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# Pembuatan dan Karakterisasi Elektrode indikator dari bahan PPy+H<sub>2</sub>SO<sub>4</sub> dan PPy+Asam sulfonat sebagai sensor urea dengan teknik immobilisasi Enzim urease pada PVA.

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## Abstraks

Telah dilakukan penelitian tentang elektroda indikator (1) PVA-Enzim/PVC-KTpCIPB, sensitivitas 19.069 mV/decade, rentang deteksi  $1.10^{-5}$  -  $5.10^{-4}$  M limit deteksi  $1.10^{-5}$  M. Lebar puncak absorbansi Uv-vis sempit (2) PVA-Enzim/GA-2.9%/PVC-KTpCIPB lebar puncak absorbansi UV-vis besar tetapi puncak absorbansi menurun, (3) PVA-Enzim/GA-2.9%/PVC-KTpCIPB-o-NPOE analisis XRD pola spektrum amorf muncul (4) PVA-Enzim/GA-2.9%/PPy+H<sub>2</sub>SO<sub>4</sub>/PVC-KTpCIPB-o-NPOE (5) PVA-Enzim/GA-2.9%/PPy+Asam Sulfonat/PVC-KTpCIPB-o-NPOE, pola spektrum amorf pada (4) dan (5) sangat berkurang untuk variasi enzim 0.6 g dalam 0.5 mL (50% air + 50% alkohol). GA berperan menambah rentang deteksi, o-NPOE membentuk amorf, variasi enzim meningkatkan intensitas pola spektrum XRD. Metode pengembangan modifikasi membran elektroda indikator secara bertahap dengan ikatan silang GA, o-NPOE, polimer konduktif. Hasil terbaik diperoleh pada elektroda indikator PVA-Enzim/GA-2.9%/PPy+Asam Sulfonat/PVC-KTpCIPB-o-NPOE. Analisis kurva linear sampel As-1 sensitivitas 41.56 mV/dekade, rentang deteksi  $10^{-4}$  -  $10^{-1}$  M serta limit deteksi  $10^{-4}$  M,  $R^2 = 97.51$  %. Elektrode indikator terbaik adalah EI<sub>5</sub>.

Key word : PVA-Enzim/GA-2.9%/PPy+H<sub>2</sub>SO<sub>4</sub> atau Asam Sulfonat/PVC-KTpCIPB-o-NPOE, Potensiometri biosensor, Immobilisasi enzim urease

## Introduction

Modifikasi lapisan membran elektroda indikator dimulai dari (1) PVA-Enzim/PVC-KTpCIPB [Dana Vlascici 2008], (2) PVA-Enzim/GA-2.9%/PVC-KTpCIPB [Aparicio-Collado, 2021; Dana Vlascici 2008], (3) PVA-Enzim/GA-2.9%/PVC-KTpCIPB-o-NPOE, (4) PVA-Enzim/GA-2.9%/PPy+ H<sub>2</sub>SO<sub>4</sub>/PVC - KTpCIPB-o-NPOE, PVA-Enzim/GA-2.9%/PPy+Asam Sulfonat/PVC-KTpCIPB-o-NPOE.

Masing masing elektroda indikator diberi notasi EI<sub>1</sub>, EI<sub>2</sub>, EI<sub>3</sub>, EI<sub>4</sub> dan EI<sub>5</sub>. EI<sub>1</sub> pola spektrum absorbansi sempit menghasilkan rentang deteksi kecil dimodifikasi menjadi EI<sub>2</sub>. EI<sub>2</sub> dimodifikasi dengan menambahkan lapisan GA pada variasi 2.6%, 2.9% dan 3.0% di PVA-Enzim dari EI<sub>1</sub>. Larutan GA dianalisis dengan UV-Vis pola spektrum absorbansi meningkatkan lebar puncak absrbansi yang berpengaruh pada rntang deteksi (lihat gambar 1a). Modifikasi EI<sub>2</sub> dilanjutkan lagi dengan menambahkan larutan o-NPOE pada variasi 61% dan 66% di larutan PVC-KTpCLPB. Larutan PVC-KTpCLPB-o-NPOE dianalisis dengan UV-Vis mengasilkan pola spektrum absorbansi terlihat gambar 1b. Analisis EI<sub>2</sub> dengan XRD

menghasilkan pola difraksi spektrum amorf di sekitar sudut  $2\theta$  20 – 25 derajat disertai turunnya intensitas energi lihat gambar 2a. Terbentuknya pola spektrum amorf dan menurunnya intensitas.

Atas dasar gambar 1 dan 2, peneliti lanjutkan modifikasi membran elektroda indikator dengan bahan polimer konduktor yaitu PPy. PPy ini hanya bisa larut di  $H_2SO_4$  dan Asam sulfonat.  $H_2SO_4$  larut pada konsentrasi 8 M sedangkan asam sulfonat larut pada konsentrasi 1 M. Modifikasi membran elektroda secara berurutan (1) PVA-Enzim/GA-2.9%/PPy+ $H_2SO_4$ /PVC-KTpCIPB-o-NPOE, dinotasi EI<sub>4</sub>-1(2) PVA-Enzim/GA-2.9%/PPy+Asam Sulfonat/PVC-KTpCIPB-o-NPOE dinotasi EI<sub>5</sub>-1. Prosedur modifikasi EI<sub>4</sub>-1 dan EI<sub>5</sub>-1, variasi jumlah tetes enzim urease yaitu satu tetes dan tiga tetes, hasil analisis dapat dilihat gambar 2b, 2c dan tabel 1. Lapisan membran elektroda terdiri dari empat lapisan, lapisan pertama PVA-Enzim, lapisan kedua GA 2.9%, lapisan ketiga PPy+ $H_2SO_4$  atau PPy+Asam Sulfonat, lapisan keempat PVC-KTpCIPB-o-NPOE 61%. Dipilihnya o-NPOE 61% dari analisis UV-Vis gambar 1. Dihubungkan dengan gambar 2b, 2c dan tabel 1, analisis pola spektrum difraksi XRD menunjukkan bekurang sangat banyak pola spektrum amorf diikuti juga meningkatnya pola spektru kristal.

Terseleksinya sampel elektroda indikator ini untuk memilih elektroda terbaik menurut modifikasi lapisan yang telah dianalisis pola spektrum absorbansi dari lapisan satu immobilisasi PVA-enzim; lapisan dua ikatan silang GA; lapisan tiga polimer konduksi PPy; dan lapisan empat plastesier o-NPOE pada PVC-KTpCIPB [El-Naby, E.H., 2019]. Setelah analisis XRD dari sampel EI<sub>5</sub>-1, EI<sub>5</sub>-3, EI<sub>4</sub>-1, EI<sub>4</sub>-3 dipilih sampel terbaik yaitu EI<sub>5</sub>-1 dan EI<sub>4</sub>-1. kedua sampel dianalisis dengan FTIR, respon waktu sel potensiometer dan analisis kurva linear menentukan sensitivitas, rentang deteksi, limit deteksi dan  $R^2$ .

## Methods

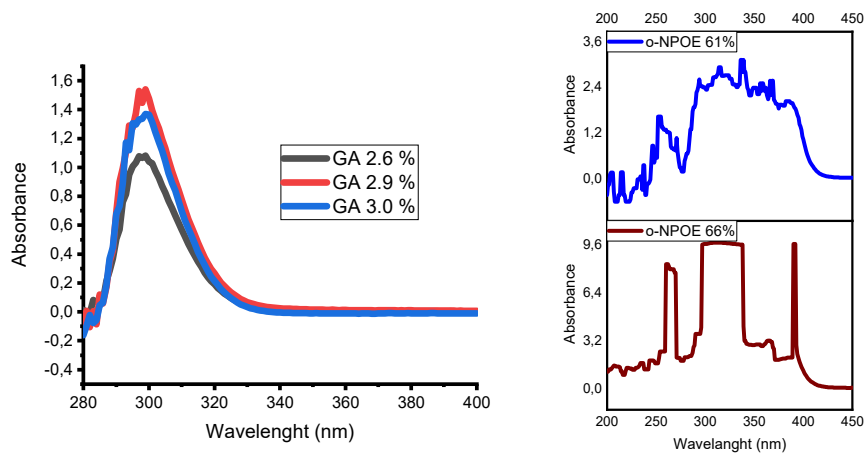
Metode dalam penelitian ini adalah metode potensiometri biosensor [Thakur dan Ragavan, 2013]; Amal M. Al-Mohaimed, at. All 2021; Linda Bertel 2021] teknik immobilisasi [Nimse, 2014] enzim urease yang analit urea, dengan menggunakan sel potensiometri menentukan kelayakan sensor urea berdasarkan (1) respon waktu sampel EI<sub>5</sub>-1 dan EI<sub>4</sub>-1, (2) melalui analisis kurva linear. Bahan terdiri dari tungsten diameter 1,0 mm 267 562 99,99%, PVA [-CH<sub>2</sub>CHOH-]<sub>n</sub>, enzim EC 3.5.1.5 (Urease) U4002, Glutaraldehyde (GA), PPy,  $H_2SO_4$ , asam sulfonat, PVC (CH<sub>2</sub>CHCl)<sub>n</sub>, potassium tetrakis 4-chlorophenyl borate (ClC<sub>6</sub>H<sub>4</sub>)<sub>4</sub>BK, tetrahydrofuran C<sub>4</sub>H<sub>8</sub>O, o-NPOE, KCl. Potensiometer (Keithley 199 DMM, USA), elektroda indikator tungsten (W), Ag/AgCl MF-2052 Elektroda referensi RE-5B dalam sel elektrokimia yang dirakit dengan mikrokomputer (instrumen Powerlab ADI, Australia), pengaduk magnet serta injeksi aliran (FIA).

