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APPENDICES

APPENDIX A

Rotating disk cyclic voltammetric results (pH dependence)

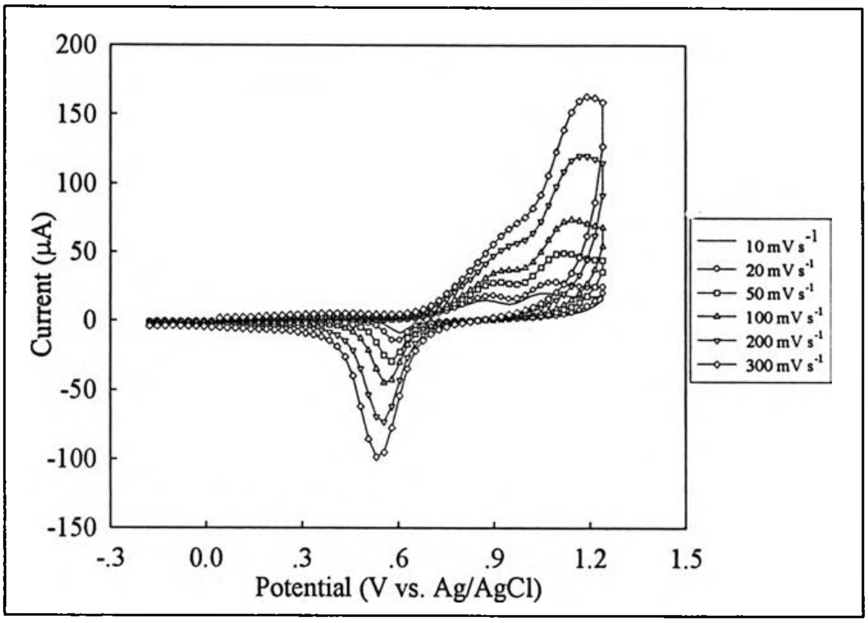

Figure A1 Rotating disk cyclic voltammograms of 1 mM tetracycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2) at Au RDE. The scan rate was varied from 10 to 300 mV s^{-1}. The rotation speed was 250 r.p.m.

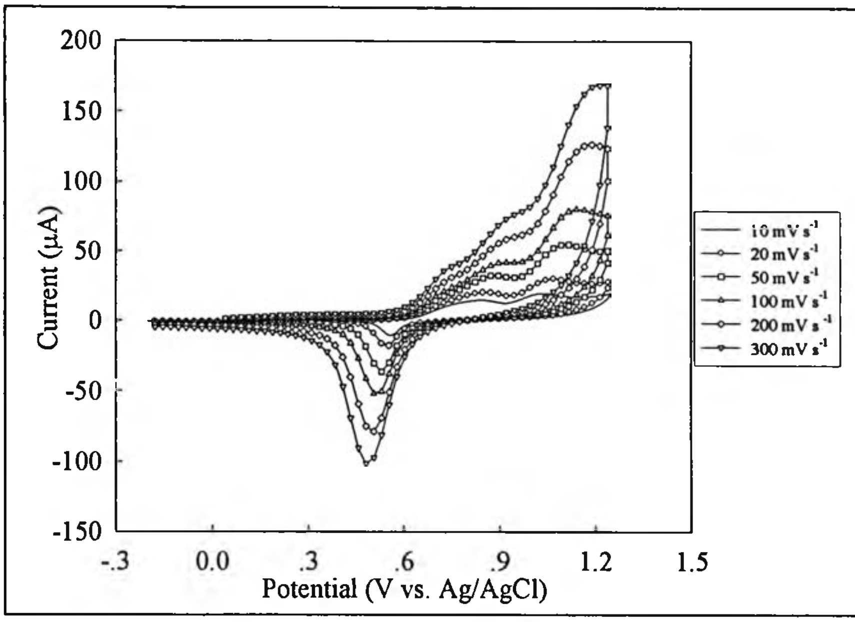

Figure A2 Rotating disk cyclic voltammograms of 1 mM chlortetracycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2.5) at Au RDE. The scan rate was varied from 10 to 300 mV s^{-1}. The rotation speed was 250 r.p.m.

