ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and Characterization of Polypyrrole Polyphenol Oxidase (PPy/PPO) on Platinum Electrode

Anceu Murniati[1,3*], Buchari Buchari[1], Suryo Gandasasmita[1] and Zeily Nurachman[2]

[1]Analytical Chemistry Research Group, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, Jl Ganesha No.10 Bandung 40132. Indonesia
[2]Biochemistry Research Group, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, Jl Ganesha No.10 Bandung 40132. Indonesia
[3]Department of Chemistry, University of Jenderal Achmad Yani, Jln. Terusan Jenderal Sudirman PO.BOX 148 Cimahi. Indonesia

ABSTRACT

Polyphenol oxidase (PPO) has been immobilized on the polypyrrole (PPy) film via entrapment method by electropolymerization. Platinum electrode coated PPy/PPO were synthesized by using of 0.1M pyrrole and 2µg/mL PPO in the 50mM phosphate buffer solution pH 6.5 at 25^{o}C. Based on the cyclic voltammograms of PPy/PPO have a spesific profiles in the number of 5 and 10 cycles, respectively, with a potential windows of -0.4 to 1.0V versus Ag/AgCl, at a scan rate of 100mV.s^{-1}. The result of PPy and PPy/PPO films were characterized by FTIR.

Corresponding author:

ISSN: 0975-8585

INTRODUCTION

Polypyrrole (PPy) is a conducting polymer which can be modified with some materials in various ways for special interests, such as merging with a variety of counter ions, derived monomers, through the incorporation of the functional group of aliphatic chain, the inclusion of neutral molecules through chemical reactions e.g. crown ethers as complexing compound and incorporation of the noble metal nanoparticles as catalysts [1]. One technique for the synthesis of conducting polymers modified by electropolymerization. Immobilization of enzymes on the polymer film by entrapping widely used for the development of biosensors [2]. PPO is an enzyme that has the potential to immobilized in PPy films can be developed as a biosensor that can respon to a sample containing phenol. PPO has an active site which consists of two copper atoms involving two reaction step, i.e the active site of the first reaction involves hydroxilation from monofenol to o-diphenol, then the second stage of the reaction of hydrogen release into to quinone. [3-5]. Immobilization of biomolecules in polymerized films with electrochemically by applied potential at an electrode in a solution of water containing monomers and biomolecules. The enzyme trapped in polymer film does not involve chemical interactions that may affect the activity of biomolecules. The advantage of the electrochemical polymerization in the polymer film can be made easily and fast procedure [6]. Polymers films such as polypyrrole can be used to immobilized enzymes by electropolymerization method. Immobilization of enzyme can be achieved during the electropolymerization when growth and covalently binding occurs to film deposition. [7]. Glukosa oxidase (GOD) and PPO has succesfully immobilized in a conducting copolymer P(PstPy-co-Py) by synthesis of copolymerization of pyrrole which is a combination of pyrrole with polystyrene (PS) [5]. PPO has been successfully immobilized in PPy matrix and the functional group of thiophene from menthyl monomer/polypyrrole (MM/Py), to study the effect of pH conditions, temperature and operational stability [8]. The use of conducting polymer biotinylated, for construction of glucose and catechol with electropolymerization for biotynil-pyrrole monomer1 at a platinum and carbon minielectrode [2].

Immobilization of PPO bound covalently to the copolymer films of poly(N-3-aminopropyl pyrrole-co-pyrrole), PAPCP films onto an electrode indium-tin-oxide (ITO) coated glass plate, with electropolymerization by cyclic voltammetry method [9]. Cyclic voltammetry is one of the measurement technique most widely used to obtain qualitative information of the electrochemical reactions. This technique provides enough information of the thermodinamycs associated with redox process, the kinetics of heterogeneous electron tranfer reaction or adsorption process. In particular, cyclic voltammetry is able to measured accurately from the electroactive species during the redox process. As a result of plot of potential versus current relationship is termed a cyclic voltammogram [7]. The purpose of this research is to create a platinum working electrode coated PPy/PPO films by electropolymerization with cyclic voltammetry method at a potential window -0.4 to 1.3 V versus Ag/AgCl with a scan rate of 100 $mV.s^{-1}$. PPy film growth occurs on the surface of the electrode studied by cyclic voltammograms by the number of cycles at 25 cycles, which is obtained from a solution of 0.1 M Py in KCl solution as supporting electrolyte. It has been observed on insertion and trapping of the enzyme in PPy films during electropolymeryzation by the number of cycles at 5 and 10 cycles. Interactions between PPy structure and PPO has been studied by functional group at a

particular wave number through FTIR observations. This is related to the FTIR spectra of PPy have studied the formation mechanism and doping PPy films in aqueous media by voltammetry, chronoamperometry and other methods combined with IR spectroscopy, previously [10].

