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CHARACTERIZATION OF UREA ANALYTE SOLUTION AND ELECTROLYTE SOLUTION USING NON-IMMOBILIZED ELECTRODE INDICATORS AND PVA-ENZYME COATED PVC-KTpClPB IMMOBILIZATION

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ABSTRACT

Electrolyte solutions used in potentiometric cells from ion-selective electrode (ISE) sensors urea contribute to a good enzymatic reaction. This study aims to obtain electrolyte solutions that have the best response to ISE on the electrode modification membrane electrode 35 mg PVA-enzyme coating 35 mg PVC-500 mg KTpClPB. Solution analysis begins with the characterization of ISE electrode membranes using UV-vis, SEM-EDX, and XRD, the best results on electrode membranes are used as ISE. Analyzing the solution sequentially is a phosphate buffer solution, KCl phosphate buffer solution and phosphate buffer KCl injection with urea using a potentiometer, power lab, and

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computer to see the solution response. Based on the results of the computer response obtained a phosphate buffer solution pH 5.5, 0.001 M, 0.001 M KCl, and 0.001 M urea.

Keywords: Solution; characterization of ISE; Electrode Indicator; PVA-enzyme Coated PVC-KTpClPB.

INTRODUCTION

A membrane as a semipermeable thin film-shaped porous media which functions to separate particles in a solution system. Analytes are chemicals that are tested on water, air or blood samples. For example, if the analyte is urea, laboratory tests will determine the amount of urea in the sample. The membrane allows the analyte analyzed to diffuse and come into direct contact with the indicator electrodes located in the electrolyte solution. Analytic diffusion is important because it has to go through the membrane, besides that the membrane removes the redox-active substances that interfere so as not to be trapped in the sample.

Biosensors are defined as integrated devices that can provide specific quantitative or semi-quantitative analytic information. Biosensors usually consist of analytes in the sample, a bioreceptor (biorecognition element), transducer and signal amplification and analysis circuit.

Biosensors are used as analytical tools that respond to certain concentrations of chemicals in biological samples. Biosensors, using biological components as catalysts in electrodes. The analyte is usually detected through physical binding or chemical reactions that occur at the electrodes. Enzymes are biocatalysts that are useful for increasing reaction rates without substantial consumption during reactions. Enzymes have been widely used in applications because of their high specificity, selectivity, biocompatible properties, and when immobilized on the electrode surface resulting in higher current density and power. They are precision and show high selectivity. Effective cycles at low current densities of 0.1 mA cm^{-2} to 5,000 cycles from periodic voltage plots containing electrolytes [1]. It can be seen in Figure 1 (a) in the 0.001 M phosphate buffer solution pH 5.5 urea injection and 1 (b) in the 0.001 M solution phosphate buffer pH 5.5 KCl urea injection there are differences in charge/discharge cycles so that there are more periodic stress cycles in Figure 1 (b), [2] there is only signal attenuation [3] when adding KCl, an interactive redox-probing approach to access redox-based chemical information and convert this information into electricity by signal processing method [3].

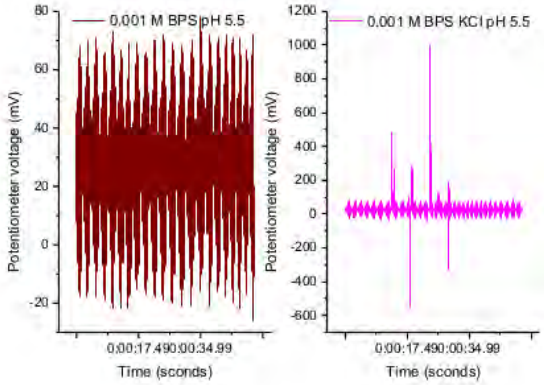

Figure 1. Voltage signals (a) LBP 0.001 M, (b) LBP KCl 0.001 M each urea injection with indicator electrodes without immobilization [4] in 0: 00: 49.94 the total signal voltage of 5,000.

