



Biosensor for uric acid determination based on the combination of polypyrrole and poly (allylamine) films

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ABSTRACT

Aim and Background: The use of polypyrrole and poly(allylamine) as a matrix of biosensor has been done separately, but the combination of this two has never existed. The purpose of this research is to produce a selective biosensor using combination of polypyrrole (PPy) and poly(allylamine) (PAA) as a polymer for the immobilization of the uricase enzyme, which is used to detect uric acid level in the blood. **Materials and Methods:** Biosensor manufacturing process began with the electropolymerization of pyrrole on platinum wire then immobilization of uricase enzyme (UOx) with glutaraldehyde as cross-linker. After that, UOx and PAA were deposited via layer-by-layer sequential deposition up to 10 UOx layers to prepare amperometric sensors for uric acid. Biosensor proved to have a linear relationship between the current response and the increase of analyte concentrations, with a correlation coefficient (r) = 0.992. **Results and Conclusion:** Presence of interferences had a little effect on uric acid analysis i.e. 1.39% glucose and 1.65% ascorbic acid. The sensors could be used up to 32 times. In the stability test, 13.4% of the initial amperometric response decreased at day 33rd. The results showed that combination of PPy and PAA can be used as a matrix for uric acid biosensor.

Key words: Biosensor, uric acid, polypyrrole, poly (allylamine)

Uric acid in human physiological fluids is of great importance in the diagnosis and therapy of patients suffering from a range of disorders associated with altered purine metabolism, most notably gout and hyperuricemia. Consequently, uric acid measurement for diagnosis and treatment of these disorders is routinely required. Electrochemical biosensors have an accurate

layer of enzymes with the function to identify biological components and provide the electroactive substances that can be detected by a transducer.¹ A conductive polymer is a material that possesses electrical conductivity and metal mechanical properties such as polymers do.² Polypyrrole (PPy) is among the good conductive polymers.³ From previous study, the use of PPy as uric acid polymers in biosensors had a detection limit of 5.0×10^{-7} M and the sensor was found to be relatively stable over the 5 weeks.⁴ Poly (allylamine)(PAA) is a polymer with a positive charge, with the presence of a positively charged membrane only anions are expected and desired to pass through.⁵ Based on the advantages of PPy as well as PAA and the absence of a uric acid biosensor manufacture from a combination of the two polymers, this research needs

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to be done. 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 Pt electrode was immersed in a solution of 0.01 M pyrrole in 0.1 M H_2SO_4 (10 mL). Electropolymerization was conducted using the cyclic voltammetry technique with a work potential range of 0.0-1.5 V and a scan rate of 50 mV/s (5 cycles). Uricase enzyme (10 units/mL, 50 μ L) was dripped on the surface of the platinum (Pt)/PPY electrode after its being washed and consequently dried at room temperature. Glutaraldehyde solution (% 0.5 m/v, 15 μ L) was dripped on the Ppy-enzyme layer, and then washed with borate buffer (0.05 M, pH 9) and dried at room temperature.⁶ The surface of the electrode was dipped in a solution of PAA (2 mg/mL) for 30 min to form a UOx/PAA coat. The surface of the Pt/Ppy/UOx/PAA electrode was dipped in a solution of uricase (0.1 mg/mL) in borate buffer (100 mM) for 10 min, and then eluted with a working buffer. The PAA/UOx layer formation was repeated 10 times. The electrode modification results was then estimated: Pt | Ppy/UOx |(PAA/UOx)₉ PAA.⁵

Working potential was used from potential that produced highest H_2O_2 oxidation current. To find out whether the current is linear against various concentration of uric acid, calibration curves measurement was done. After having a calibration curve, the next step is find out the interference effect, reused number and storage stability of the sensor. Interference with a known concentration of 5×10^{-3} M that is 1.0×10^{-4} M of glucose and ascorbic acid added to a uric acid solution of 0.36 M was observed for occurring current changes. An increase in flow was generated when there was additional interference and the effect was noted in percent (%). 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 made, and the current response produced was observed. To find out the stability storage, the response of the biosensors prepared was measured for a period of time at constant uric acid concentration at 0.36 M (Table 1).

