# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 Rotating disk voltammetry

### 4.1.1 pH dependence study

Cyclic voltammetry at a gold rotating disk electrode was used to study the influence of pH on the electrochemical oxidation of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride. A potassium dihydrogen orthophosphate solution was employed as a supporting electrolyte for the rotating disk voltammetric studies of these antibiotic drugs. The variation of pH values from the acidic values to the basic values were carried out by using 0.1 M sodium hydroxide solution or 85 % phosphoric acid. The oxidative voltammetric results of each analyte are discussed below.

#### 4.1.1.1 Tetracycline hydrochloride

Table 4.1 summarizes the electrochemical data obtained from cyclic voltammogram of 1 mM tetracycline hydrochloride oxidation at pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, and 10 at the gold rotating disk electrode. The experimental results showed that the analyte oxidation peak potential, $Ep^{ox}$ (positive scan) and oxide reduction peak potential, $Ep^{red}$ (negative scan) shifted to more negative values as the pH of the solutions increased. These phenomena may be due to

tetracycline hydrochloride, which was easier to epimerize to the anhydrotetracycline in acidic media or to isotetracycline in basic media [56]. The proposed mechanisms of the epimerization of this compound are shown in Figure 4.1. Both in acid and alkaline solutions, the epimerization were occurred at C-6 hydroxyl group. From the literature discussion, these occurrences implied that the oxidation process of tetracycline hydrochloride released hydrogen ion into the solution, and the reduction process took up hydrogen ion from the solution [57]. From the electrochemical data displayed in Table 4.1, the highest oxidation current response at the oxidation peak potential about 1.104 V vs. Ag/AgCl was obtained at the pH 2. Therefore, this pH value was chosen as the optimum pH for the study of tetracycline hydrochloride. The rotating disk cyclic voltammograms of tetracycline hydrochloride in different pH solutions are shown in Figure 4.2 - 4.5, respectively.

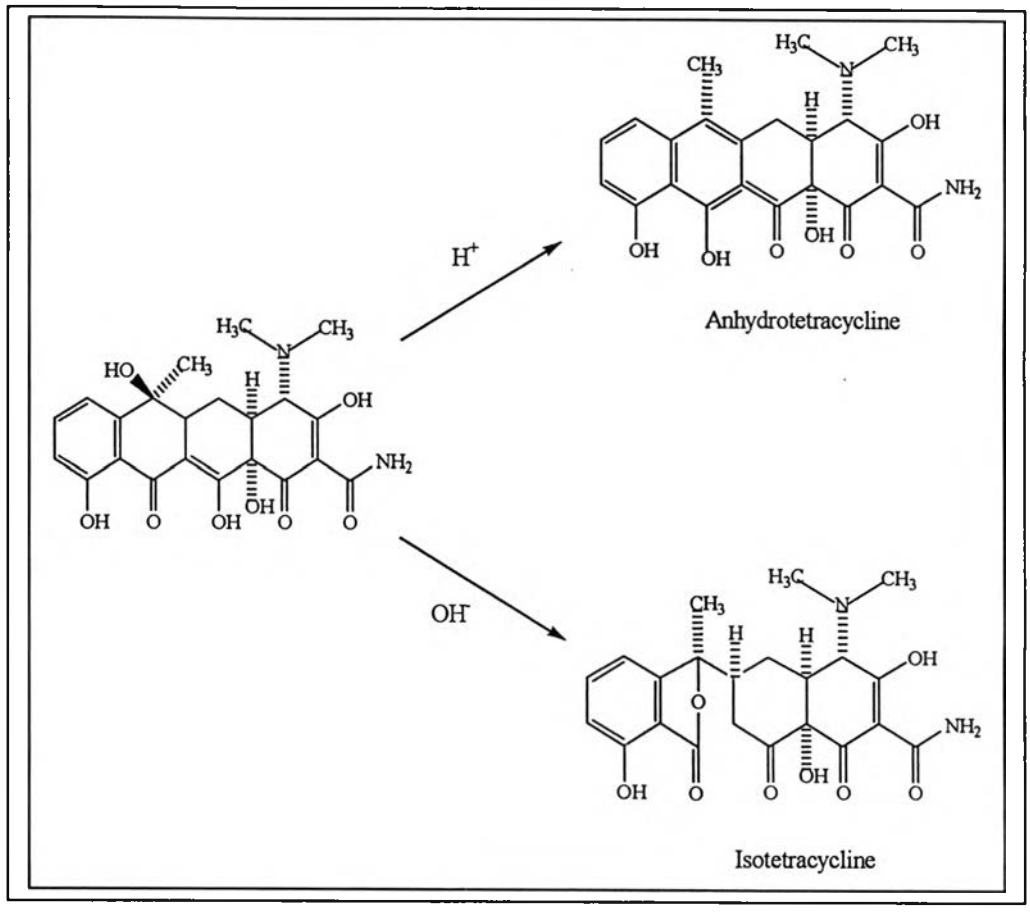


Figure 4.1 The proposed epimerization mechanism of tetracycline

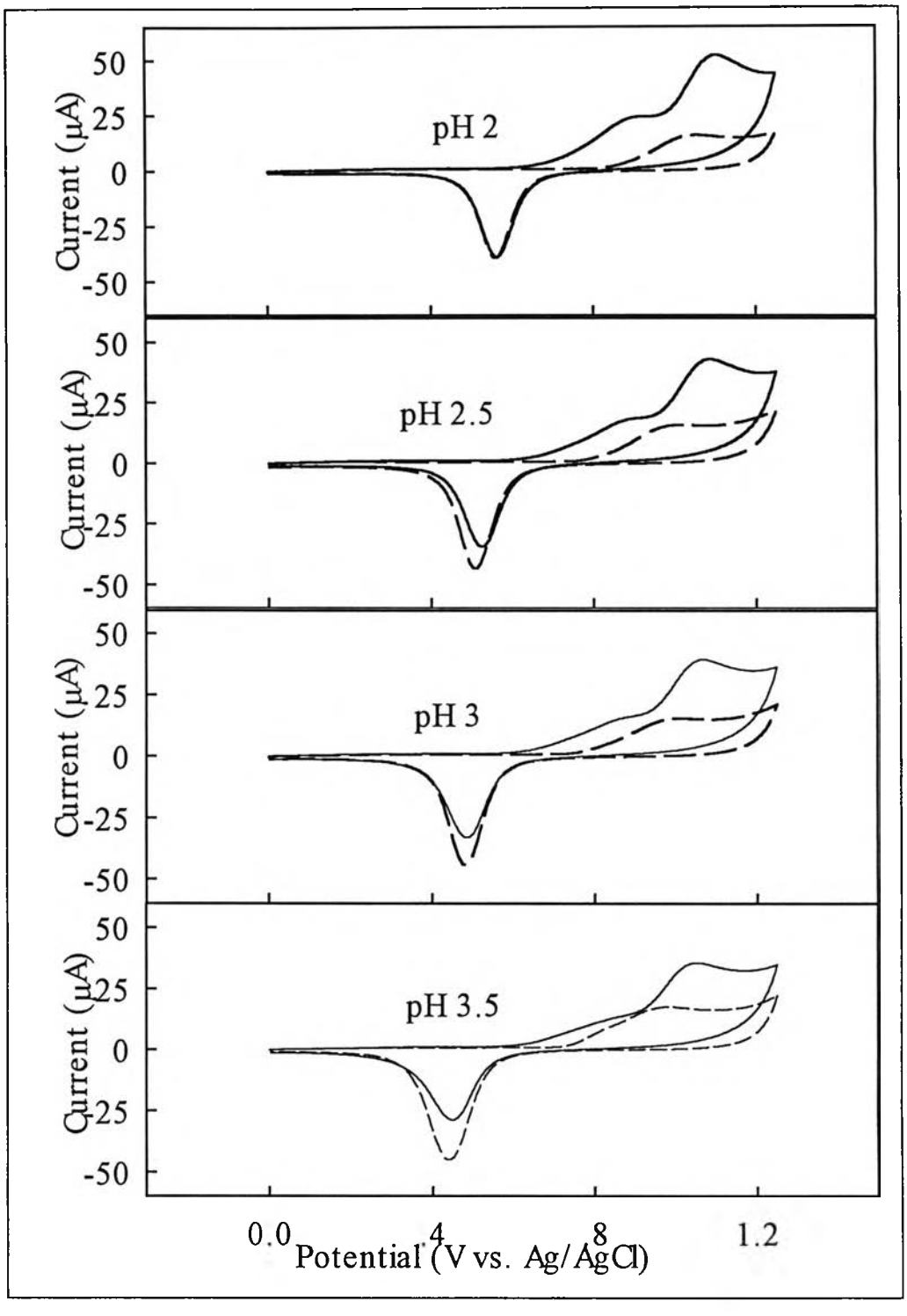


Figure 4.2 Rotating disk cyclic voltammogram of 1 mM tetracycline hydrochloride in 0.1 M $KH_2PO_4$ (pH 2, 2.5, 3 and 3.5) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

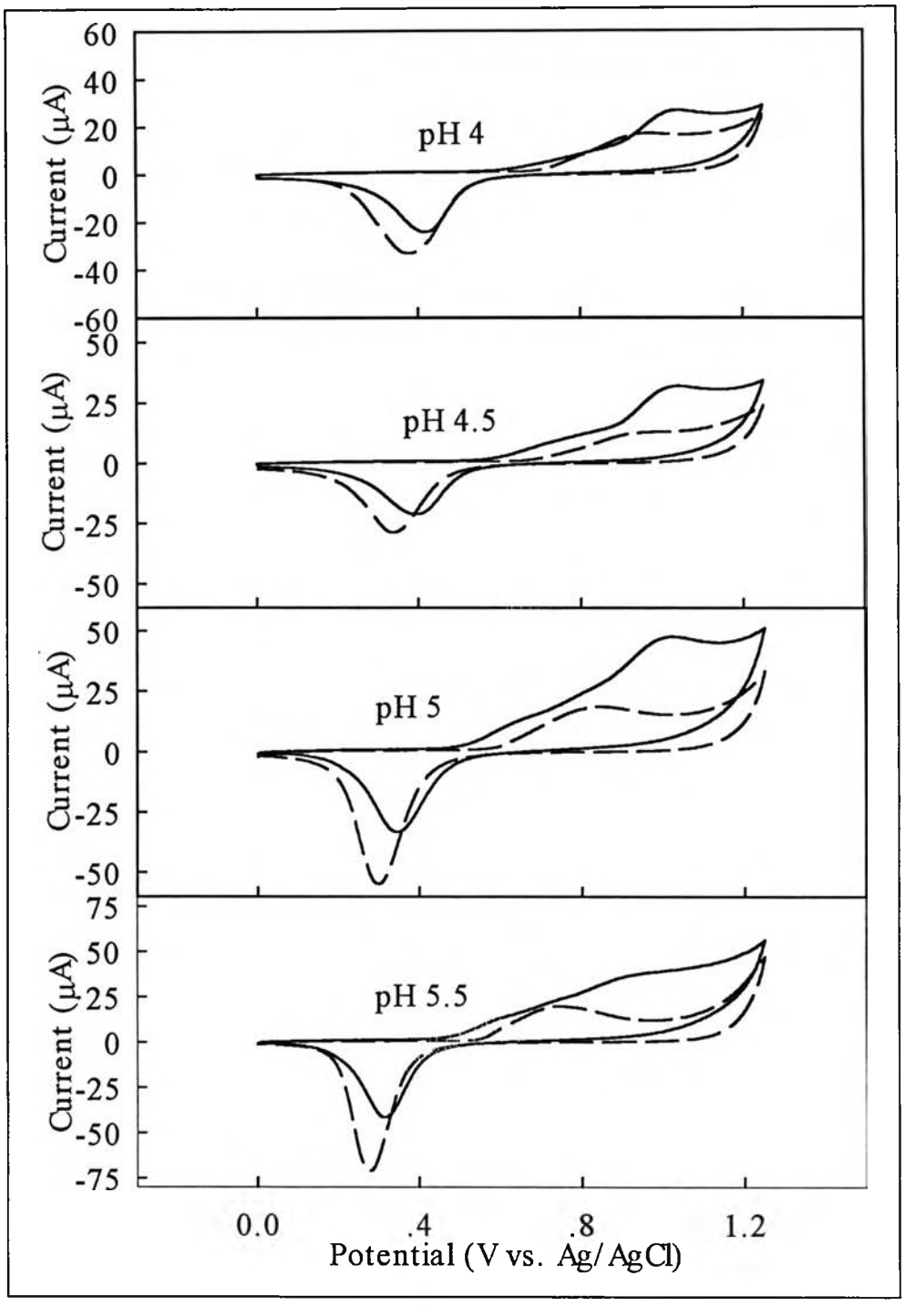


Figure 4.3 Rotating disk cyclic voltammogram of 1 mM tetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 4, 4.5, 5, and 5.5) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

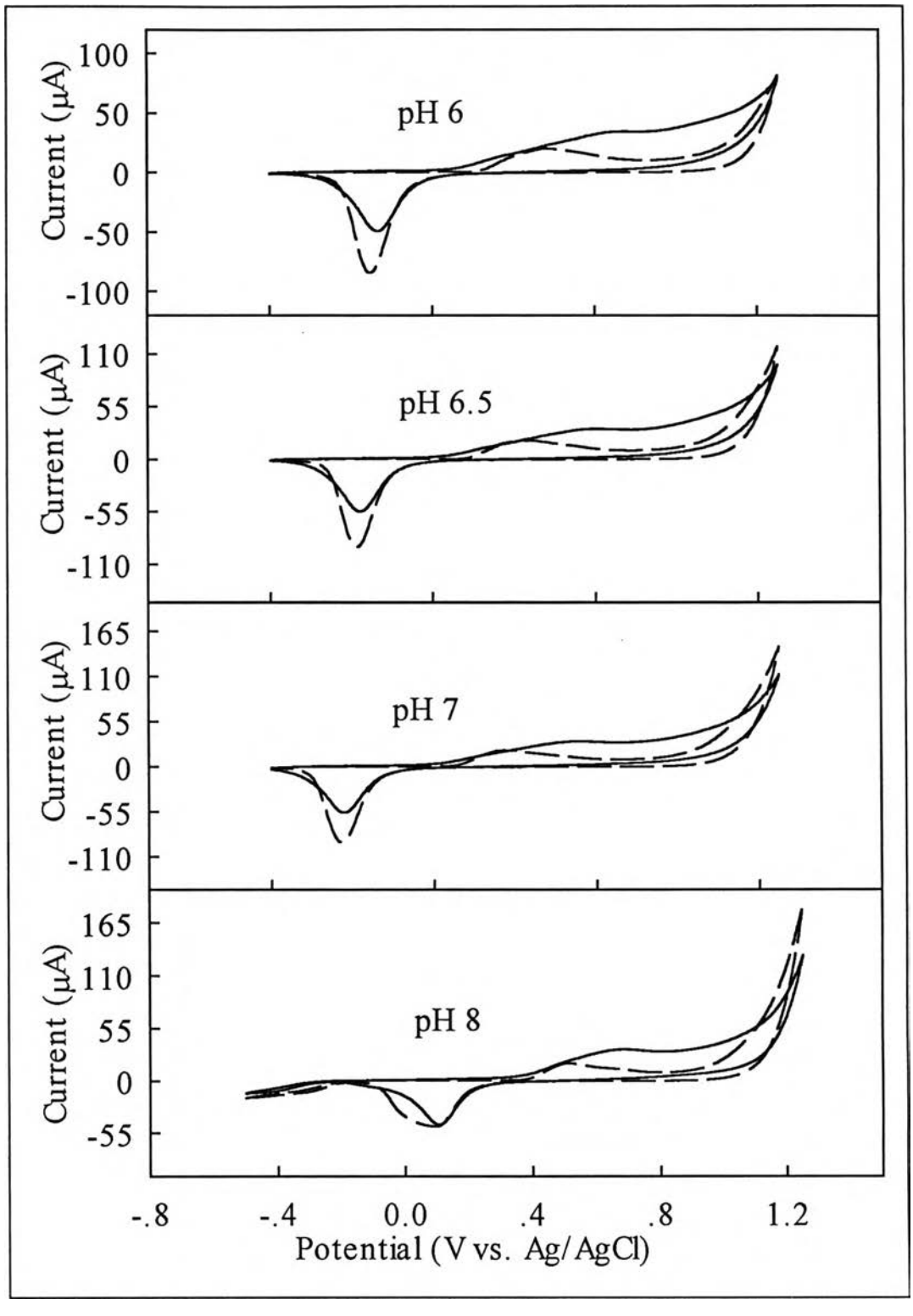


Figure 4.4 Rotating disk cyclic voltammogram of 1 mM tetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 6, 6.5, 7, and 8) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