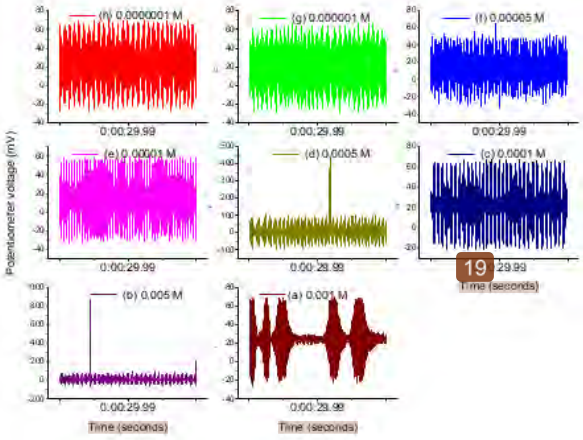

Figure 2. Response time of urea with electrode potentiometer cells without immobilisation in molarity variations (a) 0.001 M, (b) 0.005 M, (c) 0.0001 M, (d) 0.0005 M, (e) 0.00001 M and (f) 0.00005 M , (g) 0.000001 M, (h) 0.0000001 M within 0: 00: 59.94 the number of 6,000 voltage signals.

The addition of phosphate buffer solution with KCl pH 5.5 and molarity of 0.001 M changes the potentiometer voltage cycle to an asymmetrical cyclone, as can be seen in Figure 1 and Figure 3. Figure 2 is a signal of urea solution at the urea response time with an electrode potentiometer cell without

immobilization in variations of molarity (a) 0.001 M, (b) 0.005 M, (c) 0.0001 M, (d) 0.0005 M, (e) 0.00001 M and (f) 0.00005 M, (g) 0.000001M, (h) 0.0000001 M. The evolution of electrode potential as a function of time for various concentrations of urea in a solution without buffering [5], the area that has the best time response is urea 0.001 M and symmetric cell cycle symmetry [4]. The voltage signal profile depends on the charge/discharge time (voltage response to time) by [4] can be seen in Figures 1-3.

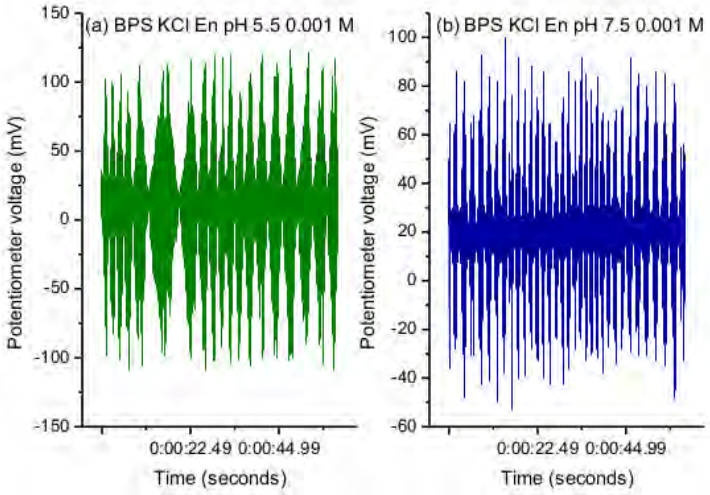

Figure 3. Voltage signal (a) (LBP) pH 5.5, (b) (LBP) pH 5.5 plus KCl Enzyme for each urea injection with indicator electrodes without immobilization according to [4] within 0:00: 59.94 number of 6.000 voltage signals.

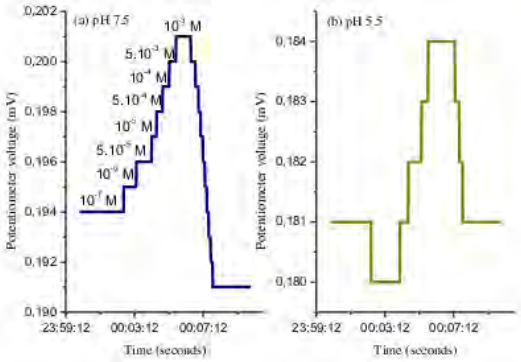

Figure 4. Potentiometric response curves [6] from selective membrane sensors (a) pH 7.5, (b) pH 5.5 plus KCl for each injection of urea with immobilized enzymes on indicator electrodes within 09:59.0 the number of voltage signals 6,000.

The response and reproducibility of the selective membrane urea sensors [7] at pH 7.5 (a) pH 5.5 (b) from Figure 4 is better at pH 7.5 can also be seen in Figure 3 (b) voltage signal phosphate buffer solution urea sensor. Response time [8] active signal of potentiometer cells at 10-3 M or 0.001 M molarity with pH 7.5 according to Figure 4, if electrodes are prevented from polarization, electrolytes show ohmic behavior [9] with a molarity of $10^{-7} - 10^{-3}$ M. The action potential is positive and negative ionic flow that moves in the cell membrane. The formation of polarization can be seen in Figure 5-7 when the indicator electrode without immobilization is immersed in the potentiometer cell while in Figure 8-12 when the immobilized indicator electrode immersed in the potentiometer cell shows selectively [10] there are differences in voltage response patterns potentiometer concerning time.

