Magnetite Nanospheres as Carbon Paste Electrode Modifier for Xanthine Biosensor

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Abstract. Magnetite Nano spheres was prepared and used to fabricate a modified carbon paste electrode as xanthine biosensor and applied to determine inhibition kinetics of Syzygium polyanthum extract toward xanthine oxidase (XO). The magnetite Nano spheres was characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD). Electrochemical behaviour of xanthine was investigated by immobilizing the XO on the surface of carbon paste electrode (CPE), modified magnetite Nano spheres-CPE (MCPE), and modified 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q₀)-CPE (QCPE) employing cyclic voltammetry (CV) technique. The result showed that the MCPE was the best electrode to determine the analytical performance under optimum condition, based on lower limit of detection (0.005 mM), wider linearity range (0.01-1 mM with R²= 99.24%), and higher sensitivity (5.16 µM⁻¹) than the two other electrodes (CPE and QCPE). The MCPE successfully improve the analytical performance on xanthine biosensor and to be applied for determining inhibition kinetics of S. polyanthum extract. Inhibition kinetics of the extract has caused increase of K_M and V_MAX (I_MAX) constant. Based on the result, the type of inhibition kinetics was a competitive inhibition.

1. Introduction

Xanthine is metabolic precursors of uric acid (UA; 2,6,8-trihydroxypurine) [1]–[3]. The concentration of xanthine in the blood and urine can be used as an indicator in clinical diagnosis to xanthinuria (a genetic disorder of the metabolism of xanthine), renal failure, hyperuricemia and gout [4], [5]. Hyperuricemia and gout can lead to other diseases such as kidney stones [6], hypertension [7], and cardiovascular damage [7], [8]. Therefore, xanthine determination is important in clinical diagnosis including for early detection of gout disease. A commonly used to determine of xanthine concentration is spectroscopy method; however, this method is expensive, less specific, very sensitive to light and affected by turbidity [9], [10]. Therefore, alternative method should be find to determine the type of inhibition kinetics quickly, accurately, low cost, and simple such as electrochemical method [11]. One of very simple technique is voltammetry using three conventional electrodes such as working electrode, auxiliary electrode, and a reference electrode. Carbon paste electrode (CPE) is one of the working electrodes that are very simple, low cost, easy to prepare, and the materials are easily obtained.
Xanthine biosensor have been developed extensively to measure the concentration of xanthine which shows the concentration of uric acid with xanthine basic principles of the biosensor in accordance with Eq. 1-3 [2]. However, xanthine biosensor performance must be improved to achieve better activity and sensitivity to be widely applied. The sensitivity of the electrochemical biosensor is determined by its ability to create micro space for biomolecules or analyte to exchange electrons directly with the electrode. Nano-sized materials are the necessary ingredients to create the micro space, due to the high surface area and better dispersion which is useful as a modifier of the working electrode in the biosensor. One interesting Nano-sized material is Nano magnetite (Fe₃O₄), because of its special characteristics such as good biocompatibility, easy preparation, low toxicity, and super paramagnetic property [12], [13].

\[
\text{Xanthine} + \text{O}_2 + \text{H}_2\text{O} \xrightleftharpoons{XOD} \text{Uric acid} + \text{H}_2\text{O}_2 \tag{1}
\]

\[
\nu vs(Ag) \quad \text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2e^- \tag{2}
\]

\[
2e^- \rightarrow \text{Working electrode} \tag{3}
\]

