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Judul artikel : Imprinting Zeolite-Modified Glassy Carbon As A Voltammetric Sensor For Uric Acid

Penulis : Miratul Khasanah*, Muji Harsini, Alfa Akustia Widati

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No : 273/IJC/04/2013

Subject : Reviewing of paper ID No: 2012-12-537

Encl. : 1

Yogyakarta, 19 April 2013

To:

Miratul Khasanah

Chemistry Department, Faculty of Sciences and Technology, Airlangga University, Kampus C Unair, Mulyorejo Surabaya, 60115, Indonesia

Dear Madam,

This is to acknowledge and inform you that the paper entitles:

Imprinting Zeolite-Modified Glassy Carbon as A Voltammetric Sensor for Uric Acid Analysis

written by:

Miratul Khasanah, Muji Harsini, Alfa Akustia Widati

has been reviewed and could be published in Indo. J. Chem. after major revision. The author should make some correction as follows:

Reviewer I:

1. To claim that zeolite has been successfully synthesized from TEOS, and TBOT, XRD and FT-IR spectra should be included in the manuscripts! Why authors did not use commercially available zeolite which its structure is already confirmed?
2. Table 2 is not clear! The unit of deviation should be accompanied (%???). Also the statement of "not observed" is ambiguous. If the experiments were not done for those ratios it should be stated as "not done"
3. Revise according to note/correction in the manuscripts.
4. Abstract and keyword in *Bahasa Indonesia* should be accompanied.

Reviewer II:

1. The paper needs more data to complete. Data required to enhance the quality of the manuscript include the XRD of the zeolite before and the XRD of the zeolite after imprinting with the uric acid.
2. The elemental analysis results of the zeolite should also be appended.
3. In work using voltammetry, it is always good to have voltammograms to be included in the writing.

4. The word "Analysis" in the title must be removed; it has been represented in the word "Sensor".
5. Please be specific about the method, is it cyclic voltammetry, pulse voltammetry, stripping voltammetry or anything else?
6. In the paragraph 3 of the introduction should have a paper or two to cite. In general more reference is need related to the works on voltammetric analysis of uric acid.
7. For writing source of chemical used in this work, write in a narrative form, not to put in the parenthesis. For example, uric acid was purchased from Fluka etc.
8. Number written in Table 1 should have consistent significant figures (3 or 4)
9. In Table 1 there is a number written as 4.281y, what does y mean?
10. For Tables 1 and 2, is it anodic or cathodic current? Must be specific.
11. Finnaly, the paper must be read by an English-competent reader. There are grammatical errors and typos throught the manuscript. Many sentences have syntax errors.

Please kindly send your revised manuscript by 3 May 2013, so that the article could be published in the upcoming Indo. J. Chem. Also **kindly send your response to the reviewers' comments in a separate letter**. Thank you for your submission and we are waiting for the other articles from you and your colleagues.

Editor Indonesian Journal of Chemistry

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Please kindly send your revised manuscript by 3 May 2013, so that the article could be published in the upcoming Indo. J. Chem. Also kindly send your response to the reviewers' comments in a separate letter. Thank you for your submission and we are waiting for the other articles from you and your colleagues.

Sincerely yours,


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IMPRINTING ZEOLITE-MODIFIED GLASSY CARBON AS A VOLTAMMETRIC SENSOR FOR URIC ACID ANALYSIS

ABSTRACT

Development of the uric acid sensor through the coating of glassy carbon (GC) electrode with imprinting zeolite (IZ) was carried out. Zeolite was synthesized by mixing TEOS, TBOT, TPAOH, and water through hydrothermal process. Zeolite was molded by uric acid to produce IZ. The deposition potential of IZ and uric acid to the GC surface was -0.6 V during 150 s with addition of KNO₃ 0.02 M as supporting electrolyte. The method validity obtained were linearity 0.9834 (concentration $5.6 \times 10^{-9} \text{ M} - 2.8 \times 10^{-8} \text{ M}$), precision 1.89-7.65%, sensitivity $0.33 \mu\text{A/nM/cm}^2$, limit of detection $5.9 \times 10^{-9} \text{ M}$, and accuracy $96.26 \pm 0.55\%$ ($n=5$). The developed sensor showed a high selectivity toward uric acid. Presence of ascorbic acid, creatine and creatinine with an equal concentration with uric acid did not interfere on the uric acid analysis.

Key words : *sensor, uric acid, voltammetry, imprinting zeolite, glassy carbon*

INTRODUCTION

Uric acid is the end product of purine metabolism. The high concentration of uric acid in the body is symptoms of some diseases such as diabetes, high blood pressure, kidney and liver disease, and can increase risk of cardiovascular [1]. Method that has been used to determine uric acid in the biomedical field is spectrophotometry. Analysis of uric acid using this method requires a large number of samples (2-3 mL). In addition, the method has a low sensitivity and high detection limit (mM).

Voltammetry has been used to determine concentration and redox properties of many compounds in the body fluids, such as uric acid in the blood serum. Analysis of uric acid by voltammetry has been developed through the development of electrode for special-purpose lowering the limit of detection and increasing sensitivity and selectivity because of uric acid coexist with other compounds whose structure are similar to that of uric acid in serum samples [2-4].

In the recent study, the zeolite was used as an alternative material to modify electrode in order to obtain a selective sensor for detecting uric acid voltammetrically. Imprinting zeolite (IZ) was synthesized by mixing of tetraethyl ortho silicate (TEOS), tetrabutyl orthotitanate (TBOT), tetrapropyl ammonium hydroxide (TPAH) and uric acid. Uric acid was then extracted from the zeolite framework so as to produce prints. The sensor was synthesized by coating IZ on the glassy carbon (GC) electrode at optimum potential and time.

Conformity between the pore size of synthesized zeolite and molecular size of uric acid could increase the adsorption capacity of zeolite, which can provide high sensitivity in uric acid determination. Zeolite has rigid form, so that in the aqueous media can maintain the shape and size of its pore (selective) [5]. The performance and validity of the sensor to analyze uric acid have been studied in this research.

EXPERIMENTAL SECTION

Materials

Chemicals used in this study were uric acid (Fluka), creatine, creatinine (Sigma-Aldrich), tetraethyl ortho silicate, tetrapropyl ammonium hydroxide, tetrabutyl orthotitanate, 2-propanol, ethanol, sodium acetate, acetic acid, sodium dihydrogen phosphate, sodium hydrogen phosphate and potassium nitrate (Merck). All chemicals were analytical grade (p.a). The stock solution of uric acid 6.0×10^{-3} M was prepared by dissolving 0.1000 g uric acid in about 10 mL sodium hydroxide 50% and diluted with water until 100 mL in volumetric flask. The solutions of creatine and creatinine were prepared by dissolving the compounds using water in the room temperature. The water used in this research is ultra high pure (UHP) water.

Instrumentation

The instrumentations used in this study were 797 Computrace Voltammetry (MVA system-1) equipped with a sample container, stirrer, processor units, PCs, glassy carbon (BAS MF-2012, d= 3mm), reference electrode Ag/AgCl (KCl 3M) and Pt

auxiliary electrodes; FTIR (Shimadzu), N₂ adsorption-desorption/BET (Quantachrome NovaWin version 2.1); x-ray diffractometer (XRD), pH meter; and other supporting equipments.

Procedure

Synthesis and characterization of zeolite

MFI zeolite was synthesized by mixing ^{zeolite with} TEOS into TBOT, and stirred for 30 minutes at room temperature. TPAH was added to the mixture, and then stirred for 15 hours, so that the mixture obtained has a mole ratio of TEOS:TiO₂:TPAH:H₂O = 1:0.017:0.24:21.2. The mixture was further heated hydrothermally at 80 ° C during 4 days. Non imprinting zeolite (NIZ) was synthesized by adding uric acid to the mixture with a molar ratio of uric acid/Si = 2.98x10⁻⁴. Subsequently, the mixture allowed aging for 3 hours [6]. The uric acid was then extracted from the zeolite framework using warm water to produce IZ. The synthesized zeolite (Z), IZ and NIZ were characterized using FTIR, XRD, and N₂ adsorption-desorption.

where is the zeolite?

Synthesis and performance test of the sensor

The IZ was *in situ* coated on GC electrode. A number of 20 mL of uric acid solution was inserted into the electrochemical cell, added with 5 mg IZ and coated to the GC electrode at the potential range between +1 V and -1 V (vs. Ag/AgCl). Coating time varied from 30-180 s, while the pH of the solution was conducted from pH 4-7. The Z and NIZ were coated on the GC electrode surface using optimum potential and time. The GC-IZ, GC, GC-zeolite (GC-Z) and GC-NIZ sensors were then applied to analyze uric acid and observed the peak potential and current to determine the sensors performance.

Method validity

A series of uric acid standard solution with concentration of 5.6x10⁻⁹, 1.1x10⁻⁸, 1.7x10⁻⁸, 2.3x10⁻⁸ and 2.8x10⁻⁸ M were analyzed by voltammetry using GC-IZ sensor. The data of standard solution current was used to determine the linearity, limit of

detection, sensitivity, and precision. Accuracy was studied by analyzing uric acid solution 2.8×10^{-8} M. The selectivity of the sensor was studied through the addition of ascorbic acid, creatine, and creatinine (matrices which are always coexist with uric acid in serum sample) on the uric acid analysis. The molar ratios of the uric acid and the each matrix were 1:1, 1:2; 1:3; 1:4 and 1:5, respectively. The current responses of the mixing compound were compared to the result of the current response of uric acid without the addition of matrix.

