

ELECTROANALYTICAL STUDIES OF GLYPHOSATE AND
AMINOMETHYLPHOSPHONIC ACID IN AQUEOUS MEDIUM USING
COPPER AND HANGING MERCURY DROP ELECTRODES

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*Specially dedicated to my beloved father (Yasin Abu Bakar),
mother (MekTeh Ali), brothers and sisters
for their love, concern,
encouragement and continuous prayer
for my success
in completing this research.*

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ABSTRACT

Glyphosate (GLYP) is commonly used in agriculture to control weeds. GLYP residue and its main metabolite, aminomethylphosphonic acid (AMPA), can be found in crops, vegetables, fruits, soil and also ground water which raised concern over the problems regarding to the environmental contaminations. These compounds can be detected and determined by gas chromatography (GC), high performance liquid chromatography (HPLC), ion chromatography (IC), capillary electrophoresis (CE) or enzyme linked immunosorbent assay (ELISA). However, most of these methods are time consuming, involve the use of toxic solvents, require complicated pre-preparation and high cost of instrumentation. Therefore, in the present study voltammetric method based on the use of copper electrode (CuE) and hanging mercury drop electrode (HMDE) were used to investigate the electrochemical behaviour and quantitative analysis of GLYP and AMPA. Investigations were carried out by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and differential pulse cathodic stripping voltammetry (DPCSV) techniques based on the reduction (cathodic current) and oxidation (anodic current) of Cu(II) in phosphate buffer at pH 6.4. Based on anodic and cathodic currents of the CuE working electrode using DPV and DPCSV techniques, linear calibration curves were obtained in the range of 1.18 up to 5.92×10^{-6} M and 7.20 up to 36.02×10^{-6} M for GLYP and AMPA respectively. The limits of detection (LOD) based on anodic current for GLYP at -80 mV vs saturated calomel reference electrode (SCE) and AMPA at -96 mV vs SCE by DPV technique were 3.24×10^{-7} M and 2.68×10^{-6} M respectively. The LOD based on cathodic current for GLYP at -180 mV vs silver/silver chloride reference electrode (Ag/AgCl) and AMPA at -190 mV vs Ag/AgCl were 4.69×10^{-7} M and 2.67×10^{-6} M respectively. The LOD by DPCSV with CuE working electrode were 3.11×10^{-7} M and 2.09×10^{-6} M for GLYP at -180 mV vs Ag/AgCl and AMPA at -190 mV vs Ag/AgCl respectively. Determination of GLYP and AMPA by HMDE requires addition of Cu(II) into the test solution. Linear calibration curves by HMDE were obtained in the range of 2.96 up to 14.79×10^{-7} M and 1.35 up to 6.75×10^{-6} M for both DPV and DPCSV. The LOD of GLYP at 0 mV vs Ag/AgCl and AMPA at -3 mV vs Ag/AgCl by HMDE were 7.41×10^{-8} M and 6.05×10^{-7} M respectively using DPV. The LOD by DPCSV technique for GLYP at -3 mV vs Ag/AgCl and AMPA at 0 mV vs Ag/AgCl by HMDE were 5.40×10^{-8} M and 2.64×10^{-7} M respectively. The DPCSV technique with CuE and HMDE working electrodes for the determination of GLYP and AMPA have good recoveries (94 - 98%) based on application to real samples.