MATERIALS AND METHODS

Instrumentation

Ultrasonic Branson 3510; a set of vacuum distillation equipment with thermostat Buchi GKR-50 USA and Welch Gem 8890 Vacuum Pump; Fourier Tranform Infra-Red (FTIR) Prestige 21 Shimadzu Japan with MTG-21 detector and BASi-Epsilon Potentiostat (version 1.60.70).

Reagents and Chemicals

Pyrroles 98% were obtained from Sigma and purified by vacuum distillation at low pressure before use. Poliphenol oxidase, PPO (tyrosinase EC 1.14.18.1, 3933 units/mg solid from mushroom) were purchased from Sigma. KCl is used as the supporting electrolyte, NaH_2PO_4 and Na_2HPO_4 were obtained from Merck. The enzyme was dissolved in 50 mM phosphate buffer pH 6.5. Preparation of phosphate buffer by dissolving NaH_2PO_4 and Na_2HPO_4 in aquabides.

Experimental

Electropolymerization has been conducted in a set of mini cell electrolysis consists of a glass cell of 5 mL, containing of 0.1 M pyrrole, 2 µg /mL PPO and both of these compounds are dissolved in a 50 mM phosphate buffer solution pH 6.5. A set of mini cell electrolysis are then fitted platinum working electrode with a diameter of 2 mm, a platinum wire as auxiliary electrode and a Ag/AgCl (3M KCl) as references electrode. Electrolysis cell is connected to potentiostat equipped with a set of computers.

RESULTS AND DISCUSSION

Cyclic voltammograms of PPy and PPO combined

Cyclic voltammograms of PPy and PPO combined with the number of cycles at one cycle each providing a specific profile on the condition of the current value and potential value specified, the cyclic voltammograms of PPy obtained from 0.1 M Py in 0.1 M KCl as supporting electrolyte and voltammograms cyclic of PPO obtained from 2µg/mL PPO in 50 mM phosphate buffer solution pH 6.5 at a temperature of 25°C with a potential window -1.0 to 1.5V versus Ag/AgCl at a scan rate 100 $mV.s^{-1}$ as shown in Fig. 1. The influence of the concentration of monomer and PPO is one of the factors that affect of the peak current value during the electropolymerization [11]. Based on Table 1. there are the oxidation of peak current value (Ip_a) and the reduction of peak current value (Ip_c) at the cyclic voltammograms of PPy are as follows

ISSN: 0975-8585

(Ip_a=0.49mA; Ip_c=-0.05 mA) and the oxidation peak potential value (Ep_a) and reduction peak potential value (Ep_c) are as follows (Ep_a=0.92V;Ep_c=0.15V). The current value and potential value at the cyclic voltammograms from PPO are as follows (Ip_a=0.207mA; Ip_c=-0.07 mA; Ep_a=1.420V; Ep_c=0.015V).

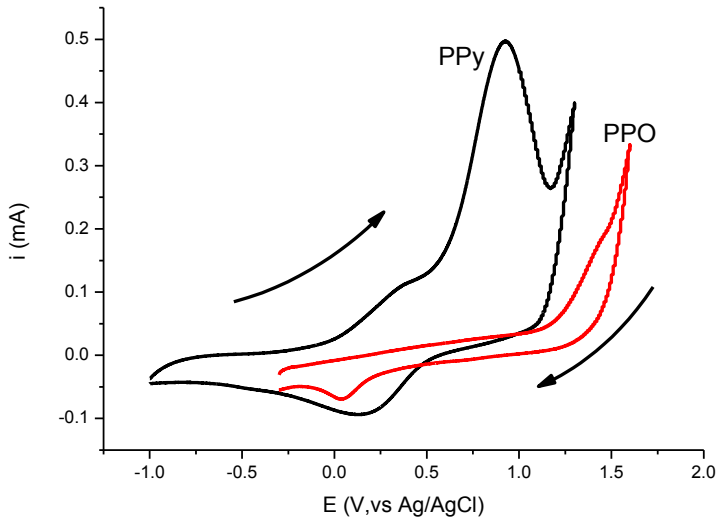


Figure 1.Cyclic voltammograms of PPy and PPO combined

Table 1. Data of the peak potential value and peak current value in the cyclic voltammogram of PPy and PPO combined

