American Journal of Analytical Chemistry, 2020, 11, 205-212
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Preparation of New Uric Acid Sensors Based on Iodide Selective Electrode

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How to cite this paper: Kalaycı, Ş. (2020) Preparation of New Uric Acid Sensors Based on Iodide Selective Electrode. *American Journal of Analytical Chemistry*, **11**, 205-212.
https://doi.org/10.4236/ajac.2020.115016

Received: April 20, 2020
Accepted: May 18, 2020
Published: May 21, 2020

Abstract

An electrode for uric acid has been prepared by using an iodide selective electrode with the uricase enzyme. The iodide selective electrode used was prepared from 10% TDMAI and PVC according to our previous study. The enzyme was immobilized on the iodide electrode by holding it at pH 7 phosphate buffer for 20 min at room temperature. The H_2O_2 formed from the reaction of uric acid was determined from the decrease of iodide concentration that was present in the reaction cell. The potential change was linear in the 2×10^{-5} to 2×10^{-4} M uric acid concentration (3 - 34 mg uric acid/100ml blood) range. Uric acid contents of some blood samples were determined with the new electrode and consistency was obtained with a colorimetric method. The effects of pH, iodide concentration, the amount of enzyme immobilized and the operating temperature were studied. No interference of ascorbic acid, glucose and urea was observed.

Keywords

Uric Acid Electrode, Uricase, Enzyme, Iodide Selective Electrode, Uric Acid in Blood

1. Introduction

Enzyme electrodes are being used for the measurement of different organic substrates. In most of them potentiometric sensors, e.g. oxygen [1] carbon dioxide [2], or ammonium ion electrodes [3] have been used, but some of them use different voltammetric measuring techniques.

Determination of uric acid in body fluids is a clinically valuable diagnostic indicator. The presence of elevated uric acid levels is a sign of gout, hyperuricemia, or Lesch-Nyhan syndrome [4]. The development of an electrochemical uric acid biosensor with an immobilized enzyme on an electrode surface has been the aim

of several recent studies [5] [6] [7]. In some of these procedures the enzyme uricase is used. This enzyme catalyzes the oxidation of uric acid to allontoin in the presence of carbon dioxide and hydrogen peroxide is formed. Hydrogen peroxide formed during this reaction can be determined with amperometric [5] [6] [8] or potentiometric sensors [9] [10] [11] [12].

A novel optical detection system consisting of combination of uricase/HRP-CdS quantum dots (QDs) for the determination of uric acid in urine sample is described. The QDs were used as an indicator to reveal fluorescence property of the system resulting from enzymatic reaction of uricase and HRP (horseradish peroxidase), which is involved in oxidizing uric acid to allaintoin and hydrogen peroxide. The linearity of the system toward uric acid was in the concentration range of 125 - 1000 μM with detection limit of 125 μM [13].

An electrochemical biosensor based on gold and palladium nano particles-modified nanoporous stainless steel (Au-Pd/NPSS) electrode has been introduced for the simultaneous determination of levodopa (LD) and uric acid (UA). Differential pulse voltammetry (DPV) was used for the simultaneous determination of LD and UA [14].

In this study, uricase was trapped in plasticized PVC and iodide ion selective electrode was used to monitor iodide. This electrode has an average slope of 63 mV/ten uric acid. When this electrode is used 4 times a day, it has a life of 70 days. The electrode is not sensitive to glucose and ascorbic acid. This study describes the preparation and application of a new potentiometric uric acid sensor.

2. Experimental

2.1. Apparatus and Reagents

Potential measurements were made with JENWAY 3030 Ion Analyser. "A double junction Ag/AgCl electrode 9240368" was used as the outer reference electrode. The enzyme was immobilized on our previously prepared iodide electrode. For the pH measurements, the ion analyzer with 924005 combined pH electrode is used. All measurements were made with a 30 ml glass cell prepared for this purpose. A magnetic stirrer was used throughout the experiments. All reagents used were analytical reagent grade (Merck). Triply distilled water was used for the preparation of solutions.

2.2. Preparation of Electrode

The iodide electrode in which the enzyme was fixed was prepared according to the procedure developed by us [15]. For this purpose, approximately 180 mg of PVC and 60 mg of ion exchanger (tridodecylmethylammonium iodide) are dissolved in 5 ml of tetrahydrofuran (THF). Then 0.2 ml of plasticizer (dibutyl phthalate) is added and mixed. After evaporation of the solvent the film membrane is cemented to a PVC tube with inner diameter of 10 mm, the tube is filled with 0.1 M KI and 0.1 M NaCl solution. A home-made Ag/AgCl electrode is immersed as the inner reference.

