ORIGINAL ARTICLE

Determination of uric acid level by polyaniline and poly (allylamine): Based biosensor

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ABSTRACT

The uric acid biosensor has been much developed by immobilizing uricase enzyme into the membrane of conductive polymer and the membrane of polyelectrolyte such as polyaniline (PANI) and poly (allylamine) (PAA) respectively. The purpose of this research was to create a new amperometric uric acid biosensor by immobilization of uricase in combination between PANI and PAA membranes. The working electrode was Pt plate (0.5 mm). The auxiliary and the reference electrode were Pt wire 0.4 mm and Ag/AgCl respectively. Uricase, uric acid, PAA, pyrrole and glutaraldehyde were supplied from Sigma. All other chemical was obtained from Merck. The biosensor was created by immobilizing of uricase by a glutaraldehyde crosslinking procedure on PANI composite film on the surface of a platinum electrode while the polyelectrolyte layer of PAA were prepared via layer-by-layer assembly on the electrode, functioning as H_2O_2-selective film. Standard of deviation, coefficient of variation (CV) and coefficient of correlation (*r*) analysis were used in this study. The biosensor had a good linearity with a correlation coefficient of 0.993 and it could be used up to 27 times with the CV value of 3.97%. The presence of other compounds such as glucose and ascorbic acid gave 1.3 ± 1.13% and 3.27 ± 2.29% respectively on the interference effect toward the current response of uric acid biosensor. The polymer combination of PANI and PAA can be used as a selective matrix of uric acid biosensor.

Key words: Biosensor, electropolymerization, poly (allylamine), polyelectrolyte such as, polyaniline, uric acid, uricase

INTRODUCTION

Uric acid is the substance of the final product of nucleic acid or purine metabolism in human body.[1] In the event of irregularities in this process the uric acid levels increases and it will cause hyperuricemia and gout diseases.[2] Therefore, uric acid measurement for diagnosis and treatment of these disorders is routinely required. Many of the methods are available for the determination of uric acid and one of the method that provide many advantages is amperometric biosensors.[3]

The determination of uric acid level with biosensor method has been developed by the immobilization of the enzyme uricase in both the conductive polymer and polyelectrolyte membrane such as polypyrrole, polyaniline (PANI) and polyfenilendiamina.[3-6]

Conductive polymers are widely used in enzyme immobilization, which has better as conductor in electricity and has a variety of structures at a relatively inexpensive price and also easy to be made.[7] PANI, for instance, is widely used as a matrix for the immobilization of several enzymes due to its stability, easy synthesis and high conductivity.[7,8] A research was conducted by Kavita Arora *et al*. (2007) regarding the immobilization of uricase into PANI with a cross-linking method with the addition of glutaraldehyde. Moreover, PANI can also be combined with polypyrrole as a matrix for immobilization of uricase in production of uric acid biosensor.[53]

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The Immobilization of enzymes can also be done by polymer electrolytes. A polymer electrolyte is a polymer which has both a positive and negative charges, such as Poly (Allylamine) (PAA), poly (vinyl sulfat) (PVS) and polyacrylate. PAA is a positively charged polymer that can only be penetrated by anions, like uric acid. The use of PAA as a matrix in the immobilization of uricase had been accomplished by combining it with PVS.[6]

MATERIALS AND METHODS

Experimental condition

The electrochemical studies were carried out using potentiostat (μ AUTOLAB) with three-electrode cell. The working electrode was Pt plate (0.5 mm). The auxiliary and the reference electrode were Pt wire 0.4 mm and Ag/AgCl respectively. The pH of the solution was measured with mettler toledo pH meter. Uricase, uric acid, PAA, pyrrole and glutaraldehyde were supplied from Sigma. All other chemical was obtained from Merck. All the solution was prepared using the distilled water.

Preparation of biosensor

The preparation of multilayer film was adopted and modified from Arslan (2008) for immobilizing uricase by a glutaraldehyde crosslinking procedure on PANI composite film on the surface of a platinum electrode. Meanwhile, the polyelectrolyte layer of PAA were prepared through layer-by-layer assembly on the electrode, functioning as H_2O_2-selective film as described in Hoshi *et al*. study (2003).