Cyclic voltammogram	Potential (V)		Current (mA)	
	Ep_a	Ep_c	Ip_a	Ip_c
PPy	0.92	0.15	0.49	-0.05
PPO	1.42	0.02	0.21	-0.07

Effect of pH on the peak current value on the cyclic voltammograms of PPO

Measurement of pH is one of the factors affecting the performance of the enzyme, may all be changed when an enzyme is immobilized. It can also relate to the function of the enzyme structure, the charge of the enzyme or solvent [7,12]. Electrochemical measurements by cyclic voltammetry method on a platinum electrode was observed from 2µg/mL PPO in 50 mM phosphate buffer solution were studied by the variation of pH from pH 6.0 to pH 7.0 at 25^{o}C. The result is cyclic voltammograms of PPO with a potential window from -0.3 to 1.8V versus Ag/AgCl, at a scan rate of 100 mV.s^{-1} (Fig. 2) and plot a graph of the correlation between the peak current response of cyclic voltammograms PPO versus variation of pH (Fig. 3). Based on the current value of Ip_a and Ip_c for each pH give different values from each other. The stability of the structure of the PPO, in phosphate buffer solution greatly influenced by current value which is relationship with the diffusion ability of PPO to cover the surface of a platinum

ISSN: 0975-8585

electrode. Based on Fig.2 (Ip_a=0.24 mA; Ip_c=-0.09 mA) and the structure of the PPO, in a phosphate buffer solution is most stable at pH 6.5 (Fig.3).

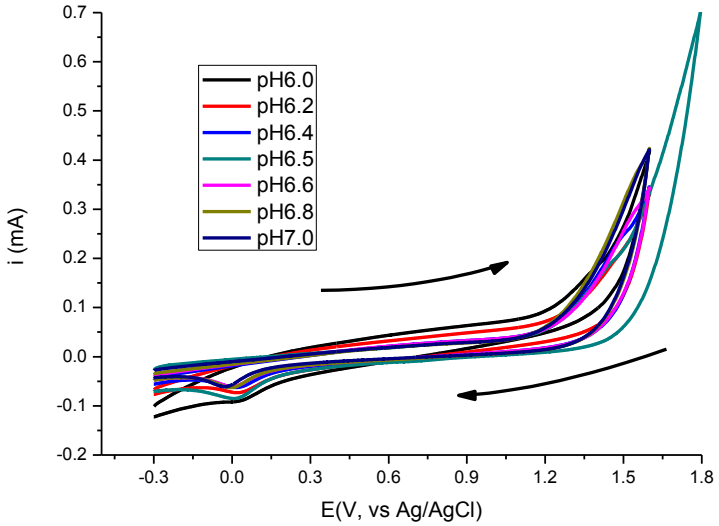


Figure 2. Effect of pH on the cyclic voltammogram of PPO

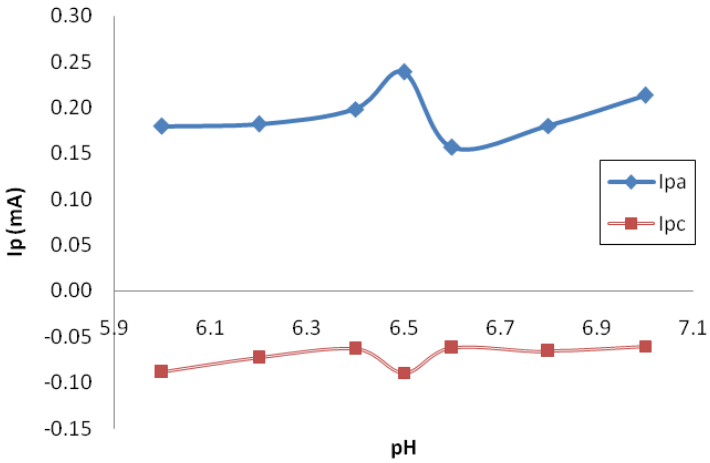


Figure 3. Response of the peak current value of the cyclic voltammogram PPO versus pH

