EGE UNIVERSITY GRADUATE SCHOOL OF
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(MSc. THESIS)

VOLTAMMETRIC AND CENTRI –VOLTAMMETRIC

BEHAVIOR OF TERBUTALINE AT

GLASSY CARBON AND CARBON PASTE

ELECTRODES

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Eylem ERDUGAN tarafından **Yüksek Lisans** tezi olarak sunulan "**Camımsı Karbon ve Karbon Pasta Elektrotlarda Terbütalinin Voltammetrik ve Santri-Voltammetrik Davranışının İncelenmesi" (Voltammetric and Centri -Voltammetric Behavior of Terbutaline at Glassy Carbon and Carbon Paste Electrodes)** başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve **27.10.2010**.tarihinde yapılan tez savunma sınavında aday oybirliği ile başarılı bulunmuştur.

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IV

ÖZET

CAMIMSI KARBON VE KARBON PASTA ELEKTROTLARDA TERBÜTALİNİN VOLTAMMETRİK VE SANTRİ-VOLTAMMETRİK DAVRANIŞININ İNCELENMESİ

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Santri-voltammetrik yöntemin kapsamını genişletmek amacıyla test maddesi olarak seçilen Terbütalinin (TB) voltammetrik ve santri-voltammetrik davranışı camımsı karbon (GCE) ve karbon pasta (CPE) elektrotlarda incelenmiştir. Yöntemin duyarlığını etkileyebilen parametreler ve optimum koşullar değerlendirilmiştir. Denemeler santrifüj işlemine ve voltammetrik ölçüme uyumlu santri-voltammetrik hücrede gerçekleştirilmiştir.

Çalışma elektrotlarının koşullandırılması, hücre içindeki konumu ve karbon pastanın bileşiminin yanı sıra ortamın pH'sı, potansiyel tarama hızı, santrifüj hızı ve süresi gibi etkin parametreler incelenmiştir. Pik akımı ve pik potansiyeline ilişkin sonuçlar pH etkisinin TB derişimi ile değiştiğini ve TB'in elektro-yükseltgenmesinin GCE ve CPE'da aynı mekanizma ile yürüdüğünü düşündürmüştür. GCE ve CPE kullanılarak TB pik akımlarının değişimi 6.4×10^{-8} - 5.6×10^{-5} M derişim aralığında voltammetrik olarak izlenmiş ve kalibrasyon grafikleri optimum koşullarda oluşturulmuştur. Voltammetrik olarak CPE'da ölçülebilen en küçük terbütalin sülfat (TBS) 35 ng mL^{-1} düzeyindedir.

Tablet formundaki Bricanyl adlı ilacın içeriğindeki (tablet başına 2.5 mg TBS) TBS'ın çözünürlüğü su ve alkollü ortamlarda incelendikten sonra voltammetrik ve santri-voltammetrik yolla analizine geçilmiştir. Her iki yöntemde de standart katma uygulanmıştır. Voltammetrik ve santri-voltammetrik yolla saptanan TBS miktarı tablet başına sırasıyla 2.38 mg ve 2.66 mg olarak hesaplanmıştır.

Anahtar sözcükler: Terbütalin, Santri-Voltammetri, Santrifüj, Karbon Pasta Elektrot, Camımsı Karbon Elektrot, Diferansiyel Puls Voltammetrisi.

ABSTRACT

VOLTAMMETRIC AND CENTRI -VOLTAMMETRIC BEHAVIOR OF TERBUTALINE AT GLASSY CARBON AND CARBON PASTE ELECTRODES

ERDUGAN, Eylem

Terbutaline (TB) was chosen as a test substance to enlarge the scope of centri-voltammetric method. Investigations of both voltammetric and centri-voltammetry behavior of TB at a glassy carbon (GCE) and carbon paste (CPE) electrodes were aimed in this study. The parameters which can affect sensitive of the method and optimal conditions were evaluated. A centri-voltammetric cell was used to be compatible with both centrifugation and voltammetry.

Conditioning and position of working electrodes, and also composition of carbon paste were examined. The effective parameters such as pH, scan rate, centrifugation speed and time were investigated. Results related to peak current and peak potential pointed out that effect of pH changed depending on TB concentration and the electro-oxidation reaction of TB occurred with the same mechanism at CPE and GCE. The change of TB peak currents was examined in the concentration range of 6.4×10^{-8} to 5.6×10^{-5} M, by using GCE and CPE in voltammetric procedure. Calibration graphs were plotted under the optimum conditions. The minimum measurable terbutaline sulphate (TBS) at CPE is as low as $35\,\mathrm{ng\,mL^{-1}}$ in voltammetric procedure.

The solubility of TBS in alcohol and non-alcohol medium were evaluated by going peak currents. Then, the content of TBS was tried to be determined in Bricanyl Drug (2.5 mg TBS in per tablet) by applying both voltammetric and centri-voltammetric procedure. Standard addition method was performed in two sections. Amount of TBS detected was calculated as 2.38 mg and 2.66 mg in per tablet in voltammetric and centri-voltammetric procedures, respectively.

Keywords: Terbutaline, Centri-Voltammetry, Centrifugation, Carbon Paste Electrode, Glassy Carbon Electrode, Differential Pulse Voltammetry.

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LIST OF SYMBOLS

Symbol	Explanation
A	Surface area of the electrode
C_O^0 and C_R^0	Redox species at the electrode surface
$C_{M(Hg)}$	Concentrations of metal atoms in mercury
d	Density
D	Diffusion coefficient
D_O	Diffusion coefficient of the oxidized forms of the electroactive species
D_R	Diffusion coefficient of the reduced forms of the electroactive species
ΔE	Amplitude
E	Application of a potential
E^0	Standard electrode potential for the redox couple
$E_{1/2}$	Half-wave potential
E_{dep}	Deposition potential
E_i	initial potential
E_p	Peak potential
E_{pa}	Anodic peak potential
E_{pc}	Cathodic peak potential
F	Faraday constant
g	Earth's gravitational acceleration
i_d	Limiting diffusion current
i_{for}	forward current
i_p	Peak current
i_{pa}	Anodic peak current
i_{pc}	Cathodic peak current

LIST OF SYMBOLS (continued)

Symbol	Explanation
i_{rev}	reverse current
k^0	Rate of the reaction
K_a	Dissociation constant for acid
n	Number of electrons transferred, successive replicates
$\mathbf{O}$	The oxidized species
$\mathbf{R}$	The reduced species
R	Gas constant
s	Standard deviation
t	Time
T	The absolute temperature
T	Squarewave period
t_{acc}	Duration of accumulation
t_{cent}	Time of centrifugation
t_{dep}	Deposition time
v_{cent}	Speed of centrifugation
α	Transfer coefficient
δ	thickness of the diffusion layer
φ	Flux of matter
ω	Frequency
x	The distance from the electrode surface

LIST OF ABBREVIATIONS

Abbreviation	Explanation
AC	Alternating current
AdSV	Adsorptive stripping voltammetry
AE	Auxiliary electrode
ASV	Anodic stripping voltammetry
BDD	Boron-doped diamond
cAMP	Cyclic adenosine monophosphate
CL	Chemiluminescence
CME	Chemically modified electrode
CSV	Cathodic stripping voltammetry
CV	Cyclic voltammetry
DC	Direct current
DME	Dropping mercury electrode
DPV	Differential pulse voltammetry
FI	Flow injection
NPV	Normal Pulse Voltammetry
PSA	potentiometric stripping analysis
GCE	Glassy Carbon Electrode
PAD	pulsed amperometric detection
SAM	self-assembled monolayer
GC	Gas chromatography
HMDE	Hanging mercury drop electrode
SWV	Square-Wave Voltammetry
MFE	mercury-film electrode
SMDE	static mercury drop electrode

ISE	ion-selective electrode
TB	Terbutaline
TBS	Terbutaline Sulphate

1. INTRODUCTION

Drug analysis is undertaken during various phases of pharmaceutical development, such as formulation and stability studies, quality control and toxicology and pharmacological testing in animals and man. In hospitals, drug analysis is performed on patients' samples in support of clinical trials, i.e. bioavailability and pharmacokinetic studies and in monitoring therapeutic drugs and drugs abuse. All these investigations require reliable and validated analytical methods in order to measure drugs in complex media such as formulation and biofluids (El-Maali, 2004).

The resorcinol-type drug terbutaline sulphate (1-[3,5-dihydroxyphenyl]-2-[tert-butylamino] ethanol sulphate) is a β_2 adrenoceptor agonist, primarily used in the treatment of bronchial asthma and other forms of allergic airway diseases. In some respiratory diseases, it can often be taken in overdoses, which can cause tremor, tachycardia, hypokalamia, and sometimes fatal consequences. It is for this reason that the analysis of terbutaline (TB) is important in pharmaceutical research and clinical chemistry.

In addition, β_2 agonists that known as a doping material due to the stimulation on the central nervous system and certain anabolic-like effects obtained are on the list of prohibited substances in sport. In official doping controls, about 300 drugs and metabolites have to be screened for each sample. Moreover, the number of determinations to be routinely processed increases continuously as the number of both samples and potential illicit drugs keeps growing. As a consequence, increasingly specific, sensitive, and, above all, fast methods for doping controls are needed (Brunelli et al., 2006). Because of this importance, it has also been studied for developing of sensitive TB analysis methods nowadays.

Terbutaline sulphate (TBS) is salt form of TB, an ethanolamine derivative with bronchodilating and tocolytic properties (Figure 1.1). TBS selectively binds to and activates beta-2 adrenergic receptors, leading to intracellular adenyl cyclase

activation via a trimeric G protein and subsequent increase in cyclic adenosine monophosphate (cAMP) production. Increased cAMP levels result in relaxation of bronchial and vascular smooth muscle mediated through the activation of protein kinase A, which phosphorylates proteins in control of muscle tone. cAMP also inhibits calcium ion release from intracellular stores, reduces calcium entry into cells and induces the sequestration of intracellular calcium all of which aids the relaxation of airway muscles. TBS also increases mucociliary clearance and reduces release of inflammatory cell mediators (Medications, 2010).

1.1. Physicochemical Properties of Terbutaline Sulphate

TB is given as the sulphate salt. It is a white to gray-white, crystalline powder; odorless or with a faint odor of acetic acid; and slightly bitter. It is unstable in light and melts at about 247ºC; three pK_a values are given: 8.8, 10.1, 11.2. It is soluble in 0.1 N hydrochloric acid, slightly soluble in methanol, and insoluble in chloroform (McEvoy, 2002; Hoover, 1990; Wade, 1986).

Chemical Formula	$(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$
Molecular Weight	548.60 g mol^{-1}
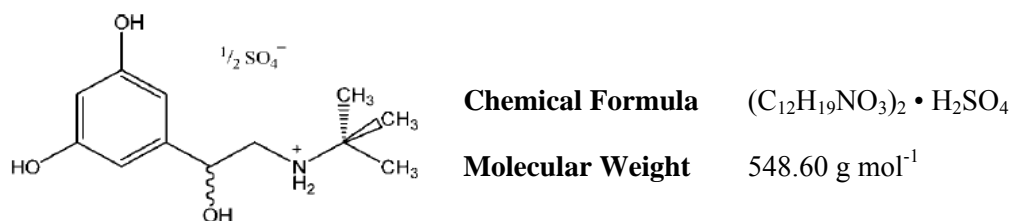
Figure 1.1. Structural formula of TBS (Harris et al., 2007).

1.2. Previous Studies for Terbutaline Determination

The TB drug is given orally and by injection or inhalation for the therapeutic management of chronic as well as prophylaxis of asthma and nocturnal asthma in particular. It is a drug of choice for the treatment of asthma but it has several drawbacks such as short biological half-life of about 3.6 hours, it is readily metabolized in the gut wall and liver when given orally. It has a short duration of action, low peak plasma level of 1.2 μg mL^{-1} and poor bioavailability

of only 14.8%. These factors necessitated formulation of controlled release transdermal drug delivery system for TBS, as this route of drug administration would reduce the dosing frequency hence better patient compliance (Rathore et al., 2006).

Many studies have been carried out relating with TB to achieve a controlled release and improved bioavailability of this drug and to determine the level of it in body. Some of these studies, were done for determination of TB, was mentioned in below.

High performance liquid chromatographic (HPLC) methods with ultraviolet (Daraghmeh et al., 2002; Reverchon and Porta, 2003), electrochemical (Sagar et al., 2002), fluorescence (Kim et al., 2001) and photodiode array (Yamini et al., 2006) detectors have been used mainly for the analysis of TBS and quantification of its dosage forms.

The chromatographic system used consisted a Hypersil 100 $C_{18,}$ 150×4.6 mm (5 μm) column, a mobile phase of ammonium acetate (0.15 M) and glacial acetic acid (pH of 4.0, 96:4 v/v) with a flow rate of 2 mL min^{-1} and a UV detector set at 270 nm. Statistical values such as recovery%, relative standard deviation (RSD), limit of detection (LOD) and limit of quantification (LOQ) calculated from the calibration curves were found within the acceptable limits. LOD and LOQ values were calculated as 0.11 and 0.37 μg mL^{-1}, respectively (Daraghmeh et al., 2002)

Sagar et al. described for the determination of TB in human plasma in the range 1–35 ng mL^{-1}. Detection was achieved using a carbon fibre micro-electrochemical detector and a column-switching system. LOD of 0.8 ng mL^{-1} was obtained for TB.

A coupled achiral–chiral HPLC system with fluorescence detection at excitation/emission wavelengths of 276/306 nm has been developed for the determination of the enantiomers of TB, (*S*)-(+)- TB and (*R*)-(−)- TB in urine. For

each enantiomer the assay was found linear between 1 and 250 ng mL^{-1}. Also, LOD was 0.3 ng mL^{-1} (Kim et al., 2001).

Another study presented a new three-phase liquid-phase microextraction strategy for extraction and preconcentration of salbutamol and TB from aqueous samples, including urine. Dynamic linear ranges of 20-5000 and 10-5000, and limits of detection of 2.5 and 0.5 ng mL^{-1} were obtained by using this method for salbutamol and TB, respectively. Also, determination of drugs in environmental water and urine samples in the range of nanograms per millilitre with RSDs<10% was possible using HPLC-photodiode array detection or HPLC-MS (Yamini et al., 2006).

There are few reported methods for the simultaneous determination of TB and related compound, salbutamol, clenbuterol, acetazolamide, althiazide, indapamide in plasma based and urine on gas chromatography- mass spectrometry (GC-MS) (Brunelli et al., 2006; Nakajima et al., 2001).

Methods based on spectrophotometric measurements are used for the assay of TB in pharmaceutical preparations and dosage forms. A simple colorimetric method for the determination of TBS by using phenanthro[9,10-d]imidazole-2-N-chloroimide has been developed. The product of reaction between this special reactant and TB gave an absorption maximum at 525 nm. A linear calibration plot for TB was obtained over the concentration range of 0.7-270 μg mL^{-1} (Tanabe and Kawanabe, 1989).

