# Detection of vitamin $b_1$ (thiamine) using modified carbon paste electrodes with polypyrrole

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**Abstract**. Vitamin $B_1$ (thiamine) is oxidized in alkaline medium and can be detected by cyclic voltammetry technique using carbon paste electrode (CPE) as a working electrode. polypyrrole-modified CPE were used in this study to increase sensitivity and selectivity measurement of thiamine. Molecularly imprinted polymers (MIP) of the modified CPE was prepared through electrodeposition of pyrrole. Measurement of thiamine performed in KCl 0.05 M (pH 10, tris buffer) using CPE and the modified CPE gave an optimum condition anodic current of thiamine at 0.3 V, potential range (-1.6–1 V), and scan rate of 100 mV/s. Measurement of thiamine using polypyrrole modified CPE (CPE-MIPpy) showed better result than CPE itself with detection limit of $6.9\times10^{-5}$ M and quantitation limit $2.1\times10^{-4}$ M. CPE-MIPpy is selective to vita min $B_1$. In conclusion, CPE-MIPpy as a working electrode showed better performance of thiamine measurement than that of CPE.

## 1. Introduction

Vitamin $B_1$ or thiamine (3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2hydroxyethyl)-4_methylthiazol-3-ium chloride) (figure 1) have a role in the development of brain and neurons [1]. There are at least 30 g Stock of vitamin $B_1$ in human body [2]. Lack of vit B1 could cause beriberi deseas, which could deffect the neuron system. It could be prevented by consuming food containing vit $B_1$ from any resource or suplement.

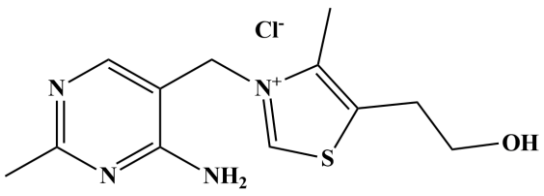


**Figure 1**. Structure of Vitamin $B_1$.

The importance of vitamin $B_1$ roles in human body, lead to development of methods to detect vit $B_1$ in foods or certain suplements. Some of the developed methods including high performance liquid chromatography [1], chemiluminesence [3], dan fluoresence [4]. The eminence of those methods are high accuracy and coefficient of determination, also low detection limit. However, those methods also

have some flaws including complicated sample preparation, requiring high tech instrument, and costly. Those problems could be avoided with electrochemical method, particulary voltammetry. The advantages of these technique including sensitivity, and the ability to produce interpretable data even in a low concentration level, fast, easy, and low cost anlysis [5,6]. Vitamin $B_1$ could be detected electrochemically due to oxidizable character into thiochrome in basic [6].

Carbon paste electrode (CPE) producing low current and selectivity, thus need to be modified to upgrade the performance. CPE Modifier used in this study is moleculary imprinted polymers (MIP) polypyrola. The previlege of using MIP polypyrola has been proved by Koirala et al. [7]. MIP polypyrola could be electropolymerized in various material with electro conductivity and give selectivity measurement. Molecules print formed as the result of interaction between polymers and molecule filters that makes MIP plypyrola selective. Pyrrole coating on CPE used electropolymerization method with eminence including, ability to coat narrow and uneven surface of CPE and thickness of polypyrrole coat could be adjusted with variation of time in polymerization [8]. This study intended to create CPE mofied by MIP polypyrrole (CPE-MIPpy) that could detect vitamin $B_1$ using voltammetry method. Modification expected to increase sensitivity and selectivity of CPE compared to the unmodified CPE.

## 2. Experimental

### *2.1 Materials*

The materials needed are graphite, paraffin, standard vitamin $B_1$-HCl (Himedia), KCl, $K_3Fe(CN)_6$ , $CoCl_2.6H_2O$, pyrrole (Sigma Aldrich), NaOH, $H_2SO_4$, HCl, KOH, $H_3PO_4$ , $H_3BO_3$, $CH_3COOH$, $CH_3OH$, $N_2$, tris buffer, copper wire, waxed paper, Pt electrode, an electrode Ag / AgCl, deionized water, filter paper, and distilled water.

### *2.2 Apparatus*

The tools used are glass tools common in laboratories, potentiostat EDAQ, Sonicator 42 Hz (As One), analytical balance (Sartorius), pH meter (Hanna Instruments), scanning electron microscope JEOL JSM-6360LA, glass tube diameter 2.5 mm, mortar and pestle, and software E-CHEM, Origin Pro v 2.1.0 and 7.0.

