Carbon Paste Electrode Modified Imprinted Zeolite as a Selective Sensor for Creatine Analysis by Potentiometry

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2019

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The 12th Congress of Indonesian Soc. for Biochemistry and Molecular Biology in Conjunction With The 2nd Int. Conf. "Collaboration Seminar of Chemistry and Industry (CoSCI)" and AnMicro Workshop 11-12 October 2018, Universitas Airlangga, Indonesia

Accepted papers received: 21 November 2018
Published online: 09 January 2019

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Carbon Paste Electrode Modified Imprinted Zeolite as a Selective Sensor for Creatine Analysis by Potentiometry

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Abstract. Carbon paste electrode modified imprinted zeolite have been developed as a sensor to analyze creatine by potentiometry. Zeolite used in this study was TS-1 type zeolite that was synthesized with mole ratio of TEOS, TBOT, TPAOH, and H_2O of 1:0.017:0.24:21.2. Imprinted zeolite (IZ) was synthesized by mole ratio of creatine/Si of 0.0306. The developed electrode showed the optimum performance on pH 5, range of measurement of 10^{-9}–10^{-4} M, linearity of 0.973, and Nernst factor of 27.31 mV/decade. The upper and lower detection limits were $5.5x10^{-5}$ M and $1.3x10^{-8}$ M, respectively. The electrode showed a good precision which is expressed by coefficient of variation of 1.13% - 1.43%. The response time of the electrode in 10^{-9}–10^{-4} M creatine was 82-199 seconds, while its life time was 7 weeks (83 times used). Urea, glucose, and uric acid in various concentrations did not interfere on creatine analysis by potentiometry using the modified electrode. Application of the modified electrode in two serum samples with spiking technique resulted in recovery of 76.8% and 81.8%. Comparative testing with the standard clinical laboratory method showed accuracy of 73%.

Keyword: creatine, imprinted zeolite, selective sensor, potentiometry

1. Introduction

Creatine is compound that produce energy in the body formed in adenosine triphosphate (ATP). About 95% of amount of creatine has been found in muscle and the rest find in brain and heart [1; 2]. Normal concentration of creatine in the blood is 10^{-5} mg/L. Excessive of creatine concentration in body can cause hypertension because creatine can increase number of blood vessel in body, so the presence of creatine in body must be control early. The most common method to determine creatine concentration in the blood is using UV-Vis spectrophotometer [3]. Another method that has been developed for creatine analysis are high performance liquid chromatography (HPLC) [4-5], liquid chromatography tandem mass spectrometry (LC/MS) [6], and gas chromatography tandem mass spectrometry (GC-MS) [7- 8]. Determination of creatine concentration using this methods require a large volume of blood sample, relatively long time analysis, complex sample preparation, and the high cost of operating instruments. Biosensor enzym based has been developed for determination of creatine and creatinine with fluoresence assay [9]. This method has narrow linear dynamic range (10^{-4}-10^{-3} M) and higher limit of detection ($1.5x10^{-5}$ M) than spectrophotometric method. Electrometry method have been developed for creatine detection, especially potentiometry, amperometry [10] and stripping voltammetry [11], Potential current technique (I-V) using modified glassy carbon electrodes of CdO nano.particles (GC-CdO NPs) has been developed to determine creatine. This method can detect creatine concentration up to 10^{-10} M, but it has low reproducibility (RSD > 15%) [12].

In previous research, imprinting zeolite (IZ) modified carbon paste electrode was developed for determination of concentration creatine by potentiometry [13]. Zeolite that is used was LTA zeolite. The research resulted linear dynamic range of 10^{-3}-10^{-6} M and detection limit of 3.41×10^{-6} M.

In this research, we studied development of carbon paste electrode modified IZ for determination creatine in blood serum by potentiometry. Zeolite that is used in this research is TS-1 zeolite. IZ synthesized from tetraethyl orthosilicate (TEOS), tetrapropylammonium hydroxide (TPAOH), tetrabutyl orthotitanate (TBOT), and creatine as a template. The parameters studied in this study are

electrode response time and life time, linear dynamic range (measurement range), Nernst factor, limit of detection, accuracy and selectivity of the electrode.