RESULT AND DISCUSSION

Synthesis and characterization of zeolite

Precursors that used to synthesize zeolite are TBOT and TEOS as titanium and silica sources, respectively. TPAH was used as a structure steering agent and alkaline condition. Uric acid was used as template and a directional pore size of zeolite. A number of uric acid as a template was added to the mixture of TBOT, TEOS, and TPAH so as the molar ratio of uric acid/Si = 2.98×10^{-4} . The next stage was aging of the mixture for 3 hours to trap the uric acid into the framework of zeolite and produce NIZ. Uric acid was then extracted from the zeolite framework by repeatedly washing with warm water to produce IZ. The residue and the filtrate were separated by centrifugation. Zeolite, NIZ and IZ obtained in this study are the solids white.

XRD diffractogram data showed that diffraction peaks of the overall synthesized zeolite appear at 2θ of 7.96; 8.85; 23.2; 23.9, and 24.4° . These peaks correspond to the characteristic of MFI zeolite framework [7]. At 2θ around 24.4° there is a single reflection peak. This shows that there has been change in the material structure of the monoclinic (silicalite) to orthorhombic (MFI zeolite) [8]. Characterization using FTIR spectroscopy showed the peak at wave number 765 cm^{-1} , 970 cm^{-1} , and 1050 cm^{-1} which is the typical peak of the zeolite. Peak at 765 cm^{-1} indicates the symmetric stretching of Si-O-Si, at 970 cm^{-1} indicates the presence of titanium (Si-O-Ti) in the zeolite framework, and at 1050 cm^{-1} indicates the presence of stretching

asymmetric Si-O-Si [9]. The NIZ spectra showed characteristic spectra of zeolite, and at 840 cm^{-1} , 1650 cm^{-1} , and 3450 cm^{-1} are the characteristic peak of uric acid. Compared to the NIZ spectra, there is a reduction in the intensity of peak at 1650 cm^{-1} in IZ spectra which is the typical peak of the carbonyl group derived from uric acid. It can be concluded that uric acid was extracted from the zeolite framework, although not overall uric acid has been successfully extracted. The results of N_2 adsorption-desorption characterization was found that zeolite has a pore diameter of 3.836 nm, while the IZ has a pore diameter of 2.186 nm.

Coating of GC electrode with imprinting zeolite

In this research, IZ was coated *in situ* and deposited together with uric acid to the GC electrode. The optimum deposition potential of IZ and uric acid on the GC electrode surface was -0.6 V during 150 s and yield the stripping peak potential -0.376 V. Potential deposition of uric acid was more negative than its stripping peak potential. This indicates that reduction reaction occurred during the deposition step and oxidation reaction occurred during the stripping step occurs. So that it can be concluded that uric acid analysis techniques using GC sensor in this study belong anodic stripping voltammetry. At pH optimization, the best voltammogram obtained in a solution with pH 7 without buffer addition. KNO_3 0.02 M was added as supporting electrolyte in the solution to minimize the non faradic current.

The pH vs. E_p curve (Fig. 1) shows relationship between the pH of the solution and the stripping potential with slope of -59.5 mV. It is clear that the redox reaction of uric acid on the GC-IZ surface involves two electrons and two protons (H^+). Redox reaction of uric acid was shown at Fig. 2.

Performance of the GC-IZ sensor

The sensor performance test showed that GC-IZ has good repeatability compared to the three other sensors. Analysis of uric acid using GC-IZ sensor produces a low current, but not as low as when using GC-Z, because the IZ layer has

pores that have been printed by uric acid so that uric acid molecules can diffuse through the IZ porous toward GC surface. The highest current was generated using GC-NIZ sensor, then successively smaller the GC, GC-IZ and GC-Z (Table 1).

Method validity

Measurement of uric acid standard solution 5.6×10^{-9} M - 2.8×10^{-8} M produce the regression equation of $y = 136.7x - 4728$ with a correlation coefficient (r) = 0.9834. The t-test on r showed t_{count} (6.3846) greater than the t_{table} (2.920). It can be concluded that there is a linear relationship between the concentrations of uric acid to the current. Precision (RSD) obtained was 1.89% -7.65%. Uric acid analysis method using developed sensor in this study provide a smaller precision than $2/3\text{RSD}_{\text{Horwitz}}$ (30% to $\mu\text{g/L}$ or 10^{-9} M level) [10-12]. The value of precision is statistically acceptable. Sensitivity of the developed method was $0.33 \mu\text{A/nM/cm}^2$. Sensitivity of the method is good enough, but lower than sensitivity obtained using GC and GC-imprinting polymer that have been developed previously [13]. Detection limit resulted was 5.9×10^{-9} M. The detection limit obtained in this study was much smaller than the results obtained from previous studies [14-20]. The detection limit is about 10^4 times lower than using spectrophotometry method (1.2×10^{-5} M). With a low detection limit, the analysis of uric acid in a very small amount of serum sample (μL) can be done. The accuracy of 2.8×10^{-8} M uric acid obtained was $96.26 \pm 0.55\%$ ($n=5$). The accuracy value is very good and statistically acceptable (accuracy for 10^{-9} M concentration level is 70-125%) [11,12].

Selectivity

One of the major problems on the determination of uric acid in the serum sample by the voltammetry method is the presence of other compounds which are usually found together with uric acid in serum sample. In this study, to determine the selectivity of the developed sensor, the interference of ascorbic acid, creatine and creatinine on determination of the uric acid has been studied. From the Table 2 can be

concluded that the analysis of uric acid voltammetrically using the developed sensor was not interfered by ascorbic acid, creatine and creatinine, because according the previous report, the normal concentration of ascorbic acid and creatine/creatinine in the serum sample were one tenth and one fourth of uric acid concentration, respectively [21-23]. Compared to the GC sensor, the selectivity of GC-IZ sensor toward uric acid in the ascorbic acid, creatine and creatinine matrices with equal concentration to uric acid were 5, 18 and 2 times higher, respectively.

CONCLUSIONS

Imprinting zeolite-modified glassy carbon showed good performance as voltammetric sensor for uric acid analysis. Analysis of uric acid 5.6×10^{-9} - 2.8×10^{-8} M by voltammetry using the sensor produced linearity of calibration curve (r) 0.9834, precision 1.89-7.65%, sensitivity $0.33 \mu\text{A/nM/cm}^2$, and limit of detection 5.9×10^{-9} M. The limit of detection obtained is low enough for the purpose of uric acid determination in natural samples such as blood serum. Accuracy of uric acid 2.8×10^{-8} M obtained was $96.26 \pm 0.55\%$ ($n=5$). Analysis of uric acid using the developed sensor was not interfered by ascorbic acid, creatine and creatinine with equal concentration with uric acid. Thus, voltammetry method using the developed sensor is suggested to be used as an alternative method to determine of uric acid in the medical field.

ACKNOWLEDGEMENT

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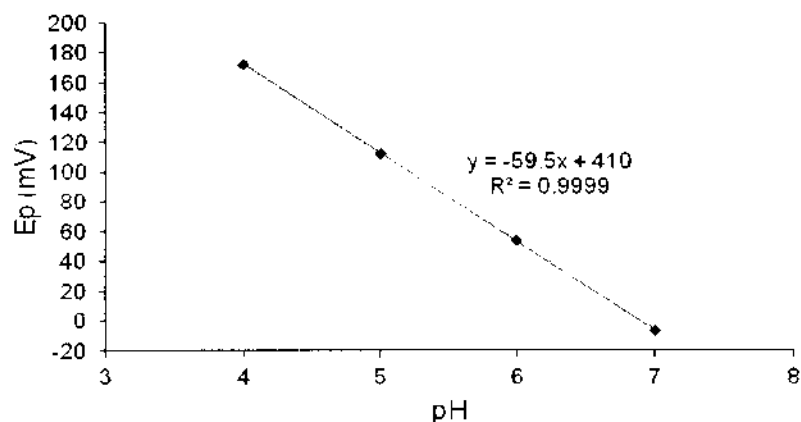


Fig 1. Stripping peak potential (E_p) versus pH of uric acid solution

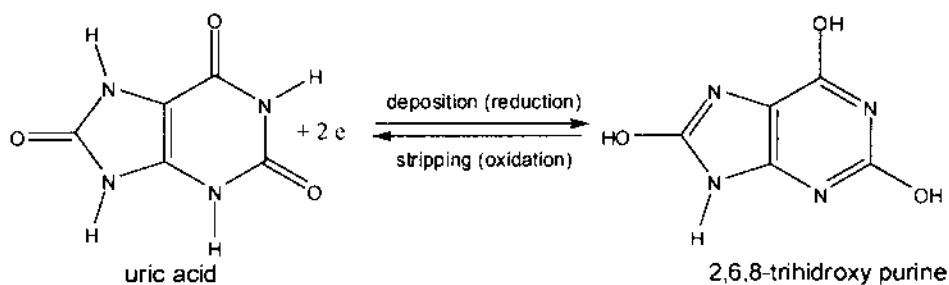


Fig 2. Redox reaction of uric acid

Table 1. The uric acid current measured using GC-IZ, GC, GC-Z, and GC-NIZ sensor

Sensor	Current (nA)	RSD (%) (n=3)
GC-IZ	619.9	1.891
GC	3082.0	4.281y
GC-Z	489.6	13.443
GC-NIZ	3643.7	10.167

uric acid concentration = 3.0×10^{-8} M

Table 2. Deviation of the uric acid current caused by adding ascorbic acid, creatine and creatinine

[UA]:[matrix]	Deviation of the current					
	Ascorbic acid		Creatine		Creatinine	
	GC	GC-IZ	GC	GC-IZ	GC	GC-IZ
1:1	14.20	3.49	6.87	0.38	5.42	2.76
1:2	-	4.44	16.60	4.14	12.62	4.52
1:3	-	6.59	30.60	17.28	18.95	5.00
1:4	-	7.06	95.29	19.63	25.79	6.81
1:5	16,10	9.14	97.57	19.77	28.46	6.38

[UA] = concentration of uric acid = 5.0×10^{-7} M ; [matrix] = concentration of ascorbic acid, creatine or creatinine, - = not observed

not done ??