One application of biosensors that should be developed is in the determination of inhibition kinetics by natural products. Iswantini et al. [11] reported that this method is successfully used to determine the inhibition kinetics of xanthine oxidase by *Sida rhombifolia* extract on uric acid biosensor. Thus, it is hope that this modifier will be able to improve the analytical performance of the xanthine biosensor by using carbon paste electrode. One of herbs that are traditionally used by Indonesian community to treat uric acid disorder is bay leaf (*Syzygium polyanthum*). This medicinal plant is commonly used as a spice in cooking as well as for treating obesity [14], bacterial and skin diseases [15], ulcers, hypertension, inflammatory, and hangover, including uric acid or gout [16], [17]. As for gout medication, allopurinol is commonly administered to reduce level of uric acid through XO inhibition. On the other hand, allopurinol tends to cause adverse side effects, such as allergies, fever, chills, leukopenia, kidney and liver failure, indigestion, headache, hair loss, even death due to vascular diffusion. The many side effects of synthetic drugs such as allopurinol encourage the community to turn to traditional medicine by utilizing herbs. Natural compounds to be used as drug candidate must be studied for their inhibition kinetics to look at the mechanism of inhibition that occurs.

This study aims to improve the analytical performance (sensitivity, linearity, and limit of detection) in xanthine biosensor using magnetite Nano spheres as a modifier of carbon paste electrode and apply this biosensor for determining the type of inhibition kinetics of xanthine oxidase by water extract of bay leaf.

2. Materials and Methods

2.1. Reagents and materials

Xanthine oxidase (XO) from bovine milk (specific activity of > 7 units/mg solid), xanthine, and glutaraldehyde (GA) were purchased from Sigma-Aldrich, (USA); ferric chloride (FeCl₃.6H₂O), sodium citrate, urea, graphite powder, paraffin oil, ethanol, 2,3-dimethoxy-5-metil-1,4-benzoquinone (Q0), bovine serum albumin (BSA), NaH₂PO₄ and Na₂HPO₄ purchased from Merck (Germany); bay leaves from plantation in Biopharmaca Research Center, Bogor Agricultural University (Indonesia).

2.2. Apparatus and measurement

The electrochemical measurements have been conducted by the potentiostat equipped with software Echem v2.1.0 (eDAQ) with a conventional three-electrode, which has been using the Ag/AgCl electrode as the reference electrode, a platinum electrode as auxiliary electrode, and carbon paste
electrode as the working electrode. Atomic absorption spectroscopy (AAS) (Shimadzu AA – 6300), X-ray diffraction (XRD) (Shimadzu 7000) and scanning electron microscopy (SEM) from Zeiss.

2.3. Synthesis of magnetite Nano spheres
Magnetite Nano spheres was synthesized by hydrothermal method in a Teflon-lined 250 mL autoclave for 12 h at 200 °C, referring to Saprudin et al. [18]. The synthesized powder was characterized by XRD and SEM, while the iron content of the synthesized filtrate was measured using AAS.

2.4. Preparation of working electrode
In this study, three types of working electrode were prepared, namely: carbon paste electrode without modification (CPE), carbon paste electrode modified Q₀ (QCPE), and carbon paste electrode modified magnetite Nano spheres with various concentrations (5%, 10%, 15% w/w). The working electrode was prepared following the procedure of Saprudin et al. [18] by hand-mixing the graphite powder and then adding paraffin oil until homogenous. The CPE was prepared from graphite (100 mg) and paraffin oil (35μL), the QCPE was prepared from graphite (90%), Q₀ (10%) and paraffin oil (35 mL), and the composition of graphite powder was decrease by increasing Nano spheres magnetite (5%, 10%, 15%) with fixed paraffin oil composition (MCPE). All types of working electrode were characterized with a solution of KCl and 5 mM K₃[Fe(CN)₆] probe at scan rate of 100mV/s.

XO was immobilized onto the surface of the working electrode after the cross-linking with BSA and GA. XO 25 U/mL (15 µL), BSA 10% (w/v) (5 µL), and glutaraldehyde 5% (v/v) (5 µL) was added and homogeneously mixed [19]. Then, 10 µL XO-GA-BSA solution was dripped onto the surface of the working electrode, dried at room temperature (27°C), covered with a dialysis membrane, and fastened using nylon tissue. Finally, XO-GA-BSA-working electrode was washed using phosphate buffer of optimum pH. When the electrodes were not in use, they were kept in a phosphate buffer at 4°C.

