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Electrochemical behavior of N-[[(1-methylethyl)amino]carbonyl]-4-[(3- methylphenyl)amino]-3-pyridinesulfonamide on Glassy carbon and Platinum electrodes in Protic media

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ABSTRACT

A voltammetric study of the oxidation and reduction of torasemide has been carried out at the glassy carbon and platinum electrode, respectively. The electrochemical behavior of torasemide was investigated by cyclic, differential pulse stripping and square wave stripping voltammetry using glassy carbon and platinum electrode. Different parameters were tested to optimize the conditions for the determination of torasemide. The dependence of intensities of currents and potentials on concentration, scan rate was investigated. For analytical purposes, a very well resolved diffusion controlled voltammetric peak was obtained in Phosphate and Acetate buffer at pH 4 and 6 for differential pulse and square wave voltammetric techniques, on glassy carbon and platinum electrode, respectively. Based on this study, simple, rapid, selective and sensitive two voltammetric methods were developed for the determination of the torasemide in tablet dosage form.

Keywords: torasemide, glassy carbon and platinum electrode, voltammetric study, differential and stripping study.

INTRODUCTION

Torasemide, N-[[(1-Methylethyl)amino]carbonyl]-4-[(30- methylphenyl)amino]-3-pyridinesulfonamide, is a lipophilic anilinopyridine sulphonylurea derivative with pharmacological properties of a high ceiling loop diuretic and Cl^- channel blocker. Its nearly complete bioavailability over a broad dose range from 2.5 up to 200 mg matches the requirements for the treatment of both acute and chronic congestive heart failure and hypertension.[1-6]

Compared to other loop diuretics, torasemide has a more prolonged diuretic effect than equipotent doses of furosemide and relatively decreased potassium-loss. There is no evidence of torasemide induced ototoxicity demonstrated in humans.[7]

Torasemide is more potent natriuretic and more potassium sparing than the most often used loop diuretic furosemide[8] . Therefore, its use is favored compared with other loop diuretics.

Diuretics have been one of the very much worked upon group of drugs and lot of study is available on them[9-17].Torasemide is studied extensively through various methods and techniques, such as determination of torasemide and its metabolites in biological fluids through HPLC with UV detection[18-20]. Electrochemical study of torasemide is also available but on HMDE and glassy carbon in universal Britton-Robinson buffer[21,22]. In this paper complete

electrochemical profile of torasemide on glassy carbon and platinum electrode is established in Phosphate and Acetate buffer in acidic pH.

MATERIALS AND METHODS

Apparatus

The voltammetric measurements were carried out on a CH Instruments, USA made (Model CHI 1230) Electrochemical Analyzer equipped with a 10ml single compartment three-electrode glass cell. These systems were connected to a processor. All experiments were carried out in three-electrode system. Glassy carbon electrode (Part No CHI 104) and platinum electrode (Part No CHI 102) were used as the working electrodes, a platinum wire as counter electrode and Ag/AgCl electrode as reference electrode. All experiments were carried out at room temperature of 25 ± 1ºC.

All the solutions examined by electrochemical technique were purged for 10 minutes with purified nitrogen gas, after which a continuous stream of nitrogen was passed over the solutions during the measurements.

All pH-metric measurements were made on a Decible DB-1011 digital pH meter fitted with a glass electrode and a saturated calomel electrode as reference, which was previously standardized with buffers of known pH.

Reagents and material

Pharmaceutical formulation Demadex tablets, labeled as 50 mg of torasemide content per tablet, were obtained from commercial sources. For the preparation of standard torasemide stock solution (1 mg/ml), 100 mg torasemide was accurately weighed, dissolved in methanol by shaking for 20 minutes. Then the solution was filtered. The filtrate was diluted with methanol to give the appropriate concentration. Standard working solutions were prepared by appropriate dilution of the stock solution. All other chemicals were of analytical reagent grade, supplied by Sigma or Merck. Double distilled water was used throughout.

Pretreatment of the working electrodes

The glassy carbon and platinum electrodes were polished with 0.5 μm alumina powder on a polishing cloth prior to each electrochemical measurement. Then, they were thoroughly rinsed with methanol and double distilled water and gently dried with a muslin cloth, until a mirror like was obtained.

Procedure

Known volumes of working standard solution of torasemide were pipetted into 10 ml volumetric flasks and then made up to volume with phosphate. The pH was adjusted at 4.0 and the solution was transferred into the voltammetric cell. Then purified nitrogen was passed to remove the dissolved oxygen under stirred conditions for 10 minutes in the first cycle and for 30 seconds for each successive cycle. The stirring was then stopped, and after a rest period, a cyclic voltammetry was initiated in the anodic direction, over the range of 0.4 to 1V on glassy carbon electrode vs Ag/AgCl/KCl reference electrode at room temperature.

Same procedure is followed for the platinum electrode where cyclic voltammetry was initiated in acetate buffer at pH 6, in the cathodic direction, over the range -0.2 to -0.8 V vs Ag/AgCl/KCl reference electrode at room temperature.