IMPRINTING ZEOLITE-MODIFIED GLASSY CARBON AS A VOLTAMMETRIC SENSOR FOR URIC ACID ANALYSIS

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EXPERIMENTAL SECTION

Materials

Chemicals used in this study were uric acid (Fluka), creatine, creatinine (Sigma-Aldrich), tetraethyl ortho silicate, tetrapropyl ammonium hydroxide, tetrabutyl orthotitanate, 2-propanol, ethanol, sodium acetate, acetic acid, sodium dihydrogen phosphate, sodium hydrogen phosphate and potassium nitrate (Merck). All chemicals were analytical grade (p.a). The stock solution of uric acid 6.0×10^{-3} M was prepared by dissolving 0.1000 g uric acid in about 10 mL sodium hydroxide 50% and diluted with water until 100 mL in volumetric flask. The solutions of creatine and creatinine were prepared by dissolving the compounds using water in the room temperature. The water used in this research is ultra high pure (UHP) water.

} write in narrative form

Instrumentation

The instrumentations used in this study were 797 Computrace Voltammetry (MVA system-1) equipped with a sample container, stirrer, processor units, PCs, glassy carbon (BAS MF-2012, d= 3mm), reference electrode Ag/AgCl (KCl 3M) and Pt

auxiliary electrodes; FTIR (Shimadzu), N₂ adsorption-desorption/BET (Quantachrome NovaWin version 2.1); X-ray diffractometer (XRD), pH meter; and other supporting equipments.

Procedure

Synthesis and characterization of zeolite

MFI zeolite was synthesized by mixing TEOS into TBOT, and stirred for 30 minutes at room temperature. TPAH was added to the mixture, and then stirred for 15 hours, so that the mixture obtained has a mole ratio of TEOS:TiO₂:TPAH:H₂O = 1:0.017:0.24:21.2. The mixture was further heated hydrothermally at 80 ° C during 4 days. Non imprinting zeolite (NIZ) was synthesized by adding uric acid to the mixture with a molar ratio of uric acid/Si = 2.98x10⁻⁴. Subsequently, the mixture allowed aging for 3 hours [6]. The uric acid was then extracted from the zeolite framework using warm water to produce IZ. The synthesized zeolite (Z), IZ and NIZ were characterized using FTIR, XRD, and N₂ adsorption-desorption.

Synthesis and performance test of the sensor

The IZ was *in situ* coated on GC electrode. A number of 20 mL of uric acid solution was inserted into the electrochemical cell, added with 5 mg IZ and coated to the GC electrode at the potential range between +1 V and -1 V (vs. Ag/AgCl). Coating time varied from 30-180 s, while the pH of the solution was conducted from pH 4-7. The Z and NIZ were coated on the GC electrode surface using optimum potential and time. The GC-IZ, GC, GC-zeolite (GC-Z) and GC-NIZ sensors were then applied to analyze uric acid and observed the peak potential and current to determine the sensors performance.

Method validity

A series of uric acid standard solution with concentration of 5.6x10⁻⁹; 1.1x10⁻⁸; 1.7x10⁻⁸; 2.3x10⁻⁸ and 2.8x10⁻⁸ M were analyzed by voltammetry using GC-IZ sensor. The data of standard solution current was used to determine the linearity, limit of

detection, sensitivity, and precision. Accuracy was studied by analyzing uric acid solution 2.8×10^{-8} M. The selectivity of the sensor was studied through the addition of ascorbic acid, creatine, and creatinine (matrices which are always coexist with uric acid in serum sample) on the uric acid analysis. The molar ratios of the uric acid and the each matrix were 1:1, 1:2; 1:3; 1:4 and 1:5, respectively. The current responses of the mixing compound were compared to the result of the current response of uric acid without the addition of matrix.

RESULT AND DISCUSSION

Synthesis and characterization of zeolite

Precursors ~~that~~ used to synthesize zeolite are TBOT and TEOS as titanium and silica sources, respectively. TPAH was used as a structure steering agent and alkaline condition. Uric acid was used as template and a directional pore size of zeolite. A number of uric acid as a template was added to the mixture of TBOT, TEOS, and TPAH so as the molar ratio of uric acid/Si = 2.98×10^{-4} . The next stage was aging of the mixture for 3 hours to trap the uric acid into the framework of zeolite and produce NIZ. Uric acid was then extracted from the zeolite framework by repeatedly washing with warm water to produce IZ. The residue and the filtrate were separated by centrifugation. Zeolite, NIZ and IZ obtained in this study are the solids white.

XRD diffractogram data showed that diffraction peaks of the overall synthesized zeolite appear at 2θ of 7.96; 8.85; 23.2; 23.9, and 24.4°. These peaks correspond to the characteristic of MFI zeolite framework [7]. At 2θ around 24.4° there is a single reflection peak. This shows that there has been change in the material structure of the monoclinic (silicalite) to orthorhombic (MFI zeolite) [8]. Characterization using FTIR spectroscopy showed the peak at wave number 765 cm^{-1} , 970 cm^{-1} , and 1050 cm^{-1} which is the typical peak of the zeolite. Peak at 765 cm^{-1} indicates the symmetric stretching of Si-O-Si, at 970 cm^{-1} indicates the presence of titanium (Si-O-Ti) in the zeolite framework, and at 1050 cm^{-1} indicates the presence of stretching

asymmetric Si-O-Si [9]. The NIZ spectra show ~~the~~ characteristic spectra of zeolite, and at 840 cm^{-1} , 1650 cm^{-1} , and 3450 cm^{-1} are the characteristic peak of uric acid. Compared to the NIZ spectra, there is a reduction in the intensity of peak at 1650 cm^{-1} in IZ spectra which is the typical peak of the carbonyl group derived from uric acid. It can be concluded that uric acid was extracted from the zeolite framework, although not overall uric acid has been successfully extracted. The results of N_2 adsorption-desorption characterization was found that zeolite has a pore diameter of 3.836 nm, while the IZ has a pore diameter of 2.186 nm.

Coating of GC electrode with imprinting zeolite

In this research, IZ was coated *in situ* and deposited together with uric acid to the GC electrode. The optimum deposition potential of IZ and uric acid on the GC electrode surface was -0.6 V during 150 s and yield the stripping peak potential -0.376 V. Potential deposition of uric acid was more negative than its stripping peak potential. This indicates that reduction reaction occurred during the deposition step and oxidation reaction occurred during the stripping step occurs. So that it can be concluded that uric acid analysis techniques using GC sensor in this study belong anodic stripping voltammetry. At pH optimization, the best voltammogram obtained in a solution with pH 7 without buffer addition. KNO_3 0.02 M was added as supporting electrolyte in the solution to minimize the non faradic current.

The pH vs. E_p curve (Fig. 1) shows relationship between the pH of the solution and the stripping potential with slope of -59.5 mV. It is clear that the redox reaction of uric acid on the GC-IZ surface involves two electrons and two protons (H^+). Redox reaction of uric acid was shown at Fig. 2.

Performance of the GC-IZ sensor

The sensor performance test showed that GC-IZ has good repeatability compared to the three other sensors. Analysis of uric acid using GC-IZ sensor produces a low current, but not as low as when using GC-Z, because the IZ layer has

pores that have been printed by uric acid so that uric acid molecules can diffuse through the IZ porous toward GC surface. The highest current was generated using GC-NIZ sensor, then successively smaller the GC, GC-IZ and GC-Z (Table 1).

Method validity

Measurement of uric acid standard solution 5.6×10^{-9} M - 2.8×10^{-8} M produce the regression equation of $y = 136.7x - 4728$ with a correlation coefficient (r) = 0.9834. The t-test on r showed t_{count} (6.3846) greater than the t_{table} (2.920). It can be concluded that there is a linear relationship between the concentrations of uric acid to the current. Precision (RSD) obtained was 1.89% -7.65%. Uric acid analysis method using developed sensor in this study provide a smaller precision than $2/3\text{RSD}_{\text{Horwitz}}$ (30% to $\mu\text{g/L}$ or 10^{-9} M level) [10-12]. The value of precision is statistically acceptable. Sensitivity of the developed method was $0.33 \mu\text{A/nM/cm}^2$. Sensitivity of the method is good enough, but lower than sensitivity obtained using GC and GC-imprinting polymer that have been developed previously [13]. Detection limit resulted was 5.9×10^{-9} M. The detection limit obtained in this study was much smaller than the results obtained from previous studies [14-20]. The detection limit is about 10^4 times lower than using spectrophotometry method (1.2×10^{-5} M). With a low detection limit, the analysis of uric acid in a very small amount of serum sample (μL) can be done. The accuracy of 2.8×10^{-8} M uric acid obtained was $96.26 \pm 0.55\%$ ($n=5$). The accuracy value is very good and statistically acceptable (accuracy for 10^{-9} M concentration level is 70-125%) [11,12].

Selectivity

One of the major problems on the determination of uric acid in the serum sample by the voltammetry method is the presence of other compounds which are usually found together with uric acid in serum sample. In this study, to determine the selectivity of the developed sensor, the interference of ascorbic acid, creatine and creatinine on determination of the uric acid has been studied. From the Table 2 can be

concluded that the analysis of uric acid voltammetrically using the developed sensor was not interfered by ascorbic acid, creatine and creatinine, because according the previous report, the normal concentration of ascorbic acid and creatine/creatinine in the serum sample were one tenth and one fourth of uric acid concentration, respectively [21-23]. Compared to the GC sensor, the selectivity of GC-IZ sensor toward uric acid in the ascorbic acid, creatine and creatinine matrices with equal concentration to uric acid were 5, 18 and 2 times higher, respectively.