The influence of the number of cycles from the cyclic voltammograms of PPy and PPy/PPO

The growth of PPy spread evenly on the surface of the electrode has been achieved by the number of cycles at 25 cycles with the value of the current in the oxidation state is about 0.4 mA and the current value of the reduction state is about-0.6mA at a potential window of 0 to 1V versus Ag/AgCl as reference electrode with a scan rate of 100mV.s^{-1}, as shown in Fig. 4. In the half-cycle of the oxidation state, the growth of PPy achieved on the potential value after 0.2 to 0.8V, then after a potential value above 0.8V PPy has reached the peak of oxidation (Fig. 4) with a value of Ep_a = 0.92 V, and the value of Ip_a = 0.49 mA (Fig. 1 and Table 1). When the scan

is reversed, on the half-cycle of the reduction state, the polymer growth has been achieved at a potential of 0.4 to 0V. The state of the polymer growth, starting from the initial stage of polymerization during the half-cycle starting potential of -0.5 to +0.5V is in a state of neutral lead discharged anion [13]. One method of immobilization of an enzyme on the electrode surface, which is based on the oxidation polymerization of the monomers in the presence of the enzyme [14].

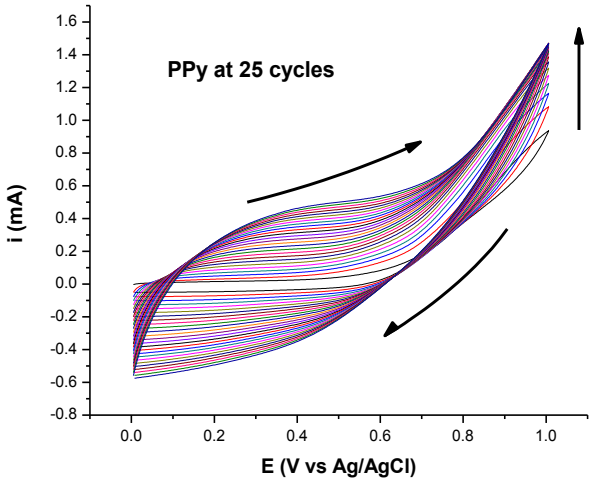
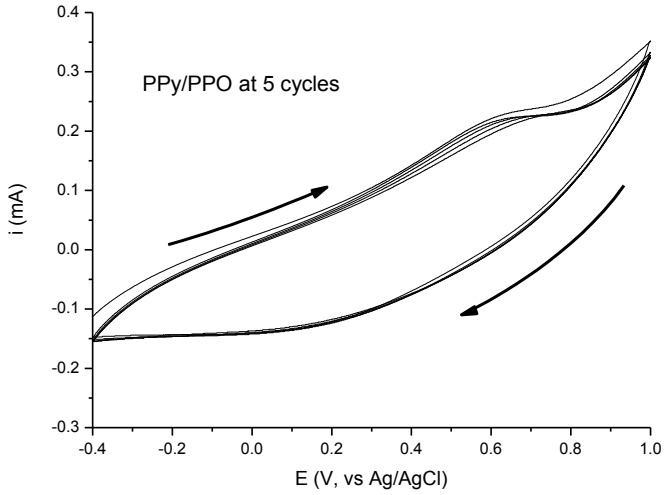


