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by Abd Hakim S, Krista Tarigan Manihar Situmorang

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Abd Hakim S[1]., Krista Tarigan[2], Manihar Situmorang[3], and Timbangen Sembiring[2]

[1]Physics Department,Faculty of Math and Science Universitas Negeri Medan, Medan, Indonesia
[2]Physics Department,Faculty of Math and Science Universitas Sumatera Utara, Medan. Indonesia
[3]Chemistry Department, Faculty of Math and Science, Universitas Negeri Medan, Medan, Indonesia

abdhakims@unimed.ac.id; fmipa@usu.ac.id, msitumorang@unimed.ac.id

Abstract. Potentiometric sensors provide an opportunity to perform biomedical, environmental analysis, and industry analysis, these sensors are generally easy to , portable, simple, and inexpensive. The potentiometric method is a method of measuring the potential difference of the balance between the indicator electrode and the reference electrode. Chemical sensors are a device that can convert chemical quantities into electrical quantities. Chemical sensors sensitive to one analyte involve a combination of molecular recognition elements PVA-enzyme PVC-KTpClPB and transducer to detect an analytical bond through a voltage signal. The urease enzyme is used for the determination of urea, the urea sensor means an accurate determination of urea essential with respect to renal function and biomedical applications. ISE is a good approach to build chemical sensors in detecting urea analyte. The immobilized product as a molecular recognition element of PVC-enzyme PVC-KTpClPB has a sensitivity of 19,069 mV / decade R^2 = 0.94 with a range of 1.10-5 - 5.10-4 M in electrolyte solution consisting of buffer KH_2PO_4 0.001 M and KCl 0.001 M.

Keyword : urea sensor, sensitivity, PVC-enzyme, PVC-KTpClPB, and buffer KH_2PO_4 and KCl

1. Introduction

Sensors are devices and technologies developed by utilizing several properties of materials or electrical and electronic elements [1]. The output variable of the sensor is converted into electrical quantities called transducers. The potentiometric sensor is a device that measures the electromotive force (voltage) generated between two electrodes, measurably dependent on the concentration of the analyte [2]. The sensor fabrication method uses a conductive lay of copolymer and a sensitive membrane [3]. Electrochemical conductor polymer condensing and biosensors play an important role in improving public health and the environment because of rapid detection, high sensitivity, small size, and affordable for environmental monitoring and clinical diagnostics [4].

Urea is one of the protein metabolism products, an important accurate determination of urea with respect to renal function and other biomedical applications [5]. The determination of urea is important in terms of clinical research and industrial application [6], the method for determining urea is a potentiometric ion potentiometric biosensor (ISE). ISE is a good approach to build chemical sensors in detecting analyt [7]. Biosensor is a combination of bioelement and elemental sensor, bioelement, functions as biochemical transducer such as enzyme, tissue / tissue, bacteria, yeast, antibodies / antigens, liposomes, organelles and others. Biomolecular recognition biosensors have selectivity but are sensitive to extreme conditions such as temperature, pH and ionic strength.

Biosensors are analytical devices, which alter the modification of the physical or chemical properties of a biomatric (enzyme, antibody, receptor, organelle, microorganism) into electricity whose amplitude depends on the concentration of the analyte defined in solution [8]. The biosensor contains three components: bioelements, detection elements and signal processing elements [9].
The development of a biosensor system follows the following rules [10] ie selectivity, sensitivity, linearity response, signal reproducibility response, fast response time, stability.

2. Materials and Methods

2.1. Materials

The material used in the study is a standard urea 56 180 Sigma-Aldrich, the enzyme EC 3.5.1.5 (Urease) U4002, 50-100 ku ix types , tungsten diameter of 1.0 mm 267 562 99.99% Aldrich, Phosphate Buffer KH_2PO_4, tris-HClbuffer, PVA [- CH_2CHOH-] n, PVC (CH_2CHCl) n, potassium tetrakis 4-chlorophenyl borate $(ClC_6H_4)_4BK$, Tetrahydrofuran C_4H_8O, KCl were from Sigma-Aldrich and the method used is the potentiometric method.

2.2. Tools

The equipment used is from the Laboratory of Chemical Laboratory from Universitas Negeri Medan in accordance with its use as follows: The equipment used in Chemical Laboratory is potentiometer (Keithley 199 DMM, USA), tungsten indicator electrode (W), RE-5B Ag / AgCl MF-2052 reference electrode in electrochemical cell assembled with microcomputer (Powerlab ADI instrument, Australia). Supporting equipment are 0.1 - 0.2 mm diameter silica hose pipe (Fison Co), and micro syringe (Hamilton Co) and magnetic stirrer. Flow injection system (FIA) coupled with integrating the electrochemical cell and detector.