CONCLUSIONS

Imprinting zeolite-modified glassy carbon showed good performance as voltammetric sensor for uric acid analysis. Analysis of uric acid 5.6×10^{-9} - 2.8×10^{-8} M by voltammetry using the sensor produced linearity of calibration curve (r) 0.9834, precision 1.89-7.65%, sensitivity $0.33 \mu\text{A/nM/cm}^2$, and limit of detection 5.9×10^{-9} M. The limit of detection obtained is low enough for the purpose of uric acid determination in natural samples such as blood serum. Accuracy of uric acid 2.8×10^{-8} M obtained was $96.26 \pm 0.55\%$ ($n=5$). Analysis of uric acid using the developed sensor was not interfered by ascorbic acid, creatine and creatinine with equal concentration with uric acid. Thus, voltammetry method using the developed sensor is suggested to be used as an alternative method to determine of uric acid in the medical field.

ACKNOWLEDGEMENT

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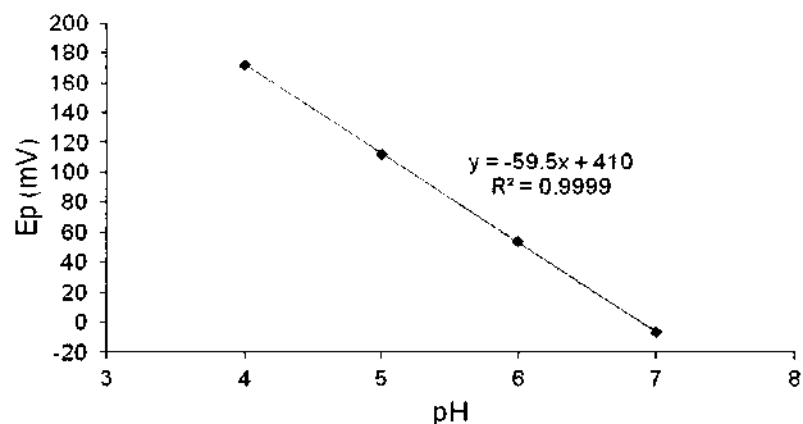


Fig 1. Stripping peak potential (E_p) versus pH of uric acid solution

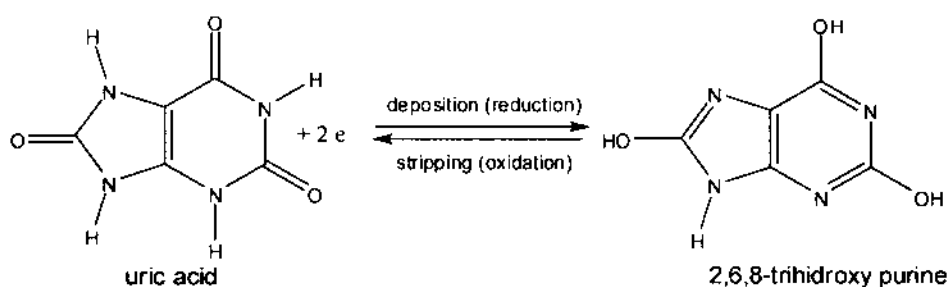


Fig 2. Redox reaction of uric acid

Table 1. The uric acid current measured using GC-IZ, GC, GC-Z, and GC-NIZ sensor

Sensor	Current (nA)	RSD (%) (n=3)
GC-IZ	619.9	1.891
GC	3082.0	4.281y
GC-Z	489.6	13.443
GC-NIZ	3643.7	10.167

uric acid concentration = 3.0×10^{-8} M

Table 2. Deviation of the uric acid current caused by adding ascorbic acid, creatine and creatinine

[UA]:[matrix]	Deviation of the current					
	Ascorbic acid		Creatine		Creatinine	
	GC	GC-IZ	GC	GC-IZ	GC	GC-IZ
1:1	14.20	3.49	6.87	0.38	5.42	2.76
1:2	-	4.44	16.60	4.14	12.62	4.52
1:3	-	6.59	30.60	17.28	18.95	5.00
1:4	-	7.06	95.29	19.63	25.79	6.81
1:5	16,10	9.14	97.57	19.77	28.46	6.38

[UA] = concentration of uric acid = 5.0×10^{-7} M ; [matrix] = concentration of ascorbic acid, creatine or creatinine, - = not observed



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3 Mei 2013 pukul 09.51

Kepada: "Indo. J. Chem." <ijcugm@yahoo.com>

Dear Editor of Indo. J. Chem.

I submit (attachment) of our revised paper entitled "Imprinting zeolite-modified glassy carbon as a voltammetric sensor for uric acid", according to the correction suggestions from the reviewers. The detail comments and response of the corrections are mentioned below:

Reviewer 1

1. The paper has been revised according to the reviewer
2. The paper has been completed with FTIR spectra and X-ray diffractogram of zeolite
3. The commercial zeolite was not used in this study because we need zeolite with a defined pore size and shape (specific for uric acid).
4. We have written abstract and keywords in Bahasa Indonesia as an attachment, because the Indo. J. Chem. guideline was not required an abstract in Bahasa Indonesia

Reviewer 2

1. The paper has been revised according to the reviewer suggestions
2. The paper is now completed with X-ray diffractogram of zeolite before and after imprinted with uric acid, FTIR spectra of zeolite and voltammogram of uric acid

I am looking forward for your next reply soon. Thank you.

Sincerely yours,
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2013/4/19 Indo. J. Chem. <ijcugm@yahoo.com>

[Kutipan teks disembunyikan]

3 lampiran

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IMPRINTING ZEOLITE-MODIFIED GLASSY CARBON AS A VOLTAMMETRIC SENSOR FOR URIC ACID

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ABSTRACT

Development of the uric acid sensor through coating of glassy carbon (GC) electrode with imprinting zeolite (IZ) was carried out. Zeolite was synthesized by mixing TEOS, TBOT, TPAOH, and water followed by hydrothermal process. Zeolite was molded together with uric acid to produce IZ. The deposition potential of IZ and uric acid to the GC surface was -0.6 V during 150 s with addition of KNO_3 0.02 M as supporting electrolyte. The method gives linearity 0.9834 (concentration $5.6 \times 10^{-9} \text{ M}$ - $2.8 \times 10^{-8} \text{ M}$), precision 1.89-7.65%, sensitivity $0.33 \mu\text{A/nM/cm}^2$, limit of detection $5.9 \times 10^{-9} \text{ M}$, and accuracy $96.26 \pm 0.55\%$ ($n=5$). The developed sensor showed a high selectivity toward uric acid. The presence of ascorbic acid, creatine, and creatinine with an equal concentration with uric acid did not interfere on the uric acid analysis

Key words : *sensor, uric acid, voltammetry, imprinting zeolite, glassy carbon*

INTRODUCTION

Uric acid is end product of purine metabolism. The high concentration of uric acid in the body is indication of some diseases such as diabetes, high blood pressure, kidney and liver disease, and can increase risk of cardiovascular [1]. To date, spectrophotometry method is used to determine uric acid in the biomedical field. Analysis of uric acid using this method requires a large number of samples (2-3 mL) and produce a low sensitivity and high detection limit (mM).

Voltammetry has been applied to determine concentration and redox properties of many compounds in the body fluids, such as uric acid in the blood serum. Voltammetry methods to detect uric acid by varying electrodes are developed attempting to lower the limit detection and to increase sensitivity and selectivity. This is because of coexist of other compounds whose structure are similar with uric acid in the

matrix of sample, that decrease the performance of the analytical method [2-4]. Therefore, a new more sensitive and selective analysis protocol is in demand.

In recent study, we used IZ as alternative material to modify the glassy carbon electrode in attempt to produce a selective sensor for uric acid detection voltametrically. The IZ was synthesized by mixing of TEOS, TBOT, TPAH and uric acid as printing material employing hydrothermal process [5]. After the synthesis, the trapped uric acid was then extracted leaving uric acid and leave prints in the zeolite framework. The sensor was made by coating the synthesized IZ on the glassy carbon electrode surface at the optimum potential and time.

Conformity between the pore size of synthesized zeolite and molecular size of uric acid could increase the adsorption capacity of zeolite, which can provide high sensitivity for uric acid determination. Zeolite has rigid structure, so that in the aqueous media can maintain the shape and size of its pore (selective) [6]. The performance and validity of the sensor for uric acid analysis have been studied in this research.

EXPERIMENTAL SECTION

Materials

Chemicals used in this study were uric acid purchased from Fluka; creatine and creatinine purchased from Sigma-Aldrich; tetraethyl ortho silicate, tetrapropyl ammonium hydroxide, tetrabutyl orthotitanate, 2-propanol, ethanol, sodium acetate, acetic acid, sodium dihydrogen phosphate, sodium hydrogen phosphate and potassium nitrate purchased from Merck. All chemicals were analytical grade (p.a). The stock solution of uric acid 6.0×10^{-3} M was prepared by dissolving 0.1000 g uric acid in about 10 mL sodium hydroxide 50% and diluted with water until 100 mL in a volumetric flask. The solutions of creatine and creatinine were prepared by dissolving the compounds using water in the room temperature. The water used in this research is ultra high pure (UHP) water.

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Zeolite was synthesized by mixing TEOS into TBOT and stirred for 30 minutes at room temperature. TPAH was then added dropwise to the mixture, and stirred for 15 hours, so that the mixture obtained has a mole ratio of TEOS:TiO₂:TPAH:H₂O = 1:0.017:0.24:21.2. The mixture was further heated hydrothermally at 80 °C during 4 days. Non imprinting zeolite (NIZ) was synthesized by adding uric acid to the mixture with a molar ratio of uric acid/Si = 2.98x10⁻⁴. Subsequently, the mixture allowed aging for 3 hours [5]. The uric acid was then extracted from the zeolite framework using warm water to produce IZ. The synthesized zeolite (Z), IZ and NIZ were characterized using FTIR, XRD, and N₂ adsorption-desorption.

