

Imprinted zeolite modified carbon paste electrode as a selective potentiometric sensor for blood glucose

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Preface: The 14th Joint Conference on Chemistry 2019

Thank you very much for this opportunity to hold the international conference of JCC 14 on 10-11 September 2019, by Chemistry Department, Faculty of Math and Natural Science, Universitas Sebelas Maret. This JCC conference is an annually program of the consortium of five chemistry departments in the region of Middle Java (Universitas Sebelas Maret, Universitas Diponegoro, Universitas Jenderal Sudirman, Universitas Negeri Semarang, Universitas Kristen Satya Wacana) and a guest member from Malaysia (Universitas Malaysia Sabah)

On the behalf of organizing committee of JCC 2019, I appreciate to all participants to meet in this scientific conference on chemistry 2019, 14th JCC. It was my pleasure to facilitate the ideas in development of chemistry and education chemistry within the region of middle Java also worldwide. I expect that our conference gives good impact to the chemist society not only in the region but in the world through the scientific ideas or publications outcoming from this conference. Besides, I hope that our collaboration can be intensively done by joint research between the members of this consortium in the near future.

I would like to thank to Scientific Committee for the publishing articles of the proceeding of the 14th JCC conference in AIP publishing.

Surakarta, 20 December 2019

Dr. rer.nat. Atmanto Heru Wibowo, S.Si., M.Si
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Imprinted Zeolite Modified Carbon Paste Electrode as A Selective Potentiometric Sensor for Blood Glucose

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Abstract. Imprinted zeolite modified carbon paste electrode as a potentiometric sensor for glucose analysis in blood serum sample has been developed. In this study, the type of used zeolite was TS-1. Imprinted zeolite (IZ) was synthesized with mole ratio of glucose/Si of 0.0306. The modified electrode prepared by mixing activated carbon, imprinted zeolite, and paraffin granule with mass ratio 9:4:7 from the measurement in range of 10^{-5} – 10^{-2} M is with Nernst factor of 28.6 mV/decade, and the detection limit of 4.79×10^{-5} M. The electrode was able to respond the glucose molecules in solution quickly (<30 s), stable for more than 9 weeks (130 times usage) and selective to glucose molecules. The urea, uric acid, and creatine with various concentrations did not interfere to glucose analysis by potentiometry using the electrode. Applying of the electrode for glucose analysis in spiked blood serum samples showed that it had recovery of $91.73 \pm 3.48\%$ (n=5), while its accuracy against spectrophotometry method as commonly used method in medical field was $90.58 \pm 4.65\%$ (n=5). Based on its performance, potentiometry using the imprinted zeolite modified carbon paste electrode is recommended as an alternative method for routine blood glucose analysis in the medical field.

INTRODUCTION

Carbon paste electrodes are the type of carbon electrode most widely used in electrochemical studies because of its inertness, low manufacturing costs, and suitability for various applications [1]. Carbon paste electrodes are easily renewed, porous, easy to miniaturized [2] and the response is fast [3]. Carbon paste electrodes can chemically modified to improve their performance in detecting samples at very small levels by mixing the modifier as one of the electrodes (bulk modified) or by coating the surface of the electrode with a thin film of the modifier (surface modified).

Among inorganic materials, zeolite has been widely used to modify carbon electrodes to form zeolite modified electrode (ZME) due to its unique porosity size, selectivity, high ion exchange ability, high thermal stability, adsorption capacity and resistance to extreme conditions [4]. The carbon-based electrodes including glassy carbon electrodes, graphite electrodes, diamond electrodes modified with boron-doped (BDE), screen printed electrodes, polymer [5] and zeolites modified electrode (ZME) [6, 7] have been widely developed.

Zeolites are crystalline aluminosilicates which have pores in molecular dimensions, unique micro pore structure, catalytic activity and good hydrothermal stability [8]. Titanium silicate-1 (TS-1) is a zeolite with MF-1 structure, usually used for selective oxidation reactions under mild conditions using hydrogen peroxide as an oxidant [9]. TS-1 type zeolites are superior types of zeolites due to nanoscale pores [10], excellent adsorption properties [11] and photocatalytic activity. The inherent activity of Ti atoms in TS-1 is higher than photocatalytic TiO_2 .