2.5. Preparation of Syzygium polyanthum extract
The S. polyanthum extraction refers to the Indonesian Herbal Pharmacopoeia Agency/FHI [20] by maceration with ethanol and water solvent. The filtrate was dried using a rotary evaporator and freeze dried to obtain dry S. polyanthum crude extract, and stored in a refrigerator.

2.6. Electrochemical measurement and optimization of xanthine oxidase activity
Mode of electrochemical measurements was as follow: potential range: -500 up to 1200 mV at a rate of 100mV/s with the cyclic voltammetry technique. Optimization was performed at temperature of 20-30°C, pH 6-9, xanthine concentration 0.1-1.0 mM, and concentration of magnetite Nano spheres 5-15%. Response surface method (RSM) was used for optimization of XO activity [21]. Electrode performance was evaluated for the three types of electrode at the optimum condition. Analytical performance such as electrode sensitivity, linearity, and limit of detection were determined following Devi et al. [2] procedure.

2.7. Application

2.7.1. Measurement of enzyme activity test (inhibition of XO power and determination of IC₅₀).
Inhibition power test of S. polyanthum crude extract toward the XO was done in optimum conditions previously acquired in accordance with the procedure of electrochemical measurements. However, S. polyanthum extract in various concentrations was added to the electrochemical cell before the addition of xanthine [11]. Allopurinol was used as a positive control, and spectrophotometry method was also used as a comparison.
2.7.2. Inhibition kinetics test of bay leaf extract toward XO. The procedure of inhibition kinetics test is similar with the determination of inhibition, except that it used substrate (xanthine) at concentration from 0.01 to 1.00 mM. Furthermore, bay leaf extract (selected concentration) was added in order to obtain the kinetics of XO inhibition [11].

3. Results and Discussion

3.1. Nano spheres magnetite characteristics

The black powder and clear yellow filtrate are indicate that the formation of magnetite is successful [22]. AAS analysis was performed to determine the residual Fe ion concentration in the filtrate, and it was found only 0.01%, meaning that 99.99% of Fe ions have been converted into the product. Diffractogram of the synthesized black powder shows the appearance of diffraction peaks that are consistent with JCPDS standard magnetite No: 19-0629 in the range of 10-80 degrees 2θ (Figure 1). The highest peaks of the spectrum correspond to the hkl (311) and (440) reflections of magnetite at 2θ angle, which are 35.52° and 62.66°, respectively. The medium peak at 30.16°, 43.25°, and 57.03°, correspond to (220), (400), and (333) crystallographic planes of face-centered cubic magnetite crystals, respectively (FCC) [24]. The magnetite crystallinity is 76.2%, and the average size of the individual or the domain diameter (D) of the Nano spheres magnetite is 38.21 nm according to the Debye-Scherer equation [23]. It concludes that the powder is absolutely the synthesized magnetite.

![Figure 1. XRD Diffractogram of magnetite Nano spheres (blue) and magnetite standard (JCPDS) No. 19-0629 (red).](image)

The magnetite was synthesized using hydrothermal method as this method has been known for synthesizing Nano spheres magnetite at high temperature (200°C) and autogenously pressure [24]. Cheng et al. [22] reported a synthesis of Nano magnetite with hydrothermal method using four chemical substances, namely ferric chloride hexahydrate, sodium citrate, polyacrylamide, and urea. However, polyacrylamide is an expensive substance. Saprudin et al. [18] were successful prepare the similar product without using polyacrylamide. The perfect Nano magnetite was formed in 12 hours at 200°C heating [18].

Figure 2 shows the synthesized Nano spheres magnetite has spherical shape, indicating the effect of magnetization [22]. Upon 1000× magnification (Figure 2a), the particles are uniformity in shape. In addition, upon 12500× magnification (Figure 2b), one set of spherical from primary Fe₃O₄ crystals
aggregates to form spherolite as a consequence of attractive force of the magnetite particles with the magnetic force to form magnetite Nano spheres accordance with Kumari et al. [25]. The average particle diameter of magnetite spheres was 121.5 nm size and 38.21 nm crystal size with “magnetite Nano spheres” particle.

