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Assay of Nimodipine - an Anti Hypertensive drug, in Bulk Form and Pharmaceutical Formulations by Cathodic Adsorptive Stripping Voltammetry

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Adsorpitive Stripping square-wave voltammetry (SWV) and differential-pulse voltammetry (DPV) methods were developed, evaluated, and compared for the assay of nimodipine in pharmaceutical formulations. The proposed method allows determination of nimodipine in linear concentration range 2 x 10^{-5} to 2 x 10^{-6} mol L^{-1}. The Limit of detection (LOD) and limit of quantification (LOQ) were noticed to be 7.11 ng/mL and 32.92 ng/mL respectively. The procedure was applied to the assay of the drug in tablets form with mean percentage recoveries of 100.1% with SWCAdSV and 99.99% with DPCAdSV. Precision and accuracy were also checked and were within the limits.

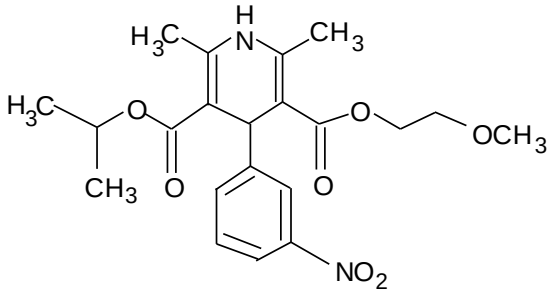
Keywords: Nimodipine, cathodic adsorptive striping voltammetry, pharmaceutical formulations, cyclic voltammetry.

1. INTRODUCTION

Nimodipine is a cardio selective drug used in the treatment of hypertension. It has been the most widely used calcium channel antagonist in the treatment of acute cerebral ischemia. The rationale for its use in acute focal cerebral ischemia relates to its ability to produce selective cerebral vasodilatation and block calcium entry into neurons by a direct action on L-type voltage-sensitive calcium channels (VSCCs) [1, 2]. Its efficacy in improving neurological outcome has been

demonstrated in animal models of focal cerebral ischemia [3-10], and this may be due primarily to improved cerebral blood flow (CBF) or to a direct cytoprotective effect.

Chemically Nimodipine is [1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridinedicarboxylic acid 2- methoxyethyl 1-methylethyl ether] (A).



(A)

For clinical investigations such as pharmacokinetic studies, development of sensitive and selective analytical methods for the determination of drugs in biological fluids is required. A thorough literature search has revealed that analytical methods have been used including spectrophotometry [11, 12], spectrofluorometry [13], gas chromatography, thin layer chromatography, high performance liquid chromatography [14], for the determination of nimodipine in pharmaceutical preparations and in biological fluids. The reported methods for quantitation of nimodipine are all expensive and time consuming. Electroanalytical techniques have been very efficient for the determination of various species [15-75]. The proposed method does not require sample pretreatment, time-consuming extraction prior to the analysis, and expensive reagents and equipment which makes the method of choice for routine pharmaceutical analysis. Although adsorptive stripping voltammetry is a simple and extremely sensitive technique [76-96], it has not been used to date for assay of nimodipine.

In the present paper a simple, sensitive and inexpensive procedure for the determination of nimodipine based on cathodic stripping voltammetry is reported.

2. MATERIALS AND METHODS

2.1. Instrumentation

Voltammetric measurements were performed with a Metrohm Computrace Voltammetric Analyzer μ AUTOLAB TYPE III Potentiostat Ecochemie (Utrecht, Netherlands). A conventional three-electrode system was used consisting of an Ag/AgCl reference electrode, a hanging mercury drop electrode (HMDE) as a working electrode and a graphite rod as auxillary electrode. The whole measurements were automated and controlled through the programming capacity of the apparatus. The data were treated through a PC connected to the Electrochemical Analyzer version-757 VA computerizes. Controlled potential coulometric experiments were performed using an Autolab Potentiostat/Galvanostat PGSTAT Metrohm. Coulometric experiments were performed in the

potentiostatic mode using Pt foil with large surface area as working electrode and a Pt wire, counter electrode.