Determination of the working potential

Determination of the working potential was conducted using a H_2O_2 solution (0.3 M) that was oxidized using the following varying potentials; 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 V. The potential which produces the highest H_2O_2 oxidation current was used as a working potential.[5]

Calibration curve

Uric acid solution with the concentration of 0.0625 M, 0.125 M, 0.25 M, 0.5 M and 0.75 M were measured using amperometric technique at 0.6 V and a potential scan rate at 50 mV/s. Current responses were plotted against the concentration of uric acid solution, then the equation of correlation coefficient (r) was calculated in the linear regression equation $Y = ax + b$.[9]

Interference effect and reuse number

Interference with a known concentration of 5 M × 10^{-3} M, 1,0 M × 10^{-4} M of glucose and ascorbic acid in a uric acid solution of 0.36 M, was observed for occurring current changes. In order to test the reuse number of the biosensors, the uric acid concentration was fixed at 0.36 M and was measured repeatedly using the biosensors, which had been made and the production of current response was observed.[5]

Storage stability

The response of the biosensors preparation was measured at constant uric acid concentration of 0.36 M for a period of time. The result of measurements during this period is plotted between current and day.

RESULTS

Preparation of biosensor

It can be seen from Figure 1 that the electropolimerization of PANI occurred at a potential oxidation of 0.2075 V.

The immobilization of uricase enzyme (10 units/ml, 50 μL) on the working electrode Pt | PANI had been done by the help of glutaraldehyde and it resulted the electrode Pt | PANI | UOX.

The electrode Pt | PANI | UOX had been resulted by dipping in the PAA solution and solution of 0.1 mg/ml uricase in 0.1 M borate buffer pH 9. The repetition of PAA coating process and immobilization performed at 10 times to obtain a working electrode "Pt | PANI | UOX (PAA/UOX) 10."

Determination of the working potential

Based on Figure 2, the potential at 0.6 V produced the highest oxidation current of H_2O_2.

Calibration curve

The calibration curve can be seen in Figure 3. The linearity obtained linear relationship with a correlation coefficient (r) = 0.993 and the regression equation $Y = 4.6175 \times 10^{-4}X = 1.064 \times 10^{-5}$.

Interference effect and reuse number

The interference test showed that the influence of glucose and ascorbic acid in uric acid analysis had the interference effect of 1.3 ± 1.13% and 3.27 ± 2.29% respectively. Results of the interference effect on acid biosensor response can be seen in Table 1.

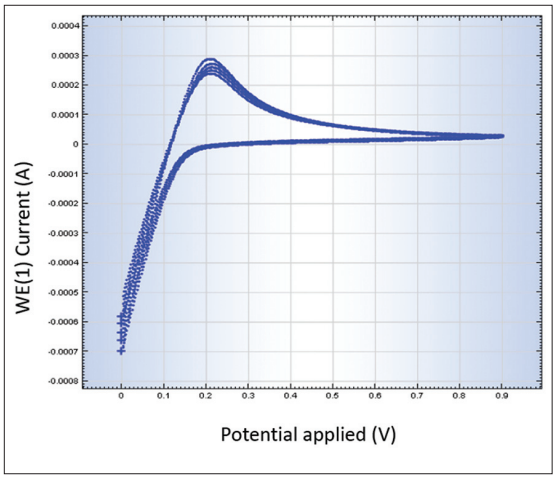

Figure 1: Voltamogram of electropolimerization polyaniline

From Figure 4 showed that the current response had a significant reduction in repetition of 28 and 29. Meanwhile in the repetition of 1 to 27, the current response relatively equal to the coefficient of variation (CV) value of 3.97%.

Storage stability

The result of storage stability was showed in Figure 5. It indicates that 16.73% of the initial amperometric response decreased at day 26th.

