DETERMINATION OF NIFEDIPINE, AMPICILLIN AND PENICILLIN G
AND THEIR ELECTRO-OXIDATION PRODUCTS BY VOLTAMMETRIC
TECHNIQUES

MOHD DZUL HAKIM BIN WIRZAL

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Specially Dedicated To,

My Mama, Nur Syamsi Ahmad and My Papa, Wirzal Munchak for your prayers, words of encouragement, love and care.

My brothers, Sharezal Wirzal and Azizi Wirzal for your support, care and encouragement.
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ABSTRACT

The study of the electrochemical behaviour of ampicillin (AMP), penicillin G (PG) and nifedipine (NFD) were carried out using three different working electrodes namely hanging mercury drop electrode (HMDE), mercury meniscus modified silver amalgam electrode (m-AgSAE) and boron-doped diamond electrode (BDDE). All measurements were made versus Ag/AgCl (3 M KCl) using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and adsorptive stripping voltammetry (AdSV). Due to the toxicity of mercury, m-AgSAE was constructed as an alternative electrode for HMDE. The DPV determination of NFD were conducted using mixtures of Britton-Robinson (BR) buffer pH 8 with methanol in three different volume/volume (v/v) ratios (1:1, 9:1, and 99:1) in the concentration range of 0.2 to 20 μmol/L. The ratio 9:1 was chosen as the optimal ratio for NFD determination. The limit of quantification (LOQ) was 0.12 μmol/L for (HMDE) and 1.2 μmol/L (m-AgSAE). Attempts to increase the sensitivity using AdSV at both electrodes were not successful. For the determination of AMP and PG, the optimal conditions have been performed in Britton-Robinson (BR) buffer in the concentration range of 1 to 9 μmol/L (HMDE) and 10 to 90 μmol/L (m-AgSAE). The limit of detection (LOD) for AMP and PG at HMDE were 0.09 and 0.065 μmol/L, respectively and the LOD for AMP and PG at m-AgSAE were 3.8 and 2.5 μmol/L, respectively. PG also was successfully determined using the BDDE working electrode where PG showed well developed oxidation peak at potential between 800 to 1100 mV vs Ag/AgCl (3 M KCl) in BR buffer. The highest and best developed peak was obtained at pH 4 at a potential of +980 mV vs Ag/AgCl (3 M KCl). Attempts to increase the sensitivity using adsorptive stripping DPV at BDDE were not successful. The LOD and LOQ for PG were 0.23 μmol/L and 1.5 μmol/L, respectively. The practical application of the newly developed method was verified on the determination of NFD, AMP and PG in spiked samples of drinking and river water using optimum conditions. Voltammetry and LC-MS method was used for monitoring the electro-oxidation mechanism of AMP, PG and NFD. The electro-oxidation process was performed in three different mediums of electrolyte which were tap water (pH~6.5) and BR buffer of pH 4 and pH 10, while the electro-oxidation time were set to 15, 30 and 60 minutes. A series of mixed metal oxides (MMO) titanium-based electrodes (TiO₂/Ti, IrO₂-TiO₂/Ti, RuO₂-TiO₂/Ti and two RuO₂-IrO₂-TiO₂/Ti with different ratios of the metal oxide) were used as an anode. AMP, PG and NFD were successfully degraded using electro-oxidation process with the best MMO electrode for the degradation was RuO₂-IrO₂-TiO₂/Ti in tap water (pH~6.5) medium. LC-MS analysis was performed to determine the by-product and mechanism of the degradation process of the drugs were proposed.
Kajian sifat elektrokimia ampisilin (AMP), penisilin G (PG) dan nifedipin (NFD) telah dijalankan dengan menggunakan tiga elektrod kerja yang berbeza iaitu elektrod titisan raksa tergantung (HMDE), elektrod perak amalgam terubahsuai meniskus raksa (m-AgSAE) dan elektrod berlian terdopkan boron (BDDE). Semua pengukuran dibuat terhadap Ag/AgCl (3 M KCl) menggunakan voltametri kitaran (CV), voltametri denyut pembeza (DPV) dan voltametri jerapan pelucutan (AdSV). Oleh kerana ketoksikan raksa, m-AgSAE telah dibina sebagai elektrod alternatif untuk HMDE. Penentuan NFD secara DPV telah dilakukan dengan menggunakan campuran larutan penimbal Britton-Robinson (BR) pH 8 dengan metanol dalam tiga nisbah isipadu per isipadu (v/v) yang berbeza (1: 1, 9: 1, 99: 1) dalam julat kepekatan 0.2-20 μmol/L. Nisbah 9:1 dipilih sebagai nisbah optimum bagi penentuan NFD. Had kuantitatif (LOQ) bagi NFD ialah 0.12 μmol/L (HMDE) dan 1.2 μmol/L (m-AgSAE). Percubaan untuk meningkatkan kepekaan menggunakan AdSV di kedua-dua elektrod tidak berjaya. Untuk penentuan AMP dan PG, keadaan optimum telah dilakukan dalam larutan penimbal Britton-Robinson (BR) dalam julat kepekatan 1 hingga 9 μmol/L (HMDE) dan 10 hingga 90 μmol/L (m-AgSAE). Had pengesanan (LOD) bagi AMP dan PG di HMDE masing-masing ialah 0.09 μmol/L dan 0.065 μmol/L sementara LOD untuk AMP dan PG di m-AgSAE masing-masing ialah 3.8 dan 2.5 μmol/L. PG juga telah berjaya ditentukan dengan menggunakan elektrod kerja BDDE di mana PG menunjukkan puncak voltammogram tertinggi dan terbaik pada keupayaan antara 800 mV hingga 1100 mV vs Ag/AgCl (3 M KCl). Percubaan untuk meningkatkan kepekaan dengan menggunakan jerapan pelucutan DPV di BDDE tidak berjaya. LOD dan LOQ bagi PG masing-masing ialah 0.23 μmol/L dan 1.5 μmol/L. Penggunaan praktis kaedah baharu yang dibangunkan ini telah ditentusahkan bagi penentuan NFD, AMP dan PG yang ditambah ke dalam sampel air minuman dan air sungai menggunakan keadaan optimum. Kaedah voltametri dan LC-MS telah digunakan untuk memantau mekanisme elektro-pengoksidaan bagi AMP, PG dan NFD. Proses elektro-pengoksidaan telah dilakukan dalam tiga medium elektrolit berbeza iaitu air paip (pH~6.5) dan larutan penimbal BR padapH 4 dan pH 10, manakala masa elektro-pengoksidaan telah ditetapkan pada 15, 30 dan 60 minit. Elektrod berasaskan titanium bagi satu siri campuran logam oksida (MMO) (TiO2/Ti, IrO2-TiO2/Ti, RuO2-TiO2/Ti dan dua RuO2-IrO2-TiO2/Ti dengan nisbah yang berbeza oksida-logam) digunakan sebagai anod. AMP, PG dan NFD telah berjaya didegradasi menggunakan proses elektro-pengoksidaan dengan elektrod MMO terbaik untuk degradasi ialah RuO2-IrO2-TiO2/Ti dalam medium air paip (pH~6.5). Analisis LC-MS telah dijalankan untuk menentukan hasil sampingan daripada proses degradasi dan mekanisma penguraian dadah bagi AMP, PG dan NFD dicadangkan.
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4.15 DP voltammograms of nifedipine at m-AgSAE in BR-buffer pH 8-MeOH (9:1), \(\text{CNFD} (1) 0\) (supporting electrolyte), (2) 2, (3) 4, (4) 6, (5) 8, (6) 10 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