2. Experimental Method

2.1 Materials and equipment

Creatine anhydrous (Fluka, 98%), tetraethyl orthosilicate (TEOS, Merck, 99%), tetrabutyl orthotitanate (TBOT, Merck, 98%), tetrapropylammonium hydroxide (TPAOH, Merck, 40%), sodium hydroxide (NaOH, Merck, 99%), glacial acetic acid (Merck, 100%), sodium acetic trihydrate (Merck), sodium dihydrogen phosphate dihydrate (Merck, 99%), sodium hydrogen phosphate dihydrate (Merck, 99%), 2-propanol (Merck, 99%), urea (Merck, 98%), glucose (Sigma Aldrich, 99%), uric acid (Sigma Aldrich, 99%), silver wires with 100% of purity, solid paraffin, carbon powder, and distilled water. All chemicals used have a purity degree of pro analysis. The potentiometric was performed on Cyberscan 510 using Ag/AgCl as reference electrode.

2.2 Preparation of imprinted zeolite (IZ)

Zeolite was synthesized by mixing TEOS, TBOT, and 2-propanol. The mixture was stirred for 30 minutes. TPAOH was then added into mixture and stirred for 15 min, and added distilled water to the mixture until the mole ratio of TEOS, TiO_2, TPAOH, and H_2O is 1: 0.017: 0.24: 21.2 [14]. The mixture was annealed at 80 ^oC for 4 x 24 h. Non imprinting zeolite (NIZ) was synthesized by adding creatine to the mixture to produce molar ratio of creatine/ Si of 0.0306. The mixture was aged for 3 h to trap the creatine into zeolite pores. Creatine was then extracted from the NIZ using hot water (80 ^oC) using a centrifugation with a speed of 6000 rpm for 10 minutes. The extraction is carried out repeatedly until the pH is neutral. The residue was dried at 80 ^oC to produce a powder called imprinted zeolite.

2.3 Preparation of carbon paste electrode-IZ

Electrode are made by filling three-quarters of the micropipette tube with melted paraffin in which Ag wire has been put into the tube. An Ag wire serves as connector between carbon paste-IZ electrode and potentiometer. The remaining portion in a micropipette tube was filled with a mixture consisting of solid paraffin, carbon, and IZ with variations of the composition (E1-E6) as shown in **Table 1**.

Table 1. The composition of activated carbon, imprinting zeolite and solid paraffin in the preparation of carbon paste electrode-imprinted zeolite

Electrode	Composition (%weight)		
	Activated carbon	Imprinted zeolite	Solid Parafifn
E1	60	0	40
E2	0	60	40
E3	55	5	40
E4	50	10	40
E5	45	15	40
E6	40	20	40

The mixture had been previously heated to form a paste, then the paste was inserted into the remaining portion of the micropipette tube with emphasis to be solid and full filled. An electrode surface is rubbed with HVS paper to make it flat and smooth.

To discover the optimum performance of the electrode, the measurement potential of creatine solution with a concentration of 10^{-11}-10^{-2} M. The pH was varied of 4,5,6,7, and 8 by adding buffer solution. The optimum composition of electrode was determined based on Nernst factor and linear dynamic range (measurement range).

2.4 Determination of method validity

Concentration of cretaine solution 10^{-11}-10^{-2} M with pH optimum were measured for their potential using optimum composition of carbon paste electrode-IZ, with each concentration measured three times in replication. From the data potential, we made curves showing the relationship between

potential and log concentration (log C) of creatine solution. The curve is a straight line (linear), which is the standard curve of a creatine solution. The data of standard curve was used to test of method validity that include linear dynamic range, linearity, Nernst factor, accuracy, presision, and limit of detection.