RESULTS AND DISCUSSION

Voltammetric behavior of torasemide on glassy carbon electrode

Torasemide species were readily adsorbed onto the glassy carbon electrode. Fig 1 displays cyclic voltammograms of 0.5 mM torasemide in phosphate buffer (0.1 M, pH 4) on a glassy carbon electrode for successive scan rates ranging from 50-800 Vs^{-1}. A large definite anodic peak, corresponding to the oxidation of the adsorbed drug is observed in potential range + 0.70 to +0.805 V. no peaks are observed in the cathodic branch, indicating that the torasemide oxidation is an irreversible process. The anodic peak may be attributed to the irreversible oxidation of the diarylamine moiety of torasemide molecule, in accordance with the redox mechanism

The peak potential shifted to the more positive potentials in the anodic direction when the scan rate (Fig 2) increased according to the following equation

$y\ (E_p) = 0.083(\log \upsilon) + 0.557,\ R^2 = 0.978$

Scan rate studies were then carried out to assess whether the processes on glassy carbon electrode were under diffusion or adsorption control. When the scan rate was varied a linear dependence of the intensity peak i_p upon the square root of the scan rate ($\upsilon^{1/2}$) (Fig 3)was found, demonstrating a diffusional behavior.

The equation representing the above said phenomenon

$i_p = 0.145\ \upsilon^{1/2} + 0.061,\ R^2 = 0.996$

A plot of logarithm of peak current versus logarithm of scan rate (Fig 4)gave a straight line with a slope of 0.516 very close to the theoretical value of 0.5, which is expressed for an ideal reaction for the diffusion controlled electrode process [23]

Value of αn was deduced from slope of graph between log of scan rate vs potential, and the value obtained is 0.7116, and thus, no of electrons involved in the process can be calculated, for α = 0.4, as 2. The diffusion coefficient is found to be 1.16×10^{-2} cm^2s^{-1} for this system from equation

$i_{pc} = (2{\cdot}99 \times 105)\ n\ \alpha^{1/2}\ A\ C_0^*\ D_0^{1/2}\ \upsilon^{1/2}$,

where, n is the number of electrons exchanged during reduction, α is electron transfer coefficient, A is apparent surface area of the electrode (cm^2), and C_0^* is the concentration of the electroactive species (mMol dm^{-3}).

These values together with the absence of cathodic wave in cyclic voltammetry indicated the irreversibility of the oxidation reaction of torasemide.

Voltammetric behavior of torasemide on platinum electrode

Voltammetric study of this drug is not available on platinum electrode. On platinum electrode, 0.5 mM solution of the drug in acetate buffer (0.1 M, pH 6) show single cathodic peak in the range 0.57 to -0.656 V, corresponding to reduction of the drug. No anodic peak appears, thus, pointing to the irreversible nature of the drug reduction.

The reduction peak can be related to the reduction of the sulfonylamide group of all the other functional groups present in the torasemide molecule.

Linearity between scan rate and peak potential was observed till scan rate 500 Vs^{-1}, (Fig 1a), with the shifting of peak potential towards more negative value. Plot between logarithm of scan rate and peak potential (Fig 2a) gives a straight line with a slope of 0.72, and thus, no of electrons can be calculated using α = 0.4 as 2. The following equation represents the relation

$y\ (E_p) = 0.0844(\log \upsilon) + 0.428,\ R^2 = 0.993$

As for glassy carbon when the scan rate was varied, on platinum electrode, a linear dependence of the intensity peak i_p upon the square root of the scan rate ($\upsilon^{1/2}$) (Fig 3a) was found, demonstrating a diffusional behavior.

$i_p = 0.059\ \upsilon^{1/2} + 0.294,\ R^2 = 0.974$

Plot between logarithm of peak current versus logarithm of scan rate (Fig 4a) shows straight line with slope of 0.38, much close to the theoretical value of 0.5 for diffusion controlled process.

The diffusion coefficient is found to be 3.9×10^{-2} cm^2s^{-1} for this system from equation

$i_{pc} = (2{\cdot}99 \times 105)\ n\ \alpha^{1/2}\ A\ C_0^*\ D_0^{1/2}\ \upsilon^{1/2}$,

where, n is the number of electrons exchanged during reduction, α is electron transfer coefficient, A is apparent surface area of the electrode (cm^2), and C_0* is the concentration of the electroactive species (mMol dm^{-3}).

These values together with the absence of anodic wave in cyclic voltammetry indicated the irreversibility of the reduction reaction of torasemide.