4.16 DP voltammograms of nifedipine at HMDE in BR-buffer pH 8-MeOH (9:1), \(\text{CNFD} (1) 0\) (supporting electrolyte), (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

4.17 DP voltammograms of nifedipine at HMDE in BR-buffer pH 8-MeOH (99:1), \(\text{CNFD} (1) 0\) (supporting electrolyte), (2) 2, (3) 4, (4) 6, (5) 8, and (6) 10 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

4.18 DP voltammograms of nifedipine at m-AgSAE in BR-buffer pH 8-MeOH (99:1), \(\text{CNFD} (1) 0\) (supporting electrolyte), (2) 2, (3) 4, (4) 6, (5) 8, (6) 10 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

4.19 Adsorptive stripping DP voltammograms of nifedipine with accumulation time (1) 0 sec (2) 10 sec, (3) 30 sec, (4) 60 sec, (5) 90 sec at HMDE in BR buffer pH 8-MeOH (9:1). Concentration of nifedipine = 1 µmol/L. Reference electrode: Ag/AgCl (3 M KCl).

4.20 DP voltammograms of nifedipine \(\text{CNFD} (1) 0\) (supporting electrolyte) (2) 1.8, (3) 3.6, (4) 5.4, (5) 7.2, (6) 9.0 (µmol/L) at HMDE in drinking water sample - BR buffer pH 8 (9:1) mixtures. Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

4.21 DP voltammogram of nifedipine at HMDE in river sample- BR buffer pH 8 (9:1) mixtures. \(\text{CNFD} (1) 0\) (supporting electrolyte) (2) 1.8, (3) 3.6, (4) 5.4, (5) 7.2, (6) 9.0 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).
4.22 DP voltammograms of nifedipine ($c_{NFD}$) (1) 0 (supporting electrolyte) (2) 1.8, (3) 3.6, (4) 5.4, (5) 7.2, (6) 9.0 (µmol/L) at m-AgSAE in drinking water sample - BR buffer pH 8 (9:1) mixtures. Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

4.23 DP voltammograms of nifedipine ($c_{NFD}$) (1) 0 (supporting electrolyte) (2) 1.8, (3) 3.6, (4) 5.4, (5) 7.2, (6) 9.0 (µmol/L) at m-AgSAE in river water sample - BR buffer pH 8 (9:1) mixtures. Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

4.24 Cation and anion interference on nifedipine in drinking and river water sample at HMDE. Reference electrode: Ag/AgCl (3 M KCl). Concentration of cation/anion = 5 µmol/L.

