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Judul : The influence of ascorbic acid, creatine, and creatinine on the uric acid analysis by potentiometry using a carbon paste modified imprinting zeolite electrode

Penulis : Miratul Khasanah, Muji Harsini, Alfa Akustia Widati, Prihantari Mukti Ibrani

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Paper Review Result [COSCI 2016]

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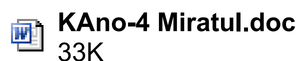

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Title	The Influence of Ascorbic Acid, Creatine, and Creatinine on the Uric Acid Analysis by Potentiometry using Carbon Paste Modified Imprinting Zeolite Electrode
Abstract	fine
Introduction	Author need inform more advantage on using Zeolite instead of other material used.
Methodology	fine
Results	Reviewer curious about equation 1, it was true that accuracy have similar equation with recovery? It was important to show the equation of % accuracy
Discussion	fine
How wellis the paperintegrated withcurrentresearch :	This paper is novel, mainly on application of zeolite on potentiometric detection
Bibliography/References:	ok
Adequacy of literature review	

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Figures:	**fine**
Tables:	**fine**
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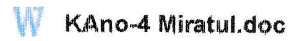

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ASSESMENT OF ARTICLE
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Title	THE INFLUENCE OF ASCORBIC ACID, CREATINE, AND CREATININE ON THE URIC ACID ANALYSIS BY POTENTIOMERY USING CARBON PASTE MODIFIED IMPRITING ZEOLITE ELECTRODE (Miratul, Alfa, Prihantari)
Abstract	Good
Introduction	Good
Methodology	Good
Results	Good
Discussion	Good
How well is the paper integrated with current research :	It was quite novel
Bibliography/References:	Need carefull for thereference format
Adequacy of literature review	Quite relevant
Figures:	Good
Tables:	Good
Overall evaluation on the paper:	Your english should be improved and it's worthy to be published in journal of International

The Influence of Ascorbic Acid, Creatine, and Creatinine on the Uric Acid Analysis by Potentiometry using Carbon Paste Modified Imprinting Zeolite Electrode

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ABSTRACT

The high level of uric acid in the body is often associated with some diseases such as hyperuricemia, hypertension, renal and cardiovascular disease. Therefore early detection of the levels of uric acid in the body is required. The method commonly used in the medical field to determine uric acid levels is spectrophotometry. The high detection limit of spectrophotometry method confined it to be used to determine low level uric acid in serum sample, while the complexity of serum matrices causing lower its selectivity. The development of imprinted zeolite-based sensor to analyze uric acid by potentiometry has been conducted. The carbon paste-IZ electrode showed a range of measurement of 10^{-6} – 10^{-4} M, Nernst factor of 28.2 mV decade^{-1}, and response time of 34-44 s. The developed electrode has a high selectivity towards uric acid. Ascorbic acid, creatine, or creatinine did not interfere on the uric acid analysis by potentiometry using the electrode, expressed by value of selectivity coefficient (Kij) less than 1. Accuracy of the method was (113.18±14.79)% (n=3). Comparison test of the method with the spectrophotometric method showed the recovery of (94.47±14.64)% (n=7).

Keywords: *uric acid, carbon paste-IZ, selectivity, potentiometry*

INTRODUCTION

Uric acid is the end product of purine metabolism. The normal level of uric acid in the blood is in the range of 2.4 to 5.7 mg/dL in women and 3.4 to 7.0 mg / dL in men [1]. Uric acid has been used as a biomarker to diagnose health problems because of its ability to cause some dangerous diseases. Thus, controlling the levels of uric acid in the body should always be done. The common curent method used to analyze uric acid in the medical field is spectrophotometry using chemical reagent or enzymatic method. The method used to determine the levels of uric acid must be selective because of uric acid found together with another compounds in the sample

such as ascorbic acid that interfere the uric acid analysis [2]. Compounds such as creatine and creatinine are also potentially interfered the analysis of uric acid because of the similarity of it functional groups or structure. These compounds cause result of uric acid analysis not accurate and do not represent actual levels of uric acid in the sample.

Various electrochemical methods were developed to solve the problem on uric acid analysis method. The determination of uric acid by voltammetry method have received much interest because of less chemical need and less time consuming compared to colorimetric and enzymatic methods [3,4]. However, a major problem encountered in this method to determine uric acid is the interference from ascorbic acid in blood and urine which can be oxidized at the potentials close to the uric acid [5].

The previous study have developed a method of determining uric acid by potentiometry using ZnO nano wires electrode immobilized uricase enzyme [6]. The method resulted measurement range of $1.0 \times 10^{-6} - 6.5 \times 10^{-4}$ M. The measurement range was the range of uric acid concentration in the blood, so the method is very suitable applied to analyze of uric acid in the blood serum. Glucose, ascorbic acid and urea do not interfere on uric acid analysis using the developed method. The other study has reported the use a ZnO nanoflakes-based sensor immobilized uricase enzyme for uric acid analysis by potentiometry [7]. The results showed that the developed sensor has a measuring range of $5.0 \times 10^{-7} - 1.5 \times 10^{-3}$ M, the detection limit of 5.0×10^{-7} M. The sensor is not interfered by the presence of ascorbic acid, glucose and urea. Potentiometric method using carbon paste electrodes modified imprinting zeolite (carbon paste-IZ) has been developed to measure the levels of uric acid [8]. The method was not interfered by the presence of urea.

In this work, we studied the influence of ascorbic acid, creatinine and urea on uric acid analysis by potentiometry using carbon paste electrode modified imprinting zeolite. Imprinted zeolites was manufactured with the mass ratio of carbon, IZ, solid paraffin resulted from previous studies [8]. The influence of ascorbic acid, creatinine and creatine was determined by adding each compound on the uric acid standard solution with the variation of concentration.

EXPERIMENTAL

Material and instrumentation

Chemical used were uric acid (Fluka), creatine and creatinine (Sigma-Aldrich), ascorbic acid, acetic acid, sodium acetate, and sodium hydroxide trihydrate, carbon powder, solid paraffin (Merck). All chemicals were analytical grade. The solvent used was distilled water. The stock solution of uric acid 10^{-2} M was prepared by dissolving 0.1680 g uric acid in about 10 mL sodium hydroxide 50% (w/w) and diluted with water until 100 mL in volumetric flask. Standard solution of uric acid 10^{-8}-10^{-3} M were prepared by diluting appropriate working solutions, and their pH were adjusted with the addition of acetate buffer pH 5. Sample used was urine from patients of a local pathological clinic.

The instrumentations used were potentiometer Cyberscan 510, reference electrode Ag/AgCl, hotplate magnetic stirrer Lab Tech, micropipette tube, pH meter Seven Easy Mettler-Teledo GmbH, sentrifuge Hittech EBA 20, oven NAPCO Vacuum Oven Model 5851, polypropylene bottle, and glassware.

Procedure

Fabrication of carbon paste – IZ electrode

Carbon paste electrode was manufactured by mixing carbon powder, imprinting zeolite and paraffin with a mass ratio of 40:25: 35 [8] assisted by heating. Zeolite was synthesized by mixing

TEOS, TBOT and TPAH with a mole ratio reported in the previous study [9]. The uric acid was then extracted from the zeolite framework using warm water to produce imprinting zeolite. The modified electrode was made by inserting a wire of silver (Ag) to the micropipette tube and fill micropipette by solid paraffin as much as 3/4 tube. Furthermore, the remaining part of the tube was filled by a paste made previously.

Determination of measurement range, Nernst factor and response time of the electrode

Measurement range is obtained by measuring the electrode potential on uric acid solution 10^{-8} to 10^{-3} M, subsequently made curve relationship between log concentration of uric acid (log $C_{uric\ acid}$) and electrode potential. Range of concentration that result the linear curve is called measurement range, while the slope of the linear curve represents the value of Nernst factor. The response time of electrode was determined by calculating the time required by the electrodes in response to the analyte until provide the potential value.

Selectivity of the electrode

The selectivity of the electrode, expressed by selectivity coefficient (K_{ij}), was studied through the addition of ascorbic acid, creatine and creatinine, compounds which are always coexist with uric acid in urine or serum sample, on the uric acid solution. Uric acid used was 10^{-4} M, while ascorbic acid, creatine and creatinine was added with various concentration. The K_{ij} value was calculated by Matched Potential Method (MPM) [10].

Accuracy and comparative test of the method

Accuracy of the method was obtained through measuring potential of urine sample, uric acid standard solution, and urine samples spiked by uric acid standard solution. Urine samples were taken from the urine of adults collected over 24 hours. The third solution is then measured using

carbon paste-IZ electrode and its value substituted into the linear regression equation of the standard curve to obtain the concentration of each solution. Accuracy/recovery (R) calculated by substituting concentration of each solution into the equation 1.

$$R = \frac{Css - Csp}{Cstd} \times 100\% \quad (1)$$

Where R is recovery, Css is concentration of spiked sample, Csp is concentration of urine sample, Cstd is concentration of standard solution.

RESULT AND DISCUSSION

Performance of the electrode

Performance of the electrode studied by applying electrodes to measure the potential of the electrode on the solution of uric acid 10^{-8} M to 10^{-3} M using a reference electrode Ag/AgCl . Curve relationship between log $C_{uric\ acid}$ with the potential presented in Figure 1a.

