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

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Abstract: Imprinted zeolite modified carbon paste (carbon paste-IZ) electrode had been developed as a sensor to analyze blood glucose content by potentiometry. The used zeolite was Lynde Type A (LTA) that synthesized with a mole ratio of Na_2O , Al_2O_3 , SiO_2 and H_2O of 4:1:1.8:270, respectively while non-imprinted zeolite was prepared with a mole ratio of glucose/Si of 0.0306. Glucose was then extracted from the zeolite framework using hot water (80 °C) to produce imprinted zeolite (IZ). The carbon paste-IZ electrode prepared from activated carbon, paraffin pastilles, and IZ with a mass ratio of 5:4:1 showed the best performance. The modified electrode demonstrated the measurement range of 10^{-4} - 10^{-2} M, the Nernst factor of 29.55 mV/decade, the response time less than 120 s, and the detection limit of 5.62×10^{-5} M. Ascorbic acid, uric acid, urea and creatinine did not interfere on the glucose analysis by potentiometry. Comparison test with spectrophotometry showed an accuracy of $(90.7 \pm 1.4)\%$ ($n = 5$), while the application of the electrode to analyze five spiked serum samples showed recovery of $(92.2 \pm 1.3)\%$ ($n = 5$). The electrode was stable for up to 9 weeks (168 times usage). Based on its performance, the developed electrode can be applied to analyze glucose in human serum sample and recommended for used in the medical field.

Keywords: blood glucose; carbon paste electrode; imprinted zeolite; potentiometric sensor

■ INTRODUCTION

Diabetes mellitus has become a serious threat to human health because it can cause kidney failure, heart disease, and stroke. An increase in high blood glucose levels (> 200 mg/dL) is a major cause of diabetes mellitus [1]. In the body, normal blood glucose values are 60–100 mg/dL (3.3×10^{-3} – 5.5×10^{-3} M), while serum glucose is 70–110 mg/dL (3.88×10^{-3} – 6.1×10^{-3} M) [2]. Based on the effects that can be caused by high blood glucose levels, controlling the glucose level in the blood is necessary. The general method for determining glucose levels is spectrophotometry using chemical or enzymatic, such as hexokinase or glucose 6-phosphate dehydrogenase [3]. A few studies have reported about the use of some methods for glucose determination including GOx (glucose oxidase) based colorimetric methods [4-5], high performance liquid chromatography (HPLC) [6], liquid chromatography-

mass spectrometry (LC-MS) [7], and potentiometry using modified carbon-based electrodes [8]. Modified carbon-based electrodes have previously been developed including zeolite-modified electrodes for the analysis of uric acid [9], Cr^{3+} ions [10], creatinine [11], creatine [12]; and polymer-based electrodes for phenol analysis [13]. Nanoparticle material has also been shown to increase the selectivity and sensitivity of electrodes to analytes [14].

In this study, an imprinted zeolite modified carbon paste electrode has been developed for potentiometric glucose detection. The zeolite used was LTA zeolite that synthesized from the basic materials of SiO_2 , NaAlO_2 , and water with a mole ratio of Na_2O , Al_2O_3 , SiO_2 , H_2O of 4:1:1.8:270 [15]. Glucose solution was added to the mixture of these materials to produce non imprinted zeolites (NIZ). Next, the glucose molecule was extracted, so that it leaves a mold on the zeolite framework called

imprinted zeolite (IZ). The imprinted is expected only match the size and shape to glucose molecules so that they can selectively detect glucose molecules when it is used for sample analysis.

Electrode performance was studied from the measurement range value, Nernst factor, detection limit, response time and the life time of the electrode. The use of electrodes was studied through the accuracy value compared with the spectrophotometry method as a standard method for determining blood glucose levels in the medical field. Electrode selectivity was investigated through the effect of another component in serum samples including ascorbic acid, uric acid, creatinine, and urea on the glucose analysis using the electrode.