There is a difference in the response of the potentiometer voltage to time images 5-7 with Figures 8-12 on the potentiometric cell when the reference electrode and indicator electrode are immersed in the urea solution as a sensor that analyzes the urea from the print screen display of the computer by the power lab.

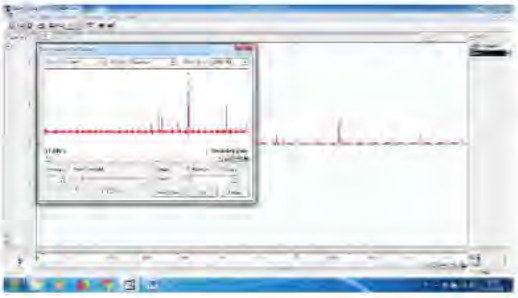

Figure 5. Voltage response to time in a 0.001 M urea solution with indicator electrodes without immobilization on a 5:1 scale potentiometer cell [4].

Increasing the pH from the phosphate buffer solution [11,12] using 0.1 M NaOH from pH 5.5 to pH 7.5.

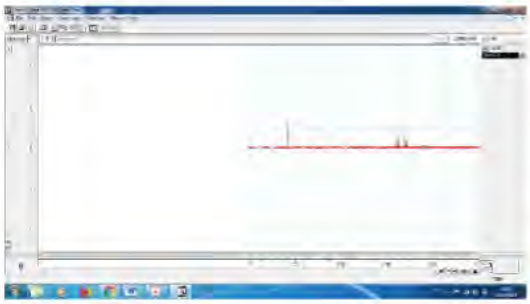

Figure 6. Voltage response to time in a 0.001 M urea solution with indicator electrodes without immobilization on a 100:1 scale potentiometer cell.

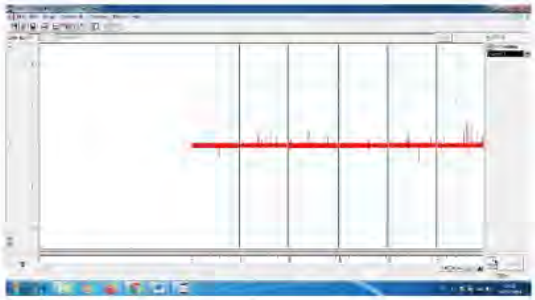

Figure 7. Voltage response to time in a phosphate KCl buffer solution of 0.001 M urea injection enzyme with an immobilized indicator electrode on a 500:1 scale potentiometer cell.

Potentiometric signals can be measured shown by direct correlation with observations of the activity and oxidation of electroactive compounds that determine cell potential increases in the growth phase is a change in cell potential Ecell = Eanode - Ecathode [13] can be seen in Figures 5, 6 and 7. Figure 5-7 shows the voltage to time signal on the potentiometric cell concentration of 0.001 M phosphate buffer pH 7.5 with reference electrodes and indicator electrodes not immobilized [12], while Figures 8-12 shows the pattern of voltage response to time, where the composition of the electrolyte solution or supporting solution under optimum conditions. The first procedure is to study the urea solution first so that the urea analyte in the best

composition gets a higher voltage signal as shown in Figures 5 and 6; the two buffer solutions were characterized by injection of urea at a molarity of 10^{-7} - 10^{-3} M as shown in Figure 7; all three buffer solutions at the chosen molarity and pH plus KCl with molarity variations of 10^{-7} - 10^{-3} M are characterized by further urea injection; and the four selected buffer and KCl solutions plus enzymes were characterized by injection of urea in a molarity variation of 10^{-7} - 10^{-3} M.

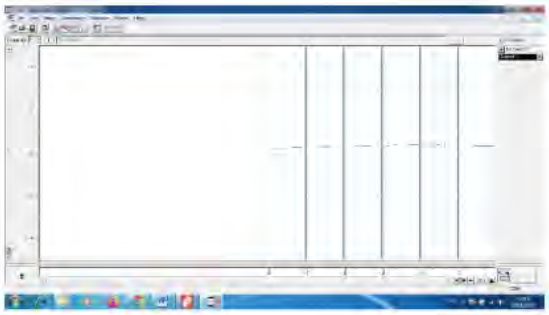

Figure 8. Voltage response to time in a 0.001 M urea solution with PVA-enzyme immobilized indicator electrodes on a 20:1 scale potentiometer cell.