Figure 4. Cyclic voltammograms of PPy by the number of cycles at 25 cycles

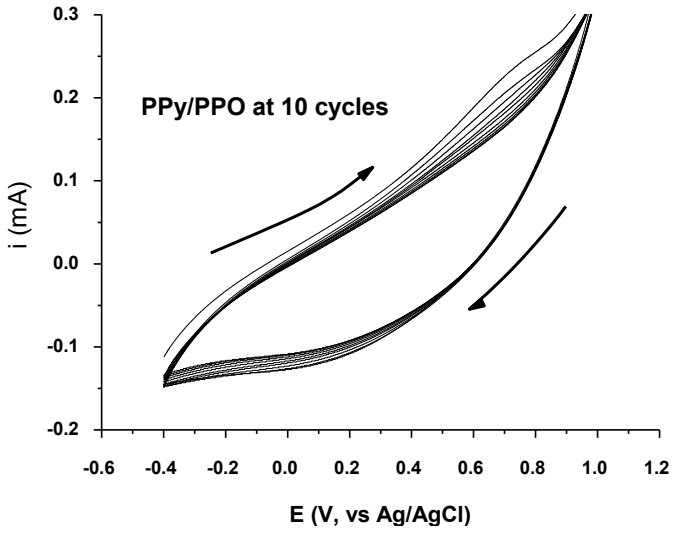
The growth of the polymer films were regularly expected to immobilize the enzyme insertion more perfect. The presence of enzymes trapped inside the polymer film of the cyclic voltammograms of PPy/PPO with a specific profile by the number of cycles at 5 and 10 cycles in the potential window of -0.4 to 1.0 V as shown in Fig. (5A and 5B). There were a condition of oxidation state at early electropolymerization polymer growth, and the half-cycle of the applied potential, as shown by the first cycle were achieved with high current value, while the second cycles and the next cycles, obtained by current value changes more regularly. According to Reynold mechanism, the electrode surface begins with the formation of radical cation, followed by a solid polymer growth. Strong interactions occur between the solvent water, the radical cation and anion of the supporting electrolyte or the solvent environment is the first step elektropolymerization, requiring a high energy process on the surface of a platinum working electrode [11]. Based on Fig.4, the polymer growth in decreased, initially with the current growth of PPy, at about 0.4 mA at an applied potential, the presence of the enzyme after the current value of approximately 0,2mA at an applied potential from Fig. (5A and 5B). In the cyclic voltammograms of PPy/PPO the polymer growth (Fig. 5B) followed by the insertion of the enzyme at a half-cycles of the potential oxidation of 0.4 to 0.8 V and the reduction potential of -0.4 to 0.4 V. This shows the number of immobilized enzyme to be involved in biological activity on the surface of the electrode. On the contrary, narrowing the area of polymer growth, the half-cycle occurs in oxidation potential of -0.4 to 0.4V and the reduction potential of 0.4 to 0.8V, and the condition is suspected the enzyme inserts accumulate during growth of the polymer on the electropolymerization. In these conditions, the polymerization process is not

ISSN: 0975-8585

good, as a result some tucked of PPO that has accumulated in the polymer films, rather than on the surface of the polymer film. As shown in Fig. 6A, PPO immobilization on PPy films has been achieved by the correlation coefficient is R^2 = 0.988 and R^2 = 0.922, respectively, as a plot of graph on the current value of redox conditions of the growth of PPy/PPO, versus the number of cycles at 5 cycles. Then, immobilization of PPO in PPy films is achieved with a correlation coefficient is R^2 = 0.944 and R^2 = 0.981, respectively, as a plot of graph on the current value of redox conditions of the growth of PPy/PPO versus the number of cycles, at 10 cycles, as shown in Fig. 6B



(A)



(B)

Figure 5. Cyclic voltammograms of PPy/PPO on the number of cycles as follows: [A]. 5 cycles and [B]. 10 cycles