Modified electrodes as sensors for electrometric analysis of organic or inorganic compound have been developed including zeolite modified electrodes for the analysis of uric acid [12], Cr^{3+} ions [2], creatinine [7], creatine [13]; and polymer-based electrodes for phenol analysis [14]. Nanoparticle material has also been used to increase the selectivity and sensitivity of electrodes [15].

In this study, an imprinted zeolite modified carbon paste electrode has been developed to detect glucose in blood serum. The development of glucose detection method still interesting to be studied because the glucose level act as indicator a diabetes mellitus disease. As we know, diabetes mellitus has become a threat to human health in the world. World Health Organization (WHO) predicted an increase in the number of people with diabetes mellitus in Indonesia from 8.4 million in 2000 to around 21.3 million in 2030. Increasing the blood glucose levels (> 200 mg/dL or equivalent to 2×10^{-2} M) is a major cause of diabetes mellitus. In the body, normal blood glucose levels are 60-100 mg/dL (6×10^{-3} – 1×10^{-3} M) while serum glucose is 70–110 mg/dL (3.88×10^{-3} – 6.1×10^{-3} M) [16]. Determination of blood glucose levels in the medical field is generally done using a spectrophotometer or glucometer. Spectrophotometers use venous blood sample, while glucometers use capillary blood sample [17].

The electrodes were fabricated from a paste made by combining activated carbon, paraffin granule and imprinted zeolite with varying compositions. Zeolites were synthesized by the mole ratio of TEOS, TBOT, TPAOH, H_2O namely 1:0.017:0.24:21.2 [18]. The parameters studied in this study were the optimization of the ratio of activated carbon, paraffin and IZ as electrodes material and the optimum pH of glucose solution. Electrode performance was expressed from the measurement range (linear dynamic range), Nernst factor value, detection limit, selectivity, accuracy, response time and electrode life time (stability). Furthermore, the sensor was applied for the analysis of glucose levels in blood serum samples and compared with the spectrophotometric method as a commonly method used in the medical field.

EXPERIMENTAL

Materials

The chemicals used in this study were glucose (Sigma Aldrich, 99.5%), creatine (Sigma Aldrich 98%), urea (Sigma Aldrich, 99.5%), and uric acid (Fluka, 99%), tetraethyl orthosilicate (Merck, 99%), tetra butyl ortho titanate (TBOT; Merck, 98%), tetra propyl ammonium hydroxide (TPAH; Merck, 40%), isopropanol (Merck, 98%), paraffin granule (Merck, 99%). Creatine and urea solution were prepared using distilled water, while the 10^{-2} M uric acid solution was prepared by dissolving uric acid powder in 1: 1 NaOH (w/w). Acetate buffer was prepared from glacial acetic acid (Merck 100%) and sodium acetate trihydrate (Merck, 99.5%) while phosphate buffers are prepared from sodium dihydrogen phosphate dihydrate (Merck, 98.5%) and sodium hydrogen phosphate dihydrate (Merck, 99%) in distilled water. Carbon powder was chemically activated by immersing it in 0.01M H_3PO_4 and n-hexane, respectively. Furthermore, the carbon powder was heated at a temperature of 300 °C for 2 hours [19]. Ag wire was used as connector of carbon paste and potentiometer.

The equipments used in this study were a Cyberscan 510 potentiometers with Ag/AgCl as a reference electrode, fourier transform infrared (FTIR) spectrophotometer (Shimadzu), X-ray diffraction (Shimadzu), Gas Sorption Quantachrome ASIQwin, Analytical balance Mettler AE 200, hotplateTermolyne S46410-2, pH meter Cyberscan Eutech instruments pH 510, HITTECH EBA 20 centrifuge, Vacuum Oven Model 5851, magnetic stirrer, polypropylene bottle, agate mortar, 1000 μL micropipette tip and glassware commonly used in laboratories.

Procedure

Synthesis and Characterization of Zeolite

Synthesis of zeolite TS-1 was carried out by mixing TEOS, TBOT and TPAOH according to the previous research [18]. Amount of 5.0 mL of isopropanol was mixed with 0.2361 g of TBOT in a polypropylene bottle. Then, a 9.0 mL of TEOS was added dropwise and stirred for 30 minutes at room temperature; 4.8 g TPAOH was added dropwisely and stirred for 15 hours. Finally, 12.2 mL of water was added to the mixture. Furthermore, the mixture formed was heated hydrothermally at 80 °C for 4 days. As much as $\frac{1}{3}$ of the mixture was neutralized by washing using distilled water, then it was dried at 80 °C. As much as $\frac{2}{3}$ part of the mixture was added by 0.2205 g of glucose in 1 mL of distilled water. Then the mixture was allowed to stand for 3 hours. As much as $\frac{1}{2}$ part of this mixture was

dried (NIZ) and the other was extracted using hot water (80 °C) by centrifugation and dried. The dried powder was named imprinting zeolite (IZ).

