

# Characterization of manufacturing indicator electrodes coated PVAenzyme coated 1X GA and 3X GA 2.9 % coated PVC-KTpClPB coated using potentiometry method

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## Characterization of manufacturing indicator electrodes coated PVA-enzyme coated 1X GA and 3X GA 2.9 % coated PVC-KTpCIPB coated using potentiometry method

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**Abstract.** Research has been carried out on the manufacture of tungsten indicator electrodes with a diameter of 1 mm coated with PVA-Enzyme coated with PVC-KTpCIPB using the biosensor potentiometer method. It has a sensitivity of 19,096 mV/decade, a detection range of  $1 \cdot 10^{-5}$  -  $5 \cdot 10^{-4}$  M, a detection limit of  $1 \cdot 10^{-5}$  M and a correlation coefficient of  $R^2 = 0.9431$ . This detection range is very small, only a difference of one order to the negative power. On the basis of this small range, the PVA-Enzyme and PVA-Enzyme-GA solutions have been characterized by UV-vis showing a very clear difference in the width of the absorbance peak at wavelength. The indicator electrodes B1-4 and B3-4 have been analyzed using UV-vis, FTIR, SEM-eds and XRD. The method used is the biosensor potentiometric method, urease enzyme immobilization technique, which analytes urea in determining sensitivity, detection range and detection limit, as well as variable signal analysis of 60 signals/second. The results obtained from the indicator electrode B1-4 have a sensitivity of 46.67 mV/decade, a detection range of  $10^{-4}$  -  $10^{-2}$  M and a detection limit of  $10^{-4}$  M,  $R^2 = 99.62$  %. The indicator electrode B3-4 has a sensitivity of 38.16 mV/decade, a detection range of  $10^{-6}$  -  $10^{-4}$  M and a detection limit of  $10^{-6}$  M,  $R^2 = 95.42$  %. The best results on electrodes B1-4.

### 1. Introduction

The manufacture of indicator electrodes with the biosensor potentiometric method of urease enzyme immobilization technique. The substrate material is a PVA polymer for immobilizing the urease enzyme and PVC water-resistant material or a solution as a water-soluble PVA-Enzyme protector. Research has been carried out on indicator electrodes coated with PVA-enzyme coated with PVC-KTpCIPB with a sensitivity of 19,069 mV/decade in a detection range of  $1 \cdot 10^{-5}$  -  $5 \cdot 10^{-4}$  M with a detection limit of  $1 \cdot 10^{-5}$  M and a correlation coefficient of  $R^2 = 0.9431$  [1]. This electrode analysis has been characterized using SEM-EDX [2]. Synthesis of buffer solution  $\text{KH}_2\text{PO}_4 + \text{KCl}$  [3]. The sensitivity, detection range and detection limit are problems in this study. First, I analyzed this small detection range by adding Glutaraldehyde (GA) variation (2.6 – 3.0 ) % into a solution of 0.0500 Gr PVA-Enzyme. The best result of UV-Vis analysis was obtained at GA 2.9%. After analyzing the solution, the indicator electrodes were made in two ways. The first method was that the indicator electrodes were coated with PVA-E-GA (1x, 2x, and 3x) and then coated with PVC-KTpCIPB 1x



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were analyzed using XRD types A1-4, A2-4 A3-4. The way the two indicator electrodes were coated with PVA-E (1x, 2x, and 3x) were coated with GA and then coated with PVC-KTpCIPB 1x were analyzed using XRD types B1-4, B2-4, B3-4. The best analysis results in the second method are indicator electrodes B1-4 and B3-4. The best result of XRD analysis used SEM-EDS advanced analysis. The results of SEM morphology analysis showed that the electrodes denoted B1-4 had more pores and the electrodes denoted B3-4 had large pores but there were cracks or fractures. The best results of the next B1-4 and B3-4 analysis were analyzed using FTIR [4]. Based on the four-step sequence of analysis [5; 6] this is where the researchers continued to analyze the indicator electrode using a potentiometric cell with the biosensor potentiometric method of the urease enzyme immobilization technique. This analysis will characterize the sensitivity, detection range and detection limit of samples B1-4 and B3-4. The potentiometer cell uses an Ag/AgCl reference electrode and indicator electrodes B1-3 and B3-4 with a buffer solution of  $\text{KH}_2\text{PO}_4 + \text{KCl}$  with a concentration of 0.001 M pH 7.5. This solution has been analyzed [7]; [8].