ISSN: 0975-8585

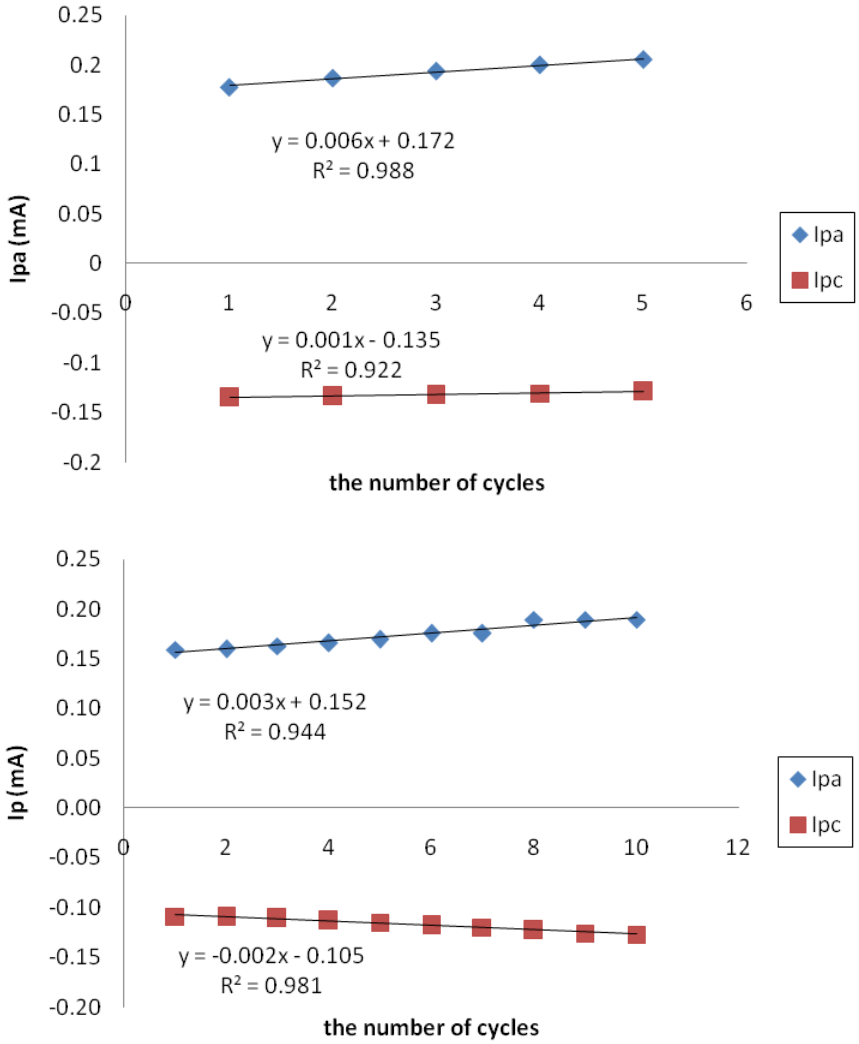
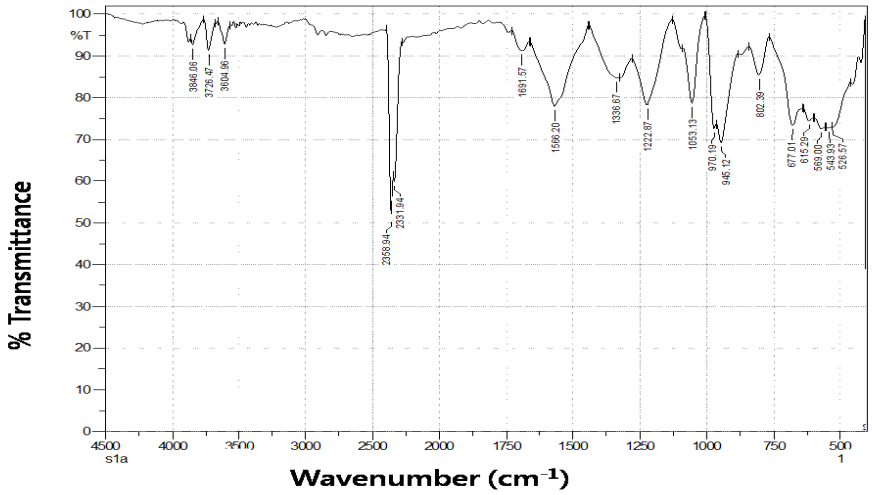


Figure 6. The relationship of the current value on the cyclic voltammograms of PPy/PPO versus the number of cycles as follows: [A]. 5 cycles and [B].10 cycles

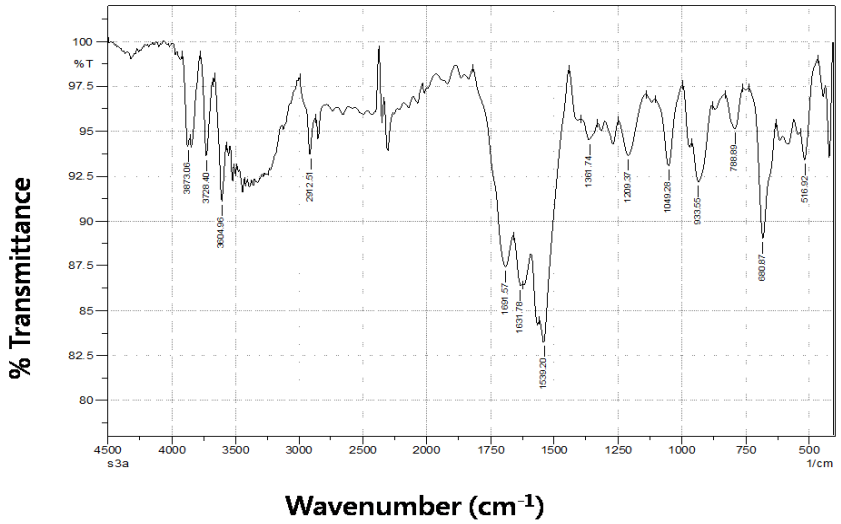
Characterization the films of PPy and PPy/PPO with FTIR

