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ELECTROCHEMICAL BEHAVIOR AND DETERMINATION OF FAMOTIDINE IN PHARMACEUTICAL FORMULATIONS

Şeniz VATANSEVER

Supervisor: Prof. Dr. H. İsmet GÖKÇEL

Department of Chemistry

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E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliğinin ilgili hükümleri uyarınca Yüksek Lisans Tezi olarak sunduğum "**Electrochemical Behavior and Determination of Famotidine in Pharmaceutical Formulations**" başlıklı bu tezin kendi çalışmam olduğunu, sunduğum tüm sonuç, doküman, bilgi ve belgeleri bizzat ve bu tez çalışması kapsamında elde ettiğimi, bu tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara atıf yaptığımı ve bunları kaynaklar listesinde usulüne uygun olarak verdiğimi, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını, bu tezin herhangi bir bölümünü bu üniversite veya diğer bir üniversitede başka bir tez çalışması içinde sunmadığımı, bu tezin planlanmasından yazımına kadar bütün safhalarda bilimsel etik kurallarına uygun olarak davrandığımı ve aksinin ortaya çıkması durumunda her türlü yasal sonucu kabul edeceğimi beyan ederim.

06 / 02 / 2015

Şeniz VATANSEVER

ÖZET

FAMOTİDİNİN ELEKTROKİMYASAL DAVRANIŞI VE İLAÇ TABLETLERİNDE TAYİNİ

VATANSEVER, Şeniz

Yüksek Lisans Tezi, Kimya Anabilim Dalı

Tez Danışmanı: Prof. Dr. H. İsmet GÖKÇEL

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Famotidinin voltammetrik tayinine ilişkin literatürdeki eksikliğe katkıda bulunmak ve az sayıda yapılmış olan voltammetrik çalışmaları iyileştirmek amacıyla test maddesi olarak seçilen famotidin (FMD) döngüsel ve diferansiyel puls voltammetrisi teknikleriyle kalem ucu elektrotta (PGE) incelenmiştir.

Famotidinin voltammetrik davranışı öncelikle döngüsel ve diferansiyel puls voltammetrisi kullanılarak farklı çalışma elektrotlarıyla araştırılmıştır. Başlangıç potansiyeli ve tarama hızı gibi voltammetrik parametreler optimize edilmiştir. FMD pik akımlarının destek elektrolitinin pH değeriyle ve FMD derişimiyle değişimi DP voltammetrisi ile PGE'da izlenmiştir. Optimum koşullardaki kalibrasyon grafikleri 8.0×10^{-7} – 3.0×10^{-5} M ve 5.0×10^{-5} – 1.0×10^{-3} M derişim aralıklarında doğrusal bulunmuştur.

Tablet formundaki Famoser® adlı ilacın içeriğindeki (40 mg FMD/tablet) FMD'in optimum koşullarda voltammetrik yolla tayini gerçekleştirilmiştir. Bu amaçla standart katma yöntemi kullanılmıştır. Elde edilen sonuçlar üretici firmanın bir Famoser tabletinin FMD içeriği olarak beyan ettiği değerle uyumludur.

Anahtar sözcükler: Famotidin, Kalem Ucu Elektrot, Döngüsel Voltammetri, Differansiyel Puls Voltammetrisi

ABSTRACT

ELECTROCHEMICAL BEHAVIOR AND DETERMINATION OF FAMOTIDINE IN PHARMACEUTICAL FORMULATIONS

VATANSEVER, Şeniz

MSc Thesis in Chemistry
Supervisor: Prof. Dr. H. İsmet GÖKÇEL
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Voltammetric behavior of Famotidine (FMD) selected as test material was investigated on a pencil graphite electrode (PGE) by using cyclic and differential pulse voltammetric techniques for the purpose of contributing to voltammetric determination of Famotidine to improve very few voltammetric studies in the literature.

First of all the voltammetric behavior of FMD was examined by using cyclic and differential pulse voltammetric techniques at different working electrodes. Voltammetric parameters, such as, starting potential, scan rate were optimized. The change of FMD peak currents at PGE was examined against the pH of supporting electrolyte and the concentration of FMD by using DP voltammetry. The calibration graph under the optimized conditions consisted of two linear segments of 8.0×10^{-7} – 3.0×10^{-5} M and 5.0×10^{-5} – 1.0×10^{-3} M.

The content of FMD in Famoser Drug (40 mg FMD in per tablet) was tried to be determined by using DP procedure under the optimum conditions. For this aim, standard additions methods were used. The obtained results were in good agreement with the manufacturer's declared FMD content in a Famoser tablet.

Keywords: Famotidine, Pencil Graphite Electrode, Cyclic Voltammetry, Differential Pulse Voltammetry

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Şeniz VATANSEVER

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CONTENTS

CONTENTS (continued)

Page

CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF SYMBOLS

Symbol	Explanation
ΔE	Amplitude
ΔE_p	Fixed or variable pulse potential
ΔE_s	Fixed change in potential per cycle
Δi	Current sampling
E	Potential
E_p	Peak potential
F	Faraday constant
L	The real film thickness
i	Peak current
i_l	Limiting current
t_p	Pulse time
t_{acc}	Duration of accumulation
t_m	The time after application of the pulse
δ	The thickness of the diffusion layer
n	Number of electrons transferred, successive replicates
t	Time
τ	Cycle time

LIST OF ABBREVIATIONS

Abbreviation	Explanation
AC	Alternating current
AdSV	Adsorptive stripping voltammetry
ASV	Anodic stripping voltammetry
CMEs	Chemically modified electrodes
CSV	Cathodic stripping voltammetry
CV	Cyclic voltammetry
CZE	Capillary zone electrophoresis
DC	Direct current
DME	Dropping mercury electrode
DPV	Differential pulse voltammetry
FMD	Famotidine
GCE	Glassy Carbon Electrode
HMDE	Hanging mercury drop electrode
HPLC	High Pressure Liquid Chromatography
LOD	Limit of detection
LOQ	Limit of qualification
LS AdSV	Linear sweep adsorptive stripping voltammetry
MWCNT	Multiwalled carbon nanotubes
MFE	Mercury film electrode
NPV	Normal Pulse Voltammetry
PGE	Pencil graphite electrode
PSA	Potentiometric stripping analysis
PVC	Poly(vinyl chloride)
RSD	Relative standard deviation

LIST OF ABBREVIATIONS (continued)

Abbreviation	Explanation
SCE	Saturated calomel electrode
SV	Staircase voltammetr
SW AdSV	Square wave adsorptive stripping voltammetry
SWV	Square-Wave Voltammetry
TPB	tetraphenyl borate
$U_{ampl.}$	Pulse amplitude
UV	Ultra-violet
MOPS	3-(N-morpholino)propansulfonic acid

1. NTRODUCTION

Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely, the measurements of electrical quantities, such as current, potential, or charge and their relationship to chemical parameters. Such use of electrical measurements for analytical purposes has found a vast range of applications, including environmental monitoring, industrial quality control, or biomedical analysis (Wang, 2006).

Electrochemistry has always provided analytical techniques characterized by instrumental simplicity, accuracy, low cost, and portability. These techniques have introduced the most promising methods for specific applications. Especially, biologically important molecules and toxic metals can be investigated electroanalytically by voltammetric methods for their determination. Additionally, the mechanisms of electrode reactions can be also thrown light on by these methods. As a result of these, electroanalytical methods have many advantages that make them an appealing choice for pharmaceutical and environmental analysis (Wang, 2006, Gupta et al., 2011).

In addition, the electroanalytical techniques have been shown to be excellent for the determination of pharmaceutical compounds in different matrices. Many of the electroactive constituents of formulations, in contrast to excipients, can be readily oxidized or reduced at the electrode surface. The selectivity of these methods is ordinarily excellent because the analyte can be readily identified by its voltammetric peak potential. Advances in experimental electrochemical techniques in the field of analysis of drugs are in demand because of their simplicity, and relatively short analysis time compared to other techniques. The use of various working electrodes such as mercury, carbon, and chemical modified electrodes for electroanalytical measurements has increased in recent years because of their applicability to the determination of electroactive compounds that undergo oxidation or reduction reactions, which is a matter of great importance in the field of clinical and pharmaceutical analysis (Gupta et al., 2011). These defining and distinguishing characteristics were an important factor

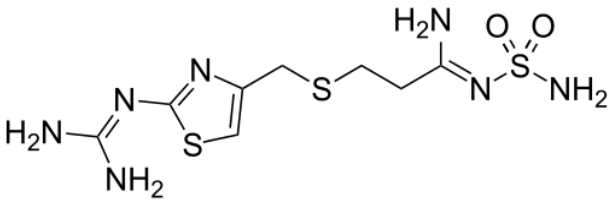
to decide on to use of voltammetric method in this thesis, because it was aimed to develop a sensitive analysis method for famotidine (FMD) and determine the FMD content in a selected drug.

FMD is a histamine H_2 - receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of peptic ulcer disease and a backward flow of stomach acid into the esophagus (gastroesophageal reflux disease). It may be used to prevent intestinal ulcers from returning after treatment. This medication is also used to treat certain stomach and throat problems caused by too much acid (Humphries, 1999).

In recent years, FMD has been investigated as an adjunct in treatment-resistant schizophrenia. In one trial, it caused a 10% reduction in schizophrenic symptom severity in treatment-resistant patients (Meskanen et al., 2013). Therefore the determination of this molecule is significantly important.

1.1 Physicochemical Properties of Famotidine

IUPAC name of FMD is 3-[[2-(diaminomethylideneamino)-1,3-thiazol-4-yl]methylsulfanyl]-N'-sulfamoylpropanimidamide and its chemical structure is given in Figure 1.1. FMD is a white to pale yellow crystalline powder with a density of 1.838 g cm^{-3} and its melting point is 163 - 164ºC at 760 mmHg. The solution of FMD is light-sensitive and should be protected from light (http://pubchem.ncbi.nlm.nih.gov/compound/3325#section=Chemical-and-Physical-Properties, http://en.wikipedia.org/wiki/Famotidine).



Chemical Formula: $C_8H_{15}N_7O_2S_3$ **Molecular Weight:** 337.4454 g mol^{-1}

Figure 1.1 Structural formula of famotidine.

1.2 Previous Studies for Famotidine Determination

FMD is widely used in the treatment and prevention of peptic ulcer disease. After intravenous administration the plasma FMD concentration-time profile exhibits a biexponential decay, with a distribution half-life of about 0.18 to 0.5 h and an elimination half-life of about 2 to 4 h. The volume of distribution of the drug at steady-state ranges from 1.0 to 1.3 L kg^{-1}; plasma protein binding is low (15 to 22%). FMD is 70% eliminated unchanged into urine after intravenous administration (http://www.ncbi.nlm.nih.gov/pubmed/1764869).

On a weight-to-weight basis, the anti-secretory effect of FMD is about 20 and 7.5 times more potent than those of other drugs such as cimetidine and ranitidine, respectively. Plasma FMD concentrations correlate with its anti-secretory effect: values of about 13 and 20 μg L^{-1} produce a 50% reduction in the gastrin-stimulated gastric acid secretion and a fasting intragastric pH>4, respectively (http://www.ncbi.nlm.nih.gov/pubmed/1764869).

Analytical methods such as high pressure liquid chromatography (HPLC) (Zhong and Yeh, 1998; Dowling and Frye, 1999; Zarghi et al., 2005; Ashiru et al., 2007), spectrophotometry (Kelani et al., 2002; Rahman and Kashif, 2003; Wani and Patıl, 2013), spectrofluorimetry (Alamgir et al., 2015), capillary electrophoresis (Helali et al., 2008), and potentiometry (Ayad et al., 2002) were reported in related to the determination of FMD in literature. However, only one study has been found on the voltammetric analysis of FMD (Skrzypek et al., 2005). The analytic parameters of these studies are given in Table 1.1.

Some studies in Table 1.1 in which used different analysis method for FMD determination have been summarized following section. For example, a rapid and sensitive HPLC method was developed about the determination of FMD in human plasma using a monolithic column by Zarghi et al. (2005). The assay enabled the measurement of FMD for therapeutic drug monitoring with a minimum detectable limit (LOD) of 5 ng mL^{-1}. The method involved simple and one-step extraction procedure. The separation was carried out in reversed-phase conditions

using a Chromolith column with an isocratic mobile phase consisting of 0.03 M disodium hydrogen phosphate buffer-acetonitrile adjusted to pH 6.5. The calibration curve plotted with values measured at 267 nm was linear over the concentration range 20 – 400 ng mL^{-1}. The coefficients of variation for inter-day and intra-day assay were found to be less than 8% (Zarghi et al., 2005).

In resent years, two simple, accurate, precise, reproducible and an economical spectrophotometric methods were also developed for the simultaneous estimation of ibuprofen and FMD in pharmaceutical bulk and synthetic mixture by Wani and Patıl (2013). The first one was developed on the basis of Q-absorbance ratio method (method I) for analysis of both the drugs. Wavelengths selected for analysis in Q-absorbance ratio method were 263 nm (λ_{max} of ibuprofen) and 273.80 nm (iso-absorptive wavelength) in 0.1N NaOH, respectively. The second one was based on derivative spectrophotometric method (method II) involving the determination of both the drugs at their respective zero crossing point. The determinations were made at 252.8 nm (zero crossing point of FMD) and 304 nm (zero crossing point of ibuprofen) in 0.1N NaOH. Both the method obeyed Beer-Lambert's law in the concentration range of 150 – 750 μg mL^{-1} for ibuprofen and 5 – 25 μg mL^{-1} for FMD. The mean percentage recovery was found to be 96.28 ± 1.44 for IBU and 98.97 ± 0.57for FMD by method I and 98.86 ± 1.27 for IBU and 97.32 ± 0.82 for FMD by method II. The proposed methods were validated. It was emphasized that the proposed methods were suitable for the routine quality control analysis of ibuprofen and FMD in pharmaceutical formulation (Wani and Patıl, 2013).

Another procedure based on spectrofluorimetry was described by Alamgir et al. (2015) for the determination of FMD from pharmaceutical preparations and biological fluids. After derivatization with benzoin in alkaline medium, fluorescence intensity was measured at 446 nm with excitation wavelength at 286 nm. Linear calibration was obtained with 0.5 – 15 μg mL^{-1} with coefficient of determination 0.997. The parameters affecting the fluorescence intensity were optimized. The pharmaceutical additives and amino acid did not interfere in the determination. The mean percentage recovery (n = 4) calculated by standard addition

Table 1.1 Some reported studies for the determination of FMD and the obtained results.
LOD: limit of detection, LOQ : limit of qualification, RSD: relative standard deviation, NR: not reported

Method	**Linear Range**		**LOD**		**LOQ**		**RSD %**		**Sample**	**Reference**
HPLC	1–100 ng mL^{-1}		NR		1 ng mL^{-1}		NR		human plasma	Zhong and Yeh, 1998
HPLC	Plasma	75.0–1500 ng mL^{-1}	NR		Plasma	75 ng mL^{-1}	NR		human plasma and urine	Dowling and Frye, 1999
	Urine	1.0–20.0 µg mL^{-1}			Urine	1.0 µg mL^{-1}				
HPLC	20–400 ng mL^{-1}		NR		15 ng mL^{-1}		NR		human plasma	Zarghi et al., 2005
HPLC	0.5–500 µg mL^{-1}		0.3 µg mL^{-1}		0,3 µg mL^{-1}		NR		human urine	Ashiru et al., 2007
Spectrophotometry	10–60 µg mL^{-1}		11.6 µg mL^{-1}		43.56 µg mL^{-1}		0.871–0.976		drug formulations	Kelani et al., 2002
Spectrophotometry	5–30 µg mL^{-1}		0.16 µg mL^{-1}		NR		0.60–0.94		drug formulations	Rahman and Kashif, 2003
Spectrophotometry	5–25 µg mL^{-1}		**Method 1**	0.3346 µg mL^{-1}	**Method 1**	1.0138 µg mL^{-1}	0.57		pharmaceutical bulk and synthetic mixture	Wani and Patıl, 2013
			Method 2	0.6954 µg mL^{-1}	**Method 2**	2.0862 µg mL^{-1}	2.13			
Spectrofluorimetry	0.5–15 µg mL^{-1}		0.022 µg mL^{-1}		0.074 µg mL^{-1}		intra-day 2.11 inter-day 0.74		pharmaceutical formulations	Alamgir et al., 2015
Electrophoresis	1.5–48 µg mL^{-1}		0.09 µg mL^{-1}		NR		0.98–1.94		pharmaceutical formulations	Helali et al., 2008
Potentiometry	1×10^{-3}–1×10^{-5} M		NR		NR		0.803		pharmaceutical formulations	Abdellatef et al., 2002
Voltammetry	1×10^{-9}–4×10^{-8}M		LS AdSV	1.8×10^{-10}M	LS AdSV	6.2×10^{-10}M	LS AdSV	19	urine	Skrzypek et al., 2005
			SW AdSV	4.9×10^{-11}M	SW AdSV	1.6×10^{-10}M	SW AdSV	9		
DP Voltammetry	2×10^{-6}–9×10^{-5} mol L^{-1}		3.73×10^{-7}mol L^{-1}		1.24×10^{-1} mol L^{-1}		NR		urine	Yagmur et al., 2014

from pharmaceutical preparation was 94.8 – 98.2% with relative standard deviation (RSD) 1.56 – 3.34% and recovery from deproteinized spiked serum and urine of healthy volunteers was 98.6 – 98.9% and 98.0 – 98.4% with RSD 0.34 – 0.84% and 0.29 – 0.87%, respectively (Alamgir et al., 2015).

