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ISSN: 0974-1496 (Print) | ISSN: 0976-0083(Online)

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CHARACTERIZATION OF GLUTARALDEHYDE COMPOSITION ON PVA-ENZYME COATED PVC-KTPCLPB MEMBRANE WITH XRD, UV-VIS, SEM-EDS AND FTIR

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ABSTRACT

This experimental research was conducted in two ways to characterize Glutaraldehyde (GA) composition on the indicator of the electrode membrane. First, PVA-GA-Enzim was coated with PVC-KTpCIPB. Second, PVA-E was coated with GA, further with PVC-KTpCIPB. Using XRD, SEM-eds, and FTIR, the electrodes were analyzed through a spectrum pattern. UV-Vis analyzed the spectral patterns of 2.6%- 3 %PVA-GA-Enzyme solution. GA concentration contributed to the increase or decrease in the activity. The sample in the first method is notated A1, A2, and A3, while the sample in the second method is marked as B1-N, B2-N, and B3-N; old B1-O, B2-O, and B3-O. The increase or decrease in the activity was analyzed using XRD diffraction spectrum 0patterns; A1-1, A1-2, A1-3, A1-4, A1-5; A2-1, A2-2, A2-3, A2-4, A2-5; A3-1, A3-2, A3-3, A3-4, A3 sample electrodes. The analysis showed the three best samples; A1-4, A1-5, A2-4, A2-5, A3-4, and A3-5 which were 29% and 30% GA composition. The results of UV-Vis analysis of the PVA-GA-Enzyme solution were between 2.6% to 3% of the GA concentration. The best results were at a concentration of 2.6%, 2.9%, and 3% with 2.9% concentration as the highest absorbance peak. The analysis of the XRD intensity spectrum pattern of the three GA compositions indicated different activity. So, it was concluded that the best composition of 2.9% was in the indicator electrode coated with PVA-Enzim GA, coated with PVC-KTpCIPB for one time each.

Keywords: Characterization, PVA-GA-Enzim, PVC-KTpCIPB, XRD; UV-Vis; SEM-EDS and FTIR.

RASĀYAN J. Chem., Vol. 15, No.2, 2022

INTRODUCTION

Enzyme immobilization is a process of enzyme physical localization to certain surfaces that helps to improve some of the enzymatic properties and their operational performance without disturbing their catalytic activity, thus enabling the recovery and reuse of enzymes so that the whole process can be controlled and economical [1].

The indicator electrode research has been carried out as a urea sensor using a biosensor potentiometric method with urease enzyme immobilization technique on a PVA membrane, namely PVA-Enzyme coated with PVC-KTpCIPB labelled PVA-E-1-2x with Ar notation. 0.0350 g of PVA dissolved in 10 mL of warm water is called PVA-Enzyme. 0.0350 g PVC-0.0500 g KTpCIPB dissolved in 10 mL THF is called PVC-KTpCIPB. It has obtained a sensitivity of 19,069 mV/decade, a detection range of 1.10⁻⁵ – 5.10⁻⁴ M, a detection limit of 1.10⁻⁵ M, and a correlation coefficient of 0.9431 [2]. Based on the analysis, this detection range is small concerning the width of the peak of the absorbance spectrum to the wavelength. For this reason, researchers want to analyze interactions in the form of molecular adsorption to increase the detection limit and selectivity of biosensors [3]. Increasing or decreasing glutaraldehyde concentration from 3% causes a decrease in activity [4], and the optimal concentration of 2.5-3% glutaraldehyde is used for urease immobilization. The technique used is absorption, entrapment, and crosslinking. The cross-linking technique [5] contributes to strengthening the biocatalyst bond, preventing leakage, reducing desorption and increasing the stability of the biocatalyst. Based on this contribution, the authors chose glutaraldehyde to strengthen the detection range of the urea sensor with an indicator electrode made of

tungsten immobilized by the urease enzyme on the indicator electrode with PVA-Enzyme-GA solution coated with PVC-KTpCIPB in two ways. In the first method, glutaraldehyde was mixed with PVA-Enzyme with the symbol PVA-E-GA, immobilized on the indicator electrode with PVA-E-GA solution after drying, coated with PVC-KTpCIPB. In the second method, having been dried, PVA-Enzyme was immobilized on the indicator electrode, it was coated with glutaraldehyde solution, and after drying, it was coated again with PVC-KTpCIPB solution. It aims to get the best way to immobilize the urease enzyme. The success of enzyme immobilization and surface modification was measured using a UV-Visible spectrophotometer [6]. The immobilization strategy was then applied to the application of biosensors for urea detection.

EXPERIMENTAL

Material and Methods

The materials used in this study were the enzyme Uriase, EC 3.5.1.5 (Urea) U4002, 50-100 ix Sigma-Aldrich type, PVA:PVC was 1:1 with a mass of 0.0350 g, GA with variations (0.26, 0.27, 0.28, 0.29, 0.30) g in 10 mL solvent, KTpCIPB 0.0500 g. This material is used in the form of a solution manufacturing indicator electrodes from potentiometer cells using the biosensor potentiometric method modification of the PVA-Enzyme-GA and PVC-KTpCIPB indicator electrode layers. The equipment used in the Physics Laboratory is XRD-6100 Shimadzu, UV-Vis Leigh UV-1601, SEM Evo MA 10 Ziess, Coating from SEM Q150RES Quorum and FTIR from the Medan Customs and Excise Laboratory.

General procedure

PVA 0.0350 g dissolved in 10 mL of hot water until cold. The urease 1 mg enzyme was dissolved in 0.5 mL of water mixed with alcohol within the ratio of 50%: 50%. Adding one drop of the enzyme, stirring with a stirrer until blended into the cold 10 mL of 0.0350 g PVA. 10 mL solution of THF mixed with PVC 0.0350 g and KTpCIPB 0.0500 g.

The indicator electrode is fabricated in two ways. The first way, Glutaraldehyde is mixed with PVA-E to become PVA-E-GA coated with PVC-KTpCIPB labelled PVA-E-GA-1. The second method is PVA-E coated with GA coated again with PVC-KTpCIPB labelled PVA_E_GA_1. The method is to obtain the best method in the manufacture of indicator electrodes. Composition of polymer membranes on ion-selective electrodes (ISE) according to [7] ionophore weight 1%, PVC polymer matrix: plasticizer (1: 2). Synthesis of polymers in the development of ISE sensors according to [8], membranes at the ratio of Ionophores: PVC: KTCIPB: Plasticizers 10: 165: 5: 330. An increase or decrease in glutaraldehyde concentration of more than 3% causes a decrease in activity, determining the optimal concentration of glutaraldehyde between 2.5 and 3% for the immobilization of the enzyme urease.

Detection Method

The manufacture of indicator electrodes in the first and second methods is according to the rules mentioned. The indicator electrode is made up of fifteen tungsten wires. In the first method, fifteen indicator electrodes were immobilized by the enzyme urease 1x in PVA into PVA-E mixed with GA denoted PVA-E-GA 1x with variations of glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%). Ten of the fifteen electrodes were immobilized with PVA-E-GA 2x and five of the ten indicator electrodes were immobilized with PVA-E-GA 3x. Simply put, the first fifteen electrodes of the first group were immobilized by PVA-E-GA 1x until dry. In the second group, only ten indicator electrodes were immobilized once again to obtain 2x immobilization of PVA-GA-E until dry. In the third group, only five indicator electrodes were immobilized once again to obtain 3x immobilization of PVA-GA-E until dry. Thus, five electrodes were immobilized by PVA-E-GA 1x according to variations in glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%), five

electrodes were immobilized by PVA-E-GA 2x according to with variations of glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%) and five electrodes immobilized PVA-E-GA 3x corresponding to variations of glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%). Then from the three groups of indicator electrodes immobilized 1x, 2x and 3x by PVA-E-GA coated with a solution of 0.0350 g PVC-0.0500 g KTpCIPB 1x until dry, respectively labelled PVA-E-GA-1-1x, PVA-E-GA-1-2x and PVA-E-GA-1-3x are denoted as A1, A2, and A3. If A1 is associated with GA concentrations (2.6%, 2.7%, 2.8%, 2.9% and 3%) it becomes A1-1, A1-2, A1-3, A1-4, A1-5; Likewise, A2 becomes A2-1, A2-2, A2-3, A2-4, A2-5 and A3 becomes A3-1, A3-2, A3-3, A3-4, A3-5. The indicator electrode can already be analyzed as an XRD test sample, which can be seen in Figures 1, 2 and 3.

Analytical Discussion

Before proceeding with the second method, the PVA-E-GA solution according to variations in glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%) was analyzed by UV-Vis to obtain the absorbance spectrum pattern of the solution to the wavelength variation of the PVA-E-GA solution. For reference analysis using distilled water because PVA-E-GA is soluble in water and the analysis couplet is PVA-E-GA with variations in glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%), the results can be seen in Figure 4. The best results were obtained from the first method, followed by the manufacture of indicator electrodes with the best GA concentration from the first method to save the cost of sample testing. The second method of PVA-E coated with GA was re-coated with PVC-KTpCIPB labelled PVA_E_GA_1-1x, PVA_E_GA_1-2x and PVA_E_GA_1-3x denoted by B1, B2, and B3. If B1 is associated with (2.6%, 2.7%, 2.8%, 2.9% and 3%) it becomes B1-1, B1-2, B1-3, B1-4, B1-5 and so on. Sample B from XRD spectrum pattern analysis was associated with UV-Vis absorbance spectral pattern analysis as PVA-E-GA solution analysis. UV-vis spectrum pattern analysis was analyzed by FTIR transmittance spectrum pattern and ended with SEM pore analysis and supporting element spectral pattern with EDS.

RESULTS AND DISCUSSION

Based on table 1 samples A1, A2, A3 and Ar with variations in GA (2.6%, 2.7%, 2.8%, 2.9% and 3%), the intensity changes with the angle 2Theta. The height of the peak intensity with respect to the 2Theta angle of each sample can be seen in table 1. The peak height of the intensity of A1-1 is 270 au, 238 a.u, 242 a.u, 110 a.u; A1-2 is 102 a.u, 134 a.u, 146 a.u, 46 a.u; A1-3 is 136 a.u, 190 a.u, 138 a.u, 56 a.u; A1-4 are 198 a.u, 236 a.u, 176 a.u, 80 a.u; A1-5 is 278 a.u, 256 a.u, 256 a.u, 130 a.u; Ar is 330 a.u, 452 a.u, 754 a.u, 6306 a.u, with respect to 2theta at 44.28 degrees, 44.32 degrees, 44.36 degrees, 44.52 degrees, respectively. Highest peak intensity A1-1 270 a.u at 44.28 degrees; A1-2 146 a.u at 44.36 degrees; A1-3 190 a.u at 44.32; A1-4 236 a.u at 44.32 degrees; A1-5 278 a.u at 44.28 degrees; Ar 6306 a.u at 44.52 degrees.

The peak intensity of A2-1 is 150 a.u, 120 a.u, 52 a.u; A2-2 is 184 a.u, 160 a.u, 76 a.u; A2-3 is 212 a.u, 180 a.u, 88 a.u; A2-4 is 228 a.u, 212 a.u, 84 a.u; A2-5 is 220 a.u, 228 a.u, 82 a.u; Ar is 452 a.u, 754 a.u, 6306 a.u, to 44.32 degrees, 44.36 degrees and 44.52 degrees, respectively. The highest intensity peak of A2-1 was 150 a.u at 44.32 degrees; A2-2 184 a.u at 44.32 degrees; A2-3 212 a.u at 44.32 degrees; A2-4 228 a.u at 44.32 degrees; A2-5 228 a.u at 44.36 degrees, Ar 6306 a.u at 44.52 degrees.

The peak intensity of A3-1 is 216 a.u, 220 a.u, 86 a.u; A3-2 is 162 a.u, 196 a.u, 62 a.u; A3-3 is 352 a.u, 338 a.u, 126 a.u; A3-4 is 340 a.u, 382 a.u, 118 a.u; A3-5 is 278 a.u, 256 a.u, 130 a.u, against 2Theta 44.28 degrees, 44.32 degrees and 44.52 degrees, respectively. Highest peak intensity A3-1 220 a.u at 44.32 degrees; A3-2 196 a.u at 44.32 degrees; A3-3 352 a.u at 44.28 degrees; A3-4 382 a.u at 44.32 degrees; A3-5 278 a.u at 44.28 degrees.

Based on data from table 1, the peak intensity height is greater than 200 au, the samples are (a) A1-1, (b) A1-4, (c) A1-5 (d) A2-3, (e) A2-4, (f) A2-5, (g) A3-1, (h) A3-3 (i) A3-4, (j) A3-5 and (k) Ar can be seen in Figure 1.

Table 1. The height of the peak with respect to angles 2Theta A1, A2, A3 and Ar.