All the solutions examined by electrochemical technique were purged for 10 min. 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 in acidic and alkaline medium.

2.2. Procedure

Nimodipine (99% pure) was provided by USV Pharmaceutical Ltd., Mumbai (India). Tablets containing nimodipine labeled 30 mg were obtained from commercial sources. KCl (1 mol L^{-1}) solution was prepared in distilled water and used as supporting electrolyte. The mass of ten tablets was determined and finely powdered, and then the required amount of sample to prepare a stock solution of 1 x 10^{-3} M nimodipine in dioxane was transferred into a 50 mL of standard flasks. After that 40 mL of dioxane was added separately to each flask to dissolve the active material. The contents of flasks were stirred magnetically for 30 min. and then diluted to volume with same solvent. After dilution the solution were centrifuged. An aliquot of the supernatant liquid was then transferred into a 10 mL of calibrated flasks and a series of dilutions were prepared with phosphate buffers in pH range 2.5 to 12.0 and mixed 1.0 mL potassium chloride as supporting electrolyte. The contents of the drug in pharmaceutical formulation were determined using calibration graph.

3. RESULTS AND DISCUSSION

The electrochemical behaviour of nimodipine was studied by cyclic voltammetry, differential pulse voltammetry and cathodic adsorptive stripping voltammetric techniques on HMDE. In all electrochemical methods nimodipine gave one well defined reduction peak in acidic medium, which is attributed to the reduction of $–NO_2$ group at HMDE.

3.1. Cyclic voltammetric behaviour

The reversibility of the reduction process was investigated by using cyclic voltammetry. The cyclic voltammograms of nimodipine with dioxane (2 x 10^{-6} mol L^{-1}) in phosphate buffers (pH 2.5 – 10) at the hanging mercury drop electrode (HMDE) exhibits a single well defined peak in the potential range -0.69 to -0.72 V, at all concentrations due to the reduction of $–NO_2$ group. The peak potential shifted to a more negative value on the increase of the scan rate, confirming the irreversible nature of the reduction process. For a totally irreversible electrode reaction the relationship between the peak potential (Ep) and the scan rate (ν) is expressed as $Ep = (2.303\ RT\alpha_nF) \log (RT/\alpha_nF) – (2.303\ RT\alpha_nF)$

log ν. A straight line is observed when Ep is plotted against log ν at a particular concentration in pH 3.2 can be expressed by the equation:

$$y\ (E_p) = 0.0296(\log \nu) + 0.6448\ (V), \quad r^2 = 0.9921$$

From the slope of the straight line ($\Delta E/ \log\nu$), the α_n value is calculated by using the expression $\Delta E/ \log\nu = -30/\alpha_n$. The α_n value is found 1.6. Fractional α values confirm the irreversible reduction of nimodipine.

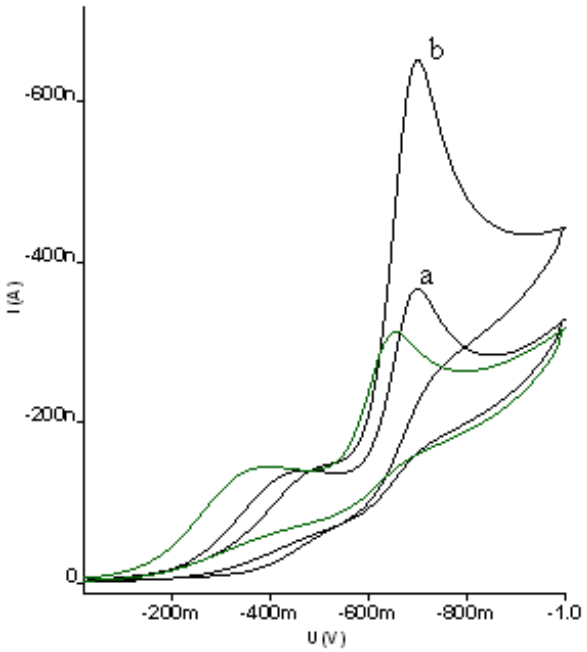


Figure 1. Cyclic voltammograms for concentration 2.5 x 10^{-4} mol L^{-1} nimodipine in phosphate buffer (pH 3.2) containing dioxane at a scan rate of 100 mV s^{-1}; (a) without accumulation; (b) after a preconcentration step, t_{acc} = 40 s, E_{acc} = -0.5 V and stirring speed 2000 rpm.