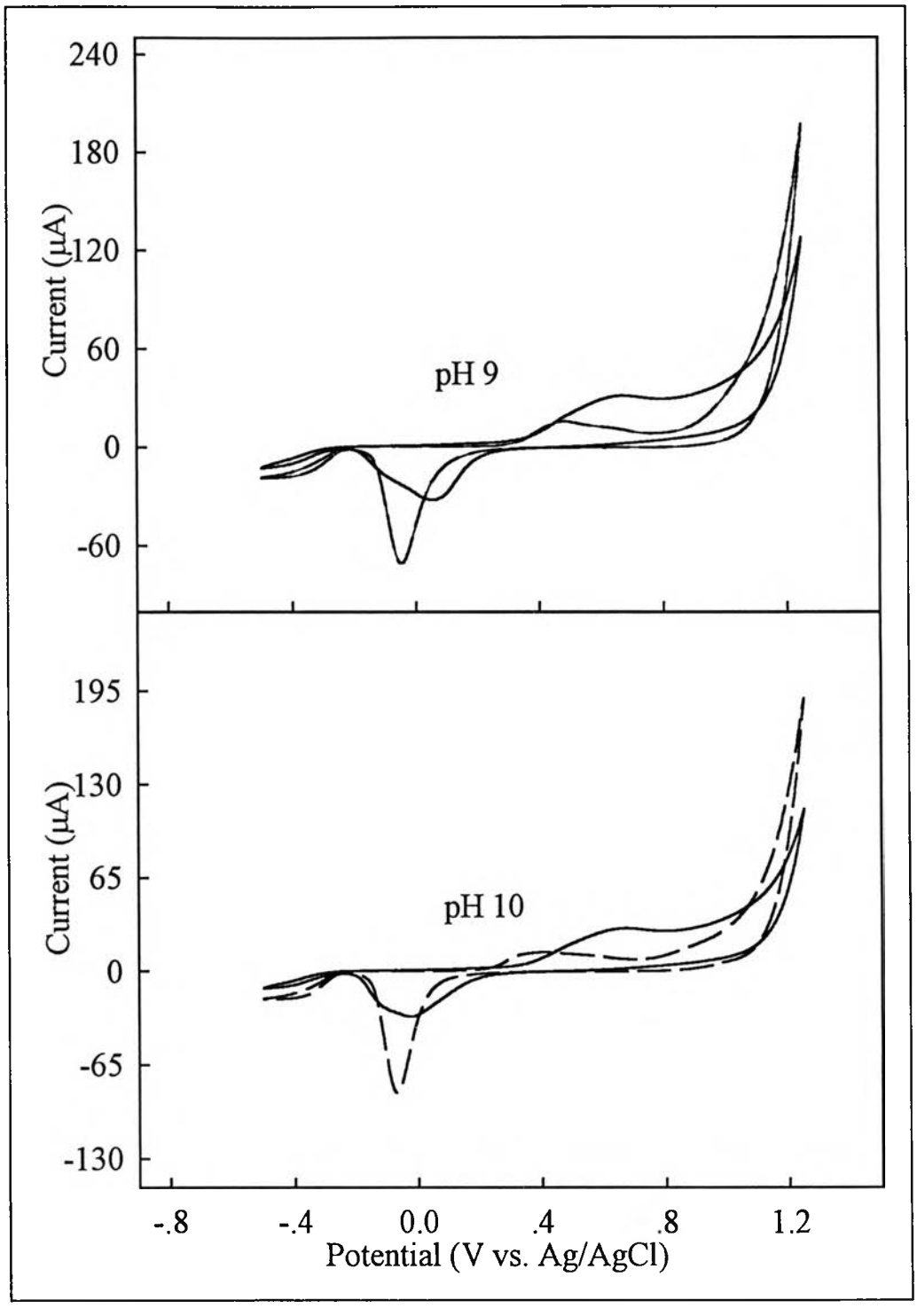


Figure 4.5 Rotating disk cyclic voltammogram of 1 mM tetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 9 and 10) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

Table 4.1 Comparison of electrochemical data obtained from rotating disk voltammograms of the 1 mM tetracycline hydrochloride at pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, and 10

| pH | $E_p^{ox}$ * (V vs. Ag/AgCl) | $I_p^{ox}$ ** (μA) | $E_p^{oxide\text{-}red}$ *** (V vs. Ag/AgCl) | $I_p^{oxide\text{-}red}$ **** (μA) |
|---|---|---|---|---|
| 2 | 1.104 | 50.90 | 0.562 | -39.99 |
| 2.5 | 1.086 | 39.50 | 0.525 | -35.35 |
| 3 | 1.064 | 38.40 | 0.483 | -34.38 |
| 3.5 | 1.050 | 34.60 | 0.452 | -29.86 |
| 4 | 1.042 | 25.90 | 0.417 | -26.35 |
| 4.5 | 1.033 | 32.30 | 0.396 | -22.11 |
| 5 | 1.011 | 48.00 | 0.347 | -34.08 |
| 5.5 | 0.955 | 40.70 | 0.315 | -42.78 |
| 6 | 0.838 | 35.00 | 0.269 | -50.32 |
| 6.5 | 0.777 | 33.00 | 0.220 | -55.73 |
| 7 | 0.733 | 31.00 | 0.181 | -56.89 |
| 8 | 0.664 | 33.00 | 0.108 | -47.33 |
| 9 | 0.648 | 32.00 | 0.059 | -33.24 |
| 10 | 0.641 | 29.00 | -0.022 | -32.22 |

* Oxidation peak potential of tetracycline hydrochloride

**Oxidation peak current of tetracycline hydrochloride

*** Reduction peak potential of surface oxide

**** Reduction peak current of surface oxide

4.1.1.2 Chlortetracycline hydrochloride

The influence of the supporting electrolyte pH on chlortetracycline hydrochloride oxidation was investigated at pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, and 10 at Au RDE. From Table 4.2, it was found that the changing of solution pH affected the analyte oxidation peak potential, oxide reduction potential, and analyte oxidation peak current. Similar to tetracycline hydrochloride, the oxidation peak potential and oxide reduction peak potential shifted to more negative values as the pH of the solutions increased. The highest analyte oxidation current response at the peak potential about 1.094 V vs. Ag/AgCl was obtained at the solution pH 2.5. Hence, the optimal pH value for the study of chlortetracycline hydrochloride was pH 2.5. The rotating disk cyclic voltammograms of chlortetracycline hydrochloride in different pH solutions are shown in Figure 4.6 - 4.9, respectively.

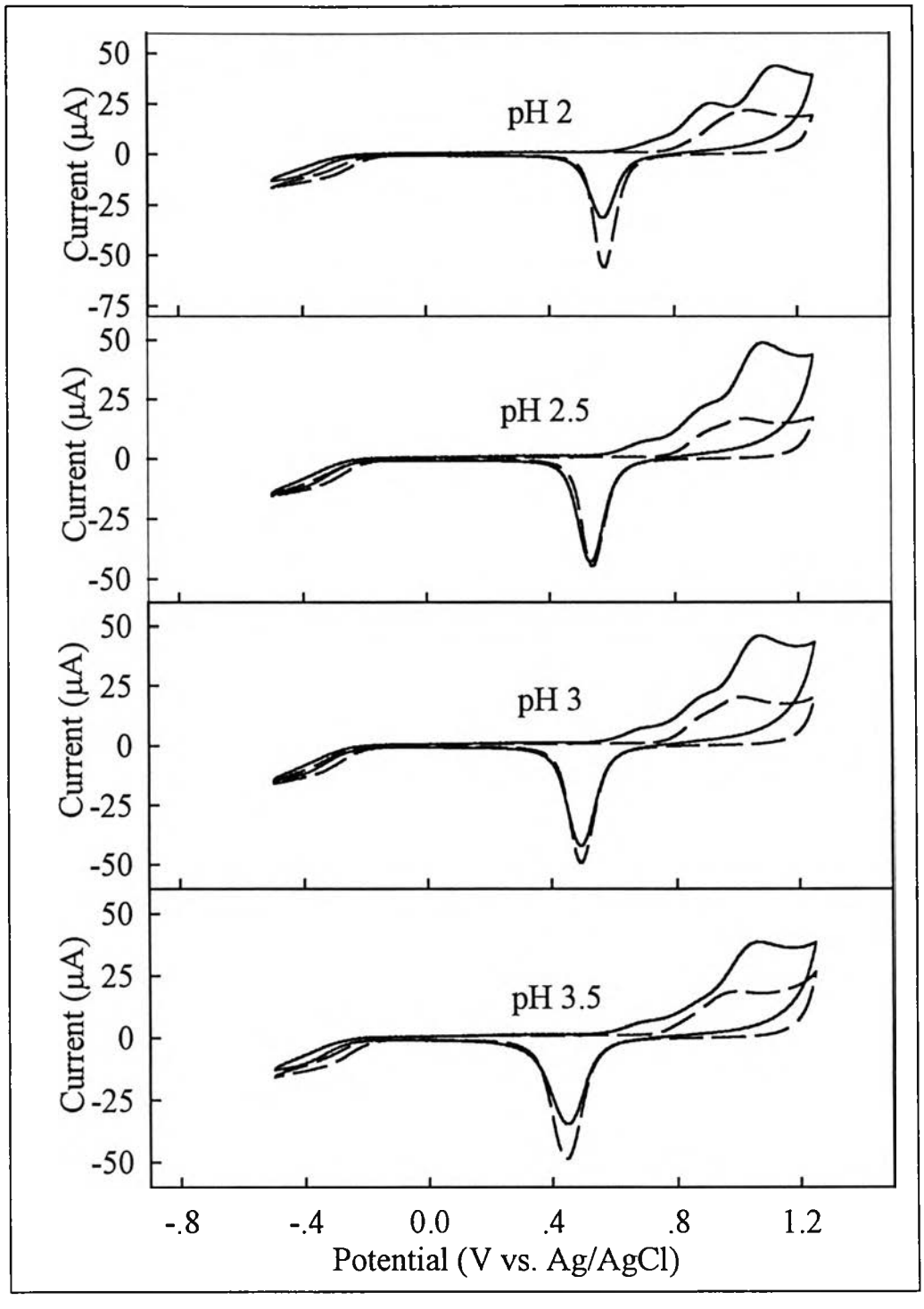


Figure 4.6 Rotating disk cyclic voltammogram of 1 mM chlortetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2, 2.5, 3 and 3.5) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

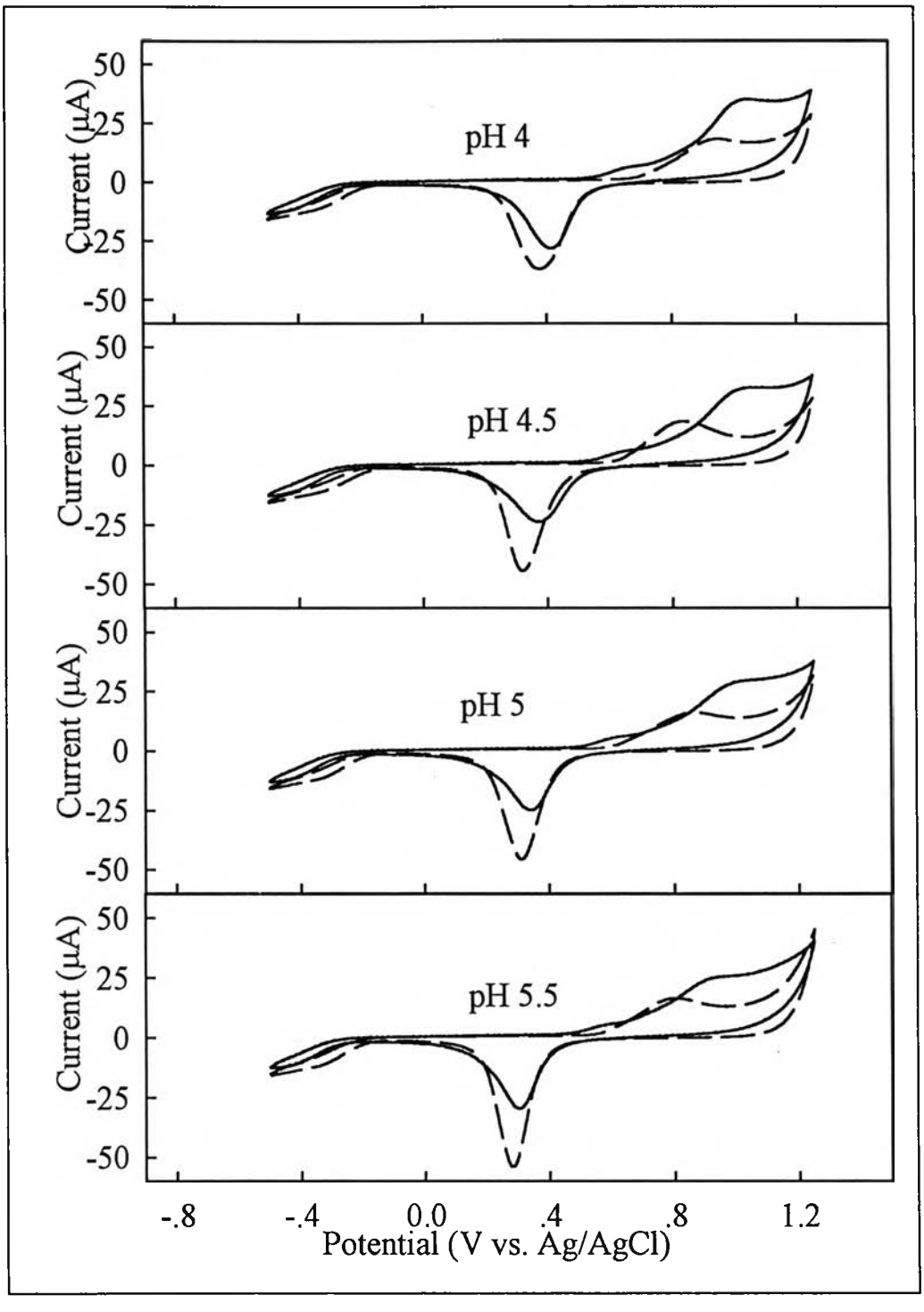


Figure 4.7 Rotating disk cyclic voltammogram of 1 mM chlortetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 4, 4.5, 5 and 5.5) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

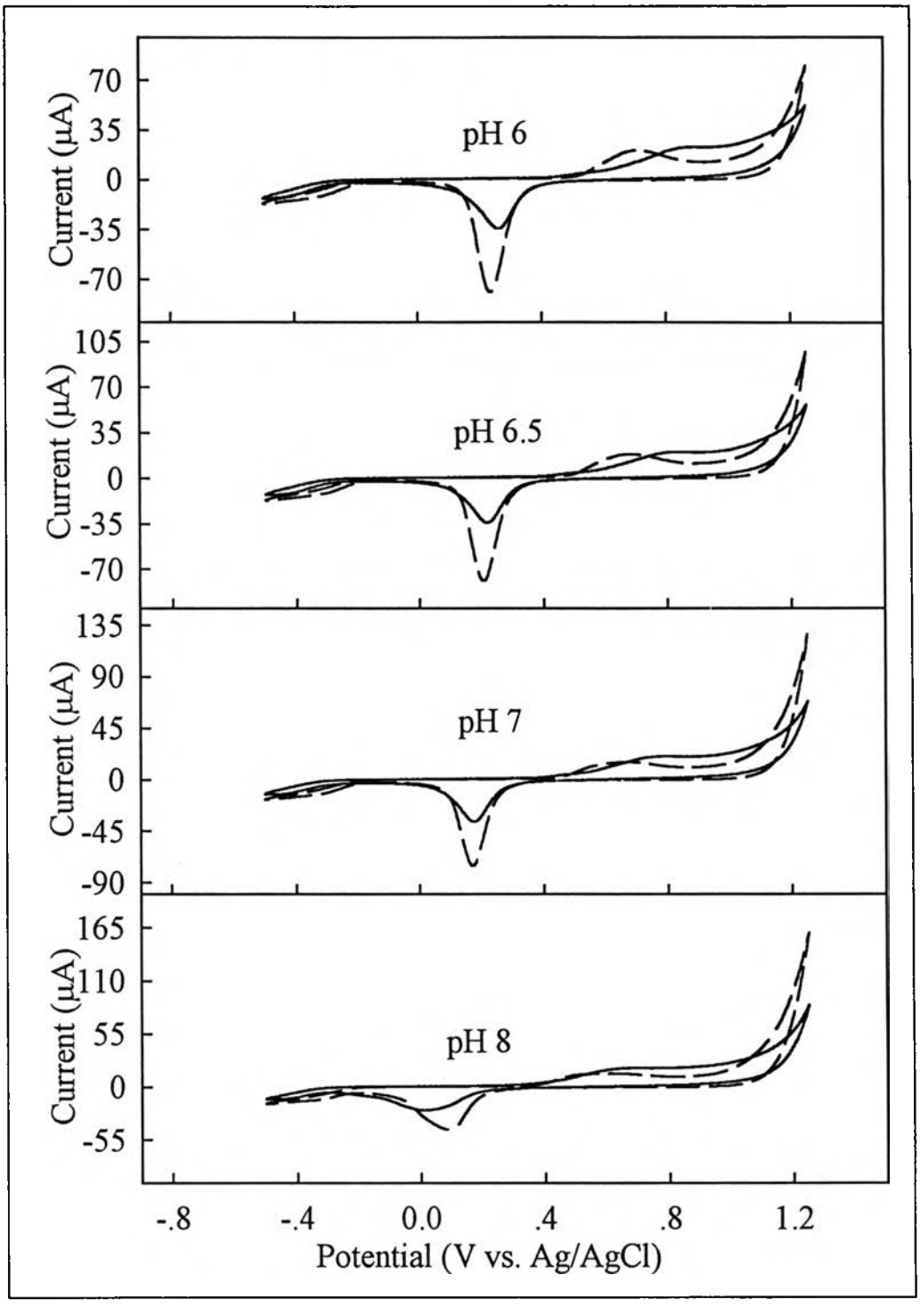


Figure 4.8 Rotating disk cyclic voltammogram of 1 mM chlortetracycline hydrochloride in 0.1 M potassium dihydrogen orthophosphate solution (pH 6, 6.5, 7 and 8) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