### *2.3 Preparation of carbon paste electrode*

Graphite and paraffin mixed with a ratio of 7: 3 (w/w). The mixture was homogenized using a sonicator for 15 minutes, then crushed and compacted using a mortar and pestle for 30 minutes to form a paste. Then the mixture was put in a 2.5 mm diameter glass tube that has been inserted copper until the remaining vacant space of about 3 mm from the tip of the tube and the tube base glued. Once solid, the electrode surface is flattened by means of unidirectional rubbed oil paper. Rubbing suspended when the black color of the carbon is no longer attached to the waxed paper. Carbon paste electrodes can be measured after being stored ± 2 days at room temperature.

### *2.4 Determination of the measurement conditions*

The determination of the optimum range potential for measuring vitamin $B_1$ uses a variation of the range potential -1.6-1 V each 0.2 V. While the hose scan rate varied from 50 to 250 mV/s each multiple of 50 mV / s. For the determination of pH, vitamin $B_1$ in KCl solution mixed with a solution of NaOH 0.1 M Tris buffer pH 10 and pH 10 separately. Each mixture scaning on the range potential and optimum scan rate.

### *2.5 Preparation of polypyrrole modified CPE (CPE-MIPpy)*

MIP polipyrrole made through electropolymerization using the potentiostat. The working electrode that has been made is connected with a potentiostat along with Ag/AgCl as a reference electrode and Pt as counter electrode. The third electrode is dipped into a solution of vitamin $B_1$ 0.01 M pyrrole

monomer and 0.1 M in Britton-Robinson buffer of pH 3, and then do electrodeposition at a potential of 0.9 V for 180 seconds. After electropolymerization completed, a layer that is formed is washed and soaked with deionized water for 24 hours. Deionized water used to extract vitamin $B_1$ that is embedded in the film. The electrode is hereinafter referred CPE-MIPpy.

*2.6 Analytical procedure*

Techniques used for the voltammetric determination of vitamin $B_1$ are cyclic voltammetry (CV). The measurements were performed in electrolyte (KCl) with tris buffer (pH 10) at laboratory temperature. The 0.001M stock solution was prepared by diluting vitamin B1 in deionized water. The calibration curves were measured in triplicate and their statistical parameters (e.g., slope, intercept, correlation coefficient, and limit of detection) were calculated. The detection limits were calculated as the concentration of an analyte using 3s/m where s is the standard deviation of intercept and m is slope.

## 3. Result and Discussion

*3.1 Optimization conditions for the determination of vitamin $B_1$*

3.1.1 *Electrolyte*. One of the factors that need to be considered when measurements voltammetry is an electrolyte. Electrolyte serves as an electron transfer medium so that electrons move to the electrode surface and unreadable as current. In this study KCl selected as an electrolyte because it does not provide background currents that influence the reaction of vitamin $B_1$. KCl has a wide potential range on the CPE [9], the redox reaction between $K^+$ and Cl occur in a very positive potential, that is 2.93 V and 1.36 V [10].

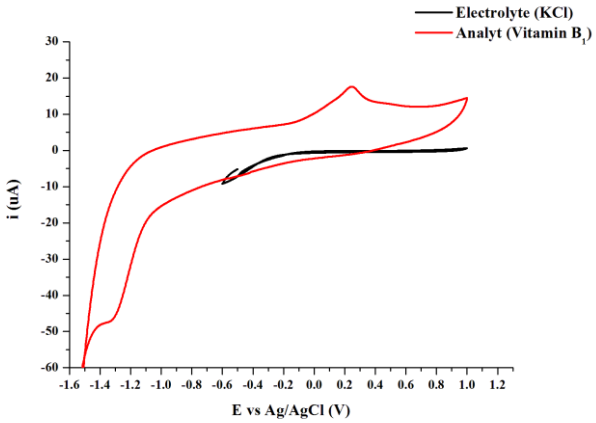


**Figure 2**. Cyclic voltammogram of vitamin B1 5 mM in KCl 50 mM and tris buffer pH 10. Scan rate 100 mV/s.

KCl as electrolyte concentration varied at 3, which is 50, 100, and 500 mM. The results showed that the concentration electrolyte of 50 mM KCl most stable because background current low value. At a concentration of 100 mM KCl, and 500 mM background currents higher, probably derived from the non-Faraday current form of charging current. Figure 2 shows the cyclic voltammogram (CV) of vitamin $B_1$ 5 mM in 50 mM KCl. It can be observed that the peak of vitamin $B_1$ (0.3 V) is not disturbed by the presence of background current of 50 mM KCl electrolyte.