For finding the adsorptive character of the drug at HMDE a cyclic voltammogram (fig.1, curve b) was recorded after 40 s preconcentration at -0.5 V and with zero second (fig.1, curve a) preconcentration time. The peak current (ip) increases after preconcentration of the drug on the electrode surface for 40 s.

According to the Randles-Sevick equation, in a linear diffusion-controlled process, $i_p\ \alpha\ \upsilon^{1/2}$ and for the adsorptive process, $i_p\ \alpha\ \upsilon$. The peak currents of nimodipine are plotted against the scan rate. The peak current i_p increases linearly with increasing scan rate υ. This points out to the adsorptive nature of the peak. A linear relationship was obtained when i_p is plotted against υ, which may be expressed by the equation:

$$y(i_p) = 0.0172\ \upsilon\ (mV/s) + 4.0272\ (\mu A), \quad r^2 = 0.9921$$

3.2. Effect of pH

For controlling pH various buffer systems such as Britton Robinson buffer, acetate buffer, borate buffer, citrate buffer and phosphate buffer were used. The best results with respect to sensitivity accompanied with sharper response were obtained with phosphate buffer. Thus study was made in the pH range 2.5-12 in phosphate buffers at a target concentration of $1.5x10^{-5}$ mol L^{-1} aqueous nimodipine solution. CV, SWCAdSV, DPCAdSV records show one reduction wave with half wave potential -0.677 V at pH 3.2. With the rise in pH the peak potential shifted towards more negative potential indicating the existence of a protonation reaction coupled with nimodipine reduction process. Fig.2 shows the influence of the pH on the peak height. Sharp response was observed at pH 3.2, so this pH value was chosen as the working pH for further studies.

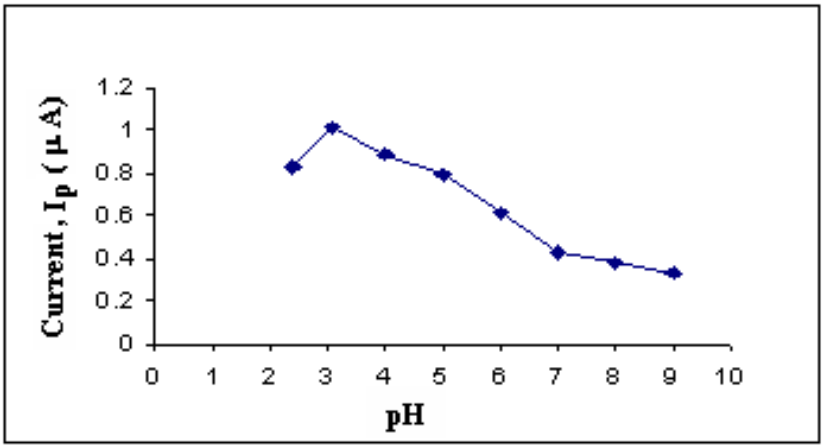


Figure 2. Influence of pH on the cathodic adsorptive peak current response for $1.5x10^{-5}$ mol L^{-1} nimodipine in phosphate buffer (pH 2.5-12) after 40 s preconcentration time; frequency (f) = 140 Hz, Δs = 10 mV and pulse amplitude is 50 mV at E_{acc} = -0.5 V.

Linear pH dependence of the peak potential for reduction wave in the range 2.4 to 9.0 shows that protons participate directly in the reduction process. After pH 9.0 no significant shift in peak potential was observed.

3.3. Controlled potential electrolysis and coulometry

By using controlled potential coulometry, the number of electrons transferred, n values were calculated from the charge consumed by the desired concentration of nimodipine. The charge consumed was determined in acidic medium. For this purpose 2 mL of 5 mg/mL solution of the electroactive species was placed in the cell and electrolysis was carried out at a potential of -0.5 to -0.8 V against Ag/AgCl reference electrode. Number of electrons n was calculated using the equation Q =

nFN, where Q is charge in coulombs, F is Faradays constant and N is number of moles of the substrate. The value is found to be four for cathodic peak of nimodipine in dioxane.