## 2. Methods

### 2.1. Material

The material consists of standard urea 56 180, enzyme EC 3.5.1.5 (Urease) U4002, tungsten diameter 1.0 mm 267 562 99.99%, Phosphate Buffer  $\text{KH}_2\text{PO}_4$ , PVA  $[-\text{CH}_2\text{CHOH}-]_n$ , PVC  $(\text{CH}_2\text{CHCl})_n$ , potassium tetrakis 4-chlorophenyl borate  $(\text{ClC}_6\text{H}_4)_4\text{BK}$ , tetrahydrofuran  $\text{C}_4\text{H}_8\text{O}$ , KCl, Glutaraldehyde derived from Sigma-Aldrich and method. As well as the tools used with the cell potentiometer, the cell potentiometric method, the biosensor, the urease enzyme immobilization technique. Potentiometer (Keithley 199 DMM, USA), tungsten indicator electrode (W), Ag/AgCl MF-2052 RE-5B reference electrode in a microcomputer assembled electrochemical cell (ADI Powerlab instruments, Australia), magnetic stirrer and flow injection (FIA).

### 2.2. Manufacture

The indicator electrodes are made in two notations B1-4 and B3-4. The materials used are the same, only different in the first layer of immobilization of the urease enzyme on the PVA-Enzyme-GA substrate, while the second layer is the same, namely PVC-KTpCIPB.

The composition of the ISE (ion selective electrode) polymer membrane is [9] 1% by weight of ionophore (I), polymer matrix (PVC): plasticizer (1:2) was used for the first layer of PVA-Enzyme. Polymer synthesis in the development of ISE sensors according to [10] with a ratio of I:PVC:KTpCIPB:Plasticizer 10:165:5:330, for the second layer of PVC-KTpCIPB. Glutaraldehyde concentration of 2.5-3% [11] results of the analysis of the optimal concentration of 2.9% glutaraldehyde for urease immobilization.

PVA 0.0350 g was dissolved in 10 mL of hot water to cool in a glass tube. PVC 0.0350 g + KTpCIPB 0.0500 g dissolved in 10 mL THF in a glass tube and covered with a plastic/aluminum foil cover. 2.9% GA solution is 0.29 g GA in 10 mL of distilled water. To coat the indicator electrode, dip into each of the instructions solutions B1-4 and B3-4.

## 3. Result and Discussion

Likewise in Figure 2, analysis of the signal display from the B3-4 indicator electrode with a potentiometer cell, namely the signal display at a 2k:1 scale in the image on the left and the signal display at a frequency of 10000 Hz. If you pay close attention to Figure 2, there is a time response to the 0.001 M KCl phosphate buffer solution pH 7.5 with urea molarity variations ranging from  $10^{-7}$  -  $10^{-2}$  M, see Figure 3b.

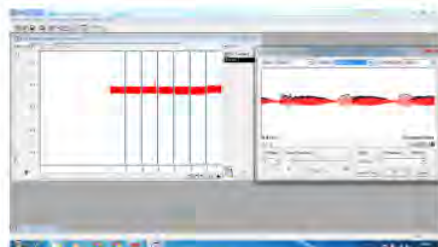
Figures 3a for B1-4 and 3b for B3-4 have a time response to the potentiometer cell voltage ranging from  $10^{-7}$  -  $10^{-1}$  M urea variation in  $\text{KH}_2\text{PO}_4$  buffer solution with 0.001 M KCl pH 7.5. Analysis of images 3a and 3b can be used as a guide to determine sensitivity, detection range and detection limit.

On the basis of the relationship of  $-\log(\text{urea concentration})$  M to the potentiometer voltage of the potentiometer cell, Figure 3a in the researcher's analysis there is only a range of  $10^{-4}$  -  $10^{-2}$  M.

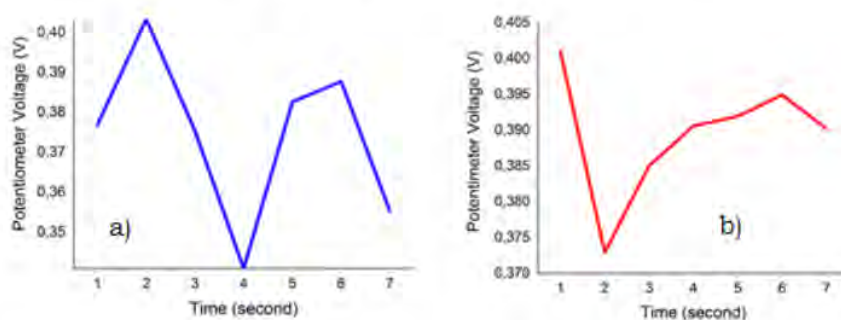
According to signal analysis, the variables in Figures 4a and 4b have symmetry in the molarity of urea  $10^{-6}$  -  $10^{-2}$  M in phosphate buffer  $\text{KH}_2\text{PO}_4$  and  $\text{KCl}$  0.001 M pH 7.5. This symmetry range is used as the following conclusions. Analysis of this variable signal was taken as many as 60 signals/second each from the cell signal of the indicator electrode potentiometer B1-4 and B3-4.