2.3. Methods

The materials used are 1 (g). The membrane composite (W_{PVA}: $W_{PVC\text{-}plastisisier}$ = 1: 1) [11] where the mass of PVA and PVC are equal, according to (Vlascici et al, 2010) comparison of plasticizer: PVC = 2: 1, the researchers use 1: 1 PVC plastisisier.

The urease enzyme 1 mg is dissolved in 0.5 mL of mixed alcohol with a ratio of 50%: 50%. PVA 0.0350 g dissolved in 10 mL hot to cold water, as well as PVC 0.0350 g + KTpClPB 0.0500 g dissolved in 10 mL THF in glass tube and covered with plastic / alumunium poil cover as coating solution. The PVA-enzyme prepared from one drop of the enzyme solution is inserted into a 0.5 mL glass beaker of PVA solution derived from 10 mL of PVA solution stirred until homogeneous.

The first step of the 5 cm long tungsten indicator electrode is 5 electrodes, each electrode is labeled 1x, 2x, 3x, 4x, and 5x subsequent 1x immersion for immobilization as deep as 4 cm into a small PVA-enzyme tube as shown in Figure 1 (a) then lift to let dry. The second step after the dry take the electrode labeled 1x dip 1x into the THF solution mixed with PVC 0.0350 g and the plastisis 0.0500 g as the coating as picture 1 (b). Repeat the first step for the 2x, 3x, 4x, and 5x labeled electrodes with each of the 2x, 3x, 4x, and 5x subsequent dipping of all 1x dicoating electrodes according to the second step as shown in Figure 1 (c). After being finished and can be used as ISE-urea as shown in Figure 1 (d) it is connected to a potentiometer (Keithley 199 DMM, USA) and microcomputer (Powerlab ADI instrument, Australia) as shown in Figure 1 (e) (f).

[12] The optimization of the membrane for analytical detection involves consideration of various parameter effects, such as the influence of the supporting electrolyte, the analytical concentration, the polymer matrix concentration, the applied current density, the polymerization time and the buffer concentration.

[13] Potentiometric biosensors are used to detect mixtures with respect to parmasi, this tool presents a quick and simple methodology, high sensitivity, low detection limits and minor annoyances of the matrix. Electrochemical detection focuses on the optimization of the biosensor structure in achievement and stability. Potentiometric biosensors are based on the use of ISE to obtain analytical information.

To study the urea sensors in this research via indicator electrode response PVC-enzym Coating PVC-KTpClPB: (1) in urea solution, (2) in variation of KCl concentration with urea injection, (3) in Tris-HCl Buffer an Phosphate Buffer KH_2PO_4 with variation of pH, (4) effect of urea concentration Tris-HCl buffer pH 6.5 and Phosphate buffer pH 7.5, (5) Urea Time Response in Tris-HCl buffer pH 6.5 and Phosphate buffer pH 7.5, (6) membrane optimization, KCl, buffer, (7) Stability test, (8) Selectivity of urea sensor, (9) urea calibration curve (sensitivity), (10) application of urea potentiometer sensor.

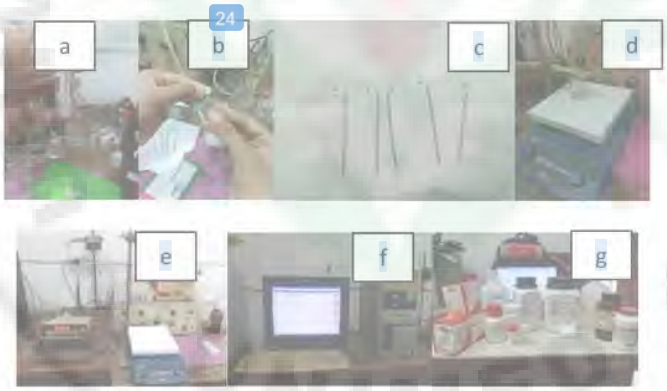

Figure 1. Potentiometric sensor design (a) immobilized PVA-enzyme, (b) Coating PVC-KTpClPB after the indicator electrode is immobilized PVA-enzyme, (c) Electrode indicator PVA-coating enzyme KTpClPB, (d) ISE-urea sensor, (e)) potentiometers (Keithley 199 DMM, USA) and microcomputers (Powerlab ADI instruments, Australia), (f) computers, (g) chemicals.

[14] The optimization of experimental variables that need to be considered for analysis using biosensors is the voltage used, the membrane thickness, the concentration of the enzyme, the concentration of the polymer matrix for immobilization, temperature, pH and buffer concentration. Research experiments have not yet conducted variations of enzyme concentration, plasticizers concentration, temperature.

