STRIPPING VOLTAMMETRIC METHODS FOR THE DETERMINATION OF AFLATOXIN COMPOUNDS

MOHAMAD HADZRI BIN YAACOB

UNIVERSITI TEKNOLOGI MALAYSIA

STRIPPING VOLTAMMETRIC METHODS FOR THE DETERMINATION OF AFLATOXIN COMPOUNDS

MOHAMAD HADZRI BIN YAACOB

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy

Faculty of Science
Universiti Teknologi Malaysia

APRIL 2006

ABSTRACT

Aflatoxin, which is produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi is one of the compounds in the mycotoxin group. The main types of aflatoxins are AFB1, AFB2, AFG1 and AFG2 which have carcinogenic properties and are dangerous to human health. Various techniques have been used for their measurements such as the high performance liquid chromatography (HPLC), enzyme linked immunosorbant assay (ELISA) and radioimmunoassay (RIA) but all these methods have disadvantages such as long analysis time, consume a lot of reagents and expensive. To overcome these problems, the voltammetric technique was proposed in this study using controlled growth mercury drop (CGME) as the working electrode and Britton Robinson buffer (BRB) as the supporting electrolyte. The voltammetric methods were used for investigating the electrochemical properties and the quantitative analysis of aflatoxins at the mercury electrode. The experimental conditions were optimised to obtain the best characterised peak in terms of peak height with analytical validation of the methods for each aflatoxin. The proposed methods were applied for the analysis of aflatoxins in groundnut samples and the results were compared with those obtained by the HPLC technique. All aflatoxins were found to adsorb and undergo irreversible reduction reaction at the working mercury electrode. The optimum experimental parameters for the differential pulse cathodic stripping voltammetry (DPCSV) method were the BRB at pH 9.0 as the supporting electrolyte, initial potential (E_i): -0.1 V, final potential (E_f): -1.4 V, accumulation potential (E_{acc}): -0.6 V, accumulation time (t_{acc}): 80 s, scan rate: 50 mV/s and pulse amplitude: 80 mV. The optimum parameters for the square wave stripping voltammetry (SWSV) method were E_i = -0.1 V, E_f = -1.4 V, E_{acc}: -0.8 V, t_{acc}: 100 s, scan rate: 3750 mV/s, frequency: 125 Hz and voltage step: 30 V. At the concentration of 0.10 μM, using DPCSV method with the optimum parameters, AFB1, AFB2, AFG1 and AFG2 produced a single peak at -1.21 V, -1.23 V, -1.17 V and -1.15 V (versus Ag/AgCl) respectively. Using the SWSV method, a single peak appeared at -1.30 V for AFB1 and AFB2 while -1.22 V for AFG1 and AFG2. The calibration curves for all aflatoxins were linear with the limit of detection (LOD) of approximately 2.0 ppb and 0.50 ppb obtained by the DPCSV and SWSV methods respectively. The results of aflatoxins content in individual groundnut samples do not vary significantly when compared with those obtained by the HPLC technique. Finally, it can be concluded that both proposed methods which are accurate, precise, robust, rugged, fast and low cost were successfully developed and are potential alternative methods for routine analysis of aflatoxins in groundnut samples.

ABSTRAK

Aflatoksin adalah sejenis sebatian yang dihasilkan oleh kulat *Aspergillus flavus* dan *Aspergillus parasiticus* yang digolongkan di dalam kumpulan mikotoksin. Jenis utama aflatoksin adalah AFB1, AFB2, AFG1 dan AFG2 yang bersifat karsinogen serta merbahaya kepada kesihatan manusia. Pelbagai teknik telah digunakan untuk menentukan aflatoksin seperti kromatografi cecair prestasi tinggi (HPLC), asai serapan imuno berikatan enzim (ELISA) dan radioamunoasai (RIA) tetapi teknik-teknik ini mempunyai kelemahan seperti masa analisis yang panjang, melibatkan reagen yang banyak dan kos yang mahal. Untuk mengatasi masalah ini, teknik voltammetri telah dicadangkan untuk kajian aflatoksin menggunakan titisan raksa pembesaran terkawal (CGME) sebagai elektrod bekerja dan larutan penimbal Britton-Robinson (BRB) sebagai elektrolit penyokong. Pelbagai kaedah voltammetri telah digunakan untuk mengkaji sifat elektrokimia aflatoksin pada elektrod raksa dan analisis kuantitatifnya. Parameter kajian telah dioptimumkan untuk memperolehi puncak yang elok berdasarkan ketinggian puncak serta pengesahan analisis untuk kaedah yang dibangunkan bagi setiap aflatoksin. Kaedah ini telah digunakan untuk menentukan kandungan aflatoksin di dalam sampel kacang tanah di mana keputusan yang diperolehi telah dibandingkan dengan keputusan HPLC. Semua aflatoksin yang dikaji didapati terjerap dan menjalani proses tindakbalas penurunan tidak berbalik pada elektrod raksa. Parameter optimum untuk kaedah voltammetri perlucutan kathodik denyut pembeza (DPCSV) adalah larutan BRB pada pH 9.0 sebagai larutan elektrolit, keupayaan awal (E_i): -1.0 V, keupayaan akhir (E_f): -1.4 V, keupayaan pengumpulan (E_{acc}): -0.6 V, masa pengumpulan (t_{acc}): 80 s, kadar imbasan: 50 mV/s dan amplitud denyut: 80 mV. Untuk kaedah voltammetri perlucutan gelombang bersegi (SWSV), parameter optimum adalah E_i : -1.0 V, E_f : -1.4 V, E_{acc}: -0.8 V, t_{acc}: 100 s, kadar imbasan: 3750 mV/s, frekuensi: 125 Hz dan beza keupayaan: 30 mV. Menggunakan parameter optimum untuk DPCSV, 0.10 μM AFB1, AFB2, AFG1 dan AFG2 menghasilkan puncak tunggal pada keupayaan -1.21 V, -1.23 V, -1.17 V dan -1.15 V (melawan Ag/AgCl) masing-masingnya. Menggunakan kaedah SWSV, puncak terhasil pada -1.30 V untuk AFB1 dan AFB2, -1.22 V untuk AFG1 dan AFG2. Keluk kalibrasi adalah linear untuk semua aflatoksin dengan had pengesanan (LOD) pada 2.0 dan 0.5 ppb diperolehi dari kaedah DPCSV dan SWSV masing-masingnya. Keputusan analisis kandungan aflatoksin di dalam sampel kacang tanah tidak memberi perbezaan ketara berbanding dengan yang diperolehi menggunakan teknik HPLC. Kesimpulannya, kedua-dua kaedah yang dikaji yang merupakan kaedah yang tepat, jitu, cepat, sesuai digunakan dengan pelbagai model voltammetri dan kos yang murah telah berjaya dibangunkan dan berpotensi besar menjadi kaedah alternatif untuk analisis kandungan aflatoksin di dalam kacang tanah secara berkala.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AAS	Atomic absorption spectrometry
Abs	Absorbance
ACP	Alternate current polarography
ACV	Alternate current voltammetry
AD	Amperometric detector
AdCSV	Adsorptive cathodic stripping voltammetry
AE	Auxiliary electrode
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
AFP1	Aflatoxin P1
AFQ1	Aflatoxin Q1
Ag/AgCl	Silver/silver chloride
ASV	Anodic stripping voltammetry
β-CD	β-cyclodextrin
BFE	Bismuth film electrode
BLMs	Bilayer lipid membranes
BRB	Britton Robinson Buffer
C_A	Concentration of analyte
CE	Capillary electrophoresis
CGME	Controlled growth mercury electrode
CME	Chemically modified electrode
CPE	Carbon paste electrode
CSV	Cathodic stripping voltammetry
CV	Cyclic voltammetry

DC	Direct current
DCP	Direct current polarography
DME	Dropping mercury electrode
DMSO	Dimethyl sulphonic acid
DNA	Deoxyribonucliec acid
DPCSV	Differential pulse cathodic stripping voltammetry
DPP	Differential pulse polarography
DPV	Differential pulse voltammetry
E_{acc}	Accumulation potential
E_i	Initial potential
E_f	Final potential
E_{high}	High potential
E_{low}	Low potential
E_p	Peak potential
ECS	Electrochemical sensing
Et_4NH_4 OH	Tetraethyl ammonium hydroxide
ELISA	Enzyme linked immunosorbant assay
FD	Fluorescence detector
FDA	Food and Drug Administration
GCE	Glassy carbon electrode
GC-FID	Gas chromatography with flame ionisation detector
HMDE	Hanging mercury drop electrode
HPLC	High performance liquid chromatography
HPTLC	High pressure thin liquid chromatography
IAC	Immunoaffinity chromatography
IACLC	Immunoaffinity column liquid chromatography
IAFB	Immunoaffinity fluorometer biosensor
IARC	International Agency for Research Cancer
I_c	Charging current
I_d	Diffusion current
I_f	Faradaic current

I_p	Peak height
ICP-MS	Induced coupled plasma-mass spectrometer
IR	Infra red
IUPAC	International Union of Pure and Applied Chemistry
KGy	Kilogray
LD_{50}	Lethal dose 50
LOD	Limit of detection
LOQ	Limit of quantification
LSV	Linear sweep voltammetry
MFE	Mercury film electrode
MS	Mass spectrometer
MECC	Micellar electrokinetic capillary chromatography
MOPS	3-(N-morpholino)propanesulphonic
MOSTI	Ministry of Science, Technology and Innovation
NP	Normal polarography
NPP	Normal pulse polarography
NPV	Normal pulse voltammetry
OPLC	Over pressured liquid chromatography
PAH	Polycyclic aromatic hydrocarbon
PLL	Poly-L-lysine
ppb	part per billion
ppm	part per million
PSA	Potentiometric stripping analysis
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
RE	Reference electrode
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RSD	Relative standard deviation
S/N	Signal to noise ratio
SCE	Standard calomel electrode
SCV	Stair case voltammetry

SDS	Sodium dodecyl sulphate
SHE	Standard hydrogen electrode
SIIA	Sequential injection immunoassay
SMDE	Static mercury drop electrode
SPE	Solid phase extraction
SPCE	Screen printed carbon electrode
SWP	Square-wave polarography
SWV	Square-wave voltammetry
SWSV	Square-wave stripping voltammetry
SV	Stripping voltammetry
t_{acc}	Accumulation time
TBS	Tris buffered saline
TEA	Triethylammonium
TLC	Thin layer chromatography
TFA	Trifluoroacetic acid
UME	Ultra microelectrode
ν	Scan rate
v/v	Volume per volume
UVD	Ultraviolet-Visible detector
UV-VIS	Ultraviolet-Visible
WE	Working electrode
WHO	World Health Organisation
λ_{max}	Maximum wavelenght
ε_{max}	Maximum molar absorptivity

LIST OF APPENDICES

CHAPTER I

LITERATURE REVIEW

1.1 Overview

Humans are continuously exposed to varying amounts of chemicals that have been shown to have carcinogenic or mutagenic properties in environmental systems. Exposure can occur exogenously when these agents are present in food, air or water, and also endogenously when they are products of metabolism or pathophysiologic states such as inflammation. Great attention is focused on environmental health in the past two decades as a consequence of the increasing awareness over the quality of life due to major environment pollutants that affect it. Studies have shown that exposure to environmental chemical carcinogens have contributed significantly to cause human cancers, when exposures are related to life style factors such as diet (Wogan *et al.,* 2004).

The contamination of food is part of the global problem of environmental pollution. Foodstuffs have been found contaminated with substances having carcinogenic, mutagenic, teratogenic and allergenic properties. As these substances can be supplied with food throughout the entire life-time of a person, it is necessary to deal with the chronic action of trace amounts of such substances. Hence the systematic determination of the foreign substances in nutritional products and feedstock plays an important role. The determination of trace impurities presents considerable difficulties owing to the fact that food is a complex system containing thousands of major and minor compounds (Nilufer and Boyacio, 2002). Increasing environment pollution by toxic substances such as toxic metals, organometallic and organic pollutants in air,

water, soil and food, calls for reliable analytical procedures for their control in environmental samples which needs reliable and sensitive methods (Fifield and Haines, 2000). The choice of the method of analysis depends on the sample, the analyte to be assayed, accuracy, limit of detection, cost and time to complete the analysis (Aboul-Eneim *et a.,* 2000). For development of this method, emphasis should be on development of simplified, cost-effective and efficient method that complies with the legislative requirements (Stroka and Anklam, 2002; Enker, 2003).

The widespread occurrence of aflatoxins producing fungi in our environment and the reported naturally occurring of toxin in a number of agricultural commodities has led the investigator to develop a new method for aflatoxin analysis (Creepy, 2002). An accurate and sensitive method of analysis is therefore required for the determination of these compounds in foodstuffs that have sustained mould growth.

Numerous articles concerning methods for determination of aflatoxins have been published. However, with regard to electroanalytical technique, only one method of determination was reported using the differential pulse pulse polarographic (DPP) technique which was developed by Smyth *et al.* (1979). In this experiment, the obtained limit of detection of aflatoxin B1 was 25 ppb which was higher as compared to the common amount of aflatoxin in contaminated food samples which is 10 ppb as reported by Pare (1997) or even less. In Malaysia, the regulatory limit for total aflatoxins in groundnut is 15 ppb. The regulatory for other foods and milk is 10 ppb and 0.05 ppb respectively (Malaysian Food Act, 1983).

1.2 AFLATOXINS

1.2.1 Aflatoxins in General

Aflatoxins are a group of heterocyclic, oxygen-containing mycotoxins that possess the bisdifuran ring system. It was discovered some 43 years ago in England

following a poisoning outbreak causing 100,000 turkeys death (Miller, 1987 and Cespedez and Diaz, 1997). The aflatoxins are the most widely distributed fungal toxins in food. The occurrence of the aflatoxins in food products demonstrated that the high levels of aflatoxins are significant concern both for food traders and food consumers (Tozzi *et al.* 2003; Herrman, 2004; Haberneh, 2004). Aflatoxin is a by-product of mold growth in a wide range of agriculture commodities such as peanuts (Urano *et al.*, 1993), maize and maize based food (Papp *et al.*, 2002; Mendez-Albores *et al.,* 2004), cottonseeds (Pons and Franz, 1977), cocoa (Jefferey *et al.,* 1982), coffee beans (Batista *et al.,* 2003), medical herbs (Reif and Metzger, 1998; Rizzo *et al.*, 2004), spices (Erdogen, 2004; Garner *et al.,* 1993; Akiyama *et al.*, 2001; Aziz *et al.*, 1998), melon seeds (Bankole *et al.,* 2004) and also in human food such as rice (Shotwell *et al.*, 1966; Begum and Samajpati, 2000), groundnut (Bankole *et al.*, 2005), peanut products (Patey *et al.*, 1990), corn (Shotwell and Goulden, 1977; Urano *et al.,* 1993), vegetable oil (Miller *et al.*, 1985), beer (Scott and Lawrence, 1997), dried fruits (Abdul Kadar *et al.,* 2004, Arrus *et al*. (2004), milk and dairy products (Kamkar, 2004; Aycicek *et al.* 2005; Sarimehmetoglu *et al.,* 2004; Martin and Martin, 2004). Meat and meat products are also contaminated with alfatoxins when farm animals are fed with aflatoxin contaminated feed (Miller, 1987 and Chiavaro *et al.,* 2001).

The molds that are major producers of aflatoxin are *Aspergillus flavus* (Bankole *et al.* 2004) and *Aspergillus parasiticus* (Begum and Samajpati, 2000; Setamou *et al.*, 1997; Erdogen, 2004; Gourama and Bullerman, 1995). *Aspergillus flavus,* which is ubiquitous, produces B aflatoxins (Samajphati, 1979) while *Aspergillus parasiticus,* which produces both B and G aflatoxins, has more limited distribution (Garcia-Villanova *et al.*, 2004). A picture of *Aspergillus flavus* seen under an electron microscope is shown in Figure 1.0.

Black olive is one of the substrate for *Aspergillus parasiticus* growth and aflatoxin B1 production as reported by Leontopoulos *et al.,* (2003). Biosynthesis of aflatoxins by this fungi depends on the environmental condition such as temperature and humidity during crop growth and storage (Leszczynska *et al.,* 2000; Tarin *et al.,*

2004 and Pildain *et al.,* 2004). The optimum temperatures for aflatoxins growth are 27.84 ^{0} C and 27.30 ^{0} C at pH=5.9 and 5.5 respectively.

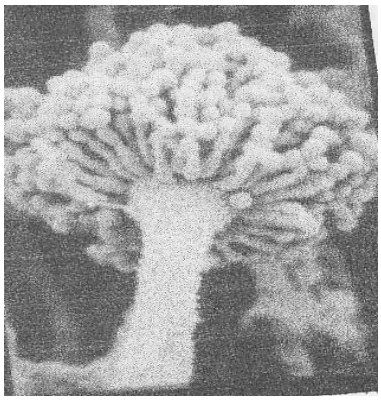


Figure 1.0: *Aspergillus flavus* seen under an electron microscope

Before harvest, the risk for the development of aflatoxins is greatest during major drought (Turner *et al*., 2005). When soil moisture is below normal and temperature is high, the number of *Aspergillus* spores in the air increases. These spores infect crops through areas of damage caused by insects and inclement weather. Once infected, plant stress occurs, which favor the production of aflatoxins. Fungal growth and aflatoxins contamination are the sequence of interactions among the fungus, the host and the environment. The appropriate combination of water stress, high temperature stress and insect damage of the host plant are major determining factors in mold infestation and toxin production (Faraj *et al.*, 1991; Koehler, 1985; Park and Bullerman, 1983). Additional factors such as heat treatment, modified-atmosphere packaging or the presence of preservative, also contribute in increasing growth rate of the aflatoxins.

Farmers have minimal control over some of these environmental factors. However appropriate pre-harvest and post-harvest management and good agricultural practice, including crop rotation, irrigation, timed planting and harvesting and the use

of pesticides are the best methods for preventing or controlling aflatoxins contamination (Turner *et al.,* 2005). Timely harvesting could reduce crop moisture to a point where the formation of the mould would not occur. For example harvesting corn early when moisture is above 20 percent and then quickly drying it to a moisture level of at least 15 percent will keep the *Aspergillus flavus* from completing its life cycle, resulting in lower aflatoxin concentration. Aflatoxins are to be found in agricultural products as a consequence of unprosperous storage conditions where humidity of 70 - 90 % and a minimum temperature of about 10° C. Commodities that have been dried to about 12 to 0.5 % moisture are generally considered stable, and immune to any risk of additional aflatoxins development. Moreover, the minimum damage of shells during mechanized harvesting of crop reduces significantly the mould contamination. Biocontrol of aflatoxin contamination is another way to reduce this contamination. The natural ability of many microorganisms including bacteria, actinomycetes, yeasts, moulds and algae has been a source for bacteriological breakdown of mycotoxins. The most active organism such as *Flavobacterium aurantiacum* which in aqueous solution can take up and metabolise aflatoxins B1, G1 and M1 (Smith and Moss, 1985).

Production of aflatoxins is greatly inhibited by propionic acid as revealed by Molina and Gianuzzi (2002) when they studied the production of aflatoxins in solid medium at different temperature, pH and concentration of propionic acid. It also can be inhibited by essential oil extracted from thyme as found by Rasooli and Abnayeh (2004). Other chemicals that can inhibit the growth of this fungus are ammonia, copper sulphate and acid benzoic (Gowda *et al.,* 2004).

1.2.2 Chemistry of Aflatoxins

Aflatoxins can be classified into two broad groups according to chemical structure which are difurocoumarocyclopentenone series and ifurocoumarolactone (Heathcote, 1984). They are highly substituted coumarin derivatives that contain a fused dihydrofuran moiety. The chemical structure of coumarin is shown in Figure 1.1.

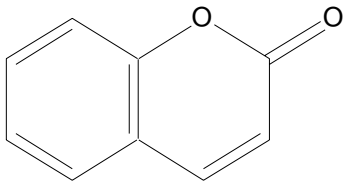


Figure 1.1 Chemical structure of coumarin

There are six major compounds of aflatoxin such as aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG2), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) (Goldblatt, 1969). The former four are naturally found aflatoxins and the AFM1 and AFM2 are produced by biological metabolism of AFB1 and AFB2 from contaminated feed used by animals. They are odorless, tasteless and colorless. The scientific name for these aflatoxin compounds are listed in Table 1.0. Aflatoxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. The chemical structures of these aflatoxins are shown in Figure 1.2. The G series of aflatoxin differs chemically from B series by the presence of a β-lactone ring, instead of a cyclopentanone ring. Also a double bond that may undergo reduction reaction is found in the form of vinyl ether at the terminal furan ring in AFB1 and AFG1 but not in AFB2 and AFG2. However this small difference in structure at the C-2 and C-3 double bond is associated with a very significant change in activity, whereby AFB1 and AFG1 are carcinogenic and considerably more toxic than AFB2 and AFG2. The dihydrofuran moiety in the structure is said to be of primary importance in producing biological effects. Hydroxylation of the bridge carbon of the furan rings for AFM1 does not significantly alter the effects of the compounds. The absolute configuration of AFB2 and AFG2 follows from the fact that it is derived from the reduction of AFB1 and AFG1 respectively.

AFB is the aflatoxin which produces a blue color under ultraviolet while AFG produces the green color. AFM produces a blue-violet fluorescence while AFM2 produces a violet fluorescence (Goldblatt, 1969). Relative fluorescence of aflatoxins in several organic solvents are shown in Appendix A (White and Afgauer, 1970). The

Table 1.0 Scientific name for aflatoxin compounds

Aflatoxin B1 (AFB1)	2,3,6a,9a-tetrahydro-4-methoxycyclopenta[c] furo[3',2':4,5]furo[2,3-*h*][l] benzopyran-1,11-dione
Aflatoxin B2 (AFB2)	2,3,6a,8,9,9a-Hexahydro-4-methoxycyclopenta[c] furo[3',2':4,5]furo[2,3-*h*][l] benzopyran-1,11-dione
Aflatoxin G1 (AFG1)	3,4, 7a,10a-tetrahydro-5-methoxy-1H, 12H furo[3',2':4,5]furo[2,3-*h*]pyrano[3,4-c]l]- benzopyran-1,12-dione
Aflatoxin G2 (AFG2)	3,4,7a,8,9,10, 10a-Hexahydro-5-methoxy-1H,12H-furo[3',2':4,5]furo[2,3-*h*]pyrano[3,4-c][l]- benzopyran-1,12-dione
Aflatoxin M1 (AFM1)	4-Hydroxy AFB1
Aflatoxin M2	4-Hydroxy AFB2

natural fluorescence of aflatoxins arises from their oxygenated pentaheterocyclic structure. The fluorescence capacity of AFB2 and AFG2 is ten times larger than that of AFB1 and AFG1, probably owing to the structural difference, namely double bond on the furanic ring. Such a double bond seems to be very important for the photophysical

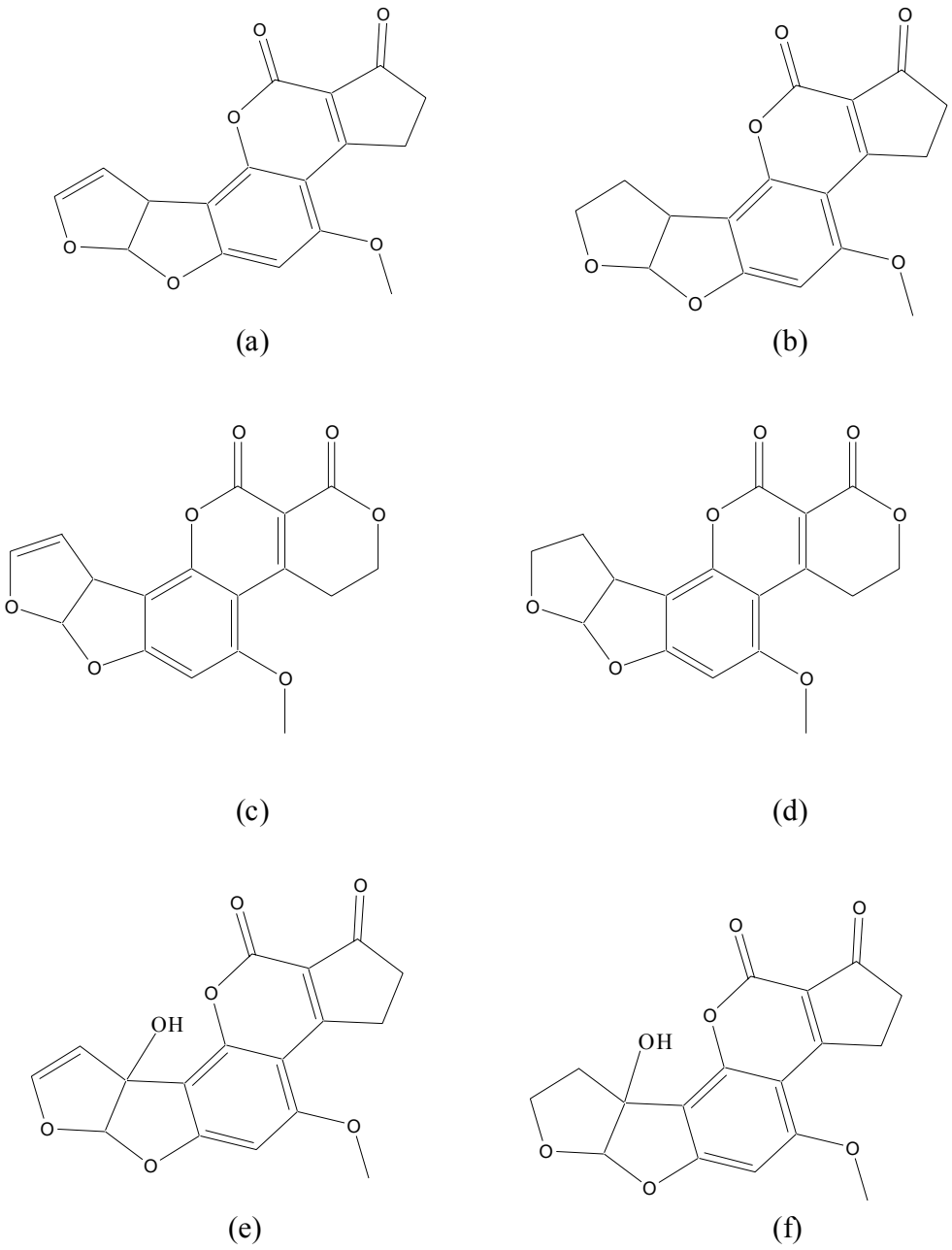


Figure 1.2 Chemical structures of (a) AFB1, (b) AFB2, (c) AFG1, (d) AFG2, (e) AFM1 and (f) AFM2.

properties of these derivatives measured just after spectroscopic studies (Cepeda *et al.,* 1996). The excitation of the natural fluorescence of AFB1 and AFG1 can be promoted

in many different ways such as post-column iodination (Tuinstra and Haasnoot, 1983; Davis and Diener, 1980), post-column bromination (Kok *et al.,*1986; Kok, 1994 ; Versantroort *et al.,* 2005), use of cyclodextrin compound (Cepeda *et al.,* 1996; Chiavaro *et al.,* 2001; Franco *et al.,* 1998) and trifluoroacetic acid, TFA (Stack and Pohland, 1975; Takahashi, 1977a; Haghighi *et al.*, 1981; Nieduetzki *et al.,* 1994).

AFB1 and AFG1 form hemiacetals, AFB2a and AFG2a when reacted with acidic solution such as triflouroacetic acid (TFA) as represented in Figure 1.3 (Joshua, 1993). The hydroxyaflatoxins are unstable and tend to decompose to yellow products in the presence of air, light and alkali. Their UV and visible spectra are similar to those of the major aflatoxins.

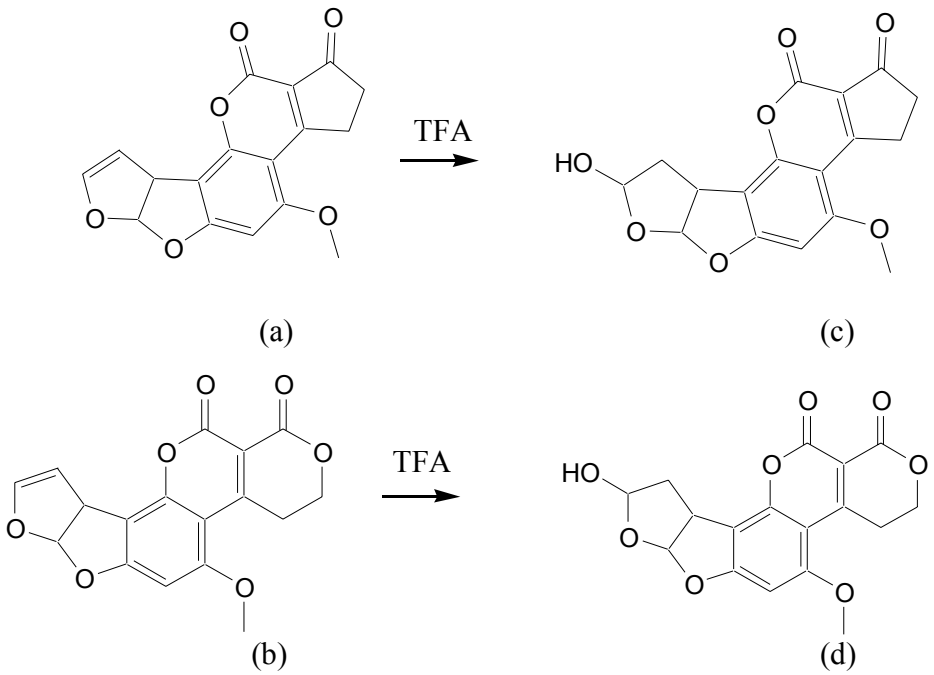


Figure 1.3 Hydration of AFB1 (a) and AFG1 (b) by TFA produces AFB2a (c) and AFG2a (d) (Joshua, 1993).

The close relationship between AFB1, AFG1, AFB2a and AFG2a was shown by the similarities in their IR and UV spectra. The main difference between AFB2a and AFG2a with AFB1 and AFG1 are found in the IR spectra, where an additional band at

3620 cm^{-1} indicates the presence of a hydroxyl group in AFB2a and AFG2a. The absence of bands at 3100, 1067 and 722 cm^{-1} (which arise in AFB1 and AFG1 from the vinyl ether group) indicates that the compounds are hydroxyl derivatives of AFB2 and AFG2. Some chemical and physical properties of aflatoxin compounds are listed in Table 1.1 (Heathcote and Hibbert, 1978; Weast and Astle, 1987). The close relationship between these aflatoxins was shown by the similarities in their UV and IR spectra as shown in Appendix B and C respectively.

Table 1.1 Chemical and physical properties of aflatoxin compounds

	AFB1	**AFB2**	**AFG1**	**AFG2**
Molecular formula	$C_{17}H_{12}O_6$	$C_{17}H_{14}O_6$	$C_{17}H_{12}O_7$	$C_{17}H_{14}O_7$
Molecular weight	312	314	328	330
Crystals	Pale yellow	Pale yellow	Colorless	Colorless
Melting point (° C)	268.9	286.9	244.6	237.40
Fluorescence under UV light	Blue	Blue	Green	Green
Solubility	Soluble in water and polar organic solvent. Normal solvents are: methanol, water: acetonitrile (9:1), trifluoroacetic acid, methanol: 0.1N HCl (4:1), DMSO and acetone			
Other properties	Odorless, colorless and tasteless in solution form. Incompatible with strong acids, strong oxidising agents and strong bases. Soluble in water, DMSO, 95% acetone or ethanol for 24 hours under ambient temperature			

Many researches have studied the stability of aflatoxins. For example, AFB1 was not found in fruit samples after being irradiated with 5.0 Kgy or more of gamma-irradiation as reported by Aziz and Moussa (2002). Gonzalez *et al.* (1998) have studied the effect of electrolysis, ultra-violet irradiation and temperature on the decomposition of AFB1 and AFG1. UV irradiation caused an intense effect on aflatoxins where after 60 min of radiation, AFG1 suffers practically with no more decomposition. However, when the aflatoxin solutions were placed in a 90° C bath for 3 min, a decrease of 20% from total amount of both AFB1 and AFG1 was obtained. A greater extent of decomposition (50%) was found for treatment at 100° C during longer time. Levi (1980) reported that experimental roasting under conditions simulating those of the typical roasting operation (20 min at 200 ± 5° C) destroyed about 80% of AFB1 added to green coffee. It was also found that AFB2 decomposed to a larger extent than AFG1 indicating a lower stability against prolonged heat treatment. During food fermentation which involved other fungi such as *Rhizopus* oryzae and *R. oligosporus,* cyclopentanone moiety of AFB1 was reduced resulting in the formation of aflatoxicol A as shown in Figure 1.4 which is non-toxic compound. It retains the blue-fluorescing property of AFB1 under UV light. It has been considered to be one of the most important B1 metabolites because there is a correlation between the presence of this metabolite in animal tissues and body fluids with toxicity of AFB1 in different animals (Lau and Chu, 1983). Aflatoxin solution prepared in water, dimethyl sulphonic acid (DMSO), 95% acetone or ethanol is stable for 24 hours under ambient conditions. AFM1 is relatively stable during pesteuring, sterilisation, preparation and storage of various dairy products (Gurbay *et al.,* 2004).

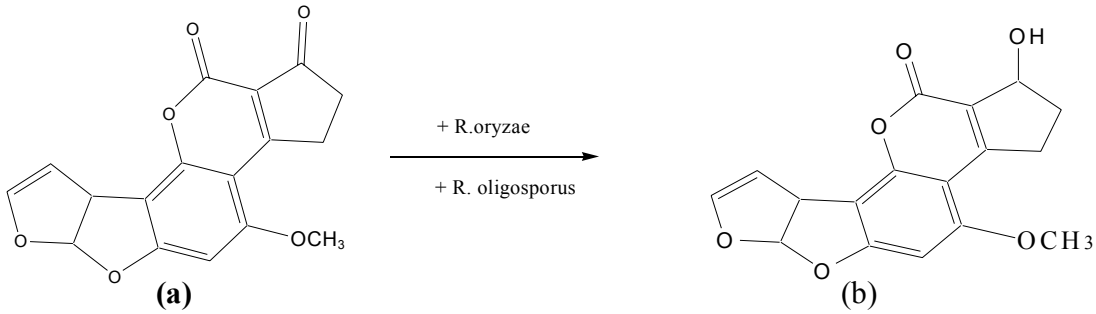


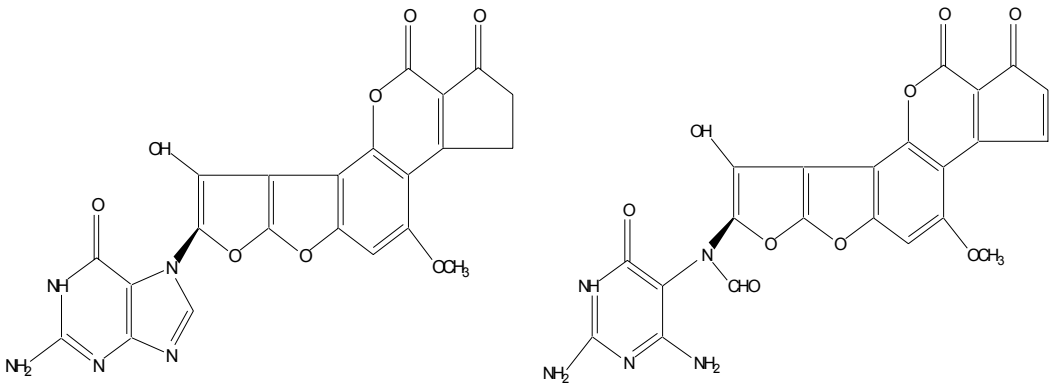
Figure 1.4 Transformation of toxic (a) AFB1 to non-toxic (b) aflatoxicol A

1.2.3 Health Aspect of Aflatoxins

Aflatoxins have received greater attention than any other mycotoxins. There are potent toxin and were considered as human carcinogen by The International Agency for Research on Cancer (IARC) as reported in World Health Organisation (WHO)'s monograph (1987). These mycotoxins are known to cause diseases in man and animals called aflatoxicosis (Eaton and Groopman, 1994). Human exposure to aflatoxins is principally through ingestion of contaminated foods (Versantroort *et al.,* 2005). Inhalation of the toxins may also occur occasionally due to the occupational exposure. After intake of contaminated feedstuffs aflatoxins cause some undesirable effect in animals, which can range from vomiting, weight loss and acute necrosis to various types of carcinoma, leading in many cases to death (Pestka and Bondy, 1990 and Bingham *et al.,* 2004). Even at low concentration, aflatoxins diminish the immune function of animals against infection. Epidemiological studies have shown a correlation between liver cancer and the prevalence of aflatoxins in the food supply.

In views of occurrence and toxicity, AFB1 is extremely carcinogenic while others are considered as highly carcinogenic as reported by Carlson (2000). It is immunosuppressive and a potent liver toxin; less than 20 μg of this compound is lethal to duckling (Hussein and Brasel, 2001). AFB1 is biochemically binding to DNA, inhibit DNA, RNA, and protein syntheses, and effects DNA polymerase activity as reported by Egner *et al.* (2003). Previous research results have demonstrated the covalent binding of highly reactive metabolite of AFB1 to the N-7 atom of guanine residues, resulting in major DNA adducts (Moule, 1984; Johnson *et al.,* 1997; Egner *et al.,* 2003). Major DNA adducts of AFB1 are shown in Figure 1.5.

The four main aflatoxins display decreasing potency in the order AFB1 > AFG1 > AFB2 > AFG2 as reported by Betina (1984). This order of toxicity indicates that the double bond in terminal furan of AFB1 structure is a critical point for determining the degree of biological activity of this group of mycotoxins (Hall and Wild, 1994). It appears that the aflatoxins themselves are not carcinogenic but rather some of their



(a) (b)

Figure 1.5 Major DNA adducts of AFB1; (a) 8,9- Dihydro-8-(N^7-guanyl)-9-hydroxy-aflatoxin B1 (AFB1-Gua) and (b) 8,9-Dihydro-8-(N^5- Formyl-2'.5'.6'-triamino-4'-oxo-N^5-pyrimidyl)-9-hydroxy- Aflatoxin B1 (AFB1-triamino-Py)

metabolites (de Vries, 1996). For example, metabolite transformation of AFB1 by cytochrome P-450 enzyme produces aflatoxin Q1 (AFQ1), AFM1, AFB1-epoxide and aflatoxin P1 (AFP1) as shown in Figure 1.6. For hamiacetals, AFB2a and AFG2a, however, are relatively non-toxic despite the close similarity of their structure to those of AFB1 and AFG1 even at the highest dosages (1200 µg for AFB2a and 1600 µg for AFG2a).

AFM1 is the main metabolic derivatives of aflatoxins in several animal species It is found in the cow milk due which had consumed feed which contaminated with AFB1 as reported by Chang *et al.* (1983), Yousef and Marth (1985), Van Egmond (1989), Tuinstra (1990) and Lopez *et al*. (2002). The relative amount of AFM1 excreted is related to the amount of AFB1 in the feed, and about 0.1% of AFB1 ingested is excreted into milk as AFM1 (Miller, 1987). There was a linear relationship between the amount of AFM1 in milk and AFB1 in feeds consumed by animals as reported by Dragacci (1995). AFM1 is produced by hydroxylation of AFB1 in the liver

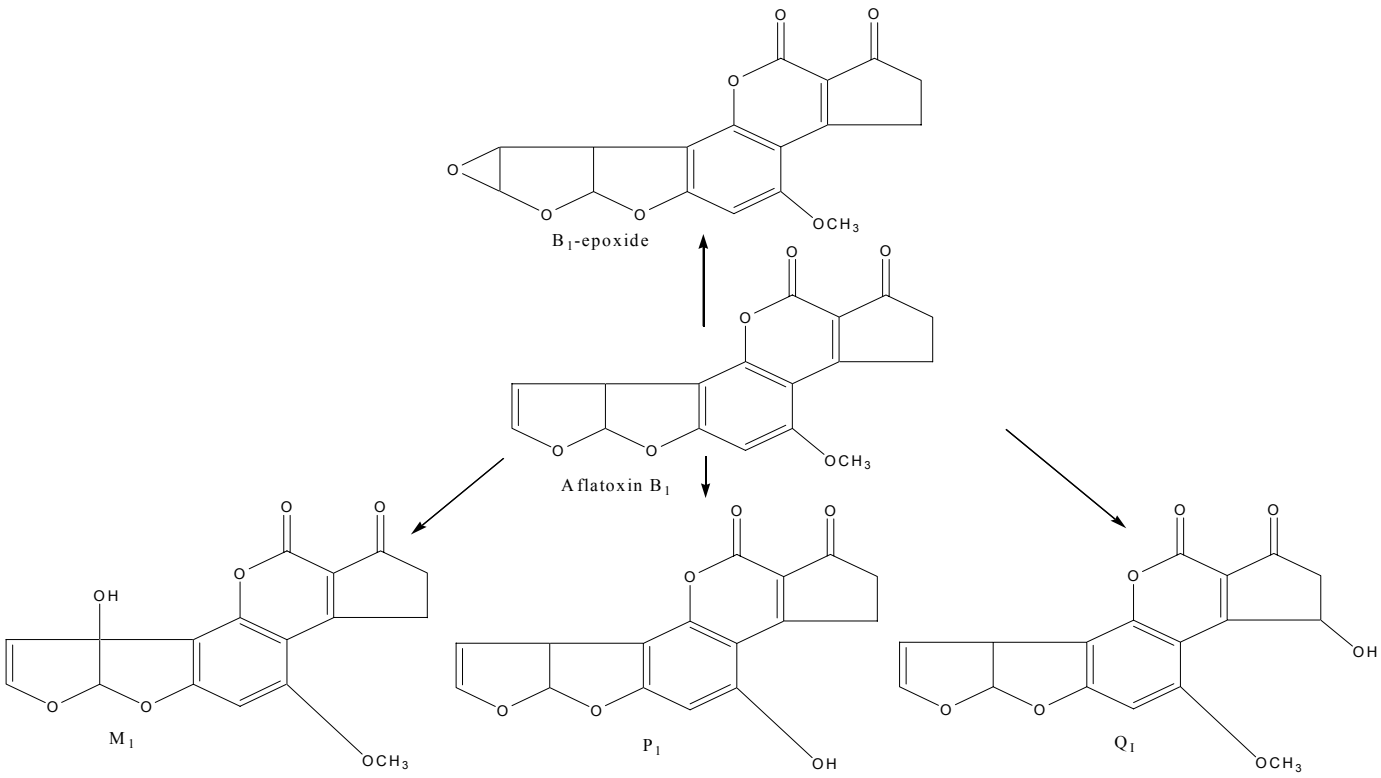


Figure 1.6 Metabolic pathways of AFB1 by cytochrom P-450 enzymes; B1-epoxide = AFB1 epoxide, M1 = aflatoxin M1, P1 = aflatoxin P1 and Q1 = aflatoxin Q1

of lactating animals, including humans. It is also known as milk toxin which is much less carcinogenic and mutagenic than AFB1. It has been classified by the International Agency For Research Cancer (IARC) as a Group 2 carcinogen (IARC, 1993). It can generally be found in milk and milk products such as dry milk, whey, butter, cheese, yogurt and ice cream. AFM2 is the analogous metabolic derivatives of AFB2.

Aflatoxins have been considered as one of the most dangerous contaminant in food and feed. The contaminated food will pose a potential health risk to human such as aflatoxicosis and cancer (Jeffrey and Williams, 2005). Aflatoxins consumption by livestock and poultry results in a disease called aflatoxicosis which clinical sign for animals include gastro intestinal dysfunction, reduced reproductivity, reduced feed utilisation, anemia and jaundice. Humans are exposed to aflatoxins by ingestion, inhalation and dermal exposure as reported by Etzel (2002). LD_{50} which is the amount of a materials, given all at once, causes the death of 50% (one half) of a group of test animal (www.aacohs.ca/oshanswers) for most animal and human for AFB1 is between 0.5 to 10.0 mg kg^{-1} body weight (Smith and Moss, 1985; Salleh, 1998). Clinical features were characterised by jaundice, vomiting, and anorexia and followed by ascites, which appeared rapidly within a period of 2-3 weeks.

Besides causing health problems to humans, aflatoxin also can cause adverse economic effect in which it lowers yield of food production and fiber crops and becoming a major constraint of profitability for food crop producer countries, an example of which is given in Rachaputi *et al.* (2001). It has been estimated that mycotoxin contamination may affect as much as 25% of the world's food crop each year (Lopez, 1999) resulting in significant economic loss for these countries. Aflatoxins also inflict losses to livestock and poultry producers from aflatoxin-contaminated feeds including death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency.

Due to the above reason, aflatoxin levels in animal feed and various human food products is now monitored and tightly regulated by the most countries. In Malaysia,

the action level for total aflatoxins is 15ppb (Malaysian Food Act, 1983). The European Commission has set limits for the maximum levels of total aflatoxins and AFB1 allowed in groundnuts, nuts, dried fruit and their products. For foods ready for retail sale, these limits are 4 ppb for total aflatoxins and 2 ppb for AFB1, and for foods that are to be processed further the limits stand at 15 ppb for total aflatoxins and 8 ppb of AFB1 (European Commission Regulation, 2001). The Food and Drug Administration (FDA) in USA has established an action level of 0.5 ppb of mycotoxin, AFM1 in milk for humans and 20 ppb for other aflatoxins in food other than milk (www.ansci.cornel.edu/toxiagents).