## Result and Discussion

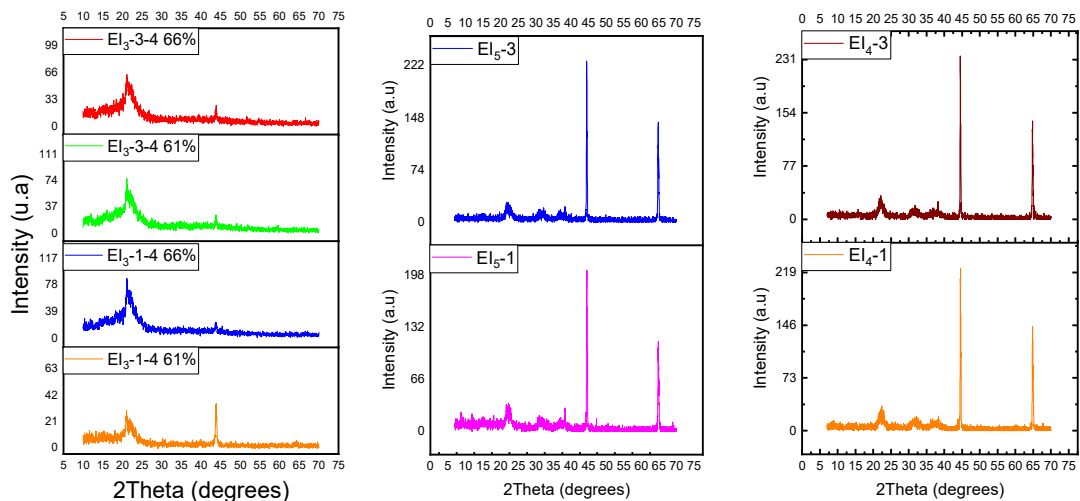
Gambar 1, menunjukkan analisis elektroda indikator dengan UV-Vis [Rezayi dkk, 2012; Singh dkk, 2013; Sharma dkk, 2016; Alarfaj, N.A. and El-Tohamy, M. F., 2020] terhadap larutan GA [Aparicio-Collado, 2021] dan larutan o-NPOE dalam PVC-KTpCIPB (PVC-KTpCIPB-o-NPOE) [Dana Vlascici 2008, Ulianas, Heng and Ahmad, 2011; Huang, 2014; Mir, Lugo, Tahirbegi and Josep Samitier, 2014, Kaur, Chhibber and Mittal, 2017; Elbeherly, 2019; Alarfaj, N.A. and El-Tohamy, M. F., 2020; Amr, 2021]. Pola spektrum absorbansi terhadap bilangan gelombang keduanya menunjukkan pelebaran pola spektrum absorbansi baik untuk GA maupun PVC-KTpCIPB-o-NPOE. Analisis gambar 1a dan 1b

terilih GA 2.9 % terbaik dan PVC-KTpCIPB-o-NPOE (o-NPOE 61). atas dasar elektroda indikator dilapisi untuk lapisan kedua dan keempat.

Gambar 2, menunjukkan analisis pola spektrum difraksi XRD [Shawky, 2021] untuk elektroda indikator (a) EI<sub>3</sub>-1-4 61%, EI<sub>3</sub>-1-4 66%, EI<sub>3</sub>-3-4 61% dan EI<sub>3</sub>-3-4 66%, (b) EI<sub>5</sub>-1, EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, EI<sub>4</sub>-3. Pada gambar 2a pola spektrum difraksi XRD dari EI<sub>3</sub>-1-4 61%, EI<sub>3</sub>-1-4 66%, EI<sub>3</sub>-3-4 61%, terbentuknya pola spektrum amorf dan kristal yang rendah intensitasnya. Berbeda dengan gambar 2b EI<sub>5</sub>-1 dan EI<sub>5</sub>-3, 2c EI<sub>4</sub>-1 dan EI<sub>4</sub>-3 [Hakim et al, 2021], terbentuk pola spektrum amorf yang rendah dan kristal yang tinggi intensitasnya. Jadi sudah jelas pola lapisan elektroda indikator EI<sub>5</sub>-1, EI<sub>5</sub>-3 dan EI<sub>4</sub>-1, EI<sub>4</sub>-3, masing-masing dalam variasi jumlah tetes enzim urease satu tetes dan tiga tetes. Tinggi puncak intensitas energi terhadap sudut difraksi 2theta dapat dilihat tabel 1 (a) EI<sub>5</sub>-1 tinggi intensitas 204 (a.u) pada sudut difraksi 44.54 derajat, (b) EI<sub>5</sub>-3 tinggi intensitas 228 (a.u) pada sudut difraksi 44.5 derajat, (c) EI<sub>4</sub>-1 tinggi intensitas 224 (a.u) pada sudut difraksi 44.54 derajat, (d) EI<sub>4</sub>-3 tinggi intensitas 236 (a.u) pada sudut difraksi 44.48 derajat.



Gambar 1. Analisis UV-Vis larutan (a) GA 2.6%, 2.9% dan 3.0%, (b) PVC-KTpCIPB-o-NPOE 61%, 66%.



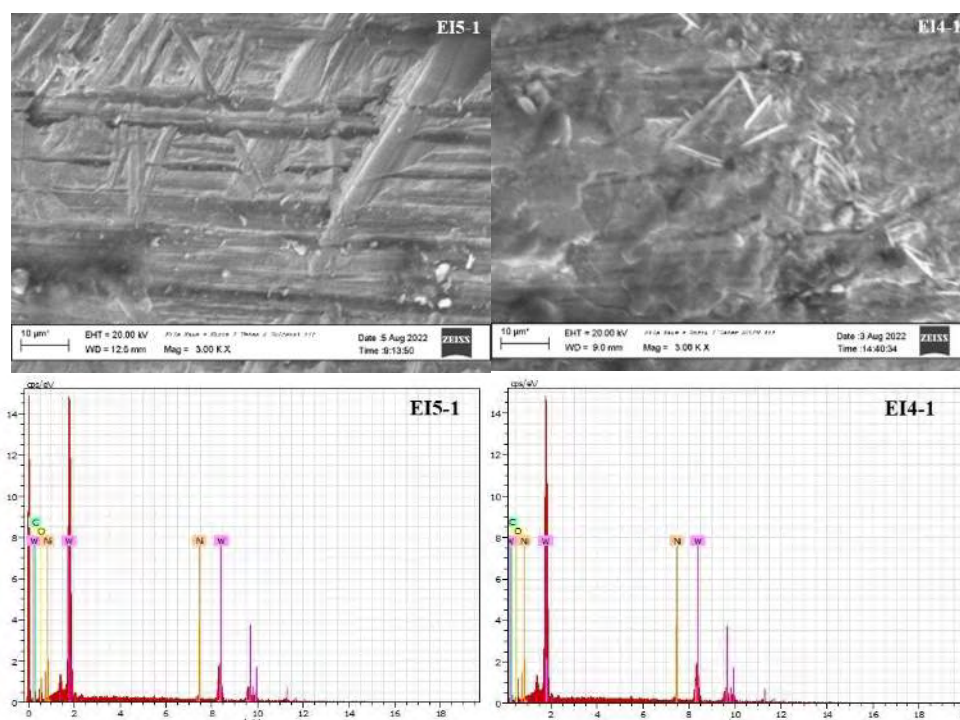
Gambar 2. Analisis pola spektrum difraksi XRD elektroda indikator (a) EI<sub>3</sub>-1-4 61%, EI<sub>3</sub>-1-4 66%, EI<sub>3</sub>-3-4 61% dan EI<sub>3</sub>-3-4 66%, (b) EI<sub>5</sub>-1, EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, EI<sub>4</sub>-3

**Table 1.** Analisis pola spektrum EDX elektroda indikator (a) EI<sub>5</sub>-1, (b) EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, (d) EI<sub>4</sub>-3

2Theta	EI <sub>5</sub> -1	EI <sub>5</sub> -3	EI <sub>4</sub> -1	EI <sub>4</sub> -3
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44.48	140	220	166	236
44.5	164	228	210	216
44.54	204	176	224	172

Peneliti masih melanjutkan analisis dengan SEM-EDX dan FTIR [Alharthi, S.S., Fallatah, A.M. and Al-Saidi, H.M., 2021] untuk kepastian yang jelas dari material yang digunakan pada elektroda indikator (a) EI<sub>5</sub>-1, (b) EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, (d) EI<sub>4</sub>-3 sebagai satu sampel terbaik. Analisis morfologi SEM dengan perbesaran 3 Kx pada 10 μm tegangan 20 Kv menghasilkan perbedaan intensitas energi 13.5 cps/keV 1M sampel EI<sub>5</sub>-1 dan intensitas energi 14.7 cps/keV, 8 M sampel EI<sub>4</sub>-1. Atas dasar tabel 1 diperoleh peningkatan intensitas analisis XRD, tabel 2 diperoleh ketidak sesuaian data lebih besar persen berat EI<sub>4</sub>-1 dari pada persen berat EI<sub>5</sub>-1. Sedangkan analisis FTIR gambar 4 dan tabel 3 transmitansi meningkat untuk elektroda indikator EI<sub>5</sub>-1 dan EI<sub>5</sub>-3, sebaliknya transmitansi menurun untuk elektroda indikator EI<sub>4</sub>-1 dan EI<sub>4</sub>-3. Analisis XRD, FTIR dan SEM-EDX [Amal M. Al-Mohaimed, at. All 2021; Linda Bertel, 2021; Wei Chen 2022] maka terpilih sampel terbaik adalah EI<sub>5</sub>-1 dan EI<sub>4</sub>-1.



