EINSTEIN (e-Journal)
Jurnal Hasil Penelitian Bidang Fisika
Available online http://jurnal.unimed.ac.id/2012/index.php/einsten
e-issn: 2407 – 747x, p-issn 2338 – 1981

ENHANCEMENT OF SENSITIVITY, DETECTION RANGE, AND DETECTION LIMIT USING GA METHOD POTENTIOMETRIC BIOSENSOR WITH UREASE ENZYME IMMOBILIZATION TECHNIQUE ON PVA

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Submit: August 2024. Approved: September 2024. Published: October 2024.

ABSTRACT

This study aims to increase the sensitivity range of the width of the UV-Vis absorbance peak with Glutaraldehyde (GA) crosslinking and useful to determine the level of urea in the urine. The potentiometer cell consists of an indicator electrode and a reference electrode. The indicator electrode is denoted PVA-Enzyme/GA/PVC-KTpCIPB, meaning PVA-Enzim as the first layer, GA as the second layer and PVC-KTpCIPB as the third layer. Biosensor with urease enzyme immobilization technique analyzed variations of urea 10-7-10-1 M. The potentiometric method of the biosensor detects signal and voltage (potentiometer cell). The signals that were analyzed for signal variables were symmetrical up and down signals of 2000 signals/second. Voltage was carried out by linear curve analysis, the results of linear curve analysis were the range of detection from a concentration of 10-4-10-2 M with cross-linked GA which increased the absorbance, the detection limit was 10-4 M, the sensitivity was 46.67 mV/ war reversibly and R squared (R2) which is 0.9839 is close to linear.

Keywords: Urease Enzyme Immobilization Technique, Indicator Electrode, Potentiometric Biosensor

INTRODUCTION

The enzyme immobilization technique is the process of physically localizing enzymes on a specific surface, which helps enhance certain enzyme properties and operational performance without disrupting their catalytic activity. This allows for the recovery and reuse of enzymes, making the entire process controllable and economical (Singh et al., 2013).

Research on the indicator electrode as a urea sensor was conducted using a potentiometric biosensor method with urease enzyme immobilization on a PVA membrane, referred to as PVA-Enzyme, coated with PVC-KTpCIPB and labelled PVA-E-1-2x with the notation Ar. 0.0350g of PVA dissolved in 10mL of warm water is denoted as PVA-E, while 0.0350g of PVC and 0.0500 g of KTpCIPB dissolved in 10mL of THF is denoted as PVC-KTpCIPB. The study yielded a sensitivity of 19.069mV/decade, a detection range of 1.10^{-5} – 1.10^{-4} M, a detection limit of 1.10^{-5} M, and an **R-squared (R^2) value of 0.9431. According to the analysis, the spectrum pattern showed narrow peak widths, resulting in a limited detection range (Hakim et al., 2019).

Researchers analyze molecular adsorption interactions to enhance detection range, detection limits, and sensitivity. An optimal increase in glutaraldehyde

concentration from 2.5-3% is used for urease immobilization. The techniques employed include absorption, trapping, and cross-linking (Ismael et al., 2013). Glutaraldehyde serves as a cross-linking agent that can form cross-links, resulting in a denser matrix with higher tensile strength (Purwatiningsih et al., 2007).

The characterization of glutaraldehyde has been conducted using XRD, UV-VIS, SEM-EDS, and FTIR tests. Indicator electrodes were fabricated using two methods to determine the best approach. In the first method, glutaraldehyde was mixed with PVA-E to form PVA-E/GA/PVC-KTpCIPB. In the second method, PVA-E was coated with GA and further coated with PVC-KTpCIPB. Variations in GA concentration (2.6%, 2.7%, 2.8%, 2.9%, and 3%) were applied either once or three times (coated and mixed). Based on the analysis, the best results are as follows: (1) For the first method, PVA enzyme coated with 2.9% GA, noted as PVA-E 1x and GA 3x /PVC-KTpCIPB 1x. (2) For the second method, PVA enzyme coated with 2.9% GA, noted as PVA-E 1x and GA 1x /PVC-KTpCIPB (Hakim et al., 2022).