Figure 2. Morphology of synthesized magnetite powder: (a). magnification of 3.000×; (b) magnification of 12.500×.

SEM characterization is in line with Cheng et al. [22] report regarding the uniformity of the spherical magnetite. This study shows that even without polyacrylamide, the synthesized Nano magnetite has almost similar size and shape. However, aggregation amongst the spheres is observed. Liang et al. [26] also reported Nano magnetite having long shape such as rods, rounded shape with agglomeration granules, and round with the uniform particle. Based on the observations on the shape, color of powder, and filtrate, through AAS, XRD, and SEM analysis, the synthesis of magnetite Nano spheres have been successfully carried out.

3.2. Characteristics of the modified electrodes
Three type of electrodes: unmodified electrode (CPE), electrode with mediator Q₀ (QCPE), and modified electrode with magnetite Nano spheres (MCPE) were able to detect the current from KCl electrolyte solution (no peak) and were able to detect the current of K₃[Fe(CN)₆] electrolyte solution (existence of a peak at approximately 0.5 V). This phenomenon indicates that the electrodes are good to be applied in analyte measurement. The average of oxidation and reduction or anodic and catodic peak of K₃[Fe(CN)₆] by MCPE gives 1.52× and 3.27× higher measurement than that of the QCPE and CPE, respectively. The best electrode gives Ipa/Ipc (anodic peak current/catodic peak current) value was approaching 1. Base on the Table 1, can be seen anodic peak on the QCPE and CPE much higher than catodic peak, this is because the existence of a layer on the surface of the electrode prevents electron transfer. However, on the MCPE was gives the anodic and catodic peak almost the same (Ipa/Ipc approaching 1). It indicates that the magnetite as a modifier to carbon paste electrodes is able to accelerate electron transfer process and that the electro active surface area is greater than that of the QCPE and CPE [27].
Table 1. The value of the potential (E) and the current (I) changes to catodic and anodic of $K_3[Fe(CN)_6]$.

| Potential and Current | Type of the electrode |
|-----------------------|-----------------------|
| Epa (Volt)            | CPE                   |
| Epc (Volt)            | QCPE                  |
| ∆E (Volt)             | MCPE                  |
| Ipa (µA)              | CPE                   |
| Ipc (µA)              | QCPE                  |
| Ipa/Ipc(µA)           | MCPE                  |

3.3. Electrochemical properties and the optimum xanthine oxidase activity

All types of working electrode are able to measure either uric acid or H$_2$O$_2$ as the result of oxidation of the xanthine (Eq. 1). Xanthine oxidase activity was optimized using response surface method (RSM). The result from Minitab contour output (data not shown) were 10% magnetite concentration, 1mM xanthine concentration, pH 7.5, and 20ºC. The success of immobilization is marked by the detection of current caused from the process between xanthine as the analyte and xanthine oxidase enzyme immobilized on the electrode surface. In this study, there is a peak in 0.5-0.7 V [28], [29] as compared with the buffer as blank. A possible scheme of reaction during the immobilization in working electrode is shown in (Figure 3). Cross-linking is the best method which provides very strong immobilization of XO to stabilize XO adsorption, and preventing its leakage compared with the other methods (physical adsorption, entrapment, covalent coupling, and electro polymerization) [3]. Glutaraldehyde is used as bifunctional reagent which forms covalent bonds with bovine serum albumin (BSA) and with XO molecules. A possible chemical reaction is that the magnetite Nano spheres is adsorbed by the paste carbon electrode and interacted with BSA then bonded with GA, and finally formed covalent bonds with the NH$_2$ group by the XO enzyme (Figure 3).
Electrochemical measurement tested on the optimum condition of the all type working electrode were performed. The optimum condition obtained for best enzyme activity was on the pH 7, 10% magnetite concentration, 1 mM xanthine concentration and at 20°C. The optimum condition was then used for the next analyte measurement condition. The optimum temperature has been reported by Iswantini and Darusman [30] under different pH 7.5 and 0.7 mM xanthine concentration. The same xanthine concentration is also reported by Iswantini et al. [11] under pH 7.5 and 30 °C. Cengiz et al. [1] reported the optimum condition of xanthine oxidase activity under pH 9 and 37 °C. The difference of xanthine oxidase optimum activity can be caused by the difference of working environment conditions and the employed method.