**Gambar 3.** SEM morphology and Spectrum pattern of intensity to EDX energy from indicator electrode (a) EI<sub>5</sub>-1, (b) EI<sub>4</sub>-1

**Tabel 2.** Persen berat elektroda indikator dari pola spektrum EDX sampel EI<sub>5</sub>-1 dan EI<sub>4</sub>-1

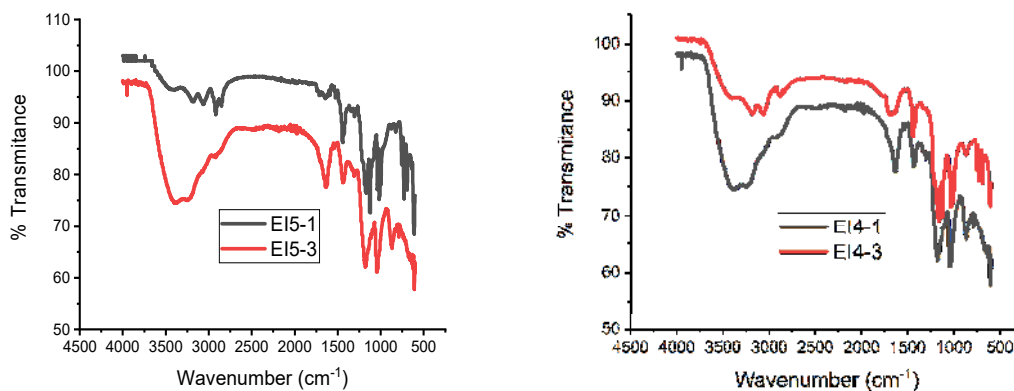
Elektroda Indikator	Material	unn. C	norm. C	Atom. C	Error (1 Sigma)
		[wt.%]	[wt.%]	[at.%]	[wt.%]
EI <sub>5</sub> -1	W 74 L-series	69.07	76.86	29.23	2.28
EI <sub>4</sub> -1	W 74 L-series	81.95	83.86	32.85	2.65

**Table 3.** Table of transmittance ranges for sample wave numbers (a) EI<sub>4</sub>-1, (b) EI<sub>5</sub>-1

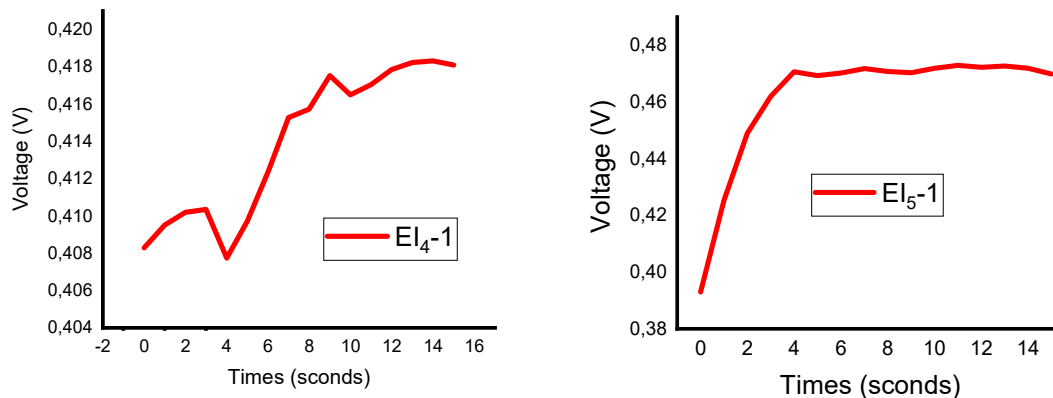
Wavenumber (cm <sup>-1</sup> )	Transmittance (%)			
	EI <sub>4</sub> -1	EI <sub>4</sub> -3	EI <sub>5</sub> -1	EI <sub>5</sub> -3

600	60.8895	75.5583	75.9339	48.5782
4000	97.9351	100.9955	102.5625	100.8259

Setelah terpilih dua sampel elektroda indikator EI<sub>5</sub>-1 dan EI<sub>4</sub>-1, sesuai dengan metode potensiometri biosensor digunakan sel potensiometer untuk uji kelayakan respon waktu elektroda indikator (gambar 5). Analisa respon waktu [Linda Bertel 2021] gambar 5a dan 5b terbaik diperoleh pada sampel EI<sub>5</sub>-1 juga didukung oleh data pada tabel 4 bahwa elektroda indikator EI<sub>5</sub>-1 memiliki sensitivitas 41.56 mV/dekade, rentang deteksi 10<sup>-4</sup> - 10<sup>-1</sup> M, limit deteksi 10<sup>-4</sup> M dan R<sup>2</sup> = 97.51% [Rahman, 2008; Hakim, 2019; Amal M. Al-Mohaimed, et. Al 2021]. Lebih besar rentang deteksi EI<sub>5</sub>-1 daripada EI<sub>4</sub>-1 rentang deteksi 10<sup>-3</sup> - 10<sup>-1</sup> M, limit deteksi 10<sup>-3</sup> M sedangkan R<sup>2</sup> = 98.83% [Ibupato dkk, 2012; Huang dkk, 2014; Elhag dkk, 2014].



**Figure 4.** The pattern of the transmittance spectrum against the wavenumber of the indicator electrodes (a) EI<sub>5</sub>-1, EI<sub>5</sub>-3, (b) EI<sub>4</sub>-1, EI<sub>4</sub>-3



**Gambar 5.** Respon waktu (a) EI<sub>4</sub>-1, (b) EI<sub>5</sub>-1

**Tabel 4.** Kurva linear elektroda indikator EI<sub>4</sub>-1 dan EI<sub>5</sub>-1

Lapisan membran elektroda indikator	Sensitivitas mV/dekade	Rentang deteksi (M)	Limit deteksi (M)	R-square (R <sup>2</sup> ) (%)
EI <sub>4</sub> -1	43.79	10 <sup>-3</sup> - 10 <sup>-1</sup>	10 <sup>-3</sup>	98.83
EI <sub>5</sub> -1	41.56	10 <sup>-4</sup> - 10 <sup>-1</sup>	10 <sup>-4</sup>	97.51

Dalam deteksi elektrokimia, sinyal terkait dengan interaksi analit di ukur melalui elektroda. Pengukuran dapat dilakukan dengan cara (a) menghubungkan arus dan tegangan, yaitu biosensor voltametri dan metrik konduktor; (b) arus atau tegangan terhadap waktu, yaitu amperometrik atau potensiometri; (c) imajiner versus bagian nyata dari impedansi, yaitu, impedometrik; (d) mengalirkan arus versus tegangan saluran dalam biosensor FET [Linda Bertel, 2021], kesepakatan dengan analisis yang dilaporkan sebelumnya (yaitu, FTIR, XRD, dan SEM) [Wei Chen, 2022] dan mengkonfirmasi interaksi dengan pengubah organik yang ditambahkan.

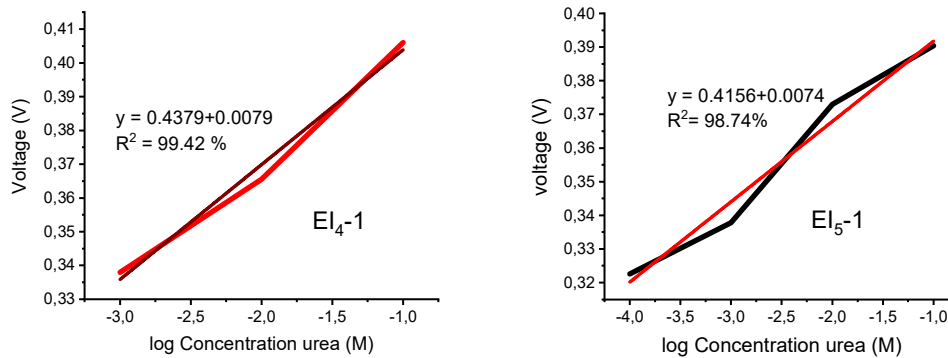


Figure 7. Analisis kurva linear (1) EI<sub>5</sub>-1, (b) EI<sub>4</sub>-1

Prosedur penelitian ini mengikuti (a) Analisis transmitansi, edx, xrd [Bibi, Aamna 2022], edx, xrd, kurva linear, sensitivitas dan rentang deteksi [Mashuni, Mashuni, 2022], transmitansi, konsentrasi, sensitivitas [Er-Yuan Chuang, 2021; Hui-Tzung Luh, 2022; Sriwichai, Saengrawee 2022]; (b) Sistem biosensor [Thakur dan Ragavan, 2013] yaitu (1) selektivitas, (2) sensitivitas, (3) respons linearitas yaitu rentang konsentrasi dari analit target yang akan diukur, (4) reproduksibilitas respons sinyal, sampel yang memiliki konsentrasi yang sama dianalisis beberapa kali harus memberikan respons yang sama, (5) waktu respons cepat dan waktu pemulihan untuk dapat digunakan kembalinya sistem biosensor, (6) Stabilitas dan masa operasi; (c) Teknik imobilisasi [Nimse dikembangkan didasarkan pada tiga mekanisme penting yaitu (1) adsorpsi fisik, (2) imobilisasi kovalen, (3) imobilisasi streptavidin-biotin. Pencapaian sensitivitas tinggi dan selektivitas membutuhkan minimalisasi adsorpsi nonspesifik dan stabilitas.

## Conclusion

Sebagai kesimpulan dari analisis XRD, SEM-EDX dan FTIR, respon waktu serta analisis kurva linear diperoleh sampel terbaik adalah elektroda indikator EI<sub>5</sub>-1 dengan modifikasi empat lapisan yaitu PVA-Enzim/GA-2.9%/PPy+Asam Sulfonat/PVC-KTpCIPB-o-NPOE.

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Marta-Usu

# Manufacture and characterization of indicator electrodes from PPy+H<sub>2</sub>SO<sub>4</sub> and PPy+Sulfonic acid as a urea sensor using urease enzyme immobilization technique in PVA.

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## Abstraks

Research has been carried out on indicator electrodes (1) PVA-Enzyme/PVC-KTpCIPB, sensitivity 19,069 mV/decade, detection range  $1.10^{-5}$  -  $5.10^{-4}$  M, detection limit  $1.10^{-5}$  M. The width of the peak UV-vis absorbance is narrow (2) PVA-Enzyme/GA-2.9%/PVC-KTpCIPB wide UV-vis absorbance peak but the absorbance peak decreased, (3) PVA-Enzyme/GA-2.9%/PVC-KTpCIPB-o-NPOE XRD analysis amorphous spectral pattern appeared (4) PVA-Enzyme/GA-2.9%/PPy+H<sub>2</sub>SO<sub>4</sub>/PVC-KTpCIPB-o-NPOE (5) PVA-Enzyme/GA-2.9%/PPy+Sulfonic Acid/PVC-KTpCIPB-o-NPOE, amorphous spectrum pattern in (4) and (5) were greatly reduced for the enzyme variation of 0.6 g in 0.5 mL (50% water + 50% alcohol). GA plays a role in increasing the detection range, o-NPOE forms amorphous, enzyme variations increase the intensity of the XRD spectrum pattern. The method of developing a gradual modification of the indicator electrode membrane by cross-linking GA, o-NPOE, conductive polymer. The best results were obtained at the indicator electrode PVA-Enzyme/GA-2.9%/PPy+Sulfonic Acid/PVC-KTpCIPB-o-NPOE. Analysis of the linear curve of the sample EI<sub>5</sub>-1 with a sensitivity of 41.56 mV/decade, a detection range of  $10^{-4}$  -  $10^{-1}$  M and a detection limit of  $10^{-4}$  M,  $R^2 = 97.51\%$ . The best indicator electrode is EI<sub>5</sub>-1.