4.25 Cation and anion interference on nifedipine in drinking and river water sample at m-AgSAE. Reference electrode: Ag/AgCl (3 M KCl). Concentration of cation/anion = 5 µmol/L.

4.26 Voltammograms for electro-oxidation of nifedipine in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. TiO$_2$/Ti electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

4.27 The percentage of degradation of nifedipine using voltammetry monitoring in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. TiO$_2$/Ti electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

4.28 Voltammograms for electro-oxidation of nifedipine in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. IrO$_2$-TiO$_2$/Ti electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).
4.29 Voltammograms for electro-oxidation of nifedipine in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. RuO$_2$-TiO$_2$/Ti electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

4.30 The percentage degradation of nifedipine using voltammetry monitoring in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. (1) IrO$_2$-TiO$_2$/Ti and (2) RuO$_2$-TiO$_2$/Ti electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

4.31 Voltammograms for electro-oxidation of nifedipine in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. RuO$_2$-IrO$_2$-TiO$_2$/Ti (40:10:50) electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

4.32 Voltammograms for electro-oxidation of nifedipine in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. RuO$_2$-IrO$_2$-TiO$_2$/Ti (10:40:50) electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

4.33 The percentage degradation of nifedipine using voltammetry monitoring in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. (1) RuO$_2$-IrO$_2$-TiO$_2$/Ti (10:40:50) and (2) RuO$_2$-IrO$_2$-TiO$_2$/Ti (40:10:50) electrode as anode; Initial concentration for nifedipine = 10 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).
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5.8 DPCSV for ampicillin at pH 6 using HMDE, BRB 0.04 M as electrolyte, $E_i = 0 \text{ mV}$, $E_{acc} = 0 \text{ mV}$, $t_{acc} = 30 \text{ s}$, scan rate $= 20 \text{ mV s}^{-1}$ where, (1) BR buffer (2) 1 (3) 3 (4) 5 µmol/L of ampicillin. Reference electrode: Ag/AgCl (3 M KCl).

5.9 DPCSV for ampicillin at pH 7 using HMDE, BRB 0.04 M as electrolyte, $E_i = 0 \text{ mV}$, $E_{acc} = 0 \text{ mV}$, $t_{acc} = 30 \text{ s}$, scan rate $= 20 \text{ mV s}^{-1}$ where, (1) BR buffer (2) 1 (3) 3 (4) 5 µmol/L of ampicillin. Reference electrode: Ag/AgCl (3 M KCl).

5.10 DPCSV for ampicillin at pH 7 using m-AgSAE, BRB 0.04 M as electrolyte, $E_i = 0 \text{ mV}$, $E_{acc} = 0 \text{ mV}$, $t_{acc} = 30 \text{ s}$, scan rate $= 20 \text{ mV s}^{-1}$ where, (1) BR buffer (2) 10 (3) 30 (4) 50 µmol/L of ampicillin. Reference electrode: Ag/AgCl (3 M KCl).

5.11 DPCSV for ampicillin at pH 8 using m-AgSAE, BRB 0.04 M as electrolyte, $E_i = 0 \text{ mV}$, $E_{acc} = 0 \text{ mV}$, $t_{acc} = 30 \text{ s}$, scan rate $= 20 \text{ mV s}^{-1}$ where, (1) BR buffer (2) 10 (3) 30 (4) 50 µmol/L of ampicillin. Reference electrode: Ag/AgCl (3 M KCl).

5.12 DPCSV for ampicillin at pH 9 using m-AgSAE, BRB 0.04 M as electrolyte, $E_i = 0 \text{ mV}$, $E_{acc} = 0 \text{ mV}$, $t_{acc} = 30 \text{ s}$, scan rate $= 20 \text{ mV s}^{-1}$ where, (1) BR buffer (2) 10 (3) 30 (4) 50 µmol/L of ampicillin. Reference electrode: Ag/AgCl (3 M KCl).

5.13 The effect of initial potential on ampicillin reduction peak at (A) HMDE and (B) m-AgSAE. $t_{acc} = 30 \text{ s}$, $E_{acc} = 0 \text{ mV}$, scan rate $= 20 \text{ mV s}^{-1}$ and BRB 0.04 M as electrolyte. Concentration of ampicillin at HMDE $=5$ µmol/L, while, 50 µmol/L at m-AgSAE. Reference electrode: Ag/AgCl (3 M KCl).