ABSTRAK

Glifosat (GLYP) biasanya digunakan dalam bidang pertanian untuk mengawal rumpai. Sisa GLYP dan metabolit utama, asid aminometilfosfonik (AMPA) boleh didapati di dalam tanaman, sayur-sayuran, buah-buahan, tanah dan juga air bawah tanah yang menimbulkan kebimbangan terhadap masalah mengenai pencemaran alam sekitar. Sebatian ini boleh dikesan dan ditentukan menggunakan kromatografi gas (GC), kromatografi cecair berprestasi tinggi (HPLC), kromatografi ion (IC), elektroforesis rerambut (CE) atau esei imunoserapan berikatan enzim (ELISA). Walau bagaimanapun, kebanyakan kaedah ini mengambil masa yang lama, melibatkan penggunaan pelarut toksik, memerlukan pra-persediaan yang rumit dan menggunakan peralatan yang mahal. Oleh itu, dalam kajian ini, kaedah voltametri berasaskan elektrod kuprum (CuE) dan elektrod titisan merkuri tergantung (HMDE) digunakan untuk mengkaji sifat elektrokimia dan analisis kualitatif bagi GLYP dan AMPA. Kajian dijalankan menggunakan teknik voltametri kitaran (CV), voltametri denyut pembeza (DPV) dan voltametri perlucutan katodik denyut pembeza (DPCSV) yang berasaskan penurunan (arus katodik) dan pengoksidaan (arus anodik) bagi Cu(II) didalam penimbal fosfat pada pH 6.4. Berdasarkan arus anodik dan katodik bagi elektrod kerja CuE menggunakan teknik DPV dan DPCSV, keluk penentuan linear telah diperoleh dalam julat 1.18 sehingga 5.92×10^{-6} M dan 7.20 sehingga 36.02×10^{-6} M untuk masing-masing GLYP dan AMPA. Had pengesanan (LOD) berdasarkan arus anodik GLYP pada -80 mV vs elektrod rujukan kalomel tepu (SCE) dan AMPA pada -96 mV vs SCE menggunakan teknik DPV masing-masing adalah 3.24×10^{-7} M dan 2.68×10^{-6} M. LOD berdasarkan arus katodik bagi masing-masing GLYP pada -180 mV vs elektrod rujukan argentum/argentum klorida (Ag/AgCl) dan AMPA pada -190 mV vs Ag/AgCl adalah 4.69×10^{-7} M dan 2.67×10^{-6} M. LOD bagi masing-masing GLYP pada -180 mV vs Ag/AgCl dan AMPA pada -190 mV vs Ag/AgCl menggunakan teknik DPCSV dengan elektrod kerja CuE adalah 3.11×10^{-7} M dan 2.09×10^{-6} M. Penentuan GLYP dan AMPA menggunakan HMDE memerlukan penambahan Cu(II) ke dalam medium ujian. Keluk penentuan linear telah diperoleh dalam julat 2.96 sehingga 14.79×10^{-7} M dan 1.35 sehingga 6.75×10^{-6} M untuk DPV dan DPCSV. LOD bagi GLYP menggunakan HMDE pada 0 mV vs Ag/AgCl dan AMPA pada -3 mV vs Ag/AgCl masing-masing adalah 7.41×10^{-8} M dan 6.05×10^{-7} M menggunakan DPV. LOD bagi teknik DPCSV untuk GLYP pada -3 mV vs Ag/AgCl dan AMPA pada 0 mV vs Ag/AgCl menggunakan HMDE masing-masing adalah 5.40×10^{-8} M dan 2.64×10^{-7} M. Teknik DPCSV dengan elektrod kerja CuE dan HMDE bagi penentuan GLYP dan AMPA mempunyai perolehan yang baik (94-98%) berdasarkan aplikasi terhadap sampel sebenar.

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ABBREVIATIONS

%	-	Percent
μm	-	Micrometer
μgL^{-1}	-	Microgram per Liter
2,4-D	-	2,4-dichlorophenoxyacetic acid
Ag/AgCl	-	Silver/Silver Chloride
$\text{Al}(\text{NO}_3)_3$	-	Aluminium Nitrate
ACP	-	Alternate Current Polarography
ACV	-	Alternate Current Voltammetry
AMPA	-	Aminomethylphosphonic Acid
ASV	-	Anodic Stripping Voltammetry
BRB	-	Britton-Robinson Buffer
CD	-	Coulometric Detector
CE	-	Capillary Electrophoresis
CGS	-	Capillary Gas Chromatography
$\text{C}_3\text{H}_8\text{NO}_5\text{P}$	-	Glyphosate
CH_3COOH	-	Glacial Acetic Acid
$\text{CH}_6\text{NO}_3\text{P}$	-	Aminomethylphosphonic Acid
CNLSD	-	Condensation Nucleation Light Scattering Detection
CSV	-	Cathodic Stripping Voltammetry
CuE	-	Copper Electrode
CV	-	Cyclic Voltammetry
DME	-	Dropping Mercury Electrode
DPASV	-	Differential Pulse Anodic Stripping Voltammetry
DPP	-	Differential Pulse Polarography
DPV	-	Differential Pulse Voltammetry
E_{acc}	-	Accumulation Potential
E_i	-	Initial Potential
E_f	-	Final Potential