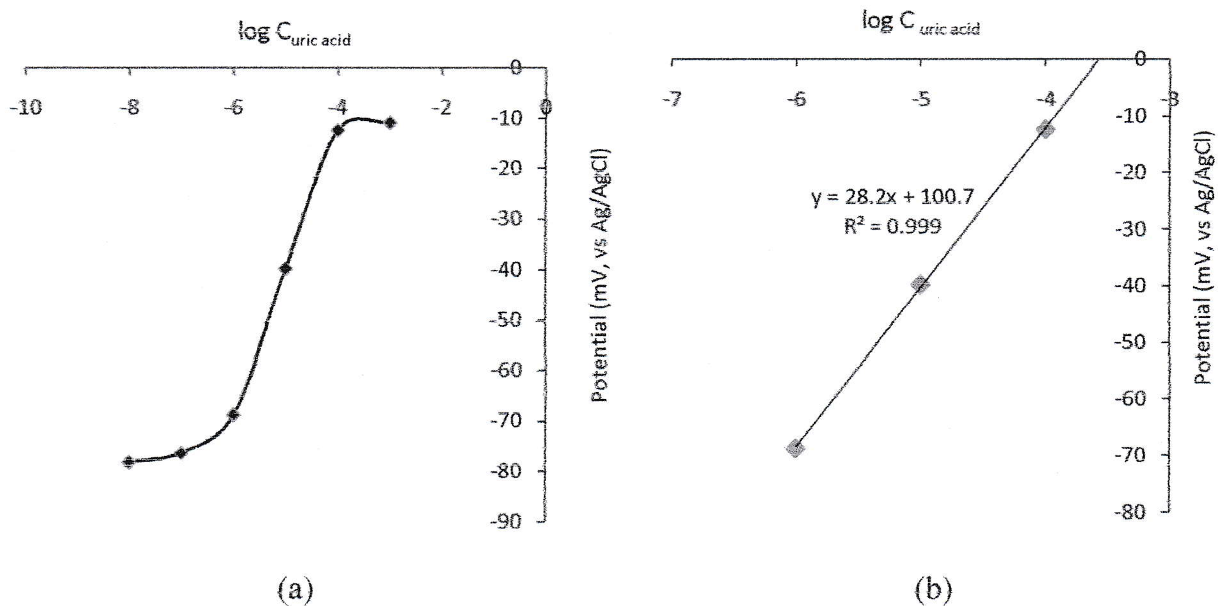

Figure 1 Curve of log $C_{uric\ acid}$ versus potential of the electrode (a) and calibration curve (b)

The range of concentrations that provide linear curve is referred to as a measurement range that was 10^{-6}-10^{-4} M (Figure 1b), with a linearity of the calibration curve (r) was 0.9995.

The response time of the concentration range was 34-44 s. The slope of the calibration curve, known as Nernst factor, was 28.2 mV $decade^{-1}$. According to the research that has been previously reported that uric acid is a divalent molecule [11, 12], thus this method should produce the Nernst factor of (29.6±2) mV $decade^{-1}$. As reported in previous study, the method had a limit of detection of $5.86x10^{-6}$ M, precision of 98.6 to 98.8 %(n=3), and a life time of 10 weeks [8].

Selectivity of the electrode

One of the most important characteristic of electrode on the potentiometric analysis is its response for the primary compound (i) in presence of other compounds (j), which is measured in terms of potentiometric selectivity coefficient (K_{ij}). Selectivity is the ability of electrodes to measure an analyte selectively wherein the analyte coexist with other components in a sample. Measurement of the coefficient of selectivity is required due to the presence of uric acid in the body generally together with other molecules that has functional group similar to uric acid, such as urea, creatine, creatinine, and ascorbic acid [13]. The coefficient of selectivity in this research was determined by the Matched Potential Method (MPM)[9]. If the selectivity coefficient value is less than 1, the analysis of uric acid was not bothered by the presence of the interfering compound.

The compounds used to study the effect of the matrix in this study were creatine, creatinine, and ascorbic acid. To determine the effect of creatine in the analysis of uric acid by potentiometric using carbon paste electrode was used a normal concentration of uric acid in the body which is 10^{-4} M. While concentration of creatine and creatinine used of $1.0x10^{-5}$, $3.0x10^{-5}$, $1.0x10^{-4}$, and $3.0x10^{-4}$ M, respectively. Ascorbic acid concentration used in this study were $5.0x10^{-6}$, $1.0x10^{-5}$, $1.0x10^{-4}$, and $5.0x10^{-4}$ M, respectively. Selected concentration of creatine,

creatinine and ascorbic acid in this study are based on its low, normal and high level in the real urine and serum sample. The value of selectivity coefficient (K_{ij}) for each concentration is shown in Table 1.

The each component that suspected to interfere to the analysis of uric acid shows selectivity coefficient less than 1 for each concentration, which means that either creatine, creatinine, as well as ascorbic acid did not interfere to uric acid analysis by potentiometry using carbon paste electrodes modified imprinting zeolite. This occurs because the electrodes have mold and only recognize the specific molecules, namely uric acid.

Tabel 1 Coefficient of selectivity (K_{ij}) of carbon paste-IZ electrode to analyze uric acid in the presence of ascorbic acid, creatine, or creatinin

Interfering compound (M)	K_{ij}
Ascorbic acid	
5.0×10^{-6}	0.1769
1.0×10^{-5}	0.3847
1.0×10^{-4}	0.7273
5.0×10^{-4}	0.2597
Creatine	
1.0×10^{-5}	0.2002
3.0×10^{-5}	0.1004
1.0×10^{-4}	0.0870
3.0×10^{-4}	0.1749
Creatinine	
1.0×10^{-5}	0.3267
3.0×10^{-5}	0.2093
1.0×10^{-4}	0.1023
3.0×10^{-4}	0.2059

Concentration of uric acid used was 10^{-4}M

Comparative test and accuracy of the method

The developed method has been applied to analyze of uric acid in the urine samples and studied its accuracy. Determining accuracy was done by measuring the electrode potential for the analysis of uric acid standard solution, urine sample, and urine sample that was spiked by uric acid standard solution. The concentration of uric acid standard solution used was 10^{-6}-10^{-4} M. Data of the potential of electrode on the measurement of each solution is presented in Table 2.

Table 2 Data of the electrode potential on the analysis of urine sample

Solution	Potential (mV, vs Ag/AgCl)
Urine	23.90
Urine + uric acid 10^{-6} M	23.91
Uric acid 10^{-6} M	-62.70
Urine + uric acid 10^{-5} M	24.01
Uric acid 10^{-5} M	-37.20
Urine + uric acid 10^{-4} M	25.00
Uric acid 10^{-4} M	-7.60

Accuracy of the potentiometric method to analyze uric acid and comparing the potentiometric method using carbon paste electrodes-IZ with spectrophotometric method commonly used in the medical field had been studied. Data of accuracy value obtained by the method are presented in Table 3. Data listed in Table 3 shows that the potentiometric method using carbon paste-IZ electrode has an accuracy of 96.18-123.04%. The range of accuracy required by the Association of Official Analytical Chemist (AOAC) for the concentration 10^{-6}-10^{-4} M is 80-110% [14]. While the results of comparative testing of potentiometric method with spectrophotometric methods for 7 samples solution showed the value of accuracy of 79.00-121.89%.

Tabel 3 Accuracy of the potentiometric method and compatarive test with spectrophotometry

Sample	Concentration (M)		Accuracy (%)	
	Potentiometry	Spectrophotometry*)	Potentiometry	Toward spectrophotometry
Urine	$1.89x10^{-3}$	$2.39x10^{-3}$		79.00
Urine + uric acid 10^{-6} M	$1.89x10^{-3}$	$1.94x10^{-3}$	96.18	97.28
Uric acid 10^{-6} M	$1.61x10^{-6}$	$1.92x10^{-6}$		83.51
Urine + uric acid 10^{-5} M	$1.91x10^{-3}$	$2.30x10^{-3}$	120.34	82.80
Uric acid 10^{-5} M	$1.29x10^{-5}$	$1.31x10^{-5}$		98.42
Urine + uric acid 10^{-4} M	$2.08x10^{-3}$	$2.10x10^{-3}$	123.04	98.41
Uric acid 10^{-4} M	$1.44x10^{-4}$	$1.18x10^{-4}$		121.89

*) *data from clinical laboratory*

CONCLUSIONS

The carbon paste modified imprinting zeolite electrode has a high selectivity towards uric acid. Presence of ascorbic acid, creatine, or creatinine did not interfere on the uric acid analysis by potentiometry using the electrode. Accuracy of the potentiometry method using the electrode was (113.18±14.79) % (n=3). Comparison test of the method with the spectrophotometric method showed the recovery of (94.47±14.64) % (n=7), therefore the method can be used to determine uric acid in the urine or serum sample.

ACKNOWLEDGMENT

The authors thank to Ministry or Research, Technology and Higher Education, Indonesia under Universitas Airlangga RUPT Grant No. 583/UN3/2016 for financial support and Chemistry Department, Faculty of Science and Technology, Universitas Airlangga for laboratory facilities.

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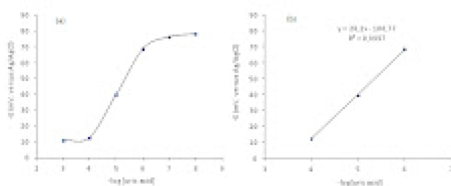

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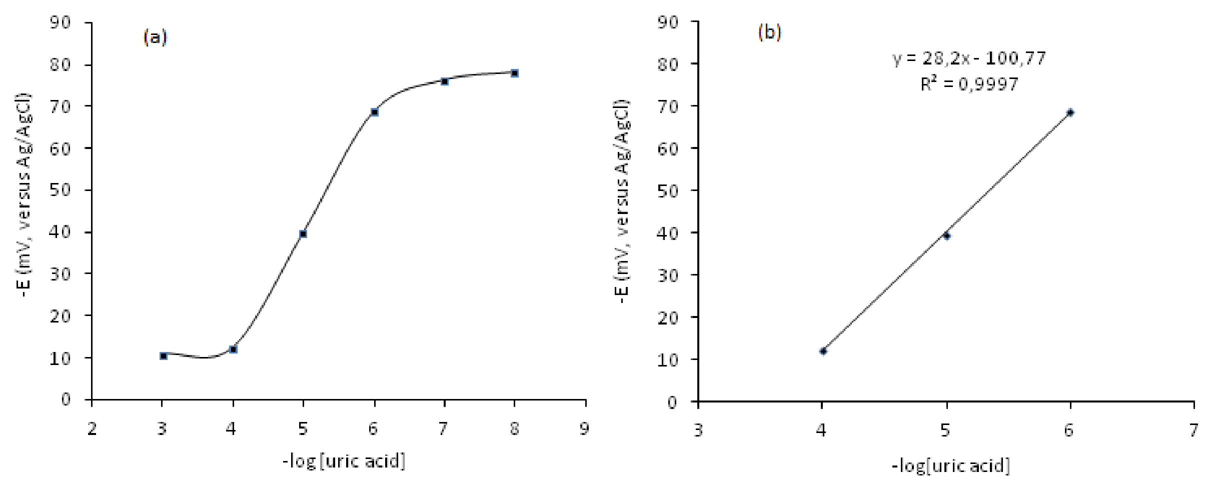