Fabrication of Carbon Paste-Imprinted Zeolite Electrode

Fabrication of carbon paste-IZ electrodes was initiated by filling $\frac{3}{4}$ of the micropipette tip with melting paraffin. Silver wire (Ag) used as a connector between the electrodes and the potentiometer was first inserted into the micropipette tip. The remaining part of the micropipette tip was filled with a paste made from mixture of solid paraffin, carbon, and IZ (the composition in Table 1). The electrode surface was rubbed using HVS paper, and immersed in a 10^{-3} M glucose solution for 24 hours for conditioning.

TABLE 1. The composition of activated carbon, IZ, and paraffin in the fabrication of carbon paste-IZ electrodes

Electrode code	Composition (% weight)		
	Activated carbon	IZ	Paraffin
E1	65	0	35
E2	60	5	35
E3	58	7	35
E4	55	10	35
E5	50	15	35
E6	45	20	35
E7	40	25	35

Determination of Electrode Performance

The carbon paste-IZ electrode was used to measure the glucose solution 10^{-8} - 10^{-2} M and determined for its performance including the measurement range, the Nernst factor, the limit of detection, precision, accuracy, response time and life time. Electrode selectivity which was expressed by the coefficient of selectivity ($K_{i,j}^{pot}$) was studied by applying the electrodes to measure a series of standard solutions of urea, uric acid, and creatine (compounds that coexist with glucose in serum samples) and calculated using matched potential methods (MPM). The potential obtained was substituted into the linear regression equation of standard glucose. $K_{i,j}^{pot}$ values were calculated using equation (1) [20].

$$(K_{i,j}^{pot} = \frac{\Delta a_i}{a_j}) \quad (1)$$

Where Δa_i is the concentration obtained by substitute the potential in interferent solution on the regression equation of the glucose solution, while a_j is the measured interferent concentration.

Furthermore, electrodes were applied to measure glucose levels in blood serum samples and compared with the results of the analysis using spectrophotometric methods as a commonly used method for the determination of blood glucose levels in the medical field. The influence of other components in the serum sample on the determination of glucose levels was tested with recovery values. Recovery (R) was determined by measuring the potential of standard glucose solution 10^{-3} M (C_1), diluted serum samples (C_2), and diluted serum samples containing standard glucose 10^{-5} M (C_3) by spiking technique. The potential obtained was then substituted into the linear regression equation of standard glucose. The recovery value was calculated using equation (2).

$$R = \frac{C_1 - C_2}{C_3} \times 100 \% \quad (2)$$

RESULTS AND DISCUSSION

Synthesis and Characterization of Zeolite

Synthesis of zeolite TS-1 was carried out from tetraethyl orthosilicate (TEOS) as a source of silica, tetrabutyl orthotitanate (TBOT) as a titania source, tertrapropylammonium hydroxide (TPAOH) as structure directing agent and provide the alkaline conditions needed for hydrolysis reactions, and water as a solvent. Glucose molecules are

trapped in the zeolite pores and it is thought that the formation of hydrogen bonds between –OH functional groups on the glucose molecules with Ti-O-Si on zeolites. on the glucose molecules with Ti-O-Si on zeolites. The hydrothermal process was intended to form a zeolite framework so that the size of the zeolite pores will conform to the size of the glucose molecule. Glucose was then extracted from the zeolite framework using hot water so that the side bonding was expected to be selective to glucose. An illustration of the synthesis process is shown in Fig. 1.