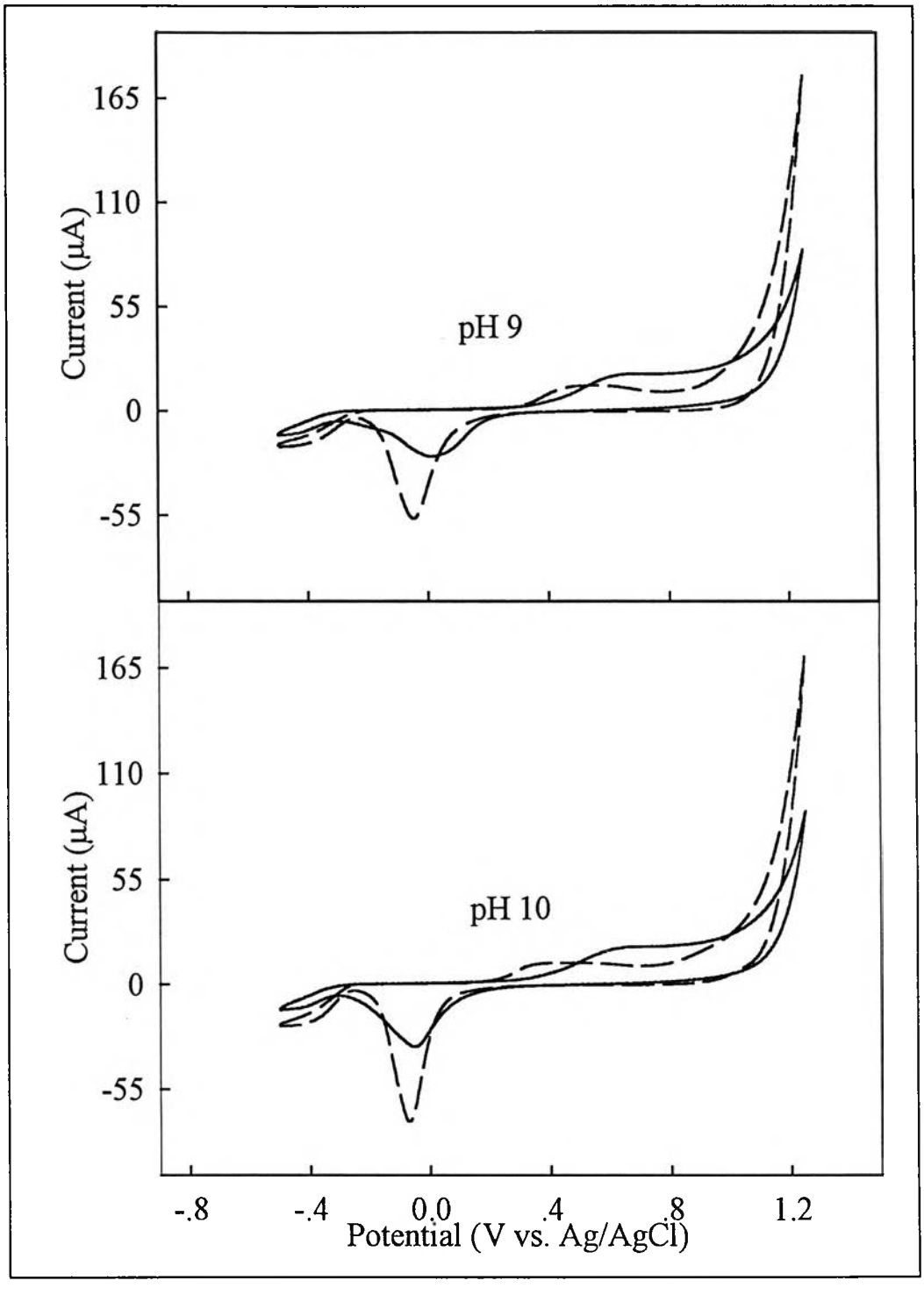


Figure 4.9 Rotating disk cyclic voltammogram of 1 mM chlortetracycline hydrochloride in 0.1 M potassium dihydrogen orthophosphate solution (pH 9 and 10) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

Table 4.2 Comparison of electrochemical data obtained from rotating disk voltammograms of the 1 mM chlortetracycline hydrochloride at pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, and 10

| pH | $E_p^{ox}$ * (V vs. Ag/AgCl) | $I_p^{ox}$ ** (μA) | $E_p^{oxide\text{-}red}$ *** (V vs. Ag/AgCl) | $I_p^{oxide\text{-}red}$ **** (μA) |
|---|---|---|---|---|
| 2 | 1.131 | 44.40 | 0.572 | -32.31 |
| 2.5 | 1.094 | 48.90 | 0.533 | -44.65 |
| 3 | 1.075 | 46.40 | 0.494 | -43.25 |
| 3.5 | 1.07 | 38.80 | 0.445 | -36.18 |
| 4 | 1.042 | 35.30 | 0.420 | -29.30 |
| 4.5 | 1.037 | 32.70 | 0.374 | -24.90 |
| 5 | 0.994 | 29.50 | 0.345 | -25.91 |
| 5.5 | 0.933 | 25.50 | 0.303 | -30.72 |
| 6 | 0.862 | 23.20 | 0.259 | -35.26 |
| 6.5 | 0.805 | 20.60 | 0.220 | -35.03 |
| 7 | 0.767 | 20.60 | 0.176 | -37.45 |
| 8 | 0.678 | 20.00 | 0.098 | -34.37 |
| 9 | 0.659 | 20.00 | 0.020 | -24.85 |
| 10 | 0.649 | 20.00 | -0.048 | -33.60 |

* Oxidation peak potential of chlortetracycline hydrochloride

**Oxidation peak current of chlortetracycline hydrochloride

*** Reduction peak potential of surface oxide

**** Reduction peak current of surface oxide

4.1.1.3 Doxycycline hydrochloride

The electrochemical data obtained from the rotating disk cyclic voltammograms of 1 mM doxycycline hydrochloride at pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, and 10 was shown in Table 4.3. Like tetracycline hydrochloride and chlortetracycline hydrochloride, the changing of solution pH affected the analyte oxidation peak potential, oxide reduction peak potential, and analyte oxidation peak current. From the electrochemical data displayed in Table 4.3, the negative shift of both analyte oxidation peak potential and oxide reduction peak potential was occurred as the solution pH increased. At the solution pH 2, the obtained oxidation current response was the highest value. Thus, the optimal pH value for the study of doxycycline hydrochloride was pH 2. The rotating disk cyclic voltammograms of doxycycline hydrochloride in different pH solutions are shown in Figure 4.10 - 4.13, respectively.

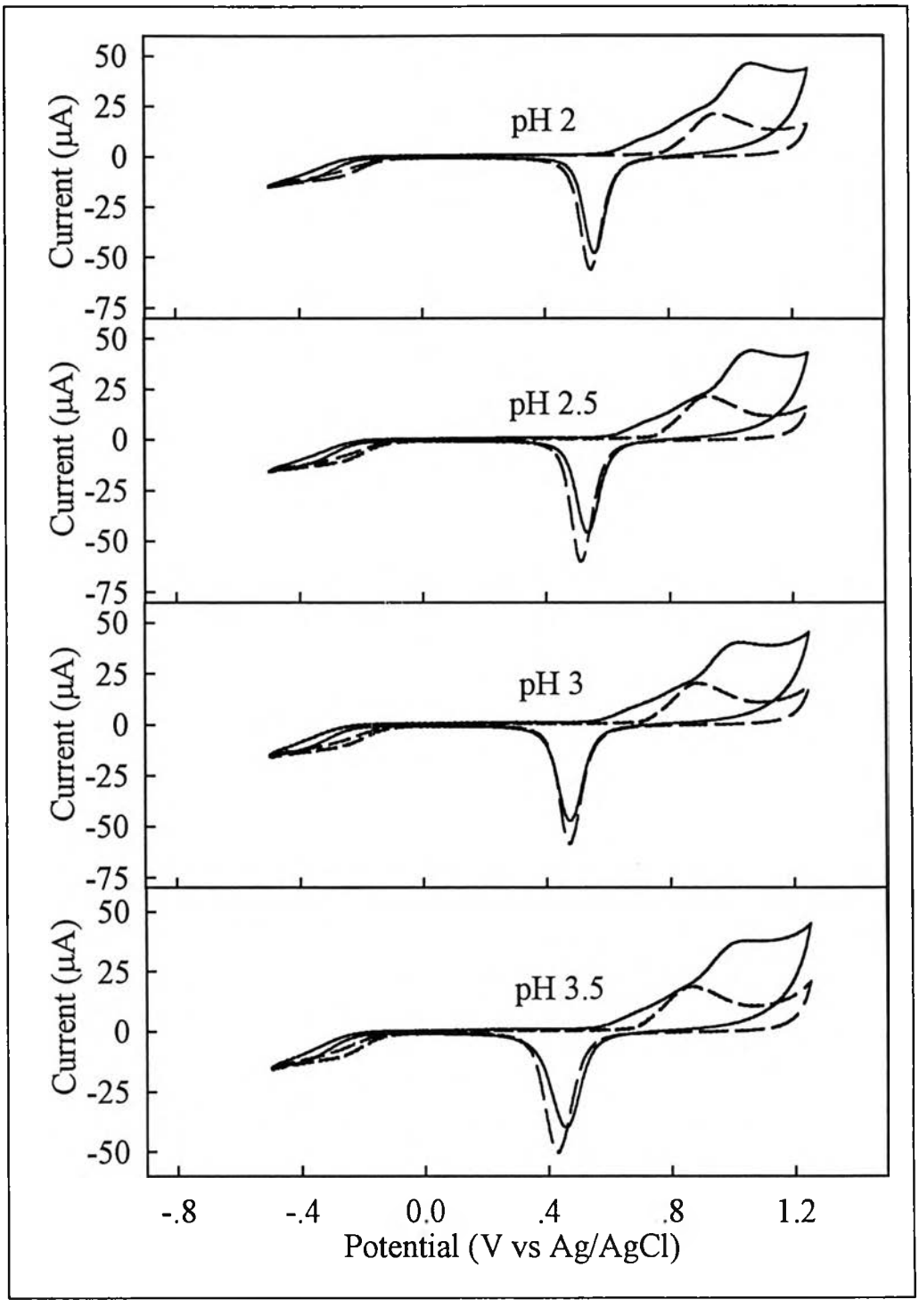


Figure 4.10 Rotating disk cyclic voltammogram of 1 mM doxycycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2, 2.5, 3, and 3.5) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

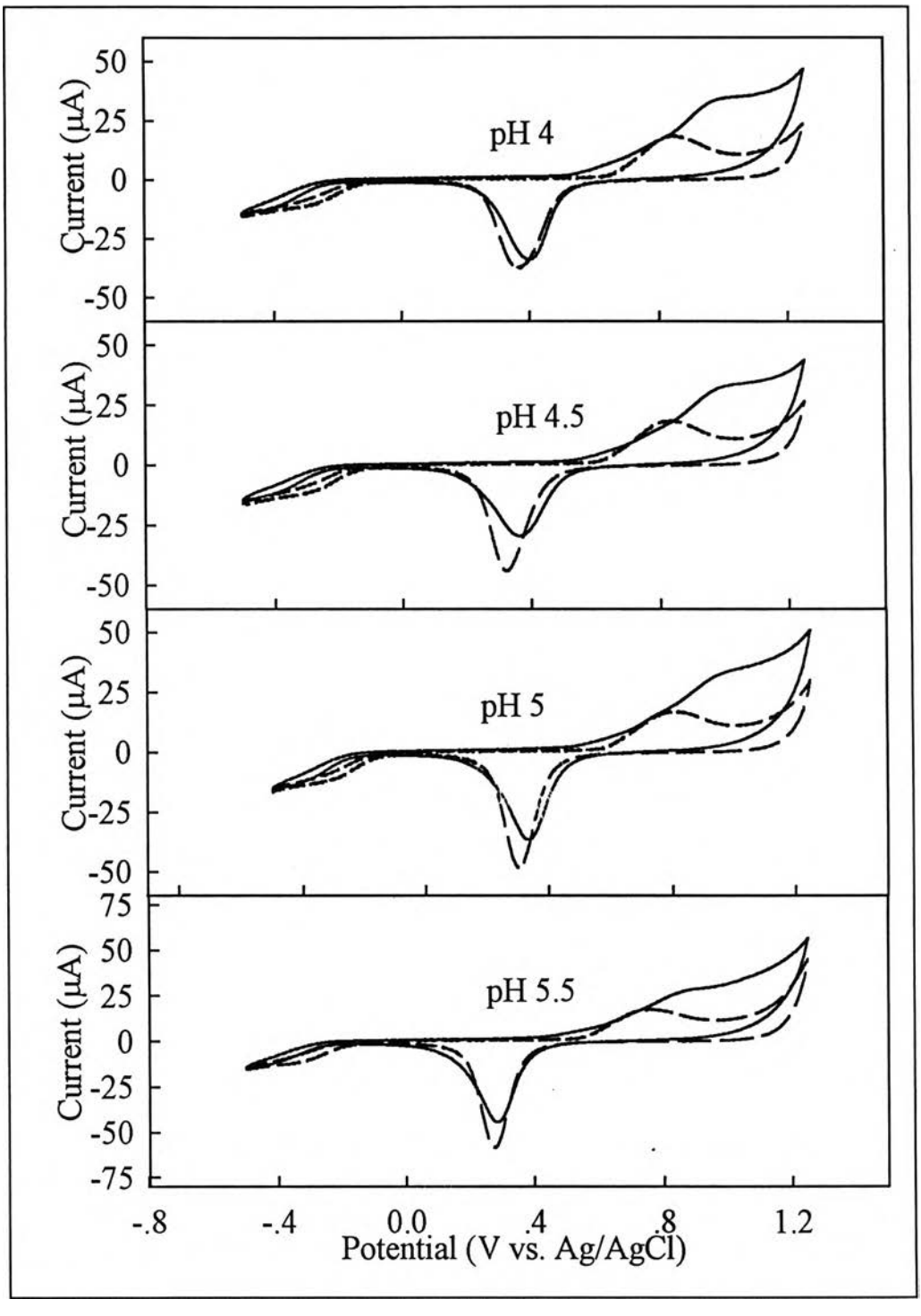


Figure 4.11 Rotating disk cyclic voltammogram of 1 mM doxycycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 4, 4.5, 5, and 5.5) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