THE INFLUENCE OF ASCORBIC ACID, CREATINE, AND CREATININE ON THE URIC ACID ANALYSIS BY POTENTIOMETRY USING CARBON PASTE MODIFIED IMPRINTING ZEOLITE ELECTRODE

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ABSTRACT

The high level of uric acid in the body is often associated with some diseases such as hyperuricemia, hypertension, renal and cardiovascular disease. Therefore early detection of the levels of uric acid in the body is required. The method commonly used in the medical field to determine uric acid levels is spectrophotometry. The high detection limit of spectrophotometry method confined it to be used to determine low level uric acid in serum sample, while the complexity of serum matrices causing lower its selectivity. The development of imprinted zeolite based sensor to analyze uric acid by potentiometry has been conducted. The carbon paste-IZ electrode showed a range of measurement of 10^{-6} *–* 10^{-4} *M, Nernst factor of 28.2 mV decade*$^{-1}$*, and response time of 34-44 s. The developed electrode has a high selectivity towards uric acid. Ascorbic acid, creatine, or creatinine did not interfere on the uric acid analysis by potentiometry using the electrode, expressed by value of selectivity coefficient* (K_{ij}) *less than 1. Recovery of the method was (113.18±14.79)% (n=3). Comparison test of the developed method with the spectrophotometric method showed the accuracy of (94.47±14.64)% (n=7).*

Keywords: uric acid, carbon paste-IZ, selectivity, potentiometry

INTRODUCTION

Uric acid is the end product of purine metabolism. The normal level of uric acid in the blood is in the range of 2.4 to 5.7 mg/dL in women and 3.4 to 7.0 mg/dL in men [1]. Uric acid has been used as a biomarker to diagnose health problems because of its ability to cause some dangerous diseases. Thus, controlling the levels of uric acid in the body should always be done. The common curent method used to analyze uric acid in the medical field is spectrophotometry using chemical reagent or enzymatic method. The method used to determine the levels of uric acid must be selective because of uric acid found together with another compounds in the sample such as ascorbic acid that interfere the uric acid analysis [2]. Compounds such as creatine and creatinine are also potentially interfered the analysis of uric acid because of the similarity of it functional groups or structure. These compounds cause result of uric acid analysis not accurate and do not represent actual levels of uric acid in the sample.

Various electrochemical methods were developed to solve the problem on uric acid analysis method. The determination of uric acid by voltammetry method have received much interest because of less chemical need and less time consuming compared to colorimetric and enzymatic methods [3,4]. However, a major problem encountered in this method to determine uric acid is the interference from ascorbic acid in blood and urine which can be oxidized at the potentials close to the uric acid [5].

The previous study have developed a method of determining uric acid by potentiometry using ZnO nano wires electrode immobilized uricase enzyme [6]. The method resulted measurement range of 1.0×10^{-6} – 6.5×10^{-4} M. The measurement range was the range of uric acid concentration in the blood, so the method is very suitable applied to analyze of uric acid in the blood serum. Glucose, ascorbic acid and urea do not interfere on uric acid analysis using the developed method. The other study has reported the use a ZnO nanoflakes-based sensor immobilized uricase enzyme for uric acid analysis by potentiometry [7]. The results showed that the developed sensor has a measuring range of 5.0×10^{-7} – 1.5×10^{-3} M, the detection limit of 5.0×10^{-7} M. The sensor was not interfered by the presence of ascorbic acid, glucose and urea. Potentiometric method using carbon paste electrodes modified imprinting zeolite (carbon paste-IZ) has been developed to measure the levels of uric acid [8]. The method was not interfered by the presence of urea. Zeolite has rigid structure, so that in the aqueous media can maintain the shape and size of its pore (to be selective) [9]. Conformity of the size and shape between the print in zeolite and uric acid molecule could increase the adsorption capacity of zeolite, which can provide high sensitivity for uric acid determination.

In this work, we studied the influence of ascorbic acid, creatinine and urea on uric acid analysis by potentiometry using carbon paste electrode modified imprinting zeolite. Imprinted zeolites was manufactured with the mass ratio of carbon, IZ, solid paraffin resulted from previous studies [8]. The influence of ascorbic acid, creatinine and creatine was determined by adding each compound on the uric acid standard solution with the variation of concentration.

EXPERIMENTAL

Material and methods

Chemical used were uric acid (Fluka), creatine and creatinine (Sigma-Aldrich), ascorbic acid, acetic acid, sodium acetate, and sodium hydroxide trihydrate, carbon powder, solid paraffin, tetraethyl orthosilicate (TEOS), tetrabuthyl orthotitanate (TBOT), tetrapropyl ammonium hydroxide (TPAH) (Merck). All chemicals were analytical grade. The solvent used was distilled water. The stock solution of uric acid 10^{-2} M was prepared by dissolving 0.1680 g uric acid in about 10 mL sodium hydroxide 50% (w/w) and diluted with water until 100 mL in volumetric flask. Standard solution of uric acid 10^{-8}-10^{-3} M were prepared by diluting appropriate uric acid working solutions, and their pH were adjusted with the addition of acetate buffer pH 5. Sample used was urine from patients of a local pathological clinic.

Fabrication of carbon paste – IZ electrode

Carbon paste electrode was manufactured by mixing carbon powder, imprinting zeolite and paraffin with a mass ratio of 40:25:35 [8] assisted by heating. Zeolite was synthesized by mixing TEOS, TBOT and TPAH with a mole ratio reported in the previous study [10]. The uric acid was then extracted from the zeolite framework using warm water to produce imprinting zeolite. The modified electrode was made by inserting a wire of silver (Ag) to the micropipette tube and fill micropipette by solid paraffin as much as 3/4 tube. Furthermore, the remaining part of the tube was filled by a paste made previously.

Determination of measurement range, Nernst factor and response time of the electrode

Measurement range is obtained by measuring the electrode potential on uric acid solution 10^{-8} to 10^{-3} M, subsequently made curve relationship between log concentration of uric acid (log [uric acid]) and electrode potential (E). Range of concentration that result the linear curve is called measurement range, while the slope of the linear curve represents the value of Nernst factor. The response time of electrode was determined by calculating the time required by the electrodes in response to the uric acid until provide the potential value.

Selectivity of the electrode

The selectivity of the electrode, expressed by selectivity coefficient (K_{ij}), was studied through the addition of ascorbic acid, creatine and creatinine, compounds which are always coexist with uric acid in urine or serum sample, on the uric acid solution. Uric acid used was 10^{-4} M, while each ascorbic acid, creatine and creatinine was added with various concentrations. The K_{ij} value was calculated by Matched Potential Method (MPM) [11].

Recovery and comparative test of the method

Recovery of the method was obtained through measuring potential of urine sample, uric acid standard solution, and urine samples spiked by uric acid standard solution. Urine samples were taken from the urine of adults collected over 24 hours. The third solution is then measured using carbon paste-IZ electrode and its value substituted into the linear regression equation of the standard curve to obtain the concentration of each solution. Recovery (R) value was calculated by substituting concentration of each solution into the equation 1. Where R is recovery, C_{ss} is concentration of spiked sample, C_{sp} is concentration of urine sample, C_{std} is concentration of standard solution.

$$R = \frac{C_{ss} - C_{sp}}{C_{std}} x100\% \qquad (1)$$

This developed method was compared with spectrophotometry UV Vis as commonly used method in medical field to determine the accuracy. Accuracy was calculated by comparing the concentration resulted from the potentiometry method (C_{dev}) and spectrophotometry method (C_{std}) as shown in equation 2.

$$A = \frac{C_{dev}}{C_{std}} x100\% \qquad (2)$$

RESULTS AND DISCUSSIONS

Performance of the electrode

Performance of the electrode studied by applying electrodes to measure the potential of the electrode on the solution of uric acid 10^{-8} M to 10^{-3} M using a reference electrode Ag/AgCl. Curve relationship between –log [uric acid] with –E (potential) presented in Fig. 1a.

The range of concentrations that provide linear curve is referred to as a measurement range that was 10^{-6}-10^{-4} M (Fig. 1b), with a linearity of the calibration curve (r) was 0.9995. The response time of the concentration range was 34-44 s. The slope of the calibration curve, known as Nernst factor, was 28.2 mV decade^{-1}. According to the research that has been previously reported that uric acid is a divalent molecule [12, 13], thus this method should produce the Nernst factor of (29.6±2) mV decade^{-1}. As reported in previous study, the method had a limit of detection of 5.86x10^{-6} M. precision of 98.6 to 98.8 % (n=3), and a life time of 10 weeks [8].

