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ORIGINAL RESEARCH

Conductimetric Biosensor for the Detection of Uric Acid by Immobilization Uricase on Nata de Coco Membrane—Pt Electrode

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Abstract: A conductimetric enzyme biosensor for uric acid detection has been developed. The uricase, as enzyme, is isolated from *Candida utilis* and immobilized on a nata de coco membrane-Pt electrode. The biosensor demonstrates a linear response to urate over the concentration range 1–6 ppm and has good selectivity properties. The response is affected by the membrane thickness and pH change in the range 7.5–9.5. The response time is three minutes in aqueous solutions and in human serum samples. Application of the biosensor to the determination of uric acid in human serum gave results that compared favourably with those obtained by medical laboratory. The operational stability of the biosensor was not less than three days and the relative error is smaller than 10%.

Keywords: biosensor, conductimetry, uric acid, nata de coco membrane

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Introduction

Uric Acid is a final product in the catabolism processes of purin nucleotide in the human body. In human blood plasma, the reference range of uric acid is among 3.6 mg/dL and 8.3 mg/dL. Excess serum accumulation of uric acid can lead to a type of arthritis known as gout. Elevated serum uric acid (hyperuricemia) is a result from high intake of purine-rich foods, high fructose intake (regardless of fructose's low glycemic index (GI) value) or impaired excretion by the kidneys. Saturation levels of uric acid in blood may result in one form of kidney stones when the urate crystallizes in the kidney. Gout can occur where serum uric acid levels are as low as 6 mg/dL, but an individual can have the serum values as high as 9.6 mg/dL and not have gout. Because of that, the analysis of uric acid level is very important to control human health.[1,2]

The uric acid can be determined by the spectrophotometric method, the method used 2,4,6-tribromophenol and 4-aminoantipirin as reactants, and uricase is used as a biocatalyst. The absorbance is measured at 492 nm in the range concentrations of 10–20 mg/dL, so this method is not applicable to determine the uric acid in a normal human body.[3] The potentiometric uric acid biosensor has been developed.[4,5] The biosensor is based on the oxidation of uric acid calatyzed by uricase to produce CO_2. In water, CO_2 can be hidrolyzed to form HCO_3^- and H_3O^+. In a previous work, we developed a potentiometric uric acid biosensor with uricase immobilized on a chitosan membrane. The limit of detection of that biosensor is 5 ppm and accuracy is more than 95%. However, the biosensor needs large sample volume.[5] In this work, we developed a coductimetric uric acid biosensor. Uricase is immobilized on a nata de coco membrane—Pt electrode, the biosensor has a simple design and is smaller than the potentiometric biosensor.

Conductometric biosensors were developed since 1961 to determine urea. The method was based on the electrical conductovity change. Formaldehyde, pesticides, insecticides and nitrate biosensors were also developed conductometrically.[6] The urea biosensor was improved by a platinum electrode as a matrix for urease immobilization.[7] This research was adapted from the principle of the conductometric urea biosensor.

A conductimetric biosensor measures small changes in conductivity of solution by using a conductimetric transducer, ie, a conductivity meter. Conductivity measurement is based on the biocatalytic reaction of the sample on an electrode. The reaction will produce ions which will result in the change of conductivity.[8] The conductimetric transducer, consists of two electrodes, a reference and a working electrode. Both electrodes are coated with a nata de coco membrane. The enzyme is immobilized on the working electrode but not on the reference electrode. During reaction, CO_2 is produced on the working electrode, which is soluble in water to form HCO_3^- and H_3O^+. Moreover, on the reference electrode no reaction occurs, so the mobility of ions on the two electrodes is different and the conductivity is changed.

The matrix of enzyme immobilization commonly used, is cellulose acetate, polyacrylamide, gelatin and chitosan.[9,10] Nata de coco is a fermentation product from coconut water by *Acetobacter xylinum*. Nata de coco is a bacterial cellulose that has high purity, elastic, and a biodegradable.[11] Therefore, nata de coco can be applicated as a matrix for the enzyme immobilization.

Experimental

Materials and reagents

Uric acid (analytical grade) was obtained from Merck. Stock solutions of uric acid (200 ppm) were prepared by dissolving the acid in a phosphate buffer, and stored at 4 °C. Low concentration standard solutions of uric acid (1–40 ppm) were freshly prepared from the stock solution before an experiment. Uricase (16 mg/mL) was isolated from *Candida utilis*, dissolved in a Tris buffer (pH 8), and stored at 4 °C. Nata de coco membranes were obtained by fermentation of coconut water with *Acetobacter xylinum*. All other chemicals were of analytical grade (Merck) and deionized water (<0.1 µS).

Apparatus

The conductivity-meter was fabricated by WTW, Germany (WTW LF91) and modified with an additional amplifier. A pair of electrodes was made from Pt wire (5 mm × 5 mm, Fig. 1).

Procedure

Production of nata de coco

The coconut water was filtered and boiled. When the solution cooled down, sugar (100 grams/L coconut

water), acetic acid (20 ml/L) until the pH is at 3–4, and *Acetobacter xylinum* (170 ml/L) were added. The solution was filled in a chamber up to 4–5 cm from the bottom, and then wrapped with cotton gauze. The nata de coco membrane was produced in 3–4 days.

