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Selective Identification for Glucose in the Presence of Fructose Using Imprinted Polyeugenol Modified Graphite Paste Electrode

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Abstract: *High blood glucose levels indicate diabetes mellitus. The conventional way was carried out to determine glucose levels, but this method just shows the total reducing sugar levels. An electrode was developed to analyze glucose in the presence of fructose by potentiometry. The optimum graphite paste electrodes-Imprinted based on polyeugenol and Ethylene Glycol Dimethacrylate (EGDMA) as crosslinkers, had been used as a glucose sensor. Polyeugenol was made by the polymerization reaction with a BF_3 catalyst with a molecular weight of 6451.338 g/mol. A total of 2135.99 glucose was contacted with polyeugenol, and the result was crosslinked with EGDMA and 2,2'-Azobis(2-methylpropionitrile) (AIBN) initiator, then Molecularly Imprinted Polymer (MIP) was generated. The optimization of electrodes showed that the best composition of MIP: paraffin: graphite is 20:35:45 with the 19.16 mV/decade Nernstian Factor, which can measure samples at the range 10^{-5}–10^{-1} M with a detection limit of 1.0334 × 10^{-5} M, a response time of 6–15 s, a lifetime (use) of 19 times and has good selectivity on fructose sample with K_{ij} less than 1. Measurements using this electrode showed that honey contains 28.78% of glucose, not much different from the UV-Vis spectrophotometry, which is 29.68%, and HPLC is 30.42%.*

Keywords: *polyeugenol; imprinted; glucose; fructose; selective electrode*

INTRODUCTION

Diabetes mellitus is of chronic disease caused by the increase in glucose levels, or called hyperglycemia which makes the regulation of glucose homeostasis not work perfectly [1]. The values of blood glucose in the normal body are 60–100 mg/dL (6 × 10^{-3}–1 × 10^{-3} M), while in diabetics, blood glucose levels can increase to > 200 mg/dL or equivalent to 2 × 10^{-2} M. The conventional way was carried out to determine glucose levels, but this method just shows the form of total reducing sugar levels and cannot determine reducing sugar individually [2]. An alternative method that can be used to measure glucose levels is potentiometry. Potentiometry has two kinds of working electrodes there reference electrode and the working electrode. In potentiometry, a working electrode is used as a sensor to detect the analyzed compound. It is important to determine the type of sample because it can produce an accurate and selective analytical instrument sensor component. In a previous study, the synthesis of a polyeugenol-based Imprinted Polymer (IP) selective electrode with a Polyethylene glycol diglycidyl ether (PEGDE) crosslinker agent as a glucose sensor showed good performance as a glucose sensor [3].

Selective electrodes are made using eugenol derivative compounds because eugenol has a large abundance in Indonesia; and is found in almost 80% of plants in Indonesia. Eugenol can be used as a starting material for the synthesis because of the presence of three functional groups attached to it; there are allyl, hydroxy, and methoxy groups; from this allyl group, eugenol can be polymerized into polyeugenol [4]. The working electrode is based on a molecularly imprinted polymer. The functional monomers, templates, initiators, solvents, and crosslinker agents will react around the template molecules through the polymerization process and produce a polymer called polyeugenol [5-6]. Compounds of Imprinted from polyeugenol have high selectivity because it only identifies the molecule template and also has good mechanical strength against organic solvents, bases, acids, high pressure, and temperature. Therefore, Molecularly Imprinted Polyeugenol (MIP) has great potential as a working electrode material for glucose sensors [7].

In this research, the synthesis of MIP was prepared with eugenol, BF_3 as a catalyst, EGDMA as a crosslinker, glucose as a MIP template, and a graphite electrode that had the ability to conduct the current from a sample. An electrode was made with various compositions and used to analyze glucose with varying concentrations and pH to get the optimum working electrode to enhance the performance of potentiometric sensors in glucose determination, such as Nernst factor, range of measurement, coefficient selectivity, response time, lifetime, and measurement glucose in the presence of fructose.

