

**ASCORBIC ACID DETERMINATION IN NATURAL AND
COMMERCIAL FRUIT JUICES BY DIFFERENTIAL PULSE ANODIC
STRIPPING VOLTAMMETRIC TECHNIQUE AT A GLASSY
CARBON ELECTRODE**

ZAIHASRA BINTI RAZIS

**Final Year Project Report Submitted in
Partial Fulfilment of the Requirement for the
Degree of Bachelor of Science (Hons.) Chemistry
In the Faculty of Applied Science
Universiti Teknologi MARA**

JANUARY 2017

ABSTRACT

ASCORBIC ACID DETERMINATION IN NATURAL AND COMMERCIAL FRUIT JUICES BY DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRIC TECHNIQUE AT A GLASSY CARBON ELECTRODE

Vitamins are important in human diet because they will give sufficient amount of nutrient that needed by human body. Humans cannot synthesize the ascorbic acid, but this vitamin is commonly found in the varieties of vegetables and fruits. Hence, these vegetables and fruits become their main sources of ascorbic acid to meet requirement of dietary intake. The contents of ascorbic acid in the natural and commercial fruit juices must be analyzed. The differential pulse anodic stripping voltammetry (DPASV) technique using glassy carbon electrode (GCE) as a working electrode and phosphate buffer at pH 4.2 as a supporting electrolyte has been proposed to be developed. The experimental voltammetric parameters were optimized in order to obtain a maximum response with analytical validation of the technique. The optimum instrumental conditions for electroanalytical determination of ascorbic acid in phosphate buffer solution at pH 4.2 by the proposed DPASV technique were initial parameter $E_i = 0$ V, end parameter $E_f = 0.7$ V, accumulation time $t_{acc} = 60$ s, scan rate $\nu = 0.125$ V/s, accumulation potential $E_{acc} = 0$ V and pulse amplitude = 0.150 V. The anodic peak was appeared at 0.3598 V. The curve was linear from 0.028 to 1.703 mM ($R^2=0.999$) with detection limit of 0.0114 mM. The precisions in terms of relative standard deviation (RSD) were 1.3%, 0.5% and 0.06%, respectively on the same day precision. The recoveries for the spiked 0.0852 mM (in commercial fruit juice samples) and 0.039 mM (in natural fruit juice samples) concentration of the ascorbic acid standard were 101.93 ± 1.65 % for pineapple sample by squeezing method while in commercial fruits sample; blackcurrent was 80.00 ± 6.25 %, orange was 73.65 ± 1.70 % and mango sample was 97.48 ± 16.90 %. The concentration of ascorbic acid in the commercial fruit juice samples; blackcurrent was 2.0213 mM, orange was 1.8286 mM and mango was 2.9798 mM. Meanwhile, there was no content of ascorbic acid detected for the lychee and guava commercial juice sample. For the natural fruit juice samples, the content of ascorbic acid in the orange was 0.800 mM and pineapple was 0.698 mM. It can be concluded that the developed technique is precise, accurate, rugged, low cost, fast and has potential to be an alternative method for routine analysis of ascorbic acid in the natural and commercial fruit juices.

TABLE OF CONTENT

TABLE OF CONTENTS	iii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
LIST OF SYMBOLS	xii
ABSTRACT	xiv
ABSTRAK	xv

CHAPTER 1 INTRODUCTION

1.1	Tropical Fruits	1
1.2	Commercial Fruits Juices	2
1.3	Ascorbic Acid	7
	1.3.1 Chemistry of Ascorbic Acid	8
	1.3.2 Biological Function of Ascorbic Acid	10
1.4	Problem Statement	10
1.5	Significant Study	11
1.6	Objectives of Study	12

CHAPTER 2 LITERATURE REVIEW

2.1	Analytical Method for Ascorbic Acid Determination in Fruit and Commercial Fruits Juices	13
	2.1.1 High Performance Liquid Chromatography (HPLC)	13
	2.1.2 Titrimetry	14
	2.1.3 Spectrophotometry	16
2.2	Voltammetric Determination of Ascorbic Acid in Fruits and Commercial Fruits Juices	18
2.3	Voltammetric Technique	22
	2.3.1 Instrumentation in Voltammetric Measurement	22

2.3.1.1 Working Electrode	23
2.3.1.2 Reference Electrode	24
2.3.1.3 Auxillary Electrode	25
2.3.2 The Supporting Electrolyte	25

CHAPTER 3 MATERIALS AND METHOD

3.1 Materials	26
3.1.1 Instrumentations	26
3.1.2 Equipment and Apparatus	27
3.1.3 Chemical and Reagents	27
3.2 Methods	28
3.2.1 Reagent and Chemical Preparation	28
3.2.1.1 Ascorbic Acid Stock Solution	28
3.2.1.2 Reagents	28
3.2.1.3 Phosphate buffer solutions	28
3.2.1.4 Sodium Hydroxide (NaOH) solution, 0.1 M	29
3.2.1.5 Hydrochloric Acid (HCl) Solution, 0.1M	29
3.2.2 General Procedure for Voltammetric Technique Analysis	29
3.2.3 Differential Pulse Stripping Voltammetry for Ascorbic Acid Analysis	30
3.2.3.1 Method Optimization	30
3.2.3.1a Effect of Accumulation Time (t_{acc})	30
3.2.3.1b Effect of Scan Rate (v)	30
3.2.3.1c Effect of Accumulation Potential (E_{acc})	31
3.2.3.1d Effect of Pulse Amplitude	31
3.2.3.2 Method Validation	31
3.2.3.2a Linearity	31
3.2.3.2b Limit of Detection (LOD) and Limit of Quantification (LOQ)	32
3.2.3.2c Precision	32

3.2.3.2.d	Accuracy	32
3.2.3.2e	Ruggedness	33
3.2.4	Ascorbic Acid Determination in the Natural Fruit Juices and Commercial Fruits Juices	33
3.2.4.1	Sample Collection and Pre-treatment	33
3.2.4.2	Recovery of Ascorbic Acid Determination in the Natural Fruit Juices and Commercial Fruits Juices	33
3.2.4.3	Determination of Ascorbic Acid in the Natural Fruit Juices and Commercial Fruits Juices	34

CHAPTER 4 RESULTS AND DISCUSSION

4.1	Optimization of Instrumental Conditions	35
4.1.1	Effect of Accumulation Time (t_{acc})	36
4.1.2	Effect of Scan Rate (ν)	37
4.1.3	Effect of Accumulation Potential (E_{acc})	38
4.1.4	Effect of Pulse Amplitude	39
4.1.5	Summary of the Overall Optimization Procedure	40
4.2	Calibration Curve of Ascorbic Acid and Validation of the Proposed DPASV method in Phosphate Buffer Solution at pH 4.2	41
4.2.1	Calibration Curve Ascorbic Acid Standard Solution	42
4.2.2	Determination of Limit of Detection (LOD) and Limit Of Quantification (LOQ)	42
4.3	Validation of the Proposed DPASV Method	43
4.3.1	Precision	43
4.3.2	Accuracy	44
4.3.3	Ruggedness	46
4.3.4	Recovery Studies of Ascorbic Acid in the Natural Fruit Juices and Commercial Fruits Juices	48
4.3.5	Volumetric Determination of Ascorbic Acid in the Natural Fruit Juices and Commercial Fruits Juices	49