*3.1.2 Optimun pH*. Oxidation of vitamin $B_1$ to tiokrom (Figure 3) is influenced by the pH of the electrolyte solution. According to Oni et al. [6]. Vitamin B1 can be oxidized to tiokrom in alkaline medium (pH 8-10). In this study measured vitamin $B_1$ measured in KCl electrolyte and electrolyte KCl pH conditioned using NaOH and Tris. The results indicate that there is a difference of current and peak

potential of oxidation of vitamin $B_1$. Response vitamin $B_1$ in the KCl and KCl-tris buffer pH 10 have the same value of oxidation potential (0.25 V), whereas the oxidation potential of vitamin $B_1$ in 0.1 M KCl-NaOH pH 10 was observed at 0.72 V (Figure 4). Oxidation potential shifts toward more positive potential indicates the oxidation reaction occurs more difficult.

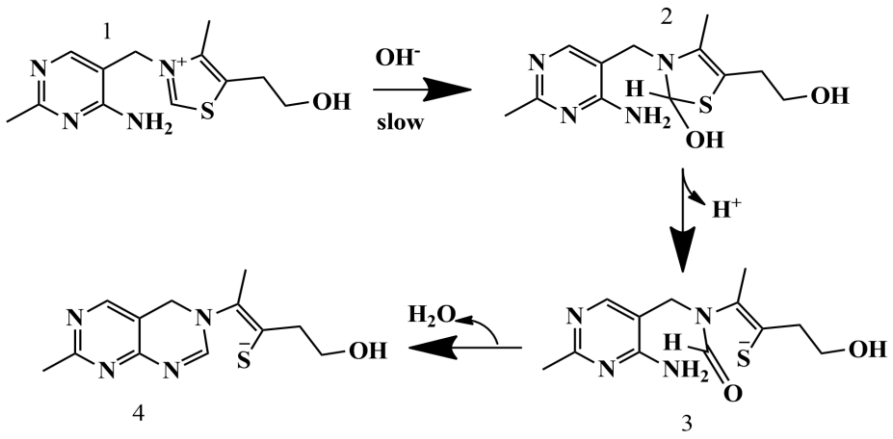


**Figure 3**. The mechanism of the oxidation reaction of vitamin $B_1$ to tiokrom.

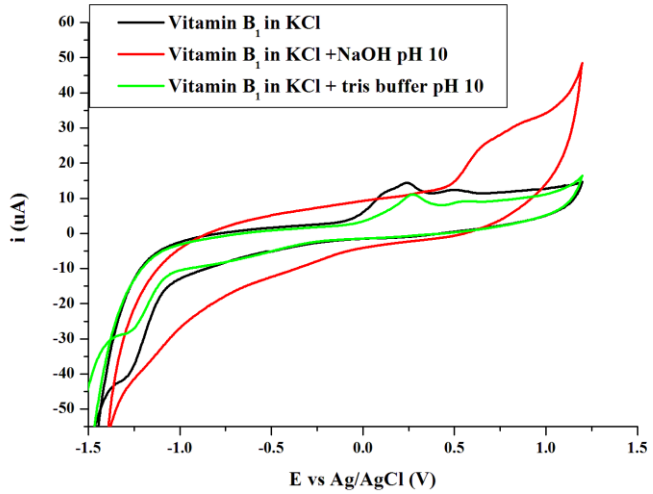


**Figure 4**. Cyclic voltammogram of vitamin B1 5 mM in KCl 50 mM, adding NaOH pH 10 dan tris buffer pH 10. scan rate 100 mV/s.

Vitamin $B_1$ in KCl give the current 1.6 times greater compared with KCl-tris buffer pH 10. However, in repeat measurements made, the intensity of the oxidation peak current inconsistent (data not shown). Based on these results, KCl-tris buffer pH 10 is used as the electrolyte in the next measurement.