A flow injection analysis method with chemiluminescence detection has also been developed for TB determination. The binding of TBS to bovine serum albumin was studied in vitro using the technique of microdialysis sampling combined with flow-injection chemiluminescence analysis (FIA-CL). In the presence of formaldehyde, TBS can be oxidized by KMnO$_4$ to produce high chemiluminescence emission in sulphate acid media. The concentration of TBS is proportional with the CL intensity in the range of 1×10^{-7} - 2×10^{-5} mol L^{-1} with a detection limit of 3×10^{-8} mol L^{-1} (Wang et al., 2003).

In recent years, capillary zone electrophoresis has been applied to determine the compound in pharmaceutical formulations and biological fluids (Shuting et al., 2009; Kim et al., 2003). In these studies, LOD values were reported as 3.0×10^{-8} mol L^{-1} and 0.8 ng mL^{-1}, respectively.

The above mentioned methods for determination of TB require expensive instrumentation, and the operations are time-consuming. On the other hand, voltammetric methods are highly selective, sensitive, rapid, and economical. Nevertheless, the voltammetric characteristics of TB have not been reported sufficiently. Limited numbers of studies are related to voltammetric determination of TB.

One of the important voltammetric studies related to determination of TB based on irreversibly oxidation of TB at an activated glassy carbon electrode (GCE). The peak current, at about 0.8 V (*vs.* a saturated calomel electrode), was proportional to the TB concentration in the range of 8×10^{-6}–8×10^{-4} M in phosphate buffer pH 6.0. This method was applied, without any interference from the excipients, to determine the drug in a tablet dosage form (Yılmaz et al., 1997).

Recently, there has been growing interest in using self-assembled monolayer (SAM) technique as a method of formation of ultra thin organic films. Because of high stability, good orientation, and easy preparation, the SAM technique has become well-established methodology in electroanalytical chemistry. In this context, a novel method for preparing 1,4-benzenedimethanethiol (BDT)–Au colloid–ferrocene (Fc) layer-by-layer self-assembled electrode by a self-assembled technique was described. Aminylferrocene was successfully immobilized on nanosized gold colloid particles associated with a 1,4-benzenedimethanethiol monolayer on a gold electrode surface and characterized by cyclic voltammograms and electrochemical impedance spectroscopy. The immobilized Fc gave an excellent electrocatalytic response to the TB oxidation. The catalytic-current response of differential pulse voltammograms increased linearly with the TB concentration from 1.75×10^{-7} to 5.62×10^{-4} mol L^{-1} and LOD was 2.30×10^{-8} mol L^{-1}. The reported method is simple, quick, and sensitive (Deng et al., 2006).

The construction and application of boron-doped diamond (BDD) thin film electrode as sensor for the determination of three β-agonists, salbutamol, TB and clenbuterol was reported by Karuwan et al. (2006). Although well-known as a chemically inert material, BDD film however shows fouling in detection of these compounds using fixed-potential mode amperometry. A suitable waveform for pulsed amperometric detection (PAD) was developed and used to determine the agonist compounds. It was seen that the developed PAD significantly refreshed the BDD surface for long-term detection in flow injection analysis. Linear working ranges were 0.5-100 μM, 1.0-100 μM and 0.5-50 μM for salbutamol, TB and clenbuterol, respectively. The developed PAD-BDD system was applied to successfully determine salbutamol and TB in commercial pharmaceutical products. The methods were validated with a capillary electrophoresis method.

In another study, a fully validated, simple, sensitive and precise square-wave adsorptive anodic stripping voltammetric procedure was described for the assay of TB in bulk form, pharmaceutical formulation and spiked human serum at a glassy carbon electrode. For 5×10^{-8} mol L^{-1} bulk TB a mean recovery of 98.78±0.94% (n = 5) was achieved following its preconcentration by adsorptive accumulation onto the glassy carbon electrode at a +0.15V (vs. Ag/AgCl/KCls) for 180 s. LOD of 6×10^{-9} mol L^{-1} and LOQ of 2×10^{-8} mol L^{-1} TB were achieved in the bulk form or its formulations (Bricanyl ® tablets). This procedure was successfully applied for the determination of TB in human serum following medium exchange. LOD of 1.41×10^{-8} mol L^{-1} (3.173 ng mL^{-1}) and LOQ of 4.70×10^{-8} mol L^{-1} (10.575 ng mL^{-1}) were achieved in human serum with a mean recovery of 98.11±1.13% (Beltagi et al., 2007). Our literature search demonstrated that the more sensitive voltammetric method for TB determination was reported by Beltagi et al.

1.3. Voltammetry

Historically, the branch of electrochemistry we now call voltammetry developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, for which he received the 1959 Nobel Prize in chemistry. The early voltammetric methods experienced a number of difficulties, making

them less than ideal for routine analytical use. However, in the 1960s and 1970s significant advances were made in all areas of voltammetry (theory, methodology, and instrumentation), which enhanced the sensitivity and expanded the repertoire of analytical methods. The coincidence of these advances with the advent of low-cost operational amplifiers also facilitated the rapid commercial development of relatively inexpensive instrumentation.

The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i, and t. They 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 analytical advantages of the various 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 (seconds), 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.

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. General theory

In voltammetry, the effects of the applied potential and the behavior of the redox current are described by several well-known laws. The applied potential controls the concentrations of the redox species at the electrode surface ($C_O{}^0$ and $C_R{}^0$) and the rate of the reaction (k^0) as described by the Nernst or Butler–Volmer equations, respectively. In the cases where diffusion plays a controlling part, the current resulting from the redox process (known as the faradaic current) is related to the material flux at the electrode–solution interface and is described by Fick's law. The interplay between these processes is responsible for the characteristic features observed in the voltammograms of the various techniques.

For a reversible electrochemical reaction (that is, a reaction so fast that equilibrium is always reestablished as changes are made), which can be described by $O + ne^- \rightleftharpoons R$, the application of a potential E forces the respective concentrations of O and R at the surface of the electrode (that is, $C_O{}^0$ and $C_R{}^0$) to a ratio in compliance with the Nernst equation:

$$E = E^0 - \frac{RT}{nF} \ln \frac{C_R^0}{C_O^0}$$

where R is the molar gas constant (8.3144 J mol^{-1}K^{-1}), T is the absolute temperature (K), n is the number of electrons transferred, F Faraday constant (96,485 C/equiv), and E_0 is the standard reduction potential for the redox couple. If the potential applied to the electrode is changed, the ratio $C_R{}^0/C_O{}^0$ at the surface will also change. If the potential is made more negative the ratio becomes larger (that is, O is reduced) and, conversely, if the potential is made more positive the ratio becomes smaller (that is, R is oxidized).

For some techniques it is useful to use the relationship that links the variables for current, potential, and concentration, known as the Butler–Volmer equation:

$$\frac{i}{nFA} = k^0 \{C_O^0 \exp[-\alpha\theta] - C_R^0 \exp[(1-\alpha)\theta]\}$$

where $\theta = nF(E\text{-}E^0)/RT$, k^0 is the heterogeneous rate constant, α is known as the transfer coefficient, and A is the area of the electrode. This relationship allows us to obtain the values of the two analytically important parameters, i and k^0.

Finally, in most cases the current flow also depends directly on the flux of material to the electrode surface. When new O or R is created at the surface, the increased concentration provides the force for its diffusion toward the bulk of the solution. Likewise, when O or R is destroyed, the decreased concentration promotes the diffusion of new material from the bulk solution. The resulting concentration gradient and mass transport is described by Fick's law, which states that, the flux of matter (φ) is directly proportional to the concentration gradient:

$$\varphi = -AD_o \left(\frac{\partial C_o}{\partial x}\right)$$

where D_o is the diffusion coefficient of O and x is the distance from the electrode surface. An analogous equation can be written for R. The flux of O or R at the electrode surface controls the rate of reaction, and thus the faradaic current flowing in the cell. In the bulk solution, concentration gradients are generally small and ionic migration carries most of the current. The current is a quantitative measure of how fast a species is being reduced or oxidized at the electrode surface. The actual value of this current is affected by many additional factors, most importantly the concentration of the redox species, the size, shape, and material of the electrode, the solution resistance, the cell volume, and the number of electrons transferred.

In addition to diffusion, mass transport can also occur by migration or convection. Migration is the movement of a charged ion in the presence of an electric field. In voltammetry, the use of a supporting electrolyte at concentrations 100 times that of the species being determined eliminates the effect of migration.

Convection is the movement of the electroactive species by thermal currents, by density gradients present in the solution, or by stirring the solution or rotating the electrode. Convection must be eliminated or controlled accurately to provide controlled transport of the analyte to the electrode.

Many voltammetric techniques have their own unique laws and theoretical relationships that describe and predict in greater detail the various aspects of the i–E behavior (such as curve shape, peak height, width, and position). When appropriate, these are discussed in more detail (Kounaves, 1997).

1.3.2. Polarography

Polarography is a voltammetric measurement whose response is determined by combined diffusion/convection mass transport. Polarography is a specific type of measurement that falls into the general category of linear-sweep voltammetry where the electrode potential is altered in a linear fashion from the initial potential to the final potential. As a linear sweep method controlled by convection/diffusion mass transport, the current vs. potential response of a polarographic experiment has the typical sigmoidal shape. What makes polarography different from other linear sweep voltammetry measurements is that polarography makes use of the dropping mercury electrode (DME) or the static mercury dropping electrode (http://en.wikipedia.org/wiki/Polarography).

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.

A number of different pulse techniques are available on the epsilon, which differ in their potential pulse wave forms, the number of sampling points, and whether a solid electrode (voltammetry) or a mercury drop electrode (polarography) is used. These are listed 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 quantitative analysis

(http://www.basinc.com/mans/EC_epsilon/Techniques/Pulse/pulse.html).

1.3.3.1. Normal Pulse Voltammetry (NPV)

This technique uses a series of potential pulses of increasing amplitude. The current measurement is made near the end of each pulse, which allows time for the charging current to decay. It is usually carried out in an unstirred solution at either DME (called normal pulse polarography) or solid electrodes.

The potential is pulsed from an initial potential E_i. The duration of the pulse, τ, is usually 1 to 100 m sec and the interval between pulses typically 0.1 to 5 s (Figure 1.2). The resulting voltammogram displays the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis.
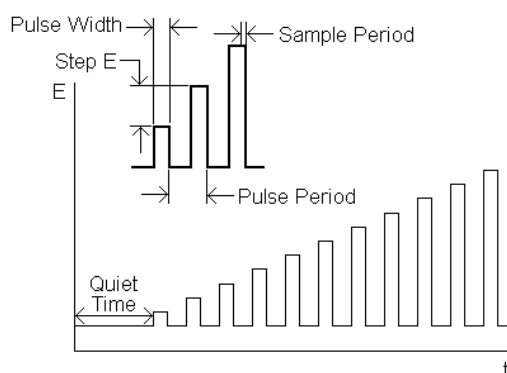
Figure 1.2. Potential waveform for normal pulse voltammetry.
(http://www.basinc.com/mans/EC_epsilon/Techniques/Pulse/pulse.html)

1.3.3.2. Differential Pulse Voltammetry (DPV)

This technique is comparable to normal pulse voltammetry 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. Current is measured at two points for each pulse, the first point (1) just before the application of the pulse and

the second (2) at the end of the pulse (Figure 1.3). These sampling points are selected to allow for the decay of the nonfaradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential.
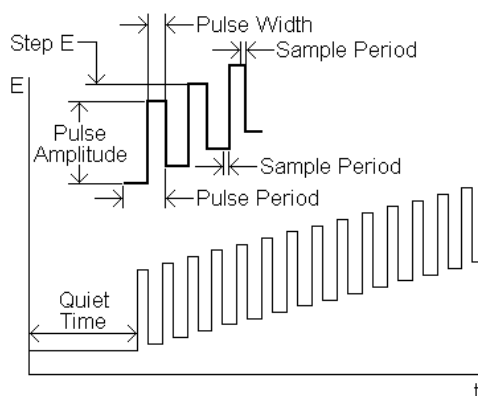
Figure 1.3. Potential waveform for differential pulse voltammetry.
(http://www.basinc.com/mans/EC_epsilon/Techniques/Pulse/pulse.html)

1.3.3.3. Square-Wave Voltammetry (SWV)

The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude E_{sw} superimposed on a staircase waveform of step height ΔE, where the forward pulse of the square wave coincides with the staircase step (Figure 1.4). The net current, i_{net}, is obtained by taking the difference between the forward and reverse currents ($i_{for} - i_{rev}$) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electroactive species and direct detection limit as low as 10^{-8} M is possible.

SWV has several advantages. Among these are its excellent sensitivity and the rejection of background currents. Another is the speed (for example, its ability to scan the voltage range over one drop during polarography with the DME). This speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal to noise ratio. Applications of SWV include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, determination of some species at trace levels, and its use with electrochemical detection in HPLC (http://www.basinc.com/mans/EC_epsilon/Techniques/Pulse/pulse.html).
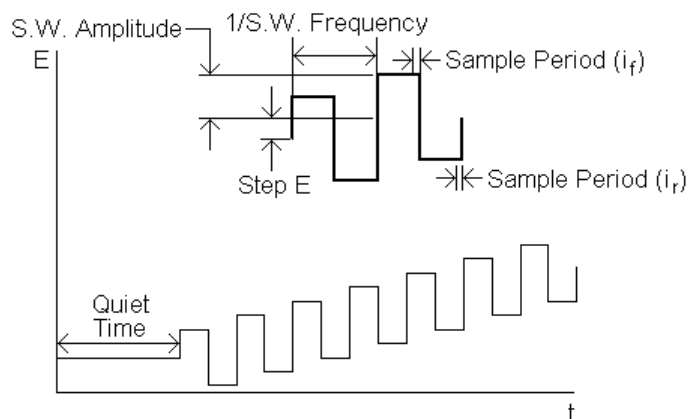
Figure 1.4. Potential waveform for square wave voltammetry.
(http://www.basinc.com/mans/EC_epsilon/Techniques/Pulse/pulse.html)

1.3.3.4. Staircase Voltammetry

Staircase voltammetry is a derivative of linear sweep voltammetry. In linear sweep voltammetry the current at a working electrode is measured while the potential between the working electrode and a reference electrode is swept linearly in time. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced. In staircase voltammetry the potential sweep is a series of stair steps (Figure 1.5). The current is measured at the end of each potential change, right before the next, so that the contribution to the current signal from the capacitive charging current is minimized
(http://en.wikipedia.org/wiki/Staircase_voltammetry).
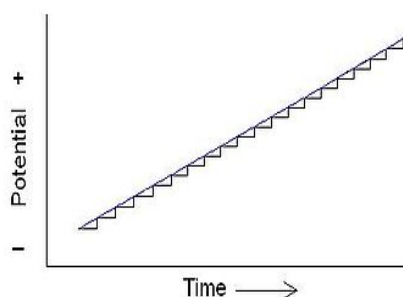
Figure 1.5. Staircase potential sweep set against a linear potential sweep.
(http://en.wikipedia.org/wiki/Staircase_voltammetry)

1.3.4. Alternating Current (AC) Voltammetry

AC Voltammetry typically involves the application of a sinusoidal oscillating voltage to an electrochemical cell. The AC experiment when used in conjunction with a lock in amplifier or frequency analyzer offers considerably increased sensitivity over the early described techniques and can also reveal important mechanistic and kinetic information not easily available using more tradition voltammetric techniques.