5.14 The effect of accumulation potential on ampicillin reduction peak at (A) HMDE and (B) m-AgSAE. $t_{acc} = 30 \text{ s}$, $E_i = 0 \text{ mV}$, scan rate $= 20 \text{ mV s}^{-1}$ and BRB 0.04 M as electrolyte. Concentration of ampicillin at HMDE $=5$ µmol/L, while, 50 µmol/L at m-AgSAE. Reference electrode: Ag/AgCl (3 M KCl).
The effect of accumulation time on ampicillin reduction peak at (A) HMDE and (B) m-AgSAE. $E_i = 0 \text{ mV}$, $E_{acc} = 0 \text{ mV}$, scan rate $= 20 \text{ mVs}^{-1}$ and BRB 0.04 M as electrolyte. Concentration of ampicillin at HMDE = 5 µmol/L, while, 50 µmol/L at m-AgSAE. Reference electrode: Ag/AgCl (3 M KCl).

Voltammograms for ampicillin at HMDE in BR-buffer pH 7, ($c_{AMP}$) (1) 0 (supporting electrolyte), (2) 1, (3) 3, (4) 5, (5) 7, (6) 9 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

Voltammograms for ampicillin at m-AgSAE in BR-buffer pH 8, ($c_{AMP}$) (1) 10, (2) 30, (3) 50, (4) 70, (5) 90 (6) 110 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

Voltammograms of ampicillin ($c_{AMP}$ (1) 0 (supporting electrolyte) (2) 0.9, (3) 2.7, (4) 4.5, (5) 6.3, (6) 8.1 (µmol/L) at HMDE in (A) drinking water sample (B) river water sample with mixture of BR buffer pH 7. Reference electrode: Ag/AgCl (3 M KCl).

Voltammograms of ampicillin ($c_{AMP}$ (1) 9, (2) 27, (3) 45, (4) 63, (5) 81 (µmol/L) at m-AgSAE in (A) drinking water sample (B) river water sample with mixture of BR buffer pH 8. Reference electrode: Ag/AgCl (3 M KCl).

Cation and anion interference on ampicillin in drinking and river water sample at HMDE. Reference electrode: Ag/AgCl (3 M KCl). Concentration of cation/anion = 5 µmol/L.

Cation and anion interference on ampicillin in drinking and river water sample at m-AgSAE. Reference electrode: Ag/AgCl (3 M KCl). Concentration of cation/anion = 5 µmol/L.
5.22 Voltammograms for electro-oxidation of ampicillin in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. TiO$_2$/Ti electrode as anode; Initial concentration for ampicillin = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

5.23 The percentage of degradation of ampicillin using voltammetry monitoring in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. TiO$_2$/Ti electrode as anode; Initial concentration for ampicillin = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

5.24 Voltammograms for electro-oxidation of ampicillin in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. IrO$_2$-TiO$_2$/Ti electrode as anode; Initial concentration for ampicillin = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

5.25 Voltammograms for electro-oxidation of ampicillin in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. RuO$_2$-TiO$_2$/Ti electrode as anode; Initial concentration for ampicillin = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

5.26 The percentage degradation of ampicillin using voltammetry monitoring in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. (1) IrO$_2$-TiO$_2$/Ti and (2) RuO$_2$-TiO$_2$/Ti electrode as anode; Initial concentration for ampicillin = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).
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5.28 Voltammograms for electro-oxidation of ampicillin in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. RuO₂-IrO₂-TiO₂/Ti (10:40:50) electrode as anode; Initial concentration for ampicillin = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

5.29 The percentage degradation of ampicillin using voltammetry monitoring in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. (1) RuO₂-IrO₂-TiO₂/Ti (40:10:50) and (2) RuO₂-IrO₂-TiO₂/Ti (10:40:50) electrode as anode; Initial concentration for ampicillin = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

5.30 The chromatograms of ampicillin (A) before electro-oxidation (B) after electro-oxidation and (C) overlay between chromatograms (A) and (B).

5.31 Mass spectra in product ion scan mode of ampicillin at a 0.5 µmol/L in ES⁺.

5.32 Mass spectral profiles of electro-oxidation of ampicillin (A) N-[(E)-3-ethylhex-3-enyl]-3,3-dimethyl-4-thia-1-azabicyclo[3.2.0]heptan-6-amine (B) 3-[2-[(3,3-dimethyl-4-thia-1-azabicyclo[3.2.0]heptan-6-yl)amino]ethyl]hexanedioic acid.