2Theta	Intensity (a.u)					
	A1-1	A1-2	A1-3	A1-4	A1-5	Ar
44.28	270	102	136	198	278	330
44.32	238	134	190	236	256	452
44.36	242	146	138	176	256	754
44.52	110	46	56	80	130	6306
2Theta	A2-1	A2-2	A2-3	A2-4	A2-5	Ar
44.30	128	166	170	212	230	378
44.32	150	184	212	228	220	452
44.52	52	76	88	84	82	6306
2Theta	A3-1	A3-2	A3-3	A3-4	A3-5	Ar
44.28	216	162	352	340	278	330
44.32	220	196	338	382	256	452
44.52	86	62	126	118	130	6306

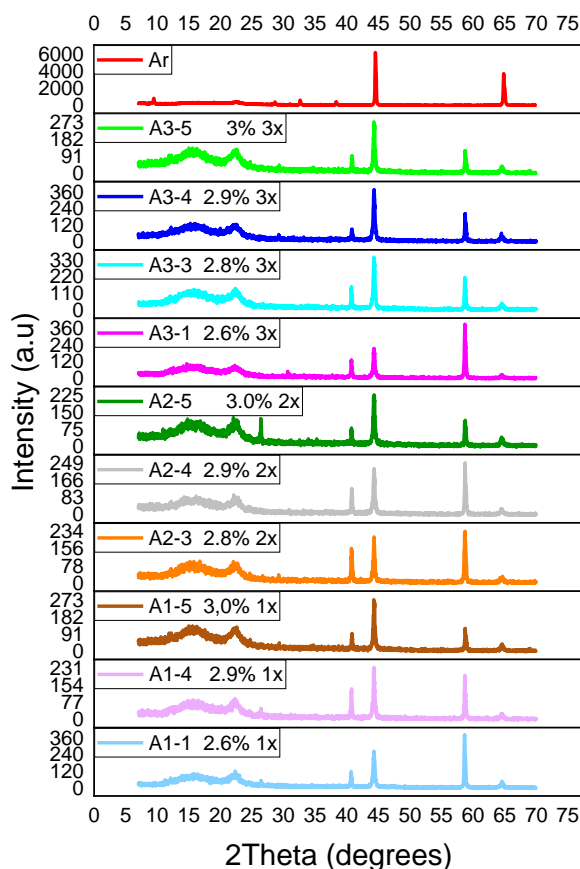


Figure 1. XRD diffraction spectrum pattern (a) A1-1, (b) A1-4, (c) A1-5 (d) A2-3, (e) A2-4, (f) A2-5, (g) A3-1, (h) A3-3 (i) A3-4, (j) A3-5 and (k) Ar.

Based on table 1, the highest intensity peaks with respect to 2Theta angle are A1-5, A2-5, A3-3, A3-4, A3-5 and Ar, the XRD diffraction spectrum pattern has a peak height above 200 au is 278 a.u; 230 a.u, 352 a.u, 382 au, 278 a.u and 6306 a.u at angles 2Theta 44.28 degrees, 44.30 degrees, 44.28 degrees, 44.32 degrees, 44.28 degrees and 44.52 degrees, the highest peak in the composition A3-4 see table 2 figure 2.

Based on the analysis of table 2 and Figure 2, the obtained best samples were in samples A1-5, A2-5 and A3-4 or groups A4 GA 2.9% and A5 GA 3.0%. Analysis of the intensity diffraction spectrum pattern against the 2Theta angle according to [9;] with the designation of the diffraction peak at the Bragg angle, table 2 and figure 2 show A4 has a higher intensity peak than A5. The highest peak intensity is A3-4 382 a.u 44.32 degrees than A1-5 278 a.u at 44.28 degrees. The intensity diffraction spectrum pattern for angle 2Theta of A2-5 230 a.u at 44.30 degrees lower than A3-4 and A1-5. The intensity spectrum pattern to the 2Theta angle of A4 and A5 can change or improve the diffraction spectrum pattern of the Ar intensity. The best sample is A3-4 in table 2 and figure 2 with a 2.9% GA composition where the electrodes coated with PVA-E mixed with GA coated with PVC-KTpCIPB. PVA-E mixed with GA labelled PVA-E-GA is the first method of making indicator electrode membranes. According to Figure 2, the intensity spectrum pattern concerning 2theta of Ar 6306 au at 44.52 degrees, while A3-4 decreased in intensity to 382 a.u at 44.32 degrees due to the addition of 2.9% GA. The optimum concentration of glutaraldehyde at GA is 2.5% [10]. The effect of GA on the biosensor response in the case of

a low concentration of 1.5% makes the substrate easy to leak from the gelatin membrane due to less cross-linking with the gelatin membrane and for the concentration as high as 3.5%, the biosensor response is less due to more cross-linking, which prevents the substrate from passing through the gelatin membrane.

Table 2. The highest intensity peak with respect to the 2Theta angle of A1-5; A2-5, A3-4 and Ar

2Theta	Intensity (a.u)	
	A1-5 3,0% 1x	Ar
44.28	278	330
44.32	256	452
44.52	130	6306
2Theta	Intensity (a.u)	
	A2-5 3.0% 2x	Ar
44.30	230	378
44.32	220	452
44.52	82	6306
2Theta	Intensity (a.u)	
	A3-4 2.9% 3x	Ar
44.28	340	330
44.32	382	452
44.52	118	6306

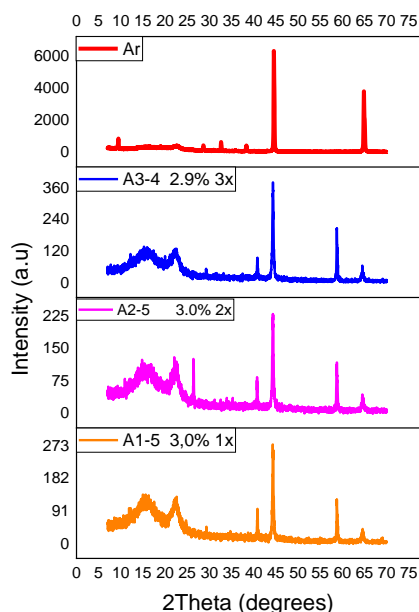


Figure 2. XRD diffraction spectrum pattern (a) A1-5, (b) A2-5, (c) A3-3 (d) A3-4 and (G) Ar.

Glutaraldehyde cross-linking produces large enzyme aggregates that can serve as catalysts and support for many substrate molecules. Enzyme cross-linking harms enzyme leaching and the entrapment followed by crosslinking disrupts the total binding activity also greatly enhances the stability of the enzyme in extreme chemical environments [11]. Appropriate addition of glutaraldehyde to produce higher enzyme stability at 0.03% glutaraldehyde concentration, in 0.1 M phosphate buffer with a pH variation of 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0 for 30 minutes [12].

Table 3. The height of the intensity peaks with respect to the 2Theta angle of B1-4-O, B2-4-O, B3-4-O; B1-4-N; B2-4-N, A3-4-N and Ar

2Theta (degrees)	Intensity (a.u)			
	B1-4-O	B2-4-O	B3-4-O	Ar
44.30	436	280	278	378
44.32	452	262	280	452
44.34	428	276	286	582
44.52	188	118	116	6.306
	B1-4-N	B2-4-N	B3-4-N	Ar
44.30	286	260	127	378
44.52	188	118	116	6.306

There is also a 2Theta angular shift of the intensity spectrum pattern to 2theta in Figure 2 on a two-decimal scale of 44 degrees. The change in the height of the intensity peak to the 2Theta angle can occur due to the addition of material to PVA, some previous research results (1) PVA-doped ZnO composites are between 2 theta angles of 30-40 degrees [13], (2) the intensity of the angle PVA/SiO₂-TiO₂ around 2Theta 20 degrees [14], (3) nanostructures with CA-doped ZnO composite intensity and PVA between 2 theta angles 40-50 degrees [15], (4) PVA hybrid nanofibers are between 2 theta angles of 30-40 degrees [16], (5) PVA/PAA/Fe₃O₄ intensity between 2 theta angles of 15-20 degrees [17]. (6) hydroxyapatite-gelatin (HAp-GEL) composites were cross-linked with different amounts of GA between 2Theta 25-55 degrees [18]. When compared to the PVA-E-1-2x (Ar) indicator electrode, the spectrum pattern of the PVA-E-GA-1-indicator electrode (1x, 2x, 3x) did not experience a 2Theta angle shift on a scale without decimals of about 44 degrees. In connection with the background in the method of making this indicator electrode, there are two ways; the first method has been completed, the following is the second method consisting of a 2.9% GA sample with the last coating, namely the old PVC-KTpCIPB notated B-4-O and the new PVC-KTpCIPB notated B-4-N. PVA-E coated GA coated again with the old PVC-KTpCIPB coded B1-4-O. The GA-coated PVA-E was re-coated with a new PVC-KTpCIPB given the notation B1-4-N.

The intensity of the diffraction spectrum pattern against the 2Theta angle in a second way, the height of the peak intensity of the 2Theta angle from B1-4-O, B2-4-O, B3-4-O (old sample) B1-4-N, B2-4-N, B3-4-N (a new sample) and Ar can be seen in table 3 and figure 3.

Table 3 and figure 3 illustrate the height of the intensity peak against the 2Theta angle, the XRD diffraction spectrum pattern of B1-4-O and B1-4-N. There are differences in the intensity spectrum pattern with the 2Theta angle. Samples B1-4-O whose peak elevation is above 200 a.u are 452 a.u, 280 a.u, 286 a.u and Ar 6306 a.u at 2Theta angles 44.32 degrees, 44.30 degrees, 44.34 degrees. The highest peak intensity at B1-4-O was 452 a.u at 44.32 degrees. Sample B1-4-N in which the peak elevation is above 200 a.u is 286 a.u; 260 a.u; and Ar 6306 a.u at an angle of 2 Theta 44.30 degrees; 44.30 degrees; and 44.52 degrees, the highest peak in the composition B1-4-N was 286 a.u 44.30 degrees.

Table 3 and Figure 3 analyze the second method chosen for the composition of B1-4-O and B1-4-N, which means that PVA-E coated with GA was 1x further coated with the old PVC-KTpCIPB 1x notation B1-O and given PVC- The new KTpCIPB marked as B1-N. PVA-E coated with GA 2.9 % 1x coated again with the old PVC-KTpCIPB 1x with the notation B1-4-O and coated with the new PVC-KTpCIPB 1x with the notation B1-4-N. The best two samples are the second method on the B1-4-O composition, namely the second method of making indicator electrodes where 1x PVA-E is coated with 2.9% GA 1x is layered again with 1x old PVC-KTpCIPB.

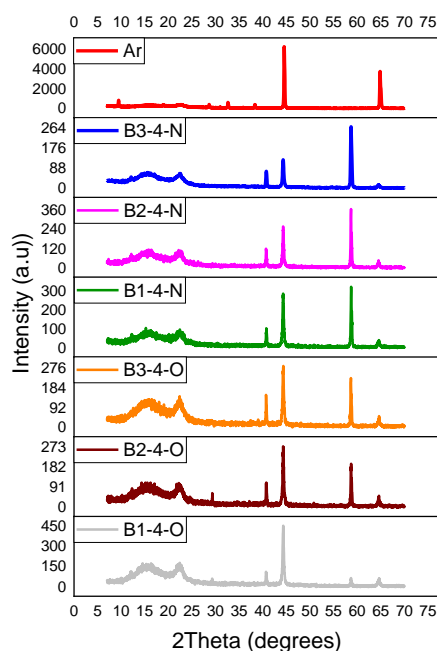


Figure 3. XRD diffraction spectrum pattern (a) B1-4-O, (b) B2-4-O, (c) B3-4-O (d) B1-4-N, (e) B2-4-N, (f) A3-4-N and (g) Ar.

After analyzing the method of making indicator electrodes with XRD, the next step was to amplify the data from the second method using UV-Vis, FTIR, and SEM-eds analysis. Based on the analysis of UV-Vis analysis, the GA concentration test between variations of 2.6 – 3.0% of A1, A2, A3, A4, and A5 are in Table 4 and Figure 4, geometric shapes [19; 20). There are three

samples of A1, A4, and A5 whose absorbance spectrum pattern is above 1000 a.u with a wavelength in the range between 250-400 nm from the PVA-E-GA solution.

Based on Figure 4 (a) the absorbance spectrum pattern of Ar has a high absorbance peak of 7,246 a.u at a wavelength of 291 nm, while with a variation of (A) A1; (B) A4; and (C) A5 absorbance peak height 1.083 a.u; 1540 a.u; 1,370 a.u at a wavelength of about 299 nm. This wavelength shift occurred with the addition of glutaraldehyde. Figure 4 (a), (b), and (c) have large peak widths, while Figure 4 (d) has a small peak width.

Figure 4 Pattern of UV-Vis absorbance spectrum for the wavelength of PVA-GA-E solution with variations in the symmetrical spectrum pattern (a) A1; (b) A4; and (c) A5 in Figure 4. The symmetrical pattern of the absorbance spectrum to wavelength is supported by [21]. If Figure 4 (a, b and c) is compared with the configuration of the PVA-E solution in Figure 4 (d), then there is a decrease in the absorbance peak height of the solution with variations of (a) A1; (b) A4; and (c) A5, followed by widening of the absorbance peak see figure 4.