EXPERIMENTAL SECTION

Materials

Eugenol p.a, $BF_3(C_2H_5)_2$, Chloroform p.a, anhydrous Na_2SO_4, D-Glucose, L-Fructosa Ethylene glycol dimethacrylate (EGDMA), AIBN (2,2'-Azobis(2-methylpropionitrile), Paraffin, graphite, NaOH, Ethanol, HCl, KCl, Na_2HPO_4, NaH_2PO_4, CH_3COOH, CH_3COONa, Kaliumnatriumtartrat-Tetrahydrat were purchased from Merck-Sigma Aldrich (Jakarta, Indonesia), aqua demineralization was purchased from Bratachem (Semarang, Indonesia), 3,5-Dinitrosalicylic acid was purchased from HIMEDIA (Jakarta, Indonesia), and Randu Honey from Mabruuk (Semarang, Indonesia).

Instrumentation

The characterizations of the functional group were carried out using FTIR Shimadzu Prestige 21 (Semarang, Indonesia), for the surface morphology MIP, NIP, Electrode MIP (EMIP), and Electrode NIP (ENIP) were analyzed using SEM Phenom Pro X Desktop with EDX (Semarang, Indonesia), and Electrode performance that was synthesized before measured with Eutech PC510 potentiometer and Ag/AgCl reference electrode (Semarang, Indonesia).

Honey glucose levels containing fructose and glucose were measured by potentiometry. The result was then compared with UV-Vis Spectrophotometry LW-V-200-RS method using DNS complexing reagent (Semarang, Indonesia) and HPLC that consisted of a carbohydrate column at ambient temperature, a flow rate of 0.769 mL/min, and 80% acetonitrile eluent with a refractive index detector (Bogor, Indonesia).

Procedure

Polymerization of eugenol

In a three-necked flask, 5.8 g of eugenol was polymerized using BF_3-diethyl ether as a catalyst under stirring for 16 h. The polymerization then ended by adding methanol. The result was dissolved with chloroform and neutralized by adding water. Afterward, anhydrous Na_2SO_4 was used to remove the water completely and then filtered. The precipitate formed was dried, crushed, weighed, and analyzed by FTIR.

Synthesis of molecularly imprinted polyeugenol (MIP)

The synthesized polyeugenol was contacted with glucose by adding 0.5 g of polyeugenol with 10 mL glucose solution of 7500 ppm and stirred for 24 h. The product is then filtered, dried, and characterized by FTIR. A total of 0.222 g of polyeugenol-glucose was then crosslinked with 0.4 mL of EGDMA, 1.67 mL of chloroform, and 0.48 mL of AIBN as initiator. Then the mixture was refluxed at 60–70 °C for 30 min, and the results were dried in an oven and sieved with a 100 mesh sieve. Polyeugenol-glucose-EGDMA was then eluted with ethanol for 24 h. The contact and release of glucose were analyzed with a spectrophotometer UV-Vis. MIP is then characterized by FTIR and SEM-EDX.

Synthesis of non-imprinted polyeugenol (NIP)

NIP synthesis is done in the same way with MIP but without contacted polyeugenol with glucose, then characterized by FTIR and SEM-EDX.

Construct and characterization of electrode

The electrodes were made by filling the micropipette tube with sanded silver wire to connect the electrode to the potentiometer; as much as the micropipette was filled with melted paraffin, then the remaining tube was filled with pasta that forms by mixing the graphite solid paraffin and MIP. The filling of pasta in the tube is done by pressing to make it completely filled and then rubbed using *Houtvrij Schrijfpapier* (HVS) paper. This process is summed up in Fig. 1. The electrode is then used to determine the glucose level on the sample.

■ RESULTS AND DISCUSSION

Polymerization of Eugenol

Eugenol polymerization was carried out for 16 h through a cationic addition process because the allyl group in eugenol underwent an addition reaction. In eugenol polymerization, the initiation step uses a $BF_3(C_2H_5)_2$ catalyst so that the double in the allyl group of eugenol breaks up and produces a carbocation. Then at the propagation stage, a polymer chain will be formed, while at the termination stage, the polymer chain will end through the addition of methanol.

The resulting polyeugenol formed in a solid gel was then dissolved in chloroform and neutralized with aqua demineralization to remove the acid from the remaining catalyst. After neutral conditions, anhydrous Na_2SO_4 was added to bind the water. The result of polyeugenol has a reddish-brown with a molecular weight of 6451.338 g/mol and a yield of 89.9%. Eugenol and polyeugenol have been synthesized and analyzed using FTIR, and the result can be seen in Fig. 2.

At wavenumbers 994 cm^{-1} and 910 cm^{-1}, it appears that the polyeugenol has lost a vinyl group more than previously in eugenol, where there is a vinyl group. The vinyl group has been lost because it has been modified and binds to other eugenols to form polyeugenol. This is in accordance with the research before[3,8-9], so it can be concluded that the polymerization reaction was successfully carried out.