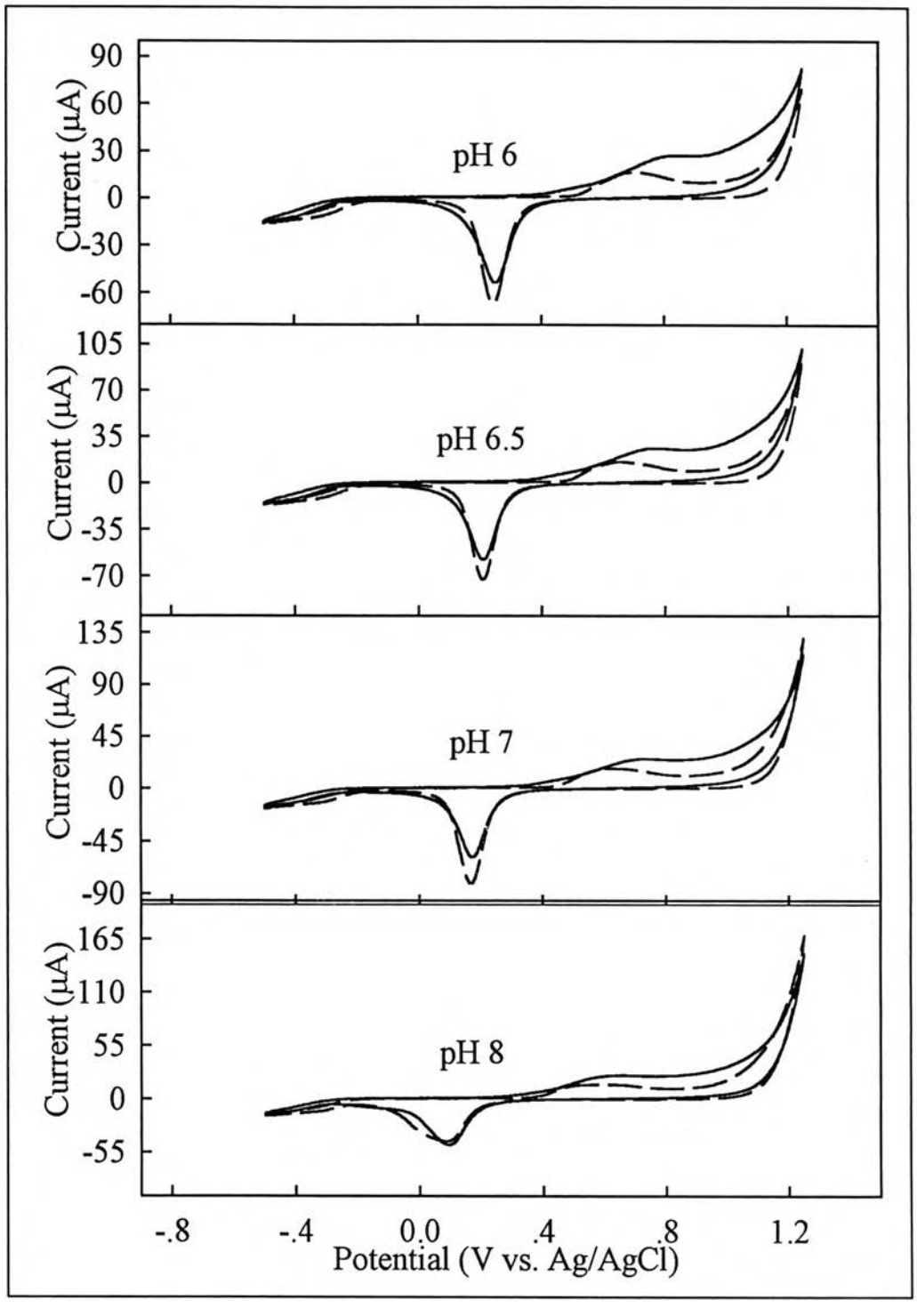


Figure 4.12 Rotating disk cyclic voltammogram of 1 mM doxycycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 6, 6.5, 7, and 8) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

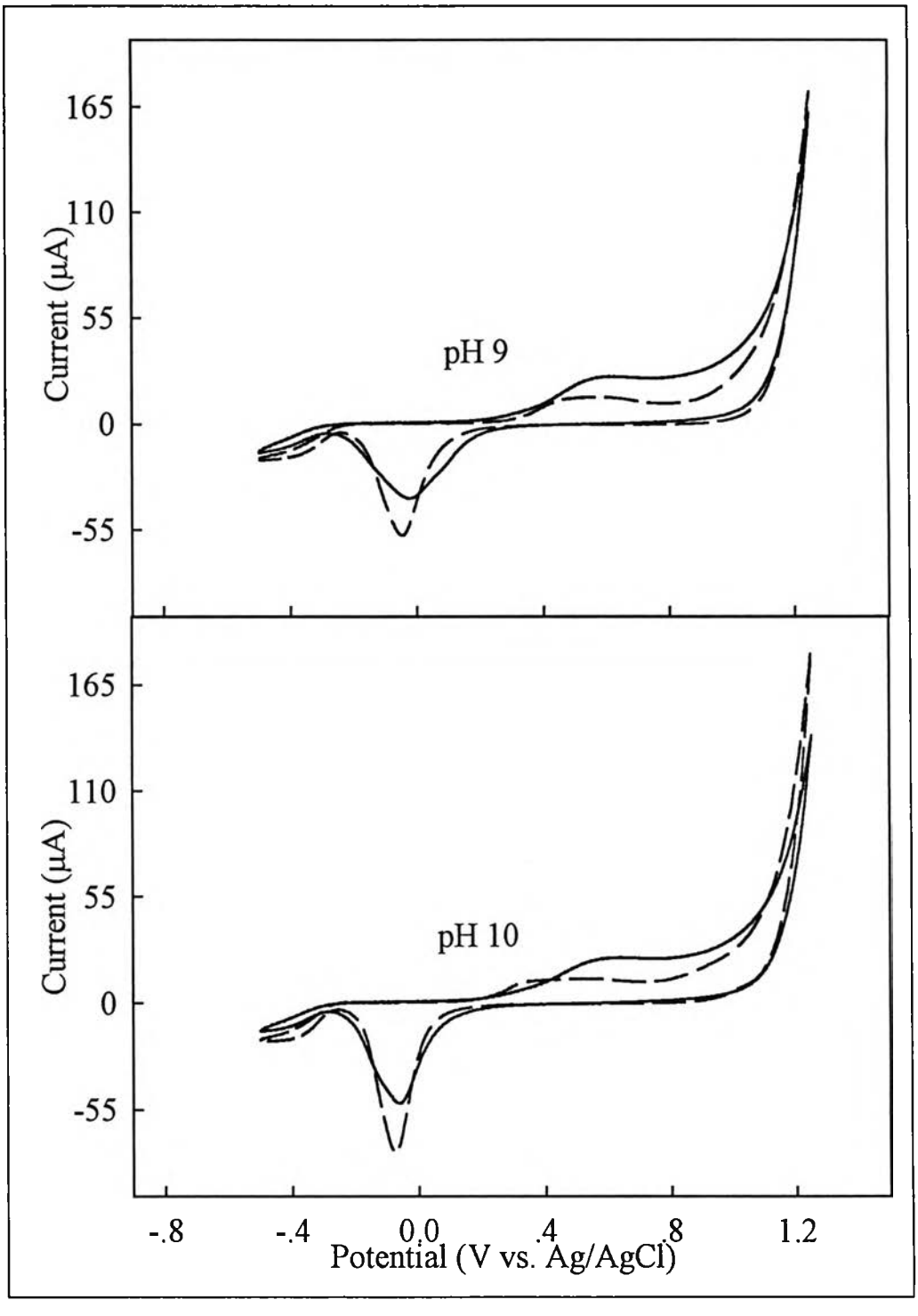


Figure 4.13 Rotating disk cyclic voltammogram of 1 mM doxycycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 9 and 10) at Au RDE (solid line). The scan rate was 50 mV $s^{-1}$. The rotation speed was 250 r.p.m. Background voltammogram is also shown in this Figure (dash line).

Table 4.3 Comparison of electrochemical data obtained from rotating disk voltammograms of the 1 mM doxycycline hydrochloride at pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, and 10

| pH | $E_p^{ox}$ * (V vs. Ag/AgCl) | $I_p^{ox}$ ** (μA) | $E_p^{oxide\text{-}red}$ *** (V vs. Ag/AgCl) | $I_p^{oxide\text{-}red}$ **** (μA) |
|---|---|---|---|---|
| 2 | 1.079 | 46.90 | 0.557 | -48.75 |
| 2.5 | 1.075 | 44.80 | 0.535 | -46.73 |
| 3 | 1.032 | 41.20 | 0.474 | -48.19 |
| 3.5 | 1.023 | 38.40 | 0.457 | -40.99 |
| 4 | 1.004 | 34.60 | 0.398 | -35.08 |
| 4.5 | 0.994 | 33.40 | 0.367 | -30.94 |
| 5 | 0.957 | 33.00 | 0.335 | -37.19 |
| 5.5 | 0.881 | 33.10 | 0.284 | -45.49 |
| 6 | 0.829 | 27.70 | 0.254 | -54.64 |
| 6.5 | 0.758 | 27.00 | 0.213 | -58.47 |
| 7 | 0.711 | 26.00 | 0.171 | -59.71 |
| 8 | 0.611 | 26.00 | 0.096 | -48.33 |
| 9 | 0.578 | 25.00 | -0.024 | -39.98 |
| 10 | 0.569 | 25.00 | -0.058 | -52.30 |

* Oxidation peak potential of doxycycline hydrochloride

**Oxidation peak current of doxycycline hydrochloride

*** Reduction peak potential of surface oxide

**** Reduction peak current of surface oxide

4.1.2 The electrochemical oxidation of the analyte

The electrochemical oxidation of 1 mM tetracycline hydrochloride at pH 2, 1 mM chlortetracycline hydrochloride at pH 2.5, and 1 mM doxycycline hydrochloride at pH 2 at Au RDE was studied. Cyclic voltammetry at the gold rotating disk electrode was used to obtain the electrochemical data of all above analytes. The obtained electrochemical data for all analytes are shown in Table 4.4. The rotating disk cyclic voltammograms obtained for 1 mM tetracycline hydrochloride at pH 2 in Figure 4.2, 1 mM chlortetracycline hydrochloride at pH 2.5 in Figure 4.6, and 1 mM doxycycline hydrochloride at pH 2 in Figure 4.10 in which the well-defined irreversible cyclic voltammograms were obtained for these analytes.

In the presence of these analytes, the anodic waves were observed on the positive scan beginning at ca. +0.8 to +1.1 V vs. Ag/AgCl for tetracycline hydrochloride, ca. +0.7 to +1.1 V vs. Ag/AgCl for chlortetracycline hydrochloride, and ca. +0.7 to +1.1 V vs. Ag/AgCl for doxycycline hydrochloride.

Table 4.4 The electrochemical data of 1 mM tetracycline hydrochloride, 1 mM chlortetracycline hydrochloride, and 1 mM doxycycline hydrochloride at Au RDE

| Analytes | $E_p^{ox}$ * (V vs. Ag/AgCl) | $I_p^{ox}$ ** (μA) | S/B [a] |
|---|---|---|---|
| Tetracycline hydrochloride | 1.104 | 50.9 | 3.305 |
| Chlortetracycline hydrochloride | 1.131 | 48.9 | 3.135 |
| Doxycycline hydrochloride | 1.079 | 46.9 | 3.752 |

[a] calculated from $I_p^{ox}$/ background current

* Oxidation peak potential

**Oxidation peak current

4.1.3 The scan rate dependence study

The effect of the scan rate on the electrochemical behaviors of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride was investigated by variation of the scan rate values from 10 to 300 mV $s^{-1}$. The rotation speed was held constant at 250 r.p.m. Figure 4.14, 4.15, and 4.16 show the relationship between the current responses and the square root of the varied scan rate values for tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride, respectively. From these results, the current responses of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride were proportional to the square root of the scan rate. It can be concluded that the diffusion process controlled the transportation of these analytes. From the rotating disk voltammograms displayed in these Figures, the oxidation of these selected analytes underwent the irreversible reaction.

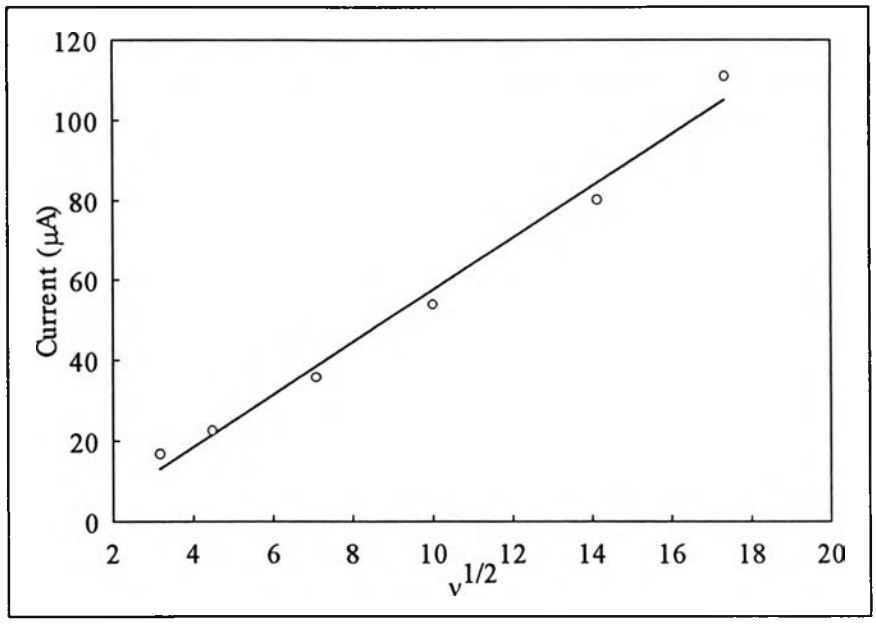


Figure 4.14 The current vs. square root of scan rate ($\upsilon^{1/2}$) curve of 1 mM tetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2) at Au RDE

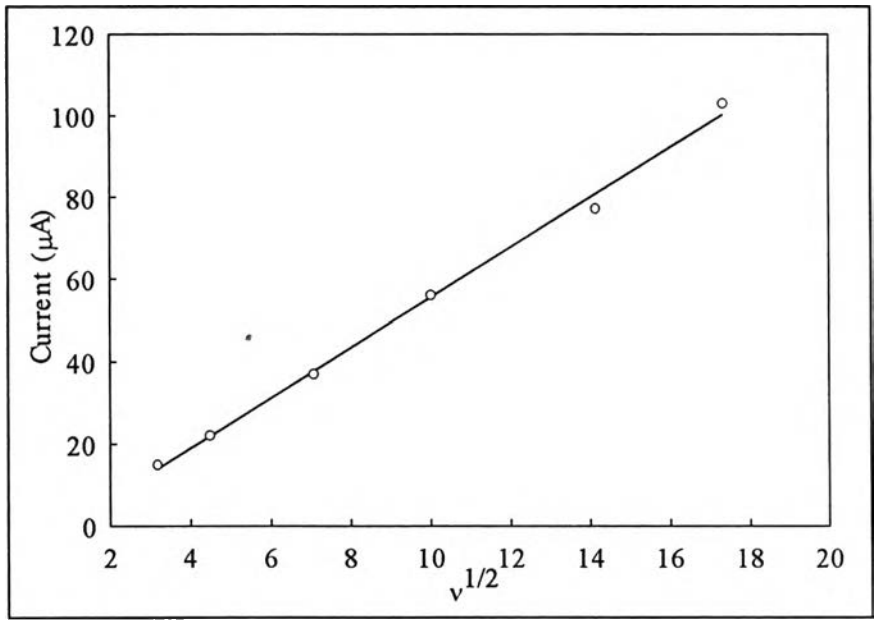


Figure 4.15 The current vs. square root of scan rate ($\upsilon^{1/2}$) curve of 1 mM chlortetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2.5) at Au RDE

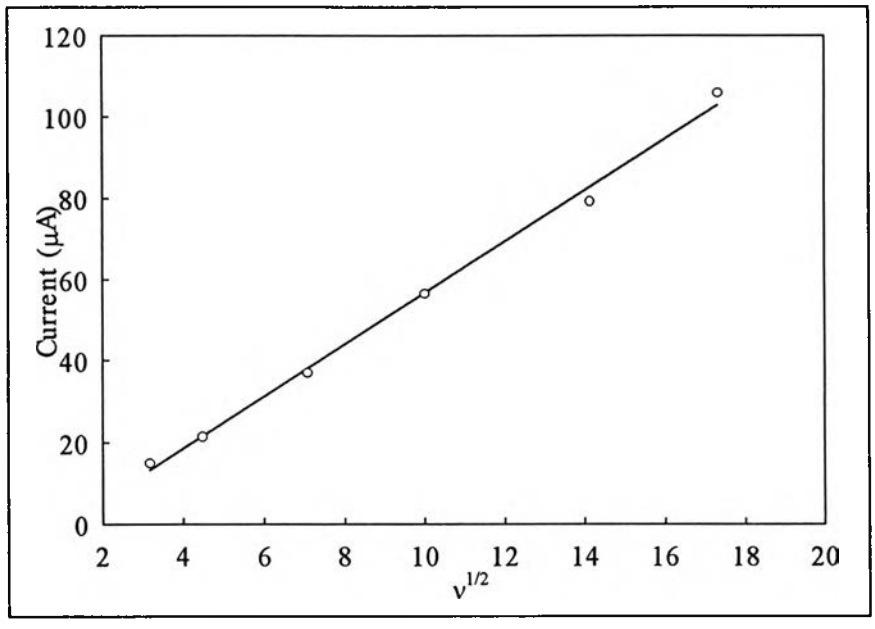


Figure 4.16 The current vs. square root of scan rate ($\upsilon^{1/2}$) curve of 1 mM doxycycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2) at Au RDE

4.1.4 The rotation speed dependence study

The electrochemical behaviors of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride, which were affected by the rotation speed, were studied. These investigations were performed by variation of the rotation speed values from 100 to 300 r.p.m. in the intervals of 50 r.p.m. The varied rotation speed values were then converted from r.p.m. to radians per second and plotted against current response. Figure 4.17, 4.18, and 4.19 show the relationship between the current responses and the varied rotation speed values for tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride, respectively.