For the immobilization procedure, first 10 mg of enzyme is dissolved in 5 ml phosphate buffer (pH = 7). The iodide electrode prepared as the above given procedure is kept in it for 2 h at room temperature. This electrode was stored in pH 7 buffer at +2˚C when not in use. The measurements are made in 19 ml pH 7 phosphate buffer, 1 ml 1×10^{-3} M Mo(VI) and in the presence of 0.01 M iodide solution.

3. Result and Discussion

Uric acid is oxidized by air oxygen in the presence of uricase enzyme and hydrogen peroxide is formed, which reacts with iodide ion quantitatively. Thus, this reaction can be used for the determination of uric acid when known concentration of iodide is present. The decrease of iodide concentration will be proportional to uric acid concentration according to the following reactions.

$$\text{Uric acid} + O_2 \xrightarrow{\text{uricase}} \text{allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + 2I^- \xrightarrow{\text{Mo(VI)}} I_2 + H_2O$$

Molybdenum (VI) or peroxidase enzyme can be employed as the catalyst the last has the advantage of higher efficiency [9] [10] [11]. As can be seen two moles of iodide are used for one mole of uric acid. Thus the change of concentration of iodide after reaction with uric acid can be used for the determination of uric acid. In this work we used iodide electrode for the determination of iodide concentration before and after the reaction with uric acid.

3.1. Effect of Iodide Concentration

Since one mole of uric acid uses two moles of iodide its concentration has to be higher than uric acid concentration. The optimum concentration of iodide has to be determined for a uric acid concentration that is in the range of glucose present in blood. For this purpose, solutions with 19 ml pH 7 phosphate buffer and 1ml 1×10^{-3} M (Mo(VI)) have been prepared containing various iodide concentrations of 1×10^{-4}, 1×10^{-3} and 1×10^{-2} M. Their potentials were measured and then after each uric acid addition once more the potentials were measured. The uric acid concentrations in the cell were in the range of blood serum, changing from 2×10^{-5} M (3 mg/100ml) to 2×10^{-4} M (34 mg/100ml blood). As can be seen from **Figure 1** the slope was the highest for 10^{-2} M iodide concentration.

3.2. Effect of pH and Buffer Concentration

Adjustment of pH is important both for the immobilization of enzyme and for the reaction between hydrogen peroxide and iodide. At different pH values changing from 9 to 5.5 the response of electrode has been measured against uric acid concentration when 0.01 M iodide was present. Whereas there was nearly no response at pH values of 5.5, 6, 6.5, 8 and 9 the slope was the largest at pH 7. At this pH the ion exchanger (mentioned in Section 3.3) is positive and enzyme

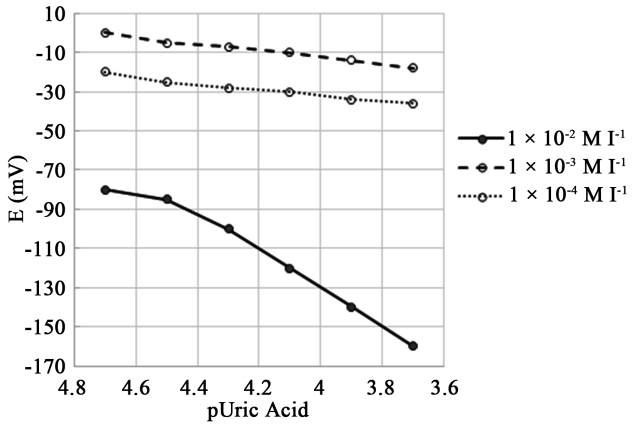

Figure 1. The effect of iodide concentration on the slope of the electrode.

is negative, thus it is the most convenient pH for immobilization and for the reaction.

Phosphate buffer has been chosen because of its pH working area. Its response for uric acid has been investigated at buffer concentrations changing from 1.0 to 10^{-3} M. The change of potential against uric acid concentration is given in **Figure 2**. As can be seen 0.1 M buffer concentration had the largest slope.