Table 4. Absorbance for PVA-GA-E and PVA-E solution wavelengths

Wavelength (nm)	Absorbance (a.u)			
	A1	A4	A5	Ar
291	0,571	0,901	0,732	7.246
299	1.083	1.540	1.370	0.23

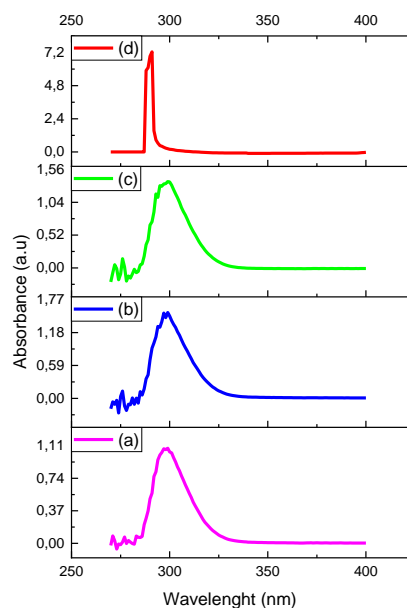


Figure 4. Pattern of UV-Vis absorbance spectrum with variations in glutaraldehyde; (a) A1; (b) A4; (c) A5 and (d) Ar.

Based on the analysis of the XRD diffraction spectrum pattern for the 2Theta angle and the UV-Vis absorbance spectrum pattern for the above wavelengths, it shows that the best glutaraldehyde composition was 2.9% of A3-4-O group. A3-4-O is the first method of making indicator electrodes where PVA-E and GA are mixed, morphology by SEM analysis can be seen in Figure 5a.

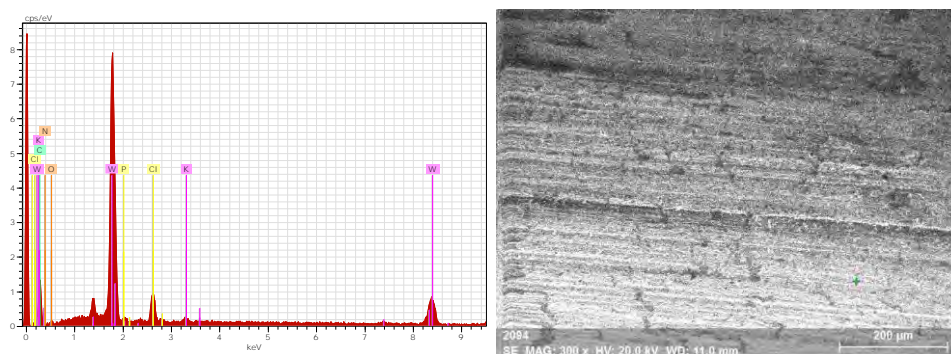


Figure 5a. EDS spectrum pattern and Morphology indicator electrode A3-4-O

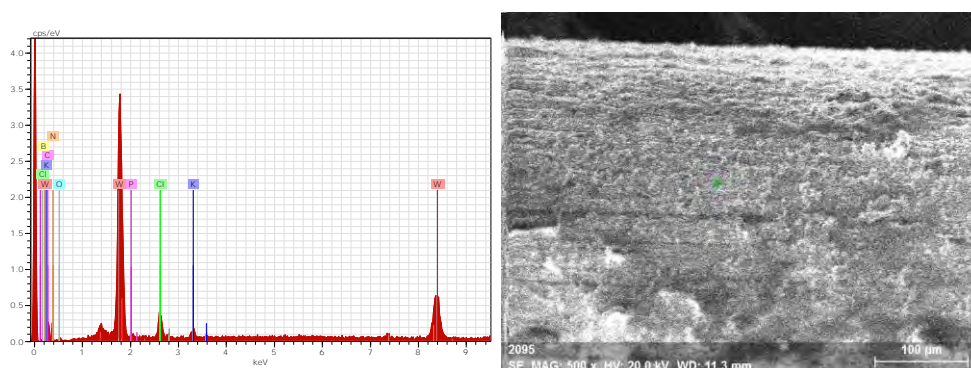


Figure 5b. EDS spectrum pattern and morphology of indicator electrode B1-4-O

There are differences in the spectrum pattern of EDS A3-4, B1-4 and the morphology of A3-4-O with B1-4-O. B3-4-O cracks while B1-4-O looks many pores at 100 m magnification 500x acceleration stress 20.00 Kv. A3-4 is an indicator electrode coated with PVA-E mixed with GA 29% 3x layered again with PVC-KTpCIPB 1x cross-linked method. B1-4 is an indicator electrode coated with PVA-E 1x coated with GA 2.9% 1x coated again with PVC-KTpCIPB 1x entrapment method. There are differences found in the cross method, so it is better to use the entrapment method [11]. The cross-linking technique [5] strengthens the biocatalyst bond, preventing leakage.

Several SEM analyses using GA to study surface morphology and energy dispersive X-ray (EDS) analysis are [1] at an accelerating voltage of 10.00 kV, magnifications of 750x, 1500x, and 3500x. SEM magnification 5000 x good performance GA 1.25% [22;23]. The analysis of the use of enzyme immobilization and its feasibility in industrial-scale biocatalysis [24] used SEM.

Based on chemical structure analysis $(ClC_6H_4)_4BK$ is KTpCIPB functional group K and Cl, $(CH_2CHCl)_n$ is PVC functional group is Cl, $[-CH_2CHOH-]_n$ is PVA functional group is OH, NH_2CONH_2 is urease enzyme functional group is C=O, $OHC(CH_2)_3CHO$ is glutaraldehyde the functional group is OH. C_4H_8O is Tetrahydrofuran the functional group is O.

Based on Figure 6b and analysis of spectroscopic table data, there are two C-H hydrogen bands in the aldehyde group with absorption frequencies of $2800-2860\text{ cm}^{-1}$ and $2700-2760\text{ cm}^{-1}$. The results obtained at B1-4-O absorption frequency are at 2852.16 cm^{-1} , New absorption frequency of 2852.61 cm^{-1} and B3-4-A absorption frequency of 2832.16 cm^{-1} . Similarly, the determination of the group function in the mixture for the urease enzyme functional group C=O at $1720-1740$

cm^{-1} at B1-4-O absorption frequency 1737.11 cm^{-1} . PVA coated with GA functional group is O-H at absorption frequency between $3330\text{--}3350 \text{ cm}^{-1}$ at B1-4-O absorption frequency 3327.02 cm^{-1} . Mixture of PVC-KTpCIPB in THF the functional group is CO at $1100\text{--}1350 \text{ cm}^{-1}$ at B1-4-O absorption frequency $1165.90\text{--}1326.89 \text{ cm}^{-1}$ and acetal ring (COC) at absorption frequency $1000\text{--}1140 \text{ cm}^{-1}$ at B1-4-O absorption frequency $1011.06\text{--}1083.60 \text{ cm}^{-1}$. sp^2 C-H band pattern of alkenes in the cis group with absorption frequency of $675\text{--}730 \text{ cm}^{-1}$ and the trisubstituent alkene group with absorption frequency of $790\text{--}840 \text{ cm}^{-1}$. In B1-4-O the absorption frequency is 688.80 cm^{-1} for the cis group with alkene substitution, while the absorption frequency is 798.83 cm^{-1} for the trisubstituent alkene group.

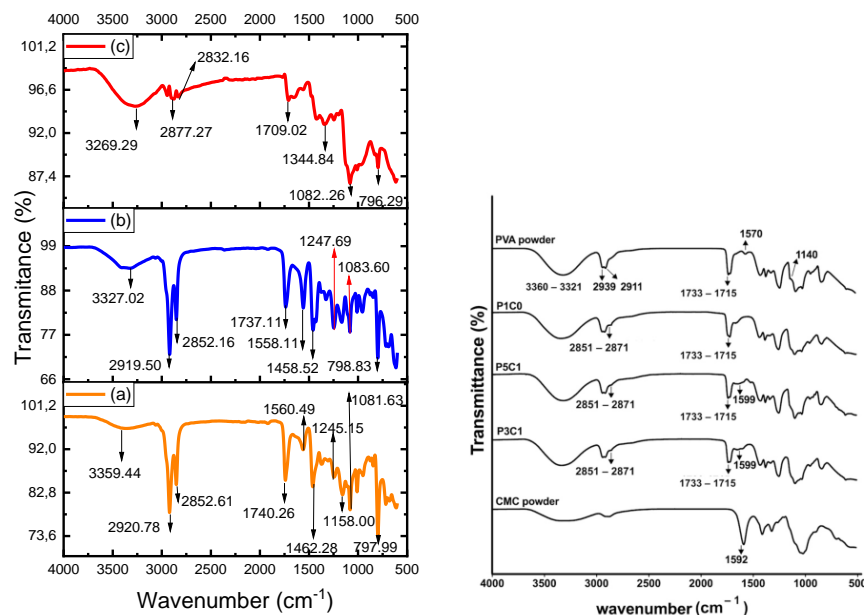


Figure 6. FTIR spectrum pattern of the sample (a) New, (b) B1-4-O, (c) A3-4-O, (d) PVA [Namkaew, J., et al, 2021]

FTIR analysis showed a complete loss of the measuring frequency of organic species and other functional groups in the template belonging to the peak [25] characterized functional group changes [26]. FTIR analysis of PVA and GA powders were influenced by the ratio [27] from the FTIR Spectrum of pure PVA membranes, PVA membranes crosslinked with glutaraldehyde (PVA/GA), and other mixtures [28]. Glutaraldehyde (GA) is a PVA crosslinking agent, substrate characterization of the FTIR spectrum pattern and XRD spectrum pattern showed the relationship between functional groups and crystal (metal) properties with the highest intensity [29]. It is similar to the analysis of the EDS spectrum pattern and the FTIR spectrum pattern [30]. Based on the structural analysis of the EDS spectrum pattern, the elements K, Cl, O, C, P, B and tungsten contained in the indicator electrode were found, supported by the presence of the C-H, C=O, O-H and C-O-C functional groups. To obtain the best sample, B1-4-O is an indicator electrode coated with PVA-enzyme coated with GA, further with the old PVC-KTpCIPB.

CONCLUSION

In this research paper, the first way of characterizing Glutaraldehyde on the urease enzyme immobilization of indicator electrode is by coating indicator electrode with PVA-E mixed with

2.9% GA 3x coated again with PVC-KTpCIPB 1x that can increase sensor activity and the best sample is A3-4. The second way is Characterizing Glutaraldehyde on the indicator electrode of the urease enzyme immobilization of the indicator electrode by coating PVA-E 1x coated with 2.9% GA 1x further with PVC-KTpCIPB and the best sample was B1-4-O. Thus, it can increase sensor activity. The best sample is the A4 sample with 2.9% GA composition with UV-Vis analysis.

There is a change in the intensity spectrum pattern by coating the membrane with XRD, SEM-EDS, and FTIR by PVC-KTpCIPB old samples.

ACKNOWLEDGEMENT

The author would like to thank the Chancellor of the State University of Medan who has provided research grants for BASIC RESEARCH, the chairman of the LPPM State University of Medan, the Physics Laboratory of the State University of Medan and LIPI-Bandung.

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CHARACTERIZATION OF GLUTARALDEHYDE COMPOSITION ON PVA-ENZYME COATED PVC-KTPCLPB MEMBRANE WITH XRD, UV-VIS, SEM-EDS AND FTIR

Abd Hakim. S¹, Satria Mihardi¹, Abdul Rais¹

¹ Physics Department, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan 20221, Indonesia

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ABSTRACT

This experimental research was conducted in two ways to characterize Glutaraldehyde (GA) composition on the indicator of the electrode membrane. First, PVA-GA-Enzim was coated with PVC-KTpCIPB. Second, PVA-E was coated with GA, further with PVC-KTpCIPB. Using XRD, SEM-eds, and FTIR, the electrodes were analyzed through a spectrum pattern. UV-Vis analyzed the spectral patterns of 2.6% - 3 %PVA-GA-Enzyme solution. GA concentration contributed to the increase or decrease in the activity. The sample in the first method is notated A1, A2, and A3, while the sample in the second method is marked as B1-N, B2-N, and B3-N; old B1-O, B2-O, and B3-O. The increase or decrease in the activity was analyzed using XRD diffraction spectrum 0patterns: A1-1, A1-2, A1-3, A1-4, A1-5; A2-1, A2-2, A2-3, A2-4, A2-5; A3-1, A3-2, A3-3, A3-4, A3 sample electrodes. The analysis showed the three best samples; A1-4, A1-5, A2-4, A2-5, A3-4, and A3-5 which were 29% and 30% GA composition. The results of UV-Vis analysis of the PVA-GA-Enzyme solution were between 2.6% to 3% of the GA concentration. The best results were at a concentration of 2.6%, 2.9%, and 3% with 2.9% concentration as the highest absorbance peak. The analysis of the XRD intensity spectrum pattern of the three GA compositions indicated different activity. So, it was concluded that the best composition of 2.9% was in the indicator electrode coated with PVA-Enzim GA, coated with PVC-KTpCIPB for one time each.

Keywords: Characterization, PVA-GA-Enzim, PVC-KTpCIPB, XRD; UV-Vis; SEM-EDS and FTIR.

RASAYAN J. Chem., Vol. 15, No. 2, 2022

INTRODUCTION

Enzyme immobilization is a process of enzyme physical localization to certain surfaces that helps to improve some of the enzymatic properties and their operational performance without disturbing their catalytic activity, thus enabling the recovery and reuse of enzymes so that the whole process can be controlled and economical [1].