In Brazil, the limits allowed for food destined for human consumption are 20 µg/kg of total aflatoxin for corn in grain, flours, peanut and by-products, and 0.5 µg/l of AFM1 in fluid milk (Sassahara *et al.*, 2005). Australia has established a minimum level of 15 ppb for aflatoxin in raw peanut and peanut product (Mackson *et al.*, 1999). In Germany, regulatory levels of total aflatoxins are 4 ppb and 2 ppb for AFB1. For human dietary products, such as infant nutriment, there are stronger legal limits, 0.05 ppb for AFB1 and the total aflatoxins (Reif and Metzger, 1995). The Dutch Food Act regulates that food and beverages may contain no more than 5 µg of AFB1 per kg (Scholten and Spanjer, 1996).

Regulatory level set by Hong Kong government is 20 ppb for peanuts and peanut products and 15 ppb for all other foods (Risk Assessment Studies report, 2001). Because of all these reasons, systematic approaches to sampling, sample preparation and selecting appropriate and accurate method of analysis of aflatoxin are absolutely necessary to determine aflatoxins at the part-of-billion (ppb) level as reported by Park and Rua (1991).

1.2.4 Analytical Methods for the Determination of Aflatoxins

Monitoring the presence of aflatoxin in various samples especially in food is not only important for consumer protection, but also for producers of raw products

prior to cost intensive processing or transport. Several methods for aflatoxins determination in various samples have been developed and reported in the literature. Method based on thin-layer chromatography (TLC) (Lafont and Siriwardana, 1981; AOAC, 1984) and high performance liquid chromatography (HPLC) with ultraviolet absorption, fluorescence, mass spectrometry or amperometry detection, have been reported (Kok *et al.,* 1986; Taguchi *et al.*, 1995; Ali *et al.,* 2005; Manetta *et al.,* 2005).

TLC and HPLC techniques are well proven and widely accepted; however, both techniques have their own disadvantages. These methods require well equipped laboratories, trained personnel, harmful solvents and time consuming. Moreover, instrumentations used are expensive (Badea *et al.*, 2004). The main disadvantage associated with TLC is the lack of precision where a possible measurement error of ± 30 – 50 % is indicated when standard and unknown aflatoxins spot are matched, and ± 15 -25 % when the unknown is interpolated between two standards (Takahashi, 1977b). In this technique, the greater effect was caused by sample matrix which is usually very complicated (Lin *et al.,* 1998). Poor repeatability is associated with the sample application, development and plate interpretation steps. HPLC technique is often viewed as laborious and time intensive, needs complex gradient mobile phase, large quantity of organic solvent, requiring a significant investment in equipments, materials and maintenance (Pena *et al*., 2002).

Pre-column derivatisation technique could improve the sensitivity of the measurement however, it requires chemical manipulations which are time consuming, involves aggressive reagents such as bromine, TFA and iodine and also it is difficult to automate. Other disadvantages of this procedure include the requirement to prepare iodine solution daily, the necessity for two pumps, dilution of the eluent stream, the need to thermostat the reactor coil and insufficient day-to-day reproducibility (Kok *et al.,* 1986). The method using TFA as derivatising agent has poor reproducibility and is difficult to automate (Reif and Metzger, 1995).

Other methods such as enzyme linked immunosorbant assay (ELISA) (Chu *et al.*, 1987; Lee *et al.*, 1990; Pesavento *et al.,* 1997 and Aycicek *et al.,* 2005), radioimmunoassay (RIA) (Stroka *et al.,* 2000) and immunoaffinity clean up (Garner, 1993 and Niedwetzki *et al.*, 1994) have also been developed for the detection of these compounds. The simplicity, sensitivity and rapid detection of aflatoxin by ELISA has made it possible to monitor several samples simultaneously but ELISA and other immunochemical methods require highly specific polyclonal or monoclonal sera for specific and sensitive detection of antigen. The production of specific antibodies for aflatoxins has allowed the development of this technique based on direct or indirect competition. This method, however, presents some drawbacks such as long incubation time, washing and mixing steps (Badea *et al.*, 2004) and is labor intensive (Carlson *et al.,* 2000). It also requires highly specific polyclonal or monoclonal sera which is expensive (Rastogi *et al.*, 2001). Blesa *et al*. (2003) reported that the ELISA method is a good screening method for investigation of aflatoxins. RIA is very sensitive but has several disadvantages. The radioisotopes are health hazards, disposable difficulties and may have short half life (Trucksess *et al.*, 1991).

Cole *et al*., (1992) have used micellar electrokinetic capillary chromatography (MECC) technique for rapid separation of aflatoxins. Pena *et al.,* (2002) proposed capillary electrophoresis (CE) for determination of aflatoxins. They found that the limit of detection (LOD) of this technique was 0.02 to 0.06 ppb with analysis time of 50 minutes. Due to long duration time and involving use of many reagents such as benzene and acetonitrile for preparation of aflatoxin stock solution and sodium dodecyl sulphate (SDS), sodium dihydrogenphosphate, sodium borate and γ-cyclodextrin for preparing the buffer solution in performing the analysis, this technique may not be suitable for routine analysis of aflatoxins. A summary of techniques used for the determination of aflatoxins compounds in various samples together with its detection limit is shown in Table 1.2.

Table 1.2 Summary of analysis methods used for the determination of aflatoxins in various samples

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
1	HPLC-FD	B1, B2, G1 and G2 in spices	0.06	Garner *et al.* 1993)
2	HPLC-FD	B1, B2, G1 and G2 in spices	0.5	Akiyama *et al.* (2001)
3	HPLC-FD	B1, B2, G1 and G2 in peanut butter	5.0	Beebe (1978)
4	HPLC-FD	B1, B2, G1 and G2 in peanut butter	0.5	Duhart *et al.* (1988)
5	HPLC-FD	B1, B2, G1 and G2 in peanut butter	Not stated	Patey *et al.* (1991)
6	HPLC-FD	B1, B2, G1 and G2 in airborne dust	B1, G1, G2 = 3.1 B2 = 1.8	Kussak *et al.* (1995a)
7	HPLC-FD	B1, B2, G1 and G2 in corn, peanuts and peanut butter	Not stated	Trucksess *et al.* (1991)
8	HPLC-FD	B1, B2, G1 and G2 in medicinal herbs and plant extract	0.05	Reif and Metzger (1995)
9	HPLC-FD	B1, B2, G1 and G2 in airborne dust and human sera	0.08	Brera *et al.* (2002)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
10	HPLC-FD	B1, B2, G1 and G2 in groundnut, peanut and peanut butter	2.0	Chemistry Department, MOSTI (1993)
11	HPLC-FD	B1, B2, G1 and G2 in standard samples	Not stated	Holcomb *et al.* (2001)
12	HPLC-FD	B1, B2, G1 and G2 in wine	0.02	Takahashi (1977a)
13	HPLC-FD	B1, B2, G1 and G2 in corn, almonds, peanuts, milo,rice, pistachio, walnuts, corn and cottonseed	Not stated	Wilson and Romer (1991)
14	HPLC-FD	M1 in milk and non fat dry milk	0.014	Chang and Lawrence (1997)
15	HPLC-FD	B1, B2, G1 and G2 in beer	B1, G1= 0.019, B2, G2 = 0.015	Scot and Lawrence (1997)
16	HPLC-FD	B1, B2, G1 and G2 in poultry and pig feeds and feedstuffs	1.0	Cespedes and Diaz (1997)
17	HPLC-FD	M1 in milk	0.05	Yousef and Marth (1985)
18	HPLC-FD	M1 in whole milk	0.014	Fremy and Chang (2002)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
19	HPLC-FD	B1, B2, G1 and G2 in pistachio kernels and shells	B1 = 0.8, B2 = 0.29 G1 = 0.9 G2 = 1.1	Chemistry Department, MOSTI (1993)
20	HPLC-FD	B1, B2, G1 and G2 in urine	0.0068	Kussak *et al.* (1995b)
21	HPLC-FD	M1 in milk	10	Gurbey *et al.* (2004)
22	HPLC-FD	M1 in urine and milk	0.0025	Simon *et al.* (1998)
23	HPLC-FD	B1, B2, G1 and G2 in sesame butter and tahini	Not stated	Nilufer and Boyacio (2002)
24	HPLC-FD	B1 and ochratoxin A in bee pollen	B1= 0.2	Garcia-Villanove *et al.* (2004)
25	HPLC-FD and UVD	B1, B2, G1 and G2 in contaminated cocoa beans	1.0	Jefferey *et al.* (1982)
26	HPLC-FD and UVD	B1 and B2 in wine and fruit juices	0.02	Takahashi (1997b)
27	HPLC-UVD	B1, B2, G1 and G2 in corn	B1, G1 = 1.0, B2, G2 = 0.25	Fremy and Chang (2002)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
28	HPLC-UVD	B1in standard sample	Not stated	Rastogi *et al.* (2001)
29	HPLC-UVD	B1and B2 in cottonseed	5.0	Pons and Franz (1977)
30	HPLC-UVD	B1 in egg	1.0	Trucksess *et al.* (1977)
31	HPLC-UVD	B1 and M1 in corn	B1 = 3.0 -5.0 M1 = not stated	Stubblefield and Shotwell (1977)
32	HPLC-UVD	B1, B2, G1 and G2 in soy sauces and soybean paste	1.0	Wet *et al.* (1980)
33	HPLC-AD	B1, B2, G1 and G2 in standard sample	7.0	Gonzalez *et al.* (1998)
34	HPTLC	B1, B2, G1 and G2 in corn, buckwheat, peanuts and cheese	B1 = 0.2 B2, G1, G2 = 0.1	Kamimura *et al.* (1985)
35	HPTLC-SPE	B1, B2, G1 and G2 in palm kernel	B1 = 3.7, B2 = 2.5, G1 = 3.0, G2 = 1.3	Nawaz *et al.* (1992)
36	TLC	B1and M1in artificial contaminated beef livers	B1= 0.03, M1 = 0.1	Stabblefield *et al.* (1982)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
37	TLC	B1in milk and milk powder	0.05	Bijl and Peteghem (1985)
38	TLC	Total aflatoxin in wheat and soybean	Not stated	Shotwell *et al.* (1977b)
39	TLC	B1, B2, G1 and G2 in apples, pears, apple juice and pear jams	B1, G1 = 2.0-2.8, B2, G2 = 2.0	Gimeno and Martins (1983)
40	TLC	B1, B2, G1 and G2 in corn and peanut meal	Corn = 2.0 Peanut meal = 3.0	Bicking *et al.* (1983)
41	TLC	M1 in milk and condensed, evaporated and non-fat powdered milk	Milk = 0.1 Others = 0.2	Fukayama *et al.* (1980)
42	TLC	B1, B2, G1 and G2 in ginger root and oleoresin	Not stated	Trucksess and Stoloff (1980)
43	TLC	B1in black olives	3000-7000	Tutour *et al.* (1984)
44	TLC	B1, B2, G1 and G2 in cereal and nuts	Not stated	Saleemullah *et al.* (1980)
45	TLC-FDs	M1in milk	0.005	Gauch *et al.* (1982)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
46	OPLC-FD	B1, B2, G1 and G2 in maize, fish, wheat, peanuts, rice and sunflower seeds	Not stated	Papp *et al.* (2002)
47	LC-MS	B1, B2, G1 anf G2 in figs and peanuts	1.0	Vahl and Jargensen (1998)
48	ELISA	M1 in milk, yogurt, cheddar and Brie	0.01 – 0.05	Fremy and Chu. (1984)
49	ELISA	M1 in infant milk products and liquid milk	Not stated	Rastogi *et al.* (1977)
50	ELISA	M1in curd and cheese	Not stated	Grigoriadou *et al.* (2005)
51	ELISA	M1 in cheese	Not stated	Sarimehtoglu *et al.* (1980)
52	ELISA	B1, B2, G1 and G2 in sesame butter and tahini	Not stated	Nilufar and Boyacio (2000)
53	ELISA ST	B1in corn	5.0	Beaver *et al.* (1991)
54	MECC	B1, B2, G1 and G2 in standard samples	0.05 – 0.09	Cole *et al.* (1992)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
55	MECC FS	B1, B2, G1 and G2 in feed samples	0.02 – 0.06	Pena *et al.* (2002)
56	IAC	Total aflatoxins and B1 in nuts	Total = 1.0 B1 = 0.2	Leszezynska *et al.* (1998)
57	IAC	B1 in mixed feeds	2.0	Shannon *et al.* (1984)
58	FI-IA-AD	M1 in raw milk	0.011	Badea *et al.* (1977)
59	GC-FID	B1, B2, G1 and G2 in culture medium	Not stated	Goto *et al.* (2005)
60	ECS with BLMs	M1 in skimmed milk	761	Androu *et al.* (1997)
61	DPP	B1 in rice, milk, corn and peeletised rabbit feed	25	Smyth *et al.* (1979)
62	SIIA	M1in an artificial contaminated food	0.2	Garden and Strachen (2001)
63	MS	B1, B2, G1 and G2 in contaminated corns	10	Plattner *et al.* (1984)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
64	IAFB	B1in standard sample	0.1	Carlson *et al.* (2002)
65	IACLC	B1 and total aflatoxin in peanut butter, pistachio paste, fig paste and paprika powder	Not stated	Stroka *et al.* (2000)
66	IACLC	B1, B2, G1 and G2 in maize and peanut butter	B1, B2, G1, G2 = 2.0	Chan *et al.* (1984)
67	ICA	B1 in rice, corn and wheat	2.5	Xiulan *et al.* (2006)
68	TLC	B1, B2, G1 and G2 in peppers	Not stated	Erdogan (2004)
69	HPLC-FD	B1, B2, G1 and G2 in traditional herbal medicines	Not stated	Ali *et al.* (2005)
70	LC-MS	M1 in bovine milk	0.02 – 0.15	Sorensen and Elbaek (2005)
71	LC-MS	M1in sidestream cigarette smoke	3.75	Edinboro and Karnes (2005)
72	TLC	M1in milk and milk products	0.0125	Bakirci (2001)

Table 1.2 Continued

No	Method	Aflatoxin / samples	Detection limit (ppb)	Reference
73	ELISA	B1and M1 in food and dairy products	Not stated	Aycicek *et al.* (2005)
74	TLC	M1 in Iranian feta cheese	0.015	Kamkar *et al.* (2005)
75	Ridascreen Test	M1 in pasteurised milk	Not stated	Alborzi *et al.* (2005)
76	Ridascreen Test	M1 in raw milk	0.245	Sassahara *et al.* (2005)
77	TLC	B1in melon seeds	2.0	Bankole *et al.* (2005b)
78	ECS-ELISA	B1in barley	0.02 – 0.03	Ammida *et al.* (2004)
79	HPLC-FD	B1, B2, G1 and G2 in bee pollen	Not stated	Gonzalez *et al.* (2005)
80	HPLC-FD	M1in milk and cheese	Milk = 0.001, Cheese = 0.005	Manetta *et al.* (2005)
81	LC-MS	B1, B2, G1 and G2 in peanuts	B1, B2, G1, G2 = 0.125 – 2.50	Blesa *et al.* (2001)
82	ELISA-SPE	M1 in milk	0.025	Micheli *et al.* (2005)

Table 1.2 Continued

Notes:

AD:	Amperometric detector
IAC:	Immunoaffinity chromatography
IACLC:	Immunoaffinity column liquid chromatogtaphy
IAFB:	Immunoaffinity fluorometer biosensor
BLMs:	Bilayer lipid membranes
DPP:	Differential pulse polarography
ECS:	Electrochemical sensing
ECS-ELISA:	Electrochemical sensing based on indirect ELISA
ELISA:	Enzyme linked immunosorbant assay
ELISA ST:	Enzyme linked immunosorbant assay for screening
ELISA-SPE:	Enzyme linked immuno sorbant assay combined with screen printed electrode
FD:	Fluorescence detector
FI-IA-AD:	Flow-injection immunoassay with amperometric detector
GC-FID:	Gas chromatography with flame ionization detecter
HPLC:	High pressure liquid chromatography
HPTLC:	High pressure thin liquid chromatography
HPTLC-SPE:	High pressure thin liquid chromatography with solid phase extraction
ICA:	Immnuochromatographic assay
MECC:	Micellar electrokinetic's capillary chromatography
MS:	Mass spectrometry
OPLC:	Over pressured liquid chromatography
SIIA:	Sequential injection immunoassay test
TLC:	Thin layer chromatography
TLC-FDs:	Thin layer chromatography with fluorescence densitometer
UVD:	Ultraviolet detector

1.2.5 Electrochemical Properties of Aflatoxins

Polarographic determination of aflatoxin has been studied by Smyth *et al.* (1979) using DPP technique to determine AFB1, AFB2, AFG1 and AFG2 in food samples using Britton-Robinson buffer solution as the supporting electrolyte. They found that all aflatoxins exhibited similar polarographic behaviour over a pH range 4 –

11. They also obtained that the best-defined waves for analytical purposes were in Britton-Robinson buffer at pH 9. Slight differences in the potential of reduction of aflatoxins have been observed owing to their slight differences in structure. They found that the half wave potentials of reduction of the various aflatoxins were AFB1 = -1.26 V, AFB2 = -1.27 V, AFG1 = -1.21 V and AFG2 = -1.23 V (all versus SCE). The limit of detection for AFB1 in pure solutions was about 2 x 10^{-8} M (25 ppb).

Gonzalez *et al.* (1998) have studied the cyclic voltammetry of AFG1 in methanol: water (1:1, v/v) solution on different electrodes such as glassy carbon, platinum and gold electrodes. They found that AFG1 gave high peak current at a potential of about +1.2V using glassy carbon electrode compared to other types of working electrode. The electro activity of the studied aflatoxins increased in the order AFG2 < AFB1 < AFB2 < AFG1. This order reflects the ability of the aflatoxins molecules to undergo electrochemical oxidation on glassy carbon electrode.

Duhart *et al.* (1988) have determined all types of aflatoxin using HPLC technique with electrochemical detection. They have used differential-pulse mode at the dropping mercury electrode (DME) with 1 s drop time for detection system, large drop size, current scale of 0.5 μA and modulation amplitude of 100 mV. They have found that using mobile phase which consisted of 62.7% BRB at pH 7, 17.9% methanol and 19.4% acetonitrile, the peak potentials for all aflatoxins were slightly different which were AFB1 = -1.37 V, AFB2 = -1.36 V, AFG1 = -1.30 V and at -1.30 V for AFG2. Due to the fairly close together of peak potentials, a potential of -1.28 V has been selected for detection purposes of these aflatoxins together with HPLC technique. They found that using this technique average percentage recoveries for peanut butter samples (n = 4) which have been spiked with a mixture of the four aflatoxins were AFB1 (76%), AFB2 (77%), AFG1 (87%) and AFG2 (81%). This results show that a major advantage of this technique was it did not require a derivatisation step as is common for fluorescent detection.

1.3 Voltammetric Techniques

1.3.1 Voltammetric Techniques in General

Voltammetry is an electrochemical method in which current is measured as a function of the applied potential. It is a branch of electrochemistry in which the electrode potential, or the faradaic current or both are changed with time. Normally, there is an interrelationship between all three of these variables (Bond *et al.*, 1989). The principle of this technique is a measurement of the diffusion controlled current flowing in an electrolysis cell in which one electrode is polarisable (Fifield and Kealey, 2000). In this technique a time dependent potential is applied to an electrochemical cell, and the current flowing through the cell is measured as a function of that potential. A plot of current which is directly proportional to the concentration of an electroactive species as a function of applied potential is called a voltammogram. The voltammogram provides quantitative and qualitative information about the species involved in the oxidation or reduction reaction or both at the working electrode.

Polarography is the earliest voltammetric technique which was developed by Jaroslav Heyrovsky (1890-1967) in the early 1920s, for which he was awarded the Nobel Prize in Chemistry in 1959. It was the first major electro analytical technique (Barek *et al*., 2001a). Since then many different forms of voltammetry have been developed such as direct current polarography (DCP), normal polarography (NP), differential pulse polarography (DPP), square-wave polarography (SWP), alternate current polarography (ACP), cyclic voltammetry (CV), stripping voltammetry (SV), adsorptive stripping voltammetry (AdSV) and adsorptive catalytic stripping voltammetry (AdCSV) techniques. Working electrodes and limit of detection (LOD) for these modern voltammetric techniques are shown in Table 1.3 (Barek et *al.,* 2001a).

The advantage of this technique include high sensitivity where quantitative and qualitative determination of metals, inorganic and organic compounds at trace levels, $10^{-4} – 10^{-8}$ M (Fifield and Kealey, 2000), selectivity towards electro active species

(Barek *et al.*, 2001b), a wide linear range, portable and low-cost instrumentation, speciation capability and a wide range of electrode that allow assays of many types of samples such as environmental samples (Zhang *et al.*, 2002; Ghoneim *et al.*, 2000; Buffle *et al.,* 2005), pharmaceutical samples (Yaacob, 1993; Yardimer *et al.,* 2001; Abdine *et al.,* 2002; Hilali *et al.,* 2003 and Carapuca *et al.,* 2005), food samples (Ximenes *et al.,* 2000; Volkoiv *et al.,* 2001); Karadjova *et al.,* 2000; Sabry and Wahbi, 1999 and Sanna *et al.,* 2000), dye samples (Mohd Yusoff *et al.,* 1998 and Gooding *et al.,* 1997) and forensic samples (Liu *et al.,* 1980; Wasiak *et al.*, 1996; Pourhaghi-Azar and Dastangoo, 2000; Woolever *et al.*, 2001).

Various advances during the past few years have pushed the detectability of voltammetric techniques from the submicromolar level for pulse voltammetric techniques to the subpicomolar level by using an adsorptive catalytic stripping voltammetry (Czae and Wang, 1999). The comparison of using polarographic and other analytical techniques in different application fields is depicted in Table 1.4 (Barek *et al.*, 2001a).

1.3.2 Voltammetric Measurement

1.3.2.1 Instrumentation

Voltammetry technique makes use of a three-electrode system such as working electrode (WE), reference electrode (RE) and auxiliary electrode (AE). The whole system consist of a voltammetric cell with a various volume capacity, magnetic stirrer and gas line for purging and blanketing the electrolyte solution.

Table 1.3 Working electrodes and LOD for modern polarographic and voltametric techniques

Technique	**Working electrode**	**LOD**
TAST	DME	~ 10^{-6} M
Normal pulse polarography (NPP) Normal pulse voltammetry (NPV)	DME HMDE	~ 10^{-7} M ~ 10^{-7} M
Stair case voltammetry (SCV)	HMDE	~ 10^{-7} M
Differential pulse polarography (DPP) Differential pulse voltammetry (DPV)	DME HMDE	~ 10^{-7} M ~ 10^{-8} M
Square wave polarography (SWP) Square wave voltammetry (SWV)	DME HMDE	~ 10^{-8} M ~ 10^{-8} M
Alternate current polarography (ACP) Alternate current voltammetry (ACV)	DME HMDE	~ 10^{-7} M ~ 10^{-8} M
Anodic stripping voltammetry (ASV) Cathodic stripping voltammetry (CSV)	HMDE MFE	~ 10^{-10} M ~ 10^{-9} M
Potentiometric stripping analysis (PSA)	MFE	~ 10^{-12} M

Table 1.4 The application range of various analytical techniques and their concentration limits when compared with the requirements in different fields of chemical analysis.

Field of chemical analysis	**Concentration range**
Environmental monitoring	10^{-12} M to 10^{-4} M
Toxicology	10^{-11} M to 10^{-2} M
Pharmacological studies	10^{-10} M to 10^{-4} M
Food control	10^{-8} M to 10^{-4} M
Forensic	10^{-7} M to 10^{-3} M
Drug assay	10^{-5} M to 10^{-2} M
Analytical techniques	**Application range**
Adsorptive stripping voltammetry	10^{-12} M to 10^{-7} M
Anodic / cathodic stripping voltammetry	10^{-11} M to 10^{-6} M
Differential pulse voltammetry	10^{-8} M to 10^{-3} M
Differential pulse polarography	10^{-7} M to 10^{-3} M
Tast polarography	10^{-6} M to 10^{-3} M
d.c.polarography	10^{-5} M to 10^{-3} M
spectrophotometry	10^{-6} M to 10^{-3} M
HPLC with voltammetric detection	10^{-7} M to 10^{-3} M
HPLC with fluorescence detection	10^{-9} M to 10^{-3} M

Table 1.4 Continued

Analytical techniques	**Application range**
Spectrofluorometry	10^{-9} M to 10^{-3} M
Atomic absorption spectrometry	10^{-8} M to 10^{-3} M
Atomic fluorescence spectrometry	10^{-9} M to 10^{-3} M
Radioimmunoanalysis	10^{-13} M to 10^{-3} M
Neutron activation analysis	10^{-9} M to 10^{-3} M
x-ray fluorescence analysis	10^{-7} M to 10^{-3} M
mass spectrometry	10^{-9} M to 10^{-3} M

A typical arrangement for a voltammetric electrochemical cell is shown in Figure 1.7 (Fifield and Haines, 2000).

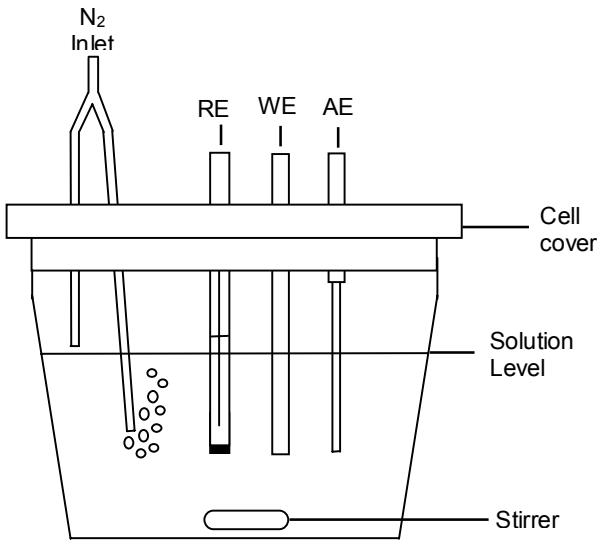


Figure 1.7 A typical arrangement for a voltammetric electrochemical cell (RE: reference electrode, WE: working electrode, AE: auxiliary electrode)

The arrangement of the electrodes within the cell is important. The RE is placed close to the WE and the WE is located between the RE and the AE. Using the three-electrode-cell concept, a potentiostat monitors the voltage over the WE and AE which is automatically adjusted to give the correct applied potential. This is obtained by continuously measuring the potential between the WE and the RE, by comparing it to the set voltage and by adjusting the applied voltage accordingly if necessary. The cell material depends on application that it is usually a small glass beaker with a close fitting lid, which includes ports for electrodes and a nitrogen gas purge line for removing dissolved oxygen and an optional stir bar.

The WE is the electrode where the redox reaction of electroactive species takes place and where the charge transfer occurs. It is potentiostatically controlled and can minimise errors from cell resistance. It is made of several different materials including mercury, platinum, gold, silver, carbon, chemically modified and screen printed electrode. The performance of voltammetry is strongly influenced by the WE. The ideal characteristics of this electrode are a wide potential range, low resistance, reproducible surface and be able to provide a high signal-to-noise response. The WE must be made of a material that will not react with the solvent or any component of the solution over as wide a potential range as possible. The potential window of such electrodes depends on the electrode material and the composition of the electrolyte as summarised in Table 1.5 below (Wang, 2000). Majority of electrochemical methods use HMDE and MFE (Economou and Fielden, 1995) for use in the cathodic potential area, whereas solid electrode such as gold, platinum, glassy carbon, carbon paste are used for examining anodic processes.

Mercury has been used for the WE in earlier voltammetry techniques, including polarography. Since mercury is a liquid, the WE often consists of a drop suspended from the end of a capillary tube. It has several advantages including its high over potential for the reduction of hydronium ion to hydrogen gas. This allows for the application of potential as negative as -1.0 V versus SCE in acidic solution, and -2.0V versus SCE in basic solution.

Table 1.5 List of different type of working electrodes and its potential windows.

ELECTRODE	ELECTROLYTE	POTENTIAL WINDOWS
Hg	1 M H_2SO_4 1 M KCl 1 M NaOH 0.1 M Et_4NH_4 OH	+0.3 to -0.1V 0 to -1.9V -0.1 to -2.0V -0.1 to -2.3V
Pt	1 M H_2SO_4 1 M NaOH	+1.0 to -0.5V +0.5 to -1.0V
C	1 M $HClO_4$ 0.1M KCl	+1.5 to -1.0V +1.0 to -1.3V

Other advantages of using mercury as the working electrode include the ability of metals to dissolve in the mercury, resulting in the formation of an amalgam. The greatest advantages of this electrode is that new drops or new thin mercury films can be readily formed, and this cleaning process removes problems that could be caused by contamination as a result of previous analysis. In contrast, this is not the generally case for electrodes made from other materials, with the possible exception of carbon electrodes, where the electrode cleaning is made of cutting off a thin layer of the previous electrode surface. Another advantage is the possibility to achieve a state of pseudostationary for linear sweep voltammetry (LSV) using higher scan rate. Miniaturised and compressible mercury electrode offer new possibilities in voltammetry especially for determination of biologically active species and surfactant. One limitation of mercury electrode is that it is easily oxidised at + 0.3 V and cannot be used at potential more positive than + 0.4 V versus the SCE, depending on the composition of the solution (Dahmen, 1986 and Fifield and Haines, 2000). At this potential, mercury dissolves to give an anodic polarographic wave due to formation of mercury (I) (Skoog *et al.,* 1996).

There are three main types of mercury electrode used in voltammetry techniques including hanging mercury drop electrode (HMDE), dropping mercury drop electrode (DME) and static mercury drop electrode (SMDE). In the HMDE, a drop of the desired size is formed and hanged at the end of a narrow capillary tube. In the DME, mercury drops form at the end of the capillary tube as a result of gravity. The drop continuously grows and has a finite lifetime of several seconds. At the end of its lifetime the mercury drop is dislodged, either manually or by the gravity, and replaced by a new drop. In the SMDE, it uses a solenoid-driven plunger to control the flow of mercury. It can be used as either HMDE or DME. A single activation of solenoid momentarily lifts the plunger, allowing enough mercury to flow through the capillary to form a single drop. To obtain a dropping mercury electrode the solenoid is activated repeatedly. The diagram of HMDE is shown in Figure 1.8 (Metrohm, 2005).

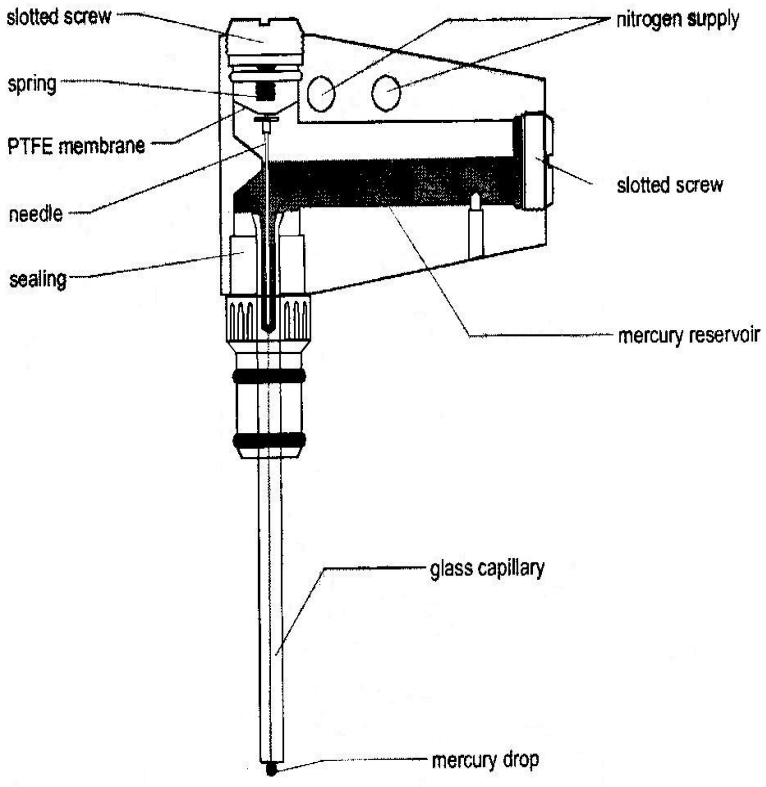


Figure 1.8 A diagram of the HMDE

Other type of mercury electrode is the controlled growth mercury drop electrode (CGME). In this electrode, a fast response valve controls the drop growth. The cross sectional view of the CGME is shown in Figure 1.9 (BAS, 1993).

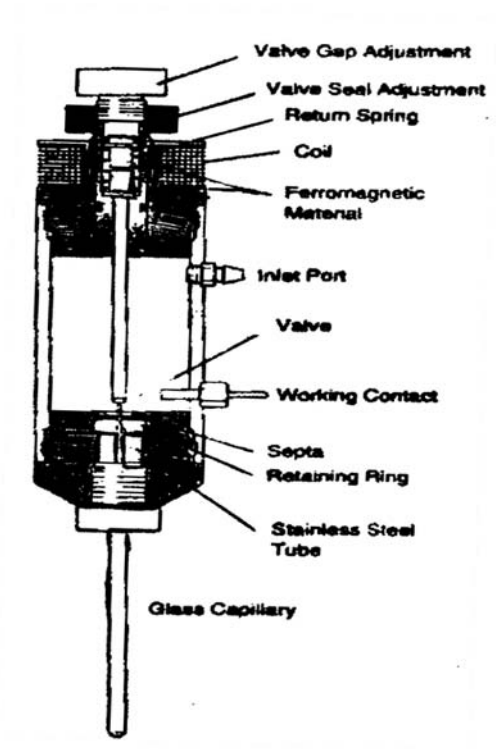


Figure 1.9 A diagram of the CGME

The capillary has a stainless stell tube embedded in the top end of the capillary. Mercury flow through the capillary is controlled by a fast response valve. The valve is rubber plug at the end of a shaft which when displaced slightly up will allow mercury to flow. Since the contact between filament of mercury in the capillary and the reservoir is a stainless steel tube, the electrode has low resistance. The total resistance from the contact point to the mercury is about 7 ohm. The valve seal is controlled by the valve seal adjustment knob. The opening of this valve is controlled by a computer-generated pulse sequence, which leads to a stepped increase in the drop size. Changing the number of pulse and/or the pulse width, so a wide range of drop sizes is available can therefore vary the drop size. Varying the time between the

pulses can control the rate of growth of the mercury drop. Therefore, a slowly growing mercury drop suitable for stripping experiments can be generated. An important advantage of the CGME compared to HMDE is no contamination of the capillary due to back diffusion (Dean, 1995).

In addition to the mercury drop electrode, mercury may be deposited onto the surface of a solid electrode to produce mercury film electrode (MFE). The MFE is based on an electrochemically deposited mercury film on conventional substrate electrode such as a solid carbon, platinum, or gold electrode. The solid electrode is placed in solution of mercury ion (Hg^{2+}) and held at a potential at which the reduction of Hg^{2+} to Hg is favorable, forming a thin mercury film. It displays the properties of a mercury electrode, having various electro analytical advantageous such as a high hydrogen evolution over potential and simple electrochemistry of many metals and other species of analytical interest. For example, Castro *et al.* (2004) have used thin-film coated on a glassy carbon electrode (GCE) for quantitative determination of polycyclic aromatic hydrogen (PAH). In this experiment, they plated the mercury over 5 min at a cell voltage of -0.9 V. Economou and Fielden (1995) have developed a square wave adsorptive stripping voltammetric technique on the MFE for study of riboflavin. In this work, riboflavin was absorbed on the MFE at a potential of 0.0 V (vs. Ag/AgCl) in pH 12.0 of electrolyte solution.

However, the increased risks associated with the use, manipulation and disposal of metallic mercury or mercury salt, have led to general trend for more environmental-friendly analytical methods such as using bismuth film electrode (BFE) instead of mercury (Kefala *et al., 2*003; Economou, 2005; Lin *et al.,* 2005a and Lin *et al.,* 2005b). The BFE consists of a thin bismuth film deposited on a carbon paste substrate that has shown to offer comparable performance to the MFE. Morfobos *et al.* (2004) have studied square wave adsorptive stripping voltammetry (SWAdSV) on a rotating-disc BFE for simultaneous determination of nickel (II) and cobalt (II). Hocevar *et al.* (2005) have developed a novel bismuth-modified carbon paste (Bi-CPE) for a convenient and

reliable electrochemical sensor for trace heavy metals detection in conjunction with stripping electroanalysis.

In another study, using pencil-lead BFE as the working electrode, Demetriades *et al.* (2004) have determined trace metals by anodic stripping voltammetry (ASV). They revealed that pencil-lead BFE were successfully applied to the determination of plumbum and zinc in tap water with results in satisfactory agreement with atomic absorption spectrometry (AAS). Zahir and Abd Ghani (1997) have developed a pencil 2B graphite paste electrode which was fabricated with polymerized 4-vinylpyridine for glucose monitoring.

Other solid or metal electrode commonly used as WE are carbon, platinum (Salavagione *et al.,* 2004; Santos and Machado, 2004; Aslanoglu and Ayne, 2004), gold (Hamilton and Ellis,1980; Parham and Zargar, 2001; Parlim and Zarger, 2003; Moressi *et al.,* 2004; Munoz *et al.*, 2005;), graphite (Orinakova *et al.,* 2004; Pezzatini *et al.,* 2004; Jin and Lin, 2005), diamond (Sonthalia *et al.,* 2004) and silver (Iwamoto *et al.*, 1984). Solid electrodes based on carbon are currently in widespread use in voltammetric technique, primarily because of their broad potential window, low background current, rich surface chemistry, low cost, chemical inertness, and suitability for various sensing and detection application (Wang, 2000). It includes glassy carbon electrode (GCE), carbon paste electrode (CPE), chemically modified electrode (CME) and screen-printed electrode (SPE).

For the GCE, the usual electrode construction is a rod of glassy carbon, sealed into an inert electrode body, a disc of electrode material is exposed to the solution. It is the most commonly used carbon electrode in electro analytical application (Ozkan and Uslu, 2002; Ibrahim *et al.*, 2003; Erk, 2004). The cleaning of this electrode is important, to maintain a reactive and reproducible surface. Pre-treated electrochemically GCE have increased oxygen functionalities that contribute to more rapid electron transfer. Wang *et al.* (1997) have used in adsorptive potentiometric stripping analysis of tamoxifen, a nonsteriodal anti-estrogen used widely for the

treatment of hormone-dependent breast cancer. The electrode was anodised at + 1.7 V for 1 min in the electrolyte containing tomixifen. Using cyclic voltammetric technique, they found that a large definite anodic peak corresponding to the oxidation of the adsorbed drug at GCE at + 0.985 V. Other workers using GCE as the WE were Wang *et al.* (2004), Shi and Shiu (2004) and Ji *et al.* (2004).

The CPE, a mixture of carbon powder and a pasting medium at certain ratio, were the result of an attempt to produce electrode similar to the dropping mercury electrode (Kizek *et al.,* 2005). They are particularly useful for anodic studies, modified electrode and also for stripping analysis. CMEs are electrodes which have been deliberately treated with some reagents, having desirable properties, so as to take on the properties of the reagents (Arrigan, 1994; Kutner *et al.,* 1998). A few examples of these applications were reported by Abbaspour and Moosavi, (2002), Abbas and Mostafa (2003); Ciucu *et al.* (2003); Ferancova *et al.* (2000) and Padano and Rivas (2000).

SPE are increasingly being used for inexpensive, reproducible and sensitive disposable electrochemical sensors for determination of trace levels of pollutant and toxic compounds in environmental and biological fluids sample (Wring *et al.*, 1991). A disposable sensor has several advantages, such as preventing contamination between samples, constant sensitivity and high reproducibility of different printed sensors (Kim *et al.*, 2002). The most useful materials for printing electrochemical sensors could be carbon-based inks because they have a very low firing temperature (20-120° C) and can be printed on plastic substrate. Carbon can also be directly mixed with different compounds, such as mediator and enzymes. A few examples of the experiments which used SPE as the WE were reported by Kim *et al.* (2002), for detection of phenols using α-cyclodextrin modified screen printed graphite electrodes. Ohfuji *et al.* (2004) have constructed a glucose sensor based on a SPE and a novel mediator pyocyanin from *Pseudomonas aeruginosa*. Carpini *et al.* (2004) have studied oligonucleotide-modified screen printed gold electrodes for enzyme-amplified sensing of nucleic acid. Lupu *et al.* (2004) have developed SPE for the detection of marker analytes during wine

making. In this work they have developed biosensors for malic acid and glucose with a limit of detection of 10^{-5} M and 10^{-6} M for malic acid and glucose respectively. The sensors were applied in the analysis of different samples of wine.

Besides using macro-electrode, voltammetric technique also utilizes microelectrode (Lafleur *et al.* 1990) with the size of electrode radius much smaller than the diffusion-layer thickness, typically between 7 to 10 μM (Hutton *et al.,* 2005 and Buffle and Tercier-Waeber, 2005) as the WE. It is constructed from the same materials as the macro-electrode but with a smaller diameter to enhance mass transport of analyte to the electrode surface due to smaller electrode than the diffusion layer. Hence, increasing signal-to noise ratio and measurement can be made in highly resistive media due to decrease of the ohmic drop that results when the electrode size reduced (Andrieux *et al.,* 1990). The microelectrode with diameter as small as 2 μm, allow voltammetric measurements to be made on even smaller sample (Harvey, 2002). Aoki (1990) reported that ultra microelectrodes influence electrode kinetics more specifically than conventional electrode because of lateral diffusion promotes mass transport. Since it minimise uncompensated resistance, they are useful for determination of kinetic parameters. Almeida *et al.* (1998) have done voltammetric studies of the oxidation of the anti-oxidant drug dipyridamole (DIP) in acetonitrile and ethanol using ultra microelectrode (UME). In this work they studied cyclic voltammetric technique of the drug with the UME which was 12.7 μm diameters. They found that with that electrode, the diffusion current of DIP is proportional to the electrode radii for low scan rates in cyclic voltammetry.

The second electrode used in the voltammetric system is auxiliary electrode (AE). The AE is made of an inert conducting material typically a platinum electrode wire (Wang, 2000). It provides a surface for a redox reaction to balance the process that occured at the surface of the WE. It does not need special care, such as polishing. In order to support the current generated at the WE, the surface area of the AE must be equal to or larger than of the WE. The function of the AE is to complete the circuit, allowing charge flow through the cell (Fifield and Haines, 2000). In an electro

analytical experiment, there is no need to place the AE in a separate compartment since the diffusion of product that was produced by a redox reaction at the surface of the AE does not significantly interfere the redox reaction at the WE.

The third electrode used in the voltammetric technique is reference electrode (RE). This RE provides a stable potential so that any change in cell potential is attributed to the working electrode. The major requirement for the RE is that the potential does not change during the recording voltammetric curve at different applied voltage (Heyrovsky and Zuman, 1968). The common RE are standard hydrogen electrode (SHE), calomel electrode (SCE) and silver/silver electrode (Ag/AgCl). The SHE is the reference electrode used to establish standard-state potential for other half reaction. It consists of a platinum electrode immersed in a solution in which the hydrogen ion activity is 1.00 and in which hydrogen gas is bubbled at a pressure of 1 atm. The standard-state potential for the reaction;

$$2H^+ (aq) \quad + \quad 2e^- \quad \rightarrow \quad H_2 (g) \qquad (1.0)$$

is 0.00 V for all temperatures. It is rarely used because it is difficult to prepare and inconvenient to use.

The second reference electrode is the Standard Calomel Electrode (SCE) which is based on the redox couple between mercury chloride (Hg_2Cl_2) and Hg as below;

$$Hg_2Cl_2(s) + 2e \rightleftharpoons 2Hg(l) + 2Cl^-(aq) \qquad (1.1)$$

The potential of this electrode is determined by the concentration of chloride ion. It is constructed using an aqueous solution saturated with potassium chloride (KCl) which has a potential of + 0.2444 V at 25^0 C. It consists of an inner tube packed with a paste of Hg, Hg_2Cl_2 and saturated KCl. A small hole connects the two tubes, an asbestos fiber serves as a salt bridge to the solution in which the SCE is immersed.

Other type of reference electrode is the Ag/AgCl electrode which is the most common RE since it can be used at higher temperature. This electrode is based on the redox couple between silver chloride (AgCl) and silver (Ag) as illustrated below;

$$AgCl(s) + 2e \rightleftharpoons Ag(s) + Cl^- \qquad (1.2)$$

The potential of this electrode is determined by the concentration of Cl^-. For saturated KCl the potential is + 0.197 V whereas for 3.5 M KCl the potential electrode is + 0.205 V at 25° C (Harvey, 2000).

1.3.2.2 Solvent and Supporting Electrolyte

Electrochemical measurements are commonly carried out in a medium which consists of solvent containing a supporting electrolyte. Sometimes in most cases, supporting electrolyte has to be added to the dissolved sample in an attempt to achieve the following (Heyrovsky and Zuman 1968);

a) To make solution conductive
b) To control the pH value so that organic substances are reduced in a given potential range and inorganic substances are not hydrolysed
c) To ensure the formation of such complexes that give well developed and well separated waves
d) To shift the hydrogen evaluation towards more negative potentials and to eliminate catalytic effects on hydrogen evolution
e) To suppress unwanted maxima by addition of surface-active substances to the supporting electrolyte.