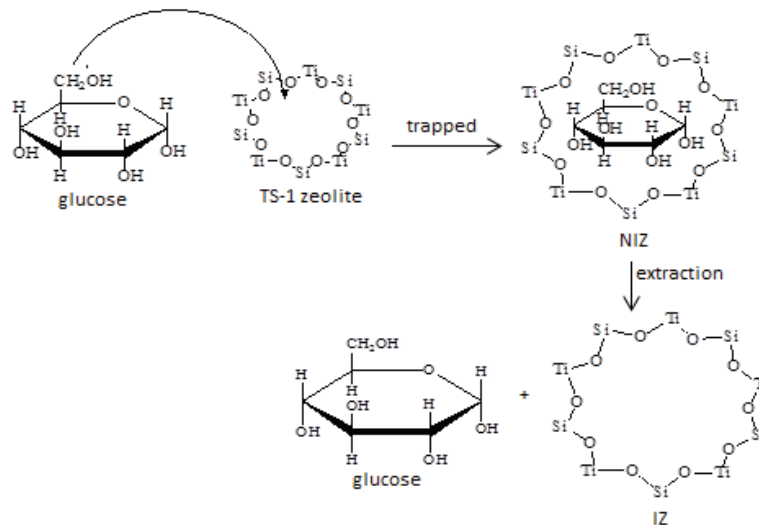


FIGURE 1. Illustration of the bonding process between zeolite and glucose

Based on the XRD pattern of Fig. 2a, the formation of TS-1 zeolite was marked by the peak at 2θ of 7.95; 8.83; 23.14; 23.25; 23.72; and 23.94°. The diffractogram was corresponded to the MFI structures. The MFI framework structure on the TS-1 zeolite was compared with the standard ZSM-5 pattern taken from the Collection of Simulated XRD Powder Patterns for the Zeolites, International Zeolite Association [21] which also has an MFI framework structure. The standard structural pattern shows the peaks at 2θ 7.95°; 8.83°; 23.18°; 23.23°; 23.74°; and 23.99°.

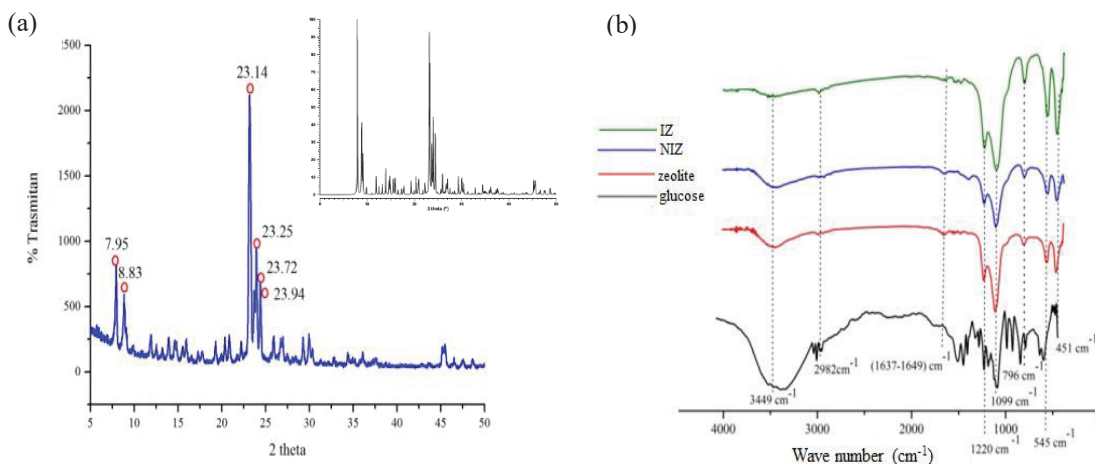


FIGURE 2. (a) The XRD pattern of zeolite TS-1 (ZSM-5, inset) and (b) FTIR spectra of glucose, zeolite, NIZ, and IZ

Figure 2b showed the typical spectra of zeolite TS-1, namely the band at wavenumbers around 451, 545, 796, 1100, and 1220 cm^{-1} . The peaks at 451 and 1220 cm^{-1} indicated Si-O-Si asymmetry stretching vibrations. The stretching vibration of the Si-O-Si symmetry was confirmed by characteristic band at 796 cm^{-1} . The MFI structure in zeolites were revealed at 451, 545 and 1222 cm^{-1} . The characteristic bands at 545 and 451 cm^{-1} were corresponded

to the rocking vibrations [22]. The absorption band at 1099 cm^{-1} were the asymmetric stretching vibrations of Si-O-Ti and the band at 1600 cm^{-1} was the peak of the -OH vibrations from Si-OH, Ti-OH, TPAOH, and H₂O [23].