Key words : PVA-Enzyme/GA-2.9%/PPy+H<sub>2</sub>SO<sub>4</sub> or Sulphonic Acid/PVC-KTpCIPB-o-NPOE, Biosensor potentiometry, Immobilization of urease enzyme

## Introduction

Modification of the indicator electrode membrane layer started from (1) PVA-Enzyme/PVC-KTpCIPB [Dana Vlascici 2008], (2) PVA-Enzyme/GA-2.9%/PVC-KTpCIPB [Aparicio Collado, 2021; Dana Vlascici 2008], (3) PVA-Enzyme/GA-2.9%/PVC-KTpCIPB-o-NPOE, (4) PVA-Enzyme/GA-2.9%/PPy+ H<sub>2</sub>SO<sub>4</sub> /PVC - KTpCIPB-o-NPOE, PVA- Enzyme/GA-2.9%/PPy+Sulfonic Acid/PVC-KTpCIPB-o-NPOE.

Each indicator electrode is given the notation EI<sub>1</sub>, EI<sub>2</sub>, EI<sub>3</sub>, EI<sub>4</sub> and EI<sub>5</sub>. EI<sub>1</sub> narrow absorbance spectrum pattern resulting in a small detection range modified to EI<sub>2</sub>. EI<sub>2</sub> was modified by adding a GA layer at variations of 2.6%, 2.9% and 3.0% in PVA-Enzyme from EI<sub>1</sub>. The GA solution was analyzed by UV-vis. The absorbance spectrum pattern increases the width of the absorption peak which affects the detection range (see Figure 1a). EI<sub>2</sub> modification was continued by adding o-NPOE solution at variations of 61% and 66% in PVC-KTpCIPB solution. The PVC-KTpCIPB-o-NPOE solution was analyzed by UV-vis to

produce the absorbance spectrum pattern seen in Figure 1b. EI<sub>2</sub> analysis with XRD produces an amorphous spectral diffraction pattern around the 2theta angle of 20-25 degrees accompanied by a decrease in energy intensity, see Figure 2a. The formation of an amorphous spectrum pattern and a decrease in intensity.

Based on Figures 1 and 2, the researchers continued to modify the indicator electrode membrane with a conducting polymer material, namely PPy. This PPy can only dissolve in H<sub>2</sub>SO<sub>4</sub> and Sulphonic Acid. H<sub>2</sub>SO<sub>4</sub> is soluble at a concentration of 8 M while sulfonic acid is soluble at a concentration of 1 M. Modification of the electrode membrane in sequence (1) PVA-Enzyme/GA-2.9%/PPy+H<sub>2</sub>SO<sub>4</sub>/PVC-KTpCIPB-o-NPOE, denoted EI<sub>4</sub>-1 (2) PVA-Enzyme/GA-2.9%/PPy+Sulfonic Acid/PVC-KTpCIPB-o-NPOE denoted EI<sub>5</sub>-1. Modification procedures EI<sub>4</sub>-1 and EI<sub>5</sub>-1, variations in the number of drops of urease enzyme are one drop and three drops, the results of the analysis can be seen in Figures 2b, 2c and table 1. The electrode membrane layer consists of four layers, the first layer is PVA-Enzyme, the second layer GA 2.9%, third layer PPy+H<sub>2</sub>SO<sub>4</sub> or PPy+Sulfonic Acid, fourth layer PVC-KTpCIPB-o-NPOE 61%. Selected o-NPOE 61% from the UV-Vis analysis of Figure 1. Compared to Figures 2b, 2c and table 1, the XRD diffraction spectrum pattern analysis showed a very large decrease in the amorphous spectrum pattern followed by an increase in the crystal spectral pattern.

This indicator electrode sample was selected to select the best electrode according to the layer modification which had analyzed the absorbance spectrum pattern of the PVA-enzyme immobilized layer; layer two GA crosslinks; PPy conduction polymer triple layer; and a layer of four o-NPOE plasticizers on PVC-KTpCIPB [El-Naby, E.H., 2019]. After XRD analysis of samples EI<sub>5</sub>-1, EI<sub>5</sub>-3, EI<sub>4</sub>-1, EI<sub>4</sub>-3, the best samples were EI<sub>5</sub>-1 and EI<sub>4</sub>-1. Both samples were analyzed by FTIR, cell response potentiometer and linear curve analysis determining sensitivity, detection range, detection limit and R<sup>2</sup>.

## Methods

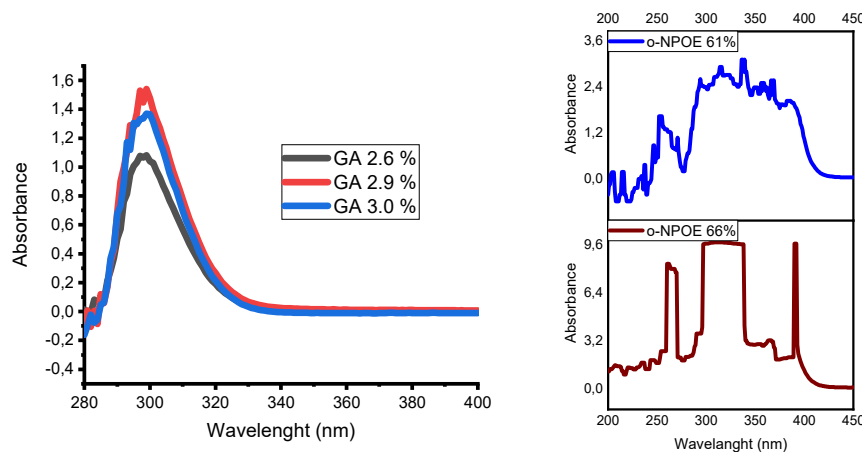
The method in this study is the biosensor potentiometric method [Thakur and Ragavan, 2013]; Amal M. Al-Mohaimed, et al. 2021; Linda Bertel 2021] immobilization technique [Nimse, 2014] urease enzyme which analytes urea, using potentiometric cells to determine the feasibility of urea sensors based on (1) response time of samples EI<sub>5</sub>-1 and EI<sub>4</sub>-1, (2) through linear curve analysis. Materials consist of 1.0 mm diameter tungsten 267 562 99.99%, PVA [-CH<sub>2</sub>CHOH-]<sub>n</sub>, enzyme EC 3.5.1.5 (Urease) U4002, Glutaraldehyde (GA), PPy, H<sub>2</sub>SO<sub>4</sub>, Sulphonic acid, PVC (CH<sub>2</sub>CHCl)<sub>n</sub>, potassium tetrakis 4-chlorophenyl borate (ClC<sub>6</sub>H<sub>4</sub>)<sub>4</sub>BK, tetrahydrofuran C<sub>4</sub>H<sub>8</sub>O, o-NPOE, KCl. Potentiometer (Keithley 199 DMM, USA), tungsten indicator electrode (W), Ag/AgCl MF-2052 RE-5B reference electrode in a microcomputer assembled electrochemical cell (ADI Powerlab instruments, Australia), magnetic stirrer and flow injection (FIA)

## Result and Discussion

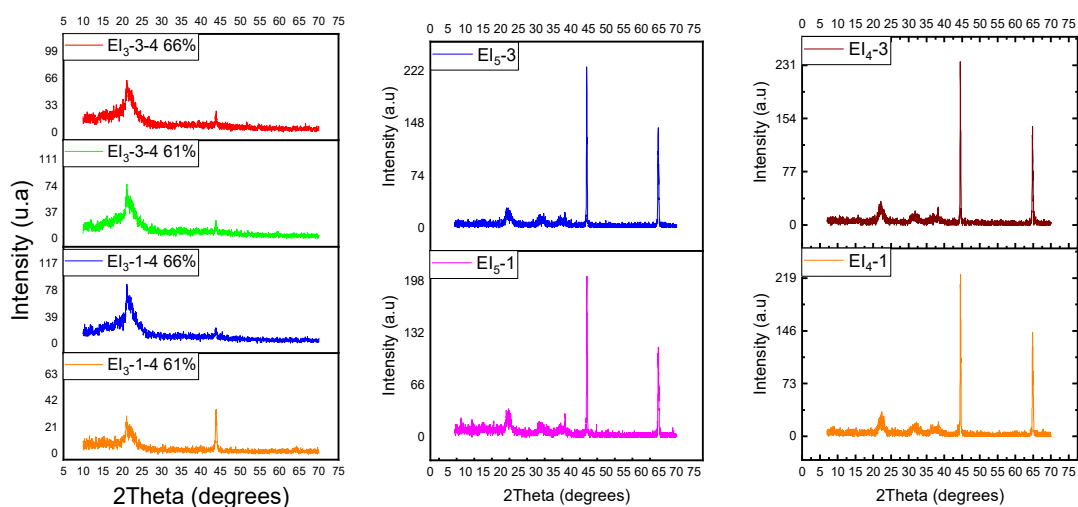
Figure 1, shows the analysis of the indicator electrode with UV-Vis [Rezayi et al, 2012; Singh et al, 2013; Sharma et al, 2016; Alarfaj, N.A. and El-Tohamy, M. F., 2020] on GA solution [Aparicio-Collado, 2021] and o-NPOE solution in PVC-KTpCIPB (PVC-KTpCIPB-o-NPOE) [Dana Vlascici 2008, Ulianas, Heng and Ahmad, 2011; Huang, 2014; Mir, Lugo, Tahirbegi and Josep Samitier, 2014, Kaur, Chhibber and Mittal, 2017; Elbeheri, 2019; Alarfaj, N.A. and El-Tohamy, M. F., 2020; Amr, 2021]. The absorbance spectrum pattern with

respect to the wavenumber of both shows a widening of the absorbance spectrum pattern for both GA and PVC-KTpCIPB-o-NPOE. Analysis of Figures 1a and 1b selected the best 2.9% GA and PVC-KTpCIPB-o-NPOE (o-NPOE 61%). on the basis of coated indicator electrodes for the second and fourth layers.