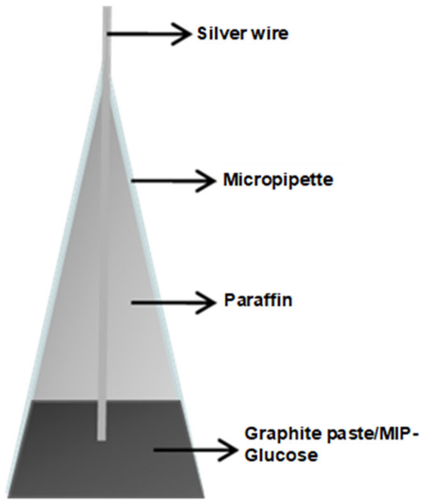

Fig 1. Electrode construction

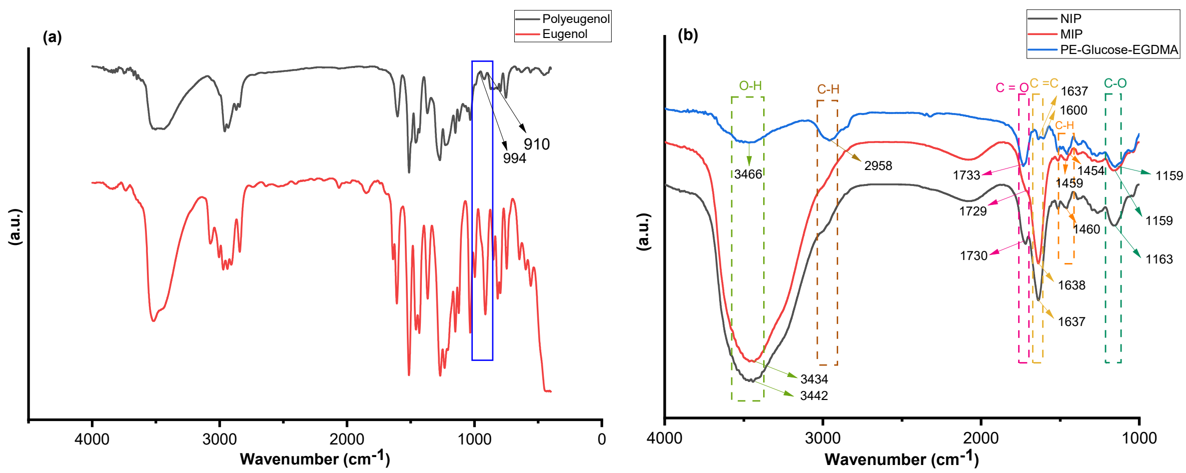

Fig 2. FTIR Comparison (a) Polyeugenol and eugenol (b) MIP, NIP, and polyeugenol (PE)-Glucose-EGDMA

Synthesis and Characterization of MIP and NIP

The amount of glucose in contact was analyzed by UV-Vis spectrophotometer and was obtained at 2135.99 ppm. The glucose released was 95.34%. The differences in functional groups and peaks that appear in NIP and MIP can be seen from the results of the FTIR spectra. A comparison of FTIR spectra between MIP, NIP, and PE-Glucose-EGDMA is shown in Table 1.

The presence of a CH group found in 2958 cm^{-1} PE-Glucose-EGDMA was not much different from the study before, which identified the presence of CH_3 vibrations in 2960 cm^{-1} EGDMA samples [10]. The MIP and NIP absorption were not clearly visible because they overlap with OH at 1600 cm^{-1}, which shows the presence of C=O aldehyde from glucose [11] which is found in PE-Glucose-EGDMA but not seen in MIP. It is because glucose has been released. The PE-Glucose-EGDMA spectra also showed a reduction in the intensity of the OH peak compared to the MIP due to the release of glucose with ethanol.