Sensor selectivity in this study was determined by value the coefficient of selectivity (K_{ij}) in the creatine solution as the main component (i) and in the urea, glucose and uric acid solution as compounds that are suspected to interfere (j) on creatine analysis. Determination of Kij was carried out using matched potential method (MPM) Eq (1) [15].

$$K_{i,j}^{pot} = \frac{\Delta ai}{aj} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Recovery (R) was determined by measuring the potential of standard creatine solution 10^{-5} M (C3), serum sample (C2), and serum sample that was spiked by standard creatine 10^{-5} M (C1). The obtained electrode potential was the substituted into equation of linear regression creatine solution. The recovery value was calculated by Eq (2)

$$R = \frac{C1-C2}{C3} \times 100\% \ldots\ldots\ldots\ldots\ldots (2)$$

2.5 Response time and life time of sensor

The response time of the carbon paste electrode-IZ against creatine is obtained by measuring of standard solution of of creatine. Electrode response time is the time needed by the electrode to recognize creatine to provide a constant potential value [16]. Life time of the electrode is the range of time in which the electrode shows good performance (linear dynamic range and Nernst factor) until significant deviations occur in the performance.

3. Result and Discussion

Optimization of electrode composition and pH of solution

The working electrode is the most important equipment in the potentiometric measurement. The electrode can be modified to improve its performance. In this research, working electrode was composed of activated carbon powder, solid paraffin, and IZ that used to increase the selectivity of electrode. The first analytic, parameter optimization is done to maximize the electrode performance. In this reasearch two kinds of optimization were carried out, that are electrode composition material and pH optimization of creatine solution.

Optimization of electrode composition is done to obtain electrode that are able to work optimally. Composition selection of optimum electrode is based on good value of Nernst factor, linearity value of calibration curve and wide of linear dynamic range. According to[17], potentiometric method fulfills of Nernst equation if Nernst factor is valued (59.2/n ± 2) mV, which n is molecule valence. Creatine is divalent molecule so that Nernst factor value should be 29.6 mV/decade [18]. Based on **Table 2**, it can be seen that electrode that results the closest theoritical Nernst factor is E3 with linear dynamic range of 10^{-9}-10^{-4} M and optimum pH 5.

Tabel 2. Nernst factor value for measuring creatine solution in various electrode composition and pH solution

pH	Nernst Factor (mV/decade)					
	E1	E2	E3	E4	E5	E6
4	2.7	15.6	7.0	11.0	2.6	1.6
5	7.9	11.5	27.3	11.3	2.4	2.4
6	22.9	18.6	23.4	18.8	21.1	17.3
7	19.1	16.6	15.6	12.5	19.2	14.0
8	12.5	13.8	18.8	10.4	16.5	9.6

Optimization of pH to determine range of pH that resulting stable potential value. Measurement of creatine solution with pH range of 4-8 using E3. Based on **Table 2**, it can be seen that standard solution with pH 5 results the best of Nernst factor value, that is 27.3 mV/decade.

The results of pH optimization show that change in pH affects to the electrode potentital generated. At pH 5, the resulting potential is relatively constant and in the pH 5 creatine as a molecule species

(pKa creatine = 3.8). Furthermore pH 5 is used as working pH for creatine analysis using carbon paste electrode-IZ by potentiometry.
As a comparison, carbon paste electrode modified TS-1 zeolite (E-Z) and non imprinted zeolite (E-NIZ) were made to study effect of IZ on electrode performance. The electrodes were made by variation in mass ratio of carbon, Z or NIZ, and solid paraffin equal with E3. The Nernst factor, linear dynamic range, and linearity of each electrode obtained can be seen in **Table 3.**

Tabel 3. Nernst factor, linear dynamic range, and coefficient of correlation on measurement standard solution of creatine using carbon paste electrode modified zeolite, NIZ, and IZ

Electrode	Nernst Factor (mV/decade)	Linear dynamic range (M)	Coefficient of correlation (r)
E3	27.3	$10^{-9} - 10^{-4}$	0.9731
E-NIZ	20.7	$10^{-9} - 10^{-4}$	0.9967
E-Z	23.6	$10^{-9} - 10^{-4}$	0.9928

Table 3 shows that E3 performance is better than E-Z and E-NIZ. This is caused by the presence of creatine mold in IZ. The resulted of imprinting technique was material pore that adjust with analyte size, so analyte can be trapped in material pore. This causes the analyte to be easily measured [19].