■ EXPERIMENTAL SECTION

Materials

Chemicals used in this study were glucose (Sigma Aldrich, 99.5%), SiO₂ (Sigma Aldrich, Ludox 40%), sodium aluminate (Sigma Aldrich, 50%), sodium dihydrogen phosphate (Merck, 99%), disodium hydrogen phosphate (Merck, 99.5%), uric acid (Fluka, 98%), urea, creatinine, H₃PO₄, AgNO₃, Ag wire, *n*-hexane, paraffin pastilles and activated carbon with pore size of 3.835 nm. The sample used was blood serum from a clinical laboratory in Surabaya.

Instrumentation

The equipment used in this study was a Cyberscan 510 potentiometers with Ag/AgCl as a reference electrode, Fourier transform infrared (FTIR) spectrophotometer (Shimadzu IR Prestige 21), X-ray diffraction (PANalytical Expert Pro), Spectrophotometer (UV 1800 Pharmaspec), hotplate (Termolyne S46410-2), pH meter (Cyberscan Eutech pH 510), centrifuge (HITTECH EBA) 20, vacuum oven (NAPCO Model 5851), magnetic stirrer, polypropylene bottle, agate mortar, 1000 µL micropipette tip and glassware.

Procedure

Synthesis of zeolite, non-imprinted zeolite (NIZ), and imprinted zeolite (IZ)

The LTA zeolite was synthesized by mixing Na₂O, Al₂O₃, SiO₂, and H₂O with a mole ratio of 4:1:1.8:270 [15].

The mixture was stirred for 3 h. A one third part was heated in an oven at 100 °C for 45 h. Next, it is washed using distilled water with the help of a centrifuge. The remaining 2/3 parts of the mixture were added with glucose to produce a glucose/Si mole ratio of 0.0306. The obtained mixture was allowed to stand for 3 h so that glucose can be binded and trapped in the zeolite framework. A half part of the mixture was dried at 80 °C and the other part was added with hot water to extract glucose and form imprinted zeolite.

Fabrication of carbon paste-imprinted zeolite and pH optimization

The carbon paste electrodes were prepared by inserting Ag wires in a 1 mL micropipette tube. Three quarter part of the micropipette tip was filled with melted paraffin. Then a paste made from a mixture of activated carbon, solid paraffin, and IZ with various compositions (Table 1) was filled into the ¼ part remaining of the micropipette tube. The surface of the electrodes was rubbed on the HVS paper and immersed in a 10⁻² M glucose solution overnight for conditioning.

Furthermore, each electrode was used to measure the glucose standard solution 10⁻⁸–10⁻¹ M pH 6 (without pH adjustment). Then a graph between the concentration and electrode potential was created and determined the value of the Nernst factor and linear measurement range. The composition of carbon paste-IZ electrodes that produce optimum performance is used as the basis for preparing zeolite-modified carbon paste electrodes (EZ) and NIZ-modified carbon paste electrodes (ENIZ). The performance of EZ and ENIZ were compared with carbon paste-IZ to observe the effect of glucose mold on the electrode performance. The effect of pH solution was

Table 1. Composition of activated carbon, paraffin, and IZ on electrode fabrication

Electrode	% weight		
	Activated carbon	paraffin	IZ
E1	60	40	0
E2	55	40	5
E3	50	40	10
E4	45	40	15
E5	40	40	20

studied through the Nernst factor value and linear measurement range resulting from the measurement of glucose solution with varies pH.

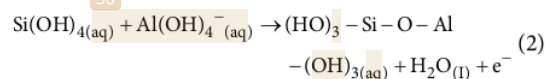
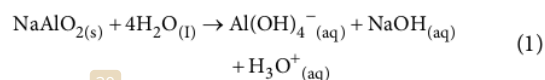
Performance of the electrode and method validity

Parameters for expressing electrode performance and method validity in this study include linear measurement range, Nernst factor, detection limit, precision, accuracy, response time, life time and selectivity. The selectivity of the electrode was stated by the selectivity coefficient (K_{ij}) value determined by the matched potential method (MPM) [16]. The accuracy was determined through a comparison test with the results of blood glucose analysis using the spectrophotometric method as a commonly used method in the medical field.