There is a difference in the display of the potentiometer voltage signal between Figure 5-7 of the non-immobilized indicator electrode and the Figure of 8-12 of the immobilized indicator electrode using a power lab and computer potentiometer the best signal pattern in Figure 11 that the response time is in the form of plateue [12], besides that it also takes a long time in the process of urea injection in potentiometric cells.

Figure 9. Voltage response to time in a phosphate KCl buffer solution of 0.001 M urea injection with a 3x immobilized PVA-enzyme indicator electrode on a 500:1 potentiometer cell.

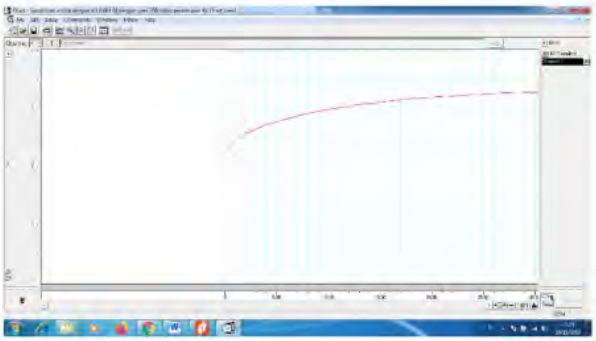

Figure 10. Voltage response to time in a 0.001 M Enzyme-KCl solution with 3x immobilized PVA-enzyme indicator electrodes on a 100:1 scale potentiometer cell.

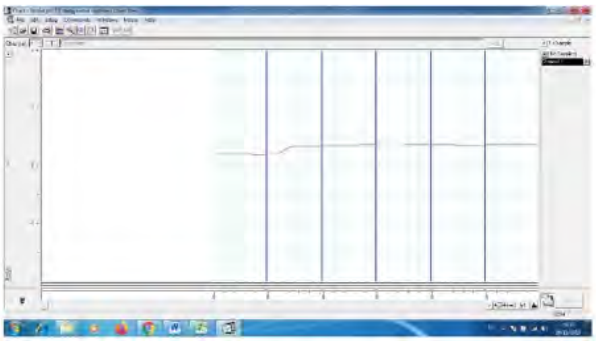

Figure 11. Voltage response to time in a phosphate buffer solution 0.001 M pH 7.5 KCl 0.001 M urea injection enzyme with 2x immobilized PVC-KTpClPB indicator electrodes on a 500:1 scale potentiometer cell [14].

Figure 12. Voltage response to time in a phosphate buffer solution 0.001 M pH 5.5 KCl urea injection enzyme 0.001 M with a 2x electrode immobilized PVC-KTpClPB indicator on a potentiometer cell [14] with a scale of 500:1.

Optimization of pH as shown in Figure 12 of the 2x-immobilized PVC-KTpClPB indicator electrodes in potentiometer cells with 0.001 M phosphate buffer solution pH 5.5 KCl urea injection enzyme 0.001 M indicator membrane 2x dyeing electrode [15]. The composition of the electrolyte solution used in a potentiometric cell is the same for an immobilized electrode as that which is immobilized using a long step. To shorten the determination of the composition of the electrolyte solution, characterization using UV-vis and FTIR was used in addition to the voltage response to the time of the immobilized potentiometer cell as shown in Figure 13, which is the voltage response to the time of the molarity phosphate buffer urea injection for 11 seconds with indicator electrodes without immobilization on the potentiometer cell. at the molarity of 0.001 M, 0.005 M and 0.0001 M. The procedure still follows the procedures one to four mentioned above.

EXPERIMENITAL

Material and Methods

The materials used in this study were KH_2PO_4, KCl, urea, and enzymes used in the research were Uriase, EC 3.5.1.5 (Urea) U4002, 50-100 ix type Sigma-Aldrich, as an electrolyte solution from potentiometric cells. The tools used are potentiometric cells consisting of Ag / AgCl reference electrodes, immobilized,

and immobilized PVA-Enzyme coating PVC-KTpClPB coating electrodes, indicator electrodes derived from tungsten. Potentiometers, Power Labs and computers to view voltage signals as a function of time showing the response of an electrolyte solution.