Figure 2, shows the analysis of the XRD diffraction spectrum pattern [Shawky, 2021] for the indicator electrodes (a) EI<sub>3</sub>-1-4 61%, EI<sub>3</sub>-1-4 66%, EI<sub>3</sub>-3-4 61% and EI<sub>3</sub>-3-4 66 %, (b) EI<sub>5</sub>-1, EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, EI<sub>4</sub>-3. In Figure 2a the XRD diffraction spectrum pattern from EI<sub>3</sub>-1-4 61%, EI<sub>3</sub>-1-4 66%, EI<sub>3</sub>-3-4 61%, amorphous and crystalline spectrum patterns are formed with low intensity. In contrast to Figures 2b EI<sub>5</sub>-1 and EI<sub>5</sub>-3, 2c EI<sub>4</sub>-1 and EI<sub>4</sub>-3 [Hakim et al, 2021], a low amorphous spectrum pattern and high intensity crystals are formed. So it is clear that the pattern of the indicator electrode layers EI<sub>5</sub>-1, EI<sub>5</sub>-3 and EI<sub>4</sub>-1, EI<sub>4</sub>-3, varies in the number of drops of urease enzyme, one drop and three drops, respectively. The peak height of energy intensity with respect to the diffraction angle of 2theta can be seen in table 1 (a) EI<sub>5</sub>-1 high intensity 204 (a.u) at a diffraction angle of 44.54 degrees, (b) EI<sub>5</sub>-3 high intensity 228 (a.u) at a diffraction angle of 44.5 degrees, (c) EI<sub>4</sub>-1 high intensity 224 (a.u) at a diffraction angle of 44.54 degrees, (d) EI<sub>4</sub>-3 high intensity 236 (a.u) at a diffraction angle of 44.48 degrees.



**Figure 1.** UV-Vis analysis of solutions (a) GA 2.6%, 2.9% and 3.0%, (b) PVC-KTpCIPB-o-NPOE 61%, 66%.



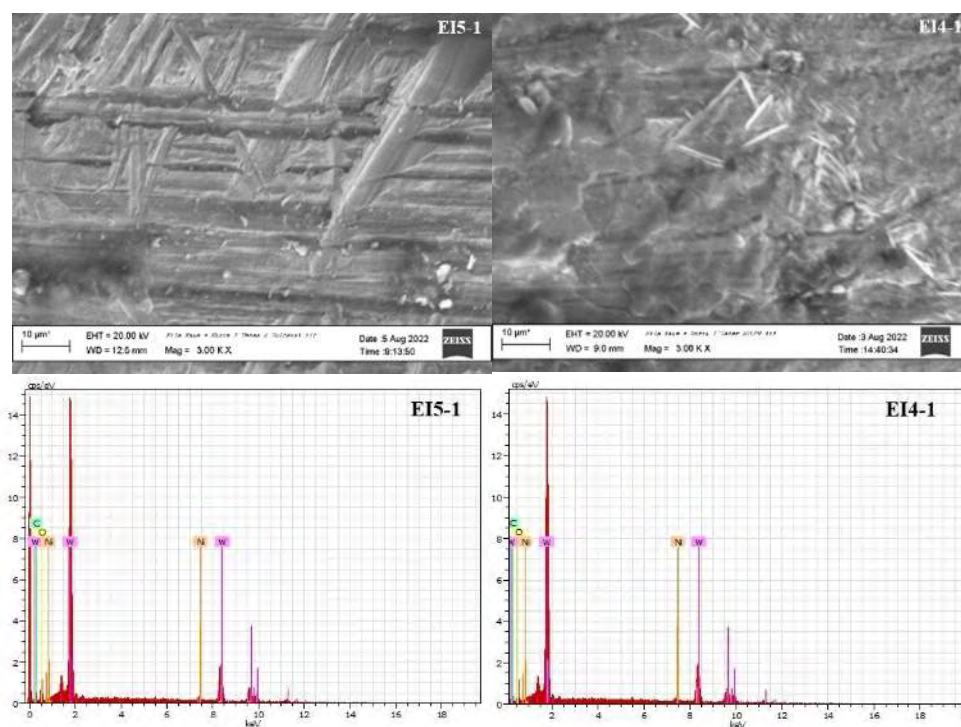
**Figure 2.** Analysis of the XRD diffraction spectrum pattern of the indicator electrode (a) EI<sub>3</sub>-1-4 61%, EI<sub>3</sub>-1-4 66%, EI<sub>3</sub>-3-4 61% and EI<sub>3</sub>-3-4 66%, (b) EI<sub>5</sub>-1 and EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1 and EI<sub>4</sub>-3.

**Table 1.** Analysis of the EDX spectrum pattern of the indicator electrode (a) EI5-1, (b) EI5-3, (c) EI4-1, (d) EI4-3

2Theta	EI5-1	EI5-3	EI4-1	EI4-3
44.48	140	220	166	236
44.5	164	228	210	216
44.54	204	176	224	172

Researchers are still continuing the analysis with SEM-EDX and FTIR [Alharthi, S.S., Fallatah, A.M. and Al-Saidi, H.M., 2021] for clear assurance of the material used in the indicator electrodes (a) EI5-1, (b) EI5-3, (c) EI4-1, (d) EI4-3 as one sample best.

SEM morphology analysis with 3 Kx magnification at a voltage of 10 m 20 Kv resulted in a difference in energy intensity of 13.5 cps/keV 1M sample EI5-1 and energy intensity 14.7 cps/keV 8 M sample EI4-1. Based on table 1, it was obtained that the intensity of XRD analysis increased, table 2 obtained that the data discrepancy was greater in weight percent EI4-1 than weight percent EI5-1. While the FTIR analysis in Figure 4 and Table 3 increases the transmittance for the indicator electrodes EI5-1 and EI5-3, on the contrary, the transmittance decreases for the indicator electrodes EI4-1 and EI4-3. XRD, FTIR and SEM-EDX analysis [Amal M. Al-Mohaimed, et. All 2021; Linda Bertel, 2021; Wei Chen 2022] then the best samples are EI5-1 and EI4-1.



**Figure 3.** SEM morphology and Spectrum pattern of intensity to EDX energy from indicator electrode (a) EI5-1, (b) EI4-1

**Table 2.** Weight percentage of indicator electrodes from the EDX spectrum pattern of samples EI5-1 and EI4-1

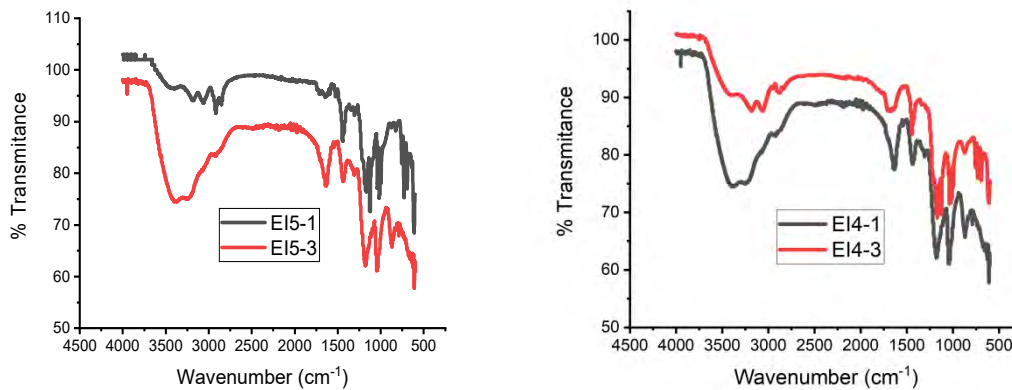
Indicator Electrodes	Material	unn. C	norm. C	Atom. C	Error (1 Sigma)
		[wt.%]	[wt.%]	[at.%]	[wt.%]

EI <sub>5</sub> -1	W 74 L-series	69.07	76.86	29.23	2.28
EI <sub>4</sub> -1	W 74 L-series	81.95	83.86	32.85	2.65

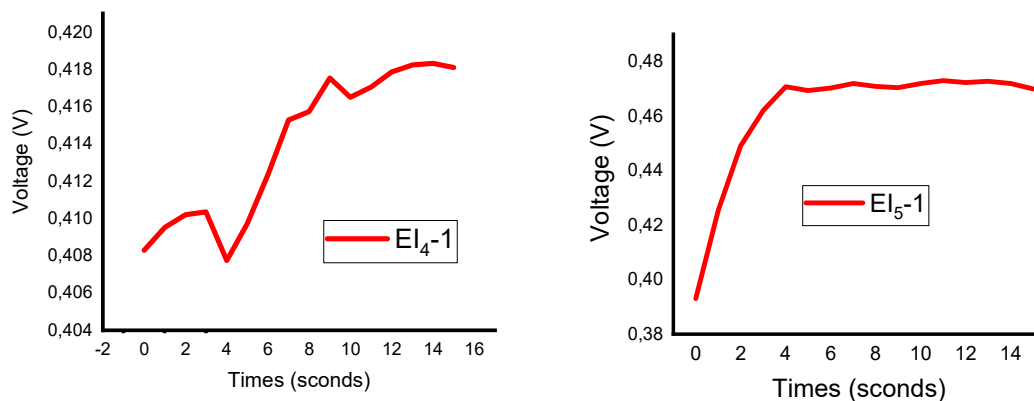
**Table 3.** Table of transmittance ranges for sample wave numbers (a) EI<sub>4</sub>-1, (b) EI<sub>5</sub>-1

Wavenumber (cm <sup>-1</sup> )	Transmittance (%)			
	EI <sub>4</sub> -1	EI <sub>4</sub> -3	EI <sub>5</sub> -1	EI <sub>5</sub> -3
600	60.8895	75.5583	75.9339	48.5782
4000	97.9351	100.9955	102.5625	100.8259

After selecting two samples of indicator electrodes EI<sub>5</sub>-1 and EI<sub>4</sub>-1, according to the biosensor potentiometry method, a potentiometer cell was used to test the feasibility of the indicator electrode response time (figure 5). The best response time analysis [Linda Bertel 2021] Figures 5a and 5b were obtained on the EI<sub>5</sub>-1 sample also supported by the data in table 4 that the EI<sub>5</sub>-1 indicator electrode has a sensitivity of 41.56 mV/decade, a detection range of 10<sup>-4</sup> - 10<sup>-1</sup> M, detection limit is 10<sup>-4</sup> M and R<sup>2</sup> = 97.51% [Rahman, 2008; Judge, 2019; Amal M. Al-Mohaimed, et. Al 2021]. The detection range of EI<sub>5</sub>-1 is greater than that of EI<sub>4</sub>-1, the detection range is 10<sup>-3</sup> - 10<sup>-1</sup> M, the detection limit is 10<sup>-3</sup> M, while R<sup>2</sup> = 98.83% [Ibupato et al, 2012; Huang et al, 2014; Elhag et al, 2014].