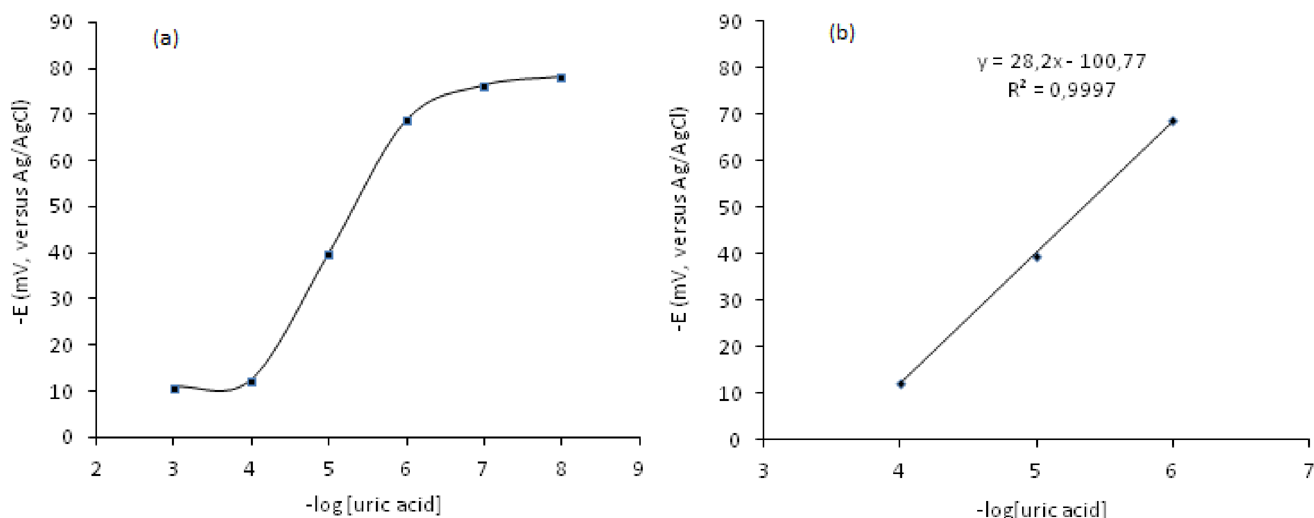

Fig. 1. Curve of–E versus –log [uric acid] (a) and calibration curve (b)

Selectivity of the electrode

One of the most important characteristic of electrode on the potentiometric analysis is its response for the primary compound (i) in presence of other compounds (j), which is measured in terms of potentiometric selectivity coefficient (K_{ij}). Selectivity is the ability of electrodes to measure an analyte selectively wherein the analyte coexist with other components in a sample. Measurement of the coefficient of selectivity is required due to the presence of uric acid in the body generally together with other molecules that has functional group similar to uric acid, such as urea, creatine, creatinine, and ascorbic acid [14]. The selectivity coefficient in this research was determined by the Matched Potential Method (MPM) [11]. If the selectivity coefficient value is less than 1, the analysis of uric acid was not bothered by the presence of the interfering compound.

The compounds used to study the effect of the matrix in this study were creatine, creatinine, and ascorbic acid. To determine the effect of creatine in the analysis of uric acid by potentiometric using carbon paste electrode was used a normal concentration of uric acid in the body which is 10^{-4} M. While concentration of creatine and creatinine used were 1x10^{-5}, 3x10^{-5}, 1x10^{-4}, and 3x10^{-4} M, respectively. Ascorbic acid concentrations used in this study were 5x10^{-6}, 1x10^{-5}, 1x10^{-4}, and 5x10^{-4} M, respectively. Selected concentration of creatine, creatinine and ascorbic acid in this study are based on its low, normal and high level in the real urine and serum sample. The value of selectivity coefficient (K_{ij}) for each concentration is shown in Table 1.

The each component that was suspected to interfere on the analysis of uric acid shows selectivity coefficient less than 1 for each concentration, which means that creatine, creatinine, as well as ascorbic acid did not interfere to uric acid analysis by potentiometry using carbon paste electrodes modified imprinting zeolite. This occurs because the electrodes have mold and only recognize the specific molecules, namely uric acid.

Table 1. Coefficient of selectivity (K_{ij}) of carbon paste-IZ electrode to analyze uric acid in the presence of ascorbic acid, creatine, or creatinine as interfering compound

Interfering compound (M)	Kij
Ascorbic acid	
$5.0x10^{-6}$	0.1769
$1.0x10^{-5}$	0.3847
$1.0x10^{-4}$	0.7273
$5.0x10^{-4}$	0.2597
Creatine	
$1.0x10^{-5}$	0.2002
$3.0x10^{-5}$	0.1004
$1.0x10^{-4}$	0.0870
$3.0x10^{-4}$	0.1749
Creatinine	
$1.0x10^{-5}$	0.3267
$3.0x10^{-5}$	0.2093
$1.0x10^{-4}$	0.1023
$3.0x10^{-4}$	0.2059

**) Concentration of uric acid used was 10^{-4} M*

Comparative test and recovery of the method

The developed method has been applied to analyze of uric acid in the urine samples and studied its recovery. Determining recovery was done by measuring the electrode potential for the analysis of uric acid standard solution, urine sample, and urine sample that was spiked by uric acid standard solution. The concentration of uric acid standard solution used was 10^{-6}-10^{-4} M. Data of the potential of electrode on the measurement of each solution is presented in Table 2.

Table 2. Data of the electrode potential on the analysis of urine sample

Solution	E (mV, vs Ag/AgCl)
Urine	23.90
Urine + uric acid 10^{-6} M	23.91
Uric acid 10^{-6} M	-62.70
Urine + uric acid 10^{-5} M	24.01
Uric acid 10^{-5} M	-37.20
Urine + uric acid 10^{-4} M	25.00
Uric acid 10^{-4} M	-7.60

Recovery of the potentiometric method to analyze uric acid and comparing the potentiometric method using carbon paste electrodes-IZ with spectrophotometric method commonly used in the medical field had been studied. Recovery of the method was obtained by spiking of urine sample by uric acid standard solution. Data of recovery values obtained are presented in Table 3. Data listed in Table 3 shows that spiking three uric acid standard solutions to an urine sample generate recovery of 96.18-123.04%. While the results of comparative testing of potentiometry method with spectrophotometric methods for 7 samples solution generated accuracy of 79.00-121.89%. The range of accuracy required by the Association of Official Analytical Chemist (AOAC) for the concentration 10^{-6}-10^{-4} M is 80-110% [15].

Table 3. Recovery of the potentiometric method and comparative test with spectrophotometric method

Sample	Concentration (M)		Recovery (%)	Accuracy (%)
	Potentiometry	Spectrophotometry*)	Potentiometry	Toward spectrophotometry
Urine	$1.89x10^{-3}$	$2.39x10^{-3}$		79.00
Urine + uric acid 10^{-6} M	$1.89x10^{-3}$	$1.94x10^{-3}$	96.18	97.28
Uric acid 10^{-6} M	$1.61x10^{-6}$	$1.92x10^{-6}$		83.51
Urine + uric acid 10^{-5} M	$1.91x10^{-3}$	2.30x10-3	120.34	82.80
Uric acid 10^{-5} M	$1.29x10^{-5}$	$1.31x10^{-5}$		98.42
Urine + uric acid 10^{-4} M	$2.08x10^{-3}$	$2.10x10^{-3}$	123.04	98.41
Uric acid 10^{-4} M	$1.44x10^{-4}$	$1.18x10^{-4}$		121.89

**) data from clinical laboratory*

CONCLUSIONS

The carbon paste modified imprinting zeolite electrode has a high selectivity towards uric acid. Presence of ascorbic acid, creatine, or creatinine did not interfere on the uric acid analysis by potentiometry using the electrode. Recovery of the potentiometry method using the electrode was (113.18±14.79) % (n=3). Comparison test of the method with the spectrophotometry method showed the accuracy of (94.47±14.64) % (n=7), therefore the method can be applied to determine uric acid in the urine or serum sample.

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THE INFLUENCE OF ASCORBIC ACID, CREATINE, AND CREATININE ON THE URIC ACID ANALYSIS BY POTENTIOMETRY USING A CARBON PASTE MODIFIED IMPRINTING ZEOLITE ELECTRODE

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ABSTRACT

The high level of uric acid in the body is often associated with some diseases such as hyperuricemia, hypertension, renal and cardiovascular disease. Therefore early detection of the levels of uric acid is required. Spectrophotometry is the method commonly used in the medical field to determine the uric acid level. Its detection limit refers to a low uric acid level in a serum sample as the complexity of the serum matrices decreases its selectivity. The development of an imprinted zeolite based sensor to analyze uric acid by potentiometry is conducted. The carbon paste-IZ electrode shows a range of measurement of 10^{-6} M - 10^{-4} M, a Nernst factor of 28.2 mV decade^{-1}, and response time of 34 s - 44 s. The developed electrode has a high selectivity towards uric acid. Ascorbic acid, creatine, or creatinine do not interfere with uric acid analysis using the electrode suggested as the value of the selectivity coefficient (K_{ij}) is less than 1. The method recovery equals (113.18±14.79) % (n = 3). The developed method is compared to the spectrophotometric one and shows an accuracy of (94.47±14.64) % (n = 7).

Keywords: uric acid, carbon paste-IZ, selectivity, potentiometry.

INTRODUCTION

Uric acid is the end product of purine metabolism. The normal level of uric acid in the blood is in the range of 2.4 mg/dL to 5.7 mg/dL for women and 3.4 mg/dL to 7.0 mg/dL for men [1]. Uric acid has been used as a biomarker diagnosing health problems because of its ability to cause some dangerous diseases. Thus, the levels of uric acid in the body should always be controlled. Spectrophotometry

using a chemical reagent or the enzymatic method are the common current methods used in field of medicine to analyze uric acid content. The method used to determine uric acid levels must be selective because uric acid is found together with other interfering compounds [2]. Creatine and creatinine are also potentially interfering uric acid analysis because of the similarity of their functional groups and structure. These compounds presence hamper obtaining accurate results in respect to uric acid levels.

Various electrochemical methods are developed to solve the problem of uric acid analysis. Voltammetry application presents a definite interest because it requires less chemicals and time compared to the colorimetric and enzymatic methods [3, 4]. However, the major problem encountered by this method is the interference of ascorbic acid and urine which can be oxidized at the potentials close to that of the uric acid [5].

A previous study [6] have advances a method of uric acid content determination by potentiometry using ZnO nano wires electrode immobilized by the enzyme uricase. Its measurement range refers to $1.0x10^{-6}$ M – $6.5x10^{-4}$ M which makes it very suitable for uric acid determination in the blood serum. Glucose, ascorbic acid and urea do not interfere in case of this method application. Another study reports the potentiometric use of a ZnO nanoflakes-based sensor immobilized by the enzyme uricase [7]. The results show that the sensor has a measuring range of $5.0x10^{-7}$ M – $1.5x10^{-3}$ M and a detection limit of $5.0x10^{-7}$ M. The sensor function is not interfered by the presence of ascorbic acid, glucose and urea. A potentiometric method using carbon paste electrodes modified by imprinting zeolite (carbon paste-IZ) is also developed to measure uric acid levels [8]. Its application is not affected by the presence of urea. The zeolite has a rigid structure and it can maintain the shape and size of its pores (i. e. its selectivity) [9] in an aqueous medium. The conformity of the zeolite print size and shape and those of the uric acid molecule increases zeolite's adsorption capacity which in turn provides high sensitivity in respect to uric acid determination.