Preparation and performance test of the sensor

The IZ was coated *in situ* on GC electrode. A number of 20 mL of uric acid solution was inserted into the electrochemical cell, added with 5 mg IZ and coated to the GC electrode at the potential range between +1 V and -1 V (vs. Ag/AgCl). Coating time was varied from 30-180 s, while the pH of the solution was arranged from pH 4-7. The Z and NIZ were coated on the GC electrode surface at the optimum potential and time. The GC-IZ, GC, GC-zeolite (GC-Z) and GC-NIZ sensors were then applied to analyze uric acid and the peaks of potential and current are observed to determine the sensor performances.

Method validity

A series of uric acid standard solution with concentration of 5.6×10^{-9} , 1.1×10^{-8} , 1.7×10^{-8} , 2.3×10^{-8} , and 2.8×10^{-8} M were analyzed by voltammetry using GC-IZ sensor. The current value of standard solution was used to determine the linearity, limit of detection, sensitivity, and the precision. Accuracy was studied by analyzing of uric acid solution 2.8×10^{-8} M. The selectivity of the sensor was studied through the addition of ascorbic acid, creatine, and creatinine (matrices which are always coexist with uric acid in serum sample) on the uric acid analysis. The molar ratios of the uric acid and the each matrix were 1:1, 1:2, 1:3, 1:4, and 1:5, respectively. The current responses of the mixing compounds were compared to the results of the current response of uric acid without the addition of matrix.

RESULTS AND DISCUSSION

Synthesis and characterization of zeolite

Precursors used to synthesize zeolite were TBOT and TEOS as titanium and silica sources, respectively, whereas TPAH served as a structure directing agent and alkaline condition. The addition of uric acid in the synthesis of IZ and NIZ served as template and a directional pore size of zeolite. Zeolite, NIZ, and IZ were obtained as white powder.

X- ray diffractogram (Fig. 1) showed that diffraction peaks of the synthesized zeolite appear at 2θ of 7.96, 8.85, 23.2, 23.9, and 24.4° . These peaks correspond to the characteristic of zeolite framework [7]. A single reflection 2θ around 24.4° indicated a transformation of monoclinic silicalite into orthorhombic zeolite (8). Both Z and IZ exhibited a similar peaks and no reflection peaks of uric acid occurred. These data indicated that uric acid which associated to the zeolite (NIZ) has been successfully extracted.

Characterization of zeolite using FTIR spectroscopy (Fig. 2) showed the peak at wave number of 800, 960, and 1100 cm^{-1} which is the typical peak of the zeolite [9].

Peak at 800 cm^{-1} indicates the symmetric stretching of Si-O-Si, at 960 cm^{-1} indicates the presence of titanium in the zeolite framework, whereas peak at 1100 cm^{-1} indicates the presence of stretching asymmetric Si-O-Si [10]. The FTIR spectra of NIZ show both characteristic peaks of zeolite, and characteristic peaks of uric acid at 840, 1650, and 3450 cm^{-1} . Comparing FTIR spectra of NIZ with IZ at the wave number 1650 cm^{-1} specific for carbonyl group of uric acid, a reduced intensity was observed which indicated that most of the uric acid was successfully extracted from the zeolite framework. Based on the physisorption analysis employing N_2 adsorption-desorption methods, the synthesized zeolite (Z) possessed a pore diameter of 3,836 nm, while IZ has a pore diameter of 2.186 nm.

Coating of GC electrode with imprinting zeolite

In this research, IZ was coated *in situ* and deposited together with uric acid on the surface of the GC electrode, and KNO_3 0.02 M was added as supporting electrolyte in the uric acid solution to minimize the non faradic current. The optimum deposition potential of IZ and uric acid on the GC electrode surface was -0.6 V during 150 s and yield the stripping peak potential of -0.376 V. Deposition potential of uric acid was more negative than its stripping peak potential. This indicates that reduction reaction occurred during the deposition step and oxidation reaction occurred during the stripping step. This feature leads to the conclusion that uric acid analysis technique using GC-IZ sensor in this study belongs to anodic stripping voltammetry.

Performance of the GC-IZ sensor

The sensor performance test showed that GC-IZ exhibit the best repeatability compared to the three other sensors. Both sensor, GC-IZ and GC-Z are porous material, however the GC-IZ possess imprinted pores mimic to uric acid shape and size. Consequently, the analyte can diffuse freely on the GC surface and induce current. This feature generated a slightly higher current of the GC-IZ sensor compare

to the GC-Z sensor. The GC-NIZ exhibited the highest current response, due to the existence of uric acid in the NIZ layer on the GC surface (Table 1). Voltammograms resulted from the uric acid analysis using GC, GC-Z, GC-NIZ and GC-Z sensors were shown in Fig.3.

Method validity

The standard curve of uric acid was made from uric acid solution of 5.6×10^{-9} M - 2.8×10^{-8} M, generated regression equation of $y = 136.7x - 4728$ with a correlation coefficient (r) of 0.9834. The t-test of r showed t_{count} (6.3846) greater than the t_{table} (2.920). It can be concluded a linear relationship between the concentrations of uric acid against the current. The obtained precision (RSD) was 1.89% -7.65%. This data lead to the conclusion that the developed sensor provide a smaller precision than $2/3 \times \text{RSD}_{\text{Horwitz}}$ (30% to $\mu\text{g/L}$ or 10^{-9} M level) [11-12]. However, this value is statistically acceptable. Sensitivity of the developed method was $0.33 \mu\text{A/nM/cm}^2$ and it is lower than using sensors of GC and GC-imprinting polymetacrylic acid from the previous study [13]. The resulted detection limit was 5.9×10^{-9} M. This value is about 10^4 times lower than using spectrophotometry method (1.2×10^{-5} M) and smaller than the results obtained from the previous studies [14-20]. With a low detection limit, the analysis of uric acid in a very small amount of serum sample (μL) can be done. The obtained accuracy of 2.8×10^{-8} M uric acid was $96.26 \pm 0.55\%$ ($n=5$). The accuracy value is very good and statistically acceptable (accuracy for 10^{-9} M concentration level is 70-125%) [11-12]. This superior new sensor offer a new alternative for uric acid analysis voltammetrically.

Selectivity

One of the major problems on the determination of uric acid in the serum sample by the voltammetry method is the presence of other compounds which are usually found together with uric acid in serum sample. In this study, to determine the

selectivity of the developed sensor, the interference of ascorbic acid, creatine, and creatinine on determination of the uric acid has been studied. From the Table 2 can be concluded that the analysis of uric acid voltammetrically using the developed sensor was not interfered by ascorbic acid, creatine and creatinine, because according the previous report, the normal concentration of ascorbic acid and creatine/creatinine in the serum sample were one tenth and one fourth of uric acid concentration, respectively [21-23]. Compared to the GC sensor, the selectivity of GC-IZ sensor toward uric acid in the ascorbic acid, creatine, and creatinine matrices with equal concentration to uric acid were 5, 18 and 2 times higher, respectively.

Redox reaction mechanism

The reaction mechanism of the redox process occurred in this research was revealed by conducting an experiment which delved the influence of pH toward stripping potential (E_p) of uric acid. The pH vs. E_p curve (Fig. 4) shows relationship between the pH of the solution and the stripping potential with slope of -59.5 mV. This value revealed that the redox reaction of uric acid on the GC-IZ surface involved 2 electrons and two protons (H^+). The redox reaction of uric acid on the GC-IZ surface is shown in Fig. 5.

CONCLUSIONS

The GC-IZ showed good performance as sensor for uric acid analysis voltammetrically. The uric acid analysis method using GC-IZ sensor has a high sensitivity, precision, accuracy, and low detection limit. The limit of detection obtained is low enough for the purpose of uric acid determination in natural samples such as blood serum. Analysis of uric acid using the developed sensor was not interfered by ascorbic acid, creatine, and creatinine with an equal concentration with uric acid. Thus, voltammetry method using the developed sensor is suggested to be used as an alternative method to determine of uric acid in the medical field.

ACKNOWLEDGEMENT

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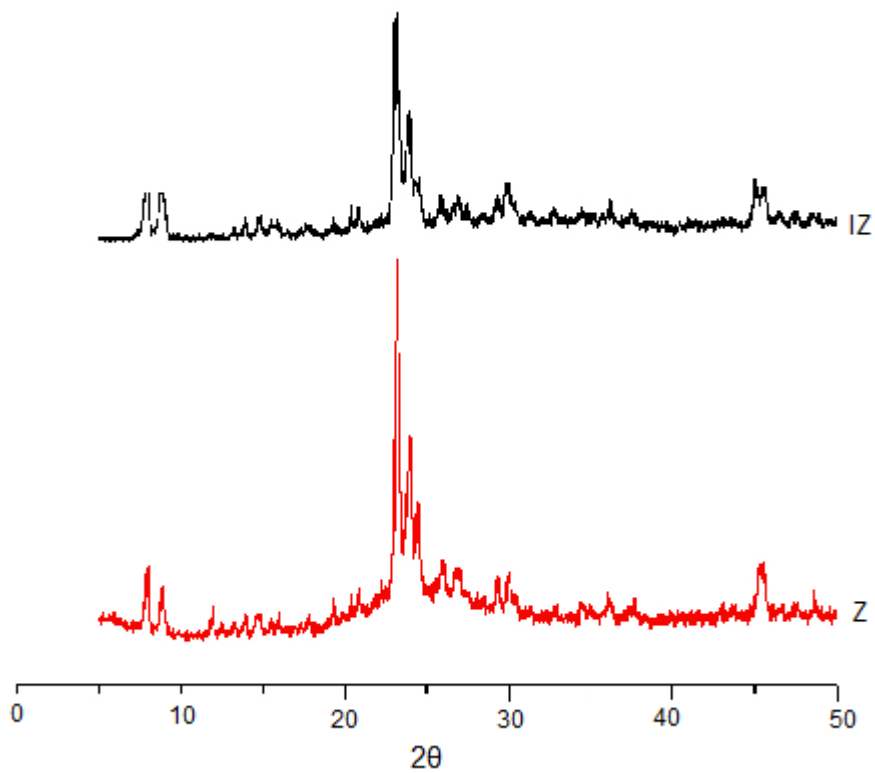