Two new potentiometric methods for determination of FMD in pure form and in its pharmaceutical tablet form were developed by Ayad et al. In the first method, the construction of plasticized poly(vinyl chloride) (PVC) matrix-type FMD ion-selective membrane electrode and its use in the potentiometric determination of FMD in pharmaceutical preparations were described. It was based on the use of the ion-associate species, formed by FMD cation and tetraphenyl borate (TPB) counterion. The electrode exhibited a linear response for 1×10^{-3} – 1×10^{-5} M of FMD solutions over the pH range 1 – 5 with an average recovery of 99.26% and mean standard deviation of 1.12%. Common organic and inorganic cations showed negligible interference. In the second method, the conditions for the oxidimetric titration of FMD were studied. The method was dependent on using lead(IV) acetate for oxidation of the thioether contained in FMD. The titration was carried out in presence of catalytic quantities of potassium bromide. Direct potentiometric determination of 1.75×10^{-2} M FMD solution showed an average recovery of 100.51% with a mean standard deviation of 1.26%. Both methods were applied successfully to commercial tablet (Ayad et al., 2002).

A simple and rapid capillary zone electrophoresis (CZE) method with UV detection was developed for the determination of FMD and its potential impurities in pharmaceutical formulations. The electrophoretic separation of these compounds was performed using a fused silica capillary and 37.5 mM phosphate buffer (pH 3.5) as the electrolyte. Under the optimized conditions, six impurities could be resolved from the FMD peak in less than 7 min. The calibration curves obtained for the seven compounds were linear over the concentration range investigated (from 1.5 to 78.5 $\mu g\ mL^{-1}$). The intra- and inter-day relative standard deviations for the migration times and corrected peak areas were less than 2% and 5%, respectively. The detection limits were found to be 0.09 $\mu g\ mL^{-1}$ for FMD, and from 0.1 to 0.62 $\mu g\ mL^{-1}$ depending on the impurities. The method has been

successfully applied to the determination of FMD in commercial dosage forms (Helali et al., 2008).

The above mentioned methods for determination of FMD require expensive instrumentation, and the operations are time-consuming such as derivatization reaction. On the other hand, voltammetric methods are highly selective, sensitive, rapid, and economical. Nevertheless, the voltammetric characteristics of FMD have not been reported sufficiently. There are very few published studies related to voltammetric determination of FMD (Skrzypek et al., 2005, Yağmur et al., 2014).

Skrzypek et al. (2005) carried out electrochemical studies on determination of FMD using various voltammetric techniques, such as cyclic voltammetry (CV), linear sweep and square wave adsorptive stripping voltammetry (LS AdSV, SW AdSV) at a controlled growth mercury drop electrode. The dependence of the current on pH, buffer concentration, nature of the buffer, and scan rate was investigated. The best results for the determination of FMD were obtained in 3-(N-morpholino)propansulfonic acid (MOPS) buffer solution at pH 6.7. This procedure enabled to determine FMD in the concentration range $1\times10^{-9} - 4\times10^{-8}$ M by LS AdSV and $5\times10^{-10} - 6\times10^{-8}$ M by SW AdSV. The value of LOD and LOQ were found to be 1.8×10^{-10} and 6.2×10^{-10} M for LS AdSV and 4.9×10^{-11} and 1.6×10^{-10} M for SW AdSV, respectively. This method was also applied for the determination of FMD in urine (Skrzypek et al., 2005).

When all this is taken into account, to make a significant contribution to the literature in this field, the voltammetric determination of FMD is the focus in this thesis.

1.3 Voltammetry

In voltammetry 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 as a function of applied potential is called a voltammogram providing quantitative and qualitative information about the

species involved in the oxidation or reduction reaction. All voltammetric techniques are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it. The earliest voltammetric technique to be introduced was polarography, which was developed by Jaroslav Heyrovsky in 1922. Many different forms of voltammetry (Fig. 1.2) have been developed (Harvey, 2000; Kounaves, 1997).

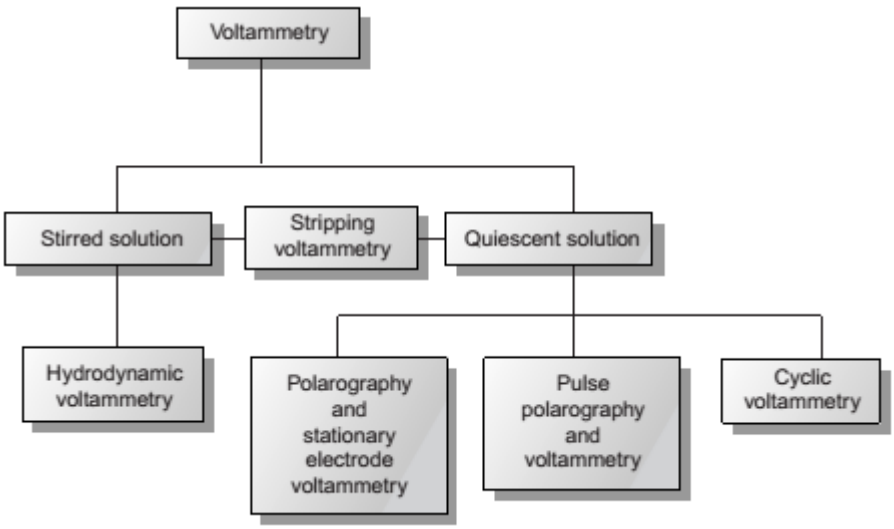


Figure. 1.2 Voltammetry and its different forms (Harvey, 2000).

The early voltammetric methods experienced a number of difficulties for routine analytical use. However, in the 1960s and 1970s significant advances were made in theory, methodology, and instrumentation areas of voltammetry. The coincidence of these advances with the advent of low-cost operational amplifiers also facilitated the rapid commercial development of relatively inexpensive instrumentation (Kounaves, 1997).

Although early voltammetric methods relied on the use of only two electrodes, modern voltammetry makes use of a three-electrode potentiostat. A time-dependent potential excitation signal is applied to the working electrode, changing its potential relative to the fixed potential of the reference electrode.

The resulting current between the working and auxiliary electrodes is measured. The auxiliary electrode is generally a platinum wire, and the SCE and Ag/AgCl electrode are common reference electrodes. Several different materials have been used as working electrodes, including mercury, platinum, gold, silver, and carbon (Harvey, 2000).

The analytical advantages of the voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1} M), a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times, simultaneous determination of several analytes, the ability to determine kinetic and mechanistic parameters, a well-developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential waveforms can be generated and small currents measured (Kounaves, 1997).

Analytical chemists routinely use voltammetric techniques for the quantitative determination of a variety of dissolved inorganic and organic substances. Inorganic, physical, and biological chemists widely use voltammetric techniques for a variety of purposes, including fundamental studies of oxidation and reduction processes in various media, adsorption processes on surfaces, electron transfer and reaction mechanisms, kinetics of electron transfer processes, and transport, speciation, and thermodynamic properties of solvated species. Voltammetric methods are also applied to the determination of compounds of pharmaceutical interest (Kounaves, 1997).

1.3.1 Polarography

Polarography is a specific type of linear-sweep voltammetry where the electrode potential is linearly altered from the initial potential to the final potential. Polarography differs from the others in the respect that a dropping mercury electrode (DME) serves as the microelectrode. As a linear sweep method controlled by convection/diffusion mass transport, the current vs. potential response of a polarographic experiment has the typical wave shape (Tural et al., 2010; http://en.wikipedia.org/wiki/Polarography).

1.3.2 Cyclic voltammetry

CV is a type of potentiodynamic electrochemical measurement. In a CV experiment the working electrode potential is ramped linearly versus time in one direction, either to more positive potentials or to more negative potentials (like linear sweep voltammetry). In cyclic voltammetry we complete a scan in both directions. This cycle can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage (Fig. 1.3). CV is generally used to study the electrochemical properties of an analyte in solution and throw light on electrode reaction mechanism (http://chemwiki.ucdavis.edu; http://en.wikipedia.org/wiki/File:Cyclovoltammogram.jpg).

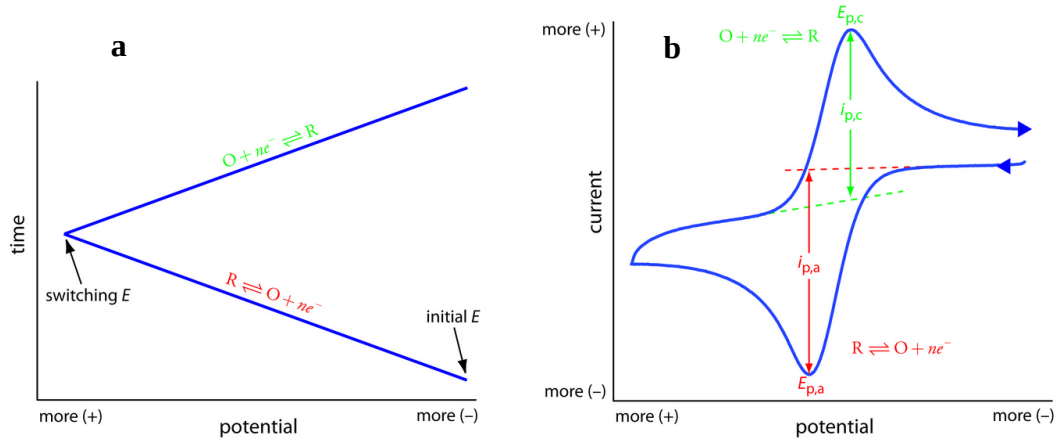


Figure 1.3 a) One cycle of the triangular potential-excitation signal showing the initial potential and the switching potential, b) the resulting cyclic voltammogram showing the measurement of the peak currents and peak potentials for a reversible electrode process (http://chemwiki.ucdavis.edu).

1.3.3 Pulse voltammetric techniques

The basis of all pulse techniques is the difference in the rate of the decay of the charging and the faradaic currents following a potential step (or "pulse"). The charging current decays exponentially, whereas the faradaic current (for a diffusion-controlled current) decays as a function of $1/(\text{time})^{1/2}$; that is, the rate of decay of the charging current is considerably faster than the decay of the faradaic current. The charging current is negligible at the end of a potential pulse. Therefore, the measured current consists solely of the faradaic current; that is, measuring the current at the end of a

potential pulse allows discrimination between the faradaic and charging currents (http://www.basinc.com/mans/EC_epsilon/Techniques/Pulse/pulse.html).

A number of different pulse techniques which differ in their potential pulse wave forms, the number of sampling points are developed. These techniques at a solid electrode or mercury drop electrode can be successfully applied. In this case, they are called as polarography or voltammetry, respectively. These are briefly explained below. The discrimination against the charging current that is inherent in these techniques leads to lower detection limits (when compared to linear sweep techniques), which makes these techniques suitable for sensitive quantitative analysis (http://www.basinc.com/mans/EC_epsilon/Techniques/Pulse/pulse.html).

1.3.3.1 Normal pulse voltammetry

Normal pulse voltammetry (NPV) consists of a series of pulses of increasing amplitude applied to successive drops (or cycle of time) at a preselected time near the end of each drop lifetime (or cycle of time). Such a normal pulse train is shown in Figure 1.4a. Between the pulses, the electrode is kept at a constant (base) potential at which no reaction of the analyte occurs (Wang, 2006).

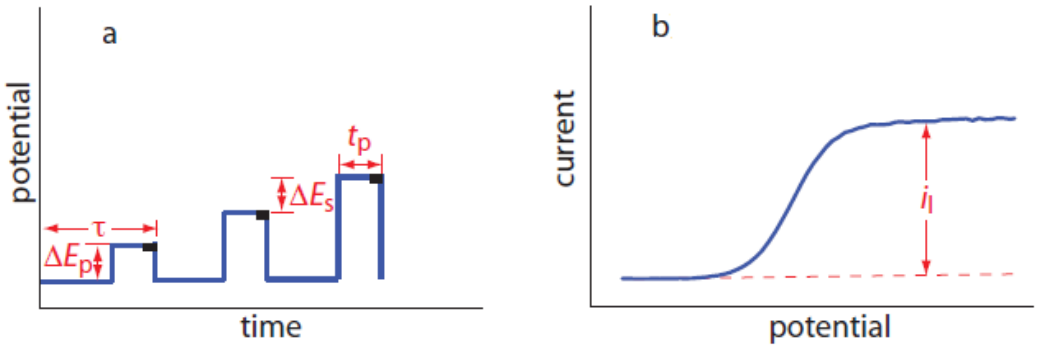


Figure 1.4 a) Potential-excitation signal and b) resulting voltammogram for NPV. ▪: Current sampling, τ : cycle time, t_p : pulse time, ΔE_p: fixed or variable pulse potential, ΔE_s: fixed change in potential per cycle, i_l : limiting current (http://chemwiki.ucdavis.edu).

The height of the potential pulse (amplitude of the pulse) increases linearly with each drop or cycle of time in the polarographic or voltammetric technique, respectively. The current is measured about 40 ms after the pulse is applied, at which time the contribution of the charging current is nearly zero. In addition, because of the short pulse duration, the diffusion layer is thinner than that of

direct current (DC) polarography and hence the faradaic current is increased. The resulting voltammogram has a sigmoidal shape (Fig. 1.4b), with a limiting current given by a modified Cottrell equation:

$$i_l = \frac{nFAD^{1/2}C}{\sqrt{\pi t_m}}$$

where t_m is the time after application of the pulse where the current is sampled. When this current can be compared to that measured in DC polarography it is predicted that normal-pulse polarography will be 5 – 10 times more sensitive than DC polarography. Normal - pulse polarography may be advantageous also when using solid electrodes. In particular, by maintaining a low initial potential during most of the operation, it is possible to alleviate surface fouling problems (due to adsorbed reaction products) (Wang, 2006).

1.3.3.2 Differential pulse voltammetry

Differential pulse voltammetry (DPV) is comparable to NPV in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential (Kounaves, 1997).

A potential waveform for DP voltammogram is shown in Figure 1.5a. In DP polarography, a dropping mercury electrode is used and the old drop is dislodged after each pulse. Similar to NPV, simple theoretical formulas can only be obtained if the initial boundary conditions are renewed after each pulse. However, since the DP voltammogram technique is mainly used in electroanalysis, just one drop or a solid electrode is used to obtain a voltammogram (Stojek, 2010).

The importance of DP voltammogram in chemical analysis is based on its superior elimination of the capacitive/background current. This is achieved by sampling the current twice: once before pulse application and then at the end of the pulse. The output from the potentiostat/ voltammograph is equal to the difference in the two current values. The double current sampling allows the analyst to detect the analytes present in the solution at a concentration as low as 0.05 μM. Another

consequence of double sampling is that the DP voltammograms are peak – shaped as shown in Figure 1.5b (Stojek, 2010).

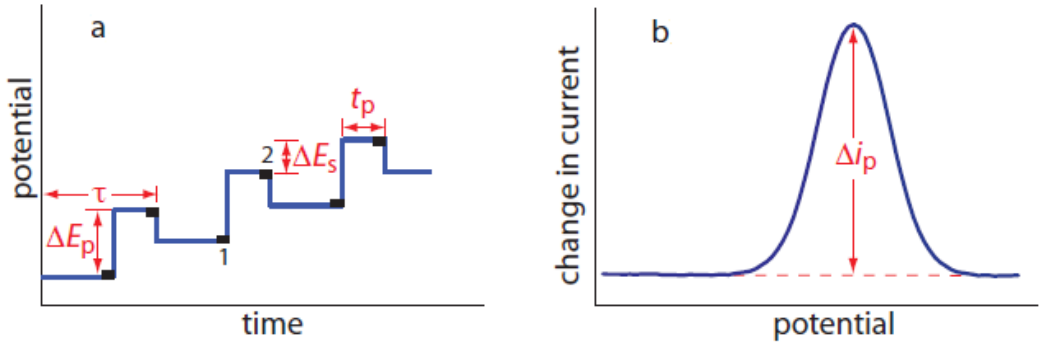


Figure 1.5 a) Potential-excitation signal and b) resulting voltammogram for DPV. ▪: Current sampling, τ : cycle time, t_p : pulse time, ΔE_p: fixed or variable pulse potential, ΔE_s: fixed change in potential per cycle, i_p : peak current (http://chemwiki.ucdavis.edu).

1.3.3.3 Square - wave voltammetry

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 (Fig. 1.6a) (Wang, 2006)..