3.1.3 *Potential Range*. Potential window illustrates the potential range of the redox reaction of the analyte. Good potential range can describe analyte peak clearly without interference peak of the redox electrolyte. Figure 5 shows the cyclic voltammogram vitamin $B_1$ measured at various potential window. In -1.2-1 potential -1-1 V and V have not seen the peak oxidation of vitamin $B_1$. Oxidation of vitamin $B_1$ peak of the new look as a potential window widened from oxidation peak -1.4-1 V. Vitamin $B_1$ is also observed during use a potential window -1.6-1 V. Range potential selected for

subsequent measurement is -1.6-1 V, because it generates a current the highest (17.9 uA at a potential of 0.3 V).

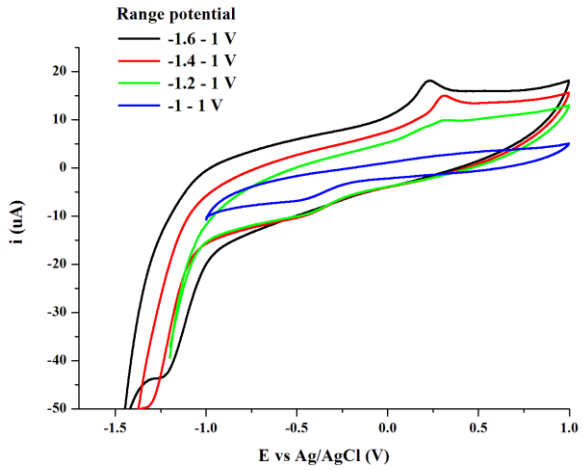


**Figure 5**. Cyclic voltammogram of vitamin $B_1$ 5 mM in KCl 50 mM with tris buffer pH 10. Scan rate 100 mV/s on a variety of potential range.

3.1.4 *Scan Rate*. Scan rate effect on the peak intensity of the oxidation of vitamin $B_1$. Selection of the optimum speed Payar need to notice the easy nature or absence of vitamin $B_1$ to undergo oxidation reactions and oxidation of high peak currents generated. Increased scan rate current proportional to the intensity of oxidation of vitamin $B_1$ (Figure 6). scan selected speed is 100 mV / s. It is considering the speed of the oxidation reaction of vitamin $B_1$ to tiokrom which is slow. At higher scan rate, feared oxidation of vitamin $B_1$ to tiokrom can not happen perfectly.

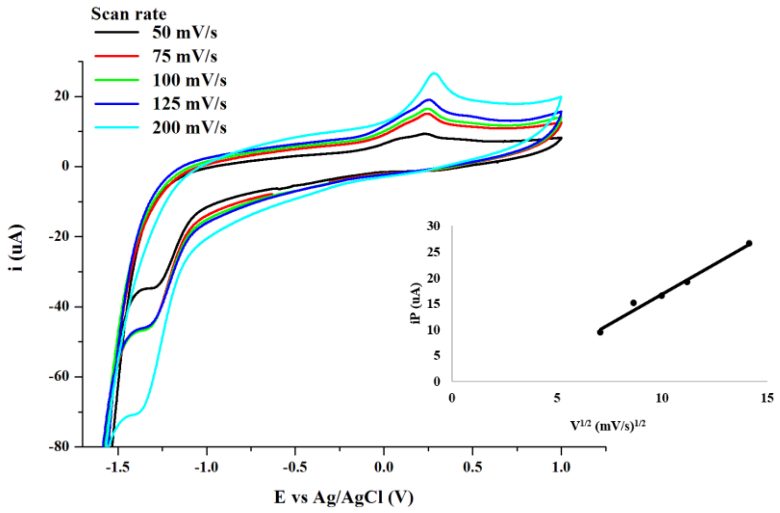


**Figure 6**. Cyclic voltammogram of vitamin B1 5 mM in KCl 50 mM with tris buffer pH 10 potential range –1.6–1 V on a variety of scan rate. Inset: scan rate root relationship between oxidation current peak curve

Based on the Randles-Sevcik equation, if the peak current (Ip) is proportional to the square root scan rate $V^{1/2}$ is proportional, then the charge transfer that occurs from the solution to the surface of the

electrode under the influence of diffusion. Meanwhile, if the relationship between the peak current (Ip) and scan velocity V is proportional to the charge transfer that occurs from the solution to the surface of the electrode under the influence of adsorption [11]. The peak current generated by vitamin $B_1$ is directly proportional to the square root of the speed scan given to the value of $R^2 = 0.9832$ (inset in Figure 6). This indicates that the charge transfer is under the influence of diffusion. Diffusion occurs from the bulk solution to the electrode surface due to the concentration gradient and flux increases on the electrode with increasing scan rate [12].