3. Results and Discussion

3.1. Membrane response of indicator electrode from ISE-urea in potentiometric system

To obtain an optimum membrane electrode response [15] of ISE-urea modified membranes [16] were immobilized PVA-coating enzyme PVC-KTpClPB at the indicator electrode. Immobilized PVA-enzyme using PVA 0.0350 g in 10 mL ades given 1 drop from 6 mg enzyme (U4002-100 KU, type IX) in 50% water solvent and 50% alcohol.

Each immobilized and coating elektoda was tested for response with urea injection at 1.10^{-5} M, 5.10^{-5} M, 1.10^{-4} M, 5.10^{-4} M, 1.10^{-3} M, 5.10^{-3} M, shown in Figure 2 best results were obtained on immersion electrode.

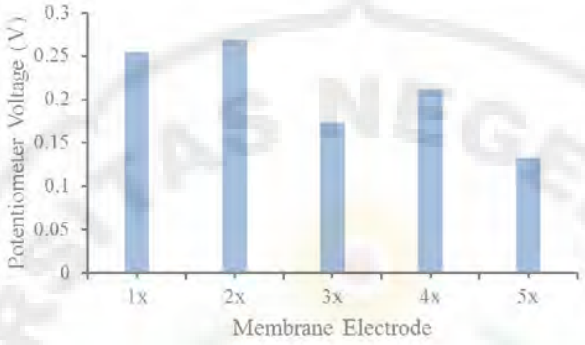

Figure 2. Response electrode indicator PVA-coating enzyme PVC-KTpClPB with variation of urea injection 1.10^{-5} M, 5.10^{-5} M, 1.10^{-4} M, 5.10^{-4} M, 1.10^{-3} M, 5.10^{-3} M. Optimization occurs at number of 2x electrode immersion with potentiometer voltage of electrode W and reference electrode Ag / AgCl of 0.268 V.

The thickness of an non-conductive layer at unummnya between 10 - 100 nm enables rapid diffusion of the substrate to and from the membrane 12, the membrane thickness reduces the sensitivity. To obtain a thickness of 10 - 100 nm the indicator electrode is only dipped briefly into the solution and then removed from the solution. [16] Membrane modification indicates potentiometric response causing this event to be completely reversible.

[17], an evaluation of the electrode voltage (indicator and reference) as a function of time for variation of urea (M) concentration in a bufferless solution, this experiment has been conducted in the study.

3.2. Influence of KCl Concentration in Potentiometric System

After the electrode response test with the optimum urea was found on the 2x immersion electrode and 1x coating, followed by the effect of KCl concentration to be used on buffer phosphate and tris-HCl buffer as an additive to electrolyte solution [16] from ISE-urea. The sensor response [18] is evaluated by measuring the voltage of the following electrochemical cell potentiometers: Ag, AgCl, / Electroda Membrane PVA coating / KCl solution.

[12] Supporting electrolytes commonly used potassium chloride KCl and potassium nitrate KNO3 are always added in the monomer solution increasing the electrical conductivity and sensitivity of the biosensor. The supporting electrolyte used in this study is potassium chloride KCl.

The concentration of KCl solution used varies 0.1 M, 0.01 M, 0.05 M, 0.001 M, 0.005 M injected urea with variations of 1.10^{-5} M, 5.10^{-5} M, 1.10^{-4} M, 5.10^{-4} M, 1.10^{-3} M, 5.10^{-3} M. The optimization result of KCl effect using ISE-urea is shown in table 1 shows that the concentration of KCl has an effect on urea response, that is at the concentration of 0.005 M potentiometer voltage 0.353 V next 0.01 M potentiometer voltage 0.305 V, 0.05 M potentiometer voltage 0.106 V, 0.1 M potentiometer voltage is 0.042 V, so it can not be ascertained sequentially according to the increase in concentration. KCL plays a protective layer [19].

Table 1. Response of the indicator electrode in potentiometric system of urea injection 1.10^{-5} M, 5.10^{-5} M, 1.10^{-4} M, 5.10^{-4} M, 1.10^{-3} M, 5.10^{-3} M within 10 minutes in KCl solution.

KCl Concentration (M)	Potentiometer Voltage (V)
0.1	0.042
0.01	0.305

0.05	0.106
0.001	0.026
0.005	0.353

According to [17], the sensor electrode down 1.0 M KCl (40 mL) in the beaker glass is stirred slowly with stirred, the voltage is measured to stable for 5 min. Then 1.0 M urea solution (40 µL) was added to 1.0 M KCl (40 mL), the voltage measured for 10 min. Next prepared urea concentrations of 0.99, 1.99, 2.99, 3.98, 4.97, 5.96 and 6.95 mM measured voltages for each solution. This experiment was conducted in a study using 0.1 M KCl in the potentiometric system of ISE-urea in each urea analysis of 1.10^{-5} M, 5.10^{-5} M, 1.10^{-4} M, 5.10^{-4} M, 1.10^{-3} M, 5.10^{-3}M within 10 minutes.