3.4. Reaction Mechanism

On the basis of CV, SWCAdSV, DPCAdSV, coulometry and spectral studies following mechanism have been postulated for the reduction of nimodipine.

$$R\text{-}NO_2 + 4e^- + 4H^+ \longrightarrow R\text{-}NHOH + H_2O$$

The single reduction peak of nimodipine is attributed to the four electron reduction of nimodipine to the corresponding hydroxylamine.

3.5. Cathodic Adsorptive Stripping Voltammetric Behaviour

In order to quantitate the adsorption current resulting from the electro-reduction of nimodipine with dioxane at HMDE, the sensitive differential pulse and square-wave cathodic adsorptive stripping voltammetric techniques were applied. Both the techniques gave comparable results; but the simple stripping square-wave voltammetry is much more sensitive than the other one but both techniques require some parameters adjustment. The optimum instrumental conditions for eg. frequency (*f*), scan increment (Δs), pulse amplitude (E_{sw}), pre-concentration time (t_{acc}), accumulation potential (E_{acc}) etc. were examined.

3.5.1. Effect of accumulation time (t_{acc}) on the square-wave cathodic adsorptive stripping peak current (i_p) response

The square-wave cathodic adsorptive stripping peak height for $1.5x10^{-4}$ mol L^{-1} nimodipine depends strongly on accumulation time suggesting an effective adsorption of nimodipine on the HMDE. The effect of accumulation time for $1.5x10^{-4}$ mol L^{-1} nimodipine was investigated from 0 to 200 s. The peak current increases with the accumulation time up to 60 s then the peak current leveled off showing that the adsorptive equilibrium on the mercury electrode surface was finally achieved. Thus a considerable increase in sensitivity can be achieved by application of adsorptive stripping voltammetric determination of nimodipine. But an accumulation time of 40 s was selected as an optimum in order to shorten analysis time.

3.5.2. Effect of accumulation potential (E_{acc}) on the square-wave cathodic adsorptive stripping peak current (i_p) response

The influence of the adsorptive potential on the peak intensity was also evaluated for $1.5x10^{-4}$ mol L^{-1} nimodipine solution following 40s pre-concentration time, over the range 0.1 to -1.0 V. The

responses were highly influenced by the accumulation potential. The peak height was maximum when the adsorption potential lied between -0.3 to –0.6 V vs. Ag/AgCl. At more cathodic values a decrease in peak current was observed indicating that the drug is no longer strongly adsorbed at potentials where the mercury is negatively charged with respect to the point of zero charge potential. Therefore, the optimal accumulation potential was fixed at –0.5 V vs. Ag/AgCl for all further experimental measurements.

3.5.3. Influence of frequency

The variation of the peak current for $1.5x10^{-4}$ mol L^{-1} nimodipine over the frequency range of 50-140 Hz was linear in phosphate buffer (pH 3.2) at accumulation time 40 s, scan increment 10 mV and pulse amplitude 50 mV that can be represented by the equation:

$$y(i_p) = 0.0101\ (f\ (\text{Hz})) - 0.8041(\mu\text{A}),\ r^2 = 0.998$$

A linear relationship was obtained between the peak current and frequency of the signal up to 140 Hz and this value was chosen to improve the sensitivity without any distortion of the peak or the baseline.

3.5.4. Influence of scan increment and pulse amplitude

Study of the effect of scan increment on the square-wave stripping peak current of the drug in phosphate buffer of pH 3.2 revealed that the peak current enhanced linearly upon the increase of scan increment (2-10 mV). A scan increment of 10 mV was preferable in the present study. At pulse amplitude of 50mV, the peak current was found to be much more sharp and defined.

Several instrumental parameters, those directly affect voltammetric response, were also optimized for e.g. mercury drop size, stirring rate and the rest period. The working conditions decided upon were: drop size 4 cm^2 and 2000 rpm. The stripping was not significantly affected when varying the rest period, since it was found that 10 s was sufficient for the formation of a uniform concentration of the reactant onto the mercury drop.