An AC voltammetric measurement is usually performed in an electrochemical cell where diffusion is the dominate mode of transport. The AC voltage is often combined with either a steady direct current (DC) signal or voltage sweep, for example, the following Figure 1.6 shows a cyclic voltammetric signal with an AC perturbation
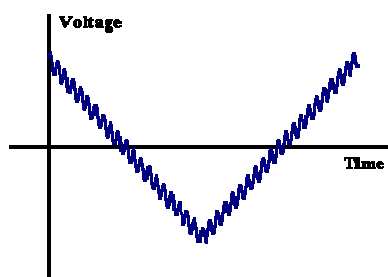
Figure 1.6. A cyclic voltammetric signal with an AC perturbation.

The most important aspect of this Figure 1.6 is the magnitude of the AC perturbation which is seen to be small in comparison to the overall change in voltage occurring during the sweep. Typically amplitude of 5 mV or less is employed in AC measurements. This small perturbation ensures only slight changes in concentrations occur close to the electrode surface and allows mathematical analysis to assume that the effect on the electrode kinetics can be calculated in a linear manner, even though the electrode kinetics strictly have an exponential dependence on the applied voltage. The Figure 1.7 below shows how the current varies when an AC component is added to a normal CV experiment. In this case the sine wave amplitude (peak to peak) was set as 40 mV, far larger than a true experiment in order to illustrate the current variation.
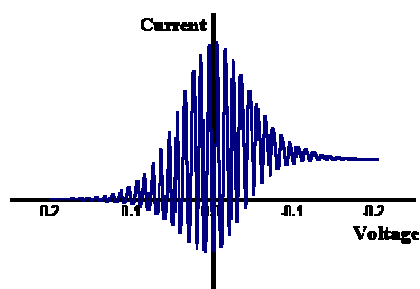
Figure 1.7. The current variation when an AC component is added to a normal CV.

However this is not the signal that is usually recorded or presented from the experiment, since a lock in amplifier or frequency response analyzer allows the component of the current which is varying sinusoidally to be separated from the DC signal. Passing the signal Figure 1.7 through the lock in amplifier provides the magnitude of the change over each cycle and appears as Figure 1.8:
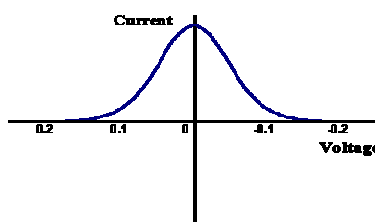
Figure 1.8. The magnitude of the change over each cycle.

The maximum change is seen to occur at E^0 as this is the region where the electrode kinetics are most sensitive to voltage changes, whereas at the two extremes of the voltage range there is no variation since the electrode kinetics are insignificantly affected and so the current doesn't vary. Next we will focus on an individual cycle, to establish the phase relationship between the current and voltage (Figure 1.9).
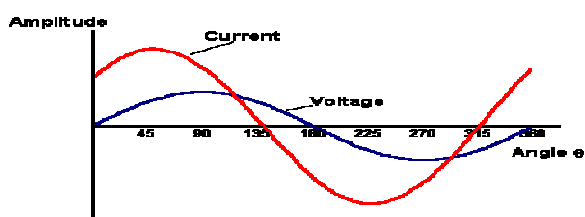
Figure 1.9. The phase relationship between the current and voltage.

It is clear that the current maximum is shifted from the applied voltage maximum by 45° and this phase shift can be understood by studying the concentration profiles close to the electrode surface during a voltage cycle. Again for illustration purposes large voltage amplitude has been employed and the concentration profiles generated are shown in Figure 1.10.
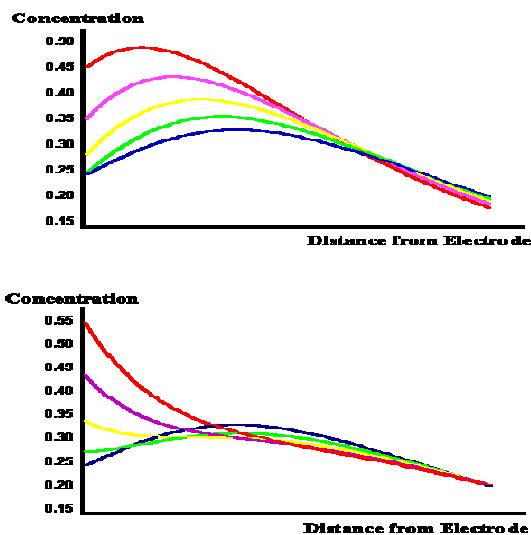
Figure 1.10. The concentration profiles.

In Figure 1.10, the voltage is dropping from zero to the negative minimum and the surface concentration of the reactant is dropping as expected. However the current is dependent upon the flux of material to the surface and the maximum of this flux occurs between the yellow and mauve curves. The origin of the phase shift can therefore be seen to result from the diffusion process occurring.

Mathematical analysis of the phase relationship by solution of Fick's laws for the reversible AC voltammogram shows that the phase angle seen in the above voltammogram is exactly as predicted. The lower figure shows the concentrations as the voltage moves from the minimum back up towards the zero line. The gradient can be seen to change direction and consequently a current begins to flow in the opposite direction to that in the top figure. Also a ripple in the concentration profile is seen as the diffusion is unable to keep up with the changes induced on the surface, hence again the phase difference between the voltage and current. (http://www.cartage.org.lb/en/themes/Sciences/Chemistry/Electrochemis/Electrochemical/ACCyclic/ACCyclic.html)

1.3.5. Cyclic Voltammetry (CV)

CV is a type of potentiodynamic electrochemical measurement. In a CV experiment the working electrode potential is ramped linearly versus time like linear sweep voltammetry. CV takes the experiment a step further than linear sweep voltammetry which ends when it reaches a set potential. When CV reaches a set potential, the working electrode's potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage (Figure 1.11). CV is generally used to study the electrochemical properties of an analyte in solution (http://en.wikipedia.org/wiki/File:Cyclovoltammogram.jpg).
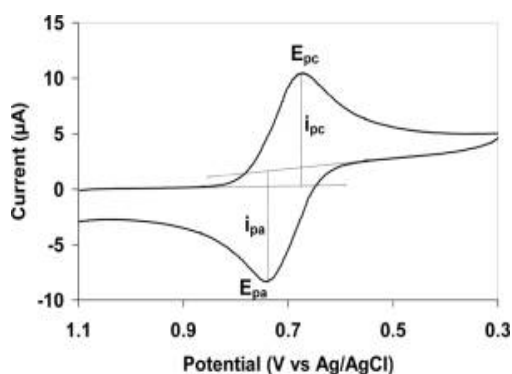
Figure 1.11. Cyclic voltammogram for a reversible electrode process.
(http://en.wikipedia.org/wiki/File:Cyclovoltammogram.jpg)

1.3.6. Preconcentration and stripping techniques

Voltammetry can be carried out using commercially available polarographic instruments employing the classical polarographic method (DC) as well as pulse methods. Modern voltammetric instruments with automatic timing of the individual operations are useful for controlling the individual steps in stripping voltammetric measurements (accumulation time, solution stirring, rest period, initiation of polarization); a computerized instrument is useful for this purpose (El-Maali, 2004).

Stripping voltammetry comprises a variety of electrochemical approaches, having a step of preconcentration onto the electrode surface prior to the voltammetric measurement. The major advantage of stripping voltammetry

compared with direct voltammetric measurements is the preconcentration factor. For trace analysis of organic compounds, the accumulation of the compound to be determined on the working electrode will be followed by voltammetric oxidation of the accumulated substance (anodic stripping voltammetry, ASV) or by electrochemical reduction (cathodic stripping voltammetry, CSV) of by chemical oxidation (potentiometric stripping analysis, PSA). In drug analysis, adsorptive stripping voltammetry (AdSV) is popular because of the low limit of determination (reaching few ppb concentrations) its accuracy and precision, as well as the low cost of instrumentation relative to other analytical methods of analysis.

1.3.6.1. Anodic stripping voltammetry

ASV 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. Second, the electrode potential is swept to positive potentials, inducing the oxidation of the metal in the mercury electrode. 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 (Mirceski et al, 2007).

1.3.6.2. Cathodic stripping voltammetry

CSV is the mirror image of ASV. This method is used to determine a variety of anions that form insoluble salts with mercury(I) ions. In CSV the mercury electrode is not inert. A relatively positive potential is applied to mercury electrode where mercury is oxidized to mercury(I):

$$2\text{Hg} \rightarrow \text{Hg}_2^{2+} + 2\text{e}$$

Then in the presence of an anion X^- an insoluble film forms on the electrode surface following the general reaction:

$$\text{Hg}_2^{2+} + 2\text{X}^- \rightarrow \text{Hg}_2\text{X}_2$$
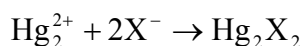
Stripping in CSV consists of a negative-going potential scan, which reduces the mercury salt to Hg and X^-:

$$\text{Hg}_2\text{X}_2 + 2\text{e} \rightarrow 2\text{Hg} + 2\text{X}^-$$
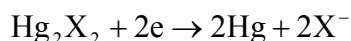
The stripping peak position and height are characteristic of the type of anion and its concentration in solution, respectively. CSV procedures have been developed for determination of ionic species. A few metal cations [e.g., cerium(III), iron(II), manganese(II), and thallium(I)] can be concentrated as insoluble hydroxides by oxidation on a carbon electrode. The hydroxides are then stripping from the electrode by reduction with a negative-going potential scan (Wang, 1994; Dewald, 1996).

1.3.6.3. Adsorptive stripping voltammetry

AdSV is applied to stripping voltammetric techniques in which accumulation is effected by adsorption of (mainly) organic determinants. The use of the term can be justified less in the case of the adsorption of metal complexes in determining metal ions, although Kalvoda (personal communication) has pointed out that metal complexes adsorb by virtue of adsorption by their ligands. The term should not be applied when there is a change of oxidation state of the

metal ion during the accumulation (e.g. in the accumulation of copper (I) complexes or salts) Orin other cases where an organic compound is being accumulated, and determined indirectly, as a metal salt or complex (e.g. as mercury salts or nickel complexes) (Fogg et al., 1999).

1.3.6.4. Potentiometric stripping analysis

PSA, known also as stripping potentiometry, differs from ASV in the method used for stripping the amalgamated metals. In this case, the potentiostatic control is disconnected following the preconcentration, and the concentrated metals are reoxidized by an oxidizing agent [e.g., O_2, Hg(II)] that is present in the solution:

$$M(Hg) + oxidant \rightarrow M^{n+}$$
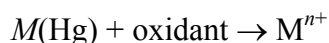
A stirred solution is used also during the stripping step to facilitate the transport of the oxidant. Alternately, the oxidation can be carried out by passing a constant anodic current through the electrode. During the oxidation step, the variation of the working electrode potential is recorded, and a stripping curve, like the one shown in Figure 1.12 (curve a), is obtained. When the oxidation potential of a given metal is reached, the potential scan is slowed down as the oxidant (or current) is used for its stripping. A sharp potential step thus accompanies the depletion of each metal from the electrode (Wang, 2006).

Modern PSA instruments use microcomputers to register fast stripping events. Such differential display of dt/dE versus E is shown in Figure 1.12 (curve b). The use of nondeaerated samples represents an important advantage of PSA (over analogous ASV schemes), particularly in field applications. In addition, such potential-time measurements eliminate the need for amplification when microelectrodes are concerned. By obviating the need for stirring or deoxygenating the solution, the coupling of PSA with microelectrodes permits convenient trace analysis of very small samples. PSA is also less susceptible to interfering surfactant effects, and hence can simplify the pretreatment of biological samples (Wang, 2006).
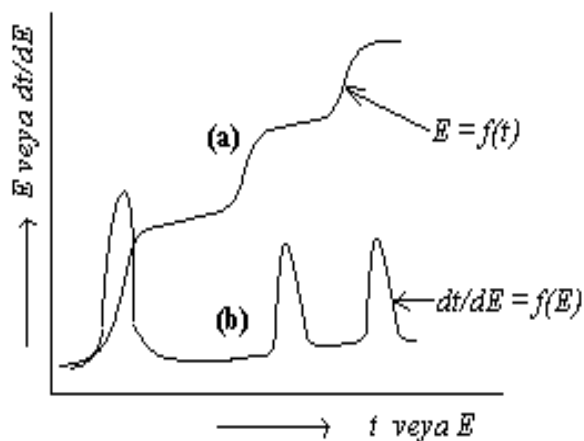
Figure 1.12. Stripping potentiograms for a solution containing three components (Tural et al., 2003).

1.3.7. Types of working electrodes used in voltammetry

Voltammetry can be carried out practically at all types of electrodes designed for voltammetry and for which a completely reproducible constant surface area can ensure reproducible results over the whole measuring period or during a series of measurements.

The working electrode is the electrode at which the reaction of interest occurs. Generally, the working electrode in voltammetry is characterized by its small surface area, which enhances polarization. Another reason for using very small electrodes is to minimize depletion (by electrolysis) of the analyte.

The choice of the working electrode is very important for the sensitivity and reproducibility of stripping analysis in particularly. Stationary working electrodes used in stripping measurement fall into two large groups, mercury electrodes and inert solid electrodes. There are two types of mercury electrodes that have gained wide acceptance for stripping analysis: the hanging mercury drop electrode (HMDE) or static mercury drop electrode (SMDE) and the mercury-film electrode (MFE). There are several kinds of solid electrodes, e.g. glassy carbon electrode (GCE), graphite electrode, carbon paste electrode (CPE), platinum electrode (Pt), gold electrode, etc (El-Maali, 2004).

Mercury electrodes

In the first half of the twentieth century, mercury electrodes were widely used for investigation of adsorption and other phenomena in electrochemical systems. This was due to the following features of these electrodes, connected with their liquid nature: (1) the true working surface area coincides with the geometric surface of the electrode, in contrast to solid electrodes, which always have a well-pronounced surface roughness; (2) the surface can be easily renewed (e.g., in dropping electrodes) and cleaned from impurities; (3) the ESE (surface tension) and its dependence from potential and solution composition can easily be determined (Bagotsky, 2006).

Carbon electrodes

Solid electrodes based on carbon are currently in wide spread 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 (e.g., graphite epoxy, wax-impregnated graphite, Kelgrap). The properties of different types of carbon electrodes are discussed below (Wang, 2006).

Glassy-carbon electrodes

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).

Carbon-paste electrodes

A CPE is made from a mixture of conducting graphite powder and a low volatility organic liquid. Not only are they suitable for a variety of organic oxidations, but they also have an extremely low background current. The low background is a major advantage over Pt and Au electrodes, which have large backgrounds in water from oxide formation and reduction (Bard, 1966).