Fig 1. X-ray diffractogram of Z and IZ

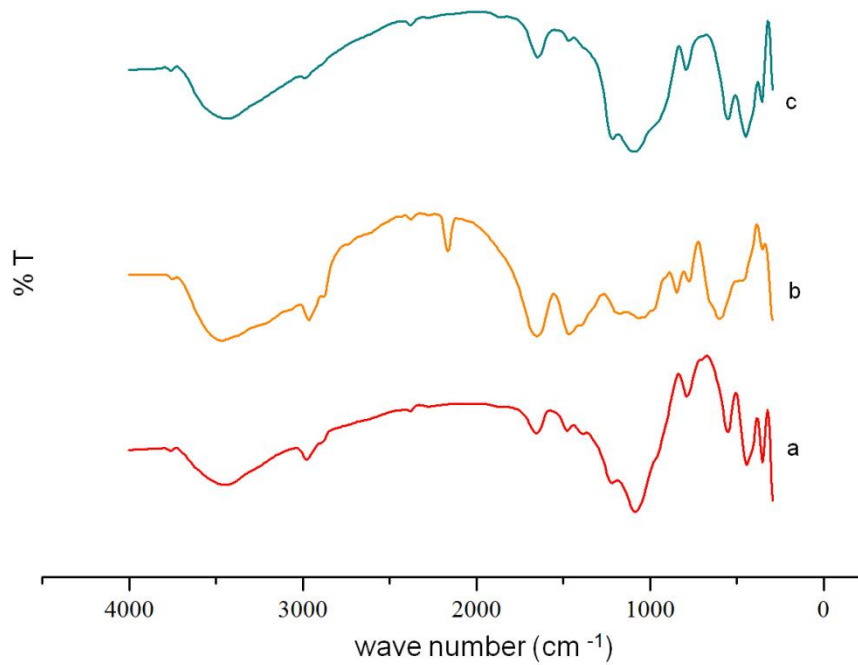


Fig 2. FTIR spectra of (a) Z, (b) NIZ, and (c) IZ

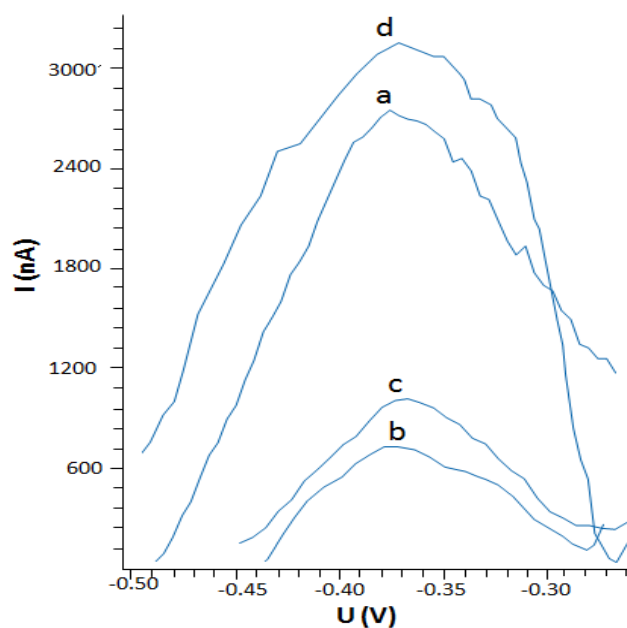


Fig 3. Stripping voltammogram of uric acid using sensor of (a) GC, (b) GC-Z, (c) GC-IZ and (d) GC-NIZ

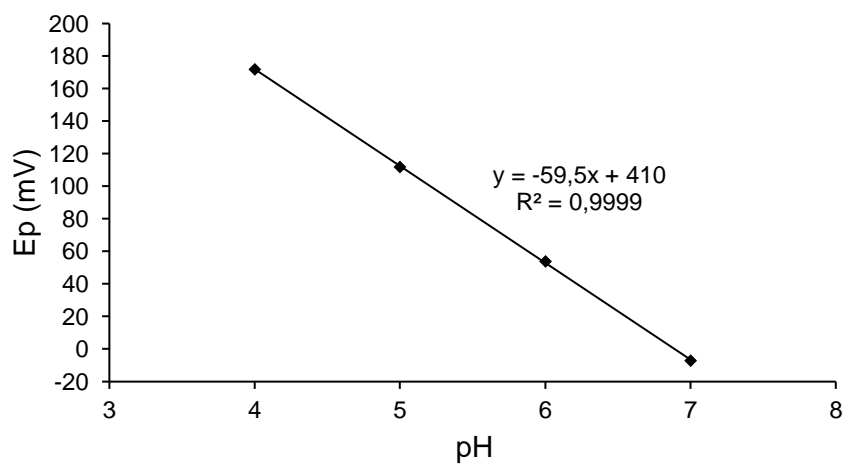


Fig 4. Stripping peak potential (E_p) versus pH of uric acid solution

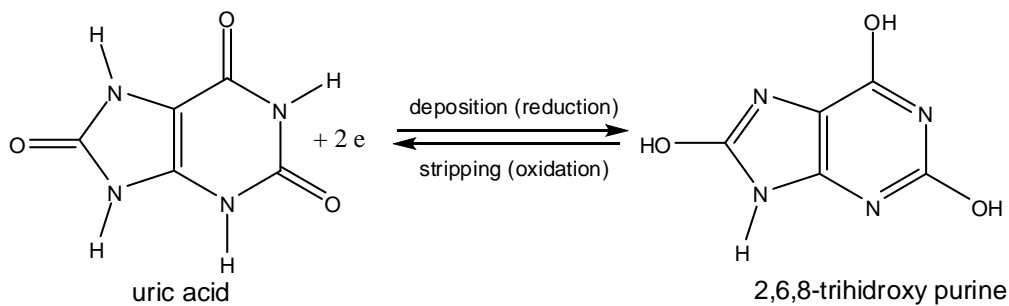


Fig 5. Redox reaction of uric acid

Table 1. The uric acid current measured using GC-IZ, GC, GC-Z, and GC-NIZ sensor

Sensor	Anodic current (nA)	RSD (%) (n=3)
GC-IZ	619.90	1.89
GC	3082.00	4.28
GC-Z	489.63	13.44
GC-NIZ	3643.67	10.17

uric acid concentration = 3.0×10^{-8} M

Table 2. Deviation of the uric acid current caused by adding ascorbic acid, creatine, and creatinine

[UA]:[matrix]	Deviation of the anodic current (%)					
	Ascorbic acid		Creatine		Creatinine	
	GC	GC-IZ	GC	GC-IZ	GC	GC-IZ
1:1	14.20	3.49	6.87	0.38	5.42	2.76
1:2	-	4.44	16.60	4.14	12.62	4.52
1:3	-	6.59	30.60	17.28	18.95	5.00
1:4	-	7.06	95.29	19.63	25.79	6.81
1:5	16,10	9.14	97.57	19.77	28.46	6.38

[UA] = concentration of uric acid = 5.0×10^{-7} M ; [matrix] = concentration of ascorbic acid, creatine or creatinine, - = not done

IMPRINTING ZEOLITE-MODIFIED GLASSY CARBON AS A VOLTAMMETRIC SENSOR FOR URIC ACID

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ABSTRAK

Pengembangan sensor asam urat melalui pelapisan elektroda GC dengan *imprinting* zeolit (IZ) telah dilakukan. Zeolit (Z) disintesis dengan cara mencampurkan TEOS, TBOT, TPAOH, dan air melalui proses hidrotetal. Pada saat sintesis zeolit ditambahkan asam urat, kemudian asam urat diekstraksi dari struktur zeolit sehingga menghasilkan zeolit yang tercetak molekul asam urat (IZ). Deposisi zeolit dan asam urat pada permukaan elektroda GC dilakukan pada potensial -0,6 V selama 150 detik dengan penambahan KNO_3 0,02 M sebagai elektrolit pendukung. Metode yang dikembangkan ini memiliki linieritas sebesar 0,9834 (konsentrasi $5,6 \times 10^{-9}$ M – $2,8 \times 10^{-8}$ M); presisi 1,89-7,65%; sensitivitas $0,33 \mu\text{A/nM/cm}^2$; batas deteksi $5,9 \times 10^{-9}$ M, dan akurasi sebesar $96,26 \pm 0,55\%$ ($n=5$). Selektivitas sensor yang dikembangkan ini cukup tinggi. Keberadaan asam askorbat, kreatin dan kreatinin dengan konsentrasi sama dengan asam urat tidak mengganggu analisis asam urat.

Kata kunci : *sensor, asam urat, voltammetri, zeolit, glassy carbon*



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IMPRINTING ZEOLITE-MODIFIED GLASSY CARBON AS A VOLTAMMETRIC SENSOR FOR URIC ACID

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ABSTRACT

Development of the uric acid sensor through coating of glassy carbon (GC) electrode with imprinting zeolite (IZ) was carried out. Zeolite was synthesized by mixing TEOS, TBOT, TPAOH, and water followed by hydrothermal process. Zeolite was molded together with uric acid to produce IZ. The deposition potential of IZ and uric acid to the GC surface was -0.6 V during 150 sec with addition of KNO_3 0.02 M as supporting electrolyte. The method gives linearity of 0.9834 (concentration 5.6×10^{-9} M – 2.8×10^{-8} M), precision 1.89 – 7.65%, sensitivity $0.33 \mu\text{A/nM/cm}^2$, limit of detection 5.9×10^{-9} M, and accuracy $96.26 \pm 0.55\%$ ($n = 5$). The developed sensor showed a high selectivity toward uric acid. The presence of ascorbic acid, creatine, and creatinine with an equal concentration with uric acid did not interfere on the uric acid analysis.

