

Development of Chlorpyrifos Sensor Using Molecularly Imprinted Polymer (MIP) Polyvinyl Alcohol (PVA)-Fe₃O₄ as Receptor

Affiah Muhimmatul Mustaghfiroh, Ani Mulyasuryani* and Ulfa Andayani

Chemistry Department, Faculty of Mathematics and Natural Sciences, University of Brawijaya

*Corresponding email: mulyasuryani@ub.ac.id

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ABSTRACT

Development of a chemical sensor to detect chlorpyrifos has been carried out using a molecularly imprinted polymer (MIP) polyvinyl alcohol (PVA)-Fe₃O₄ as a membrane receptor. The MIP-Fe₃O₄ receptor is composed of polyvinyl alcohol (PVA) polymer, glutaraldehyde crosslinking reagent, citric acid catalyst, chlorpyrifos template, and Fe₃O₄. The MIP-Fe₃O₄ receptor is coated on the working surface of the screen-printed carbon electrode (SPCE) with a size of 1.5 x 3 mm². In this study, the effect of adding concentrations of chlorpyrifos and citric acid into membrane receptor was studied. The chlorpyrifos concentrations applied were 0.05, 0.1, 0.5 and 1% (w/w) and the concentrations of citric acid were 9.2, 16.8 and 23.3% (w/w). Sensor performance is also influenced by pH and type of electrolyte. The best sensitivity of the sensor is produced in the concentration range of 10⁻¹³ - 10⁻⁶ M at 24 mV/decade with a response time of 150 seconds.

Keyword: chemical sensor, chlorpyrifos, molecularly imprinted polymer, Fe₃O₄, screen-printed carbon electrode

INTRODUCTION

A chemical sensor generally consists of receptor, transducer, and detector. The receptor serves to selectively respond to the analytes. Some receptors that have been developed in chemical sensors are selective membranes, enzymes, antibodies, nucleic acids, and tissues [1]. The receptor used is generally the selective membrane. The selective membrane consists of glass membrane, liquid membrane, gas membrane, solid membrane and coated membrane [2]. The selective membrane contains active ingredients equal to the analytes, thus, it can selectively respond to analytes [3]. One of the selective membranes that can be developed is molecularly imprinted polymer (MIP). MIP as a receptor in chemical sensors has been used to detect clenbuterol [4], hydroxyzine [5], cocaine [6], 2-aminopyridine [7], and melamine [8]. In this study, the MIP membrane has been developed as a receptor to detect chlorpyrifos.

Chlorpyrifos is an organophosphate insecticide commonly used in agriculture [9]. The maximum residual limit for chlorpyrifos is 0.5 mg/kg in celery [10]. Acute exposure of chlorpyrifos in the body can inhibit the performance of acetylcholinesterase enzymes and cause various diseases such as headache, shortness of breath, vomiting, and diarrhea [9]. Chemical sensors based-MIP to detect chlorpyrifos have been developed. MIP is generally composed of the template molecule, functional monomer and crosslinking reagent [11]. In

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2017, Xu et.al. [12] have developed a voltammetry sensor based-MIP to detect chlorpyrifos. In this study, MIP was composed of chlorpyrifos as template, methacrylate acid as functional monomer, ethylene glycol dimethacrylate (EGDMA) as the crosslinking reagent and 2,2-azobisisobutyronitrile (AIBN) as initiator [12]. Previously, Li et al. [13] had developed a potentiometric method based-MIP to detect chlorpyrifos. In the study, MIP was composed of chlorpyrifos as template, methacrylate acid as functional monomer, ethylene glycol dimethacrylate (EGDMA) as the crosslinking reagent and 2,2-azobisisobutyronitrile (AIBN) as initiator [13]. However, the use of functional monomer in the formation of MIP requires volatile solvent, therefore, the use of functional monomer can be replaced with a polymer. In addition, the use of the polymer in the development of MIP can eliminate the polymerization process, as a result, molecular printing becomes simpler and faster. Polymer used in the development of MIP is polyvinyl alcohol (PVA) since PVA is biodegradable, biocompatible, and polar [14]. In this study, MIP has been developed as a receptor using PVA polymer, glutaraldehyde crosslinking reagent, citric acid catalyst, and chlorpyrifos template.