RESULTS AND DISCUSSION

Synthesis and Characterization of Zeolite, NIZ, and IZ

The LTA zeolite is prepared through a sol-gel method using silica sol as the silica source and sodium aluminate as the aluminium source. The precursor solution is combined under vigorous stirring. The mixture is then sealed in polypropylene bottles for the hydrothermal process. The reactions that occur in zeolite synthesis are as shown in Eq. (1) and (2).



SiO_2 was added to $\text{NaAl}(\text{OH})_4$ solution dropwise and stirred for 1 h until it became a white gel. This process is an aging step which is the process of forming a zeolite crystal core [18]. The aging process aims the breaking of silica monomers into a stronger silica gel with the expected pore [19].

Furthermore, the mixture was heated hydrothermally at 100°C for 45 h in a polypropylene bottle to accelerate the growth of zeolite crystals [20]. From this process, a white suspension is produced. A total of 1/3 parts of the suspension are centrifuged at 4000 rpm for 5 min. Then the precipitate was separated and washed with distilled water to remove the remaining NaOH and dried it in an oven at 80°C for 24 h.

Non imprinted zeolite (NIZ) is zeolite which contains templates/mold in the pores. Non imprinted zeolite was prepared by taking as much as 2/3 parts of a mixture of SiO_2 , NaAlO_2 , and H_2O (after the hydrothermal process). Then the mixture was added with glucose solution dropwise and stirred with a magnetic stirrer for

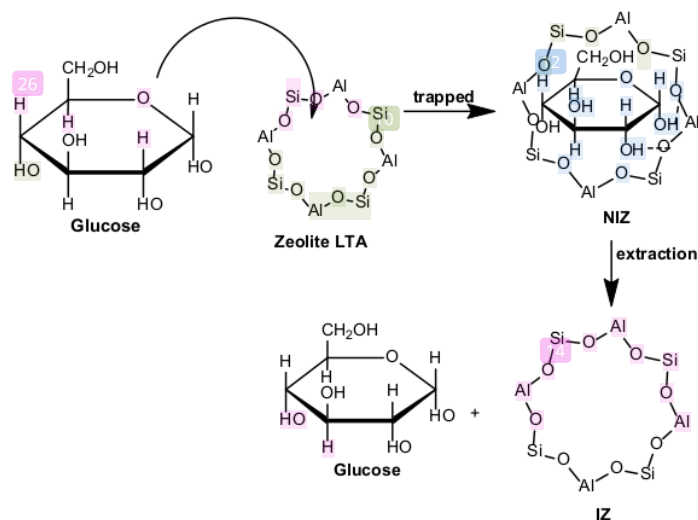


Fig 1. An illustration of trapping glucose molecules into a zeolite framework

30 min. An illustration of trapping glucose molecules into a zeolite framework can be seen in Fig. 1. The imprinted zeolite (IZ) was obtained by extracting glucose molecules from the NIZ framework using hot water. Benedict tests were performed to ensure that all glucose has been extracted from the NIZ framework (Fig. 2).

Characterization of zeolites by XRD was carried out to determine the formation of synthesized LTA zeolites. Fig. 3 is an X-ray diffraction pattern of LTA zeolites.

Based on the XRD pattern of Fig. 3, there is a peak with high intensity at position 2θ i.e. 7.23; 10.16; 12.46; 16.09; 21.67; 23.97; 27.11; 29.93; and 34.17°. At position 2θ 12.46°, it shows the orientation of the cubic structure of the LTA zeolite [22]. Furthermore, these peaks were compared with the LTA zeolite diffraction pattern in the Collection of Simulated XRD Powder Patterns for Zeolites International Zeolite Association (IZA) data base [21] and American Society for Testing and Materials (ASTM) standard no. 39-222 [23]. There is a similarity between the peaks of the synthesized zeolite with the zeolite diffractogram in the data base. (Table 2) It can be concluded that synthesized LTA zeolite has formed. The peak at 2θ 13.79° which is a typical peak of silicon dioxide (SiO_2), indicates that there is remaining unreacted SiO_2 to form zeolites.