Performance of carbon paste -IZ electrode and method validity
Sensor performance of carbon paste-IZ was expressed with response time and life time of sensor. A sensor is more sensitive when the time needed to response the analyte is shorter [16]. In this research, determination of response time used concentration in the linear dynamic range, that is 10^{-9}-10^{-4} M and resulting the time response of 82-199 s. Standard curve of linear dynamic range of creatine is showed in **Figure 1**.

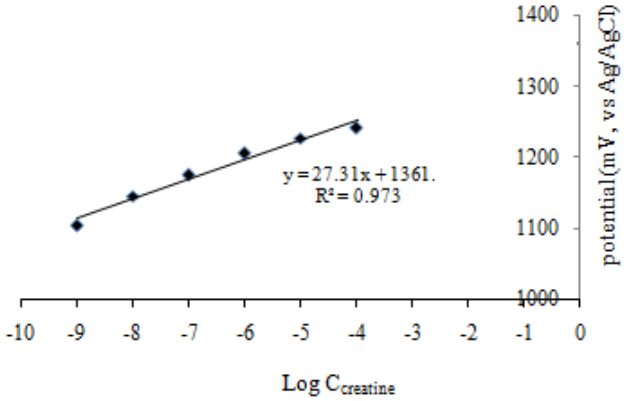

Figure 1. Calibration curve of creatine

Figure 1 shows that Nernst factor value is 27.3 mV/decade, in the measurement range of 10^{-9}-10^{-4} M. The modified electrode showed precision which is expressed by coefficient of variation of 1.13% - 1.43%. Creatine is divalent molecule (**Figure 2**), therefore it should has Nernst factor value of (29,6 ± 2) mV/decade.

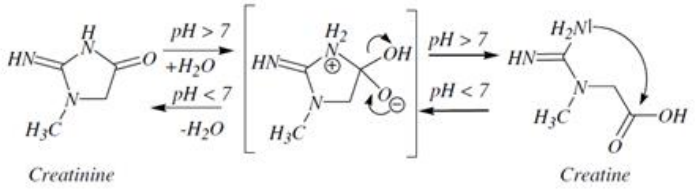

Figure 2. Prediction of of creatine reduction reaction [8]

Selectivity of the electrode toward creatine in urea, glucose, and uric acid matrices is displayed in **Table 4**. It can be seen that the carbon paste -IZ electrode exhibits higher selectivity toward creatine in the urea, glucose, and uric acid matrices compared to the bare carbon paste electrode, which is indicated by it more less K_{ij}.

Table 4. Comparison of K_{ij} value of carbon paste -IZ electrode and bare carbon paste electrode in urea, glucose, and uric acid solution

Interferent	Concentration (M)	K_{ij}	
		Carbon paste-IZ	Bare carbon paste
Urea	10^{-6}	5.47×10^{-5}	0.0648
	10^{-5}	3.05×10^{-6}	0.9755
	10^{-4}	3.72×10^{-7}	0.3589
	10^{-3}	4.32×10^{-8}	1.0898
	10^{-2}	2.60×10^{-9}	0.8014
Glucose	10^{-6}	6.80×10^{-5}	0.3108
	10^{-5}	7.17×10^{-6}	0.5948
	10^{-4}	5.97×10^{-7}	0.9187
	10^{-3}	5.01×10^{-8}	0.3670
	10^{-2}	4.21×10^{-9}	0.3514
Uric acid	10^{-6}	3.56×10^{-7}	0.6011
	10^{-5}	1.04×10^{-7}	0.9755
	10^{-4}	1.60×10^{-7}	1.1573
	10^{-3}	6.40×10^{-8}	1.0898