Chemical sensors based-MIP have been widely modified by adding supporting materials, such as Fe_3O_4 . Fe_3O_4 is superparamagnetic, conductive and pseudo-capacitive material [15]. Modifications of MIP with Fe_3O_4 have been developed to detect gemifloxacin mesylate [16], glyphosate [17], and hydrochlorothiazide [18]. Addition of Fe_3O_4 increases the conductivity of MIP [19]. Increased conductivity facilitates electrical interactions between MIP and transducer, thus, sensor sensitivity increases [11]. The transducer that can be used is a screen printed carbon electrode (SPCE). The main advantages of SPCE transducer are simplicity, versatility, low cost, portability, ease of operation, small size, and mass production capabilities [20]. The SPCE transducer consists of a working electrode and a reference electrode Ag/AgCl [1]. In this study, the MIP PVA- Fe_3O_4 receptor is coated on the working surface of the SPCE to produce signals, the scheme of this arrangement is shown in Figure 1.

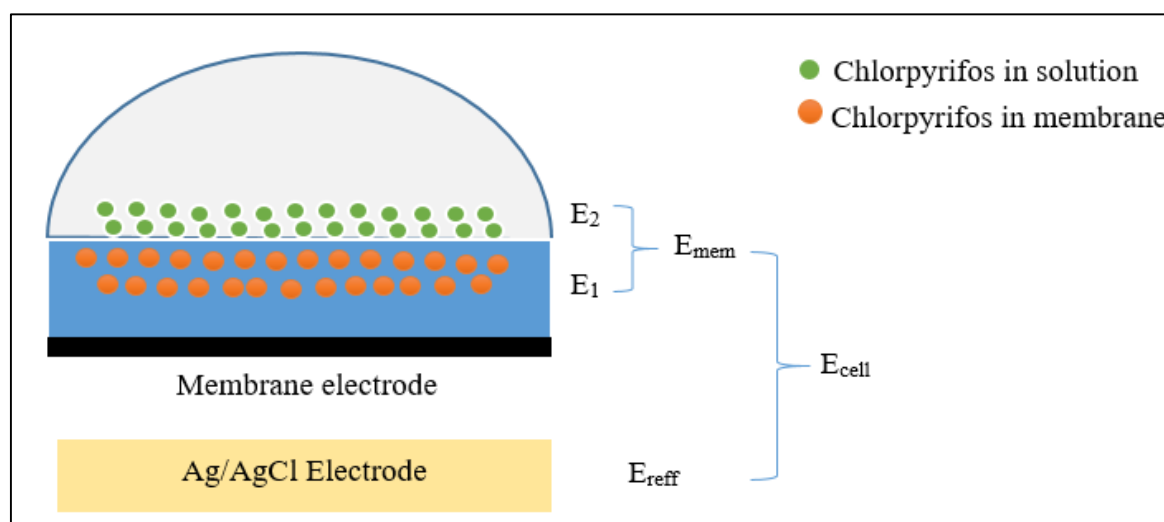


Figure 1. The mechanism of signal formation on chlorpyrifos sensor.

The cell potential as a signal resulted from the potential difference between membrane potential difference and reference potential Ag/AgCl (E_{ref}). Membrane potential difference (E_{mem}) resulted from the difference between the inner membrane potential (E_1) and the outer membrane potential (E_2). Based on Figure 1, the amount of chlorpyrifos in the membrane plays an important role in increasing sensor sensitivity. However, excessive chlorpyrifos in

the membrane produces a non-uniform membrane and causes decreasing sensor sensitivity [4]. The amount of chlorpyrifos printed in the membrane is also influenced by the citric acid catalyst. The excessive citric acid in the membrane can act as a crosslinking reagent. The excessive crosslinking reagent in the membrane can increase the rigidity of the membrane [21]. In addition, chlorpyrifos in the solution is also influenced by the pH of the solution and the electrolyte environment. Therefore, in this study, the effect of chlorpyrifos and citric acid concentrations on the membrane on sensor sensitivity and the effect of pH 3-6 and the electrolyte environment on the signal are investigated.