ECD	-	Electron Capture Detector
EI-MS	-	Electron Impact Mass Spectrometry
ES-MS	-	Electrospray Mass Spectrometry
ESI-MS	-	Electrospray Ionization Mass Spectrometry
ELISA	-	Enzyme-Linked Immunosorbant Assays
EPA	-	Environmental Protection Agency
EPSPS	-	5-enolpyruvylshikimate-3-phosphatesynthase
FD	-	Fluorescence Detection
FMOC-Cl	-	9-fluorenyl-methyloxycarbonyl chloroformate
FPD	-	Flame Photometric Detector
g	-	Gram
GC	-	Gas Chromatography
GLYP	-	Glyphosate
H ₃ BO ₃	-	Boric Acid
HCl	-	Hydrochloric Acid
H ₃ PO ₄	-	Orthophosphoric Acid
HILIC	-	Hydrophilic Interaction Chromatography
HMDE	-	Hanging Mercury Dropping Electrode
HPIC-ICP-DRC-MS-		High Performance Ion Chromatography coupled to Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometry
HPLC	-	High Performance Liquid Chromatography
HPR	-	Horseradish Peroxidase
I _p	-	Peak Current
IC	-	Ion Chromatography
ICP-MS	-	Inductive Coupled Plasma Mass Spectrometry
IMAC	-	Immobilized Metal Ion Affinity
IT-MS	-	Ion Trap Mass Spectrometry
K _a		Acid Dissociation Constant
KCl	-	Potassium Chloride
KH ₂ PO ₄	-	Potassium Dihydrogen Phosphate
KNO ₃	-	Potassium Nitrate
LC	-	Liquid Chromatography
L'ELISA	-	Linker Enzyme Linked Immunosorbant Assay

LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
LQ	-	Liquid Chromatography
M	-	Molar
mL	-	Milliliter
Mv/s	-	Milivolt per Second
MS	-	Mass Chromatography
N ₂	-	Nitrogen Gas
NaCl	-	Sodium Chloride
Na ₂ HPO ₄	-	Disodium Hydrogen Phosphate
NaOH	-	Sodium Hydroxide
NPD	-	Nitrogen Phosphorus Detector
NPP	-	Normal Pulse Polarography
NPV	-	Normal Pulse Voltammetry
ng ml ⁻¹	-	Nanogram per Mililiter
Ni(NO ₃) ₂		Nickel Nitrate
NiAl-NO ₃ -LDH		Nickel Aluminium Nitrate Layered Double Hydroxide
OPA-MERC	-	<i>o</i> -phthaldehyde-mercaptoethanol
pKa		- log ₁₀ Ka
<i>p</i> -value		Probability value
POEA	-	Polyoxyethylene Amine
PBS	-	Phosphate buffer saline
ppm	-	Part per Million
PF-PD	-	Pulsed Flame Photometric Detector
R ²	-	Correlation Coefficient
SCE	-	Saturated Calomel Electrode
SCV	-	Stair Case Voltammetry
SME	-	Supported Liquid Membrane
SPE	-	Solid Phase Extraction
SPE	-	Screen Printed Electrode
SWP	-	Square Wave Polarography
SWV	-	Square Wave Voltammetry
SWSV	-	Square Wave Stripping Voltammetry
TFAA	-	Trifluoroacetic Anhydride

TFE	-	Trifluoroethanol
t_{acc}	-	Accumulation Time
UME	-	Ultramicroelectrode
US	-	United State
UVD	-	Ultraviolet Detector
ν	-	Scan Rate

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research

Since the discovery of its high herbicidal activity by John E. Franz, a Monsanto's scientist in 1970, glyphosate (GLYP) has been used as an active ingredient in herbicides (Roundup or Rodeo) and widely used to control weeds in agriculture, landscape and forest applications (Williams *et al.*, 2000). Due to its extensive application, the residue can be found in crops, vegetables, fruits, soil and also ground water. During 1990-91, glyphosate was ranked eleventh among the conventional pesticides used in the U.S (EPA, 1993). The extensive use of glyphosate has raised a global concern because of many problems related to the environmental contamination, health issues and negative effect to non-targeted organisms or plants.

