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Electroanalytical study of nifedipine using activated glassy carbon electrode[1]

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Abstract

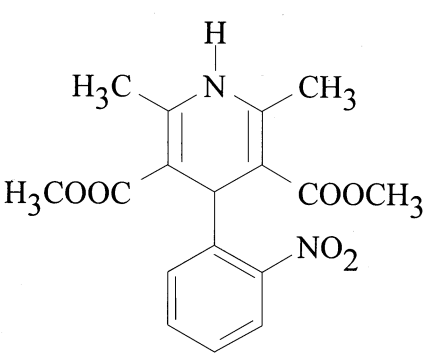
The electrochemical properties of nifedipine have been investigated in aqueous solution by linear sweep and cyclic voltammetry. The method is based both on the reduction and on the oxidation of the drug at a glassy carbon electrode activated by applying a new pre-treatment. The voltammograms of nifedipine on pH, concentration and scan rate have been carefully examined. Both the electroreduction and electrooxidation of nifedipine allow its determination at pH 1.5 in the concentration range of 2×10^{-5}–6×10^{-4} M and 8×10^{-5}–1×10^{-3} M, respectively. The method has been applied to commercial samples (tablets and capsules). © 1998 Elsevier Science B.V.

Keywords: Nifedipine; Voltammetry; Activated glassy carbon electrode; Pharmaceutical formulations

1. Introduction

Nifedipine, a calcium channel blocker belonging to the dihydropyridine family, has commonly been used as a potent arterial vasodilator in the management of angina and cardiovascular diseases [1].

Nifedipine and other dihydropyrine calcium antagonists in pharmaceuticals can undergo photodegradation process [2–6] accompanied by losing their pharmacological activity. This process involves the reduction of aromatic nitro group to a nitroso group and/or the oxidation of the dihydropyridine ring to a pyridine ring [3,4,6]. In addition, these molecules are extensively biotransformed into the inactive metabolites due to the dihydropyridine ring aromatization [7,8]. Since these unfavorable physical and pharmacokinetic characteristics are related to the redox processes on the dihyropyridine moiety, the knowledge of the



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electroanalytical properties of these compounds is of biological interest.

From its electrochemical reduction point of view, nifedipine has been studied using mercury electrode [3,9,10]. A survey of the literature indicates that studies on the reductive behaviors of nifedipine and other structurally related molecules at solid electrodes have been limited to their cyclic voltammetric investigation [11]. However, to the best of our knowledge, no study based on the reduction at solid electrodes has been devoted to this drug in dosage forms. On the other hand, little attention has been paid to its electrooxidation [9,11,12].

Gas chromatography [13–15] and liquid chromatography with UV or electrochemical detector [16–20] have been mainly used for the analysis of nifedipine in biological fluids.

The aim of this work was to study both the reductive and oxidative properties of nifedipine at activated glassy carbon electrode to throw more light on the mechanism of the redox reactions. The methods were then applied to the analysis of the drug in pharmaceuticals.

2. Materials and methods

2.1. Apparatus

Voltammetric analysis was performed with a Tacussel type PRG 3 polarograph and an EPL 2 recorder (Tacussel). The three electrode cell was equipped with a glassy carbon working electrode (Tacussel XM 540, area:1.013 cm^2). A platinum wire was used as counter electrode. All potentials were reported versus to a saturated calomel electrode (SCE). For the electrode pre-treatment, a Wenking Model HP 70 potentiostat and exact-type 250 function generator were used. To minimize the photodegredation of nifedipine, all containers used were well wrapped with aluminium foil. Except when the influence of scan rate was investigated, all voltammograms were recorded using a scan rate of 100 mVs^{-1}. When necessary (reduction), dissolved oxygen was removed from the analyzed solution by passing purified nitrogen through the cell.

HPLC experiments were carried out on a Waters chromatograph (Model 510) equipped with a UV detector (Model 481). The chromatograms were analyzed with a chromatographic workstation (Baseline 810).

2.2. Reagents

Nifedipine was kindly provided by Fako Drugs, İstanbul, Turkey and was used without prior purification. Chemicals for preparation of buffers and supporting electrolytes were analytical grade.