DISCUSSION

Electropolimerization PANI performed using cyclic voltammetry technique with a potential range of 0.0 to 0.9 V and the scan rate of 50 mV/s by 5 cycles. By cyclic voltammetry technique, the thickness and perfection polymer can be controlled by adjusting the number of cycles, potential range and scan rate during the electropolimerization ongoing process. The emergence of the oxidation peak in Figure 2 associated with the presence of sulfate anion in the transition state semiconductive (leucoemeraldin shape) become conductive PANI (form polaronicemeraldin).[10]

Immobilization of uricase enzyme (10 units/ml, 50 µL) on the working electrode Pt | PANI was done with addition of glutaraldehyde. Glutaraldehyde cross-linker serves as a way to form a covalent bond with the amine group on the PANI matrix, while the other side of glutaraldehyde binds to the amino groups on the enzyme. After immobilization, the electrode Pt | PANI | aquademine UOX washed to remove non-covalent bonding to the surface of the electrode. In addition, aquademine also selected as the wash solution to minimize the enzyme electrode and enzyme inactivity due to metal ions in water non aquademine.[3,11]

PAA is a polyelectrolyte polymer that can dissociate to form the perfect solution with a positive charge at pH 11.[6] PAA dissolved in Dulbecco buffer pH 11 containing NaCl salt which will affect the shape of PAA conformation. In solution with salt concentration is too high, the PAA will tend to form a thicker and circular due to the effects of charge neutralization of the polyelectrolyte counterion by the presence of excessive salts in solution.