**Figure 4.** The pattern of the transmittance spectrum against the wavenumber of the indicator electrodes (a) EI<sub>5</sub>-1, EI<sub>5</sub>-3, (b) EI<sub>4</sub>-1, EI<sub>4</sub>-3

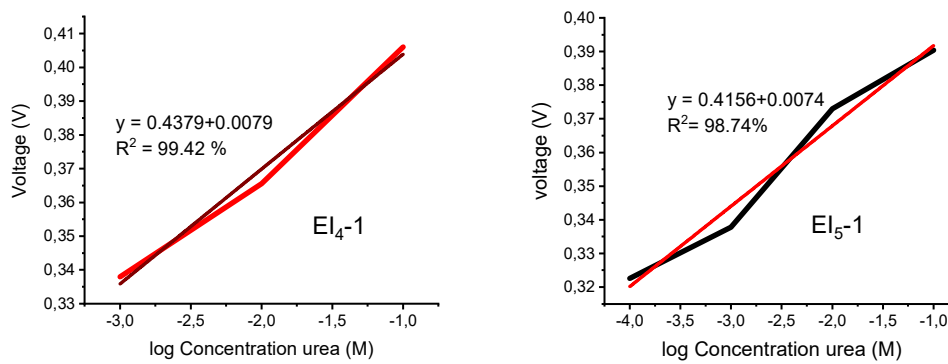


**Figure 5.** Response time (a) EI<sub>4</sub>-1, (b) EI<sub>5</sub>-1

**Table 4.** Linear curve of indicator electrodes EI<sub>4</sub>-1 and EI<sub>5</sub>-1

Indicator electrode membrane layer	Sensitivity mV/decade	Detection range (M)	Detection limit (M)	R-square (R <sup>2</sup> ) (%)
EI <sub>4</sub> -1	43.79	10 <sup>-3</sup> - 10 <sup>-1</sup>	10 <sup>-3</sup>	98.83
EI <sub>5</sub> -1	41.56	10 <sup>-4</sup> - 10 <sup>-1</sup>	10 <sup>-4</sup>	97.51

In electrochemical detection, the signal associated with the interaction of the analyte is measured through the electrode. Measurements can be made by (a) connecting current and voltage, namely voltammetric and conductor metric biosensors; (b) current or voltage with respect to time, ie, amperometric or potentiometric; (c) the imaginary versus the real part of the impedance, ie, impedometric; (d) drain current versus line voltage in a FET biosensor [Linda Bertel, 2021], agreement with previously reported analyzes (i.e., FTIR, XRD, and SEM) [Wei Chen, 2022] and confirming interactions with added organic modifiers.

**Figure 7.** Analisis kurva linear (1) EI<sub>5</sub>-1, (b) EI<sub>4</sub>-1

The procedure of this study followed (a) Analysis of transmittance, edx, xrd [Bibi, Aamna 2022], edx, xrd, linear curve, sensitivity and detection range [Mashuni, Mashuni, 2022], transmittance, concentration, sensitivity [Er-Yuan Chuang, 2021; Hui-Tzung Luh, 2022; Sriwichai, Saengrawee 2022]; (b) The biosensor system [Thakur and Ragavan, 2013], namely (1) selectivity, (2) sensitivity, (3) linearity response, namely the concentration range of the target analyte to be measured, (4) reproducibility of signal response, samples having a different concentration. the same analyzed several times should give the same response, (5) fast response time and recovery time for reusability of the biosensor system, (6) stability and operating life; (c) The [Nimse 2014] immobilization technique was developed based on three important mechanisms, namely (1) physical adsorption, (2) covalent immobilization, (3) streptavidin-biotin immobilization. Achieving high sensitivity and selectivity requires minimization of nonspecific adsorption and stability.

## Conclusion

As a conclusion from XRD, SEM-EDX and FTIR analysis, response time and linear curve analysis, the best sample is the EI<sub>5</sub>-1 indicator electrode with four layer modifications, namely PVA-Enzyme/GA-2.9%/PPy+Sulfonic Acid/PVC-KTpCIPB-o -NPOE.

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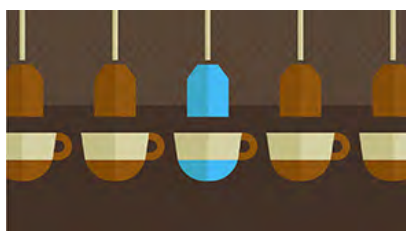
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# Manufacture and characterization of indicator electrodes from PPy + H<sub>2</sub>SO<sub>4</sub> and PPy + Sulfonic acid as a urea sensor using urease enzyme immobilization technique in PVA

Q1 Q2

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## Abstract

Research has been carried out on indicator electrodes (1) PVA-Enzyme/PVC-KTpCIPB, sensitivity 19,069 mV/decade, detection range  $1.10^{-5}$ – $5.10^{-4}$  M, detection limit  $1.10^{-5}$  M. The width of the peak UV–vis absorbance is narrow (2) PVA-Enzyme/GA-2.9 %/PVC-KTpCIPB wide UV–vis absorbance peak but the absorbance peak decreased, (3) PVA-Enzyme/GA-2.9 %/PVC-KTpCIPB-o-NPOE XRD analysis amorphous spectral pattern appeared (4) PVA-Enzyme/GA-2.9 %/PPy + H<sub>2</sub>SO<sub>4</sub>/PVC-KTpCIPB-o-NPOE (5) PVA-Enzyme/GA-2.9 %/PPy + Sulfonic Acid/PVC-KTpCIPB-o-NPOE, amorphous spectrum pattern in (4) and (5) were greatly reduced for the enzyme variation of 0.6 g in 0.5 mL (50 % water + 50 % alcohol). GA plays a role in increasing the detection range, o-NPOE forms amorphous, enzyme variations increase the intensity of the XRD spectrum pattern. The method of developing a gradual modification of the indicator electrode membrane by cross-linking GA, o-NPOE, conductive polymer. The best results were obtained at the indicator electrode PVA-Enzyme/GA-2.9 %/PPy + Sulfonic Acid/PVC-KTpCIPB-o-NPOE. Analysis of the linear curve of the sample EI<sub>5</sub>-1 with a sensitivity of 41.56 mV/decade, a detection range of  $10^{-4}$ – $10^{-1}$  M and a detection limit of  $10^{-4}$  M,  $R^2 = 97.51$  %. The best indicator electrode is EI<sub>5</sub>-1.

## Keywords:

PVA-enzyme/GA-2.9%/PPy+H<sub>2</sub>SO<sub>4</sub> or sulphonic acid/PVC-KTpCIPB-o-NPOE, Biosensor potentiometry, Immobilization of urease enzyme


## Abbreviations

No keyword abbreviations are available

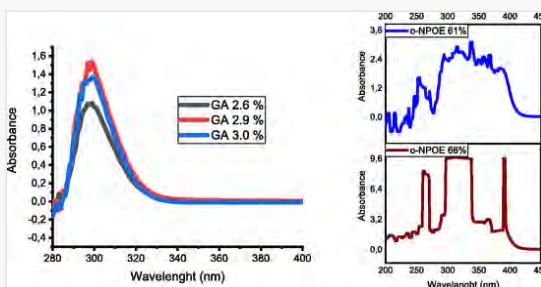
## 1 Introduction

Modification of the indicator electrode membrane layer started from (1) PVA-Enzyme/PVC-KTpCIPB [1,2] PVA-Enzyme/GA-2.9 %/PVC-KTpCIPB [1-3] PVA-Enzyme/GA-2.9 %/PVC-KTpCIPB-o-NPOE, [4] PVA-Enzyme/GA-2.9 %/PPy + H<sub>2</sub>SO<sub>4</sub>/PVC - KTpCIPB-o-NPOE, PVA-Enzyme/GA-2.9 %/PPy + Sulfonic Acid/PVC-KTpCIPB-o-NPOE.


Each indicator electrode is given the notation EI<sub>1</sub>, EI<sub>2</sub>, EI<sub>3</sub>, EI<sub>4</sub> and EI<sub>5</sub>. EI<sub>1</sub> narrow absorbance spectrum pattern resulting in a small detection range modified to EI<sub>2</sub>. EI<sub>2</sub> was modified by adding a GA layer at variations of 2.6 %, 2.9 % and 3.0 % in PVA-Enzyme from EI<sub>1</sub>. The GA solution was analyzed by UV-vis. The absorbance spectrum pattern increases the width of the absorption peak which affects the detection range (see Fig. 1a). EI<sub>2</sub> modification was continued by adding o-NPOE solution at variations of 61 % and 66 % in PVC-KTpCIPB solution. The PVC-KTpCIPB-o-NPOE solution was analyzed by UV-vis to produce the absorbance spectrum pattern seen in Fig. 1b. EI<sub>2</sub> analysis with XRD produces an amorphous spectral diffraction pattern around the 2theta angle of 20–25 degrees accompanied by a decrease in energy intensity, see Fig. 2a. The formation of an amorphous spectrum pattern and a decrease in intensity.