Membrane electrodes [16] from PVA-KTpClPB immobilization enzymes from PVA composition analysis [17,18,19]: PVA:PVC composition is 1:1 with mass 0.0350 g enzyme 1 mg and KTpClPB 0.0500 g, KTpClPB 0.0700 g, KTpClPB 0.0100 g KTpClPB 0.0500 g.

General procedure

The method used is the biosensor potentiometric method of potentiometric cells through analysis of (1) urea analyte, (2) urea phosphate buffer, (3) KCl and urea phosphate buffer, (4) KCl and urea phosphate buffer, (5) KCl urea phosphate buffer immobilized enzymes with a pH variation of 5.5 and 7.5, were analyzed in advance of the pH buffer phosphate variation. The following steps (1) - (5) are characterized by spectrum patterns and FTIR functional groups and absorbance and UV-Vis polarization patterns. Urea analyzers were analyzed from the voltage response to time with molarity variations of 0.001 M, 0.005 M and 0.0005 M for maximum.

Detection Method

The urea cell analyzer potentiometer sensor analyzer has been analyzed namely 0.001 M phosphate buffer pH 7.5 the best conditions with KCl 0.001 M solution and 0.001 M urea as potentiometer cell electrolyte solution [12].

RESULTS AND DISCUSSION

After testing the material by characterizing the PVA-enzyme immobilization membrane PVC-KTpClPB coating, followed by a reversible test [12] through linear curve characterization with criteria obtained sensitivity is 60 mV/decade using phosphate buffer KH2PO4 0.001 M pH 7.5, KCl 0.001 M injection urea [17] based on the results of this study continued characterization of electrolyte solutions to see the best response. Retrieval of data begins with the print screen display of the response signal from urea solution

ranging from 0.0000001 M to 0.01 M, urea injection buffer from 0.0005 M to 0.01 M, urea injection buffer and KCl.

The degree of acid solubility [13] (or the degree of acid dissociation between pH 0 – 7, even though the H^+ concentration is increasing, there is still dissolved OH, for the base the concentration is between pH 7 and pH 14. The pKa value is defined as "minus the logarithm of the concentration of H^+ ions in solution". A phosphate buffer solution KH_2PO_4 0.001 M pH 7.5, KCl 0.001 M urea injection means the potentiometer cell electrolyte solution is alkaline [12].

The response time and duration of the electrodes in the range of solution 10^{-5} - 10^{-2} mol / L for each potentiometric sensor [20], the time response curve pattern is in accordance [21] for images 11 (immobilized indicator electrode) and Figure 13 (non-immobilized indicator electrode). Potentiometric methods have advantages such as high sensitivity, high selectivity, low cost, and fast determination. To determine the electrode reversibility, the technique was completed in the reverse concentration order, starting with the highest concentration and ending with the lowest concentration [20].

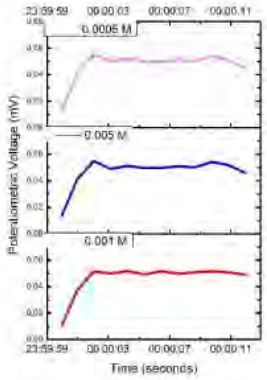

Figure 13. Response time of molarity of urea injection phosphate buffer for 11 seconds indicator electrode without immobilization in potentiometer cells with a molarity of 0.001 M, 0.005 M and 0.0001 M.

Observations in electrolytes are evaluated using Cu | cells Li type coin separator and causes an internal short circuit, which results in safety problems. Highly sTable voltage polarization up to 330 hours cycle symmetric cells using electrolytes produces greatly increased voltage polarization and random voltage oscillations. Electrolyte compatibility with various cathodes to cathode material [4] has been shown Reversible cycle trends [17] for electrode indicators of PVA immobilization-PVC-KTpClPB coating enzymes with linear curves have a sensitivity of 19.069 mV/decade meets reversible criteria using a phosphate buffer of KH_2PO_4 0.001 M pH 7.5. The pH value of the phosphate buffer solution can be increased to 7.40 by HCl and NaOH solutions, the longest immersion time is 33 weeks [22]. A 0.05 M phosphate buffer pH = 7.4 containing 0.1 M KCl, is used as a carrier solution for amperometric measurements [23]. The effect of phosphate buffer solution concentration on the potentiometric response [24] can be seen in Figure 11 after the indicator electrodes were immobilized by PVA-KTpClPB coating enzymes according to membrane composition [17], if before immobilized as Figure 7.