These electrodes are simple to make and offer an easily renewable surface for electron exchange. CPEs belong to a special group of heterogeneous carbon electrodes. These electrodes are widely used mainly for voltammetric

measurements; however, carbon paste-based sensors are also applicable in coulometry (both amperometry and potentiometry).

In general, CPEs are popular because carbon pastes are easily obtainable at minimal costs and are especially suitable for preparing an electrode material modified with admixtures of other compounds thus giving the electrode certain pre-determined properties. Electrodes made in this way are highly selective sensors for both inorganic and organic electrochemistry.

The biggest disadvantage of CPEs, which limits their applicability in practical analysis, is that success in working with carbon paste-based electrodes depends on the experience of the user. While this is true for any type of solid electrodes, CPEs are rather an exceptional case. In contrast to commercially available solid electrodes for which basic electrochemical characteristics are comparable for almost all products from each manufacturer, each carbon paste unit is an individual, where the physical, chemical and electrochemical properties may differ from one preparation to another. For this reason each probe must be calibrated individually. (http://en.wikipedia.org/wiki/Carbon_paste_electrode; Uslu and Özkan, 2007).

Carbon-fiber electrodes

The growing interest in ultramicroelectrodes has led to widespread use of carbon fibers in Electroanalysis. Such materials are produced, mainly in connection with the preparation of high-strength composites, by high-temperature pyrolysis of polymer textiles or via catalytic chemical vapor deposition. Different carbon fiber microstructures are available, depending upon the manufacturing process. They can be classified into three broad categories, namely, low, medium and high-modulus types. The last type is most suitable for electrochemical studies because of its well-ordered graphite-like structure and low porosity.

Improved electron transfer performance can be achieved by various electrode pretreatments, particularly "mild" and "strong" electrochemical activations, or heat treatment. Most electroanalytical applications rely on fibers of

5-20 μm diameters that provide the desired radial diffusion. Such fibers are typically mounted at the tip of a pulled glass capillary with epoxy adhesive, and are used in cylindrical or disk configurations. Precautions should be taken not to contaminate the carbon surface with the epoxy. The main advantage of carbon-fiber microelectrodes is their small size (5-30μm diameter for commercially available fibers), which makes, them very attractive for anodic measurements in various microenvironments-for example, the detection of neurotransmitter release in the extra cellular space of the brain. Nanometer-sized carbon fibers can be prepared by etching the fiber in flame or under ion beam (Wang, 2006).

Chemically modified electrodes

Chemically modified electrodes (CMEs) comprise a relatively modem approach to electrode systems that finds utility in 1) a wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity to electrode surface chemistry, electrostatic phenomena at electrode surfaces, and electron and ionic transport phenomena in polymers, and 2) the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection, and electro-organic syntheses.

Compared with other electrode concepts in electrochemistry, the distinguishing feature of a CME is that a generally quite thin film (from a molecular monolayer to perhaps a few micrometers-thick multilayer) 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 (ISEs) 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).

1.3.8. Centri - voltammetry

Centri-voltammetry is a novel method, which allows combining the advantages of centrifugation and voltammetry. The method offers a practical way for application of coprecipitation in trace analysis allowing direct voltammetric scan hence prevents the loss of the analyte, which usually is the case for other preconcentration techniques.

Centri-voltammetric analysis method is based on bulk deposition at the electrode surface and preconcentration ratio of analyte improves with the effect of centrifugation process. This method shows similarity with stripping voltammetric techniques; only the preconcentration step differs as the centrifugation is employed. Carefully controlled and optimized experimental conditions were implemented to prove reproducibility of the result of analysis.

Centrifugation is a process that involves the use of the centrifugal force for separating materials in certain types, used in industry and in laboratory settings. If a liquid (or solution) contains materials of different densities or widely differing molecular weights, these materials may be separable by using a centrifuge. In chemistry and biology, centrifugation increases the effective gravitational force on a mixture in a test tube, to rapidly and completely bring the precipitate ("pellet") to the bottom of the tube. The remaining solution is called the supernatant or supernatant liquid. The supernatant liquid is then separated from the precipitate by decantation or withdrawal with a Pasteur pipette (http://www.scribd.com/doc/180299/centrifugation).

Centrifuge is a piece of equipment, generally driven by a motor that puts objects in rotation around a central, fixed axis, applying a force perpendicular to the axis. The equipment consists of a fixed base and a rotating component, called a rotor that holds the objects or samples to be spun (http://www.newworldencyclopedia.org/entry/Centrifuge).

Previous centri-voltammetric studies are related with the analysis of some metal cations. Pioneering part of the study includes collection of lead ions by

utilizing a carrier precipitate (Al(OH)$_3$) on a microelectrode by means of centrifugation (Kirgöz et al, 2004). In the second part of this study Amberlite XAD-7 resin was used as a carrier material for the purpose of preconcentration of Pb(II) ions (Kirgöz et al, 2005). The detection limits of these methods under optimal conditions were reported as 2.2×10^{-9} and 5.2×10^{-9} M, respectively.

The determination of mercuric ions was achieved by using centrifugal forces without carrier material to collect mercuric ions effectively onto gold film electrode surface following their reduction to metallic mercury with sodium borohydride or hydrazine. Another centri-voltammetric method for mercury determination was described by using Purolite C100 as a carrier material and applied to spring water samples. In latter method, used bare GCE, the use of a bare GCE was considerable shortened the analysis procedure. The values of LOD and LOQ was calculated as 1.0×10^{-13} and 5.8×10^{-12} M, respectively (Ürkmez, 2004, 2010; Ürkmez et al., 2009).

The simultaneous centri-voltammetric behavior of cadmium, lead, and copper ions was investigated in the presence of carrier precipitate, Al(OH)$_3$, on mercury film electrode. The linear concentration ranges were obtained as 7.0×10^{-8} - 2.0×10^{-6} M for lead, 5.0×10^{-8} - 2.0×10^{-6} M for cadmium, and 2.0×10^{-7} - 3.0×10^{-6} M for copper ions (Vardar, 2008).

Analysis of some cations, which can not be detected by ASV because of that they are insoluble in mercury, can be achieved by centri-voltammetry as shown by Koçak et al. In this respect, the behavior of Mo(VI) cation was investigated in the absence and presence of complexing ligand (8-Hydroxyquinoline). Calibration graph of Mo(VI) cation could be obtained in the range of 1×10^{-7} to 5×10^{-6} M and LOD of 1.4×10^{-8} M was reached by using pyrogallol red as complexing ligand In addition, the giant polyoxomolybdate (POM) molecule that formed on the electrode surface diffusing from the molybdenum ion and reducing at the electrode surface was observed in chloroacetic acid medium (Koçak et al, 2008, Koçak 2009).

1.4. Purpose of Thesis

Previous studies related to centri-voltammetric method were purposeful to analysis of some metal cations as mentioned in Section 1.3.8. However, there is no study about application of this method for organically or biological molecules that is vital importance. Because of this reason, investigations of both voltammetric and centri-voltammetry behavior of TB at a GCE and CPE were aimed in this study too. Terbutaline was chosen as a test substance because of making a contribution to enrich the field of application of this new developed method. The investigation of parameters which can affect sensitive of the method and determination of optimal conditions were planned.

2. MATERIALS AND METHODS

2.1. Apparatus

Voltammetry experiments in differential pulse (DPV) and cyclic (CV) waveforms were performed at room temperature. Voltammetric measurements in DP mode were carried out with a Metrohm 693 Voltammetric Analyser (VA) processor and a 694 VA stand that consisted of a three-electrode system (Figure 2.1). Cyclic voltammograms were obtained by using a Palm Sens PSLite v1.7 model. The three-electrode system for both of using methods was the same. A platinum wire of large area was used as the auxiliary electrode; a miniature home made Ag/AgCl (saturated KCl) (Hassel, et al, 1999) electrode as the reference electrode. Bare glassy carbon (GCE) and carbon paste (CPE) electrodes (2 ± 0.1 mm diameter) were used as a working electrode to compare the results of experiments

Figure 2.1. Metrohm 693 Voltammetric Analyzer (VA) processor and a 694 VA Stand with its own cell.

A "centri-voltammetric cell" (Vardar, 2008) which has a large volume (~ 40 mL) was used as to be compatible with both centrifugation and voltammetry, and it was made up from Plexiglas (Figure 2.2). The cell consisted of two parts (part 1and 2) which were screwed together. GCE supplied from Metrohm was cut in the middle to be accord with designed cell geometry. This working electrode was placed in its cavity of the part 1 facing upward to collect the analyte on the electrode surface effectively.

A   B
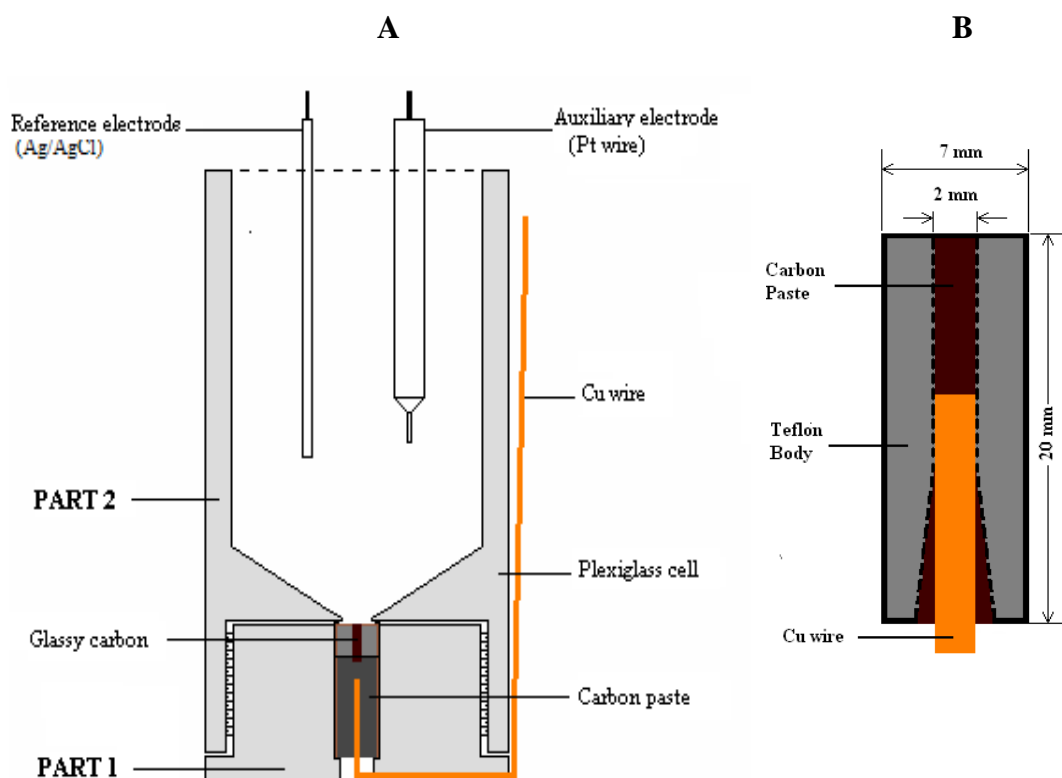
Figure 2.2. A) The mounted centri-voltammetric cell with GCE, B) CPE.

pH values of solutions were measured and adjusted by using a Thermo Orion 3 Star with a combined glass electrode. Nüve NF 800 was used for centrifugation. An ELMA Transsonic 460/H ultrasonic bath was used for the cleaning procedure of the GCE surface before every measurement.

2.2. Chemicals and Solutions

The deionized water obtained from the Milli-Q-18.2 MΩ Ultrapure Water system was used throughout the study. The using chemicals and their properties were listed in below (Table 2.1). All chemicals were supplied from Merck with the exception of graphite, Bricanyl drug and Standart Terbutaline. Synthetic graphite powder of 1-2 micron was supplied from Aldrich (CAS number: 7782-42-5; EEC number: 231-955-3; LOT number: AQ14201LG).

Bricanyl drug contained 2.5 mg TBS per tablet was supplied from pharmacy. Analytical Reference Standard TBS (ADM no: 20531J04) was supplied

from AstraZeneca (UK Limited) by via an approval of the Pre-clinical Material Evaluation Agreement. The properties of this reference material were given in Table 2.2 according to its certificate of analysis and safety data sheet.

Table 2.1. The used chemicals and their properties.

Chemicals	Percentage %	Density (g mL^{-1})
HCl	30 (suprapur grade)	1.15
HClO$_4$	60 (GR for analysis)	1.53
NaOH	99 (pellets GR for analysis)	solid
CH$_3$OH	99.8 (extra pure)	0.79
CH$_3$CH$_2$OH	96 (v/v)	
CH$_3$COOH	99-100 (for synthesis)	1.05
H$_3$BO$_3$	99.8 (for analysis)	crystalline
H$_3$PO$_3$	85 (suitable for use excipient)	1.71
NaH$_2$PO$_4$.2H$_2$O	98-100.5 (extra pure)	solid
Bricanyl Tablet	---	2.5 mg (C$_{12}$H$_{19}$NO$_3$)$_2$.H$_2$SO$_4$/Tablet
(C$_{12}$H$_{19}$NO$_3$)$_2$.H$_2$SO$_4$	99.7 (CAS No: 23031-32-5)	crystalline powder

Table 2.2. The properties of Standard TBS.

Properties	Results
Solubility	
Water	Freely soluble
Acetonitrile	Practically insoluble
Ethanol (95%)	Practically insoluble
Acetic Acit	Practically insoluble
Chloroform	Practically insoluble
Ether	Practically insoluble
Melting Point	253°C
Identification	
UV	Complies
Sulphate	Complies
pH	4.4

Color and Clarity of Solution	Complies
Chloride	<100 µg/g
Acetic Acid	0.2%
3,5-Dihydroxy-ω-t-butylamino-acetophenone sulphate (AF)	<0.005% w/w
Heavy Metals (JP)	<10 ppm
Heavy Metals (Ph Eur)	<25 µg Pb/g
Arsenic	<2 ppm
Water Content *	0.2%
Sulphated Ash	<0.1%
Assay by Titration	100.4% w/w on dry basis

Other Related Substances:*
  Largest
  Total
	None detected>0.02%
	None detected

Organic Volatile Impurities:
  1,4-Dioxane
  Dichloromethane
	<100 ppm
	<100 ppm

Loss on Drying *	0.14% w/w
Crystal Modification	Crystal Modification B, <2% of A-form
Storage	Keep container tightly closed. Protect from light.
Storage Temperature	Room temperature
Exposure Limit Value	0.01 mg/m^3
Stability	Stable under normal conditions
Hazardous Reactions	None known
Hazardous Decomposition Product(s)	None known
Inhalation	Harmful by inhalation. May cause tremor and palpitation of the heart. Symptoms may include headache, restlessness, rash and cramps in hands and feet.
Skin Contact	Unlikely to cause skin sensitization
Eye Contact	May cause slight eye irritation
Ingestion	May produce effects similar to inhalation
Long Term Exposure	Harmful: danger of serious damage top health by prolonged exposure if swallowed. Repeated exposure of animals to high levels produces adverse effects on the heart.
Risk Phrases	R20: Harmful by inhalation R48/22: Harmful danger of serious damage to health by prolonged exposure if swallowed.