In the potentiometric method, the equilibrium potential difference between the indicator electrode and the reference electrode will be measured (Tamba, 2016). The potentiometric ISE (Ion Selective Electrode) cell uses an electrolyte solution consisting of phosphate buffer, KCl, urease enzyme, urea, and glutaraldehyde (GA). A common issue with the potentiometric method lies in the fabrication of membrane components for biosensor samples (Goncalves, 2014). The electrode membrane parameters are determined by the type of supporting material or matrix selected (Fauziyah, 2012). One of these materials is the PVA polymer, which is water-soluble, biocompatible, and offers good chemical and thermal stability. The large number of hydroxyl groups in PVA provides a biocompatible microenvironment for the presence of enzymes (such as urease) (Kale et al., 2016).

Polyvinyl Chloride (PVC) is a thermoplastic polymer, meaning it deforms under stress and does not return to its original shape once the stress is released (Wirjosentono, 1998). PVC is waterproof and has good porosity, making it suitable for use as a membrane. A plasticizer is required to enhance the elasticity of PVC. The ratio of PVC to plasticizer in the membrane composition affects the sensitivity and selectivity of the ISE membrane, as the PVC membrane will coat the PVA membrane (Tamba, 2016). A potentiometric cell consists of an indicator electrode and a reference electrode. It requires an electrolyte solution containing a buffer to stabilize the voltage, urease enzyme as a catalyst, and urea as the analyte, with specific compositions to achieve optimal results (Hakim et al., 2018).

Based on this contribution, we selected glutaraldehyde cross-linking to enhance the detection range, detection limit, and sensitivity using an indicator electrode (PVA-E 1x coated with GA 1x and further coated with PVC-KTpCIPB 1x) immobilized with the urease enzyme.

RESEARCH METHOD

Sample testing was conducted at the Chemistry Laboratory of Universitas Negeri Medan.

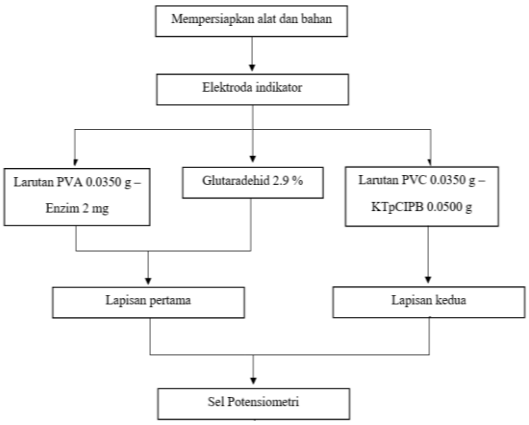


Figure 1. Research flowchart

Before conducting the research, the researchers must prepare the equipment and materials, which consist of the enzyme EC 3.5.1.5 (urease), urea standard (56 180), tungsten with a diameter of 1 mm, KCl, phosphate buffer, PVA (-CH_2CHOOH-)n,

PVC $(CH_2CHCl)n$, and glutaraldehyde. The equipment used includes a potentiometer (Keithley 199 DMM, USA), tungsten indicator electrode (W), reference electrode (Ag/AgCl), microcomputer assembly (ADI Powerlab instrument, Australia), and a magnetic stirrer (Sterier).

The fabrication of the indicator electrode (tungsten) begins with the first layer of PVA-Enzyme, which is dipped once. The second layer, a solution of glutaraldehyde (GA), is also dipped once, while the third layer, PVC-KTpCIPB, undergoes one dipping as well.

The composition of the polymer membrane for the ISE (Ion Selective Electrode) is 1% ionophore (I), with a polymer matrix (PVC) and plasticizer in a 1:2 ratio, used for the first layer of PVA enzyme. The optimal concentration of glutaraldehyde for urease immobilization is 2.9%.

PVA 0.0350 g is dissolved in 10 mL of hot water until it cools in a glass tube. PVC 0.0350 g + KTpCIPB 0.0500 g is dissolved in 10 mL of THF in a glass tube and covered with plastic/aluminum foil. The GA solution at 2.9% consists of 0.29 g of GA in 10 mL of distilled water.

The immobilization technique of the urease enzyme in the PVA solution is represented as PVA-E. Cross-linking of PVA-E is achieved by coating PVA-E with GA on the indicator electrode, denoted as PVA-E/GA. The success of enzyme immobilization and surface modification is measured using a UV-VIS spectrophotometer and applied in biosensor applications for urea sensing.

