

An-Najah National University

Faculty of Graduate Studies

**Spectrophotometric and Electroanalytical
Determination of Prilocaine**

By

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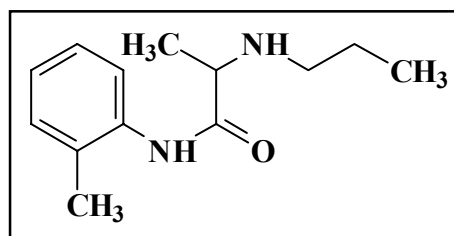
Chapter One

Introduction

Introduction

1.1 Prilocaine

Prilocaine hydrochloride, (M.Wt. 256.5) N-(2-Methylphenyl)-2-(propylamino)-propanamide; 2-(propylamino)-o-propionotoluidide; N-propylaminopropionyl)-o-toluidine; α - propylamino-2-methylpropionanilide; propitocaine¹⁵ was prepared by Lofgre¹ in the 60's as a local anesthetic of the amide type with an intermediate duration of action.



Prilocaine

Prilocaine could be found as in pharmaceutical preparation in different forms as in *EMLA*^R Cream, and *Citanest* both produced by Astra.

The duration of anesthesia produced by Prilocaine is longer than that of an equal dose of lidocaine. Following infiltration of 0.75 –1.0 ml of 4% Prilocaine hydrochloride solution for dental anesthesia, the onset of anesthesia averages less than 2 min and the drug provides pulp analgesia of 10-15 min duration.²

Depending on the dose administered, the duration of soft tissue anesthesia averages about 1-2 hours, when 4% solution is used for inferior alveolar nerve block, the onset of anesthesia averages less than 3 min and the duration of soft tissue anesthesia averages about 2.0 hours. At a concentration of 0.5 – 1 μ g/ml,

approximately 55% of Prilocaine is bound to plasma proteins, Prilocaine crosses the blood brain barrier and the placenta.²

1.2 Elimination

Prilocaine hydrochloride is metabolized principally in the liver to o-toluidine and 1-N-n-propylamine which may undergo further degradation, some metabolism of Prilocaine may also occur in the kidneys, Prilocaine is extracted in the urine as various metabolites and less than 1% as unchanged drug.²

1.3 Adverse Effects

Prilocaine hydrochloride shares the toxic potentials of local anesthetics and the usual precautions of local anesthetic thereby should be observed. A metabolite of Prilocaine hydrochloride, probably an N-hydroxy metabolite of o-toluidine may produce methemoglobin concentrations up to 15% and cyanosis, particularly when 600 mg or more of Prilocaine is administered in adults, other clinical symptoms of methemoglobinemia (namely: tachycardia, fatigue, headache, lightheadedness, and dizziness) may occur with higher doses. If the recommended dose has not been exceeded, symptoms of hypoxia are probably related to improper ventilation and should be treated with oxygen. If hypoxia persists following alveolar ventilation with oxygen, then methylene blue therapy should be instituted.²

1.4 Safety of Prilocaine application

Several studies have been set recently to check up the safety of Prilocaine preparations on adults and neonates^{16.17.18}. A recent pilot study prepared by Martine C. et al¹⁹, showed that application of an eutectic mixture of Lidocaine – Prilocaine (*EMLA*^R cream) to the heel under occlusion four times a day during 30 min is safe in neonates. The study established on determining methamoglobin levels and plasma concentrations of Lidocaine, Prilocaine and o-toluidine after 24 hour of final application using a co-oximeter for methamoglobin dtermination, and HPLC for analysis of Lidocaine, Prilocaine and o-toluidine.

1.5 Chromatographic determination of Prilocaine

Watanabe et al.³ described a method for the determination of Prilocaine in blood using GC-MS. Blood (0.2 g), 5 μ L deuterated lidocaine (internal standard; 0.1 mg/ml) and 0.8 ml of 5.0 M NaOH were heated in a sealed vial to 120⁰C. After heating, the needle of an SPME device containing an extraction fiber was inserted through the septum of the vial. After 45 min exposure to the headspace, the needle was inserted into the injection port of the GC-MS. The compounds on the fiber were desorbed by exposing the fiber for 5 min in the GC injection port then analyzed on a fused-silica DB-1 column (30 m x 0.32 mm i.d.; 0.25 μ m film thickness), operated with temperature programming from 100-280⁰C at 20⁰C /min. Calibration graphs were linear from 0.01-20 μ g/g.

Grouls et al.⁴ described a method for the determination of 11 anaesthetics among which is Prilocaine using HPLC. The solutions were analysed on a 10 μ m Waters Radial Pack Resolve C18 column with methanolic 33% phosphate buffer at pH 11.0 as the mobile phase (1 ml/min) and UV detection at 220 nm. Calibration graphs were linear for methyl-p-aminobenzoate, benzocaine, procaine, N-butyl-p-aminobenzoate, mepivacaine, prilocaine, lidocaine, bupivacaine, etidocaine, tetracaine and oxybuprocaine.

Siluveru and Stewart⁵ described a method for the stereoselective determination of R-(-) and S-(+)-prilocaine in human serum by capillary electrophoresis using a derivatized cyclodextrin and ultra-violet detection, Plasma was prepared , and were injected over 24 s into a fused-silica capillary (72 cm

x 50 μm i.d.) with 100 mM sodium dihydrogen phosphate buffer of pH 2.5 using 15 mM – heptakis (2 , 6 – di – O – methyl) – beta – cyclodextrin / 0.03 mM-hexadecyltrimethylammonium bromide as background electrolyte, and an applied current of 71 μA . Detection was at 215 nm. Calibration graphs were linear from 45-750 ng/ml for R-(-)- and S-(+)-Prilocaine, with a detection limit of 38 ng/ml and RSD of 8.5 % and 9.17% for R-(-)- and S-(+)-Prilocaine, respectively.

Siluveru and Stewart ⁶ described a method for the stereoselective determination of R(-)- and S(+)-prilocaine in human serum using LC and UV detection, Human serum (1 ml) was mixed with 0.5 ml phosphate buffer and 4 μL 2 mg/ml S-bupivacaine solution (internal standard) and applied to a 1.0 mm C18 Bond-Elut SPE column. After washing with H_2O and acetonitrile, R- and S-Prilocaine were eluted with 2% HCl in methanol. The eluate was evaporated to dryness and the residue was reconstituted in 1 ml hexane. A portion (50 μL) was analysed by LC on a 5 μm naphthyl ethylamine column (25 cm x 4 mm i.d.; Sumichiral DA-4700; YMC Inc; Wilmington, NC, USA) with hexane/ethylenechloride/methanol/TFA (850:100:50:1) as mobile phase (0.8 ml/min) and detection at 220 nm. Baseline separation was achieved in <10 min. No interference was observed. The calibration graphs were linear for 10-1000 ng/ml and the detection limits were 4 and 5 ng/ml for R- and S-prilocaine, respectively. The corresponding mean absolute recoveries were 90.1 +/- 5.2% and 89.3 +/- 4.3%, the corresponding intra-day RSD (n = 3) were 5.4-5.5% and 4.8-5.1%, and the corresponding inter-day RSD (n = 9) were 4-5.8% and 2.8-5.7%.

Klein et al ⁷ described a method for simultaneous determination of some local anesthetics in plasma by HPLC. Plasma (200 μ L) was vortex mixed with 10 μ L of bupivacaine solution. (Internal standard; 15 mg/ml) and 100 μ L of 2.0 M NaOH and the mixture was extracted with 5 ml of anhydrous ethyl ether by vortex mixing, mixing on a rotator and centrifugation. A 4.5 ml portion of the ether layer was re-extracted with 250 μ L of 12.5mM H₂SO₄ in a similar manner. A portion of the acid phase (50 μ L) was analysed on a column (15 cm x 4.6 mm i.d.) of Octyl 1B (5 μ m) with elution (1 ml/min) with acetonitrile/0.05M-phosphate buffer of pH 5.8 and detection at 210 nm. Calibration graphs for Prilocaine, lidocaine (lignocaine) and o-toluidine were linear from 20-2000 ng/ml and the detection limit was 4 ng/ml (200 pg on column). Recoveries were 68-98% and 75-103% at 20 and 1000 ng/ml, respectively.⁷

Koeppel et al ⁸ used methanol as a solvent for extracts of urine or plasma for the determination of drugs by GC - MS associated with the formation of artifacts. Artifacts with a molecular ion of [M + 12] (where M is the molecular weight of the drug) were formed by various drugs, e.g., amphetamine, propafenone, flecainide, beta-blockers and Prilocaine. The mechanism involves addition of formaldehyde, that may be formed from methanol by thermal dehydrogenation in the GC injection port, and subsequent loss of H₂O.⁹

Bjork et al ⁹ described a method for the simultaneous determination of local anaesthetics in plasma samples using GC, Plasma was mixed with internal standard, sodium carbonate solution and hexane - CH₂Cl₂ (4:1). The organic

phase was evaporated to dryness, and the residue was dissolved in hexane - ethanol (9:1) and analysed by GC on a column (17 m x 0.2 mm) of cross-linked methyl silicone with temperature programming from 150⁰C (held for 1 min) at 40⁰C min⁻¹ to 240⁰C (held for 6.8 min). He as carrier gas (0.5 ml min⁻¹) and N - P detection. The internal assay coefficients of variation were 1.5 to 13.8%. The limit of detection was 40 nM and the calibration graphs were rectilinear for up to 3 μM. Recoveries were found to be 79 to 103%.

Whelpton et al ¹⁰ described a method for the determination of Prilocaine in human plasma samples using HPLC, Plasma (0.2 ml), containing 0.1 μg of etidocaine as internal standard, was extracted with toluene (1 ml) by vortex-mixing for 60 s and centrifugation at 12,000 rpm for 1 min. The extract was cleaned up on an AASP diol cartridge by elution with acetonitrile (0.5 ml), and Prilocaine was determined by HPLC on a column (15 cm x 4.6 mm i.d.) of Spherisorb 5 CN equipped with a guard column (2 cm x 2 mm i.d.) of Co:Pell ODS. The mobile phase (1 ml min⁻¹) was 0.01M-H₃PO₄ in acetonitrile - H₂O , and detection was with an Environmental Science Association Model 5100 A Coulochem detector at +0.7 and +0.9 V vs. a Pd reference electrode. Calibration graphs were rectilinear for 5 to 500 and 10 to 1000 ng/ml. Inter-assay coefficients of variation were 0.9 to 15.7%; intra-assay values were 1.6 to 5.7% (n = 10).

Adams et al ¹¹ described a method for the determination of Prilocaine using HPLC, Plasma was made alkaline and extracted with ethyl ether, and the organic phase was back-extracted with 250 μL of 0.025 M H₂SO₄. A 50 μL aliquot of the extract was analysed by HPLC on a C18 column (30 cm x 3.9 mm i.d.), with a mobile phase (1 ml min⁻¹) of acetonitrile - 0.05M-sodium phosphate buffer (3:7) and detection was at 210

nm. For determination of lignocaine, mepivacaine and Prilocaine, the buffer was adjusted to pH 5.8; for bupivacaine and etidocaine it was adjusted to pH 3.5. Separation of Prilocaine and mepivacaine was not satisfactory. Recovery was ~90%, and coeff. of variation were 2%. The detection limit was ~30 ng/ml. Minor modifications are necessary for the detection of other amide local anaesthetics in current clinical use.

Prat, M. and Bruguerolle, B.¹², described a method for GC determination of five local anaesthetic drugs in serum. Serum was mixed with trichloroacetic acid and internal standard solution, the mixture was centrifuged and the supernatant solution was mixed with NaOH before extraction of Prilocaine, etidocaine, lignocaine, mepivacaine and bupivacaine into CH₂Cl₂. The extract was evaporated to dryness under Nitrogen, and the residue was dissolved in CS₂ for GLC at 230⁰C on a column (1.8 m x 2.0 mm i.d.) of 3% of OV-17 on Chromosorb W AW-DMCS with nitrogen as carrier gas (35 ml/min) and FID. Calibration graphs were rectilinear and coefficients of variation were <3%.

1.6 Electrochemical Methods for the determination of Prilocaine

A single electrochemical method has been reported in the literature for the determination of Prilocaine, Fernandez-Marcote and co-workers¹ described a method for the determination of Prilocaine as its N-nitrosamine derivative. The method based on the polarographic (DP) determination of Prilocaine derivatives. The calibration graph was linear over the range 1M to 0.1mM, with detection and quantitation limits of 0.6 μ M and 2 μ M, respectively, and RSD was 1.0%.

1.7 Limitations for the reported methods

Most of the methods reported in the literature for the determination of Prilocaine suffer from many advantages, they are almost need chemical treatment for Prilocaine samples before the determination step, time consuming, need sophisticated instruments and special training.

On the other hand, all the methods reported concentrate in the determination of Prilocaine in serum, while our goal is to determine the drug in pharmaceutical preparations.

The only reported method for determination of Prilocaine in pharmaceutical preparations was described by Fernandez-Marcote and co-workers¹ in 1998, the method was based on DPP determination of Prilocaine as its N-nitrosamine derivative, while the present method aim to setup a direct determination of Prilocaine using DP-AdCSV.