The present work reports a study on the influence of ascorbic acid, creatinine and urea on uric acid analysis by potentiometry using a carbon paste electrode modified by an imprinting zeolite. The preparation of latter is already described [8]. The lof ascorbic acid, creatinine and creatine is followed by introducing each compound of a varying concentration to the uric acid standard solution.

EXPERIMENTAL

Material and methods

Uric acid (Fluka), creatine and creatinine (Sigma-Aldrich), ascorbic acid, acetic acid, sodium acetate, and sodium hydroxide trihydrate, carbon powder, solid paraffin, tetraethyl orthosilicate (TEOS), tetrabuthyl orthotitanate (TBOT), tetrapropyl ammonium hydroxide (TPAH) (all products of Merck) were used. They were of an analytical grade. Distilled water was used as a solvent The stock solution of 10^{-2} M uric acid was prepared by dissolving 0.1680 g uric acid in about 10 mL 50 % (w/w) aqueous solution of sodium hydroxide (a 100 mL volumetric flask was used for the preparation of the latter). The standard 10^{-8} M- 10^{-3} M solutions of uric acid were prepared by diluting appropriate uric acid working solutions, and their pH were adjusted through the addition of acetate buffer of pH 5. Urine from patients of a local pathological clinic was used as a sample.

Fabrication of carbon paste – IZ electrode

Carbon paste electrode was manufactured by mixing carbon powder, imprinting zeolite and paraffin in a mass ratio of 40:25:35 [8]. The process was assisted by heating. The zeolite was synthesized by mixing TEOS, TBOT and TPAH in mole ratios reported in a previous study [10]. The uric acid was then extracted from the zeolite framework using warm water to produce imprinting zeolite. The electrode investigated was prepared by inserting a silver (Ag) wire in a micropipette tube filled to its 3/4 by solid paraffin and subsequent introduction of by the paste already described.

Determination of the measurement range, the Nernst factor and the response time of the electrode

Measurement range was determined by measuring the electrode potential in a uric acid solution of a concentration ranging from 10^{-8} M to 10^{-3} M and the subsequent presentation of the dependence of the logarithm of uric acid concentration (log [uric acid]) on the electrode potential (E). The measurement range referred to the concentration interval where the relation pointed above was linear, while the slope of that line was equal to the Nernst factor value. The response time of electrode was determined by the time required to obtain a potential value referring to uric acid presence.

Selectivity of the electrode

The selectivity of the electrode, expressed by the selectivity coefficient (K_{ij}), was studied through the addition of ascorbic acid, creatine and creatinine, compounds which always coexist with uric acid

in urine or in a serum sample. Uric acid used was 10^{-4} M, while the ascorbic acid, the creatine and the creatinine were added in varying concentrations. The K_{ij} value was calculated by Matched Potential Method (MPM) [11].

Method recovery and comparative test

The recovery of the method was followed by measuring the potential in a urine sample, a uric acid standard solution, and urine samples spiked by a uric acid standard solution. The urine samples were taken from the urine of adults collected over 24 hours. The potential of the carbon paste-IZ electrode was read and its value was substituted into the linear regression equation describing the standard curve aiming to obtain the concentration of each solution. The recovery (R) value was calculated by substituting the concentration of each solution into Eq. 1:

$$R = \frac{C_{ss} - C_{sp}}{C_{std}} x100\% \qquad (1)$$

where R was the recovery, C_{ss} was the concentration of the spiked sample, C_{sp} was the concentration of the urine sample, while C_{std} was the concentration of the standard solution.

The method described was compared to the UV-Vis spectrophotometrical one to determine its accuracy. The latter was calculated by referring the concentration determined potentiometrically (C_{dev}) to that obtained spectrophotometrically (C_{std}) (Eq. 2):

$$A = \frac{C_{dev}}{C_{std}} x100\% \qquad (2)$$

RESULTS AND DISCUSSION

Performance of the electrode

The performance of the electrode assembled is studied by measuring its potential in solutions of uric acid of a concentration ranging from 10^{-8} M to 10^{-3} M using Ag/AgCl as a reference electrode. The relationship between –log [uric acid] and –E (potential) is presented in Fig. 1a.

The measurement range found refers to the concentration interval of 10^{-6} M - 10^{-4} M (Fig. 1b), where the linearity is characterized by r value of 0.9995. The response time is found equal to 34 s - 44 s, while the Nernst factor estimated is equal to 28.2 mV decade^{-1}. In view of the fact that uric acid is a divalent molecule [12, 13], the Nernst factor is expected to be equal to (29.6±2) mV decade^{-1}. As

previously reported the method has a limit of detection of 5.86×10^{-6} M. precision of 98.6 to 98.8 % (n=3), and a life time of 10 weeks [8].

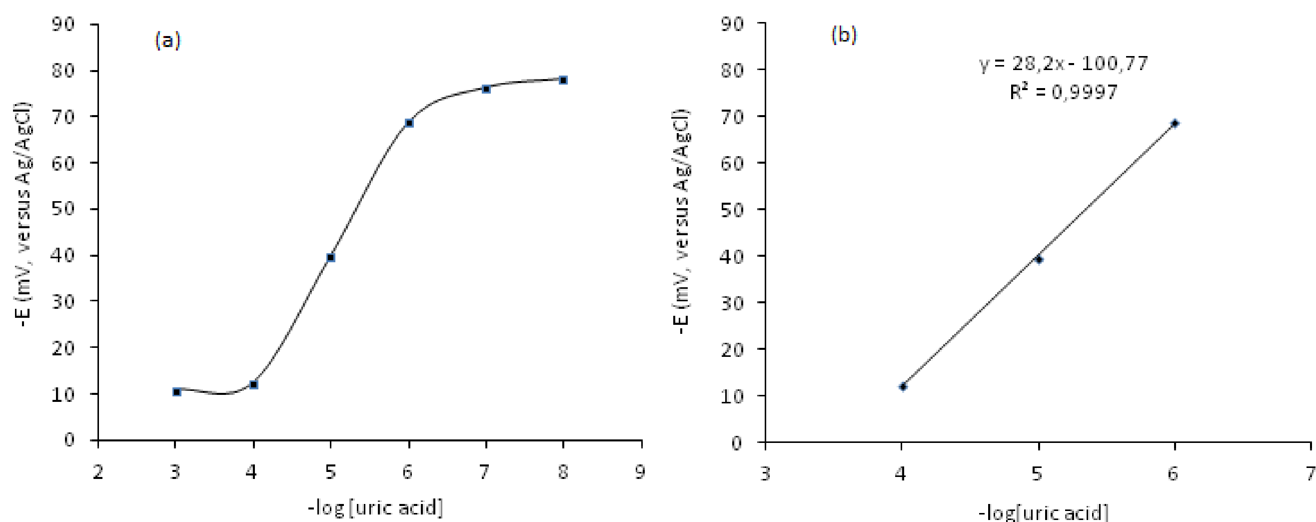

Fig. 1. Curve of–E versus –log [uric acid] (a) and calibration curve (b).

Selectivity of the electrode

Selectivity is the ability of electrodes to measure an analyte selectively wherein the analyte coexist with other components in a sample. One of the most important characteristics in this respect electrode response to primary compound (i) in presence of other compounds (j), which is measured in terms of the potentiometric selectivity coefficient (K_{ij}). The estimation of the coefficient of selectivity is required due to the presence of uric acid in the body together with other molecules of functional groups similar to those of uric acid, such as urea, creatine, creatinine, and ascorbic acid [14]. The selectivity coefficient is determined in this research by the Matched Potential Method (MPM) [11]. If the selectivity coefficient value is less than 1, the analysis of uric acid is not hampered by the presence of the interfering compounds.

Creatine, creatinine, and ascorbic acid are the compounds used to study the effect of the matrix in this investigation. The concentration of uric acid used coincides with that in the body which is 10^{-4} M. The concentrations of creatine and creatinine used are 1×10^{-5} M, 3×10^{-5} M, 1×10^{-4} M, and 3×10^{-4} M, while those of ascorbic acid refer to 5×10^{-6} M, 1×10^{-5} M, 1×10^{-4} M, and 5×10^{-4} M. The choice of the concentrations values pointed above is determined by these substances low, normal and high levels in real urine and serum samples. The value of the selectivity coefficient (K_{ij}) for each concentration is shown in Table 1.

It is seen that the selectivity coefficient is less than 1 for each concentration of the compounds suspected to interfere with the analysis of uric acid. This means that creatine, creatinine, as well as ascorbic acid do not hamper uric acid analysis by potentiometry using the electrode assembled. It is so because the electrode recognizes specific molecules, namely uric acid.

Table 1. Coefficient of selectivity (K_{ij}) of carbon paste-IZ electrode obtained in the course of analysis of uric acid in presence of ascorbic acid, creatine, and creatinine as interfering compounds.

Interfering compound (M)	Kij
Ascorbic acid	
$5.0x10^{-6}$	0.1769
$1.0x10^{-5}$	0.3847
$1.0x10^{-4}$	0.7273
$5.0x10^{-4}$	0.2597
Creatine	
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Creatinine	
$1.0x10^{-5}$	0.3267
$3.0x10^{-5}$	0.2093
$1.0x10^{-4}$	0.1023
$3.0x10^{-4}$	0.2059

**) Concentration of uric acid used was 10^{-4} M*

Comparative test and recovery of the method

The recovery of the method applied is identified by measuring the electrode potential in a uric acid standard solution, a urine sample, and a urine sample spiked by a uric acid standard solution. The concentration of the uric acid standard solution used is 10^{-6} M - 10^{-4} M. The electrode potential values obtained are listed in Table 2.

Table 2. Values of the electrode potential obtained in the course of analysis of a urine sample.