Optimization of Research Parameter

Carbon paste-IZ electrodes were prepared from a mixture of activated carbon powder, paraffin granule and IZ powder. The activated carbon used had a surface area of $984.330\text{ m}^2/\text{g}$. The pore size of the carbon was 3.835 nm , this showed that the carbon was classified as mesoporous material [24]. IZ served to increase the electrode selectivity to glucose.

In accordance with the composition in Table 1, the electrodes showed the optimum performance, namely E6 which was fabricated with a mass ratio of carbon, paraffin and IZ of 9:7:4. The indicator used to determine the electrode performance was the Nernst factor value which approached the theoretical value ($59.2/n\text{ mV}$) [25]. The E6 showed a Nernst factor of 24.83 mV/decade . Table 2 presented Nernst factor and measurement range of electrode and linearity of the calibration curve of glucose solution.

TABLE 2. Data of electrode measuring range, Nernst factor, and linearity of calibration curve of glucose solution at pH 7

Electrode	Linear dynamic range (M)	Nernst (mV/decade)	Factor	Linearity (r)
E1	10^{-5} - 10^{-2}	21.11		0.998
E2	10^{-5} - 10^{-2}	16.57		0.865
E3	10^{-5} - 10^{-2}	17.28		0.858
E4	10^{-5} - 10^{-2}	22.21		0.947
E5	10^{-5} - 10^{-2}	21.65		0.919
E6	10^{-5} - 10^{-2}	24.83		0.986
E7	10^{-5} - 10^{-2}	23.71		0.957

In this study, the pH optimization of the solution was carried out to determine the effect of acid and base conditions on the measurement of glucose solution. In an alkaline media, glucose was present as an open chain carboxylic ion whereas in an acidic media glucose turned into lactone. The pH optimization was carried out by measuring the potential of a 10^{-8} - 10^{-2} M glucose solution with a pH of 5, 6, 7, and 8 using the E6 electrode (Fig. 3). The measurement of glucose solution using E6 at a pH 7 obtained the Nernst factor 28.6 mV/decade . This value is closest to the theoretical value. Glucose is a divalent molecule, so that theoretically it will produce a Nernst factor of 29.6 mV/decade [26].

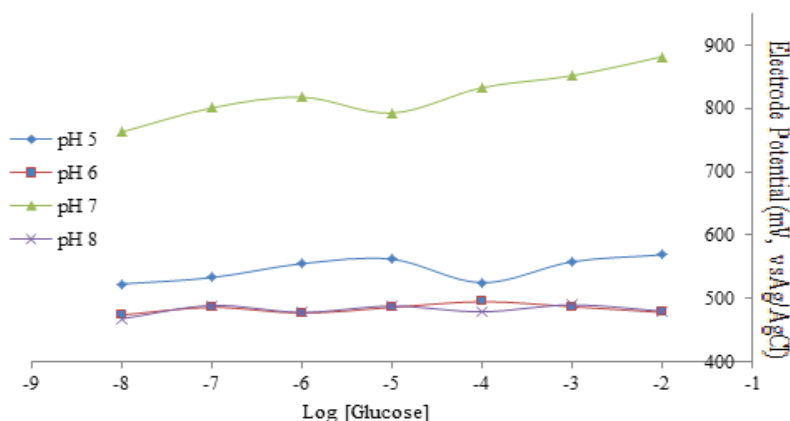


FIGURE 3. Plot electrode potential and log[Glucose] at varying pH

In order to know the effect of the mold on the performance of the IZ modified electrodes, zeolite (EZ) and NIZ (ENIZ) modified electrodes with the same composition as the E6 electrodes were fabricated and applied to measure glucose standard solution. Data of the electrodes potential the E1, E6, EZ, and ENIZ on measurements of standard glucose solutions and the Nernst factor were shown in Table 3.

TABLE 3. Data of electrode potential and Nernst factor using E1, E6, EZ, and ENIZ

[Glucose] (M)	Potential (mV) of the electrode			
	E1	E6	ENIZ	EZ
10^{-5}	102.5	793	976	977
10^{-4}	103.8	833	985	988
10^{-3}	127.7	852	1083	935
10^{-2}	146.5	882	1035	1028
Nernst factor (mV/decade)	15.6	28.6	27.5	10.0

Performance of The Electrode and Method Validity

The results of glucose analysis using E6 at pH 7 provided a measurement range of 10^{-5} - 10^{-2} M with a Nernst factor value of 28.6 mV/decade and a response time of 8.9-21.5 s. The response time of E6 was faster than that of non-enzymatic sensor based on the poly (o-phenylenediamine) [27] and previous research of imprinted zeolite-based electrode [7].