3.6. Validation of the proposed method

3.6.1. Linearity

The applicability of the proposed square-wave cathodic adsorptive stripping (SWCAdS) voltammetric and differential pulse cathodic adsorptive stripping (DPCAdS) voltammetric procedures as an analytical method for the determination of nimodipine was examined by measuring the stripping peak current as function of concentration of the bulk drug for at least three times under the optimized operational parameters. A calibration graph for the nimodipine was recorded to estimate the analytical

characteristics of the developed method when the most ideal and suitable chemical conditions and instrumental parameters for the voltammetric determination were established. The linearity was checked by preparing standard solutions at 10 different concentrations. The calibration graph can be represented by the following equation:

$$\text{SWCAdSV: } ip(\mu A) = (6.3x10^3)\ C\ (\text{mol } L^{-1}) + 0.5169,\ r^2 = 0.996$$

$$\text{DPCAdSV: } i_p\ (\mu A) = (1.3x10^3)\ C\ (\text{mol } L^{-1}) + 0.2643,\ r^2 = 0.999$$

The regression plots showed that there was a linear dependence of the current intensity on the concentration in both DPCAdSV and SWCAdSV modes over the range is given in Table 1. The table also shows the detection limits and the results of the statistical analysis of the experimental data such as slopes, intercept, the correlation coefficients obtained by the linear least squares treatment of the results along with standard deviation (S.D.) of the intercept (s_a) on the ordinate. The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the values of the correlation coefficient and S.D. The specificity of the method was investigated by observing any interference encountered from the excipients of the tablets mass. It was shown that in the proposed method co-administered drugs did not interfere.

Table 1. Analytical parameters for voltammetric determination of nimodipine with dioxane using SWCAdSV and DPCAdSV modes.

Parameter	**SWCAdSV**	**DPCAdSV**
Concentration Range (µg mL^{-1})	2 – 500	20 – 500
Minimum detectability (ng mL^{-1})	7.11	32.92
Sensitivity (ng mL^{-1})	23.76	109.75
Correlation coefficient (r^2)	0.996	0.999
Slope (µA/mol L^{-1})	$6.3x10^3$	$1.3x10^3$
Intercept (µA)	0.5169	0.2643
s_a	3.6×10^{-5}	3.9×10^{-5}
Applications	Tablets	Tablets

3.6.2. Sensitivity /Detection limit

The detection limit was calculated by equation:

$$LOD = 3S_a / b$$

Where S_a is the standard deviation of intercept and b is the slope of the regression line. The calculated detection limit of standard solution for SWCAdSV was 7.11 ng/mL and for DPCAdSV was 32.92 ng/mL. The peak is not resolved from the noise at concentration lower than LOD.

The quantitation limits were estimated by equation:

$$LOQ = 10\ S_a / b$$

Where S_a is the standard deviation of intercept and b is the slope of the regression line. The lower limit of quantitation for standard solution was 23.76 ng/mL for SWCAdSV and 109.75 ng/mL for DPCAdSV.

3.6.3. Specificity/selectivity

The specificity of the optimized procedure for estimation of nimodipine was examined in presence of excipients such as colloidal silicon dioxide, lactose anhydrous and stearic acid or alternatively; lactose, starch, silica precipitated, talc and magnisium stearate were added to dosage form. Samples containing 0.1 µg mL^{-1} bulk nimodipine and different concentrations of the excipient under evaluation were analyzed by means of the proposed procedure. The obtained mean percentage recoveries (%R) and the relative standard deviations (%RSD) based on the average of five replicate measurements (99.7 ± 0.6 to 100.1 ± 0.9) for SWCAdSV and (99.4 ± 1.1 to 99.99 ± 0.7) for DPCAdSV showed that no significant interference from excipients. Thus the proposed procedure can be considered specific.

3.6.4. Repeatability

The repeatability was examined by performing five replicate measurements for 0.1 µg mL^{-1} bulk drug followed pre-concentration for 60s under the same operational conditions. Percentage recoveries (%R) of 99.2, 99.6, 99, 99.7 and 100.2 were achieved with a mean value of 99.54 and (%R.S.D.) of 0.6, which indicates repeatability and high precision of the proposed procedure.