The choice of the solvent is primarily by the solubility of the analyte, its redox activity and also by solvent properties such as electrical conductivity, electrochemical activity and chemical reactivity. The solvent should not react with the analyte and

should not undergo electrochemical reaction over a wide potential range. In aqueous solution the cathodic potential is limited by the reduction of hydrogen ions:

$$2\,H^{+}(aq) + 2\,e^{-} \rightarrow H_2(g) \qquad (1.3)$$

resulting hydrogen evolution current. The more acidic the solution the more positive is the potential of this current due to the reaction expressed by;

$$E = E^{0}_{H2/H+} - 0.059\ pH \qquad (1.4)$$

The composition of the electrolyte may affect the selectivity of voltammetric measurements. The ideal electrolyte should give well-separated and well-shaped peaks for all the analytes sought, so that they can be determined simultaneously. For example Kontoyannis *et al.* (1999) used tris buffered saline (TBS) at pH 7.4 as the supporting electrolyte for simultaneous determination of diazepam and liposome using DPP technique. Inam and Somer (1998) have determined selenium (Se) and lead (Pb) simultaneously in whole blood sample by the same technique using 0.1 M HCl as the supporting electrolyte. They observed that there were three peaks at -0.33 V, -0.54 V and -0.41 V which belonged to an intermetallic compound (PbSe), Se and Pb respectively. Barbiera *et al.* (1997) have developed anodic stripping voltammetric technique for simultaneous determination of trace amounts of zinc, lead and copper in rum without pre-treatment and in the absence of supporting electrolyte. They observed that there were three peaks at -0.92 V, -0.42 V and 0.05 V which belong to Zn, Pb and Cu respectively.

Because of the sensitivity of the voltammetric method, certain impurities in supporting electrolyte can affect the accuracy of the procedures. It is thus necessary to prepare the supporting electrolyte from highly purified reagents and should not easily oxidised and reduced. To obtain acceptable ionic strength of supporting electrolyte, certain concentration should be prepared which is usually about 0.1 M. This level is a compromise between high conductivity and minimum contamination (Wang, 2000).

The low ionic strength which is 0.01 M of supporting electrolyte ($HClO_4$ – $NaClO_4$) was very effective for the adsorptive accumulation of analyte on the electrode as found by Berzas *et al.* (2000) when they developed adsorptive stripping square wave technique for determination of sildenafil citrate (Viagra) in pharmaceutical tablet.

Dissolved oxygen must be removed from supporting electrolyte first since the reduction of dissolved oxygen will cause two cathodic peaks at -0.05 V and -0.9 V (versus SCE) as reported by Fraga *et al.* (1998) and Reinke and Simon (2002). With increasing pH, the waves due to reduction of oxygen are shifted to more negative potential. The oxygen reduction generates a large background current, greater than that of the trace analyte, and dissolved oxygen therefore tends to interfere with voltammetric analysis (Colombo and van den Berg, 1998). The common method for the removal of dissolved oxygen is by purging with an inert gas such as nitrogen or argon where longer time may be required for large sample volume or for trace measurements. To prevent oxygen from reentering, the cell should be blanketed with the gas while the voltammogram is being recorded. However, this conventional procedure is time consuming and not suitable for flow analysis. Due to this reason, Colombo and van den Berg (1998) have introduced in-line deoxygenating for flow analysis with voltammetric detection. They have used an apparatus which is based on the permeation of oxygen through semi-permeable silicone tubing into an oxygen free chamber and enables the determination of trace metals by flow analysis with voltammetric determination.

1.3.2.3 Current in Voltammetry

When an analyte is oxidised at the working electrode, a current passes electrons through the external electric circuitry to the auxiliary electrode, where reduction of the solvent or other components of the solution matrix occurs. Reducing an analyte at the working electrode requires a source of electrons, generating a current that flows from the auxiliary electrode to the cathode. In either case, a current resulting from redox reaction at the working electrode and auxiliary electrode is called a faradaic current. A current due to the analyte's reduction is called a cathodic current. Anodic currents are

due to oxidation reaction. The magnitude of the faradaic current is determined by the rate ofthe resulting oxidation or reduction reaction at the electrode surface. Two factors contribute to the rate of the electrochemical reaction which are the rate at which the reactants and products are transported to and from the electrode, and the rate at which electron pass between the electrode and the reactants and products in solution.

There are three modes of mass transport that influence the rate at which reactant and products are transported to and from the electrode surface which are diffusion, migration and convection. Difussion from a region of high concentration to region of low concentration occurs whenever the concentration of an ion or molecule at the surface of electrode is different from that in bulk solution. When the potential applied to the WE is sufficient to reduce or oxidise the analyte at the electrode surface, a concentration gradient is established. The volume of solution in which the concentration gradient exists is called the diffusion layer. Without other modes of mass transport, the width of the diffusion layer increases with time as the concentration of reactants near electrode surface decreases. The contribution of diffusion to the rate of mass transport is time-dependent.

Convection occurs when a mechanical means is used to carry reactants toward the electrode and to remove products from the electrode. Ther most common means of convection is to stirr the solution using a stir bar. Other methods include rotating the electrode and incorporating the electrode into a flow cell.

Migration occurs when charged particles in solution are attracted or repelled from an electrode that has a positive or negative surface charge. When the electrode is positively charged, negatively charged particles move toward the electrode, while the positive charged particles move toward the bulk solution. Unlike diffusion and convection, migration only affects the mass transport of charged particles.

The rate of mass transport is one factor influencing the current in voltammetry experiment. When electron transfer kinetics are fast, the redox reaction is equilibrium,

and the concentrations of reactants and products at the electrode are those psecified by Nersnt Equation. Such systems are considered electrochemically reversible. In other system, when electron transfer kinetics are sufficiently slow, the concentration of reactants and products at the electrode surface, and thus the current, differ from that predicted by the Nersnt euqation. In this case the system is electrochemically irreversible.

Other currents that may exist in an electrochemical cell those are unrelated to any redox reaction are nonfaradaic and residual currents. The nonfaradaic current must be accounted for if the faradaic component of the measured current is to be determined. This current occurs whenever the electrode's potential is changed. Another type of non-faradaic current is charging current which occur in electrochemical cell due to the electrical double layer's formation. Residual current is a small current that inevitably flows through electrochemical cell even in the absence of analyte (Harvey, 2000).

1.3.2.4 Quantitative and Qualitative Aspects of Voltammetry

Quantitative information is obtained by relating current to the concentration of analyte in the bulk solution and qualitative information is obtained from the voltammogram by extracting the standard-state potential for redox reaction. The concentration of the electroactive species can be quantitatively determined by the measurement of limiting current which is linear function of the concentration of electro active species in bulk solution.

Half- potential serves as a characteristic of a particular species which undergoes reduction or oxidation process at the electrode surface in a given supporting electrolyte, and it is independent of the concentration of that species. Its function in the qualitative determination is the same as retention time in chromatographic technique.

1.3.3 Type of Voltammetric Techniques

1.3.3.1 Polarography

Polarography is a subclass of voltammetry in which the WE is the DME. This technique has been widely used for the determination of many important reducible species since the DME has special properties particularly its renewable surface and wide cathodic potential range. In this technique, it takes place in an unstirred solution where a limiting current is diffusion limiting current (Harvey, 2000). Each new mercury drop grows in a solution whose composition is identical to that of the initial bulk solution. The relationship between the concentration of analyte, C_A, and limiting current (I_d) is given by Ilikovic equation (Ewing, 1997; Heyrosky, 1968; Ilkovic, 1934 and Dahmen,1986).

$$I_d = 708nD^{\frac{1}{2}}m^{\frac{2}{3}}t^{\frac{1}{6}}C_A \qquad (1.5)$$

where:

n = number of electrons transferred in the redox reaction

D = analyte's diffusion coefficient ($cm^2 sec^{-1}$)

m = flow rate of mercury drop ($g\ sec^{-1}$)

t = drop time (sec)

C_A = concentration of depolariser ($mol\ l^{-1}$)

The above equation represents the current at the end of the drop life. The average current (I_{ave}) over the drop life is obtained by integrating the current over this time period:

$$I_{ave} = 607nD^{\frac{1}{2}}m^{\frac{2}{3}}t^{\frac{1}{6}}C_A \qquad (1.6)$$

From the above equation, there is a linear relationship between diffusion current and concentration of electroactive species. It also indicates that the limiting diffusion

current is a function of the diffusion coefficient which depends on the size and shape of the diffusion particle. As compared to another technique such as cyclic voltammetry technique, in this technique, the peak current is directly proportional to concentration and increases with the square root of the scan rate as given by the Randles-Sevcik equation (Equation 1.7) for a reversible system (Wang, 2000).

$$I_p = (2.69 \times 10^5)\, n^{3/2}\, ACD^{1/2}\upsilon^{1/2} \qquad (1.7)$$

where;

n	=	number of electron
A	=	electrode area (cm^2)
C	=	concentration (mol cm^{-3})
D	=	diffusion coefficient ($cm^2\ s^{-1}$)
υ	=	scan rate ($V\ s^{-1}$)

There are several types of polarographic techniques as was mentioned earlier. Polarography is used extensively in the analysis of metal ion, inorganic anions and organic compounds containing easily reducible or oxidisable functional group. A list of electroreducible and electrooxidisable organic functional groups is shown in Table 1.6 (Dean, 1995).

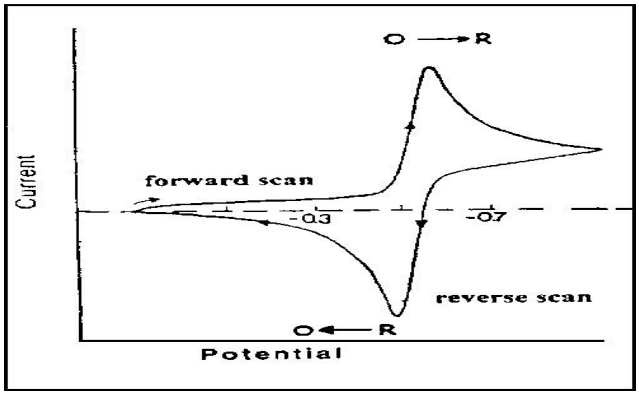
Table 1.6 Electroreducible and electrooxidisable organic functional groups

Electroreducible organic functional groups	Aromatic carboxylic acid, azomethines, azoxy compounds conjugated alkene, conjugated aromatic, conjugated carboxylic acid conjugated halide, conjugated ketone, diazo compounds, dienes, conjugated double bond, nitroso compounds, organometallics, disulfide, heterocycles, hydroquinones, acetylene, acyl sulfide aldehyde, hydroxylamines, imines, ketones, nitrates, nitriles nitro compounds, oximes, peroxides, quinones, sulfones, sulfonium salts and thiocyanates.
Electrooxidisable organic functional groups	Alcohols, aliphatic halides, amines, aromatic amines, aromatic ides, carboxylic acids, ethers, heterocyclic amines, heterocyclic aromatics, olefins, organometallic and phenols.

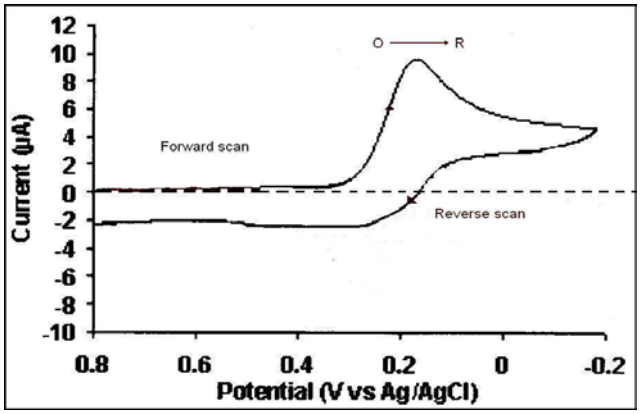
1.3.3.2 Cyclic Voltammetry

Cyclic voltammetry (CV) is a potential-controlled reversal electrochemical experiment. A cyclic potential sweep is imposed on an electrode and the current response is observed (Gosser, 1993). CV is an extension of linear sweep voltammetry (LSV) in that the direction of the potential scan is reversed at the end of the first scan (the first switching potential) and the potential range is scanned again in the reverse direction. The experiment can be stopped at the final potential, or the potential can be scanned past this potential to the second switching potential, where the direction of the potential scan is again reversed. The potential can be cycled between the two switching potentials for several cycles before the experiment is ended at the final potential. CV is the most widely used technique for acquiring qualitative information about electrochemical reactions.

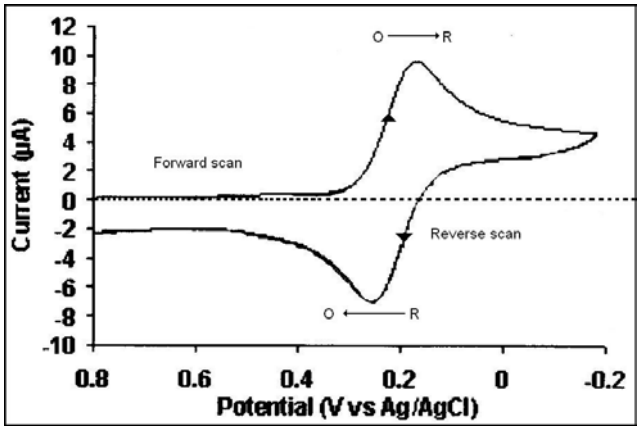
Analysis of the current response can give considerable information about the thermodynamics of redox processes, the kinetics of heterogeneous electron-transfer reaction and the coupled chemical reactions or adsorption processes. It is often the first electrochemical experiment performed in an electrochemical study especially for any new analyte since it offers a rapid location of redox potentials of the electro active species and convenient evaluation of the effect of media upon the redox process. In the CV technique, the applied potential sweep backwards and forwards between two limits, the starting potential and the switching potentials. In this technique, the potential of the WE is increased linearly in an unstirred solution. The resulting plot of current versus potential is called a cyclic voltammogram. Figure 1.10a to 1.10c show the cyclic voltammograms for reversible, irreversible and quasireversible reactions. For a typical reduction and oxidation process in reversible reaction (as in Figure 1.10a), during the forward sweep the oxidised form is reduced, while on the reverse sweep the reduced form near the electrode is reoxidised. Chemical reaction coupled to the electrode reaction can drastically affect the shape of the CV response. In the case of irreversible reaction, no reverse peak is observed (Figure 1.10b).



(a)



(b)



(c)

Figure 1.10 Cyclic voltammograms of (a) reversible, (b) irriverisble and (c) quasireversible reactions at mercury electrode (O = oxidised form and R = reduced form)

The cyclic voltammogram shows characteristics of an analyte by several important parameters such as peak currents and peak potentials that can be used in the analysis of the cyclic voltammetric response either the reaction is reversible, irreversible or quasi-reversible as listed in Table 1.7. Cyclic voltammogram will guide the analyst to decide the potential range for the oxidation or reduction of the analyte and that it can be a very useful diagnostic tool.

Table 1.7 The characteristics of different type of electrochemical reaction

Type of reaction	**Characteristics**
Reversible	Cathodic and anodic peak potential are separated by 59/n mV. The position of peak voltage do not alter as a function of voltage scan rate The ratio of the peak current is equal to one The peak currents are proportional to square root of the scan rate The anodic and cathodic peaks are independent of the scan rate
Irreversible	Disappearance of a reverse peak The shift of the peak potential with scan rate (20 – 100 mV/s) Peak current is lower than that obtained by reversible reaction.
Quasi-reversible	Larger separation of cathodic and anodic peak potential (> 57/ n mV) compared to those of reversible system.

1.3.3.3 Stripping Voltammetry

Stripping technique is one of the most important and sensitive electrochemical technique for measuring trace metals and organic samples. The term stripping is applied to a group of procedures involving preconcentration of the determinant onto the working electrode, prior to its direct or indirect determination by means of a potential sweep (Wang, 1985). The preconcentration (or accumulation) step can be adsorptive, cathodic or anodic. Its remarkable sensitivity is attributed to the addition of an effective preconcentration step with advanced measurement procedures that generate an extremely favorable signal-to-noise ratio as reported by Blanc *et al.* (2000). Brainina *et al.* (2000) list advantages of this technique such as high sensitivity, low detection limit, wide spectrum of test materials and analytes, both of organic and inorganic origin, insignificant effect of matrix in certain instances, compatibility with other methods, relative simplicity and low cost of equipment and finally, it can be automatic on-line and portable options. This technique is able to measure many analytes simultaneously such as reported by Ghoneim *et al.* (2000). They have determined up to eleven metals in water sample simultaneously using this technique. Stripping voltammetry technique utilises a few steps as follows;

a) Deposition step:

In this step, analyte will be preconcentrated on the WE within a certain time while solution is stirred. The deposition potential imposed on the WE is chosen according to the species to be determined and is maintained for a deposition period depending on their concentration. The choice of deposition potential can provide some selectivity in the measurement (Sun *et al.,* 2005). Deposition time must be controlled since the longer the deposition time, the larger the amount of analye available at the electrode during stripping. During deposition step, the solution is stirred to facilitate transportation of ions of interest to the surface of WE.

b) Rest step:

In this step, it allows formation of a uniform concentration of the ions of interest on the mercury. As the forced convection is stopped at the end of the deposition period, the deposition current drops almost to zero and a uniform concentration is established very rapidly. It also insures that the subsequent stripping step is performed in a quiescent solution.

c) Stripping step:

This step consists of scanning the potential anodically for anodic stripping and cathodically for cathodic stripping. When the potential reaches the standard potential of a certain ion of interest–metal ion complex, the particular ion of interest is reoxidised or reduced back into solution and a current is flowing. The resultant voltammogram recorded during this step provide the analytical information of the ions of interest. The stripping step after preconcentration gives the possibility for selective determination of different substances assuming that they also have different peak potential as reported by Kubiak *et al.* (2001). The potential-time sequence in stripping analysis is shown in Figure 1.11 which shows all three steps that were mentioned earlier.

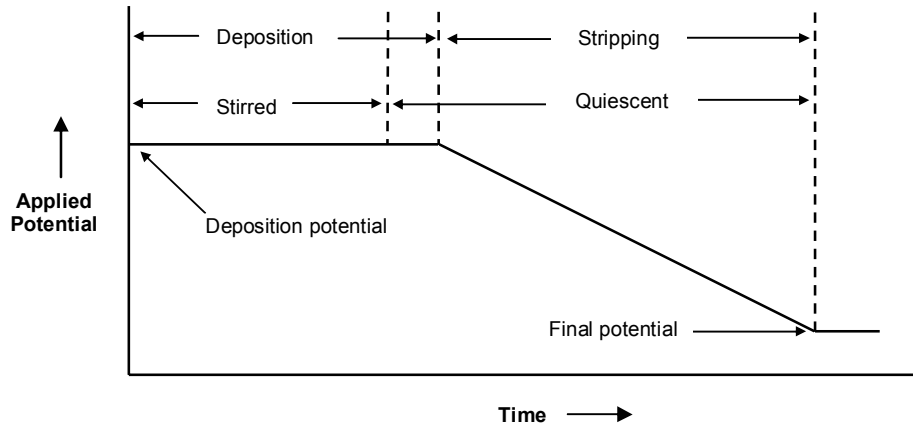


Figure 1.11 The potential-time sequence in stripping analysis

Most stripping measurements require the addition of appropriate supporting electrolyte and removal of dissolved oxygen. The former is needed to decrease the resistance of the solution and to ensure that the metal ions of interest are transported toward the electrode by diffusion and not by electrical migration. Contamination of the sample by impurities in the reagents used for preparing the supporting electrolyte is a serious problem. Dissolved oxygen severely hampers the quantitation and must be removed.

The main types of interference in stripping analysis are overlapping stripping signals, the adsorption of organic surfactants on the electrode surface, the presence of dissolved oxygen and the formation of intermetallic compounds by metals such as copper and zinc co-deposited in the mercury electrode. Overlapping signals cause problems in the simultaneous determination of analytes with similar redox potential such as lead and tin. Intermetallic-compounds formation and surfactant cause a depression of the stripping response and also shifting the signal location. Stripping voltammetry is composed of three related techniques that are anodic, cathodic and adsorptive stripping voltammetry.

1.3.3.3a Anodic Stripping Voltammetry (ASV)

ASV is mainly used in the determination of metal ions that can be reduced and then re-oxidised at a mercury electrode. Voltammetric measurements of numerous metal ions in various types of samples have been reported (Arancibia *et al.,* (2004) and Shams *et al.,* (2004)). The term ASV is applied to the technique in which metal ions are accumulated by reduction at an HMDE held at a suitable negative potential. The deposition potential is usually 0.3 to 0.5 V more negative than a standard potential for reduced metal ion to be determined. ASV consists of two steps. The first step is a controlled potential electrolysis in which the working electrode is held at a cathodic potential sufficient to deposit the metal ion (M^{n+}) on the electrode to form an amalgam, M(Hg). This step is called accumulation step which is represented by an equation;

$$M^{n+} + ne^- + Hg \rightarrow M(Hg) \quad (1.8)$$

The solution is stirred during this process to increase the rate of deposition. Near the end of the deposition time, stirring is stopped to eliminate convection as a mode of mass transport. The duration of the deposition step is selected according to the concentration level of the metals ion.

In the second step, the potential is scanned anodically toward more positive potential. When the potential of the WE is sufficiently positive the analyte is stripped from the electrode, returning to solution as its oxidised form. This step is called stripping which is represented by an equation

$$M(Hg) \rightarrow M^{n+} + ne^- \quad (1.9)$$

The current during the stripping step is monitored as a function of potential giving rise to a peak-shaped voltammogram. The peak current is proportional to the analyte's concentration.

1.3.3.3b Cathodic Stripping Voltammetry (CSV)

CSV is used to determine a wide range of organic compounds, and also inorganic compounds that form insoluble salts with the electrode material. It has been found to be widely applicable to many problems of clinical and pharmaceutical interest (Wang, 1988). Voltammetric measurements of numerous electro active species of biological significance such as drugs (Ghoneim *et al.,* 2003; Arranz *et al.*, 1999; Rodriguez *et al.*, 2004; Ghoneim and Beltagi, 2003 and Cabanillas *et al.*, 2003) and toxic substances (Hourch *et al.,* 2003; Safavi *et al.*, 2004) have been reported.

The term CSV was used originally for the indirect trace determination of organic compounds as mercury salt, involving anodic oxidation of mercury and subsequently cathodic reduction of mercury. This technique is similar to the previous

technique with two exceptions. First, the deposition step involves the oxidation of the mercury electrode from Hg to Hg^{2+}, which then reacts with the analyte to form an insoluble salt at the surface of the electrode. For example, when chloride ion (Cl^-) is the analyte, the deposition step is

$$2Hg(l) + 2Cl^-(aq) \rightarrow Hg_2Cl_2(s) + 2e^- \quad (1.10)$$

Secondly, stripping is accomplished by scanning cathodically toward a more negative potential, reducing Hg^{2+} back to Hg and returning the analyte to the solution.

$$Hg_2Cl_2(s) + 2e^- \rightarrow 2Hg(l) + 2Cl^-(aq) \quad (1.11)$$

1.3.3.3c Adsorptive Stripping Voltammetry (AdSV)

The term AdSV seems to have been first used by Lam *et al.* in 1983. It is a powerful analytical technique for the determination of nmol levels of a wide range of organic compounds (Smyth and Smyth, 1978; Smyth and Vos, 1992). In technical report for International Union Of Pure And Applied Chemistry under Analytical Chemistry Division Commission On Electro analytical Chemistry (Fogg and Wang 1999), suggested that AdSV technique is applied to stripping voltammetric technique in which accumulation is effected by adsorption of mainly organic determinants and that can be justified less in case of the adsorption of metal complexes in determining metal ions. The AdSV term should not be applied when there is a change of oxidation state of the metal ion during the accumulation for example in the accumulation of copper (I) complexes or salts or in other cases where an organic compounds is being accumulated, and determined indirectly, as a metal salts or complex such as mercury salts or nickel complexes.

In this technique the deposition step occurs without electrolysis process. Instead, the analyte adsorbs on to the electrode's surface. During deposition, the electrode is maintained at a potential that enhances adsorption. When deposition is

sufficient the potential is scanned in an anodic or cathodic direction depending on whether we wish to oxidise or reduce the analyte. In recent years, AdSV which improves sensitivity and selectivity, have promoted the development of many electrochemical methods for ultra-trace measurement of a variety of organic (Ghoneim *et al.,* 2003; Ghoniem and Taufik, 2004; Farias *et al.,* 2003 and Hourch *et al.,* 2003) and inorganic species (Ensafi *et al.,* 2004; Zimmerman *et al.,* 2001) and Jurado-Gonzalez *et al.* (2003). Barek *et al.* (2001a) reported that AdSV on HMDE is much more sensitive with a typical LOD between 10^{-9} and 10^{-10} M.

1.3.3.4 Pulse voltammetry

This technique uses pulse waveform in recording its voltammogram which offers enhanced sensitivity and resolution. The advantage of pulse techniques is that the waveform is designed so as to discriminate against non-faradic current hence, increase sensitivity. The enhanced resolution is particularly useful when several electroactive species are being analysed simultaneously (Fifield and Haines, 2000). In this technique, current sampling takes place at the end of the pulse and utilise the different time dependence of faradic (i_f) and charging current (i_c), as shown in Figure 1.12.

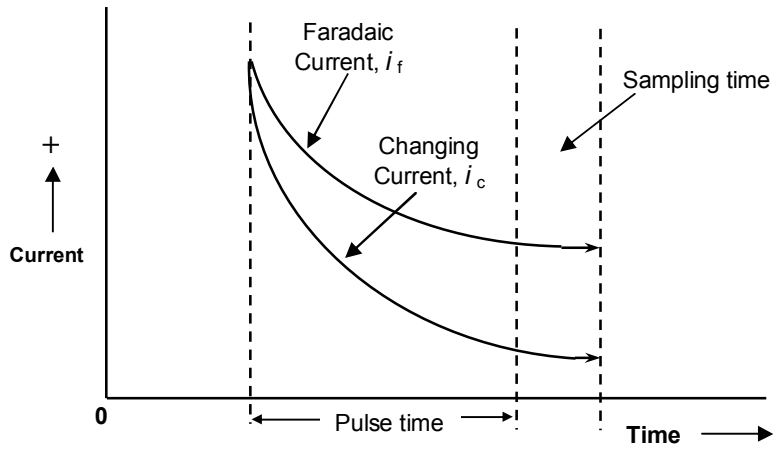


Figure 1.12 Schematic drawing showing the i_f and i_c versus pulse time course

This technique, is aimed at lowering the detection limits of voltammetric measurement down to 10^{-8} M in its differential pulse mode. Increasing the ratio between the faradic and non-faradic current permit convenient quantitation down to the 10^{-8} M concentration level (Wang, 2000). Differential pulse and square wave techniques are the most commonly used pulse technique. Differential pulse polarograms or voltammograms are peak shaped because current difference is measured. The following section gives an overview of three important waveforms in pulse technique.

1.3.3.4a Differential Pulse Voltammetry (DPV)

In DPV, fixed magnitude pulse (10 to 100 mV) superimposed on a linear potential ramp are applied to the working electrode at a time just before the end of the drop as shown in Figure 1.13. The current is sampled twice, just before the pulse application and again late in the pulse life normally after 40 ms, when the charging current has decayed. Subtraction of the first current sampled from the second provides a stepped peak-shape derivative voltammogram. The resulting differential pulse voltammogram consists of current peaks, the height of which is directly proportional to the concentration of corresponding analyte. The differential-pulse operation results in a very effective correction of the charging background current.

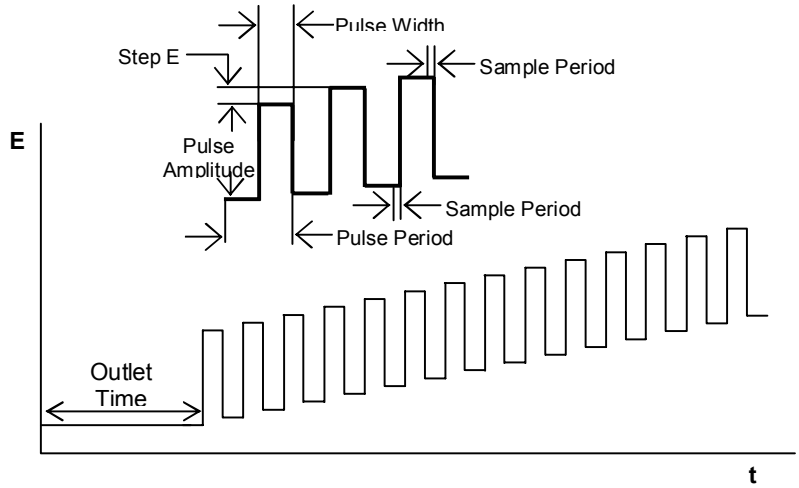


Figure 1.13 The schematic diagram of steps in DPV by superimposing a periodic pulse on a linear scan

1.3.3.4b Square Wave Voltammetry (SWV)

SWV is a large-amplitude differential technique in which a waveform composed of a symmetrical square wave, superimposed on a base staircase potential, is applied to the working electrode. The current is sampled twice during each square-wave cycle, once at the end of forward pulse and another at the end of the reverse pulse. Since the square-wave modulation amplitude is very large, the reverse pulses cause the reverse reaction of the product of the forward pulse. The difference between the two measurements is plotted versus the base staircase potential as shown in Figure 1.14. The resulting peak current is proportional to the concentration of the analyte. Excellent sensitivity accrues from the fact that the net current is larger than either the forward or reverse components. Coupled with the effective discrimination against the charging current, very low detection limits can be attained.

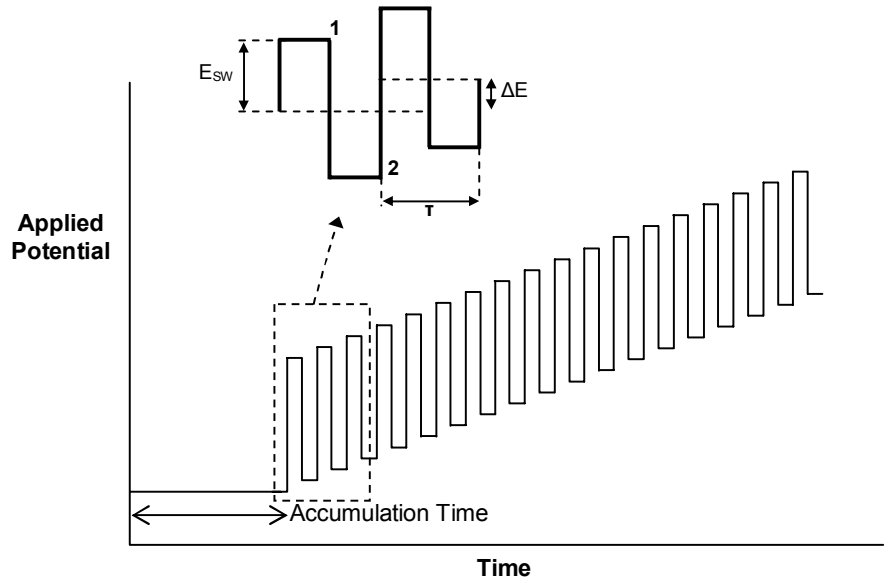


Figure 1.14 Waveform for square-wave voltammetry

The advantage of SWV is that a response can be found at a high effective scan rate, thus reducing the scan time. Because of its high scan rate, it provides a great economy of time (Arranz *et al.,* 1999 and Ghoneim and Tawfik., 2004). There are

several reports on application of the SWV technique for determination of several samples as listed in Table 1.8 which involves deoxygenation step prior analysis. However, in certain case beside it offers the additional advantage of high speed; it can increase analytical sensitivity and relative insensitivity to the presence of dissolved oxygen as reported by Economou *et al.* (2002).

Table 1.8 Application of SWV technique

No	Sample	Supporting electrolyte	Reference
1	Ketorolac in human serum	Acetate buffer, pH 5.0	Radi *et al.* (2001)
2	Imidacloprid in river water	BRB, pH 7.2	Guiberteau *et al.* (2001)
3	Cocaine and its metabolite	Phosphate buffer, pH 8.5	Pavlova *et al.* (2004)
4	Amlodipine besylate in tablets and biological fluids	BRB, pH 11.0	Gazy (2004)
5	EDTA species in water	Diluted HCl, pH 2.8 and 0.05 M NaCl	Zhao *et al.* (2003)
6	RDX in soil	0.1 M acetate buffer, pH 4.5	Ly *et al.* (2002)
7	Levofloxacin in human urine	0.05 M acetate buffer, pH 5.0	Radi and El-Sherif (2002)
8	Sertraline in commercial products	0.1 M borate, pH 8.2	Nouws *et al.* (2005a)

Table 1.8 continued

No	Sample	Supporting electrolyte	Reference
9	Cadmium in human hair	0.01 M TEA, pH 11.0	Arancibia *et al.* (2004)
10	Copper, stannous, antimony, thallium, and plumbum in food and environmental matrices	0.1 M dibasic ammonium citrate, pH 6.3	Locatelli (2005)
11	Imatinib (Gleevec) and its metabolite in urine	0.012 M $HClO_4$, pH 2.0	Rodriguez *et al.* (2005)
12	Famotidine in urine	0.02 M MOPS buffer, pH 6.7	Skrzypek *et al.* (2005)
13	Haloperidol in bulk form, pharmaceutical formulation and biological fluids	BRB, pH 9.0	El-Desoky *et al.* (2005)
14	Copper, cadmium and zinc complexes with cephalosporin antibiotic	Acetic acid, pH 7.34	El-Maali *et al.* (2004)
15	Triprolidine in pharmaceutical tablets	0.04 M BRB, pH 11.0	Zayed and Habib (2005)
16	Metoclopramide in tablet and urine	0.4 M HCl-sodium acetate, pH 6.2 and 0.2 M KCl	Farghaly *et al.* (2005)
17	Cefoperazone in bacterial culture, milk and urine	BRB, pH 4.4	Billova *et al.* (2005)

Table 1.8 continued

Notes:

BRB: Britton-Robinson buffer
EDTA: Ethylene diamine tetraacetic acid
$HClO_4$: Perchloric acid
MOPS: 3-(N-morpholino)propanesulphonic
RDX: Hexahydro-1,3,5-trinitro-1,3,5-triazine
TEA: Triethylammonium

1.4 Objective and Scope of Study

1.4.1 Objective of Study

The development of methods for the determination of aflatoxins has been constantly in demand due to the fact that aflatoxins are a major concern as the toxic contaminants of foodstuffs and animal feed, and have been recognised as a potential threat to human health since the early 1960s resulting in frequent economic losses. The widespread occurrence of aflatoxins producing fungi in our environment and the reported natural occurrence of toxin in a number of agricultural commodities has led the investigator to develop a new method for aflatoxins analysis.

In order to maintain an effective control of aflatoxins in food and foodstuffs proper analytical procedures must be applied. Different analytical methods such as thin layer, liquid chromatography or enzyme immunoassay test which were mentioned in Chapter I, have been developed for aflatoxins determination. However all these proposed techniques have their disadvantages. For HPLC technique, the method requires well equipped laboratories, trained personnel, harmful solvents as well as time consuming, and costly in buying and maintenance.

For immunological methods such as ELISA technique, it has a few disadvantages such as long incubation time, washing and mixing steps and also labour intensive. This method requires highly specific polyclonal or monoclonal sera which is costly. For radioimmunoassay (RIA) technique, it uses radioisotope which raises concerns in radiation safety in dealing with and also in disposing radioactive waste. For micellar electrokinetic capillary chromatography (MECC) technique, it needs preparing a lot of reagents for the buffering system and takes considerable time to complete the analysis.

With regards to voltammetric analysis, no stripping voltammetric study of aflatoxins is reported until now. This study has been proposed in order to develop a new alternative technique for determination of aflatoxins. This technique is the most promising method for determination of aflatoxins due to its main advantages compared to competing analytical techniques such as excellent sensitivity, reasonable speed, minimal sample pre-treatment, satisfactory selectivity, wide applicability, ability to undertake speciation analysis and low cost of instrumentation and maintenance as reported by Economou *et al.* (2002) and Radi (2003). Stripping analysis has been proven to be a powerful technique for the determination of both organic and inorganic electroactive species (Bond, 1980). Differential pulse cathodic stripping voltammetry satisfies the requirement of an efficient and senstitive technique for the determination of aflatoxin compounds because it has been shown to be a powerful technique in determination of other organic compounds in various sample origins down to ppb level as reported by Zima *et al.* (2001), Sun and Jiao (2002), Yardimer and Ozaltin (2004) and Nouws *et al.* (2005b).

In this research, the voltammetric behaviour of these compounds would be studied in great detail and the stripping voltammetry especially differential pulse stripping voltammetry (DPSV) and square wave stripping voltammetry (SWSV) techniques could provide accurate and sensitive methods for aflatoxins determination especially in food sample such as groundnuts, would be investigated.

The groundnut is selected for the sample to be analysed in this study since among foods and foodstuff, peanut and peanuts products are widely utilized as health food (www.copdockmill.co.uk/aflatoxin). It seem to be significantly contaminated with aflatoxins. It contains high content of carbohydrate which provides a substrate that is particularly suitable for toxin production (Neal, 1987). This will affect the quality and safety of humans life. Futhermore, Aspergillus moulds which produces aflatoxins grow easy on groundnut.

AFB1, AFB2, AFG1 and AFG2 were selected as the subjects in this study due to the regulatory requirement for their determination in imported raw grounnuts as imposed by the Malaysian government to assure that this commodity is free from any toxin contamination before being supplied for the domestic market. AFM1 and AFM2 are not involved in this study since currently no such regulation imposed by the Malaysian government even though a regulatory standard has already been set which is not more 0.05 ppb AFM1 and AFM2 should be present in milk and milk products. For the time being, the analysis on AFM1 and AFM2 are not being carried out in Malaysia. The other reason, globally, AFB1, AFB2, AFG1 and AFG2 are more concerned with their contamination in many food samples compared to AFM1 and AFM2 which contaminate milk and milk products only.

The objectives of this study are:

a) To investigate the electrochemical behaviour of aflatoxins B1 (AFB1), AFB2, AFG1 and AFG2 on the mercury electrode in appropriate supporting electrolyte.

b) To establish optimum conditions for the determination of those aflatoxins by the method of differential pulse cathodic stripping voltammetry (DPCSV) and square wave stripping voltammetry (SWV) techniques with a HMDE as the working electrode using Britton-Robinson buffer (BRB) solution as the supporting electrolyte.

c) To develop an accurate, sensitive, fast and simple method for the determination of all studied aflatoxins in food samples such as ground nut and comparing the results with the established method such as HPLC.

1.4.2 Scope of Study

The studies are as follows:

a) Studies on the voltammetric behaviour of aflatoxin compounds using cyclic voltammetry (CV) technique as an introductory step. Using this technique, the effect of increasing concentration of aflatoxins, scan rate and repetitive scanning on the peak height (I_p) and peak potential (E_p) of each aflatoxin will be investigated.

b) Studies on the differential pulse cathodic and anodic stripping voltammetriy (DPCSV) of all aflatoxins. Parameters optimisation include pH of supporting electrolyte, accumulation potential (E_{acc}), accumulation time (t_{acc}), scan rate (υ), initial potential (E_i), final potential (E_f) and pulse amplitude.

c) Using optimised analytical parameters and experimental conditions, the effect of increasing concentration of aflatoxins to the I_p of the compounds will be studied. Regression equation, R^2 value, linearity range, limit of detection (LOD), limit of quantification (LOQ), accuracy and reliability of the method will be obtained. Ruggedness and robustness tests also will be studied for the proposed technique.

d) The proposed technique will be further investigated in terms of interference where each aflatoxin will be reacted with increasing amounts of metals ion such as zinc, aluminum, nickel, lead and copper, and with organic compounds such as ascorbic acid, L-cysteine and β-cyclodextrin.

e) The optimised parameters for DPCSV technique will be applied for square wave stripping voltammetry (SWSV) including optimisation steps such as the E_{acc}, t_{acc}, pulse amplitude, scan rate, voltage step and frequency. The last 3 parameters are interrelated to each other in the SWSV technique.

f) Both DPCSV and SWSV techniques that were successfully developed will be applied in the determination of aflatoxins content in real samples such as groundnut. The recovery studies also will be carried out for the accuracy test of the developed method. The results will be compared with that obtained by accepted technique such HPLC. For the HPLC analysis, the final solutions from the extraction and clean-up procedures of groundnut in chloroform were sent to the Chemistry Department, Penang Branch, Ministry of Science, Technology and Innovation (MOSTI).

g) The stability of aflatoxins will be determined for aflatoxin stock and standard solutions according to the following procedures

   i) Aflatoxin stock solutions prepared in benzene: acetonitrile (98:2) will be studied using ultra-violet-visible spectrophotometer. In this analysis, each aflatoxin stock solution will be monitored every month (from 0 to 12 months) and the concentrations of aflatoxins will be calculated from the measured absorbance.

   ii) Aflatoxin standard solutions in BRB which was kept in the freezer at -4.0^0 C will be measured using voltammetric technique monthly up to 6 months

   iii) Aflatoxin standard solutions in BRB which have been added into the voltammetric cell and exposed to ambient temperature will be onitored every hour (from 0 to 8 hours) by measuring their peak height and peak potential

iv) Aflatoxin standard solution in BRB which was added into the voltammetric cell containing BRB at different pH (6.0, 7.0, 9.0 and 11.0) and exposed to ambient temperature will be monitored every hour (from 0 to 3 hours) by measuring their peak height and peak potential.

REFERENCES

Abbas, A.N. and Mostafa, G.A.E. (2003). New Triiodomercurate-Modified Carbon Paste Electrode for the Potentiometric Determination of Mercury. *Anal. Chim. Acta*. **478**:329-335.

Abbaspour, A. and Moosavi, S.M.M.(2002). Chemically Modified Carbon Paste Electrode for Determination of Copper (II) by Potentiometric Method. *Talanta*. **52**: 91-96.

Abdine, H. and Belal, F. (2002). Polarographic Behaviour and Determination of Acrivastine Capsules and Human Urine. *Talanta.* **56**: 97-104

Abdulkadar, A.H.W., Abdulla, A. A. A., Afrah M. A. and Jassim, H.A. (2004). Mycotoxins in Food Products Available in Qatar. *Food Control.* **15**: 543-548.

Aboul-Eneim, H. Y., Stefan, R-I. and Baiulescu, G-E. (2000). *Quality and Realibility in Analytical Chemistry*. New York: CRC Press.

Abu Zuhri, A. Z., Zatar, N. A., Shubeitah, R.M. and Arafat, H.H. (2000). Voltammetric and Spectrophotometric Determination of Nizatidine in Pharmaceutical Formulations. *Mikrochim. Acta.***134**:153-160.

Ahmad, M. (1993). *Pengenalan Statistik Dalam Kimia Analisis*. Kuala Lumpur: Dewan Bahasa Dan Pustaka.

Akiyama, H., Goda, Y. Tanaka. T, and Toyoda, M. (2001). Determination of Aflatoxins B1, B2, G1 and G2 in Spices Using a Multifunctional Column Clean-up, *J. of Chromatography A*. **932**: 153-157.

Alborzi, S., Pourabbas, B., Rashidi, M. and Astaneh, B. (2005). Aflatoxin M1 Contaminated in Pasteurised Milk in Shiraz (south of Iran). *Food Control*. Article in press.

Al-Ghamdi, A.H., Al-shadokhy, M.A. and Al-watan, A.A. (2004). Electrochemical Determination of Cephalothin Antibiotic by Adsorptive Stripping Voltammetric Technique. *J.Pharm. Bioanal. Anal.* **35(5)**:1001-1009.

Ali, N., Hashim, N.H., Saad, B., Safan, K., Nakajima, M. and Yoshizawa, T. (2005). Evaluation of a Method to Determine the Natural Occurence of Aflatoxins in Commercial Traditional Herbal Medicines From Malaysia and Indonesia. *Food and Chemical Toxicology.* **43(12)**: 1763-1772.

Almeida, l.E., Castilho, M., Maza, L.H. and Tabak, M.(1998). Voltammetric and Spectroscopic Studies of the Oxidation of the Anti-oxidant Drug Dipyridamole in Acetonitrile and Ethanol. *Anal. Chim. Acta*. **375**: 223-231.

Ammida, N.H.S., Micheli, L. and Palleschi, G. (2004). Electrochemical Immunosensor for Determination of Aflatoxin B1 inBarley. *Anal. Chim. Acta*. **520**: 159-164.

Andrieux, C.P., Hapiot, P. and Saveant, J.M.(1990). Ultramicroelectroded for Fast Electrochemical Kinetics. *Electroanalysis.* **2**:183-193.

Androu, V.G., Nikolelis, D. and Tarus, B. (1997). Electrochemistry Investigation of Transduction of Interactions of Alfatoixin M1 With Bilayer Lipid Membranes (BLMs), *Anal. Chim. Acta.* **350**:121-127.

Aoki, K. (1990). Theory of Stationary Current Potential Curves at Interdigitated Microarray Electrodes for Quasi-reversible and Totally Irreversible Electrode Reactions. *Electroanalysis.* **2**: 229-233.

Arancibia, V., Alarcon, L. and Sefura, R. (2004). Supercritical Fluid Extraction of Cadmium as Cd-Oxime Complex From Human Hair; Determination of Square-Wave Anodic or Adsorptive Stripping Voltammetry. *Anal. Chim. Acta.* **502**: 189-194.

Arranz, A., Dolara, I., de Betono, S.F., Moreda, J. M., Cid, A. and Arranz, J.F. (1999). Electroanalytical Study and Square Wave Voltammetric Techniques for the Determination of β-blocker Timolol at the Mercury Electrode. *Anal. Chim .Acta*. **389**: 225-232.

Arranz, P., Arranz, A., Moreda, J. M., Cid, A. and Arranz, J.F. (2003). Stripping Voltammetric and Polarographic Techniques for Determination of Anti-fungal Ketoconazole on the Mercury Electrode. *J. Pharm. Biomed. Anal.* **33**: 589-596.