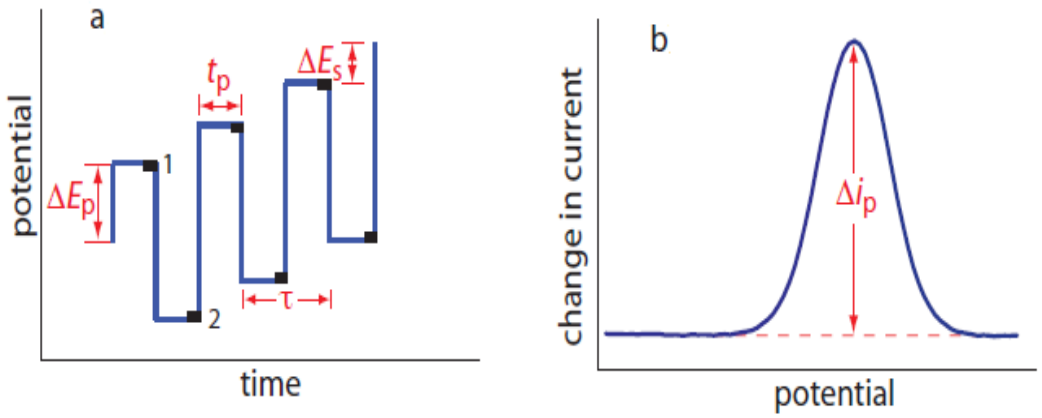


Figure 1.6 a) Potential-excitation signal and b) resulting voltammogram for SWV. ▪: Current sampling, τ : cycle time, t_p : pulse time, ΔE_p: fixed or variable pulse potential, ΔE_s: fixed change in potential per cycle (http://chemwiki.ucdavis.edu).

The current is sampled twice during each square-wave cycle, once at the end of the forward pulse and once 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 vs. the base staircase potential (Fig. 1.6b).

The resulting peak-shaped voltammogram for a rapid reversible redox system is symmetrical about the half-wave potential, and the peak current is proportional to the concentration (Fig. 1.7). 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 background current, very low detection limits near 10^{-8} M can be attained. Comparison between square-wave and differential-pulse voltammetry for reversible and irreversible cases indicated that the square-wave currents are 4 and 3.3 times higher, respectively, than the analogous differential-pulse response (Wang, 2006)..

The major advantage of square-wave voltammetry is its speed. The analysis time is drastically reduced; a complete voltammogram can be recorded within a few seconds, as compared with about 2–3 min in differential-pulse voltammetry (Wang, 2006).

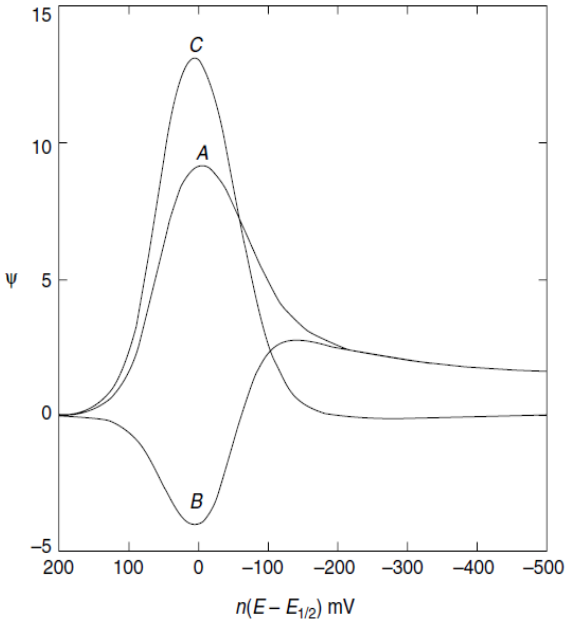


Figure 1.7 SW voltammograms for reversible electron transfer. A: forward current; B: reverse current; C: net current (Wang, 2006)

1.3.3.4 Staircase voltammetry

This is the simplest pulse voltammetric technique; however, it is probably also the one most often used for a dynamic electrochemical examination of various compounds. The sequence of pulses in staircase voltammetry (SV) forms a potential staircase. An appropriate potential waveform is given in Figure 1.8a.

SV is in fact a modified, discrete linear scan (or cyclic) voltammetry. The potential scan can be reversed in SV, similarly as it is done in cyclic voltammetry, and then a cyclic staircase voltammogram can be obtained. Staircase voltammograms (Fig.1.8b) are peaked-shaped the same as linear scan voltammograms. There are some differences between these voltammetric techniques anyway. A linear scan (or cyclic) voltammogram forms a continuous current vs. potential curve, while each staircase voltammogram consists of a number of i – E points. Also, the peak heights obtained under conditions of identical scan rates in linear scan and staircase voltammetries may differ considerably (Stojek, 2010).

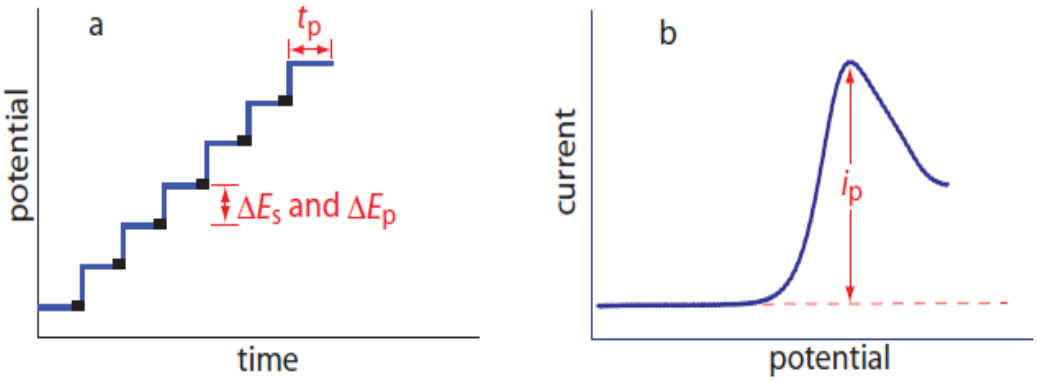


Figure 1.8 a) Potential-excitation signal and b) resulting voltammogram for SV. ▪: Current sampling, t_p : pulse time, ΔE_p: fixed or variable pulse potential, ΔE_s: fixed change in potential per cycle (http://chemwiki.ucdavis.edu).

1.3.4 Alternating current voltammetry

Alternating current (AC) voltammetry is frequency-domain technique which involves the superimposition of a small-amplitude AC voltage on a linear ramp.

Usually the alternating potential has a frequency of 50-100 Hz and an amplitude of 10-20 mV. The background current and noise caused by capacitance effects can be diminished by phase-sensitive measurement of the in-phase component of the alternating current. The resulting AC current is plotted against the potential and a peak-shaped voltammogram obtains. The peak potential is the same as that of the polarographic half-wave potential. The peak current is proportional to the concentration of the analyte (Wang, 2006).

This mode is suitable for application of detection limits ($5x10^{-7}$ M) lower than DC mode. Furthermore, the possibility of measuring the change in the drop capacity allows determining adsorbed but non-electroactive species (Wang, 1994).

1.3.5 Stripping analysis

Stripping analysis is an extremely sensitive electrochemical technique for measuring trace metals. Its remarkable sensitivity is attributed to the combination of an effective preconcentration step with advanced measurement procedures that generates an extremely favorable signal-to-background ratio. Since the metals are preconcentrated into the electrode by factors of 100-1000, detection limits are lowered by 2-3 orders of magnitude compared to solution-phase voltammetric measurements. Hence, four to six metals can be measured simultaneously in various matrices at concentration levels down to 10^{-10} M, utilizing relatively inexpensive instrumentation. The ability to obtain such low detection limits strongly depends on the degree to which contamination can be minimized. (Wang, 2006).

Stripping analysis is a two-step technique. The first, or deposition step, involves the electrolytic deposition of a small portion of the metal ions in solution into the electrode to pre-concentrate the metals. This is followed by the stripping step (the measurement step), which involves the dissolution (stripping) of the deposit (Fig. 1.9). Different versions of stripping analysis, which are briefly summarized below, can be employed, depending upon the nature of the deposition and measurement steps (Wang, 2006).

1.3.5.1 Anodic stripping voltammetry

Anodic stripping voltammetry (ASV), which is the most widely used form of stripping analysis, is commonly applied to the analytical determination of a wide range of trace metals capable of forming an amalgam. The method has two stages: first, a preconcentration step is performed in which electrodeposition of metal ions in solution leads to the accumulation of metal as an amalgam. In this stage the potential is held at a negative potential. Second, the electrode potential is swept to positive potentials, inducing the oxidation of the metal in the mercury electrode.

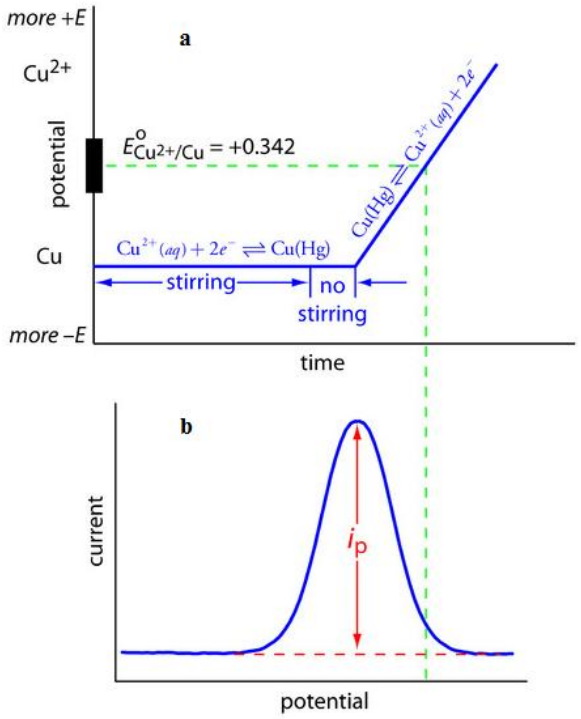


Figure 1.9 a) Potential-excitation signal and b) voltammogram for determination of Cu(II) at a hanging mercury drop electrode or a mercury film electrode using ASV (http://chemwiki.ucdavis.edu).

The highest sensitivity is obtained if a thin mercury film covered rotating disk electrode is used in the combination with SWV as a stripping technique. On this electrode the accumulation is performed under hydrodynamic conditions, which provide effective and stable mass transfer during this step but usually the rotating of the electrode must be stopped before the stripping peaks are recorded in order to decrease the electrical noise. So, a short rest period is introduced between two steps to allow the solution to calm down. The factor of preconcentration is inversely proportional to the film thickness:

$C_{M(Hg)} / C = D\ t_{acc} / L\delta$

where $C_{M(Hg)}$ and C are concentrations of metal atoms in mercury and metal ions in the bulk of the solution, respectively, t_{acc} is a duration of accumulation and δ is the thickness of the diffusion layer at the rotating electrode during the accumulation period, L is the real film thickness (Mirceski et al, 2007).

A particular advantage of ASV is that speciation of the metal in solution is possible compared to AAS which only yields the total metal concentration (Wang, 2006).

1.3.5.2 Cathodic stripping voltammetry

Cathodic stripping voltammetry (CSV) is the mirror image of ASV. When the potential is held at a positive value (anodic deposition) followed by scanning in a negative direction, the technique is called as CSV. Electrode reactions are:

$$A^{n-} + Hg \underset{\text{stripping}}{\overset{\text{deposition}}{\rightleftharpoons}} HgA + ne^-$$

The resulting reduction peak current provides the desired quantitative information. CSV is used to measure a wide range of organic and inorganic compounds, capable of forming insoluble salts with mercury. Among these are various thiols or penicillin's, halide ions such as cyanide, and sulfide. Highly sensitive measurements can thus be performed (Wang, 2006).

1.3.5.3 Adsorptive stripping voltammetry

When the preconcentration step is adsorption, the technique is adsorptive stripping voltammetry (AdSV). AdSV broadens even more the range of species that can be analyzed by the stripping techniques. It often offers a significant improvement in sensitivity and selectivity of metal ions analysis. The principle of the method is the formation of surface-active metal complex which is subsequently adsorbed and undergoes reduction at the electrode surface. The electrochemical process may involve the ligand as well as the metal center. The

method is also suitable for numerous important organic compounds (polycyclic hydrocarbons, nucleic acids and drugs). For different species, either cathodic or anodic stripping can be utilized. While very useful for trace analysis, the technique has limitations for higher concentrations due to the limited number of adsorption sites at the electrode surface (Zoski, 2007).

1.3.5.4 Potentiometric stripping analysis

Potentiometric stripping analysis (PSA) is an alternative for metal ions analysis. As with ASV, the pre-electrolysis is carried out under potential-controlled conditions. Amalgamated metals are subsequently stripped by either applying a controlled anodic current or addition of an oxidizing agent to the solution. The resulting chrono-potentiogram presents stripping plateaus corresponding to the different metals. These plateaus are qualitatively identified using the Nernst equation for each $M^{n+}/M(Hg)$ couple. The quantitative analysis can be facilitated by differentiating the E vs. t curve yielding a peak shaped response.

1.3.6 Working electrodes used in voltammetry

The performance of the voltammetric procedure is strongly influenced by the working electrode material. The working electrode should provide high signal-to-noise characteristics and reproducible responses. Thus, its selection depends on some factors: the redox behavior of the target analyte, the background current over the potential window, electrical conductivity, surface reproducibility, mechanical properties, cost, availability, and toxicity. A range of materials have found application as working electrodes for electroanalysis such as mercury, carbon or noble metals (particularly platinum and gold) (Wang, 2006).

Mercury Electrodes: Mercury is widely used in the practice of electroanalytical chemistry, both for working electrodes and for reference electrodes. The use of mercury is nearly ideal for working electrode construction for several reasons. Mercury has a large liquid range (-38.9 to 356.9 ºC at normal pressure), and therefore electrodes of various shapes may be easily prepared either in pure form or by deposition of mercury on the conducting support. The surface

of such electrodes is highly uniform and reproducible if the mercury is clean (Kissinger and Heineman, 1996).

One of the most important reasons for the application of mercury to the construction of working electrodes is the very high over potential for hydrogen evolution on such electrodes. Relative to a platinum electrode, the over potential of hydrogen evolution under comparable conditions on mercury will be –0.8 to –1.0 V. It is therefore possible in neutral or (better) alkaline aqueous solutions to reduce alkali metal cations at mercury electrodes, giving relatively well defined polarographic waves at potentials more negative than –2.0 V vs. saturated calomel electrode (SCE). In some non-aqueous systems even –0.3 V vs. SCE (aqueous) is accessible (Kissinger and Heineman, 1996).

There are several types of mercury electrodes. Of these, the dropping mercury electrode (DME), the hanging mercury drop electrode (HMDE), and mercury film electrode (MFE) are the most frequently used (Wang, 2006).

Unfortunately, mercury electrodes have serious limitations in applications at positive potentials and this has led to extensive research in the development of solid metal and carbon electrodes. In addition, the toxicity of mercury makes it less and less popular. The risk associated with mercury electrodes is their use, handling and disposal of waste because of its toxicity (Kissinger and Heineman, 1996; Jaimez et al., 2013).

Carbon electrodes: Solid electrodes based on carbon are currently in widely use in electroanalysis, primarily because of their broad potential window, low background current, rich surface chemistry, low cost, chemical inertness, and suitability for various sensing and detection applications. In contrast, electron transfer rates observed at carbon surfaces are often slower than those observed at metal electrodes. The electron-transfer reactivity is strongly affected by the origin and history of the carbon surface. While all common carbon electrode materials share the basic structure of a six-membered aromatic ring and sp^2 bonding, they differ in the relative density of the edge and basal planes at their surfaces. The

edge orientation is more reactive than the graphite basal plane toward electron transfer and adsorption. Materials with different edge-to-basal plane ratios thus display different electron-transfer kinetics for a given redox analyte. The edge orientation also displays undesirably high background contributions. A variety of electrode pretreatment procedures have been proposed to increase the electron-transfer rates. The type of carbon, as well as the pretreatment method, thus has a profound effect upon the analytical performance. The most popular carbon electrode materials are those involving glassy carbon, carbon paste, carbon fiber, screen printed carbon strips, carbon films, or other carbon composites. (Wang, 2006, McCreery, 1991).

Glassy-carbon electrodes (GCE): Glassy (or "vitreous") carbon has been very popular because of its excellent mechanical and electrical properties, wide potential window, chemical inertness (solvent resistance), and relatively reproducible performance. The material is prepared by means of a carefully controlled heating program of a remodeled polymeric (phenol-formaldehyde) resin body in an inert atmosphere. The carbonization process is carried out very slowly over the 300-1200°C temperature range to insure the elimination of oxygen, nitrogen, and hydrogen.