Precision value (coefficient of variation) obtained is better than the rule made by Association of Official Analytical Chemist (AOAC) which is 7.3–30% for 10^{-9} to 10^{-4} M [20]. The upper limit of detection of creatine by potentiometry using carbon paste electrode-IZ is 5.5×10^{-5} M. **Table 5** shows recovery value of potentiometry method to analyze creatine in two blood serum samples with standard addition technique of 76.8 and 81.8%. This value is lower than the set limit by AOAC for concentration of the order 10^{-5} M (80-110%). Comparison test against analysis result by spectrophotometry in clinical laboratory shows accuracy of 71.3 and 74.6%. This value is lower than set limit by AOAC (80-110%).

Tabel 5. Data of accuracy and recovery value of creatine analysis in serum sample

Sample	Concentration of creatine by (M)		Accuracy	*Recovery*
	Potentiometry	Spectrophotometry*)	(%)	(%)
Creatine 10^{-5} M	1.03×10^{-5}	-	-	-
Blood serum 1	4.91×10^{-5}	6.90×10^{-5}	71.3	-
Blood serum 1 + creatine 10^{-5} M	4.02×10^{-5}	-	-	76.8
Blood serum 2	5.70×10^{-5}	5.40×10^{-5}	74.6	-
Blood serum 2 + creatine 10^{-5} M	5.04×10^{-5}	-	-	81.8

*) *data obtained from local clinical laboratory*

This developed method shows lower limit of detection namely 1.3×10^{-8} M. It is possible to analyze creatine in sample with much smaller volume compare creatine analysis by spectrophotometry. The complete of electrode performance is summarized in **Table 6**.

Table 6. Performance of the carbon paste-IZ electrode to analyze creatine by potentiometry

No	Parameter	Value
1	Linear dynamic range (M)	$10^{-9} – 10^{-4}$
2	Correlation coefficient	0.9731
3	Nernst factor (mV/decade)	27.31
4	Response time(s)	82 – 199
5	Lower and upper detection limit (M)	1.3×10^{-8} and 5.5×10^{-5}
6	Coefficient of variation (%)	1.13 – 1.43
7	Accuracy toward spectrophotometry (%)	71.3 and 74.6
8	Recovery (standard addition technique) (%)	76.8 and 81.8
9	Lifetime(s)	83 times of usage (7 weeks)
10	Selectivity	Not be interfered by urea, uric acid, and glucose (Kij<1)

4. Conclusion

Imprinted zeolite made from TS-1 zeolite has increased performance of carbon paste electrode to analyze creatine by potentiometry. Carbon paste-IZ electrode showed optimum performance in a creatine solution pH 5. Response time of the electrodes was less than 4 minutes and exhibits high selectivity toward creatine than urea, glucose, and uric acid. The electrodes showed a long life time and limit of detection around 1000 times lower than normal concentrations of creatine in blood serum. Therefore, the proposed electrode can be recomended for creatine detection in serum samples by potentiometry.

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Acknowledgment
The authors thank to Chemistry Department, Faculty of Science and Technology, Universitas Airlangga for laboratory facilities provided.

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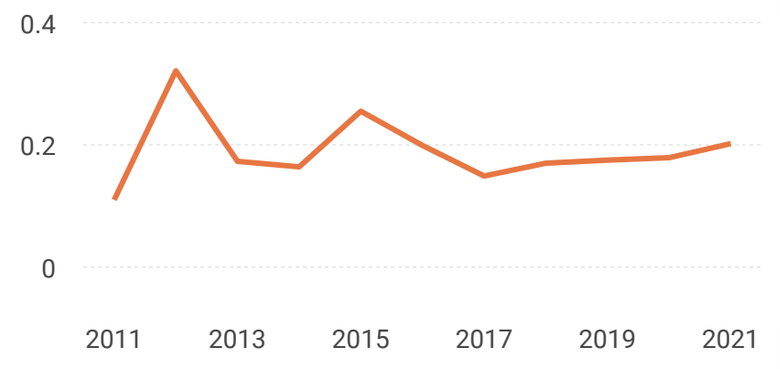