5.33 A proposed reaction pathway for the electro-oxidation of ampicillin by MMO electrode.

6.1 Schematic reduction of Penicillin G.
6.2 DPCSV of 5 µmol/L penicillin G at HMDE in BR buffer. The numbers indicate pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.3 DPCSV of 50 µmol/L penicillin G at m-AgSAE in BR buffer. The numbers indicate pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.4 The dependence of peak potential of penicillin G ($c_{PG} = 5$ µmol/L) at HMDE on pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.5 The dependence of peak potential of penicillin G ($c_{PG} = 50$ µmol/L) at m-AgSAE on pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.6 The dependence of peak current of penicillin G ($c_{PG} = 5$ µmol/L) at HMDE on pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.7 The dependence of peak current of penicillin G ($c_{PG} = 50$ µmol/L) at m-AgSAE on pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.8 DPCSV for penicillin G at pH 9 using HMDE, BRB 0.04 M as electrolyte, $E_i = 0$ mV, $E_{acc}= 0$ mV, $t_{acc}= 30$ s, scan rate = 20 mVs$^{-1}$ where, (1) BR buffer (2) 1 (3) 3 (4) 5 (µmol/L) of penicillin G. Reference electrode: Ag/AgCl (3 M KCl).

6.9 DPCSV for penicillin G at pH 10 using HMDE, BRB 0.04 M as electrolyte, $E_i = 0$ mV, $E_{acc}= 0$ mV, $t_{acc}= 30$ s, scan rate = 20 mVs$^{-1}$ where, (1) BR buffer (2) 1 (3) 3 (4) 5 (µmol/L) of penicillin G. Reference electrode: Ag/AgCl (3 M KCl).

6.10 DPCSV for penicillin G at pH 11 using HMDE, BRB 0.04 M as electrolyte, $E_i = 0$ mV, $E_{acc}= 0$ mV, $t_{acc}= 30$ s, scan rate = 20 mVs$^{-1}$ where, (1) BR buffer (2) 1 (3) 3 (4) 5 (µmol/L) of penicillin G. Reference electrode: Ag/AgCl (3 M KCl).

6.11 DPCSV for penicillin G at pH 12 using HMDE, BRB 0.04 M as electrolyte, $E_i = 0$ mV, $E_{acc}= 0$ mV, $t_{acc}= 30$ s, scan rate = 20 mVs$^{-1}$ where, (1) BR buffer (2) 1 (3) 3 (4) 5 (µmol/L) of penicillin G. Reference electrode: Ag/AgCl (3 M KCl).
6.12 The effect of initial potential on penicillin G reduction peak at pH 12 using (A) HMDE and (B) m-AgSAE. $E_{acc} = 0$ mV, $t_{acc} = 30$ s, scan rate = 20 mVs$^{-1}$ and BRB 0.04 M as electrolyte. Concentration of penicillin G = 5 µmol/L (HMDE) and 50 µmol/L (m-AgSAE). Reference electrode: Ag/AgCl (3 M KCl).

6.13 The effect of accumulation potential on penicillin G reduction peak at pH 12 using (A) HMDE and (B) m-AgSAE. $E_i = 0$ mV, $t_{acc} = 30$ s, scan rate = 20 mVs$^{-1}$ and BRB 0.04 M as electrolyte. Concentration of penicillin G = 5 µmol/L (HMDE) and 50 µmol/L (m-AgSAE). Reference electrode: Ag/AgCl (3 M KCl).

6.14 The effect of accumulation time on penicillin G reduction peak at pH 12 using (A) HMDE and (B) m-AgSAE. $E_i = 0$ mV, $E_{acc} = 0$ mV, scan rate = 20 mVs$^{-1}$ and BRB 0.04 M as electrolyte. Concentration of penicillin G = 5 µmol/L (HMDE) and 50 µmol/L (m-AgSAE). Reference electrode: Ag/AgCl (3 M KCl).

6.15 Voltammograms for penicillin G at HMDE in BR-buffer pH 12, ($c_{PG}$ (1) 0 (supporting electrolyte), (2) 1, (3) 3, (4) 5, (5) 7, (6) 9 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

6.16 Voltammograms for penicillin G at m-AgSAE in BR-buffer pH 12, ($c_{PG}$ (1) 0 (supporting electrolyte), (2) 10, (3) 30, (4) 50, (5) 70, (6) 90 (µmol/L). Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).

6.17 Voltammograms of penicillin G ($c_{PG}$ (1) 0 (supporting electrolyte) (2) 0.9, (3) 2.7, (4) 4.5, (5) 6.3, (6) 8.1 (µmol/L) at HMDE in (A)drinking water sample (B) river water sample with mixture of BR buffer pH 12 Reference electrode: Ag/AgCl (3 M KCl).
6.18 Voltammograms of penicillin G (c<sub>PG</sub>) (1) 0 (supporting electrolyte) (2) 9, (3) 27, (4) 45, (5) 63, (6) 81 (µmol/L) at m-AgSAE in (A)drinking water sample (B) river water sample with mixture of BR buffer pH 12. Reference electrode: Ag/AgCl (3 M KCl).

6.19 Cyclic voltammograms of 500 µmol/L penicillin G at BDDE in BR buffer. The numbers indicate pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.20 The dependence of CV peak potential of penicillin G (c<sub>PG</sub> =500 µmol/L) at BDDE on pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.21 The dependence of CV peak current of penicillin G (c<sub>PG</sub> =500 µmol/L) at BDDE on pH of BR-buffer. Reference electrode: Ag/AgCl (3 M KCl).