The structure of glassy carbon involves thin, tangled ribbons of cross-linked graphite-like sheets. Because of its high density and small pore size, no impregnating procedure is required. However, a surface pretreatment is usually employed to create active and reproducible GCEs and to enhance their analytical performance. Such pretreatment is usually achieved by polishing (to a shiny "mirror-like" appearance) with successively smaller alumina particles (down to 0.05μm) on a polishing cloth. The electrode should then be rinsed with deionized water before use. Additional activation steps, such as electrochemical, chemical, heat, or laser treatments, have also been used to enhance the performance. The improved electron-transfer reactivity has been attributed to the removal of surface contaminants, exposure of fresh carbon edges, and an increase in the density of surface oxygen groups (which act as interfacial surface mediators) (Wang, 2006).

Chemically modified electrodes: Chemically modified electrodes (CMEs) comprise a relatively modern approach to electrode systems that finds utility in a wide spectrum of basic electrochemical investigations, and the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electro chromic displays, corrosion protection, and electro-organic syntheses (Durst et al., 1997).

Compared with other electrode concepts in electrochemistry, the distinguishing feature of a CME is that a generally quite thin film of a selected chemical is bonded to or coated on the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner. The range of electrode surface properties includes, but is more diverse than, that of ion-selective electrodes which also involve, in their highest forms, rational design of the phase-boundary, partition and transport properties of membranes on or between electrodes (Durst et al., 1997).

Pencil graphite electrode (PGE): In recent years, it draws the attention that PGEs have been used as the bare or modified working electrode in many studies. When compared with other carbon based electrodes, PGEs have some advantages such as high electrochemical reactivity, commercial availability, good mechanical rigidity, disposability, low costs, low technology, and easy of modification. Additionally, Gowda and Nandibewoor (2014) reported that PGEs offer a renewable surface, which is simpler and faster than polishing procedures and results in good reproducibility for the individual surfaces. Due to their useful and important functions, recently many scientists have focused on the usage of these electrodes in many electroanalytical applications. For example, Özcan and Şahin (2010) reported that electrochemically treated PGEs could successfully be used for the determination of low levels of dopamine in blood serum. Their results showed that electrochemically treated PGEs were very promising as a good adsorbent for organic molecules. Furthermore the electrocatalytic oxidation of NADH was investigated using a pencil graphite electrode modified with quercetin by Dilgin et al. (2013) and good results were obtained.

1.4 Purpose of Thesis

The main purpose of this thesis is the investigation of voltammetric behavior of FMD especially using PGE and then, to develop an electrochemical procedure for the sensitive, simple and low-cost for determination of this substance.

The voltammetric determination of FMD is the focus on this thesis because very few studies (Skrzypek et al., 2005, Yağmur et al., 2014) have been reported on this topic according our literature research. To make a significant contribution to the literature in this field, cyclic and DP voltammetric study was planned. After the effective parameters such as pH of supporting electrolyte solution, scan rate of potential, type of working electrode are optimized; the developed voltammetric procedure will be applied to determination of FMD in a pharmaceutical sample.

2. MATERIALS AND METHODS

2.1 Apparatus

All electrochemical experiments were carried out using a Compactstat Electrochemical Interface (Ivium Technologies, Eindhoven, The Netherlands) (Fig. 2.1A) and a voltammetric cell with a small working volume (Fig. 2.1B).

Cyclic and differential pulse (DP) voltammetric experiments were performed in a traditional three-electrode system using a platinum wire as auxiliary electrode, an $Ag/AgCl/KCl_{sat}$ as reference electrode (BASi Corporate Headquarters, West Lafayette, USA) and a pencil graphite electrode (PGE) as working electrode. A Bandelin Sonorex RK 100H ultrasonic bath was used to clean the surface of glassy carbon electrode (GCE) when it was used as working electrode in some sections of the thesis

A HI 221 Hanna pH-meter with a combined glass electrode (Hanna HI 1332) was used to adjust the pH values of the solutions. The solutions throughout this work were all prepared using deionized water from a Milli-Q (Millipore, Bedford, USA) device.

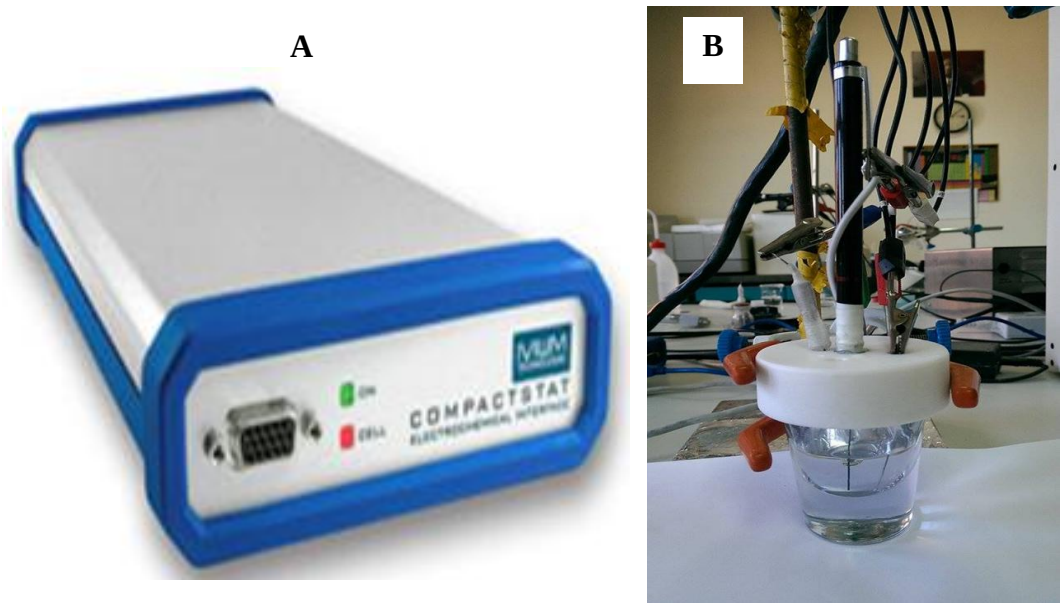


Figure 2.1 **A**) Compactstat Electrochemical Interface (Ivium Technologies), **B**) voltammetric cell equipped electrodes.

2.2 Chemicals and Solutions

The chemicals used throughout this study and listed below were of analytical-reagent grade and supplied from Merck. The properties of these chemicals were given in Table 2.1.

Table 2.1 Some chemicals used in the study and their properties.

Chemicals	**Percentage %**	**Density (g mL^{-1})**
H_3BO_3	99.5-105.5	-
NaOH	99 (pellets GR for analysis)	-
CH_3COOH	96	1.05
H_3PO_4	85 (suitable for use excipient)	1.71
HNO_3	65	1.40
KCl	99.5	-

Chemical reference material for famotidine was purchased as solid from Dr. Reddy's (Medak, India). The properties of this reference material are given in Table 2.2 according to its certificate of analysis. Famoser® commercial drug contained 40 mg FMD per tablet was supplied from the local pharmacy in Turkey (barcode number: 8699578091026, expiration date: April 2016).

The standard stock solution of FMD (1.0×10^{-2} M), containing about 0.1 M HNO_3 was prepared by dissolving 0.0101 g of famotidine and diluting to 3.0 mL with 0.1 M HNO_3 solution. The working solutions with lower concentrations were prepared by further dilution of them via transfer with a digital adjustable transfer pipettes.

The sample solution of Famoser® drug was carefully prepared. For this purpose, five tablets were grounded to a fine powder and homogenized. A Famoser tablet has a weight of 0.2063 g (average of 5 tablets) and involves 40 mg FMD per tablet. Then, this obtained powder (0.2063 g) was added into 50 mL water containing about 0.2 M HNO_3 for dissolving. The obtained heterogenic tablet mixture solution was filtered and washed. Then obtained supernatant solution was diluted 100 mL water. The solutions of standard FMD and Famoser® tablet were stored in the dark and refrigerated.

Table 2.2 The properties of chemical reference material of FMD.

TEST	**RESULTS**	**SPECIFICATIONS**
DESCRIPTION	Almost white crystalline powder, sensitive to light	White to pale yellowish white, crystalline powder, sensitive to light
SOLUBILITY	Freely solube in dimethlyformamide and in glacial acetic acid, slightly soluble in methanol, very slightly soluble in water, practically insoluble in acetone, in alcohol, in chloroform, in eter and in ethyl acetate	Freely solube in dimethlyformamide and in glacial acetic acid, slightly soluble in methanol, very slightly soluble in water, practically insoluble in acetone, in alcohol, in chloroform, in eter and in ethyl acetate
IDENTIFICATION A. Infrared absorption B. Ultraviolet absorption	Matches with working standart Complies	To match with working standard Shall comply to working standard
LOSS AND DRYING	0.1% w/w	Not more than 0.5% w/w
RESIDUE ON IGNITION	0.05% w/w	Not more than 0.1% w/w
HEAVY METALS	Less than 0.001%	Not more than 0.001%
CHROMATOGRAPHIC PURITY BY TLC	Complies	No secondary spot more than 0.3% and some of the secondary spots not more than 1.0%
ORGANIC VOLATILE IMPURITIES	Complies	It meets UPS requirements
ASSAY(on dried basis)	99.8% w/w	Not less than 98.5% w/w and not more than 101.0% w/w

Britton-Robinson (BR) buffer solutions containing 0.1 M KCl were used as supporting electrolyte that has different pH values throughout the study. BR buffer solutions in the pH range 2.0 – 10.0 were prepared from mixture solution of 0.04 M H_3PO_4, 0.04 M H_3BO_3 and 0.04 M CH_3COOH in deionized water. For each buffer solution, the pH of 50 mL mixture solution was adjusted to the desired value by adding 0.2 M NaOH, and then it was diluted to 100 mL with deionized water.

Multiwalled carbon nanotube (MWCNT) was purchased from J.T. Baker. 100 mg MWCNT was kept waiting in the mixture HNO_3 (33 mL, 1/1) and H_2SO_4 (17 mL, 1/1) during 20 h. After it was kept waiting in ultrasonic bath during 1 h, it was heated by stirring at 100^oC for 4 h. Then it was washed with deionized water

many times and was dried 4 h with IR lamp. Finally, MWCNT was dispersed in DMF and a 10 μL aliquot of the obtained suspension was dropped on the bare GCE surface.

2.3. Procedure

All glassware was kept in a HNO_3 solution bath (1/10 v/v) and rinsed with ultrapure water prior to use. Voltammetric measurements were performed at ambient conditions and voltammetric cell contents were kept under the highly pure argon atmosphere during the measurements.

Before every measurement GCE was mechanically polished using a BAS-polishing kit with aqueous 1.0, 0.3 and 0.05 μm alumina (Al_2O_3) slurry on a polishing cloth for three minutes to a mirror-like surface. After they were washed with deionized water, they were cleaned by sonication in ethanol and deionized water for 5 min, respectively.

2.3.1 Preparation of pencil graphite electrode

A pencil lead with a diameter of 0.5 mm (Ultra-Polymer, 2B) and a total length of 60 mm (Tombow, Japan), and a mechanical pencil Model T 0.5 (Rotring, Germany) were purchased from a local bookstore. The latter was used as the holder for the pencil lead. Electrical contact to the lead was obtained by wrapping a metallic wire to the metallic part of the holder.

The surface of PGE was conditioned by applying a potential of +1.45 V for 60 s in the supporting electrolyte (0.04M BR buffer solution containing 0.1 M KCl, pH 7.0). This conditioning procedure was applied all used PGE before electroanalytical measurement in order to obtain more sensitive and stable analytical signals.

2.3.2 Voltammetric procedure

The supporting electrolyte was deaerated with argon for 5 min prior to all electrochemical measurements. Between consecutive additions, the voltammetric cell content was also deaerated with argon for 30 s.

In order to control the presence of impurities, the voltammograms of supporting electrolytes were recorded throughout this study.

The electrochemical behavior of FMD at PGE was investigated by recording both cyclic and DP voltammograms in the BR buffer solution in the pH range of 2.0 - 10.0. For this, 5 mL of supporting electrolyte was placed in the electrochemical cell and after a total of 1 cm part of pencil graphite was immersed into the supporting electrolyte. Than the solution was degassed by argon and the cyclic and DP voltammograms of supporting electrolyte were recorded in the range of 0.5 to 1.2 V vs. $Ag/AgCl/KCl_{sat}$, in quiescent solution at a scan rate of 50 mV s^{-1} and 25 mV s^{-1}, respectively.

The effect of pH on the anodic peak current values resulting from the oxidation of FMD were investigated using both cyclic and DP voltammetry techniques. Firstly, the cyclic voltammograms of 1.0×10^{-4} M FMD were recorded under the same conditions after that the required volume of FMD standard solution was added to the cell containing supporting electrolyte which has different pH values in the range of 2.0 - 10.0 for each measurement.

Secondly, the DP voltammograms of 1.0×10^{-5} M FMD were similarly recorded in the BR buffer solution in the pH range of 2.0 - 10.0 as follows unless otherwise specified: 20 mV pulse amplitude, 30 ms pulse time, scan rate of 25 mVs^{-1}.

To compare the obtained peak currents, both CV and DP voltammograms were also recorded at bare and MWCNT modified GCEs in similar conditions.

In addition, anodic stripping voltammetric studies were carried out by using different deposition time. To investigate the effect of deposition potential (E_{dep}) on the stripping peak current of FMD, it was deposited on PGE in BR buffer solution (pH=3.0) containing 0.1 M KCl, under stirring condition for 60 s at varying E_{dep} values from 800 mV to 1000 mV.

The potential scan was consecutively repeated several times during the CV and DP measurements and the peak current and peak potential values obtained from first scan for FMD was evaluated.

The effective parameters for analytical performance, such as dynamic calibration range, limit of detection, limit of quantification, repeatability, etc. were optimized by considering the peak current of FMD obtained from DP voltammograms recorded between 0.5 V and 1.2 V.

2.3.3 Famotidine analysis in Famoser® drug

The content of FMD was tried to be determined in Famoser® drug by applying the voltammetric procedure under optimum experimental conditions. For this purpose, standard additions method was used.

The standard addition is a useful method when the analyte is present in a complicated matrix and no ideal blank is available. A typical procedure involves preparing several solutions containing the same amount of unknown, but different amounts of standard (Wake Forest University, 2015).

Under the optimum experimental condition, 25 μL tablet solution was added into the voltammetric cell containing 5.0 mL BR buffer solution (pH 3.0) and DP voltammogram was recorded. Then, the known volumes of the standard FMD solution were consecutively added into the same voltammetric cell and then the voltammogram was recorded after each addition.

The standard addition calibration curve of the obtained current values from proposed method against concentrations of FMD standard solution added was plotted. Then, linear regression is performed; the slope (m) and y-intercept (b) of the calibration curve were used to calculate the concentration of analyte in the sample.

3. RESULTS AND DISCUSSION

The investigation of voltammetric behavior of FMD by using both DPV and CV techniques and the sensitive determination of FMD at PGE was aimed within the scope of this thesis. This section outlines of the efforts to investigate the electrochemical behaviour of FMD and to seek an applicable techniques.

After preliminary studies, the voltammetric behavior of FMD was investigated on PGE in BR buffer solutions ($2.0 < pH < 10.0$) containing 0.1 M KCl in cyclic and DP modes. The effective parameters such as pH of supporting electrolyte solution and scan rate (ν) were examined in detail. The optimum conditions were examined for voltammetric procedure. Then, this procedure was applied to determine FMD in a tablet form of Famoser® drug.

3.1 Preliminary Investigations

In connection with the aim, type of working electrode was examined in detail by using CV and DPV techniques. Then, the resulting peak currents, which were obtained in the same supporting electrolyte (BR buffer solution containing 0.1 M KCl, pH 3.0) at bare GCE, carbon nanotubes modified GCE (MWCNT/GCE) and bare PGE, were compared with each other. Finally, the performance of anodic stripping voltammetric technique was also investigated for determination of FMD

3.1.1 Cyclic voltammetric studies using different working electrode

3.1.1.1 Working electrode: Glassy carbon electrode

To investigate the electrochemical behaviour of FMD, CV voltammograms (5 cycles) of 1.0×10^{-4} M FMD solution were recorded at scan rate (ν) of 50 mV s^{-1} in the BR buffer solution containing 0.1 M KCl (pH 3.0) by using a bare GCE as a working electrode. CV measurements were repeated twice under the same conditions (Fig. 3.1). The results obtained from these voltammograms (in first scan) are given in Table 3.1. As shown in Table 3.1, when GCE was used as a working electrode, the peak current value of FMD was found low according to its concentration.