The electrode detection limit was the lowest or highest concentration that could be responded by electrode. The detection limit was determined from the intersection of linear curve with non-linear curve on the plot of log [glucose] and electrode potential. In this study, the linear equation obtained was $y = 18.6x + 940.1$ and the nonlinear equation was $y = 14.66x^2 + 172x + 12866$. From the both equations obtained the lower detection limit was 4.79×10^{-5} M. The value of the measurement range and detection limit explained that the potentiometric method using imprinted zeolite modified carbon paste electrodes in this study could be used for glucose analysis with a normal concentration to glucose concentrations of diabetics [16].

In this study, precision was expressed by the coefficient of variation (CV). At concentrations of 10^{-5} - 10^{-2} M, the CV values were 0.92%-4.89%. The CV value was better than the limit set by AOAC [28].

Electrode selectivity tests were carried out through the application of electrodes for measurement of urea, uric acid, and creatine solutions. Electrode selectivity was expressed by the coefficient of selectivity ($K_{i,j}^{pot}$) which was calculated using matched potential methods (MPM). The coefficient of selectivity value in Table 4 showed that zeolite modified electrodes had a higher selectivity to glucose (i) than uric acid, urea and creatine (j).

TABLE 4. Selectivity coefficient ($K_{i,j}^{pot}$) of electrode on uric acid, urea and creatine solution

Solution	Concentration (M)	$K_{i,j}^{pot}$	
		Bare carbon paste	Carbon paste-IZ
Uric acid	10^{-5}	0.96	2.35×10^{-6}
	10^{-4}	0.99	4.04×10^{-6}
	10^{-3}	1.12	4.67×10^{-6}
	10^{-2}	1.31	4.75×10^{-6}
Urea	10^{-5}	1.39	9.77×10^{-7}
	10^{-4}	2.14	2.37×10^{-7}
	10^{-3}	2.29	1.24×10^{-6}
	10^{-2}	2.45	1.46×10^{-6}
Creatine	10^{-5}	1.18	6.49×10^{-7}
	10^{-4}	1.23	7.87×10^{-7}
	10^{-3}	1.34	1.08×10^{-6}
	10^{-2}	1.39	1.24×10^{-6}

Accuracy test was used to determine whether the potentiometric using developed electrode is as good as spectrophotometric, the commonly used method for determining blood glucose levels in the medical field, while the recovery test was intended to see whether there is interference caused by the sample matrices. The accuracy value obtained from the analysis of five blood samples in this study was $90.58 \pm 4.65\%$ (Table 5). This value was slightly lower than the accuracy range accepted for an analysis method according to the Association of Official Analytical Chemist (AOAC) [28]. The recovery of potentiometric methods using carbon paste-IZ electrodes was $(91.73 \pm 3.48)\%$ ($n=5$). This value fulfilled the criteria of a chemical analysis method according to AOAC [28]. Based on the recovery value and accuracy, it was assumed that there are several components in blood samples that have the

potential to interfere with glucose analysis such as fructose, sucrose, maltose [29] and ions (Na^+ , Cl^- , K^+ , PO_4^{3-} , SO_4^{2-} , NH_4^+) [30].

TABLE 5. Data accuracy and *recovery* potentiometry method to analyse blood glucose

Sample	Glucose concentration (M)		Accuracy (%)	Recovery (%)
	Potentiometry	Spectrophotometry		
Glucose 10^{-3} M	9.28×10^{-4}	-	-	-
Serum sample 1	4.86×10^{-3}	5.62×10^{-3}	86.53	-
Serum sample 1 + Glucose 10^{-3} M	5.71×10^{-3}	-	-	91.59
Serum sample 2	4.94×10^{-3}	5.44×10^{-3}	90.79	-
Serum sample 2 + Glucose 10^{-3} M	5.84×10^{-3}	-	-	96.98
Serum sample 3	4.63×10^{-3}	4.76×10^{-3}	97.27	-
Serum sample 3 + Glucose 10^{-3} M	5.44×10^{-3}	-	-	87.28
Serum sample 4	9.07×10^{-3}	9.81×10^{-3}	92.40	-
Serum sample 4 + Glucose 10^{-3} M	9.92×10^{-3}	-	-	92.10
Serum sample 5	10.57×10^{-3}	12.40×10^{-3}	85.90	-
Serum sample 5 + Glucose 10^{-3} M	11.41×10^{-3}	-	-	90.70

Electrode life time was investigated to know the stability of the electrodes. The life time of electrodes depended on the mechanical properties of an electrode material. This mechanical property was influenced by the flexibility of the material, the pH and the level of solubility of the material. Imprinted zeolite modified carbon paste electrodes presented a good performance up to 9 weeks (130 times usage). This life time was longer than the previous research [7, 13].