Subsequent analysis using SEM-EDX, which aims to see the surface morphology of MIP and NIP (Fig. 3) and to see the levels of C and O elements contained in MIP and NIP (Table 2). The SEM results in Fig. 3 show the surface morphology at 5000× magnification. It can be seen that the two samples have different surface morphology. The MIP sample has more pores, which is thought to be a mold of glucose compounds, while the NIP

Table 1. FTIR Comparison MIP, NIP, and PE-Glucose-EGDMA

Wavenumber (cm^{-1})	Functional group	NIP	MIP	PE-Glucose-EGDMAG
3400–3500	O–H	Presence	Presence	Presence
2954.5	C–H	Not Presence	Not Presence	Presence
1715–1730	C=O ester	Presence	Presence	Presence
1600	C=O stretching, aldehyde	Not Presence	Not Presence	Presence
1638–1648	C=C stretching	Presence	Presence	Presence
1450–1465	C–H bending, aromatic	Presence	Presence	Presence
1100	C–O stretching, ether	Presence	Presence	Presence

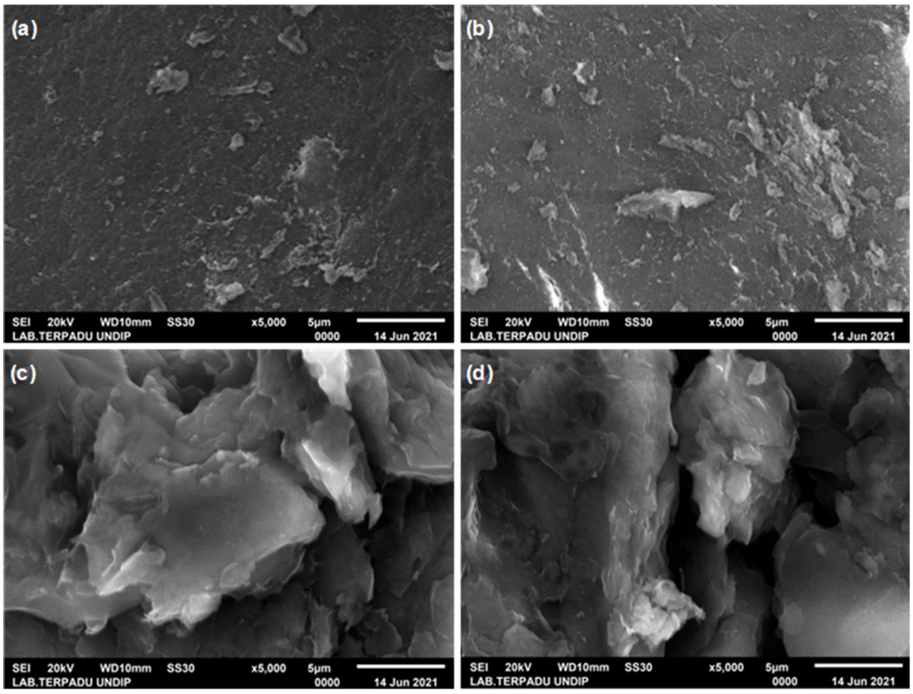

Fig 3. SEM result of (a) NIP (b) MIP (c) ENIP (d) EMIP

sample has a smoother surface. Both MIP and NIP used Image J software to show the amount of pore. The results of pore measurements using Image J are shown in Table 3.

Characterization of Graphite Paste/MIP-Glucose Electrodes (EMIP)

The synthesis of the electrode by mixing various compositions (% mass) of MIP, graphite, paraffin, and pH variations. Tables 4, 5, and 6 show the optimization results, with optimum pH being 7 with a Nernst factor of 19.16 mV/decade. E5 (EMIP) also shows a linearity value of 0.991, which is close to 1. So, E5 is an optimum electrode and suitable for analyzing glucose samples.

The ENIP was prepared with a composition such as E5 as control, and measurements were made in comparison to the E5 electrode (EMIP) (Table 7). The ENIP shows a Nernstian factor of 2.29 mV/decade. The result of this low Nernstian factor is that there is no

Table 2. Element mass percentage

Unsure	Mass (%)			
	MIP	NIP	EMIP	ENIP
C	71.82	72.72	94.51	96.06
O	27.76	26.79	4.67	3.87

Table 3. Pore measurements using Image J

	MIP	NIP	EMIP	ENIP
Count	185151	115917	51.91	44.60
Total area	130394	99909	1434799	1381.25
Area (%)	28.63	22.14	32.68	31.20

Table 4. Nernstian factor and measurement range in pH 3

Electrode	Composition (wt.%)			Nernstian factor (mV/decade)	Measurement range (M)	Linearity (r)
	MIP	Paraffin	Graphite			
E1	0	35	65	-14.84	10^{-8}–10^{-3}	0.95
E2	5	35	60	-9.99	10^{-8}–10^{-3}	0.92
E3	10	35	55	-8.11	10^{-7}–10^{-3}	0.98
E4	15	35	50	-5.88	10^{-7}–10^{-1}	0.92
E5	20	35	45	-3.33	10^{-7}–10^{-2}	0.83
E6	25	35	40	-4.64	10^{-8}–10^{-2}	0.96