Preparation of the biosensor[12]

Platinum wire 5 × 5 mm (Fig. 1A) was coated by a nata de coco membrane. The Pt-wire is inserted between two layers of the membrane which is then fastened to each other. The electrode is dried at 50 °C for 30 minutes (Fig. 1B). For the uricase immobilization, 2 mL enzyme is used; the electrode is immersed in the enzyme solution for 24 hours at 5 °C. The enzyme is immobilized on the working electrode but not on the reference electrode.

Conductivity measurement

Measurements were conducted at room temperature (25 °C) in a glass cell. The electrodes are fixed in place and are immersed in a phosphate buffer solution until stabilization occurs in the output signal, then the cell is used to measure standard solutions of uric acid. The solution is stirred during the measurement of the conductivity. Conductivities were corrected by the constant cell (K = 0.92 cm^{-1}).

Results and Discussion

The effect of nata de coco membrane thickness

The Pt-wire is coated by nata de coco membranes one, two and three times, resulting a 5, 10 and 15 µm dry membrane thickness. The membrane thickness is measured using a micrometer-screw. The conductivity is measured at pH 9, based on previous work result.[5] The optimum performance biosensor is resulted at a 10 µm of membrane thickness (Fig. 2).

The sensitivity of the biosensor with a 10 µm thickness is smaller compared to the biosensor with a 15 µm thickness, but is opposite for the linearity. The performance of the biosensor depends on the amount of enzyme, and is affected by the size and number of membrane pores. The number of membrane pores increase proportionally with the thickness of the membrane. The immobilized uricase on the 10 µm thickness (28.68 mg) is greater than on the 15 µm thickness (25.10 mg). Hence the rate of diffusion of the ions (which is produced by oxidation of the uric acid) from membrane to the electrode surface is slower at 10 µm thickness than at 15 µm thickness. The research used the 10 µm membrane thickness for evaluation of the biosensor.

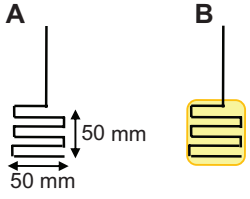

Figure 1. The biosensor was designed from Pt-wire (**A**), coated by nata de coco membrane (**B**).

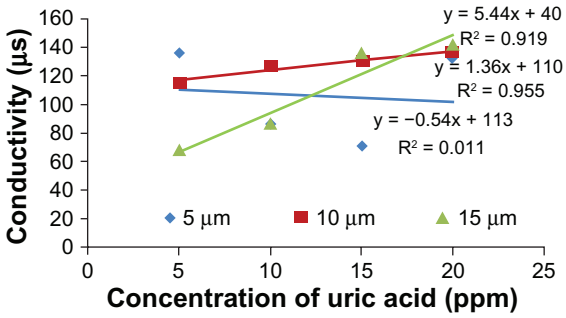

Figure 2. Curve of relation of the uric acid concentration to conductivity for various membrane thickness of the biosensor.

The effect of pH solution

In this research, the pH range used is 7.5 up to 9.5. There are two reasons. First, in previous works, the maximum immobilized uricase activities are at pH 8–9. Second, the number of HCO_3^- ions as an oxidation product of uric acid is present (>90%) at pH 7.5–9.5. Figure 3 illustrates that the conductivity is not significantly different for all concentrations of uric acid (1–10 ppm) at pH 8–9.5. At pH 7.5, conductivities show significant dynamic linear correlations with the uric acid concentrations at 1–3 ppm.

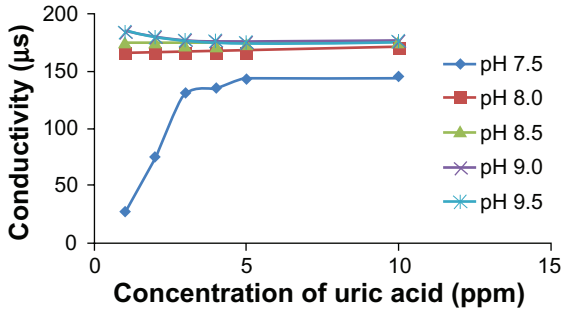

Figure 3. Curve of relation uric acid concentration to conductivity in various solution of pH.

The nata de coco membrane is an acetic cellulose with a —OH function group. That may cause a cross-linking between the —OH and the —NH groups of the uricase. This influences side active conformations and change of the isoelectric pH protein in the uricase. We concluded the optimum pH of immobilized uricase on nata de coco (pH 7.5) is different than on chitosan (pH 9).

Characterization of biosensor

Response time

Response time is the time to allow the system to come to equilibrium, which is indicated by the signal stability.[13] Figure 4 illustrates the relation of measuring time and conductivity at several concentrations of uric acid. The conductivity decreases consistently for all concentrations. This phenomenon is caused by the amount of the uric acid loading on the membrane pores. Initially, the ions to arrive to the electrode surface are ions from the buffer solution (Na^+, K^+, OH^-, $HPO_4^=$), so the conductivity is caused by these ions. The conductivity of the blank solution (phosphate buffer) averages at 250 µS, which is higher than the conductivity of the uric acid solution. The response time was determined from Figure 4, which is the starting time where the signal is constant; the response time for all concentrations is 3 minutes.