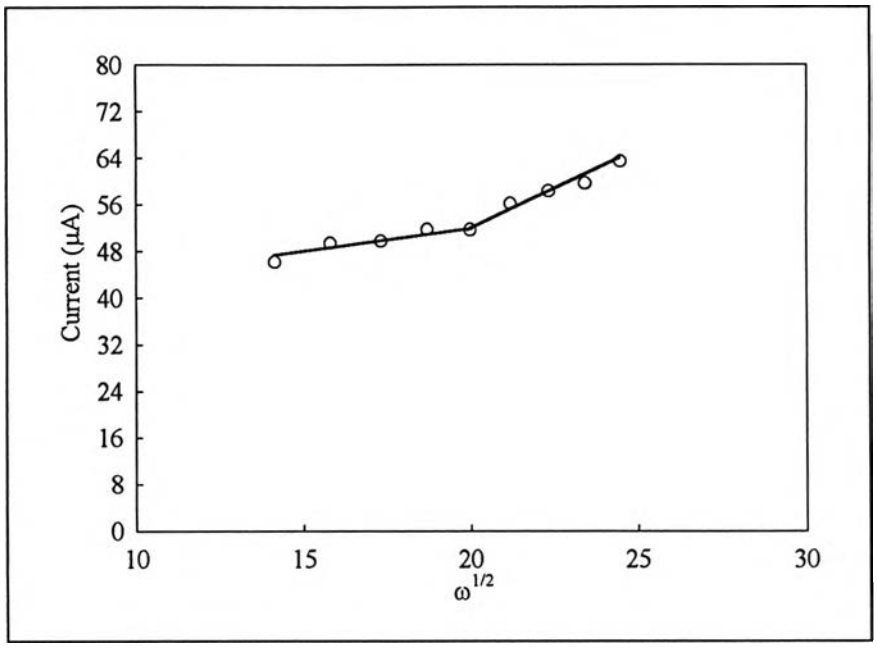


Figure 4.17 The current vs. square root of rotation speed ($\omega^{1/2}$) curve of 1 mM tetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2) at Au RDE

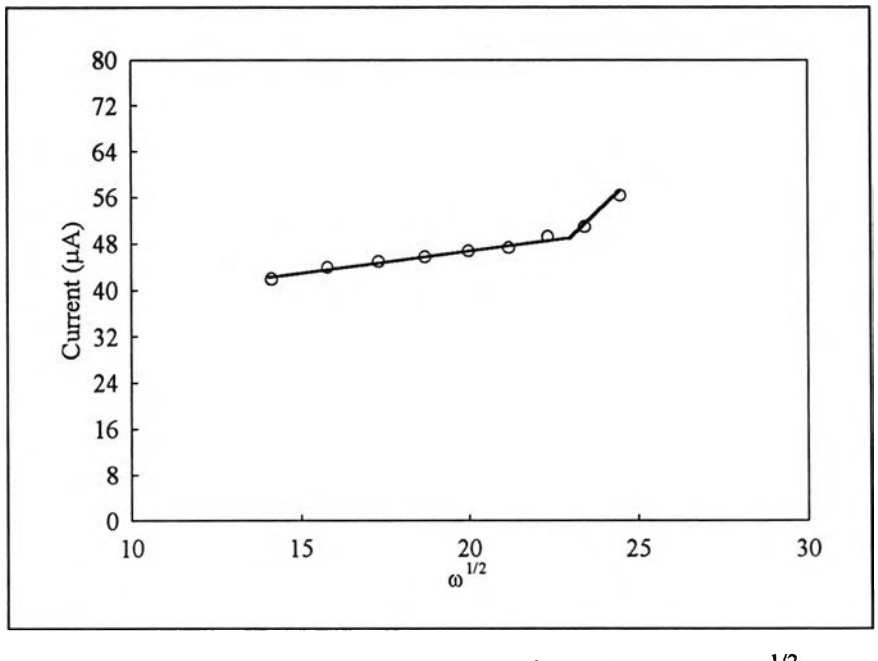


Figure 4.18 The current vs. square root of rotation speed ($\omega^{1/2}$) curve of 1 mM chlortetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2.5) at Au RDE

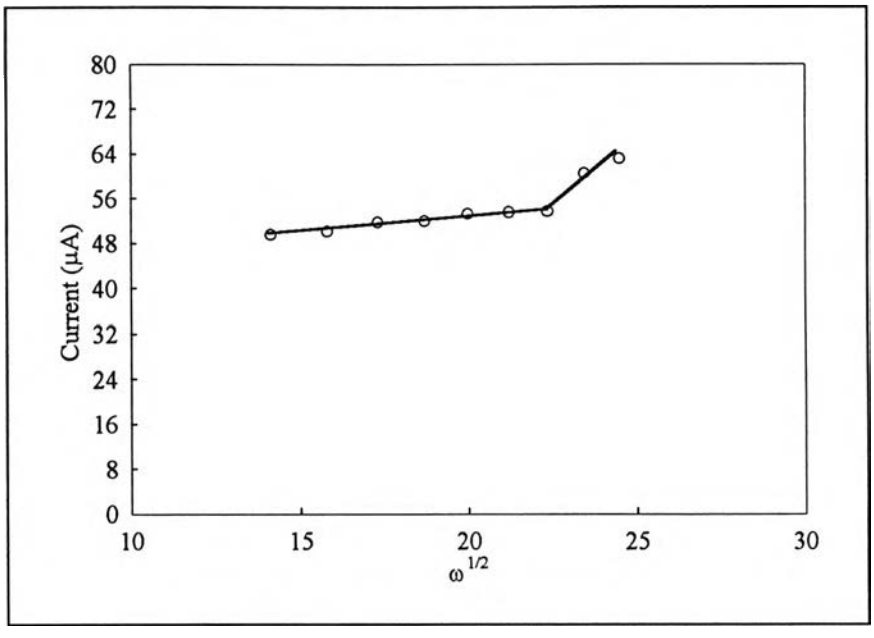


Figure 4.19 The current vs. square root of rotation speed ($\omega^{1/2}$) curve of 1 mM doxycycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2) at Au RDE

From the results displayed on Figure 4.17, 4.18, and 4.19, the anodic currents of all three antibiotics increase little change as a result of the variations in electrode rotation speed. From these results, the reactions of these compounds at Au RDE can be assumed that the electrode surface process controls them. In other word, the surface oxide catalyzes these reactions. On the other hand, if the anodic response increased with increasing in electrode rotation speed, the anodic response is under the mass transfer control.

## 4.2 Flow injection with pulsed amperometric detection

### 4.2.1 Optimization of PAD waveform parameters

This part was carried out in the flow injection system. The variation of potential parameters for the PAD waveform was obvious from the voltammetric (i-E) response of the analyte. The variation of timing parameters for the potential applications was not obvious from the voltammetric responses. These variations were based on the literature review values. The selected value of each waveform parameter was based on the value providing the highest averaged current signal. The order of PAD waveform parameter selection was detection potential ($E_{det}$), delay time ($t_{del}$), integration time ($t_{int}$), oxidation potential ($E_{oxd}$), oxidation time ($t_{oxd}$), reduction potential ($E_{red}$), and reduction time ($t_{red}$), respectively. The results of PAD waveform parameter selection of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycyline hydrochloride are shown in Figure 4.20, 4.21, and 4.22, respectively.

#### 4.2.1.1 Tetracycline hydrochloride

This compound was the first of antibiotic drugs using in this study. The variation of $E_{det}$ was first performed while the other parameters were held constant. These varied potentials were chosen from the potential region of cyclic voltammogram where the oxidation of tetracycline was occurred. The detection potential was varied from +0.8 to +1.2 V vs. Ag/AgCl in the intervals of 50 mV. The averaged oxidation peak currents obtaining from the triplicate injection of 1 mM tetracycline hydrochloride were plotted against the varied potential values. Figure 4.20 (a) shows the FI-PAD response variations for 1 mM tetracycline hydrochloride according to $E_{det}$ variation. From these results, the current responses increased with

the $E_{det}$ increased from +0.8 to +1.1 V vs. Ag/AgCl. The averaged current response at +1.15 V vs. Ag/AgCl was not different from that obtained at +1.1 V vs. Ag/AgCl detection potential. For the detection potential over +1.2 V vs. Ag/AgCl, the same relationship between the averaged current responses and detection potential was obtained. The potentials higher than +1.2 V vs. Ag/AgCl were not considered as the selected detection potential because those current responses were contributed from the evolution of oxygen. Therefore, the potential at +1.15 V vs. Ag/AgCl was chosen as the optimal detection potential due to the highest oxidation response.

The second PAD waveform parameter, detection time ($t_{det}$), consisting of delay time ($t_{del}$) and integration time ($t_{int}$) was next optimized. This parameter was the time period for the application of $E_{det}$. The first timing parameter was $t_{del}$. The range of the varied $t_{del}$ was 100 to 600 ms in the intervals of 100 ms. Figure 4.20 (b) shows the FI-PAD response variations for 1 mM tetracycline hydrochloride according to $t_{del}$ variation. It shows that the current responses increased from 100 to 500 ms of delay time and the response decayed beyond 500 ms. The value of $t_{del}$ 500 ms was selected as the optimal value. The variation of $t_{int}$ was started from 40 to 140 ms in the intervals of 20 ms. Figure 4.20 (c) shows the FI-PAD response variations for 1 mM tetracycline hydrochloride according to $t_{int}$ variation. Clearly, the averaged current response obtaining at 100 ms was the highest. Consequently, the $t_{int}$ value of 100 ms was selected as the optimal value.

The optimization of oxidation potential ($E_{oxd}$) and reduction potential ($E_{red}$) could not be considered as independent of the choice of their time periods [58]. The next optimized PAD waveform parameters were $E_{oxd}$ and $t_{oxd}$. Both $E_{oxd}$ and $t_{oxd}$ were varied for several values of them. The variation of this potential was

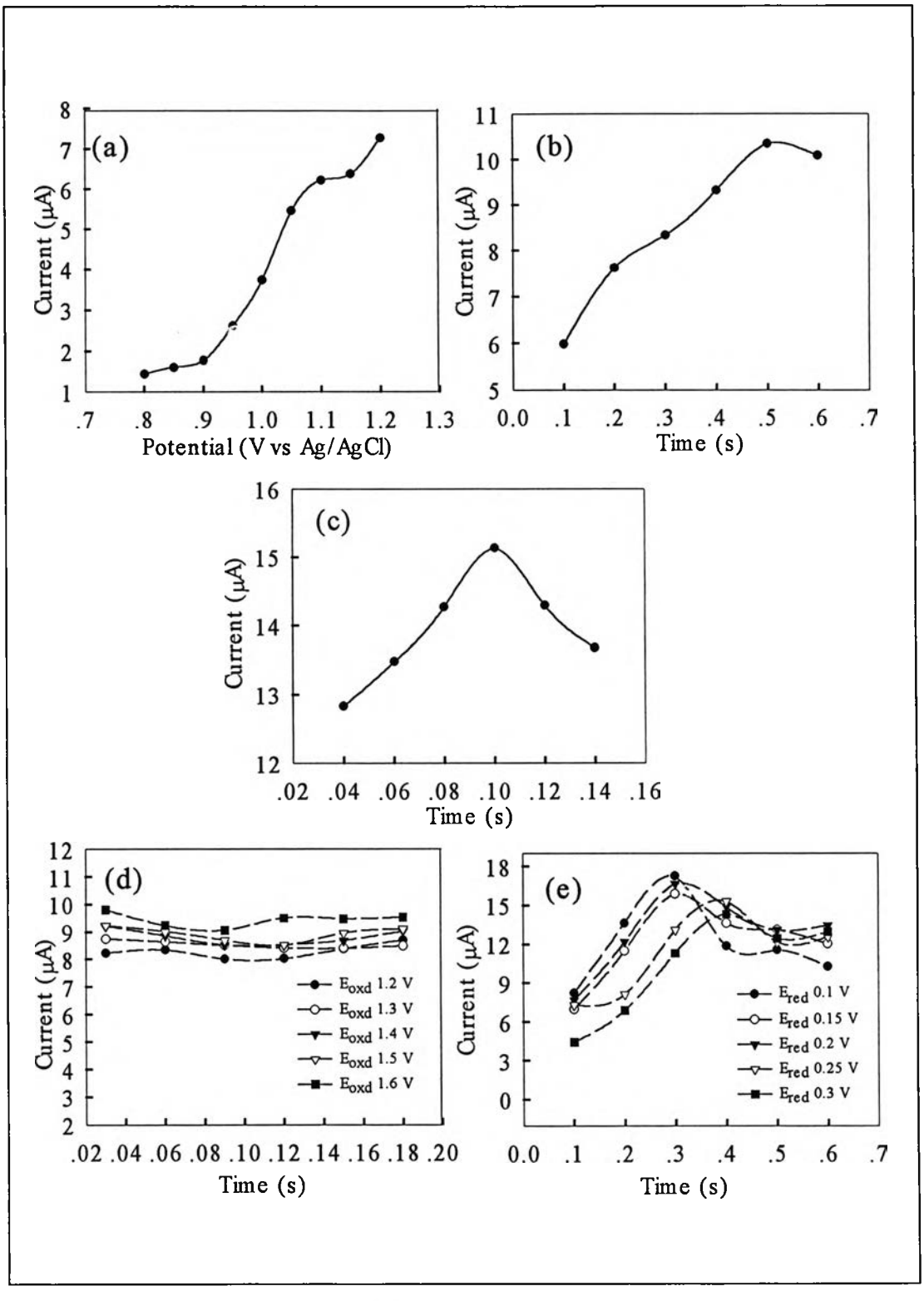


Figure 4.20 FI-PAD response as a function of (a) $E_{det}$, (b) $t_{del}$, (c) $t_{int}$, (d) $E_{oxd}$ and $t_{oxd}$, (e) $E_{red}$ and $t_{red}$ for 1 mM tetracycline hydrochloride at an Au RDE in 0.1 M $KH_2PO_4$ solution (pH 2)

started from the potential value more positive than +1.15 V vs. Ag/AgCl, $E_{det}$. The duration time of oxidation time application was based on the literature value. During the application of high positive potentials, the gold oxide layer was formed. This oxide layer was then cleaned off from the electrode surface at high negative potentials. The oxide dissolution was the cause of the electrode recession. For minimizing the dissolution and recession of the electrode surface, the shorter $t_{del}$ was recommended [59]. The varied $E_{oxd}$ range was +1.2 to +1.6 V vs. Ag/AgCl in the intervals of 100 mV. The varied $t_{oxd}$ range was 30 to 180 ms in the intervals of 30 ms. Figure 4.20 (d) shows the FI-PAD responses for 1 mM tetracycline hydrochloride as a function of $t_{oxd}$ for several values of $E_{oxd}$. Clearly, the averaged current response obtaining at $E_{oxd}$ +1.6 V vs. Ag/AgCl for every $t_{oxd}$ values were the highest current signal. For the value of $E_{oxd}$ at +1.6 V vs. Ag/AgCl, the values of $t_{oxd}$ from 120 to 180 ms provided the highest averaged current response. The values of $E_{oxd}$ at +1.6 V vs. Ag/AgCl and $t_{oxd}$ at 150 ms were selected as the optimal values.

The last optimized PAD waveform parameters for tetracycline hydrochloride were $E_{red}$ and $t_{red}$. The variation of reduction potential was started from the potential value more negative than +1.15 V vs. Ag/AgCl, $E_{det}$. The duration of reduction time was based on the literature value, which is similar to the detection time and oxidation time. The varied $E_{red}$ range was +0.1 to +0.3 V vs. Ag/AgCl in the intervals of 50 mV. The varied $t_{red}$ range was 100 to 600 ms in the intervals of 100 ms. Figure 4.20 (e) shows the FI-PAD responses for 1 mM tetracycline hydrochloride as a function of $t_{red}$ for several values of $E_{red}$. These results show that the highest averaged current response was obtained at $E_{red}$ +0.1 V vs. Ag/AgCl for the $t_{red}$ of 300 ms. Hence, these potential and timing values were chosen as the optimal values. The overall optimal PAD waveform parameters are shown in Table 4.5.