Figure (4b) shows the comparison of oxidation peak current produced from the three types of modified magnetite electrode using various concentrations; 5%, 10%, and 15%. The performance in detecting the current is MCPE 10% > MCPE 15% > MCPE 5%. Therefore, the MCPE 10% was used for further experiment. This was in accordance with the optimum result of RSM. The chosen parameters: MCPE then was compared to the modified electrode Q0 (QCPE) and the electrode without modification (CPE) (Figure 4a).

![Figure 4. Cyclic voltammogram of xanthine measurement on the optimum condition.](image)

3.4. Electrode Performance
The highest the xanthine concentration, the higher the current produced; and obtained high linear regression (99.24%) with the wider range than CPE and QCPE. Figure 5 shows linearity and sensitivity of the three working electrodes, while Table 2 shows a difference of analytical performance (limit of detection, linearity, and sensitivity). Based on Figure 5 and Table 2, MCPE exhibits the low limit of detection with wider linearity range and higher sensitivity than that of CPE and QCPE. Using xanthine concentration of 1µM to 1mM, the LOD (limit of detection) are 0.025 mM, 0.010 mM, and 0.005 mM, respectively. The MCPE gives the best LOD as compared to the others. However, there is a report indicating higher limit of detection 0.2 mM [31] and lower limit of detection for xanthine biosensor as of 0.1 µM [2], [32] and 0.75 µM [33].

The graph (Figure 5) shows the current difference produced from the xanthine measurement using CPE, QCPE, and MCPE. The linearity and sensitivity linearity as perceived from it’s the value of (R²). R² of MCPE 10% > QCPE > CPE with their value are (0.01-1mM) with R²=99.24%; (0.1-1mM) with R²=97.77%; (0.1-1.0mM) with R²=94.15%, respectively. Basically, the three electrodes are in the linear area, but MCPE is the best electrode based on observation for the three electrodes performance.
observed. The result of linearity were obtained for Nano sphere magnetite modified carbon paste electrode better than linearity of Iswantini et al. were reported [11] on same range with lower R² value (0.978). The other study reported of the lower linearity range were 0.0015-0.07mM [34] and 0.1-300 μM [2].

The sensitivity can be seen from the calculated line equation. MCPE is the electrode with the highest sensitivity (5.16 µA mM⁻¹) among the others. CPE has 4× lower sensitivity than that of MCPE (1.29 µA mM⁻¹) and of QCPE 1.3× smaller than that of MCPE (3.99 µA mM⁻¹). The other study of lower sensitivity is reported 0.95 µA mM⁻¹ [11] and higher sensitivity (29.5 µA mM⁻¹) is reported [35].

The electrode producing the highest oxidation current is the carbon paste electrode modified with magnetite Nano spheres, followed by carbon paste electrode with a mediator Q₀, and the least is the electrode without any modification (Figure 5). We believe that the magnetite increases the xanthine oxidation current to the uric acid, as compared with Q₀ which is commonly used mediator for uric acid biosensor [11]. The magnetite Nano spheres increases the peak current due to large surface area of the magnetite Nano spheres and therefore, high electron transfer rate. Dervisevic et al. [28] shows that addition of Fe₂O₄ Nano composite decreases the electrode resistance and increases electron transfer efficiency.