3.6.5. Precision and Stability

The intra-day and inter-day precision of the proposed procedure was estimated by analyzing 0.1 µg (100 ng) bulk nimodipine solutions for four times in four successive days using SWCAdSV and DPCAdSV. The percentage recoveries based on the average of four separate determinations are abridged in Table 2. The results confirmed both the good precision of the proposed procedure and stability of the drug's solution.

3.6.6. Robustness

The robustness was examined by evaluating the influence of small variation of some of the most important procedure variables including pre-concentration potential (E_{acc}) and pre-concentration

time (t_{acc}), pH etc. The obtained results provided an indication of the reliability of the proposed procedure for the assay of nimodipine and hence it can be considered robust.

Table 2. Precision and accuracy for assay of nimodipine in tablets samples by the proposed procedure.

SWCAdSV				
Nominal Concentration (μg mL^{-1})	Measured Concentration (μg mL^{-1})	%R	Precision (%R.S.D)	Accuracy (Mean relative error)
1.0	1.0006	100.0	0.5	0.06
3.0	3.003	100.1	0.84	0.1
7.0	6.99	99.91	1.1	-0.14
10.0	9.93	99.32	0.68	-0.7
	DPCAdSV			
Nominal Concentration (μg mL^{-1})	Measured Concentration (μg mL^{-1})	%R	Precision (%R.S.D)	Accuracy (Mean relative error)
1.0	0.999	99.9	0.7	-0.1
3.0	2.996	99.86	0.49	-0.13
7.0	6.94	99.2	1.3	-0.85
10.0	9.96	99.6	0.9	-0.4

a Average of five replicate measurements.

As shown in Table-3, the obtained mean percentage recoveries based on the average of five replicate measurements were not significantly affected within the studied range of variations of some operational parameters, and consequently the proposed procedure can be considered robust.

3.6.7. Ruggedness

Two analysts analyzed the same standard with SWCAdSV and DPCAdSV methods using the same instrument. The methods were found to be rugged with the results of variation coefficients 1.03

and 0.8% for SWCAdSV, 1.6 and 1.1% for DPCAdSV methods for first and second analysts, respectively. The results show no statistical differences between different analysts.

Table 3. Influence of variation of some of the operational parameters of the proposed procedure on the mean percentage recovery of 1.0 µg mL^{-1} bulk nimodipine; frequency, f = 140Hz, and scan increment, Δs = 10mV.

Variables	**Condition**	**(%)R ± S.D (n = 3)***
i) pH of the medium		
3.2	t_{acc} = 40 s	101.7 ± 0.5
3.6	E_{acc} = -0.5 V	100.3 ± 0.3
ii) Pre-concentration potential, E_{acc} (V)		
-0.4	pH = 3.2	99.9 ± 0.7
-0.5	t_{acc} = 40 s	100.8 ± 0.5
iii) Pre-concentration time, t_{acc} (s)		
35	pH = 3.2	101.2 ± 0.6
40	E_{acc} = -0.5V	101.5 ± 0.9
iv) Pulse amplitude, E_{sw} (mV)		
40	t_{acc} = 40 s	100.9± 0.25
50	E_{acc} = -0.5V	101.4 ± 1.1
v) Concentration of solution (µg/mL)		
1	pH = 3.2	100.5 ± 1.4
2	E_{sw} = 50	101.4 ± 0.8

*Each value is the mean of 5 experiments.

3.7. Application of method to the pharmaceutical dosage forms

The optimized procedure was successfully applied for determination of nimodipine drug in tablets. There was no need for filtration of tablets extracts from un-dissolved excipients; just dilution of an aliquot from the supernatant layer with the supporting electrolyte is required before measurement. Voltammograms of nimodipine in phosphate buffer exhibit well defined cathodic peak. The current is mainly adsorption-controlled and proportional to the concentration. The good linearity of the calibration graph and the negligible scatter of the experimental points are clearly evident by the correlation coefficients. The precision was estimated using the calibration graph and standard addition method. Representative voltammograms are shown in Fig. 3.