The indicator electrode research has been carried out as a urea sensor using a biosensor potentiometric method with urease enzyme immobilization technique on a PVA membrane, namely PVA-Enzyme coated with PVC-KTpCIPB labelled PVA-E-1-2x with Ar notation, 0.0350 g of PVA dissolved in 10 mL of warm water is called PVA-Enzyme. 0.0350 g PVC-0.0500 g KTpCIPB dissolved in 10 mL THF is called PVC-KTpCIPB. It has obtained a sensitivity of 19,069 mV/decade, a detection range of 1.10⁻⁵ - 5.10⁻⁴ M, a detection limit of 1.10⁻⁵ M, and a correlation coefficient of 0.9431 [2]. Based on the analysis, this detection range is small concerning the width of the peak of the absorbance spectrum to the wavelength. For this reason, researchers want to analyze interactions in the form of molecular adsorption to increase the detection limit and selectivity of biosensors [3]. Increasing or decreasing glutaraldehyde concentration from 3% causes a decrease in activity [4], and the optimal concentration of 2.5-3% glutaraldehyde is used for urease immobilization. The technique used is absorption, entrapment, and crosslinking. The cross-linking technique [5] contributes to strengthening the biocatalyst bond, preventing leakage, reducing desorption and increasing the stability of the biocatalyst. Based on this contribution, the authors chose glutaraldehyde

to strengthen the detection range of the urea sensor with an indicator electrode made of tungsten immobilized by the urease enzyme on the indicator electrode with PVA-Enzyme-GA solution coated with PVC-KTpCIPB in two ways. In the first method, glutaraldehyde was mixed with PVA-Enzyme with the symbol PVA-E-GA, immobilized on the indicator electrode with PVA-E-GA solution after drying, coated with PVC-KTpCIPB. In the second method, having been dried, PVA-Enzyme was immobilized on the indicator electrode, it was coated with glutaraldehyde solution, and after drying, it was coated again with PVC-KTpCIPB solution. It aims to get the best way to immobilize the urease enzyme. The success of enzyme immobilization and surface modification was measured using a UV-Visible spectrophotometer [6]. The immobilization strategy was then applied to the application of biosensors for urea detection.

EXPERIMENTAL

Material and Methods

The materials used in this study were the enzyme Uriase, EC 3.5.1.5 (Urea) U4002, 50-100 ix Sigma-Aldrich type, PVA:PVC was 1:1 with a mass of 0.0350 g, GA with variations (0.26, 0.27, 0.28, 0.29, 0.30) g in 10 mL solvent, KTpCIPB 0.0500 g. This material is used in the form of a solution manufacturing indicator electrodes from potentiometer cells using the biosensor potentiometric method modification of the PVA-Enzyme-GA and PVC-KTpCIPB indicator electrode layers. The equipment used in the Physics Laboratory is XRD-6100 Shimadzu, UV-Vis Leigh UV-1601, SEM Evo MA 10 Ziess, Coating from SEM Q150RES Quorum and FTIR from the Medan Customs and Excise Laboratory.

General procedure

PVA 0.0350 g dissolved in 10 mL of hot water until cold. The urease 1 mg enzyme was dissolved in 0.5 mL of water mixed with alcohol within the ratio of 50%: 50%. Adding one drop of the enzyme, stirring with a stirrer until blended into the cold 10 mL of 0.0350 g PVA. 10 mL solution of THF mixed with PVC 0.0350 g and KTpCIPB 0.0500 g.

The indicator electrode is fabricated in two ways. The first way, Glutaraldehyde is mixed with PVA-E to become PVA-E-GA coated with PVC-KTpCIPB labelled PVA-E-GA-1. The second method is PVA-E coated with GA coated again with PVC-KTpCIPB labelled PVA_E_GA_1. The method is to obtain the best method in the manufacture of indicator electrodes. Composition of polymer membranes on ion-selective electrodes (ISE) according to [7] ionophore weight 1%, PVC polymer matrix: plasticizer (1: 2). Synthesis of polymers in the development of ISE sensors according to [8], membranes at the ratio of Ionophores: PVC: KTCIPB: Plasticizers 10: 165: 5: 330. An increase or decrease in glutaraldehyde concentration of more than 3% causes a decrease in activity, determining the optimal concentration of glutaraldehyde between 2.5 and 3% for the immobilization of the enzyme urease.

Detection Method

The manufacture of indicator electrodes in the first and second methods is according to the rules mentioned. The indicator electrode is made up of fifteen tungsten wires. In the first method, fifteen indicator electrodes were immobilized by the enzyme urease 1x in PVA into PVA-E mixed with GA denoted PVA-E-GA 1x with variations of glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%). Ten of the fifteen electrodes were immobilized with PVA-E-GA 2x and five of the ten indicator electrodes were immobilized with PVA-E-GA 3x. Simply put, the first fifteen electrodes of the first group were immobilized by PVA-E-GA 1x until dry. In the second group, only ten indicator electrodes were immobilized once again to obtain 2x immobilization of PVA-GA-E until dry. In the third group, only five indicator electrodes were immobilized once again to obtain 3x immobilization of PVA-GA-E until dry. Thus, five electrodes were immobilized by PVA-E-GA 1x according to variations in glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%), five electrodes

were immobilized by PVA-E-GA 2x according to with variations of glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%) and five electrodes immobilized PVA-E-GA 3x corresponding to variations of glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%). Then from the three groups of indicator electrodes immobilized 1x, 2x and 3x by PVA-E-GA coated with a solution of 0.0350 g PVC-0.0500 g KTpCIPB 1x until dry, respectively labelled PVA-E-GA-1-1x, PVA-E-GA-1-2x and PVA-E-GA-1-3x are denoted as A1, A2, and A3. If A1 is associated with GA concentrations (2.6%, 2.7%, 2.8%, 2.9% and 3%) it becomes A1-1, A1-2, A1-3, A1-4, A1-5; Likewise, A2 becomes A2-1, A2-2, A2-3, A2-4, A2-5 and A3 becomes A3-1, A3-2, A3-3, A3-4, A3-5. The indicator electrode can already be analyzed as an XRD test sample, which can be seen in Figures 1, 2 and 3.

Analytical Discussion

Before proceeding with the second method, the PVA-E-GA solution according to variations in glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%) was analyzed by UV-Vis to obtain the absorbance spectrum pattern of the solution to the wavelength variation of the PVA-E-GA solution. For reference analysis using distilled water because PVA-E-GA is soluble in water and the analysis couplet is PVA-E-GA with variations in glutaraldehyde (2.6%, 2.7%, 2.8%, 2.9% and 3%), the results can be seen in Figure 4. The best results were obtained from the first method, followed by the manufacture of indicator electrodes with the best GA concentration from the first method to save the cost of sample testing. The second method of PVA-E coated with GA was re-coated with PVC-KTpCIPB labelled PVA_E_GA_1-1x, PVA_E_GA_1-2x and PVA_E_GA_1-3x denoted by B1, B2, and B3. If B1 is associated with (2.6%, 2.7%, 2.8%, 2.9% and 3%) it becomes B1-1, B1-2, B1-3, B1-4, B1-5 and so on. Sample B from XRD spectrum pattern analysis was associated with UV-Vis absorbance spectral pattern analysis as PVA-E-GA solution analysis. UV-vis spectrum pattern analysis was analyzed by FTIR transmittance spectrum pattern and ended with SEM pore analysis and supporting element spectral pattern with EDS.

RESULTS AND DISCUSSION

Based on table 1 samples A1, A2, A3 and Ar with variations in GA (2.6%, 2.7%, 2.8%, 2.9% and 3%), the intensity changes with the angle 2Theta. The height of the peak intensity with respect to the 2Theta angle of each sample can be seen in table 1. The peak height of the intensity of A1-1 is 270 a.u., 238 a.u., 242 a.u., 110 a.u.; A1-2 is 102 a.u., 134 a.u., 146 a.u., 46 a.u.; A1-3 is 136 a.u., 190 a.u., 138 a.u., 56 a.u.; A1-4 are 198 a.u., 236 a.u., 176 a.u., 80 a.u.; A1-5 is 278 a.u., 256 a.u., 256 a.u., 130 a.u.; Ar is 330 a.u., 452 a.u., 754 a.u., 6306 a.u., with respect to 2theta at 44.28 degrees, 44.32 degrees, 44.36 degrees, 44.52 degrees, respectively. Highest peak intensity A1-1 270 a.u at 44.28 degrees; A1-2 146 a.u at 44.36 degrees; A1-3 190 a.u at 44.32; A1-4 236 a.u at 44.32 degrees; A1-5 278 a.u at 44.28 degrees; Ar 6306 a.u at 44.52 degrees.

The peak intensity of A2-1 is 150 a.u., 120 a.u., 52 a.u.; A2-2 is 184 a.u., 160 a.u., 76 a.u.; A2-3 is 212 a.u., 180 a.u., 88 a.u.; A2-4 is 228 a.u., 212 a.u., 84 a.u.; A2-5 is 220 a.u., 228 a.u., 82 a.u.; Ar is 452 a.u., 754 a.u., 6306 a.u., to 44.32 degrees, 44.36 degrees and 44.52 degrees, respectively. The highest intensity peak of A2-1 was 150 a.u at 44.32 degrees; A2-2 184 a.u at 44.32 degrees; A2-3 212 a.u at 44.32 degrees; A2-4 228 a.u at 44.32 degrees; A2-5 228 a.u at 44.36 degrees, Ar 6306 a.u at 44.52 degrees.

The peak intensity of A3-1 is 216 a.u., 220 a.u., 86 a.u.; A3-2 is 162 a.u., 196 a.u., 62 a.u.; A3-3 is 352 a.u., 338 a.u., 126 a.u.; A3-4 is 340 a.u., 382 a.u., 118 a.u.; A3-5 is 278 a.u., 256 a.u., 130 a.u., against 2Theta 44.28 degrees, 44.32 degrees and 44.52 degrees, respectively. Highest peak intensity A3-1 220 a.u at 44.32 degrees; A3-2 196 a.u at 44.32 degrees; A3-3 352 a.u at 44.28 degrees; A3-4 382 a.u at 44.32 degrees; A3-5 278 a.u at 44.28 degrees.

Based on data from table 1, the peak intensity height is greater than 200 au, the samples are (a) A1-1, (b) A1-4, (c) A1-5 (d) A2-3, (e) A2-4, (f) A2-5, (g) A3-1, (h) A3-3 (i) A3-4, (j) A3-5 and (k) Ar can be seen in Figure 1.

Table 1. The height of the peak with respect to angles 2Theta A1, A2, A3 and Ar.

2Theta	Intensity (a.u)					Ar
	A1-1	A1-2	A1-3	A1-4	A1-5	
44.28	270	102	136	198	278	330
44.32	238	134	190	236	256	452
44.36	242	146	138	176	256	754
44.52	110	46	56	80	130	6306
2Theta	A2-1	A2-2	A2-3	A2-4	A2-5	Ar
44.30	128	166	170	212	230	378
44.32	150	184	212	228	220	452
44.52	52	76	88	84	82	6306
2Theta	A3-1	A3-2	A3-3	A3-4	A3-5	Ar
44.28	216	162	352	340	278	330
44.32	220	196	338	382	256	452
44.52	86	62	126	118	130	6306

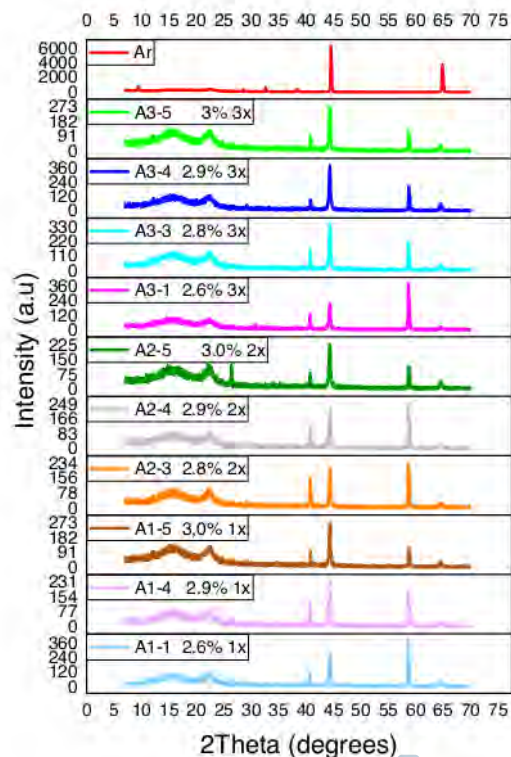


Figure 1. XRD diffraction spectrum pattern (a) A1-1, (b) A1-4, (c) A1-5 (d) A2-3, (e) A2-4, (f) A2-5, (g) A3-1, (h) A3-3 (i) A3-4, (j) A3-5 and (k) Ar.

Based on table 1, the highest intensity peaks with respect to 2Theta angle are A1-5, A2-5, A3-3, A3-4, A3-5 and Ar, the XRD diffraction spectrum pattern has a peak height above 200 au is 278 a.u; 230 a.u, 352 a.u, 382 au, 278 a.u and 6306 a.u at angles 2Theta 44.28 degrees, 44.30 degrees, 44.28 degrees, 44.32 degrees, 44.28 degrees and 44.52 degrees, the highest peak in the composition A3-4 see table 2 figure 2.