EXPERIMENT

Chemicals and instrumentations

The materials used in this study include chlorpyrifos (200 EC Dursban, DOW), water (Hydrobath aqua demineralization), polyvinyl alcohol, citric acid (CV. Kridatama, Malang, Indonesia), ammonium sulfate (Sigma-Aldrich), iron sulfate tetra-hydrate (Sigma-Aldrich), sodium hydroxide (Merck), iron trichloride hexa-hydrate (Sigma-Aldrich), ethanol (Merck), glutaraldehyde 50% (Sigma Aldrich), potassium dihydrogen phosphate trihydrate (Merck), (Merck), potassium chloride (Merck).

The equipment used in this study include Screen-Printed Carbon Electrode (SPCE) BI 1302 (Quasense Inc.) consisting of carbon as working electrode ($1.5 \times 3 \text{ mm}^2$) and Ag/AgCl as reference electrode, oven (Memmert), micropipette (Accumax Pro), glassware, analytical balance (AL204 Mettler Toledo), magnetic stirrer, potentiometer SANWA CD800a, and connector (Quasense Inc.).

Procedure

The MIP PVA-Fe₃O₄ receptor was made by optimizing the concentration of chlorpyrifos and citric acid in the membrane. Optimization of chlorpyrifos concentration was carried out by adding 0.6% chlorpyrifos 4.6, 9.1, 46 and 91 μL in four different containers containing 5% PVA 0.9 mL, 0.5% suspension Fe₃O₄ 5.5 μL , 5% citric acid 100 μL and 4% glutaraldehyde 100 μL . Optimization of the concentration of citric acid was carried out by adding 100 μL of citric acid with concentrations of 5, 10 and 15% into three different containers containing 5% PVA 0.9 mL, 0.5% suspension Fe₃O₄ 5.5 μL , 0.6% chlorpyrifos 9.1 μL and 4% glutaraldehyde 100 μL . The MIP PVA-Fe₃O₄ receptor was taken as much as 5 μL and coated on the surface of the working electrode from SCPE ($1.5 \times 3.0 \text{ mm}^2$). SPCE is dried in the oven for 1 hour at 50°C.

The effect of pH was studied by measuring the solution with concentrations of 10^{-13} - 10^{-6} M at pH 3-6. pH is adjusted to the required value by adding 0.1 M HCl solution. The characterization of chlorpyrifos sensor was carried out by preparing a MIP PVA-Fe₃O₄ receptor using the optimum concentrations of chlorpyrifos and citric acid. The solutions were measured at concentrations of 10^{-13} - 10^{-6} M in HCl pH 6, phosphate buffer pH 6, and phosphate buffer-KCl pH 6.

Signal measurement was carried out by connecting the SPCE with a potentiometer. The working electrode is connected to the positive pole and the reference electrode Ag/AgCl is connected to the negative pole. Signal measurement was carried out by dripping 50 μL of chlorpyrifos solution on the surface of the two electrodes. The signal was detected against Ag/AgCl. The signal was read every 10 seconds for 180 seconds. The sensitivity of the sensor can be known by making a graph between $-\log[\text{chlorpyrifos}]$ and signal. The graph results the equation $y = ax + b$. Slope (a) shows the sensitivity of the sensor.

RESULT AND DISCUSSION

Effect of chlorpyrifos concentration in membranes on the sensor sensitivity

In the development of chlorpyrifos sensor, chlorpyrifos in MIP membrane acts as an active ingredient that will selectively respond to analytes. When the chlorpyrifos solution is dropped on the surface of SPCE, the MIP membrane will be hydrated and chlorpyrifos in the solution will interact with the active ingredients. In this study, the effect of the addition of chlorpyrifos with concentrations of 0.05, 0.1, 0.5 and 1% (w/w) on the sensor sensitivity was studied.

The graph in Figure 2a shows the relationship between $-\log[\text{chlorpyrifos}]$ on the signal at four different chlorpyrifos concentrations. Based on the graph, the signal continues to increase when the concentration of the solution decreases. This indicates that the signal has a quantitative relationship with the concentration of chlorpyrifos in the solution. In addition, the addition of chlorpyrifos in the membrane with a concentration of 1% gives the greatest signal. Increased chlorpyrifos concentration in the membrane is directly proportional to the increased inner membrane potential (E_1). If the inner membrane potential (E_1) is greater than the outer membrane potential (E_2), membrane potential difference (E_{mem}) will increase. Thus, the resulted signal becomes larger. During signal measurement, low concentrations of solution produce a greater signal difference and vice versa. At low concentrations, all chlorpyrifos in the solution can interact with chlorpyrifos in the membrane. Meanwhile, not all chlorpyrifos in the solution can interact with chlorpyrifos in the membrane when using high concentration solutions, leading to the unmeasured signal. Hence, the use of high concentration solutions will produce a lower signal difference than low concentration solutions.