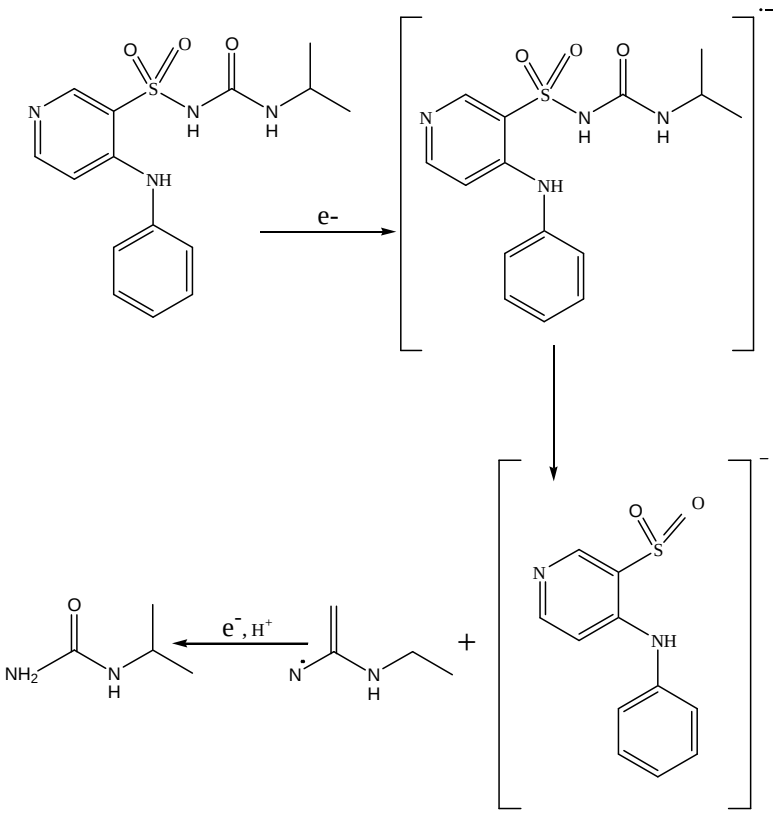
Electrode reaction

On glassy carbon electrode

Structure of torasemide clearly shows that the most susceptible oxidizable group of all the functional groups is diarylamine. Diarylamines oxidize through formation of radical cation by the loss of one electron . Since nitrogen here is electron deficient thus, loses proton and forming radical. This radical structure is much stable than the original structure due to delocalization of the radical charge on the two benzene rings along with oxygens of the sulfonyl group conjugated with pyridine ring. The evolution of the oxidation process could be a multiple process, taking into account the different functional groups present in the molecule. Hence intramolecular processes in addition to coupling reactions known as tail-tail or para-para coupling, characteristic of diarylamines, can be produced. [21]

On platinum electrode

Pyridine and sulfonamide are two potential reducible groups present in torasemide. Reduction of pyridine is too cathodic for aqueous media thus; its reduction cannot take place. The electrochemical reduction of arylsulfonamides leads to cleavage of the S-N bond and involves two electrons Reduction follows through homolytic cleavage of sulfur-nitrogen bond leading to the formation of two radicals [22,24,25]. The reduction occurs as



Validation of the proposed techniques

For the quantitative determination of torasemide two techniques, namely differential pulse and square wave stripping voltammetry were developed. Both of these techniques are effective and rapid electroanalytical techniques

with well established advantages, including good discrimination against background currents and low determination limits.

Validation of the optimized procedure for the quantitative assay of torasemide was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility, accuracy, precision and recovery.

Linearity
The applicability of the DPSV and SWSV (Fig 5, 5a and Fig 7, 7a) procedures as analytical methods for the determination of torasemide was examined by measuring the stripping peak current as a function of the bulk drug at least three times under the operational parameters (Table 1)

For glassy carbon electrode
The calibration plots (Fig 6, 6a and Fig 8, 8a) showed that there was linear dependence of the peak current and peak area ratio on the concentration in both DPSV and SWSV. Linear regression equation being expressed as

For DPSV

$i_{pc} = 1.198C + 1.712,\ R^2 = 0.978$

For SWSV

$i_{pc} = 1.253C + 6.109,\ R^2 = 0.986$

For platinum electrode

Linear equation for platinum electrode

For DPSV

$i_{pc} = 2.211C + 0.147,\ R^2 = 0.985$

For SWSV

$i_{pc} = 0.941C + 6.407,\ R^2 = 0.972$

Here also linearity is followed by peak area ratio and peak current on the concentration of drug.

Specificity
The specificity was evaluated in the presence of various substances that are usually found in the pharmaceutical tablets and formulations. For these investigations, interfering species, such as, sucrose, lactose, cellulose, starch, magnesium stearate, were added at different concentrations. None of them cause any significant effects on the activity of the drug, thus, these analytical studies can be considered specific.

Sensitivity and detection limit
Detection limit was calculated using equation,

$LOD = 3S_a/b$

Where, S_a is the standard deviation of intercept and b is the slope of the regression line.

Values of LOD for both glassy carbon and platinum electrodes, for both the stripping techniques are summarized in table 2 and 3, respectively.

Quantitation limits are calculated using equation

LOQ = $10S_a/b$

Where, S_a is the standard deviation of intercept and b is the slope of the regression line.

Values are summarized in table 2 and 3.

Accuracy, precision and stability
Accuracy and the precision of the proposed method was also evaluated by recovery studies after adding known amounts of the pure drug to phosphate and acetate buffer solutions and analyzed through the proposed stripping voltammetric procedure, repeated for five times. The values of mean recovery obtained by the standard addition method, with relative standard deviation (RSD) are given in table 2 and 3.

Standard solution of torasemide was stable for 24 hrs at room temperature, studied though stripping study.