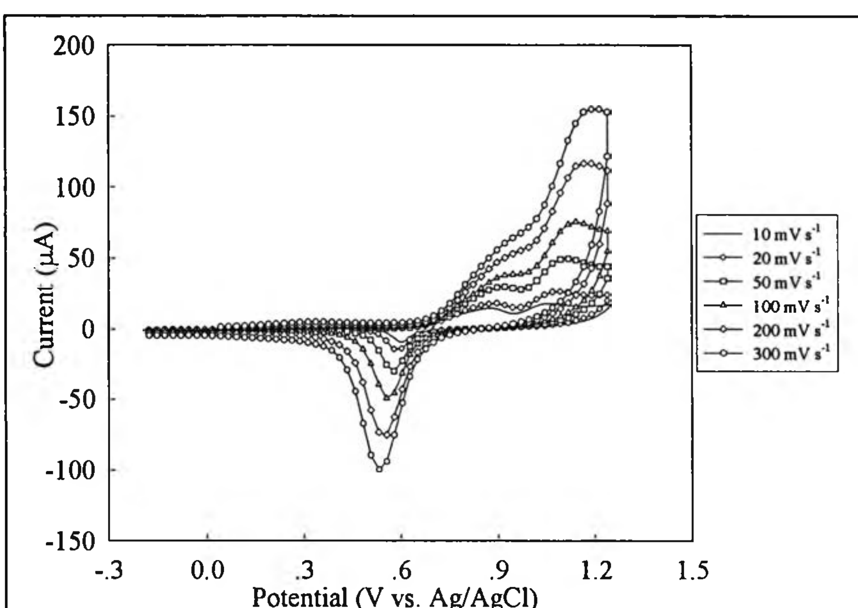

Figure A3 Rotating disk cyclic voltammograms of 1 mM doxycycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2) at Au RDE. The scan rate was varied from 10 to 300 mV s^{-1}. The rotation speed was 250 r.p.m.

APPENDIX B

Rotating disk cyclic voltammetric results (pH dependence)

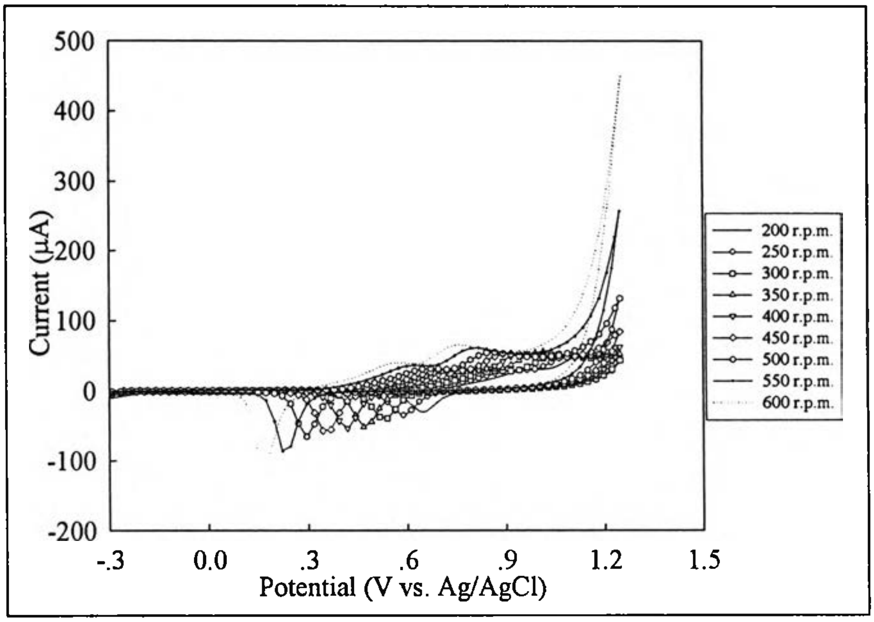

Figure B1 Rotating disk cyclic voltammograms of 1 mM tetracycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2) at Au RDE. The rotation speed was varied from 200 to 600 r.p.m. The scan rate was 50 mV s^{-1}.