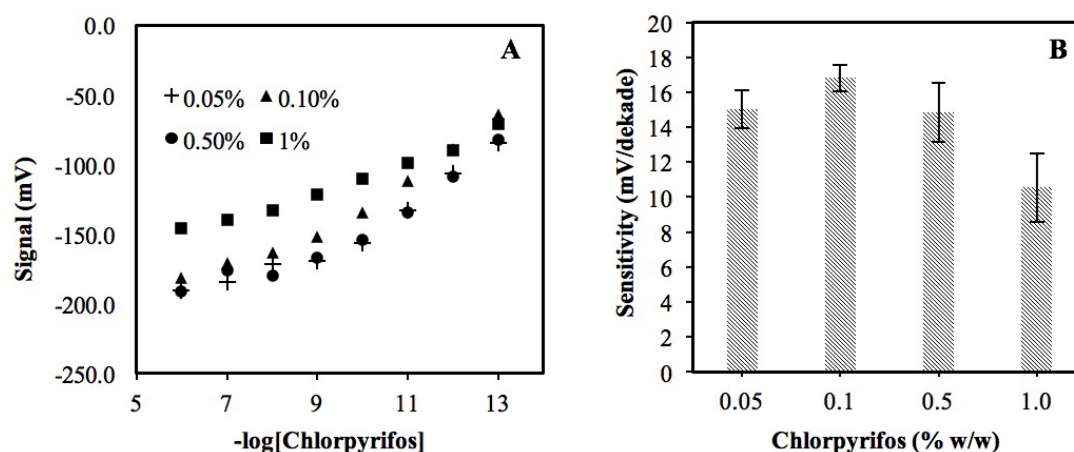


Figure 2. (a) The effect of the addition chlorpyrifos in membranes with concentrations of 0.05, 0.10, 0.50 and 1% (w/w); (b) the effect of chlorpyrifos concentrations on the sensor sensitivity.

The graph in Figure 2b shows the relationship between the addition of chlorpyrifos concentration in the membrane on the sensor sensitivity. Based on the graph, the addition of chlorpyrifos concentration up to 0.1% produces an increase in sensor sensitivity. However, the addition of chlorpyrifos at concentrations greater than 0.1% produces a decrease in sensor sensitivity. It is caused by excessive chlorpyrifos in the membrane. This is in accordance with the result of study Liang et.al. which states that the sensitivity of the sensor will increase with an increase in active ingredients. However, the addition of excess active ingredients will

reduce the sensitivity of the sensor. The excessive active ingredients produce the non-uniform membrane. The non-uniform membrane causes a decrease in sensor sensitivity [4]. In this study, the addition of chlorpyrifos which produced the highest sensitivity and lowest measurement error was at a concentration of 0.1% w/w with a sensitivity of 16.8 mV/decade.

Effect of citric acid concentration in membranes towards sensor sensitivity

In the development of the chlorpyrifos sensor, citric acid serves as a catalyst in the crosslinking process between glutaraldehyde and polyvinyl alcohol (PVA). The excessive concentration of citric acid in MIP can increase the rigidity of the MIP membrane. In this study, the effect of the addition of citric acid with a concentration of 9.2, 16.8 and 23.3% (w/w) towards sensor sensitivity was studied.