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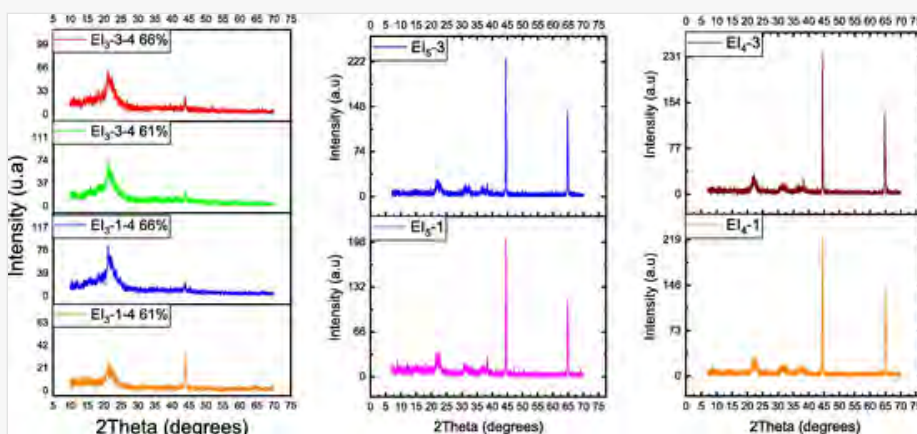
**Fig. 1**



UV-vis analysis of solutions (a) GA 2.6%, 2.9% and 3.0%, (b) PVC-KTpCIPB-o-NPOE 61%, 66%.

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**Fig. 2**



Analysis of the XRD diffraction spectrum pattern of the indicator electrode (a) EI<sub>3</sub>-1-4 61%, EI<sub>3</sub>-1-4 66%, EI<sub>3</sub>-3-4 61% and EI<sub>3</sub>-3-4 66%, (b) EI<sub>5</sub>-1 and EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1 and EI<sub>4</sub>-3.

Based on Figs. 1 and 2, the researchers continued to modify the indicator electrode membrane with a conducting polymer material, namely PPy. This PPy can only dissolve in H<sub>2</sub>SO<sub>4</sub> and Sulphonic Acid. H<sub>2</sub>SO<sub>4</sub> is soluble at a concentration of 8 M while sulfonic acid is soluble at a concentration of 1 M. Modification of the electrode membrane

in sequence (1) PVA-Enzyme/GA-2.9 %/PPy + H<sub>2</sub>SO<sub>4</sub>/PVC-KTpCIPB-o-NPOE, denoted EI<sub>4</sub>-1 (2) PVA-Enzyme/GA-2.9 %/PPy + Sulfonic Acid/PVC-KTpCIPB-o-NPOE denoted EI<sub>5</sub>-1. Modification procedures EI<sub>4</sub>-1 and EI<sub>5</sub>-1, variations in the number of drops of urease enzyme are one drop and three drops, the results of the analysis can be seen in Fig. 2b, c and Table 1. The electrode membrane layer consists of four layers, the first layer is PVA-Enzyme, the second layer GA 2.9 %, third layer PPy + H<sub>2</sub>SO<sub>4</sub> or PPy + Sulfonic Acid, fourth layer PVC-KTpCIPB-o-NPOE 61 %. Selected o-NPOE 61 % from the UV-vis analysis of Fig. 1. Compared to Fig. 2b, c and Table 1, the XRD diffraction spectrum pattern analysis showed a very large decrease in the amorphous spectrum pattern followed by an increase in the crystal spectral pattern (see Fig. 3).

Table 1

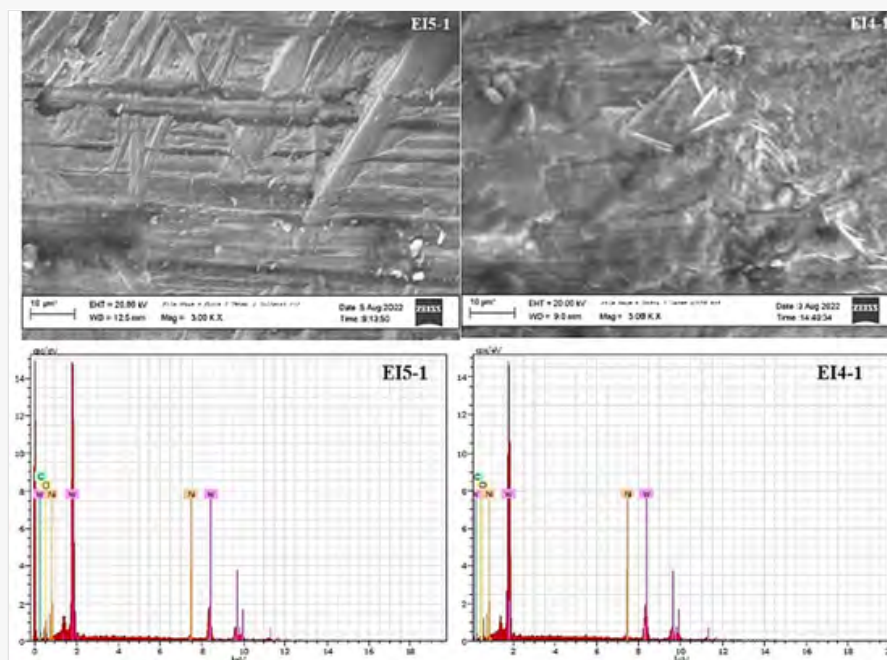
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Analysis of the EDX spectrum pattern of the indicator electrode (a) EI<sub>5</sub>-1, (b) EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, (d) EI<sub>4</sub>-3.

2Theta	EI <sub>5</sub> -1	EI <sub>5</sub> -3	EI <sub>4</sub> -1	EI <sub>4</sub> -3
44.48	140	220	166	236
44.5	164	228	210	216
44.54	204	176	224	172

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Fig. 3



SEM morphology and Spectrum pattern of intensity to EDX energy from indicator electrode (a) EI<sub>5</sub>-1, (b) EI<sub>4</sub>-1.

This indicator electrode sample was selected to select the best electrode according to the layer modification which had analyzed the absorbance spectrum pattern of the PVA-enzyme immobilized layer; layer two GA crosslinks; PPy conduction polymer triple layer; and a layer of four o-NPOE plasticizers on PVC-KTpCIPB [3]. After XRD analysis of samples EI<sub>5</sub>-1, EI<sub>5</sub>-3, EI<sub>4</sub>-1, EI<sub>4</sub>-3, the best samples were EI<sub>5</sub>-1 and EI<sub>4</sub>-1. Both samples were analyzed by FTIR, cell response potentiometer and linear curve analysis determining sensitivity, detection range, detection limit and R<sup>2</sup>.

## 2 Methods

The method in this study is the biosensor potentiometric method [4–6] immobilization technique [7] urease enzyme which analytes urea, using potentiometric cells to determine the feasibility of urea sensors based on (1) response time of samples EI<sub>5</sub>-1 and EI<sub>4</sub>-1, (2) through linear curve analysis. Materials consist of 1.0 mm diameter tungsten 267 562 99.99 %, PVA [–CH<sub>2</sub>CHOH–]<sub>n</sub>, enzyme EC 3.5.1.5 (Urease) U4002, Glutaraldehyde (GA), PPy, H<sub>2</sub>SO<sub>4</sub>, Sulphonic acid, PVC (CH<sub>2</sub>CHCl)<sub>n</sub>, potassium tetrakis 4-chlorophenyl borate (C<sub>10</sub>H<sub>4</sub>)<sub>4</sub>BK, tetrahydrofuran C<sub>4</sub>H<sub>8</sub>O, o-NPOE, KCl. Potentiometer (Keithley 199 DMM, USA), tungsten indicator electrode (W), Ag/AgCl MF-2052 rE-5B reference electrode in a microcomputer assembled electrochemical cell (ADI Powerlab instruments, Australia), magnetic stirrer and flow injection (FIA).

## 3 Result and discussion


Fig. 1, shows the analysis of the indicator electrode with UV–vis [8–11] on GA solution [2] and o-NPOE solution in PVC-KTpCIPB (PVC-KTpCIPB-o-NPOE) [1,16–18]. The absorbance spectrum pattern with respect to the wavenumber of both shows a widening of the absorbance spectrum pattern for both GA and PVC-KTpCIPB-o-NPOE. Analysis of Fig. 1a and 1b selected the best 2.9 % GA and PVC-KTpCIPB-o-NPOE (o-NPOE 61 %). on the basis of coated indicator electrodes for the second and fourth layers.

Fig. 2, shows the analysis of the XRD diffraction spectrum pattern [18] for the indicator electrodes (a) EI<sub>3</sub>-1-4 61 %, EI<sub>3</sub>-1-4 66 %, EI<sub>3</sub>-3-4 61 % and EI<sub>3</sub>-3-4 66 %, (b) EI<sub>5</sub>-1, EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, EI<sub>4</sub>-3. In Fig. 2a the XRD diffraction spectrum pattern from EI<sub>3</sub>-1-4 61 %, EI<sub>3</sub>-1-4 66 %, EI<sub>3</sub>-3-4 61 %, amorphous and crystalline spectrum patterns are formed with low intensity. In contrast to Fig. 2b EI<sub>5</sub>-1 and EI<sub>5</sub>-3, 2c EI<sub>4</sub>-1 and EI<sub>4</sub>-3 [19], a low amorphous spectrum pattern and high intensity crystals are formed. So it is clear that the pattern of the indicator electrode layers EI<sub>5</sub>-1, EI<sub>5</sub>-3 and EI<sub>4</sub>-1, EI<sub>4</sub>-3, varies in the number of drops of urease enzyme, one drop and three drops, respectively. The peak height of energy intensity with respect to the diffraction angle of 2theta can be seen in Table 1 (a) EI<sub>5</sub>-1 high intensity 204 (a.u) at a diffraction angle of 44.54 degrees, (b) EI<sub>5</sub>-3 high intensity 228 (a.u) at a diffraction angle of 44.5 degrees, (c) EI<sub>4</sub>-1 high intensity 224 (a.u) at a diffraction angle of 44.54 degrees, (d) EI<sub>4</sub>-3 high intensity 236 (a.u) at a diffraction angle of 44.48 degrees.

Researchers are still continuing the analysis with SEM-EDX and FTIR [20] for clear assurance of the material used in the indicator electrodes (a) EI<sub>5</sub>-1, (b) EI<sub>5</sub>-3, (c) EI<sub>4</sub>-1, (d) EI<sub>4</sub>-3 as one sample best.