The standard stock solution of TBS of 2×10^{-3} M was prepared by dissolving 0.0274 g TBS analytical reference material in water and diluting to 25 mL. The working solutions with lower concentrations were prepared daily by further dilution of them via transfer with a digital adjustable Transferpette, (Brand 20-200 μL, cat. No: 7041 78).

The stock solution of Bricanyl drug (2.5 mg TBS per tablet) was prepared at various concentration (1.82×10^{-4} M - 5.47×10^{-4} M). For this purpose various numbers of tablets (1-6) were homogenized by grounding, and then this obtained powder was added into 15 mL water for dissolving. After waiting one day, the heterogenic tablet mixture solution was filtered with blue band filter paper. Insoluble component of tablets on filter paper were fairly washed and then obtained supernatant solution was diluted 25 mL or 50 mL with deionized water.

The stock solutions of standard TBS and Bricanyl drug were stored in dark and at room temperature.

Various buffer solutions and an acid solution were used to adjust pH of the working medium throughout the study. Procedures of preparation for these solutions were listed in below.

➢ 0.1 M $HClO_4$ solution (pH 1.6) was prepared by taking 0.55 mL $HClO_4$ (60%) and it was diluted to 50 mL with water.

➢ 0.25 M Phosphate buffer solution (pH 2.5) was prepared by taking 1.7 mL H_3PO_4, it was diluted to 30 mL, and was added 1.5 M NaOH until up to that pH 2.5. Then this solution was diluted to 100 mL.

➢ 0.25 M Acetate buffer solutions were prepared at different pH values in the range of 4.0 to 5.7. 1.43 mL acetic acid (99-100%) was taken and diluted to 100 mL with water. Then this solution was titrated with 1.5 M NaOH until up to that desired pH values and diluted to 250 mL at every turn.

➢ 0.25 M Phosphate buffer solution, pH 7.0, was prepared. 1.5601 g $NaH_2PO_4.2H_2O$ was dissolved in water and diluted to 50 mL. pH of this solution was arranged to 7.0 by using 1.5 M NaOH and diluted to 100 mL with water.

➢ 0.1 M Borate buffer solutions, pH values 8.5 and pH 10.0, were prepared. 1.2366 g boric acid was dissolved in water and diluted to 100 mL. Thus 0.2 M boric acid solution was firstly obtained. Then, pH value of 50 mL 0.2 M boric acid was arranged to 8.5 with 1.5 M NaOH and diluted to 100 mL. 0.1 M Borate Buffer Solution, pH 10.0, was also prepared in the same way. As a consequence, 0.1 M Borate buffer solutions were prepared at different pH.

2.3. Procedure

All glassware and centri-voltammetric cell were kept in a HNO_3 solution bath (1/10 v/v) and rinsed with ultra pure water before using. After preparing of GCE and CPE, as explained in below, they were placed in the centri-voltammetric cell and two different voltammetric procedures were applied. One of them included centrifugation step. Voltammetric cell contents were deareated with nitrogen for 300 s before measurements and kept under the nitrogen atmosphere during measurements.

2.3.1. Preparation of carbon paste electrode

Carbon paste was prepared by mixing of graphite powder and nujol as binding at different percentage ratios which were 65%, 75% and 80%. For this purpose, graphite powder and nujol was weighted at convenient amount. Then, weighted graphite and nujol were added into a mortar and homogenized. The obtained paste was packed into a Teflon pipe which was previously constructed with conventional size for centri-voltammetric cell (Figure 2.2B). The inner and out diameter of Teflon pipe was 2 mm and 7 mm respectively. The surface of the resulting carbon paste electrodes was smoothed and rinsed carefully with deionized water for renewed surface.

The electrical contact was provided by inserting a copper wire in the inner hole of the Teflon tube. The CPE, prepared by this way, was placed into the cavity of part 1. Then the parts of centri-voltammetric cell were screwed together for centrifugation and voltammetric experiments. This procedure was repeated before each measurement (Figure 2.2A).

2.3.2. Preparation of Glassy Carbon Electrode

For pretreatment of GCE whose length was shorted by cutting, its surface was first polished with Al_2O_3 on a polishing cloth and rinsed with water. It was placed into the cavity of part 1. The back side of GCE body was filled with carbon paste material until to reach GC material and a copper wire was inserted in it for electrical connection (Figure 2.2A). Then, sonication step was applied to GCE placed in Part 1 nearly for 4 minutes in water. At the end of this step, the part 1 and 2 was combined for experiments.

2.3.3. Voltammetric Procedure

Voltammetric studies were performed using GCE and CPE as working electrode to compare the results. To investigation of standard TBS behaviour was used the cell of voltammetric analyzer and the centri-voltammetric cell in which working electrode was placed and mounted. The impact of the effective parameters was investigated in order to determine the optimum conditions and the calibration graphs were generated under such conditions.

After adding buffer solution of 25 mL in cell the auxiliary and reference electrodes were inserted and deaerated. The potential was scanned in positive direction in the range of 0 to 1200 mV vs. Ag/AgCl (sat. KCl) in quiescent solution. The voltammogram was recorded for supporting electrolyte by differential-pulse voltammetry with pulse amplitude of 50 mV, pulse duration of 0.6 s and scan rate of 10 mVs^{-1}. Then, TBS was added at various amount in the cell and the anodic peak resulting from oxidation of TBS was recorded in same conditions. These resulting peak currents using both of working electrodes were compared and utilized for analytical purposes. The voltammetric operation sequence and the segment of were given in Appendix 1.

2.3.4. Centri -Voltammetric Procedure

After adding buffer solution of 25 mL in the *centri-voltammetric cell* the voltammetric procedure above mention was similarly applied. The difference between of these procedures was that centrifugation step was applied after TBS adding into this cell and then the voltammogram was recorded. The effective parameters which can affect sensitive of the method, namely centrifugation speed (v_{cent}) and time (t_{cent}) were investigated.

2.3.5. Terbutaline analysis in Bricanyl drug

The content of TBS was tried to be determined in Bricanyl drug by applying both voltammetric and centri-voltammetric procedure in the obtained optimum experimental conditions. Standard addition method was performed in two sections.

3. RESULTS AND DISCUSSION

A typical electrochemical cell consists of the sample dissolved in a solvent, an ionic electrolyte, and three electrodes. Cells come in a variety of sizes, shapes, and materials. The type used depends on the amount and type of sample, the technique, and the analytical data to be obtained. The material of the cell (glass, Teflon, polyethylene) is selected to minimize reaction with the sample and ionic electrolyte. In most cases the reference electrode should be as close as possible to the working electrode; in some cases, to avoid contamination, it may be necessary to place the reference electrode in a separate compartment (Kounaves, 1997). Because of these, the voltammetric responses of TB were examined by using GCE and CPE in various medium and voltammetric cell. Initially, the effect of these electrodes' position and geometry of voltammetric cell were considered on peak current of TB. The optimum conditions were determined for voltammetric and centri-voltammetric procedures. Then, these methods were applied to determine TBS in a tablet form of Bricanyl drug.

3.1. Conditioning of Working Electrode

Consecutive potential scans were carried out both two working electrodes (GCE, CPE) for pre-conditioning. To that end, 25 mL acetate buffer solution, pH 5.5, was added in centri-voltammetric cell and ternary electrode system was placed. Then, consecutive potential scan was applied five times in potential range of 0 to1.2 V (vs Ag/AgCl/KCl) at 20 mVs^{-1}. The obtained voltammograms was given in Figure 3.1. It was seen that in response curves of both electrodes, baseline current decreased rapidly after first scan (a curve) but this falling off decreases latter scans (b-e curves). Because of this reason, consecutive twice potential scans command was added to operation sequence for latter experiments. The previous procedure was repeated at 10 mVs^{-1} by applying the new operation sequence (Appendix 1). It was seen that from the obtained voltammograms, baseline decreased in both electrodes but baseline, given by CPE, was too low (Figure 3.2). However, previously studies were performed by using GCE (Yılmaz et al., 1998, Beltagi et al., 2007).
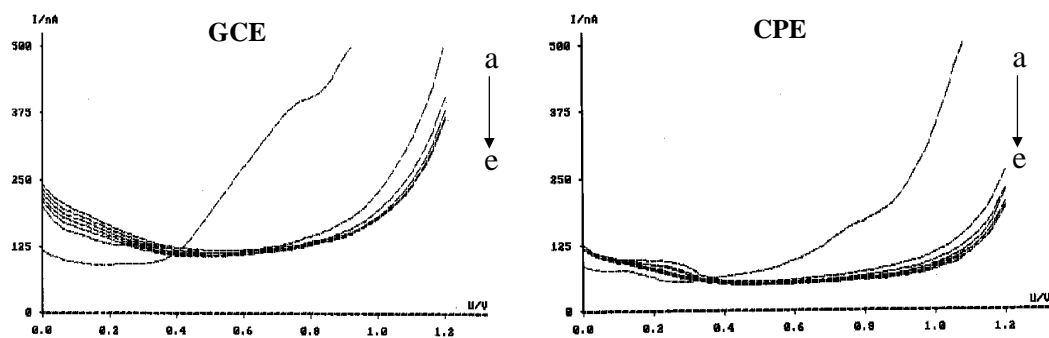
Figure 3.1. Effect of consecutive potential scans (a to e) on baseline current of both GCE and CPE at pH 4.0, scan rate: 20 mVs^{-1}.
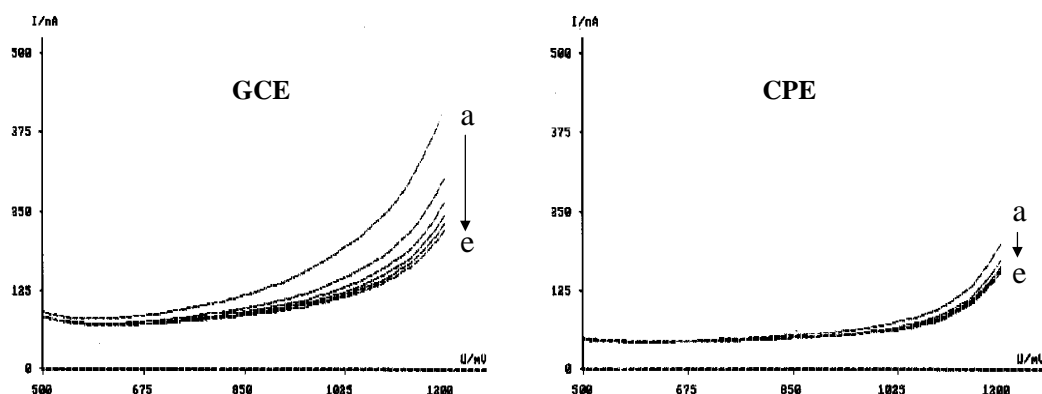
Figure 3.2. After the operation sequence was modified, effect of consecutive potential scans (a to e) on baseline current of both GCE and CPE at pH 4.0. Scan rate: 10 mVs^{-1}.

3.2. Position of Working Electrode and Potential Waveform

The effect of electrode position was examined at two different cells by using GCE as a working electrode. For this purpose, GCE was equipped with voltammetric cell, used in conventional voltammetric measurements (electrode was plunged into supporting electrolyte, Figure 2.1) and centri-voltammetric cell, constructed for this studying, (electrode was down, Figure 2.2A). Then, the voltammograms of 1.0×10^{-5} M standard TBS solution were recorded at pH 4.0. The obtained peak current and potential values (vs Ag/AgCl/KCl) from recorded voltammograms (Figure 3.3) were given in Table 3.1.
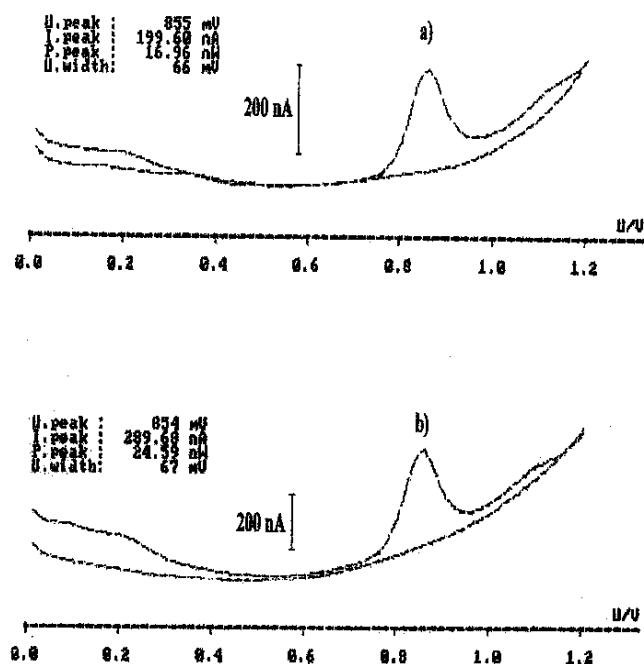
Figure 3.3. Effect of electrode position on the peak current of TBS. C_{TBS}: 1.0×10^{-5} M, pH 4.0: **a)** GCE facing downwards in the voltammetric cell, **b)** GCE facing upwards in the centri-voltammetric cell.

Table 3.1. The peak current and potential values of 1.0×10^{-5} M TBS solution for two different electrode positions at pH 4.0. Other experimental conditions were same.

Position of GCE	i_p (nA)	E (mV)
Facing downwards in the Voltammetric cell	200	855
Facing upwards in the bottom of Centri-voltammetric cell	290	854

When GCE was located at the bottom of the cell, the observed peak current of TB at 0.85 V (vs Ag/AgCl/KCl) was increased 1.5 fold under the same experimental conditions. Therefore, the centri-voltammetric cell was used in the latter studies.