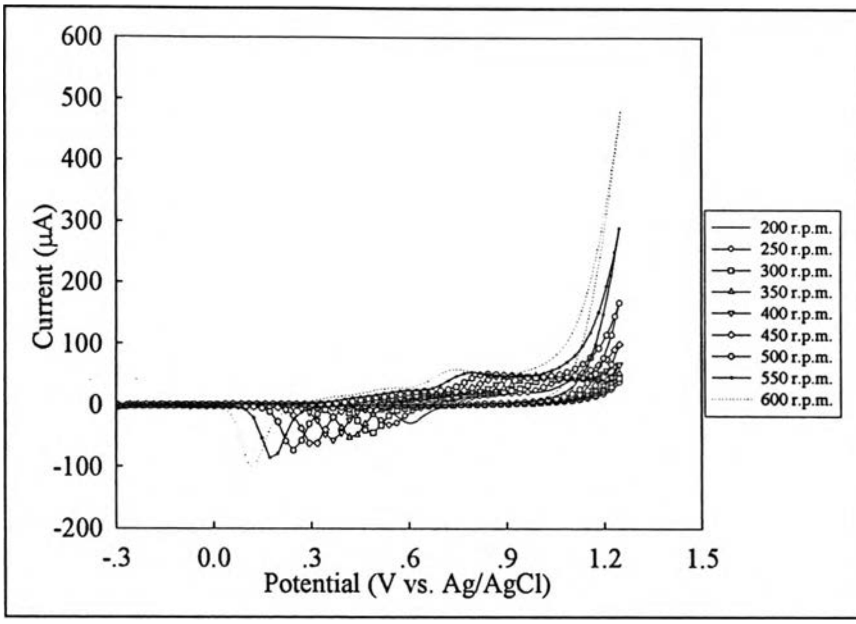

Figure B2 Rotating disk cyclic voltammograms of 1 mM chlortetracycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2.5) at Au RDE. The rotation speed was varied from 200 to 600 r.p.m. The scan rate was 50 mV s^{-1}.

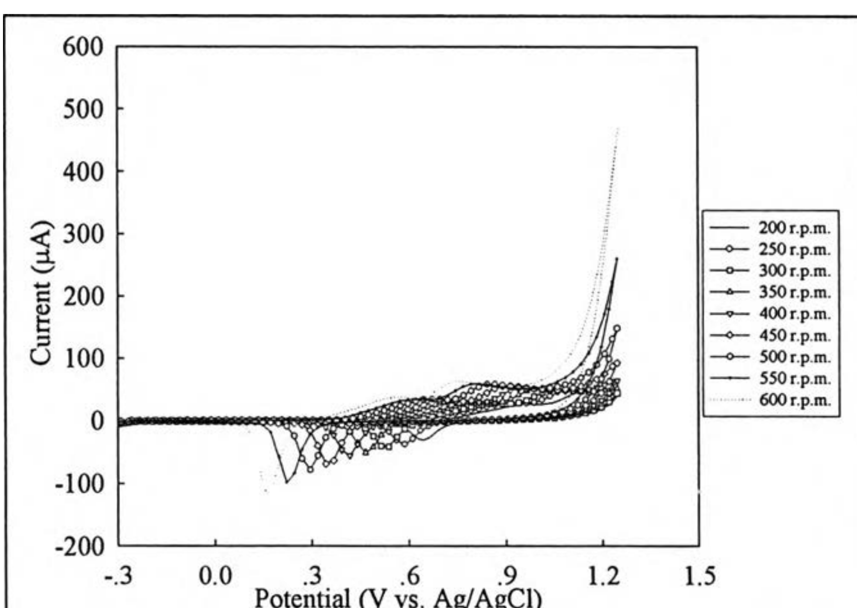

Figure B3 Rotating disk cyclic voltammograms of 1 mM doxycycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2) at Au RDE. The rotation speed was varied from 200 to 600 r.p.m. The scan rate was 50 mV s^{-1}.