Demonstrates linear suitability for buffered solutions with pH increasing from pH 3 - 11 for KCl solutions with increased concentrations from 1×10^{-5} M - 1 M [14,25] KCl FTIR spectrum patterns (a) urea, (b) Buffer pH 5.5 and urea, (c) Buffer pH 5.5 + KCl and urea, (d) Buffer pH 5.5 + Enzyme + KCl and urea, (e) Buffer pH 7.5 + Enzyme + KCl and urea image 14 left. FTIR buffer pattern phosphate image 14 right part there are 2 transmittance spectrum patterns namely (b) and (c) similar to the FTBC pattern of nBC (Nano-bacterial cellulose) immersed in phosphate buffer solution [22] also supported by XRD diffraction spectrum pattern test is also similar. In the FTIR spectrum, the absorption band at 3345 cm^{-1} stretches for hydroxyl groups and intermolecular hydrogen bonds, stretching vibration absorption bands at 2930 and 2853 cm^{-1} are each given asymmetric or epoxide CH bonds and intramolecular hydrogen bonds, and vibration absorption bands stretching from 1170 to 1060 cm is given to the CO bond [11,22]. No degradation of the spectrum [26] only shifts in vibration and changes in spectrum patterns.

The concentration of buffer solution can affect the activity of cells that are immobilized in the concentration of phosphate buffer solution. A higher buffer capacity at 20 mM phosphate buffer solution was found to provide a higher correlation coefficient value, where the regression line matches the data in a linear pattern. Higher buffer concentrations can stabilize changes in the pH of the reaction medium as the system involves

enzymatic reactions that release protons [24]. Initial concentration increases the time for the adsorption process to reach equilibrium in a longer time, high adsorption capacity is taken as the optimal value for further adsorption studies [27] Figure 4 ie 10^{-3} M.

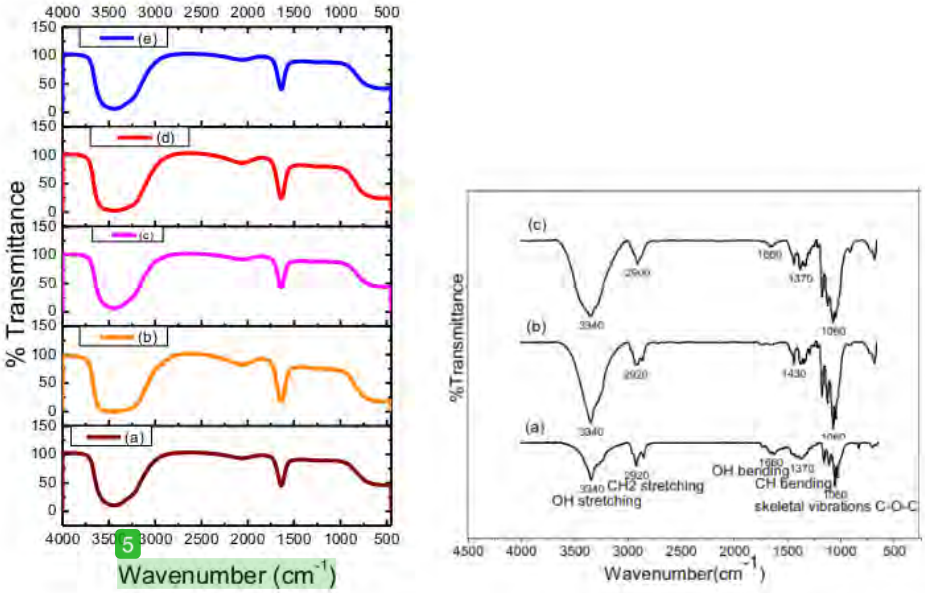

Figure 14. Spectrum pattern of FTIR (a) urea, (b) Buffer pH 5.5 and urea, (c) Buffer pH 5.5 + KCl and urea, (d) Buffer pH 5.5 + Enzim + KCl and urea, (e) Buffer pH 7.5 + Enzim + KCl and urea.