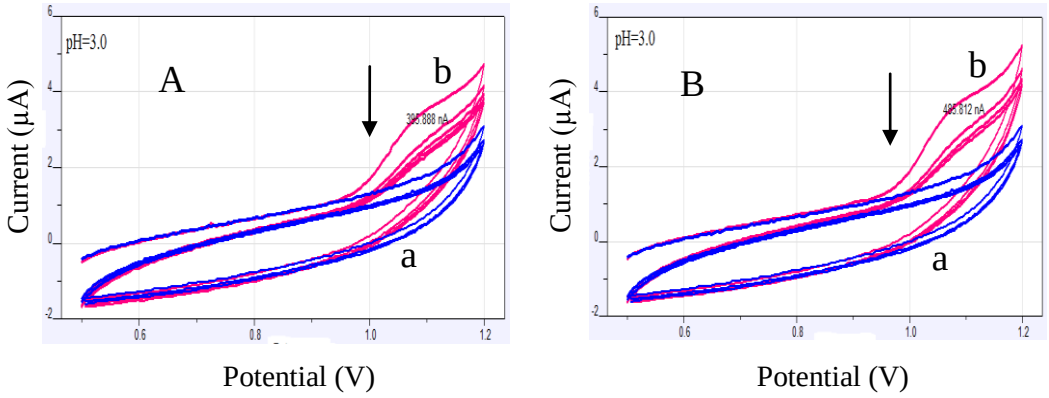


Figure 3.1 Cyclic voltammograms of first (A) and second (B) measurements at GCE (v: 50 mVs^{-1}) **a)** absence of FMD, **b)** presence of 1.0×10^{-4} M FMD for five successive cycles in BR buffer solution (pH 3.0) containing 0.1 M KCl.

Table 3.1 The peak current and peak potential values of 1.0×10^{-4} M FMD at GCE in BR buffer solution (pH 3.0) containing 0.1 M KCl.

pH	Repeated measurements			
	1		2	
	Current (µA)	Potential (mV)	Current (µA)	Potential (mV)
3.0	0.396	1065	0.486	1060

3.1.1.2 Working electrode: Multiwalled carbon nanotubes modified glassy carbon electrode

The effect of working electrodes was also investigated by using MWCNT/GCE in the BR buffer solutions containing 0.1 M KCl (pH 3.0). CV voltammograms (5 cycles) of 1.0×10^{-4} M FMD solution were recorded at scan rate of 50 mV s^{-1}. CV measurements were repeated twice under the same conditions (Fig. 3.2). The peak

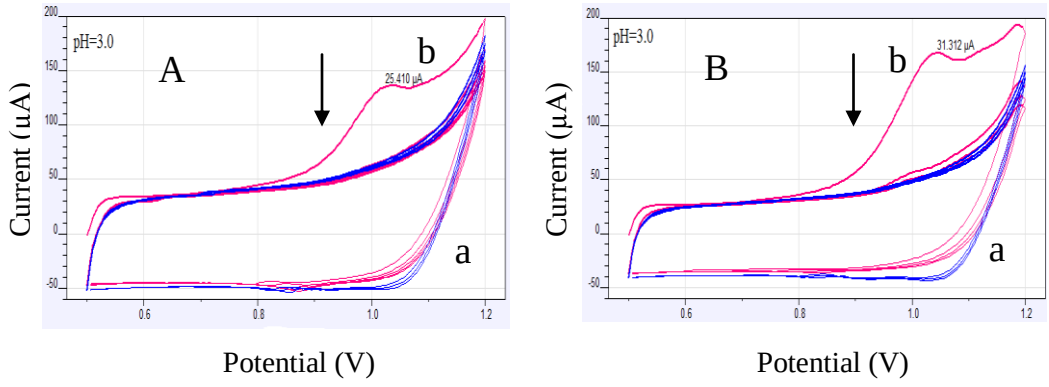


Figure 3.2 Cyclic voltammograms of first (A) and second (B) measurements at MWCNT/GCE a) absence of FMD, b) presence of 1.0×10^{-4} M FMD for five successive cycles in the same conditions.

current and peak potential values obtained from these voltammograms are given in Table 3.2. As shown in Table 3.2, when MWCNT/GCE was used as a working electrode, the peak current value of FMD was found very higher than that of bare GCE. It was observed that also the background current increased very much.

Table 3.2 The peak current and peak potential values of 1.0×10^{-4} M FMD at MWCNT/GCE in the same conditions.

pH	Repeated measurements			
	1		2	
	Current (µA)	Potential (mV)	Current (µA)	Potential (mV)
3.0	25.41	1035	31.312	1045

3.1.1.2 Working electrode: Pencil graphite electrode

To investigate the electrochemical behaviour of FMD, CV voltammograms (5 cycles) of 1.0×10^{-4} M FMD solution were recorded at scan rates of 50 mV s^{-1} in the BR buffer solutions containing 0.1 M KCl (pH 3.0) by using a bare PGE as a working electrode. CV voltammograms were recorded in the same conditions twice (Fig. 3.3). The results obtained from these voltammograms (in first scan) are given in Table 3.3. As shown in Table 3.3, when bare PGE was used as a working electrode, the peak current value of FMD was higher than that of bare GCE and lower than that of MWCNT/GCE. In addition, it was observed that the background current decreased very much according to MWCNT/GCE. It is noteworthy that also the shape of the peak was occurred much sharper than others.

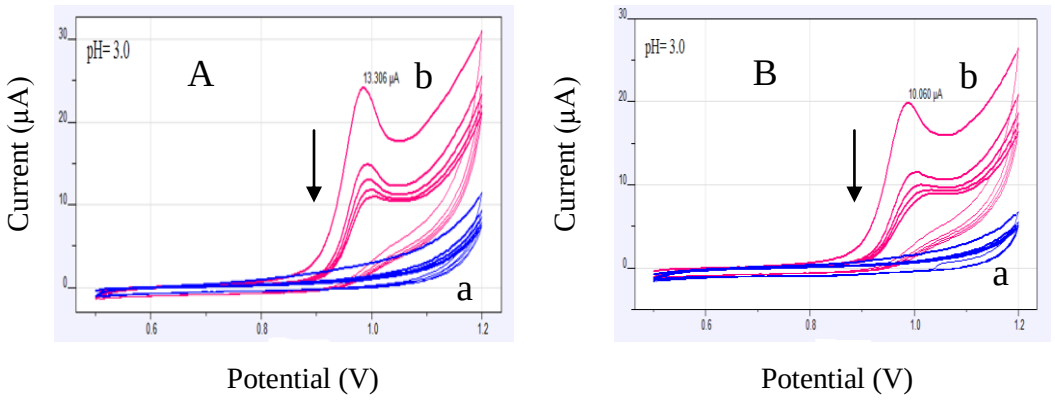


Figure 3.3 Cyclic voltammograms of first (A) and second (B) measurements at PGE a) absence of FMD, b) presence of 1.0×10^{-4} M FMD for five successive cycles in the same conditions.

Table 3.3 The peak current and peak potential values of 1.0×10^{-4} M FMD at PGE in the same conditions.

pH	Repeated measurements			
	1		2	
	Current (µA)	Potential (mV)	Current (µA)	Potential (mV)
3.0	13.306	985	10.06	990

In all of the studies in this section, the cyclic voltammograms showed that the peak current of FMD decreased for an increasing number of cycles (especially after the first cycle). It can be was attributed that the oxidation products of FMD was adsorbed on the electrode surface.

3.1.2 Differential pulse voltammetric studies using different working electrode

The effect of working electrodes was also investigated by using DP in the BR buffer solutions containing 0.1 M KCl (pH 3.0). In this section, only results with bare GCE and PGE were given because the peak current of FMD was not observed with MWCNT/GCE.

3.1.2.1 Working electrode: Glassy carbon electrode

DP measurements for 1.0×10^{-5} M FMD were repeated twice at scan rates of 25 mV s^{-1} in the same supporting electrolyte (Fig. 3.4). The peak current and peak potential values obtained for FMD are given in Table 3.4. As shown in Table 3.6, when the bare GCE was used as a working electrode, the very low peak current value of FMD was found.

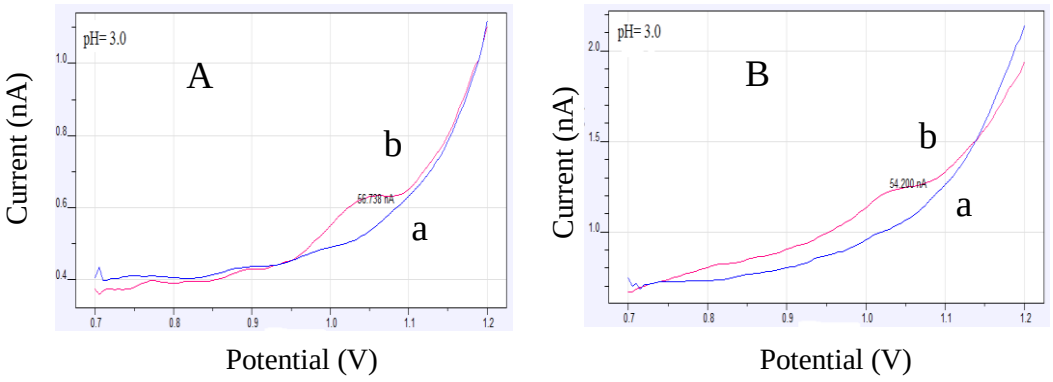


Figure 3.4 DP voltammograms of first (A) and second (B) measurements at GCE **a)** absence of FMD, **b)** presence of 1.0×10^{-5} M FMD in the same conditions (ν: 25 mVs^{-1}).

Table 3.4 The peak current and peak potential values of 1.0×10^{-5} M FMD with GCE at the same conditions (ν: 25 mVs^{-1}).

pH	Repeated measurements			
	1		2	
	Current (µA)	Potential (mV)	Current (µA)	Potential (mV)
3.0	0.057	1040	0.054	1035

3.1.2.2 Working electrode: Pencil graphite electrode

DP measurements for 1.0×10^{-5} M FMD were repeated twice at scan rates of 25 mV s^{-1} (Fig. 3.5). The potential was consecutively scan three times for each experiment. The peak current and peak potential values obtained from first scan for FMD are given in Table 3.5. As shown in Table 3.5, when the PGE was used as a working electrode the peak currents value of FMD is very higher than that of GCE. In addition, the peak shape of FMD obtained at PGE was much sharper than that of PGE.

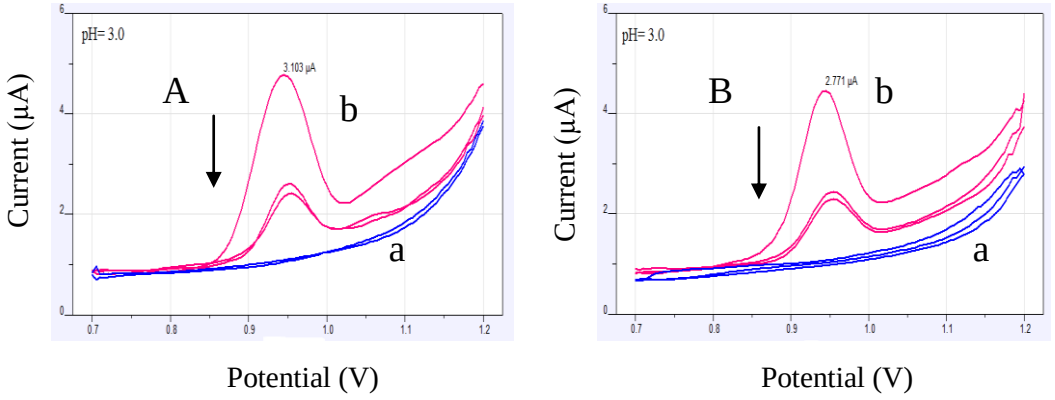


Figure 3.5 DP voltammograms of first (A) and second (B) measurements at PGE **a)** absence of FMD, **b)** presence of 1.0×10^{-5} M FMD in the same conditions (ν: 25 mVs^{-1}).

Table 3.5 The peak current and peak potential values of 1.0×10^{-5} M FMD at PGE in the same conditions (ν: 25 mVs^{-1}).

pH	Repeated measurements			
	1		2	
	Current (µA)	Potential (mV)	Current (µA)	Potential (mV)
3.0	3.103	945	2.771	945

3.1.3 Anodic stripping voltammetric studies

The preconcentration step in stripping voltammetry was examined to improve of sensitivity. In order to see the effect of deposition potential (E_{dep}) on the stripping peak current of FMD, firstly FMD was deposited on PGE in BR buffer solution (pH=3.0) containing 0.1 M KCl, under stirring condition for 60 s at varying E_{dep} values in the range of 800 mV to 1000 mV. DP anodic stripping voltammograms were recorded at 25 mVs^{-1} (Fig. 3.6). The potential was consecutively scan three times for each E_{dep}. The stripping peak currents (in first scan) which were obtained at different E_{dep} values for FMD are given in Table 3.6.

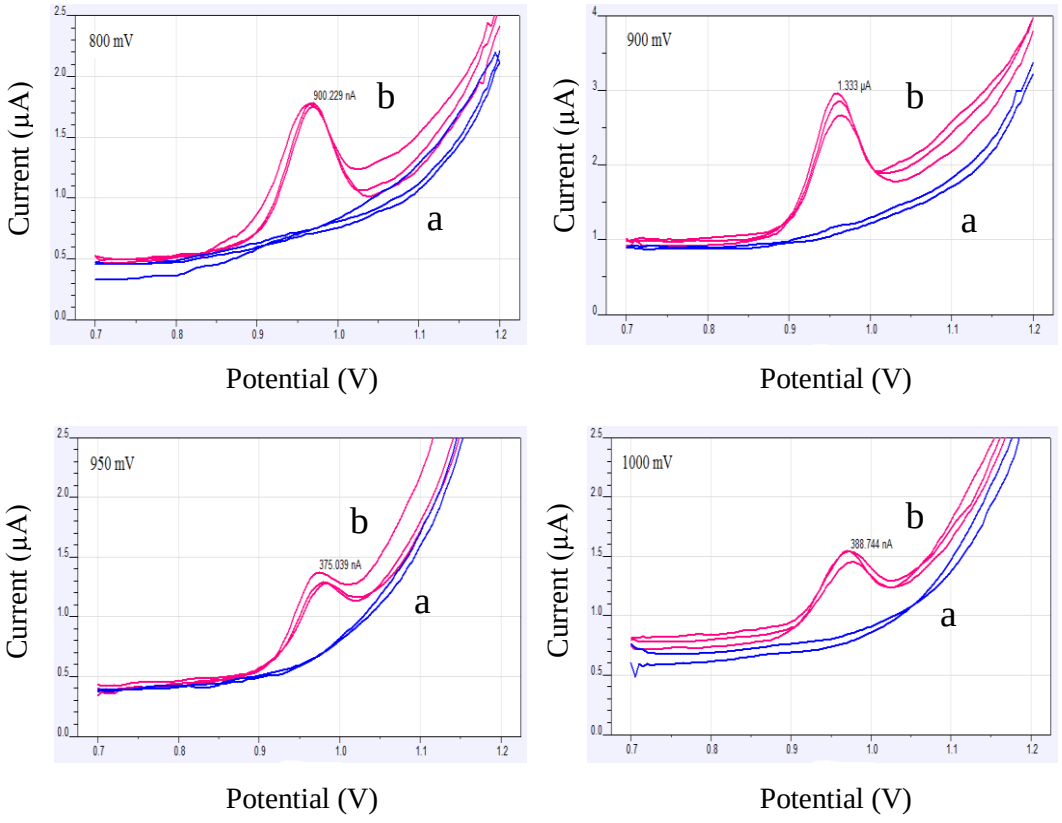


Figure 3.6 DP anodic stripping voltammograms at PGE (v: 25 mVs^{-1}) a) absence of FMD, b) presence of 1.0×10^{-5} M FMD in BR buffer solution (pH=3.0) containing 0.1 M KCl.

Table 3.6 The effect of deposition potential (E_{dep}) on the stripping peak current of 1.0×10^{-5} M FMD.

E_{dep} (mV)	i_{peak} (µA)
800	0.900
900	1.333
950	0.375
1000	0.389

As shown in Figure 3.7, when E_{dep} was 900 mV, the highest peak current for FMD was obtained. However, this value was 2 - 2.5 times lower than that of obtained without the deposition procedure for the same supporting electrolyte, scan rate, and concentration of FMD (Table 3.5 and 3.6).

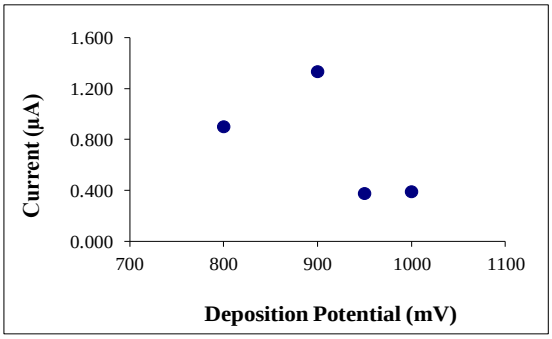


Figure 3.7 The effect of deposition potential (E_{dep}) on the stripping peak current of FMD.

As a result the highest peak current value for FMD was obtained at PGE using DP voltammetric technique. In connection with this, the studies on the investigation of cyclic and DP voltammetric behaviours of FMD were continued by using bare PGE. These studies including these optimization experiments were presented below.