Arrigan, D. W. M. (1994). Voltammetric Determination of Trace Metals and Organics After Accumulation at Modified Electrodes. *Analyst*. **119**: 1953-1966.

Arrus, K., Blank, G., Abramson, D. Clear, R. and Holley, R. A. (2004). Aflatoxins Production by *Aspergillus flavous* in Brazil Nuts. *J of Stored Products Research*. **41(5)**: 513-527.

Aslanoglu, M. and Ayne, G. (2004). Voltammetric Studies of the Interaction of Quinacrine With DNA. *Anal. Bioanal.Chem*. **380**: 658-663.

Aycicek, H., Aksoy, A. and Saygi, S.(2005). Determination of Aflatoxin Levels in Some Dairy and Food Products Which Consumed in Ankara, Turkey. *Food Control*. **16**: 263-266.

Aziz, N.H. and Moussa, L.A., (2002). Influence of Gamma-radiation on Mycotoxin Producing Moulds and Mycotoxins in Fruits. *Food Control.* **13**: 281-288.

Aziz, N.H., Youssef, Y.A., El-Fouly, M.Z. and Moussa, L.A., (1998). Contamination of Some Common Medicinal plant Samples and Spices by Fungi and Their Mycotoxins. *Bot. Bull. Acad. Sin*. **39**: 279-285.

Badea, M., Micheli, L., Messia, M. C., Candigliota, T., Marconi, E., Mottram, T., Velasco-Garcia, M., Moscone, E. and Palleschi, G. (2004). Aflatoxin M1 Determination in Raw Milk Using a Flow-injection Immunoassay System. *Anal Chim Acta*. **520**: 141-148.

Bakirci, I. (2001). A Study On the Occurence of Aflatoxin M1 in Milk and Milk Products Produced in Van Province of Turkey. *Food Control*. **12**: 47-51.

Bankole, S.A., Ogunsanwo, B.M. and Eseigbe, D.A. (2005a). Aflatoxins in Nigerian Dry-roasted Groundnuts. *Food Chemistry*. **89**: 503-506.

Bankole, S.A., Ogunsanwo, B.M. and Mabekoje, O.O.(2004) Natural Occurrence of Moulds and Aflatoxin B1 in Melon Seed From Markets in Nigeria. *Food and Chemical Toxicology*. **42**: 1309-1314.

Bankole, S.A., Ogunsanwo, B.M., Osho, A. and Adewuyi, G.O. (2005b). Fungal Contamination and Aflatoxin B1 of 'Egusi' Melon Seeds in Nigeria. *Food Control*. Article in press.

Barbeira, P.J.S. and Stradiotto, W.R., (1997). Simultaneous Determination of Trace Amounts of Zinc, Lead and Copper in Rum by Anodic Stripping Voltammetry. *Talanta*. **44**: 185-188.

Barker, G.C. and Jenkins, I.L. (1952). Square-wave Polarography. *Analyst*. **77**: 685-695.

Barek, J., Fogg, A. G., Muck, A. and Zima, J. (2001a). Polarography and Voltammetry at Mercury Electrodes. *Critical Reviews In Analytical Chemistry.* **31**: 291-309.

Barek, J., Cvacka, J., Muck, A. and Quaiserova, A. (2001b). Electrochemical Methods for Monitoring of Environmental Carcinogens. *Fresenius J Anal Chem.* **369**: 556-562.

Barek, J., Pumera, M., Muck. A., Kaderabkova, M. and Zima, J. (1999). Polarographic and Voltammetric Determination of Selected Nitrated Polycyclic Aromatic Hydrocarbons. *Anal Chim Acta*. **393**: 141-146.

BAS (1993) Controlled Growth Mercury Electrode Instruction Manual, Bioanalytical System, Inc. Indiana.

Batista, L.B., Chalfoun, S.M., Prado, G., Schwan, R.F. and Wheals, A.E. (2003). Toxigenic Fungi Associated With Processed (green) Coffee Beans (*Coffea arabica-L)*. *Int J Of Food Microbiolog*. **85**: 293 – 300.

Beaver, R.W. and Wilson, D.M. (1990). Comparison of Postcolumn Derivatisation-Liquid Chromatography With Thin-layer Chromatography for Determination of Aflatoxins in Naturally Contaminated Corn. *J. Assoc.Off. Anal. Chem.* **73(4)**: 379 – 381.

Beaver, R.W., James, M.A. and Lin, T.Y. (1991). ELISA-based Screening Test With Liquid Chromatography for the Determination of Aflatoxin in Corn. *J. Assoc.Off. Anal. Chem.* **74**: 827 – 829.

Beebe, B.M. (1978). Reverse Phase High Pressure Liquid Chromatographic Determination of Aflatoxins in Foods. *J. Assoc. Off. Anal. Chem.* **81**: 1347-1352.

Begum, F. and Samajpati, N. (2000). Mycotoxin Production on Rice, Pulses and Oilseeds. *Naturwissenschaften*. **87**: 275-277.

Berzas, J.J., Rodriguez, J., Castaneda, G. and Villasenor, M.J. (2000). Voltammetric Behavior of Sildenafil Citrate (Viagra) Using Square-wave and Adsorptive Stripping Square-wave Techniques. Determination in Pharmaceutical Products. *Anal. Chim. Acta*. **417**: 143-148.

Betina, V. (1984). *Mycotoxin – Production, Isolation, Separation and Purification.* Netherland: Elsevier Science.

Bicking, M.K.L., Kniseley, R.N and Svec, H.J. (1983). Coupled-Column for Quantitating Low Levels of Aflatoxins. *J . Assoc. Off. Anal. Chem.* **66**: 905-908.

Bijl, J. and Peteghen, C.V.(1985). Rapid Extraction and Sample Clean-up for the Fluorescence DensitometricDetermination of Aflatoxin M1in Milk and Milk Powder. *Anal. Chim. Act.* **170**: 149-152.

Billova, S., Kizek, R., Jelen, F. and Novotna, P. (2003). Square-wave Voltammetric Determination of Cefoperazone in a Bacterial Culture, Pharmaceutical Drug, Milk and Urine. *Anal Bioanal Chem*. **377**: 362-369.

Bingham, A.K., Huebner, H.J., Phillips, T.D. and Baver, J.E. (2004). Identification and Reduction of Urinary Aflatoxin Metabolites in Dog. *Food and Chemical* Toxicology. **42(11)**: 1851-1858.

Blanc, R., Gonzalez-Casado, A., Navabu, A. and Vilchez, J.L. (2000). The Estimate of Blanks in Differential Pulse Voltammetric Technique: Application to Detection Limits Evaluation as Recommended by IUPAC. *Anal. Chim. Acta*. **403**: 117-123.

Blesa, J., Soriano, J.M., Molto, J.C., Marin, R. and Manes, J. (2003). Determination of Aflatoxins in Peanuts by Matrix Solid-phase Dispersion and Liquid Chromatography, *J of Chromatography A*. **1011**: 49-54.

Bond, A.M., Oldham, K.B. and Zoski, C.G. (1989). Steady State Voltammetry. *Anal. Chim. Acta*. **216**: 177-230.

Brainina, Kh. (1974). *Stripping Voltammetry in Chemical Analysis*. New York: Wiley and Sons.

Brainina, Kh., Malakhova, N.A. and Stojko, Y. (2000). Stripping Voltammetry in Environmental and Food Analysis. *Fresenius J Anal Chem*. **368**: 307-325.

Brera, C., Caputi, R., Miraglia, M., Iavicoli, I., Salerno, A. and Carelli, G. (2002). Exposure Assessment to Mycotoxins in Workplace: Aflatoxins and Ochratoxin A Occurence in Airborne Dust and Human Sera. *Microchemical J*. **73(1-2)**: 167-173.

Bressolle, F., Bromet-Petit, M. and Audron, M. (1996). Validation of Liquid Chromatography and Gas Chromatography Methods: Application to Pharmacokinetic. *J. Chromatogr, B*. **686**: 3-10.

Buffle, J. and Tercier-Waeber, M-L. (2005). Voltammetric Environmental Trace-Metal Analysis and Speciation: From Laboratory to *in situ* Measurements. *Trend In Anal. Chem.* **24(3)**: 172-191.

Cakala, M., Mazerska, Z., Donten, M. and Stojek, Z. (1999). Electroanalytical and Acid-base Properties of Imidazoacridinanone, an Antitumor Drug (C-1311). *Anal. Chim. Acta*. **379**: 209-215.

Canabillas, A.G., Burguillos, J.M.O., Canas, M.A.M, Caceres, M.I.R. and Lopez, F.S. (2003). Square Wave Adsorptive Stripping Voltammetric Determination of Piromidic acid. Application in Urine. *J Pharm. Biomed. Anal.* **33**: 553-562.

Carapuca, H.M., Cabral, D.J. and Rocha, L.S. (2005). Adsorptive Stripping Voltammetry of Trimethoprim: Mechanistic Studies and Application to the Fast Determination in Pharmaceutical Suspensions. *J Pharm. Biomed. Anal.* **38**: 364-369.

Carlson, M.A. , Bargeron, C.B. , Benson, R.C., Fraser, A.B., Phillips, T.E., Velky, J. T., Groopman, J.D., Strickland, P.T. and Ko, H.W. (2000). An Automated, Handheld Biosensor for Aflatoxin. *Biosensors and Bioelectronic*. **14**: 841-848.

Carpini, G., Lucavelli, F., Marrazza, G. and Mascini, M., (2004). Oligonucleotide-Modified Screen-printed Gold Electrodes for Enzyme Amplified Sensing of Nucleic Acids. *Biosensors and Bioelectronic.* **20**: 167-175.

Castro, A.A., Wagener, A.L.R., Farias, P.A.M. and Bastos, M. B. (2004). Adsorptive Stripping Voltammetry of 1-hydroxypyrene at the Thin-film Mercury Electrode-basis for Quantitative Determination of PAH Metabolite in Biological Materials. *Anal. Chim. Acta*. **521**: 201-207.

CEN Report. (1999). Food analysis – Biotoxins – Criteria of Analytical Methods of Mycotoxins. European Committee for Standardisation CR 13505:99 E. 1-7.

Cepeda, A., Franco, C.M., Fente, C.A., Vazquez, B.I., Rodriguez, J.L., Prognon, P. and Mahuzier, G. (1996). Post-collumn Excitation of Aflatoxins Using Cyclodextrins in Lliquid Chromatography for Food Analysis. *J of Chromatogr. A*. **721**: 69-74.

Cespedez, A.E and Diaz, G.J. (1997). Analysis of Aflatoxins in Poultry and Pig Feeds and Feedstuffs used in Colombia. *J. Assoc. Off. Anal. Chem*. **80**: 1215-1219.

Chan, C.C. (2004). Potency method validation. In: Chan, C.C., Lam, H., Lee, Y.C. and Zhang, X-M. *Analytical Method Validation and Instrument Performance Verification.* USA: John Wiley and Sons.

Chan, D., MacDonald, S.J., Boughtflower, V. and Brereton, P. (2004). Simultaneous Determination of Aflatoxins and Ochratoxin A in Food Using a Fully Automated Immunoaffinity Column Clean-up and Liquid Chromatography-Fluorescence Detection. *J of Chromatoraphy A*. **1059**: 13-16.

Chang, H.L. and De Vries, J.W. (1983). Rapid High Performance Liquid Chromatography Determination of AFM1 in Milk and Nonfat Dry Milk. *J. Assoc. Off. Anal. Chem*. **66**: 913-917.

Chemistry Department, Ministry of Science Technology and Innovation. (2000). *Standard Procedure for Determination of Aflatoxins* (unpublished paper), Malaysia.

Chiavaro, E., Asta, D., Galaverna, G., Biancardi, A., Gambarelli, E., Dossena, A. and Marchelli, R. (2001). New Reversed-phase Liquid Chromatography Method to Detect Aflatoxins in Food and Feed with Cyclodextrins as Fluorescence Enhancers Added to the Eluent. *J of Chromatogr. A*. **937**: 31-40.

Chu, F.S., Fan, T.S.L., Zhang, G.S., Xu, Y.S. Faust, S. and Mac Mohan, P.L. (1987). Improved Enzyme-linked Immunosorbent Assay for Aflatoxin B1 in Agricultural Commodities. *J. Assoc. Anal. Chem*. **70**: 854-857.

Ciucu, A.A., Neguleseus, C. and Beldwin, R. P. (2003). Detection of Pesticides Using an Amperometric Biosensor Based on Ferophthalocyanine Chemically Modified Carbon Paste Electrode and Immobilised Enzymetic System. *Biosensors and Bioelectronic*.18: 303-310.

Cole, R.O., Holland, R.D. and Sepaniak, M.J. (1992). Factors Influencing Performance in the Rapid Separation of Aflatoxins by Micellar Electrokinetics Capillary Chromatography. *Talanta*.**39**: 1139-1147.

Colombo, C. and van den Berg, C.M.G. (1998). In-line Deoxygenated for Flow Analysis With Voltammetric Detection. *Anal. Chim. Acta*. **377**: 229-240.

Conesa, A. J., Pinilla, J. M. and Hernandez, L. (1996). Determination of Metbendazole in Urine by Cathodic Stripping Voltammetry. *Anal. Chim. Acta*. **331**: 111-116.

Creepy, E.E. (2002). Update of Survey, Regulation and Toxic Effect of Mycotoxins in Europe. *Toxicology Letter*. **127**: 19-28.

Czae, M. Z. and Wang, J. (1999). Pushing the Detectibility of Voltammetry: How Low Can Go ?. *Talanta*. **50**: 921-928.

Dahmen, E.A.M.F. (1986). *Electroanalysis: Theory and Application in Aqueous and Non-aqeuous Media and in Automated Chemical Control*. New York: Elsevier.

Davies, N.D. and Diener, U.L. (1980). Confirmatory Test for the High Pressure Liquid Chromatographic Determination of Aflatoxin B1. *J. Assoc. Off. Anal. Chem.* **63 (1)**: 107- 109.

De Botono, S.F., Moreda, J.M., Arranz, A. and Arranz, J.F. (1996). Study of the Adsorptive Stripping Voltammetric Behaviour of the Antihypertensive Drug Doxazosin. *Anal. Chim. Acta*. **329**: 25-31.

De Vries, J. (1996). *Food Safety and Toxicity*. New York: CRC.

Dean, J.A. (1995). *Analytical Chemistry Handbook*. USA: Mc Graw Hill.

Demetriades, D., Economou, A. and Voulgaropoulos, A. (2004). A Study of Pencil-Lead Bismuth-film Electrodes for the Determination of Trace Metals by Anodic Stripping Voltammetry. *Anal. Chim. Acta*. **519**: 167-172.

Dhavan, A.S. and Choudry, M.R. (1995). Incidence of Aflatoxins in Animal Feedstuffs: A decade's Scenario in India. *J. AOAC Int.* **78 (3)**: 693 – 698.

Dragacci, S., Gleizes, E., Fremy, J.M. and Candlish A.A.G. (1995). Use of Immunoaffinity Chromatography as a Purification Step for the Determination of AFM1 in Cheese. *Food Additives and Contaminants*. **12(1)**: 59-65.

Duhart, B.T., Shaw, S., Wooley, M., Allen, T. and Grimes, C. (1988a). Determination of Aflatoxins B1, B2, G1 and G2 by High Performance Liquid Chromatography With Electrochemical Detector. *Anal. Chim. Acta*. **208**: 343-346.

Eaton, D.L and Groopman, J.D. (1994).*The Toxicity of Aflatoxin*. United Kingdom: Academic Press.

Economou, A. (2005). Bismuth-film Electrode: Recent Development and Potentialities for Electroanalysis. *Trends Anal. Chem.* **24(4)**: 334-340.

Economou, A. and Fielden, P.R. (1995). A Study of Riboflavin Determination by Squarw-Wave Adsorptive Stripping Voltammetry on Mercury Film Electrode, Electroanalysis. **7**: 447 – 453.

Economou, A., Bolis, S.D., Efstathiou, C.E. and Volikakis, G.J. (2002). A Virtual Electroanalytical Instrument for Square-wave Voltammetry. *Anal. Chim. Acta*. **467**: 179-188.

Edinboro, L.E. and Karnes, H.T. (2005). Determination of Aflatoxin B1 in Sidestream Cigarette Smoke by Immunoaffinity Column Extraction Coupled With Liquid Chromatography/mass Spectrometry. *J of Chrom. A*. **1083**: 127-132.

Egner, P.A., Munoz, A and Kensler, T.W. (2003). Chemoprevention With Chlorophylin in Individuals Exposed to Dietary Aflatoxins. *Mutation Research.* **523**: 209-216.

El-Desoky, H.S. and Ghoneim, M.M. (2005). Assay of the Anti-psychotic Drug Haloperidol in Bulk Form, Pharmaceutical Formulation and Biological Fluids Using Square-wave Adsorptive Stripping Voltammetry at a Mercury Electrode. *J Pharm. Biomed. Anal.* **38**: 543-550.

El-Hady, D.A., Abdel-Hamid, M.I., Seliem, M.M., Andrisano, V. and El-Maali, N.A. (2004). Osteryoung Square Wave Stripping Voltammetry at Mercury Film Electrode for Monitoring Ultra Levels of Tarabine PFS and its Interaction With SsDNA. *J Pharm. Biomed. Anal.* **34**: 879-890.

El-Hafnawey, G.B., El-Hallag, I.S., Ghoneim, E.M. and Ghoniem, M.M. (2004), Voltammetric Behavior and Quantification of the Sedative-hypnotic Drug Chlordiazepoxide in Bulk Form, Pharmaceutical Formulation and Human Serum at a Mercury Electrode. *J of Pharm. And Biomed Anal.* **34**: 75-86.

El-Maali, N.A., Osman, A.H., Aly, A.A.A., Al-Hazmi, G.A.A. (2005). Voltammetric Analysis of Cu(II), Cd(II) and Zn(II) Complexes and Their Cyclic Voltammetry with Several Cephalosporin Antibiotics. *Bioelectrochemistry*. **65**: 95-104.

Enker, C.G. (2003). *The Art and Science of Chemical Analysis*. New York: John Wiley and Sons.

Ensafi, A.A., Khayamian, T. and Khaloo, S.S. (2004). Application of Adsorptive Cathodic Differential Pulse Stripping Method for Simultaneous Determination of Copper and Molybdenum Using Pyrogallol Red. *Anal. Chim. Acta*. **505**: 201-207.

Erdogan, A. (2004). The Aflatoxin Contamination of Some Pepper Types Sold in Turkey. *Chemosphere*. **56**: 321-325.

Erk, N. (2004). Voltammetric Behaviour and Determination of Moxifloxacin in Pharmaceutical Products and Human Plasma. *Anal. Bioanal.Chem*. **378**: 1351-1356.

Etzel, R.A. (2002). Mycotoxin. *J of Am.Med.Assoc*. **287**: 425-427.

EU Commision, Commision Regulation (EC) No 466/2001 of 8 March 2001 Setting Maximum Levels for Certain Contamination Foodstuffs. (2001). *J European Community*. L7.1

Ewing, G.W (Ed). (1997). *Analytical Instrumentation Handbook: Revised and Expanded*, 2nd Ed. New York: Marcel Dekker.

Fang, Y., Gong, F., Fang, X. and Fu, C. (1998). Separation and Determination of Cyclodextrins by Capillary Electrophoresis With Aperometric Detection. *Anal. Chim. Acta.* **369**: 39-45.

Faraj, M.K., Smith, J.E. and Harren, G. (1991). Introduction of Water Activity and Temperature on Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus* in Irradiated Maize Seeds. *Food Additives Contaminants*. **6(6)**: 731-736.

Farghaly, O.A. and Ghandour, M.A. (2005). Square Wave Stripping Voltammetry for Direct Determination of Eight Heavy Metals in Soil and Indoor-airborne Particulate Matter. *Environ. Research.* **97**: 229-235.

Farghaly, O.A., Taher, M.A., Naggar, A.H. and El-Sayed,A.Y., (2005). Square Wave Anodic Stripping Voltammetric Determination of Metoclopramide in Tablets and Urine at Carbon Paste Electrode. *J Pharm. Biomed. Anal.* **38**: 14-20.

Farias, P.A.M., Wargener, A.L.R., Bastos, M.B.R., de Silva, A.T. and Castro, A.G.(2004). Cathodic Adsorptive Stripping Voltammetric Behavior of Guanine in the Presence of Copper at the Static Mercury Drop Electrode. *Talanta*. **61**: 829-835.

Feroncova, A., Korgova, E., Miko, R. and Labuda, J., (2001). Determination of Tricyclic Antidepressent Using a Carbon Paste Electrode Modified with β-Cyclodextrin. *J. of Electroanalytical Chem.* **492**: 74-77.

Ferreira, V.S., Zanoni, M.V.B. and Fogg, A.G. (1999). Cathodic Stripping Voltammetric Determination of Ceftazidine With Reactive Accumulation at a Poly-L-lysine Modified Hanging Mercury Drop Electrode. *Anal. Chim. Acta.* **384**: 159-166.

Fifield, F.W. and Kealey, D. (2000). *Principle and Practice of Analytical Chemistry 3rd Edition*. UK: Blackwell science.

Fifield, F.W. and Haines, P.J. (2000).*Environmental Analytical Chemistry* 2nd Ed, UK: Blackwell Science.

Foog, A.G. and Wang, J. (1999).Terminology and Convention for Electrochemical Stripping Analysis. *Pure Appl. Chemistry*. **71**: 891-897.

Fraga, J.M.G., Abizanda, A.I.J., Moreno, F.J. and Leon, J.J.A. (1998). Application of Principal Component Regression to the Determination of Captopril by Differential Pulse Polarography With no Prior Removal of Dissolved Oxygen. *Talanta.* **46**: 73-82

Franco, C.M., Fente, C.A., Vazquez, B.T., Cepede, A., Mahuzier, G. and Prognon, P. (1998). Interaction Between Cyclodextrin and Alatoxins Q1, M1 and P1 Flourescence and Chromatographic Studies. *J of Chromatogr. A*. **815**: 21-29.

Fremy, J.M. and Chang, B. (1983). Rapid Determination of AFM1 in Whole Milk by Reversed-phase High Performance Liquid Chromatography. *J of Chromatogr. A*. **219**: 156-161.

Fremy, J.M. and Chu, F.S. (1984). Direct Enzyme-linked Immunosorbent Assay for Determining Aflatoxin M1 at Picogram Levels in Dairy Products. *J. Assoc. Anal. Chem.* **67**: 1098 – 1101.

Fukayama, M. Winterli, W. and Hsieh, D.P.H (1980). Rapid Method for Analysis of Aflatoxin M1 in Dairy Products. *J. Assoc. Anal. Chem.* **63**: 927 – 930.

Gao, W., Song, J. and Wu, N. (2004). Voltammetric Behavior and Square-wave Voltammetric Determination of Trepibutone at a Pencil Graphite Electrode. *J. Electroanalytical Chem.* **576(1)**: 1-7.

Garcia-Villanova, R.J., Cordon, C., Parama,s A.M.G., Aparicio, P. and Rosales, M.E.G. (2004). Simultaneous Immunoaffinity Column Clean up and HPLC Analysis of Aflatoxins and Ochratoxin A in Spanish Bee Pollen. *J Agric. Food Chem.***52**: 7235-7239.

Garden, S.R. and Strachan, N.J.C. (2001). Novel Colorimetric Immunoassay for the Detection of Aflatoxin B1, *Anal. Chim. Acta.* **444**: 187-191.

Gauch, R., Leuenberger, U. and Baumgartne, E. (1979). Rapid and Simple Determination of Aflatoxin M1 in Milk in the Low Parts per 10^{12} range. *J of Chromatogr. A.* **178**: 543-549.

Garner, R.C., Whattam, M.M. , Taylor, P.J.L. and Stow, M.W. (1993). Analysis of United Kingdom Purchased Spices for Aflatoxins Using Immunoaffinity Column Clean up Procedure Followed up by High-performance Liquid Chromatographic Analysis and Post-column Derivatization with Pyridium Bromide Perbromide. *J. Chromatography.* **648**: 485-490.

Gazy, A.A.K. (2004). Determination of Amlodipine Besylate by Adsorptive Square-Wave Anodic Stripping Voltammetry on Glassy Carbon Electrode in Tablets and Biological Fluids. *Talanta*. **62**: 575-582.

Ghoneim, M.M. Hassanein, A.M., Hamman, E. and Beltagi, A.M. (2000). Simultanoues Determination of Cd, Pb, Cu, Sb, Bi, Se, Zn, Mn, Ni, Cr and Fe in Water Sample by Differential Pulse Stripping Voltammetry at a Hanging Mercury Drop Electrode. *Fresenius J. Anal. Chem.* **367**: 378-383.

Ghoneim, M. M., Tawfik, A. and Khashaba, P.Y. (2003a). Cathodic Adsorptive Stripping Square-wave Voltammetric Determination of Nifedipine Drug in Bulk, Pharmaceutical Formulation and Human Serum. *Anal. Bioanal. Chem.* **375**: 369-375.

Ghoneim, M.M., and Tawfik, H.(2004). Assay of Anti-coagulant Drug Warfarin Sodium in Pharmaceutical Formulation and Human Biological Fluids by Square-wave Adsorptive Cathodic Stripping Voltammetry. *Anal. Chim. Acta*. **511**: 63-69.

Ghoneim, M.M., El Reis, M.A., Hamman, E. and Beltagi, A.M. (2004). A Validated Stripping Voltammetric Procedure for Quantification of the Anti-hypertensive and Benign Prostatic Hyperplasia Drug Terazosine in Tablets and Human Serum. *Talanta*. **64(3)**: 703-710.

Ghoneim, M.M., El-Baradie, K.Y. and Tawfik, H. (2003b). Electrochemical Behavior of the Antituberculosis Drug Isonianid and its Square-wave Adsorptive Stripping Voltammetric Estimation in Bulk Form, Tablets and Biological Fluids at a Mercury Electrode. *J. Pharm. Biomed. Anal.* **33**: 673-685.

Ghoniem, M.M. and Beltagi, A.M. (2003). Adsorptive Stripping Voltammetric Determination of the Anti-inflammatory Drug Celecoxib in Pharmaceutical Formulation and Human Serum. *Talanta*, **60**: 911-921.

Ghoniem, M.M. and Tawfik A. (2004). Assay of Anti-coagulant Drug Warfarin Sodium in Pharmaceutical Formulation and Human Biological Fluids by Square-wave Adsorptive Cathodic Stripping Voltammetry. *Anal. Chim. Acta*. **511**: 63-69.

Gimeno, A. and Martins, M. L (1983). Rapid Thin Layer Chromatographic Determination of Patulin, Citrinin and Aflatoxin in Apples and Pears and Their Juices and Jams. *J. Assoc. Anal. Chem.,* **66**: 85-91.

Goldblatt L.A (1969) *Aflatoxin*. New York: Academic Press.

Gonzalez, M.P.E., Mattusch, J. and Wennrich, R. (1998). Stability and Determination of Aflatoxins by High-performance Liquid Chromatography With Amperometric Detection. *J.Chromatography A*. **828**: 439-444.

Gonzalez, G., Hinojo, M.J., Mateo, R., Medina, A. and Jimenez, M. (2005). Occurrence of Mycotoxin Producing Fungi in Bee Pollen. *Int. J. Food Microbiology*. Article in press.

Gooding, J.J., Compton, R.G., Brennan, C.M. and Atherton, J.H. (1997). A New Electrochemical Method for the Investigation of the Aggregation of Dyes in Solution. *Electroanalysis*. **9(10)**: 759-764.

Gosser, D.K (1993). *Cyclic Voltammetry.* New York: Wiley-VCH.

Goto, T., Matsui, M. and Kitsuwa, T. (1988). Determination of Aflatoxins by Capillary Column gas Chromatography. *J of Chromatography*. **447**: 410-414.

Gourama, H. and Bullerman, L. (1995). *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic Fungi of Concern in Foods and Feeds: a Review. *J of Food Protection*. **58**: 1395-1404.

Gowda, N.K.S., Malathi, V. and Suganthi, R.U. (2004). Effect of Some Chemical and Herbal Compounds on Growth of *Aspergillus parasiticus* and Aflatoxin Production. *Anim. Feed Sci. Technol.* **116(3-4)**: 281- 291.

Grigoriadou, I.K., Eleftheriadou, A., Mouratidou, T. and Katikou, P. (2005). Determination of Aflatoxin M1 in Ewe's Milk Samples and the Produced Curd and Feta Cheese. *Food Control*. **16**: 257-261.

Guiberteau, A., Galcano, T., More, N., Parilla, P. and Salinas, F., (2001). Study and Determination of the Pesticide Imidacloprid by Square-wave Adsorptive Stripping Voltammetry. *Talanta*. **53**: 943-949.

Gurbay, A., Aydin, S., Girgin, G., Engin, A.B. and Sahin, G. (2004). Assessment of Aflatoxin M1 Levels in Milk in Ankara, Turkey. *Food Control*. Article in press.

Haghighi, B., Thorpe, C., Pohland, A.E. and Barnette, R. (1981). Development of a Sensitive High performance Liquid Chromatographic Method for Detection of Aflatoxins in Pistachio Nuts. *J. of Chromatography*. **206**: 101-108.

Hall, A.J. and Wild, C.P. (1994). *The toxicity of aflatoxin: Human, Health, Veterinary and Agriculture Significance.* New York: Academic.

Hamilton, F.W. and Ellis, J. (1980). Determination of Arsenic and Antimony in Electrolytic Copper by Anodic Stripping Voltammetry at a Gold Film Electrode. *Anal. Chim. Acta*. **119**: 225-233.

Harvey, D. (2000). *Modern Analytical Chemistry*. USA: McGraw Hill.

Heathcote, J.G. (1984). Aflatoxins and Related Toxins. In: Betina, V. (Ed). *Mycotoxins – Production, Isolation, Separation and Purification*. Netherland: Elsevier Science.

Heathcote, J.G. and Hibbert, J.R (1984) *Aflatoxin: Chemical and Biological Aspect*. New York: Elsevier.

Heberneh, G.G. (2004). Highlight on Plant Toxin in Toxicon. *Toxicon*. **44**: 341-344.

Hernandez, P., Dabrio-Ramos, M., Paton, F., Ballesteros, Y. and Hernandez, L. (1997). Determination of Abscisic Acid by Cathodic Stripping Square Wave Voltammetry. Talanta. **44**: 1783-1792.

Herrman, T. (2004). Mycotoxin in feed grains and ingredient in www. oznet.ksu.edu. Quote dated: 18th April 2004

Heyrovsky, J. and Zuman, P. (1968). *Practical Polarography; An Intoduction for Chemistry Students*. New York: Academic Press.

Hilali, A., Jimenez, J.C., Callejon, M., Bello, M.A. and Guirem, A. (2003). .Electrochemical Reduction of Cefminox at the Mercury Electrode and its Voltammetric Determination in Urine. *Talanta.* **59(1)**: 137-146.

Hocevar, S.B., Svancara, I., Vytras, K. and Ogorevc, B. (2005). Novel Electrode for Electrochemical Stripping Analysis Based on Carbon Paste Modified With Bismuth Powder. *Electrochim. Acta.* Article in press.

Holcomb, M. , Wilson, D.M., Truckess, M. W. and Thompson, H. C. (1992). Determination of Aflatoxins in Food Products by Chromatography. *J. of Chromatography A*. **624**: 341-352.

Hourch, M.E., Dudoit, A. and Amiard, J-C. (2003). Optimization of New Voltammetric Method for the Determination of Metallothionein. *Electrochim.Acta*, **48**: 4083-4088.

Hussien, H.S. and Brasel, J.M. (2001). Mycotoxin, Metabolism and Impact on Humans and Animals. *J Toxicology*. **167(2)**: 101-134.

Hutton, E.A., Hocevar, S.B. and Ogorevc, B. (2005). Ex situ Preparation of Bismuth Film Microelectrode for Use in Electrochemical Stripping Microanalysis. *Anal. Chim. Acta.* **537**: 285-292.

Ibrahim, M.S., Kamal, M.M. and Temerk. Y.M. (2003). Comparison of the Voltammetric Studies at Mercury Electrode and Glassy Carbon Electrodes for the Interaction of Lumichrome with DNA and Analytical Application. *Anal. Bioanal.Chem*. **375**: 1024-1030.

Ibrahim, M.S., Shehatta I.S. and Sultan, M.R. (2002). Cathodic Adsorptive Stripping Voltammetric Determination of Nalidixic Acid in Pharmaceuticals, HumanUrine and Serum. *Talanta*. **56**: 471-579.

Illkovic, D. (1934). Polarography Study With the Dropping Mercury Cathode. Part XLIV- The Dependence of Limiting Currents on the Diffusion Constant, on the Rate of Dropping on the Size Electrode. *Collection of Czechoslovak chemical communication.* **6**: 498-513.

Inam, R. and Somer, G. (1998). Simultaneous Determination of Selenium and Lead in Whole Blood Samples by Differential Pulse Polarography. *Talanta*. **46**: 1347-1355.

International Agency for Research on Cancer (1993). IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, vol. 56. World Health Organisation, Lyon.

IUPAC. (1978). Nomenclature: Symbols, Units and Their Usage in Spectrochemical Analysis-II. *Spectrochim. Acta, Part B*. **32**: 242.

Iwamoto, M., Webber, A. and Osteryoung, A. (1984). Cathodic Reduction of Thyroxine and Related Compounds on Silver Electrode. *Anal. Chem.* **56**: 1203-1206.

Jaimez, J., Fente, C.A., Vazquez, B.I., Franco, C.M., Cepeda, A., Mahuzier, G. and Prognon, P. (2000). Application of the Assay of Aflatoxins by Liquid Chromatography with Fluorescence Detection in Food Analysis. *J. Chromatography A.*. **882**: 1-10.

Janssens, M. M. T., Put, H. M. C. and Nout, M. J. R. (1996). In Vries, *Food Safety and Toxicity*. New York: CRC Press.

Jeffrey, A.M. and Williams, G.M. (2005). Risk Assessment of DNA-reactive Carcinogens in Food. *Toxicology and Appl. Pharmacology*. **207(2)**: 628-635.

Jeffery, H.W., Lenorich, L.M and Martin, R. Jr. (1982). Liquid Chromatography Determination of Aflatoxins in Artificially Contaminated Cocoa Beans. *J. Assoc. Off. Anal. Chem.* **80**: 1215-1219.

Jelen, F., Tomschik, M. and Palecek, E. (1997). Adsorptive Striping Square-wave Voltammetry of DNA. *J. Electroanal. Chem.* **423**: 141-148.

Ji, Z., Yuan, G., Cai, S., Liu, J. and Liu, G. (2004). Electrochemical Studies of the Oxidation Mechanism of Polyamide on Glassy Carbon Electrode, *J. of Electroanalytical Chem.* **570**: 265-273.

Jin, G-P. and Lin, X-Q. (2005). Voltammetric Behavior and Determination of Estrogens at Carbamylcholine Modified Paraffin-impregnated Graphite Electrode. *Electrochim. Acta*. **50**: 3556-3562.

Johnson, W.W., Harris, T.M. and Guyerich V.P. (1996). Kinetics and Mechanism of Hydrolysis of AFB1 exo-8-9-epoxide and Rearrangement of the Dihydrodiol. *J. Am. Chem. Soc.* **118**: 672-676.

Joshua, H. (1993). Determination ofAflatoxin by Reversed-phase High Performance Liquid Chromatography with Post-column in-line Photochemical Derivatization and Fluorescence Detection. *J. Chromatography A.* **654**: 247-254.

Jurado-Gonzalez, A. J., Galindo-Riano, M. D. and Garcia-Vargas, M. (2003). Experimental Designs in the Development of a New Method for the Sensitive Determination of Cadmium in Sea Water by Adsorptive Cathodic Stripping Voltammetry. *Anal. Chim. Acta*. **487**: 229-241.

Kamal, M.M., Abu Zuhri, A.Z. and Nasser, A.A. O. (1996). Adsorptive Stripping Voltammetric Analysis of 2,4,6-tri(2'-pyridyl)-1,3,5-triazine at a Mercury Electrode. *Fresenius J Anal Chem.* **356**: 500-503.

Kamimura, H., Nishijima, M., Yasuda, K., Ushiyama, H., Tabata, S., Matsumoto, S. and Nishima, T. (1985). Simple, Rapid Clean-up Method for Analysis of Aflatoxins and Comparison with Various Methods. *J. Assoc. Off. Anal. Chem*. **68**: 458-461.

Kamkar A. (2004) A Study on the Occurence of Aflatoxin M1 in Raw Milk Produced in Sarab City of Iran. *Food Control*. **16(7)**: 593-599.

Kamkar, A. (2005). A Study of the Occurrence of Aflatoxin M1 in Iranian Feta Cheese. *Food Control*. Article in press.

Karadjova, I., Girousi, S. , Illadou, E. and Stratis, I. (2000). Determination of Cd, Co, Cr, Cu, Fe, Ni and Pb in Milk, Cheese and Chocolate. *Mikrochim. Acta.* **134**: 185-191.

Kefala, G., Economou, A., Voulgaropoulos, A. and Sofoniou, M.(2003). A Study of Bismuth-film Electrode for the Detection of Trace Metals by Anodic Stripping Voltammetry and Their Application to the Determination of Pb and Zn in Tapwater and Human Hair. *Talanta*. **61**: 603-610.

Khodari, M., Ghandour, M. and Taha, A.M. (1997). Cathodic Stripping Voltammetric of the Anticancer Agent 5-fluorouracil and Determination in Urine. *Talanta*. **44**: 305-310.

Kim, H. J., Chang, S-C. and Shim, Y-B., (2002). α-cyclodextrin Modified Screen Printed Graphite Electrodes for Detection of Phenols. *Bull, Korean Chem. Soc.* **23**: 427-431.

Kizek, R., Masarik, M., Kramer, K.J., Potesil, D., Bailey, M., Howard, J.A., Klejdus, B., Mikelova, R., Adam, V., Trimkova, L. and Jelan, F. (2005). An Analysis of Avidin, Biotin and Their Interaction at Attomole Levels by Voltammetric and Chromatographic Techniques. *Anal. Bioanal. Chem.* **381**: 1167-1178.

Koehler, P.E., Benchat, L.R. and Chhinnan, M.S. (1985). Influence of Temparature and Water Activity on Aflatoxin Production by *Aspergillus flavus* in Cowpea (Vigna Ungiculata) Seeds and Meal. *J of Food Protection.* **48**: 1040-1043.

Kok, W. T., Van Neer, T.C.H., Traag, W.A. and Tuinstra, L.G.M.T. (1986). Determination of Aflatoxins in Cattle Feed by Liquid Chromtography and Post-column Derivatization with Electrochemically Generated Bromine. *J. Chromatogr.* **367**: 231 – 236.

Kok, W.Th. (1994). Derivatization Reactions for the Determination of Aflatoxins by Liquid Chromatography With Fluorescence Detection. *J Chromatography B* **659**: 127 – 137.

Komorsky-Lovric, S., Lovric, M. and Branica, M. (1992). Peak Current–frequency Relationship in Adsorptive Stripping Square-wave Voltammetry. *J. Electroanal. Chem.* **335(1-2)**: 297-308.

Kontoyannis, C. G., Antimisianis, S.G. and Douromis, D. (1999). Simultaneous Quantitative Determination of Diazepan and Liposomes using Differential Pulse Polarography. *Anal. Chim. Acta*. **391**: 83-88.

Kotoucek, M. Skopilova, J. and Michalkova, D. (1997). Electroanalytical Study of Salozosulfapyridine and Biseptol Components at the Mercury Electrode. *Anal. Chim.Acta*. **353**: 61-69.

Kubiak, W.W., Latonen, R.M., and Ivaska, A. (2001). The Sequential Injection System With Adsorptive Stripping Voltammetry Detection. *Talanta*. **53**: 1211-1219.

Kussak, A., Anderson, B. and Anderson, K. (1995a). Determination of Aflatoxins in Airborne Dust From Feed Factories by Automated Immunoaffinity Column Clean-up and Liquid Chromatography. *J Of Chromatogr. A*. **708**: 55-60.

Kussak, A., Anderson, B. and Anderson, K. (1995b). Immunity Column Clean-up for the High Performance Liquid Chromatographic Determination of Aflatoxins B1, B2, G1, G2, M1 and Q1 in Urine. *J Of Chromatogr. B*. **672**: 253-259

Kutner, W., Wang, J., L'Her, M. and Buck, R. (1998). Analytical Aspects of Chemically Modified Electrode: Classification, CriticalEvaluation and Recommendation. (IUPAC Recommendation 1998). *Pure and Appl. Chem.* **70**: 1301-1318.

Lafleur, R.D., Myland, J.C. and Oldham, K.B. (1990). Analytical Microelectrode Voltammetry with Minimal Instrumentation. *Electroanalysis*. **2**: 223-238.

Lafont, P. and Siriwardana, M. C. (1981). Assay Method for Aflatoxin in Milk. *J of Chromatogr.* **219**: 162-166.

Lam, G.K., Kalvoda, R. and Kaponica, M. (1983). Determination of Uranium by Adsorption Stripping Voltammetry. *Anal. Chim. Acta*. **154**: 79-83.

Lau, P.H. and Chu, F.S. (1983). Preparation and Characterisation of Acid Dehydration Product of Aflatoxicol. *J. Assoc. Off. Anal Chem.* **66(1)**: 98-101..

Lee, L.S., Wall, J.H., Cotty, P.J. and Bayman, P. (1990). Interpretation of Enzyme-linked Immunosorbent Assay with Conventional Chromatography Procedures for Quantitation of Aflatoxin in Individual Cotton Bolls, Seeds and Seeds Sections. *J. Assoc. Anal. Chem.* **73(4)**: 581-584.

Leontopoulos, D., Siafaka, A. and Markaki, P. (2003). Black Olives as Substrate for *Aspergillus parasiticus* Growth and Aflatoxin B1 Production. *Food Microbiology*. **20**: 119-126.

Leszezynska, J. Kucharska, U. and Zegota, H. (2000). Aflatoxin in Nuts Assayed by Immunological Methods. *Eur Food Res Technol*. **210**: 213-215.

Levi, C. (1980). Mycotoxins in Coffee. *J Assoc. Off. Anal. Chem.* **63(6)**: 1282-1285.

Lin, L., Lawrence, N.S., Thongngamdee, S., Wang, J. and Lin, Y. (2005). Catalytic Adsorptive Stripping Determination of Trace Chromium (VI) at the Bismuth Film Electrode. *Talanta*. **65**: 144-148.

Lin, L., Thongngamdee S., Wang, J., Lin, Y., Sadik, O.A. and Ly, S-Y. (2005). Adsorptive Stripping Voltammetric Measurements of Trace Uranium at the Bismuth Film Electrode. *Anal. Chim. Acta*. **535**: 9-13.

Lin, L., Zhang, J., Wang, P., Wang, Y. and Chen, J. (1998). Thin-layer Chromatography of Mycotoxins and Comparison with Other Chromatographic Methods. *J of Chromatogr. A*. **815**: 3-20.

Liu, J.H., Lin, W.F. and Nicol, J.D. (1980). The application of Anodic Stripping Voltammetry to Forensic Science. II. Anodic Stripping Voltammetric Analysis of Gunshot Residues. *Forensic Sci Int.* **16**: 53-62.

Liu, Y., Jiang, Y., Song, W., Lu, N., Zou, M., Xu, H. and Yu, Z. (2000). Voltammetric Determination of 5-hydroxydole-3-acetic Acid in Human Gastric Juice. *Talanta*. **50**: 1261-1266.

Locatelli, C. (2005). Overlapping Voltammetric Peaks – an Analytical Procedure for Simultaneous Determination of Trace Metals. Application to Food and Environmental Matrices. *Anal Bioanal Chem*. **381**: 1073-1081.

Loftsson, T. and Brewster, M.E. (1996) Pharmaceutical Applications of Cyclodextrins. 1. Drug Solubilisation and Stabilisation. *J. Pharm. Sci.* **85**: 1017-1025.

Lopez, C.E, Ramos, L.L., Ramadan, S.S. and Bulacio, L.C. (2002). Presence of Aflatoxin M1 in Milk for Human Consumption in Argentina. *Food Control*. **14(1)**: 31-34.

Lupu, A., Compagnone, D. and Palleschi, A. (2004). Screen-printed Enzyme Electrodes for the Detection of Marker Analytes During Wine Making. *Anal. Chim. Acta*. **513**: 67-72.

Ly, S-Y, Kim,D-H. and Kim, M-H. (2002). Square-wave Cathodic Stripping Voltammetry Analysis of RDX Using Mercury-film Plated Glassy Carbon Electrode. *Talanta*. **58**: 919-926.

Malaysian Food Act (1983) Act No 281, Reviewed 2002, Food Quality Control Dept, Ministry of Health, Malaysia.

Mackson, J., Wright, G., Rachaputi, N.C., Krosch, S. and Tatnell, J. (1999). Aflatoxin Research Update Workshop Proceeding. Kingaroy, Queensland. August 1999.

Martins, M.L. and Martin, H.M.,(2004). Aflatoxins M1 in Yoghurts in Portugal. *Int. J of Food Microbiology*. **91**: 315-317.

Manetta, A.C., Giuseppe, L.D., Giammarco, M.,Fusaro, I., Simonella, A., Gramenzi, A. and Formigoni, A. (2005). High-performance Liquid Chromatography With Post-column Derivatisation and Fluorescence Detection for Sensitive Determination of Aflatoxin M1 in Milk and Cheese. *J Chrom. A*. **1083**: 219-222.