3.3. Pengaruh pH Tris-HCl Buffer dan pH Phosphaet Buffer KH_2PO_4

To obtain the optimum electrode response from ISE-ure in potentiometric system, the optimum concentration of tris-HCl buffer (mass of formula $C4H_{11}NO_3$ = 157.60 g) was prepared with variation of pH 5-9 urea / 10 min and phosphate buffer (KH_2PO_4 = 136.08 g) was prepared with a variation of pH 4.5 - 10 injected urea / 10 min each with a variation of injection 1.10^{-5} M, 5.10^{-5} M, 1.10^{-4} M, 5.10^{-4} M, 1.10^{-3} M, 5.10^{-3} M.

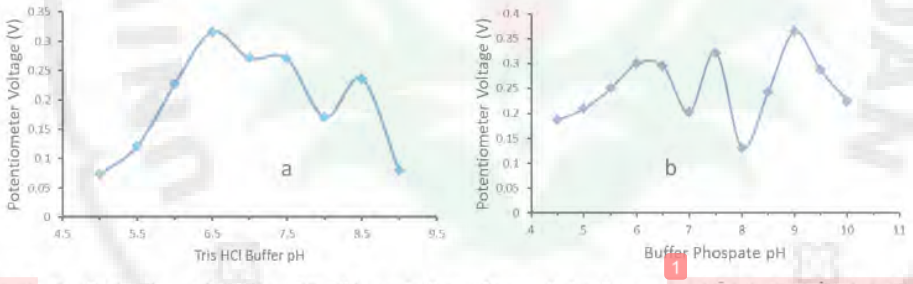

Figure 3. Optimization of buffer pH with variation of urea injection at 1.10^{-5} M, 5.10^{-5} M, 1.10^{-4} M, 5.10^{-4} M, 1.10^{-3} M, 5.10^{-3} M (a) Tris-HCl buffer and (b) Phosphaet Buffer KH2PO4 in the ISE-urea FIA system. Optimization occurs in (a) Tris-HCl buffer pH 6.5 and (b) Posphat KH2PO4 buffer pH 7.5.

[20] to investigate the urea biosensor system was studied with the effect of increasing the urea concentration in the biosensor response in the buffer. The biosensor response was optimized as a pH function, incubation time with urea and immobilization conditions, the calibration curve of the biosensor response as a function of urea concentration around 4.0-10.0 µM in 20 mM tris buffer pH effect of pH 4.5 - 8.5 optimization occurred in Tris buffer pH 7.2 . To see the biosensor response in the study, urea injection of 1.10-5 M, 5.10-5 M, 1.10-4 M, 5.10-4 M, 1.10-3 M, 5.10-3 M.

The pH optimization results on Tris-HCl Buffer and Phosphate Buffer KH2PO4 on the ISE-urea response are shown in Fig. 3, based on Figure 3 Tris-HCl Buffer pH 6.5 with an average potentiometer voltage of 0.316 V while the Phosphaet Buffer KH2PO4 pH 7.5 with average voltage potentiometer 0.321 V. On the basis of this information, researchers have a tendency to choose Phosphate Buffer KH2PO4 as a catalyst. Garcia et al. (2009), the construction and conditions of membrane ion selective electrodes incorporated with an ISE electrode containing 0.001 M KCl and analyte and buffer. Tris-HCl Buffer and Phosphate Buffer KH2PO4 in this study 0.01 M KCl. According to [21] a variation of 100 mM KCl, 20 mM, 5 mM in Tris-HCl Buffer can improve the absorption spectrum by UV-Vis. In this research, KCl optimization has not been done in buffer but optimization of KCl in urea, there is difference of procedure with [21].

3.4. Effect of urea concentration in Tris-HCl buffer pH 6.5 and Phosphate buffer pH 7.5.

After optimizing Tris-HCl buffer at pH 6.5 for 2x immersion electrode, optimized dilution of Tris-HCl buffer from stock through urea / 10 minute injection with concentration variation 0.01 M, 0.05 M, 0.001 M, 0.005 M, 0.0001 M, 0.0005 M , and 0.00001 M, the result can be seen in figure 4.

Optimization of Tris-HCl buffer concentration to pH 6.5 occurred at dilution of urea injection concentration 0,001 M with potentiometer voltage 316 Volt. The optimization of KH2PO4 phosphaet buffer concentration at pH 7.5 for 2x immersion electrode was carried out by optimizing KH2PO4 phosphaet buffer dilution from urea / 10 minute injection stock with concentration variation 0.01 M, 0.05 M, 0.001 M, 0.005 M, 0.0001 M, 0.0005 M, and 0.00001 M. Optimization of phosphate concentration of KH2PO4 buffer for pH 7.5 occurred at dilution of urea injection concentration 0,001 M with potentiometer voltage 0,321 Volt.