APPENDIX C

Flow injection with pulsed amperometric detection results

and calibration curves

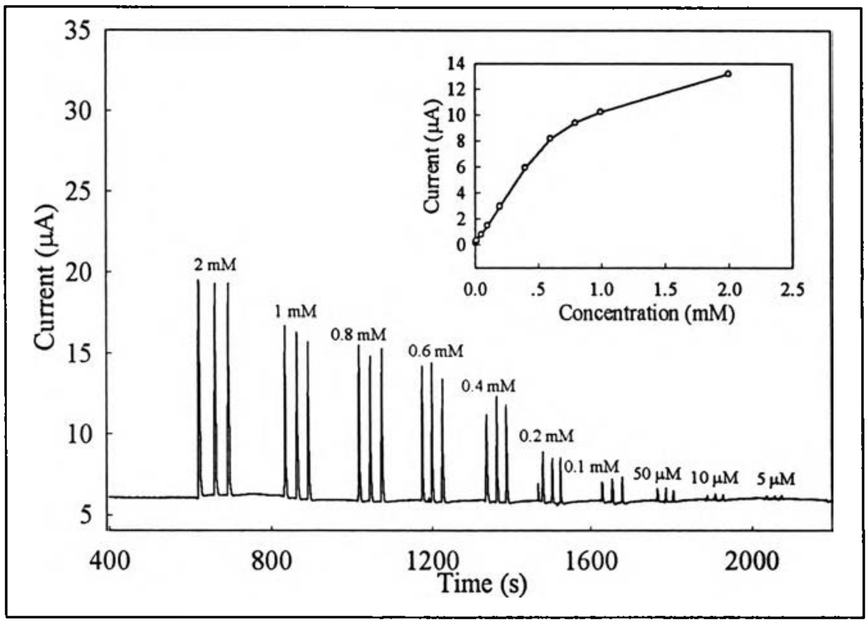

Figure C1 Flow injection with pulsed amperometric detection results of tetracycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2) at gold disk electrode. The flow rate was 1 ml min^{-1}. The corresponding calibration curve is also shown (inset Figure).

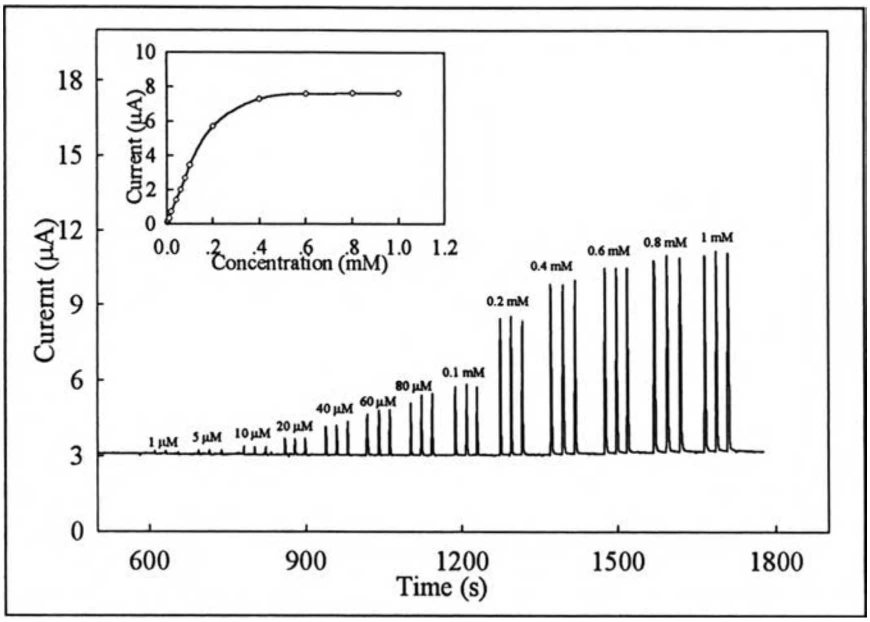

Figure C2 Flow injection with pulsed amperometric detection results of chlortetracycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2.5) at gold disk electrode. The flow rate was 1 ml min^{-1}. The corresponding calibration curve is also shown (inset Figure).