The drug was dissolved in methanol: supporting electrolyte (20:80) mixture. 0.2 M sulphuric acid, 0.2 M acetate buffer pH 3.5, 0.2 M phosphate buffer pH 7.0 and 0.2 M Britton-Robinson buffer pH 1.5–12.0 were used for the supporting electrolytes. Doubly distilled water was used throughout.

2.3. Pre-treatment of the glassy carbon electrode

Activation of the glassy carbon electrode was performed by cycling a square-wave potential with a frequency of 350 Hz followed by the application of a high frequency (3500 Hz), multi-scan triangular potential sweep. After these steps, the electrode surface was highly activated for ca. 40 experiments; the electrode was only applied a potential of $+1.5$ V for 5 min and then -1.0 V for 2 s in 0.1 M KNO_3 solution before each recording. Details of the method was described previously [21].

2.4. Assay procedure for dosage forms

2.4.1. Tablets

Not less than ten tablets were thoroughly weighed and powdered. Portion equivalent to a stock solution of concentration about 10^{-3} M was accurately weighed, transferred into 100 ml calibrated flask and dissolved in methanol. The content was allowed to settle after shaking. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with methanol:buffer solution in order to obtain a final solution of 20:80 methanol:buffer, pH 1.5. Each solution was trans-

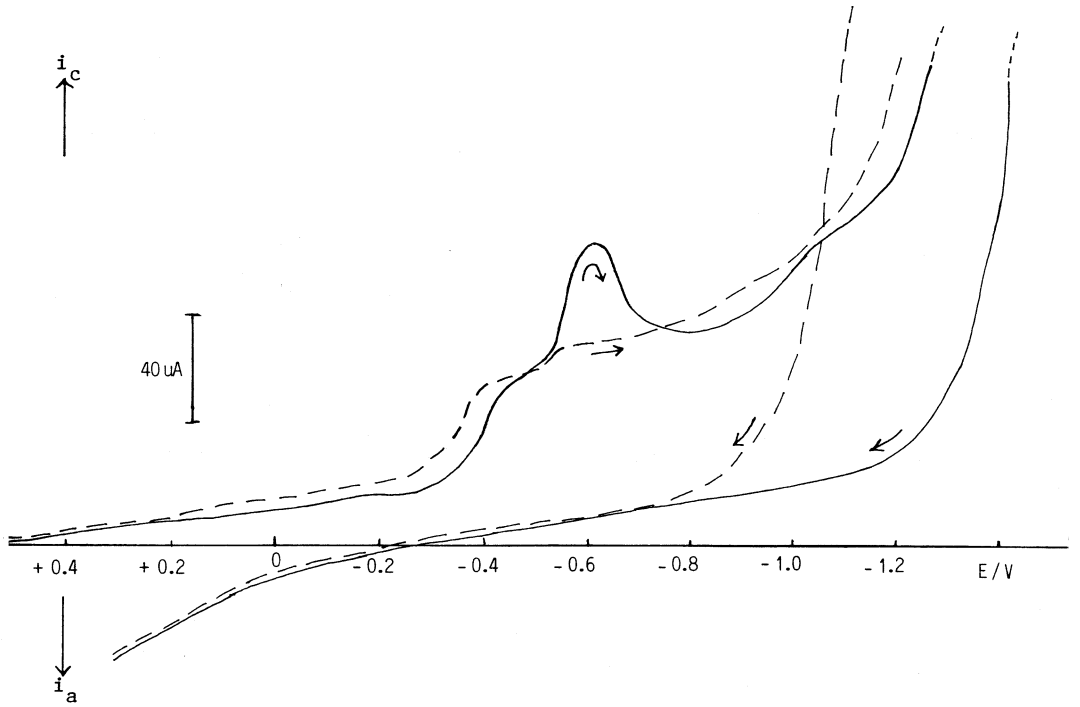

Fig. 1. Cyclic voltammogram of 2×10^{-4} M nifedipine in 0.2 M H_2SO_4 (20% MeOH). Scan rate, 100 mV s^{-1}. Dashed line represents the residual current.

ferred to a voltammetric cell and recorded as in pure drug.