Mendez-Albores J.A., Arambula-Villa G., Preciado-Ortiz R.E. and Moreno-Martinez E., (2004). Aflatoxins in Pozol, a Nixtamalized Maize-based Food. *Int J of Food Microbiology*. **94**: 211-215.

Metrohm (2005). Introduction to Polarography and Voltammetry Monograph, Metrohm Ltd, Switzerland.

Micheli, L. Grecco, R., Moscone, D. and Palleschi, G. (2005). An Electrochemical Immunosensor for Aflatoxin M1 Determination in Milk Using Screen-printed Electrodes. *Biosensors and Bioelectronic*. **21(4)**: 588-596.

Miller, N. (1987). *Toxicology Aspects of Food*. New York: Elsevier Science.

Miller, J.C. and Miller, J.N. (1993). *Statistic for Analytical Chemistry*. London: Ellis Horwood.

Miller, N., Pretorius,H.E. and Trinder, D.W. (1985). Determination of Aflatoxins in Vegetable Oils. *J. Assoc. Off. Anal. Chem*. **68**: 136-137.

Mohd. Yusoff, A.R., Ahamad, R. and Azman, S. (1998). Kaedah Voltammetri Dalam Penentuan Pewarna reaktif dan Hasil Hidrolisisnya. *Malays. J. Anal. Sci.* **4(1)**: 139-146.

Molina, M. and Gianuzzi, L. (2002). Modelling of Aflatoxin Production by *Aspergillus parasticus* in a Solid Medium at Different Temperature, pH and Propionic Acid Concentration. *Food Res. Int.* **35**: 585-594.

Moressi, M.B., Andreu, R., Calvente, J.J, Fernandez, H. and Zon, M. A. (2004). Improvement of Alterneriol Monomethyl Ether Detection at Gold Electrode Modified With a Dedocanethiol Self-assembled Monolayer. *J. of Electroanalytical Chem.* **570**: 209-217.

Morfobos, M., Economou, A. and Voulgaropoulos, A. (2004). Simultaneous Determination of Nickel(II) and cobalt(II) by Square Wave Adsorptive Stripping Voltammetry on a Rotating-disc Bismuth-film Electrode. *Anal Chim Acta*. **519**: 57-64.

Mouley, Y. (1984). Biochemical Effects of Mycotoxin. In Betina, V (Ed.). *Mycotoxin – Producton, Isolation, Separation and Purification*. Netherland: Elsevier Science.

Nawaz, S., Coker, R.D and Haswell, S.J. (1992). Development and Evaluation of Analytical Methodology for Determination of Aflatoxins in Palm Kernel. *Analyst*. **117**: 67 – 71.

Navalion, A., Blanc, R., Reyes, L., Navas, N. and Vikches, J.K. (2002). Determination of the Antibacterial Enrofloxacin by Differential Pulse Adsorptive Stripping Voltammetry. *Anal. Chim. Acta*. **454**: 83-91.

Neal, G.E. (1987). Influence of Metabolism: AflatoxinMetabolism and its Possible Relationships With Disease. In: Watson D.H. *Natural Toxicants in Foods: Progress and prospects*. London: VCH.

Niedwetzki, G. Lach, G. and Ceschwill, K. (1994). Determination of Aflatoxin in Food by Use of an Automatic Work Statio. *.J.Of Chromatography A*. **661**: 175-180.

Nilufer, D. and Boyacio, D. (2002). Comparative Study of Three Different Methods for the Determination of Aflatoxins in Tahini. *J. Agric. Food. Chem.* **50**: 3375-3379.

Nouws, H.P.A., Delerue-Matos, C., Barros, A.A. and Rodrigues, J.A., (2005a). Electroanalytical Study of the Antidepressant Sertraline. *J. Pharm. Biomed. Anal.* **39(1-2)**: 290-293.

Nouws, H.P.A., Delerue-Matos, C., Barros, A.A., Rodrigues, J.A. and Silva, A.S. (2005b). Electroanalytical Study of Fluvoxamine. *Anal and Bioanal. Chem.* **382(7)**: 1662-1668.

Official Methods of Analysis 14th Ed. (1984) AOAC, Washington D.C.

Ohfuji, K., Sato, N., Hamadansoto, N., Kabayashi, T., Imada, N., Okura, H. and Watanabe, E., (2004). Construction of a Glucose Based on a Screen-printed Electrode and a Novel Mediator Pyocyanin From Pseudomonos Aeruginosa. *Biosensors and Bioelectronic.* **19**: 1237-1244.

Orinakova, R., Tankova, L., Galova, M. and Supicova, A. (2004). Application of Elimination Voltammetry in the Study of Electroplating Processes on the Graphite Electrode. *Electrochimica Acta*. **49**: 3587-3594.

Ortiz, M.Z., Arcos, J., Juarros, J.V., Lopez-Palacios, J. and Sarabia, L.A. (1993). Robust Procedure for Calibration and Calculation of the Detection Limit of Trimipramine by Adsorptive Stripping Voltammetry at a Carbon Paste Electrode. *Anal. Chem.* **65**: 678-682.

Ozkan, S.A. and Uslu, B. (2002). Electrochemical Study of Fluvastatin Sodium – Analytical Application to Pharmaceutical Dosage Forms, Human Serum and Simulated Gastric Juice. *Anal. Bioanal.Chem*. **372**: 582-586.

Ozkan, S.A., Uslu, B., Aboul-Enein, H.Y. (2003). Voltammetric Investigation of Tamsulosin. *Talanta*. **61**: 147-156.

Pacheco, W.F., Farias, P.A.M. and Aucelio, R.Q. (2005). Square-wave Adsorptive Stripping Voltammetry for the Determination of Cyclofenil After Photochemical Derivatisation. *Anal. Chim. Acta*. **549(1-2)**: 67-73.

Padano, M. L. and Rivas, G. A., (2000). Amperometric Biosensor for the Quantification of Gentisic Acid using Poliphenol Oxidase Modified Carbon Paste Electrode. *Talanta*. **53**: 489-495.

Papp E., Otta K.H., Zaray G. and Mincsvics (2002). Liquid Chromatographic Determination of Aflatoxins. *Microchemical J* . **73(1-2)**: 39-46.

Pare, J.R.J and Belanger, J.M.R. (1997). *Instrumental Methods in Food Analysis*. N.York : Elsevier.

Parham, H and Zargar, B. (2001). Determination of Isosorbic Dinitrate in Arterial Plasma, Synthetic Serum and Pharmaceutical Formulations by Linear Sweep Voltammetry on a Gold Electrode. *Talanta*. **55**: 255-262.

Park, K.Y. and Bullerman. L.B. (1983). Effects of Cycling Temperature on Aflatoxins Production by *Aspergillus parasiticus* and *Aspergillus flavous* in Rice and Cheese. *J of Food Science*. **48**: 889-896.

Park, D.L. and Rua, S.M. (1991). Sample Collection and Preparation Techniques for Aflatoxin Determination in Whole Cottonseed. *J Assoc. Off. Anal. Chem.* **74**: 73-75.

Parlim, A. and Zerger, S. (2003). Determination of Isosorbide Synthetic Serum and Pharmaceutical Formulation by Linear Sweep Voltammetry on a Gold Electrode. *Talanta*. **55**: 255-262.

Patey A.L., Sharman M. and Gilbert J (1990). Determination of Aflatoxin B1 Levels in Peanut Butter Using an Immunoaffinity Column Cleanup Procedure: Interlaboratory Study. *Food Addit. Contam.* **7**; 515-520.

Patey, A.L., Sharman, M. and Gilbert, J. (1991). Liquid Chromatographic Determination of Aflatoxin Levels in Peanut Butters Using an Immunoaffinity Column Clean up Method: International Collaborative Trial. *J. Assoc. Off. Anal. Chem.* **74**: 76-81.

Pavlova V., Mirceski V., Lovric S.K, Javanovic S.P. and Mitrevski B. (2004). Studying Electrode Mechanism and Analytical Determination of Cocaine and its Metabolites at the Mercury Electrode Using Square-wave Voltammetry. *Anal Chim.Acta*. **512**: 49-56.

Pena, R. Alcaraz, M.C., Arce, L., Rios, A. and Valcarcel, M. (2002). Screening of Aflatoxins in Feed Samples Using a Flow System Coupled to Capillary Electrophoresis. *J Of Chromatography A*. **967**: 303-314.

Perez-Ruiz, T., Martinez-Lozano, C., Sanz, A. and Guillen, A. (2004). Successive Determination of Thiamine and Ascorbic Acid in Pharmaceuticals by Flow Injection Analysis. *J.Pharm. Biomed. Anal.* **34**: 551-557.

Pesavento, M., Domagala, S., Baldini, E. and Cucca, L. (1997). Characterisation of Enzyme Linked Immunosorbent Assay for Aflatoxin B1 Based on Commercial Reagents. *Talanta.* **45**: 91-104.

Pestka, J. and Bundy, G. (1990). Alteration of Immune Function Following Dietary Mycotoxin Exposures. *Canadian J. of Physilogy and Pharmacology*. **68**: 1009-1016.

Pezzatini, G., Midili, I., Toti, G., Loglio, F. and Innoceti, M. (2004). Determination of Chlorite in Drinking Water by Differential Pulse Voltammetry on Graphite. *Anal. Bioanal.Chem*. **380**: 650 - 657.

Pildain, M.B., Vaamonde, G. and Cabral, D. (2004). Analysis of Population Structure of *Aspergillus flavus* From Peanut Based Vegetative Compatibility, Geographic Origin, Mycotoxin and Sclerotia Production. *Int. J. Food Microb.* **93**: 31- 40.

Plattner, R.D., Bennett, G.A. and Stubblefield, R.D. (1984). Identification of Aflatoxins by Quadrupole Mass Spectrometry/Mass Spectrometry. *J. Assoc. Anal. Chem.* **67(4)**: 734 -738.

Pons, W.A. Jr. and Franz, A.O. Jr. (1977). High Performance Liquid Chromatography of Aflatoxins in Cottonseed Products. *J Assoc off Anal Chem.* **60**: 89 - 95.

Pournaghi-Azar, M.H. and Dastango, H. (2002). Differential Pulse Anodic Stripping Voltammetry of Copper in Dichloromethane: Application to the Analysis of Human Hair. *Anal. Chim. Acta*. **405**:135 - 144.

Rachaputi, N.C., Wright, G.C., Krosch, S. and Tatnell,J. (2001). Management Practice to Reduce Aflatoxin Contamination in Peanut. Proceedings of the 10th Australian Agronomy Conference, Hobart. 2001.

Radi, A. (2003). Square-wave Adsorptive Cathodic Stripping Voltammetry of Pantoprazole. *J of Pharmaceutical and biomedical analysis.* **33**: 687 – 692.

Radi, A. and El-Sherif, Z. (2002). Determination of Levofloxacin in Human Urine by Adsorptive Square-wave Anodic Stripping Voltammetry on a Glassy Carbon Electrode. *Talanta.* **58**: 319 - 324.

Radi, A., Beltagi, A.M. and Ghoniem, M.M. (2001). Determination of Ketorolac in Human Serum by Square-wave Adsorption Stripping Voltammetry. *Talanta*. **54**: 283 - 289.

Rasooli, I. and Abnayeh, M.R.(2004). Inhibitory Effect of Thyme Oils on Growth and Aflatoxin Production by *Aspergillus parasiticus. Food Control.* **15**: 479 - 483.

Rastogi, S., Das, M. and Khanna, S.K. (2001). Quantitative Determination of Aflatoxin B1-oxime by Column Liquid Chromatography With Utraviolet Detection. *J Of Chromatography A*. **933**: 91 - 97.

Rastogi, S., Dwivedi, P.D., Khanna, S.K. and Das, M. (2004). Detection of Aflatoxin M1 Contamination in Milk and Infant milk Products From Indian Market by ELISA. *Food Control*. **15**: 287 - 290.

Reif, K. and Metzger, W. (1995). Determination of Aflatoxins in Medicinal Herbs and Plant Extracts. *J Of Chromatography A*. **692**: 131 - 136.

Reinke, R. and Simon, J. (2002). The Online Removal of Dissolved Oxygen From Aqueous Solutions Used in Voltammetric Technique by Chromatomembrane Method. *Anal. Bioanal. Chem.* **374**: 1256 - 1260.

Riley, T. and Tomlinson, C. (1984). *Principles of Electroanalytical Methods (Analytical Chemistry by Open Learning)*. London: John Wiley.

Risk assesment studies report No. 5 (1991). Chemical Hazrad – Evaluation Aflatoxin in Food. Food and Environmental Hygeine Department, Hong Kong.

Rizk, M., Belal, F., Aly, F.A. and El-Enany, N.M. (1998). Differential Pulse Polarographic Determination of Ofloxacin Pharmaceuticals and Biological Fluids. *Talanta*. **46**: 83 - 89.

Rizzo I., Vedoya G., Maurutto S., Haidukowski M. and Varsavsky E., (2004). Assessment of Toxigenic Fungi on Argentinean Medicinal herbs. *Microbiological Research*. **159**: 113 - 120.

Rodriguez, J., Berzas, J.J., Castanada, G. and Rodrigues, N., (2005). Voltammetric Determination of Imanitib (Gleevec) and its Main Metabolite Using Square-wave and Adsorptive Stripping Square-wave Technique in Urine Samples. *Talanta*. **66**: 202 - 209.

Rodriguez, J., Berzas, J.J., Castanada, G. and Rodrigues, R. (2004). Determination of Seldenafil Citrate (Viagra) and its Metabolite (UK-103,320) by Square-wave and Adsorption Stripping Square-wave Voltammetry. Total Determination in Biological samples. *Talanta.* **62**: 427 - 432.

Sabry, S.M. and Wahbi, A.A.M. (1999). Application of Orthogonal Functions to Differential Pulse Voltammetric Analysis: Simultanuoes Determination of tin and Lead on Soft Drinks. *Anal. Chim.* Acta, **401**: 173 - 183.

Safavi, A., Maleki, N. and Shahbaazi, H.R.(2004). Indirect Determination of Cyanide Ion and Hydrogen Cyanide by Adsorptive Stripping Voltammetry at a Mercury Electrode. *Anal.Chim.Acta.* **503**: 213 - 221.

Salavagione. H.J., Arias. J., Garces. P., Marallon. E., Barbero. C. and Vazguez. J.L, (2004). Spectroelectrochemical Study of the Oxidation of Aminophenols on Platinum Electrode in Acid Medium. *J. Of Electroanalytical Chem.* **565**: 375 - 383.

Saleemullah, Iqbal, A., Khalil, I.A. and Shah H. (2005). Aflatoxin Contents of Stored andArtificially Inoculated Cereals and Nuts. *Food Chemistry*. Article in press.

Salleh, B. (1998). *Mikotoksin- Implikasi Terhadap Kesihatan Manusia dan Haiwan*. Pulau Pinang: Universiti Sains Malaysia.

Samajpathi, N. (1979) .Aflatoxin Produced by Five Species of *Aspergillus* on Rice. *Naturwissenschaften.* **66**: 365 - 366.

Sandulescu, R., Mirel, S. and Oprean, R. (2000). The Development of Spectrophotometric and Electroanalytical Methods for Ascorbic Acid and

Acetaminophen and Their Application in the Analysis of Effervescent Dosage Form. *J. Pharm. Biomed. Anal.* **23**: 77 - 87.

Sanna, G., Pilo, M.I., Pin, P.C. Tapparo, A. and Seeber, R. (2000). Determination of Heavy Metals in Honey by Anodic Stripping Voltammetry at Microelectrode. *Anal. Chim. Acta.* **415**: 165 - 173.

Santos, M.C. and Machado, S.A.S. (2004). Microgravimetric, Rotating Ring-disc and Voltammetric Studies of the Underpotential Deposition of Selenium on Polycrystalline Platinum Electrode. *J. of Electroanalytical Chem.* **567**: 203 - 210.

Sarimehmetoglu, B., Kuplulu, O. and Celik, T.H. (2004). Detection of Aflatoxin M1 in CheeseSamples by ELISA. *Food Control*. **15**: 45 - 49.

Sassahara, M., Netto, D.P. and Yanaka, E.K. (2005). Aflatoxin Occurence in Foodstuff Supplied to Dairy Cattle and Aflatoxin M1 in Raw Milk in the North of Parana State. *Food and Chemical Technology*. **43**: 981 - 984.

Scholten, J.M and Spanjer, M.C. (1996). Determination of Aflatoxin B1 in Pastachio Kernels and Shells. *J. Assoc. Off. Anal. Chem.* **79**: 1360 - 1364.

Scott, P.M. and Lawrence, G.A. (1997). Determination of Aflatoxins in Beer. *J. Assoc. Off. Anal. Chem.* **80**: 1229 - 1233.

Setamou, M.K., Gardwell, F., Schulthess, S. and Hell, K. (1997). *Aspergillus flavus* Infection and Aflatoxin Contaminants of Preharvest Maize in Benin. *Plant Disease.* **81**: 1327 - 1327.

Shahrokhian, S. and Karimi, M. (2004). Voltammetric Studies of a cobalt(II)-4-Methylsalophen Modified Carbon-paste Electrode and its Application for the

Simultaneous Determination of Cysteine and Ascorbic Acid. *Electrochim. Acta*. **50**: 77 - 84.

Shams, E., Barbei, A. and Soltaninezhad,M. (2004). Simultaneous Determination of Copper, Zinc and Lead by Adsorptive Stripping Voltammetry in the Presence of Morin. *Anal. Chim. Acta*. **501**: 119 - 124.

Shannon, G.M., Shotwell, O.L and Kwole, W.F. (1983). Extraction and Thin Layer Chromatography of Aflatoxin B1 in Mixed Feeds. *J. Assoc. Off. Anal. Chem*. **66**: 582 - 586

Shenasi, M., Aidoo, K.E. and Candlish, A.A.G. (2002). Microflora of Date Fruits and Production of Aflatoxins at Various Stages of Maturation. *Int.J Food Microbiology*. **79(1-2)**: 113 - 119.

Shi K. and Shiu K.K. (2004). Adsorption of Some Quinone Derivatives at Chemically Activated Glassy Carbon Electrode. *J. of Electroanalytical Chem.* **574(1)**: 63 - 70.

Shotwell, O.L and Goulden, M.L (1977). Aflatoxins: Comparison of Analysis of Corn by Various Methods. *J. Assoc. Off. Anal. Chem.* **60**: 83 - 88.

Shotwell, O.L, Goulden, M.L., Benntt, G.A., Plattner, R.D. and Hesseltine, C.W (1977). Survey of 1975 Wheat and Soybeans for Aflatoxins, Zeralenone and Ochratonin, *J. Assoc. Off. Anal. Chem.* **60**: 778 - 783.

Shotwell, O.L., Hesseltin,e C. and Stubblefield, R.D. (1966). Production of aflatoxin on rice. *Appl. Microbiol.* **14**: 425 - 428.

Simon, P., Delsaut, P., Lafontaine, M. Morele, Y. and Nicol, T. (1998). Automated Column-switching High-performance liquid chromatography for the determination of aflatoxin M1. *J of Chromatogr. B.* (1998). **712**: 95 - 104.

Skoog, D.A., West, D.M. and James, F. (1996) *Fundamentals of Analytical Chemistry*. 7th Ed. New York; Saunders College Publishing,

Skrzypek S., Ciesielski W., Sokolowski A., Yilmaz S. and Kazmierczak A. (2005). Square Wave Adsorptive Stripping Voltammetric Determination of Famotidine in Urine. *Talanta.* **66**:1146 - 1151.

Smith, J.E. and Moss M.O. (1985). *Mycotoxins – Formation, Analysis and Significance*. Chichester: John Wiley.

Smyth, M. R., Lawellin, D. W. and Osteryoung, J.G (1979) Polarographic Study of Aflatoxin B1, B2, G1 and G2: Application of Differential-pulse polarography to the determination of Aflatoxin B1 in Various Foodstuff. *Analyst.* **104**: 73 - 78.

Smyth, M.R. and Vos, J.G., (1992). In Shevla, G. (Ed). *Analytical Voltammetry in Comprehensive Analytical Chemistry*. Amsterdam: Elsevier Science.

Smyth, M.R. and Smyth, W.F. (1978). Voltammetric Methods for the Determination of Foreign Organic Compounds of Biological Significance. A review. *Analyst.* **103(1227)**: 103 - 122.

Sonthalia, P., McGaw, E., Show, Y. and Swain, G.M. (2004). Metal Ion Analysis in Contaminated Water Samples Using Anodic Stripping Voltammetry and a Nanocrystalline Diamond Thin-film Electrode. *Anal Chim Acta.* **522**: 35 - 44.

Sorensen, L.K. and Elbaek, T. (2005). Determination of Mycotoxins Bovine Milk by Liquid Chromatography Tandem Mass Spectrometry. *J Chrom. B.* **820**:183-196.

Stack, M. E. and Pohland, A.E. (1975). Collabration Study of a Method for Chemical Confirmation of the Identify of Aflatoxin. *J. Assoc.Off. Anal.Chem.* **58**: 110 - 113.

Stroka, J. and Anklem, E (2002). New Strategies for the Screening and Determination of Aflatoxins and Detection of Aflatoxin-producing Moulds in Food and Feed. *Trend In Anal. Chem.* **21**: 90 - 95.

Stroka, J., Elke, A., Urban, J., John, G., Anna, B., Carlo, B., Per-Erik, C., Fiona, G., John, G., and Loius, S. (2000). Immunoaffinity Column Cleanup With Liquid Chromatograhpy Using Post-column Bromination for Determination of Aflatoxins in Peanut Butter, Pistachio Paste, Fig Paste and Paprika Powder. Collaborative Study. *J. of AOAC International.* **83**: 320 - 340.

Stubblefield, R.D and Showell, O.L (1977). Reverse Phase Analytical and Preparative High Performance Liquid Chromatography of Aflatoxins. *J. Assoc. Off. Anal. Chem.* **60**: 784 - 790.

Stubblefield, R.D., Kwolek, W.F. and Stoloff, L (1982). Determination and Thin Layer Chromatographic Confirmation of Identity of AFB1 and AFM1 in Artificially Contaminated Beef Livers: Collaborative Study. *J. Assoc. Off. Anal. Chem*. **65**: 1435 - 14.

Sturm, J.C., Canelo, H., Nunez-Vergara, L.J. and Squella, J.A. (1997). Voltammetric Study of Ketorolac and its Differential Pulse Polarographic Determination in Pharmaceuticals. *Talanta*. **44**: 931 - 937.

Sun, N., Mo, W-M. Shen, Z-L and Hu, B-X. (2005). Adsorptive Stripping Voltammetric Technique for the Rapid Determination of Tobramycin on the Hanging Mercury Electrode. *J. Pharm. Biomed. Anal.* **38(2)**: 256 - 262.

Sun, W. and Jiao, K. (2002). Linear Sweep Voltammetric Determination of Protein Based on its Interaction With Alizarin Red S. *Talant.* **56**: 1073 - 1080.

Taguchi, S., Fukushima, S., Summoto, T., Yoshida, S. and Nishimune, T. (1995). Aflatoxins in Foods Collected in Osaka, Japan. *J AOAC Int*. **78**: 325 – 327.

Takahashi, D.M. (1977a). Reverse Phase High Pressure Liquid Chromatographic Analytical System for Aflatoxins in Wine With Fluorescence Detection. *J Chromatograph*. **131**: 147 - 156.

Takahashi, D.M. (1977b). Reverse Phase High Pressure Liquid Chromatographic Analytical System for Aflatoxins in Wine and Fruit Juices. *J. Assoc. Off. Anal. Chem.* **60**: 799 – 804.

Tarin, A, Russell, M.G. and Guardino, X. (2004). Use of High Performance Liquid Chromatography in Assess Airborne Mycotoxins. *J of Chromatogr. A.* **1047(2)**: 235 - 240.

Thompson, M. (1998). Do We Really Need Detection Limit?. *Analyst*. **123**: 405 - 407.

Torriero, A.A.J., Luco, J.M., Sereno, L. and Raba, J. (2004). Voltammetric Determination of Salicylic Acid in Pharmaceuticals Formulations of Acetylsalicylic Acid. *Talanta*. **62**: 247- 254.

Tozzi, C., Anfossi, L. Baggiani, C., Giavanalli, C. and Girandi, G. (2003). A Combinatorial Approach to Obtain Affinity Media With Binding Properties Towards the Aflatoxins. *Anal. Bioanal. Chem.* **375**: 994 - 999.

Trucksess, M. W. and Stollof, L. (1980). Thin Layer Chromatographic Determination of Alatoxins in Dry Ginger Root and Ginger Oleoresin. *J. Assoc. Off. Anal. Chem*.**63**: 1052 - 1054.

Trucksess, M. W., Stack, M. E., Nesheim, S., Page, S.W. , Albert, R.H., Hansen, T.J. and Donahue, K.F. (1991). Immunoaffinity Column Coupled with Solution Fluometry or Liquid Chromatography Postcolumn Derivatization for Determination of Aflatoxins in Corn, Peanuts and Peanut butter: Collaborative Study. *J. Assoc. Off. Anal. Chem.* **74**: 81 - 88.

Trucksess, M. W., Stollof, L., Pons, W.A., Cucullu, A.F. Lee, L.S. and Franz, A.O. (1977). Thin Layer Chromatography of AFB1 in Egg. *J. Assoc. Off. Anal. Chem.* **60**: 795 - 798.

Tuinstra L.G.M. and Hassnott, W. (1983). Rapid Determination of Aflatoxin B1 in Dutch Feedingstuffs by High Performance Liquid Chromatography and Post-column Derivatisation. *J of Chromatogr.* **282**: 457 - 462.

Tuinstra, L.G.M., Kienhuis, P.G.M. and Dols, P. (1990). Automated Liquid Chromatographic Determination of Aflatoxin M1 in Milk Using on-line Dialysis for Sample Preparation. *J. Assoc. Off. Anal. Chem.* **73**: 969 - 973.

Turner, P.C., Sylla, A., Gong, Y.Y., Diallo, M.S., Surcliffer, A.E., Hall, A. J. and Wild, C.P. (2005). Reduction in Exposure to Carcinogenic Aflatoxins by Postharvest Intervention Measures in West Africa: Community Based Intervention Study. *Lancet*. **365**: 1950 - 1956.

Tutour, L.B., Elaraki, A. T. and Aboussalim, A.(1984). Simultaneous Thin Layer Chromatographic Determination of Aflatoxin B1 and Ochratoxin A in Black Olives, *J Assoc Off Anal Chem.* **67**: 611 - 620.

Urano, T., Trucksess ,M.W. and Page, S.W., (1993). Automated Affinity Liquid Chromatography Sstem for On-line Isolation, Separation and Quantitation of Aflatoxins in Methanol-water Extracts of Corn or Peanuts. *J.Agric. Food Chem.* **41**: 1982 - 1985.

Vahl, M. and Jorgensen, F. (1998). Determination of Aflatoxins in Food Using LC/MS/MS. *Z Lebensm Unters Forsch A.*. **206**: 243 - 245.

Van Egmond, H.P. (1989). *Introduction of Mycotoxins in Dairy Products.* London: Elsevier Applied Science.

Verma, K.K., Jain, A., Verma, a. and Chaurasia, A. (1991). Spectrophotometric Determination of Ascorbic Acid in Pharmaceuticals by Background Correction and Flow Injection. *Analyst*. **116**: 641 - 645.

Versantroort, C.H.M., Oomen, A.G., and Sips, A.J.A.M. (2005). Applicability of an in Vitro Digestion Model in Assessing the Bioaccessibility of Mycotoxins From Food. *Food and Chemical Toxicology*. **43**: 31- 40.

Volke, J. and Liska, F. (1994). *Electrochemistry in Organic Synthesis.* New York: Springer-Verlag.

Volkov, A.G. and Mwesigwa, S. (2001). Electrochemistry of Soyabean: Effect of Uncouplers, Pollutant and Pesticides. *J. Of Electroanalytical Chemistry*. **496**: 153 - 157.

Wang, H. S., Huang, D. H. and Liu, R.M. (2004). Study on the Electrochemical Behavior of Epinephrine at a Poly(3-methylthiophene) Modified Glassy Carbon Electrode. *J. of Electroanalytical Chem.* **570**: 83 - 90.

Wang, J. (1985): *Stripping analysis: Principles, Instrumentation and Application.* USA: VCH Publishers.

Wang, J., Cai, X., Fernandes, J. R., Ozsoz, M. and Grant, D. H., (1997). Adsorptive Potentiometric Stripping Analysis of Trace Tomoxifen at a Glassy Carbon Electrode, *Talanta*. **45**: 273 - 278.

Wang, J. (1988). *Electroanalytical Technique in Clinical Chemistry and Laboratory Medicine.* USA: VCH publisher.

Wang, J. (2000). *Analytical Electrochemistry.* USA: WILEY-VCH.

Weast, R.C and Astle, M.J. (1981). *Handbook of Chemistry and Physics*. 61th Ed. Flourida: CRC Press.

Wasiak, W, Cissewska, W. and Ciszewski, A. (1996). Hair Analysis Part I: Differentrial Pulse Anodic Stripping Voltammetry Determination of Lead, Cadmium, Zinc and Copper in Human Hair Samples of Persons in Permanent Contact With a Polluted Workplace Environment. *Anal. Chim. Acta.* **335**: 201 - 207.

Weast, R.C and Astle, M.J. (1987). *CRC Handbook of Data On Organic Compounds,* Vol II. Flourida: CRC Press.

Wei, R.D., Chang, S.Ch. and Lee, S.S. (1980). High Pressure Liquid Chromatography Determination of Aflatoxins in Soy Sauces anf Fermented Soybean Paste. *J. Assoc. Off. Anal. Chem*. **63**:1269- 1274.

White, C.E. and Argauer, R.J. (1970). Fluorescence Analysis- A Practical Approach. New York: Marcell Dekker.

Wilson, T.J. and Romer, T.R (1991). Use of the Mycosep Multifunctional Clean-up Column for Liquid Chromatography Determination of Aflatoxins in Agriculture Products. *J. Assoc. Off. Anal. Chem.***74** : 951 - 956.

Wogan, G.N., Hecht, S. S., Felton, J. S., Conney, A.H. and Loeb, L.A. (2004). Environmental and Chemical Carcinogenesis. *Seminars In Cancer Biology*. **14**: 473 - 486.

Woolever, C.A. and Dewald,H.D. (2001). Differential Pulse Anodic Stripping Voltammetry of Barium and Lead in Gunshot Residues. *Forensic Sci Int.* **117**: 185-190.

World Health Organisation (1987) in: IARC Monograph on the Evaluation of Carcinogenic Risk to Human, WHO, Lyon, pp 82 Supl. I.

Wring S. A., Hart J.P. and Birck B.J. (1991). Voltammetric Behaviour of Screen-printed Carbon Electrode, Chemically Modified With Selected Mediatiors and Their Applications as Sensor for Detection of Reduced Gluthathione. *Analyst*. **116** : 123-129.

www.ansci.cornell.edu/toxicagents/aflatoxin. Quoted date - 30th. July 2002.

www.ccohs.ca/oshanswers/chemicals. Quoted date – 5th February 2006

www.copdockmill.co.uk/aflatoxin Quoted date – 26th August 2001

Ximenes, M.I.N., Rath, S. and Reyes, F.G.R. (2000). Polarographic Determination of Nitrate in Vegetable. *Talanta*. **51**: 49 - 56.

Xiulan S., Xiaolian, Z., Jian, T., Xiaohong, G., jun, Z. and Chu, F.S. (2006). Development of an Immunochromatographic Assay for Detection of Aflatoxin B1 in Foods. *Food Control*. **17**: 256 - 262.

Yardimer, C. and Ozaltin, N. (2001). Electrochemical Studies and Differential Pulse Polarographic Analysis of Lansoprazole in Pharmaceuticals. *Analyst*. **126**: 361-366.

Yardimer, C. and Ozaltin, N. (2004). Electrochemical Studies and Square-wave Voltammetric Determination of Fenofibrate in Pharmacetical Formulations. *Anal. Bioanal. Chem.* **378**: 495 - 498.

Yaacob, M.H. (1993). *Polarographic Determination of Sn(II) in Radiopharmaceutical Samples*. University of Manchester, Institute of Science and Technology (UMIST), United Kingdom. MSc Thesis.

Yilmaz, S., Uslu, B. and Ozkan, S.A. (2001). Anodic Oxidation of Etodolac and its Square Wave and Differential Pulse Voltammetric Determination in Pharmaceuticals and Human Serum. *Talanta*. **54**: 351 - 360.

Youmens, H.L. (1973). *Statistics for Chemistry*. USA: Bells and Howell Publishers.

Yousef, A.E and Marth, E.H. (1985). Rapid Reverse Phase Liquid Chromatography Determination of AFM1 in Milk. *J. Assoc. Off. Anal. Chem.* **68**: 462 - 465.

Zahir, M.H. and Abd. Ghani, S. (1997). Fabrication of Directly Polymerized 4-vinylpyridine onto a Pencil 2B Graphite Paste Electrode for Glucose Monitoring, *Anal Chim Acta*, **354**: 351 - 358.

Zayed, S.I.M. and Habib, I.H.I. (2005). Adsorptive Stripping Voltammetric Determination of Triprolidine Hydrochloride in Pharmaceutical Tablets. Il Farmaco. **60(6-7)**: 621 - 625.

Zhang, Z.Q., Zhang, H. and H,e G. F. (2002). Preconcentration with Membrane Cell and Adsorptive Polarographic Determination of Formaldehyde in Air. *Talanta.* **57**: 317 - 322.

Zhang, Z.Q, Li, Y.F., He, X.M. and Zhang, H. (1996). Electroanalytical Characteristics of Enoxican and Their Analytical Application. *Talanta*.**43**: 635 - 641.

Zhao, C., Pan, Y., Su, Y., Zhang, Z., Guo, Z. and Sun, L. (2003). Determination of EDTA Species in Water by Square-wave Voltammetry using a Chitosan-coated Glassy Carbon Electrode. *Water Research*. **37**: 4270 - 4274.

Zima, J., Barek, J., Moriera, J.C., Mejstrik, V. and Fogg, A.G. (2001). Electrochemical Determination of Trace Amounts of Environmentally Important Dyes. *Fresenius. J. Anal. Chem.* **369**: 567 - 570.

Zimmermann, S., Menzel A., Bernaz Z., Eckhardt J-D., Stubens D., Alt F., Messerschmidt H., Taraschewski H. and Sures B.(2001). Trace Analysis of Platinum in Biological Samples: A Comparison Between Sector field ICP-MS and Adsorptive Cathodic Stripping Voltammetry Following Different Digestion Procedures. *Anal. Chim. Acta*. **439**: 203 - 209.

APPENDIX A

Relative fluorescence of aflatoxins in different solvents

Aflatoxin	Methanol	Ethanol	Chloroform
B1	0.6 (430 nm)	1.0 (430 nm)	0.20 (413 nm)
B2	5.3 (430 nm)	2.7 (430 nm)	0.25 (413 nm)
G1	1.0 (450 nm)	1.4 (450 nm)	6.2 (430 nm)
G2	8.7 (450 nm)	4.7 (450 nm)	6.8 (430 nm)

UV spectra of the principal aflatoxins (In methanol)

Aflatoxin	λ_{max} (nm)				ε_{max} (l mol^{-1} cm^{-1})			
AFB1	223	-	266	363	20,800	-	12,960	20,150
AFB2	223	-	266	363	18,120	-	12,320	23,100
AFB2a	228	-	256	363	17,600	-	10,300	20,400
AFM1	225	-	265	360	21,000	-	11,000	21,000
AFM2	222	-	264	358	19,800	-	10,000	21,400
AFG1	226	243	264	363	15,730	11,070	10,670	17,760
AFG2	220	245	265	363	21,090	12,400	10,020	20,030
AFG2a	223	242	262	365	18,600	10,100	8,700	18,000

Relative intensities of principle bands in the IR spectra of the aflatoxins

(Strong (S), medum (M), weak (W), ** Sharply defined band)

Bands:	AFB1	AFB2	AFB2a	AFM1	AFM2	AFG1	AFG2	AFG2a
3620	-	-	M**	-	-	-	-	M**
3450	-	-	-	M	M	-	-	-
3040	W	-	-	W	-	W	-	-
2980-2860 (4 bands)	W	W	W	W	W	W	W	W
1770	S	S	S	S	S	S	S	S
1670	W	W	W	W	W	W	W	W
1600	S	S	S	M	M	S	S	S
1570	S	S	S	M	M	S	S	S
1390	S	M	S	M	M	M	S	S
1310	S	S	S	S	M	S	S	S
1250	M	M	W	W	M	M	W	W
1070	M	-	-	W	-	M	-	-
990	M	M	M	W	W	M	M	M
950	W	M	M	W	W	W	M	M
830	M	M	M	W	W	W	W	W
780	W	W	W	W	W	W	W	W
720-710	W	W	W	W	W	W	-	W

APPENDIX D

Calculation of concentration of aflatoxin stock solution

Formula used for calculation of concentration of aflatoxin stock solution by UV-VIS spectrometric technique

$$[\text{Aflatoxin}] / \mu g/ml = \frac{Abs \times MW \times 1000}{\varepsilon}$$

Where

Abs = absorbance value

MW = molecular weight

ε = molar absorbtivity ($cm^{-1} M^{-1}$)

Parameters used in this calculation;

Aflatoxin	MW	Solvent	ε	Λ (nm)
AFB1	312	Benzene:acetonitrile (98:2)	19,800	353
AFB2	314		20,900	355
AFG1	328		17,100	355
AFG2	330		18,200	357

Example: AFB1

Abs = 0.640 MW = 312 ε = 19,800

$$[AFB1] = \frac{0.640 \times 312 \times 1000}{19,800} = 10.08\ \mu g / ml = 10.08\ ppm$$

APPENDIX E

Extraction procedure for aflatoxins in real samples

Extraction procedure for aflatoxins in real samples: Chemistry Dept. Penang Branch, Ministry of Science, Technology and Innovation;

1. Groundnut with shell: remove shell of entire sample. Coarse grind. Remove 50 g and regrind this portion to finer size for drawing analytical sample.

2. Transfer 25 gm analytical sample into a waring blender and add 125 ml of MeOH-0.1N HCl (100:25) and blend for 2 min (timing). Stand for 5 min (timing).

3. Filter through whatman No. 1 paper or equivalent. Complete filtration as soon as possible

4. Collect 50 ml of filtrate in a stoppered container. (*Equivalent weight: 125 ml / 25 g sample)*

5. Pipette 20 ml of filtrate into a stoppered or screw cap bottle (*Equivalent weight: 20 ml / 4 g sample)*

6. Add exactly 20 ml of 15% $ZnSO_4$ solution. Stopper or cap tightly and shake vigorously for 30 sec. Filter through diatomaceous earth into another container. (*Equivalent weight: 40 ml / 4 g sample)*

7. Pipette 20 ml of this filtrate into a small separating funnel and add exactly 5 ml of chloroform. Stopper or cap tightly and shake vigorously for 30 sec. (*Equivalent weight: 20 ml / 2 g sample).Equivalent weight: 5 ml chloroform / 2 g sample)*

8. Stand for a few minute to let layers separate. After separated, draw chloroform layer into a suitable container and pipette 1 ml extract accurately into amber bottle for preparation of final sample before injecting into voltammetric cell.

APPENDIX F

Calculation of individual aflatoxin in groundnut sample

Aflatoxin, ng/g (ppb) =

$$\frac{P}{P'} \times C \times \frac{1000\ \mu l}{100\ \mu l} \times \frac{125\ ml}{V} \times \frac{1}{W}$$

where;

P = peak height of sample (nA)

P' = peak height of standard (after substract with peak height of sample) (nA)

C = amount of aflatoxin injected into voltammetric cell (ng)

V = effective volume;

$$= \frac{20}{2} \times \frac{\text{volume of chloroform used for sample preparation}}{\text{total volume of chloroform added}} = \frac{20}{2} \times \frac{1}{5} = 2\ ml$$

W = weight of sample (25 g)

$$\Longrightarrow \frac{P}{P'} \times C \times \frac{1000\ \mu l}{100\ \mu l} \times \frac{125\ ml}{2\ ml} \times \frac{1}{25\ g}$$

$$\Longrightarrow \frac{P}{P'} \times C \times 25 \text{ (for injected volume = 100 } \mu l)$$

For other injection volume;

Volume (µl)	Formula
200	$\frac{P}{P'} \times C \times 12.5$
300	$\frac{P}{P'} \times C \times 8.33$
400	$\frac{P}{P'} \times C \times 6.25$

Notes;

From first equation;

1000 µl	=	Volume of final solution prepared in BRB at pH 9.0
100 µl	=	Injection volume of sample
125 ml	=	Volume of solvent for mixing and blending groundnut

APPENDIX G

Cyclic voltammograms of AFB1, AFG1 and AFG2 with increasing of their concentrations

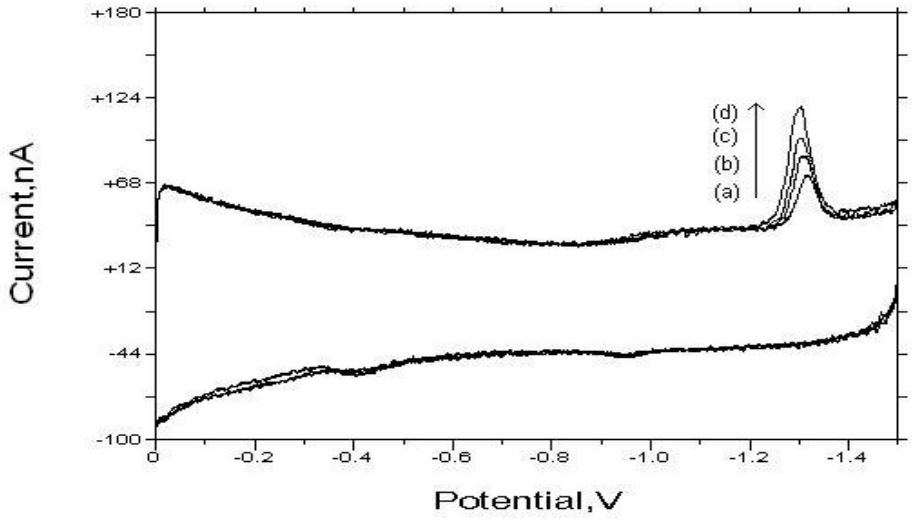


Figure G-1 Effect of increasing AFB1 concentrations on the I_p of cathodic cyclic voltammetric curves in BRB at pH 9.0. (a) 1.30 µM, (b) 2.0 µM (c) 2.70 µM and (d) 3.40 µM. Parameters conditions are E_i = 0, E_{low} = -1.5 V, E_{high} = 0, scan rate = 200 mV/s.

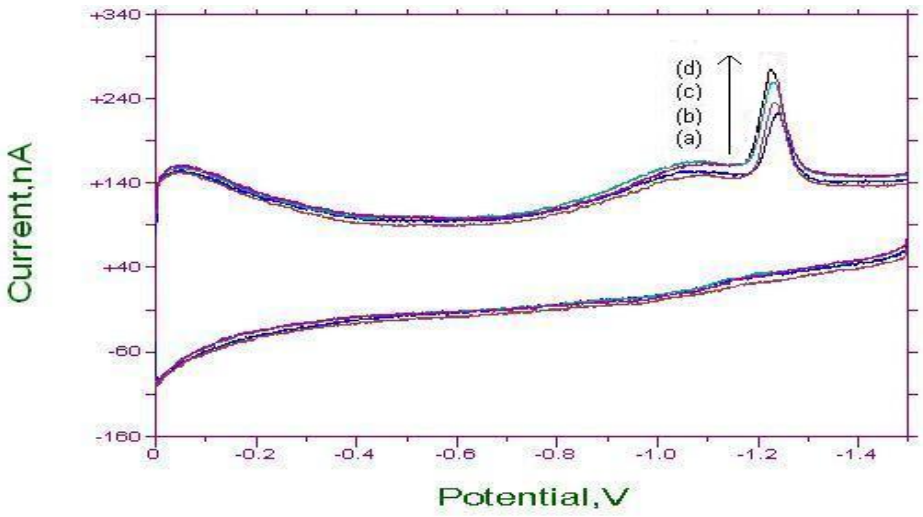


Figure G-2 Effect of increasing AFG1 concentrations on the I_p of cathodic cyclic voltammetric curves in BRB at pH 9.0. (a) 1.30 µM, (b) 2.0 µM (c) 2.70 µM and (d) 3.40 µM. Parameters conditions are E_i = 0, E_{low} = -1.5 V, E_{high} = 0, scan rate = 200 mV/s.