3.2 Voltammetric Behaviour of Famotidine at Pencil Graphite Electrode

3.2.1 Cyclic voltammetric studies

To investigate the electrochemical behavior of FMD; cyclic voltammograms of 1.0×10^{-4} M FMD were recorded at the scan rate of 100 mV s^{-1} with ten successive cycles in the BR buffer solution (pH 7.0) containing 0.1 M KCl (Fig. 3.8). As can be seen from Figure 3.8, the oxidation of FMD was irreversible because the cathodic peak of FMD did not observe. These cyclic voltammograms showed that the peak currents of FMD decreased with consecutive scans, and then they reached to stable peak current values. According to this result, the potential scanning range between 500 – 1200 mV was selected for the further studies.

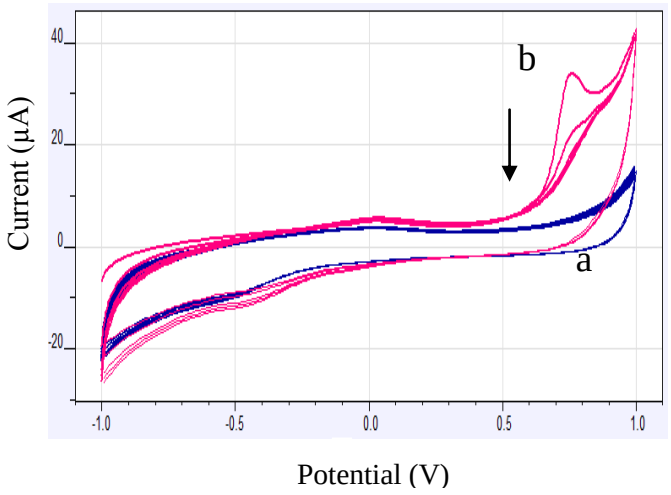
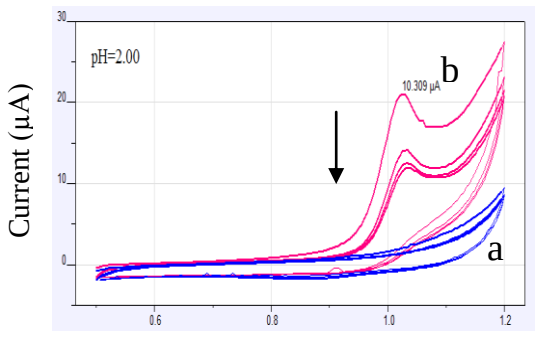
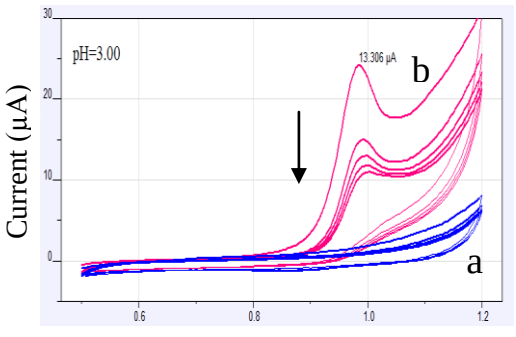


Figure 3.8 Cyclic voltammograms (v: 100 mVs^{-1}) **a)** absence of FMD, **b)** presence of 1.0×10^{-4} M FMD for ten successive cycles in BR buffer solution (pH 7.0) containing 0.1 M KCl.

3.2.1.1 Effect of pH

Effect of pH was investigated by using cyclic voltammograms in the range of 500 mV to 1200 mV in BR buffer solutions (2.0 < pH< 10.0) containing 0.1 M KCl at PGE. The cyclic measurements (5 cycles) for 1.0×10^{-4} M FMD solution were repeated twice under the same conditions by using separate pencil leads at every turn. The obtained cyclic voltammograms and results at a scan rate of 50 mVs^{-1} are given in Figure 3.9 and Table 3.7, respectively. In general seen as, the anodic peak current of FMD reduced by the increasing of pH. On the other hand, the peak shapes of FMD broadened and deteriorated. In addition, the smooth peaks were obtained at low pH values. The results showed that the highest peak currents of FMD were observed at pH 3.0.





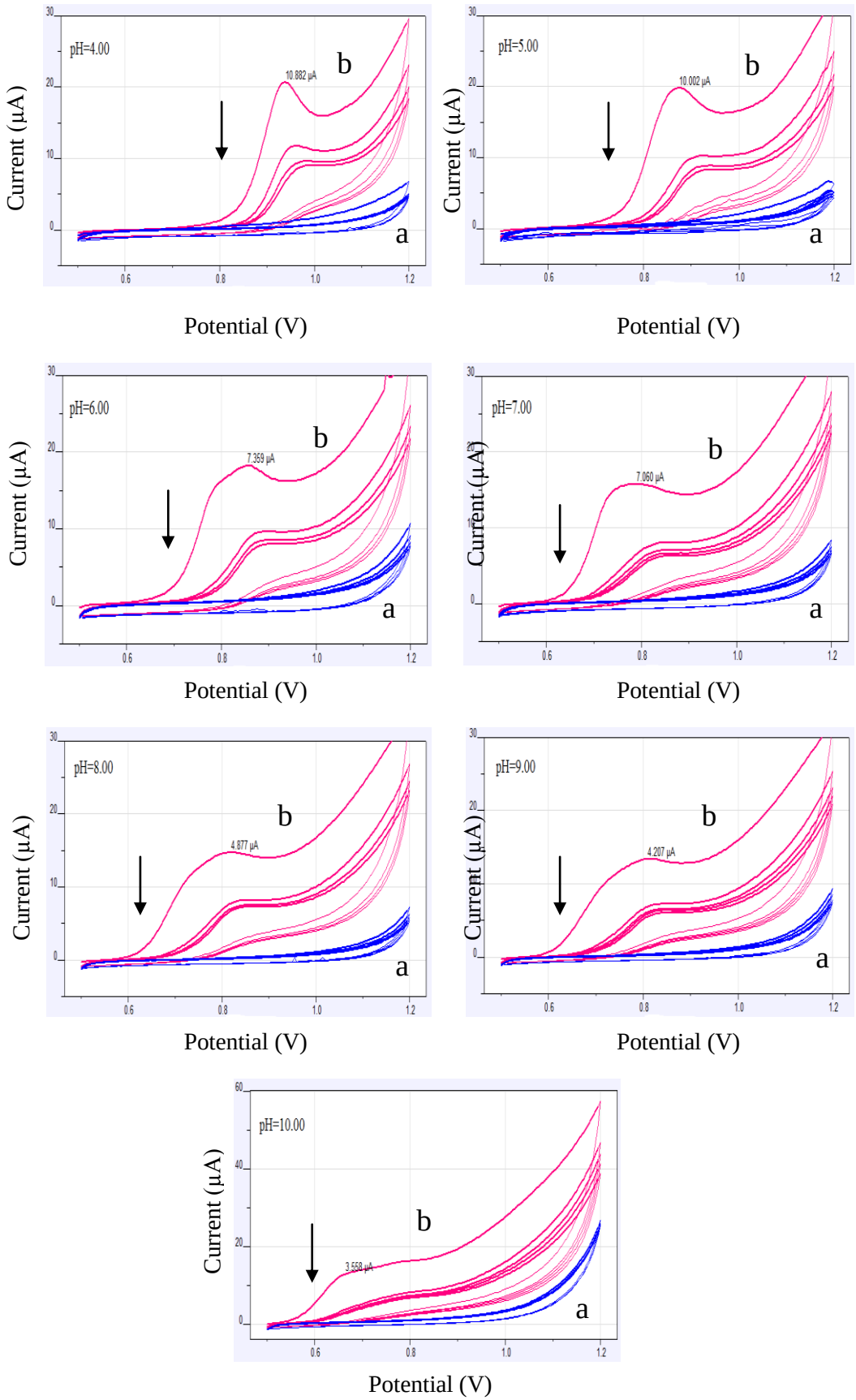
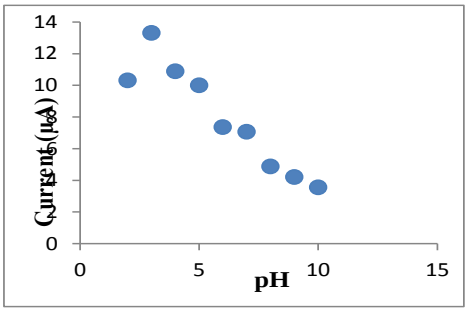


Figure 3.9 Cyclic voltammograms (v: 50 mVs^{-1}) a) absence of FMD, b) presence of 1.0×10^{-4} M FMD for five successive cycles in BR buffer solution at different pH values (2.0<pH<10.0) containing 0.1 M KCl.

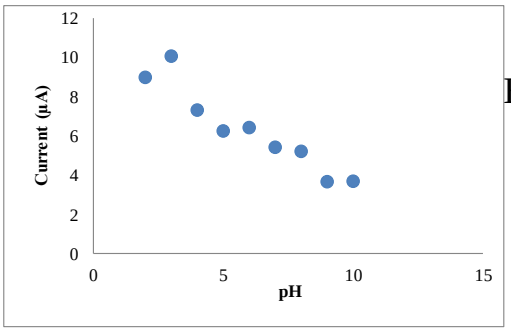
Table 3.7 The peak current and peak potential values of 1.0×10^{-4} M FMD solution at PGE in BR buffer solution (2.0<pH<10.0) containing 0.1 M KCl.

pH	Repeated measurements			
	1		2	
	Current (µA)	Potential (mV)	Current (µA)	Potential (mV)
2.0	10.309	1025	8.980	1025
30.	13.306	985	10.06	990
4.0	10.882	940	7.313	980
5.0	10.002	875	6.249	915
6.0	7.359	855	6.422	855
7.0	7.060	790	5.419	805
8.0	4.877	820	5.211	810
9.0	4.207	810	3.665	805
10.0	3.558	715	3.689	715

As can be seen in Figure 3.10, when the medium pH was increased the peak current decreased almost linearly, except the peak current obtained at pH 2.0.



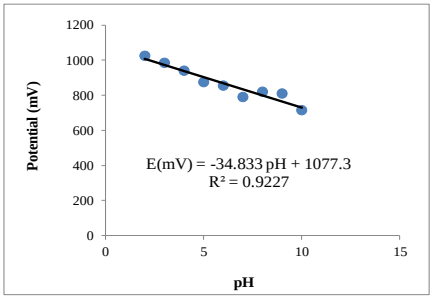
A



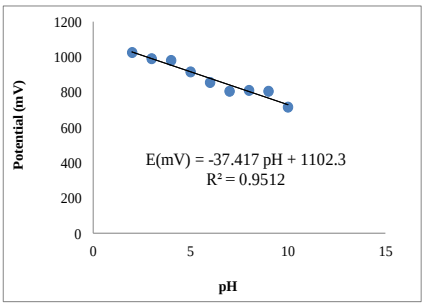
B

Figure 3.10 Effect of pH on the peak current of 1.0×10^{-4} M FMD in first (A) and second (B) CV measurements in BR buffer solution (2.0<pH<10.0) containing 0.1 M KCl. *v*: 50 mV s^{-1}.

In addition to this, with the increase of the medium pH, the peak potential shifted to less positive potential values (Fig. 3.11). This result can be attributed to the consumption of the hydrogen during the oxidation of FMD at PGE. It is known that the slope values of the linear graphs (Fig 3.11) are determined using the equation of −2.303 mRT/nF. In this equation, m and n are the number of protons and electrons involved in the redox reaction, respectively (Rieger, 1993). The slope value in the pH ranges of 2.0–10.0 was found about −35 mV/pH. In this case, the number of electron transferred is not equal to that of the hydrogen ions taking part in the electrode reaction.



A



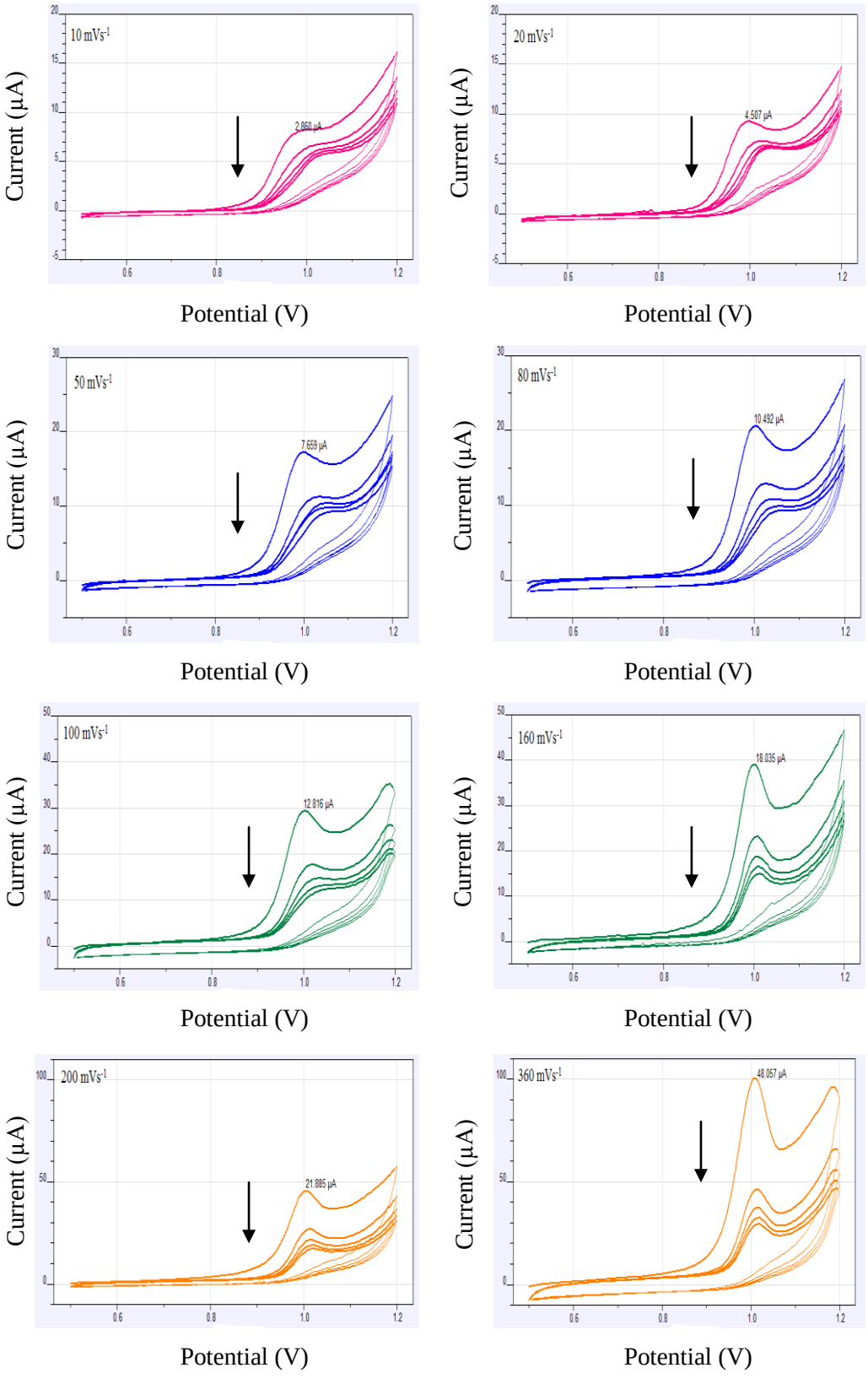
B

Figure 3.11 Effect of pH on the peak potential of 1.0×10^{-4} M FMD in first (A) and second (B) CV measurements in BR buffer solution (2.0<pH<10.0) containing 0.1 M KCl. *v*: 50 mV s^{-1}.

3.2.1.2. Effect of scan rate

The scan rate has an important effect on the peak currents. The effect of the scan rate on peak current was examined in the range 10 mVs^{-1} to 1600 mVs^{-1} for 1.0×10^{-4} M FMD. The supporting electrolyte solution was renewed before each measurement and

the voltammogram was recorded. The potential was consecutively scan five times for each scan rate. The data obtained from first scan are given in Table 3.8. As can be seen in Figure 3.12, the anodic peak of FMD grows with increasing scan rate. No cathodic peak of FMD was not observed on the reverse scan.



