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Characterization of indicator electrodes using the potentiometric biosensor method as urea sensor with XRD, SEM-EDS, UV-Vis and FTIR

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Abstract. The purpose of this study is the characteristics of the indicator electrode which has been coated with a membrane with XRD, SEM-EDS, UV-Vis and FTIR. Research has been carried out using XRD, SEM-EDS, UV-Vis and FTIR tests on immobilized indicator electrodes 0.0350 g PVA-Enzyme coated with PVC-KTpClPB 0.0120 g and 0.0500 g. The method used was the biosensor potentiometric method with the urease enzyme immobilization technique in PVA coated with PVC-KTpClPB. To see the difference in the absorbance spectrum pattern of each PVA-Enzyme composition coated with PVC-KTpClPB against wavelength with UV-Vis, PVA-Enzim solution as a reference and PVC-KTpClPB solution as the first analysis 0.0120 g and as the second analysis 0,0500 g. To see the PVA-enzyme membrane coated with PVC-KTpClPB in the two compositions above was characterized by FTIR and SEM-EDS. To see the indicator electrode that had been immobilized by PVA-enzyme coated with PVC-KTpClPB in the two compositions above, it was characterized by XRD. It was concluded that the best results were found on the immobilization indicator electrode of 0.0350 g PVA-Enzyme coated with 0.0350 g PVC-0.0500 g KTpClPB.

1. Introduction

Urea in the blood is transported to the kidneys from the liver filtered and excreted through urine [1] . Urea in milk contributes 55% of non-protein nitrogen. At normal levels of urea in natural milk are 3.0-6.0 mM, depending on the animal feed habits, the allowable limit for the concentration of urea in milk is usually ≤ 11 mM. In mammals urea is synthesized in the liver due to the end product of biochemical reactions involved in the formation of endogenous ammonia detoxification. Detection of urea through urea sensors is very much needed development, there are more rapid examples of capacitive multi-enzyme biosensor field effects based on comparative studies of Drop-Coating and Nano-Spotting Techniques [2]. Biomedical sensors used to detect metabolites, ions, and viruses are updated by [3] in the manufacture of electrochemical biosensors. Preparation of Urea Biosensor for Dynamic Liquid Measurement by [4].

A biosensor made in the form of a membrane is used for rapid detection [5]. The analysis used as follows (1) study of the membrane surface of the indicator electrode that has been modified is carried out by scanning electron microscopy (SEM) of membrane morphology [4; 6; 7], (2) FTIR spectral pattern shows immobilization of urease deposited on the electrodes [1; 8; 6; 7], (3) UV-Vis spectrum patterns [9]. (4) XRD diffraction spectrum patterns [7]. On the basis of the explanation above, the researcher performed the characterization of the electrode membrane using sequential analysis of XRD, UV-Vis, SEM-EDS and FTIR on two indicator electrodes with different compositions of KTpClPB, namely 0.0500 g and 0.0120 g.

2. Method

The indicator electrode was made using the biosensor potentiometric method with the urease enzyme immobilization technique on the PVA-Enzim membrane and then coated with PVC-KTpClPB. The PVA-Enzyme membrane was derived from a solution of 0.0350 g PVA and 1 mg urease enzyme, the PVC-KTpClPB membrane was derived from a PVC-KTpClPB solution[10;11]. The first method of making PVA solution consists of 0.0350 g of PVA dissolved in 10 mL of hot water to cool somewhere. The second method 0.0350 g PVC + 0.0500 g KTpClPB is dissolved in 10 mL THF in a glass tube and covered with a plastic / aluminum poil cover. The third method 0.0350 g PVC + 0.0120 g KTpClPB was dissolved in 10 mL THF in a glass tube and covered with a plastic / aluminum poil cover. The fourth method of 1 mg urease enzyme is dissolved in 0.5 mL of water mixed with alcohol in a ratio of 50% : 50%. The indicator electrode is made of tungsten wire with a diameter of 1 mm 99.99% Aldrech metal, amounting to three with a length of 1.5 cm.

The urease enzyme is dropped 1 drop in the PVA solution, stirred until blended to become PVA-enzyme. The tungsten indicator electrode was immersed in a 0.0350 g PVA-Enzyme solution to immobilize the urease enzyme and coated with 0.0350 g PVC + 0.0120 g KTpClPB. There are three tungsten used which will be used for dyeing 1x, 2x, and 3x which are denoted E_{A-1x}, E_{A-2x} and E_{A-3x}. Likewise, the tungsten indicator electrode was immersed in 0.0350 g PVA-Enzyme solution for immobilization of the urease enzyme and coated with PVC 0.0350 g + 0.0500 g KTpClPB, the indicator electrode was denoted E_{B-1x}, E_{B-2x} and E_{B-3x}.