Table 5. Nernstian factor and measurement range in pH 5

Electrode	Composition (wt.%)			Nernstian factor (mV/decade)	Measurement range (M)	Linearity (r)
	MIP	Paraffin	Graphite			
E1	0	35	65	-0.24	10^{-5}–10^{-2}	0.87
E2	5	35	60	-5.67	10^{-8}–10^{-1}	0.72
E3	10	35	55	1	10^{-7}–10^{-2}	0.75
E4	15	35	50	-2.57	10^{-5}–10^{-1}	0.93
E5	20	35	45	-1	10^{-4}–10^{-1}	0.83
E6	25	35	40	-1.1	10^{-4}–10^{-2}	0.89

Table 6. Nernstian factor and measurement range in pH 7

Electrode	Composition (wt.%)			Nernstian factor (mV/decade)	Measurement range (M)	Linearity (r)
	MIP	Paraffin	Graphite			
E1	0	35	65	0.68	10^{-5}–10^{-2}	0.98
E2	5	35	60	1.84	10^{-8}–10^{-1}	0.94
E3	10	35	55	1.28	10^{-7}–10^{-2}	0.89
E4	15	35	50	6.85	10^{-8}–10^{-4}	0.98
E5	20	35	45	19.16	10^{-5}–10^{-1}	0.99
E6	25	35	40	4.51	10^{-8}–10^{-2}	0.96

Table 7. Comparison of EMIP and ENIP performance

Electrode	Nernstian factor (mV/decade)	Measurement range (M)	Linearity (r)
EMIP (E5)	19.16	10^{-5}–10^{-1}	0.99
ENIP	2.29	10^{-5}–10^{-1}	0.88

imprinted molecule that is compatible with glucose.

Graphite paste from EMIP and ENIP was analyzed using SEM-EDX (Fig. 4). This analysis aims to see the surface morphology of MIP and NIP graphite paste which is a component of the electrode, and to see the levels of C and O (Table 2) elements contained in it. The SEM imaging results in Fig. 3 show the surface morphology at a magnification of 5000×.

EMIP and ENIP have a surface morphology that is not much different because both EMIP and ENIP have the same carbon composition of 45%, while the composition of MIP and NIP are 20% each, only the MIP surface has a more uneven surface. The results of pore measurements using Image J are shown in Table 3.

The result of the TGA/DTG analysis (Fig. 4) that was carried out with a temperature range of 10 to 1200 °C, the temperature increase of 10 °C/min, and using nitrogen flow rate showed the weight loss of graphite paste MIP and NIP. Both of them contained the same composition with a mass ratio of graphite:paraffin:MIP/NIP is 45:35:20 (wt.%) and have significant weight loss begins at 238 °C for MIP and 215 °C for NIP.

Limit of Detection

The lowest concentration of analyte in the sample can be reliably detected with a limit of detection [12]. In this study, the limit of detection is determined by the intersection between the linear and nonlinear lines of the standard curve [13]. The resulting linear line equation is $y = 19.16x + 251.4$ while the non-linear line $y = 7.4x^2 + 84.7x + 142.2$. The results of the measurement of the detection limit on EMIP are shown in Fig. 5. The EMIP detection limit is 1.0334×10^{-5} M so that the electrode can detect the presence of glucose theoretically up to 1.0334×10^{-5} M with the Nernst factor measured at 19.16 mV/decade. EMIP can be used to analyze the glucose level in the blood because the normal body has blood glucose levels at 60–100 mg/dL (6×10^{-3}–1×10^{-3} M), while people with diabetes mellitus have glucose levels of more than 200 mg/dL or equivalent, with 2×10^{-2} M [2]. The comparison of LOD results by our work and some previous research is reported in Table 8.

Response Time

Determination of the electrode response time is carried out to determine the time required for the electrode to respond to the analyte in solution. It can be seen (Table 9) that the greater concentration will give a faster response time. This is in accordance with the research [18] because the greater concentration will increase the molecular movements from the solution to the electrode.