Based on the analysis of table 2 and Figure 2, the obtained best samples were in samples A1-5, A2-5 and A3-4 or groups A4 GA 2.9% and A5 GA 3.0%. Analysis of the intensity diffraction spectrum pattern against the 2Theta angle according to [9;] with the designation of the diffraction peak at the Bragg angle, table 2 and figure 2 show A4 has a higher intensity peak than A5. The highest peak intensity is A3-4 382 a.u 44.32 degrees than A1-5 278 a.u at 44.28 degrees. The intensity diffraction spectrum pattern for angle 2Theta of A2-5 230 a.u at 44.30 degrees lower than A3-4 and A1-5. The intensity spectrum pattern to the 2Theta angle of A4 and A5 can change or improve the diffraction spectrum pattern of the Ar intensity. The best sample is A3-4 in table 2 and figure 2 with a 2.9% GA composition where the electrodes coated with PVA-E mixed with GA coated with PVC-KTpCIPB. PVA-E mixed with GA labelled PVA-E-GA is the first method of making indicator electrode membranes. According to Figure 2, the intensity spectrum pattern concerning 2theta of Ar 6306 au at 44.52 degrees, while A3-4 decreased in intensity to 382 a.u at 44.32 degrees due to the addition of 2.9% GA. The optimum concentration of glutaraldehyde at GA is 2.5% [10]. The effect of GA on the biosensor response in the case of a low concentration of

1.5% makes the substrate easy to leak from the gelatin membrane due to less cross-linking with the gelatin membrane and for the concentration as high as 3.5%, the biosensor response is less due to more cross-linking, which prevents the substrate from passing through the gelatin membrane.

Table 2. The highest intensity peak with respect to the 2Theta angle of A1-5; A2-5, A3-4 and Ar

2Theta	Intensity (a.u)	
	A1-5 3.0% 1x	Ar
44.28	278	330
44.32	256	452
44.52	130	6306
2Theta	A2-5 3.0% 2x	Ar
44.30	230	378
44.32	220	452
44.52	82	6306
2Theta	A3-4 2.9% 3x	Ar
44.28	340	330
44.32	382	452
44.52	118	6306

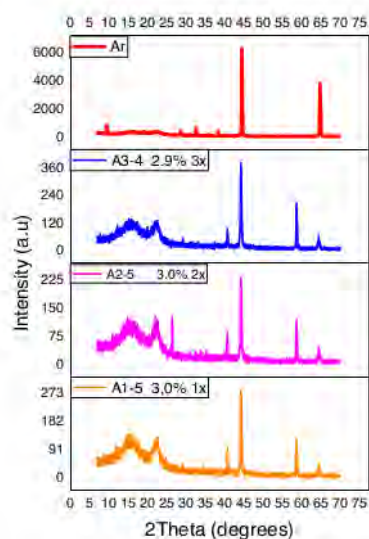


Figure 2. XRD diffraction spectrum pattern (a) A1-5, (b) A2-5, (c) A3-3 (d) A3-4 and (G) Ar.

Glutaraldehyde cross-linking produces large enzyme aggregates that can serve as catalysts and support for many substrate molecules. Enzyme cross-linking harms enzyme leaching and the entrapment followed by crosslinking disrupts the total binding activity also greatly enhances the stability of the enzyme in extreme chemical environments [11]. Appropriate addition of glutaraldehyde to produce higher enzyme stability at 0.03% glutaraldehyde concentration, in 0.1 M phosphate buffer with a pH variation of 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0 for 30 minutes [12].

Table 3. The height of the intensity peaks with respect to the 2Theta angle of B1-4-O, B2-4-O, B3-4-O; B1-4-N; B2-4-N, A3-4-N and Ar

2Theta (degrees)	Intensity (a.u)			
	B1-4-O	B2-4-O	B3-4-O	Ar
44.30	436	280	278	378
44.32	452	262	280	452
44.34	428	276	286	582
44.52	188	118	116	6.306
	B1-4-N	B2-4-N	B3-4-N	Ar
44.30	286	260	127	378
44.52	188	118	116	6.306

There is also a 2Theta angular shift of the intensity spectrum pattern to 2theta in Figure 2 on a two-decimal scale of 44 degrees. The change in the height of the intensity peak to the 2Theta angle can occur due to the addition of material to PVA, some previous research results (1) PVA-doped ZnO composites are between 2 theta angles of 30-40 degrees [13], (2) the intensity of the angle PVA/SiO₂-TiO₂ around 2Theta 20 degrees [14], (3) nanostructures with CA-doped ZnO composite intensity and PVA between 2 theta angles 40-50 degrees [15], (4) PVA hybrid nanofibers are between 2 theta angles of 30-40 degrees [16], (5) PVA/PAA/Fe₃O₄ intensity between 2 theta angles of 15-20 degrees [17]. (6) hydroxyapatite-gelatin (HAp-GEL) composites were cross-linked with different amounts of GA between 2Theta 25-55 degrees [18]. When compared to the PVA-E-1-2x (Ar) indicator electrode, the spectrum pattern of the PVA-E-GA-1-indicator electrode (1x, 2x, 3x) did not experience a 2Theta angle shift on a scale without decimals of about 44 degrees. In connection with the background in the method of making this indicator electrode, there are two ways; the first method has been completed, the following is the second method consisting of a 2.9% GA sample with the last coating, namely the old PVC-KTpCIPB notated B-4-O and the new PVC-KTpCIPB notated B-4-N. PVA-E coated GA coated again with the old PVC-KTpCIPB coded B1-4-O. The GA-coated PVA-E was re-coated with a new PVC-KTpCIPB given the notation B1-4-N.

The intensity of the diffraction spectrum pattern against the 2Theta angle in a second way, the height of the peak intensity of the 2Theta angle from B1-4-O, B2-4-O, B3-4-O (old sample) B1-4-N, B2-4-N, B3-4-N (a new sample) and Ar can be seen in table 3 and figure 3.

Table 3 and figure 3 illustrate the height of the intensity peak against the 2Theta angle, the XRD diffraction spectrum pattern of B1-4-O and B1-4-N. There are differences in the intensity spectrum pattern with the 2Theta angle. Samples B1-4-O whose peak elevation is above 200 a.u are 452 a.u, 280 a.u, 286 a.u and Ar 6306 a.u at 2Theta angles 44.32 degrees, 44.30 degrees, 44.34 degrees. The highest peak intensity at B1-4-O was 452 a.u at 44.32 degrees. Sample B1-4-N in which the peak elevation is above 200 a.u is 286 a.u; 260 a.u; and Ar 6306 a.u at an angle of 2 Theta 44.30 degrees; 44.30 degrees; and 44.52 degrees, the highest peak in the composition B1-4-N was 286 a.u 44.30 degrees.

Table 3 and Figure 3 analyze the second method chosen for the composition of B1-4-O and B1-4-N, which means that PVA-E coated with GA was 1x further coated with the old PVC-KTpClPB 1x notation B1-O and given PVC- The new KTpClPB marked as B1-N. PVA-E coated with GA 2.9 % 1x coated again with the old PVC-KTpClPB 1x with the notation B1-4-O and coated with the new PVC-KTpClPB 1x with the notation B1-4-N. The best two samples are the second method on the B1-4-O composition, namely the second method of making indicator electrodes where 1x PVA-E is coated with 2.9% GA 1x is layered again with 1x old PVC-KTpClPB.

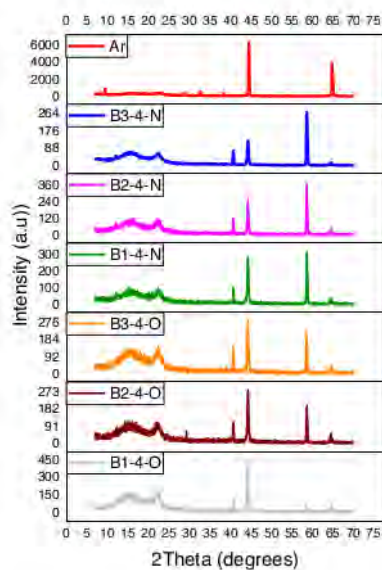


Figure 3. XRD diffraction spectrum pattern (a) B1-4-O, (b) B2-4-O, (c) B3-4-O (d) B1-4-N, (e) B2-4-N, (f) A3-4-N and (g) Ar.

After analyzing the method of making indicator electrodes with XRD, the next step was to amplify the data from the second method using UV-Vis, FTIR, and SEM-eds analysis. Based on the analysis of UV-Vis analysis, the GA concentration test between variations of 2.6 – 3.0% of A1, A2, A3, A4, and A5 are in Table 4 and Figure 4, geometric shapes [19; 20). There are three samples

of A1, A4, and A5 whose absorbance spectrum pattern is above 1000 a.u with a wavelength in the range between 250–400 nm from the PVA-E-GA solution.

Based on Figure 4 (a) the absorbance spectrum pattern of Ar has a high absorbance peak of 7,246 a.u at a wavelength of 291 nm, while with a variation of (A) A1; (B) A4; and (C) A5 absorbance peak height 1.083 a.u; 1540 a.u; 1,370 a.u at a wavelength of about 299 nm. This wavelength shift occurred with the addition of glutaraldehyde. Figure 4 (a), (b), and (c) have large peak widths, while Figure 4 (d) has a small peak width.

Figure 4 Pattern of UV-Vis absorbance spectrum for the wavelength of PVA-GA-E solution with variations in the symmetrical spectrum pattern (a) A1; (b) A4; and (c) A5 in Figure 4. The symmetrical pattern of the absorbance spectrum to wavelength is supported by [21]. If Figure 4 (a, b and c) is compared with the configuration of the PVA-E solution in Figure 4 (d), then there is a decrease in the absorbance peak height of the solution with variations of (a) A1; (b) A4; and (c) A5, followed by widening of the absorbance peak see figure 4.

Table 4. Absorbance for PVA-GA-E and PVA-E solution wavelengths

Wavelength (nm)	Absorbance (a.u)			
	A1	A4	A5	Ar
291	0,571	0,901	0,732	7.246
299	1.083	1.540	1.370	0.23

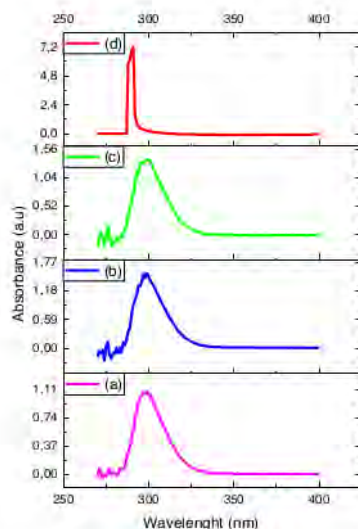


Figure 4. Pattern of UV-Vis absorbance spectrum with variations in glutaraldehyde; (a) A1; (b) A4; (c) A5 and (d) Ar.

Based on the analysis of the XRD diffraction spectrum pattern for the 2Theta angle and the UV-Vis absorbance spectrum pattern for the above wavelengths, it shows that the best glutaraldehyde composition was 2.9% of A3-4-O group. A3-4-O is the first method of making indicator electrodes where PVA-E and GA are mixed, morphology by SEM analysis can be seen in Figure 5a.

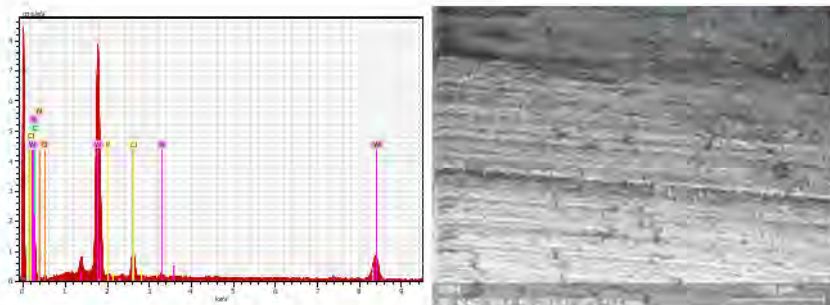


Figure 5a. EDS spectrum pattern and Morphological indicator electrode A3-4-O

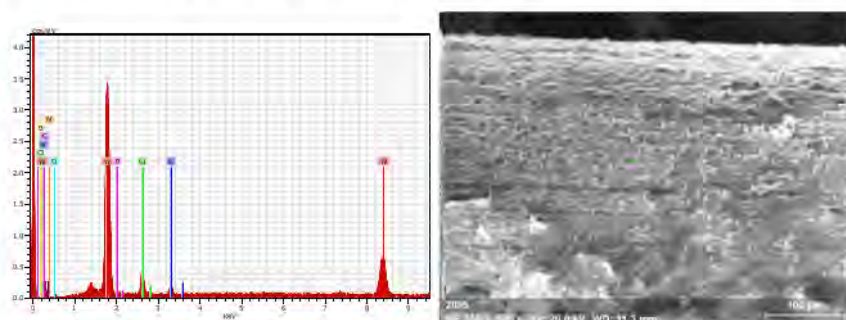


Figure 5b. EDS spectrum pattern and morphology of indicator electrode B1-4-O

There are differences in the spectrum pattern of EDS A3-4, B1-4 and the morphology of A3-4-O with B1-4-O. B3-4-O cracks while B1-4-O looks many pores at 100 m magnification 500x acceleration stress 20.00 Kv. A3-4 is an indicator electrode coated with PVA-E mixed with GA 29% 3x layered again with PVC-KTpCIPB 1x cross-linked method. B1-4 is an indicator electrode coated with PVA-E 1x coated with GA 2.9% 1x coated again with PVC-KTpCIPB 1x entrapment method, There are differences found in the cross method, so it is better to use the entrapment method [11]. The cross-linking technique [5] strengthens the biocatalyst bond, preventing leakage. Several SEM analyses using GA to study surface morphology and energy dispersive X-ray (EDS) analysis are [1] at an accelerating voltage of 10.00 kV, magnifications of 750x, 1500x, and 3500x. SEM magnification 5000 x good performance GA 1.25% [22;23]. The analysis of the use of enzyme immobilization and its feasibility in industrial-scale biocatalysis [24] used SEM.