Next, the six electrodes $E_{A-1}x$, E_{A-2x}, E_{A-3x} and E_{B-1x}, E_{B-2x} and E_{B-3x} were analyzed with XRD Figure 1. Making the indicator electrode begins with analysis of 0.0350 g PVA-Enzyme solution, 0.0350 g + PVC solution KTpClPB 0.0120 g and 0.0350 g PVC solution + 0.0500 g KTpClPB using UV-Vis. UV-Vis analysis through 0.0350 g PVA-Enzyme solution as reference, 0.0350 g PVC solution + 0.0120 g KTpClPB as analysis 1 and 0.0350 g + KTpClPB 0.0500 g PVC solution resulted in the absorbance spectrum pattern of Figures 4a and 4b.

For the manufacture of PVC-KTpClPB coated PVA-Enzim membranes, one drop of PVA-Enzim solution is dropped on clean glass using a dropper on the glass plane in two different places. After drying, add another drop once to obtain two layers of PVA-Enzyme in each place. After drying the second drop of each PVA-Enzyme layer, one of which is a drop of 0.0120 g of PVC-KTpClPB solution and the other is a drop of 0.0500 g of PVC-KTpClPB until dry so that a PVA-Enzim membrane is coated with PVC-KTpClPB 0.0120 g denoted M_1 and PVA-Enzim coated with PVC-KTpClPB 0.0500 g in M_2 denotation. Each membrane M_1 and M_2 were analyzed by SEM-EDS see Figures (2 and 3) and FTIR Figure 5.

3. Results and discussion

The six indicator electrodes E_{A-1x}, E_{A-2x}, E_{A-3x} and E_{B-1x}, E_{B-2x} and E_{B-3x} were analyzed by XRD and obtained six XRD diffraction spectrum patterns, see Figure 1. The XRD diffraction spectrum pattern of the six indicator electrodes is at intensity between the 2 theta angle of 40-50 degrees. PVA hybrid nanofibers are located between an angle of 2 theta 30-40 degrees [12]. The nanostructures with CA and PVA doped ZnO composites with intensities between 2 theta angle of 40-50 degrees [13]. PVA / SiO_2-TiO_2 is about 2 theta 20 degrees [16]. PVA between angle 2 angles 35-40 degrees [14]. PVA / PAA / Fe_3O_4 between 2 angles 15-20 degrees [15]. The best results of the XRD spectrum pattern of

the two compositions were obtained at the indicator electrodes E_{A-2x} and E_{B-2x}. The best spectral pattern was selected on the E_{B-2x} indicator electrode with immobilization of PVA-Enzim 2x coated with PVC-KTpClPB 0.0500 g 1x according to [10].
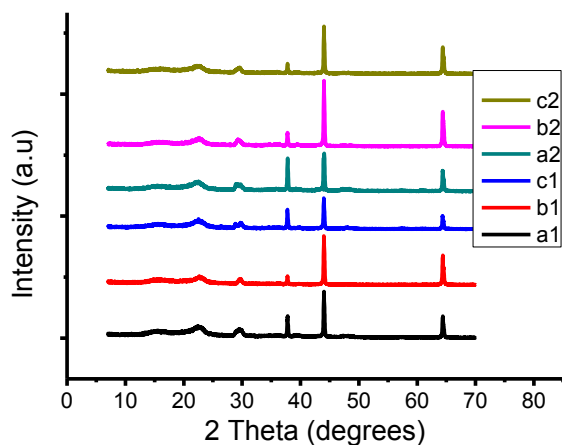

Figure 1. The XRD diffraction spectrum pattern of the indicator electrodes (a1) E_{A-1x}, (a2) E_{A-2x}, (a3) E_{A-3x}, and (a2) E_{B-1x}, (b2) E_{B-2x}, (c2) E_{B-3x}.
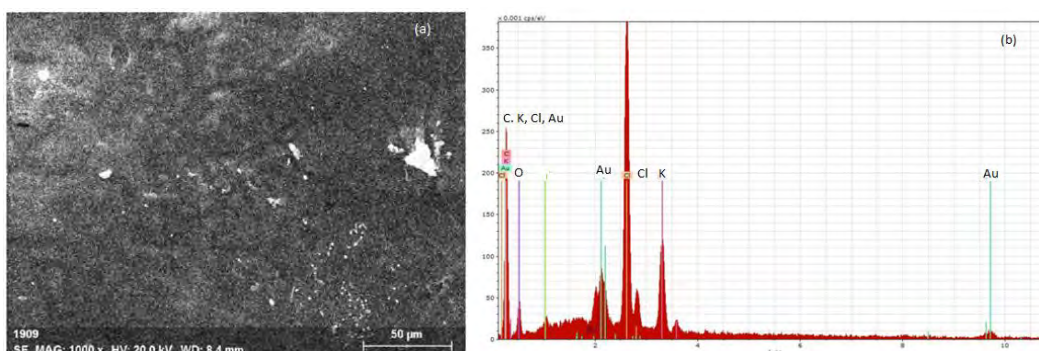

Figure 2. (a) Morphology of SEM M_2, (b) Spectrum pattern EDS M_2.