1.8 Aim of the work

Several procedures were published for the determination of Prilocaine using chromatographic methods, on the other hand, a little attention has been paid for the spectrophotometric and electroanalytical methods for the determination of Prilocaine particularly, and local anesthetics in general.

The aim of the work is to develop a new, sensitive and direct method for the determination of Prilocaine in pharmaceutical formulations, using extractional spectrophotometry, and Differential-pulse adsorptive cathodic stripping voltammetry (DP-AdCSV) methods.

Chapter Two
Experimental

2.1 Reagents and Solutions

Through out the experimental work, doubly distilled water was used, Prilocaine and all other chemicals and solvents were of analytical grade (Aldrich, Sigma, Merck, and Riedel-deHaen).

2.1.1 Prilocaine stock solution (1.0×10^{-3} M)

The stock solution was prepared by dissolving exactly 0.0256 gm of Prilocaine (obtained from SIGMA) in doubly distilled water, the volume was then completed to 100 ml with water, and stored in the refrigerator, the solution is stable for two weeks.

2.1.2 Preparation of Britton – Robinson (BR) Buffer

A mixture of acetic acid, boric acid, and phosphoric acid was prepared by mixing equal volumes of 0.04 M of each acid, the pH of the buffer was adjusted using 0.2 M sodium hydroxide solution.

2.1.3 Preparation of Bromothymol Blue (BTB) and Bromocresol Green (BCG) solutions (1.0×10^{-3} M)

0.0624 gm and 0.0690 gm of BTB and BCG (obtained from SIGMA) respectively were dissolved in 2.0 ml of 0.1 M sodium hydroxide. A 20.0 ml of ethanol (96%) (obtained from Aldrich) was added and the volume was completed to 100 ml with double distilled water.

2.2 Apparatus

2.2.1 Spectrophotometric measurements

A UV-2, UNICAM UV-Visible spectrophotometer was used for all spectrophotometric measurements. All measurements were carried out using quartz cells (10 mm), at room temperature.

2.2.2 pH Meter

In all our work HANNA pH meter of model 8521 was used.

2.2.3 Electroanalytical measurements

All the voltammograms were carried out using Model 264 B EG&G polarographic analyzer / stripping voltammeter, coupled with model 303 A stand and model 305 automatic stirrer and RE 0150 X-Y recorder.

The three electrode system was constructed using an auxiliary platinum wire electrode and Ag/AgCl reference electrode.

2.3 Recommended procedure for determination of Prilocaine

2.3.1 Spectrophotometric method

A measured amount of dye (6.0×10^{-5} M and 8.0×10^{-5} M for BTB and BCG respectively) was transferred into 100 ml separatory funnel, then 3 ml of BR-buffer solution of pH 4.5 and 3.0 for BTB and BCG respectively were added. Accurate volume of solution containing Prilocaine in the range 2.5 – 50 ppm and 4.5 – 55 ppm for BTB and BCG respectively was added and followed by 20 ml of chloroform, the mixture was shaken vigorously for 30 – 60 s and allowed to

stand for 20 min. The organic phase was collected in a 25-ml volumetric flask and the volume was completed to the mark with chloroform.

Blank solution was prepared at the same time under the same conditions, the absorbance of the formed complex was measured at 416 and 420 nm for BTB and BCG respectively, and the amount of Prilocaine was determined from the already constructed calibration curve.

2.3.2 Adsorptive cathodic stripping method

Exactly 8.0 ml of BR-Buffer was transferred into a clean and dry voltammetric cell, the voltage range set to be (-0.7) – (-1.5) V, and an accumulation time of 0.0. The operation started by switching the device on in differential pulse stripping voltammetry mode, the stirrer was switched on at a slow rate while purging with nitrogen “extra pure” for 8 min. A new drop of mercury is formed, the scan was initiated directly in the negative direction with a scan rate of 20 mV/sec and pulse amplitude of 25 mV.

A measured amount of Prilocaine solution (0.05 – 6 ml of 1.0×10^{-3} M Prilocaine) was injected into the cell, and the scan repeated under the same conditions, the purging for 30-60 sec was carried out between successive measurements. A calibration graph of concentration vs. current was constructed and used to determine an unknown concentration of the drug.

Chapter Three
Results and Discussion
Spectrophotometric method

3.1 Absorption Spectra

The absorption spectra of Prilocaine with BTB and BCG were studied in the wavelength range 350-650 nm for solutions prepared according to the recommended procedures. The BTB-Prilocaine and BCG-Prilocaine ion-pairs exhibit absorbance maxima at 416 and 420 nm, respectively. Absorption spectra of the two ion-pairs are shown in Figures (1) and (2).

To optimize the conditions for determination of Prilocaine, several factors were studied such as the effect of pH, amount of buffer used, time of equilibrium, reagent concentration, type of organic solvent, amount of dye, shaking time and number of extractions.

3.2 Effect of pH

The effect of pH was studied within the pH range 2.5 – 7.0 of Britton Robinson buffer (BR Buffer). It was found that the maximum absorption obtained at pH 4.5 and 3.0 for BTB-Prilocaine and BCG-Prilocaine, respectively. These results are summarized in Table (1), Figures (3), and (4).

Table 1 : Effect of pH on the absorbance of Prilocaine-BTB and BCG-Prilocaine ion-pairs. [BTB] = [BCG] = [Prilocaine] = 1.0×10^{-3} M, Temperature = 25°C , $\lambda = 416, 420$ nm for BTB and BCG, respectively.

pH	Absorbance	
	BTB	BCG
2.5	0.36	0.33
3.0	0.52	0.47
3.5	0.55	0.43
4.0	0.59	0.43
4.5	0.68	0.34
5.0	0.62	0.20
6.0	0.55	0.05
7.0	0.54	0.05

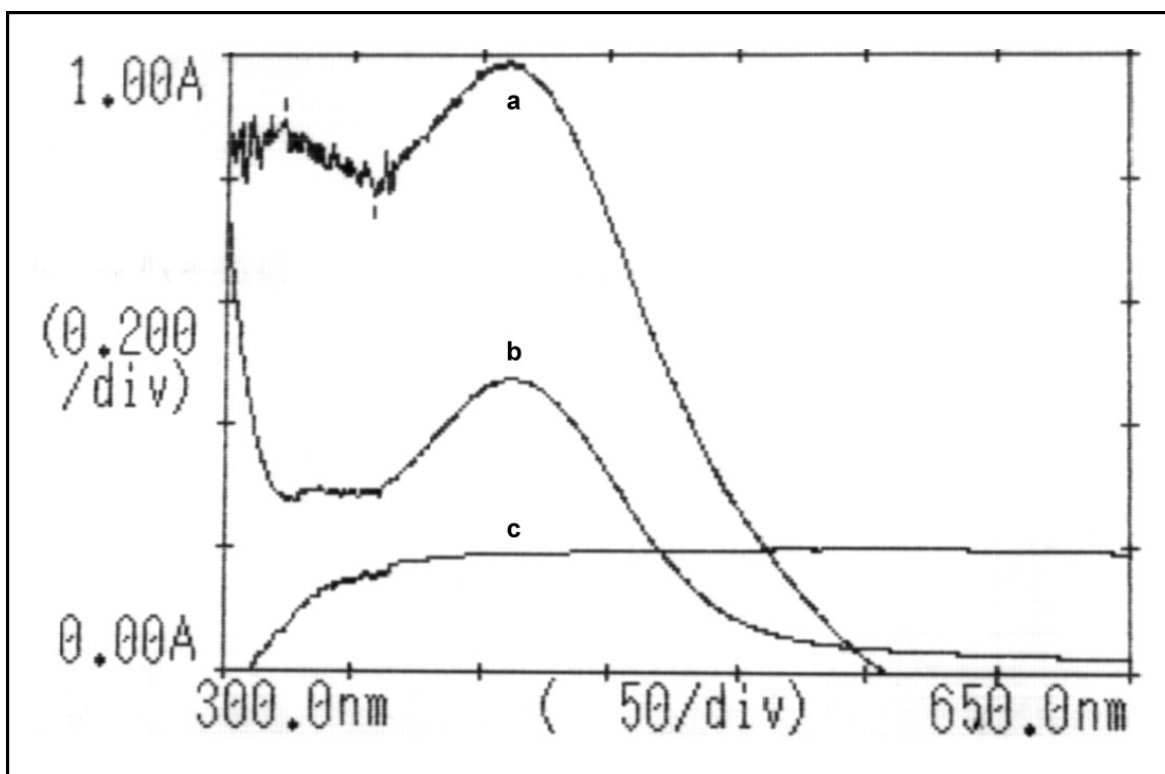


Figure (1) : Absorption spectra for Prilocaine-BTB ion pair

a: Prilocaine-BTB ion pair against reagent blank, b: reagent blank against chloroform, c: Prilocaine solution against chloroform, pH= 4.5, Temperature = 25⁰C. , λ_{\max} = 416 nm.

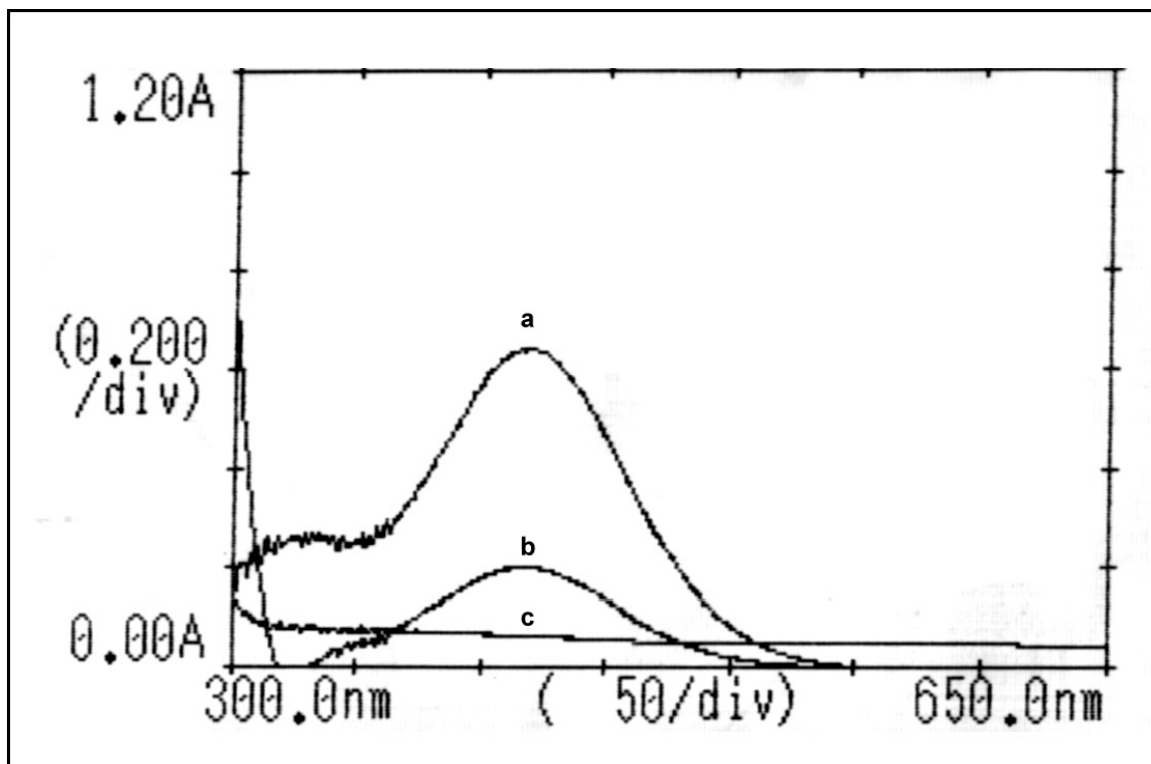


Figure (2) : Absorption spectra for Prilocaine-BCG ion pair

a: Prilocaine-BCG ion pair against reagent blank, b: reagent blank against chloroform, c: Prilocaine solution against chloroform, pH= 3.0, Temperature = 25⁰C. , $\lambda_{\text{max}} = 420 \text{ nm.}$

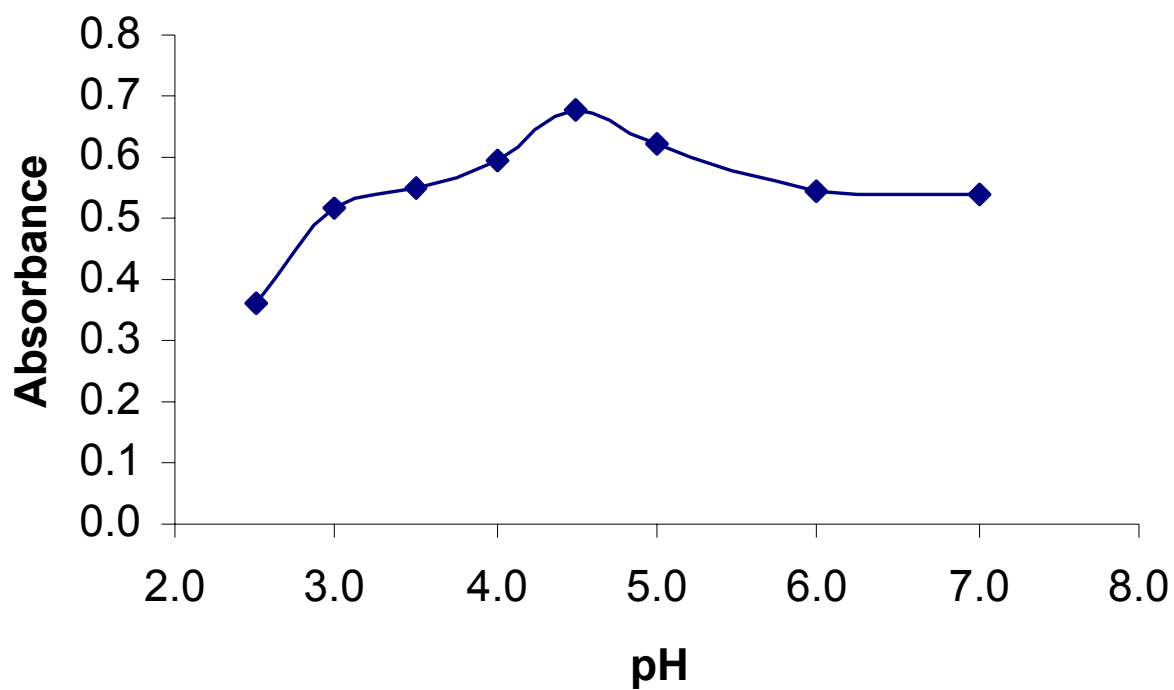


Figure (3) : Effect of pH on the absorbance of Prilocaine-BTB ion-pair complex, [BTB]=[Prilocaine] = 1.0×10^{-3} M. $\lambda=416$ nm.