Keywords: sensor; uric acid; voltammetry; imprinting zeolite; glassy carbon

ABSTRAK

Pengembangan sensor asam urat melalui pelapisan elektroda GC dengan imprinting zeolit (IZ) telah dilakukan. Zeolit (Z) disintesis dengan cara mencampurkan TEOS, TBOT, TPAOH, dan air melalui proses hidrotermal. Pada saat sintesis zeolit ditambahkan asam urat, kemudian asam urat diekstraksi dari struktur zeolit sehingga menghasilkan zeolit yang tercetak molekul asam urat (IZ). Deposisi zeolit dan asam urat pada permukaan elektroda GC dilakukan pada potensial $-0,6$ V selama 150 detik dengan penambahan KNO_3 0,02 M sebagai elektrolit pendukung. Metode yang dikembangkan ini memiliki linieritas sebesar 0,9834 (konsentrasi $5,6 \times 10^{-9}$ M – $2,8 \times 10^{-8}$ M); presisi 1,89 – 7,65%; sensitivitas $0,33 \mu\text{A/nM/cm}^2$; batas deteksi $5,9 \times 10^{-9}$ M, dan akurasi sebesar $96,26 \pm 0,55\%$ ($n = 5$). Selektivitas sensor yang dikembangkan ini cukup tinggi. Keberadaan asam askorbat, kreatin dan kreatinin dengan konsentrasi sama dengan asam urat tidak mengganggu analisis asam urat.

Kata Kunci: sensor; asam urat; voltametri; zeolit; glassy carbon

INTRODUCTION

Uric acid is end product of purine metabolism. The high concentration of uric acid in the body is indication of some diseases such as diabetes, high blood pressure, kidney and liver disease, and can increase risk of cardiovascular [1]. To date, spectrophotometry method is used to determine uric acid in the biomedical field. Analysis of uric acid using this method requires a large number of samples (2-3 mL) and produces a low sensitivity and high detection limit (mM).

Voltammetry has been applied to determine concentration and redox properties of many compounds in the body fluids, such as uric acid in the blood serum. Voltammetry methods to detect uric acid by varying electrodes are developed to attempt the lower the limit detection and to increase sensitivity and selectivity. This is because of coexisting of other compounds whose

structure is similar with uric acid in the matrix of sample, that decrease the performance of the analytical method [2-4]. Therefore, a new more sensitive and selective analysis protocol is in demand.

In recent study, we used IZ as alternative material to modify the glassy carbon electrode in attempt to produce a selective sensor for uric acid detection voltammetrically. The IZ was synthesized by mixing of TEOS, TBOT, TPAH and uric acid as printing material employing hydrothermal process [5]. After the synthesis, the trapped uric acid was then extracted leaving uric acid and leave prints in the zeolite framework. The sensor was made by coating the synthesized IZ on the glassy carbon electrode surface at the optimum potential and time.

Conformity between the pore size of synthesized zeolite and molecular size of uric acid could increase the adsorption capacity of zeolite, which can provide

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high sensitivity for uric acid determination. Zeolite has rigid structure, so that in the aqueous media can maintain the shape and size of its pore (selective) [6]. The performance and validity of the sensor for uric acid analysis have been studied in this research.

EXPERIMENTAL SECTION

Materials

Chemicals used in this study were uric acid purchased from Fluka; creatine and creatinine purchased from Sigma-Aldrich; tetraethyl ortho silicate, tetrapropyl ammonium hydroxide, tetrabutyl orthotitanate, 2-propanol, ethanol, sodium acetate, acetic acid, sodium dihydrogen phosphate, sodium hydrogen phosphate and potassium nitrate purchased from Merck. All chemicals were analytical grade (p.a). The stock solution of uric acid 6.0×10^{-3} M was prepared by dissolving 0.1000 g uric acid in about 10 mL sodium hydroxide 50% and diluted with water until 100 mL in a volumetric flask. The solutions of creatine and creatinine were prepared by dissolving the compounds using water in the room temperature. The water used in this research is ultra high pure (UHP) water.

Instrumentation

The instruments used in this study were 797 Computrace Voltammetry (MVA system-1) equipped with a sample container, stirrer, processor units, personal computer, glassy carbon (BAS MF-2012, $d = 3$ mm), reference electrode Ag/AgCl (KCl 3M) and Pt auxiliary electrodes; FTIR (Shimadzu), N_2 adsorption-desorption/BET (Quantachrome NovaWin version 2.1); X-ray diffractometer (XRD), pH meter; and other supporting equipments.

Procedure

Synthesis and characterization of zeolite

Zeolite was synthesized by mixing TEOS into TBOT and stirred for 30 min at room temperature. TPAH was then added dropwise to the mixture, and stirred for 15 h, so that the mixture obtained has a mole ratio of $TEOS:TiO_2:TPAH:H_2O = 1:0.017:0.24:21.2$. The mixture was further heated hydrothermally at $80^\circ C$ for 4 days. Non imprinting zeolite (NIZ) was synthesized by adding uric acid to the mixture with a molar ratio of uric acid/Si = 2.98×10^{-4} . Subsequently, the mixture allowed aging for 3 h [5]. The uric acid was then extracted from the zeolite framework using warm water to produce IZ. The synthesized zeolite (Z), IZ and NIZ were characterized using FTIR, XRD, and N_2 adsorption-desorption.

Preparation and performance test of the sensor

The IZ was coated *in situ* on GC electrode. A number of 20 mL of uric acid solution was inserted into the electrochemical cell, added with 5 mg IZ and coated to the GC electrode at the potential range between +1 V and -1 V (vs. Ag/AgCl). Coating time was varied from 30-180 sec, while the pH of the solution was arranged from pH 4-7. The Z and NIZ were coated on the GC electrode surface at the optimum potential and time. The GC-IZ, GC, GC-zeolite (GC-Z) and GC-NIZ sensors were then applied to analyze uric acid and the peaks of potential and current are observed to determine the sensor performances.

Method validity

A series of uric acid standard solution with concentration of 5.6×10^{-9} , 1.1×10^{-8} , 1.7×10^{-8} , 2.3×10^{-8} , and 2.8×10^{-8} M were analyzed by voltammetry using GC-IZ sensor. The current value of standard solution was used to determine the linearity, limit of detection, sensitivity, and the precision. Accuracy was studied by analyzing of uric acid solution 2.8×10^{-8} M. The selectivity of the sensor was studied through the addition of ascorbic acid, creatine, and creatinine (matrices which are always coexist with uric acid in serum sample) on the uric acid analysis. The molar ratios of the uric acid and the each matrix were 1:1, 1:2, 1:3, 1:4, and 1:5, respectively. The current responses of the mixing compounds were compared to the results of the current response of uric acid without the addition of matrix.

RESULT AND DISCUSSION

Synthesis and Characterization of Zeolite

Precursors used to synthesize zeolite were TBOT and TEOS as titanium and silica sources, respectively, whereas TPAH served as a structure directing agent and alkaline condition. The addition of uric acid in the synthesis of IZ and NIZ served as template and a directional pore size of zeolite. Zeolite, NIZ, and IZ were obtained as white powder.

X-ray diffractogram (Fig. 1) showed that diffraction peaks of the synthesized zeolite appear at 2θ of 7.96° , 8.85° , 23.2° , 23.9° , and 24.4° . These peaks correspond to the characteristic of zeolite framework [7]. A single reflection 2θ around 24.4° indicated a transformation of monoclinic silicalite into orthorhombic zeolite [8]. Both Z and IZ exhibited a similar peaks and no reflection peaks of uric acid occurred. These data indicated that uric acid which associated to the zeolite (NIZ) has been successfully extracted.

Characterization of zeolite using FTIR spectroscopy (Fig. 2) showed the peak at wave number

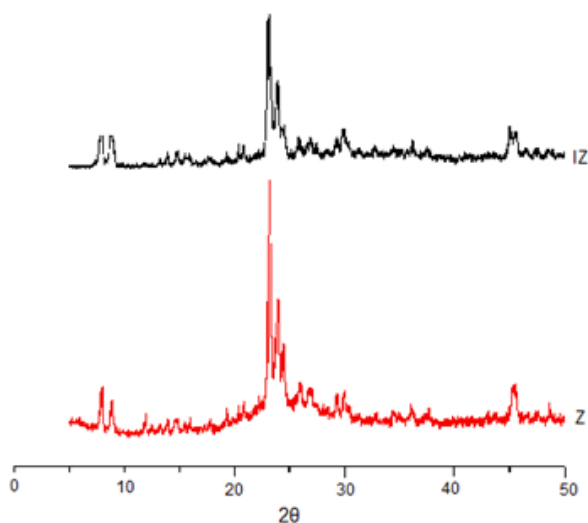


Fig 1. X-ray diffractogram of Z and IZ

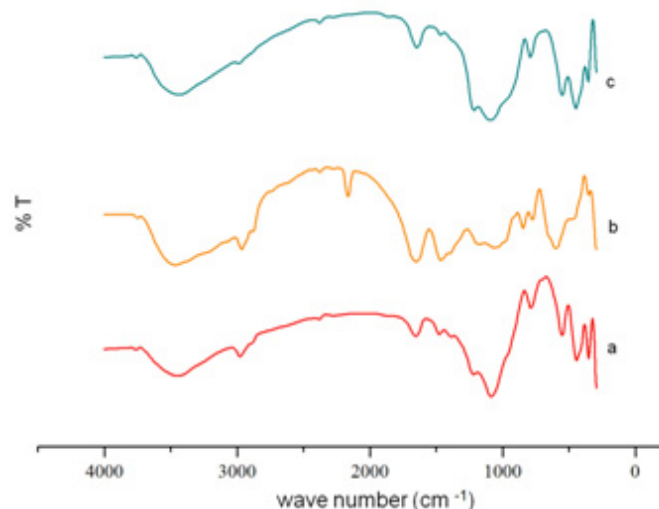


Fig 2. FTIR spectra of (a) Z, (b) NIZ, and (c) IZ

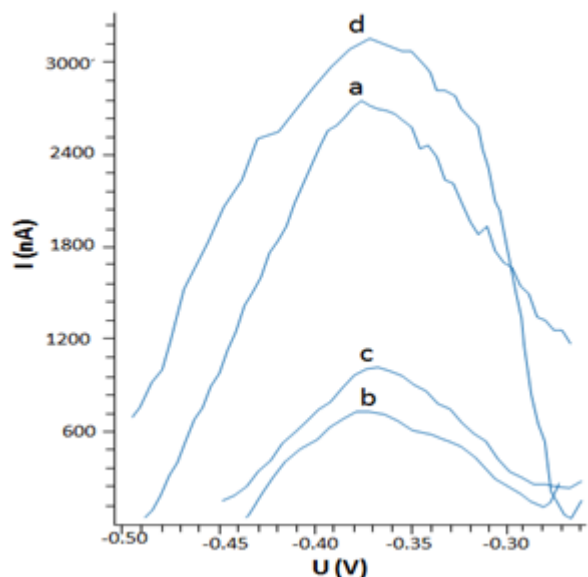


Fig 3. Stripping voltammogram of uric acid using sensor of (a) GC, (b) GC-Z, (c) GC-IZ and (d) GC-NIZ