The potentiometric cell method involved varying urea concentrations ranging from \(10^{-7}\) to \(10^{-1}\) M. To perform these variations, the dilution formula (\(V_1 \times M_1 = V_2 \times M_2\)) was used, where 0.1 mL of urea solution was added to 10 mL of phosphate buffer and placed in a beaker. The mixture was then stirred using a magnetic stirrer for 5 minutes, after which a potentiometric cell test was conducted for 15 minutes to measure the potential difference.

RESULT AND DISCUSSION

Data and results from the potentiometric cell testing. The analysis of the indicator electrode signal display shows the signal on a scale of 2k:1 in the image on the left, with the signal displayed at a frequency of 10,000 Hz for the phosphate buffer solution KCl 0.001 M at pH 7.5, with urea molarity variations ranging from 10^{-7}-10^{-2}M. The characterization testing of the indicator electrode generated a total of 630,000 data points.

Collected signal data at rates of 600, 1000, and 2000 signals per second to compare the symmetry of the signals. Data collection was performed seven times with variations in urea over a duration of 15 minutes. There were differences in the number of data points obtained for each variation because the data collection started at 0.01 seconds and did not end precisely at 15:00 minutes. Additionally, the process was conducted manually for both the start and stop of the data collection.

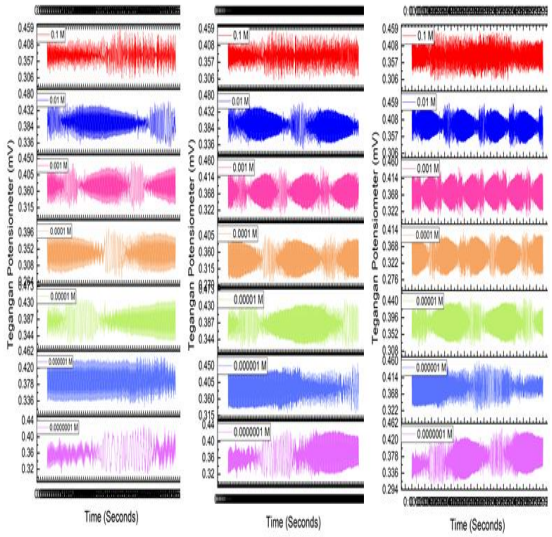


Figure 2. Analysis of Variable Signal from the Indicator Electrode

From Figure 2, the analysis of the variable signal from the indicator electrode shows symmetrical signals above and below. In the data for 600 signals, symmetry is observed at urea concentrations of 10^{-2} and 10^{-3} M, while the data for 1000 signals shows symmetry at urea concentrations ranging from 10^{-2} to 10^{-5} M. In the 2000 signal data, symmetry is also observed at urea concentrations from 10^{-2} to

10^{-5} M, with a greater number of symmetrical signals. According to Basile, Bhatt, and O'Mullane (2016), signal symmetry can be seen as symmetry above and below, followed by left-right symmetry in the variable signal pattern.

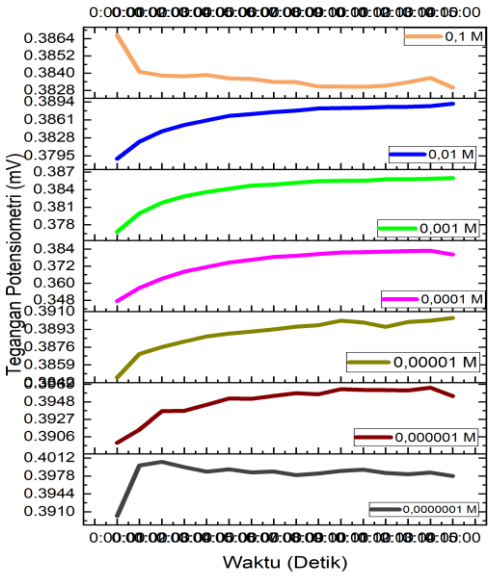
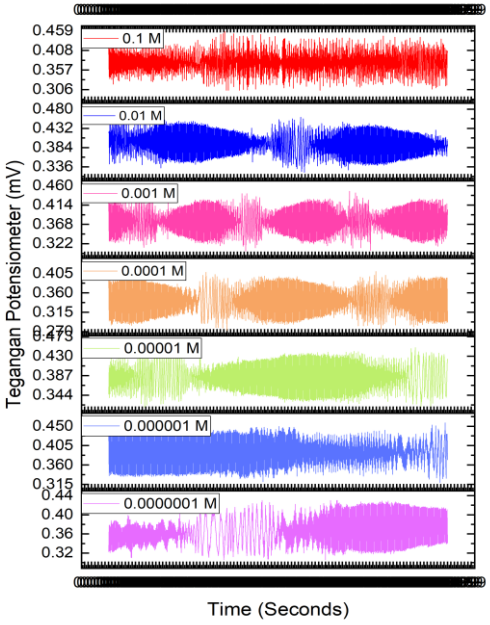