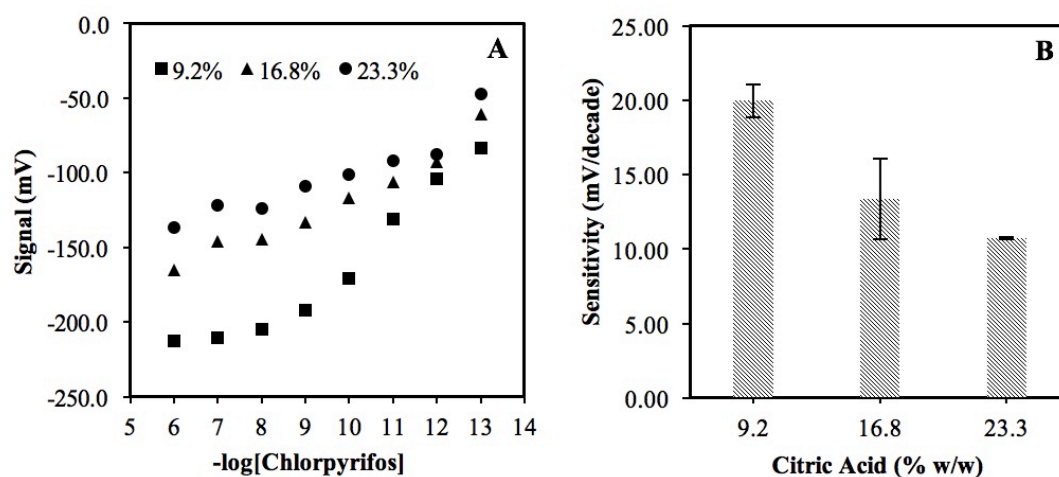


Figure 3 (a) The effect of the addition citric acid in membranes with concentrations of 9.2, 16.8 and 23.3% (w/w); (b) the effect of citric acid concentrations on the sensor sensitivity.

The graph in Figure 3a shows the relationship between $-\log$ [chlorpyrifos] on the signal at three different concentrations of citric acid. Based on the graph, the addition of citric acid with the concentration of 9.2-23.3% results in an increase in signal. If the concentration of citric acid is added excessively, the membrane will become more rigid. It causes chlorpyrifos in the solution difficult to interact with chlorpyrifos in the membrane and result in a decrease in signal. However, in this study, the signal increases when the concentration of citric acid in the membrane increases. This is caused by the interference of citric acid. Chlorpyrifos in the solution can also interact with citric acid and cause increased inner membrane potential (E_1), thus, produces a larger signal.

The graph in Figure 3b shows the relationship between the addition of citric acid concentration in the membrane on the sensor sensitivity. The increase in the concentration of citric acid is inversely proportional to the decrease in sensor sensitivity. It was caused by the increase in rigidity of the membrane. This is in accordance with the result of the study by Sridach et.al. which states that the addition of excess citric acid can increase the number of crosslinks in the membrane, thus, the membrane becomes more rigid [21]. Meanwhile, If the membrane becomes more rigid, chlorpyrifos in the solution will be difficult to interact with chlorpyrifos in the membrane, thus, the equilibrium is difficult to be reached. It causes a decrease in sensor sensitivity. In this study, the addition of citric acid which produces the

highest sensitivity and lower measurement error was at a concentration of 5% with a sensitivity of 19.8 mV/decade.

Effect of pH

In measuring signals using a chlorpyrifos sensor, the pH of the solution can affect the solubility of the solution. Chlorpyrifos is easily hydrolyzed in alkaline conditions. This shows that chlorpyrifos easily dissolves in alkaline conditions. In this study, the effects of pH towards the signal has been studied. The pHs studied were pH 3, 4, 5 and 6.

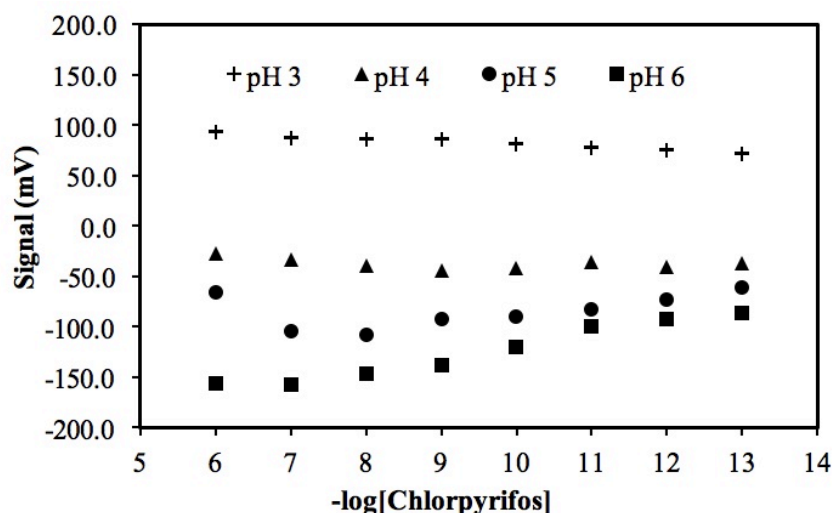


Figure 4. Effect of pH on the signal.