Table 1. The uric acid current measured using GC-IZ, GC, GC-Z, and GC-NIZ sensor

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GC-Z	489.63	13.44
GC-NIZ	3643.67	10.17

uric acid concentration = 3.0×10^{-8} M

of 800, 960, and 1100 cm^{-1} which is the typical peak of the zeolite [9]. Peak at 800 cm^{-1} indicates the symmetric stretching of Si-O-Si, at 960 cm^{-1} indicates the presence of titanium in the zeolite framework, whereas peak at 1100 cm^{-1} indicates the presence of stretching asymmetric Si-O-Si [10]. The FTIR spectra of NIZ show

both characteristic peaks of zeolite, and characteristic peaks of uric acid at 840 , 1650 , and 3450 cm^{-1} . Comparing FTIR spectra of NIZ with IZ at the wave number 1650 cm^{-1} specific for carbonyl group of uric acid, a reduced intensity was observed which indicated that most of the uric acid was successfully extracted from the zeolite framework. Based on the physisorption analysis employing N_2 adsorption-desorption methods, the synthesized zeolite (Z) possessed a pore diameter of 3.836 nm , while IZ has a pore diameter of 2.186 nm .

Coating of GC Electrode with Imprinting Zeolite

In this research, IZ was coated *in situ* and deposited together with uric acid on the surface of the GC electrode, and KNO_3 0.02 M was added as supporting electrolyte in the uric acid solution to minimize the non faradic current. The optimum deposition potential of IZ and uric acid on the GC electrode surface was -0.6 V during 150 sec and yield the stripping peak potential of -0.376 V . Deposition potential of uric acid was more negative than its stripping peak potential. This indicates that reduction reaction occurred during the deposition step and oxidation reaction occurred during the stripping step. This feature leads to the conclusion that uric acid analysis technique using GC-IZ sensor in this study belongs to anodic stripping voltammetry.

Performance of the GC-IZ Sensor

The sensor performance test showed that GC-IZ exhibit the best repeatability compared to the three other sensors. Both sensor, GC-IZ and GC-Z are porous material, however the GC-IZ possess imprinted pores mimic to uric acid shape and size. Consequently,

the analyte can diffuse freely on the GC surface and induce current. This feature generated a slightly higher current of the GC-IZ sensor compare to the GC-Z sensor. The GC-NIZ exhibited the highest current response, due to the existence of uric acid in the NIZ layer on the GC surface (Table 1). Voltammograms resulted from the uric acid analysis using GC, GC-Z, GC-NIZ and GC-Z sensors were shown in Fig. 3.

Method Validity

The standard curve of uric acid was made from uric acid solution of 5.6×10^{-9} M – 2.8×10^{-8} M, generated regression equation of $y = 136.7x - 4728$ with a correlation coefficient (r) of 0.9834. The t -test of r showed t_{count} (6.3846) greater than the t_{table} (2.920). It can be concluded a linear relationship between the concentrations of uric acid against the current. The obtained precision (RSD) was 1.89% – 7.65%. This data lead to the conclusion that the developed sensor provide a smaller precision than $2/3 \times \text{RSD}_{\text{Horwitz}}$ (30% to $\mu\text{g/L}$ or 10^{-9} M level) [11-12]. However, this value is statistically acceptable. Sensitivity of the developed method was $0.33 \mu\text{A/nM/cm}^2$ and it is lower than using sensors of GC and GC-imprinting polymetacrylic acid from the previous study [13]. The resulted detection limit was 5.9×10^{-9} M. This value is about 10^4 times lower than using spectrophotometry method (1.2×10^{-5} M) and smaller than the results obtained from the previous studies [14-20]. With a low detection limit, the analysis of uric acid in a very small amount of serum sample (μL) can be done. The obtained accuracy of 2.8×10^{-8} M uric acid was 96.26

$\pm 0.55\%$ ($n = 5$). The accuracy value is very good and statistically acceptable (accuracy for 10^{-9} M concentration level is 70 – 125%) [11-12]. This superior new sensor offer a new alternative for uric acid analysis voltammetrically.

Selectivity

One of the major problems on the determination of uric acid in the serum sample by the voltammetry method is the presence of other compounds which are usually found together with uric acid in serum sample. In this study, to determine the selectivity of the developed sensor, the interference of ascorbic acid, creatine, and creatinine on determination of the uric acid has been studied. From the Table 2, it can be concluded that the analysis of uric acid voltammetrically using the developed sensor was not

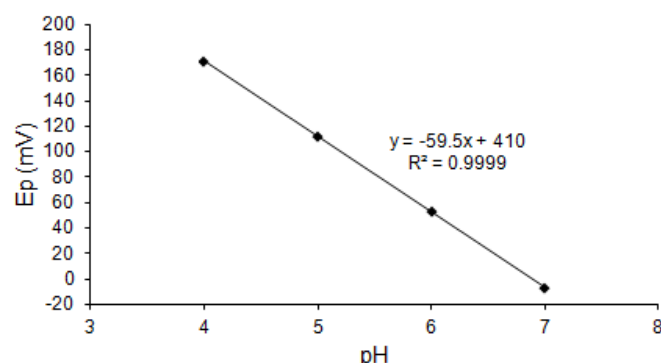


Fig 4. Stripping peak potential (E_p) versus pH of uric acid solution

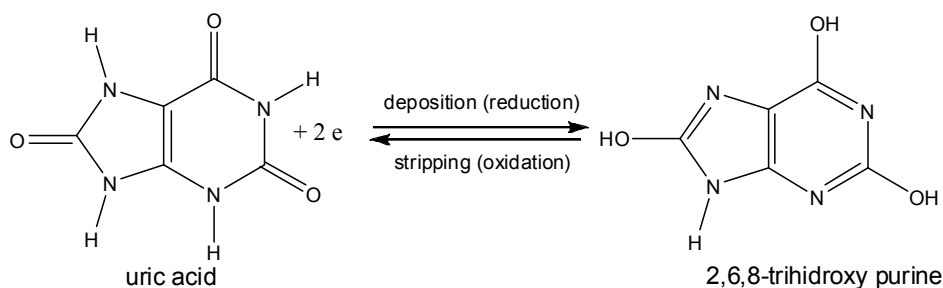


Fig 5. Redox reaction of uric acid

Table 2. Deviation of the uric acid current caused by adding ascorbic acid, creatine, and creatinine

[UA]:[matrix]	Deviation of the anodic current (%)					
	Ascorbic acid		Creatine		Creatinine	
	GC	GC-IZ	GC	GC-IZ	GC	GC-IZ
1:1	14.20	3.49	6.87	0.38	5.42	2.76
1:2	-	4.44	16.60	4.14	12.62	4.52
1:3	-	6.59	30.60	17.28	18.95	5.00
1:4	-	7.06	95.29	19.63	25.79	6.81
1:5	16,10	9.14	97.57	19.77	28.46	6.38

[UA] = concentration of uric acid = 5.0×10^{-7} M; [matrix] = concentration of ascorbic acid, creatine or creatinine, - = not done

interfered by ascorbic acid, creatine and creatinine, because according the previous report, the normal concentration of ascorbic acid and creatine/creatinine in the serum sample were one tenth and one fourth of uric acid concentration, respectively [21-23]. Compared to the GC sensor, the selectivity of GC-IZ sensor toward uric acid in the ascorbic acid, creatine, and creatinine matrices with equal concentration to uric acid were 5, 18 and 2 times higher, respectively.

Redox Reaction Mechanism

The reaction mechanism of the redox process occurred in this research was revealed by conducting an experiment which delved the influence of pH toward stripping potential (E_p) of uric acid. The pH vs. E_p curve (Fig. 4) shows relationship between the pH of the solution and the stripping potential with slope of -59.5 mV. This value revealed that the redox reaction of uric acid on the GC-IZ surface involved 2 electrons and two protons (H^+). The redox reaction of uric acid on the GC-IZ surface is shown in Fig. 5.

CONCLUSION

The GC-IZ showed good performance as sensor for uric acid analysis voltammetrically. The uric acid analysis method using GC-IZ sensor has a high sensitivity, precision, accuracy, and low detection limit. The limit of detection obtained is low enough for the purpose of uric acid determination in natural samples such as blood serum. Analysis of uric acid using the developed sensor was not interfered by ascorbic acid, creatine, and creatinine with an equal concentration with uric acid. Thus, voltammetry method using the developed sensor is suggested to be used as an alternative method to determine of uric acid in the medical field.

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