2.4.2. Capsules

Not less than ten capsules containing enteric-coated granules, were accurately weighed, emptied carefully and the mass of the collected content was determined. The empty shells were weighed and the net fill weight per capsule was calculated. The capsule contents were powdered and analysed as for tablets.

2.5. Recovery experiments

In order to know whether the excipients show any interference with the analysis, known amounts of the pure drug were added to the different pre-analysed formulations of nifedipine and the mixtures were analyzed by the proposed method. The recoveries obtained after five repeated experiments were calculated.

3. Results and discussion

A study of the effect of different supporting electrolytes on the voltammetric behavior of nifedipine was carried out at the activated glassy carbon electrode.

3.1. Electrochemical reduction

Cyclic voltammetry conducted on 2×10^{-4} M solution of nifedipine showed only a single irreversible reduction peak ($Ep_c = -0.61$ V) in sulphuric acid media (0.2 M) (Fig. 1). This peak is due to the four-electron reduction of the nitro group to a hydroxylamine derivative according to a classical equation (Eq. (1)) currently accepted [11,22–24]:

$$ArNO_2 + 4e^- + 4H^+ \rightarrow ArNHOH + H_2O \qquad (1)$$

In Britton-Robinson buffer at above pH 1.5, the electrode process split into two steps, over the

pH range investigated (Fig. 2). However, as reported by El-Jammal et al. [11], the splitting of nifedipine reduction peak at glassy carbon electrode can be observed only in strongly alkaline solution or when the dihydropyridine ring is first oxidized. On comparison of the voltammograms with those under the same conditions at non-activated glassy carbon electrode which was polished on 0.3 μm alumina after each scan, the formation of the splitting was occured more clearly in the case of activated glassy carbon electrode. Therefore, it may be assumed that the electrode activation is involved in the electrode reaction; such a pre-treatment of electrode allows interpretation of the splitting in more acidic media. Nevertheless, several authors have reported similar splitting at several solid electrodes for other nitrocompounds (even in the absence of inhibitors and aprotic solvents), depending not only on the nature of the electrode material, but also on the pre-treatment of the electrode [25–28]. These authors attributed the more positive peak to a reversible one-electron reduction of the nitro group yielding a radical anion ($ArNO_2/ArNO_2^{\cdot -}$) and the more negative one to an irreversible three-electron reduction of radical anion to the hydroxylamine. However, in the case of our study, the reoxidation peak of the first step in the reverse scan could not be detected, which confirms the observation of El Jammal et al. [11].

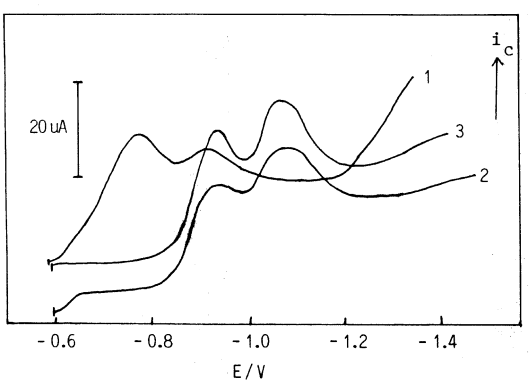

Fig. 2. Linear sweep voltammetric response of 2×10^{-4} M nifedipine in BR buffer (20% MeOH). Scan rate, 100 mV s^{-1}. (1) pH 3.2; (2) pH 7.0; (3) pH 9.0.

When using acetate (pH 3.5) and phosphate (pH 7.0) buffers instead of Britton-Robinson buffer, a similar phenomenon was observed.

The first peak (more positive) increased more rapidly than the second one with increasing scan rate, however, it decreased with increasing the nifedipine concentration. A negative shift in the peak potentials were observed when the scan rate was increased over the 10–100 mV s^{-1} range. Variation of pH of Britton-Robinson buffer from 1.5 to 12.0 indicated that the peak potentials are shifted linearly to more negative potentials up to $\sim$pH 4 and at pH > 5 they remain almost pH independent. Cyclic voltammograms presented a slight adsorption character in acidic solutions. This effect was more pronounced in alkaline media.