6.22 Mechanism of electro-oxidation of (A) Penicillin G to (B) Beta-sulfoxide of penicillin G.

6.23 Effect of initial potential on penicillin G oxidation peak on BDD electrode at BRB pH 4. Scan rate = 50 mV/s. Concentration of penicillin G = 5 µmol/L. Reference electrode: Ag/AgCl (3 M KCl).

6.24 Effect of accumulation potential on penicillin G oxidation peak on BDD electrode at BRB pH 4. Scan rate = 50 mV/s. Concentration of penicillin G = 5 µmol/L. Reference electrode: Ag/AgCl (3 M KCl).

6.25 Effect of accumulation time (t<sub>acc</sub>) on 5 µmol/L penicillin G oxidation peak on BDDE in BRB pH 4 with initial potential (E<sub>i</sub>) = 500 mV and scan rate = 50 mV/s. Reference electrode: Ag/AgCl (3 M KCl).

6.26 DP voltammogram of penicillin G at BDDE in BR buffer pH 4 (1) 0 (supporting electrolyte) (2) 1 (3) 3 (4) 5 (5) 7 (6) 9 (µmol/L) in 0.04 M BRB. Right: Corresponding calibration straight lines. Reference electrode: Ag/AgCl (3 M KCl).
6.27 DP voltammogram of penicillin G at BDDE in drinking water sample-BR buffer pH 4 (9:1) (1) 0 (supporting electrolyte) (2) 0.9 (3) 2.7 (4) 4.5 (5) 6.3 (6) 8.1 (µmol/L) in 0.04 M BRB. Reference electrode: Ag/AgCl (3 M KCl).

6.28 DP voltammogram of penicillin G at BDDE in river water sample-BR buffer pH 4 (9:1) (1) 0 (supporting electrolyte) (2) 0.9 (3) 2.7 (4) 4.5 (5) 6.3 (6) 8.1 (µmol/L) in 0.04 M BRB. Reference electrode: Ag/AgCl (3 M KCl).

6.29 Cation and anion interference on penicillin G in drinking and river water sample at HMDE. Reference electrode: Ag/AgCl (3 M KCl). Concentration of cation/anion = 5 µmol/L.

6.30 Cation and anion interference on penicillin G in drinking and river water sample at m-AgSAE. Reference electrode: Ag/AgCl (3 M KCl). Concentration of cation/anion = 5 µmol/L.

6.31 Cation and anion interference on penicillin G in drinking and river water sample at BDDE. Reference electrode: Ag/AgCl (3 M KCl). Concentration of cation/anion = 5 µmol/L.

6.32 Voltammograms for electro-oxidation of penicillin G in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. TiO₂/Ti electrode as anode; Initial concentration for penicillin G = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).

6.33 The percentage of degradation of penicillin G using voltammetry monitoring in (A) pH 4, (B) tap water and (C) pH 10; electro-oxidation time (1) 0 min (2) 15 min (3) 30 min (4) 60 min. TiO₂/Ti electrode as anode; Initial concentration for penicillin G = 5 µmol/L. Working electrode for voltammetry determination: HMDE. Reference electrode: Ag/AgCl (3 M KCl).
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6.43 A proposed reaction pathway for the electro-oxidation of penicillin G by MMO electrode.
LIST OF ABBREVIATIONS

Ag/Cl   - Argentum Chloride
AMP     - Ampicillin
BDDE    - Boron Doped Diamiond Electrode
BRB     - Britton Robinson buffer solution
CV      - Cyclic voltammetry
DPASV   - Differential pulse anodic stripping voltammetry
DPCSV   - Differential pulse cathodic stripping voltammetry
DPV     - Differential pulse voltammetry
DW      - Deionized water
HMDE    - Hanging mercury drop electrode
KCl     - Potassium chloride
LC-MS   - Liquid chromatography mass spectrometry
LOD     - Limit of detection
LOQ     - Limit of quantitative
m-AgSAE - Mercury meniscus silver solid alamalgam electrode
MeOH    - Methanol
MMO     - Mixed metal oxide electrode
NEPs    - New emerging pollutants
NFD     - Nifedipine
PG      - Penicillin G
RSD     - Relative standard deviation
SD      - Standard deviation
UV      - Ultra violet
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<table>
<thead>
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<tr>
<td>µM</td>
<td>micro molar</td>
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<tr>
<td>µm</td>
<td>micrometer</td>
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<td>µmol/L</td>
<td>micromol per litre</td>
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<tr>
<td>A</td>
<td>Ampere</td>
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<td>A/cm²</td>
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<tr>
<td>E_{acc}</td>
<td>Accumulation potential</td>
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<tr>
<td>E_i</td>
<td>Initial potential</td>
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<tr>
<td>E_{reg}</td>
<td>Regenerated Potential</td>
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<tr>
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<td>IrO₂</td>
<td>Iridium Oxide</td>
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<td>mass per charge number</td>
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<td>nanoampere</td>
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<tr>
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<td>Ruthenium Oxide</td>
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<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>t_{acc}</td>
<td>Accumulation time</td>
</tr>
<tr>
<td>TiO₂</td>
<td>Titanium Oxide</td>
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<tr>
<td>V</td>
<td>Voltage</td>
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<tr>
<td>v/v</td>
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<tr>
<td>µL</td>
<td>microliter</td>
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<tr>
<td>Ω cm</td>
<td>ohm centimeters</td>
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CHAPTER 1