*3.2 CPE modified polymers molecularly imprinted polypyrrole (CPE-MIPPy)*

Polypyrrole selected as an CPE modifier because it has good electrical conductivity and can be synthesized electrochemically on the surface of CPE through electropolymerysation [7]. Vitamin $B_1$ is mixed with pyrrole monomer before electropolymerysation process that vitamin $B_1$ is at polipirola matrix. Trapped vitamin $B_1$ in polipirola occur through hydrogen interaction between the N-H group at the pyrrole with O-H and N-H group in vitamin $B_1$. Following that, vitamin $B_1$ is extracted with a porogenic solvent in order to obtain moleculary imprinted polymer (MIP) on the surface of CPE. MIPpy existence on the CPE's surface is expected to improve the selectivity of CPE on the measurement of vitamin $B_1$. MIPpy role as mold that can only be passed by a molecule of vitamin $B_1$. So only the vitamin $B_1$ can reach the electrode surface and produce a response in the form of oxidation current.

*3.2.1 Oxidation of vitamin $B_1$ at CPE-MIPPy*. Cyclic voltammograms of vitamin $B_1$ which measured by CPE and CPE-MIPpy. show the increase of peak oxidation intensity of vitamin $B_1$ of 1.5 when measured with CPE-MIPpy (Figure 7). The process of electron transfer on the surface of CPE-MIPpy more easily occur because the electrical conductivity character owned by polypyrrole.

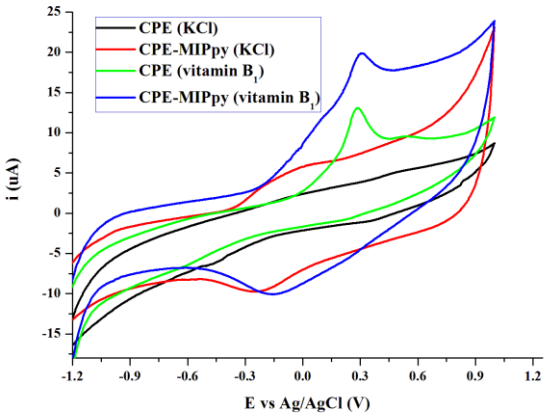


**Figure 7**. Cyclic voltammogram of vitamin $B_1$ 5 mM in KCl 50 mM with tris buffer pH 10 at CPE-MIPpy. Scan rate 100 mV/s with bubbling with N2(g).

3.2.2 *Selectivity CPE-MIPpy on measurement vitamin $B_1$*. The selectivity indicates the ability of a method to distinguish the analyte from the matrix or other nuisance. Selectivity CPE-MIPpy on the measurement of vitamin $B_1$ was evaluated using vitamin $B_6$ (pyridoxine) as a bully. CPE-MIPpy can detect vitamin $B_1$ and vitamin $B_6$ simultaneously (Figure 8). Peak oxidation of vitamin $B_6$ can be detected because vitamin $B_6$ molecules can pass through the mold vitamin $B_1$ and reaches the surface of the CPE. Escape of vitamin $B_6$ possible because the molecule size smaller than vitamin $B_1$. Even

CPE-MIPpy can be bypassed by vitamin $B_6$, vitamin $B_1$ measurement is not disturbed due to oxidation peak vitamin $B_1$ and vitamin $B_6$ are at a different potential so that its peak can be distinguished. This opens the opportunity to do the simultaneous measurement of vitamin $B_1$ and vitamin $B_6$ use CPE-MIPpy.

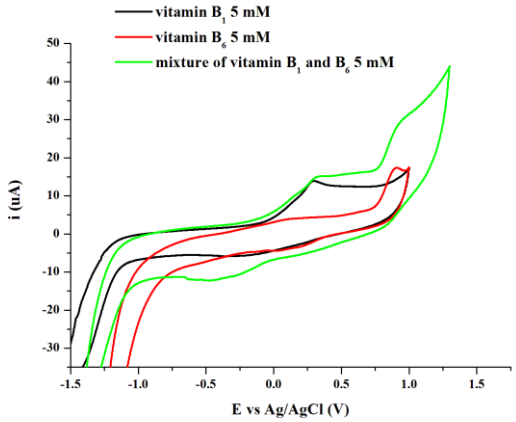


**Figure 8**. Cyclic voltammogram of vitamin B1 5 mM, Vitamin B6 5 Mm, and mixture at CPE-MIPpy. Scan rate 100 mV/s and bubbling with $N_{2\,(g)}$.