SEM morphology analysis with 3 Kx magnification at a voltage of 10 m 20 Kv resulted in a difference in energy intensity of 13.5 cps/keV 1 M sample EI<sub>5</sub>-1 and energy intensity 14.7 cps/keV 8 M sample EI<sub>4</sub>-1. Based on Table 1, it was obtained that the intensity of XRD analysis increased, Table 2 obtained that the data discrepancy was greater in weight percent EI<sub>4</sub>-1 than weight percent EI<sub>5</sub>-1. While the FTIR analysis in Fig. 4 and Table 3 increases the transmittance for the indicator electrodes EI<sub>5</sub>-1 and EI<sub>5</sub>-3, on the contrary, the transmittance decreases for the indicator electrodes EI<sub>4</sub>-1 and EI<sub>4</sub>-3. XRD, FTIR and SEM-EDX analysis [6,21,22] then the best samples are EI<sub>5</sub>-1 and EI<sub>4</sub>-1.

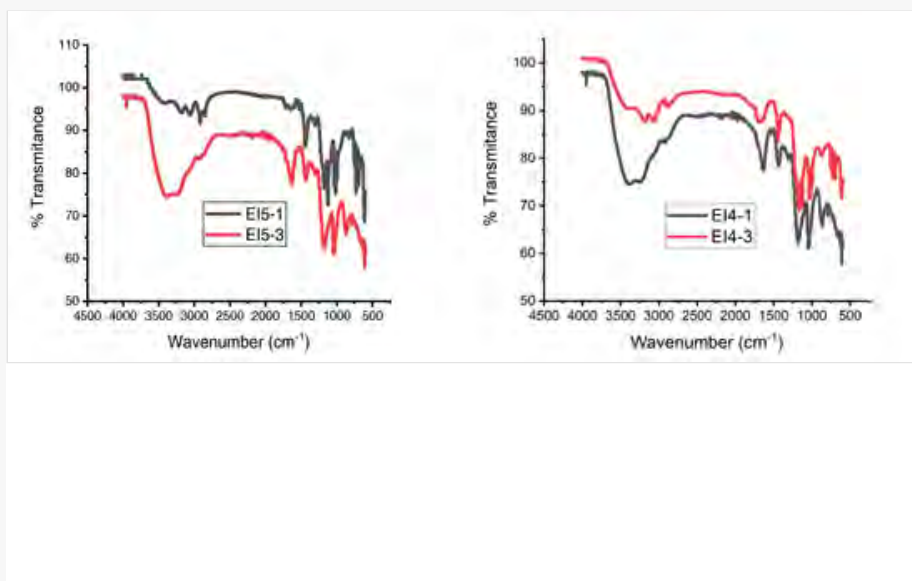
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Weight percentage of indicator electrodes from the EDX spectrum pattern of samples EI<sub>5</sub>-1 and EI<sub>4</sub>-1.

Indicator Electrodes	Material	unn. C	norm. C	Atom. C	Error (1 Sigma)
		[wt.%]	[wt.%]	[at.%]	[wt.%]
EI <sub>5</sub> -1	W 74 L-series	69.07	76.86	29.23	2.28
EI <sub>4</sub> -1	W 74 L-series	81.95	83.86	32.85	2.65

**Fig. 4**



The pattern of the transmittance spectrum against the wavenumber of the indicator electrodes (a) EI<sub>5</sub>-1, EI<sub>5</sub>-3, (b) EI<sub>4</sub>-1, EI<sub>4</sub>-3.

**Table 3**


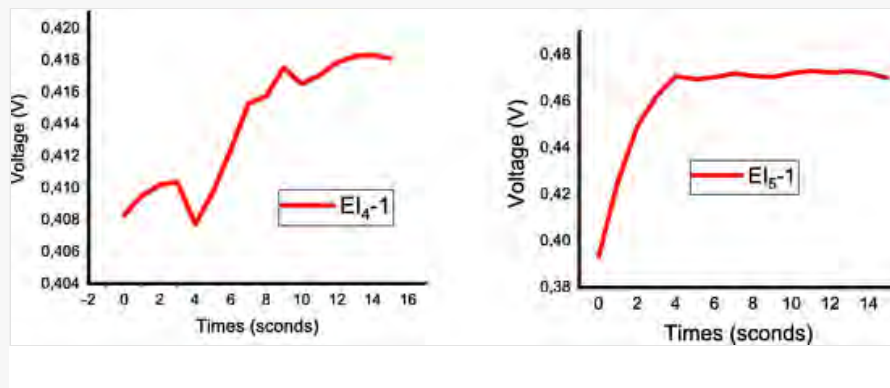
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Table of transmittance ranges for sample wave numbers (a) EI<sub>4</sub>-1, (b) EI<sub>5</sub>-1.

Wavenumber (cm <sup>-1</sup> )	Transmittance (%)			
	EI <sub>4</sub> -1	EI <sub>4</sub> -3	EI <sub>5</sub> -1	EI <sub>5</sub> -3
600	60.8895	75.5583	75.9339	48.5782
4000	97.9351	100.9955	102.5625	100.8259

After selecting two samples of indicator electrodes EI<sub>5</sub>-1 and EI<sub>4</sub>-1, according to the biosensor poenstometry method, a potentiometer cell was used to test the feasibility of the indicator electrode response time (Fig. 5). The best response time analysis [6] Fig. 5a and b were obtained on the EI<sub>5</sub>-1 sample also supported by the data in Table 4 that the EI<sub>5</sub>-1 indicator electrode has a sensitivity of 41.56 mV/decade, a detection range of 10<sup>-4</sup>–10<sup>-1</sup> M, detection limit is 10<sup>-4</sup> M and R<sup>2</sup> = 97.51 % [5,23]. The detection range of EI<sub>5</sub>-1 is greater than that of EI<sub>4</sub>-1, the detection range is 10<sup>-3</sup>–10<sup>-1</sup> M, the detection limit is 10<sup>-3</sup> M, while R<sup>2</sup> = 98.83 % [13,25,26].

**Fig. 5**



Response time (a) EI4-1, (b) EI5-1.

Table 4

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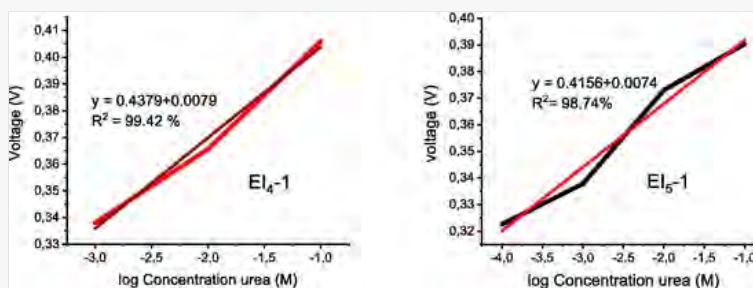
Linear curve of indicator electrodes EI<sub>4</sub>-1 and EI<sub>5</sub>-1.

Indicator electrode membrane layer	Sensitivity mV/decade	Detection range (M)	Detection limit (M)	R-square (R <sup>2</sup> ) (%)
EI <sub>4</sub> -1	43.79	10 <sup>-3</sup> -10 <sup>-1</sup>	10 <sup>-3</sup>	98.83
EI <sub>5</sub> -1	41.56	10 <sup>-4</sup> -10 <sup>-1</sup>	10 <sup>-4</sup>	97.51

In electrochemical detection, the signal associated with the interaction of the analyte is measured through the electrode. Measurements can be made by (a) connecting current and voltage, namely voltammetric and conductor metric biosensors; (b) current or voltage with respect to time, i.e., amperometric or potentiometric; (c) the imaginary versus the real part of the impedance, i.e., impedometric; (d) drain current versus line voltage in a FET biosensor [6], agreement with previously reported analyzes (i.e., FTIR, XRD, and SEM) [22] and confirming interactions with added organic modifiers (see Fig. 6).

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Fig. 6





The procedure of this study followed (a) Analysis of transmittance, edx, xrd [27], edx, xrd, linear curve, sensitivity and detection range [28], transmittance, concentration, sensitivity [29–31]; (b) The biosensor system [20], namely (1) selectivity, (2) sensitivity, (3) linearity response, namely the concentration range of the target analyte to be measured, (4) reproducibility of signal response, samples having a different concentration. the same analyzed several times should give the same response, (5) fast response time and recovery time for reusability of the biosensor system, (6) stability and operating life; (c) The [7] immobilization technique was developed based on three important mechanisms, namely (1) physical adsorption, (2) covalent immobilization, (3) streptavidin–biotin immobilization. Achieving high sensitivity and selectivity requires minimization of nonspecific adsorption and stability.

## 4 Conclusion

As a conclusion from XRD, SEM-EDX and FTIR analysis, response time and linear curve analysis, the best sample is the EI<sub>5</sub>-1 indicator electrode with four layer modifications, namely PVA-Enzyme/GA-2.9 %/PPy + Sulfonic Acid/PVC-KTpCIPB-o-NPOE.

## 4 Uncited references

[12,14,15,24].


## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

I would like to thank Medan State University for obtaining Applied Research in 2022, as well as data analysis collaboration with the Universitas Sumatera Utara.

## References


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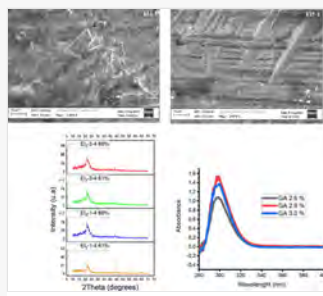
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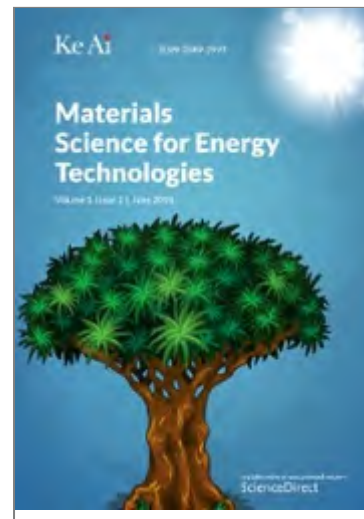
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