Table 1: The optimized experimental conditions of the proposed procedure for the determination of torasemide

	Optimized Value	
	Glassy carbon electrode	Platinum electrode
pH	4.0	6.0
Buffer Type	Phosphate Buffer	Acetate Buffer
Strength of the buffer (M)	0.1	0.1
Temperature (°C)	23-27	23-27
Initial potential (V)	0.40	-0.20
Final potential (V)	1.0	0.80
Scan increment (V)	0.004	0.004
Pulse amplitude (V)	0.025	0.025
Frequency (Hz)	15	15
Deposition time (s)	15	15

Table 2: Regression data of the calibration lines for quantitative determination of torasemide by DPSV and SWSV
For glassy carbon electrode

Parameters	Method	
	DPSV	SWSV
Concentration Range (M)	5×10^{-7}-1.3×10^{-6}	5×10^{-7}-1.3×10^{-6}
Slope	1.1985	1.2537
Intercept	1.7121	6.1099
Correlation Coefficient	0.978	0.986
SD	1.05	0.094
LOQ (M)	8.76×10^{-6}	7.49×10^{-7}
LOD (M)	2.62×10^{-6}	2.2×10^{-7}
Average Recovery (%)	99.56	100.14
R.S.D. (%)	0.68	0.15

Table 3: Regression data of the calibration lines for quantitativedetermination of torasemide by DPSV and SWSV
For platinum electrode

Parameters	Method	
	DPSV	SWSV
Concentration Range (M)	5×10^{-7}-1.3×10^{-6}	5×10^{-7}-1.3×10^{-6}
Slope	2.211	0.941
Intercept	0.147	6.407
Correlation Coefficient	0.985	0.972
SD	1.40	0.06
LOQ (M)	6.32×10^{-6}	6.37×10^{-7}
LOD (M)	1.89×10^{-6}	1.91×10^{-7}
Average Recovery (%)	100.36	100.17
R.S.D. (%)	0.457	0.523

FOR GLASSY CARBON ELECTRODE

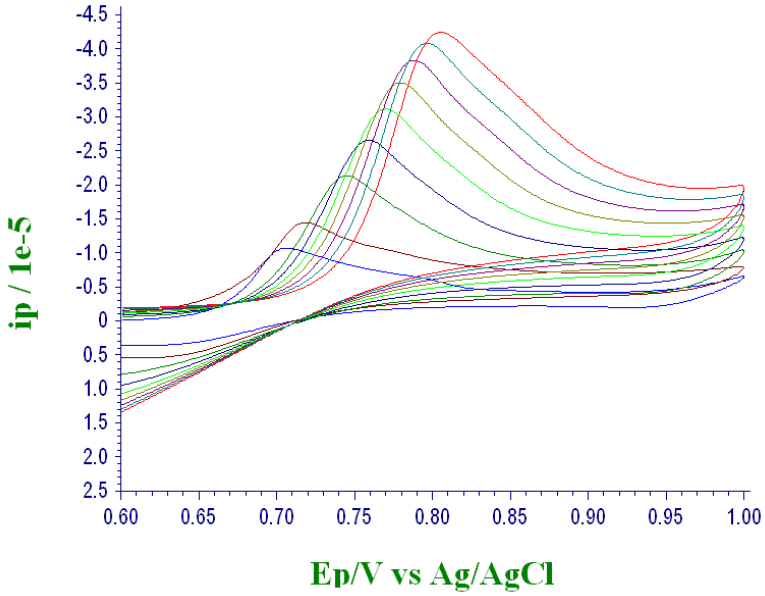


Fig 1: Cyclic voltammograms of 1.0x10-3 M torasemide at different scan rates (100, 200, 300,400, 500, 600, 700 and 800 mV s-1) using GC Electrode

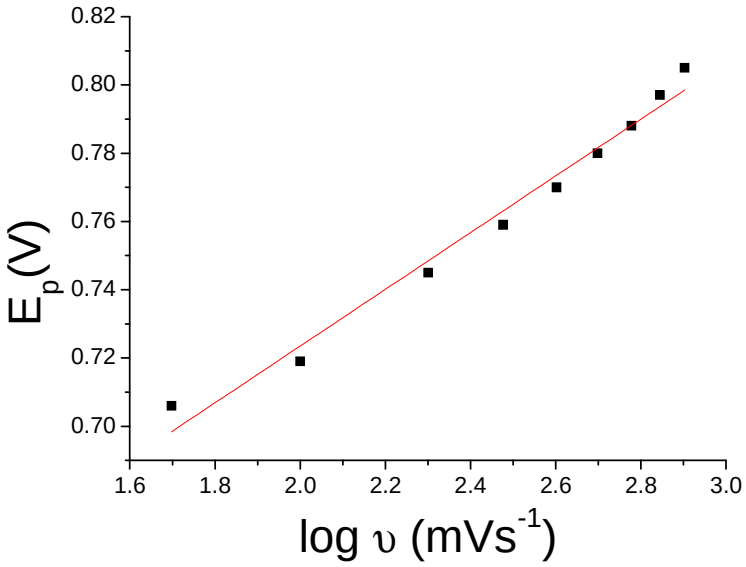


Fig 2: Plot of anodic peak potential (E_{pa}) as a function of log (υ)