*3.3 Surface morphology of CPE and CPE modified polipyrrole*

CPE surface morphology, CPE-MIPpy, and CPE-py observed using SEM. CPE has the smoothest surface structure while the CPE-MIPpy among the most rugged and irregular. Irregular structure of the CPE-MIPpy formed from the interaction of vitamin B1 with polipyrole when elektropolimerisasi then vitamin B1 is extracted, leaving a mold. CPE-py more irregular than CPE-MIPpy as contained in the surface just polipyrrole.

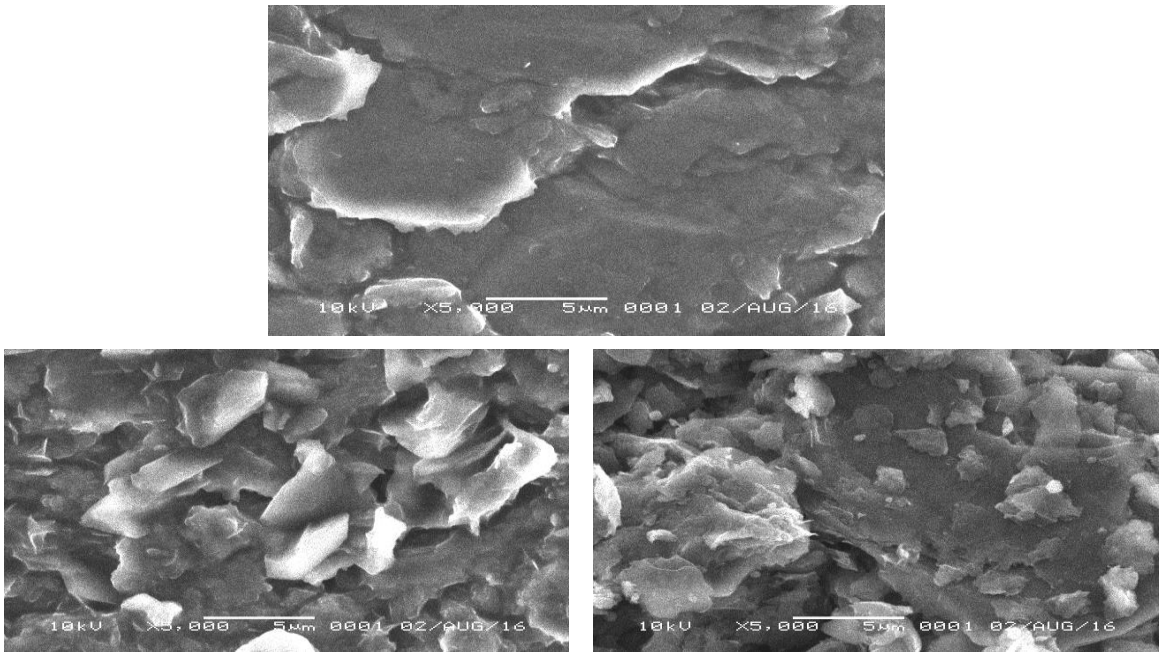


**Figure 9.** The surface morphology CPE (above), CPE-MIPpy (bottom left) and CPE-py (bottom right) were characterized using SEM with a magnification of 5.000 times.

### *3.4 Performance evaluation of CPE and CPE-MIPpy on measurement vitamin $B_1$*

3.4.1 *Linearity*. Linearity showed the relation between current response to variation of concentration at optimum measurement conditions. Linearity CPE and CPE-MIPpy measured by variations concentration of vitamin $B_1$ 0.256-10 mM with 6 times repetition. The results showed that higher concentration of vitamin $B_1$ oxidation, increasing current intensity. This indicates that more molecules of vitamin $B_1$ are oxidized (Figure 10). Linearity is evaluated based on the value of the coefficient of determination ($R^2$) obtained from concentration curve with peak current of oxidation. The linear regression equation was obtained for measurement of vitamin $B_1$ with CPE and CPE-MIPpy has ipa (uA) = 1336.9 x + 1.66 ($R^2$ = 0.9866) and ipa (uA) = 2805.8 x + 3:40 ($R^2$ = 0.9900). Relationship between concentration and oxidation current intensity of vitamin $B_1$ in measurements with CPE-MIPpy more linear than with CPE. Besides the slope value measurement of vitamin $B_1$ with CPE-MIPpy showed higher sensitivity CPE-MIPpy on the measurement of vitamin $B_1$ is better than CPE.