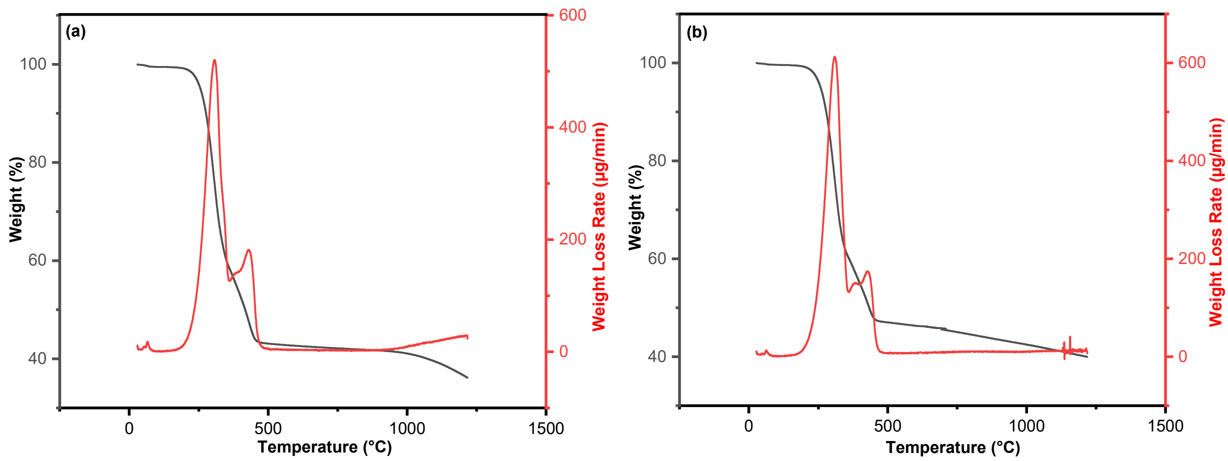

Fig 4. TGA/DTG of (a) MIP and (b) NIP

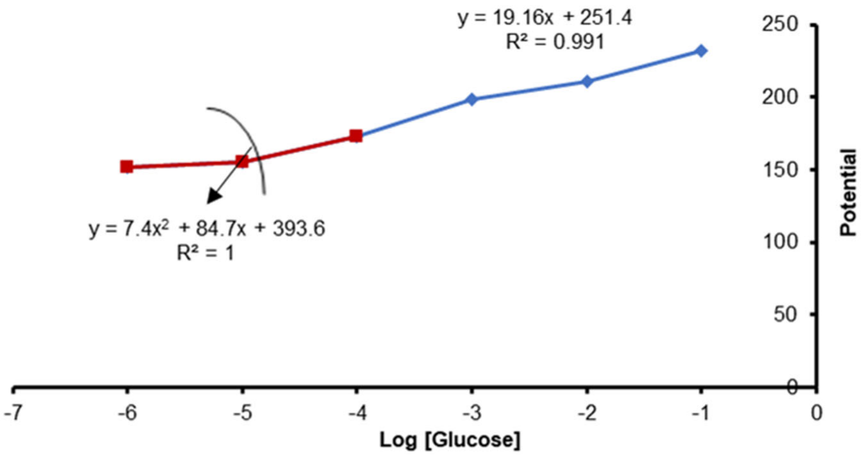

Fig 5. Detection limit measurement curve

Table 8. The comparison of the LOD result by our work and some previous research

Method	The material of the electrode	Linear range	LOD	Ref.
Amperometry	Carbon paste/GOx silica	5×10^{-4}–9×10^{-3} M	1.5×10^{-4} M	[14]
Amperometry	Carbon paste/selenium nanoparticle-mesoporous silica composite (MCM-41)	1×10^{-5}–2×10^{-3} M	1×10^{-4} M	[15]
Potentiometry	Poly (3-aminophenyl boronic acid-co-3-octylthiophene)	5×10^{-3}–5×10^{-2} M	5×10^{-4} M	[16]
Potentiometry	Carbon nanotube on gold printed	10^{-3}–10^{-1} M	1×10^{-4} M	[17]
Potentiometry	Carbon paste/IZ	10^{-4}–10^{-2} M	5.6×10^{-5} M	[13]
Potentiometry	Carbon paste/MIP Polyeugenol with crosslinker PEGDE	10^{-5}–10^{-1} M	8.363×10^{-5} M	[3]
Potentiometry	Carbon paste/MIP Polyeugenol with crosslinker EGDMA	10^{-5}–10^{-1} M	1.0334×10^{-5} M	This work