The graph in Figure 4 shows the relationship between $-\log$ [chlorpyrifos] on the signal at four different pHs. Based on the graph, the decrease in pH is inversely proportional to the increase in signal. Increased signal is caused by the solubility of chlorpyrifos in acidic pH. Chlorpyrifos is not easily hydrolyzed under acidic conditions. Therefore, chlorpyrifos activity in solution is low. The lower chlorpyrifos activity in solution causes a decrease in the outer membrane potential (E_2). If the outer membrane potential (E_2) decreases, the membrane potential difference will increase and result in an increase in signal. In this study, the highest chlorpyrifos activity was achieved at pH 6.

Effect of electrolyte

In the development of the chlorpyrifos sensor, Fe_3O_4 can attract the ions present in the solution, hence, this affects the signal formation. The solution was measured at a concentration of 10^{-13} - 10^{-6} M in pH 6 phosphate buffer, pH 6 phosphate buffer-KCl, and pH 6 HCl.

The graph in Figure 5a shows the relationship between $-\log$ [chlorpyrifos] on the signal in the solution conditioned with water and KCl 10^{-5} M. Based on Figure 5a, the resulted graphs in water and KCl 10^{-5} M are not much different. It shows that the addition of KCl does not affect the signal. The aim of adding KCl in the solution is to stabilize the reference electrode Ag/AgCl [22]. The graph in Figure 5b shows the relationship between $-\log$ [chlorpyrifos] on the signal in the solution conditioned with phosphate buffer pH 6, phosphate buffer-KCl pH 6, and HCl pH 6. Based on this graph, the signal in HCl pH 6 produces the strongest signal. The ionic strengths order is HCl pH 6 < phosphate buffer pH 6 < phosphate buffer-KCl pH 6.

If the ionic strength is low, less number of ions attracted by Fe_3O_4 at the surface of the receptor, and as a result, produce low surface capacity. The lower surface capacity will produce a larger membrane potential difference (E_{mem}) and leads to stronger signal detection. Based on Figure 5b, the resulted graphs in phosphate buffer pH 6 and phosphate-KCl buffer pH 6 are not much different. This is because their ionic strengths are not significantly different, 4×10^{-4} M in phosphate buffer pH 6, and 4.1×10^{-4} M in phosphate buffer -KCl pH 6.