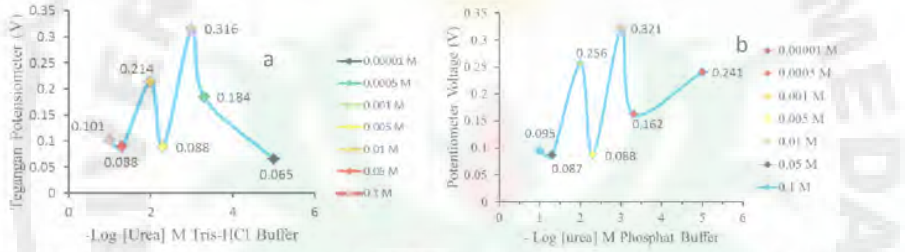

Figure 4. Effect of concentration (a) Tris-HCl buffer pH 6.5 at dilution of urea injection concentration 0.001 M with potentiometer voltage 0.316 Volt, (b) Phosphat KH2PO4 buffer pH 7.5 at dilution of urea injection concentration 0.001 M with potentiometer voltage 0.321 Volt.

3.5. Urea Time Response in Tris-HCl buffer pH 6.5 and Phosphate buffer pH 7.5

[22] the concentration of 10 mM phosphate buffer solution and urea concentration from 1.25 mg / dL - 240 mg / dL, the response achievement is independent of the amount of urease entrapped, but at the initial pH, buffer capacity and ionic strength of the solution at the surface area, porosity and physical characteristics of both enzymes and supporting materials. Therefore, urease variation was not performed in this study.

Based on the optimum optimum pH optimization for each tris-HCl Buffer and phosphaet buffer KH2PO4 the response time of urea injection to Tris-HCl Buffer after 2 min with average potentiometer voltage of 0.316 V while phosphaet buffer KH2PO4 has response after 5 minutes with average voltage potentiometer 0.199 V can be seen in figure 5.

According to [23] a response time of less than 10 seconds is good, means the response time for both buffers is good. [24] The time response after the membrane yields a stable potential, Figure 5 has shown a stable potential.

[17] The sensor time response was investigated by determining the UV-visible absorbance profile. Six small bottle samples, each containing a buffer and 5 mM of urea solution that has been marked with specific time (1.5, 2, 2.5, 3, 4, 5 and 6 min). The kinetic behavior of immobilized urease variation in absorbance is measured as a function of time during enzyme catalysis hydrolysis. The absorbance is obtained until it increases monotonically with time until a plateau has been reached (plateau).

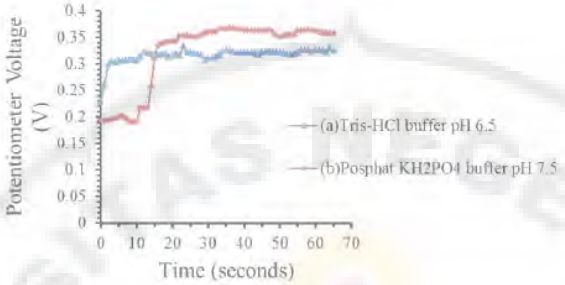

Figure 5. Response of urea time on (a) Tris-HCl Buffer and (b) Posphat KH2PO4 Buffer in FIA ISE-urea system on urea analysis 1.10-5 M, 5.10-5 M, 1.10-4 M, 5.10-4 M, 1.10-3 M, 5.10-3 M. Optimization occurs on Tris-HCl Buffer pH 6.5 after 2 seconds and Posphat KH2PO4 Buffer pH 7.5 after 5 seconds.

3.6. Effect of Tris-HCl buffer concentration at pH 6.5 and Phosphate buffer at pH 7.5

To make ISE-urea as biosensor, biosensor consists of two components [25] ie element of bio-receptor (immobilized enzyme urease) and transducer signal from ISE-urea in potentiometric system, optimization is required as follows (1) Optimization of electrode membrane from ISE- urea in potentiometry system on urea analysis 1.10-5 M, 5.10-5 M, 1.10-4 M, 5.10-4 M, 1.10-3 M, 5.10-3 M. Optimization occurs on the number of immersion of 2x electrodes with potentiometer voltage of electrode W and an Ag / AgCl reference electrode of 0.268 V. (2) Optimization of KCl solution injections of ISE-urea with potentiometric system in urea analysis 1.10-5 M, 5.10-5 M, 1.10-4 M, 5.10-4 M, 1.10- 3 M, 5.10-3 M. Optimization occurs at KCl 0.005 M potentiometer voltage of electrode W and reference electrode Ag / AgCl of 0.353 V. (3) Optimization of pH of injection concentration at (a) Tris-HCl Buffer and (b) Phosphaet Buffer KH2PO4 from ISE-urea in potentiometric system on urea analysis of 1.10-5 M, 5.10-5 M, 1.10-4 M, 5.10-4 M, 1.10-3 M, 5.10-3 M. Optimization occurs in (a) Tris-HCl Buffer pH 6.5 and (b) Posphat KH2PO4 Buffer pH 7.5. (4) Optimization of Tris-HCl buffer concentration at pH 6.5 for dilution of urea injection concentration 0.001 M with potentiometer voltage 316 Volt. Optimization of phosphaet buffer concentration of KH2PO4 for pH 7.5 at dilution of urea injection concentration 0.001 M with potentiometer voltage 0.321 Volt.