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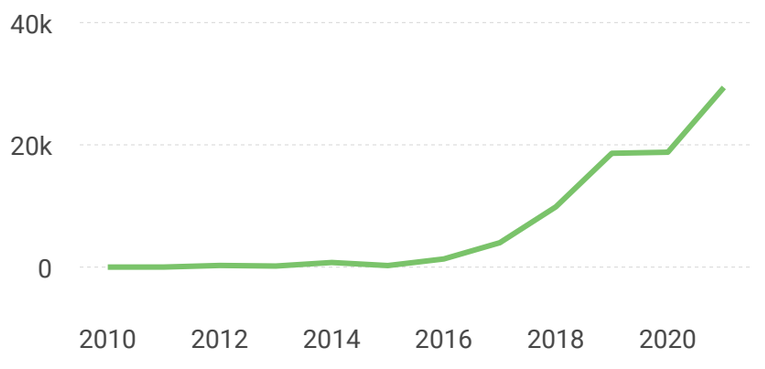

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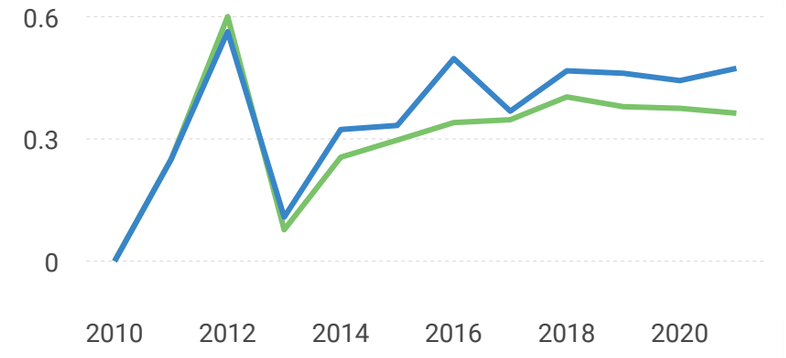

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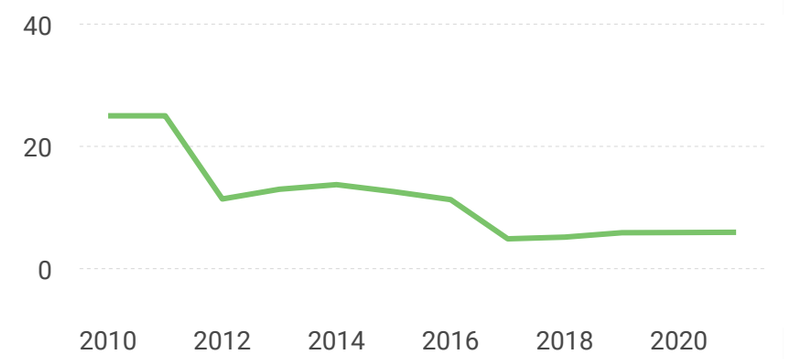

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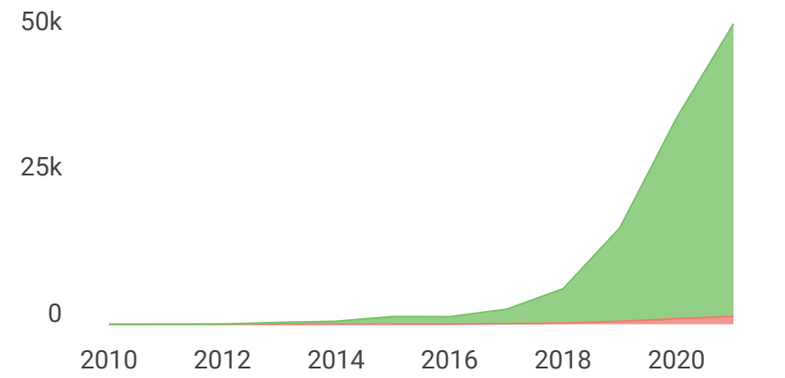

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