**Figure 1.** The display of the potentiometer cell signal and the display of the variable analyzer at a frequency of 10000Hz from the indicator electrode B1-4.



**Figure 2.** Display of the potentiometer cell signal and display of the variable analyzer at a frequency of 10000Hz from the indicator electrode B3-4.



**Figure 3.** The response time to the potentiometer cell voltage ranged from  $10^{-7}$  -  $10^{-1}$  M urea variation in  $\text{KH}_2\text{PO}_4$  buffer solution with 0.001 M  $\text{KCl}$  pH 7.5. B1-4 a), B3-4 b).

First for the B1-4 indicator electrode, the analysis pays attention to Figures 3a and 4a, it can be seen in Figures 5a and 5b that the sensitivity is 46.67 mV/decade, the detection range is  $10^{-4}$  -  $10^{-2}$  M and the detection limit is  $10^{-4}$  M,  $R^2 = 99.62\%$ . Probability of linear response time only lies in the molarity of urea  $10^{-4}$  -  $10^{-2}$  M, others do not. Second, for the B3-4 indicator electrode, the analysis looks at Figures 3b and 4b, it can be seen in Figures 6a and 6b, the sensitivity is 36.67 mV/decade, the detection range is  $10^{-6}$  -  $10^{-2}$  M and the detection limit is  $10^{-6}$  M. There are several opportunities for linearity to be obtained due to the relationship between the  $-\log$  concentration of urea (M) to the potentiometer voltage is not perfectly linear.

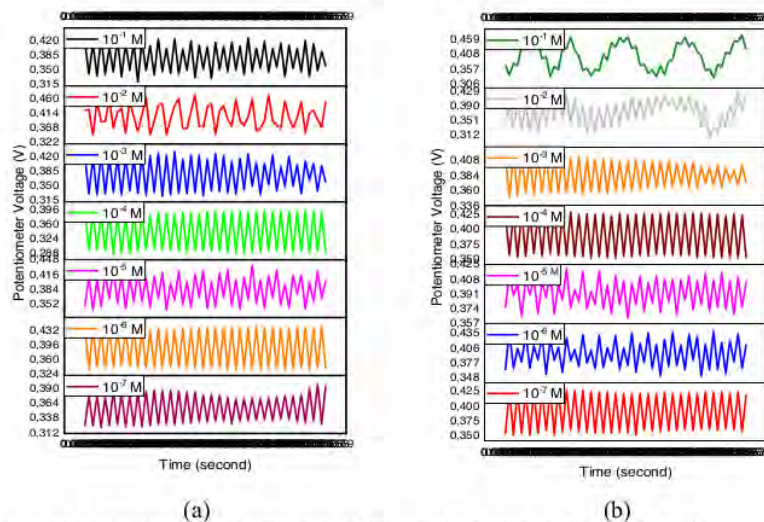


Figure 4. Variable signal analysis of indicator electrodes B1-4 and B3-4.