INTRODUCTION

1.0 Background of Study

In recent years, there has been an increasing concern about the presence of new emerging pollutants (NEPs) such as pesticides, drugs, dyes and endocrine disrupting chemicals (EDCs) waste in the aquatic environment (Deblonde et al., 2011; Rodil et al., 2012; Jiang et al., 2013). These may cause a huge effect in the environment and eventually will affect human health. NEPs had been observed in the sewage water (De la Torre et al., 2012; Peysson and Vulliet, 2013), surface water (Moreno-González et al., 2013; Meffe and de Bustamante, 2014) or even in drinking water (Schriks et al., 2010; Post et al., 2012) and the increasing concern over the risks of NEPs is reflected by a rapid increase in the number of scientific publications (Scopus, ISI journal and web of science) exploring the environmental impacts of NEPs over the past few years (Fig. 1.1).

Among the NEPs, the presence of drugs (antibiotic, anti-depression and hypertension drugs) in the aquatic environment seems to attract researchers due to their unrestricted and widely used in both human and livestock. These drugs enter the environment through many ways such as wastewater affluent, treated sewage, industrial and clinical waste and also via excretion by human and animals (through urine; as inactive polar conjugates). Some of them can disrupt the endocrine system of aquatic animals (Benjamin, et al., 2008 and Pei-Jen et al., 2007). It also can enter the food chain and can increase the risk of cancer in human (Banerjee et al., 2011; Bangalore et al., 2011; Veitonmäki et al., 2013). Antibiotic drugs for example have
several side effects ranging from hypersensitivity reaction (e.g. allergy to penicillin), soft stools or diarrhea to more serious effects such as damage of vital organs (Sørensen et al., 1999; Evaggelopoulou and Samanidou, 2013).

Figure 1.1: Number of publications with the words ‘new emerging contaminants’ in the title or abstract published since 2006.

Electroanalytical methods (e.g. voltammetric techniques) are well known as one of the alternative techniques for the determination of metals (Achterberg and Braungardt, 1999; Hwang et al., 2008; Injang et al., 2010; Trindade et al., 2012; Nascimento et al., 2014; Lin et al., 2015) and organic compounds (Zanoni et al., 1997; Guaratini et al., 2001; Guiberteau et al., 2001; Deýlová et al., 2014; Ivandini et al., 2014; Janíková-Bandžuchová et al., 2015). The advantages of the voltammetric techniques are due to its sensitivity, selectivity, simple instrument, easy to operate and low operational costs. The sensitivity of the technique can also be increased by modification of the working electrodes.

The fundamental concept of electrochemistry is basically based on the interface between the measurements of electrical quantities such as current, potential and charge and also their relationship with chemical parameters. There are two types of electro-analytical measurements namely;
(i) Potentiometry which is a static (zero current) technique in which information about the sample composition is obtained from measurement of potential established across a membrane.

(ii) Potentiostatic also known as controlled potential that based on the dynamic (non-zero current) situations. The electrode potential is used to drive an electron-transfer reaction and initial the measurement of current. This principle can measure any chemical species that is electro-active, i.e. which can be made to reduce or oxidize.

Electrochemical methods (electro-oxidation, electro-coagulation, electro-flotation, etc.) have gained interest in water treatment for the degradation/removal of NEPs. For example, electro-oxidation has been successfully applied to degrade organic pollutants (Särkkä et al., 2008; Bagastyo et al., 2011; Schaefer et al., 2015). The electro-oxidation of NEPs can be enhanced by using metal oxide (MO) and mixed metal oxides (MMO) electrodes. These electrodes have shown a promising results for several electro-oxidation processes in degradation of NEPs such as drugs (Radenovic et al., 2011; Sopaj et al., 2015), dyes (Zhou et al., 2011), pesticides (Henych et al., 2015; Madsen et al., 2015) and also other organic compounds (Särkkä et al., 2008; Schaefer et al., 2015). MMO have some advantages include a wide potential window in aqueous and non-aqueous electrolytes, chemical and physical stability and small background, which makes them a popular choice as a new electrode material.