Table 1. Bonds of phosphate buffer electrolyte solution groups, KCl, Enzymes, and urea

No	Sample	Frekuensi (cm^{-1}	Grup Prekuensi (cm^{-1})	Ikatan
1	(a)	3435.57	3100-3500	N-H Hydrogen, O-H
		2062.34	2000-2400	stretch
			2100-3400	C ≡ C
		1637.28	1600-1700	carboxylic acid O-H
			1600-1680	C=N stretch
				C=C Carbon
2	(b)	3435.26	3100-3500	N-H Hydrogen, O-H
		2065.74	2000-2400	stretch
			2100-3400	C ≡ C
		1634.04	1600-1700	carboxylic acid O-H
			1600-1680	C=N stretch
				C=C Carbon

No	Sample	Frekuensi (cm^{-1}	Grup Prekuensi (cm^{-1})	Ikatan
3	(c)	3433.92	3100-3500	N-H Hydrogen, O-H
		2066.68	2000-2400	stretch
			2100-3400	C ≡ C
		1637.12	1600-1700	carboxylic acid O-H
			1600-1680	C=N stretch
				C=C Carbon
4	(d)	3435.27	3100-3500	N-H Hydrogen, O-H
		2066.65	2000-2400	stretch
			2100-3400	C ≡ C
		1633.66	1600-1700	carboxylic acid O-H
			1600-1680	C=N stretch
				C=C Carbon
5	(e)	3434.90	3100-3500	N-H Hydrogen, O-H
		2064.35	2000-2400	stretch
			2100-3400	C ≡ C
		1637.11	1600-1700	carboxylic acid O-H
			1600-1680	C=N stretch
				C=C Carbon

Effect of pH and buffer capacity, all biosensor potentiometric cells for the detection of buffer solution concentrations that can influence activity [24]. The optimization of biosensors is influenced by cell loading carried out on the membrane surface at the electrodes with different concentrations. The buffer capacity is optimized by varying the concentration of the phosphate buffer solution between 1 mM and 50 mM.

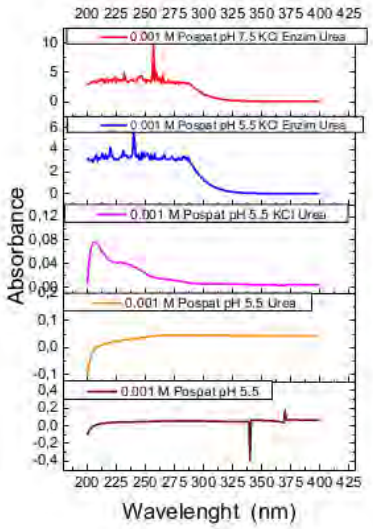

Figure 15. UV-Vis absorbance of phosphate buffer 0.001 M pH 7.5 KCl 0.001 M 300 μL enzyme 1 drop 6 mg 10 mL (50% water and 50% alcohol) [17].

Phosphate buffer solutions at the solution-membrane interface arise because the potassium in the solution selectively diffuses between the aqueous and membrane phases, doped with KTpClB lipophilic salt and ion-selective carriers [28]. The electrochemical biosensor response uses phosphate buffer pH 7.5 [17,24].

The interaction mechanism has been examined using (1) electrode membrane solution [16] from the immobilization of PVA-KTpClPB coting enzymes from the composition analysis of the composition of PVA : PVC composition is 1 : 1 with mass 0.0350 g enzyme 1 mg and KTpClPB 0.0500 g KTpClPB, 0.0700 g, KTpClPB 0.0100 g, KTpClPB 0.0500 g, analyzed by transmission spectrum membrane [17,18,19] with FTIR, (2) Urea solution 0.001 M, solution 0.001 M M phosphate buffer pH 5.5 urea, phosphate buffer solution 0.001 M pH 5.5, KCl 0.001 M and urea 0.001 M, phosphate buffer solution 0.001 M pH 5.5, KCl 0.001 M, enzyme 1 mg and urea 0.001 M, analyzed absorbance spectrum and UV polarization spectrum - Vis obtained the greatest absorption and polarization at pH 7.5 molarity of 0.001 M with the greatest polarization [29] in Figure 15.

According to FTIR analysis, the results of the study of PVA solution and PVC-KTpClPB solution have functional groups from NH in the frequency group 3100-3400 cm-1, OH in the frequency group 2700 – 3300 cm^{-1}, CN in the frequency group 1600 – 1700 cm^{-1}, CO in the frequency group 1200 – 1350 cm^{-1}, CH in the frequency group 700 – 950 cm^{-1} and Cis (included in the CH functional group) in the frequency group 650-800 cm-1 are in the PVA solution and PVC coating solution. Unless the functional group C ≡ C is only obtained in PVA solutions with a frequency of 2167,106 cm^{-1} in the frequency group 2100 – 2200 cm^{-1}. Between the electrolyte solution and the indicator electrode membrane, there is a suitability of the functional groups obtained in the electrolyte solution namely NH, OH in the frequency group 3100 – 3500 cm^{-1}, C ≡ C in the frequency group 2000 – 2400 cm^{-1}, OH in the frequency group 2100 – 3400 cm^{-1} carboxylic acid, C = N in the 1600 – 1700 cm^{-1} frequency group, C = C in the 1600 – 1680 cm^{-1} frequency group from Table 2.