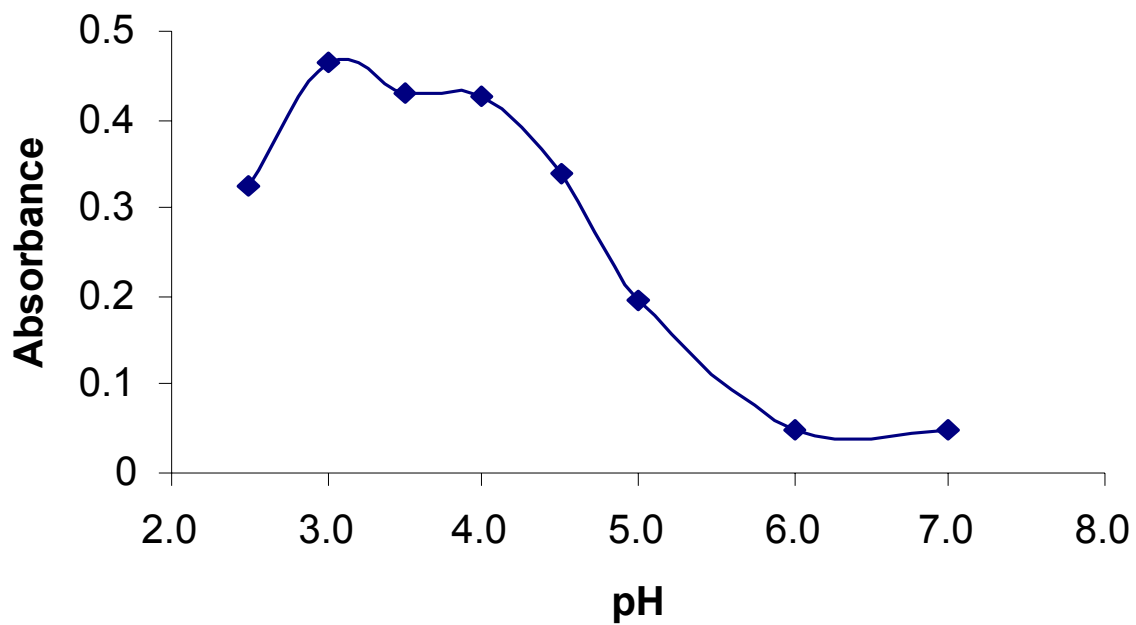


Figure (4) : Effect of pH on the absorbance of Prilocaine-BCG ion-pair complex, [BTB]=[Prilocaine] = 1.0×10^{-3} M. $\lambda=420$ nm

3.3 Choice of Organic Solvent

Several organic solvents were investigated to choose the most suitable solvent for the extraction of Prilocaine-dye ion-pair. It was found that chloroform and dichloromethane are suitable solvents for the extraction of both BTB and BCG – Prilocaine ion-pairs, respectively.

The results indicate that there is an obvious relationship between the solvent polarity and the amount of complex extracted. Table (2) show that as the polarity of the solvent increases the absorbance of the extracted complex increases for both BTB and BCG – Prilocaine ion pairs. This correlate with the results obtained by Khaled B. A ¹⁴, Chloroform was recommended as main solvent in this work.

Table 2 : Effect of type of organic solvent on the extraction of Prilocaine-Dye ion-pair complex. [BTB] =[BCG]= [Prilocaine] = 1.0×10^{-3} M, λ = 416 and 420 nm for BTB and BCG respectively, pH = 4.5 and 3.0 for BTB and BCG respectively, Temperature = 25⁰C, The solvent arranged according to decrease in polarity.

Solvent	Absorbance	
	BTB	BCG
Chloroform	0.40	0.55
Dichloromethane	0.40	0.51
Diethylether	0.30	0.31
Carbon tetrachloride	0.30	0.10
Toluene	0.05	0.13
n-Hexane	0.08	0.04

3.4 Effect of amount of solvent

The effect of amount of chloroform used for the extraction of Prilocaine-Dye ion-pairs was studied. The amount of solvent had no significant effect on the absorbance of the formed complex. Table (3) shows the effect of the amount of chloroform on the absorption of both BTB and BCG- Prilocaine ion-pairs. A 20.0-ml of solvent was recommended in this study.

Table 3 : Effect of amount of chloroform on the absorbance of Prilocaine-Dye ion-pairs. [BTB] = [BCG]=[Prilocaine] = 1.0×10^{-3} M. $\lambda = 416$ and 420 nm for BTB and BCG respectively, pH = 4.5 and 3.0 for BTB and BCG respectively, Temperature = 25⁰C.

Amount of solvent (ml)	Absorbance	
	BTB	BCG
5	0.55	0.50
10	0.61	0.54
15	0.63	0.58
20	0.70	0.59

3.5 Effect of the number of extraction times on the absorbance

The effect of multi extraction times of Prilocaine-dye ion-pair was investigated. A single and double (2×10 ml) extraction using 20.0 ml of solvent was tested, and the volume completed to 25.0 ml.

It was found that the percent of extraction using a single and double extraction were almost the same, hence, a single extraction for both BTB and BCG-Prilocaine ion-pairs using 20.0 ml of solvent was recommended in this work.

3.6 Effect of shaking time

The effect of shaking time on the absorbance of the ion-pair complex formed between Prilocaine and the investigated dyes were studied within the range 60 - 180 sec.

As shown in Table (4), there is a significant effect of shaking time on the absorbance. Shaking time of 180 and 60 sec were recommended for BTB and BCG respectively.

Table 4 : Effect of shaking time on the absorbance of Prilocaine-Dye ion pairs, . [BTB]=[BCG] = [Prilocaine] = 1.0×10^{-3} M, λ = 416 and 420 nm for BTB and BCG respectively, pH = 4.5 and 3.0 for BTB and BCG respectively, Temperature = 25⁰C.

Shaking time (s)	Absorbance	
	BTB	BCG
0	0.31	0.10
30	0.52	0.61
60	0.56	0.63
90	0.58	0.61
120	0.58	0.54
180	0.60	0.50

3.7 Effect of amount of buffer

The effect of the amount of BR buffer used in extraction of the Prilocaine-dye complex was investigated at the maximum wavelength, it was found that the amount of buffer has no significant effect on the absorbance. A 3.0 ml of buffer was recommended for both dyes, as shown in Table (5).

Table 5 : Effect of amount of buffer solution on the absorption maxima for Prilocaine – BTB ion-pairs, [BTB]=[BCG] = [Prilocaine] = 1.0×10^{-3} M, $\lambda = 416$ and 420 nm for BTB and BCG respectively, pH = 4.5 and 3.0 for BTB and BCG respectively, Temperature = 25°C .

Amount of Buffer (ml)	Absorbance	
	BTB	BCG
1.0	0.63	0.55
2.0	0.64	0.58
3.0	0.61	0.60
4.0	0.58	0.57
5.0	0.58	0.55

3.8 Effect of dye concentration

The effect of dye concentration on the absorbance of Prilocaine-dye ion pair at the optimum conditions was studied in the concentration range 1.0×10^{-5} M to 8.0×10^{-5} M.

It was found that there is general increase in the absorbance as the concentration of dye increases up to a dye concentration of 6.0×10^{-5} M and 8.0×10^{-5} M for BTB and BCG respectively. Any further increase in the dye concentration did not show any effect on the absorbance up to of 7.0×10^{-5} M and 10.0×10^{-5} M for BTB and BCG respectively. The suitable concentration of dye was found to be 6.0×10^{-5} M for BTB-Prilocaine complex, and 8.0×10^{-5} M for BCG-Prilocaine complex. Results are shown in Table (6).

Table 6 : Effect of Dye concentration on the absorption maxima for Prilocaine-dye ion-pairs, [Prilocaine] = 1.0×10^{-3} M, $\lambda = 416$ and 420 nm for BTB and BCG respectively, pH = 4.5 and 3.0 for BTB and BCG respectively, Temperature = 25°C .

Concentration of Dye (M)	Absorbance	
	BTB	BCG
1.0×10^{-5}	0.2	0.20
2.0×10^{-5}	0.33	0.26
3.0×10^{-5}	0.32	0.33
4.0×10^{-5}	0.43	0.39
5.0×10^{-5}	0.68	0.48
6.0×10^{-5}	0.73	0.57
7.0×10^{-5}	0.73	0.57
8.0×10^{-5}	0.73	0.57
12.0×10^{-5}	0.70	0.52

3.9 Stability of the complex

The stability of the ion-pair complexes in the organic layer was determined by following the absorbance of the complex formed over a period of time, Figures (5) and (6) show the absorbance of Prilocaine-Dye ion-pairs as a function of time at room temperature.

The obtained results showed that the absorbance increases gradually with time up to about 15 min for both BTB and BCG – Prilocaine ion pairs, and remain constant for at least 120 hours as shown in the Figures (5) and (6).

3.10 Stoichiometry

3.10.1 Molar Ratio Method

In this method, a series of solutions containing different concentration of the dye were prepared, where the concentration of Prilocaine and all other conditions were kept constant. The absorbance of the formed complexes were measured at λ_{\max} and plotted versus the mole ratio $[\text{dye}]/[\text{Prilocaine}]$, Figures (7) and (8) indicate that the ion-pair complex have a 1:1 stoichiometric ratio for both BTB and BCG complexes.

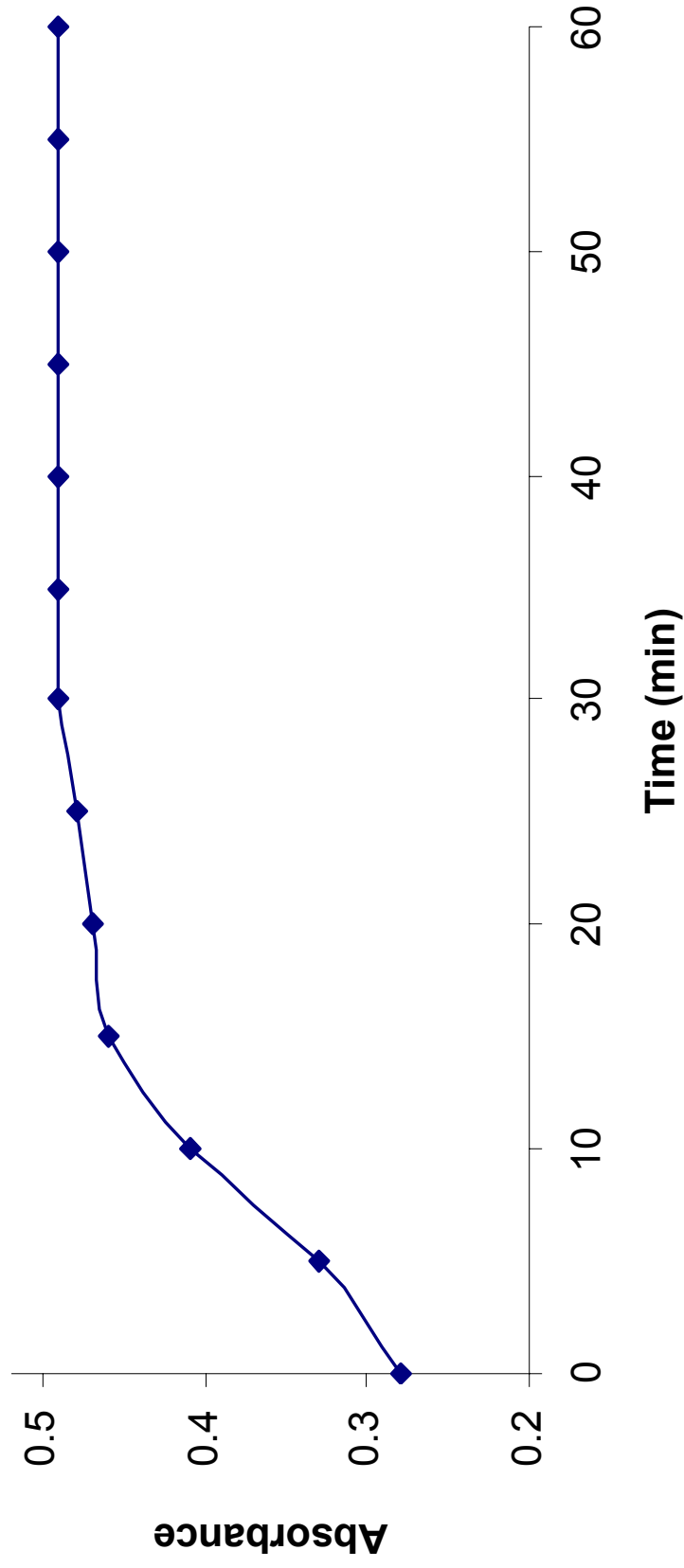


Figure 5: Effect of Time on the absorbance of the Prilocaine-BTB ion-pair in the organic phase at room temperature.