Table 2. Optimization of ISE-urea in tris-HCl buffer as urea sensor

Concentration (M)	-Log [urea] M	Potentiometer Voltage (V)
0,00001	5	0,05
0,0005	3,3	0,046
0,001	3	0,047
0,005	2,3	0,051
0,01	2	0,047
0,05	1,3	0,05

After completion of the above sequential treatment, the next step is to test ISE-urea in potentiometer system as biosensor at electrode condition 2x dipping of dicoating PVC, KCl concentration 0.005 M (a) Tris-HCl Buffer 0.001 M pH 6.5 and (b) Phosphaet Buffer KH2PO4 0.001 M pH 7.5, with urea injection of 0.05 M, 0.01 M, 0.005 M, 0.001 M, 0.0005 M, and 0.0000 M in order to obtain the optimum conditions, from the test results obtained at the concentration of urea the voltage of potentiometer 0.309 (V) for phosphaet buffer KH2PO4 can be seen in table 2 and table 3.

Tables 2 and 3 are made in graph - log [urea] as a function the voltage can be seen in figure 6 and figure 7.

Tabel 3. Optimalisasi ISE-urea dalam *phosphaet buffer* KH_2PO_4 sebagai sensor urea

Concentration (M)	-Log [urea] M	Potentiometer Voltage (V)
0,00001	5	0,254
0,0005	4,3	0,28
0,001	4	0,297
0,005	3,3	0,309
0,01	3	0,307
0,05	2,3	0,316

Optimization of Ise-urea in tris-HCl buffer according to FIG. 6 as follows urea concentration 0.00001 M tension 0.050 V, 0.0005 M tension 0.046 V, 0.001 M tension 0.047 V, 0.005 M tension 0.051 V, 0.01 M tension 0.047 V; 0.05 M of 0.050 V holds at 0.005 M urea with 0.051 V for tris-HCl buffer. The optimization of Ise-urea in phosphate KH2PO4 buffer according to figure 7 as follows: urea concentration 0.00001 M 0.254 V, 0.0005 M tension 0.280 V, 0.001 M 0.297 V, 0.005 M tension 0.309 V, 0.01 M 0.307 V, 0.05 M tension 0.316 V occurs in 0.005 M urea of 0.309 V for phosphat KH2PO4 buffer. On the basis of Figures 6 and 7 the best is Ise-ure in the phosphate buffer KH2PO4.

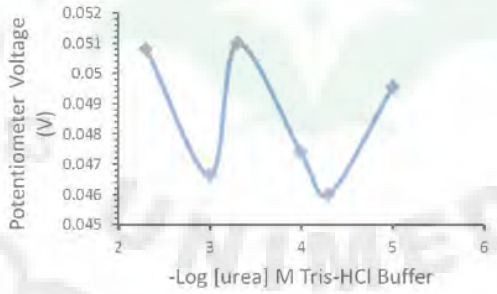

Figure 6. Optimization of ISE-urea in tris-HCl buffer as biosensor

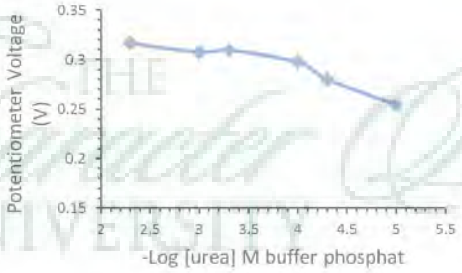

Figure 7. Optimization of ISE-urea in phosphaet buffer KH_2PO_4 as biosensor

Biosensor construction depends on modification of the electrode, immobilization enzyme, biosensor achievement (glucose, hydroperoxide, phenol, cholesterol, uric acid and urea) [26]. Tetrakis (4-chlorophenyl) borate (TpClPB) is a complex ion pair as an electroactive material [27], there are three electroactive materials namely tetraphenylborate, chlorine derivative, and dipicrylamine. The experimental study was immobilized PVA-enzyme urease coating PVC-plasticizers KTpClPB. The characterization of the potentiometric response of the ISE relies not only on ionophor but also the amount of additive used [28]. The analysis parameters of the electrode are the detection limit, the linear response range, the pH effect, the time response and the selectivity [29]. According to [29] two new pH effects, time response, urea linear response ranges from 1.10-5 - 5.10-3 M while the following selectivity has not been performed.