Solution	E (mV, vs Ag/AgCl)

Urine	23.90
Urine + uric acid 10^{-6} M	23.91
Uric acid 10^{-6} M	-62.70
Urine + uric acid 10^{-5} M	24.01
Uric acid 10^{-5} M	-37.20
Urine + uric acid 10^{-4} M	25.00
Uric acid 10^{-4} M	-7.60

The data referring to the juxtaposition of the method considered and the spectrophotometrical analysis usually applied are summarized in Table 3. It is evident that 3 the recovery of the method advanced reaches 96.18 - 123.04 % in case of spiking uric acid solutions of three different concentrations to a urine sample. The results referring to the comparative testing of potentiometry and spectrophotometry in case of 7 sample solutions show accuracy of 79.00 % - 121.89 %. The range of accuracy required by the Association of Official Analytical Chemist (AOAC) for the concentration range of 10^{-6} M - 10^{-4} M is 80 – 110 % [15].

Table 3. Data illustrating the potentiometric method recovery and its juxtaposition to spectrophotometry.

Sample	Concentration (M)		Recovery (%)	Accuracy (%)
	Potentiometry	Spectrophotometry*)	Potentiometry	Toward spectrophotometry
Urine	1.89×10^{-3}	2.39×10^{-3}		79.00
Urine + uric acid 10^{-6} M	1.89×10^{-3}	1.94×10^{-3}	96.18	97.28
Uric acid 10^{-6} M	1.61×10^{-6}	1.92×10^{-6}		83.51
Urine + uric acid 10^{-5} M	1.91×10^{-3}	2.30x10-3	120.34	82.80
Uric acid 10^{-5} M	1.29×10^{-5}	1.31×10^{-5}		98.42
Urine + uric acid 10^{-4} M	2.08×10^{-3}	2.10×10^{-3}	123.04	98.41
Uric acid 10^{-4} M	1.44×10^{-4}	1.18×10^{-4}		121.89

**) data from clinical laboratory*

CONCLUSIONS

A carbon paste electrode modified by an imprinting zeolite is prepared. It has a high selectivity towards uric acid. The presence of ascorbic acid, creatine, or creatinine does not affect uric acid analysis by potentiometry using the electrode assembled. The recovery of the potentiometry method amounts to (113.18±14.79) % (n=3). The comparison of the method to the spectrophotometrical analysis usually applied shows an accuracy of (94.47±14.64) % (n=7). It is concluded that the method can be applied to determine uric acid in urine or a serum sample.

Acknowledgements

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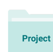

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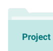

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Journal of Chemical Technology and Metallurgy, 52, 6, 2017, 1039-1044

THE INFLUENCE OF ASCORBIC ACID, CREATINE, AND CREATININE ON THE URIC ACID ANALYSIS BY POTENTIOMETRY USING A CARBON PASTE MODIFIED IMPRINTING ZEOLITE ELECTRODE

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ABSTRACT

The high level of uric acid in the body is often associated with some diseases such as hyperuricemia, hypertension, renal and cardiovascular disease. Therefore early detection of the levels of uric acid is required. Spectrophotometry is the method commonly used in the medical field to determine the uric acid level. Its detection limit refers to a low uric acid level in a serum sample as the complexity of the serum matrices decreases its selectivity. The development of an imprinted zeolite based sensor to analyze uric acid by potentiometry is conducted. The carbon paste-IZ electrode shows a range of measurement of 10^{-6} M - 10^{-4} M, a Nernst factor of 28.2 mV decade^{-1}, and response time of 34 s - 44 s. The developed electrode has a high selectivity towards uric acid. Ascorbic acid, creatine, or creatinine do not interfere with uric acid analysis using the electrode suggested as the value of the selectivity coefficient (K_{ij}) is less than 1. The method recovery equals (113.18±14.79) % (n = 3). The developed method is compared to the spectrophotometric one and shows an accuracy of (94.47±14.64) % (n = 7).

Keywords: uric acid, carbon paste-IZ, selectivity, potentiometry.

INTRODUCTION

Uric acid is the end product of purine metabolism. The normal level of uric acid in the blood is in the range of 2.4 mg/dL to 5.7 mg/dL for women and 3.4 mg/dL to 7.0 mg/dL for men [1]. Uric acid has been used as a biomarker diagnosing health problems because of its ability to cause some dangerous diseases. Thus, the levels of uric acid in the body should always be controlled. Spectrophotometry using a chemical reagent or the enzymatic method are the common current methods used in field of medicine to analyze uric acid content. The method used to determine uric acid levels must be selective because uric acid is found together with other interfering compounds [2]. Creatine and creatinine are also potentially interfering uric acid analysis because of the similarity of their functional groups and structure. These compounds presence hamper obtaining accurate results in respect to uric acid levels.

Various electrochemical methods are developed to solve the problem of uric acid analysis. Voltammetry application presents a definite interest because it requires less chemicals and time compared to the colorimetric and enzymatic methods [3, 4]. However, the major problem encountered by this method is the interference of ascorbic acid and urine which can be oxidized at the potentials close to that of the uric acid [5].

A previous study [6] have advances a method of uric acid content determination by potentiometry using ZnO nano wires electrode immobilized by the enzyme uricase. Its measurement range refers to 1.0x10^{-6} M – 6.5x10^{-4} M which makes it very suitable for uric acid determination in the blood serum. Glucose, ascorbic acid and urea do not interfere in case of this method application. Another study reports the potentiometric use of a ZnO nanoflakes-based sensor immobilized by

the enzyme uricase [7]. The results show that the sensor has a measuring range of $5.0x10^{-7}$ M – $1.5x10^{-3}$ M and a detection limit of $5.0x10^{-7}$ M. The sensor function is not interfered by the presence of ascorbic acid, glucose and urea. A potentiometric method using carbon paste electrodes modified by imprinting zeolite (carbon paste-IZ) is also developed to measure uric acid levels [8]. Its application is not affected by the presence of urea. The zeolite has a rigid structure and it can maintain the shape and size of its pores (i. e. its selectivity) [9] in an aqueous medium. The conformity of the zeolite print size and shape and those of the uric acid molecule increases zeolite's adsorption capacity which in turn provides high sensitivity in respect to uric acid determination.

The present work reports a study on the influence of ascorbic acid, creatinine and urea on uric acid analysis by potentiometry using a carbon paste electrode modified by an imprinting zeolite. The preparation of latter is already described [8]. The lof ascorbic acid, creatinine and creatine is followed by introducing each compound of a varying concentration to the uric acid standard solution.

EXPERIMENTAL

Material and methods

Uric acid (Fluka), creatine and creatinine (Sigma-Aldrich), ascorbic acid, acetic acid, sodium acetate, and sodium hydroxide trihydrate, carbon powder, solid paraffin, tetraethyl orthosilicate (TEOS), tetrabuthyl orthotitanate (TBOT), tetrapropyl ammonium hydroxide (TPAH) (all products of Merck) were used. They were of an analytical grade. Distilled water was used as a solvent. The stock solution of 10^{-2} M uric acid was prepared by dissolving 0.1680 g uric acid in about 10 mL 50 % (w/w) aqueous solution of sodium hydroxide (a 100 mL volumetric flask was used for the preparation of the latter). The standard 10^{-8} M - 10^{-3} M solutions of uric acid were prepared by diluting appropriate uric acid working solutions, and their pH were adjusted through the addition of acetate buffer of pH 5. Urine from patients of a local pathological clinic was used as a sample.

Fabrication of carbon paste – IZ electrode

Carbon paste electrode was manufactured by mixing carbon powder, imprinting zeolite and paraffin in a mass ratio of 40:25:35 [8]. The process was assisted by heating. The zeolite was synthesized by mixing TEOS, TBOT and TPAH in mole ratios reported in a previous study [10]. The uric acid was then extracted from the zeolite framework using warm water to produce imprinting zeolite. The electrode investigated was prepared by inserting a silver (Ag) wire in a micropipette tube filled to its 3/4 by solid paraffin and subsequent introduction of by the paste already described.

Determination of the measurement range, the Nernst factor and the response time of the electrode

Measurement range was determined by measuring the electrode potential in a uric acid solution of a concentration ranging from 10^{-8} M to 10^{-3} M and the subsequent presentation of the dependence of the logarithm of uric acid concentration (log [uric acid]) on the electrode potential (E). The measurement range referred to the concentration interval where the relation pointed above was linear, while the slope of that line was equal to the Nernst factor value. The response time of electrode was determined by the time required to obtain a potential value referring to uric acid presence.

Selectivity of the electrode

The selectivity of the electrode, expressed by the selectivity coefficient (K_{ij}), was studied through the addition of ascorbic acid, creatine and creatinine, compounds which always coexist with uric acid in urine or in a serum sample. Uric acid used was 10^{-4} M, while the ascorbic acid, the creatine and the creatinine were added in varying concentrations. The K_{ij} value was calculated by Matched Potential Method (MPM) [11].