The response time depends on the uric acid concentration at 1–6 ppm. The minimum response time is obtained by the lowest of uric acid concentration. This indicates the dependence of oxidation rate of uric acid to the concentration. That means that uric acid concentration range is smaller than K_M (22 ppm) and the oxidation rate is below maximum velocity (V_m).

Range of uric acid concentrations

Figure 5 is a curve to determine the concentration range of uric acid. The curve shows two linear equations and the intercept shows the maximum concentration of uric acid which can be determined by the biosensor. The concentration range of uric acid is 1–6 ppm.

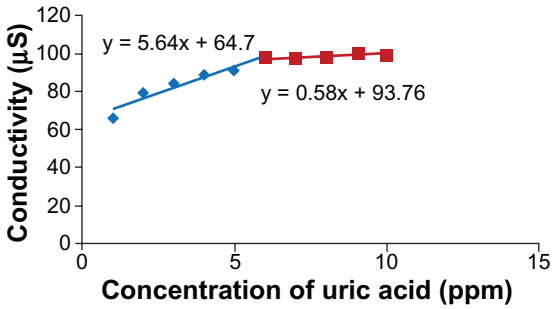

Figure 5. Determination of the concentration range of uric acid.

In the human serum, the range of uric acid concentration lies between 3.6 mg/dL (36 ppm) and 8.3 mg/dL (83 ppm). The biosensor is applicable to detect uric acid in human serum if the sample is diluted ten times.

The biosensor lifetime

Figure 6 is the relation of the uric acid concentration with conductivity using similar solutions in a five days period. The sensitivity and linearity of the curves are relatively stable at a three days period. However, the sensitivity and linearity decrease after three days. This phenomenon is caused by the release of immobilized enzyme from the nata de coco membrane.

The performance of the biosensor can be improved by changing the enzyme immobilization method and adding a *crosslinker* reagent. The immobilized enzyme can be adjusted by a certain form cross-linking structure.

Biosensor validation

Table 1 is the uric acid concentrations in fifteen samples. The uric acid concentration is determined by the biosensor and compared to results

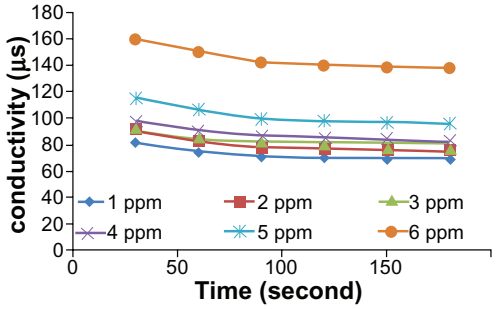

Figure 4. The relation of measuring time and conductivity at 1–6 ppm uric acid concentrations.

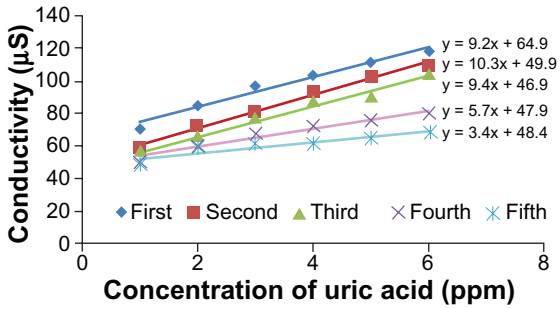

Figure 6. Relation of uric acid concentration and conductivity in five days period.

Table 1. Concentrations of uric acid in fifteen samples.

Number of samples	Uric acid concentration (ppm)		Relative error (%)
	Biosensor	Clinical lab	
1	6.7	7.0	4.3
2	5.6	5.3	5.7
3	6.2	5.8	6.8
4	7.6	8.1	6.1
5	5.4	5.7	5.3
6	6.6	6.3	4.7
7	8.1	7.7	5.2
8	5.2	4.9	6.1
9	4.4	4.6	4.3
10	7.0	7.4	5.4
11	13.6	12.4	9.7
12	15.4	13.7	12.4
13	12.2	11.0	10.9
14	16.0	14.1	13.5
15	14.4	12.9	11.6

of a clinical laboratory. Determination of uric acid concentration by the biosensor used a standard curve, with a linear equation of Y = 10.5X + 57.2. The relative error is smaller than 10% for uric acid concentrations lower than 10 ppm. However, the relative error is greater than 10%, if the uric acid is more than 10 ppm.

Conclusion

The conductimetric biosensor for uric acid detection can be made by uricase immobilized on a nata de coco membrane—Pt electrode. The maximum biosensor performances were resulted at 10 µm membrane thickness and pH 7.5. The response time is three minutes and range of concentration uric acid is 1 to 6 ppm. The biosensor has a lifetime of three days and a relative error below 10%, the sensitivity is 9 to 10 µS/ppm.

Acknowledgments

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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