3.7 *Stability Test*

The properties of stability depend on the free activity and immobilization obtained in the buffer [30]. To improve long-term stability, various influencing factors include (1) lifetime of enzyme immobilization and membrane material; (2) immobilization or entrapment forces and (3) pollution and contamination of the sample [31]. Lifetime study of immobilization of enzyme and material membrane in 6 months starting from september 2016 until march 2017 on use of tris-HCl 0.001 M buffer pH 5.5 - pH 9 and phosphate buffer 0.001 M pH 5.5 - pH 9 used for 20 minutes each buffer , every 5 minutes were injected with urea 0.05 M, 0.01 M, 0.005 M, 0.001 M, 0.0005 M, and 0.0000 M obtained urea optimization 0.0005 M for phosphate buffer and 0.005 M for HCl trism buffer, the result 8 (a) the buffer tris-HCl pH 6 - pH 7.5.

The stability position can be seen in Fig. 9 (a) for phosphate buffers at pH 7.5 and Fig. 9 (b) for the tris-HCl buffer at pH 6.5, its appearance as a signal if the phosphate buffer is diluted closer to the flat line than the tris-HCl buffer. 9 (a) Tris-HCl buffer time response pH 6.5 and figure 5 (b) phosphate buffer pH 7.5 is more stable phosphate buffer than tris-HCl buffer.

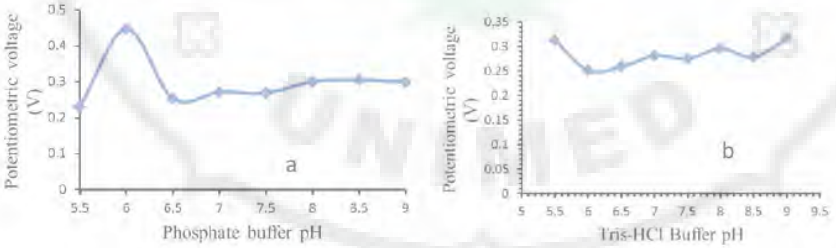

Figure 8. Stability of phosphate buffer pH 7 - 8.5 (a) and tris-HCl buffer pH 6 - 7.5 (b)

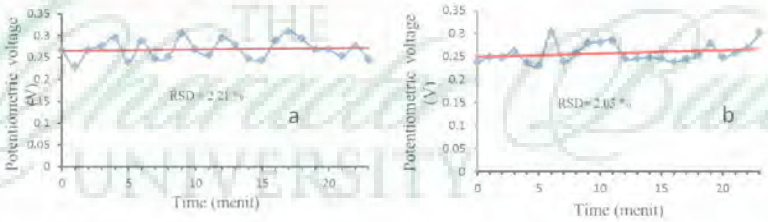

Figure 9. The position of phosphate buffer stability of pH 7.5 (a) and tris buffer HCl pH 6.5 (b)

The development stability of the sensor is tested by measuring the electrode potential as a function of time, by checking the detection limit and the calibration curve [29]. According to [29] the best pH 7.5

phosphat buffer stability is based on the time response and calibration curve of 0.0003 V / decade. Satellite 10 mM phosphate buffer pH 7.35 [33]. Each of the 9 (a) and (b) images having RSD is by consecutive 2.21% and 2.03% smaller by [33] ie 5.06%.

3.8. The selectivity of urea sensors from Ise-urea in Potentiometric systems

To find out the selectivity of electrochemical biosensors from ISE-urea in potentiometric system for urea determination have been inferential study using standard standard solution, based on stability test with urea optimization 0.0005 M for phosphate buffer and 0.005 M for buffer of HCl trisma, then selectivity test using pure urea 0.0005 M in buffer phosphat and 0.005 M for buffer of HCl trisma.

The biosensor response to the suspected intruder compounds present in the sample to be studied. To the potentiometric system was injected consecutively 50 µL standard solution of 1 mM urea which was incubated and followed by injection of 1 mM disrupting compound separately, then mixed inferent with standard urea (1 mM inferent + 1 mM urea) then incubated and analyzed using system potentiometric, potential measurement results according to [16] can be seen in Table 4 for the tris-HCl buffer and table 5 for the phosphate buffer KH2PO4.

[34] Biosensors are an analytical tool assimilated amalgam from biological elements (bioreceptor). Bioreceptors are a type of molecule that utilizes a biochemical mechanism for recognition. [15] to create CP-based (Conductive polymers = CP) used in selective chemical sensitivity sensitivity requires polymerizable and stable selective monomers under polymerization conditions. Electron transfer across the polymer phase of the solution affects the potentiometric response of the analyte in redox-active. In regard to potentiometry, the film acts as a cation or anion charge exchange and provides an anionic or cationic potentiometric response. Selective potentiometric responses are shown in Tables 4 and 5.