CONCLUSION

Imprinted zeolite modified carbon paste electrode showed a wide measurement range, low detection limit, fast response time and long life time. The developed electrode recognized selectively the presence of glucose compared to urea, uric acid and creatine in solution. Comparison of potentiometry with spectrophotometry as a commonly used method for blood glucose analysis in medical field, it showed an accuracy of $90.58 \pm 4.65\%$ ($n=5$). Based on the electrode performance, potentiometry using the carbon paste-IZ electrode is recommended as an alternative method for routine analysis of blood glucose in the medical field.

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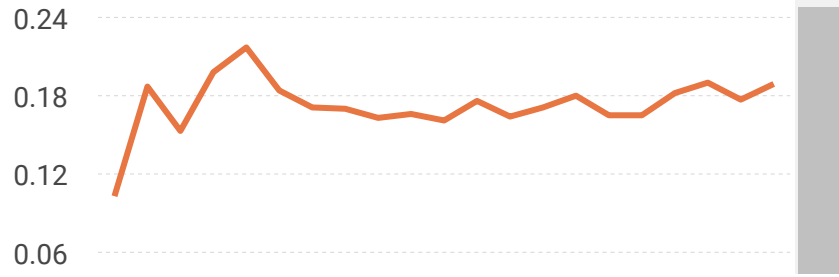
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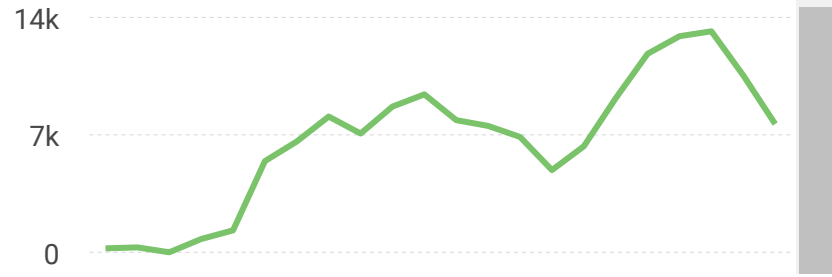


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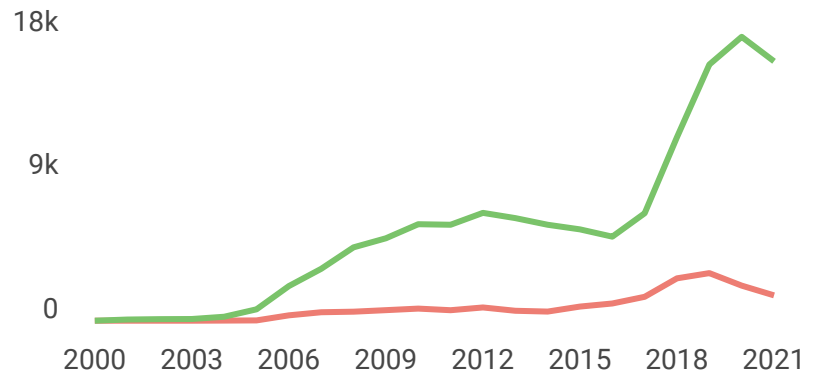