Table 9. Result of response time

Concentration (M)	Potential (mV)	Response time (sec)
10^{-5}	155.1	15
10^{-4}	173.2	12
10^{-3}	198.7	9
10^{-2}	211	8
10^{-1}	232	6

The Lifetime of the Electrode

Determination of the lifetime of the electrode aims to determine the time limit for using the electrode. Based on the measurement of the number of electrodes used, the Nernst factor results are produced as in Fig. 6. It shows that up to 19 times, the electrodes still show good performance. The weight of the electrode, after being used 25 times, was reduced by 0.12 g. Due to its weight loss, the amount of substance that functions as a mold was reduced, and the Nernstian Factor was also lowered.

Coefficient Selectivity

In this study, the potential match method (MPM) was used to determine the electrode selectivity for glucose analysis [19]. The value of the selectivity coefficient (K_{ij}) of the electrodes in a 10^{-5}–10^{-1} M fructose solution was 0.478, 0.266, 0.175, 0.219, 0.107, and 0.087. The fructose selectivity test was chosen because it has the most similar structure to glucose. From the K_{ij} value, fructose does not interfere with potentiometric glucose analysis using EMIP because the selectivity coefficient value is less than 1. The electrode will be selective for the analyte compared to the interfering compound if the

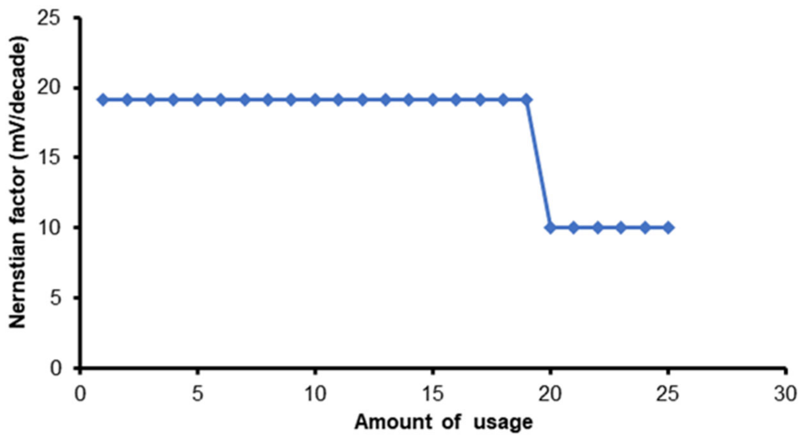

Fig 6. Lifetime electrode graph

value of $K_{ij} < 1$, otherwise the electrode will be selective for the interfering compound analyte if the value of $K_{ij} > 1$ and if the value of $K_{ij} = 0$, it means that the foreign compound does not interfere the analyte [20].

Measurement of Glucose on Honey Sample

Determination of glucose levels in honey was carried out to determine the ability of the hybrid electrodes to analyze glucose in samples containing reducing sugars (glucose and fructose). In this measurement, EMIP is used to measure the solution of raw honey. The measurement results showed that the glucose level in honey was 28.78%. Meanwhile, for measurements with UV-Vis spectrophotometry, glucose levels were 29.68%, then the measurement using HPLC where glucose and fructose are separated at retention times of 4.99 and 4.51 min, resulting in measurements for glucose of 30.42% and fructose of 38.38%.

■ CONCLUSION

The optimum composition electrode has a mass ratio of 45:35:20 (wt.%) by graphite:paraffin:MIP with an optimum pH of analyte is 7. Analysis of the performance of the electrode as a glucose sensor produces a Nernst factor of 19.16 mV/decade. The measurement range is 10^{-5}–10^{-1} M with a detection limit of 1.0334×10^{-5} M, a response time of 6–15 s, and a lifetime (use) of 19 times. It has good selectivity with a Kij of less than 1. The electrode can also measure glucose levels in honey samples and give a percentage result of glucose content is 28.78%, not much different from the measurement results on UV-Vis spectrophotometry, which is 29.68%, and HPLC, which is 30.42%. Based on this performance, the electrode is recommended as an alternative method to analyze glucose selectively on a blood sample.

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■ AUTHOR CONTRIBUTIONS

M.C.D conducted the conceptualization, methodology, and validation, L.C. conducted the resources, and investigation, wrote and revised the manuscript, M.C.D., G.G., R.A.L., M.K. supervised the research. All authors agreed to the final version of the manuscript.