3.3. Immobilization of Enzyme

The ion exchanger (TDMAI) on the iodide electrode becomes a positive charge at pH = 7, at this pH the enzyme becomes minus charge and thus the enzyme will be immobilized on the electrode surface. The quantity of enzyme will be important since only one part of it can be immobilized on the electrode surface. For this purpose, iodide electrode was dipped into pH 7 phosphate buffer solutions each containing 5, 10 and 20 mg/ml enzyme for 2 h. It was washed with distilled water and its response has been measured for uric acid concentrations in the presence of 0.01 M iodide. As can be observed from **Figure 3**, the slope was the largest for 10 mg/ml enzyme.

3.4. Effect of Temperature

Optimum temperature is very important since the enzyme activity will increase with temperature, but on the other hand at high temperatures there may be thermal deactivation of the enzyme and also decrease of O_2 concentration. In a solution containing 0.01 M iodide, the potential of a solution of 1×10^{-4} M uric acid has been followed between temperatures of 30˚C - 60˚C within 5˚C intervals. The maximum activity of the enzyme was obtained at 50˚C (**Figure 4**).

3.5. Response and Lifetime

This electrode did not lose its activity for 45 days when used 4 times a day. The response time was measured at different uric acid concentrations. As can be seen from **Figure 5** the response was almost immediate. The lifetime of the electrode is also very good compared with other electrodes [6] [8] [10] [11] [15].

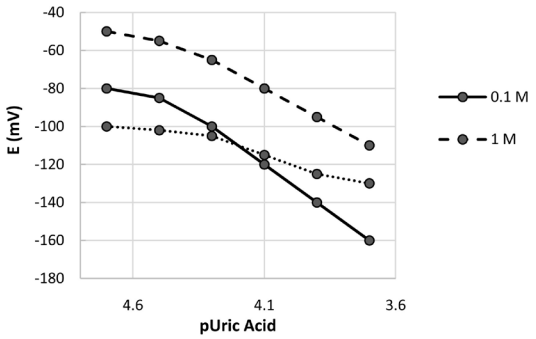

Figure 2. The change of potential with buffer concentration (1×10^{-2} M I^-).

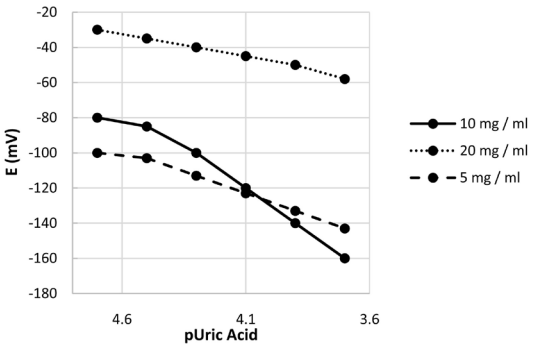

Figure 3. The effect of immobilized enzyme quantity on the slope of the electrode (1×10^{-2} M I^-).

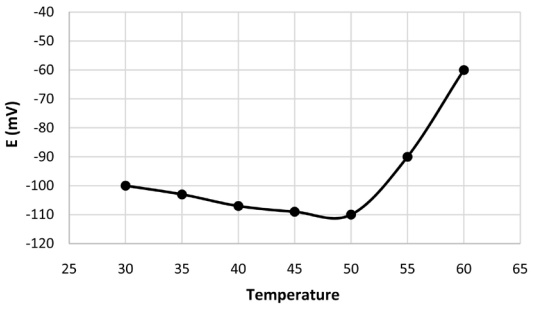

Figure 4. The effect of temperature on the activity of enzyme (2×10^{-4} M uric acid, 1×10^{-2} M I^-).

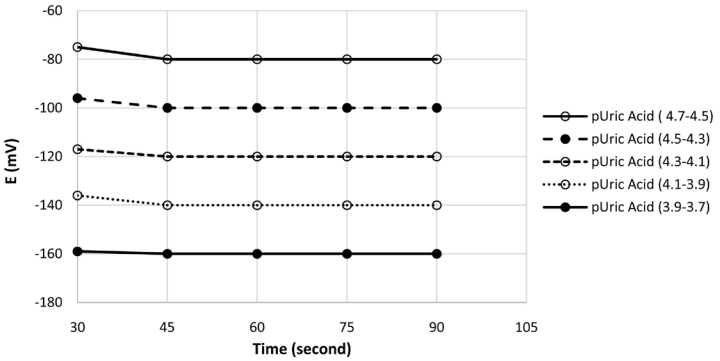

Figure 5. The dependence of response time of the electrode on the change of concentration.