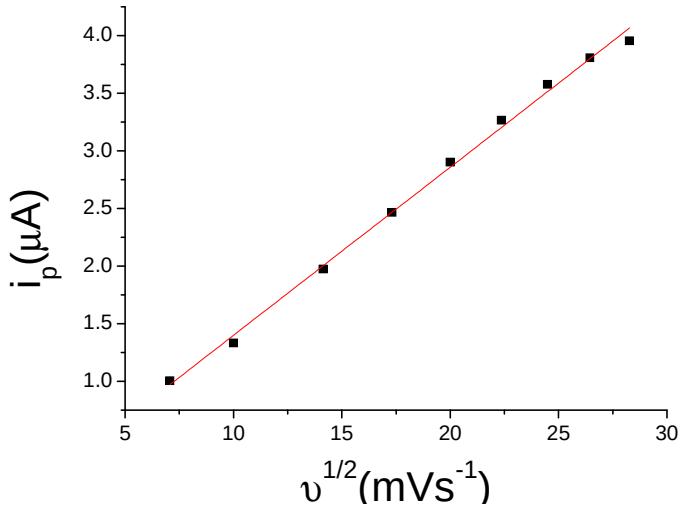


Fig 3: Plot of anodic peak current (i_{pa}) as a function of $\upsilon^{1/2}$

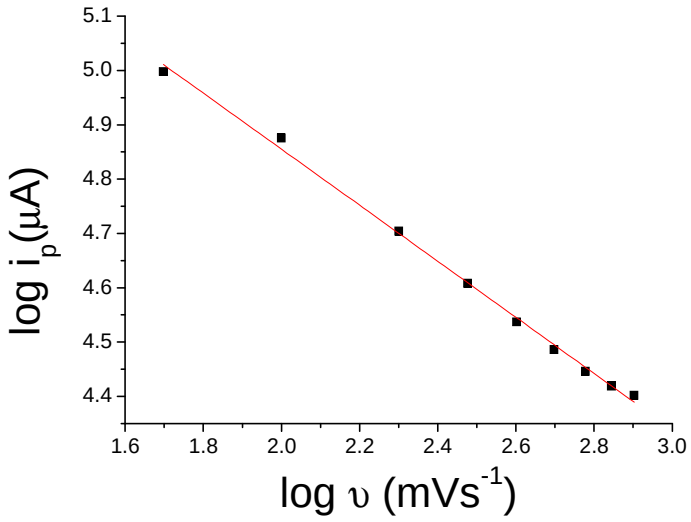


Fig 4: Plot of log (i_{pc}) as a function of log (υ) for 5×10^{-4} M Torasemide

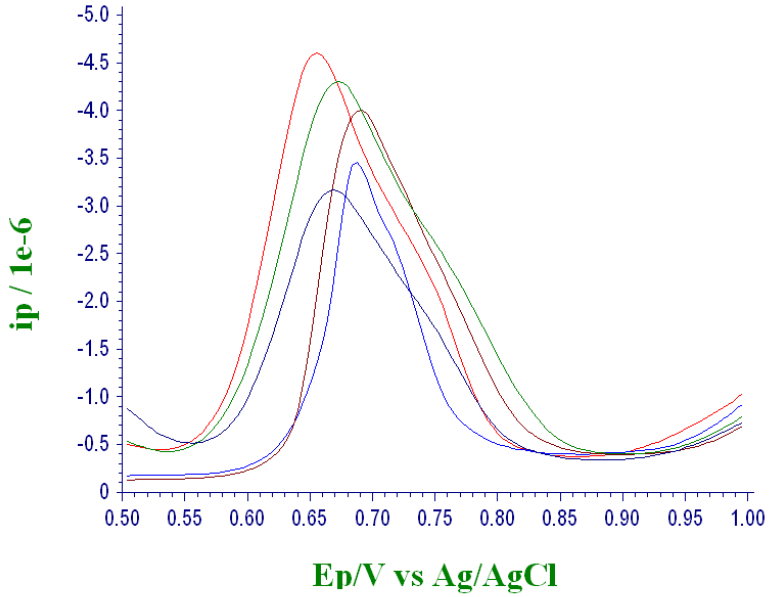


Fig 5: The DPSV for torasemide at different concentrations

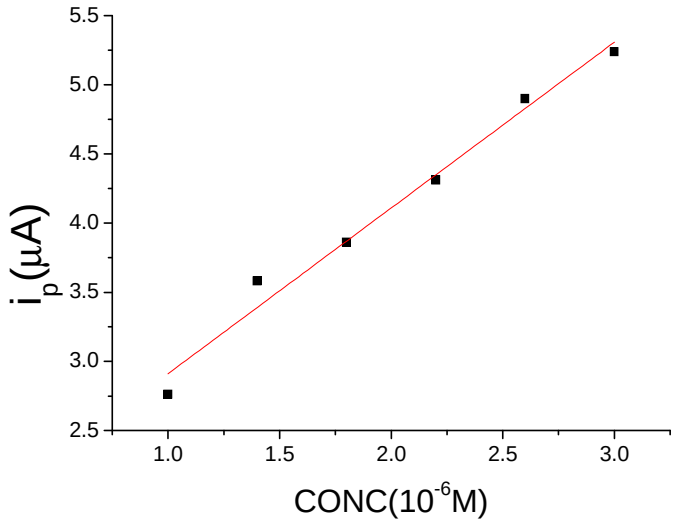


Fig 6: The dependence of the DPSV current for Torasemide at different concentrations