1.2 Problem Statement

Currently, several methods are available for the determination of NEPs, but most of these methods need expensive instrumentations, tedious sample preparation and suffer from instrument difficulty due to matrix interference. High performance liquid chromatography (HPLC) for example have a complex design (prior extraction and pre-concentration stage) and also need to combine with other instrument such as
MS-MS in order to obtain lower detection limit. Thus, a new method must be developed to overcome this problem. In the case on electrochemical studies, working electrode such as HMDE was the common and popular working electrodes used in the determination of NEPs. However, due to the toxicity (E.g. HMDE (metallic mercury)), alternative electrodes are searched possibly with performance similar to mercury electrodes but less toxic and more environmentally friendly (“Greener working electrode”).

The interest in NEPs does not only focus on the main compounds but also the degradation or transformational products. Many reports stated that the presence of NEPs in pharmaceutical effluents and surface waters have raised substantial concern in the public and regulatory agencies as it gives potential risk to humans and wildlife. It is important to have an effective primary treatment for the removal/degradation of NEPs from hospital and clinical wastewater to protect the environment. From our literature reviews, a number of methods such as bio-analytical assessments, photodegradation and activated carbon have been used to overcome this situation. However, most of these methods still do not satisfy the requirements (e.g. need longer time to degrade the compounds). Hence, a new method must be developed in order to reduce and eliminate these chemicals.

1.3 Research Objectives

1) To study the electrochemical behavior of selected drugs using voltammetric techniques with three different working electrodes (Hanging mercury drop electrode (HMDE), mercury meniscus silver solid amalgam electrode (m-AgSAE) and boron doped diamond electrode (BDDE)).

2) To develop a method for the determination of the drugs utilizing (BDDE and m-AgSAE) as new “Green” working electrode.

3) To study and evaluate the efficiency of commercial mixed metal oxide (MMO) electrodes in the electro-oxidation of the selected drugs.
4) To apply the developed method for the determination of selected drugs in real samples.

1.4 Scope of Research

The scope of the study involves the determination and the electro-oxidation of ampicillin, penicillin G and nifedipine. To achieve these, the study was divided into three parts;

1. Part I: The study of the electrochemical behavior of ampicillin, penicillin G and nifedipine using voltammetric technique with hanging mercury drop electrode (HMDE), mercury meniscus modified silver amalgam electrode (m-AgSAE) and boron doped diamond electrode (BDDE) working electrodes.

2. Part II: The employment of the developed method for the determination of ampicillin, penicillin G and nifedipine in real samples (drinking and river water) using HMDE, m-AgSAE and BDDE.

3. Part III: The study of the electro-oxidation of ampicillin, penicillin G and nifedipine using commercial mixed metal oxide electrodes (different in compositions and ratios; TiO$_2$/Ti, IrO$_2$-TiO$_2$/Ti, RuO$_2$-TiO$_2$/Ti, IrO$_2$-RuO$_2$-TiO$_2$/Ti) and the determination of the degradation (or transformational products) were carried out using voltammetric and LC-MS techniques.
1.5 Significance of Research

Due to the increasing fears in the toxicity of mercury, which can cause “mercurophobia” (Cizek et al., 2007; Fischer et al., 2007; Chorti et al., 2014), a newly developed electrode must be constructed to overcome these crucial problems; resulting in the development of mercury meniscus modified silver amalgam electrode (m-AgSAE). M-AgSAE can be compared with HMDE and it is a suitable alternative electrode for HMDE with less toxicity.

On the other hand, the electrochemical methods for removal of heavy metals in industrial wastewaters are well known. However, the application for the removal/degrade of organic pollutants is quite new. Electrochemical method is seen to be the suitable and simple method for NEPs removal/degrade because of the simultaneous oxidation-reduction process taking place at the electrodes without the need to add in any chemicals and time consuming. Indeed, the electrochemical methods have been suggested as a useful method of removing/degrade harmful NEPs in wastewater and effluent.

Fortunately, the unique properties of MMO (as anode materials) can successfully overcome these problems. MMO electrode has high catalytic activity and also has low oxygen evolution over potential which can make the electrode easily realize oxygen evolution. This research will help to develop a new method for the degradation of ampicillin, penicillin G and nifedipine using electro-oxidation method and to evaluate the effectiveness of MMO electrodes in order to degrade nifedipine, ampicillin and penicillin G.
1.6 Research Novelty

The novelty of this research includes:

a) The method for electrochemical determination of ampicillin, penicillin G and nifedipine mercury meniscus modified silver amalgam electrode (m-AgSAE). The mercury meniscus modified silver amalgam electrode (m-AgSAE) is equally sensitive compared with HMDE (i.e. still having the properties of HMDE) but more “Greener” (less amount of mercury used).

b) A novel method for the removal/degradation of ampicillin, penicillin G and nifedipine using electro-oxidation method with titanium based mixed metal oxide (MMO) as the anode electrode.
REFERENCES


Niksa, M.J. and E.J.R. (2009). Precious metal containing mixed metal oxide (MMO) electrodes have revolutionized the electrochemical industries, especially electroplating and chlorine generation. MMO electrodes have also enabled the development of other electrochemical processes by impr.