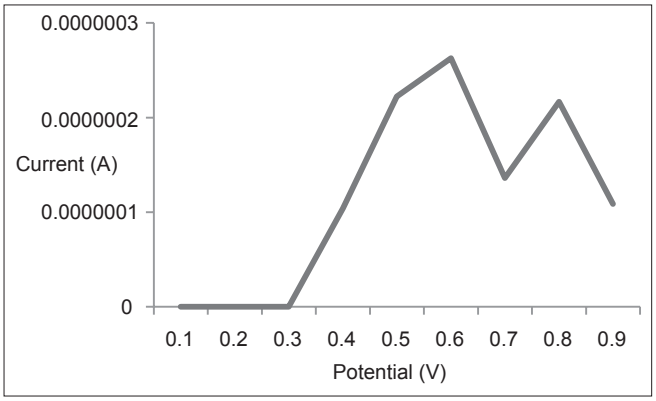

Figure 2: Oxidation current of H_2O_2 at many potentials using Pt electrode

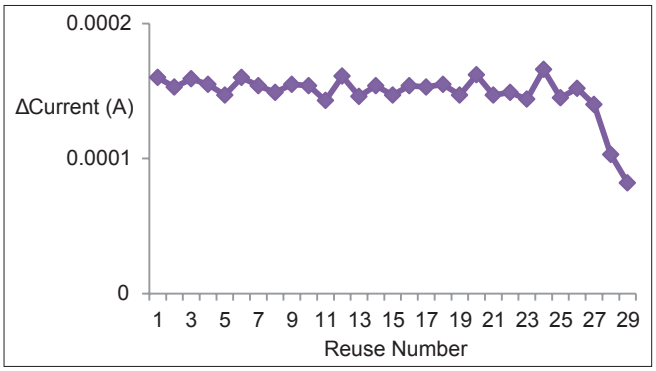

Figure 4: Test result of reuse number

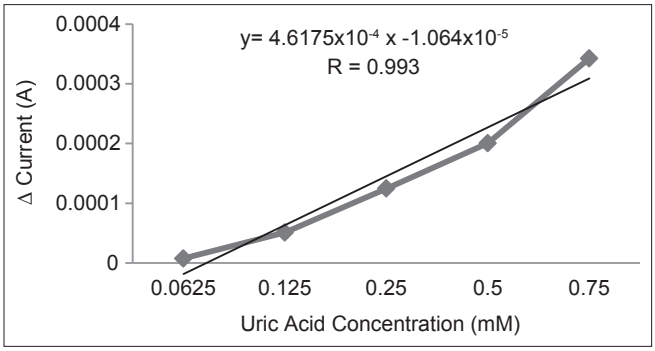

Figure 3: Calibration curve of uric acid biosensor

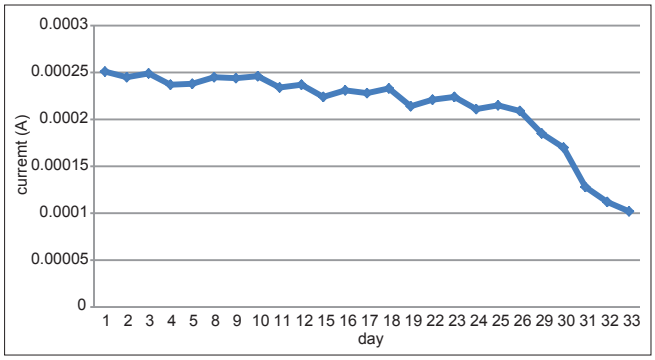

Figure 5: Storage stability of biosensor

Table 1: Test result of interference effect towards response of uric acid biosensor (*n*=3)

Sample	Concentration of interference (M)	Current (A)	Current of interference (µA)	Effect of interference (%)
Uric acid 0.36 mM	-	0.000153±0.000001	-	-
Uric acid 0.36 mM+glucose	5×10^{-3}	0.000155±0.00000173	0.000002±0.00000152	1.3±1.13
Uric acid 0.36 mM + ascorbic acid	1×10^{-4}	0.000158±0.00000351	0.000005±0.00000642	3.27±2.29

Electrode Pt | PANI | UOX | PAA uricase was dipped into a solution of 0.1 mg/ml in 0.1 M borate buffer pH 9 for 30 min. Uricase solution made by mixing the enzyme uricase with 1 M borate buffer pH 9 in aquademine. The use of borate buffer 1 M aimed to keep the uricase solution remains dissolved in 0.1 M borate buffer, which is stable in the buffer. After immobilization for 30 min, the electrode was washed with 0.1 M borate buffer pH 9 to remove the immobilized enzyme.[6]

Determination of potential work for uric acid measurements performed using H_2O_2 solution. H_2O_2 solution is the result of reaction of the enzyme uricase and uric acid to be analyzed. When the potential was given, H_2O_2 will change as electron current that can be measured. The resulting current value will be proportional to the amount of uric acid, which is being analyzed, so that H_2O_2 is used in the determination of employment potential for the measurement of uric acid.[5]

The result of coefficient of correlation showed a linear relationship between the increase in current with increase in the concentration of the analyte.[9] The result of coefficient of correlation indicates that uric acid biosensor can be used in the quantitative measurement of uric acid.

From the interference test, glucose has a smaller effect on the resulting flow biosensor. This is due to neutral charge which cannot be attracted by PAA, which is positive charge. In contrast to glucose, ascorbic acid will form anions in solution, so there are likely to be attracted by a PAA layer. Effect of ascorbic acid on the response of the biosensor in this study is smaller than the research conducted Hoshi *et al*. (2003), which used a combination of PAA and PVS as an enzyme immobilization matrix in testing uricase.

Reuse number was conducted to determine the limitations of the biosensors produced in repetitive usage. The main advantage of reusability is to reduce the cost of the treatment. From Figure 4, it was found that the current response increased and decreased during use and has a CV approximately to 3.97%. For the final usage, the biosensor described a decrease in response and the current did not return up for 2 usages, which were the 28th and 29th time of usage. The decline in current response occurred due to the enzyme activity reducing during the repeated and continuous usage.[5] To sum up, the biosensor can be used up to 27 times.

The response of the biosensors was measured every day for a period of time at constant uric acid concentration at 0.36 M. The result of the measurement during this period is plotted in Figure 5. It was observed that 16.73% of the initial amperometric response decreased at day 26th. This indicates that the preparation of biosensor can be used for quite long time.

CONCLUSIONS

The uric acid biosensor prepared in this study:

- Has good linear relationship between current and concentration (r = 0.993)
- Has good CV 3.97% (<5%) and can be used up to 27 times
- Has good current response in interference test (glucose and ascorbic acid gave 1.3 ± 1.13% and 3.27 ± 2.29% respectively)
- Has a satisfactory storage stabilization (the electrode lost 16.73% of the initial amperometric response at day 26th).

In conclusion, the polymer combination of PANI and PAA can be used as a selective matrix of uric acid biosensor.

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