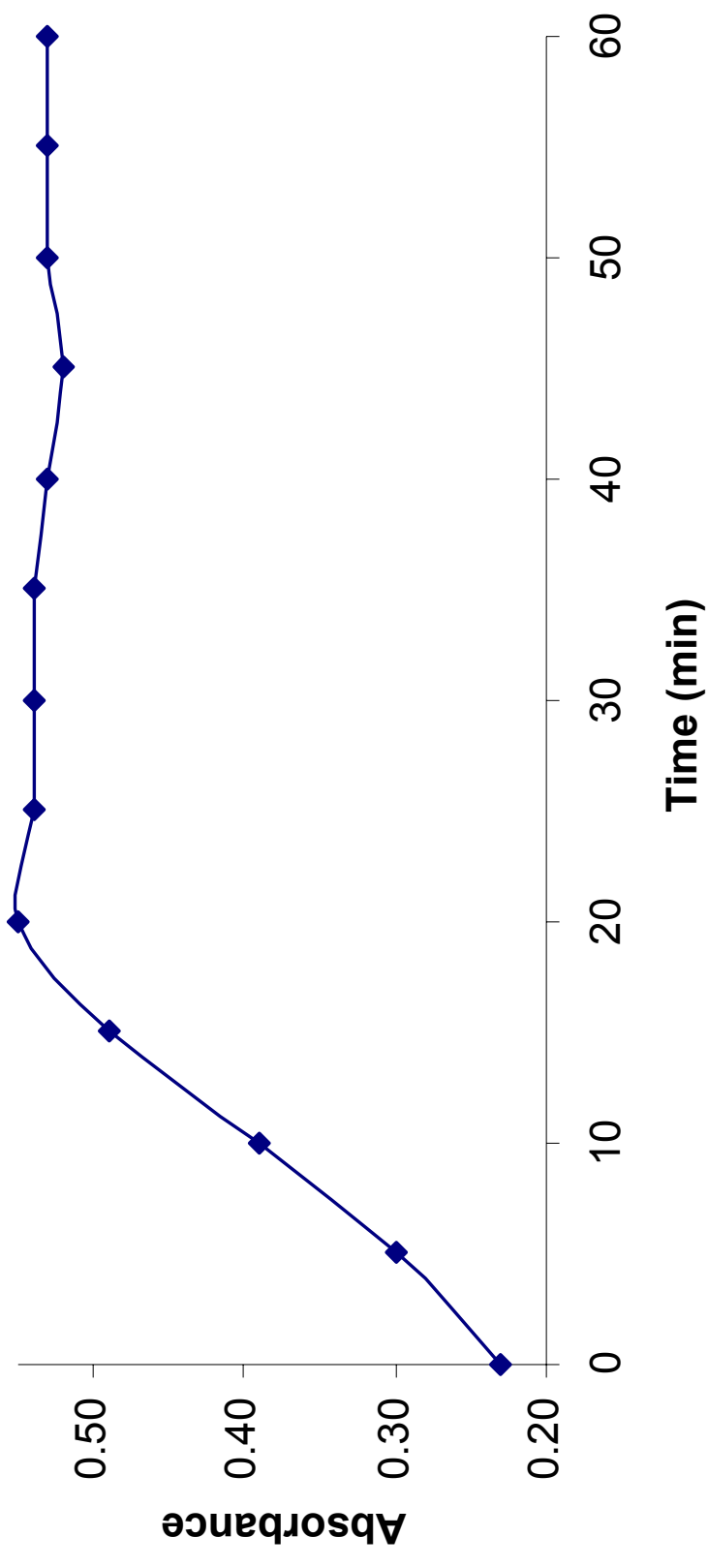


Figure 6: Effect of Time on the absorbance of the Prilocaine-BCG ion-pair in the organic phase at room temperature.

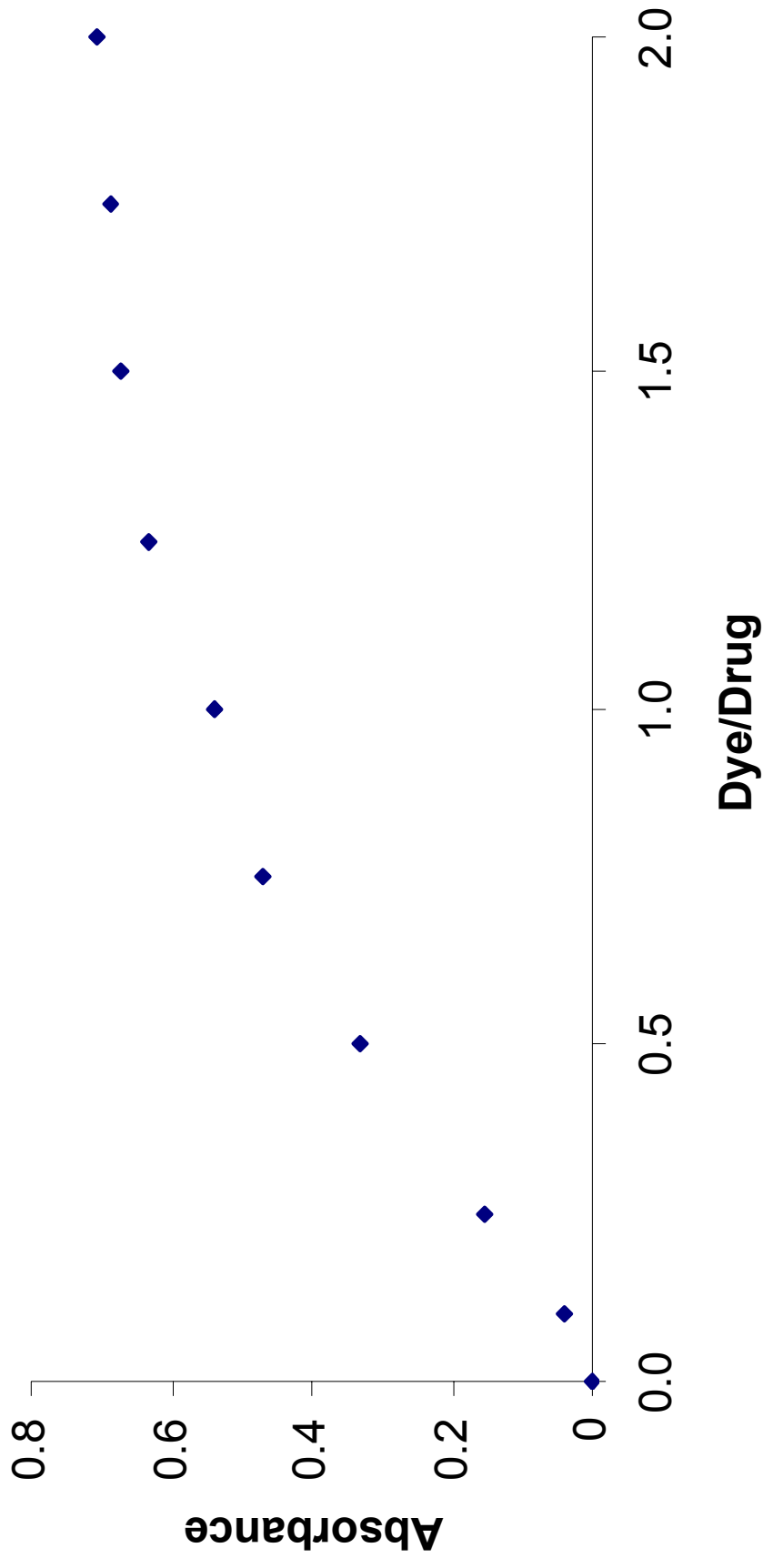


Figure 7 : Molar ratio method for the determination the stoichiometry of the ion-pair formed between Prilocaine and BTB at 416 nm.

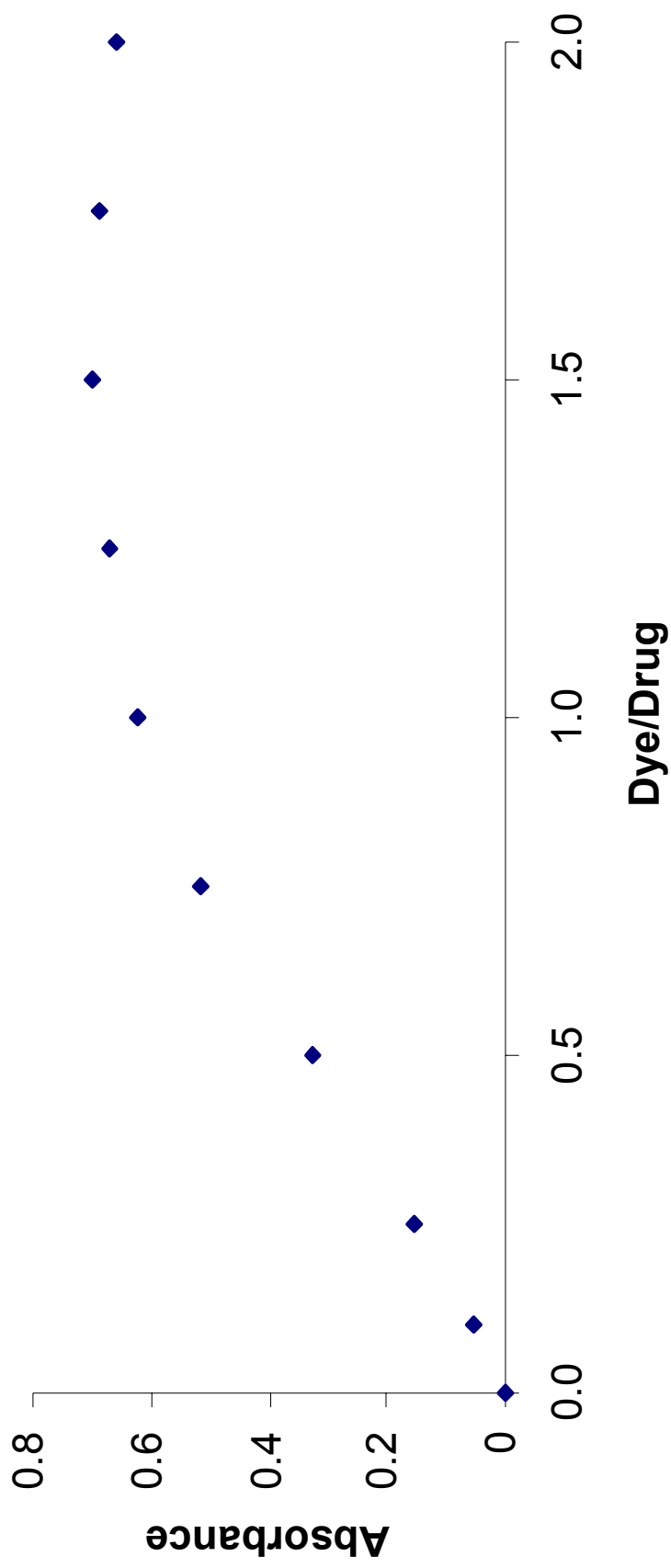


Figure 8 : Molar ratio method for the determination the stoichiometry of the ion-pair formed between Prilocaine and BCG at 420 nm.

3.11 Effect of Temperature

The effect of temperature on the absorbance of the formed complex was investigated over the range 15-50⁰C. The solution prepared as described in the general procedures and the absorbance measurements were taken in closed cells to prevent evaporation of the solvent. The results obtained show that, there is no significant effect of temperature on the absorbance of the formed complex between Prilocaine with BTB and BCG over the investigated range. During the work all measurements were carried out at room temperature.

Table 7 : Effect of temperature on the absorbance of Prilocaine-Dye ion pairs, . [BTB]=[BCG]= [Prilocaine] = 1.0×10^{-3} M.