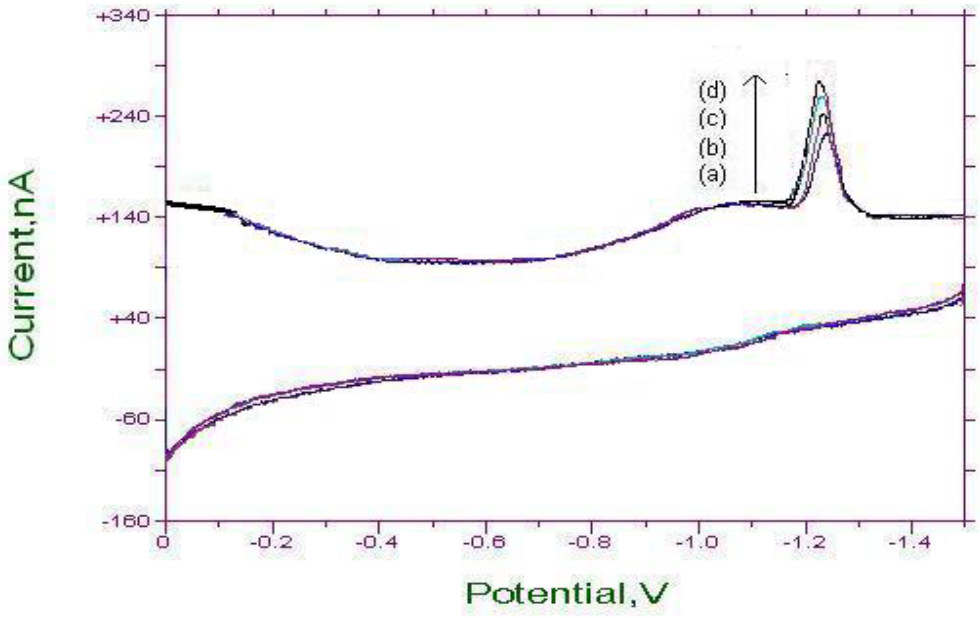


Figure G-3 Effect of increasing AFG2 concentrations on the I_p of cathodic cyclic voltammetric curves in BRB at pH 9.0. (a) 1.30 µM, (b) 2.0 µM (c) 2.70 µM and (d) 3.40 µM. Parameters conditions are E_i = 0, E_{low} = -1.5 V, E_{high} = 0, scan rate = 200 mV/s.

APPENDIX H

Dependence of the peak heights of AFB1, AFG1 and AFG2 on their concentrations

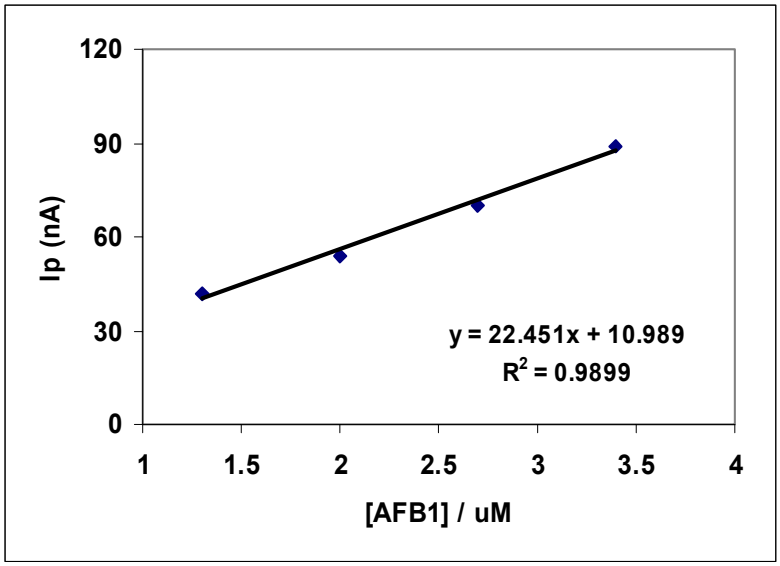


Figure H-1 Dependence of the I_p of AFB1 on concentration of AFB1 in BRB solution at pH 9.0.

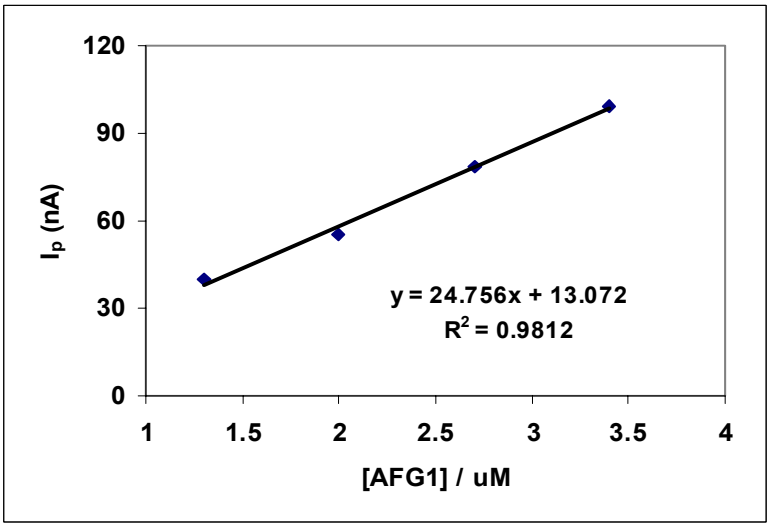


Figure H-2 Dependence of the I_p of AFG1 on concentration of AFG1 in BRB solution at pH 9.0.

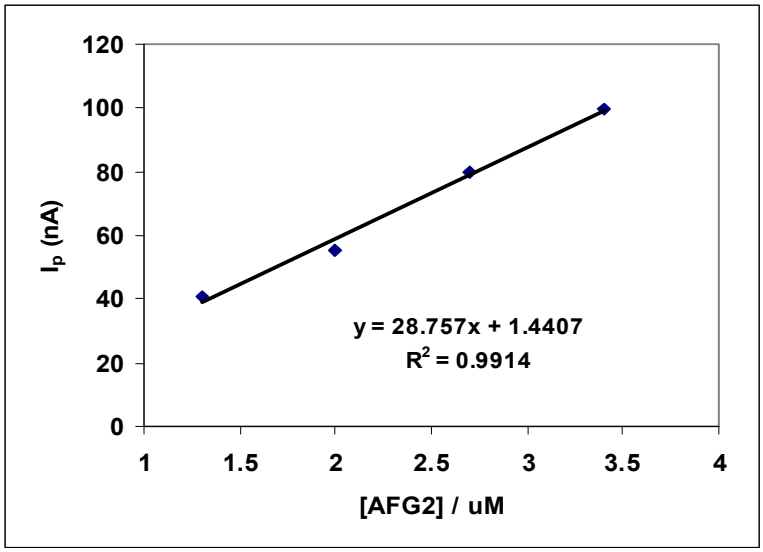


Figure H-3 Dependence of the I_p of AFG2 on concentration of AFG2 in BRB solution at pH 9.0.

APPENDIX I

Repetitive cyclic voltammograms and their peak height of AFB2, AFG1 and AFG2 in BRB at pH 9.0

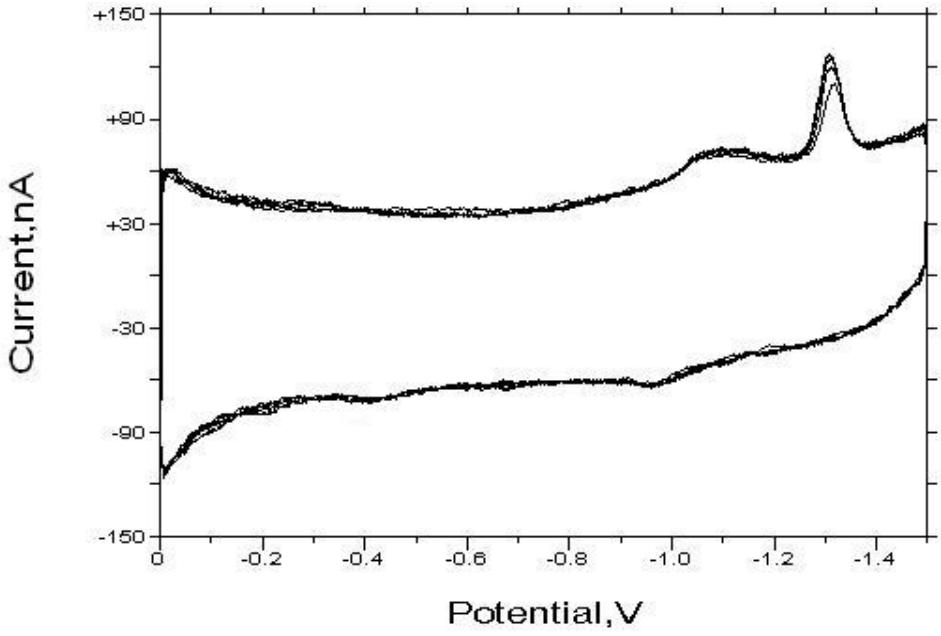


Figure I-1 Repetitive cathodic cyclic voltammograms of 1.3 μM AFB1 in BRB solution at pH 9.0. Parameter conditions are $E_i = 0$, $E_{low} = -1.5$ V, $E_{high} = 0$ and scan rate = 200 mV/s

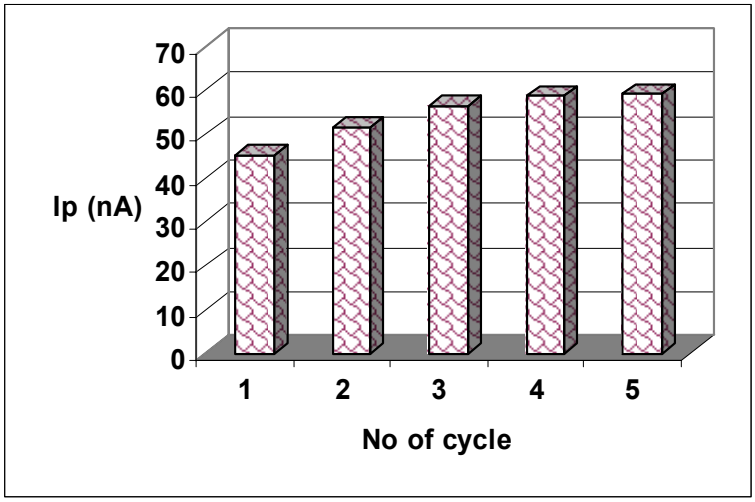


Figure I-2 Increasing I_p of AFB1 cathodic peak obtained from repetitive cyclic voltammetry

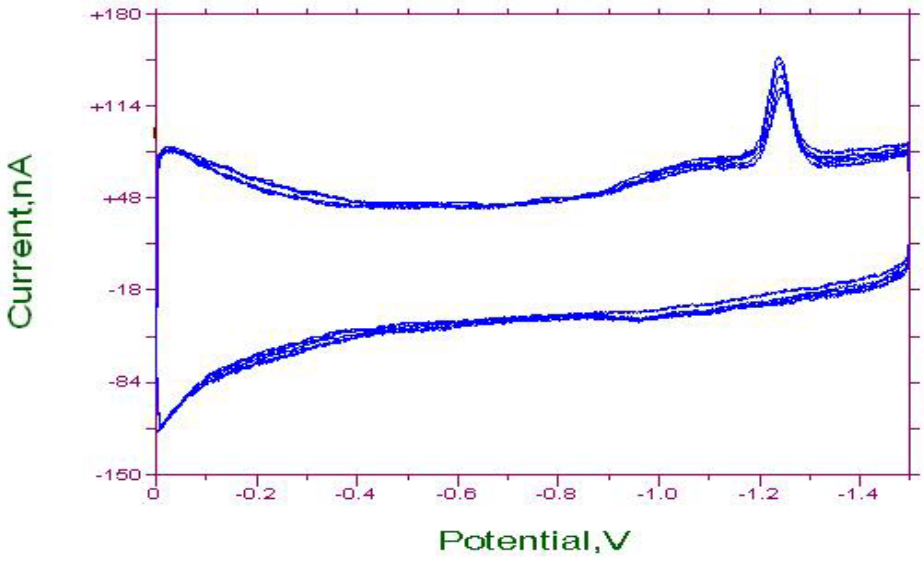


Figure I-3 Repetitive cathodic cyclic voltammograms of 1.3 µM AFG1 in BRB solution at pH 9.0. Parameter conditions are $E_i = 0$, $E_{low} = -1.5$ V, $E_{high} = 0$ and scan rate = 200 mV/s

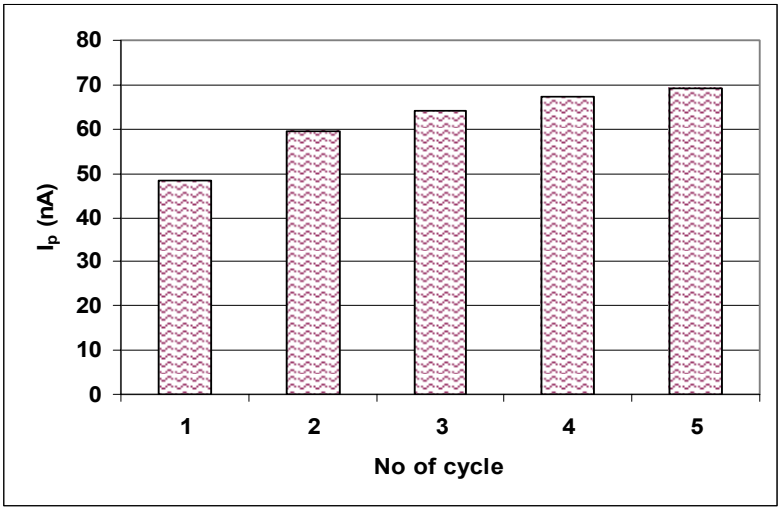


Figure I-4 Increasing I_p of AFG1 cathodic peak obtained from repetitive cyclic voltammetry

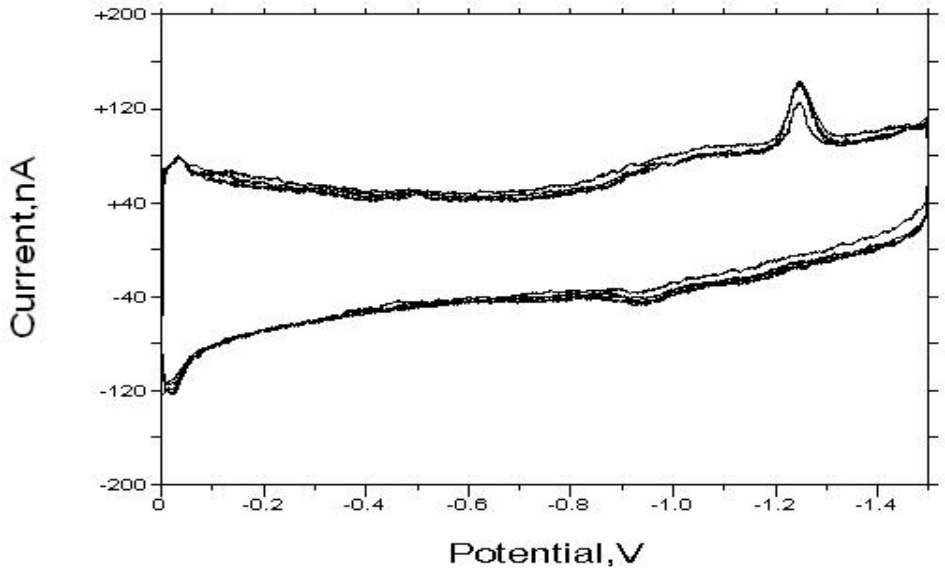


Figure I-5 Repetitive cathodic cyclic voltammograms of 1.3 μM AFG2 in BRB solution at pH 9.0. Parameter conditions are $E_i = 0$, $E_{low} = -1.5$ V, $E_{high} = 0$ and scan rate = 200 mV/s

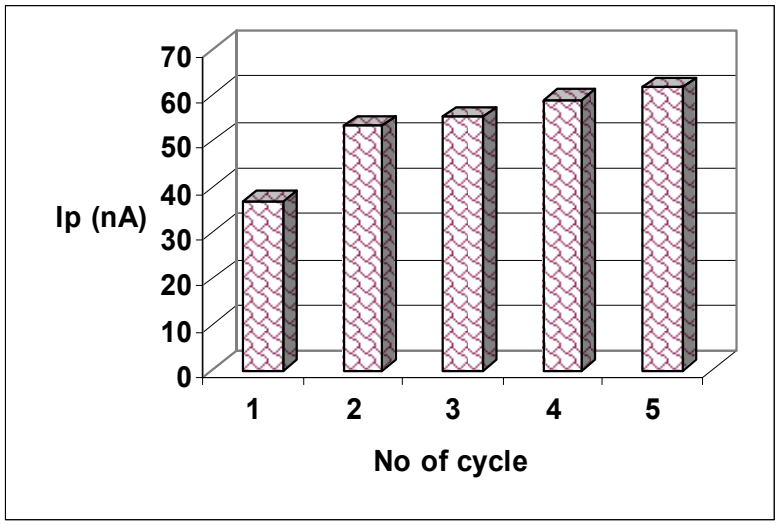


Figure I-6 Increasing I_p of AFG2 cathodic peak obtained from repetitive cyclic voltammetry

APPENDIX J

Voltammetric plot E_p-log υ for the reduction of AFB1, AFG1 and AFG2 in BRB at pH 9.0

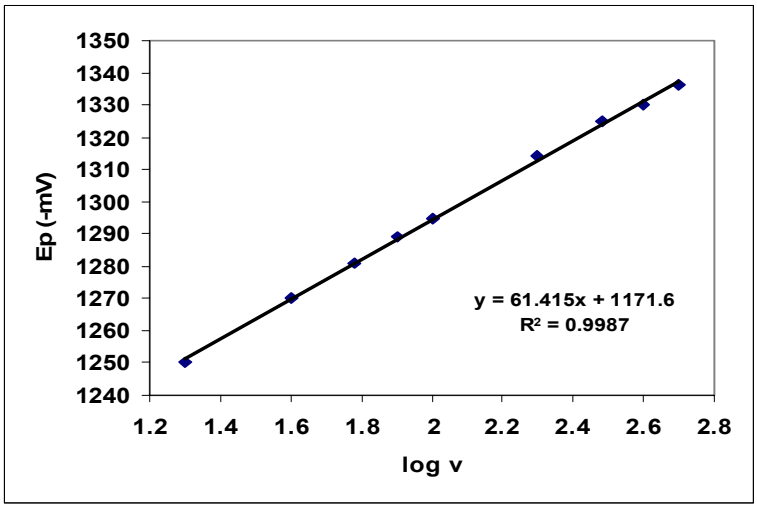


Figure J-1 The voltammetric plot E_p – log υ for the reduction of 1.3 µM AFB1 in BRB solution at pH 9.0

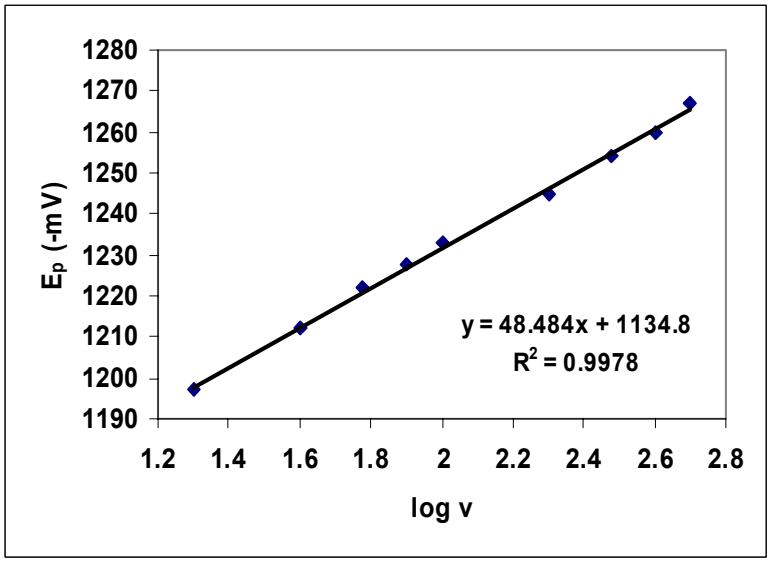


Figure J-2 The voltammetric plot E_p – log υ for the reduction of 1.3 µM AFG1 in BRB solution at pH 9.0

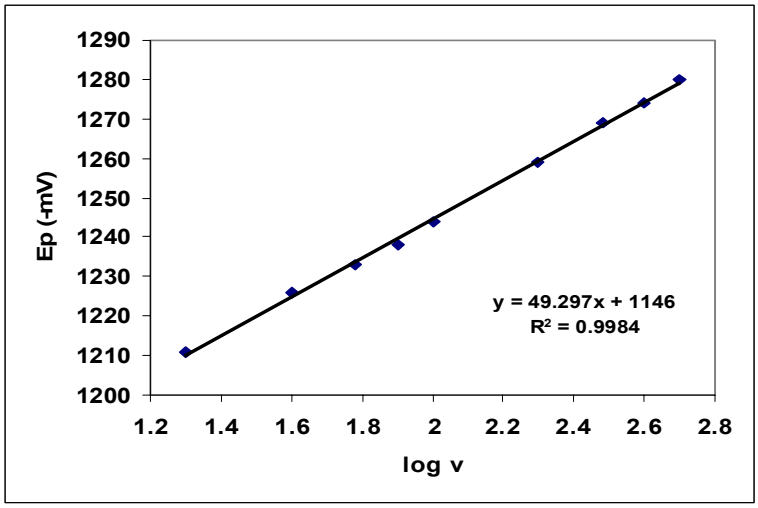


Figure J-3 The voltammetric plot E_p – log υ for the reduction of 1.3 µM AFG2 in BRB solution at pH 9.0

APPENDIX K

Plot of peak height versus scan rate for AFB1, AFG1 and AFG2 in BRB at pH 9.0

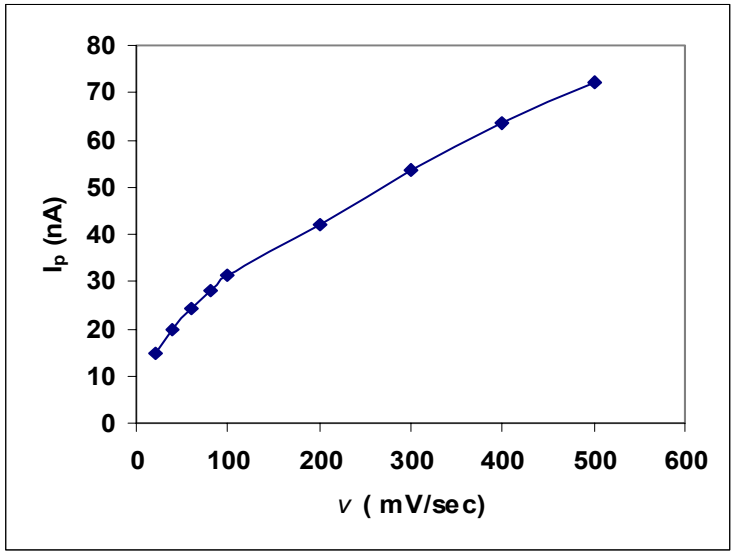


Figure K-1 Plot of I_p versus scan rate (υ) for 1.3 µM AFB1 in BRB at pH 9.0

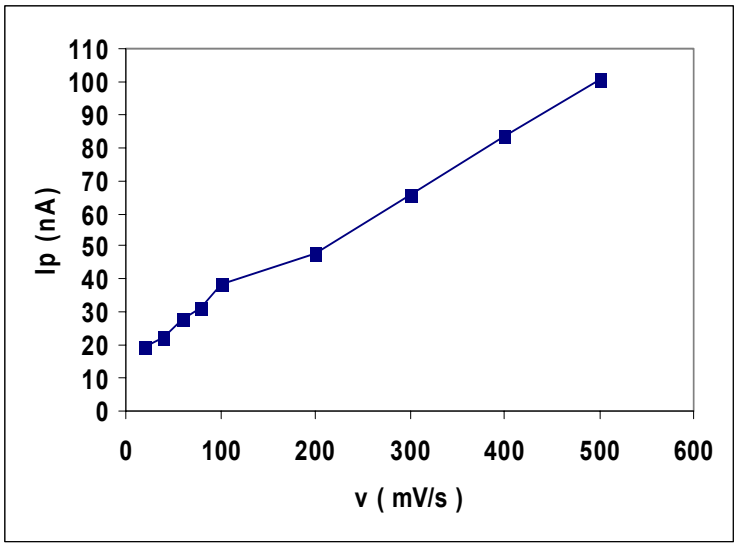


Figure K-2 Plot of I_p versus scan rate (υ) for 1.3 µM AFG1 in BRB at pH 9.0

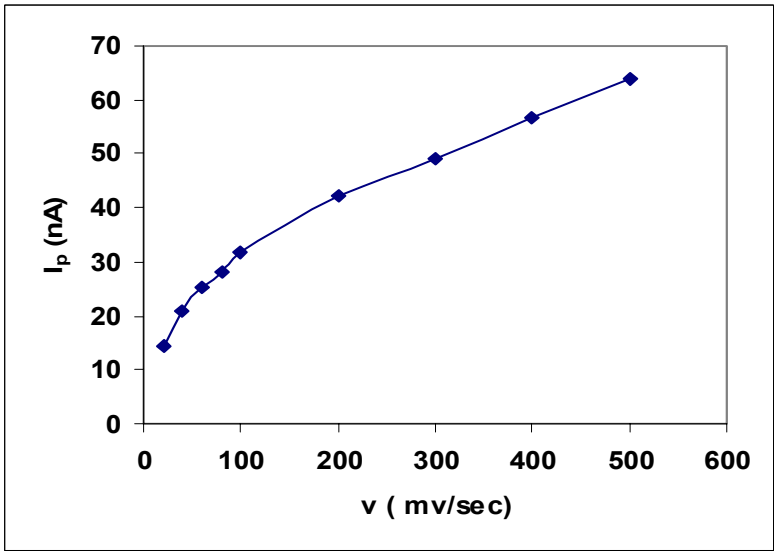


Figure K-3 Plot of I_p versus scan rate (υ) for 1.3 µM AFG2 in BRB at pH 9.0

APPENDIX L

Voltammograms of AFB2 with increasing concentrations

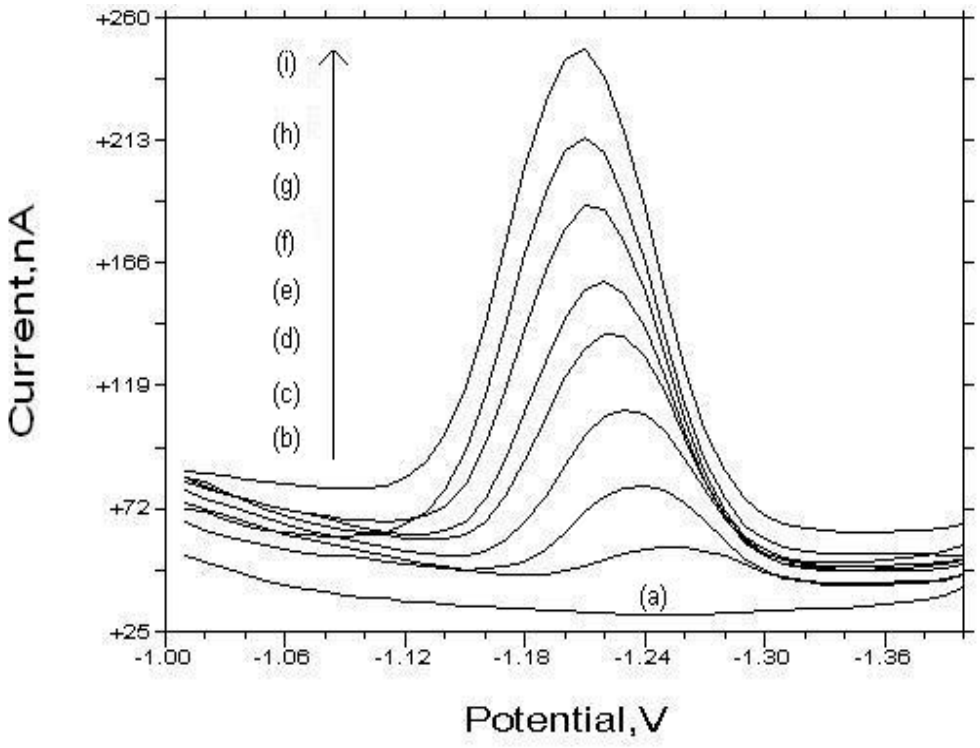


Figure L-1 Cathodic stripping voltammograms of increasing concentration of AFB2 in (a) BRB at pH 9.0, (b) 0.02 µM (c) 0.06 µM (d) 0.10 µM (e) 0.14 µM, (f) 0.18 µM (g) .22 µM (h) 0.26 µM and (i) 0.32 µM. Parameter conditions; E_i = -1.0 V, E_f = -1.4 V, E_{acc} = -0.60 V, t_{acc} = 80 s, υ = 50 mV/s and pulse amplitude = 80 mV.

APPENDIX M

Voltammograms of 0.1 μM and 0.2 μM AFB2 obtained on the same day measurements

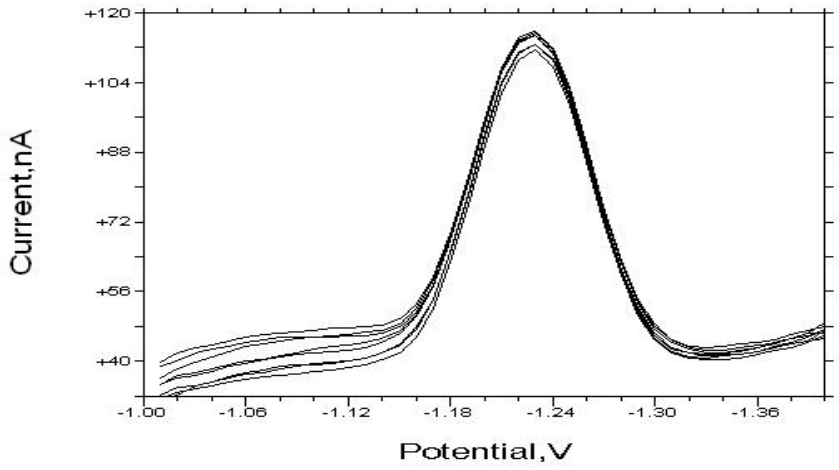


Figure M-1 Cathodic stripping voltammograms of 0.1μM AFB2 obtained in the same day (n= 8) with RSD = 2.83% E_{acc} = -6.0 V, t_{acc}= 80 s, υ = 50 mV/s and pulse amplitude = 80 mV

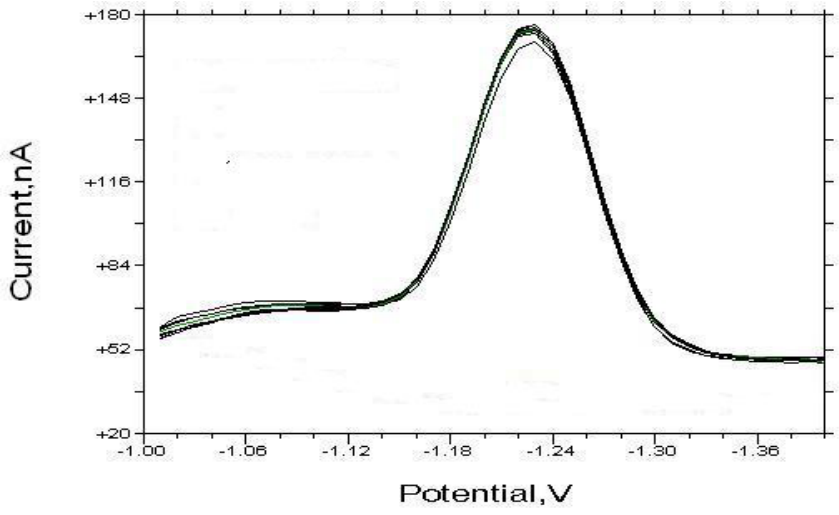


Figure M-2 Cathodic stripping voltammograms of 0.2 μM AFB2 obtained in the same day (n= 8) with RSD = 0.72%. E_{acc} = -6.0 V, t_{acc}= 80 s, υ = 50 mV/s and pulse amplitude = 80 mV

APPENDIX N

Voltammogramms of AFB2 at inter-day measurements

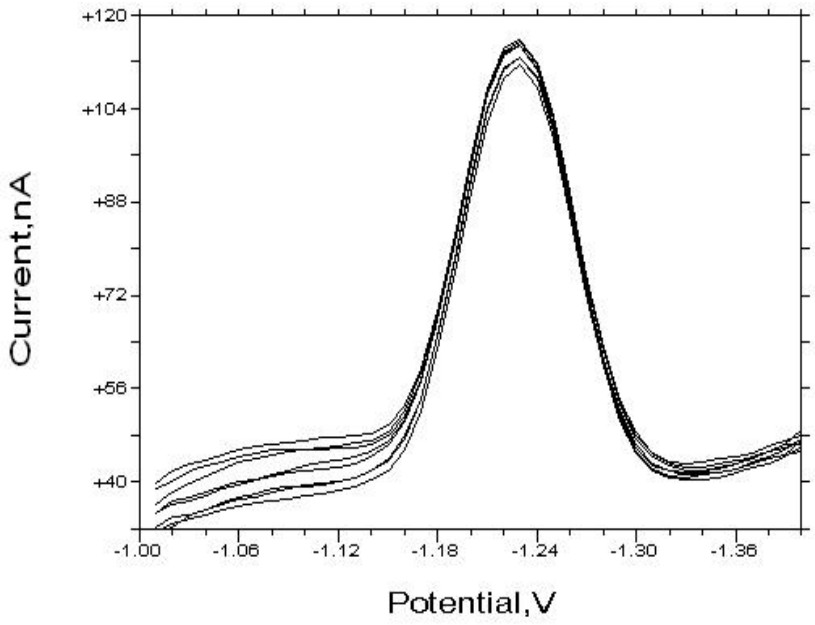


Figure N-1 Cathodic stripping voltammograms of 0.1µM AFB2 obtained at day 1 (n= 8) with RSD = 2.83% .E_{acc} = -6.0 V, t_{acc}= 80 s, υ = 50 mV/s and pulse amplitude = 80 mV

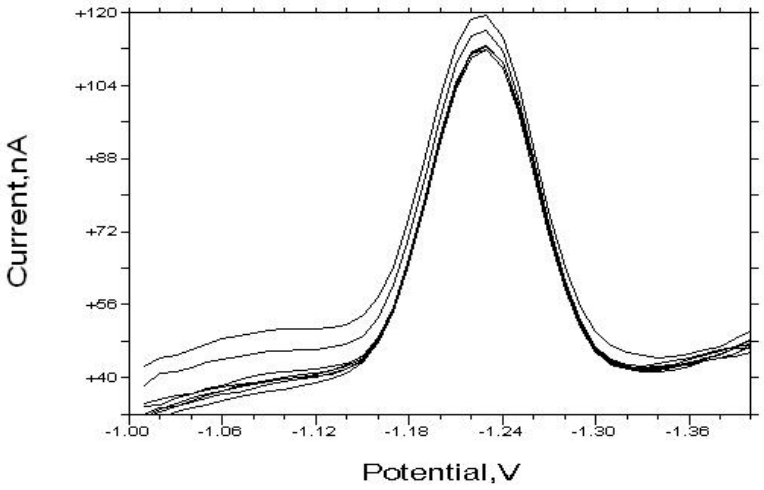


Figure N-2 Cathodic stripping voltammograms of 0.1µM AFB2 obtained at day 2 (n= 8) with RSD = 2.39% E_{acc} = -6.0 V, t_{acc}= 80 s, υ = 50 mV/s and pulse amplitude = 80 mV

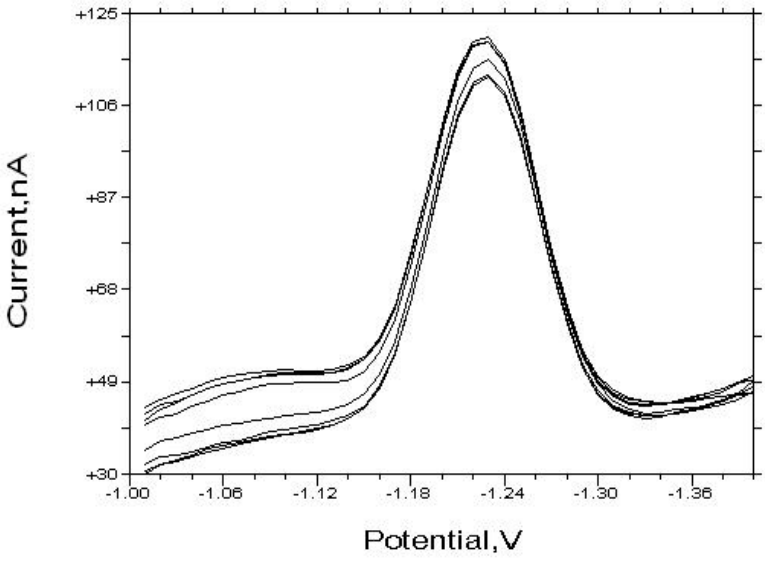


Figure N-3 Cathodic stripping voltammograms of 0.1µM AFB2 obtained at day 3 (n= 8) with RSD = 1.31% E_{acc} = -6.0 V, t_{acc}= 80 s, υ = 50 mV/s and pulse amplitude = 80 mV

APPENDIX O

F test for robustness and ruggedness tests

a) Two tailed F test was used to observe any significant different of variance by using small variation of a few important parameters such pH of buffer solution, E_{acc} and $t_{acc.}$.

Optimum parameters	Variation
pH of BRB = 9.0	8.5 and 9.5
E_{acc} = -0.60 V	-0.59 V and -0.61 V
t_{acc} = 80 s	75 and 85 s

0.1 µM AFB2 (n =5):

For optimum and small variation in pH of BRB (as an example)

pH	n	I_p	SD
9.0	5	60.62	0.88
8.5	5	58.82	0.79

$F = (0.88)^2 / (0.79)^2 = 0.7744/ 0.6241 = 1.24$ (< F tabulated at 95 % confidential level; 9.60).

No significant different for pH 9.0 and 8.5 at 95% confidential level.

pH	n	I_p	SD
9.0	5	60.62	0.88
9.5	5	59.30	0.44

$F = (0.88)^2 / (0.44)^2 = 0.7744 / 0.1936 = 4.00$ (< F tabulated at 95 % confidential level; 9.60).

No significant different for pH 9.0 and 9.5 at 95% confidential level.

b) Two tailed F test was used to observe any significant different of variance by using different voltammetric analyser which are BAS and Metrohm under optimum parameters (AFB1 as an example).

AFB1

Voltammetric analyser	n	I_p	SD
Metrohm	5	59.88	0.94
BAS	5	58.28	2.79

F(4,4) at 95% confidence level = 9.60
$F = (2.79)^2 / (0.94)^2 = 7.78 / 0.88 = 8.84$ (< F tabulated; 9.60)

No significant difference between the results obtained for 0.1 µM AFB1 by two types of voltammetric analyser.

APPENDIX P

Voltammograms of AFB1 with increasing concentration

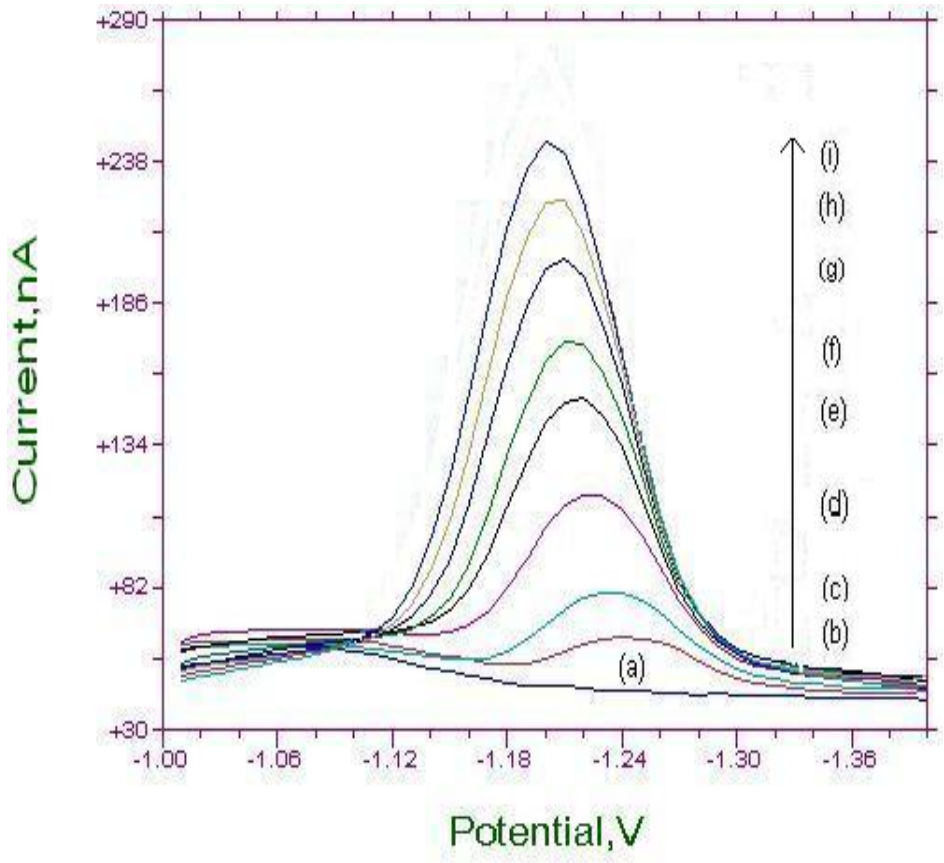


Figure P-1 Cathodic stripping voltammograms of increasing concentration of AFB1 in (a) BRB at pH 9.0, (b) 0.02 µM (c) 0.04 µM (d) 0.14 µM (e) 0.18 µM, (f) 0.22 µM (g) 0.26 µM (h) 0.30 µM and (i) 0.32 µM. Parameter conditions; E_i = -1.0 V, E_f = -1.4 V, E_{acc} = -0.60 V, t_{acc} = 80 s, υ = 50 mV/s and pulse amplitude = 80 mV.

APPENDIX Q

Voltammograms of AFG1 with increasing concentrations

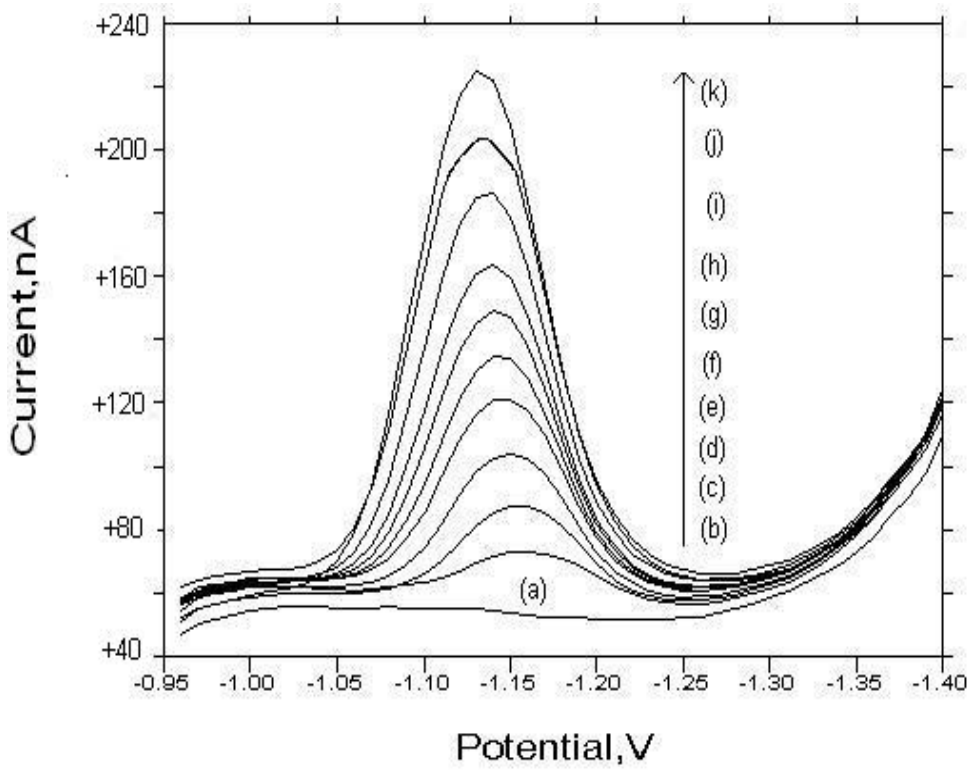


Figure Q-1 Cathodic stripping voltammograms of increasing concentration of AFG1 in (a) BRB at pH 9.0, (b) 0.02 µM, (c) 0.04 µM, (d) 0.06 µM, (e) 0.08 µM, (f) 0.10 µM, (g) 0.14 µM, (h) 0.18 µM, (i) 0.26 µM (j) 0.30 µM and (k) 0.32 µM. Parameter conditions; E_i = -0.95 V, E_f = -1.4 V, E_{acc} = -0.60 V, t_{acc} = 80 s, υ = 50 mV/s and pulse amplitude = 80 mV.

