv-Visible, FTIR, VPSEM and four point probe.

MATERIALS AND METHODS

Polyaniline (PANI) emeraldine base (Sigma Aldrich; MW 10000) was dissolved using methanol (Merck) as a solvent. The resulting solution is kept in ultra sonication bath for 1 hours. The ultra sonicated solution was filtered

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and was spread over Langmuir- Blodgett trough, at air/ water interface and a time of 30 min was allowed for solvent evaporation. Surface pressure against area/ molecule isotherm was recorded using simple filter paper as sensor (Wilhelmy plate). The transfer pressure was maintained at 12mN/m. The pH of the subphase water was maintained at pH 1 by adding *p*- toluene sulfonic acid. Langmuir Blodgett trough (Model LB 312DMC) was used to see the formation of PANI-EB monolayer. The PANI-EB monolayer that formed was deposited on Indium Tin Oxide (ITO) glass and was dried at room temperature. For electrodeposition process, PANI-EB monolayer that deposited on ITO glass will become as a cathode electrode and platinum electrode become as an anode electrode. Phosphate buffer solution was used as medium solution. Electrodeposition process was done with using 750A with voltage 15V and about 5 minutes.

RESULTS AND DISCUSSION

The UV-visible spectra of PANI-EB with and without HRP are shown in Figure 1. It indicates that PANI-EB have higher rate absorption when HRP are added. PANI-EB is an organic polymer that has ability to absorb electromagnetic radiation because of the existence of its electron valency that can be excited to higher level. The result shows, the range of wavelength is around 300nm and above because of limitation by a certain functional groups (chromophore) that have low excitation energy. The PANI-EB shows two sharp absorption peaks at 320nm and 800nm. The peak seen at 320nm can be attributed to the π-π* transition centred on the benzenoid ring and the peak at ~800nm can be understood to arise from the polaron bands.

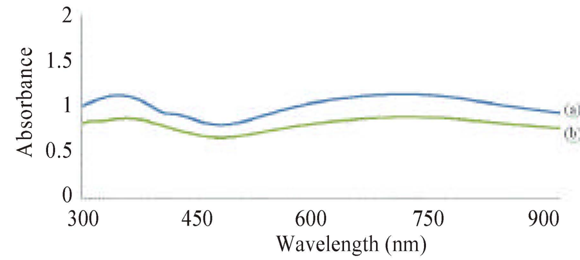

Fig. 1: Variable spectrum of U.V.-visible PANI-EB (a) with HRP (b) without HRP

Table 1: Resistivity of PANI-EB when added by HRP

	Voltage (mV)	ρ_s(ohms/ square)
PANI-EB	15.51	7.029 X 10^7
PANI-EB/ HRP	10.24	4.640 X 10^7

Figure 2 shows the FTIR spectrum of PANI-EB/ HRP dissolved in phosphate buffer solution. FTIR analysis proves the existence of the new peaks at 3448 cm-1 that show the occur interaction between PANI-EB/HRP. The peaks seen at 2450, 1070, 955, 864, 618 and 537 cm-1 are characteristics of PANI-EB that correspond to the stretching vibrations of N=quinoid=N. While peak seen at 1263 and 518 cm-1 are characteristics of HRP due to the α-helical structure

$$\text{Sheet resistivity, } \rho_s = 4.532 \frac{V}{I} \quad (1)$$

Table 1 shows resistivity value when PANI-EB was added by HRP. HRP play a role to amplify a weak voltage and increase the signal. By doing electrodeposition HRP on PANI-EB monolayer on ITO glass, we can see the different surface forms before and