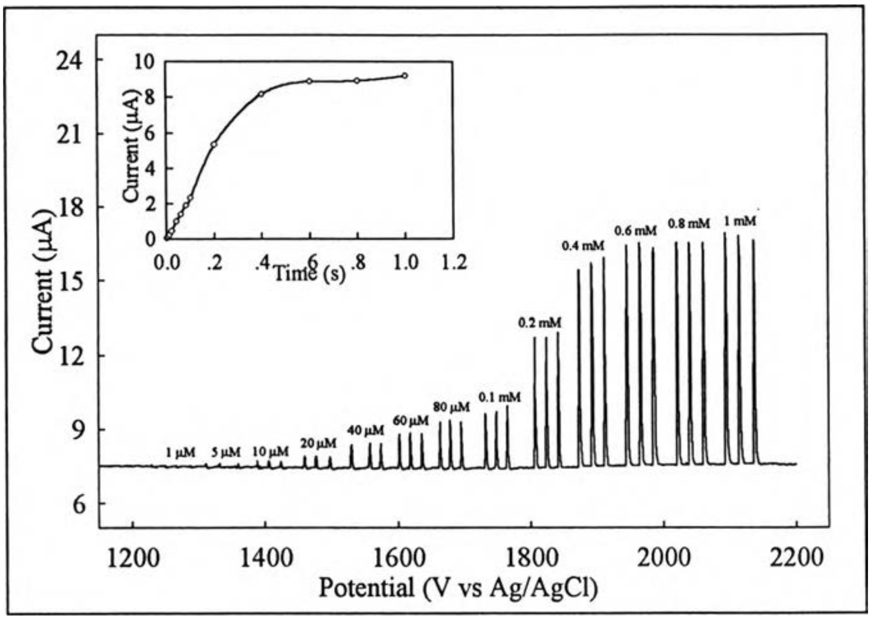

Figure C3 Flow injection with pulsed amperometric detection results of doxycycline hydrochloride in 0.1 M KH_2PO_4 solution (pH 2) at gold disk electrode. The flow rate was 1 ml min^{-1}. The corresponding calibration curve is also shown (inset Figure).

APPENDIX D

Flow injection with pulsed amperometric detection results of drug samples

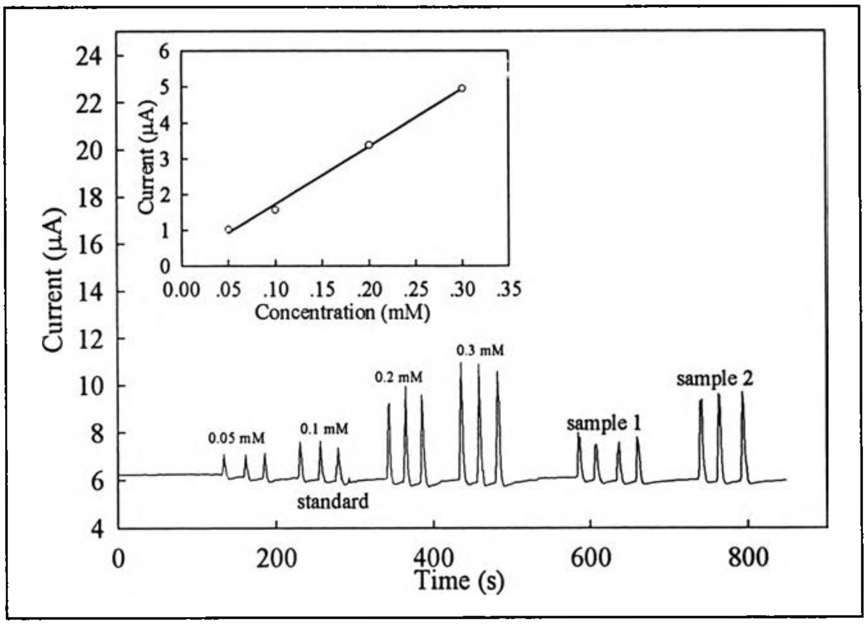

Figure D1 Flow injection with pulsed amperometric detection results of tetracycline hydrochloride capsule in 0.1 M KH_2PO_4 solution (pH 2) with three replicated injections using the optimized PAD waveform. The flow rate was 1 ml min^{-1}. The corresponding calibration curve is also shown (inset Figure).