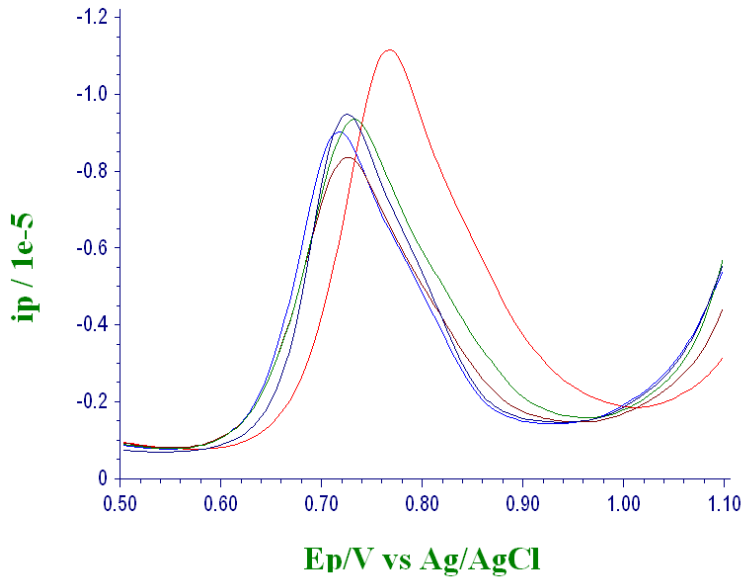


Fig 7: The SWSV for Torasemide at different concentrations

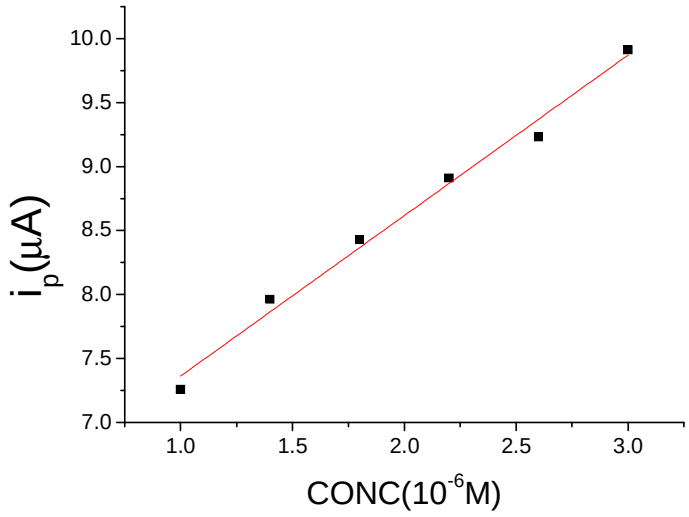


Fig 8: The dependence of the SWSV current for Torasemide at different concentrations

FOR PLATINUM ELECTRODE

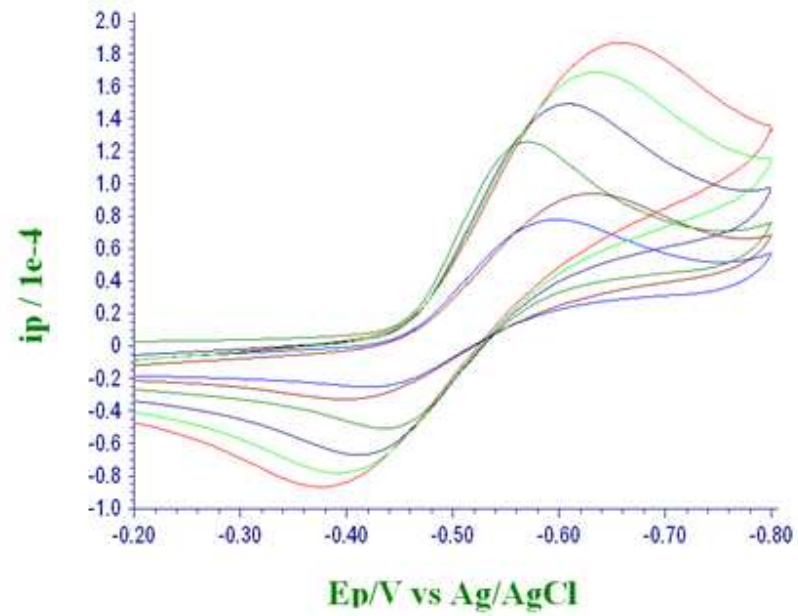


Fig 1a: Cyclic voltammograms of 1.0x10-3 M Torasemide at different scan rates (50, 100, 200, 300 and 400 mV s-1) using GC Electrode

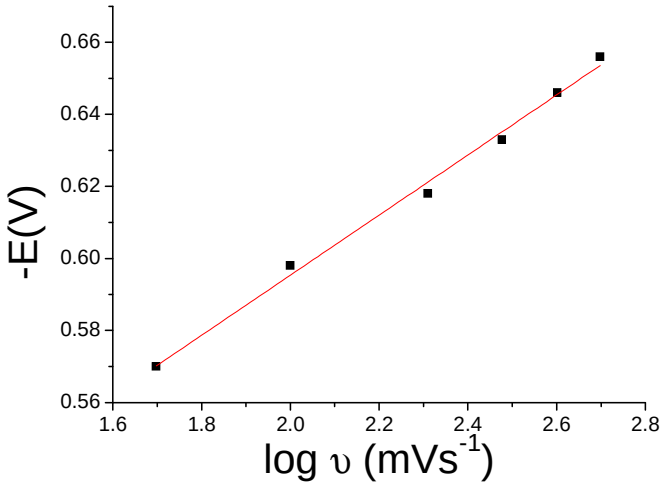


Fig 2a: Plot of cathodic peak potential (E_{pc}) as a function of log (υ)