Temperature ⁰ C	Absorbance	
	BTB	BCG
15	0.52	0.53
20	0.56	0.53
25	0.60	0.53
30	0.58	0.52
35	0.54	0.51
40	0.51	0.48
45	0.46	0.45
50	0.40	0.39

3.12 Beer's law and sensitivity

The calibration graphs were plotted under the optimum conditions recommended in the general procedures. The relation between the absorbance of the complex and Prilocaine concentration obey Beer's law over the concentration range 2.0 – 24.5 ppm using BTB, and 2 – 26.5 ppm using BCG as shown in figures (9) and (10).

The molar absorptivities (ϵ) were calculated from the linear part of the calibration curve and found to be $1.68 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $1.83 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ for BTB and BCG respectively. The relative standard deviations (RSD's) were calculated for each of the two systems and found to be 0.61% and 0.53% for BTB and BCG respectively.

These values indicated a good reproducibility of the proposed method.

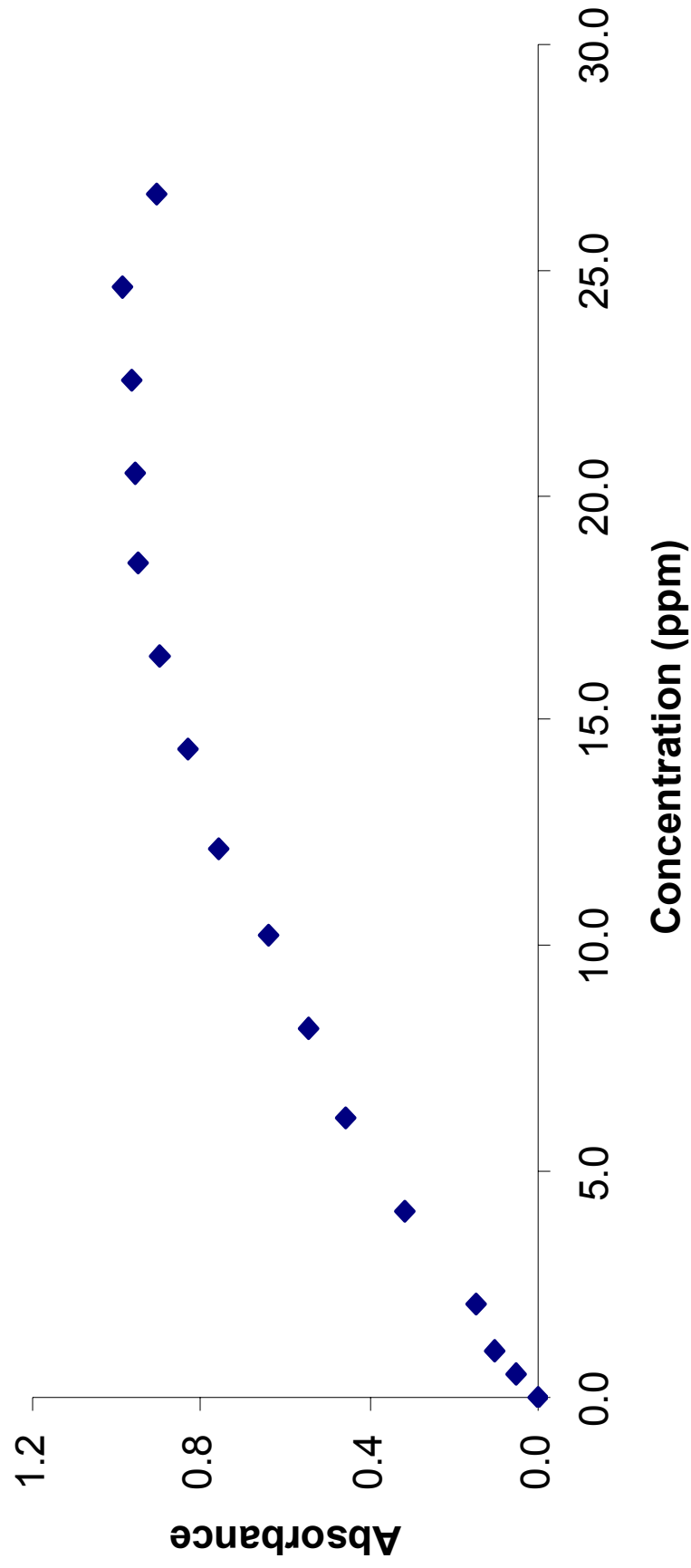


Figure 9 Calibration graph for the determination of Prilocaine using BTB reagent.

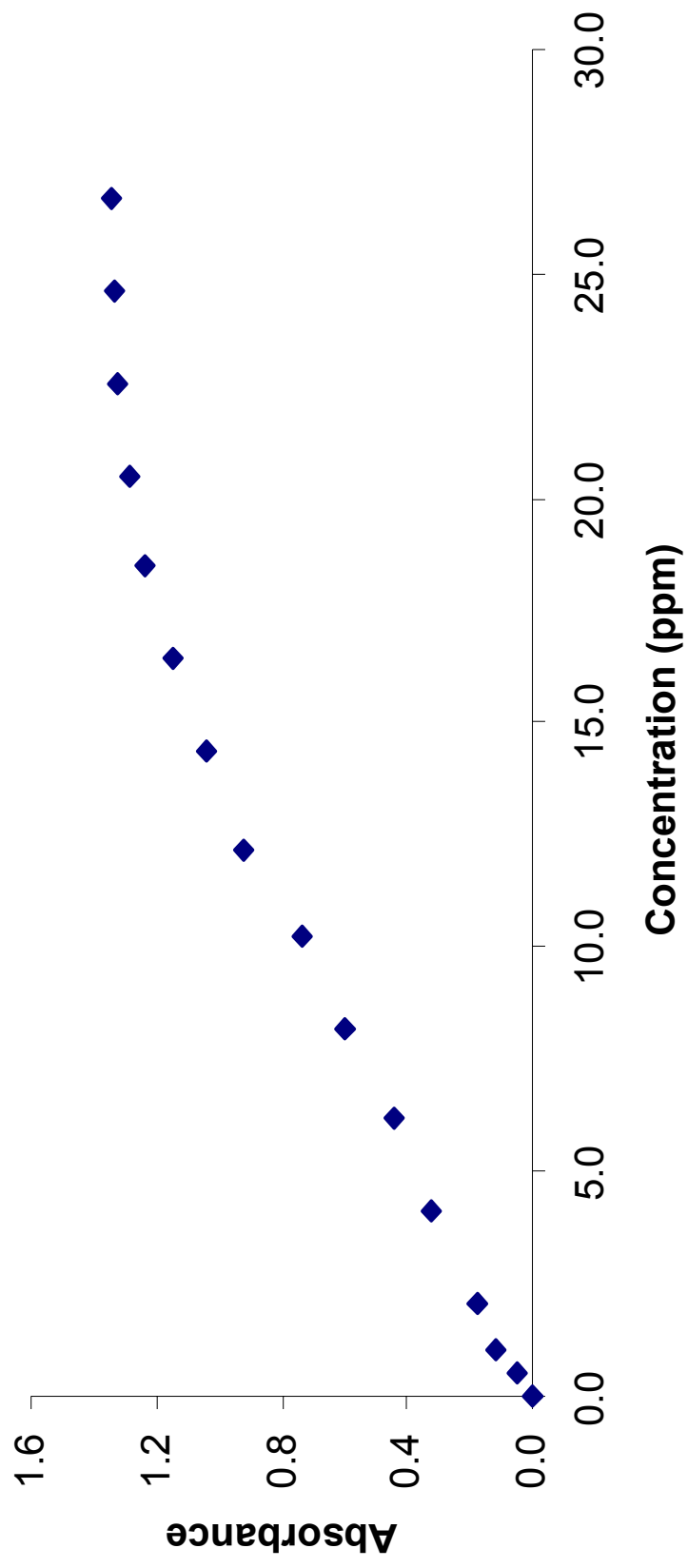


Figure 10 Calibration graph for the determination of Prilocaine using BCG reagent.

3.13 Conclusion

Table 8, list for parameters affecting the spectrophotometric method for the determination of Prilocaine, and the analytical results.. :

	BTB	BCG
Wave Length	416 nm	420 nm
pH	4.5	3.0
Solvent	Chloroform	Chloroform
Amount of solvent	20 ml	20 ml
Extraction times	Once	Once
Shaking time	180 s	60 s
Amount of buffer	3.0 ml	3.0 ml
Dye concentration	6.0×10^{-5}	8.0×10^{-5}
Stability	Days	Days
Conc. Range (ppm)	2.0 – 24.5 ppm	2.0 – 26.5 ppm
Detection Limit (ppm)	2.0 ppm	2.0 ppm
RSD	0.61%	0.53%
ϵ	$1.68 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$	$1.83 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$

Chapter Four
Results and Discussion
Electroanalytical method
for determination of Prilocaine

Results and discussion

In order to optimize the experimental conditions for the voltammetric determination of Prilocaine, various conditions have been tested such as: effect of pH, accumulation potential, accumulation time, scan rate, pulse amplitude, drop size, and cyclic voltammetric behavior.

4.1 Voltage Direction

It was found that Prilocaine exhibit a voltammetric peak at -1.2 V in both anodic and cathodic differential-pulse adsorptive stripping voltammetry, as shown in figure (11). It was found that, the height of the cathodic peak is higher than the anodic one, hence, differential-pulse adsorptive cathodic stripping voltammetry (DP-AdCSV) was used during this work.

4.2 Effect of pH

Differential-pulse adsorptive cathodic stripping voltammograms (DP-AdCSV) of 5.88×10^{-5} M was carried out over a pH range equals (5.0 –11.5) using Britton-Robinson (BR) buffer. It was found that, the voltammograms consist of one peak throughout the whole pH range studied, no voltammetric peaks were obtained at pH's below 5 due to the hydrolysis of Prilocaine¹. Peak height increase gradually as the pH increases from 5.0 to 11.0, as shown in Figure (12) and Table (9). pH of 10.5 was recommended as optimum pH in this work.

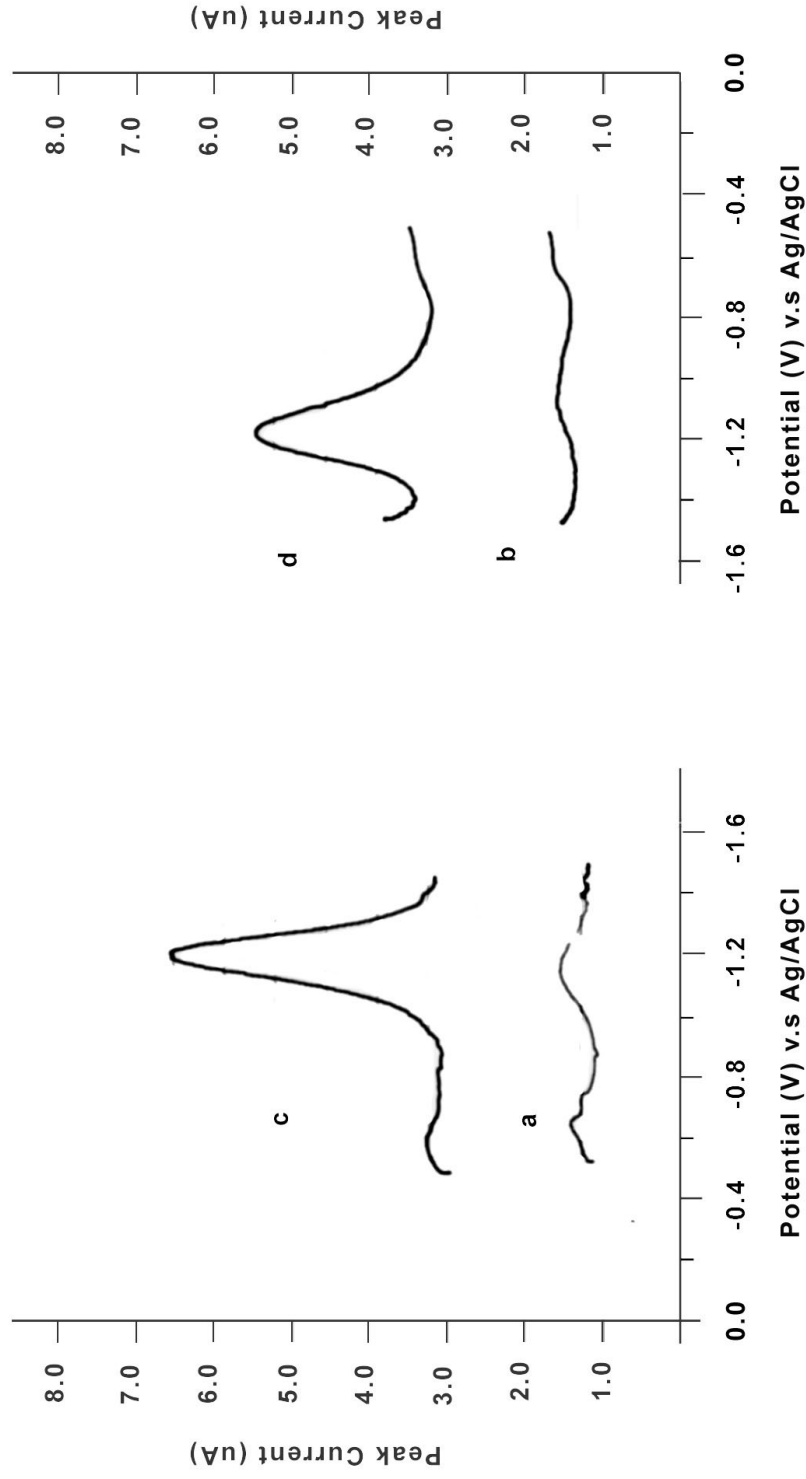


Figure 11, DP-AdCS voltammograms for 5.88×10^{-5} M Prilocaine, (a & b) solution blanks, (c) cathodic scan, (d) anodic scan, scanned from (-0.5) – (-1.5)V, pH = 10.5, drop size: medium, pulse amplitude=25mV, accumulation time = 0.0 sec, scan rate =20 mV/sec.