Characterization with FTIR spectrophotometers produced the spectrum in Fig. 4. The wave number 1640 cm^{-1} shows the peak of bending vibrations -OH

from Si-OH, Al-OH, and H_2O and the peak at wave number 990 cm^{-1} is the asymmetry stretching vibrations of Al-O and Si-O [24]. The peak at wave number 561 cm^{-1}

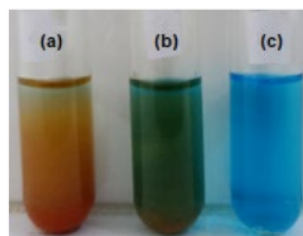


Fig 2. Benedict test on (a) glucose standard solution, (b) leached filtrate of NIZ, a (c) leached filtrate of IZ

Table 2. The data peak position of synthesized LTA zeolite and data base from IZA and ASTM

2 θ (°)		
ASTM	Synthesized zeolite	IZA
7.18	7.23	7.18
10.16	10.16	10.17
12.45	12.46	12.46
-	13.79	-
16.09	16.09	16.11
21.65	21.67	21.67
23.97	23.97	23.99
27.09	27.11	27.11
29.92	29.93	29.94
34.16	34.17	34.18

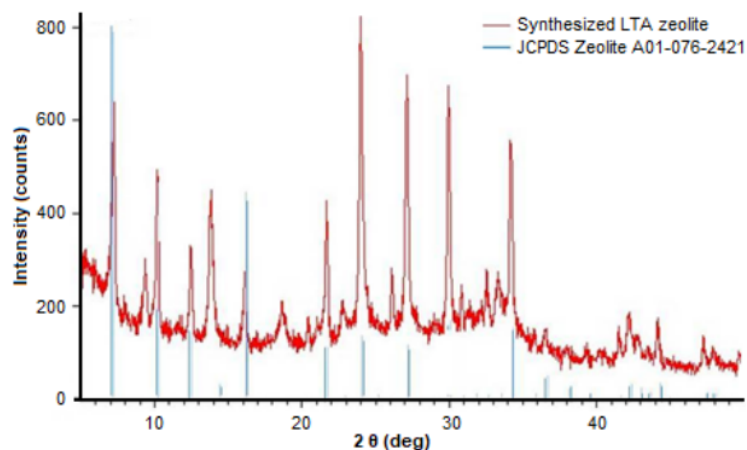


Fig 3. XRD-pattern of synthesized LTA zeolite and LTA zeolite on IZA [21]

indicates the crystallization of zeolites with a double ring [25] and the peak at wave number 440 cm^{-1} is a tetrahedral internal vibration of Si-O and Al-O [24]. The wavenumber at 3460 cm^{-1} in non imprinted zeolite spectrum is broader than the peak at zeolite or imprinted zeolite. This is due to the presence of stretching -OH from C-OH in glucose [26].

Optimization of Electrode Composition and pH Solution

The carbon paste-IZ electrodes are prepared from a mixture of activated carbon, paraffin and IZ. The presence of imprinted zeolite is expected to increase the electrode selectivity to glucose because IZ has a selective mold for glucose. The composition of activated carbon and IZ in the manufacture of electrodes may affect the performance of the electrodes, therefore the preparation of electrodes with variations in the composition of carbon and IZ were conducted. The resulted measurement range and Nernst factor from the measuring data of glucose solution using the carbon paste-IZ electrode with varies composition are shown in Table 3.

The electrode that exhibits a Nernstian graph on the electrode potential versus $\log [\text{glucose}]$ plot is the E3 (Table 3). The E3 electrodes also show good linearity in the measurement range of 10^{-4} - 10^{-2} M. The carbon paste modified zeolite (EZ) and NIZ (ENIZ) were prepared by composition of activated carbon, paraffin and zeolite or NIZ similar to the composition of the E3 electrode to determine the effect of glucose mold on the electrode performance. NIZ-modified electrodes produce curves close to Nernstian, while zeolite-modified electrodes

show the shape of sub-Nernstian curves. It is suspected that the presence of glucose molecules in NIZ enhances the electrodes to recognize free glucose molecules in solution. Thus, ENIZ functions like an ion selective electrode in recognizing glucose molecule.