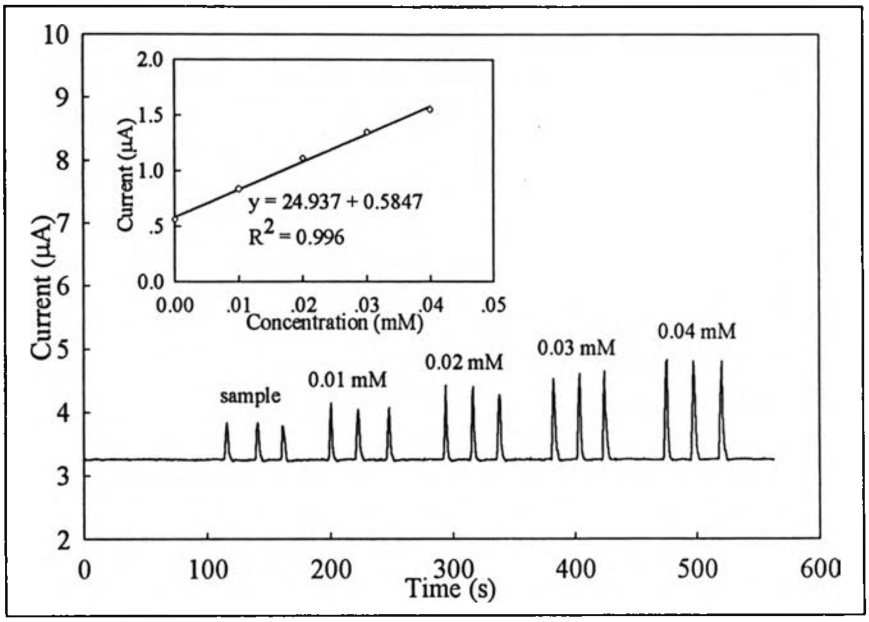

Figure D2 Flow injection with pulsed amperometric detection results of chlortetracycline hydrochloride capsule in 0.1 M KH_2PO_4 solution (pH 2.5) with three replicated injections using the optimized PAD waveform. The flow rate was 1 ml min^{-1}. The corresponding calibration curve is also shown (inset Figure).

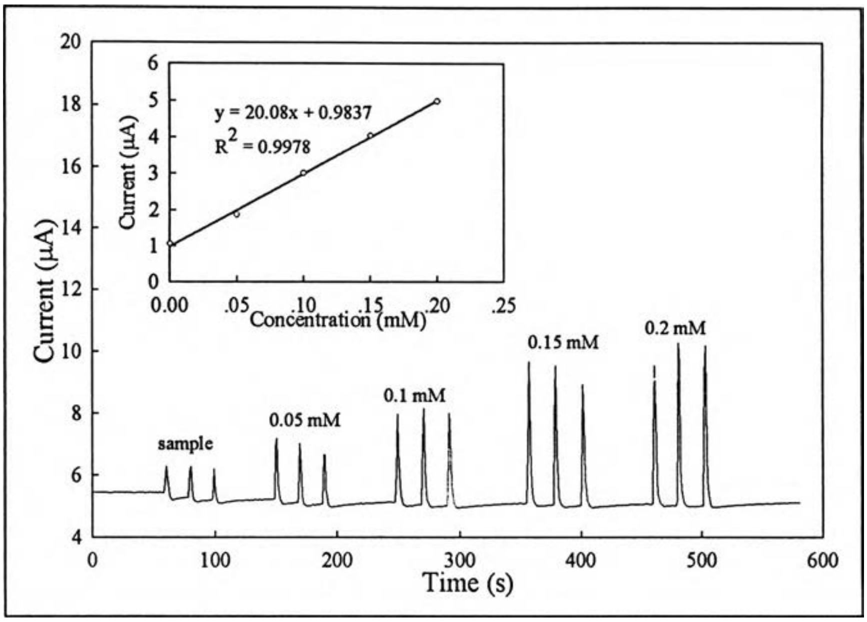

Figure D3 Flow injection with pulsed amperometric detection results of doxycycline hydrochloride capsule in 0.1 M KH_2PO_4 solution (pH 2) with three replicated injections using the optimized PAD waveform. The flow rate was 1 ml min^{-1}. The corresponding calibration curve is also shown (inset Figure).