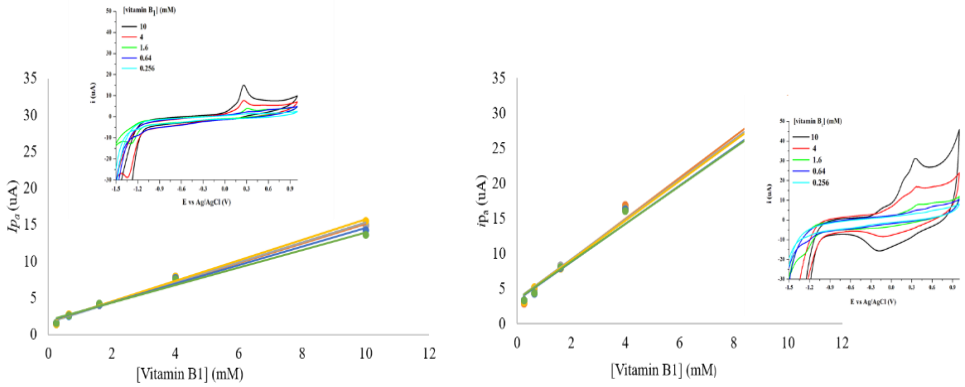


**Figure 10**. Curve relationship between the concentration of vitamin $B_1$ and vitamin B1 oxidation peak currents. Inset: cyclic voltammograms of various concentrations of vitamin $B_1$.

3.4.2 *Limit of detection and limit of quantitation*. Limit of detection (LOD) indicates the lowest concentration of an analyte that can be detected by the instruments or methode [14], in this case carbon paste electrodes, but not for quantitation. LOD for CPE-MIPpy of 6.9 x $10^{-5}$ M. This value is 9.7 more lower than the CPE, meaning the sensitivity of CPE-MIPpy in the measurement of vitamin $B_1$ is better than the CPE. Limit of quantitation (LOQ) indicates the lowest concentration of an analyte that can be determined by a method on the level of good precision and good accuracy. LOQ CPE-MIPpy on the measurement of vitamin $B_1$ 11.42 more lower than CPE. These results indicate that the CPE-MIPpy can be used well for measurement of vitamin $B_1$ at concentrations low enough.

3.4.3 *Precision*. Precision indicates the value of measurement accuracy based on the percent relative standard deviation (%RSD). The smaller %RSD increasingly rigorous methods / techniques used. Precision measured at concentrations of 5 series and each performed 6 repetitions. %SBR to CPE and CPE-MIPpy row by 3.68% and 3.90%, which means cyclic voltammetry technique has good accuracy for CPE and CPE-MIPpy.

3.4.4 *Stability and Reproducibility*. The stability test is required to determine the consistency of the response from the working electrode. Stability and reproducibility were evaluated based on %RSD from current and potential of oxidation peak vitamin B1. The stability oxidation peak of CPE better than CPE-MIPpy. CPE has stable current response to the measurement until day 7, while the current response of CPE-MIPpy decreased by 32% after using for 2 days (Figure 11). Stability oxidation potential of CPE-MIPpy lower than CPE, but still within the limits of tolerance with the value of %RSD is less than 5% [13].

Reproducibility is required to evaluate the uniformity of CPE and CPE-MIPpy. Reproducibility was evaluated based on %RSD current and oxidation peak potential of vitamin B1. Reproducibility of oxidation peak current of CPE-MIPpy better than the CPE because the current of three electrodes is uniform by 21 ± 0.7 uA, whereas CPE has fluctuate currents by 20 ± 6.3 uA (Figure 12).

Repeatability potential oxidation peak of CPE is bad with the value of %RSD is 5.40%. Less uniformity between the CPE to the others CPE for the manufacture are still conventional. Reproducibility potential oxidaton of CPE-MIPpy is very good because the polymerization of pyrrole on the surface of CPE done using the potentiostat. Overall, CPE-MIPpy has the sensitivity and reproducibility is more higher but still less stable than the CPE.