Based on chemical structure analysis $(ClC_6H_4)_4BK$ is KTpCIPB functional group K and Cl, $(CH_2CHCl)_n$ is PVC functional group is Cl, $[-CH_2CHOH-]_n$ is PVA functional group is OH, NH_2CONH_2 is urease enzyme functional group is C=O, $OHC(CH_2)_3CHO$ is glutaraldehyde the functional group is OH. C_4H_8O is Tetrahydrofuran the functional group is O.

Based on Figure 6b and analysis of spectroscopic table data, there are two C-H hydrogen bands in the aldehyde group with absorption frequencies of $2800-2860\text{ cm}^{-1}$ and $2700-2760\text{ cm}^{-1}$. The results obtained at B1-4-O absorption frequency are at 2852.16 cm^{-1} , New absorption frequency of 2852.61 cm^{-1} and B3-4-A absorption frequency of 2832.16 cm^{-1} . Similarly, the determination of the group function in the mixture for the urease enzyme functional group C=O at $1720-1740\text{ cm}^{-1}$ at B1-4-O absorption frequency 1737.11 cm^{-1} . PVA coated with GA functional group is O-H at absorption frequency between $3330-3350\text{ cm}^{-1}$ at B1-4-O absorption frequency 3327.02 cm^{-1}

¹. Mixture of PVC-KTpCIPB in THF the functional group is CO at 1100-1350 cm^{-1} at B1-4-O absorption frequency 1165.90-1326.89 cm^{-1} and acetal ring (COC) at absorption frequency 1000 - 1140 cm^{-1} at B1-4-O absorption frequency 1011.06-1083.60 cm^{-1} . sp² C-H band pattern of alkenes in the cis group with absorption frequency of 675-730 cm^{-1} and the trisubstituent alkene group with absorption frequency of 790-840 cm^{-1} . In B1-4-O the absorption frequency is 688.80 cm^{-1} for the cis group with alkene substitution, while the absorption frequency is 798.83 cm^{-1} for the trisubstituent alkene group.

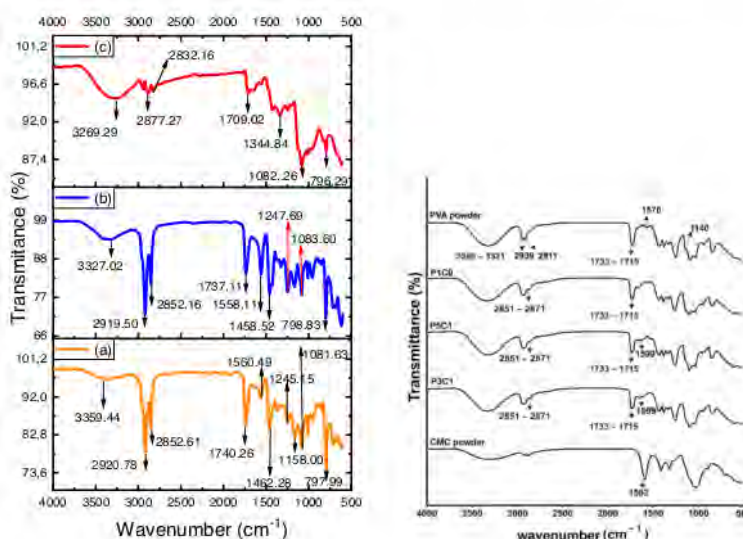


Figure 6. FTIR spectrum pattern of the sample (a) New, (b) B1-4-O, (c) A3-4-O, (d) PVA [Namkaew, J., et al, 2021]

FTIR analysis showed a complete loss of the measuring frequency of organic species and other functional groups in the template belonging to the peak [25] characterized functional group changes [26]. FTIR analysis of PVA and GA powders were influenced by the ratio [27] from the FTIR Spectrum of pure PVA membranes, PVA membranes crosslinked with glutaraldehyde (PVA/GA), and other mixtures [28]. Glutaraldehyde (GA) is a PVA crosslinking agent, substrate characterization of the FTIR spectrum pattern and XRD spectrum pattern showed the relationship between functional groups and crystal (metal) properties with the highest intensity [29]. It is similar to the analysis of the EDS spectrum pattern and the FTIR spectrum pattern [30]. Based on the structural analysis of the EDS spectrum pattern, the elements K, Cl, O, C, P, B and tungsten contained in the indicator electrode were found, supported by the presence of the C-H, C=O, O-H and C-O-C functional groups. To obtain the best sample, B1-4-O is an indicator electrode coated with PVA-enzyme coated with GA, further with the old PVC-KTpCIPB.

CONCLUSION

In this research paper, the first way of characterizing Glutaraldehyde on the urease enzyme immobilization of indicator electrode is by coating indicator electrode with PVA-E mixed with 2.9% GA 3x coated again with PVC-KTpCIPB 1x that can increase sensor activity and the best sample is A3-4. The second way is Characterizing Glutaraldehyde on the indicator electrode of the

urease enzyme immobilization of the indicator electrode by coating PVA-E 1x coated with 2.9% GA 1x further with PVC-KTpCIPB and the best sample was B1-4-O. Thus, it can increase sensor activity. The best sample is the A4 sample with 2.9% GA composition with UV-Vis analysis.

There is a change in the intensity spectrum pattern by coating the membrane with XRD, SEM-EDS, and FTIR by PVC-KTpCIPB old samples.

ACKNOWLEDGEMENT

The author would like to thank the Chancellor of the State University of Medan who has provided research grants for BASIC RESEARCH, the chairman of the LPPM State University of Medan, the Physics Laboratory of the State University of Medan and LIPI-Bandung.

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CHARACTERIZATION OF GLUTARALDEHYDE COMPOSITION ON PVA-ENZYME COATED PVC-KTPCLPB MEMBRANE WITH XRD, UV-VIS, SEM-EDS, AND FTIR

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ABSTRACT

An indicator electrode has been made in two ways using the urease enzyme immobilization technique with Glutaraldehyde (GA) crosslinking at various concentrations (2.6 - 3.0)%. The aim is to choose the best indicator electrode from both methods, this is through XRD, SEM-EDS, and FTIR analysis. The method used is a potentiometric biosensor method. The indicator electrode for the first method is denoted PVA-E-GA/PVC-KTpCIPB, meaning that PVA-E is mixed with PVA-E solution coated with PVC-KTpCIPB. The indicator electrode of the second method is denoted PVA-E/GA/PVC-KTpCIPB, meaning that PVA-E coated with GA is coated with PVC-KTpCIPB. Each in 1x, 2x, and 3x layer variations. The best results of GA crosslinking from the first and second methods were at a concentration of 2.9%. The best result of the first method of indicator electrode is PVA-E-GA3x/PVC-KTpCIPB1x. the best result of the second method is the indicator electrode PVA-E 1x/GA 1x/PVC-KTpCIPB 1x.

Keywords: Indicator Electrode, Immobilization, XRD, SEM-EDS, and FTIR Analysis.

RASAYAN *J. Chem.*, Vol. 15, No. 4, 2022

INTRODUCTION

Enzyme immobilization is the process of physical localization of enzymes on certain surfaces which helps to improve some of the properties of enzymes and their operational performance without disturbing their catalytic activity, thereby enabling the recovery and reuse of enzymes so that the whole process can be controlled and economical.¹ Research on indicator electrodes as urea sensors has been carried out using a biosensor potentiometric method with urease enzyme immobilization technique on PVA membrane, namely PVA-Enzyme coated with PVC-KTpCIPB labeled PVA-E-1-2x with Ar notation. 0.0350 g of PVA dissolved in 10 mL of warm water is denoted PVA-E. 0.0350 g PVC-0.0500 g KTpCIPB dissolved in 10 mL THF denoted PVC-KTpCIPB. It has obtained a sensitivity of 19,069 mV/decade, a detection range of $1.10^{-5} - 5.10^{-4}$ M, a detection limit of 1.10^{-5} M, and a correlation coefficient of 0.9431.² Based on the analysis, this detection range is small about the width of the peak absorbance spectrum concerning wavelength. Researchers want to analyze the interaction in the form of molecular adsorption to increase the detection limit and selectivity of the biosensor.³ Increasing or decreasing glutaraldehyde concentration from 3% causes a decrease in activity,⁴ and the optimal glutaraldehyde concentration of 2.5-3% is used for urease immobilization. The techniques used are absorption, trapping, and cross-linking. The cross-linking technique⁵ contributes to strengthening the biocatalyst bond, preventing leakage, reducing desorption, and increasing the stability of the biocatalyst. Based on these contributions, the authors chose glutaraldehyde to strengthen the detection range of the urea sensor with an indicator electrode made of tungsten immobilized by the urease enzyme on the indicator electrode. Immobilization of urease enzyme in PVA solution is denoted by PVA-E. There are two cross-links in PVA-E by (1) mixing GA with PVA-E-GA notation and (2) coating PVA-E with GA on the indicator electrode with PVA-E/GA notation. Each of the first and second methods was coated again with PVC-KTpCIPB. The design of the indicator electrode in the first method is denoted PVA-E-GA/PVC-KTpCIPB. The design of the second method is denoted by PVA-E/GA/PVC-KTpCIPB. This aims to obtain the best way to immobilize the urease enzyme. The success of enzyme immobilization and surface modification was measured using a UV-Visible spectrophotometer.⁶ The immobilization strategy was then applied to the biosensor application for urea detection.

EXPERIMENTAL

Material and Methods

The materials used in this study were the enzyme Uriase, EC 3.5.1.5 (Urea) U4002, 50-100 ix Sigma-Aldrich type, PVA:PVC 1:1 with a mass of 0.0350 g, GA with variations (0.26, 0, 27, 0.28, 0.29, and 0.30) g in 10 mL solvent, KTpCIPB 0.0500 g. This material is used in the form of a solution, the manufacture of indicator electrodes from potentiometer cells using the biosensor potentiometric method modified PVA-E indicator electrode layer crosslinked GA and PVC-KTpCIPB. Crosslinks are modified in two ways. The equipment used in the Physics Laboratory is XRD-6100 Shimadzu, UV-Vis Leigh UV-1601, SEM Evo MA 10 Zeiss, Coating from SEM Q150RES Quorum, Stirrer, and FTIR from the Medan Customs and Excise Laboratory.

General Procedure

PVA 0.0350 g dissolved in 10 mL of hot water until cold. The urease enzyme 1 mg is dissolved in 0.5 mL of water mixed with alcohol in a ratio of 50%: 50%. Add one drop of the enzyme, and stirred with a Stirrer until mixed into 10 mL cold 0.0350 g PVA. THF was mixed with 0.0350 g PVC and 0.0500 g KTpCIPB in 10 mL. The indicator electrode is made in two ways. In the first method, Glutaraldehyde is mixed with PVA-E to become PVA-E-GA/PVC-KTpCIPB denoted A. The indicator electrode for the second method is PVA-E coated with GA coated again with PVC-KTpCIPB labeled PVA-E/GA/PVC-KTpCIPB denoted B. The method is to obtain the best method in the manufacture of indicator electrodes. The composition of the polymer membrane on the ion selective electrode (ISE) according to⁷ by weight 1% ionophore, PVC polymer matrix: plasticizer (1: 2). Synthesis of polymers in the development of ISE sensors according to⁸, membranes at the ratio of Ionophores: PVC: KTCIPB: Plasticizers 10: 165: 5: 330. An increase or decrease in the concentration of glutaraldehyde by more than 3% causes a decrease in activity, determining the optimal concentration of glutaraldehyde between 2.5% -3% for urease enzyme immobilization.

Detection Method

The first method, fifteen PVA-E-GA 1x/PVC-KTpCIPB 1x indicator electrodes with GA variations (2.6%, 2.7%, 2.8%, 2.9% and 3%) are notated A1-1, A1-2, A1-3, A1-4, and A1-5. Five indicator electrodes PVA-E-GA 2x/PVC-KTpCIPB 1x are denoted A2-1, A2-2, A2-3, A2-4, and A2-5. Five indicator electrodes PVA-E-GA 3x/PVC-KTpCIPB 1x are denoted A3-1, A3-2, A3-3, A3-4, and A3-5. The second method uses the old and new PVC-KTpCIPB. Five indicator electrodes PVA-E1x/GA1x/PVC-KTpCIPB1x with variations in GA (2.6%, 2.7%, 2.8%, 2.9%, and 3%), where PVC-KTpCIPB-old is denoted B1-1-O, B1-2-O, B1-3-O, B1-4-O, and B1-5-O. Five indicator electrodes PVA-E1x/GA1x/PVC-KTpCIPB1x with variations in GA (2.6%, 2.7%, 2.8%, 2.9%, and 3%), where PVC-KTpCIPB-newly denoted B1-1-N, B1-2-N, B1-3-N, B1-4-N, and B1-5-N. All indicator electrodes analyzed by XRD can be seen in Table-1, after being selected, it can be seen in Fig.-1.