The behavior of TB was examined in acetate buffer supporting electrolyte (0.25 M), pH 4.0, at different potential waveforms. For this purpose, the voltammograms of 1.0×10^{-4} M standard TBS were taken at SW, DC and DP modes. As seen in Figure 3.4, the maximal peak value (2.13 µA) was obtained at DP mode and latter experiments were practiced at DP mode.
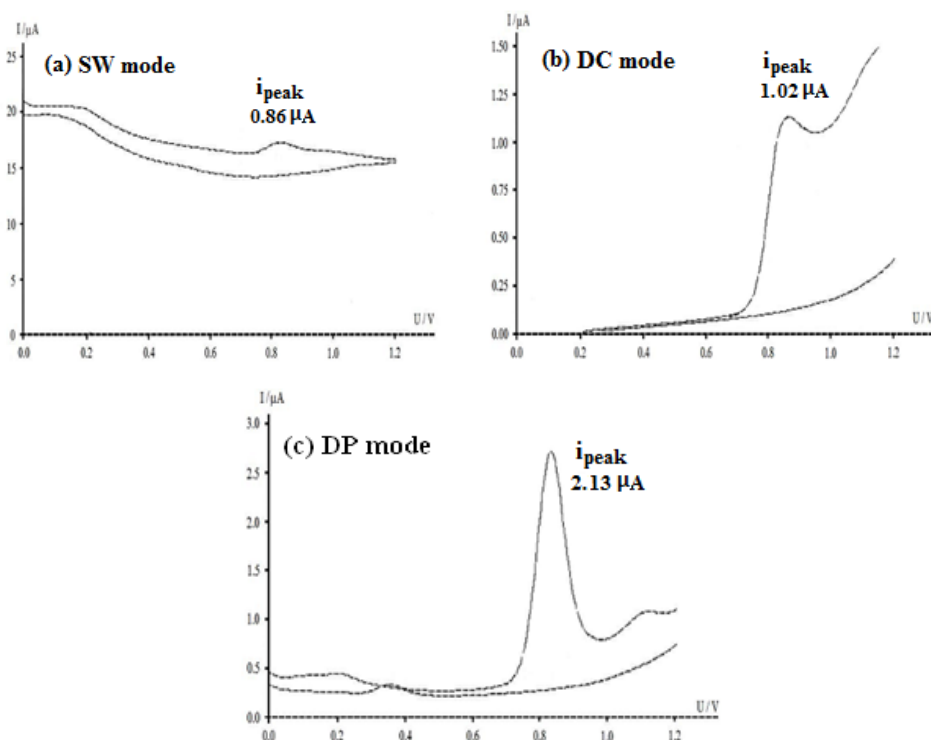
Figure 3.4. The voltammograms of 1.0×10^{-4} M standard TBS at SW, DC and DP modes. pH 4.0. Scan rate: 10 mVs^{-1}.

Besides, reversibility of TB oxidation was controlled with CV. The cyclic voltammograms of baseline firstly and then that of 1.0×10^{-4} M standard TBS were recorded consecutively three times. As seen Figure 3.5, the oxidation of terbutaline was irreversible.
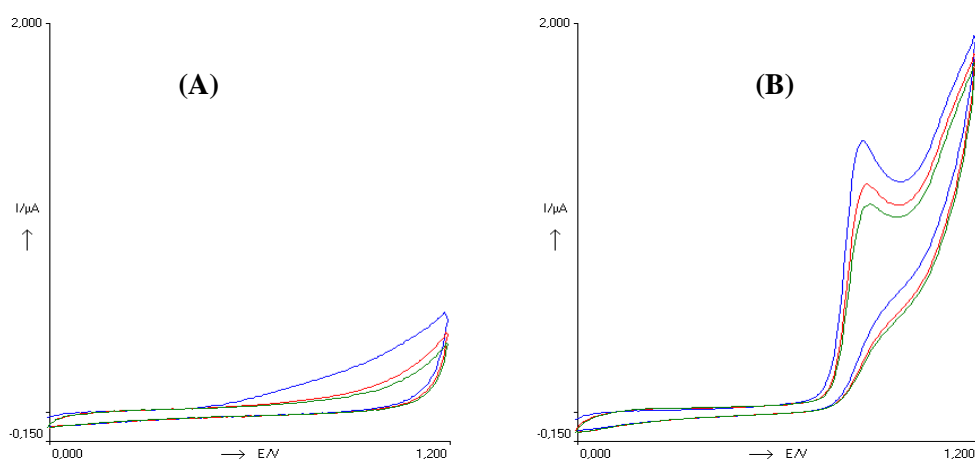
Figure 3.5. The sequence cyclic voltammograms at GCE in acetate buffer solution (0.25 M), (A) absence of TBS (B) presence of 1.0×10^{-4} M TBS. pH 4.0; scan rate 10 mVs^{-1}.

3.3. Parameters Effect in Voltammetric Method

CPE, used as a working electrode, was also investigated aspects of composition. The response curves of CPE, was prepared by using graphite powder and nujol at different ratios (80%, 75% and 60% graphite powder), up against to TB were evaluated. The maximum peak current values were obtained at CPE contained 75% graphite and 25% nujol so this composition was used in latter studies.

3.3.1. Effect of pH on Terbutaline Oxidation

TB oxidation at GCE and CPE was evaluated at different pH medium in the centri-voltammetric cell. The voltammograms related to various TB concentrations (1.6×10^{-6} to 1.4×10^{-5} M) were obtained in the pH range of 1.6 to 10.0 by using $HClO_4$ solutions; acetate, phosphate and borate buffer solutions. The obtained results for GCE and CPE were given in Table 3.2 and Table 3.3, respectively.

Table 3.2. The peak current values (nA) related to TBS oxidation at GCE. Scan rate: 10 mVs^{-1}.

C_{TBS} (M) pH	1.59×10^{-6}	4.78×10^{-6}	7.96×10^{-6}	1.11×10^{-5}	1.43×10^{-5}
1.58	24	158	272	382	485
2.50	24	146	262	361	464
4.00	39	181	346	488	615
5.50	34	159	291	416	503
7.00	28	124	215	313	405
8.54	71	211	325	423	502
10.05	26	92	165	263	383

These results pointed out that effect of pH changed depending on TB concentration. The obtained peak currents for every TB concentration were plotted versus to pH for signifying to dependence. In other words, iso-concentration curves were drawn at each pH for GCE and CPE (Figure 3.6A and B).

Table 3.3. The peak current values (nA) related to TBS oxidation at CPE. Scan rate: 10 mVs^{-1}.

C_{TBS} (M) \ pH	1.59×10^{-6}	4.78×10^{-6}	7.96×10^{-6}	1.11×10^{-5}	1.43×10^{-5}
1.58	37	157	252	335	389
2.50	32	123	204	275	362
4.00	45	173	284	404	506
5.50	34	133	272	392	537
7.00	32	108	193	292	388
8.54	67	192	301	407	514
10.05	26	71	118	171	216
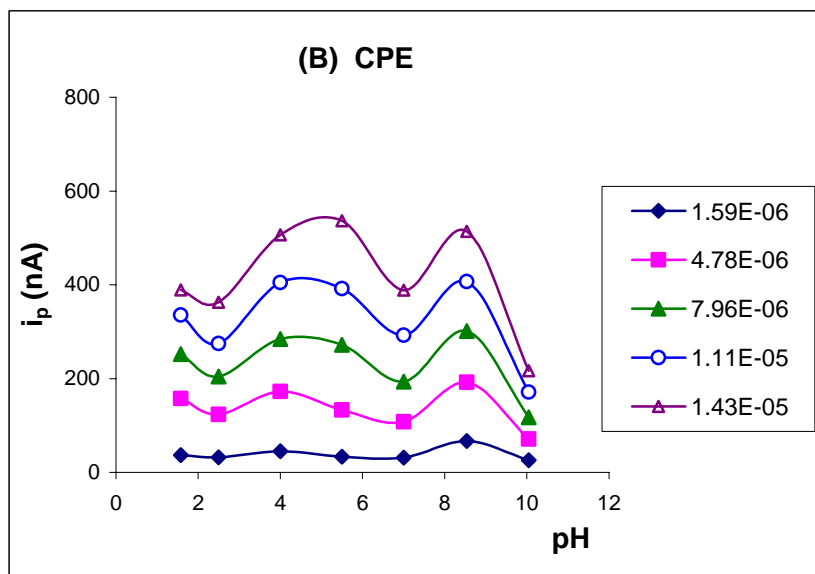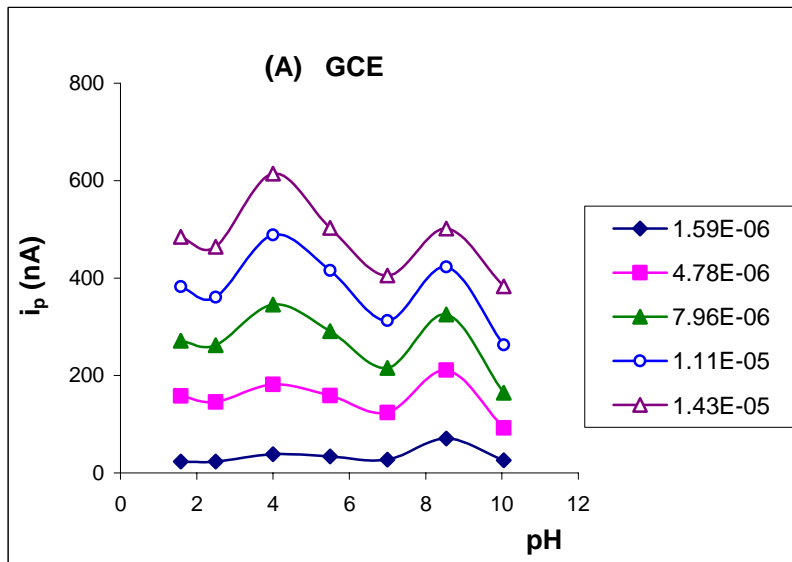
Figure 3.6. Change of TBS peak currents with pH in different concentration for (A) GCE, (B) CPE.

When Figure 3.6A and B were compared an interesting situation arisen. Peak currents around pH 8.5 were high at small concentrations, but peak currents around pH 4.0 were high at high concentrations. This situation was seen more clearly in data of GCE (Figure 3.6A). Also, current values significantly decreased in each of electrodes at pH 7.0. It was thought that the reason of the results was changing of the structure of TB molecule in solution at different pH and electrode reaction mechanism. Clarification of this situation requires advanced structural analysis and investigation of electro-oxidation mechanism for TBS.

The effect of pH on TBS oxidation can be evaluated in terms of peak potential. The variation of peak potentials values of TBS with pH in range of 1.58 to 10.05 at GCE and CPE were shown in Figure 3.7.
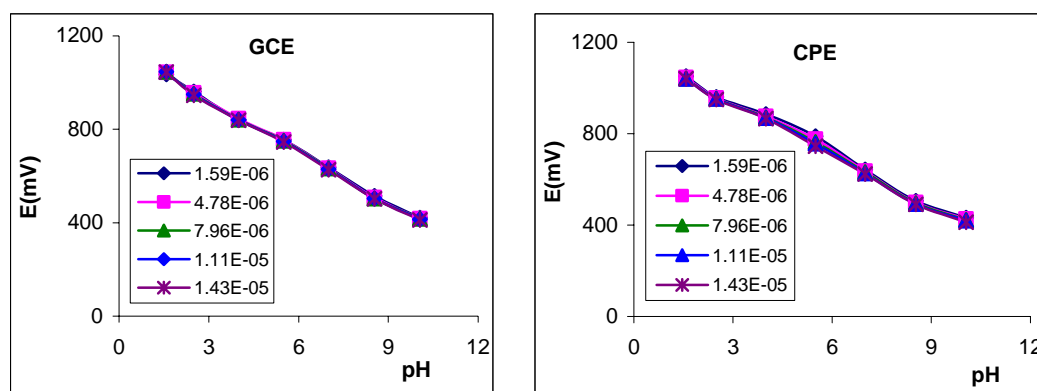
Figure 3.7. Influence of peak potentials values of TBS with pH.
TBS concentrations: 1.6×10^{-6} to 1.4×10^{-5} M.

The obtained graphs were found in a linear fashion ranging at working pH range. The average value of regression coefficients for these graphs was calculated as 0.998 approximately. Similarly founded equations were as follows:

For GCE: $E(mV) = 74.7\ pH + 1156.4$

For CPE: $E(mV) = 73.1\ pH + 1146.8$

The slope and intercept values of these equations were too close to each other. This result pointed that the electro-oxidation reaction of TB occurred to the same mechanism at CPE and GCE.

Because next investigations will be compared, it was studied with buffer solutions in which relatively high peaks were appeared at about pH 4.0, 5.5 and 8.5 in latter experiments.

3.3.2. Effect of Scan Rate

The scan rate of potential has an important impact on the peak currents. The effect of the scan rate was examined in the range of 10 to 120 mVs^{-1}. For this purpose, scan rate was changed from 10 mVs^{-1} to 120 mVs^{-1} step by step in acetate buffer solution, pH 4.0, contained 1.0×10^{-5} M TB and voltammograms were recorded. In pursuit of renewing solution in cell, scan rate operation was changed from 120 mVs^{-1} to 10 mVs^{-1}. This procedure was repeated for GCE and CPE at pH 4.0 and 8.54. The obtained data were given in Table 3.4 and 3.5, taking into account the change direction of scanning rate. The peak currents depended on scan rate and modified direction of scan rate was given in Figure 3.8 and 3.9 for every pH and electrode.

The changeability of peak currents with scan rate at CPE and GCE was similar pH 4.0 and the relative high peak currents were observed at small scan rates. Also, the obtained peak currents at CPE were higher than that of GCE for all scan rates. But, the changing of peak currents was different for GCE and CPE. The variation of peak currents at GCE was observed in a narrow range.

Table 3.4. TBS peak current values at different potential scan rate (mVs^{-1}) for GCE and CPE. C_{TBS}: 1.0×10^{-5} M, **pH 4.0**.

CPE				GCE			
Scan rate	i (nA)	Scan rate	i (nA)	Scan rate	i (nA)	Scan rate	i (nA)
10	443	120	358	10	249	120	172
20	419	80	415	20	260	80	245
30	402	60	380	30	249	60	232
40	373	40	409	40	227	40	275
60	353	30	455	60	206	30	307
80	404	20	489	80	238	20	328
120	365	10	503	120	207	10	346

Table 3.5. TBS peak current values at different potential scan rate (mVs^{-1}) for GCE and CPE. C$_{TBS}$: 1.0×10^{-5} M, **pH 8.54**.