In cyclic voltammetry, a small anodic peak was observed in the reverse scan at pH higher than 5 (e.g., $Ep_a = -0.15$ V at pH 10), which may be attributed the oxidation to nitroso compound of hydroxylamine.

3.2. Electrochemical oxidation

Nifedipine was oxidized at activated glassy carbon electrode in acidic media (pH < 2.5), producing only one anodic peak. The electrochemical oxidation can be represented by the following Eq. (2) [12]:

$$\text{Dihydropyridine} \rightarrow \text{Pyridine} + 2e^- + 2H^+ \qquad (2)$$

The peak became broader on increasing the pH and could not be observed clearly because of the poor resolution of Epa and the solvent oxidation. A well-defined peak with the highest signal was obtained in strongly acidic solution (pH 1.5). No signal was observed on scanning in the negative direction, showing the irreversible behavior of the peak.

In Fig. 3, the cyclic voltammetry of nifedipine (2×10^{-4} M) in Britton-Robinson buffer pH 1.5 is shown.

3.3. Analytical application

According to the above results, by choosing the most suitable condition (0.2 M Britton-Robinson buffer:methanol mixture (80:20), pH 1.5) for ob-

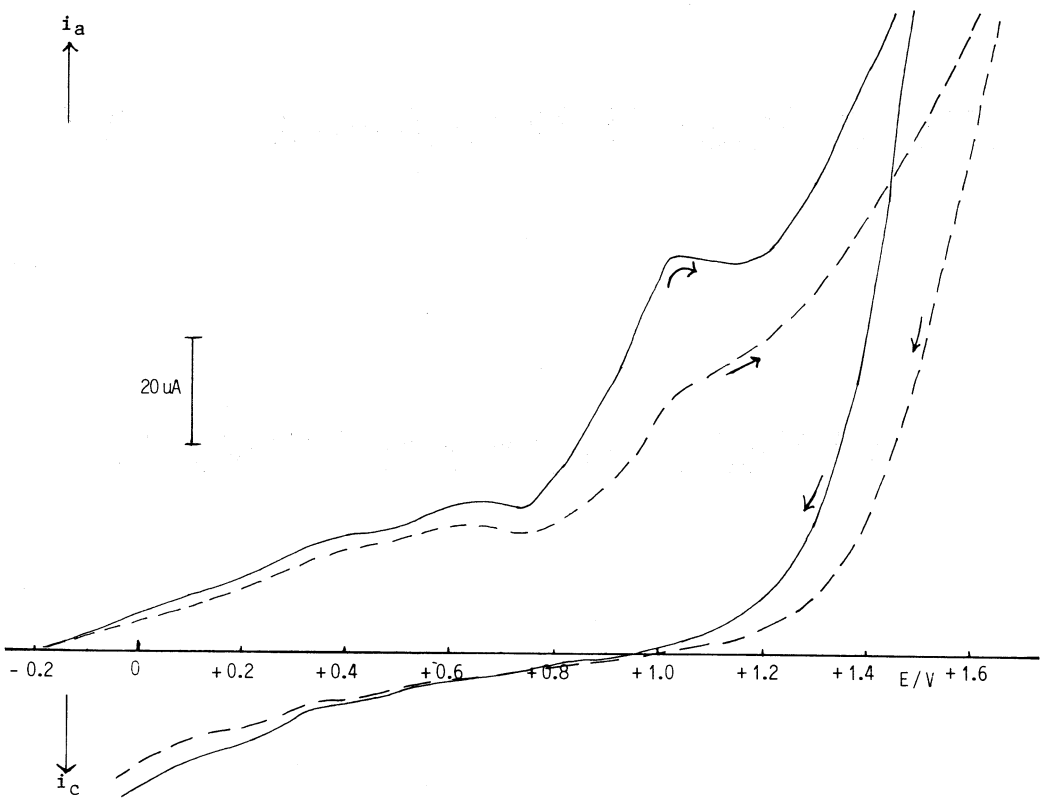

Fig. 3. Cyclic voltammogram of 2×10^{-4} M nifedipine in BR buffer pH 1.5 (20% MeOH). Scan rate, 100 mV s^{-1}. Dashed line represents the residual current.