![Figure 5. Regression equations and linearity of relation between substrate concentration and xanthine oxidase activity.](image)

| Types of working electrode | Analytical performance |
|----------------------------|------------------------|
|                            | Limit of detection (mM)| Range of linearity | Linear regression value (%) | Sensitivity (µA mM⁻¹) |
| Carbon paste electrode     | 0.025                  | 0.10–1.00          | 94.15 %                      | 1.29                  |
| without modification (CPE) |                        |                     |                               |                      |
| Carbon paste electrode     | 0.010                  | 0.10–1.00          | 97.77 %                      | 3.99                  |
| modified Q₀ (QCPE)         |                        |                     |                               |                      |
| Carbon paste electrode     | 0.005                  | 0.01–1.00          | 99.24 %                      | 5.16                  |
| modified Nano spheres      |                        |                     |                               |                      |
| magnetite (MCPE)           |                        |                     |                               |                      |

**Table 2.** Analytical performance of the working electrodes.
3.5. Application

3.5.1. Inhibition of XO power and the resulted IC$_{50}$. *S. polyanthum* water extract is more potential to inhibit the activity of xanthine oxidase than ethanol extract (Figure 6), that indicated by the IC$_{50}$ value. Based on the graph, IC$_{50}$ value of water and ethanol extracts of *S. polyanthum* were 69.47 ppm and 238.07 ppm, respectively. IC$_{50}$ of the water extract is 3.42× lower than that of the ethanol extract, with higher linear regression value. Water is the commonly used solvent to prepare of uric acid drug conventionally. Based on phytochemical test, secondary metabolites compounds in water extract are flavonoids, saponins and tannins, while based on BPOM [36], a flavonoid and tannin with fluoretin and quercetin are the main component. Schmeda *et al.* [37] informs that this leaves contain flavonoids, mircicitin, and quercetin groups has efficacy in inhibiting the activity of xanthine oxidase. Ethanol extract is known to contain alkaloids, flavonoids, saponins, and tannins; tannin is the main component, which is not less than 21.7 % [36].

Inhibition power analysis of xanthine oxidase by bay leaf extract was done by varying the concentration of the extract. Various concentrations of extract will give IC$_{50}$ value and further for determining the enzyme inhibition kinetics. The increasing concentrations of *S. polyanthum* extract also increasing the inhibition of xanthine oxidase. It is also shown by current reducing as detected by the CPE-modified magnetite Nano spheres which indicates the activity of the enzyme after the extract addition at various concentrations. Based on regression equation, which is $y = 11.217 \ln (\times) + 2.4297$ ($R^2 = 0.9268$) for electrochemical method and $y = 11.751 \ln (\times) - 4.0643$ ($R^2 = 0.9671$) for spectroscopy method (Figure 7), the IC$_{50}$ value of water extract by electrochemical method (69.47 ppm) is lower than that by spectroscopy method (99.56 ppm).

![Figure 6](image1.png)  ![Figure 7](image2.png)

The positive control (commercial allopurinol) was used to inhibit xanthine oxidase activity. The relation between allopurinol concentration and its inhibitory toward xanthine oxidase is in Table 3 for electrochemical method and Table 4 for spectroscopy method. IC$_{50}$ value of allopurinol was 2.51 ppm for electrochemical method and 3.11 ppm for spectroscopy method. Other studies reported the IC$_{50}$ of allopurinol of 6.10 ppm [38] and 3.74 ppm[39]. The electrochemical method performance in this study provides relatively small IC$_{50}$ values as compared with the spectrophotometric method reported by Apaya *et al.* [38], Azmi *et al.* [39]. However, others studies also reported low IC$_{50}$ values of allopurinol , namely 2.45 [30], and 0.60 ppm [40]. Inconsistency of IC$_{50}$ values may be caused by different testing conditions and sensitivity tests.
Table 3. Inhibitory power of Allopurinol against xanthine oxidase using electrochemical method.