CPE				GCE			
Scan rate	i (nA)	Scan rate	i (nA)	Scan rate	i (nA)	Scan rate	i (nA)
10	313	120	194	10	323	120	214
20	246	80	214	20	350	80	262
30	232	60	194	40	376	60	311
40	220	40	208	48	383	40	342
60	198	30	243	60	369	20	352
80	239	20	270	80	340	10	305
120	233	10	272	120	321		
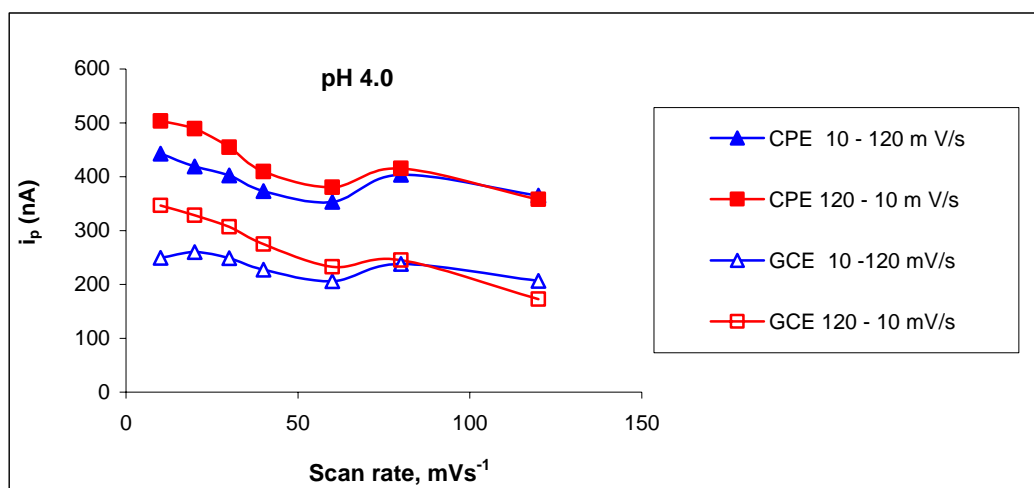
Figure 3.8. Change of TBS peak currents with potential scan rate in the same concentration for GCE and CPE. C$_{TBS}$: 1.0×10^{-5} M, **pH 4.0**.
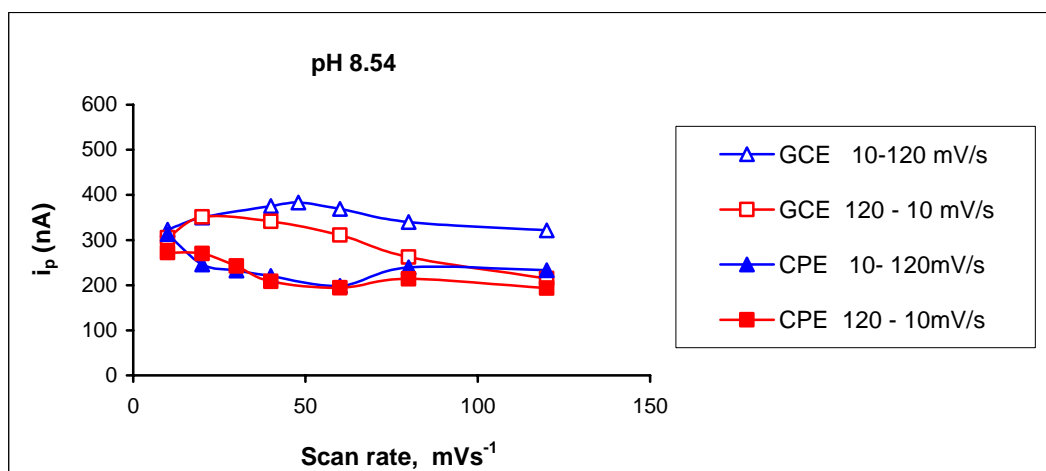
Figure 3.9. Change of TBS peak currents with potential scan rate in the same concentration for GCE and CPE. C$_{TBS}$: 1.0×10^{-5} M, **pH 8.54**.

3.3.3. Calibration Curves for Terbutaline by Voltammetric Method

The change of TB peak currents was examined in the concentration range of 1.6×10^{-6} to 5.6×10^{-5} M by using GCE and CPE (Table 3.6). The graphs of obtained peak currents against TB concentration were plotted. The calibration graph related to CPE was linear (R^2: 0.999) whereas the graph related to GCE was deviated from linearity at higher values of 4.1×10^{-5} M TBS concentration (Figure 3.10). Because of this, it was studied in more diluted conditions.

Table 3.6. The peak current (nA) values of TBS oxidation at GCE and CPE (75% C); pH 5.50.

	GCE	CPE
$C_{TBS}\times10^{-5}$ (M)	i (nA)	i (nA)
0.16	19	47
0.96	262	324
1.75	510	613
2.53	746	895
3.30	951	1170
4.08	1150	1400
4.84	1290	1690
5.60	1420	1910
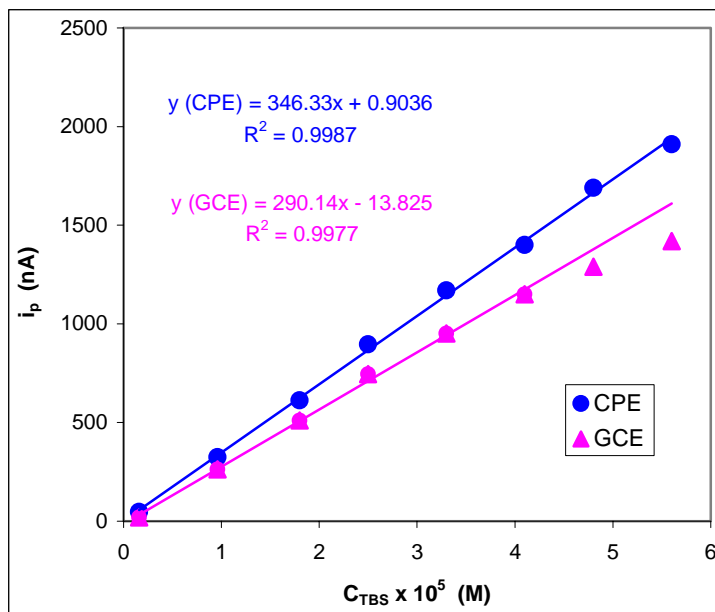
Figure 3.10. Calibration graph of TBS for GCE and CPE (75% C).
Experimental conditions: pH 5.50, scan rate: 10 mVs^{-1}.

When similar study was repeated in concentration range of 6.4×10^{-8} to 2.9×10^{-6} M, the values of Table 3.7 were obtained. The plotted calibration graphs were linear for both electrodes and regression coefficients were around 0.997. However, a peak was not observed at GCE for 6.4×10^{-8} M TBS (Figure 3.11). These results point out that CPE is better than GCE in terms of both linear concentration range and sensitivity. However, the reproducibility was better at GCE. When the results of 8 measurements on different days for 1.0×10^{-5} M TBS solutions were evaluated, the reproducibility was calculated as 9.0% for GCE. The minimum measurable TB at CPE is as low as 35 ng mL^{-1} and it came close to reported LOD value that was obtained at GCE with ASV method for determination of TB by Beltagi et al. (2007).

Table 3.7. The peak current values of TBS oxidation at CPE (75% C) and GCE; pH 5.50.

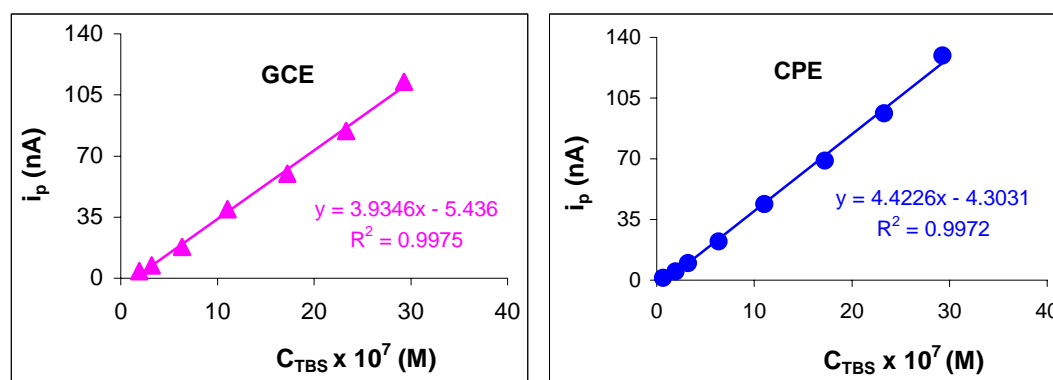|	GCE	CPE
C_{TBS} (M)	i (nA)	i (nA)
6.40×10^{-8}	Not detectable	1
1.92×10^{-7}	4	5
3.20×10^{-7}	7	10
6.35×10^{-7}	18	22
1.11×10^{-6}	39	44
1.72×10^{-6}	60	69
2.33×10^{-6}	84	96
2.93×10^{-6}	113	130

Figure 3.11. Calibration graphs of TBS for GCE and CPE. Experimental conditions: pH 5.50, scan rate: 10 mVs^{-1}.

3.4. Parameters Effect in Centri-Voltammetric Method

The centri-voltammetric behavior of TB was examined in consideration of information that was obtained from studies related to voltammetric behavior of TB. In the centri-voltammetric studies, two new parameters, which resulted from centrifugation step and could affect signals related to TB, were performed to investigate. These parameters are centrifugation speed and duration that could affect preconcentration ratio of TB on the electrode surface. Studies of in this section were performed under the best conditions of pH and scan rate determined in Section 3.3.

First of all $Al(OH)_4^-$ and Purolite C100 as carrier precipitate and carrying material as mentioned Introduction Section were performed in this section. It was expected that these materials increased pre-concentration ratio. However, there was no effect observed at TB peak currents.

So without using any carrier material, the voltammograms of 1.0×10^{-5} M TBS solution were obtained at pH 4.0 by using centrifugation or not for comparing. As seen in Figure 3.12, the measured peak current value at GCE without centrifugation was 238 nA (curve A). When centrifugation was supplied to TBS solution at 500rpm during 5 minute using same electrode, the peak current value was 278 nA (curve B). At the same condition peak current was measured as 331 nA at CPE (curve C). On the other hand, while centrifugation speed was increased to 750 rpm, the peak current values were obtained as 374 nA (curve D) and 417 nA (curve E) for centrifugation duration of 3 and 10 minutes at CPE, respectively.

As a result, higher peak current values were obtained when centrifugation speed and duration were increased by using CPE. Because the mass transfer of TB towards the electrode surface is increased via centrifugation. However, centrifugal force, created by centrifugation, caused that solution phase was got into inner layer of CPE. Thus, the response of electrode was often impaired and reproducibility was decreased.
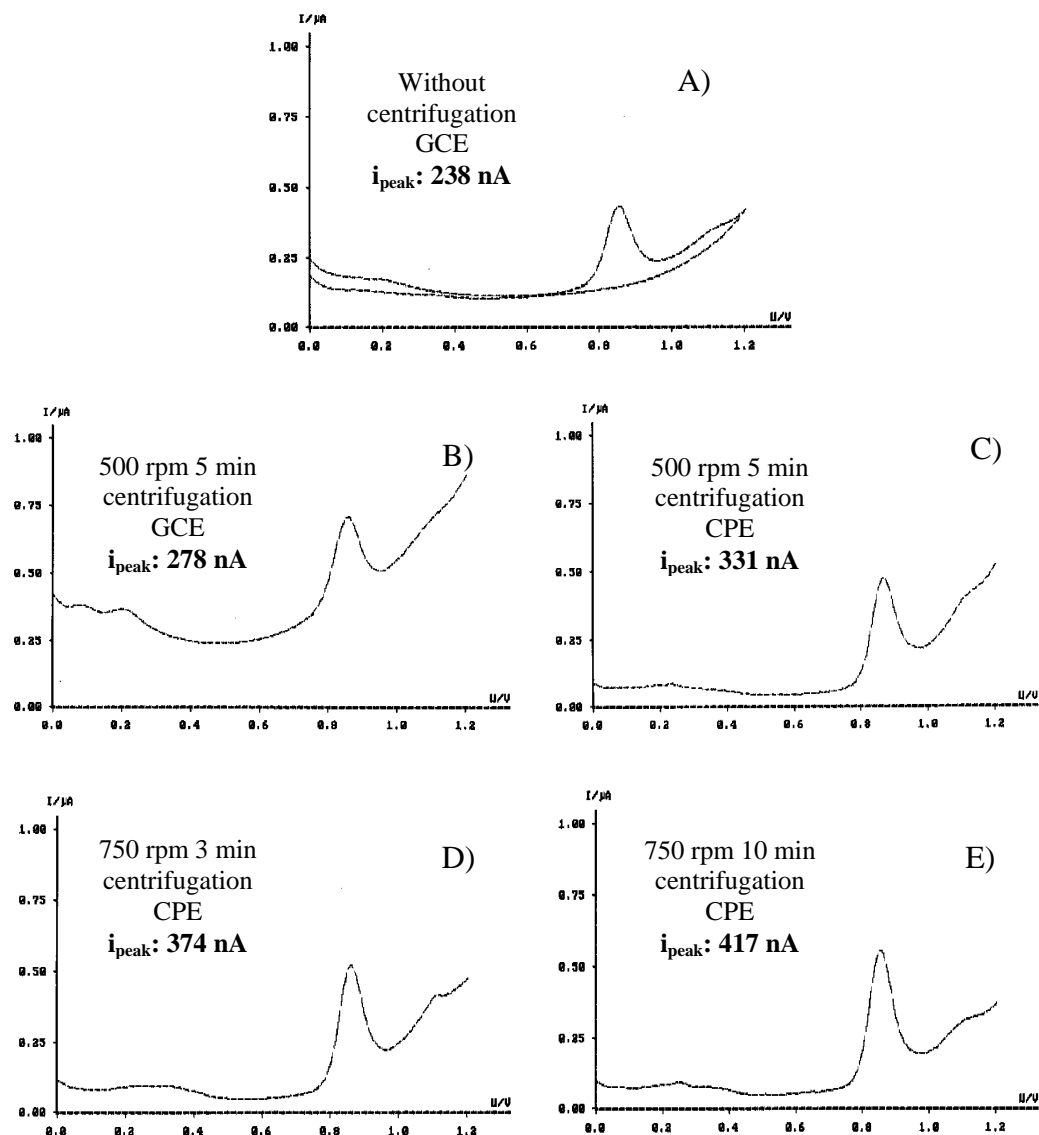
Figure 3.12. Effects of electrode type and centrifugation on peak currents of 1.0×10^{-5} M TBS, pH 4, scan rate 10 mVs^{-1}.

3.5. Determination of Terbutaline Sulphate in Bricanyl Tablet

The solubility values of TB given in literature are different from each other such as 90; 213 and 239 mg mL^{-1} in water, 252 mg mL^{-1} in phosphate buffer, pH 6.6, (Reverchon and Porta, 2003; Pramodkumar and Shivakumar, 2006; Drugbank, 2010). It was also seen that, some studies were carried out with solution of TB in alcohol (Yamini et all, 2006). For that reasons, after grinding tablet of Bricanly Drug, they were tried to dissolve by steeping in pure water or alcohol during one day. Then, TBS sample solutions, contained 60% ethanol or

60% methanol, were prepared by diluting with water. TBS concentrations of these solutions were calculated with considering prospectus of Bricanyl Drug, highlighted that one tablet contained exactly 2.5 mg TBS. The voltammograms of TBS solutions in the concentration range of 8.0×10^{-6} - 3.45×10^{-5} M, prepared with alcohol or not, were recorded in acetate buffered, pH 5.1. The peak current values, obtained from the voltammograms, were given in Table 3.8.

Table 3. 8. According to peak current (nA) value at CPE (80% C), comparison of terbutaline sulphate solubility in deionized water, methanol-water and ethanol-water mixture, pH 5.1; scan rate 20 mVs^{-1}.

$C_{TBS} \times 10^5$ (M)	i_p (water)	i_p(methanol)	i_p(ethanol)
0.80	162	225	57
1.78	367	449	209
2.66	537	629	344
3.45	711	812	435

When the obtained peak current values were drawn versus to TBS concentration, it was seen that the peak currents became differ at the same concentration in different medium and varied linearly against concentration of TBS (Figure 3.13). Thereby the solubility of TBS in alcohol and non-alcohol medium were evaluated by going peak currents (nA).