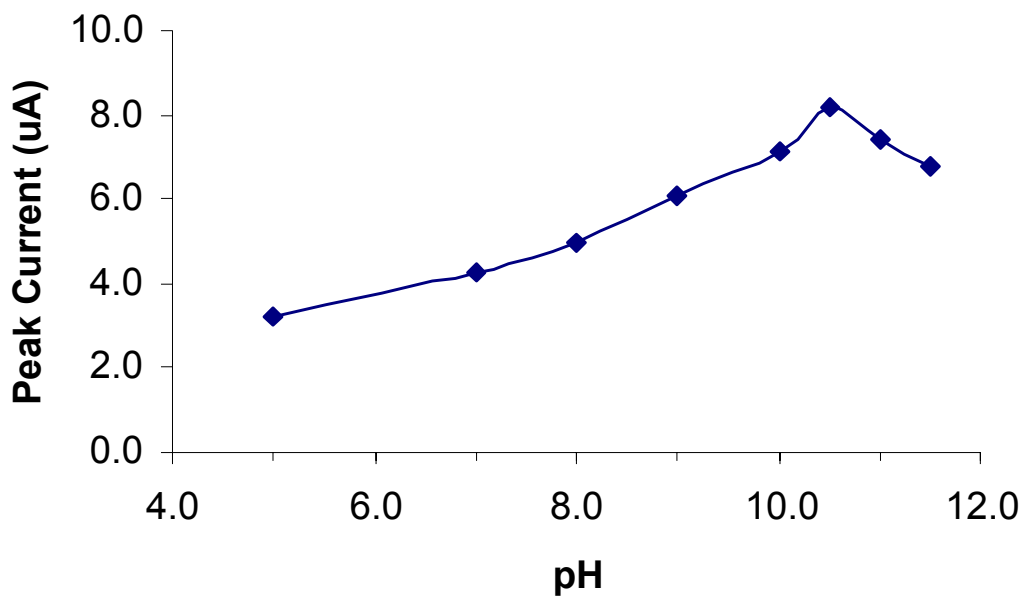


Figure 12 : Effect of pH on the DP-AdCSV of 5.88×10^{-5} M Prilocaine, other conditions as listed in figure (11).

Table 9 : Effect of pH on the DP-AdCSV of 5.88×10^{-5} M Prilocaine, other conditions as listed in figure (11).

pH	Peak Current (μ A)
5.0	3.2
7.0	4.3
8.0	5.0
9.0	6.1
10.0	7.1
10.5	8.2
11.0	7.4
11.5	6.8

4.3 Effect of accumulation potential

The effect of changing the accumulation potential on the peak height was studied over a potential range (+0.6)-(-0.8) V. The operation carried out by allowing the ions to accumulate at the desired potential, and re-zero for the potential adjusted just before the scan began.

It was found that changing the accumulation potential has no effect on the peak height or its position, a potential range of (-0.7)-(-1.5) V was recommended in this work. Figure (13)

4.4 Effect of accumulation time

The effect of accumulation time on the peak height of Prilocaine was studied over the range of 0 – 120, As shown in Figure (14), there is a markedly decrease in the peak current as the accumulation time increases. Hence, direct scan without accumulation was recommended in the procedure of this work.

Decreasing the peak current sharply with increasing the accumulation time may be due to the strong desorption of accumulated Prilocaine.

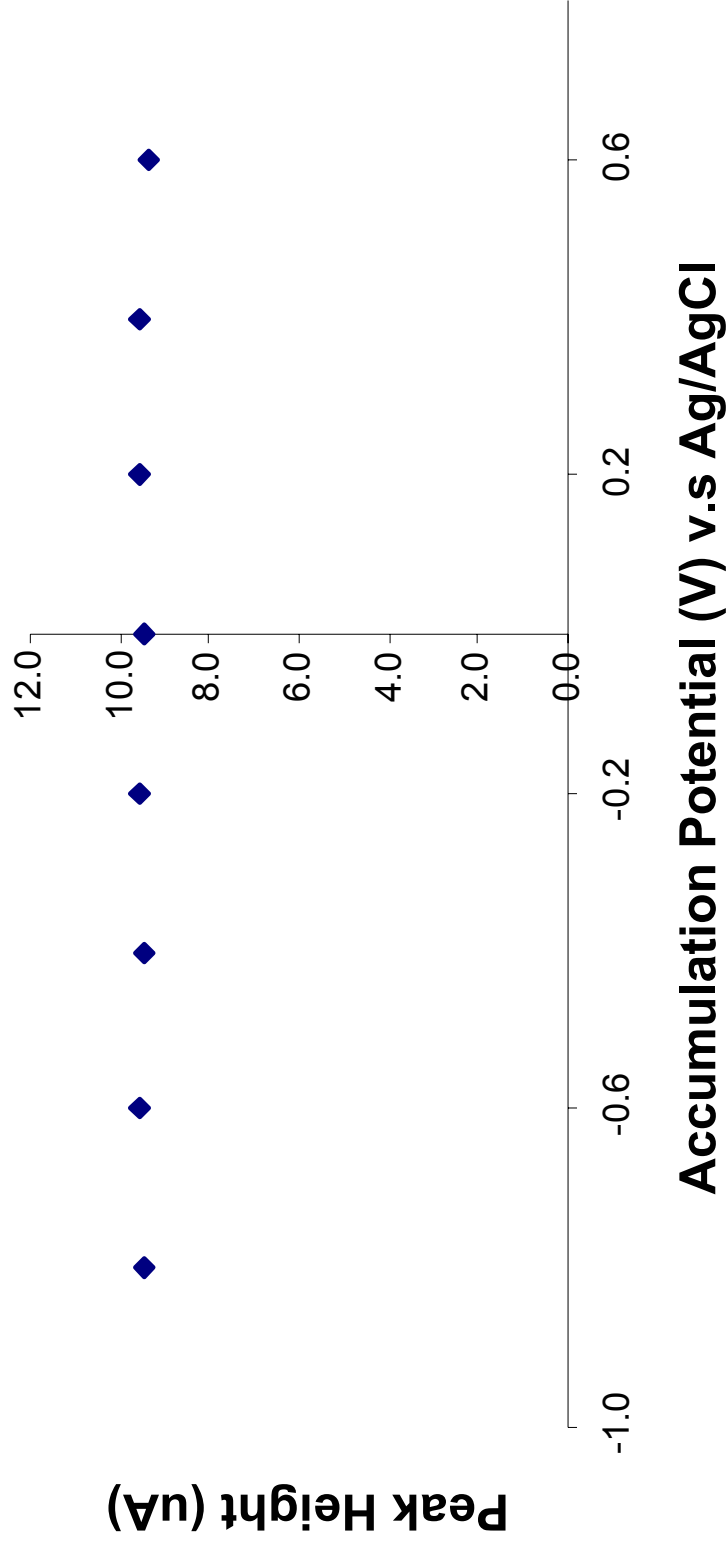


Figure 13, Effect of changing the accumulation potential on the DP-AdCS voltammograms for 5.88×10^{-5} M Prilocaine, other conditions as listed in figure (11).

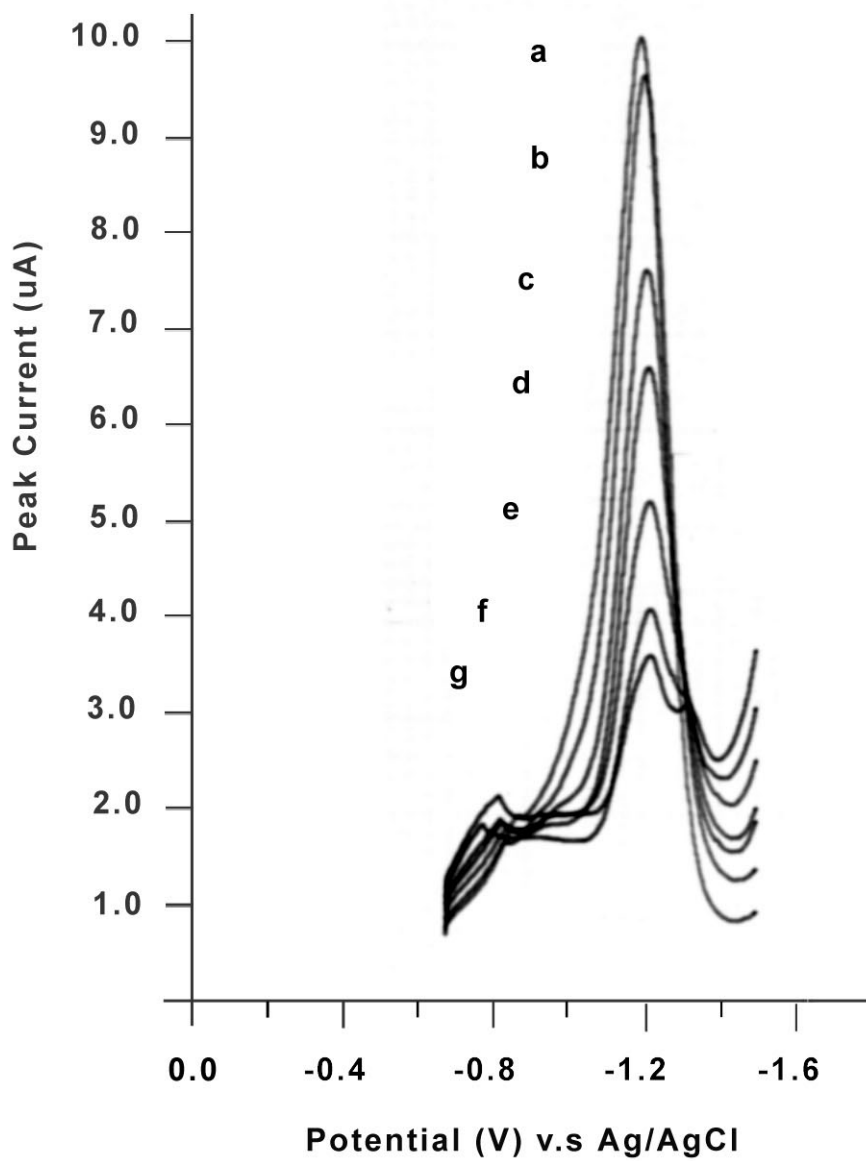


Figure 14, Effect of accumulation time on DP-AdCSV of 5.88×10^{-5} M Prilocaine, (a-g) accumulation for 0, 15, 30, 45, 60, 90, and 120 respectively, other conditions as listed in figure (11).

4.5 Cyclic voltammetry measurements

The cyclic voltammetry were carried at three concentration levels of Prilocaine as shown in Figure (15). A very small anodic peak was observed at lower scan rate and becomes developed at high scan rate (above 200 mV/sec).

4.6 Effect of scan rate

The effect of scan rate on the peak height of Prilocaine has been investigated on DC-AdCSV as shown in Figure (16), a gradual increase in the peak height was associated with the increase of scan rate, with a little shift in the peak potential to more negative direction indicating slow kinetics character of the dissolution of Prilocaine¹⁴.

4.7 Effect of drop size

The peak height of Prilocaine increased by increasing the surface area of the mercury drop (HMDE), as shown in Figure (17). However, a medium drop size was recommended in this work to assure high sensitivity and reproducibility.

4.8 Effect of pulse amplitude

The effect of pulse amplitude on the peak current of Prilocaine was investigated, Figure (18). The peak current increases by increasing the pulse amplitude.

On the other hand, the peak potential was shifted toward more positive values as increasing the pulse amplitude. Pulse amplitude of 25 mV/sec was recommended throughout this work.