3.6. Interference Studies

The product of enzymatic reaction is hydrogen peroxide, thus reducing agents such as glucose and ascorbic acid, two compounds commonly found in biological fluids, may interfere [8] [9]. The strong interference of glucose and ascorbic acidin a former study [9] was eliminated after pretreatment with hydrogen peroxide. With our new electrode in the presence of 0.01 M iodide and 2×10^{-5} M uric acid there was no interference from the above mentioned substances in the concentration ranges that are commonly encountered in biological fluids (0 - 2.5 mM). The selectivity constants determined by using the mixed solution method [16] are given in **Table 1**.

3.7. Measurement of Uric Acid in Blood Serum

It was found that, with this new electrode the uric acid could be determined with high accuracy and precision (**Table 2**). For a solution containing 5 mg/100ml the result obtained for 4 measurements was 4 ± 0.1 mg/100ml.

The blood samples that were analyzed for their uric acid quantity were obtained from the University Health Center. They were first centrifuged with a speed of 9000 round/min and these were used for uric acid determination. First the potential of a solution containing 19 ml buffer (pH = 7), 1 ml 1×10^{-3} M Mo (VI) and 0.01 M iodide was measured. A 0.1 ml of serum sample was added and the potential was once more measured. Then two standard additions of 0.1 M uric acid (each 0.1 ml) were made and potentials were measured. From the change of potentials, the amount of uric acid in blood was determined. Blood samples shall not wait long time; otherwise uric acid will be lost because of destruction. If it has to wait additions of fluoride or ascorbic acid is needed. Glucose quantities for three different blood samples are given in **Table 3** with the results of University Health Center (colorimetric) for comparison.

Table 1. Selectivity coefficient ($K_{A,B}^{pot}$) for the uric acid electrode in mixed solutions (in the presence of 1×10^{-5} M uric acid)[a].

	B	
	Glucose	Ascorbic acid
$K_{A,B}^{pot}$	2.3×10^{-4}	3.4×10^{-4}

[a]A: Uric acid; B: Interfering ion.

Table 2. Determinations of uric acid in a known samples[a].

Uric acid (mg/100ml)	Uric acid determined with the new electrode (mg/100ml)		
X_i	$\bar{X}$	S	CI*
4	4.1	0.012	4.1 ± 0.2

CI: 95%, N = 4.

Table 3. Uric acid levels in the four different blood samples.

Quantity given from health center (mg/100ml)	Uric acid levels, with the new electrode (mg/100ml)
4.5	4.4 ± 0.2
6.2	6.3 ± 0.3
3.9	3.8 ± 0.1
10.6	10.7 ± 0.5

95% CI, N = 4.

4. Conclusion

A new enzyme based electrode is prepared by using an iodide selective electrode. Here one electrode works as enzyme holder and at the same time it monitors the iodide concentration. Since the iodide electrode is constructed with an ion exchanger and not with AgI, it does not show any interference of most common ions such as chloride and sulfate. The prepared sensor displayed very good performance in regard to reproducibility, sensitivity and long lifetime. It shows linear response in the 3 - 34 mg/100ml concentration range with a slope of about 63 mV per decade change of uric acid.

Acknowledgements

The author thanks to the Gazi University research fund for the financial support of this research.

Conflicts of Interest

The author declares no conflict of interest.

References

[1] Updike, S. and Hicks, G.P. (1967) Reagentless Substrate Analysis with Immobilized Enzymes. *Science*, **158**, 270-272. https://doi.org/10.1126/science.158.3798.270

[2] Kalcher, K., Svancara, I., Buzuk, M., Vytras, K. and Walcarius, A. (2009) Electrochemical Sensors and Biosensors Based on Heterogeneous Carbon Materials. *Monatshefte für Chemie—Chemical Monthly*, **140**, 861-889. https://doi.org/10.1007/s00706-009-0131-9

[3] Raab, L.S., Decker, G.L., Jonas, A.J., Kaetzel, M.A. and Dedman, J.R. (1991) Glucocoticoid Regulation of Rat Liver Urate Oxidase. *Journal of Cellular Biochemistry*, **47**, 18-30. https://doi.org/10.1002/jcb.240470104