The IZ modified electrode with the best performance was then used to measure the glucose solutions 10^{-8} - 10^{-1} M at pH 4, 5, 6, 7, and 8. Measurement data was used to create a graph of $\log [\text{glucose}]$ versus

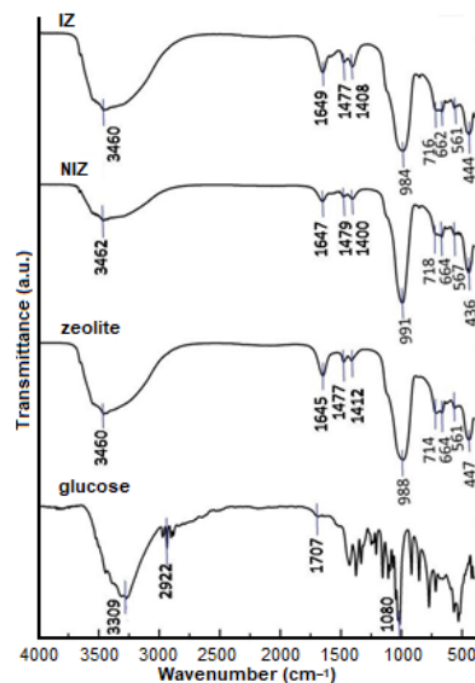


Fig 4. FTIR spectra of glucose, zeolite LTA, NIZ, and IZ

Table 3. Value of the electrode measurement range, Nernst factor and linearity of calibration graph in the glucose measurement (without pH adjustment)

Electrode	Nernst factor (mV/decade)	Linear measurement range (M)	Linearity (R^2)
E1	27.79	10^{-4} - 10^{-2}	0.9549
E2	27.71	10^{-4} - 10^{-2}	0.9512
E3	29.55	10^{-4} - 10^{-2}	0.9924
E4	24.55	10^{-4} - 10^{-2}	0.9434
E5	16.16	10^{-4} - 10^{-2}	0.9604
EZ*	13.50	10^{-4} - 10^{-2}	0.9720
ENIZ*	27.35	10^{-4} - 10^{-2}	0.9147

*) similar composition with E3

electrode potential (Fig. 5) and determined the measurement range, linearity and the Nernst factor (Table 4).

Fig. 5 explained that the best performance of E3 is shown in the measurement of glucose solution without adjusting pH (pH 6). From these measurements obtained linear and Nernstian curve with the equation of $y = 29.55x + 175.52$ in the concentration range of 10^{-4} – 10^{-2} M and non-linear curve with the equation of $y = -0.1x^2 - 3.18x + 38.43$. Based on the intersection of both lines, the detection limit value obtained was 5.62×10^{-5} M. The value is lower than the detection limit of the conductive polymer-based sensors developed previously [8]. Table 5 displayed the comparison data of the limit of detection (LOD) of our work with the previously reports.

With this low detection limit, carbon paste-IZ electrodes can be used for glucose analysis with concentrations up to 100 times lower than normal concentrations in the blood [2]. The linear curve describes

the linear measurement range of the potentiometric method using the developed electrodes, which is 10^{-4} – 10^{-2} M with a Nernst factor of 29.55 mV/decade. It shows that glucose has two valency molecules. This result is in agreement with the research report of [31] which fabricated the potentiometric glucose sensor based on glucose oxidase immobilized iron ferrite magnetic particle/chitosan composite modified gold coated glass electrode. They resulted in the Nernst factor of glucose about 27.3 mV/decade. A similar result also reported by [32] which stated glucose is a divalent molecule in the analysis of glucose using the voltammetry method. The valency of the molecule is constant in both analyses using potentiometry and voltammetry. The comparison of potentiometry and voltammetry method in the Ca^{2+} sensor is used as an analogue approach, whereas both of the methods found a similar Nernst factor value [33-34].