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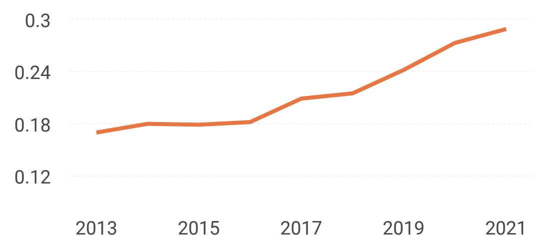

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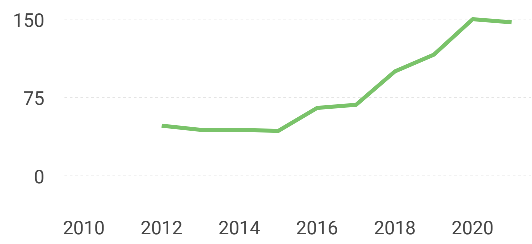

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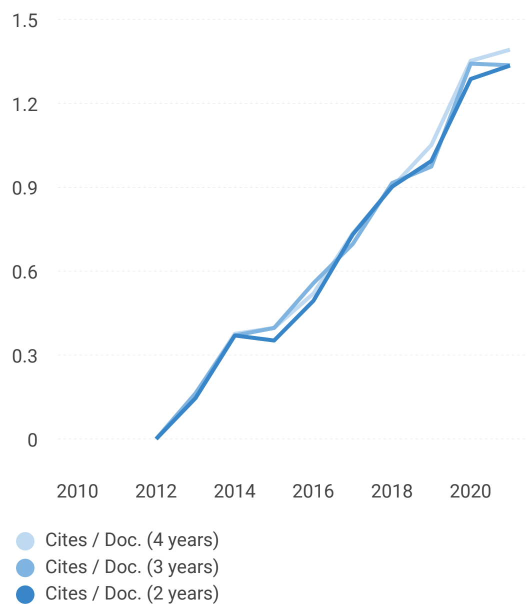

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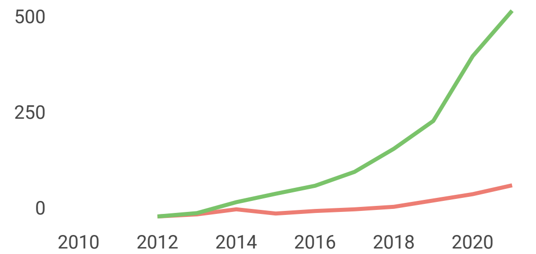

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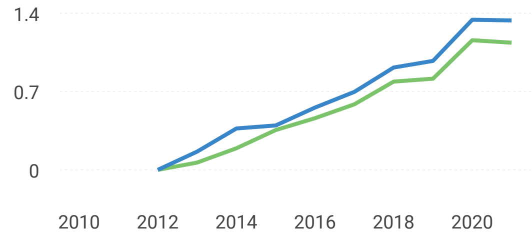

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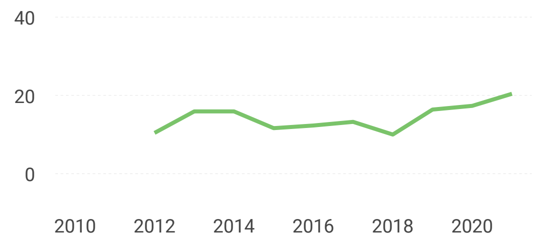

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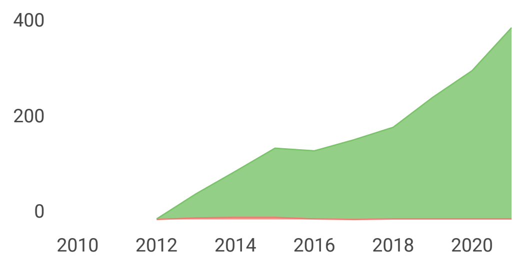

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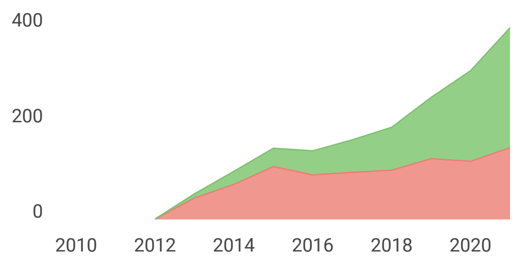

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