Based on Figure 1, the XRD diffraction spectrum pattern of the two best samples is the E_{A-2x} and E_{B-2x} indicator electrodes, the membranes of the electrodes were analyzed by SEM-EDS. Each membrane electrode indicator E_{A-2x} and E_{B-2x} is denoted by M_1 and M_2. The results obtained from SEM-EDS analysis are the morphology and spectral patterns of the indicator electrode membranes E_{A-2x} and E_{B-2x} as shown in Figure 2 and Figure 3. SEM morpology was analyzed at observation diameter 50 μm, magnification of 1000 x operating voltage 20 kV.

There are differences in SEM morphology and the EDS spectrum pattern of the indicator electrodes E_{A-2x} and E_{B-2x} from the membranes M_1 and M_2. Based on pore SEM morphology [4; 6] which is more clearly visible and brighter in Figure 2a for the indicator electrode E_{B-2x}, namely the M_2 membrane. Likewise with the EDS spectrum pattern [10; 11; 13; 14], the highest peaks of the EDS spectrum pattern are between energies 1-3 keV. The elements contained in the next EDS spectrum pattern will be linked to FTIR analysis and the chemical structure of the substance used.
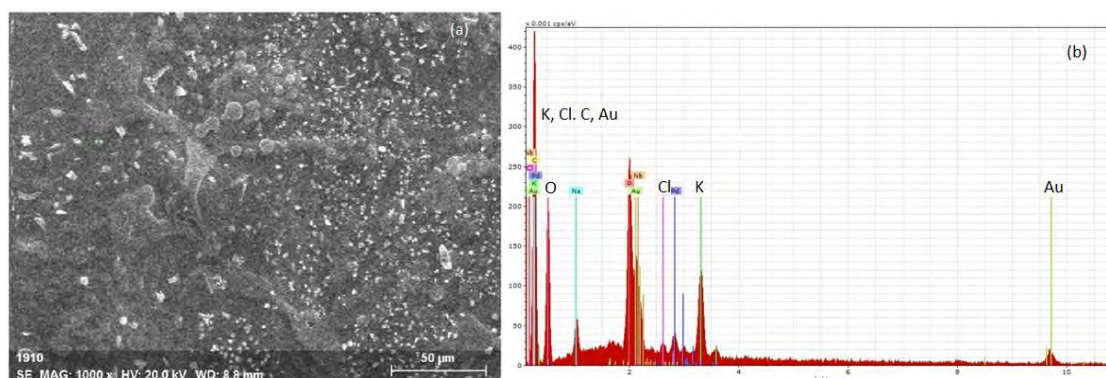
Figure 3. (a) Morphology of SEM M_1, (b) Spectrum pattern EDS M_1.

Based on the XRD diffraction spectrum pattern analysis of the indicator electrode, also the morphological analysis and EDS spectrum pattern of SEM of the indicator electrode membrane. The next step is to analyze the M_1 and M_2 indicator electrode membrane solutions using UV-Vis through the absorbance spectrum pattern of wavelengths. The analysis of the absorbance spectrum pattern was traced from a wavelength of 200-1000 nm. The analysis results obtained can be seen in Figure 4a of M_1 solution and Figure 4b of M_2 solution. Based on the spectral pattern, the highest absorbance peaks of M_1 and M_2 were 3.725 a.u and 7.246 a.u at wavelengths 315 nm and 291 nm. There was a shift in the absorbance peak of the M_1 solution and M_2 solution [12], as well as a change in the width of the absorbance peak which affected the detection range of the sensor [11].
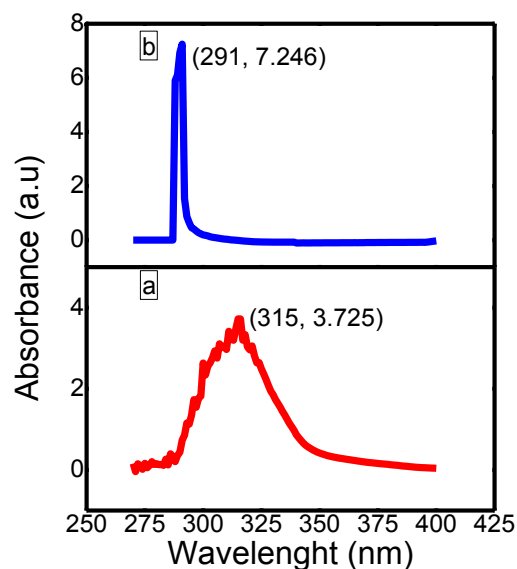

Figure 4. UV-vis absorbance spectrum pattern of
(a) M_1 (b) M_2 solution.