Table 4. Data of electrode measurement range, Nernst factor, and linearity on varies pH of glucose solution

pH	Measurement range (M)	Nernst factor (mV/decade)	Linearity (R^2)
4	10^{-3} – 10^{-1}	1.40	0.8547
5	10^{-7} – 10^{-4}	2.49	0.9208
6	10^{-6} – 10^{-4}	1.25	0.9868
6*	10^{-4} – 10^{-2}	29.55	0.9924
7	10^{-4} – 10^{-1}	3.02	0.8341
8	10^{-6} – 10^{-4}	3.35	0.9964

*) pH of glucose solution without pH adjustment

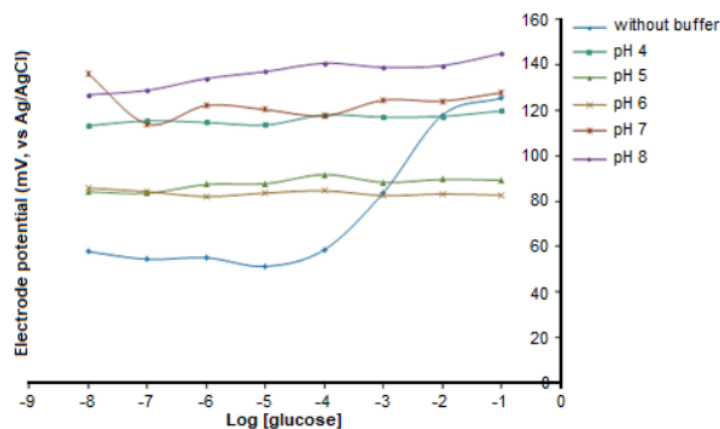


Fig 5. The plot of log [glucose] and electrode potential on varies pH

Table 5. The comparison data of the LOD of our work with the previously reports

Method	The material of the electrode	Linear range	LOD	Reference
Amperometry	Carbon paste/GOx silica	5×10^{-4} – 9×10^{-3} M	1.5×10^{-4} M	[27]
Amperometry	Carbon paste/selenium nanoparticle-mesoporous silica composite (MCM-41)	1×10^{-5} – 2×10^{-3} M	1×10^{-4} M	[28]
Potentiometry	Poly (3-aminophenyl boronic acid-co-3-octylthiophene)	5×10^{-3} – 5×10^{-2} M	5×10^{-4} M	[8]
Potentiometry	Poly (terthiophene benzoic acid) (pTBA) layered-AuZn alloy oxide (AuZnOx)	1.6×10^{-3} – 2.7×10^{-2} M	9.6×10^{-4} M	[29]
Potentiometry	Carbon nanotube on gold printed	10^{-3} – 10^{-1} M	1×10^{-4} M	[30]
Potentiometry	Carbon paste/IZ	10^{-4} – 10^{-2} M	5.6×10^{-5} M	This work

The coefficient of variation (CV) in the linear range is 0.42–2.20%. This value is within the limit of the CV value required by the Association of Analytical Chemist (AOAC) which is 5.3% for concentrations less than 10^{-2} M [35].

The Selectivity of the Electrode

Electrode selectivity to glucose molecules is expressed by the value of the selectivity coefficient (K_{ij}). This value is calculated using the matched potential method (MPM) [16]. Determination of K_{ij} is begun by measuring separately the glucose, ascorbic acid, uric acid, creatinine and urea solution with a concentration range of 10^{-4} – 10^{-2} M using bare carbon paste and carbon paste-IZ electrode, then the selectivity coefficient value of each electrode is determined. The K_{ij} values of bare carbon paste and carbon paste-IZ electrode are shown in Table 6.

Data on Table 6 illustrates that the presence of ascorbic acid, uric acid, creatinine or urea did not interfere with glucose potentiometric analysis using imprinted zeolite modified carbon paste electrodes, while the bare carbon paste electrodes are disturbed by these components. It explains that the modification of electrodes using imprinted zeolite can increase electrode selectivity, due to the presence of specific molds for glucose molecules in the zeolite. The selectivity of carbon paste-IZ electrode is as good as the zeolite-based electrodes developed previously [11-12,36].