| [Allopurinol] (ppm) | Enzyme activity (ΔIpa) (µA) | % Inhibition | [Allopurinol] (ppm) |
|---------------------|-----------------------------|--------------|---------------------|
| 0.0                 | 12.670                      | 0.000        | 0.0                 |
| 0.1                 | 11.373                      | 10.234       | 0.1                 |
| 0.5                 | 9.810                       | 22.573       | 0.5                 |
| 1.0                 | 8.246                       | 34.912       | 1.0                 |
| 2.0                 | 6.997                       | 44.778       | 2.0                 |
| 4.0                 | 3.987                       | 68.535       | 4.0                 |
| 6.0                 | 2.597                       | 68.008       | 6.0                 |

Table 4. Inhibitory power of Allopurinol against xanthine oxidase by spectroscopy method.

| [Allopurinol] (ppm) | Enzyme activity (ΔIpa) (µA) | % Inhibition | [Allopurinol] (ppm) |
|---------------------|-----------------------------|--------------|---------------------|
| 0.0                 | 99.114                      | 0.000        | 0.0                 |
| 0.5                 | 77.897                      | 21.406       | 0.5                 |
| 1.0                 | 69.306                      | 30.075       | 1.0                 |
| 2.0                 | 59.944                      | 39.519       | 2.0                 |
| 4.0                 | 49.321                      | 50.238       | 4.0                 |
| 6.0                 | 33.462                      | 66.239       | 6.0                 |

3.5.2. Inhibition kinetics of bay leaf extract toward XO. The concentration of the extract selected in the inhibition kinetic test was 100 ppm. This selection based on the ability of inhibitory (> 50%), and the concentration was also the nearest concentration IC\textsubscript{50} value of the bay leaf water extract that has been obtained previously. The inhibition kinetics of the extract was determined by the Lineweaver-Burk and the Eadie-Hofstee method. Based on the analysis (Figure 8), the coefficient of determination (\(R^2\)) obtained from Lineweaver-Burk method was relatively better than that of the Eadie-Hofstee method (data not shown). Finally, the kinetic parameters (\(K_M\) and \(V_{MAX}\)) were determined using Lineweaver-Burk method.

The type of inhibition kinetics is determined by observing the change of Michaelis-Menten constant value (\(K_M\)) and the maximum reaction rate (\(V_{MAX}\)), which indicated the maximum current (\(I_{MAX}\)). Based on Figure 8, there is a significant change of \(K_M\) values and very small change of \(I_{MAX}\) values. \(K_M\) values increased from 0.1994 mM\(^{-1}\) to 0.5638 mM\(^{-1}\) or 64.64% increasing. \(I_{MAX}\) value decreased from 3.9169 µA\(^{-1}\) to 3.9154 µA\(^{-1}\). Very small change of \(I_{MAX}\) value may be perceived as no change [41].

Competitive inhibitor will cause a change of \(K_m\), while noncompetitive inhibitor will cause a change \(I_{MAX}\) value. Increasing \(K_m\) and relatively fixed of \(I_{MAX}\) after the addition of inhibitors, indicates that the water extract of bay leaf refers to the type of competitive inhibition kinetics. At this competitive inhibition kinetics, inhibitor (bay leaf extract) reacted with the enzyme competitively toward the substrate for binding the active site of the enzyme. The level of inhibition depends on the relative concentrations of substrate and inhibitor, and in most cases the presence of a competitive inhibitor, the maximum reaction rate (\(V_{MAX}\)) can be achieved if the substrate concentration is sufficiently high.
4. Conclusion
Magnetite Nano spheres as a modifier carbon paste electrodes (MCPE) could be improved the analytical performance of xanthine biosensor, such as: 4× higher sensitivity than the carbon paste electrodes without modification (CPE) and 1.3× higher than the carbon paste electrode modified Q₀ (QCPE); 2× smaller limit of detection than QCPE and 5× smaller than CPE; and wider linearity range (0.01-1.00) than QCPE and CPE (0.10-1.00). The modified magnetite Nano spheres carbon paste electrode could be used to determine the type of inhibition kinetics of Syzygium polyanthum toward xanthine oxidase. Inhibition kinetics of the extract increased of KᵢM value and VₘAX (IₘAX) value was constant. Based on the result, the type of inhibition kinetics was a competitive inhibition.

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