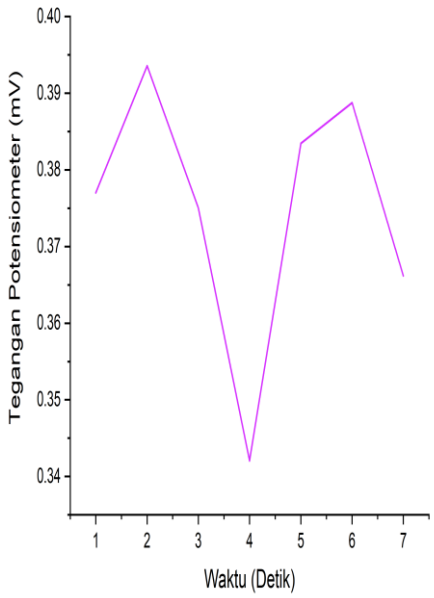
Figure 3. Curve of the Relationship between Potentiometric Voltage (mV) and Time (Seconds)

Based on Figure 3, the research results show that the response time of the potentiometric biosensor developed for urea concentrations ranging from 10^{-6} to 10^{-2} M is 6 minutes with a constant increase. In contrast, at urea concentrations of 10^{-7} and 10^{-1} M, a decrease is observed. The response time is the duration required for the potentiometric sensor to provide a stable potential response. The faster the potentiometric sensor achieves a stable potential response, the better the quality of the sensor. In this study, the response time is illustrated in Figure 3, demonstrating a steadily increasing state over 15 minutes.

To determine the quality of the urea electrode created, several factors or parameters that define the quality of the biosensor are identified, enabling its use for urea analysis (Wahab and Nafie, 2006). The factors assessed in this study include the biosensor's detection limit, detection range, and sensitivity, as shown in Figures 4 and 5.



(a)

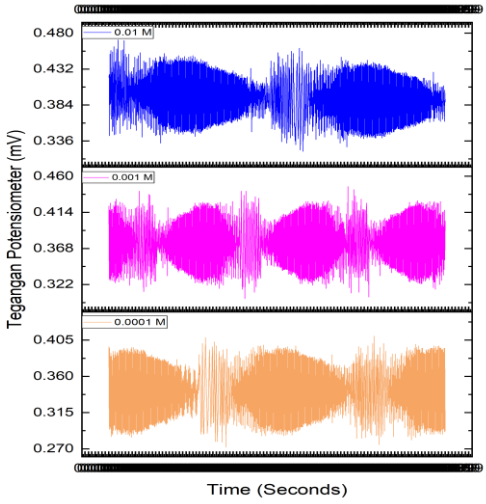


(b)

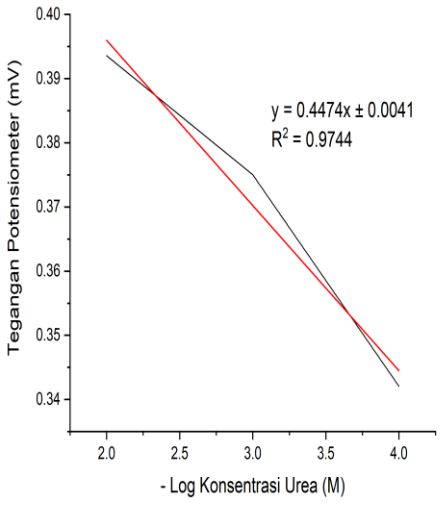
Figure 4. (a) Analysis of Variable Signal; (b) Linearity Curve of the Relationship between Time and Voltage

Based on Figure 4 (a), the analysis of the variable signal shows symmetrical signals above and below at urea concentrations ranging from 10^{-2} to 10^{-5}, while for urea concentrations of 10^{-1}, 10^{-6} and 10^{-7} the signals are not symmetrical. The variable signal from the absorbance spectrum can be analyzed through a linearity curve to determine the

detection range, detection limit, and sensitivity. Figure 4 (b) shows the response time to potentiometric voltage from urea variations of 10^{-7} to 10^{-1} M in a phosphate buffer solution of KH_2PO_4 with KCl 0.001 M at pH 7.5. This can be used as a guide to determine the detection limit, detection range, and sensitivity. Two linear lines are obtained, corresponding to urea variations of 10^{-4} to 10^{-2} M and 10^{-5} to 10^{-4} M. Based on these two linear lines, the sensitivity results can be seen in Figures 5 and 6.