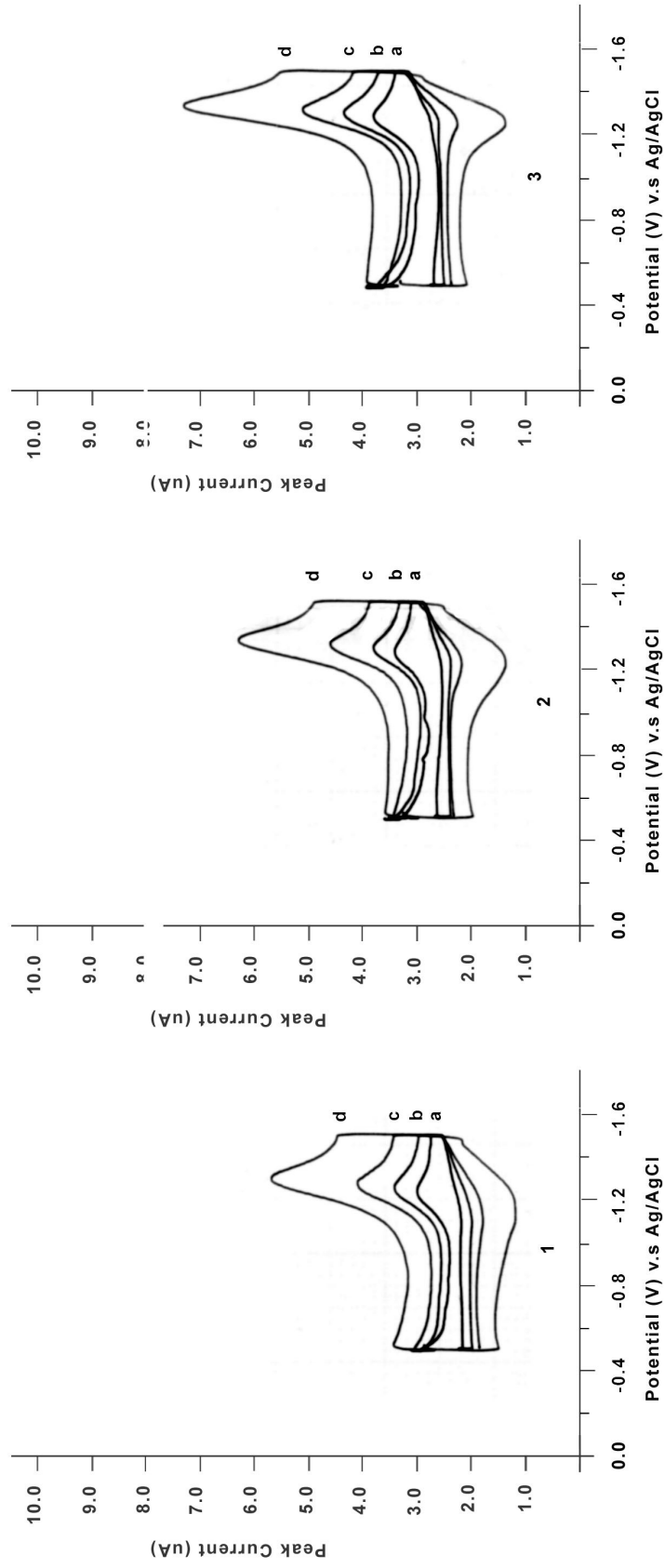


Figure (15), Repetitive cyclic voltammograms for three different concentrations of Prilocaine scanned from $(-0.5) - (-1.5)$ V, (1) 4.7×10^{-5} M Prilocaine, (2) 9.1×10^{-4} M Prilocaine, (3) 1.7×10^{-3} M Prilocaine, (a-d) scan rates 20, 50, 100, 200 mV/sec respectively, other conditions as listed in figure (11).

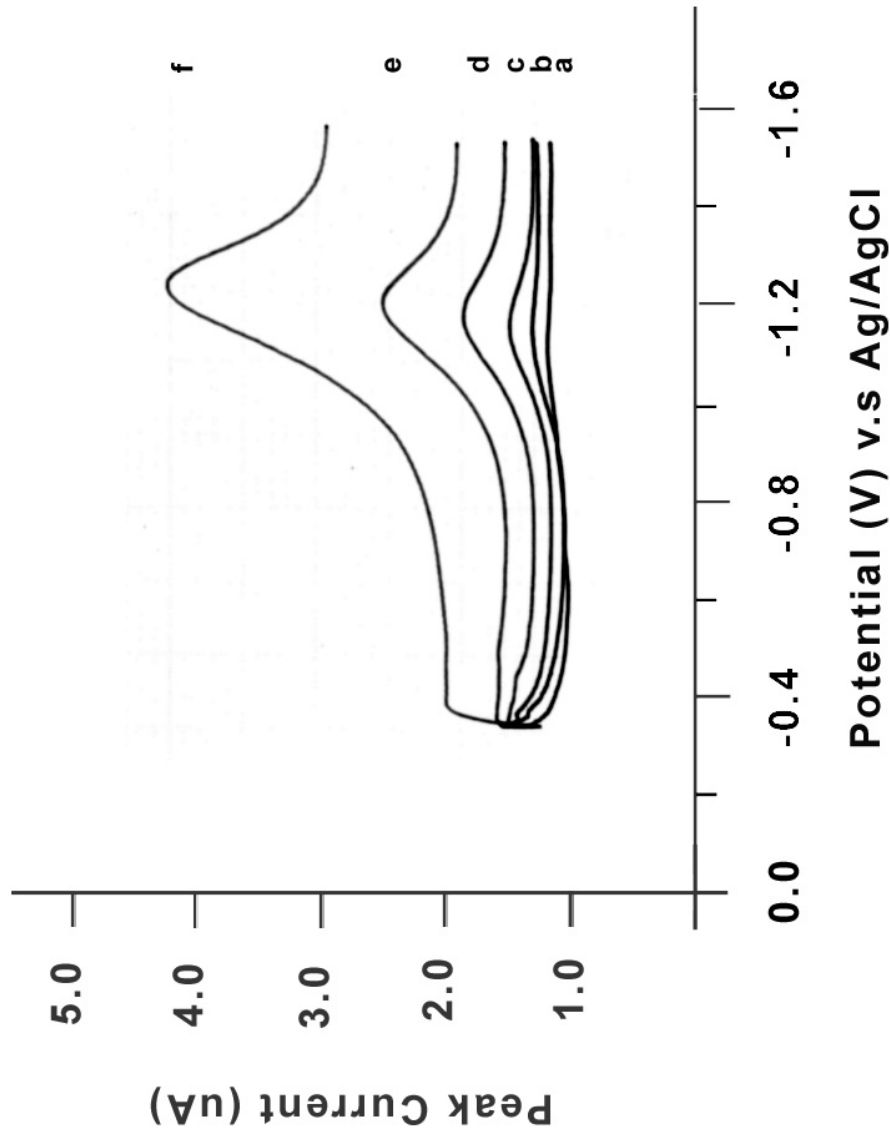


Figure (16), Effect of scan rate on the DC-AdCS voltammograms for 5.88×10^{-5} Prilocaine, (a-f) scan rates: 5, 10, 20, 50, 100, 500 mV/sec respectively, other conditions as listed in figure (11).

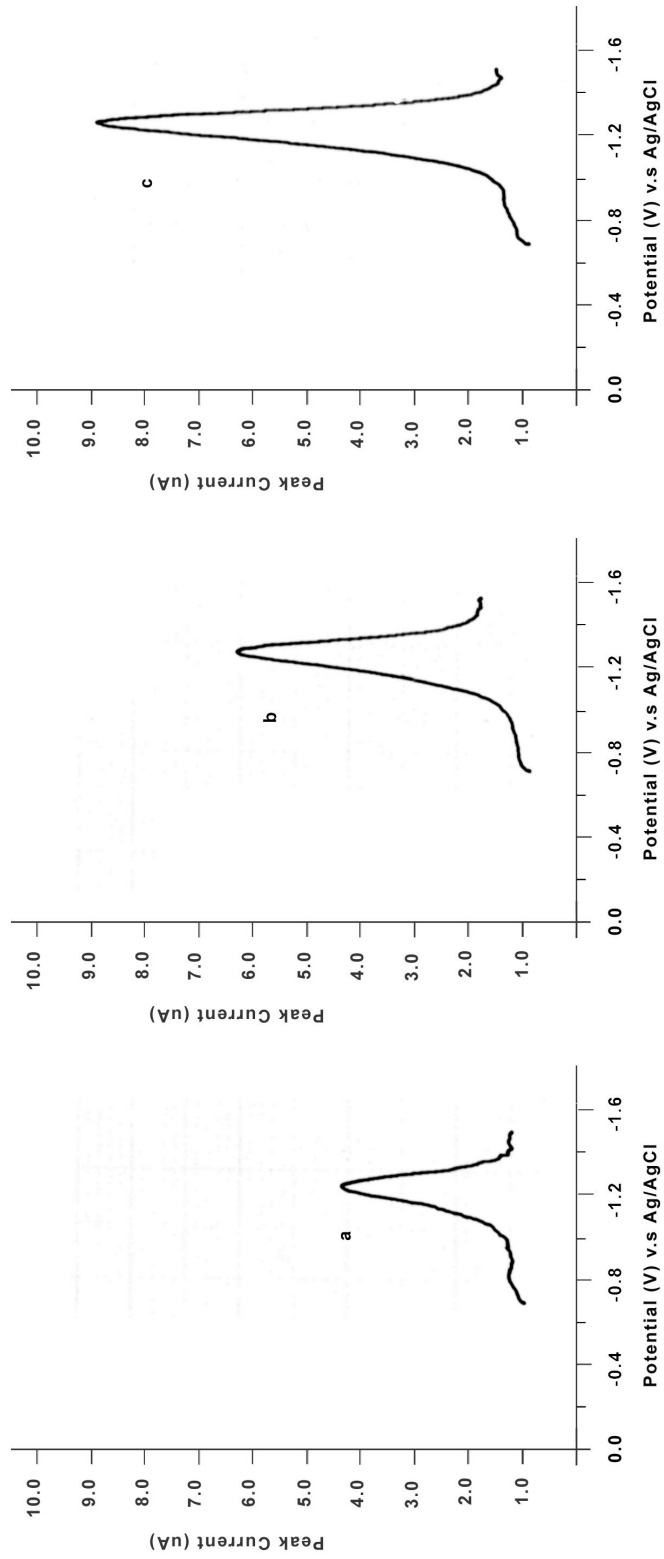


Figure (17), Effect of drop size on the DP-AdCS voltammograms for 5.88×10^{-5} Prilocaine, (a-c) drop size: small, medium, large respectively, other conditions as listed in figure (11).

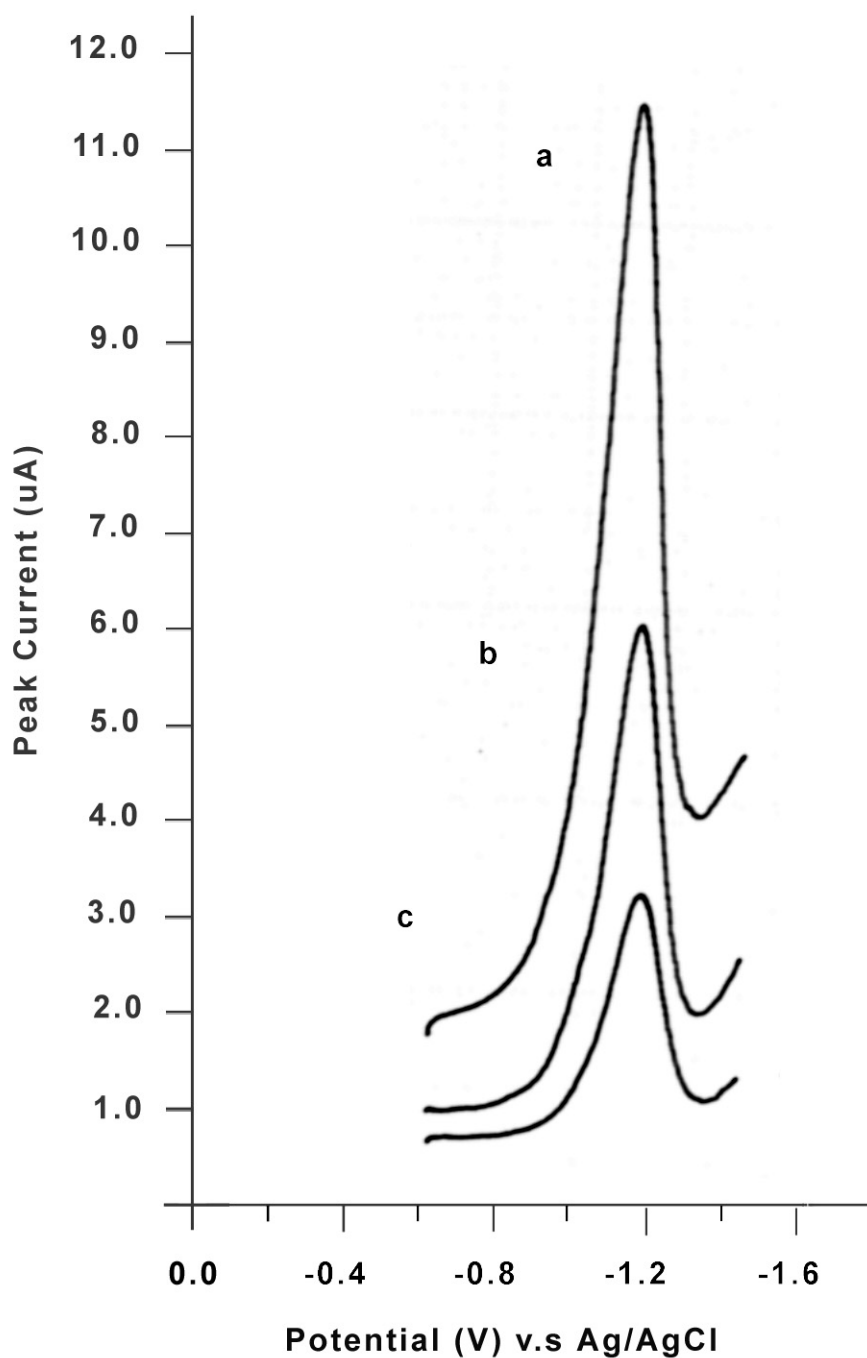


Figure (18), Effect of pulse amplitude on the DP-AdCS voltammograms for 5.88×10^{-5} Prilocaine , (a-c) pulse amplitude: 100, 50, 25 mv respectively, other conditions as listed in figure (11).