taining well-developed peaks (further, adsorption processes are less), both the electroreduction and electrooxidation were suited for the sensitive determination of nifedipine. The reproducibility of peak potential and peak current was tested by repeating four experiments on 2×10^{-4} M nifedipine.The relative standard deviations were calculated to be 1.55 and 1.24% for peak potential and 1.16 and 1.77% for peak current using the electroreduction and electrooxidation of nifedipine, respectively. The characteristics of the calibration plots are listed in Table 1. These results obtained by linear sweep voltammetry at activated glassy carbon electrode, show that the linearity ranges of calibration plots can be expanded to lower concentrations than those reported in the literature using differential pulse techniques [3,12]. The detection limits were calculated from ten replicate measurements of 3×10^{-5} M and 9×10^{-5} M nifedipine and calculated to be 1.1×10^{-5} M and 4.3×10^{-5} M for electroreduction and electrooxidation of nifedipine, respectively (according to the $3\,s/m$ definition [29], where s is the standard deviation of the signal and m is the slope of calibration graph).

An additional result obtained from the limit detections showed that the sensitivity at activated glassy carbon electrode is about five times as great as that at non-activated glassy carbon electrode.

In order to check the application of the proposed method, nifedipine was analyzed in different drug formulations. The results in Table 2 are in accordance with those obtained by the official method [30], which involves high pressure liquid

Table 1
Characteristics of nifedipine calibration plots in Britton-Robinson buffer pH 1.5 (20% MeOH)

Electrochemical investigation	Linearity range, M	Equation*	Correlation coefficient	Standard error of slope	Standard error of intercept
Electroreduction	2×10^{-5} -6×10^{-4}	$y = 21.8x - 0.5$	0.999	0.053	0.14
Electrooxidation	8×10^{-5} -1×10^{-3}	$y = 3.47x + 0.07$	0.999	0.056	0.032

* y in μA, ×in 10^{-4} M, slope in μA/10^{-4} M, intercept in μA.

Table 2
Comparative studies for nifedipine dosage forms

Formulation*	Voltammetry				HPLC [30]	
	Electroreduction		Electrooxidation		Tablet	Capsule
	Tablet	Capsule	Tablet	Capsule		
Amount found** mg	9.9	20.1	9.9	20.1	9.7	19.7
R.s.d. %	1.1	1.1	2.2	1.2	1.4	1.8
$t_{calculated}$	2.295	1.712	1.704	2.06	t theoretical: 2.306($P = 0.05$)	

R.s.d.: Relative standard deviation.
* Declared amount: 10 mg per tablet and 20 mg per capsule.
** Each value is the mean of five measurements.

Table 3
Recovery studies by proposed method

	Electroreduction		Electrooxidation	
	Tablet	Capsule	Tablet	Capsule
Recovery* %	98.6	99.0	99.7	99.2
R.s.d. %	0.7	0.9	1.3	1.6

* Each value is the mean of five measurements

chromatographic procedure. Statistical analysis of the results by using Student's *t*-test showed no significant difference between the performances of the two methods as regards to accuracy and precision. Further, according to the results obtained by recovery studies mentioned under Experimental, the mean percentage recoveries and their standard deviations indicated satisfactory accuracy for proposed method (Table 3).

4. Conclusion

It can be concluded that the voltammetric determination based both on the reduction and on the oxidation of nifedipine at activated glassy carbon electrode is accurate, cheap and easy without any preceding and time consuming separation. As can be seen in the literature of the last 10 years, high pressure liquid chromatography is the best method for the analysis of nifedipine in biological fluids and voltammetry and polarography in pharmaceutical preparations. Because of this, the proposed method represents a good analytical alternative. Furthermore, stability studies of commercial products to follow nifedipine photodecomposition at glassy carbon electrode are being carried out in detail in our laboratory.

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