APPENDIX R

Voltammograms of AFG2 with increasing concentrations

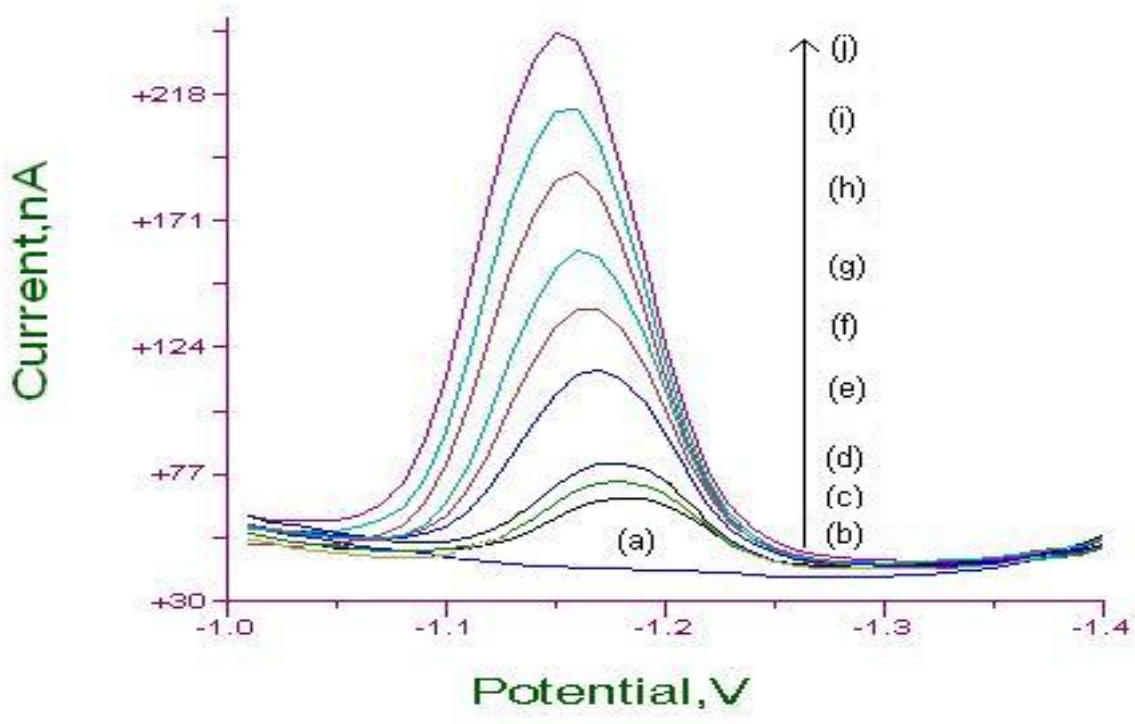


Figure R-1 Cathodic stripping voltammograms of increasing concentration of AFG2 in (a) BRB at pH 9.0, (b) 0.02 µM, (c) 0.04 µM, (d) 0.06 µM, (e) 0.10 µM, (f) 0.14 µM, (g) 0.18 µM, (h) 0.26 µM, (i) 0.28 µM and (j) 0.30 µM. Parameter conditions; E_i = -1.0 V, E_f = -1.4 V, E_{acc} = -0.60 V, t_{acc} = 80 s, υ = 50 mV/s and pulse amplitude = 80 mV.

APPENDIX S

LOD determination according to Barek *et al.* (2001a)

By standard addition of lower concentration of analyte (AFB1, AFB2, AFG1 and AFG2) until obtaining the sample response that is significantly difference from blank sample

a) AFB1; 0.5 x 10^{-8} M or 1.56 ppb gave I_p = 4.3 nA at E_p = -1.250 V

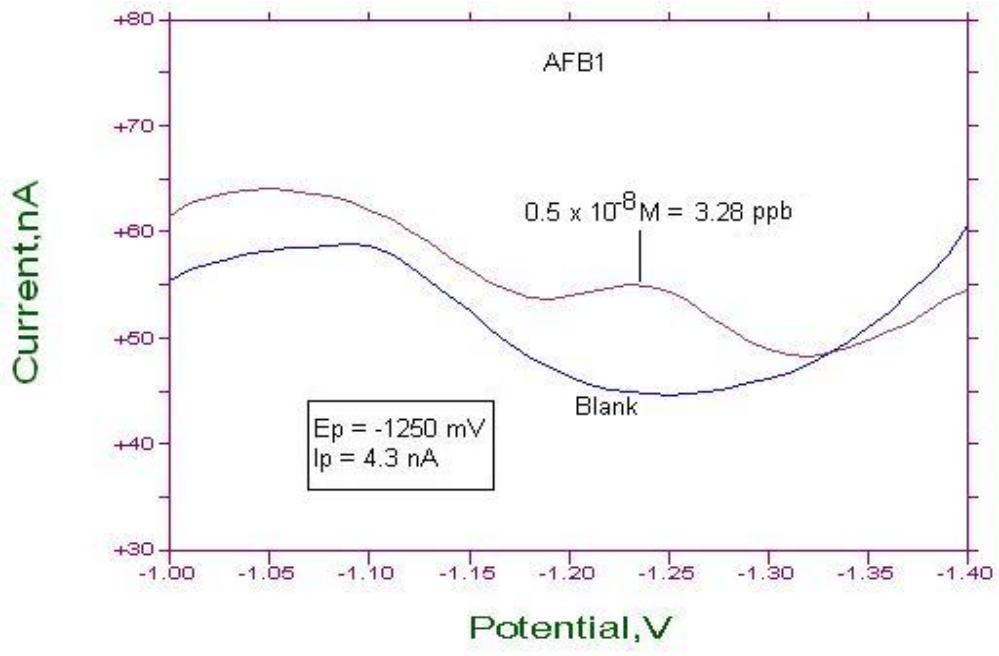


Figure S-1 Voltammogram of 0.5 x 10^{-8} M of AFB1 in BRB at pH 9.0

b) AFB2; 0.78 x 10^{-8} M or 2.5 ppb gave I_p = 5.7 nA at E_p = -1.260 V

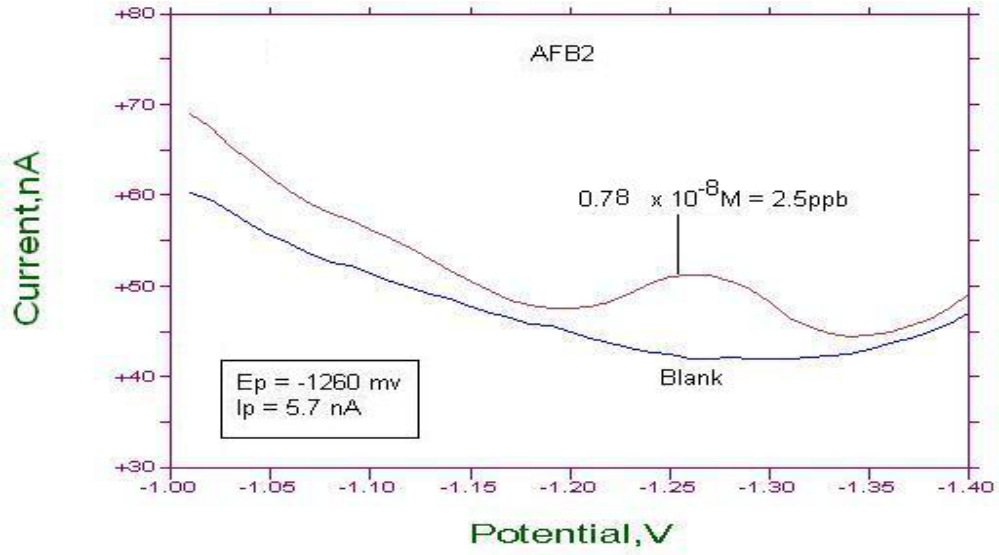


Figure S-2 Voltammogram of 0.78 x 10^{-8} M of AFB2 in BRB at pH 9.0

c) AFG1; 1.0 x 10^{-8} M or 3.28 ppb gave I_p = 4.34 nA at E_p = -1.160 V

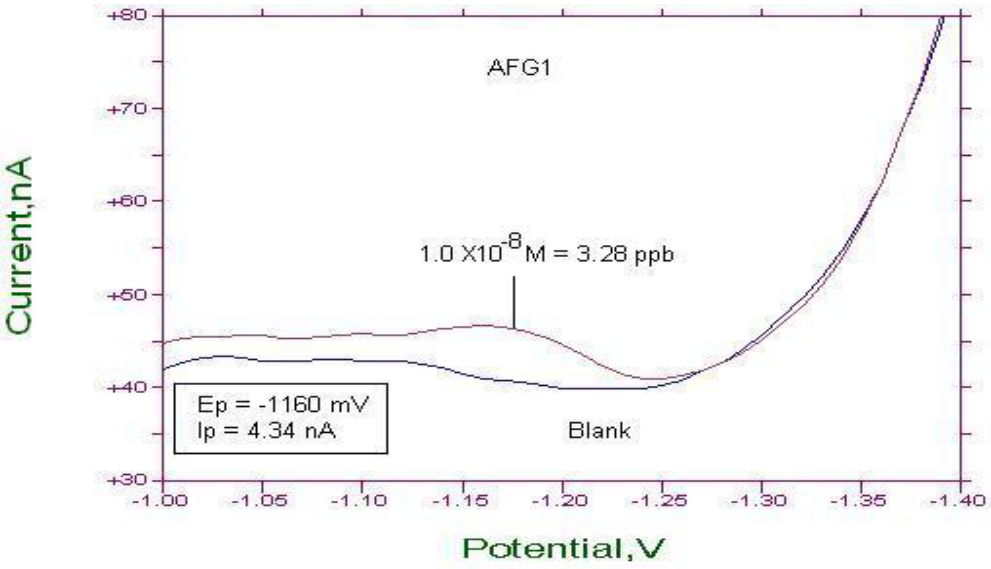


Figure S-3 Voltammogram of 1.0 x 10^{-8} M of AFG1 in BRB at pH 9.0

d) AFG2; 0.76 x 10^{-8} M or 2.5 ppb gave I_p = 6.19 nA at E_p = -1.190 V

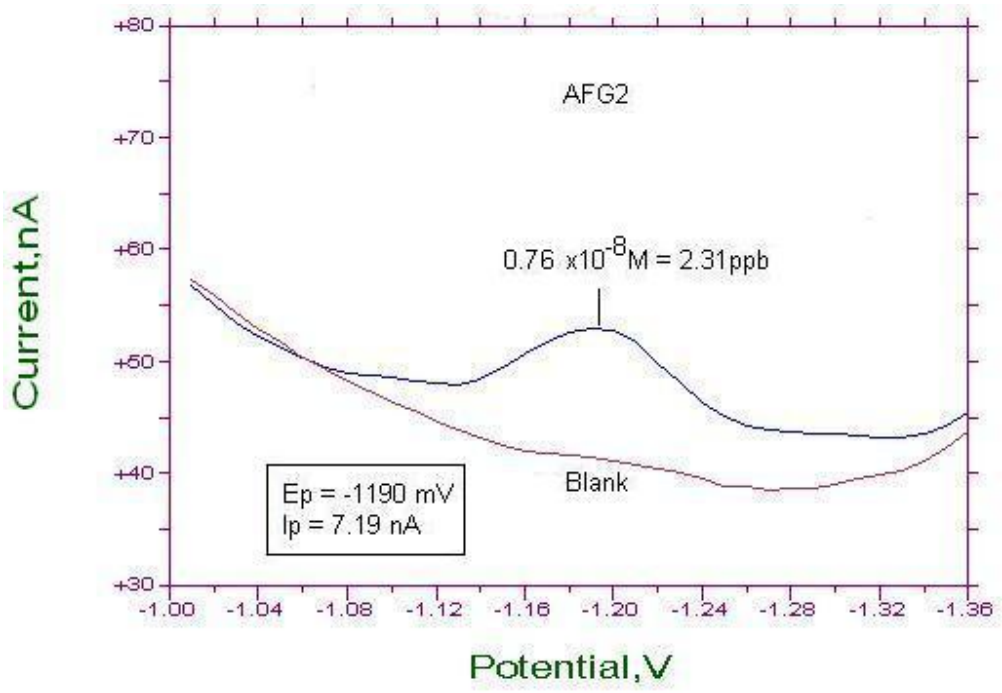


Figure S-4 Voltammogram of 0.76 x 10^{-8} M of AFG2 in BRB at pH 9.0

From the results, LOD for AFB1, AFB2, AFG1 and AFG2 are 0.5 x 10^{-8} M (1.56 ppb), 0.78 x 10^{-8} M (2.50 ppb), 1.0 x 10^{-8} M (3.28 ppb) and 0.76 x 10^{-8} M (2.50 ppb), respectively.

APPENDIX T

LOD determination according to Barek *et al.* (1999)

The limit of detection is calculated as threefold standard deviation from seven analyte determinations at the concentration corresponding to the lowest point on the appropriate calibation curve.

AFB1 (as an example);

concentration for lowest point on calibration curve is 2.0×10^{-8} M.

I_p values from seven determinations of this concentration;

11.76, 12.00, 12.40, 11.80, 11.85, 11.92, 11.92

Mean = 11.95 Standard deviation = 0.2142

$0.2142 \times 3 = 0.642 \times 10^{-8}$ M or 2.00 ppb

LOD for determination of AFB1 = 0.64×10^{-8} M or 2.00 ppb.

APPENDIX U

LOD determination according to Zhang *et al.* (1996)

The LOD is the concentration giving a signal equal to three times the standard deviation of the blank signal divided by slope from calibration curve.

AFB1 as an example

Regression equation for calibration curve is y = 5.4363x + 3.7245

Slope = 5.4363

I_p for blank from seven measurements;

40.86, 42.24, 40.22, 38.53, 42.52, 41.04, 41.64

standard deviation of blank (SD_{blk}) = 1.3533

$3SD_{blk}$ / m = (3 x 1.3533) / 5.4363 = 0.7468×10^{-8} M or 2.35 ppb

LOD for determination of AFB1 = 0.75×10^{-8} M or 2.35 ppb.

APPENDIX V

LOD determination according to Miller and Miller (1993)

LOD is the concentration giving a signal three times the standard error plus the y-intercept divided by the slope of calibration curve

Regression equation for peak height of analyte with their concentrations is;

$y_i = mx + b$

where; y_i = peak height
m = slope
b = y-intercept

y value for calculation of standard error is $y'_i = mx + b$ where x is the concentration of analyte.

Standard error is calculated based on following equation;

$S_{y/x} = \sqrt{(\sum (y_i - y_i')^2) / n - 2}$

$LOD = (3 \times S_{y/x}) / m$

As example, LOD for AFB1 is calculated as below;

Regression equation for AFB1; y = 5.4363x + 3.7245

[AFB1] = x	**Peak height = y**	**$y_i' = mx + b$**	**$y - y_i'$**	**$(y - y_i')^2$**
2	11.86	14.597	-2.737	7.491
4	23.77	25.470	-1.70	2.89
6	36.34	36.342	-0.002	4×10^{-6}
10	60.4	58.088	2.312	5.345
14	82.12	79.833	2.287	5.230
18	103	101.578	1.422	2.022
22	125.1	123.323	1.777	3.158
26	145.6	145.068	0.532	0.283
30	165.2	166.814	-1.614	2.605
32	175.4	177.686	-2.286	5.226
			$\sum (y_i - y_i')^2$	34.250

(x in 10^{-8} M and peak height is in nA)

n = 10, m = 5.4363, $\sum (y_i - y_i')^2 = 34.250$

$S_{y/x} = \sqrt{34.250/ 8} = 2.069$

LOD = (3 x 2.069) / 5.4363 = 1.142×10^{-8} M or 3.56 ppb

====> LOD for AFB1 is 1.14×10^{-8} M or 3.56 ppb.

APPENDIX W

ANOVA test (Youmens, 1973)

Table W-1 LOD (in ppb) of aflatoxins obtained from three different method

Aflatoxin		B1	B2	G1	G2	Totals
Method	1	1.56	2.50	3.28	2.50	9.84
	2	2.00	2.80	3.50	3.02	11.32
	3	2.35	2.86	3.60	2.84	11.65
Totals		5.91	8.16	10.38	8.36	32.81

Four sums of squares were calculated in order to make up the ANOVA table. They are total, sample, method and error sums of squares. The sums of squares were calculated in five steps as follows;

a) Calculation of $C = (\sum X_{ij})^2 / kn = (32.81)^2 / 12 = 89.71$

b) Calculation of total sum of squares $= \sum (X_{ij})^2 - C = 93.63 - 89.71 = 3.92$

c) Calculation of sample or block sum of squares $= \frac{1}{n} \sum^2_j - C = \frac{1}{3} \times 279.15 - 89.71$
$= 3.34$

d) Calculation of method or process sum of squares $= \frac{1}{k} \sum^2_i - C$

$= \frac{1}{4} \times 360.69 - 89.71 = 0.46$

e) Calculation of error sum of squares

Error sum of squares = total sum of squares – (block sum of squares + process sum of squares)

= 3.92 – (3.34 + 0.46) = 0.12

The ANOVA table was constructed as follows:

Table W-2 Analysis of variance

Source	Degrees of freedom (f)	Sum of squares (SS)	Mean squares (SS / f = MS)
Total	kn – 1 = 11	3.92	
Block	n – 1 = 2	3.34	
Process	k – 1 = 3	0.46	0.153
Error	kn – n – k + 1 = 6	0.12	0.02

f) Calculation of F

F = process mean square / error mean square

= 0.153 / 0.02 = 7.65

g) Test of null hypothesis

Tabular F from Fisher's F table is 8.94 for $f_1 = 6$ and $f_2 = 3$. This is larger than the calculated value of F. The hypothesis was not disproved, hence the experiment indicates that the methods are not giving significantly different of LOD of aflatoxins at 95% probability level.

This ANOVA test indicates that all three methods can be selected in determination of LOD of aflatoxins using proposed technique.

APPENDIX X

Peak height of AFB2 obtained from modification of mercury electrode with PLL

Table X-1 I_p of 10 ppb all aflatoxins in BRB at pH 9.0 in presence and absence of poly-L-lysine (PLL)

Aflatoxin	No PLL		With PLL	
	I_p (nA)	E_p (V)	I_p (nA)	E_p (V)
AFB1	15.70	-1.26	10.22	-1.22
AFB2	14.83	-1.26	12.20	-1.26
AFG1	18.77	-1.19	16.20	-1.19
AFG2	17.60	-1.22	13.90	-1.22

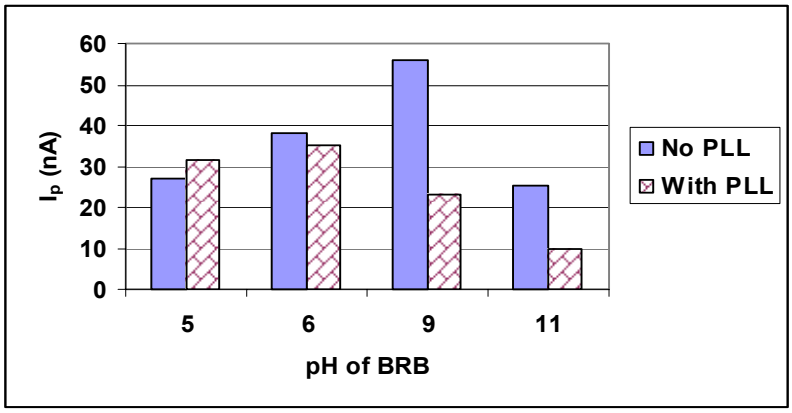


Figure X-1 I_p of 0.1 µM AFB2 (31.4 ppb) in different pH of BRB with and without PLL coated on mercury electrode.

APPENDIX Y

SWSV voltammograms of AFB1, AFB2, AFG1 and AFG2 in BRB at pH 9.0

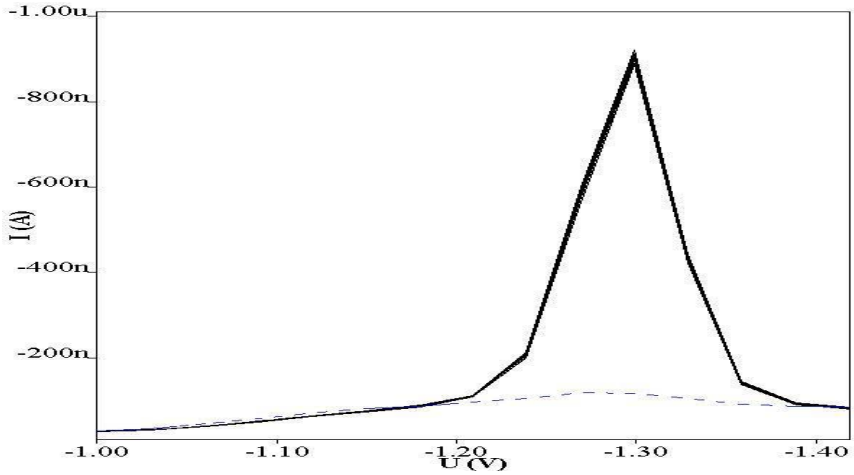


Figure Y-1 SWS voltammograms of AFB1 in BRB at pH 9.0 (n =10). Experimental parameters; E_i = -1.0 V, E_f = -1.4 V, E_{acc} = -0.8 V, t_{acc} = 100 s, υ = 3750 mV/s, frequency = 125 Hz, voltage step = 0.03 s and amplitude = 50 mV. Blank is represented by broken line.

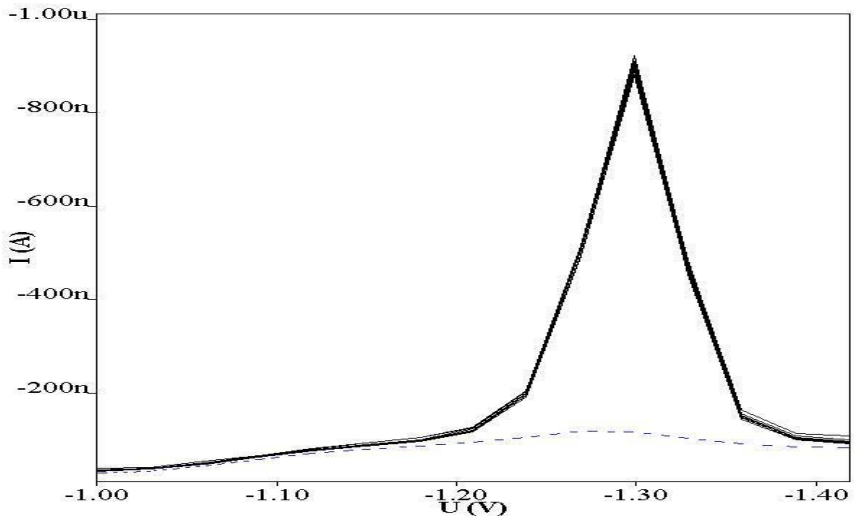


Figure Y-2 SWS voltammograms of AFB2 in BRB at pH 9.0 (n=10). All experimental conditions are the same as in the Figure Z-2.

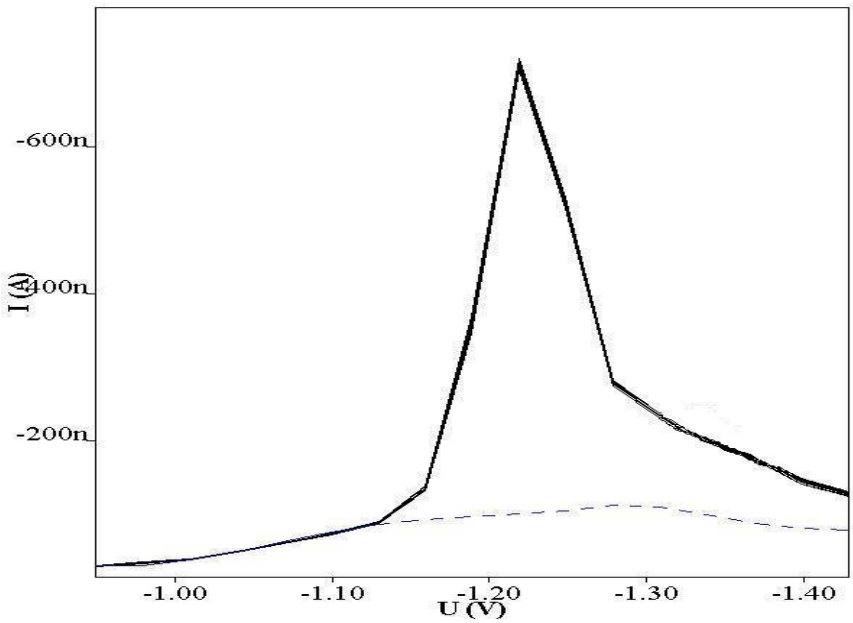


Figure Y-3 SWS voltammograms of AFG1 in BRB at pH 9.0 (n=10). All experimental conditions are the same as in the Figure Z-2 except for E_i = -0.95 V.

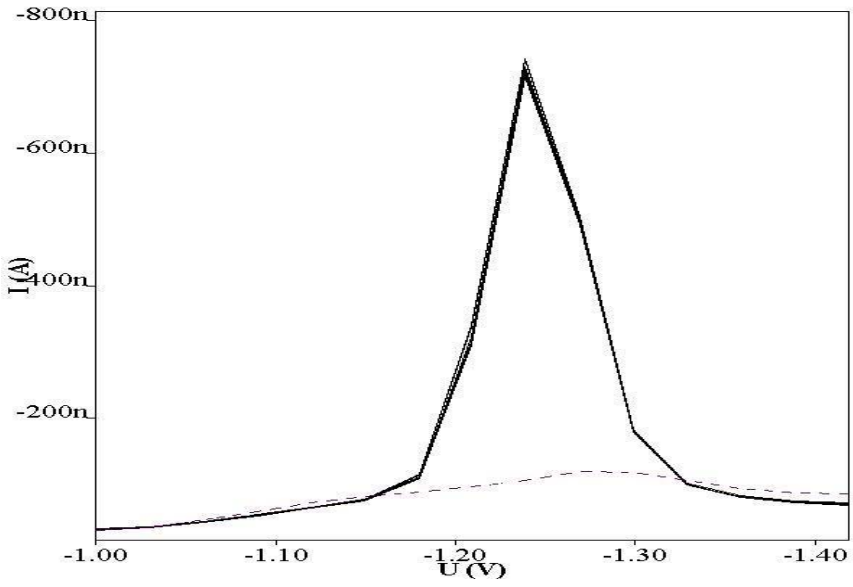


Figure Y-4 SWSV voltammograms of AFB2 in BRB at pH 9.0 (n=10). All experimental conditions are the same as in the Figure Z-2.

APPENDIX Z

SWSV voltammograms of 0.1 μM of AFB1, AFB2, AFG1 and AFG2 in BRB at pH 9.0

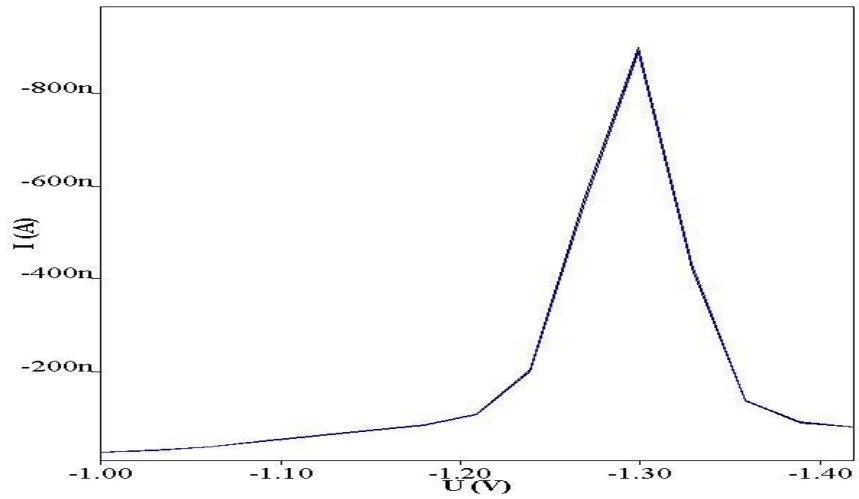


Figure Z-1 SWSV voltammograms of 0.10 μM of AFB1 (n =3) in BRB at pH 9.0. E_i = -1.0 V, E_f = -1.4 V, E_{acc} = -0.8 V, t_{acc} = 100 s, υ = 3750 mV/s, frequency = 125 Hz, voltage step = 0.03 V and amplitude = 50 mV.

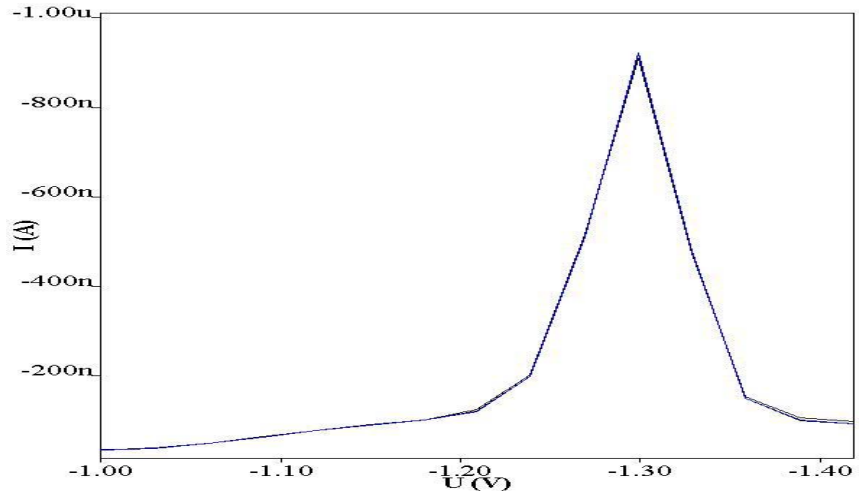


Figure Z-2 SWSV voltammograms of 0.10 μM of AFB2 (n = 3) in BRB at pH 9.0. All experimental parameters are the same as in Figure Z-1.

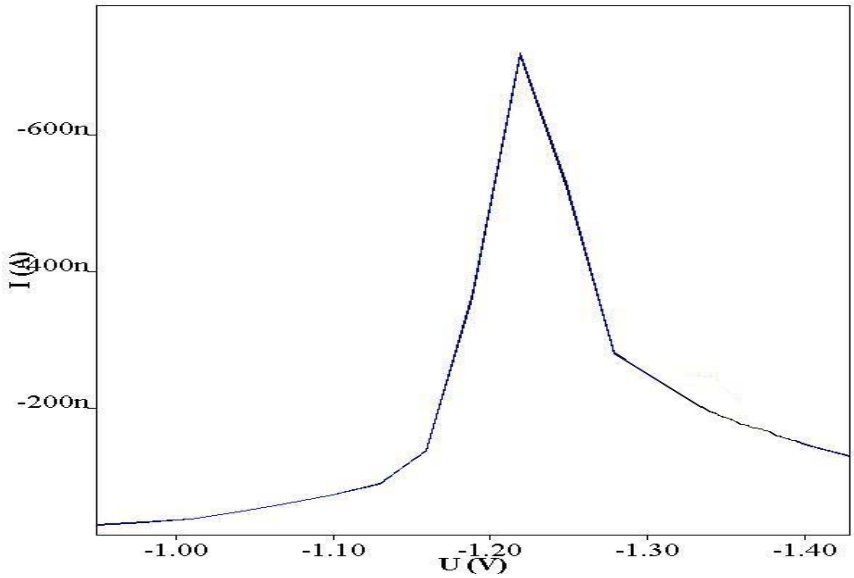


Figure Z-3 SWSV voltammograms of 0.10 µM of AFG1 (n=3) in BRB at pH 9.0. All experimental parameters are the same as in Figure Z-1 except for E_i = -0.95 V.

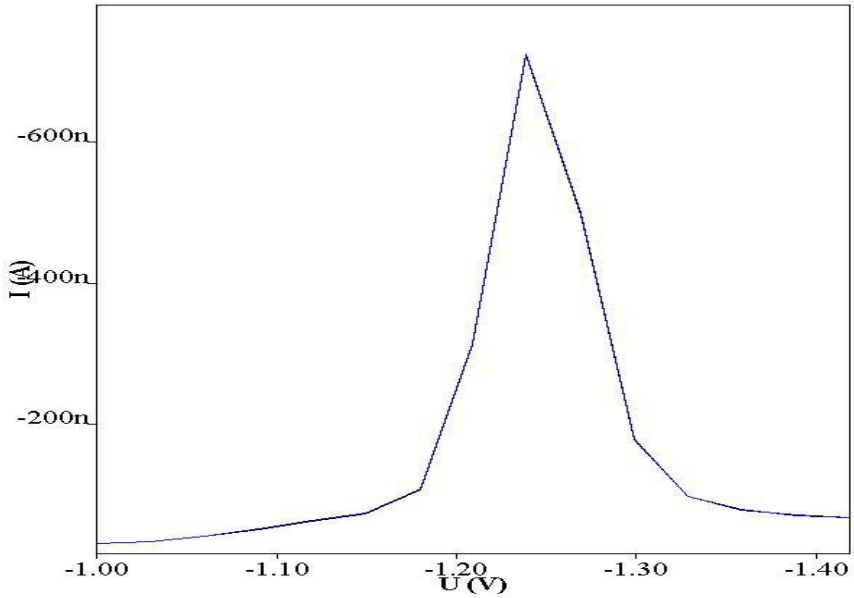


Figure Z-4 SWSV voltammograms of 0.10 µM of AFG2 (n =3) in BRB at pH 9.0. All experimental parameters are the same as in Figure Z-1.

APPENDIX AA

UV-VIS spectrums of 10 ppm AFB1, AFB2, AFG1 and AFG2 stock solutions

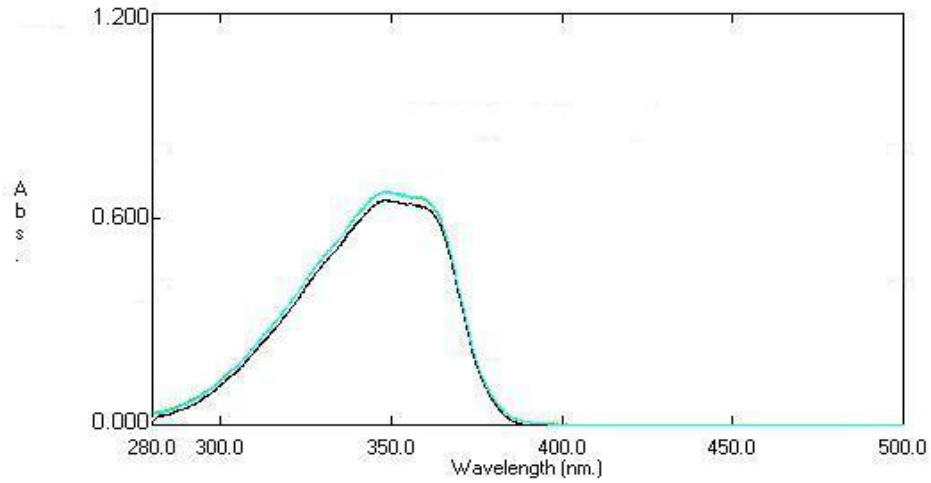


Figure AA-1 UV-VIS spectrums (n=3) of 10 ppm AFB1 in benzene: acetonitrile (98%)

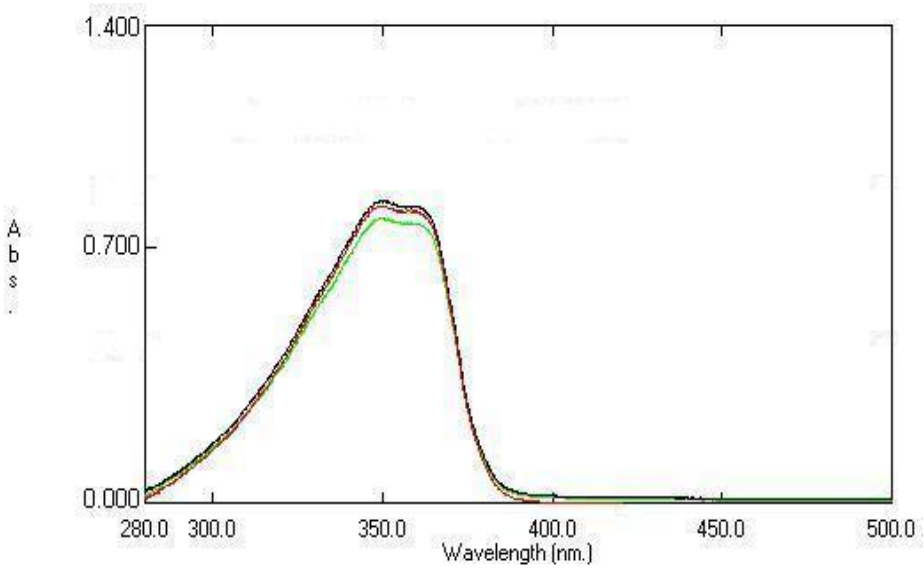


Figure AA-2 UV-VIS spectrums (n=3) of 10 ppm AFB2 in benzene: acetonitrile (98%)

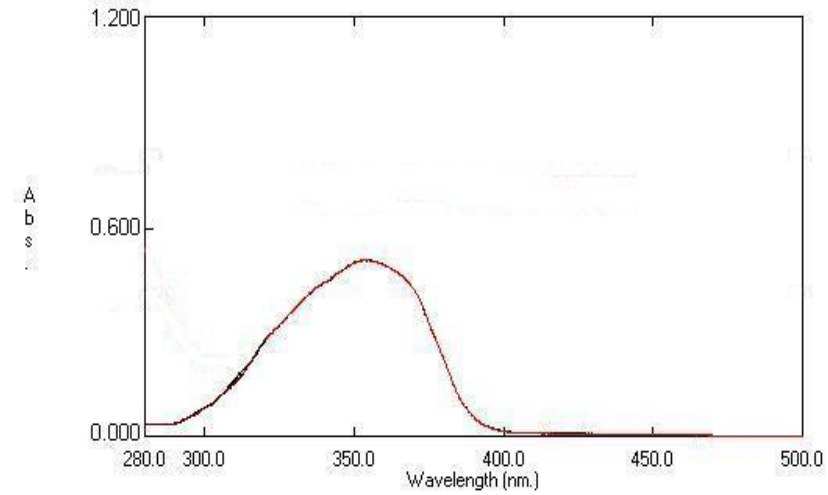


Figure AA-3 UV-VIS spectrums (n=3) of 10 ppm AFG1 in benzene: acetonitrile (98%)

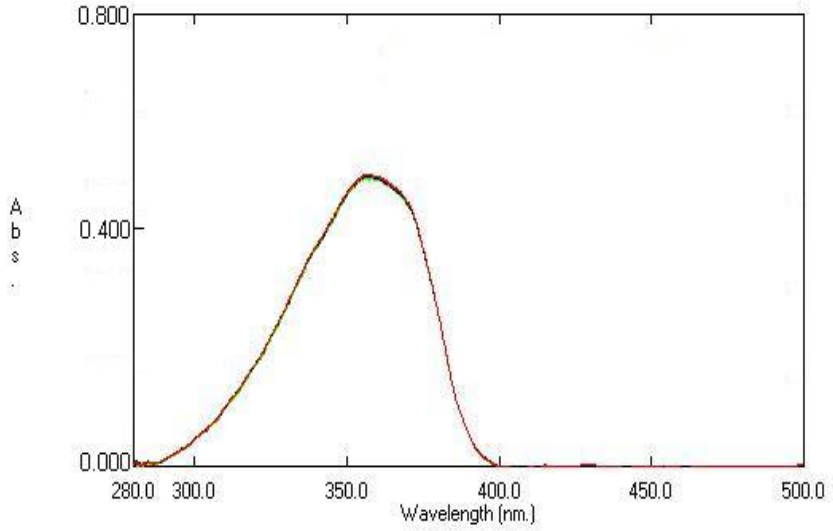


Figure AA-4 UV-VIS spectrums (n=3) of 10 ppm AFG2 in benzene: acetonitrile (98%)

APPENDIX AB

Voltammograms of AFB1, AFB2, AFG1 and AFG2 obtained from 0 to 6 months storage time in the cool and dark conditions

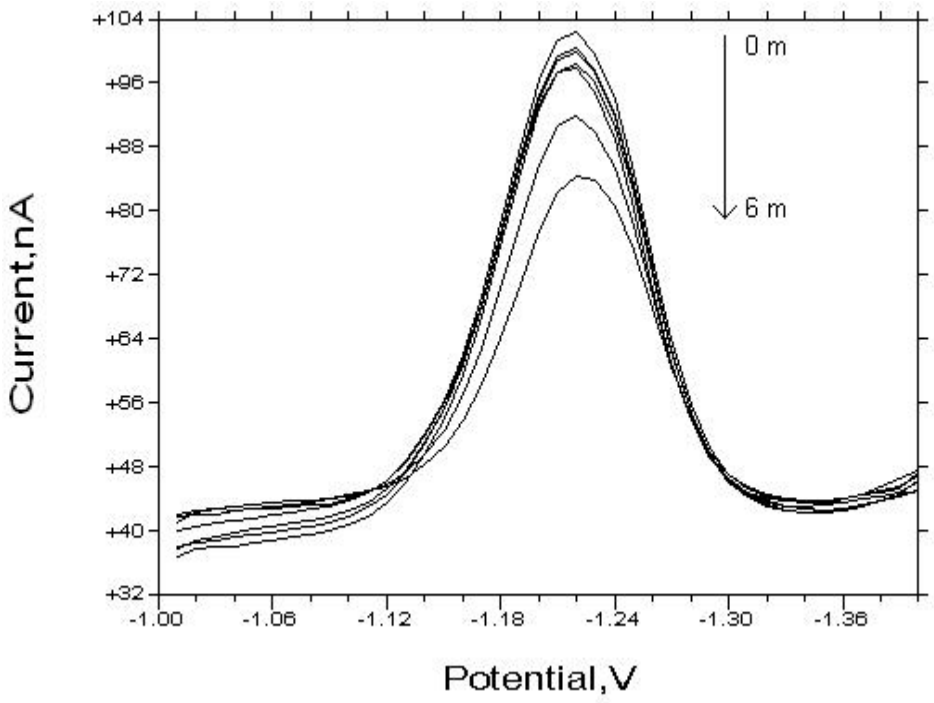


Figure AB-1 Voltammograms of 0.10 µM AFB1 in BRB at pH 9.0 obtained from difference storage time of 0 to 6 months in the dark and cool conditions. DPCSV parameter conditions: E_i = -1.0 V, E_f = -1.4 V, E_{acc} = -0.6 V, t_{acc} = 80 s, υ = 50 mV/s and pulse amplitude = 80 mV.

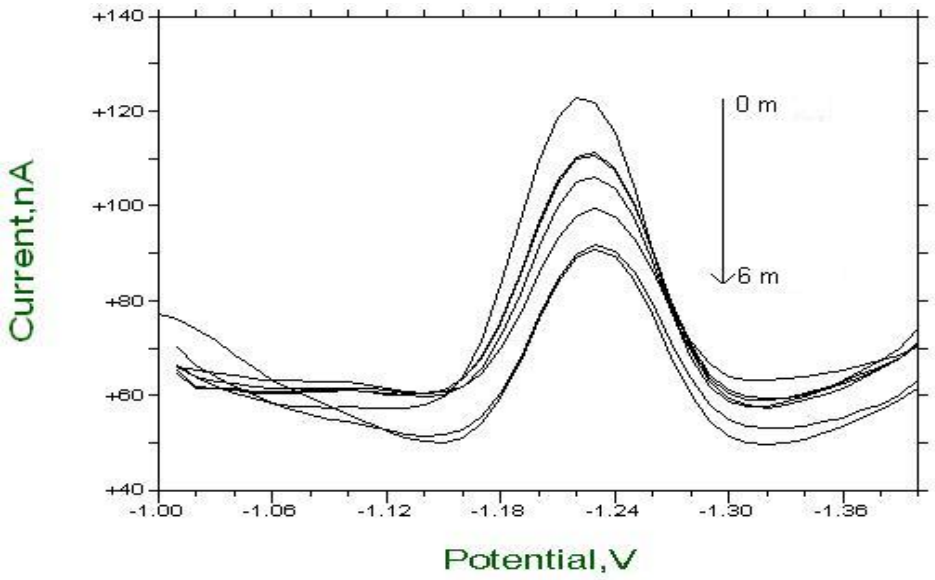


Figure AB-2 Voltammograms of 0.10 µM AFB2 in BRB at pH 9.0 obtained from difference storage time of 0 to 6 months in the dark and cool conditions. DPCSV parameter conditions are the same as in Figure AB-1.

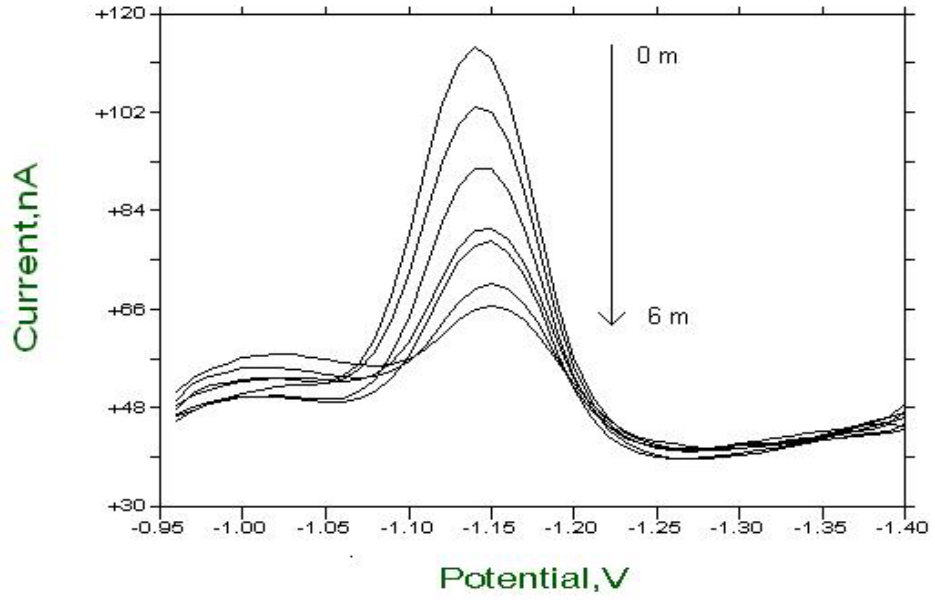


Figure AB-3 Voltammograms of 0.10 µM AFG1 in BRB at pH 9.0 obtained from difference storage time of 0 to 6 months in the dark and cool conditions. DPCSV parameter conditions are the same as in Figure AB-1 except for E_i = -0.95 V.