4.9 Calibration curve

The quantitative evaluation was based on the linear correlation between the peak current and Prilocaine concentration. The optimum conditions were found to be: BR buffer (pH=10.5), accumulation potential -0.7 V, accumulation time of 0, scan rate 20 mV/sec, pulse height 50 mV at 0.2 sec pulse interval and medium drop size.

The calibration graph was constructed by plotting the peak height vs different concentrations of Prilocaine, Figures (19) and (20) show the linear dependence was over the range 6.2×10^{-7} - 8.4×10^{-5} M (0.16 – 21.6 ppm) with detection limit equals 0.16 ppm, and RSD of 0.68%.

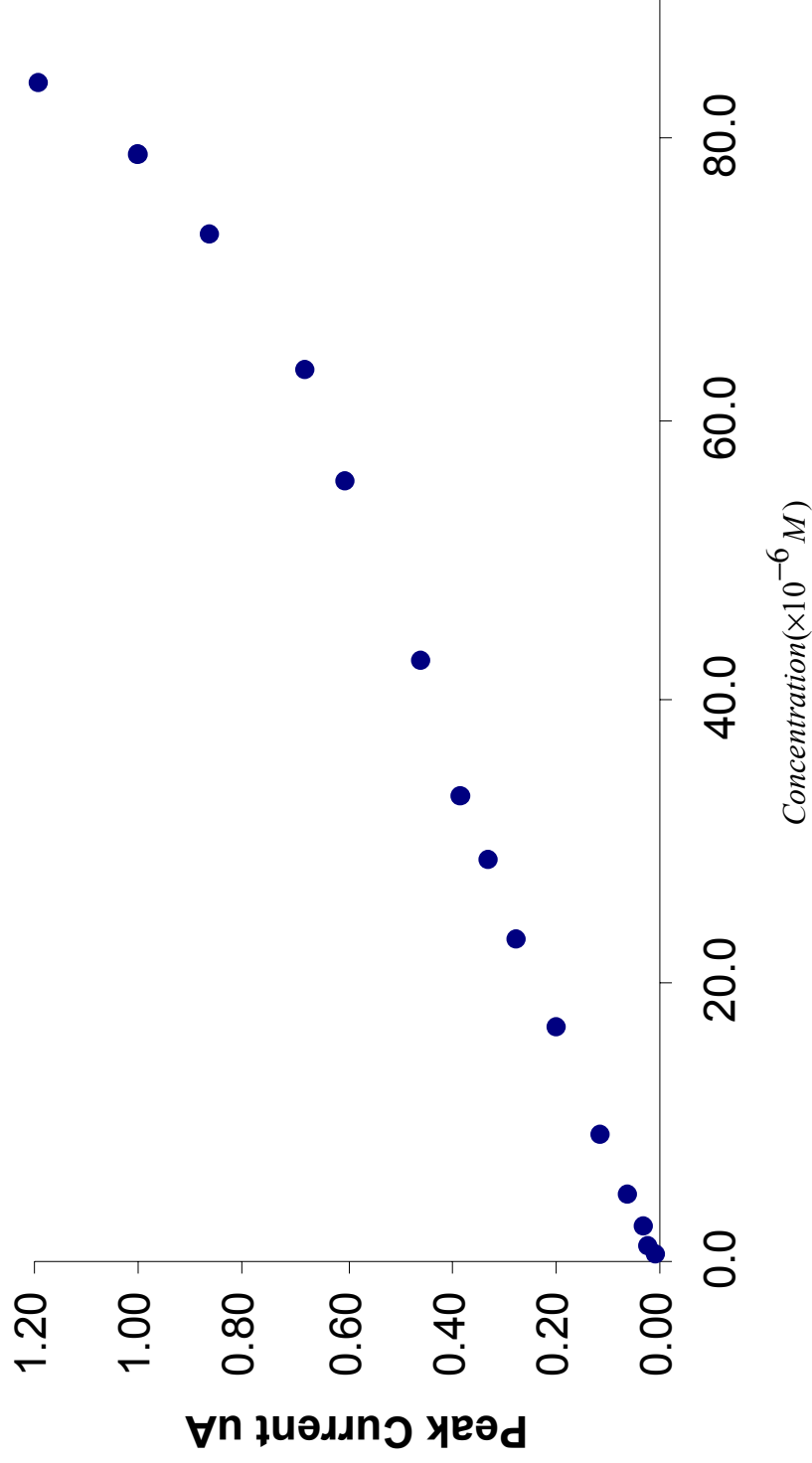


Figure (19), Calibration graph for DP-AdCSV of Prilocaine, pH = 10.5, drop size: medium, pulse amplitude=25mV at 0.2 sec, accumulation time = 0.0 sec, current = 2 μA , scan rate = 20 mV/sec. X set = 250 mV/in and Y set = 500 mV/cm.

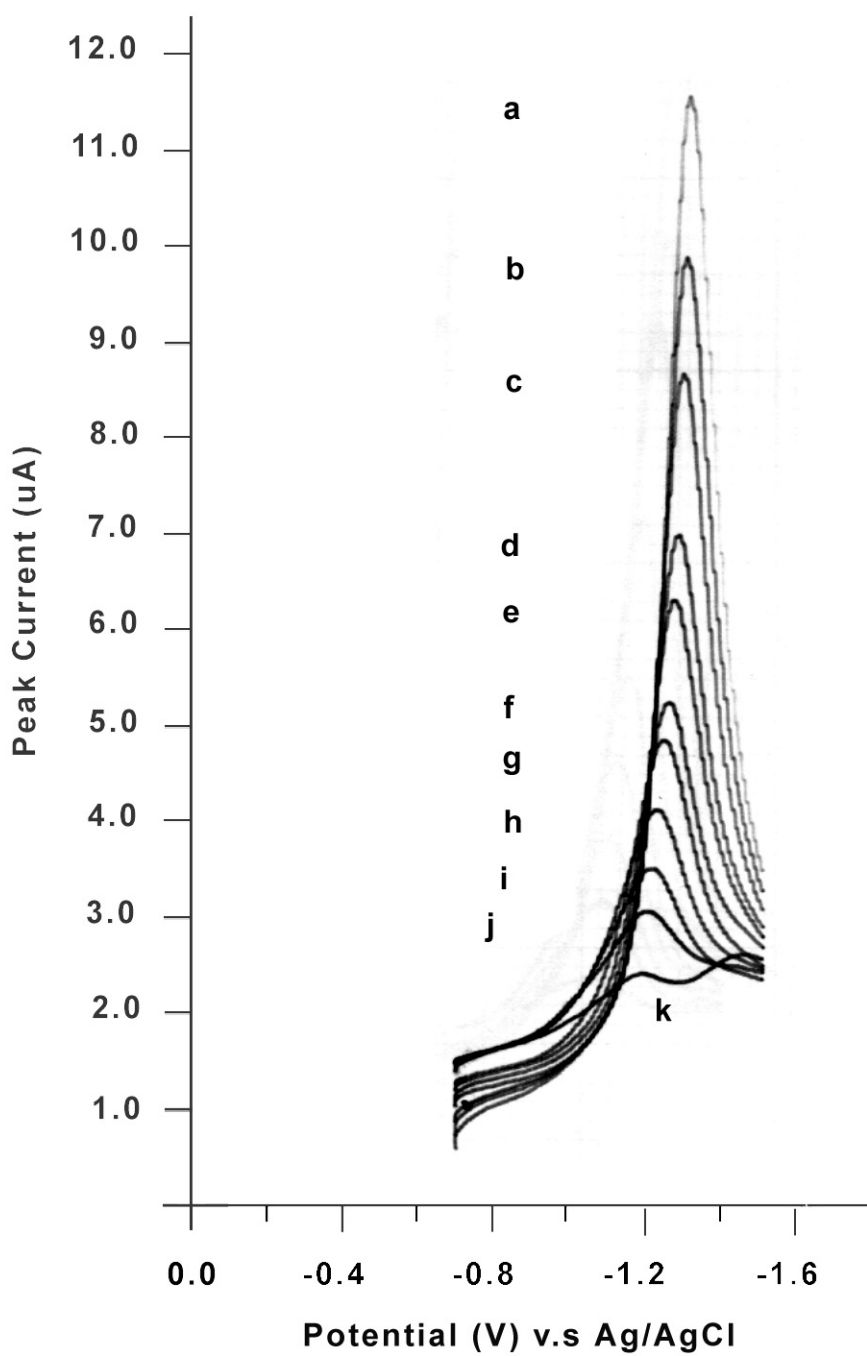


Figure (20), Calibration graph for DP-AdCSV of Prilocaine, (k): blank solution, (a-j) Prilocaine concentrations, other conditions as listed in figure (20).

4.10 Conclusion

Table (10), list for parameters affecting the electroanalytical method for the determination of Prilocaine, and the analytical results.. :

pH	10.5
Accumulation Potential	-0.7 V
Accumulation Time	0.0 s
Scan Rate	20 mV/sec
Drop size	Medium
Pulse Amplitude	50 mV
Conc. Range	$6.2 \times 10^{-7} - 8.4 \times 10^{-5}$ M
Detection Limit	6.2×10^{-7} M
RSD	0.68%

4.11 Analytical application

The validity of the proposed method was investigated for the determination of Prilocaine using the commercially available *EMLA*^R cream (Astra-France).

Exactly 1.0 gm of the cream was dissolved in distilled water, and the volume was completed to 100 ml, then the Prilocaine concentration was determined using the DP-AdCSV according to the general procedures using the pre constructed calibration curve. The determination repeated for five times, a final recovery of 98.1% was obtained, with an RSD of 0.56%.

A little shift in the potential towards positive direction was observed during the use of the commercial form compared with the standard material.

4.12 Comparison between the proposed methods with other published methods

Table (11) shows a comparison between the proposed methods and other published methods for the determination of Prilocaine. Beside the limitations of the reported methods described in section 1.6, the proposed methods show high sensitivity and reproducibility, less time consuming and do not require high sophisticated instrumentation and training.

Hence, the proposed methods are suitable for routine analysis for Prilocaine in pharmaceutical preparations.

4.13 Further Studies

This work aimed to setup analytical determination for Prilocaine, during the work, some questions and ideas have been rise up, and they are suitable for further studies.

These ideas include, the kinetics and mechanism of ion pair formation between the Prilocaine and both BTB and BCG dyes in acidic medium. And the chemistry of Prilocaine during the adsorption and stripping steps during the voltammetric determination in basic medium, noting that the unusual results obtained from studying the effect of accumulation time.

On the other hand, it's vital to study if these methods success with other local anesthetics and drugs, and also if they success in determining the Prilocaine in other mediums neither than pharmaceutical preparations.

Table (11), Comparison between the proposed methods and other published methods.

Researchers	Sample	Technique	Linear range	Detection Limit	RSD	Ref.
Watanabe et al	Blood	GC-MS	0.01 – 20 ppm	-	-	3
Grouls et al	Blood	HPLC	-	-	-	4
Siluveru and Stewart	Blood	Electrophoresis	0.045– 0.75 ppm	0.038 ppm	8.5%	5
Siluveru and Stewart	Blood	LC + UV	0.01 – 1 ppm	0.005 ppm	5.5%	6
Klein et al	Blood	HPLC	0.02 – 2 ppm	0.004 ppm	-	7
Koeppel et al	Blood	GC - MS	-	-	-	8
Bjork et al	Blood	GC	40 nM - 3 μ M	3 μ M		9
Whelpton et al	Blood	HPLC	0.005 – 0.5 ppm		0.9 – 15.7%	10
Adams et al	Blood	HPLC	-	0.03 ppm	2%	11
Prat, M. and Bruguerolle, B.	Blood	GC	-	-	-	12
Fernandez-Marcote and co-workers	Pharm. Preparations	DPP	0.1 mM – 1.0 M	2 μ M	1%	1
Proposed Method	Pharm. Preparations	UV-Visible	2.0 – 26.5 ppm	2.0 ppm	0.61%	
Proposed method	Pharm. Preparations	DP-AdCSV	0.16 – 21.6 ppm	0.16 ppm	0.68%	

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