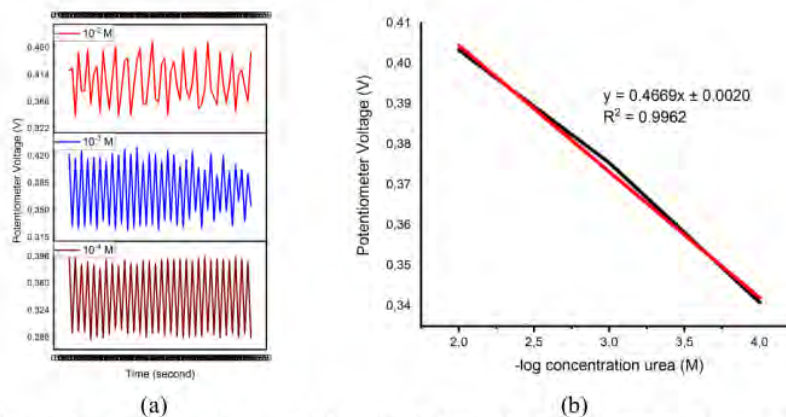
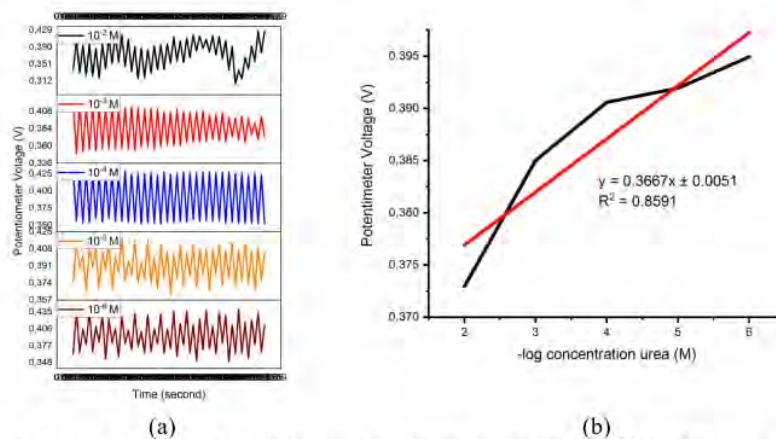


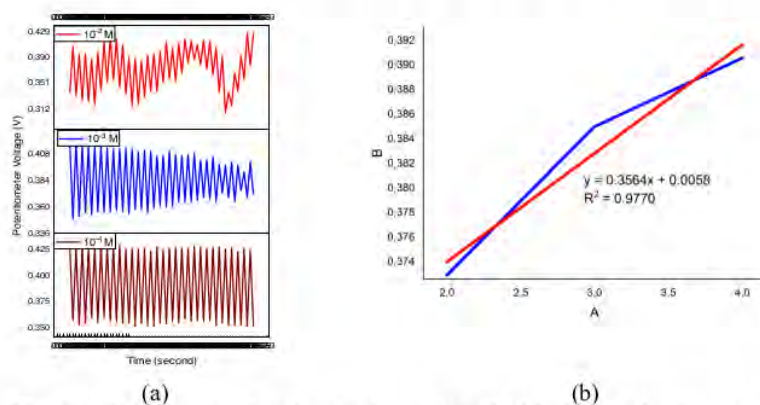
Figure 5. Analysis of the variable signal from the potentiometer cell and the sensitivity is 46.67 mV/decade, the detection range is  $10^{-4}$  -  $10^{-2}$  M and the detection limit is  $10^{-4}$  M,  $R^2 = 99.62\%$ . B1-4.

Analysis of images 6a and 6b, images 7a and 7b as well as images 8a and 8b there are differences in the sensitivity of the detection range and detection limit. The greatest sensitivity was obtained at

38.16 mV/decade at  $10^{-6}$  -  $10^{-4}$  M urea molarity for indicator electrode B3-4. This is also confirmed by the analysis of the variable signal from the potentiometer cell. The symmetry of the signal is also found in the urea molarity of  $10^{-6}$  -  $10^{-4}$  M, and the detection limit is  $10^{-6}$  M,  $R^2 = 95.42\%$ .



**Figure 6.** Analysis of variable signal and sensitivity is 36.67 mV/decade, detection range is  $10^{-6}$  -  $10^{-2}$  M and detection limit is  $10^{-6}$  M,  $R^2 = 85.91\%$ . B3-4.



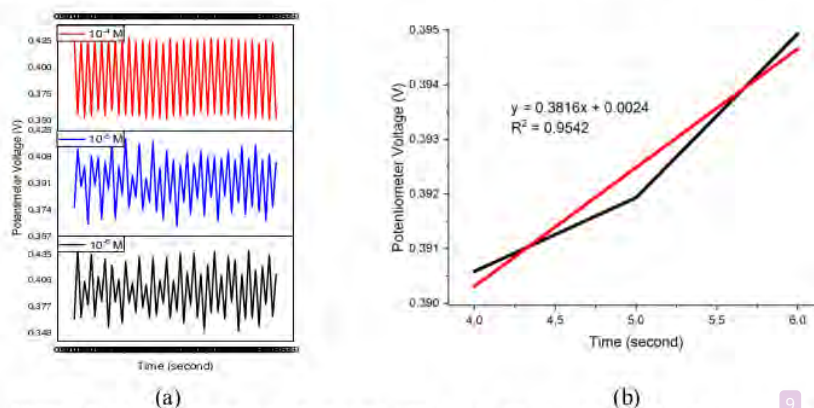
**Figure 7.** Variable signal analysis and sensitivity 35.64 mV/decade, detection range  $10^{-4}$  -  $10^{-2}$  M and detection limit  $10^{-4}$  M,  $R^2 = 97.70\%$ . B3-4.