Table 4.5 Optimal waveform parameters for pulsed amperometric detection of tetracycline hydrochloride at an Au working electrode

| Potential (V vs. Ag/AgCl) | | Time (ms) | |
|---|---|---|---|
| parameter | optimal | parameter | optimal |
| $E_{det}$ | 1.15 | $t_{del}$ | 500 |
| | | $t_{int}$ | 100 |
| $E_{oxd}$ | 1.6 | $t_{oxd}$ | 130 |
| $E_{red}$ | 0.1 | $t_{red}$ | 300 |

4.2.1.2 Chlortetracycline hydrochloride

The second of antibiotic drugs used in this study was chlortetracycline hydrochloride. The first parameter optimized for PAD waveform was $E_{det}$. The other waveform parameters were held constant whereas this parameter was varied. The potential region of cyclic voltammetric where the oxidation of chlortetracycline occurred was used for the detection potential variation. This potential variation was started from +0.8 to +1.2 V vs. Ag/AgCl in the intervals of 50 mV. The obtained oxidation peaks from the triplicate injections of 0.5 mM chlortetracycline hydrochloride were then averaged. These averaged peak currents were plotted against the varied potential values. Figure 4.21 (a) shows the FI-PAD responses belonging to $E_{det}$ variation for 0.5 mM chlortetracycline hydrochloride. The selection of $E_{det}$ was also based on the potential providing highest current response. For the $E_{det}$ from +0.8 to +1.0 V vs. Ag/AgCl, the current responses were directly proportional to the varied potential values. Unlike the $E_{det}$ from +0.8 to +1.0 V vs.

Ag/AgCl, the potentials above +1.05 to +1.2 V vs. Ag/AgCl produced the constant current responses. The potential at +1.05 V vs. Ag/AgCl was chosen as the optimal detection potential. This selected potential was appropriated because it was not higher than +1.2 V vs. Ag/AgCl where the oxygen evolution was occurred.

The detection time ($t_{det}$) consisting of $t_{del}$ and $t_{int}$ was the next optimized PAD waveform parameter. The $E_{det}$ was applied during this time period. The same as tetracycline hydrochloride, the variation of $t_{del}$ was started from 100 to 600 ms in the intervals of 100 ms. The triplicate injections of 0.5 mM chlortetracycline hydrochloride were performed. The obtained peak currents were then averaged. Figure 4.21 (b) shows the FI-PAD responses belonging to $t_{del}$ variation for 0.5 mM chlortetracycline hydrochloride. According to these results, the selected $t_{del}$ was 200 ms due to the highest current response. In the same manner, the variation of $t_{int}$ was performed in the range of 30 to 180 ms in the intervals of 30 ms. Figure 4.21 (c) shows the FI-PAD responses belonging to $t_{int}$ variation for 0.5 mM chlortetracycline hydrochloride. These results suggested that the value of $t_{int}$ at 100 ms provided the highest averaged current response. This time value was selected as the optimal value.

The next potential and timing parameters could not be considered as independent of the choice of each other. The optimization of $E_{oxd}$ and $t_{oxd}$ was performed by variation of the values of them and selection the choice of their optimal values. The variation of this positive cleaning potential was set from the potential value more positive than +1.05 V vs. Ag/AgCl, $E_{det}$. As mention above, the shorter time period of $E_{oxd}$ application was the appropriated value. The values for the $E_{oxd}$ variation were within the range of +1.2 to +1.6 V vs. Ag/AgCl in the intervals of 100 mV. The variation of $t_{oxd}$ was performed within the range of 30 to 180 ms in the

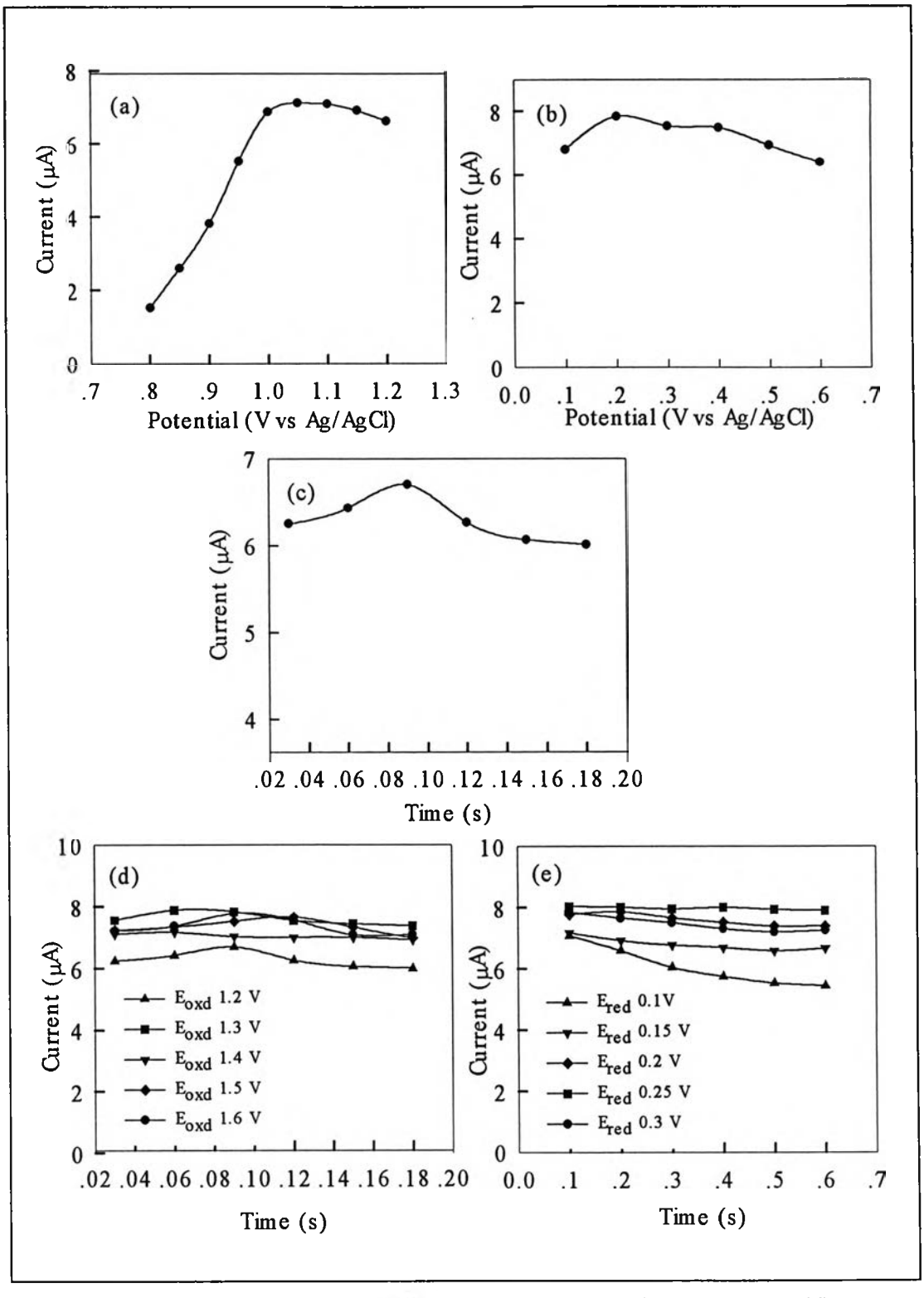


Figure 4.21 FI-PAD response as a function of (a) $E_{det}$, (b) $t_{del}$, (c) $t_{int}$, (d) $E_{oxd}$ and $t_{oxd}$, (e) $E_{red}$ and $t_{red}$ for 0.5 mM chlortetracycline hydrochloride at an Au RDE in 0.1 M $KH_2PO_4$ solution (pH 2.5)

intervals of 30 ms. Figure 4.21 (d) shows the FI-PAD responses for 0.5 mM chlortetracycline hydrochloride as a function of $t_{oxd}$ for several values of $E_{oxd}$. From these results, the highest averaged current response was obtained at $E_{oxd}$ +1.3 V vs. Ag/AgCl for the $t_{oxd}$ of 70 ms. Thus, these potential and timing values were selected as the optimal values.

The optimization of $E_{red}$ and $t_{red}$ was the last optimized parameters for chlortetracycline hydrochloride. As the $E_{oxd}$ and $t_{oxd}$ optimization, the selected $E_{red}$ value was considered together with the value of $t_{red}$. Figure 4.21 (e) shows the FI-PAD responses for 0.5 mM chlortetracycline hydrochloride as a function of $t_{red}$ for several values of $E_{red}$. From the figure as shown, the $E_{red}$ variation was started from +0.1 to +0.3 V vs. Ag/AgCl in the intervals of 50 mV. The variation of $t_{red}$ was started from 100 to 600 ms in the intervals of 100 ms. According to these results, the value of $E_{red}$ providing the highest averaged current response was +0.25 V vs. Ag/AgCl. This potential value was selected as the optimal value. For the value of this $E_{red}$ shown, the value of $t_{red}$ at 400 ms was chosen as the optimal value for PAD waveform.

Table 4.6 Optimal waveform parameters for pulsed amperometric detection of chlortetracycline hydrochloride at an Au working electrode

| Potential (V vs. Ag/AgCl) | | Time (ms) | |
|---|---|---|---|
| parameter | optimal | parameter | optimal |
| $E_{det}$ | 1.05 | $t_{del}$ | 200 |
| | | $t_{int}$ | 100 |
| $E_{oxd}$ | 1.3 | $t_{oxd}$ | 70 |
| $E_{red}$ | 0.25 | $t_{red}$ | 400 |

4.2.1.3 Doxycycline hydrochloride

The last of the antibiotic drugs used in this study was doxycycline hydrochloride. The optimization of $E_{det}$ was carried out by variation this potential and selection the potential that provided the highest averaged current response. The values of the varied potentials were chosen from the cyclic voltammogram of this antibiotic drug. The potential region of +0.8 to +1.2 V vs. Ag/AgCl of the anodic signal on the positive scan was used for the variation of this potential. The averaged peak currents obtained from the triplicate injections of 0.5 mM doxycycline hydrochloride were plotted against the varied detection potentials. Figure 4.22 (a) shows the FI-PAD response variations for 0.5 mM doxycycline hydrochloride according to $E_{det}$ variation. It was found that the highest averaged peak current was obtained at the $E_{det}$ of 1.15 V vs. Ag/AgCl. Hence, this potential value was selected as the optimal value of $E_{det}$.

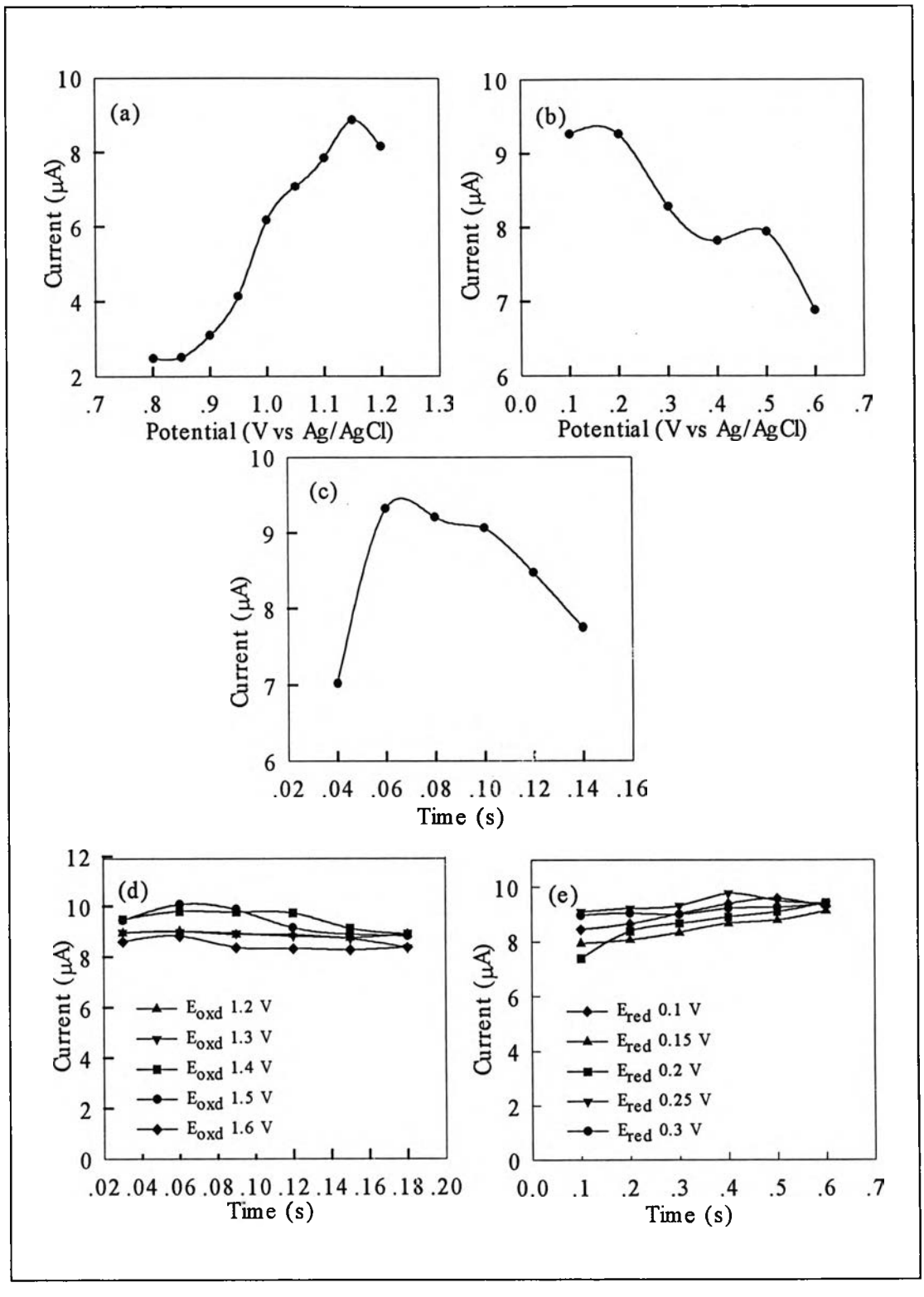


Figure 4.22 FI-PAD response as a function of (a) $E_{det}$, (b) $t_{del}$, (c) $t_{int}$, (d) $E_{oxd}$ and $t_{oxd}$, (e) $E_{red}$ and $t_{red}$ for 0.5 mM doxycycline hydrochloride at an Au RDE in 0.1 M $KH_2PO_4$ solution (pH 2)

The next optimized PAD waveform parameter was $t_{det}$. This timing parameter consisted of $t_{del}$ and $t_{int}$. The variation of $t_{del}$ and $t_{int}$ values was based on the literature survey values. The same as tetracycline hydrochloride and chlortetracycline hydrochloride, the variation of $t_{del}$ was started from 100 to 600 ms in the intervals of 100 ms. An optimal value of $t_{del}$ was chosen from the value providing the highest averaged peak current. Figure 4.22 (b) shows the FI-PAD responses belonging to $t_{del}$ variation for 0.5 mM doxycycline hydrochloride. From these results, the value of $t_{del}$ at 200 ms was selected as the optimal value for the PAD waveform of doxycycline hydrochloride determination. The time period for integration of the analyte oxidation current was the next optimized timing parameter. The duration of the variation of $t_{int}$ was started from 40 to 140 ms in the intervals of 20 ms. Figure 4.22 (c) shows the FI-PAD response variations for 0.5 mM doxycycline hydrochloride according to $t_{int}$ variation. By considering these results, the value of $t_{int}$ at 70 ms offered the highest averaged peak current. Thus, the optimal value of $t_{int}$ was 70 ms.

The optimization of the two interdependent parameters was next optimized. These two interdependent parameters were $E_{oxd}$ and $t_{oxd}$. The same as tetracycline hydrochloride and chlortetracycline hydrochloride, the variation of $E_{oxd}$ was conducted from +1.2 to +1.6 V vs. Ag/AgCl in the intervals of 100 mV. The variation of $t_{oxd}$ was set from 30 to 180 ms in the intervals of 30 ms. Figure 4.22 (d) shows the FI-PAD responses for 0.5 mM doxycycline hydrochloride as a function of $t_{oxd}$ for several values of $E_{oxd}$. These results demonstrated that the averaged current responses of each $E_{oxd}$ value were slightly affected from the variation of $t_{oxd}$. The highest averaged current response was obtained at +1.5 V vs. Ag/AgCl for 70 ms, $t_{oxd}$. Hence, these potential and timing values were chosen as the optimal values of $E_{oxd}$ and $t_{oxd}$, respectively.

The last of the two interdependent parameters was $E_{red}$ and $t_{red}$. During the optimization of these parameters, the other parameters were set at their optimal values. The range of the varied $E_{red}$ was +0.1 to +0.3 V vs. AgAg/Cl in the intervals of 50 mV. The variation of $t_{red}$ was performed within the range of 100 to 600 ms in the intervals of 100 ms. Figure 4.22 (e) shows the FI-PAD responses for 0.5 mM doxycycline hydrochloride as a function of $t_{red}$ for several values of $E_{red}$. From these results, the highest averaged peak current was achieved at $E_{red}$ +0.25 V vs. Ag/AgCl. As a result, the value of $E_{red}$ at +0.25 V vs. Ag/AgCl was chosen as the optimal value. For the value of this $E_{red}$ shown, the selected $t_{red}$ value was 40 ms due to the highest averaged current response.