Table 4. The urea response to the 1 mM disrupting compound in the presence of tris-HCl buffer 0.001 M pH 6.5 compound as a bully. The number in the table is the difference between the electrode potential of the bottom electrode potential of the buffer solution.

Inference	Measurement (ΔE, V)		Difference
	Pure State	Mixed	
Urea	0,068	0,068	0
Kolesterol	0,19	0,048	0,142
Glukosa	0,335	0,085	0,25
Protein	0,578	0,543	0,035

Tabel 5. Respon urea terhadap senyawa pengganggu 1 mM dengan kehadiran*phosphat KH_2PO_4 buffer* 0.001 M pH 7.5 senyawa pengganggu sebagai pengganggu. Angka dalam tabel adalah selisih antara potensial elektroda terhadap potensial elektroda dasar larutan buffer.

Inference	Measurement (ΔE, V)		Difference
	Pure State	Mixed	
Urea	0,195	0,195	0
Kolesterol	0,19	0,265	-0,075
Glukosa	0,335	0,047	0,288
Protein	0,578	0,53	0,048

In the selectivity method it is defined as the ratio of activity (concentration) of the major ions and the interfering ion to give the same potential change in the reference solution. The potentiometric selectivity (log K) in the electrolyte solution of the interfering compound is determined by the difference in voltage in the electrolyte by the interfering electrolyte of the interfering compound [16]. Research experiments have been conducted as in table 4 and 5 there are different stresses for compounds disrupting cholesterol, glucose and proteins in urea. The selectivity of potentiometry was

determined by the membrane composition with variation of the plasticizer or lipophilic salt additive [35, the experimental study did not vary the plasticisation. The normal concentration of intruder substances in human serum 1 × 10-2 M [36], serum experiments have not yet been performed, but the urea molarity used already meets human serum concentrations of 0.001 M. Urea in serum 1.3-3.5 mM (8- 20 mg / dL) [17].

3.9. Urea Calibration Curve (sensitivity)

Based on the results of research to obtain a linear sensor and sensitivity of 19.069 mV / decade in the range from 1.10-5 -5.10-4 M with a detection limit of 1.10-5 shown in Figure 10, the 19.7 mv / decade PVA electrode membrane membrane with a range of 5.10- 7-10-2M by [37]. The sensitivity values vary depending on the range and limit of detection [38] and [39].

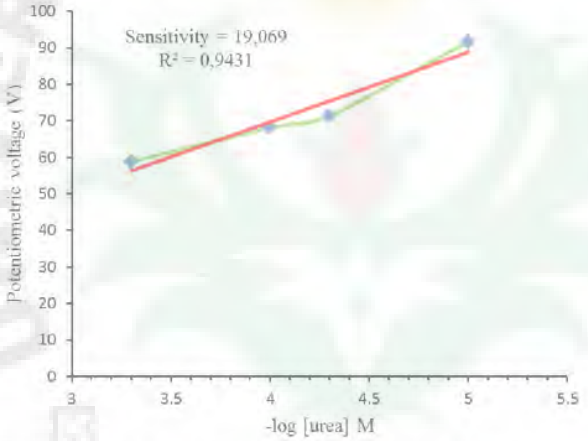

Figure 10. Calibration of urea sensor from Ise-urea with potentiometric system

The composition and slope of the calibration curve are influenced by plasticizers, the influence of dielectric constant and charge exchange mobility determines the electrode characteristic [40]. In this research, there is no variation of plastisier composition and dielectric tetpan variation in order to obtain the best calibration curve. The concentration and sensitivity range depends on polarity enzyme concentrations and buffer pH [12], the experimental studies have not yet conducted variations in enzyme concentrations. Electrode coating PVC-NPOE sensitivity 57 mV / decade, PVC-TCP-HDTMA + PS- sensitivity 58.59 mV / decade for titration potentiometry [41], experiment of this study PVA-coating electrode PVC-plastisisier 19,069 mV / decade in the range from 1.10 -5 -5.10-4 M with detection limit of 1.10-5 with R2 = 94.31% using phosphate buffer 0.001 M KCl 0.001 M.

4. Conclusion

After optimization process there was interference KCl 0.005 M in optimum condition to obtain urea sensor calibration of ISE urea with reference electrode and electrode indicator of immobilized PVA-enzyme / PVC-KTpClPB in phosphate buffer solution 0.001 M pH 7.5 KCl 0.001 M. Additionally influence, signal response reproducibility, fast response time, stability, selectivity, sensitivity and linearity response (calibration curve).

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