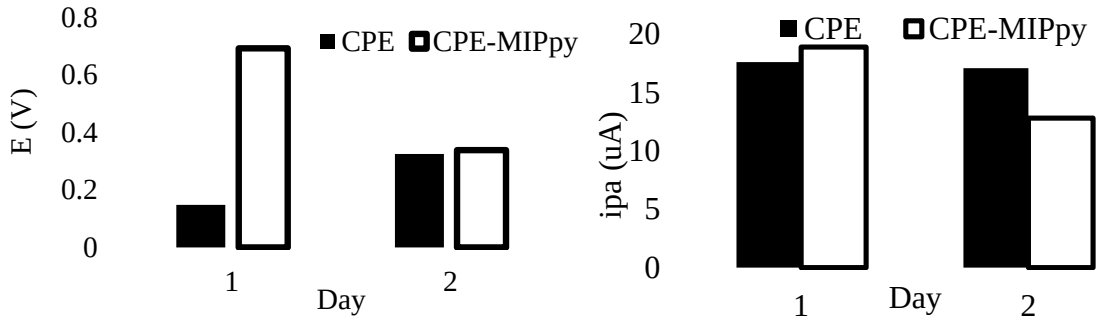


**Figure 11.** Stability current measurement (right) and potential (left) uses vitamin $B_1$ and CPE CPE MIPpy.

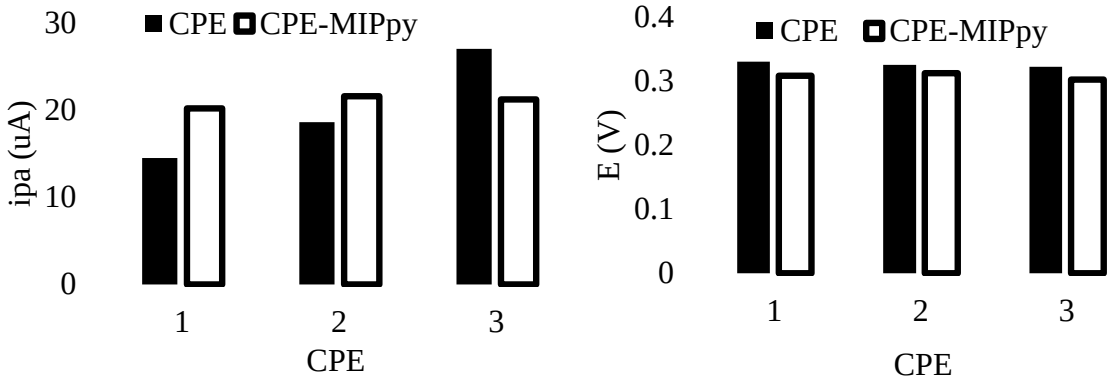


**Figure 12**. reproducibility of the current measurement (left) and potential (right) uses vitamin B1 CPE and CPE-MIPpy.

**Table 1.** Regression data of the calibration line for quantitative determination of vitamin B1 using Cyclic voltammetry.

| **Parameters** | **CPE** | **CPE-MIPpy** |
|---|---|---|
| Measured peak potential (V) | 0.32 | 0.32 |
| Linearity range (M) | 2.56 × 10-4 - 1 × 10-2 | |
| Slope (uA/M) | 1 336.9 | 2 805.8 |
| Intercept (uA) | 1.66 | 3.4 |
| Determination coefficient ($R^2$) | 0.9866 | 0.99 |
| Limit of detection (M) | $6.7 \times 10^{-4}$ | $6.9 \times 10^{-5}$ |
| Limit of quantification (M) | $2.4 \times 10^{-3}$ | $2.1 \times 10^{-4}$ |
| Stability of peak current (%RSD) | 1.64 | 8.31 |
| Stability of peak potential (%RSD) | 1.41 | 2.10 |
| Reproducibility of peak current (%RSD) | 1.89 | 1.56 |
| Reproducibility of peak potential (%RSD) | 5.41 | 0.26 |

## 4. Conclusion

Vitamin $B_1$ can be detected using methods voltammetry with carbon paste electrode as the working electrode scanning at of -1.6-1 V, scan rate at 100 mV/s and the addition of tris buffer pH 10 signal is generated in the form of oxidation peak currents that are potentially ± 0.32 V. measurement of vitamin $B_1$ with the modified polipirola CPE (CPE-MIPpy) showed better results than the CPE. CPE-MIPpy selective measurement of vitamin $B_1$. CPE-MIPpy as the working electrode shows better performance for the measurement of vitamin $B_1$ from the CPE.

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