The symmetry of the signal [7] is very dependent on the incoming signal and outgoing signal from the redox process, that the incoming signal is not the same as the outgoing signal with a total signal data of 400 signals/second [12]. Signal symmetry [13] can be seen in Figure 1 and Figure 2, Figure 4a and Figure 4b, Figure 5a, 6a, 7a and 8a. The voltage between the oxidation and reduction peaks [14] is theoretically 59 mV for a reversible reaction, as can be seen in Figures 5a and 8a. Voltage greater than 100 mV is the peak of nonsymmetric oxidation and reduction indicating a nonreversible (irreversible) reaction, as can be seen in Figures 6a and 7a.

It has been found that the PVA electrode membrane has a sensitivity of 19.7 mV/decade with a range of  $5.10^{-7}$  -  $10^{-2}$  M by [15]. The sensitivity value varies depending on the range and limit of detection [16] and [17].

The development of a biosensor system follows [18], namely (1) Selectivity, (2) Sensitivity, (3) Response linearity, namely the concentration range of the target analyte to be measured, (4) Reproducibility of signal response, samples having the same concentration analyzed several times should give the same response, (5) Fast response time and recovery time for reusability of the biosensor system, (6) Stability and operating life.

Conductive polymer is a chemical sensor in the application of metal electrodes coated with a conductive polymer film used for potentiometric measurements [19]. Conductive polymers are organic materials that have electrical and optical properties similar to those of metallic conductors and semiconductors.



**Figure 8.** Variable signal analysis and sensitivity 38.16 mV/decade, detection range  $10^{-6}$  -  $10^{-4}$  M and detection limit  $10^{-6}$  M,  $R^2 = 95.42\%$ . B3-4.

The immobilization technique was developed based on three important mechanisms, namely (a) physical adsorption, (b) covalent immobilization, (c) streptavidin-biotin immobilization. Achieving high sensitivity and selectivity requires minimization of nonspecific adsorption and stability [20].

On this basis, the researchers developed a modified PVA-enzyme coated with GA with an immobilization technique using a potentiometric biosensor method, coated again with PVC-KTpCIPB to protect the electrode from the  $\text{KH}_2\text{PO}_4 + \text{KCl}$  buffer solution. In order for a long lasting indicator electrode to be used.

Research has been carried out on PVA-enzyme electrodes coated with PVC-plasticiser 19,069 mV/decade in the range from  $1.10^{-5}$  -  $5.10^{-4}$  M with a detection limit of  $1.10^{-5}$  with  $R^2 = 94.31\%$  using 0.001 M KCl phosphate buffer 0.001 M pH 7.5 [1].

The indicator electrode coated with PVA-enzyme coated GA coated with PVA-KTpCIPB increased its detection range to two orders of B1-4, detection range was  $10^{-4}$  -  $10^{-2}$  M and B3-4 detection range was  $10^{-6}$  -  $10^{-4}$  M. So it is true that GA can increase the detection range.

#### 4. Conclusion

Based on the analysis of variable signal, sensitivity, detection range and detection limit, there is a relationship between the symmetry of the variable analysis signal with sensitivity, detection range and detection limit to determine the best indicator electrode. The results of the analysis and research showed that the indicator electrode B1-4 had a sensitivity of 46.67 mV/decade, a detection range of  $10^{-4}$  -  $10^{-2}$  M and a detection limit of  $10^{-4}$  M,  $R^2 = 99.62\%$ . B3-4 sensitivity is 38.16 mV/decade, detection range is  $10^{-6}$  -  $10^{-4}$  M and detection limit is  $10^{-6}$  M,  $R^2 = 95.42\%$ . This step is also



corroborated by analysis and characterization of UV-vis, FTIR, SEM-EDS and XRD from previous studies. Thus the best indicator electrode sample is B1-4.

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# Characterization of manufacturing indicator electrodes coated PVAenzyme coated 1X GA and 3X GA 2.9 % coated PVC-KTpCIPB coated using potentiometry method

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GRADEMARK REPORT

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FINAL GRADE

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GENERAL COMMENTS

**Instructor**

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