The shift of the absorbance peak with respect to the wavelength of the M_1 and M_2 solutions was supported by [16] using PVA / SiO_2-TiO_2. According to [8; 9; 15] it is expected that there is no shift of the absorbance peak with respect to the wavelength, which is expected to be a symmetrical UV-Vis absorbance spectrum pattern [17]. Also by FTIR %transmittance spectrum pattern and XRD diffraction spectrum pattern with cerium oxide nano samples (CeO_2NCs) as a function of urea concentration by [18].

After the M_1 and M_2 membranes were analyzed by SEM-EDS, FTIR analysis was continued to see the relationship between elements contained in the EDS spectrum pattern of SEM. This FTIR analysis is to see the functional groups which are the elements forming the chemical structure of the substances contained in the membranes M_1 and M_2. The functional groups obtained from Figure 5 and table 1 are the functional groups O-H, C-H, -C≡C-, C = C, C-O and N-H.
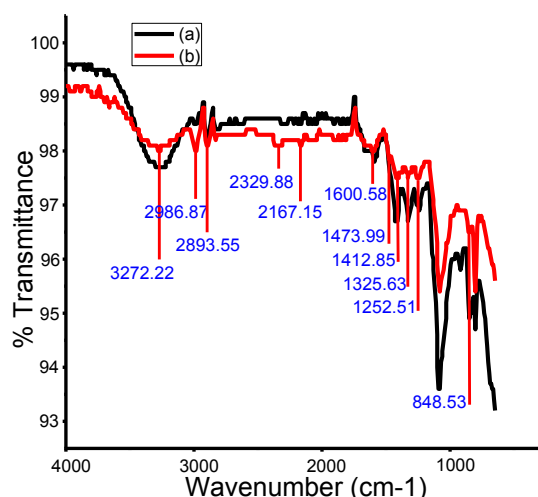

Figure 5. % Transmission spectrum pattern of wave numbers (a) M1 and (b) M2.

Based on the chemical structure of PVA, PVC, KTpClPB and THF as membranes. The chemical structure of PVA contains the O-H functional group, the PVC chemical structure contains the C-H and C-Cl functional groups, the plasticizers chemical structure (KTpClPB) contains the K-Cl functional groups, and the THF chemical structure contains the C-O and O-H functional groups. The chemical structure of PVA-enzyme and PVC-KTpClPB was supported by FTIR and SEM-EDS analysis. The functional groups obtained from FTIR are O-H, C-H, -C≡C-, C=C, C-O and N-H, from SEM-EDS obtained elements K, Cl, O, and C and Au, because when analyzed by SEM the membrane was coated with Au. So that this series of analyzes mutually reinforces the data, namely XRD, SEM-EDS, UV-Vis and FTIR.

Table 1. Formation of bonds from absorption frequency groups based on absorption frequency.

No	Absorption frequencies (cm^{-1})	Group absorption frequencies (cm^{-1})	ikatan
1	3272.22	3200-3400	alcohol O-H
2	2986.87	2850-3000	sp^3 C-H
3	2893.55	2850-3000	sp^3 C-H
4	2329.88	2000-2500	-
5	2167.15	2100-2250	sp C-X triple bonds, -C≡C-
6	1600.58	1600-1660	C=C weak or not present
		1550-1640	N-H bend, stronger in amides than amines
7	1473.99	1400-1500	C=C stretch aromatic
8	1412.85	1400-1500	C=C stretch aromatic
9	1325.63	1100-1350	acyl and phenyl C-O
		1300-1390	nitro compounds, symmetric (medium)
10	1252.51	1100-1350	acyl and phenyl C-O
11	848.53	800-900	alkene sp^2 C-H bend

4. Conclusion

The best results from the indicator electrode as a urea sensor with XRD, SEM-EDS, UV-Vis and FTIR analysis at 0.0350 g PVA-enzyme and 0.0350 PVC-0.0500 g KTpClPB. The indicator electrodes are twice coated with PVA-enzyme and one time coated with PVC-KTpClPB.

Acknowledgements

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