Chemically, glyphosate consists of three functional groups which are the amine, the carboxylate and the phosphate that can coordinate strongly to metal ions, mostly transition metals such as copper (Songa *et al.*, 2009). Hence, glyphosate possesses a high affinity and chelating capacity for transition metals, resulting in the formation of poorly soluble glyphosate-metal complexes or insoluble precipitate in soil or hard water. Glyphosate degrades rapidly in soil and slowly in water due to a number of microbial activities (Figure 1.1). The main metabolite of glyphosate degradation is aminomethylphosphonic acid, (AMPA) and other metabolites are N-methylaminomethylphosphonic acid, glycine, N,N-dimethylaminomethylphosphonic acid and hydroxymethylphosphonic acid which represent less than one percent of original total glyphosate (Rueppel *et al.*, 1977).

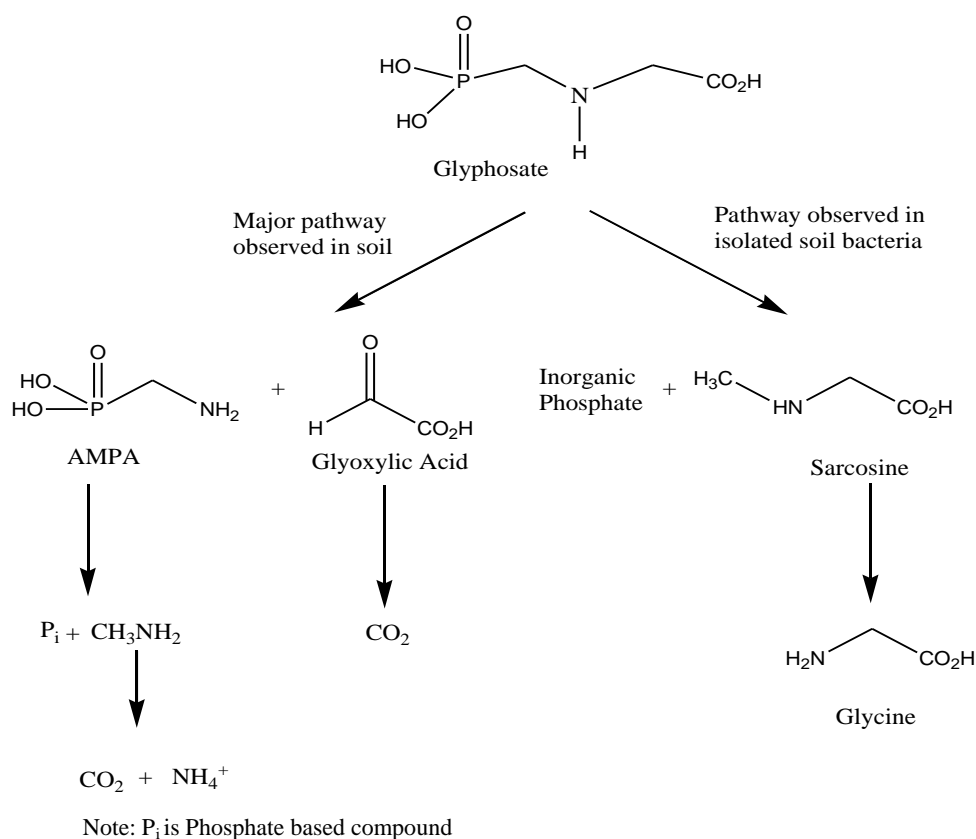


Figure 1.1: Degradation Products of Glyphosate (Giesy *et al.*, 2000)

Glyphosate and AMPA in different matrices can be detected and determined by several analytical techniques such as high performance ion chromatography, HPLC (Bot *et al.*, 2002; Popp *et al.*, 2008; Glass, R. L., 1983; Khrolenko and Wieczorek, 2005; Roseboom and Berkhoff 1982), gas chromatography, GC (Katoaka *et al.*, 1996; Motojyuku *et al.*, 2008), spectrometric method (Jan *et al.*, 2009), capillary electrophoresis, CE (Chang and Liao, 2002; Cikaló *et al.*, 1996; Kodama *et al.*, 2008), hydrophilic interaction chromatography (Coutinho *et al.*, 2007a) ion chromatography, IC (Coutinho *et al.*, 2008; Maure *et al.*, 2009; Zhu *et al.*, 1999) and enzyme-linked immunosorbant assays, ELISA (Clegg *et al.*, 1999; Rubio *et al.*, 2003). Most of these techniques are sensitive and selective but require complicated sample preparation, involving toxic solvents, time consuming and high in cost. The use of electrochemical technique for detection of glyphosate and AMPA is still limited (Songa *et al.*, 2009; Mendez *et al.*, 2007; Khenifi *et al.*, 2009). Electrochemical methods such as voltammetry have the advantages of relatively lower cost, more convenient to operate sensitive and have become the latest trend towards portable and smaller detection system of environmental contaminants.