APPENDIX E

Description of analytical performance characteristics

Accuracy [8], [9]

Accuracy denotes that closeness of a measurement or set of measurements to the accepted value. Accuracy is normally reported in terms of error. Error is the difference between the accepted and measured values. There are several ways and units in which the accuracy can be expressed. Recovery is a term often used to describe accuracy, the equation for recovery is:

$$\%\text{Recovery} = \frac{\text{Measured value}}{\text{True value}} \text{x} 100$$

Relative error is the another term that can be expressing the accuracy. The equation is shown below:

$$\%\text{error} = \frac{(\text{Measured value} - \text{True value})}{\text{True value}} \text{ x } 100$$

Precision [59], [60], [61]

Precision refers to the agreement between values in a set of data that have been carried out in exactly the same mode. It is a measure of the reproducibility of the analysis. Precision of the results can be ascertained through the use of replicate measurements. There are several popular ways to express the precision of data. Multiple injections of a homogeneous sample and calculation of the relative standard deviation (% RSD) do it. The equation for %RSD is shown below:

$$\%RSD = \frac{\text{standard deviation}}{\text{Mean}} \times 100$$

Linearity (Linear range)

A linearity is the range where the analyte response is linearly proportional to concentration. The working sample concentration and samples tested for accuracy should be in the linear range.

Sensitivity

Sensitivity is the change in the analytical response divided by the corresponding change in the concentration of a standard (calibration) curve, i.e. the slope of the analytical calibration.

Limit of Detection (LoD)

The detection limit of a method is the lowest analyte concentration that can be determined to be different from an analyte blank. There are numerous way that detection limit have been defined. An example is the lowest analyte concentration that is above the noise level of the system, typically, three time the noise level (S/N = 3). This term is used to describe low analyte concentrations (< 10 μM). For high analyte concentrations, the detection limit is defined as the lowest concentration that provides a signal to background ratio S/B of three. The equation of S/B ratio is shown below:

$$\text{S/B ratio} = \frac{(\text{total signal} - \text{blank signal})}{\text{blank signal}}$$

APPENDIX F

The proposed method for determination of tetracycline antibiotics

F1 Chemicals and reagents

F1.1 Tetracycline hydrochloride (Sigma)

F1.2 Chlortetracycline hydrochloride (Sigma)

F1.3 Doxycycline hydrochloride (Sigma)

F1.4 Potassium dihydrogen orthophosphate (Merck)

F1.5 Phosphoric acid (85% J.T. Baker)

F1.6 Tetracycline hydrochloride capsule (TC Mycin 250 mg)

F1.7 Cholrtetracycline hydrochloride capsule (Aureomycin 250 mg)

F1.8 Doxycycline hydrochloride capsule (Medomycin 100 mg)

F2 Apparatus

F2.1 Gold disk electrode (0.07 cm^2, Bioanalytical System Inc.) pretreated by polishing with 0.05 micron of aluminum/water slurry

F2.2 Ag/AgCl electrode

F2.3 Autolab Potentiostat (PGSTAT 30, Metrohm)

F2.4 Peristaltic pump (Eyela, SMP-23)

F2.5 Rheodyne injection valve, Model 7225 (Altech)

F2.6 Thin layer flow cell (Bioanalytical System Inc.

F2.7 0.2 μM Nylon membrane filter (Altech)

F2.8 0.45 μM Nylon membrane syringe filter with polypropylene (PP) housing (Orange Scientific filter)

F3 The preparation of supporting electrolyte and standard solutions

F3.1 0.1 M Potassium dihydrogen orthophosphate (KH_2PO_4)

KH_2PO_4 13.60 g was dissolved in 1.0 L of deionized water and then adjusted with 85 % phosphoric acid to the required pH (pH 2 and 2.5).

F3.2 Tetracycline hydrochloride solution

The 1 mM tetracycline hydrochloride solution was prepared by weighing 0.0481 g tetracycline hydrochloride powder and transferring into 100 ml volumetric flask. The 0.1 M KH_2PO_4 solution (pH 2) was used for diluting this aliquot to the mark.

F3.3 Chlortetracycline hydrochloride solution

The 0.5 mM chlortetracycline hydrochloride solution was prepared by weighing 0.0258 g tetracycline hydrochloride powder and transferring into 100 ml volumetric flask. The 0.1 M KH_2PO_4 solution (pH 2.5) was used for diluting this aliquot to the mark.

F3.4 Doxycycline hydrochloride solution

The 0.5 mM doxycycline hydrochloride solution was prepared by weighing 0.0240 g tetracycline hydrochloride powder and transferring into 100 ml volumetric flask. The 0.1 M KH_2PO_4 solution (pH 2.) was used for diluting this aliquot to the mark.

