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Voltammetric determination of nilvadipine in dosage forms and spiked human urine

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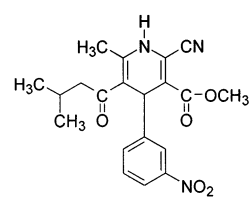
Abstract

The voltammetric behaviour of nilvadipine was studied adopting direct-current, differential-pulse and alternating current polarography. Nilvadipine-being nitroderivative-exhibited well-defined cathodic waves over the whole pH range in Britton–Robinson buffers. At pH 5, the diffusion-current constant, (Id) was 4.78. The current-concentration plots are rectilinear over the range 1.5–20 and 0.2–10 μg/ml using the direct current and differential pulse-polarographic techniques with minimum detectability of 0.05 μg/ml (1.3×10^{-7} M) using the latter technique. The proposed method was applied to commercial capsules containing the drug. The percentage recoveries were in agreement with those obtained by a reference method. Furthermore, the method was applied to spiked human urine, the percentage recovery was 95.54 ± 2.137. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Nilvadipine; Voltammetry; Polarography; Dosage-forms; Urine

1. Introduction

Nilvadipine, 2-cyano-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 3-methyl 5-(1-methylethyl) ester is a dihydropyridine derivative with calcium antagonist activity [1–3]. It is used mainly as an antihypertensive and antianginal agent.



Reviewing the literature revealed that all the reported methods rely on the use of chromatographic techniques, such as GC [4–8], HPLC [8–11] and capillary electrophoresis [12]. Although chromatographic methods offer a high degree of specificity, yet, sample clean-up and the instrumentation limitation preclude their use in routine clinical studies. In addition, no reports

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have been published concerning its determination in capsules. The proposed method was developed as an alternative substitute to the chromatographic methods, and the results obtained were promising. The presence of the reducible nitro group, initiated the present study. Compared with the reported chromatographic methods, which require lengthy extraction and clean-up procedures, the proposed method does not require a prior extraction step. Just dilution of the urine with the buffer solution eliminates its potential interference. As applied to capsules, the method is very simple, does not require the use of chemical reagents. In addition, the method can be considered a stability-indicating assay because the reduction of the nitro group to the corresponding nitroso is the major route of degradation of this group of compounds with the production of therapeutically inactive products [13]. The proposed method is based on the reduction of the nitro group.

2. Experimental

2.1. Reagents and materials

Nilvadipine Working Standard was kindly provided by Klinge Pharma, Fujisawa Group, Japan (Batch No. AN 11424) and was used as received. Capsules containing nilvadipine (Escor Capsules-labeled to contain 8 and 16 mg of nilvadipine per capsule) were obtained from commercial sources. Urine was obtained from healthy volunteers.

Britton–Robinson buffers (BRb), 0.08 M covering the pH range 2.1–12 [14].

A stock solution of nilvadipne (5×10^{-3} M) was prepared in methanol, and was further diluted with the same solvent to give the appropriate working solutions.

2.2. Apparatus

The polarographic study and the differential pulse polrographic (DPP) measurements were carried out using the Polarecord E 506 Metrohm (Herisau, Switzerland). The drop time of 1 s was electronically controlled using a 505 Stand from the same company. The polarograms were recorded using a potential scan rate of 10 mV/s. A three-electrode system, a DME as the working electrode, an Ag/AgCl reference electrode and a platinum wire as the auxiliary electrode was used. Phase selective, alternating current (ACt) polarograms of 1.5×10^{-4} M. solutions were recorded at different pH values using the same instrument; the superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°C.

2.3. Procedures

2.3.1. Recommended procedure

Transfer aliquots of nilvadipine stock solution into a set of 25-ml volumetric flasks, so that the final concentration is in the range cited in Table 1. Add sufficient methanol so that its content should be always 30% (v/v). Complete to volume with BRb of pH 5. Pass nitrogen gas for 5 min. Record the polarograms over the range -0.2 to -1.0 V. Plot the produced current in both DC_t and DPP modes versus the final concentration of the drug to get the calibration graphs. Alternatively, derive the corresponding regression equation.

2.3.2. Analysis of capsules

Empty the contents of 10 capsules and mix well. Weigh a quantity of the mixture equivalent to 16.0 mg of nilvadipine and transfer into a 50-ml volumetric flask. Add about 40 ml of methanol/water (1:1) and sonicate for 30 min. Complete to the mark with the same solvent. Centrifuge for 5 min. Transfer aliquots of the supernatant into 25-ml volumetric flask. Complete as described under Section 2.3.1. The nominal content of the capsule is determined either from the calibration graph or from the corresponding regression equation.