Table 4.7 Optimal waveform parameters for pulsed amperometric detection of doxycycline hydrochloride at an Au working electrode

| Potential (V vs. Ag/AgCl) | | Time (ms) | |
|---|---|---|---|
| parameter | optimal | parameter | optimal |
| $E_{det}$ | 1.15 | $t_{del}$ | 150 |
| | | $t_{int}$ | 70 |
| $E_{oxd}$ | 1.5 | $t_{oxd}$ | 70 |
| $E_{red}$ | 0.25 | $t_{red}$ | 400 |

4.2.2 Calibration and linearity

The concentration range of 1 μM to 2 mM of the analyte solutions was used to obtain the calibration curve and the linear dynamic range. The averaged peak currents from triplicate injections of each analyte solution were plotted versus the varied concentrations. A linear dynamic range was obtained over 2-3 order of magnitude. The linear regression equation was obtained by least square adjustment as following:

$$y = mx + c$$

where y : current signal (μA)

m : slope or sensitivity (μA $mM^{-1}$)

x : analyte concentration (mM) and c is the intercept (μA)

Figure 4.23, 4.24, and 4.25 show the effect of the concentration of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride on peak current, respectively. From these results, the linear dynamic range (LDR) of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride were 1 μM to 0.6 mM, 1 μM to 0.1 mM, and 1 μM to 0.1 mM, respectively. The sensitivity, as the slope of these calibration plots, is 13.697 μA $mM^{-1}$ for tetracycline hydrochloride, 33.758 μA $mM^{-1}$ for chlortetracycline hydrochloride, and 23.354 μA $mM^{-1}$ for doxycycline hydrochloride, with the correlation coefficients of 0.999. The regression parameters are summarized in Table 4.8.

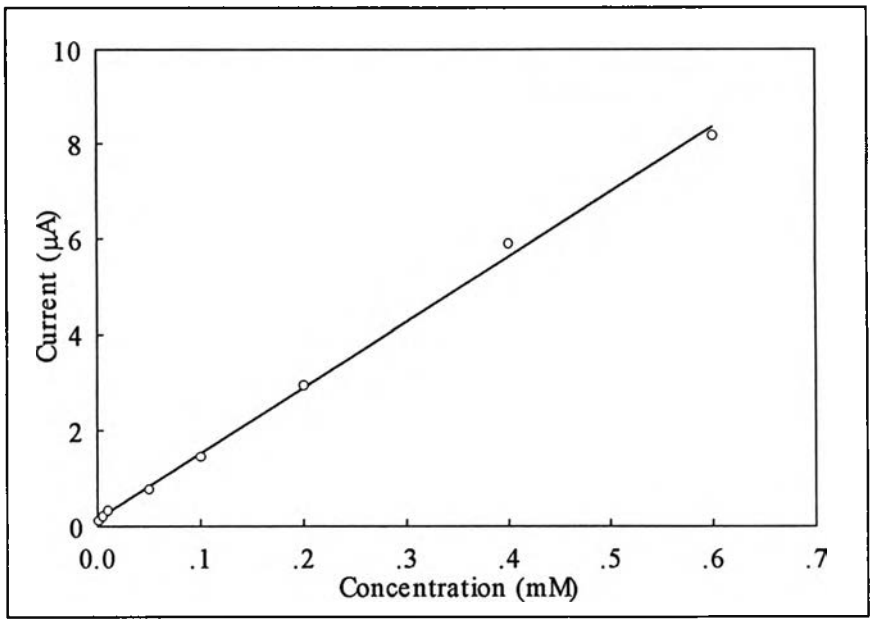


Figure 4.23 Calibration curve of tetracycline hydrochloride (0.001 – 0.6 mM) in 0.1 M $KH_2PO_4$ solution (pH 2) at the Au RDE. The scan rate was 50 mV $s^{-1}$.

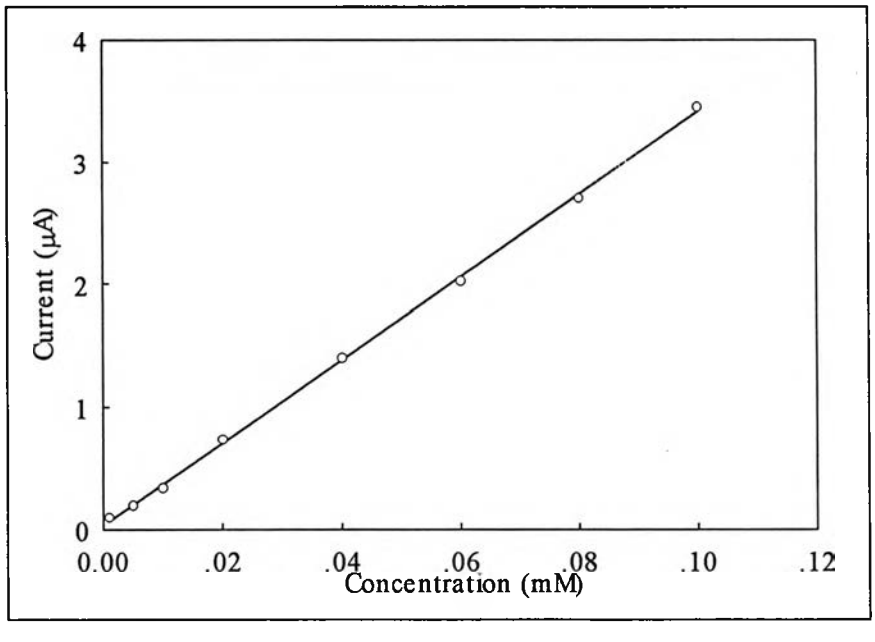


Figure 4.24 Calibration curve of chlortetracycline hydrochloride (0.001 – 0.1 mM) in 0.1 M $KH_2PO_4$ solution (pH 2.5) at the Au RDE. The scan rate was 50 mV $s^{-1}$.

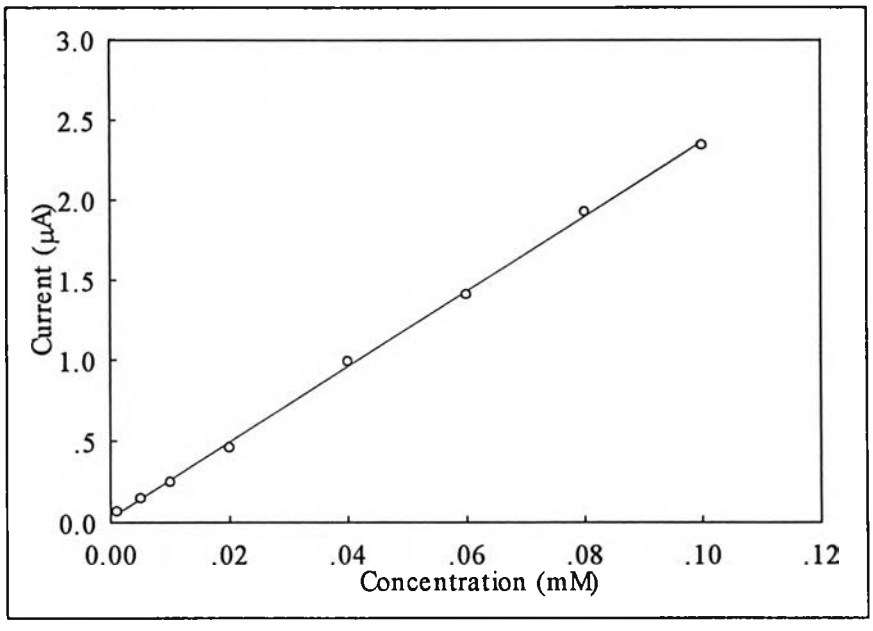


Figure 4.25 Calibration curve of doxycycline hydrochloride (0.001 – 0.1 mM) in 0.1 M $KH_2PO_4$ solution (pH 2) at the Au RDE. The scan rate was 50 mV $s^{-1}$.

Table 4.8 Regression statistics for tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride

| Analytes | LDR (mM) | Sensitivity (μA $mM^{-1}$) | Intercept (μA) | $R^2$ |
|---|---|---|---|---|
| Tetracycline hydrochloride | 0.001-0.6 | 13.697 | 0.144 | 0.9980 |
| Chlortetracycline hydrochloride | 0.001-0.1 | 33.758 | 0.037 | 0.9994 |
| Doxycycline hydrochloride | 0.001-0.1 | 23.354 | 0.027 | 0.9992 |

4.2.3 Limit of Detection (LoD)

The detection limit was investigated by examine various concentration of tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride from 1 μM to 2 mM. The limit of detection was determined under the definition of three times of signals to noise ratio that is 3S/N. Figure 4.26 shows the FI-PAD responses for three repetitive injections of 1 μM tetracycline hydrochloride in potassium dihydrogen orthophosphate solution (pH 2). It was found that the limit of detection was 1 μM. Figure 4.27 shows the FI-PAD responses for three repetitive injections of 1 μM chlortetracycline hydrochloride in potassium dihydrogen orthophosphate solution (pH 2.5). From these results, the detection limit obtained from this proposed method was 1 μM for chlortetracycline hydrochloride. Figure 4.28 shows the FI-PAD responses for three repetitive injections of 1 μM doxycycline hydrochloride in potassium dihydrogen orthophosphate solution (pH 2). The same as tetracycline hydrochloride and chlortetracycline hydrochloride, the detection limit was 1 μM.

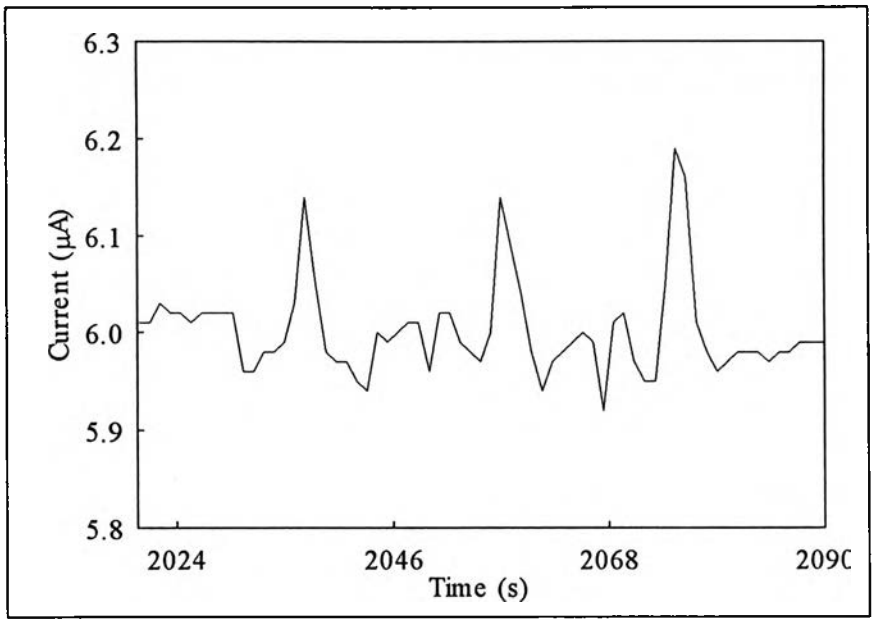


Figure 4.26 Flow injection analysis with pulsed amperometric detection results for 1 μM tetracycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2). The flow rate was 1 ml $min^{-1}$.

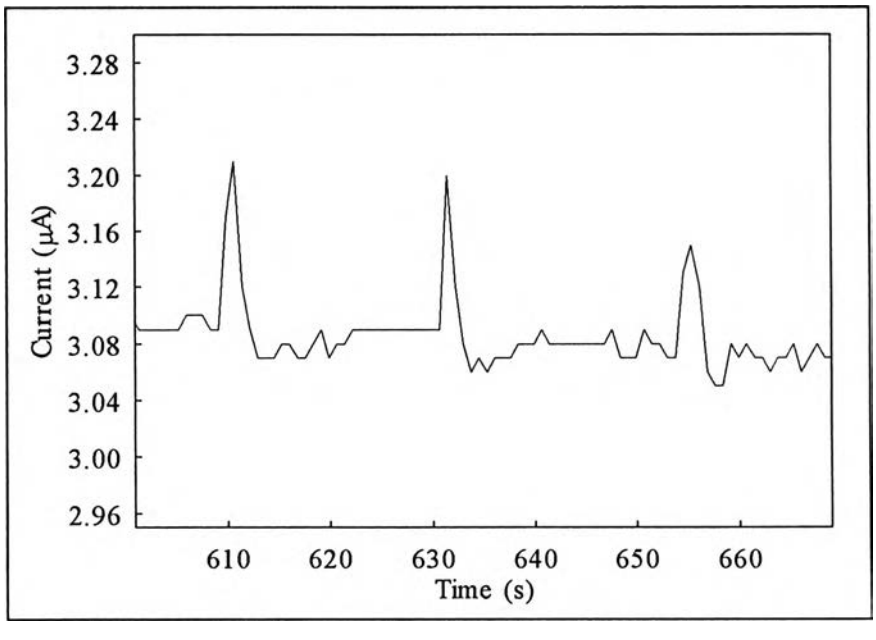


Figure 4.27 Flow injection analysis with pulsed amperometric detection results for 1 μM chlortetracycline hydrochloride in 0.1 $KH_2PO_4$ solution (pH 2.5). The flow rate was 1 ml $min^{-1}$.

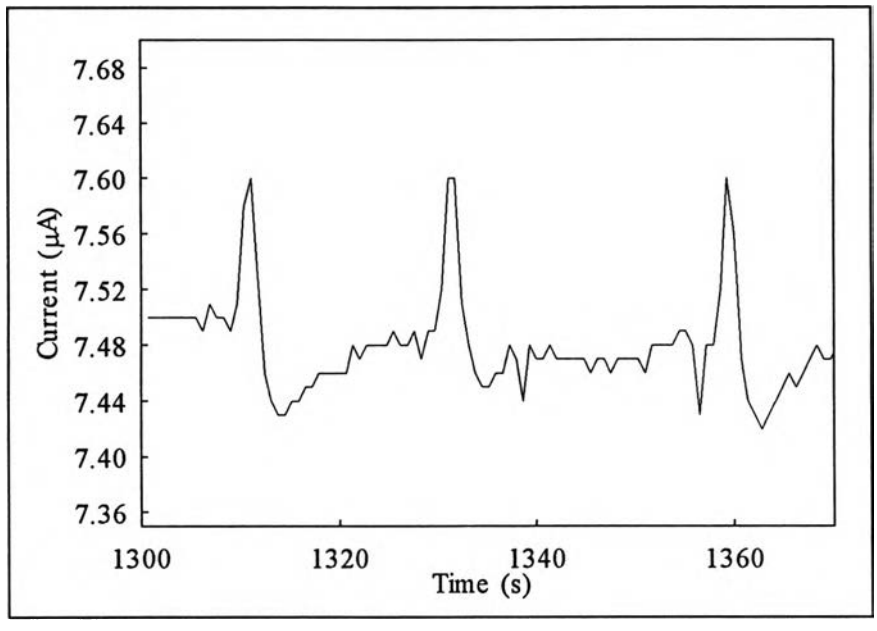


Figure 4.28 Flow injection analysis with pulsed amperometric detection results for 1 μM doxycycline hydrochloride in 0.1 M $KH_2PO_4$ solution (pH 2). The flow rate was 1 ml $min^{-1}$.

4.2.4 Repeatability

The relative standard deviation value was used to define the repeatability. Under the optimal PAD waveform parameters, 0.5 mM of the analyte solutions was injected ten times. Figure 4.29, 4.30, and 4.31 show the FI-PAD responses for 0.5 mM tetracycline hydrochloride, 0.5 mM chlortetracycline hydrochloride, and 0.5 mM doxycycline hydrochloride, respectively. The repeatability of each analyte was summarized in Table 4.9.