F4 Procedure

The FI-PAD apparatus consisted of a thin-layer flow-through cell, a 20 μl sample injection loop, a peristaltic pump and an electrochemical detector. The flow rate was 1 ml min^{-1}. The optimized waveform parameters of each analyte were shown in Table F1, F2, and F3.

Table F1 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

Table F2 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

Table F3 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

F4.1 Tetracycline hydrochloride

The stock standard solution (1 mM) volume of 0.5, 1.0, 2.0, and 3.0 ml were pipetted into each 10 ml volumetric flask and diluted with 0.1 M KH_2PO_4 solution (pH 2). The final concentrations of the standard solutions were 24.05, 48.10, 96.12, and 144.17 $\mu g\ ml^{-1}$, respectively. Each concentration was injected for three replicates. The calibration curve was obtained from the plotting between the averaged peak currents and the varied concentrations.

For real drug capsule (TC Mycin, 250 mg per capsule), a mass of powder of ten capsules was transferred to a 1000 ml volumetric flask and dissolved in 0.1 M KH_2PO_4 solution (pH 2), filtrated solution through a 0.45 μM Nylon membrane syringe filter. The filtrated was further diluted with 0.1 M KH_2PO_4 solution (pH 2) to obtain a final concentration of 50.01 $\mu g\ ml^{-1}$ (0.104 mM). The working sample solution was further injected for three replicates. The amount of tetracycline hydrochloride in drug capsule was calculated from the calibration curve.

For other real samples such as milk and body fluid, the separation of tetracycline hydrochloride from other interference by HPLC was suggested. The obtained analyte portion was injected for three replicates. If the concentration was not in the calibration range, the dilution of concentration was performed.

F4.2 Chlortetracycline hydrochloride

For real drug capsule (Aureomycin, 250 mg), a mass of powder of ten capsules was transferred to a 1000 ml volumetric flask and dissolved in 0.1 M KH_2PO_4 solution (pH 2.5), filtrated through a 0.45 μM Nylon membrane syringe filter. Then, the filtrated solution was further diluted with 0.1 M KH_2PO_4 solution to obtain a final concentration of 257.65 μg ml^{-1} (0.5 mM).

2.5 ml of sample solution (0.5 mM) was pipetted in each 10 ml volumetric flask and then 0, 1.0, 2.0, 3.0 and 4.0 ml of a standard solution was added to give final concentration of the standard solutions was 0, 25.77, 51.53, 103.06, and 206.12 μg ml^{-1}, respectively. Each working solution was injected for three replicates. The amount of chlortetracycline hydrochloride in drug capsule was calculated from the calibration curve.

For other real samples such as milk and body fluid, the obtained analyte portion, which separated from other interference by HPLC, was injected for three replicates. If the concentration was more or less than 0.5 mM (compare to the peak height of standard at the same concentration), the dilution of concentration was performed. The working sample solutions were prepared as mentioned above and injected three replicates for each concentration.

F4.3 Doxycycline hydrochloride

For real drug capsule (Medomycin, 100 mg), a mass of powder of ten capsules was transferred to a 1000 ml volumetric flask and dissolved in 0.1 M KH_2PO_4 solution (pH 2), filtrated through a 0.45 μM Nylon membrane syringe filter. Then, the filtrated solution was further diluted with 0.1 M KH_2PO_4 solution to obtain a final concentration of 240.45 μg ml^{-1} (0.5 mM).

2.5 ml of sample solution (0.5 mM) was pipetted in each 10 ml volumetric flask and then 0, 1.0, 2.0, 3.0 and 4.0 ml of a standard solution was added to give final concentration of the standard solutions 0, 24.05, 48.09, 144.27, and 192.36 μg ml^{-1}, respectively. Each working solution was injected for three replicates. The amount of chlortetracycline hydrochloride in drug capsule was calculated from the calibration curve.

For other real samples such as milk and body fluid, the obtained analyte portion, which separated from other interference by HPLC, was injected for three replicates. If the concentration was more or less than 0.5 mM (compare to the peak height of standard at the same concentration), the dilution of concentration was performed. The working sample solutions were prepared as mentioned above and injected three replicates for each concentration.

CURRICULUM VITAE

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