In this study, the biosensors were prepared by the use of the polymer polypyrrole-PPA as mentioned in the

experimental section. Working potential that is used for uric acid determination is 0.6 V; this is based on deviation from linearity that was observed after potential 0.6 V. To determine if the biosensor response is suitable with concentration of the analyte tested, a calibration curve is made with various uric acid concentrations; 0.0625 M, 0.125 M, 0.25 M, 0.50 M, 0.75 M. From the test results, the following graph is obtained.

It has been showed that the calibration curve results show a linear relationship with the correlation coefficient (r)=0.992 and the regression equation of $y = 0.0000938x + 0.0001209$. The linearity parameter is fulfilled when there is a linear relationship between the response generated by the biosensor with the increase in analyte tested (Figure 1).

The presence of other compounds in the serum or urine as a matrix in uric acid level testing can interfere with the result interpretation of the analysis. Therefore, the effect of interference was investigated as mentioned in the experimental section.

From the results, it was found that the effect of glucose on the measurement of uric acid was smaller in comparison to ascorbic acid. Glucose is a neutral compound, whereas ascorbic acid is an anionic molecule like uric acid. This phenomenon causes a bigger effect from ascorbic acid because anionic molecules are pulled closer toward the PAA layer unlike glucose that is neutral in nature.⁵ However, ascorbic acid does not influence the measurement or uric acid too much because its calculated influence is only as much as 1.65%. This is because inside the modified electrode layer, the enzyme uricase can only convert H_2O_2 and only H_2O_2 undergoes oxidation and produces a current when potential is applied during measurement.⁵ Reuse number was conducted to determine the limitations of the biosensors produced in repetitive usage.⁶ As shown in

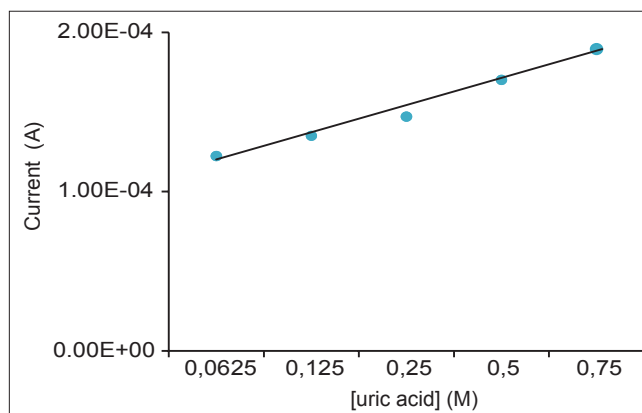


Figure 1: The calibration curve of uric acid biosensors (phosphate buffer pH 7, 4, 25°C)

Table 1: Interfering substances (constant uric acid concentration 0.36 M) on the amperometric response of the biosensors

Interfering substances	Concentration	Interference effect of substances (%)
Glucose	5×10^{-3} M	1.39
Ascorbic acid	1.0×10^{-4} M	1.65

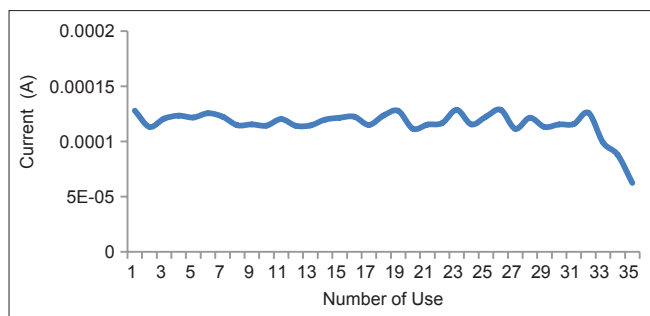


Figure 2: Reuse number of the biosensors (phosphate buffer pH 7, 4, 25°C)

Figure 2, it was found that the current response increased and decreased during use and has a coefficient variation amounting up to 4.46%. The biosensor produced can be used up to 32 times.

The response of the biosensors prepared was measured every day for a period of time at constant uric acid

concentration at 0.36 M. It was observed that 13.4% of the initial amperometric response decreased at day 33. This indicates that the biosensor prepared can be used for quite long time.

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