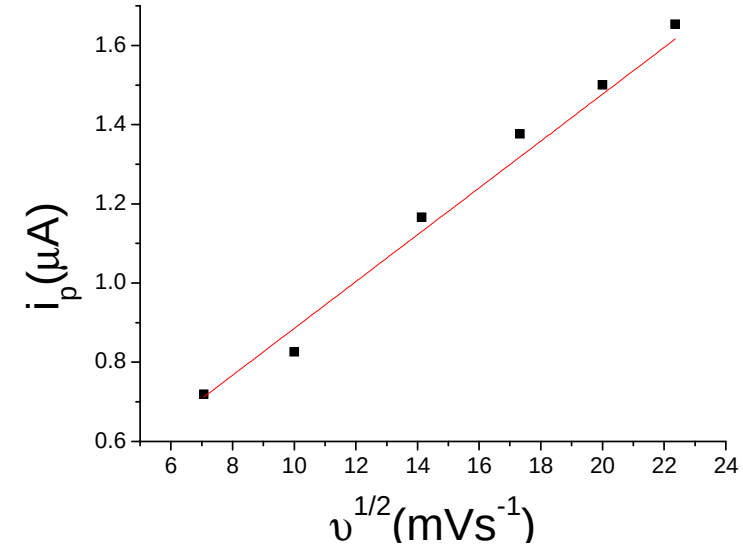
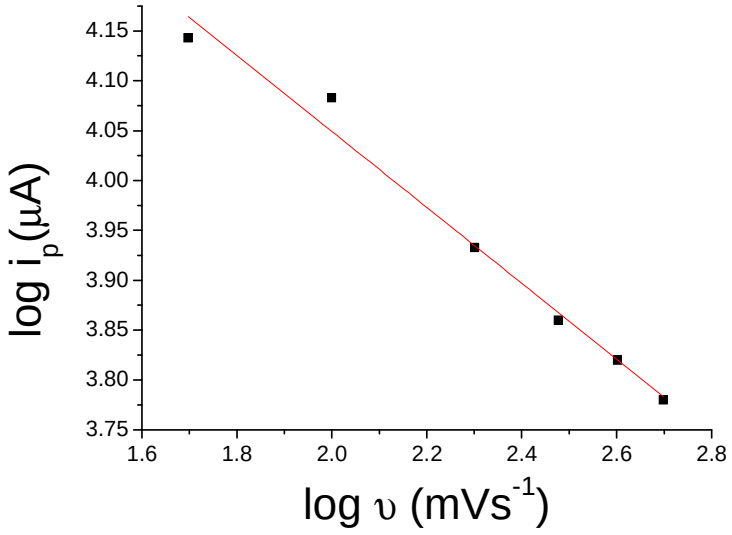