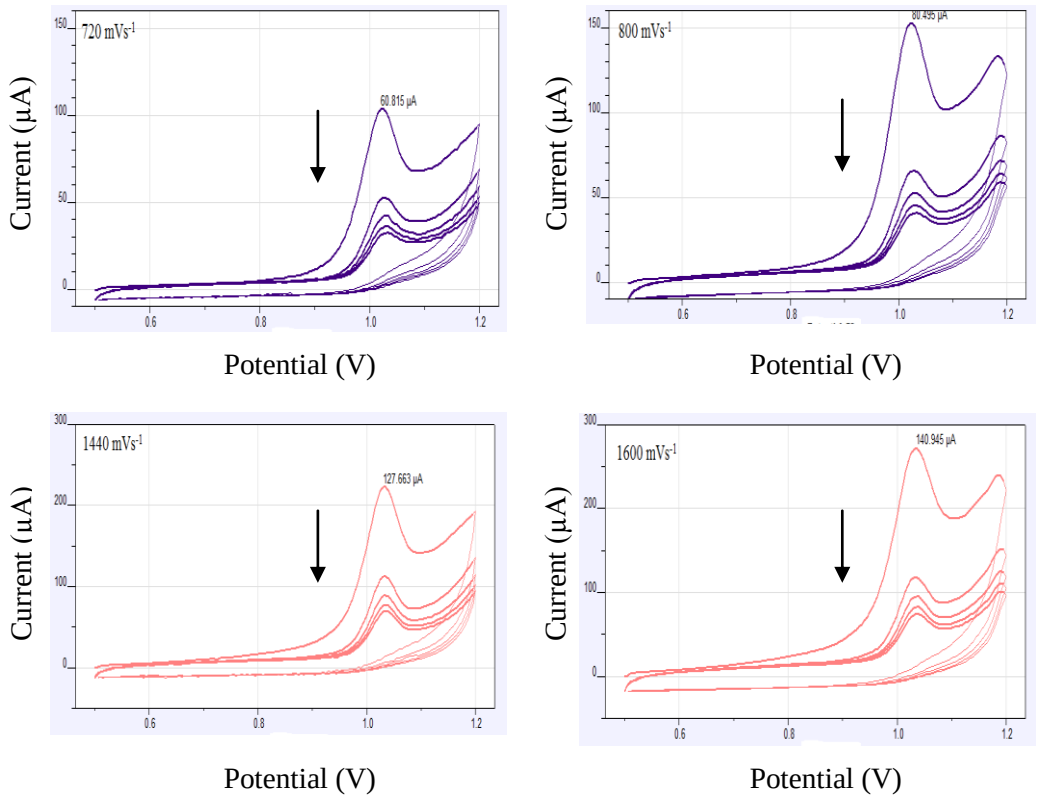


Figure 3.12 Cyclic voltammograms of 1.0×10^{-4} M FMD at the different scan rates (10 <ν<1600 mVs^{-1}) for five successive cycles in BR buffer solution (pH=3.0) containing 0.1 M KCl.

Table 3.8 Dependence of peak current on scan rate for 1.0×10^{-4} M FMD by using CV in BR buffer solutions containing 0.1 M KCl, pH 3.0.

Scan Rate (mVs^{-1})	Current (µA)	Potential (mV)	Scan Rate (mVs^{-1})	Current (µA)	Potential (mV)
10	2.860	975	360	48.057	1015
20	4.507	990	400	41.999	1015
50	7.659	995	720	60.815	1020
80	10.492	1000	800	80.495	1025
100	12.816	1000	1440	127.663	1030
160	18.035	1005	1600	140.945	1035
200	21.885	1005			

The linear change of peak current values with the square root of the scan rate was also observed in BR buffer solutions containing 0.1 M KCl (pH 3.0) in the range of 500 – 1200 mVs^{-1}. The linear regression equations were $i\,(\mu A) = 1.705\ v^{1/2}((mV\ s^{-1})^{1/2}) - 3.5429$ and $i(\mu A) = 18.471v^{1/2}(mVs^{-1})^{1/2}$-80.923 with a correlation coefficient of $R^2 = 0.9807$ and $R^2 = 0.9952$ in this range (Fig. 3.14) indicating that the oxidation

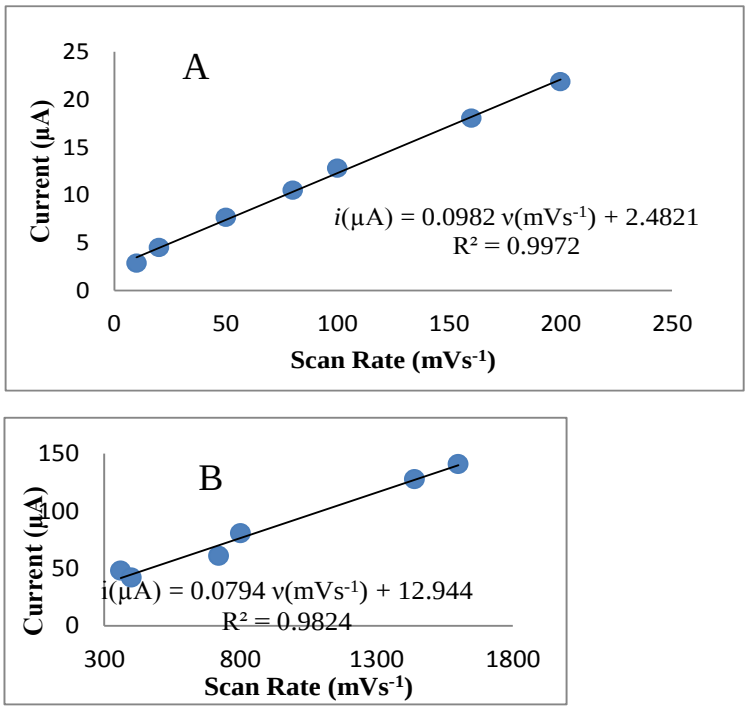


Figure 3.13 The plot of the peak currents of FMD (1.0×10^{-4} M) versus scan rate a) 10 <ν<200 mVs^{-1}, b) 360 <ν<1600 mVs^{-1}.

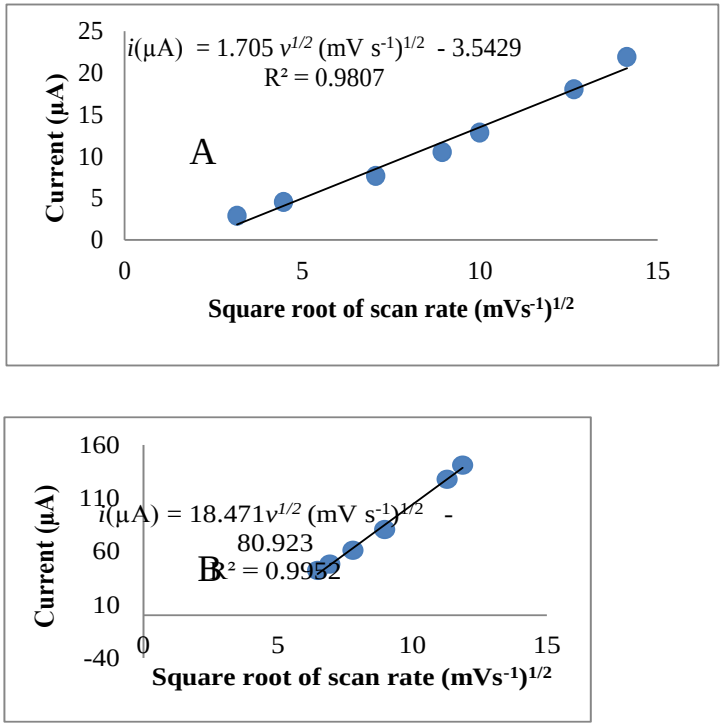
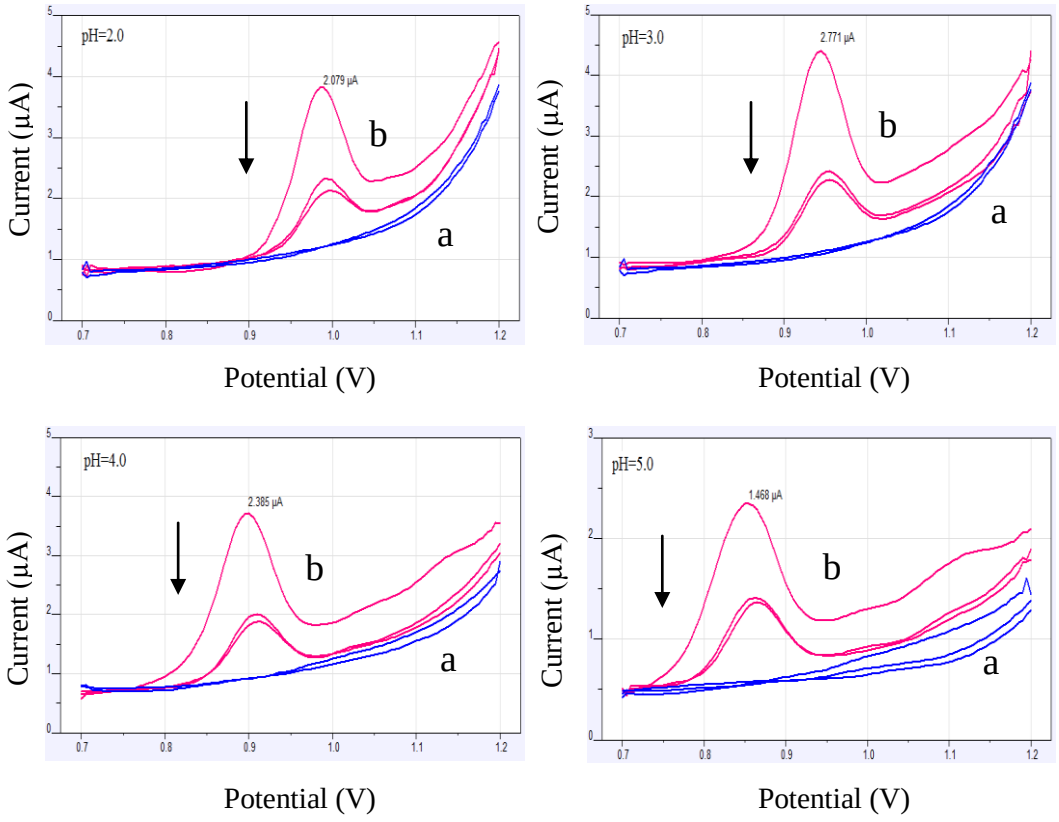


Figure 3.14 The plot of the peak currents of FMD (1.0×10^{-4} M) versus the square root of the scan rate a) 10 <ν<200 mVs^{-1}, b) 360 <ν<1600 mVs^{-1}.

reaction of FMD was also controlled by diffusion in in BR buffer solution containing 0.1 M KCl (pH 3.0). In addition, the peak potential values shifted to more positive values with increasing scan rate.

3.2.2 Differential pulse voltammetric studies

FMD oxidation at PGE was evaluated by using DP voltammograms in the range of 700 mV to 1200 mV. The voltammograms for FMD concentration of 1.0×10^{-5} M was obtained in the pH range of 2.0 to 10.0 by using BR buffer solutions containing 0.1 M KCl. DP measurements using separate pencil leads were repeated twice in the same conditions. The potential was consecutively scan three times for each experiment. The obtained DP voltammograms at a scan rate of 25 mVs^{-1} are given in Figure 3.15. As can be seen, the peaks shapes of FMD broadened and deteriorated, also the peak currents of FMD decreased with consecutive cycle, and then they reached to values stable peak current values in a manner similar to CV measurements.



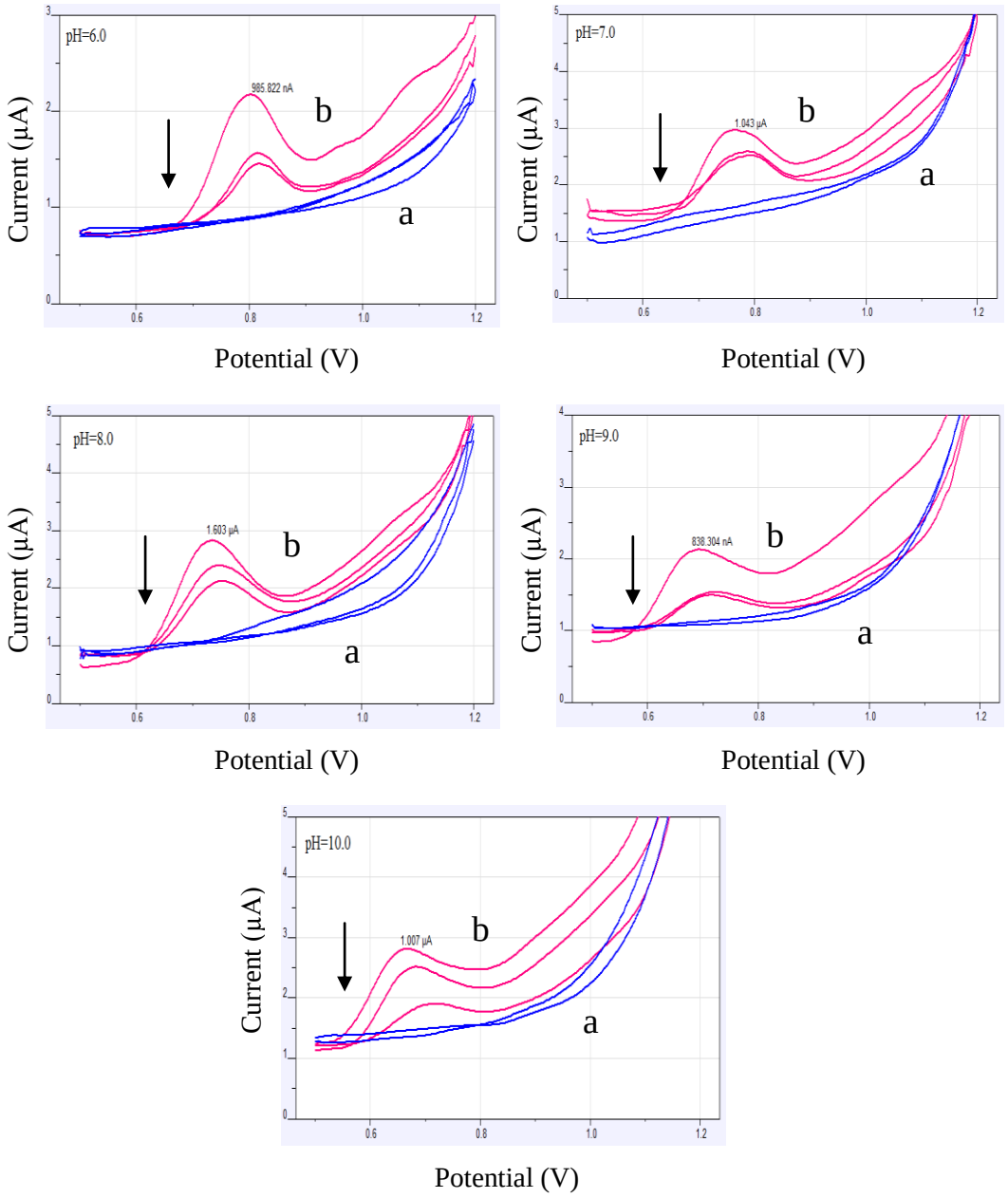


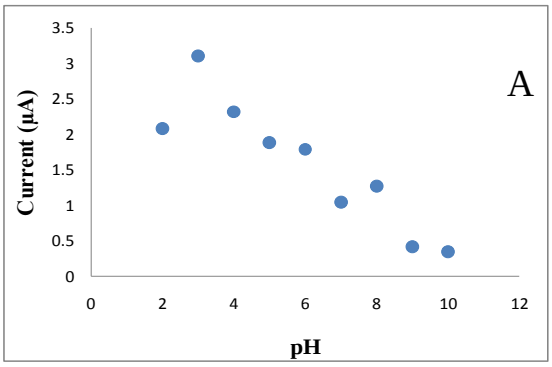
Figure 3.15 DP voltammograms (ν: 25 mVs^{-1}) a) absence of FMD, b) presence of 1.0×10^{-5} M FMD in BR buffer solution (2.0<pH<10.0) containing 0.1 M KCl.

The variation of peak current values (for first scan) of FMD with pH in range of 2.0 to 10.0 at PGE was similar to the result of CV studies (Table 3.9). The highest peak currents of FMD were observed at pH 3.0 (Fig. 3.16).

The variation of peak potential values of FMD with pH was also similar to the result of CV studies in same pH range (Fig. 3.17). Once again the peak potential shifted to less positive values with the increase in pH. However, this time the slope was greater (about 11-15%) than that of CV measurement.

Table 3.9 The peak current and peak potential values of FMD (1.0×10^{-5} M) at PGE in BR buffer solution (2.0<pH<10.0) containing 0.1 M KCl.

pH	Repeated measurements			
	1		2	
	Current (µA)	Potential (mV)	Current (µA)	Potential (mV)
2.0	2.079	990	2.079	990
3.0	3.103	945	2.771	945
4.0	2.316	905	2.385	900
5.0	1.885	850	1.468	855
6.0	2.153	795	0.986	805
7.0	1.043	765	1.043	765
8.0	2.031	700	1.603	725
9.0	0.413	730	0.838	680
10.0	0.344	695	1.007	655



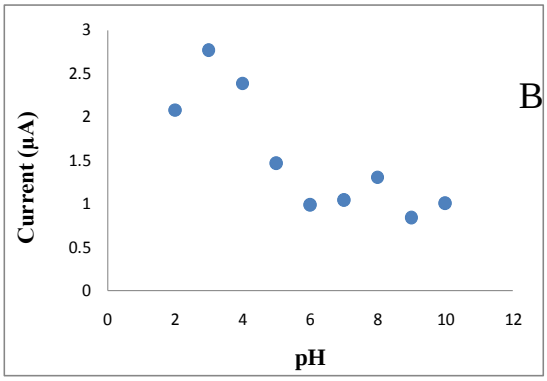


Figure 3.16 Effect of pH on the peak current of 1.0×10^{-5} M FMD in first (A) and second (B) scan DP experiments in BR buffer solution (2.0<pH<10.0) containing 0.1 M KCl. *v*: 25 mV s^{-1}.