Analytical Discussion

For selecting the best sample, the first step is XRD analysis, the second is UV-Vis analysis, the third is SEM-EDS and the fourth is FTIR. XRD analysis of selected samples with an intensity greater than 200 a, u concerning the diffraction angle of 2theta. Performed for all samples A1, A2, A3, A4, A5; B1-O, B2-O, B3-O, B4-O, and B5-O; B1-N, B2-N, B3-N, B4-N, and B5-N. After selecting the best sample from Table-3 and Fig.-3, the SEM-EDS analysis continued with the selected samples A3-4-O and B1-4-O as shown in Fig.-5a and 5b as the third step. In the second step, only the PVA-E-GA solution was analyzed by UV-Vis from the PVA-GA-E solution, the results can be seen in Fig.-4. The four membranes (a) B1-4-N, (b) B1-4-O, and (c) A3-4-O were analyzed by FTIR see Fig.-6. The final decision in selecting the sample was to find the relationship between all XRD, UV-Vis, SEM-EDS, and FTIR analyses.

RESULTS AND DISCUSSION

Based on Table-1 sample groups A1, A2, and A3 with variations in GA (2.6%, 2.7%, 2.8%, 2.9%, and 3%) and Ar, the height of the intensity peak changes with the angle of 2Theta. The height of the peak intensity concerning the 2Theta angle of each sample can be seen in Table-1. The highest peak intensity of A1-1 is 270 a.u at 44.28 degrees; A1-2 is 146 a.u at 44.36 degrees; A1-3 is 190 a.u at 44.32; A1-4 is 236 a.u at 44.32 degrees; A1-5 is 278 a.u at 44.28 degrees; Ar is 6306 a.u at 44.52 degrees.

Table-1: The Height of the Peak Concerning Angles 2Theta A1, A2, A3, and Ar

2Theta (degrees)	Intensity (a.u)					
	A1-1	A1-2	A1-3	A1-4	A1-5	Ar
44.28	270	102	136	198	278	330
44.32	238	134	190	236	256	452
44.36	242	146	138	176	256	754
44.52	110	46	56	80	130	6306

2Theta (degrees)	Intensity (a.u)					
	A2-1	A2-2	A2-3	A2-4	A2-5	Ar
44.30	128	166	170	212	230	378
44.32	150	184	212	228	220	452
44.52	52	76	88	84	82	6306

2Theta (degrees)	Intensity (a.u)					
	A3-1	A3-2	A3-3	A3-4	A3-5	Ar
44.28	216	162	352	340	278	330
44.32	220	196	338	382	256	452
44.52	86	62	126	118	130	6306

The highest peak intensity of A2-1 was 150 a.u at 44.32 degrees; A2-2 is 184 a.u at 44.32 degrees; A2-3 is 212 a.u at 44.32 degrees; A2-4 is 228 a.u at 44.32 degrees; A2-5 is 230 a.u at 44.30 degrees, Ar is 6306 a.u at 44.52 degrees. The highest peak intensity of the A3-1 was 220 a.u at 44.32 degrees; A3-2 is 196 a.u at 44.32 degrees; A3-3 is 352 a.u at 44.28 degrees; A3-4 is 382 a.u at 44.32 degrees; A3-5 is 278 a.u at 44.28 degrees. Sample selection based on Table-1 data, the height of the intensity peak with the diffraction angle 2theta greater than 200 au was taken as a sample. The samples are (a) A1-1, (b) A1-4, (c) A1-5 (d) A2-3, (e) A2-4, (f) A2-5, (g) A3-1, (h) A3-3 (i) A3-4, (j) A3-5, and (k) Ar can be seen in Fig.-1. Based on the analysis of Fig.-1, the samples were selected again according to groups, obtained in groups A1 and A3. Group A1 is A1-5 and A2-5, and group A3 is A3-4. The highest intensity peak in the composition A3-4 sees Table-2 and Fig.-2. Analysis of the intensity diffraction spectrum pattern against the 2Theta angle according to⁹ shows the diffraction peak at the Bragg angle. Table-2 and Fig.-2 show that the A4 sample group, namely A3-4, has a higher intensity peak than the A5 sample group, namely A1-5. The intensity spectrum pattern against the 2Theta angle of the sample groups A4 and A5 can change the diffraction intensity of Ar. The amorphous spectral patterns in Fig.-1 and 2 are compared with the Ar samples after the addition of GA.

PVA-E mixed with GA labeled PVA-E-GA is the first method of making indicator electrode membranes. Based on Fig.-2, the intensity spectrum pattern concerning angle 2theta, Ar is 6306 a.u at 44.52 degrees, while A3-4 decreases the peak intensity concerning angle 2theta to 382 a.u at 44.32 degrees, due to the addition of 2.9% GA. The optimum concentration of glutaraldehyde in GA is 2.5%.¹⁰ The effect of GA on the biosensor response for a low concentration of 1.5% makes the substrate easy to leak from the membrane due to less cross-linking. For concentrations as high as 3.5%, the biosensor response is less due to more cross-linking, which prevents the substrate from passing through the membrane. Glutaraldehyde cross-linking produces large enzyme aggregates that can serve as catalysts and support for many substrate molecules. Enzyme crosslinking is detrimental to enzyme leaching and the entrapment followed by crosslinking disrupts total binding activity and also greatly enhances enzyme stability in extreme chemical environments.¹¹ Appropriate addition of glutaraldehyde to produce higher enzyme stability at a concentration of 0.03% glutaraldehyde, in 0.1 M phosphate buffer with a pH variation of 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5, and 8.0 for 30 minutes.¹² Changes in the height of the peak intensity concerning the 2Theta angle can occur due to the addition of material in PVA. Several results of previous studies (1) PVA-doped

ZnO composites were at an angle of 2theta 30-40 degrees¹³; (2) PVA/SiO₂-TiO₂ around 2theta 20 degrees¹⁴; (3) CA and PVA doped ZnO composite nanostructures between 2theta angles of 40-50 degrees¹⁵; (4) PVA nanofiber hybrids are between 2theta angles of 30-40 degrees¹⁶; (5) PVA/PAA/Fe₃O₄ between 2theta angles of 15-20 degrees¹⁷; (6) The hydroxyapatite-gelatin (HAp-GEL) composite was crosslinked with different GA between 2theta angles of 25-55 degrees.¹⁸

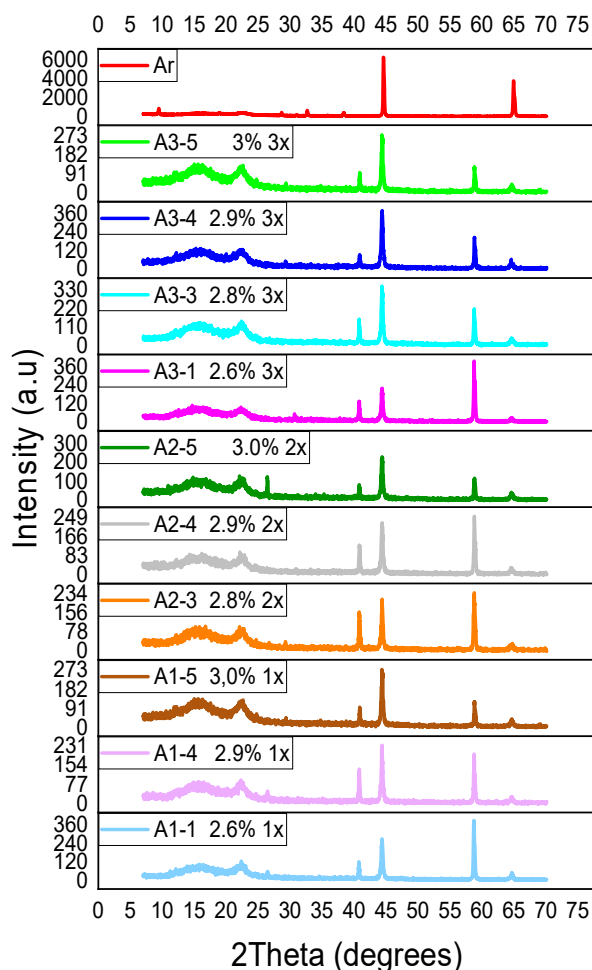


Fig.-1: XRD Diffraction Spectrum Pattern (a) A1-1, (b) A1-4, (c) A1-5 (d) A2-3, (e) A2-4, (f) A2-5, (g) A3-1, (h) A3-3 (i) A3-4, (j) A3-5, and (k) Ar

Table-2: The Highest Intensity Peak Concerning the 2 Theta angle of A1-5; A2-5, A3-4, and Ar

2Theta (degrees)	Intensity (a.u)		2Theta (degrees)	Intensity (a.u)		2Theta (degrees)	Intensity (a.u)	
	A1-5 3,0% 1x	Ar		A2-5 3,0% 1x	Ar		A3-4 3,0% 1x	Ar
44.28	278	330	44.30	230	378	44.28	340	330
44.32	256	452	44.32	220	452	44.32	382	452
44.52	130	6306	44.52	82	6306	44.52	118	6306

When compared with the indicator electrode (Ar), namely PVA-E-1-2x which is coated with PVA-enzyme 1x coated with PVC-KTpCIPB 2x. The 1x PVC-KTpCIPB coated PVA-E-GA coated indicator electrode (1x, 2x, 3x) does not undergo a 2 theta angular shift on a non-decimal scale of approximately 44 degrees.

The method of making the electrode is the first method. The following is the second method for the PVA-enzyme coated indicator electrodes with 2.9% GA coated again with PVC-KTpCIPB (1x, 2x, 3x), with the old PVC-KTpCIPB material with the notation B-4-O and the new PVC-KTpCIPB with the notation B-4-N. GA coated with PVA-E coated again with the old PVC-KTpCIPB coded B1-4-O.

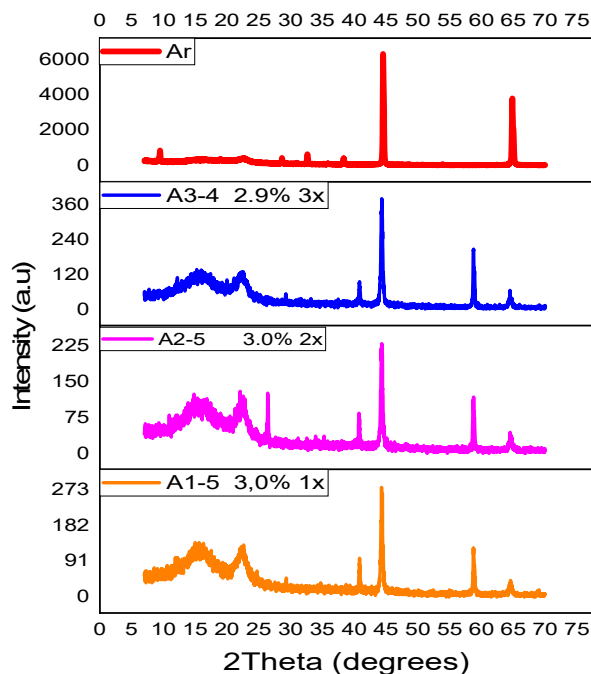


Fig.-2: XRD Diffraction Spectrum Pattern (a) A1-5, (b) A2-5, (c) A3-3 (d) A3-4 and (G) Ar

The indicator electrode was coated with PVA-E coated GA coated again with a new PVC-KTpCIPB with the notation B1-4-N. The intensity of the diffraction spectrum pattern concerning the 2θ angle in a second way from B1-4-O, B2-4-O, B3-4-O (old sample), B1-4-N, B2-4-N, B3-4-N (a new sample) and Ar can be seen in Table-3 and Fig.-3. There is a difference in the intensity spectrum pattern with a 2θ angle. The selected B1-4-O samples peaked above 200 a.u. The highest peak intensity of B1-4-O is 452 a.u at 44.32 degrees. Likewise, the sample B1-4-N was selected for peaks above 200 a.u, the highest peak in the B1-4-N composition was 286 a.u at 44.30 degrees.

Table-3: The Height of the Intensity Peaks Concerning the 2θ Angle of B1-4-O, B2-4-O, B3-4-O; B1-4-N; B2-4-N, A3-4-N, and Ar

2θ (degrees)	Intensity (a.u)			
	B1-4-O	B2-4-O	B3-4-O	Ar
44.30	436	280	278	378
44.32	452	262	280	452
44.34	428	276	286	582
44.52	188	118	116	6.306
2θ (degrees)	Intensity (a.u)			
	B1-4-N	B2-4-N	B3-4-N	Ar
44.30	286	260	127	378
44.52	188	118	116	6.306

Intensity Ar 6306 a.u at an angle of 2θ 44.30 degrees. Analysis of Table-3 and Fig.-3, using the second method, the compositions B1-4-O and B1-4-N were selected, which means that PVA-E coated with GA 1x was further coated with old PVC-KTpCIPB 1x notation B1-O; given the new PVC-KTpCIPB notated as B1-N. This means that PVA-E is coated with 2.9% GA 1x coated again with the old PVC-KTpCIPB 1x with the notation B1-4-O and coated with the new PVC-KTpCIPB 1x with the notation B1-4-N. The two best samples are the second method on the B1-4-O composition, namely the second method of making

indicator electrodes coated with PVA-E 1x coated with 2.9% GA 1x coated again with the old PVC-KTpClPB 1x.