[4] Martinez-Pérez, D., Ferrer, M.L. and Mateo, C.R. (2003) A Reagent Less Fluorescent Sol-Gel Biosensor for Uric Acid Detection in Biological Fluids. *Analytical Biochemistry*, **322**, 238-242. https://doi.org/10.1016/j.ab.2003.08.018

[5] Gülce, H., Özyörük, H., Çelebi, S.S. and Yýldýz, A. (1995) Amperometric Enzyme Electrode for Aerobic Glucose Monitoring Prepared by Glucose Oxidase Immobilized in Poly(vinylferrocenium). *Journal of Electroanalytical Chemistry*, **394**, 63-70. https://doi.org/10.1016/0022-0728(95)04013-E

[6] Sun, L.-X., Xu, F. and Okada, T. (1998) Studies on Optimization of a Platinum Cat-

alyst and Porphine Modified, Pyrolytic Graphite, Amperometric, Glucose Sensor by Sequential Level Elimination Experimental Design. *Talanta*, **47**, 1165-1174. https://doi.org/10.1016/S0039-9140(98)00195-7

[7] Campanella, L. and Tomassetti, M. (1989) Sensors in Pharmaceutical Analysis. *Selective Electrode Rev.*, **11**, 69-73.

[8] Losada, J. and Pilar, M. (1997) A Glucose Amperometric Sensor Based on Covalent Immobilization of Glucose Oxidase in Poly-2-Aminoaniline Film via Chloranil on Platinized Platinum Electrode. *Electroanalysis*, **9**, 1416-1421. https://doi.org/10.1002/elan.1140091808

[9] Nagy, G., Von Storp, L.H. and Guilbault, G.G. (1973) Enzyme Electrode for Glucose Based on an Iodide Membrane Sensor. *Analytica Chimica Acta*, **66**, 443-445. https://doi.org/10.1016/S0003-2670(01)82564-2

[10] Hitti, I.K.A., Moody, G.J. and Thomas, J.D.R. (1984) Glucose Oxidase Membrane Systems Based on Poly(vinyl chloride) Matrices for Glucose Determination with an Iodide Ion-Selective Electrode. *Analyst*, **109**, 1205-1208. https://doi.org/10.1039/an9840901205

[11] Merkoçi, A., Fabregas, E. and Alegret, S. (1999) Consolidated Biocomposite Membrane Technology for Production of Potentiometric Biosensors. *Sensors & Actuators, B*, **60**, 97-105. https://doi.org/10.1016/S0925-4005(99)00262-2

[12] Ali, U., Alvi, N.H., Ibupoto, Z., Nur, O., Willander, M. and Danielsson, B. (2011) Selective Potentiometric Determination of Uric Acid with Uricase Immobilized on ZnO Nanowires. *Sensors and Actuator B*, **152**, 241-247. https://doi.org/10.1016/j.snb.2010.12.015

[13] Azmi, N.E., Ramli, N.I., Abdullah, J., Hamid, M.A.A., Sidek, H., Rahman, S.A., Ariffin, N. and Yusof, N.A. (2015) A Simple and Sensitive Fluorescence Based Biosensor for the Determination of Uric Acid Using H_2O_2-Sensitive Quantum Dots/Dual Enzymes. *Biosensors and Bioelectronics*, **67**, 129-133. https://doi.org/10.1016/j.bios.2014.07.056

[14] Rezaei, B., Shams-Ghahfarokhi, L., Havakeshian, E. and Ensafi, A.A. (2016) An Electrochemical Biosensor Based on Nanoporous Stainless Steel Modified by Gold and Palladium Nanoparticles for Simultaneous Determination of Levodopa and Uric Acid. *Talanta*, **158**, 42-50. https://doi.org/10.1016/j.talanta.2016.04.061

[15] Somer, G., Kalayci, S. and Ekmekçi, G. (2001) Preparation and Application of Iodide-Mercury Selective Membrane Electrode Based on Ion Exchangers. *Sensors & Actuators B*, **81**, 122-127. https://doi.org/10.1016/S0925-4005(01)00942-X

[16] Srinivasan, K. and Rechnitz, G.A. (1969) Selectivity Studies on Liquid Membrane, Ion Selective Electrodes. *Analytical Chemistry*, **41**, 1203-1208. https://doi.org/10.1021/ac60279a014