Incidentally, diffusion coefficients of electroactive species may vary according to medium condition therefore it wasn't ignored that peak currents also may be changed. But in working concentration range, sample volumes, added in supporting electrolyte, was taken into account, the cell contained 2.6-11% alcohol. Despite increasing of alcohol percentage with TBS concentration, the linearity of the graph wasn't changed. So it was decided that diffusion coefficient effect wasn't decisive.

From that point of view, it was decided that TBS was mostly dissolved in methanol. But, because of harmful effects of methanol on human health and its volatility, that may easily changed the concentration; TB aqueous solution was used in the optimization studies.
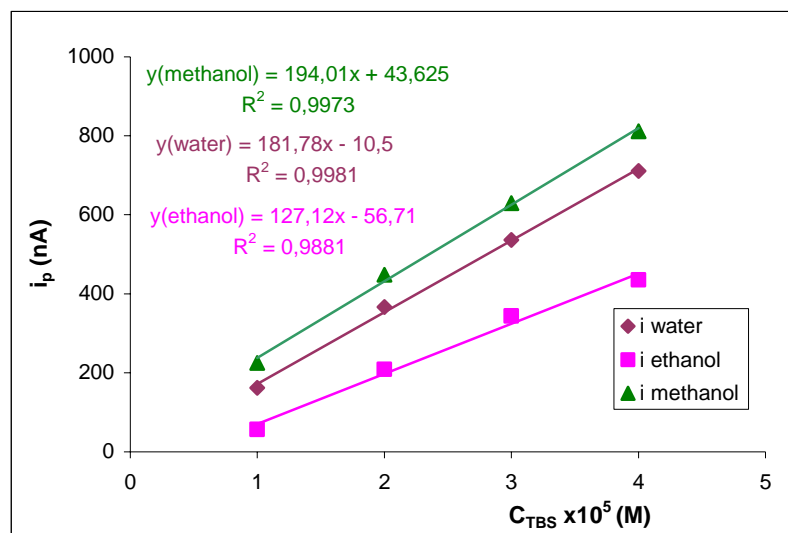
Figure 3.13. According to peak current value, comparison of TBS solubility in deionized water, methanol-water and ethanol-water mixture.

3.5.1. Terbutaline Determination Using Voltammetric Method

Sample solutions of Bricanyl Drug (in tablet form) were prepared as mentioned in above and Section 2 with two tablet for used in three days. The voltammograms were taken in acetate buffer solution, pH 4.0, by applying standard addition method. 500 µL sample solution, contained 2 tablet in 25 mL, was added into 25 mL acetate buffer solution in cell and then, various volumes of 2.0×10^{-3} M standard TBS solution were consecutively added in cell. This procedure was practiced twice using both GCE and CPE and the obtained voltammograms were given in Figure 3.14. The standard addition graphs were plotted by using concentration and peak current values in Table 3.9 and 3.10 (Figure 3.15 and 16).
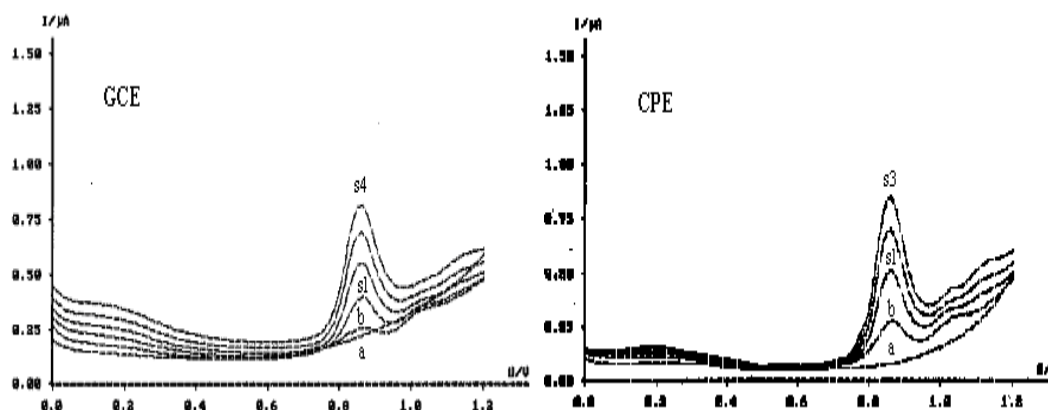
Figure 3.14. The voltammograms standard addition to sample of Bricanyl Drug at pH 4.0, using GCE and CPE. a: supporting electrolyte, b: sample solution, s: added standard TBS.

Table 3.9. The results of standard addition to sample of Bricanyl Drug, using **GCE**. pH 4.0, scan rate 10 mVs^{-1}.

GCE	$C_{standard} \times 10^{-5}$ (M)	I_{peak} (nA)	E (mV)	Result, Difference%
n=1	0	54	860	2.29 mg TBS in per tablet -8.4%
	0.80	175	860	
	1.59	303	857	
	2.37	405	855	
	3.15	483	855	
n=2	0	127	864	2.47 mg TBS in per tablet -1.2%
	1.60	541	838	
	3.15	857	850	

Table 3.10. The results of standard addition to Sample of Bricanyl Drug, using **CPE**. pH 4.0, scan rate 10 mVs^{-1}.

CPE	$C_{standard} \times 10^{-5}$ (M))	I_{peak} (nA)	E (mV)	Result, Difference%
n=1	0	138	859	2.76 mg TBS in per tablet +10.4%
	0.80	368	855	
	1.59	578	853	
	2.37	713	852	
n=2	0	145	869	2.62 mg TBS in per tablet +4.8%
	1.6	460	860	
	3.15	798	855	
	4.69	1060	853	
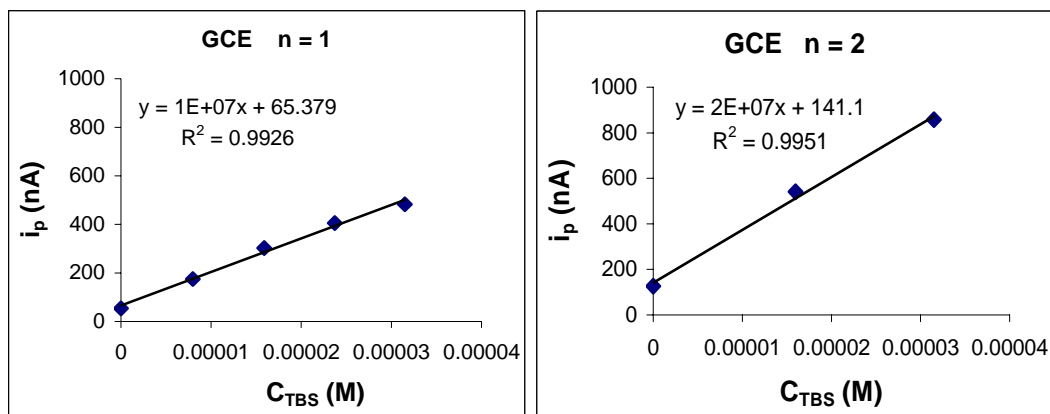
Figure 3.15. The calibration graphs of TBS at GCE at pH 4.0 (Repeated twice).
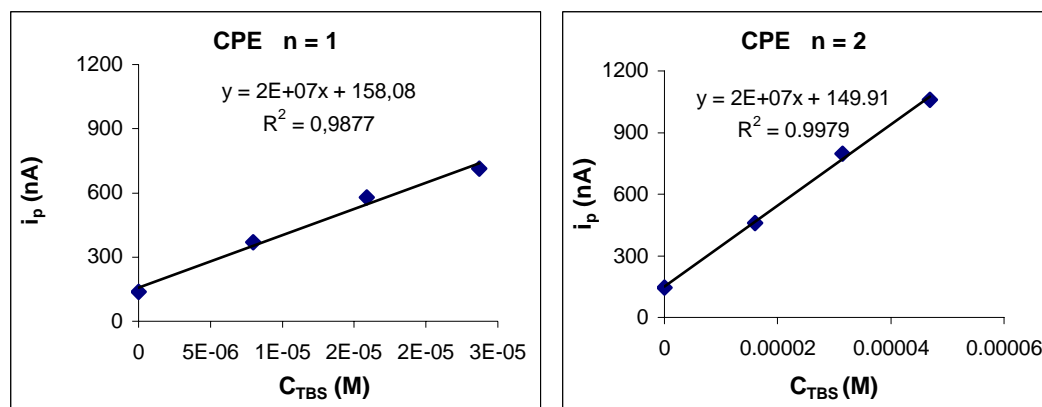
Figure 3.16. The calibration graphs of TBS at CPE at pH 4.0 (Repeated twice).

Producer firm is noted that tablet form of Bricanyl Drug is contained as 2.5 mg TBS per tablet. Amount of TBS detected by voltammetric analysis was calculated as average value 2.38 mg in per tablet. A difference of 4.8% was observed between these amounts.

3.5.2. Terbutaline Determination Using Centri-Voltammetric Method

Centrifugal force, created by centrifugation, caused that solution phase was got into inner layer of CPE. Thus, the response of electrode was often impaired. Therefore, studies in this section were only carried out with GCE.

For the application of centri-voltammetric method to TBS determination in Bricanyl Drug, supporting electrolyte (acetate buffer solution), sample solution of TBS and standard TBS solution (3 times) was added to centri-voltammetric cell, respectively. The cell content was deaerated with nitrogen gas and centrifuged at each stage. Finally, the voltammograms were recorded.

The obtained voltammograms by applying these procedures were stacked and shown in Figure 3.17. The standard addition graphs were plotted by using concentration and peak current values in Table 3.11 (Figure 3.18).

Table 3. 11. The centri-voltammetric results of standard addition to Sample of Bricanyl Drug using GCE at pH 4.0. Centrifugation: 500 rpm during 5 min. Scan rate 10 mVs^{-1}.

$C_{standard} \times 10^{-5}$ (M)	I_{peak} (nA)	E (mV)	Result, Difference%
0	136	871	
0.80	324	863	2.66 mg TBS in per tablet +6.2%
1.59	494	860	
2.37	605	858	
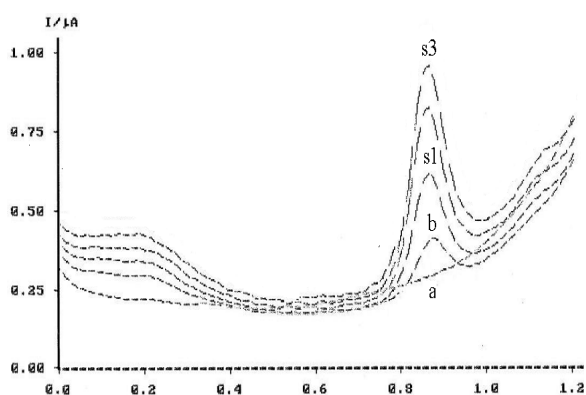
Figure 3.17. The centri-voltammograms standard addition to sample of Bricanyl Drug at pH 4.0, using GCE. a: supporting electrolyte, b: sample solution, s: standard TBS. Centrifugation: 500 rpm during 5 min.
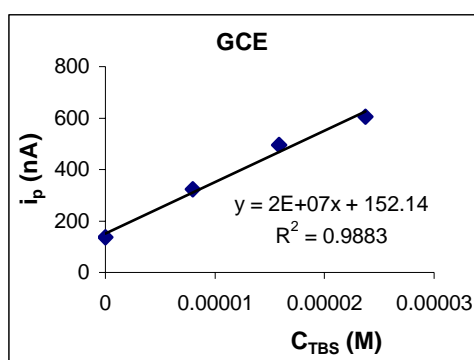
Figure 3.18. The calibration graph of TBS at GCE at pH 4.0. Centrifugation: 500 rpm during 5 min.

Amount of TBS detected by centri-voltammetric analysis was calculated as 2.66 mg in per tablet. This result was different 6.2% level from the noted value by producer.

4. CONCLUSION

Voltammetric and centri-voltammetric behaviour of TB were performed using GCE and CPE as working electrode to compare the results. A centri-voltammetric cell was used to be compatible with both centrifugation and voltammetry. The parameters which can affect sensitive of the method investigated.

The effect of pH on TB oxidation can be examined in terms of peak current and potential values. The values of pH at which relatively high peaks were appeared were determined. The variation of peak potentials values of TBS with pH result pointed that the electro-oxidation reaction of TB occurred to the same mechanism at CPE and GCE.

It was seen that from the obtained voltammograms, baseline given by CPE was too low that of GCE. The peak currents of TB about 0.8 V vs. Ag/AgCl/KCl at the CPE have been observed higher than that of GCE at same concentrations. The minimum measurable TB at CPE is as low as 35 ng mL^{-1}. As a result CPE is better than GCE in terms of both linear concentration range and sensitivity.

In the centri-voltammetric studies, two new parameters, which could affect signals related to TB, were examined. TB was preconcentrated on the CPE surface via centrifugation without any carrier material. Higher peak current values were obtained at low speed and long duration of centrifugation. However, centrifugal force, created by centrifugation (at high speed), caused that solution phase was got into inner layer of CPE. Thus, the response of electrode was often impaired and reproducibility was decreased.

The solubility of TBS in alcohol and non-alcohol medium were evaluated by going peak currents. Then, the content of TBS was tried to be determined in Bricanyl Drug (2.5 mg TBS in per tablet) by applying both voltammetric and centri-voltammetric procedure. Standard addition method was performed in two sections. Amount of TBS detected was calculated as 2.38 mg and 2.66 mg in per tablet in voltammetric and centri-voltammetric procedures, respectively.

In future, various carrier materials, whereon TB adsorbs, can be selected to improve applicability and the sensitivity of centri-voltammetric method.

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APPENDIXES

Appendix 1: The modified operation sequence and the programme of the voltammetric measurement of TB in DP mode.

OPERATION SEQUENCE

Method   : eylem. mth.
Title   : the voltammetric measurement of TB

	Instructions	t/s	Main parameters		Auxiliary parameters	
1	DOS/M		V.added	24.000 mL		
2	REM		24.0 mL buffer			
3	SMPL/M		V fraction	1.000mL	V. total	25.0 mL
4	PURGE					
5	STIR	300.0	Rot. speed	1000/min		
6	QPURGE	10.0				
7	QSTIR					
8	SEGMENT		Segm. name	on		
9	(ADD					
10	STIR		Rot. speed	1600 /min		
11	PURGE	20.0				
12	QPURGE					
13	QSTIR	10.0				
14	(REP					
15	SEGMENT		Segm. name	pol		
16	REP)2					
17	ADD >M		Soln. name	terbut	V. add	1.000 mL
18	ADD)0					
19	END					

SEGMENT pol

Method: eylem

	Instructions	t/s	Main parameters		Auxiliary parameters	
1	RDE		Rot. speed	0 /min		
2	DPMODE		U. ampl	50 mV	t. meas	20.0 ms
			t. step	0.60 s	t. pulse	40.0 ms
3	SWEEP	121.8	U. start	0 mV	U. step	6 mV
			U. end	1200 mV	Sweep rate	10 mV/s
4	END					

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