Method Comparison Test and Recovery

The stability of electrode was studied using a comparison test of the results of the analysis with the potentiometric method using a carbon paste-IZ electrode with a spectrophotometric method which is

Table 6. Data of K_{ij} value of bare carbon paste electrode and carbon paste-IZ electrode

Solution	Concentration (M)	Selectivity coefficient (K_{ij})	
		Bare carbon paste	Carbon paste-IZ
Ascorbic acid	10^{-4}	1.66	3.24×10^{-3}
	10^{-3}	2.59	3.48×10^{-3}
	10^{-2}	3.67	3.79×10^{-3}
Uric acid	10^{-4}	1.98	2.27×10^{-3}
	10^{-3}	2.70	2.64×10^{-3}
	10^{-2}	4.98	3.71×10^{-3}
Creatinine	10^{-4}	2.21	1.62×10^{-2}
	10^{-3}	3.01	1.77×10^{-2}
	10^{-2}	4.76	1.94×10^{-2}
Urea	10^{-4}	2.21	5.21×10^{-3}
	10^{-3}	3.19	6.25×10^{-3}
	10^{-2}	4.68	9.37×10^{-3}

Table 7. Data of accuracy and recovery value on the determination of blood serum glucose

Solution	Glucose concentration (M)		Accuracy (%)	Recovery (%)
	Potentiometry	Spectrophotometry [*]		
Glucose 10 ⁻³ M	8.38 × 10 ⁻⁴	-	-	-
Serum sample 1	4.19 × 10 ⁻³	4.55 × 10 ⁻³	92.02	-
Serum sample 1 + Glucose 10 ⁻³ M	4.97 × 10 ⁻³	-	-	93.4
Serum sample 2	4.18 × 10 ⁻³	4.72 × 10 ⁻³	88.60	-
Serum sample 2 + Glucose 10 ⁻³ M	4.94 × 10 ⁻³	-	-	90.3
Serum sample 3	5.85 × 10 ⁻³	6.38 × 10 ⁻³	91.70	-
Serum sample 3 + Glucose 10 ⁻³ M	6.66 × 10 ⁻³	-	-	92.5
Serum sample 4	8.11 × 10 ⁻³	8.89 × 10 ⁻³	91.20	-
Serum sample 4 + Glucose 10 ⁻³ M	8.89 × 10 ⁻³	-	-	93.10
Serum sample 5	11.09 × 10 ⁻³	12.22 × 10 ⁻³	90.72	-
Serum sample 5 + Glucose 10 ⁻³ M	11.86 × 10 ⁻³	-	-	92.18

^{*}) data from clinical laboratory

standard method for determining blood glucose levels in the medical field. Sampling and handling of blood samples were carried out by a clinical laboratory in Surabaya. The recovery test is carried out to determine the influence of the matrix in the serum sample through standard addition techniques. Data on the accuracy and recovery resulted in blood glucose analysis are shown in Table 7.

Based on Table 7, the accuracy value obtained from the analysis of blood serum samples in this study is the range of accuracy received for a chemical analysis method according to AOAC, which is 80–110% [35]. This shows that the potentiometric method to analyze glucose in serum using the developed electrodes give in good agreement results from spectrophotometric method. Recovery value in this study illustrates that there are other components contained in serum samples that interfere with glucose analysis such as fructose, sucrose, maltose [37] and ions such as Na⁺, Cl⁻, K⁺, PO₄³⁻, SO₄²⁻, NH₄⁺ [38]. However, based on the obtained recovery value, the developed potentiometric method fulfill the requirements as a chemical analysis method. Thus, the developed method can be used as an alternative method for determining blood glucose levels in the medical field.

CONCLUSION

The carbon paste-IZ electrodes for potentiometric glucose analysis showed a measurement range of 10⁻⁴–10⁻² M, Nernst factor 29.55 mV/decade, and able to detect concentrations up to 100 times lower than normal

concentrations of glucose in the blood serum. The electrode exhibited fast response and high selectivity against glucose molecules in solutions containing ascorbic acid, uric acid, creatinine, or urea. Potentiometry using developed electrodes shows high accuracy toward spectrophotometry as a standard method for blood glucose analysis in the medical field. Based on its performance, the potentiometric method using carbon paste-IZ electrodes is recommended as an alternative method for the analysis of blood glucose levels in the medical field.

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