1.2 Problem Statement

Glyphosate is a systemic, nonselective and post-emergence herbicide used for control a wide range of weed in non-crop situation. Glyphosate has been rated least dangerous in comparison to other herbicides and placed in Toxicity Category III for oral and dermal acute toxicity by the US Environmental Protection Agency (EPA, 1993). However, a recent study has shown that Roundup formulations which contained glyphosate can cause death of human embryonic, placental, and umbilical cells in vitro even at low concentrations (Benachour and Seralini, 2009). This herbicide is also has been reported to give the health hazard to human (Kodama *et al.*, 2008).

Numerous methods were reported in literature for the detection and determination of glyphosate and AMPA by HPLC, GC, IC, CE and ELISA but the derivatization procedures must be performed at either pre- or post-column to convert glyphosate into volatile, chromophore or fluorophore derivatives. In many cases these involve generation of unstable products. The separation method for these compounds has been shown to be difficult due to their properties such as high solubility in water, low solubility in organic solvents, absence of chromophore or fluorophore group, high polarity, low volatility and complexing behavior (Chang and Liao, 2002).

Considering the extensive and widespread use of glyphosate in agriculture, there is a need to develop a simple, rapid, sensitive and effective method for detection and determination of glyphosate and AMPA in the environment using copper electrode (CuE) and hanging mercury drop electrode (HMDE). Although few researchers have successfully shown qualitative application of copper electrode for the detection of glyphosate, literature information on conditions to develop an accurate and sensitive technique for determination of glyphosate and AMPA is still lacking (Coutinho *et al.*, 2007b and Coutinho *et al.*, 2007c).

1.3 Objectives of the Research

The objectives of this research are:

1. To investigate the electrochemical behavior of glyphosate and aminomethylphosphonic acid (main metabolite of glyphosate) in aqueous medium by using copper electrode (CuE) and hanging mercury drop electrode (HMDE).
2. To develop voltammetric techniques for determination of glyphosate and aminomethylphosphonic acid in aqueous samples.
3. To determine optimum conditions for the determination of glyphosate and aminomethylphosphonic acid by differential pulse voltammetry (DPV) and differential pulse stripping voltammetry (DPSV) techniques including interferences study.

1.4 Scope of the Research

This research involved voltammetric investigation of glyphosate and AMPA using copper electrode and HMDE. The investigation involves the following:

1. Cyclic voltammetry (CV) studies on the electrochemical behavior of glyphosate and aminomethylphosphonic acid at a copper electrode and HMDE with emphasis on the effect of concentration and scan rate on the peak current.
2. Differential pulse voltammetry (DPV) studies of glyphosate and aminomethylphosphonic acid under different changing parameters such as buffer, pH of buffer, scan rate (v) and initial potential (E_i).

3. Investigation on the differential pulse stripping voltammetry (DPSV) technique for determination of glyphosate and aminomethylphosphonic acid with optimization of parameters such as buffer, pH of buffer, scan rate (ν), initial potential (E_i), accumulation potential (E_{acc}) and accumulation time (t_{acc}).
4. Application of the optimized parameters for both techniques to investigate the effect of increasing concentration of glyphosate and aminomethylphosphonic acid to the peak current (I_p). From the graph, regression equation, R^2 value, linearity range, limit of detection (LOD) and limit of quantification (LOQ) were obtained.
5. Investigation on the interference using differential pulse stripping voltammetry (DPSV) technique by reacting glyphosate and aminomethylphosphonic acid with increasing amounts of metal ion such as zinc and iron.
6. Application of the differential pulse stripping voltammetry (DPSV) for determination of glyphosate and aminomethylphosphonic acid in real sample. The recoveries studies were carried out and the results were compared with HMDE.

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