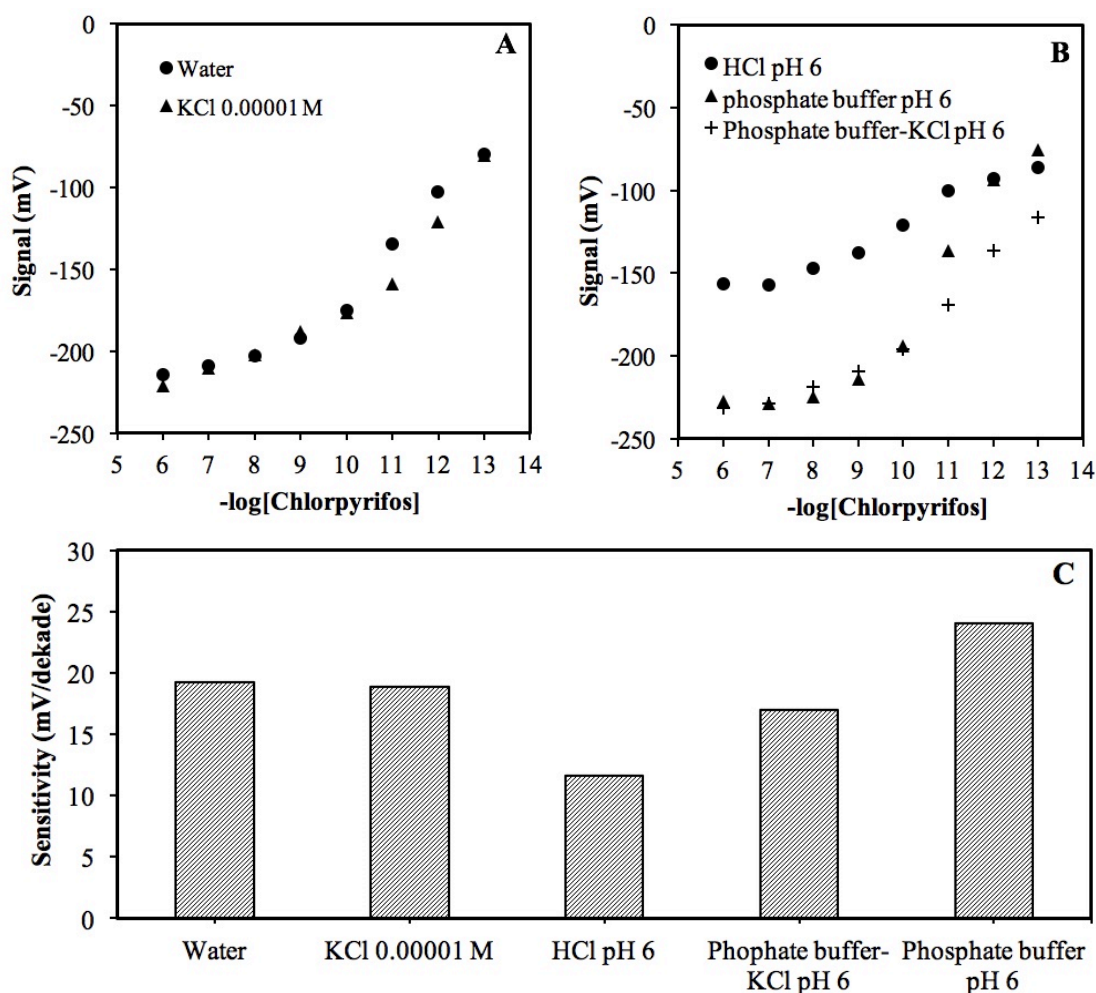


Figure 5 (a) The effect of electrolytes on the signal in the solution in water and KCl 0,00001 M; (b) the effect of electrolytes on the signal in the solution in HCl pH 6, phosphate buffer pH 6 and phosphate buffer-KCl pH 6; (c) sensor sensitivity in various solutions.

The graph in Figure 5c shows the relationship between the solution in various conditions on sensor sensitivity. Based on the graph, the sensitivities of the sensor in water and KCl 10^{-5} M are not different. This is because the ionic strength in KCl 10^{-5} M does not affect the chlorpyrifos activity in solution. If chlorpyrifos activity in KCl 10^{-5} M is the same as in water, the interactions of chlorpyrifos in solution with chlorpyrifos in the membrane will be the same. And the sensitivities of the sensor produced are not different. The sensor sensitivity order is HCl pH 6 < phosphate buffer-KCl pH 6 < phosphate buffer pH 6. Measurement in HCl pH 6 produces the lowest sensor sensitivity. This was caused by the low ionic strength of

HCl pH 6, thus, the attraction of Fe_3O_4 to ions will decrease and result in a decrease in conductivity. The decrease in conductivity is directly proportional to the decrease in sensor sensitivity. The sensor sensitivity of phosphate buffer-KCl pH 6 is lower than phosphate buffer pH 6 and greater than HCl pH 6. This is due to the higher ionic strength of buffer-KCl pH 6. The higher ionic strength of buffer-KCl pH 6 causes an increase in attraction of Fe_3O_4 to ions and form an electrical double layer. Electrical double layer causes chlorpyrifos in solution difficult to interact with an active ingredient, thus, the sensor sensitivity decreases. The best sensor sensitivity was produced in phosphate buffer pH 6 with the sensitivity value of 24 mV/decade. This sensor can detect chlorpyrifos up to 1×10^{-6} nmol L^{-1} in celery. The result is lower than in the result of the study by Abdallah et. al. which states that their potentiometric method can detect chlorpyrifos up to 0.027 nmol L^{-1} [16].

CONCLUSION

The sensitivity of the chemical sensor to detect chlorpyrifos using MIP PVA- Fe_3O_4 receptor is influenced by the concentration of chlorpyrifos and citric acid. The best sensitivity is produced at chlorpyrifos concentration of 0.1 %(w/w) and 9.2 %(w/w) citric acid concentration. The sensor's signal is also influenced by pH and electrolyte in the solution. The sensitivity of the chlorpyrifos sensor is 24 mV/decade in phosphate buffer pH 6 with the concentration range of 10^{-6} - 10^{-13} M and response time of 150 seconds.

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