Enzyme activity is calculated by measuring the amount of NH_3 ammonia production in which urease can catalyze the hydrolysis of urea as follows [30] :

$$CO(NH_2)_2 + 3H_2O \xrightarrow{\text{Urease}} HCO_3^- + 2NH_4^+ + OH^- \quad (1.1)$$

The presence of enzymes in the hydrolysis of urea produces [31]:

$$NH_2CONH_2 + 2H_2O + H^+ \xrightarrow{\text{Urease}} HCO_3^- + 2NH_4^+ \quad (1.2)$$

Urea produces enzymatic hydrolysis [5]:

$$NH_2CONH_2 + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3 \quad (1.3)$$

Ammonia in the electrode solution becomes balanced with ammonium ions and produces hydroxide ions [32]:

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (1.4)$$

Based on the functional groups, indicator electrode membranes and enzymatic reactions that produce ions with CO, NH, and OH functional groups as shown in equation (1-1) - (1-4), whereas in the electrolyte solution no CO function groups are obtained. absorption spectrum [33] from the results of FTIR sample tests as shown in Figure 16.

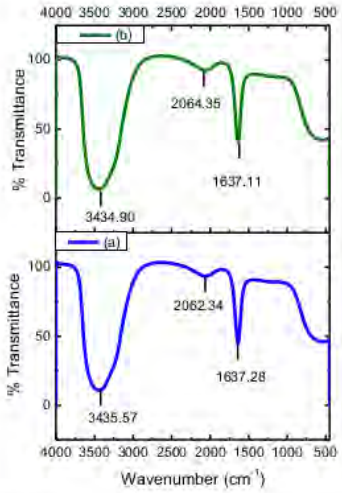

Figure 16. Pattern Spectrum FTIR (a) urea, (b) buffer pH 7.5 + enzyme + KCl and urea.

Table 2. Bonding of the electrolyte solution group of Figure 15

No	Sample	Frequency (cm^{-1})	Group frequency (cm^{-1})	Bond
1	(a)	3435.57	3100-3500	N-H Hydrogen, O-H
		2062.34	2000-2400	stretch
			2100-3400	C ≡ C
		1637.28	1600-1700	carboxylic acid O-H
			1600-1680	C=N stretch
				C=C Carbon
2	(b)	3434.90	3100-3500	N-H Hydrogen, O-H
		2064.35	2000-2400	stretch
			2100-3400	C ≡ C
		1637.11	1600-1700	carboxylic acid O-H
			1600-1680	C=N stretch
				C=C Carbon

FTIR spectrum patterns (a) urea, (b) Phosphate buffer pH 7.5 + Enzyme + KCl and urea in Figure 16 are similar to only a vibrational shift between urea and phosphate buffer solution pH 7.5 + Enzyme + KCl can be seen in Table 2. Frequency difference [34] in Table 1 between sample (a) and sample (c) producing a sample (e) phosphate buffer pH 7.5 + enzyme + KCl and urea, which is supported by (1) response time of molarity of the phosphate urea injection buffer Figure 13, (2) UV-Vis Absorption of phosphate buffer 0.001 M pH 7.5 KCl 0.001 M 300 μL enzyme 1 drop 6 mg 10 mL (50% water and 50% alcohol) Figure 15. This causes the computer screen display to display the response time of the potentiometer cell between the indicator electrodes without immobilization (unclear Figures 5-7 less selective and sensitive) and immobilized (clear pictures 8-12 selective and sensitive) immersed in a KCl phosphate buffer solution of the enzyme and urea.

CONCLUSION

The display of a computer print screen for photometer cells with indicator electrodes being immobilized with a PVA membrane-PVC-KTpClPB coating enzymes closely matches the appearance of the response time characteristics with note composition of the PVA electrode membrane: PVC is 1:1 and PVC:KTpClPB 1: 2 1% enzyme and enzyme KCl phosphate buffer solution and urea 0.001 M pH 7.5. The electrolyte solution optimization method with the old potentiometric method can be shortened by characterizing the electrolyte solution using UV-Vis and FTIR characterization first.

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