Method recovery and comparative test

The recovery of the method was followed by measuring the potential in a urine sample, a uric acid standard solution, and urine samples spiked by a uric acid standard solution. The urine samples were taken from the urine of adults collected over 24 hours. The potential of the carbon paste-IZ electrode was read and its value was substituted into the linear regression equation describing the standard curve aiming to obtain the concentration of

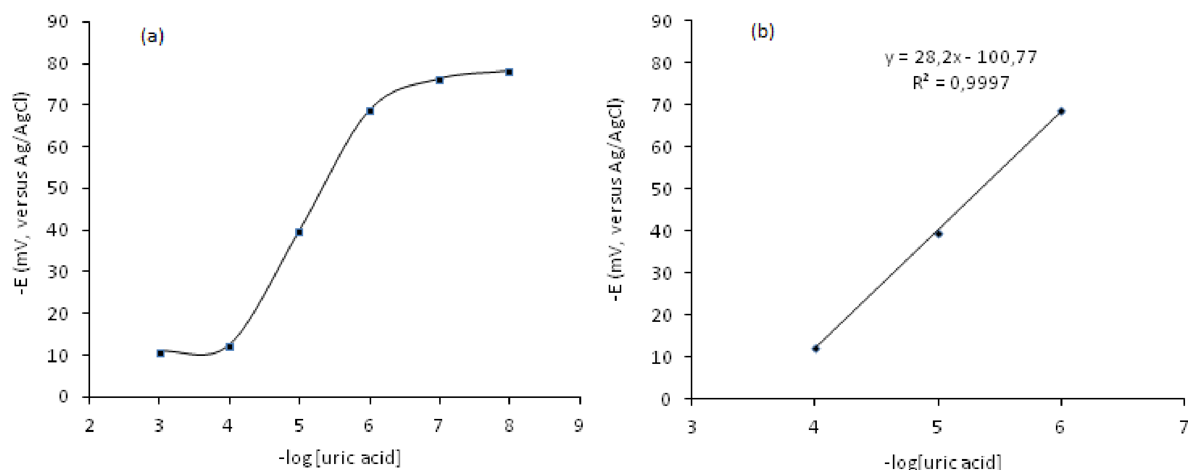

Fig. 1. Curve of –E versus –log [uric acid] (a) and calibration curve (b).

each solution. The recovery (R) value was calculated by substituting the concentration of each solution into Eq. 1:

$$R = \frac{c_{ss} - c_{sp}}{c_{std}} x100\% \quad (1)$$

where R was the recovery, C_{ss} was the concentration of the spiked sample, C_{sp} was the concentration of the urine sample, while C_{std} was the concentration of the standard solution.

The method described was compared to the UV-Vis spectrophotometrical one to determine its accuracy. The latter was calculated by referring the concentration determined potentiometrically (C_{dev}) to that obtained spectrophotometrically (C_{std}) (Eq. 2):

$$A = \frac{c_{dev}}{c_{std}} x100\% \quad (2)$$

RESULTS AND DISCUSSION

Performance of the electrode

The performance of the electrode assembled is studied by measuring its potential in solutions of uric acid of a concentration ranging from 10^{-8} M to 10^{-3} M using Ag/AgCl as a reference electrode. The relationship between –log [uric acid] and –E (potential) is presented in Fig. 1a.

The measurement range found refers to the concentration interval of 10^{-6} M - 10^{-4} M (Fig. 1b), where the linearity is characterized by r value of 0.9995. The response time is found equal to 34 s - 44 s, while the Nernst factor estimated is equal to 28.2 mV decade^{-1}. In view of the fact that uric acid is a divalent molecule [12, 13], the Nernst factor is expected to be equal to (29.6±2) mV decade^{-1}. As previously reported the method has a limit of detection of $5.86x10^{-6}$ M. precision of 98.6 to 98.8 % (n=3), and a life time of 10 weeks [8].

Selectivity of the electrode

Selectivity is the ability of electrodes to measure an analyte selectively wherein the analyte coexist with other components in a sample. One of the most important characteristics in this respect electrode response to primary compound (i) in presence of other compounds (j), which is measured in terms of the potentiometric selectivity coefficient (K_{ij}). The estimation of the coefficient of selectivity is required due to the presence of uric acid in the body together with other molecules of functional groups similar to those of uric acid, such as urea, creatine, creatinine, and ascorbic acid [14]. The selectivity coefficient is determined in this research by the Matched Potential Method (MPM) [11]. If the selectivity coefficient value is less than 1, the analysis of uric acid is not hampered by the presence of the interfering compounds.

Creatine, creatinine, and ascorbic acid are the compounds used to study the effect of the matrix in this investigation. The concentration of uric acid used coincides with that in the body which is 10^{-4} M. The

Table 1. Coefficient of selectivity (K_{ij}) of carbon paste-IZ electrode obtained in the course of analysis of uric acid in presence of ascorbic acid, creatine, and creatinine as interfering compounds.

Interfering compound (M)	K_{ij}
Ascorbic acid	
$5.0x10^{-6}$	0.1769
$1.0x10^{-5}$	0.3847
$1.0x10^{-4}$	0.7273
$5.0x10^{-4}$	0.2597
Creatine	
$1.0x10^{-5}$	0.2002
$3.0x10^{-5}$	0.1004
$1.0x10^{-4}$	0.0870
$3.0x10^{-4}$	0.1749
Creatinine	
$1.0x10^{-5}$	0.3267
$3.0x10^{-5}$	0.2093
$1.0x10^{-4}$	0.1023
$3.0x10^{-4}$	0.2059

) Concentration of uric acid used was 10^{-4} M

concentrations of creatine and creatinine used are $1x10^{-5}$ M, $3x10^{-5}$ M, $1x10^{-4}$ M, and $3x10^{-4}$ M, while those of ascorbic acid refer to $5x10^{-6}$ M, $1x10^{-5}$ M, $1x10^{-4}$ M, and $5x10^{-4}$ M. The choice of the concentrations values pointed above is determined by these substances low, normal and high levels in real urine and serum samples. The value of the selectivity coefficient (K_{ij}) for each concentration is shown in Table 1.

It is seen that the selectivity coefficient is less than 1 for each concentration of the compounds suspected to interfere with the analysis of uric acid. This means that creatine, creatinine, as well as ascorbic acid do not hamper uric acid analysis by potentiometry using the electrode assembled. It is so because the electrode recognizes specific molecules, namely uric acid.

Table 2. Values of the electrode potential obtained in the course of analysis of a urine sample.

Solution	E (mV, vs Ag/AgCl)
Urine	23.90
Urine + uric acid 10^{-6} M	23.91
Uric acid 10^{-6} M	-62.70
Urine + uric acid 10^{-5} M	24.01
Uric acid 10^{-5} M	-37.20
Urine + uric acid 10^{-4} M	25.00
Uric acid 10^{-4} M	-7.60

Comparative test and recovery of the method

The recovery of the method applied is identified by measuring the electrode potential in a uric acid standard solution, a urine sample, and a urine sample spiked by a uric acid standard solution. The concentration of the uric acid standard solution used is 10^{-6} M - 10^{-4} M. The electrode potential values obtained are listed in Table 2.

The data referring to the juxtaposition of the method considered and the spectrophotometrical analysis usually applied are summarized in Table 3. It is evident that 3 the recovery of the method advanced reaches 96.18 - 123.04 % in case of spiking uric acid solutions of three different concentrations to a urine sample. The results

Table 3. Data illustrating the potentiometric method recovery and its juxtaposition to spectrophotometry.

Sample	Concentration (M)		Recovery (%)	Accuracy (%)
	Potentiometry	Spectrophotometry *)	Potentiometry	Toward spectrophotometry
Urine	$1.89x10^{-3}$	$2.39x10^{-3}$		79.00
Urine + uric acid 10^{-6} M	$1.89x10^{-3}$	$1.94x10^{-3}$	96.18	97.28
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) data from clinical laboratory

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CONCLUSIONS

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Keywords: uric acid, carbon paste-IZ, selectivity, potentiometry.

INTRODUCTION

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Various electrochemical methods are developed to solve the problem of uric acid analysis. Voltammetry application presents a definite interest because it requires less chemicals and time compared to the colorimetric and enzymatic methods [3, 4]. However, the major problem encountered by this method is the interference of ascorbic acid and urine which can be oxidized at the potentials close to that of the uric acid [5].

A previous study [6] have advances a method of uric acid content determination by potentiometry using ZnO nano wires electrode immobilized by the enzyme uricase. Its measurement range refers to 1.0x10^{-6} M – 6.5x10^{-4} M which makes it very suitable for uric acid determination in the blood serum. Glucose, ascorbic acid and urea do not interfere in case of this method application. Another study reports the potentiometric use of a ZnO nanoflakes-based sensor immobilized by

the enzyme uricase [7]. The results show that the sensor has a measuring range of $5.0x10^{-7}$ M – $1.5x10^{-3}$ M and a detection limit of $5.0x10^{-7}$ M. The sensor function is not interfered by the presence of ascorbic acid, glucose and urea. A potentiometric method using carbon paste electrodes modified by imprinting zeolite (carbon paste-IZ) is also developed to measure uric acid levels [8]. Its application is not affected by the presence of urea. The zeolite has a rigid structure and it can maintain the shape and size of its pores (i. e. its selectivity) [9] in an aqueous medium. The conformity of the zeolite print size and shape and those of the uric acid molecule increases zeolite's adsorption capacity which in turn provides high sensitivity in respect to uric acid determination.

The present work reports a study on the influence of ascorbic acid, creatinine and urea on uric acid analysis by potentiometry using a carbon paste electrode modified by an imprinting zeolite. The preparation of latter is already described [8]. The lof ascorbic acid, creatinine and creatine is followed by introducing each compound of a varying concentration to the uric acid standard solution.

EXPERIMENTAL

Material and methods

Uric acid (Fluka), creatine and creatinine (Sigma-Aldrich), ascorbic acid, acetic acid, sodium acetate, and sodium hydroxide trihydrate, carbon powder, solid paraffin, tetraethyl orthosilicate (TEOS), tetrabuthyl orthotitanate (TBOT), tetrapropyl ammonium hydroxide (TPAH) (all products of Merck) were used. They were of an analytical grade. Distilled water was used as a solvent. The stock solution of 10^{-2} M uric acid was prepared by dissolving 0.1680 g uric acid in about 10 mL 50 % (w/w) aqueous solution of sodium hydroxide (a 100 mL volumetric flask was used for the preparation of the latter). The standard 10^{-8} M - 10^{-3} M solutions of uric acid were prepared by diluting appropriate uric acid working solutions, and their pH were adjusted through the addition of acetate buffer of pH 5. Urine from patients of a local pathological clinic was used as a sample.

Fabrication of carbon paste – IZ electrode

Carbon paste electrode was manufactured by mixing carbon powder, imprinting zeolite and paraffin in a mass ratio of 40:25:35 [8]. The process was assisted by heating. The zeolite was synthesized by mixing TEOS, TBOT and TPAH in mole ratios reported in a previous study [10]. The uric acid was then extracted from the zeolite framework using warm water to produce imprinting zeolite. The electrode investigated was prepared by inserting a silver (Ag) wire in a micropipette tube filled to its 3/4 by solid paraffin and subsequent introduction of by the paste already described.