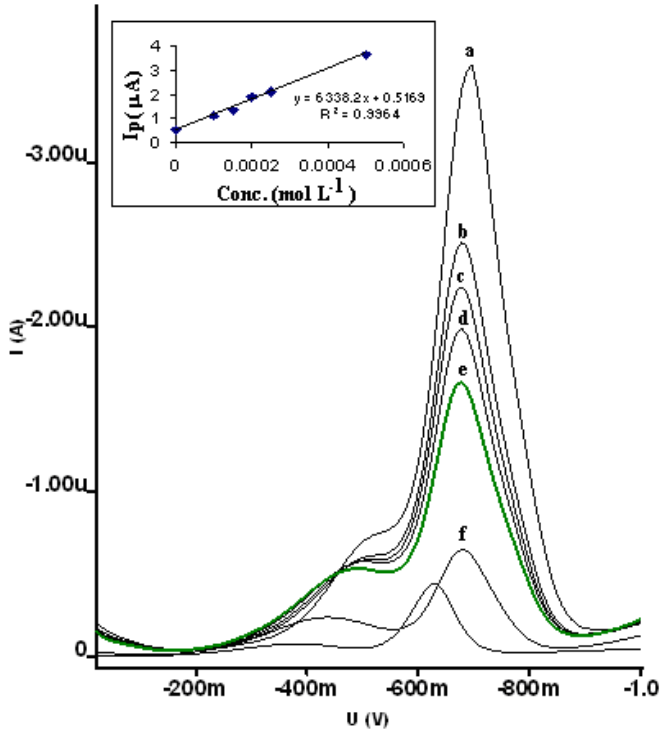


Figure 3. The dependence of the SWCAdS voltammetric current response for nimodipine with dioxane at different concentrations; (a) $5x10^{-4}$ mol L^{-1}, (b) $2.5x10^{-4}$ mol L^{-1}, (c) $2x10^{-4}$ mol L^{-1}, (d) $1.5x10^{-4}$ mol L^{-1}, (e) $1x10^{-4}$ mol L^{-1}, (f) $2x10^{-6}$ mol L^{-1}. Insert: plot of ip, μA vs. conc. (mol L^{-1}); phosphate buffer, pH = 3.2 (0.2 M), equilibrium time = 10 s; frequency f = 140 Hz; Δs = 10 mV; pulse amplitude ΔEsw = 50 mV. E_{acc} = -0.5 V; and t_{acc} = 40 s**.**

The percentage recovery of nimodipine based on the average of five replicate measurements was found as 100.1% & 99.99% for SWCAdSV and DPCAdSV respectively (Table 4). The accuracy of the proposed procedure was also judged by applying the standard addition method as excellent percentage recovery of added nimodipine was achieved. Therefore, the proposed procedure should be applicable to the analysis of this and other similar formulation products containing nimodipine.

Table 4. Application of the proposed voltammetric methods for the analysis of dosage form.

Parameter	**SWCAdSV***	**DPCAdSV***
Labeled amount (mg)	30	30
Amount founded (mg)	30.06	29.99
Standard error	0.009	0.06
Added	0.1 μg/mL	1 μg/mL
Founded	0.1001 μg/mL	0.999 μg/mL
Recovery (%)	100.1	99.99
%RSD of recovery	0.5	0.76

*Each value is the mean of 5 experiments.

4. SUMMARY

The electroactivity of nimodipine using SWCASV was established and studied for the first time. The electrochemical reduction of nimodipine under the conditions described in this work is an irreversible process controlled by adsorption. The developed method with detection limit of 7.11 ng/mL is more sensitive to already reported spectroscopic method [11] and spectrofluorometry method [13] for determination in pharmaceutical dosage form. In addition no sophisticated instrumentation is required. In these methods, high percentage of recovery shows that the compounds are completely extracted from tablet formulations and the results indicate that the developed method can be used to quantify nimodipine without interference from other ingredients. Consequently, the proposed method has a potential of a good analytical alternative for determining nimodipine in pharmaceutical formulations and it can be adopted for pharmacokinetic studies as well as for quality control laboratory studies.

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