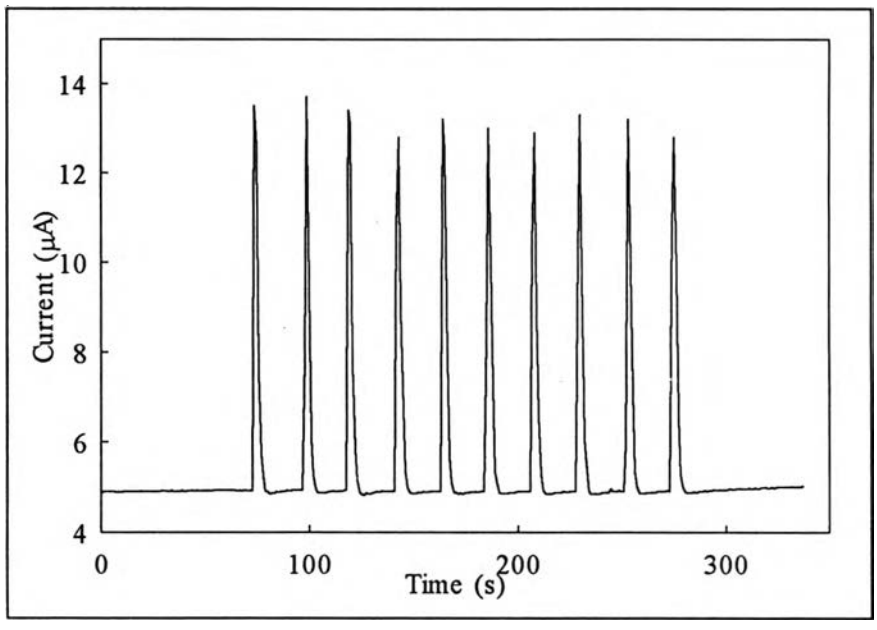


Figure 4.29 Flow injection with pulsed amperometric detection results for 0.5 mM tetracycline hydrochloride (10 injections) in 0.1 $KH_2PO_4$ solution (pH 2). The flow rate was 1 ml $min^{-1}$.

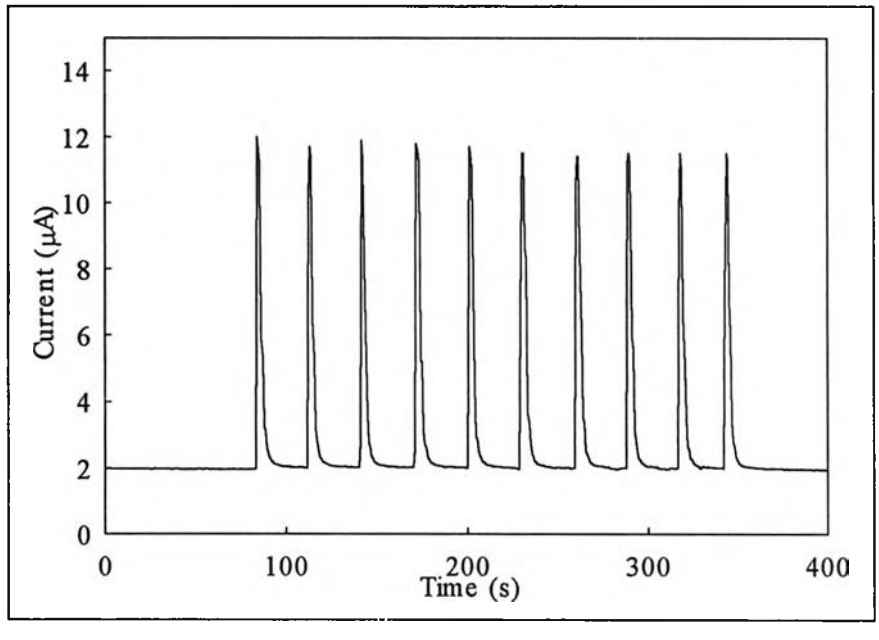


Figure 4.30 Flow injection with pulsed amperometric detection results for 0.5 mM chlortetracycline hydrochloride (10 injections) in 0.1 M $KH_2PO_4$ solution (pH 2.5). The flow rate was 1 ml $min^{-1}$.

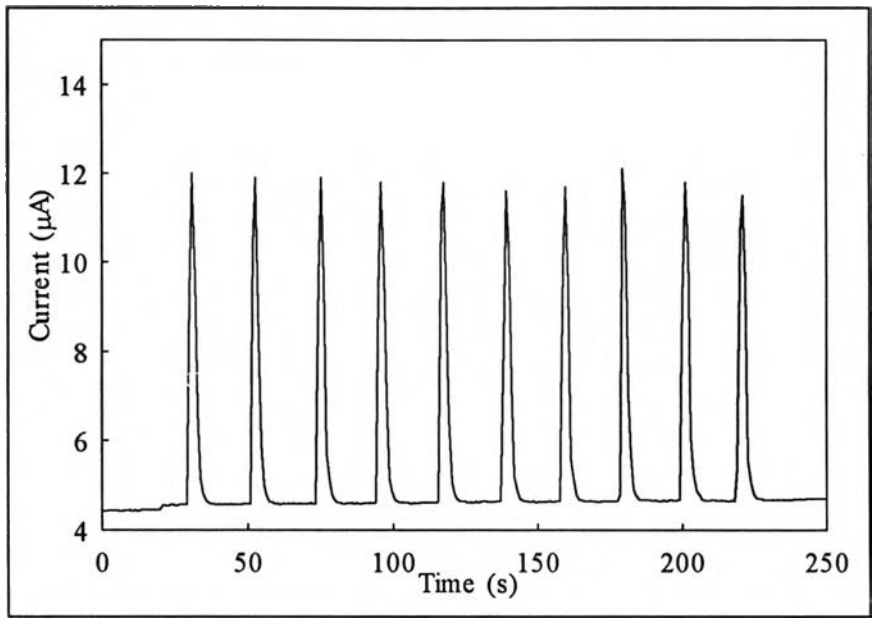


Figure 4.31 Flow injection with pulsed amperometric detection results for 0.5 mM doxycycline hydrochloride (10 injections) in 0.1 M $KH_2PO_4$ solution (pH 2). The flow rate was 1 ml $min^{-1}$.

Table 4.9 % RSD of 0.5 mM tetracycline hydrochloride, chlortetracycline hydrochloride, and doxycycline hydrochloride

| analytes | %RSD |
|---|---|
| Tetracycline hydrochloride | 3.53 |
| Chlortetracycline hydrochloride | 2.18 |
| Doxycycline hydrochloride | 3.17 |

### 4.2.5 Applications

Flow injection with pulsed amperometric detection was applied to determination of the drug capsules. The calibration method was used to determine the amount of tetracycline hydrochloride in the drug sample. The determination of chlortetracycline hydrochloride and doxycycline hydrochloride in the drug samples was performed by the standard addition method.

#### 4.2.5.1 Tetracycline hydrochloride

For the determination of tetracycline hydrochloride in real drug sample by standard addition method, the percent recovery was greater than 100 by two or three times (The results were not shown.). These results may be due to the other ingredients in drug capsule such as some additive, which added to stabilize the active ingredient. The added additive component may be interacted with the active ingradient and produced the new compound, which can be detected at the same potential as tetracycline hydrochloride. Figure 4.32 shows the calibration graph of tetracycline hydrochloride. The comparison between the amount of tetracycline hydrochloride obtained from the calibration and the labeled amount (250 mg per capsule) was carried out. The percentage of relative error compared to the labeled amount was lower than 10 %. Recovery ranging from 90-109 % was obtained. In order to evaluate the proposed method for the determination of this compound in drug capsule, the within- and between-day studies were carried out.

Table 4.10 Relative error of tetracycline hydrochloride capsule sample with PAD using the gold disk electrode applied to flow injection system of the within- and between-day studies

| Amount of prepared ($\mu g\ ml^{-1}$) | Within-day assay | | Between-day assay | |
|---|---|---|---|---|
| | Amount of founded ($\mu g\ ml^{-1}$) | Percent of relative error (%) | Amount of founded ($\mu g\ ml^{-1}$) | Percent of relative error (%) |
| 24.05 | 25.25 | 4.99 | 21.67 | -9.90 |
| 48.09 | 52.13 | 8.40 | 48.79 | 1.46 |
| 72.14 | 69.06 | -4.27 | 72.19 | 0.07 |

Relative error ranging from –4.27 to 8.40 % and -9.90 to 1.46 % were obtained for within- and between-day studies, respectively. The results of within- and between-day assays were satisfactory. This proposed method is precise for tetracycline hydrochloride determination.

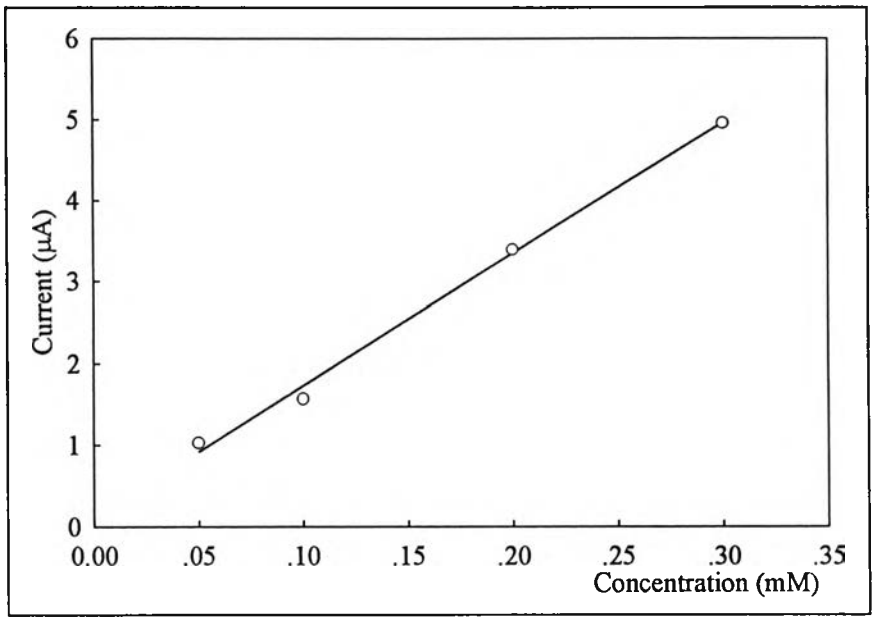


Figure 4.32 Calibration graph for tetracycline hydrochloride capsule

4.2.5.2 Chlortetracycline hydrochloride

The commercial drug capsule of chlortetracycline hydrochloride was analyzed by the proposed method. Figure 4.33 shows the standard addition graph of chlortetracycline hydrochloride capsule. It was found that the slope was 24.937 μA $mM^{-1}$ and the intercept was 0.5847 μA. The comparison between the labeled amount (250 mg per capsule) and the amount obtained by the suggested method was conducted. Recovery values greater than 107 % were found. In order to evaluate this proposed method for the determination of this compound in drug capsule, the recovery, and within- and between-day studies were carried out on sample to which the known amounts of chlortetracycline hydrochloride standards were added. The results of within- and between-day assays are summarized in Table 4.11.

Table 4.11 Recovery of chlortetracycline hydrochloride capsule sample with PAD using the gold disk electrode applied to flow injection system of the within- and between-day studies

| Amount of added ($\mu g\ ml^{-1}$) | Within-day assay | | Between-day assay | |
|---|---|---|---|---|
| | Amount of founded ($\mu g\ ml^{-1}$) | Percent of recovery (%) | Amount of founded ($\mu g\ ml^{-1}$) | Percent of recovery (%) |
| 5.15 | 4.82 | 93.59 | 5.20 | 100.97 |
| 10.31 | 11.26 | 109.21 | 10.64 | 103.20 |
| 15.50 | 15.59 | 100.32 | 15.61 | 100.97 |
| 20.61 | 20.01 | 97.09 | 19.99 | 96.99 |

Recoveries ranging from 93-109 % and 96-104 % were obtained for within- and between-day studies, respectively. The results of within- and between-day assays were satisfactory. This proposed method is precise for chlortetracycline hydrochloride determination.

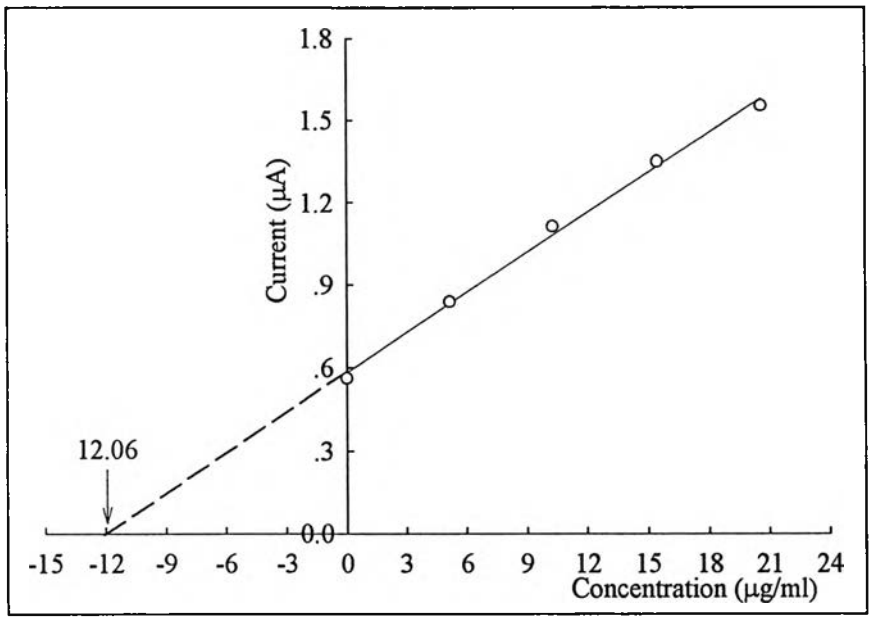


Figure 4.33 Standard addition graph for chlortetracycline hydrochloride capsule

4.2.5.3 Doxycycline hydrochloride

This proposed method was applied to the determination of doxycycline hydrochloride in commercial drug capsule. Figure 4.34 shows the standard addition graph of doxycycline hydrochloride capsule. It was found that the slope was 20.08 μA $mM^{-1}$ and the intercept was 0.9837 μA. The labeled amount (100 mg per capsule) and the amount obtained by the suggested method were compared. Recovery values greater than 94 % were found. In order to evaluate this proposed method for the determination of this compound in drug capsule, the recovery, and within- and between-day studies were carried out on sample to which the known amounts of doxycycline hydrochloride standards were added. The results of within- and between-day assays are summarized in Table 4.12.

Table 4.12 Recovery of doxycycline hydrochloride capsule sample with PAD using the gold disk electrode applied to flow injection system of the within- and between-day studies

| Amount of added ($\mu g\ ml^{-1}$) | Within-day assay | | Between-day assay | |
|---|---|---|---|---|
| | Amount of founded ($\mu g\ ml^{-1}$) | Percent of recovery (%) | Amount of founded ($\mu g\ ml^{-1}$) | Percent of recovery (%) |
| 24.05 | 21.16 | 87.98 | 24.24 | 100.79 |
| 48.09 | 48.43 | 100.71 | 49.77 | 103.49 |
| 72.14 | 73.72 | 102.19 | 70.93 | 98.32 |
| 96.18 | 95.89 | 99.70 | 95.80 | 99.60 |

Recoveries ranging from 87-103 % and 98-104 % were obtained for within- and between-day studies, respectively. The results of within- and between-day assays were satisfactory. This proposed method is precise for doxycycline hydrochloride determination.

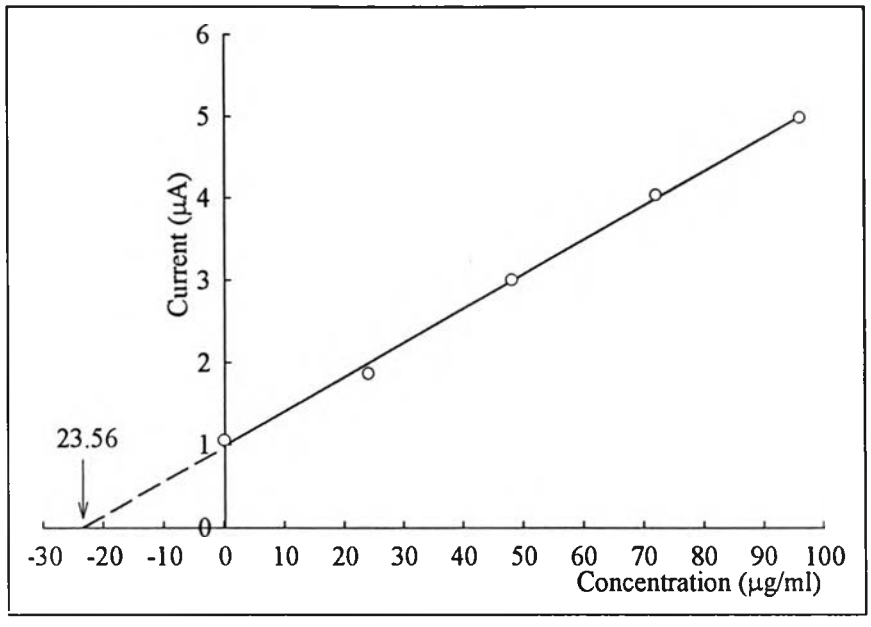


Figure 4.34 Standard addition graph for doxycycline hydrochloride capsule