Determination of the measurement range, the Nernst factor and the response time of the electrode

Measurement range was determined by measuring the electrode potential in a uric acid solution of a concentration ranging from 10^{-8} M to 10^{-3} M and the subsequent presentation of the dependence of the logarithm of uric acid concentration (log [uric acid]) on the electrode potential (E). The measurement range referred to the concentration interval where the relation pointed above was linear, while the slope of that line was equal to the Nernst factor value. The response time of electrode was determined by the time required to obtain a potential value referring to uric acid presence.

Selectivity of the electrode

The selectivity of the electrode, expressed by the selectivity coefficient (K_{ij}), was studied through the addition of ascorbic acid, creatine and creatinine, compounds which always coexist with uric acid in urine or in a serum sample. Uric acid used was 10^{-4} M, while the ascorbic acid, the creatine and the creatinine were added in varying concentrations. The K_{ij} value was calculated by Matched Potential Method (MPM) [11].

Method recovery and comparative test

The recovery of the method was followed by measuring the potential in a urine sample, a uric acid standard solution, and urine samples spiked by a uric acid standard solution. The urine samples were taken from the urine of adults collected over 24 hours. The potential of the carbon paste-IZ electrode was read and its value was substituted into the linear regression equation describing the standard curve aiming to obtain the concentration of

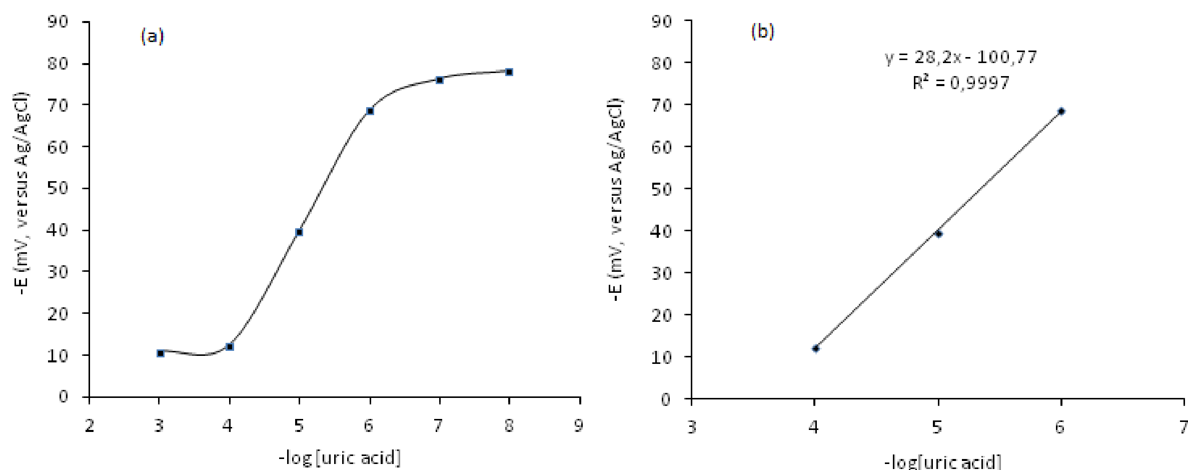

Fig. 1. Curve of –E versus –log [uric acid] (a) and calibration curve (b).

each solution. The recovery (R) value was calculated by substituting the concentration of each solution into Eq. 1:

$$R = \frac{c_{ss} - c_{sp}}{c_{std}} x100\% \quad (1)$$

where R was the recovery, C_{ss} was the concentration of the spiked sample, C_{sp} was the concentration of the urine sample, while C_{std} was the concentration of the standard solution.

The method described was compared to the UV-Vis spectrophotometrical one to determine its accuracy. The latter was calculated by referring the concentration determined potentiometrically (C_{dev}) to that obtained spectrophotometrically (C_{std}) (Eq. 2):

$$A = \frac{c_{dev}}{c_{std}} x100\% \quad (2)$$

RESULTS AND DISCUSSION

Performance of the electrode

The performance of the electrode assembled is studied by measuring its potential in solutions of uric acid of a concentration ranging from 10^{-8} M to 10^{-3} M using Ag/AgCl as a reference electrode. The relationship between –log [uric acid] and –E (potential) is presented in Fig. 1a.

The measurement range found refers to the concentration interval of 10^{-6} M - 10^{-4} M (Fig. 1b), where the linearity is characterized by r value of 0.9995. The response time is found equal to 34 s - 44 s, while the Nernst factor estimated is equal to 28.2 mV decade^{-1}. In view of the fact that uric acid is a divalent molecule [12, 13], the Nernst factor is expected to be equal to (29.6±2) mV decade^{-1}. As previously reported the method has a limit of detection of $5.86x10^{-6}$ M. precision of 98.6 to 98.8 % (n=3), and a life time of 10 weeks [8].

Selectivity of the electrode

Selectivity is the ability of electrodes to measure an analyte selectively wherein the analyte coexist with other components in a sample. One of the most important characteristics in this respect electrode response to primary compound (i) in presence of other compounds (j), which is measured in terms of the potentiometric selectivity coefficient (K_{ij}). The estimation of the coefficient of selectivity is required due to the presence of uric acid in the body together with other molecules of functional groups similar to those of uric acid, such as urea, creatine, creatinine, and ascorbic acid [14]. The selectivity coefficient is determined in this research by the Matched Potential Method (MPM) [11]. If the selectivity coefficient value is less than 1, the analysis of uric acid is not hampered by the presence of the interfering compounds.

Creatine, creatinine, and ascorbic acid are the compounds used to study the effect of the matrix in this investigation. The concentration of uric acid used coincides with that in the body which is 10^{-4} M. The

Table 1. Coefficient of selectivity (K_{ij}) of carbon paste-IZ electrode obtained in the course of analysis of uric acid in presence of ascorbic acid, creatine, and creatinine as interfering compounds.

Interfering compound (M)	K_{ij}
Ascorbic acid	
$5.0x10^{-6}$	0.1769
$1.0x10^{-5}$	0.3847
$1.0x10^{-4}$	0.7273
$5.0x10^{-4}$	0.2597
Creatine	
$1.0x10^{-5}$	0.2002
$3.0x10^{-5}$	0.1004
$1.0x10^{-4}$	0.0870
$3.0x10^{-4}$	0.1749
Creatinine	
$1.0x10^{-5}$	0.3267
$3.0x10^{-5}$	0.2093
$1.0x10^{-4}$	0.1023
$3.0x10^{-4}$	0.2059

) Concentration of uric acid used was 10^{-4} M

concentrations of creatine and creatinine used are $1x10^{-5}$ M, $3x10^{-5}$ M, $1x10^{-4}$ M, and $3x10^{-4}$ M, while those of ascorbic acid refer to $5x10^{-6}$ M, $1x10^{-5}$ M, $1x10^{-4}$ M, and $5x10^{-4}$ M. The choice of the concentrations values pointed above is determined by these substances low, normal and high levels in real urine and serum samples. The value of the selectivity coefficient (K_{ij}) for each concentration is shown in Table 1.

It is seen that the selectivity coefficient is less than 1 for each concentration of the compounds suspected to interfere with the analysis of uric acid. This means that creatine, creatinine, as well as ascorbic acid do not hamper uric acid analysis by potentiometry using the electrode assembled. It is so because the electrode recognizes specific molecules, namely uric acid.

Table 2. Values of the electrode potential obtained in the course of analysis of a urine sample.

Solution	E (mV, vs Ag/AgCl)
Urine	23.90
Urine + uric acid 10^{-6} M	23.91
Uric acid 10^{-6} M	-62.70
Urine + uric acid 10^{-5} M	24.01
Uric acid 10^{-5} M	-37.20
Urine + uric acid 10^{-4} M	25.00
Uric acid 10^{-4} M	-7.60

Comparative test and recovery of the method

The recovery of the method applied is identified by measuring the electrode potential in a uric acid standard solution, a urine sample, and a urine sample spiked by a uric acid standard solution. The concentration of the uric acid standard solution used is 10^{-6} M - 10^{-4} M. The electrode potential values obtained are listed in Table 2.

The data referring to the juxtaposition of the method considered and the spectrophotometrical analysis usually applied are summarized in Table 3. It is evident that 3 the recovery of the method advanced reaches 96.18 - 123.04 % in case of spiking uric acid solutions of three different concentrations to a urine sample. The results

Table 3. Data illustrating the potentiometric method recovery and its juxtaposition to spectrophotometry.

Sample	Concentration (M)		Recovery (%)	Accuracy (%)
	Potentiometry	Spectrophotometry *)	Potentiometry	Toward spectrophotometry
Urine	$1.89x10^{-3}$	$2.39x10^{-3}$		79.00
Urine + uric acid 10^{-6} M	$1.89x10^{-3}$	$1.94x10^{-3}$	96.18	97.28
Uric acid 10^{-6} M	$1.61x10^{-6}$	$1.92x10^{-6}$		83.51
Urine + uric acid 10^{-5} M	$1.91x10^{-3}$	2.30x10-3	120.34	82.80
Uric acid 10^{-5} M	$1.29x10^{-5}$	$1.31x10^{-5}$		98.42
Urine + uric acid 10^{-4} M	$2.08x10^{-3}$	$2.10x10^{-3}$	123.04	98.41
Uric acid 10^{-4} M	$1.44x10^{-4}$	$1.18x10^{-4}$		121.89

) data from clinical laboratory

referring to the comparative testing of potentiometry and spectrophotometry in case of 7 sample solutions show accuracy of 79.00 % - 121.89 %. The range of accuracy required by the Association of Official Analytical Chemist (AOAC) for the concentration range of 10^{-6} M - 10^{-4} M is 80-110 % [15].

CONCLUSIONS

A carbon paste electrode modified by an imprinting zeolite is prepared. It has a high selectivity towards uric acid. The presence of ascorbic acid, creatine, or creatinine does not affect uric acid analysis by potentiometry using the electrode assembled. The recovery of the potentiometry method amounts to (113.18±14.79) % (n=3). The comparison of the method to the spectrophotometrical analysis usually applied shows an accuracy of (94.47±14.64) % (n=7). It is concluded that the method can be applied to determine uric acid in urine or a serum sample.

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