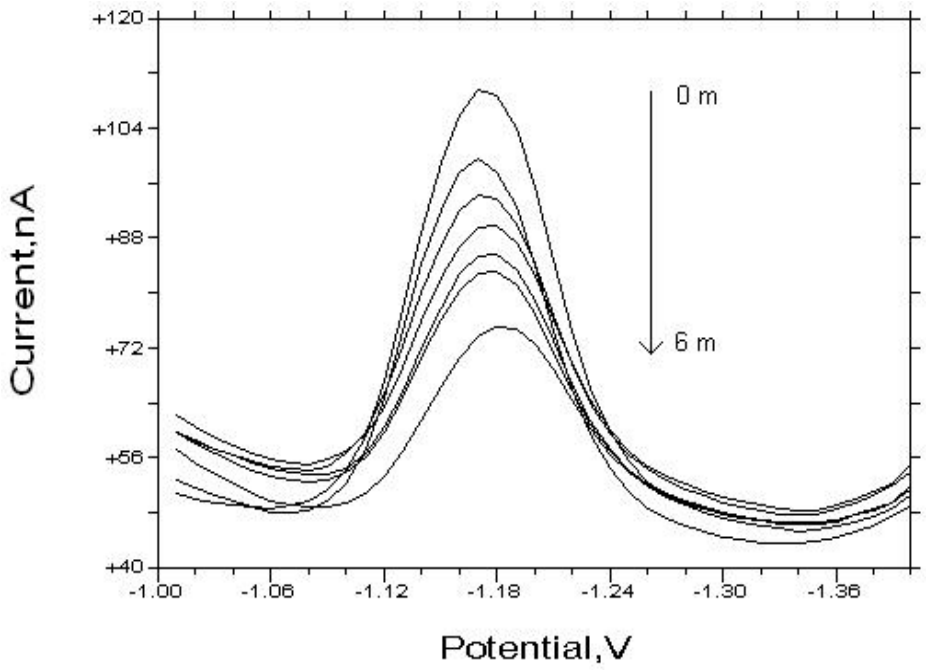


Figure AB-4 Voltammograms of 0.10 μM AFG2 in BRB at pH 9.0 obtained from difference storage time of 0 to 6 months in the dark and cool conditions. DPCSV parameter conditions are the same as in Figure AB-1.

APPENDIX AC

Voltammograms of AFB1, AFB2, AFG1 and AFG2 obtained from 0 to 8 hours exposure time

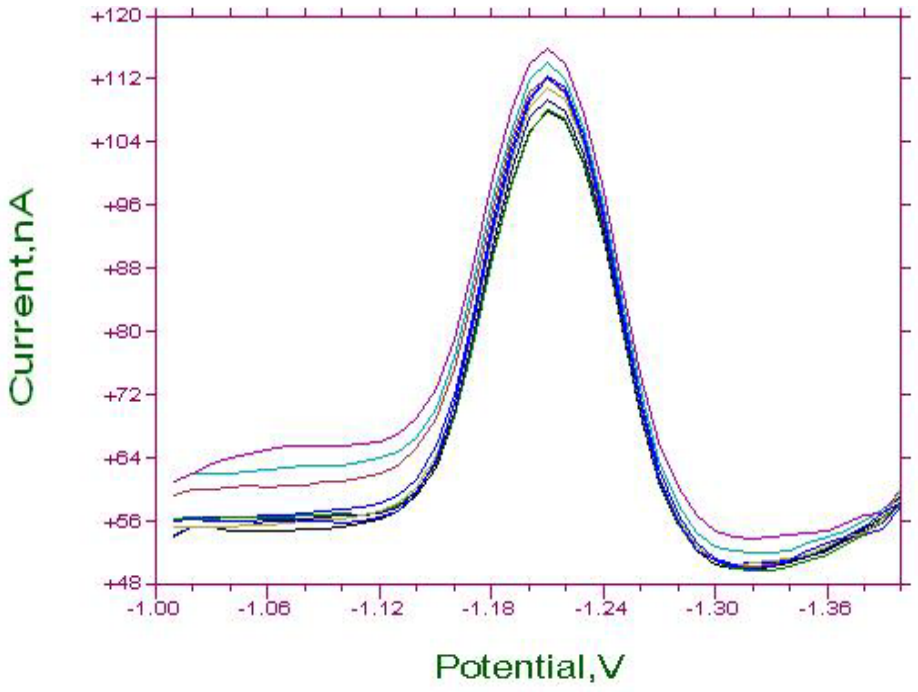


Figure AC-1 Voltammograms of 0.10 µM AFB1 in BRB at pH 9.0 exposed to normal laboratory conditions from 0 to 8 hrs. Experimental conditions: E_i = -1.0 V, E_f = -1.4 V, E_{acc} = -0.60 V, t_{acc} = 80 s, υ = 50 mV/s and pulse amplitude = 80 mV.

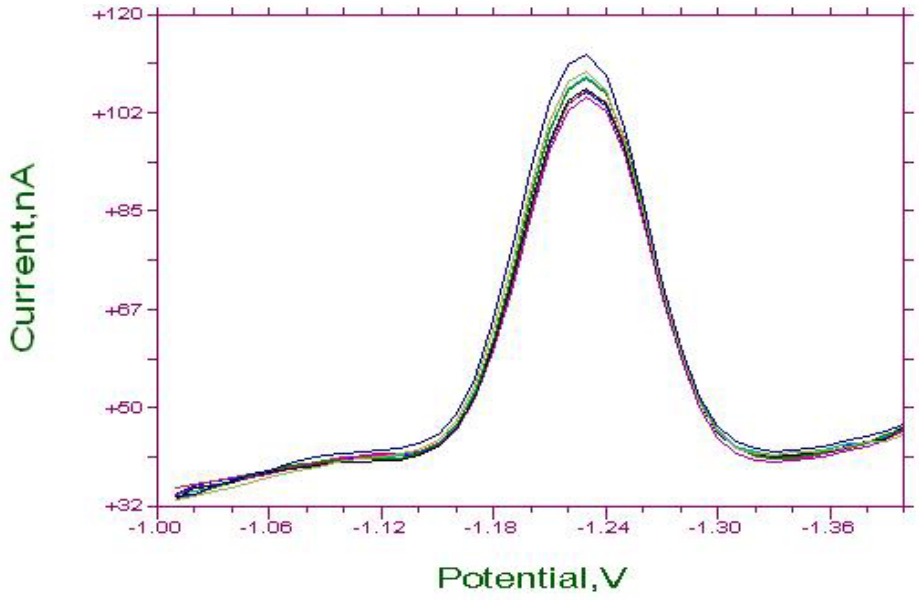


Figure AC-2 Voltammograms of 0.10 µM AFB2 in BRB at pH 9.0 exposed to normal laboratory conditions from 0 to 8 hrs. Experimental conditions are the same as in Figure AC-1.

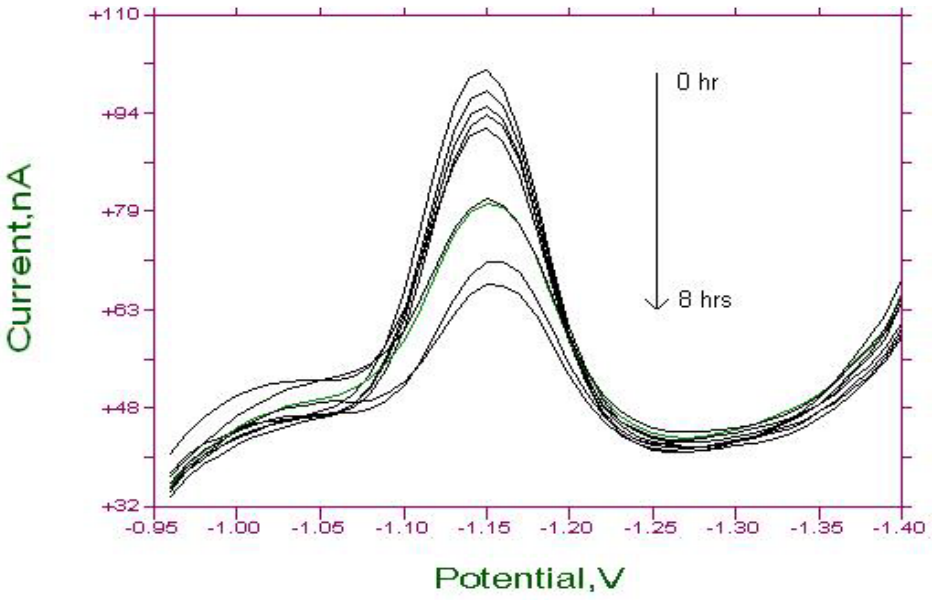


Figure AC-3 Voltammograms of 0.10 µM AFG1 in BRB at pH 9.0 exposed to normal laboratory conditions from 0 to 8 hrs. Experimental conditions are the same as in Figure AC-1 except for E_i = -0.95 V.

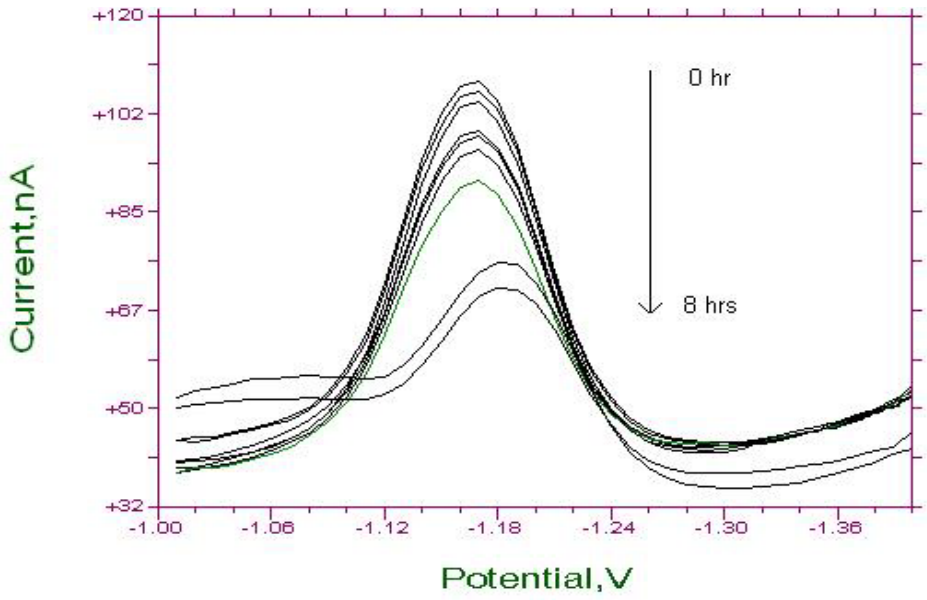


Figure AC-4 Voltammograms of 0.10 µM AFG2 in BRB at pH 9.0 exposed to normal laboratory conditions from 0 to 8 hrs. Experimental conditions are the same as in Figure AC-1.

APPENDIX AD

UV-VIS spectrums of AFB2 and AFG2 in BRB at pH 6.0 and 11.0

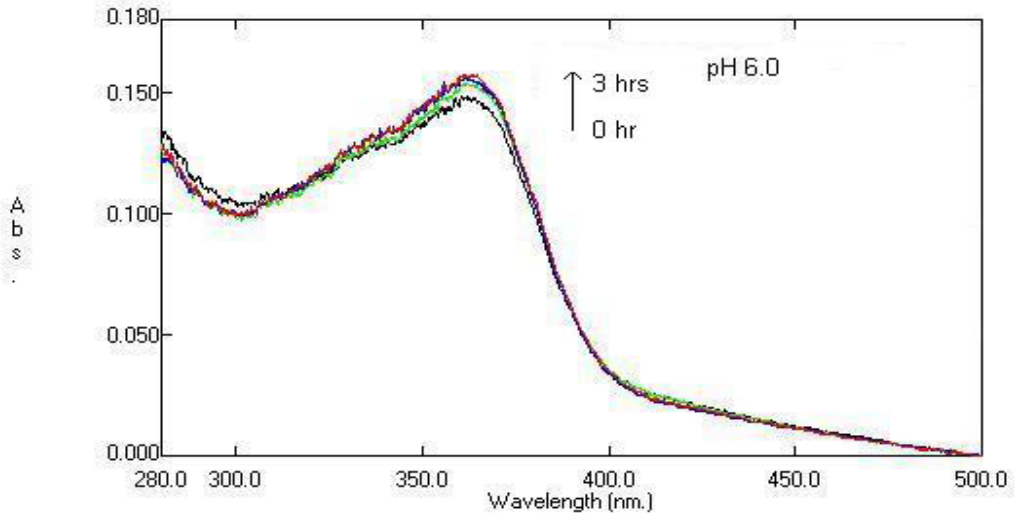


Figure AD-1 UV-VIS spectrums of 1.0 ppm AFB2 in BRB at pH 6.0 from 0 to 3 hrs exposure time

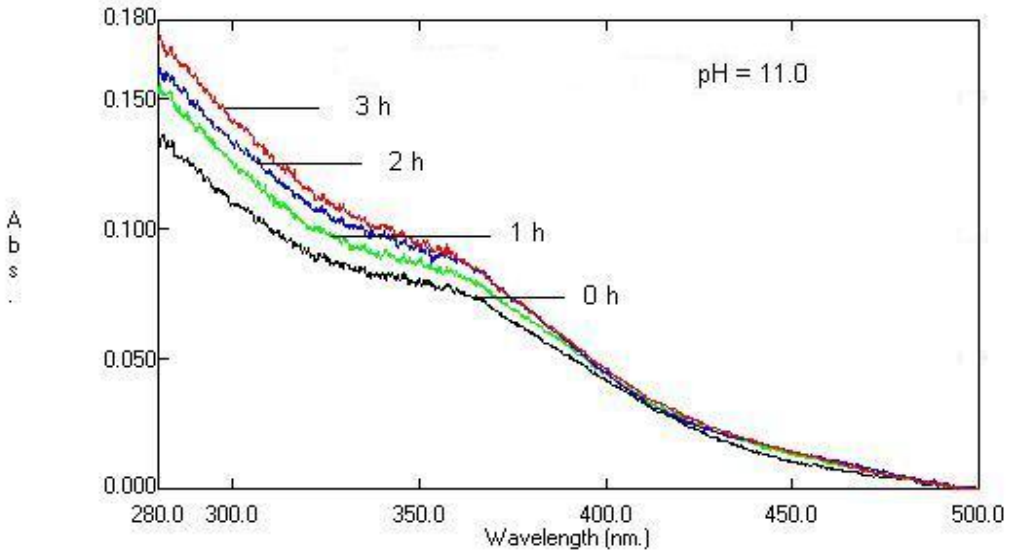


Figure AD-2 UV-VIS spectrums of 1.0 ppm AFB2 in BRB at pH 11.0 from 0 to 3 hrs exposure time

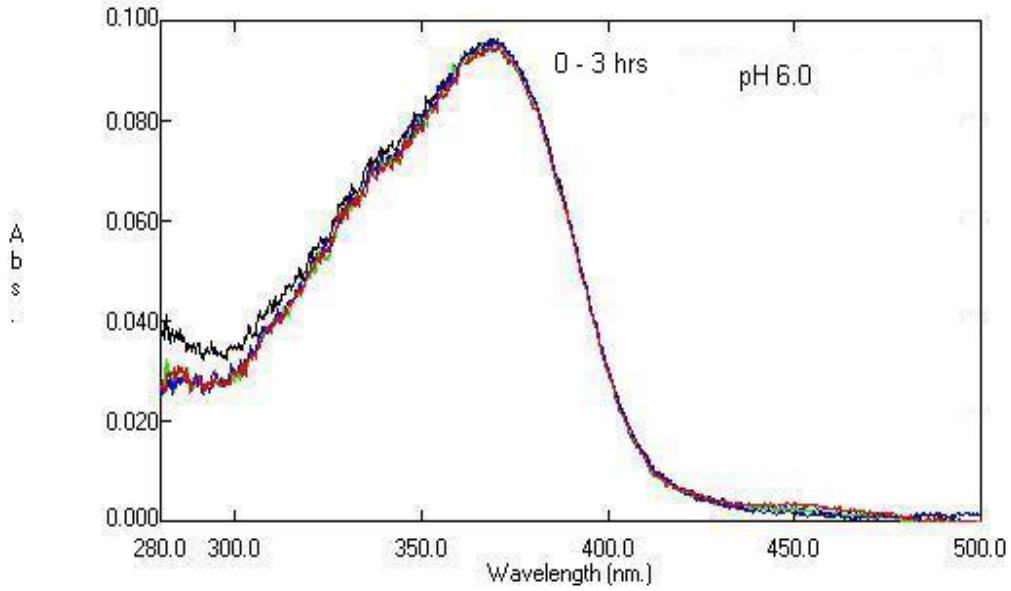


Figure AD-3 UV-VIS spectrums of 1.0 ppm AFG2 in BRB at pH 6.0 from 0 to 3 hrs exposure time

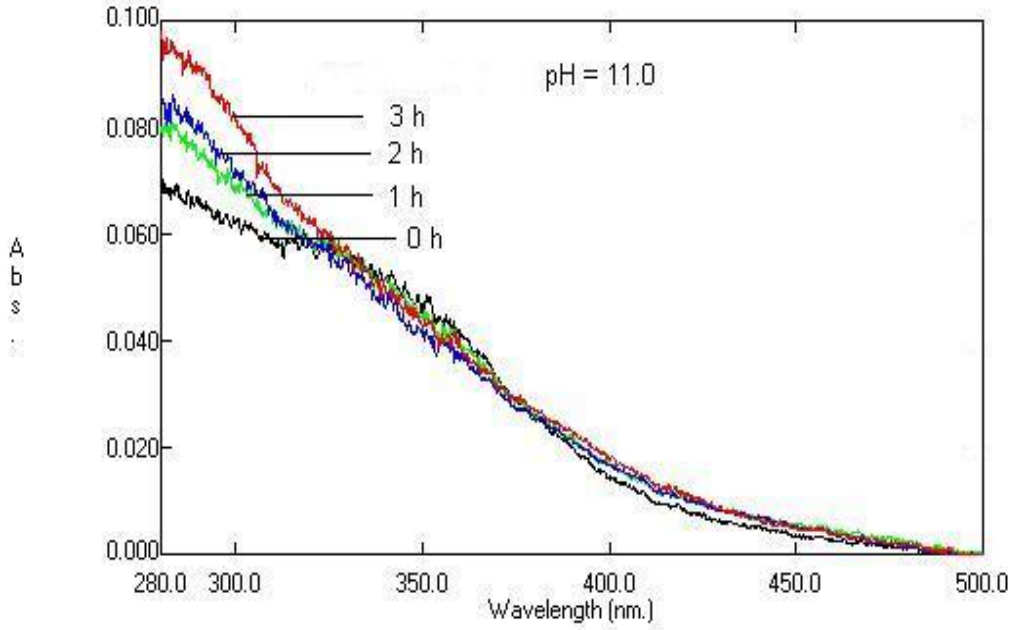


Figure AD-4 UV-VIS spectrums of 1.0 ppm AFG2 in BRB at pH 11.0 from 0 to 3 hrs exposure time

APPENDIX AE

Voltammograms of AFB1 and AFG1 in 1.0 M HCl and 1.0 M NaOH

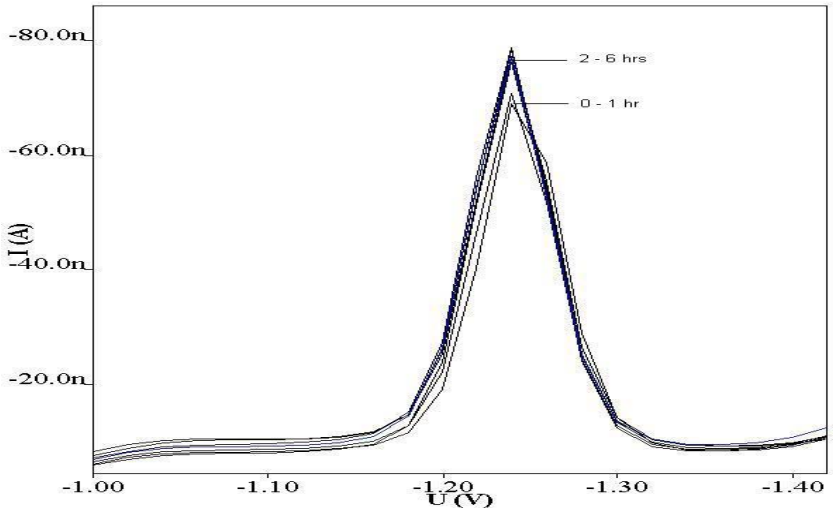


Figure AE-1 DPCSV voltammograms of AFB1 in 1.0 M HCl from 0 to 6 hours

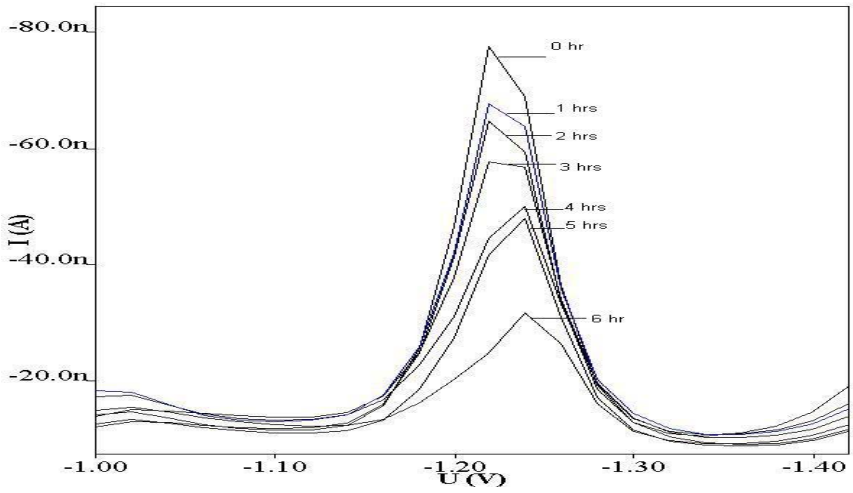


Figure AE-2 DPCSV voltammograms of AFB1 in 1.0 M NaOH from 0 to 6 hours.

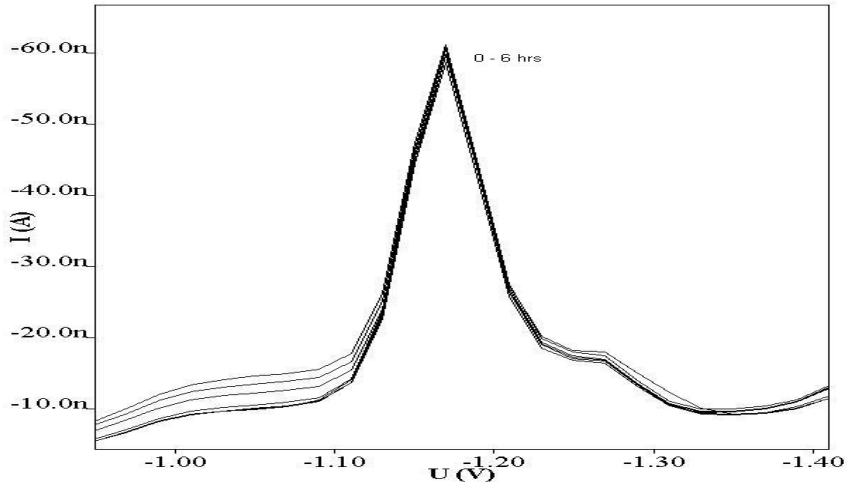


Figure AE-3 DPCSV voltammograms of AFG1 in 1.0 M HCl from 0 to 6 hours.

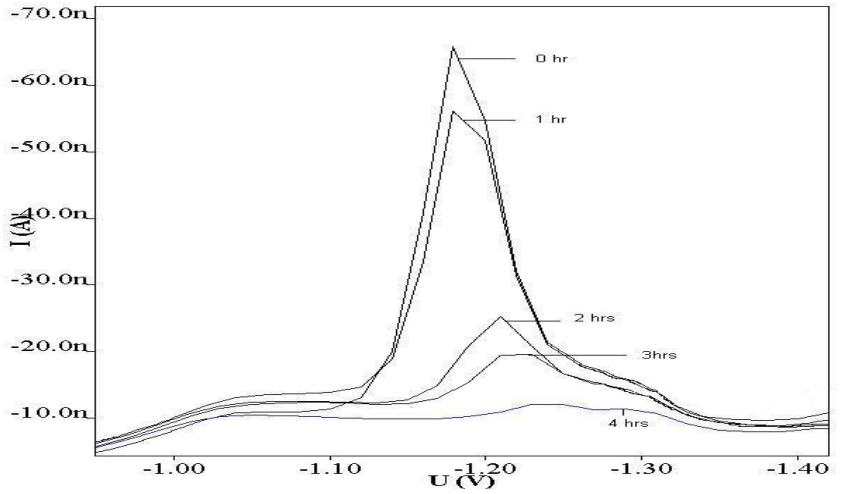
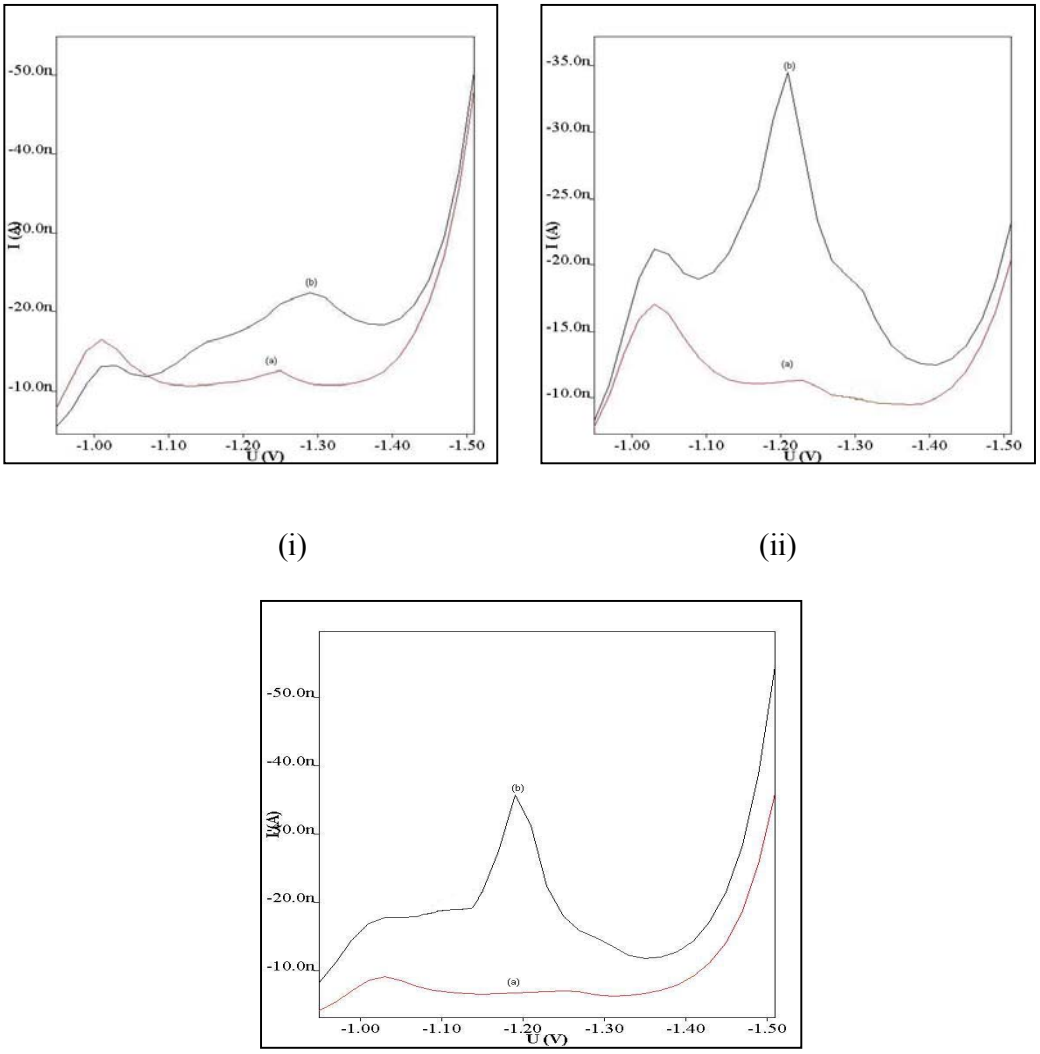


Figure AE-4 DPCSV voltammograms of AFG1 in 1.0 M NaOH from 0 to 4 hours.

APPENDIX AF

DPCS voltammograms of real samples added with various concentrations of AFG1



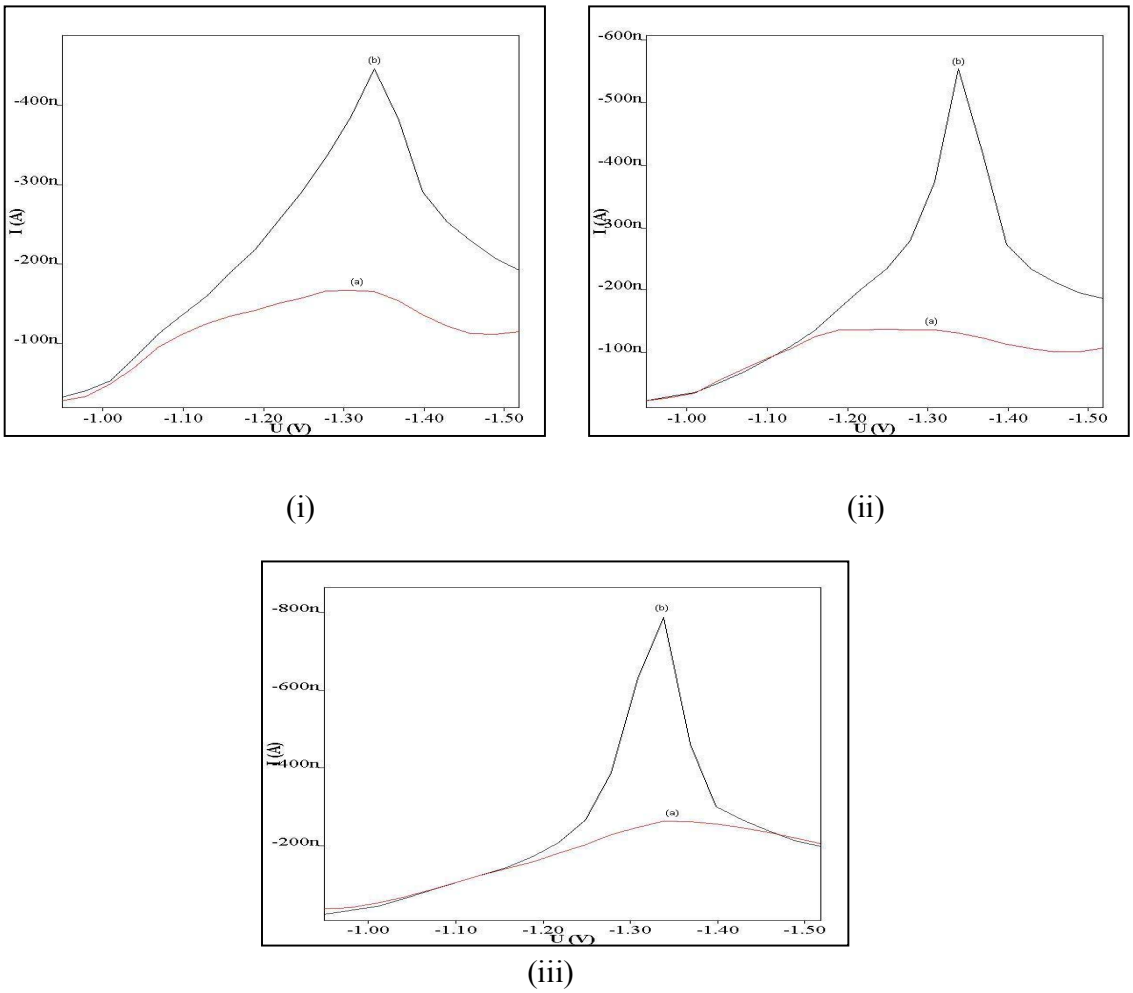
(i) (ii)

(iii)

Figure AF-1 DPCSV voltammograms of real samples (b) added with 3 ppb (i), 9 ppb (ii) and 15 ppb (iii) AFG1 obtained in BRB at pH 9.0 (a) as the blank on the first day measurement.

APPENDIX AG

SWSV voltammograms of real samples added with various concentrations of AFB1



(i) (ii)

(iii)

Figure AG-1 SWSV voltammograms of real samples (b) added with 3 ppb (i), 9 ppb (ii) and 15 ppb (iii) AFB1 obtained in BRB at pH 9.0 (a) as the blank on the first day measurement.

APPENDIX AH

Percentage of recoveries of 3 ppb and 9 ppb of all aflatoxins in real samples obtained by SWSV method.

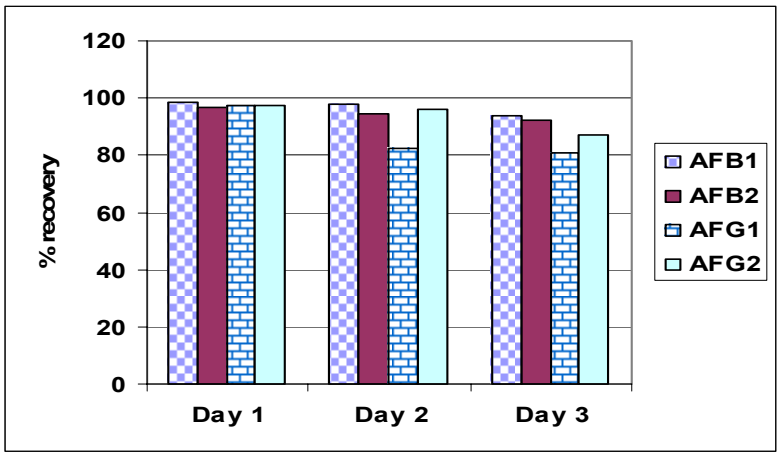


Figure AH-1 Percentage of recoveries of 3 ppb of all aflatoxins in real samples obtained by SWSV method for one to three days of measurements.

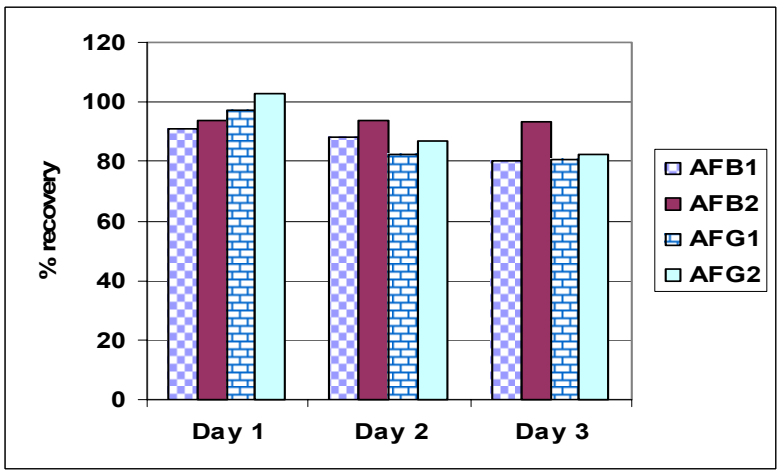


Figure AH-2 Percentage of recoveries of 9 ppb of all aflatoxins in real samples obtained by SWSV method for one to three days of measurements.

APPENDIX AI

Calculation of percentage of recovery for 3.0 ppb AFG1 added into real sample.

From voltammograms of real sample added with 3.0 ppb AFG1;

Peak height = 5.85 nA, 6.53 nA, 6.09 nA Average = 6.16 nA

Peak height for 10 ppb AFG1 in BRB pH 9.0 = 20.60 nA*. So for 1 ppb = 2.060 nA

For peak height = 6.16 nA ===> (6.16 / 2.060) x 1.0 ppb = 2.99 ppb

Injected AFG1 = 3.0 ppb
From the above calculation, found AFG1 = 2.99 ppb
Recovery (%) = (2.99 / 3.0) x 100 % = **99.67%.**

Summary:

AFG1 added = 3. 0 ppb

AFG1 found = 2.99 ppb

% recovery of AFG1 in real sample = **99.67 %**

* Notes; For other aflatoxins, the peak heights for 10 ppb of AFB1, AFB2 and AFG2 in BRB pH 9.0 is 21.93 nA, 21.33 nA and 20.23 nA respectively.

APPENDIX AJ

HPLC chromatograms of real samples: S10 and S07

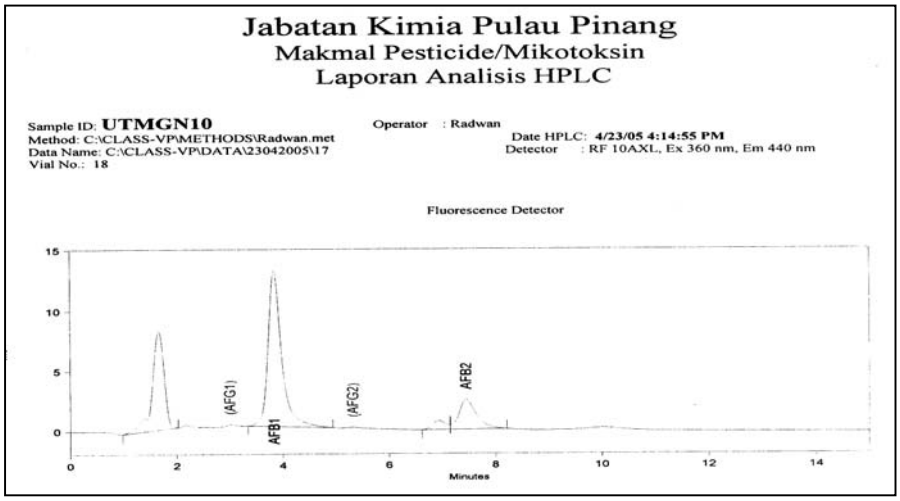


Figure AJ-1 HPLC chromatogram for S10 which contains 36.00 ppb total aflatoxins.

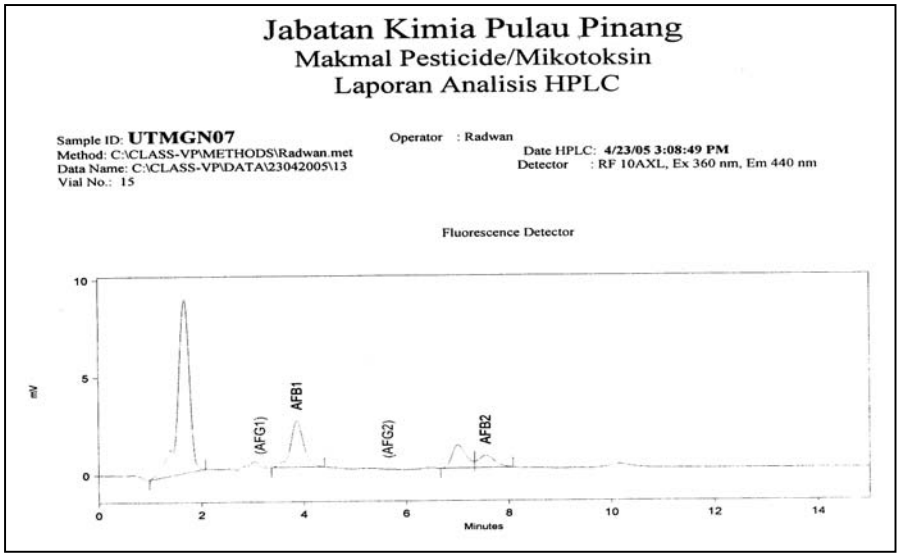


Figure AJ-2 HPLC chromatogram for S07 which contains 3.67 ppb total aflatoxins.

APPENDIX AK

Calculation of aflatoxin in real sample; S13

1st analysis

From voltammograms of real sample ;

Peak height = 1.78 nA, 1.80 nA, 1.79 nA Average = 1.79 nA

From voltammograms of real sample added with 10 ppb AFB1 standard solution.
Peak height = 22.5 nA, 22.6 nA, 22.8 nA Average = 22.63 nA

From equation as stated in Appendix F;
Aflatoxin content = [(1.79) / (22.63 – 1.79)] x 12.5 = **10 .74 ppb**

2nd analysis

From voltammograms of real sample ;

Peak height = 1.80 nA, 1.79 nA, 1.82 nA Average = 1.80 nA

From voltammograms of real sample added with 10 ppb AFB1 standard solution.
Peak height = 22.7 nA, 22.5 nA, 22.8 nA Average = 22.67 nA

From equation as stated in Appendix F;
Aflatoxin content = [(1.80) / (22.67 – 1.80)] x 12.5 = **10 .78 ppb**

Average for duplicate analysis = (10.74 + 10.78) / 2 = **10.74 ppb**

Total aflatoxins content in S13 = **10.76 ppb**

APPENDIX AL

List of papers presented or published to date resulting from this study

1. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Cylic voltammetry study of AFB2 at the mercury electrode. Paper presented at SKAM -16, Kucing, Sarawak, 9 – 11th September 2003.

2. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Cylic voltammetry study of AFB2 at the mercury electrode. *Malaysian J. Anal. Sci.* In press.

3. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Differential pulse stripping voltammetric technique for determination of AFB2 at the mercury electrode. J. *Collect. Czech. Chem. Common*. In press

4. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Stability studies of aflatoxin G1 (AFG1) using differential pulse stripping voltammetric technique. Paper presented at Symposium Life Science II, USM Penang. 31st to 3rd April 2004.

5. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Developement of differential pulse stripping voltammetric (DPCSV) technique for determination of AFG1 at the mercury electrode. *Chemical Analysis (Warsaw).* In press

6. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Cyclic voltammetry study of AFG1 at the mercury electrode. Paper presented at KUSTEM 3rd Annual Seminar on Sustainability Science and Management, Kuala Terengganu, Terengganu. 4 – 5th May 2004.

7. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Stability studies of aflatoxins using differential pulse stripping voltammetric (DPCSV) technique. Paper presented at SKAM-17, Kuantan, Pahang, 24 – 26th August 2004.

8 Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Stability studies of aflatoxins using differential pulse stripping voltammetric (DPCSV) technique. *Malaysian J. Anal. Sci.* In press.

9. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Voltammetric determination of aflatoxins: Differential pulse voltammetric (PCSV) versus Square-wave stripping voltammetry (SWSV) techniques. Paper presented at KUSTEM 4th Annual Seminar on Sustainibility Science and Management, Kuala Terengganu, 2nd – 3rd May 2005.

10. Yaacob, M.H., Mohd. Yusoff, A.R. , Ahamad, R. and Misni, M. Determination of the aflatoxin B1 in groundnut samples by differential pulse cathodic stripping voltammetry (DPCSV) technique. Paper presented at Seminar Nasional Kimia II, Universiti Sumatera Utara, Medan, Indonesia. 14th April 2005.

11. Yaacob, M.H., Mohd. Yusoff, A.R. Ahamad, R., and Misni, M. Development of differential pulse cathodic stripping voltammetry (DPCSV) technique for the determination of aflatoxin B1 in groundnut samples. *J Sains Kimia*. **9(3)**: 31-36.

12. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Application of differential pulse cathodic stripping voltammetry (DPCSV) technique in studying stability of aflatoxins. *J Sains Kimia*. **9(3)**: 64-70.

13. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Square-wave cathodic stripping voltammetric (SWSV) technique for determination of aflatoxin B1 in groundnut samples. Paper presented at SKAM-18, JB, Johor. 12 – 14th September 2005.

14. Yaacob, M.H., Mohd. Yusoff, A.R. and Ahamad, R. Square-wave cathodic stripping voltammetric (SWSV) technique for determination of aflatoxin B1 in groundnut samples. *Malaysian J. Anal. Sci.* In press.

APPENDIX AM

ICP-MS results on analysis of BRB at pH 9.0

Quantitative Analysis - Summary Report

Sample ID: mhy01
Sample Date/Time: Thursday, December 18, 2003 08:55:12
Sample Description:
Solution Type: Sample
Blank File: d:\elandata\Dataset\default\Blank.3616
Number of Replicates: 3
Peak Processing Mode: Average
Signal Profile Processing Mode: Average
Dual Detector Mode: Dual
Dead Time (ns): 35

BRB pH = 9.0 one
[Zn] = 6.494 ppb

Sample File: D:\elandata\Sample\Hazri.sam
Method File:
Dataset File: d:\elandata\Dataset\default\mhy01.3678
Tuning File: d:\elandata\Tuning\default.tun
Optimization File: d:\elandata\Optimize\default.dac
Calibration File: D:\elandata\System\17-12-03.cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
Cs	133	7180	6.005	21006.201	1.667
Zn	64	49591	10.131	32691.249	23.527
Sr	88	186886	5.585	9799.174	14.861
Sn	120	7095	8.086	316.090	9.454
Pb	208	35601	9.050	321.229	10.953
Ni	58	3727	1.190	3984.436	13.569
Ti	48	73832	1.370	-9294.857	36.440
B	11	135214077	1.287	11205.395	2.126
Rb	85	33939	5.840	372271.700	1.696
Se	82	-3	309.790	-76.465	20.462
Mn	55	15140	1.300	11416.917	8.153

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
Cs	133	-13825.765				ug/L
Zn	64	16899.586	**6.494**	1.93	29.7	ug/L
Sr	88	177087.103				ug/L
Sn	120	6778.583				ug/L
Pb	208	35279.672				ug/L
Ni	58	-257.043				ug/L
Ti	48	83126.639				ug/L
B	11	135202871.594				ug/L
Rb	85	-338332.335				ug/L
Se	82	73.959				ug/L
Mn	55	3723.254				ug/L

Page 1
Sample ID: mhy01
Report Date/Time: Thursday, December 18, 2003 08:57:42

Figure AM-1 ICP-MS results on analysis of BRB at pH 9.0