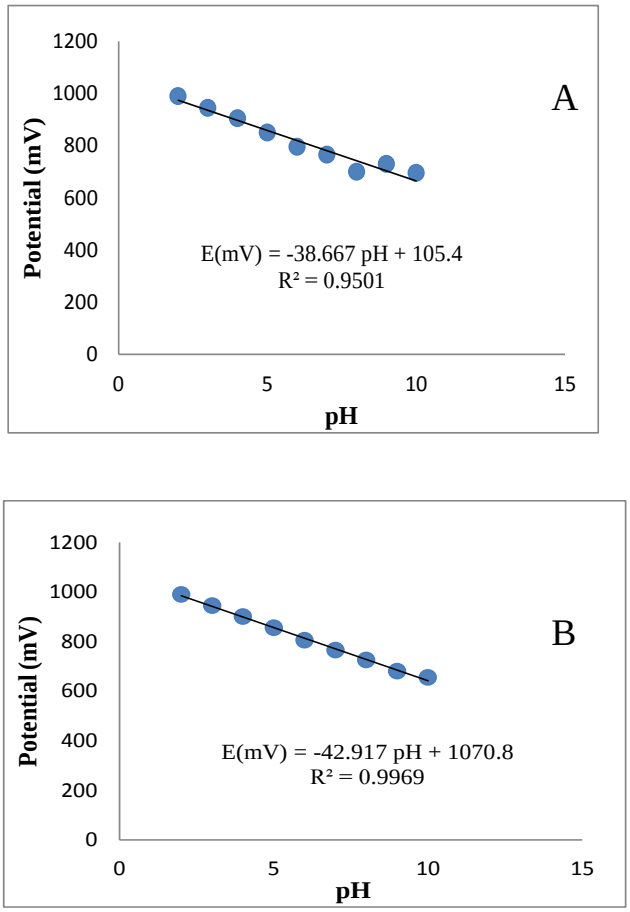


Figure 3.17 Effect of pH on the peak potentials of 1.0×10^{-5} M FMD in first (A) and second (B) scan DP experiments in BR buffer solution (2.0<pH<10.0) containing 0.1 M KCl. *v*: 25 mV s^{-1}.

3.3 Analytical Characteristics

A calibration study was carried out over the range 8×10^{-7} - 1×10^{-3} M FMD concentration to establish a reliable analytical response for the determination of FMD, under optimized conditions using a PGE. These DP experiments were carried out four times at the scan rate of 25 mVs^{-1}, the scan range of 700 - 1200 mV in in BR buffer solution (pH 3.0) containing 0.1 M KCl. The obtained data are shown in Table 3.10. When the obtained peak currents were plotted against concentrations of FMD two linear segments were observed as of 8×10^{-7} - 3×10^{-5} M (low concentrations) and 5×10^{-5} - 1×10^{-3} M (high concentrations). Their equations were as follows:

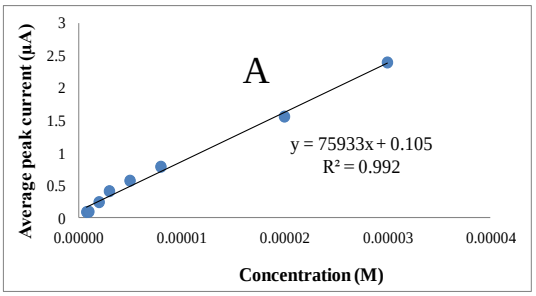
For low concentrations : $i\,(\mu A) = 75933\, C_{FMD}(M) + 0.105$ $(R^2 = 0.9920)$

For high concentrations : $i\,(\mu A) = 8384.9\, C_{FMD}(M) + 2.195$ $(R^2 = 0.9979)$

where i is the peak current and C is the concentration of FMD.

Table 3.10 Peak currents obtained with different FMD concentrations (n=4) in BR buffer solution (pH 3.0) containing 0.1 M KCl at PGE. Pulse amplitude 20mV, pulse time 30ms. *v*: 25 mV s^{-1}, potential scan range: from 700 mV to 1200 mV.

C_{FMD} (M)	Average Current (µA)	C_{FMD} (M)	Average Current (µA)
8×10^{-7}	0.089	5×10^{-5}	2.433
1×10^{-6}	0.094	8×10^{-5}	2.826
2×10^{-6}	0.241	1×10^{-4}	3.005
3×10^{-6}	0.410	2×10^{-4}	3.974
5×10^{-6}	0.571	3×10^{-4}	4.753
8×10^{-6}	0.786	5×10^{-4}	6.640
2×10^{-5}	1.558	8×10^{-4}	8.889
3×10^{-5}	2.391	1×10^{-3}	10.447



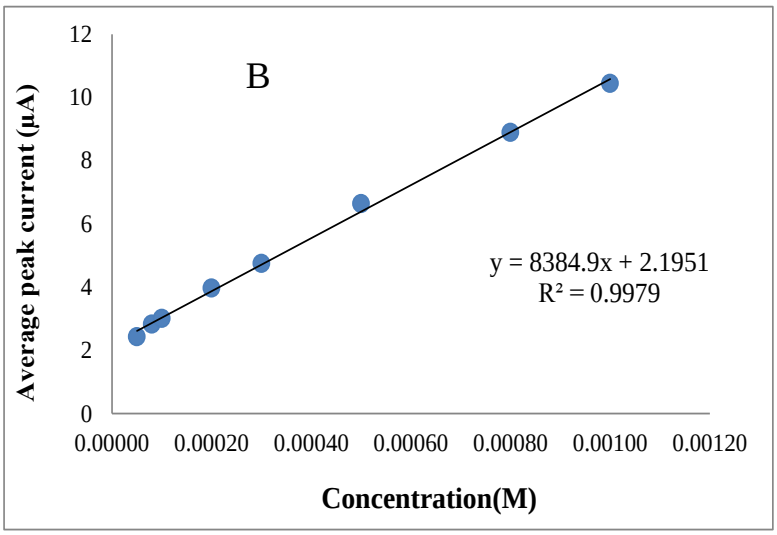


Figure 3.18 Calibration curves ($i_{aver}=f(C_{FMD})$, for n=4) for determination of FMD at PGE. **A)** 8.0×10^{-7} – 3.0×10^{-5} M and **B)** 5.0×10^{-5} – 1.0×10^{-3} M FMD.

3.4 Real Sample Analysis

Sample solutions of Famoser® Drug (in tablet form) were prepared as mentioned in Section 2.2 with five tablets. The voltammograms were recorded under optimum working conditions (BR buffer solution pH 3.0 containing 0.1 M KCl) by applying standard addition method. The procedure explained in Section 2.3.3 was practiced twice using PGE. The obtained voltammograms and data are given in Figure 3.19 and Table 3.11, respectively. The standard addition graph was plotted against the calculated concentration of standard FMD in the cell (Fig. 3.20). The results were in good agreement with the content marked in the label of Famoser®. In addition, the values of absolute and relative error were calculated by considering Famoser® tablet in which contained 40 mg FMD (Table 3.11).

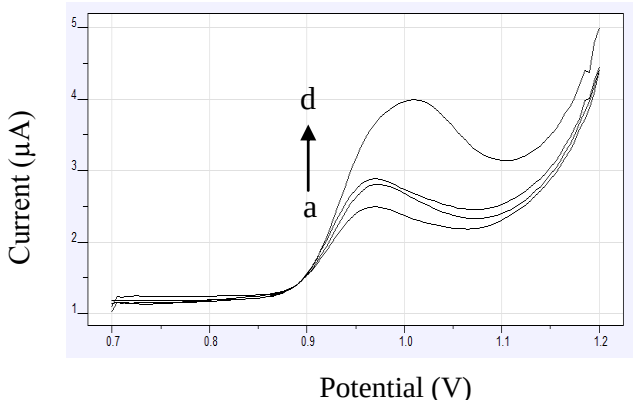


Figure 3.19 Voltammograms of FMD in Famoser® drug sample using the standard additions method (under the optimum conditions) a) presence of tablet solution (25 µL); b) 2.0×10^{-5} M, c) 4.0×10^{-5} M, d) 6.0×10^{-5} M standard FMD solution in cell.

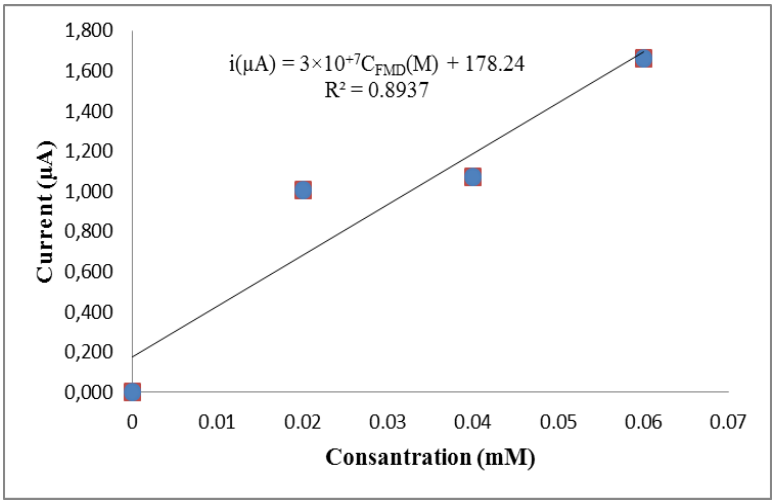


Figure 3.20 The standard addition graph for the determination of FMD in Famoser® tablet.

Table 3.11 The results of standard addition method for Famoser® tablet. *v*: 25 mV s^{-1}.

C_{FMD}(M)	Repeated measurements	
	1	**2**
	i (µA)	**i (µA)**
25 µL tablet solution*	0.770	0.834
2.0×10^{-5}	1.008	1.922
4.0×10^{-5}	1.071	2.496
6.0×10^{-5}	1.663	3.491
Equation	$i(\mu A) = 3\times10^{+7}C_{FMD}(M) + 178.24$ $R^2 = 0.8937$	$i(\mu A) = 6\times10^{+7}C_{FMD}(M) + 320.78$ $R^2 = 0.9427$
FMD amount (mg/tablet)	42.86	38.57
Absolute error (mg)	+2.86	-1.43
Relative error (%)	+7.15	-3.58

4. CONCLUSION

In this thesis, it was aimed to investigate the voltammetric behavior of FMD and develop a method for the determination of FMD, especially using PGE. For this aim, it was investigated on bare GCE, MWCNT/GCE and PGE in BR buffer solutions containing 0.1 M KCl using both the cyclic and DP modes. The effective parameters such as pH of supporting electrolyte solution, scan rate of potential, type of working electrode were optimized.

In all of the CV studies, the oxidation of FMD was irreversible and the peak currents of FMD were decreased by increasing number of cycles (especially after the first cycle) because of the adsorption of the oxidation products of FMD on the electrode surface. Then, they reached to values of stable peak current.

As a result of CV and DPV studies in the same condition, the highest peak current value for FMD was obtained at PGE using DP voltammetric technique.

Therefore, the studies on the investigation of cyclic and DP voltammetric behaviors of FMD were carried out using bare PGE. In addition, the effect of the preconcentration step in stripping voltammetry was examined to improve the sensitivity at varying E_{dep} values in the range of 800 mV to 1000 mV. When E_{dep} was 900 mV, the highest peak current was obtained for FMD at PGE. However, this value was 2 - 2.5 times lower than that of obtained without the deposition procedure for the same supporting electrolyte, scan rate, and concentration of FMD (Table 3.5, 3.6, and 3.12).

Table 3.12 Comparison of peak current density obtained for 1.0×10^{-5} M FMD at different working electrodes.

pH=3.0	Current (µA)	Area(mm^2)	$I = \frac{i}{A}$
PGE	3,103	15,9	0,195
GCE	0,054	7,07	0,008
MWCNT/GCE		7,07*	

* Physical area of MWCNT/GCE

The effect of pH was investigated by recording cyclic and DP voltammograms in BR buffer solutions (2.0 < pH< 10.0) containing 0.1 M KCl at PGE. The variations of peak potential value of FMD with pH in CV and DPV were similar to each other (Fig. 3.11 and 3.17). They have shown that proton was consumed during the oxidation of FMD. The highest peak current of FMD was also observed at pH 3.00 for both the CV and DPV. When the pH increased (pH > 5.0), the peaks shapes of FMD were broadened and deteriorated.

The effect of scan rate was examined by recording cyclic voltammograms in the range of 10 mVs^{-1} to 1600 mVs^{-1} for 1.0×10^{-4} M FMD in BR buffer solutions containing 0.1 M KCl (pH 3.0). The peak current values were linearly changed with the square root of the scan rate in the range of 500 – 1200 mV. This result has indicated that the oxidation reaction of FMD on the surface of PGE was controlled by diffusion.

Calibration curves were constructed by using the results of DP experiments at the scan rate of 25 mVs^{-1}, the scan range of 700 - 1200 mV in BR buffer solutions containing 0.1 M KCl (pH 3.0) at PGE. A linear relationship between

the FMD concentration and the peak current was obtained over the concentration range 8.0×10^{-7} to 3.0×10^{-5} M and 5.0×10^{-5} to 1.0×10^{-3} M. The linearity of the developed method was described by the following equations:

For low concentrations : $i\,(\mu A) = 75933\, C_{FMD}(M) + 0.105 \quad (R^2 = 0.9920)$

For high concentrations : $i\,(\mu A) = 8384.9\, C_{FMD}(M) + 2.195 \quad (R^2 = 0.9979)$

The RSD values for 3.0×10^{-6} M FMD were calculated as 6.07%. The LOD and LOQ were calculated as 8.83×10^{-7} M and 2.94×10^{-6} M FMD respectively, using the equation LOD = $3s_b/m$ and LOQ = $10\ s_b/m$, where s_b is the standard deviation of the solution response for 3.0×10^{-6} M FMD, m is the slope of the first calibration plot. The lowest observable peak current was for 8.0×10^{-7} M FMD (Table 3.13).

Table 3.13 Comparison of the proposed method with some reported voltammetric methods for determination of FMD.

Linear Range (M)	**LOD (M)**		**LOQ (M)**	**RSD %**	**Sample**	**Ref.**
$1\times10^{-9} - 4\times10^{-8}$	LS AdSV	1.8×10^{-10}	6.2×10^{-10}	19	urine	Skrzypek et al., 2005
	SW AdSV	4.9×10^{-11}	1.6×10^{-10}	9		
$2\times10^{-6} - 9\times10^{-5}$	3.73×10^{-7}		1.24×10^{-6}	NR	urine	Yagmur et al., 2014
8.0×10^{-7} - 1×10^{-3}	**8.83×10^{-7}**		**2.94×10^{-6}**	**6.07**	**drug**	Present work

The proposed voltammetric procedure was successfully applied to the direct determination of FMD in Famoser® drug. The obtained results were in good agreement with the content of tablet and the relative error was calculated as about 3.6-7.2%.

A very low-cost and simple DP voltammetric procedure was developed for determination of FMD. Voltammetric methods reported Skrzypek et al. (2005) have lower LOD value than the proposed method. This low LOD value arises from the use of a controlled growth mercury drop electrode. However, the use of mercury is being increasingly abolished from analytical methodology due to its toxicity in recent years. A similar voltammetric study for FMD determination was carried out at an ultra-trace graphite electrode by Yağmur et al. (2014). The linear calibration range of the proposed method is comparable to, or better than this study (Table 3.13).

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CIRRICULUM VITAE

Name	:	Şeniz VATANSEVER
Citizenchip	:	T.C.
Date of Birth	:	May, 14, 1989
Place of Birth	:	Eskişehir – Türkiye
Marital Status	:	Single
Personal Address	:	Ege University, Faculty of Science Department of Chemistry 35100, Bornova, İzmir - TURKEY
Private Address	:	Yalı Mahallesi 6440/2 Sokak No:25 Karşıyaka, İzmir - TURKEY
Business address	:	DALAN Kimya Endüstri A.Ş. Kemalpaşa Cad. No.325 35060 Pınarbaşı / İzmir
Phone Number	:	+90 505 704 89 63
e-mail	:	vatanseverseniz@hotmail.com

Education

2008-2012	:	B.Sc. Degree, Ege University, Faculty of Science Department of Chemistry
B.Sc. thesis	:	Poli(Stiren-ko-MMA) Kopolimerinin Fotopolimerizasyonu ile Sentezi ve Karekterizasyonu, 2012
2013-2015	:	M.Sc. Degree, Ege University, Faculty of Science Department of Chemistry (Analytical Chemistry)
M.Sc. thesis	:	Electrochemical Behavior and Determination of Famotidine in Pharmaceutical Formulations, 2015