Fig 3a: Plot of cathodic peak current (i_{pc}) as a function of $\upsilon^{1/2}$



4a: Plot of log (i_{pc}) as a function of log (υ) for 5 × 10^{-4} M Torasemide

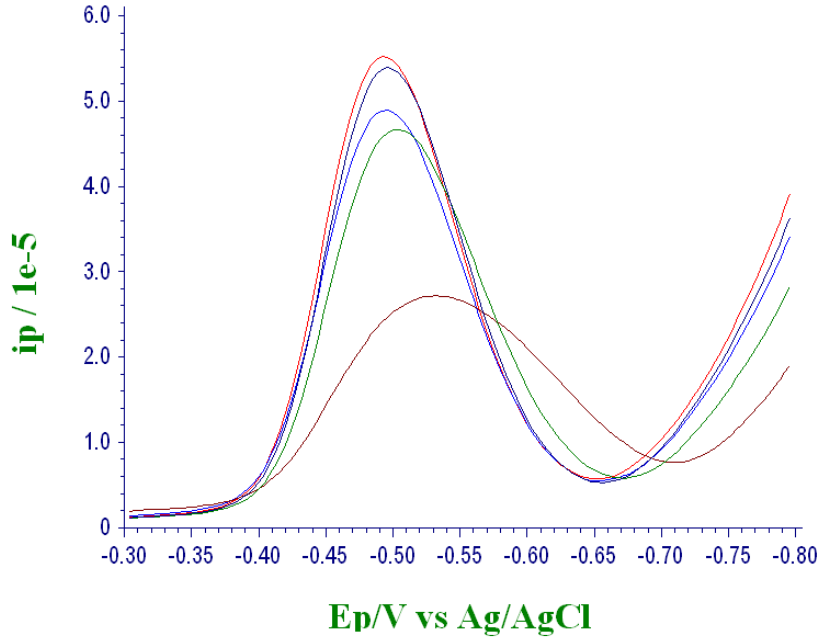


Fig 5a: The DPSV for Torasemide at different concentrations

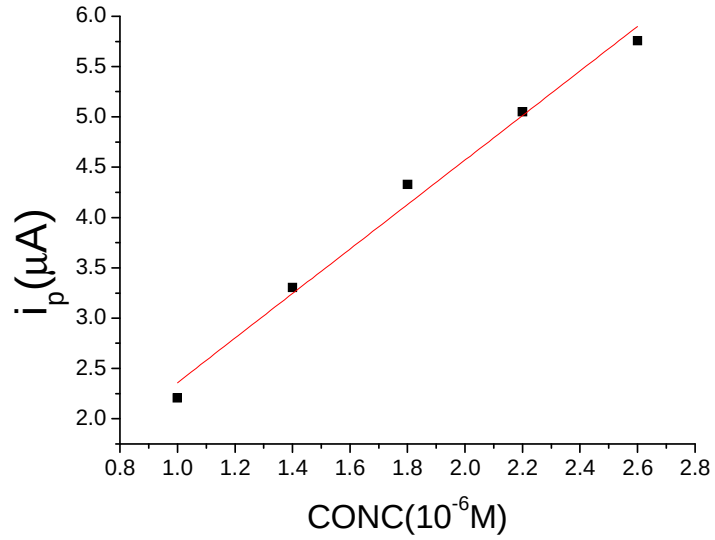


Fig 6a: The dependence of the DPSV current for Torasemide at different concentrations

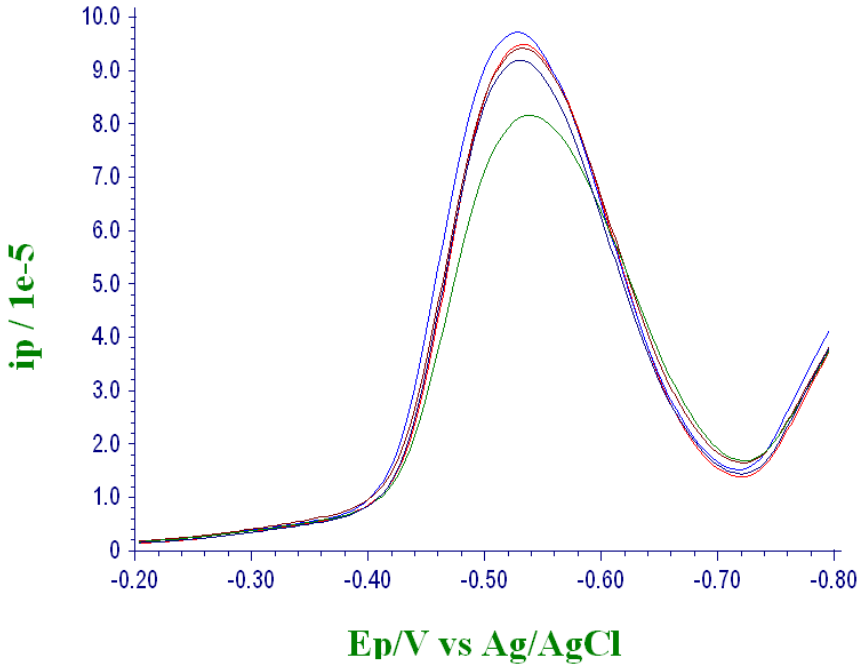


Fig 7a: The SWSV for Torasemide at different concentrations

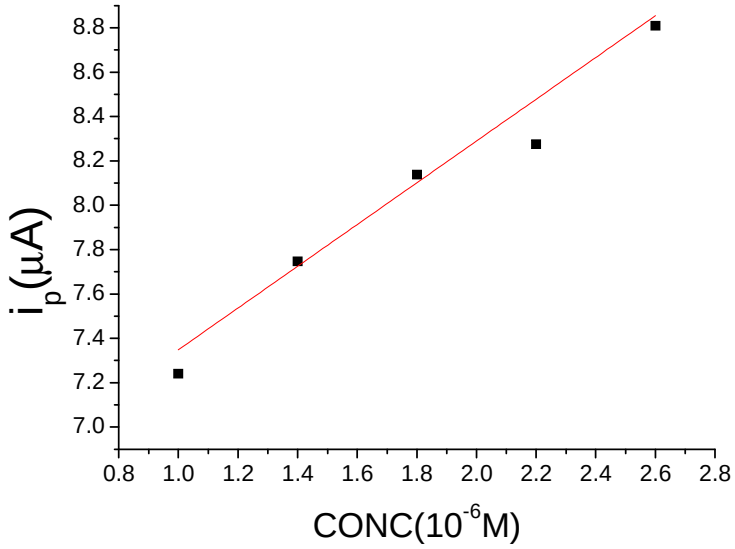


Fig 8a: The dependence of the SWSV current for Torasemide at different concentrations

CONCLUSION

Electrochemical behavior of the torasemide on glassy carbon and platinum electrodes was established and studied in phosphate and acetate buffers, respectively, for the first time. Torasemide was oxidized on glassy carbon while reduced on the platinum electrode, both involving two electron process.

The sensitive and practical DPSV and SWSV voltammetric procedure for determination of torasemide is described. The principal advantages of DPSV and SWSV over the other techniques are that they may be applied directly to the

analysis of pharmaceutical dosage forms without the need for extensive sample preparation, since there was no interference from the excipients and endogenous substances. The proposed methods are rapid, requiring less than 5 mins to run a sample and do not include time consuming extraction steps.

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