FTIR spectra of PPy can be described from the Fig. 7A. There is a functional group of the C=C stretching at the peak with the strong intensity and a weak vibration, at 1556.20 and 1691.57 cm^{-1}. Followed by the functional groups of C-N stretching, at the strong intensity and weak vibration at 1336.67 cm^{-1}. The functional group of C-H stretching at the peak with a medium intensity at 2331.94; 2358.94cm^{-1} and functional group of C-H wagging vibration at 677.01cm^{-1}, at the weak vibration. The interaction between PPy and PPO can be explained by the shift of the peak of a wave number in the FTIR PPy/PPO spectra (Fig. 7B). The presence of PPO in PPy films, there has been a shift in the peak of wave number, such as a shift in the peak of wave number from 2331.94; 2358.94 to 2912.51cm^{-1}; the functional groups of C-H, with strong intensity and a weak vibration. Followed, a shift in the peak from 1336 to 1361.74cm^{-1} as the functional groups of C-N, with medium intensity and a weak vibration.

ISSN: 0975-8585



(A)



(B)

Figure 7: FTIR spectra of (A) PPy and (B) PPO

CONCLUSION

Based on the cyclic voltammograms of PPy, the growth of polymer on the surface of a platinum electrode has been achieved by the number of cycles at 25 cycles, at a potential window of 0 to 1V versus Ag/AgCl, with a scan rate of 100 $mV.s^{-1}$. The ability of PPy film to immobilize PPO, has been studied based on the shift of current at a potential window of -0.4 to 1.0 V. The presence of the enzyme on PPy film has been achieved by the correlation coefficient with the average value is $R^2 > 0.9$, from the graph of Ip versus the number of cycles at redox condition. Interactions between PPy and PPO has been observed by FTIR with a shift of wave number from PPy spectrum to PPy/PPO spectrum.

ISSN: 0975-8585

ACKNOWLEDGMENTS

We would like to thanks of the support and funding for this research to Doctoral Program Scholarship, Vouchers ITB. We also many thanks for the Research grant of Penelitian Disertasi Doktor and Penelitian Strategis Nasional from Directorate General of Higher Education, The Ministry of National Education.

REFERENCES

[1] Jüttner K, Mangold KM, Lange M, Bouzek K. Russ J Electrochem 2004; 40: 317-325.
[2] Cosnier S, Gondran C, Le Pellec A, Senillou A. Anal Lett 2001; 34: 61-70.
[3] Burton SG. Trends in Biotech 2003; 12: 543-549.
[4] Whitaker JR. Principles of Enzymology for the Food Sciences. 2nd, Marcell Dekker, INC USA, 1994, pp. 544.
[5] Elkinci O, Boyukbayram AE, Kiralp S, Toppare T, Yagci Y. J Macrom Sci Part A 2007; 44: 801-808.
[6] Cosnier S. Appl Biochem Biotechnol 2000; 89: 127-138.
[7] Wang J. Analytical Electrochemistry. 2nd edition, John Willey and sons, New York, 2000:pp. 28,173,174.
[8] Kiralp S, Toppare L, Yagci Y. Int J Biological Macrom 2003; 33: 37–41.
[9] Rajesh, Takashima W, Kaneto K. Sens Actuators B 2004; 102: 271-277.
[10] Yakovleva *AA.* Russ J Electrochem 2000; 36: 1275-1282.
[11] Sadki S, Schottland P, Brodie N, Sabouraud G. Chem Soc Rev 2000; 29: 283-293.
[12] Chibata I. Immobilized Enzymes: Research and Development. Kodansha,Tokyo, 1978, pp.111.
[13] Cheung KM, Bloor D, Stevens GC. J Mater Sci 1990; 25: 3814-3837.
[14] Yildiz HB, Toppare L, Gursel YH, Yagci Y. Enzyme Microb Technol 2006; 39: 945-948.