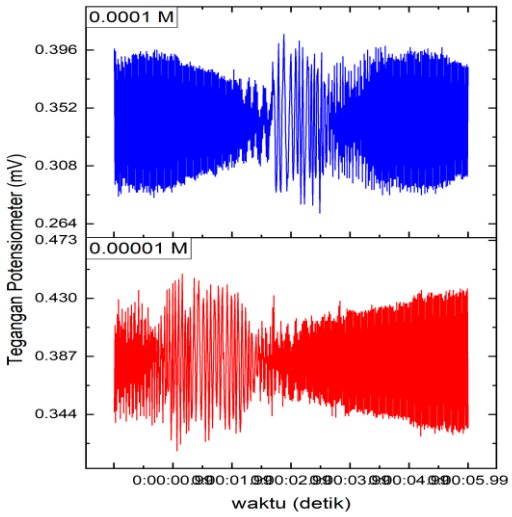


(a)

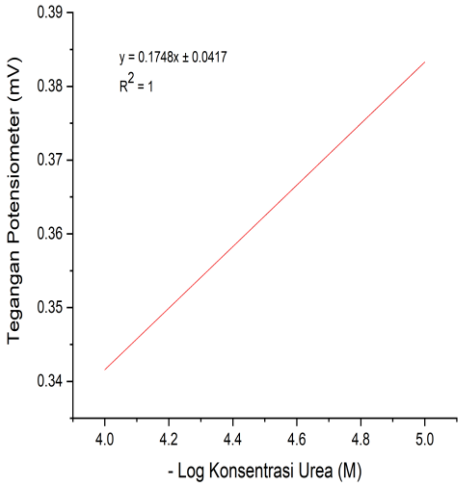


(b)

Figure 5. (a) Analysis of Variable Signal for Urea Concentration 10^{-4} to 10^{-2} M; (b) Linear Curve – log Urea Concentration 10^{-4} to 10^{-2} M



(a)



(b)

Figure 6. (a) Analysis of Variable Signal for Urea Concentration 10^{-5} to 10^{-4} M; (b) Linear Curve – log Urea Concentration 10^{-5} to 10^{-4} M

Figures 5 and 6 show differences in variable signals and linear curves. In Figure 5(a), the analysis of the variable signal displays symmetrical signals above and below, while the linear curve (b) has a range of two orders. In contrast, Figure 6(a) shows an analysis of the variable signal that is asymmetrical, and (b) the linear curve has a range of one order.

Berdasarkan perbandingan gambar 5 dan 6 dapat dilihat nilai dari Kurva linier pada gambar 5 (b) memperoleh sensitivitas 45,22 mV/dekade dengan rentang deteksi 10^{-4} – 10^{-2} M dan limit deteksi 10^{-4} M serta koefisien determinasi (R2) 0,9838 sedangkan pada gambar 6 (b) sensitivitas 17,48 mV/dekade

dengan rentang deteksi 10^{-5} – 10^{-4} M, limit deteksi 10^{-4} M dan R2 = 1.

The immobilization technique is developed based on three important mechanisms: (a) physical adsorption, (b) covalent immobilization, and (c) streptavidin-biotin immobilization. Achieving high sensitivity and selectivity requires minimizing non-specific adsorption and ensuring stability (Hakim et al., 2020). The authors obtained the best results from Figure 5, where the indicator electrode coated with GA layered on PVA-Enzyme and further coated with PVC-KTpCIPB increased the detection range to two orders, specifically 10^{-4} to 10^{-2} M.

CONCLUSION AND SUGGESTION

The analysis of the variable signal and linear curve using the indicator electrode coated with PVA-E/GA/PVC-KTpCIPB with the addition of 2.9% GA has proven to enhance the detection range of absorbance (peak width). The sensitivity result is 46.67 mV/decade, with a detection range for urea concentrations from 10^{-4} to 10^{-2} M, a detection limit of 10^{-4} M, and an R-squared (R^2) value of 0.9839.

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