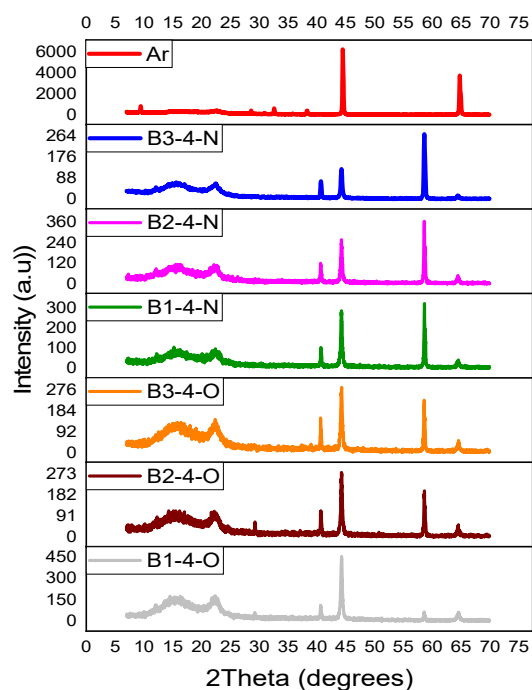


Fig.-3: XRD Diffraction Spectrum Pattern (a) B1-4-O, (b) B2-4-O, (c) B3-4-O (d) B1-4-N, (e) B2-4-N, (f) A3-4-N and (g) Ar

Table-4: Absorbance for PVA-GA-E and PVA-E Solution Wavelengths

Wavelength (nm)	Absorbance (a.u)			
	A1	A4	A5	Ar
291	0.571	0.901	0.732	7.246
299	1.083	1.540	1.370	0.23

After analyzing the method of making indicator electrodes with XRD, the next step is the explanation of the second method using UV-Vis, FTIR, and SEM-EDS analysis. Based on the analysis of UV-Vis analysis, the GA concentration test was carried out between 2.6-3.0% variations in samples A1, A2, A3, A4, and A5 as shown in Table-4 and Fig.-4, the shape is symmetrical.^{19,20} There are three samples A1, A4, and A5 whose absorbance spectrum pattern is above 1.000 a.u with wavelengths ranging from 250-400 nm from the PVA-E-GA solution. Based on Fig.-4 (a) the absorption spectrum pattern of Ar has a high absorption peak of 7.246 a.u at a wavelength of 291 nm, while with variations (A) A1; (B) A4; and (C) A5 absorption peak height, respectively 1.083 a.u; 1.540 a.u; 1.370 a.u at a wavelength of about 299 nm. This wavelength shift occurs with the addition of glutaraldehyde. Fig.-4(a), (b), and (c) have a large peak width, while Figure 4 (d) has a small peak width. The UV-Vis absorbance spectrum pattern concerning the wavelength of the PVA-GA-E solution with variations in GA concentration 2.%, 2.9%, and 3.0% formed a symmetrical spectrum pattern (a) A1; (b) A4; and (c) A5 in Fig.-4. The symmetrical pattern of the absorbance spectrum concerning wavelength is supported by.²¹ If Fig.-4 (a, b, and c) is compared with the configuration of the PVA-E solution in Fig.-4 (d), then there is a decrease in the absorbance peak height of the solution with variations in the concentration of GA (a) A1; (b) A4; and (c) A5, followed by widening of the absorbance peak see Fig.-4.

Similarly, the determination of the group function in the mixture for the urease enzyme functional group C=O at 1720-1740 cm^{-1} at B1-4-O absorption frequency 1737.11 cm^{-1} . PVA coated with GA functional group is O-H at absorption frequency between 3330-3350 cm^{-1} at B1-4-O absorption frequency 3327.02

cm^{-1} . A mixture of PVC-KTpCIPB in THF the functional group C-O between $1100\text{-}1350\text{ cm}^{-1}$ in B1-4-O absorption frequency $1165.90\text{-}1326.89\text{ cm}^{-1}$ and the acetal ring functional group (C-O-C) between absorption frequency $1000\text{ - }1140\text{ cm}^{-1}$ in B1-4-O absorption frequency $1011.06\text{-}1083.60\text{ cm}^{-1}$.

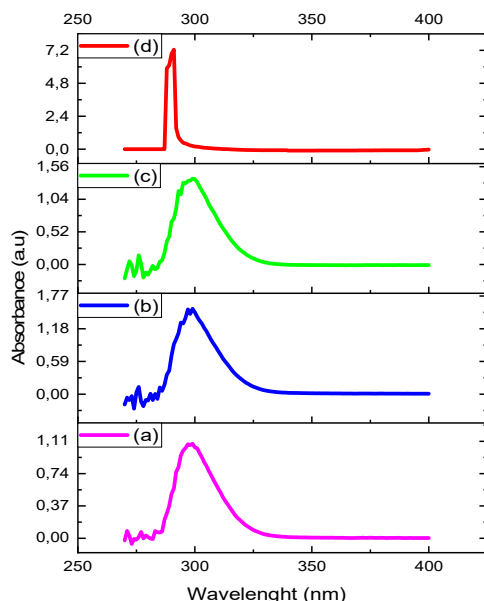


Fig.-4: Pattern of UV-Vis Absorbance Spectrum with Variations in Glutaraldehyde; (a) A1; (b) A4; (c) A5 and (d) Ar

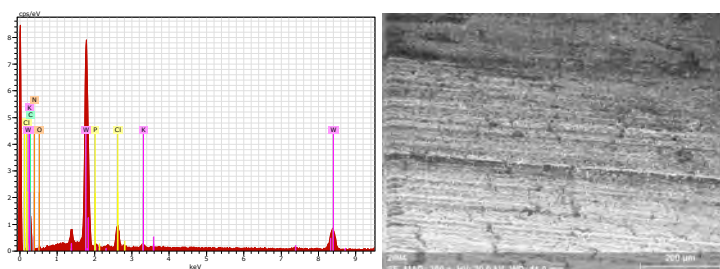


Fig.-5a: EDS Spectrum Pattern and Morphology Indicator Electrode A3-4-O

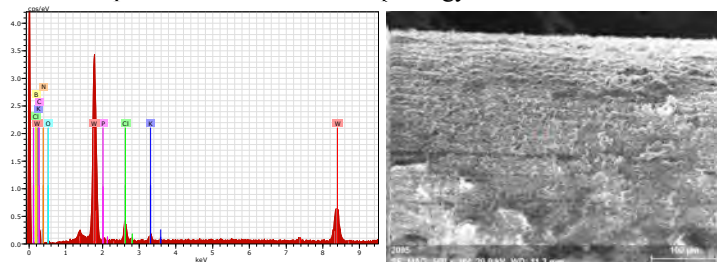


Fig.-5b: EDS Spectrum Pattern and Morphology of Indicator Electrode B1-4-O

The Sp^2 C-H functional group in the alkene pattern band in the cis group with an absorption frequency of $675\text{-}730\text{ cm}^{-1}$ and the trisubstituent alkene group with an absorption frequency of $790\text{-}840\text{ cm}^{-1}$. In B1-4-O the absorption frequency is 688.80 cm^{-1} for the cis group with alkene substitution and the absorption frequency is 798.83 cm^{-1} for the trisubstituent alkene group. Based on Fig.-6 and analysis of spectroscopic table data, there are two C-H hydrogen bands in the aldehyde group with absorption frequencies of $2800\text{-}2860\text{ cm}^{-1}$ and $2700\text{-}2760\text{ cm}^{-1}$. The results were obtained at a B1-4-O absorption frequency of 2852.16 cm^{-1} and B1-4-N absorption frequency of 2852.61 cm^{-1} and A3-4-O absorption frequency of 2832.16 cm^{-1} . Similarly, the determination of the group function in the mixture for the urease enzyme functional group C=O at $1720\text{-}1740\text{ cm}^{-1}$ at B1-4-O absorption frequency 1737.11 cm^{-1} . PVA coated with GA functional

group is O–H at absorption frequency between 3330–3350 cm^{-1} at B1-4-O absorption frequency 3327.02 cm^{-1} . A mixture of PVC-KTpCIPB in THF the functional group C–O between 1100–1350 cm^{-1} in B1-4-O absorption frequency 1165.90–1326.89 cm^{-1} and the acetal ring functional group (C–O–C) between absorption frequency 1000 - 1140 cm^{-1} in B1-4-O absorption frequency 1011.06–1083.60 cm^{-1} . The Sp^2 C–H functional group in the alkene pattern band in the cis group with an absorption frequency of 675–730 cm^{-1} and the trisubstituted alkene group with an absorption frequency of 790–840 cm^{-1} . In B1-4-O the absorption frequency is 688.80 cm^{-1} for the cis group with alkene substitution and the absorption frequency is 798.83 cm^{-1} for the trisubstituted alkene group.

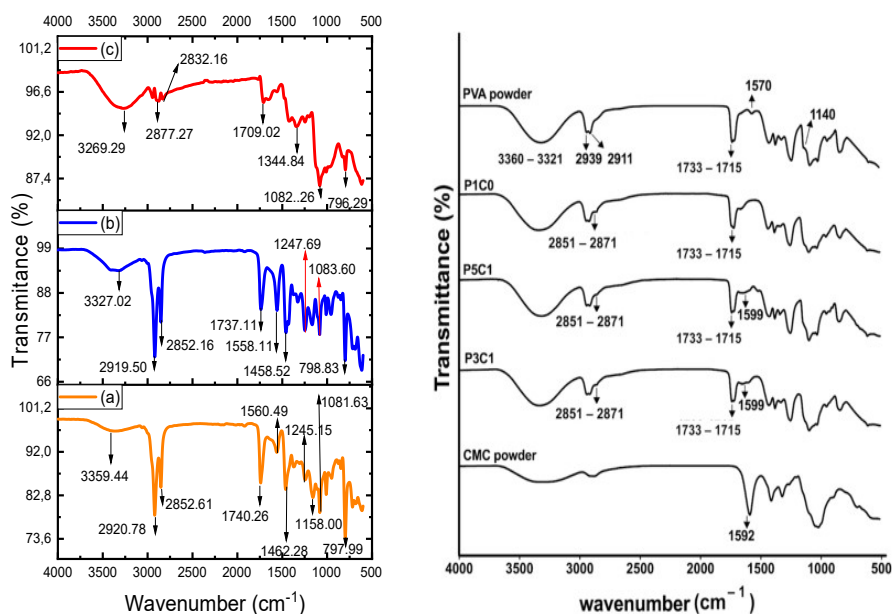


Fig.-6: FTIR Spectrum Pattern of the Sample (a) B1-4-N, (b) B1-4-O, (c) A3-4-O, (d) PVA²⁷

According to the FTIR analysis of the pattern of the transmittance spectrum to the wave number, see Fig.-6, it is symmetrical for samples B1-4-N and B1-4-O while samples A3-4-O are not symmetrical. Likewise, XRD analysis in Fig.-3 and SEM-EDS analysis in Fig.-5a and 5b, so that the best sample was selected for the manufacture of indicator electrodes coated with PVA-enzyme 1x coated with 2.9% GA, 1x coated with PVC-KTpCIPB 1x with the notation B1-4. FTIR analysis showed the loss or shift in the absorbance frequency of organic species and functional groups²⁵ marked by changes in functional groups.²⁶ FTIR analysis of PVA and GA powders was influenced by the ratio²⁷ of the FTIR spectral patterns of pure PVA membranes, PVA membranes crosslinked with glutaraldehyde (PVA/GA), and other mixtures.²⁸ Glutaraldehyde (GA) is a PVA crosslinking agent, substrate characterization of the FTIR spectrum pattern and XRD spectrum pattern showed the relationship between functional groups and crystal properties (metal) with the highest intensity.²⁹ This is similar to the analysis of the EDS spectrum pattern and the FTIR spectrum pattern.³⁰ Based on the structural analysis of the EDS spectrum pattern, it was found that the elements K, Cl, O, C, P, B, and tungsten contained in the indicator electrode were supported by the C–H, C=O, O–H, and C–O–C functions.

CONCLUSION

Based on the description above and analysis in two ways, the conclusions are obtained, the best results are as follows (1): The first method is PVA-enzyme mixed with 2.9% GA with the notation PVA-E-GA, the indicator electrode is coated with PVA-E-GA 3x coated with PVC-KTpCIPB 1x, become PVA-E-GA3x/PVC-KTpCIPB1x with the notation A3-4-O; (2) the second method is PVA-enzyme coated with 2.9% GA with the notation PVA-E/GA, the indicator electrode is coated with PVA-E 1x coated with GA 1x again coated with PVC-KTpCIPB 1x, becoming PVA-E 1x/GA 1x/PVC-KTpCIPB 1x, denoted B1-4-N. There is an analytical relationship between XRD, SEM-EDS, and FTIR in selecting samples for making indicator electrodes using the urease enzyme immobilization technique.

ACKNOWLEDGEMENT

The author would like to thank the Chancellor of the State University of Medan who has provided research grants for BASIC RESEARCH, the Chairman of the Research and Community Service Institute Universitas Negeri Medan, the Physics Laboratory of the Universitas Negeri Medan, and LIPI-Bandung.

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[RJC-7073/2022]