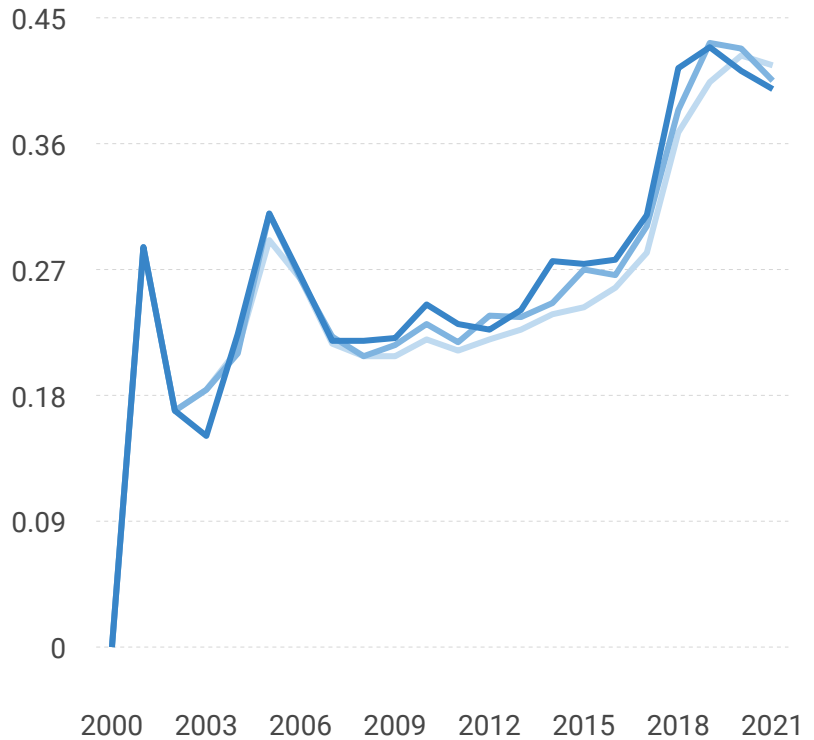
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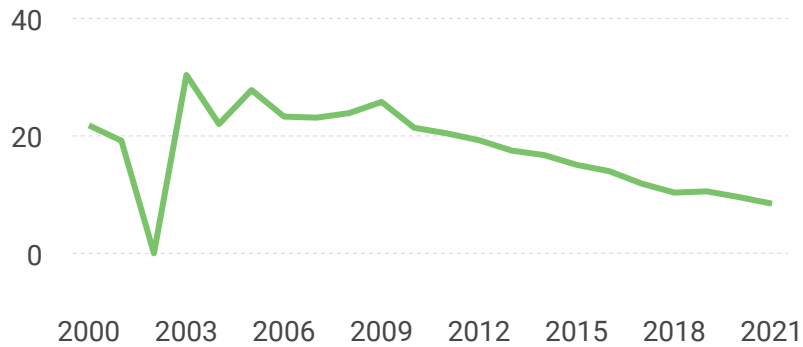


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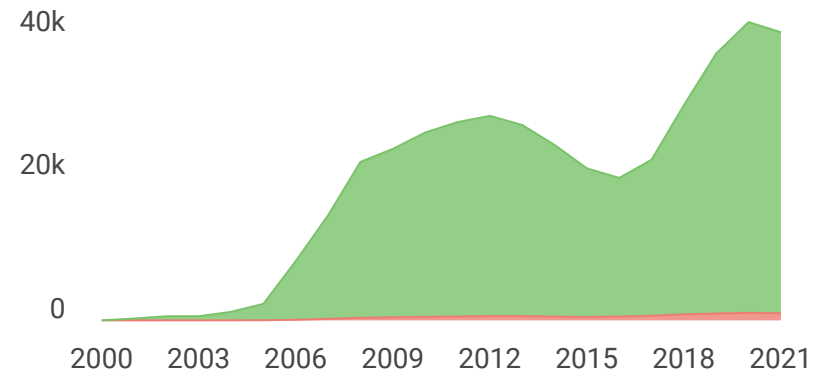
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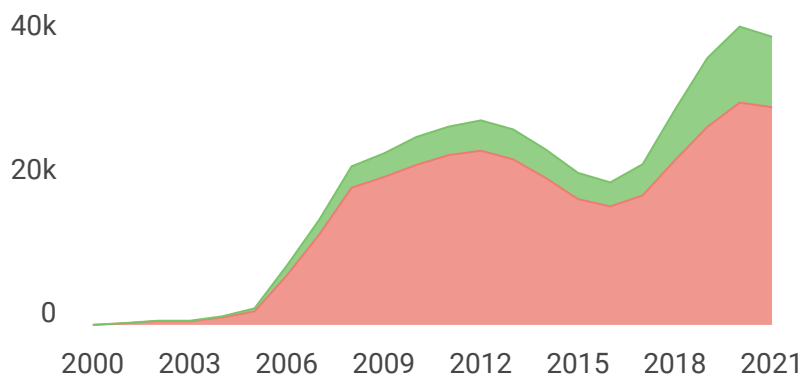
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