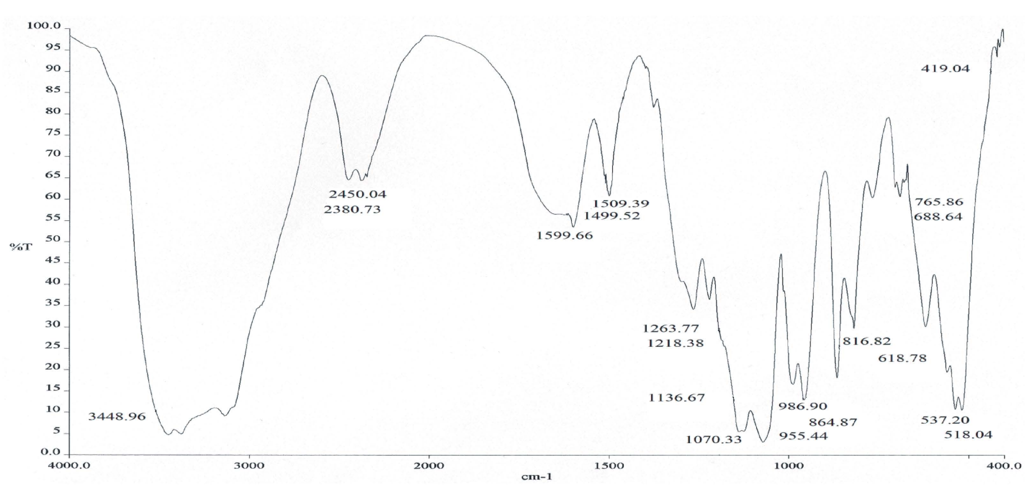

Fig. 2: FTIR spectrum PANI-EB/HRP

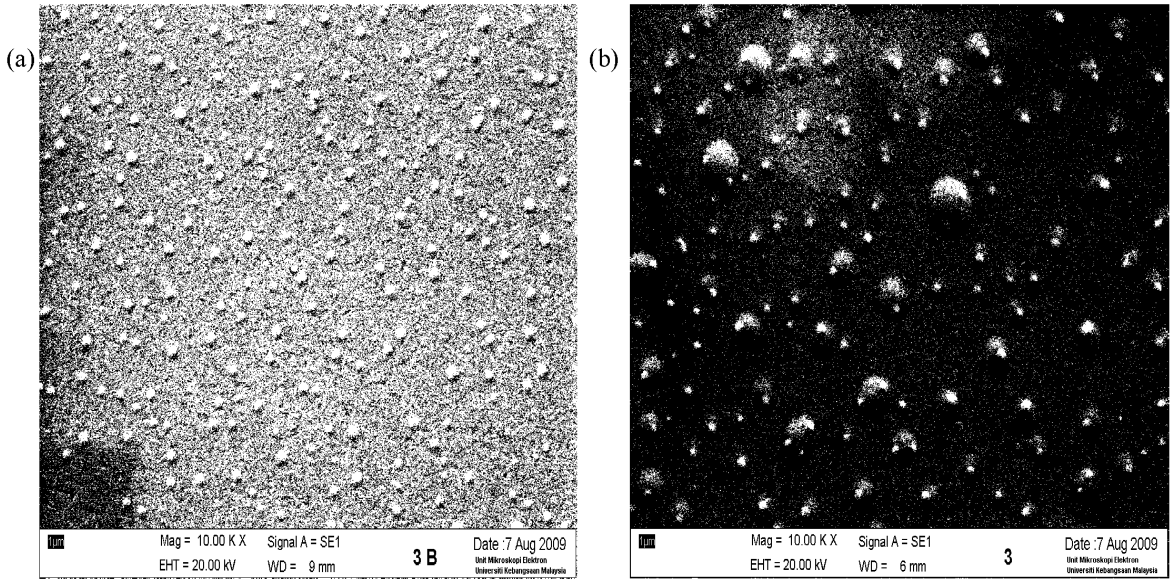

Fig. 3: Electrodeposition (a) PANI-EB (b) PANI-EB with HRP

after the deposition (Fig. 3). Smooth surface can be seen before deposition of HRP with the size of PANI-EB nanoparticle is nearly same. The big sphere that wrapped the PANI-EB nanoparticle is a phosphate buffer that indirectly deposit together with HRP. By applying the sheet resistivity, ρ_s equation (Eq.1) shown that PANI-EB/ HRP (4.640 X 10^7) have lower resistivity compared to PANI-EB (7.029 X 10^7) alone. Conductivity is an inverse of resistivity. As we know polyaniline is a conductive polymer but by adding HRP, it directly increasing the PANI-EB conductivity by reducing the resistivity of PANI-EB. It is supported by FTIR result (Fig. 2) that shows there is strong bonding interaction between PANI-EB and HRP.

CONCLUSION

This paper shows polymers are suitable for immobilization techniques. Its advantages include the ease of polymeric support, ease handling and excellent stability. By deposited HRP on PANI-EB, it will increase the conductivity of PANI-EB surface. This result supported by VPSEM, FTIR and Uv-Visible.

ACKNOWLEDGEMENT

The authors would like to thank the Nuclear Science Programme, Faculty of Science and Technology Universiti Kebangsaan Malaysia for facilities provided and gratefully acknowledged the financial support a research grant (UKM-GUP-NBT-08-27-106) that make the research possible.

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