2.3.3. Construction of calibration curve for urine

Transfer 1.0 ml aliquots of urine into a series of centrifugation tubes. Add aliquots of nilvadipine stock solution so that the final concentration is in the range of 0.13–1.114 μg/ml. Mix well using a vortex mixer. Transfer the contents of the cen-

trifugation tubes quantitatively into 25-ml measuring flasks. Wash the tubes with BRb of pH 5 and transfer the washings into the same measuring flasks. Complete to the mark with BRb of pH 5 then transfer the whole contents into the polarographic cell and pass nitrogen gas for 5 min. Record the polarogram using the DPP mode and measure the current. Plot the values of the current (ip) versus the corresponding concentration to get the calibration graph. Alternatively, derive the regression equation.

3. Discussion

3.1. Influence of pH on the reduction waves

Fig. 1 shows the typical polarogram of nilvadipine in BRb of pH 5, both in DC_t and DPP modes. Fig. 2 shows the effect of pH on the development of the cathodic waves, a well-defined wave followed by a more negative ill-defined one, were produced, a typical behaviour of nitro group at the DME. The ratio of the height of the first wave to the second one is 2:1. The two waves showed negative shift upon increasing the pH of the medium. The relation between the half-wave potentials ($E_{1/2}$) of the main reduction wave and pH is given by the following regression equations: over the pH range 2.1–9:

$$E_{1/2}\,(\text{mV}) = 101.4 + 64.4\ \text{pH} \quad (R = 0.9919)$$

and over the pH range 10–12:

$$E_{1/2}\,(\text{mV}) = 600 + 10\ \text{pH} \quad (R = 1.0).$$

Logarithmic analysis of the main reduction wave (the first one) obtained in BRb of different pH values resulted in straight lines with different slopes. Assuming that the rate-determining step involves the transfer of two electrons (a free-radical one electron transfer is not likely to occur), the values of the slopes suggest that the reduction process is irreversible in nature. The αn_a values were calculated according tot he treatment of Meites and Israel [15] and are listed in Table 1.

Table 1
Effect of pH on the development of the polarographic waves of nilvadipine

pH	$-E_{1/2}$ (mV)	$\Delta E_{1/2}/\Delta$pH	Id/C	$W_{1/2}$ (mV)	αn_a	Z (H^+)
2.1	200		5.16	70	1.35	
		90				2.0
3.0	300		5.16	60	1.2	
		80				1.63
4.0	380		5.03	65	1.08	
		60				1.09
5.0	440		5.1	60	1.2	
		60				1.2
6	500		5.16	65	1.08	
		60				2.0
7	560		5.03	65	1.54	
		50				1.04
8	610		5.03	60	1.35	
		40				0.91
9	660		4.89	65	1.2	
		40				0.81
10	700		4.64	70	1.35	
		10				0.23
11	710		5.03	65	1.2	
		10				0.26
12	720		5.16	65	1.54	

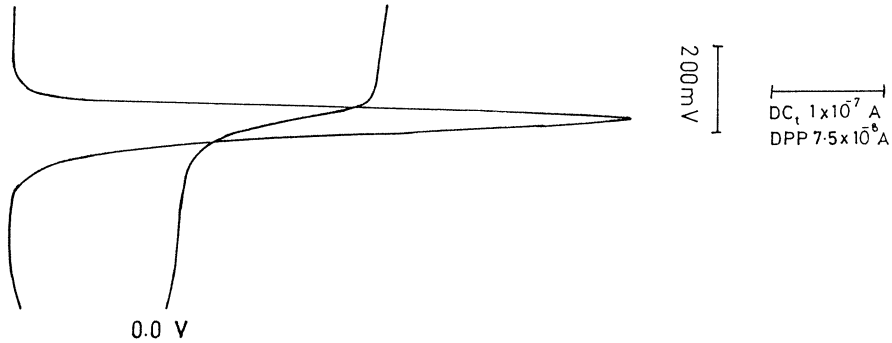

Fig. 1. Typical DC_t and DPP polarograms of nilvadipine (1×10^{-4} M) in BRb of pH 5. Scan rate: 10 mV/s.

3.2. Study of the wave characteristics

Increasing mercury height (h) resulted in a corresponding increase in waveheight (w); a plot of w versus $\sqrt{h}$ gave a straight line; also a plot of log h versus log w gave a straight line with a slope of about 0.57. Changing the buffer concentration over the range 0.01–0.08 M resulted in a negligible increase in waveheight. These two characteristics point out to a diffusion-controlled process. The alternating current (ACt) behaviour of nilvadipine (1×10^{-4} M solution) was studied using a phase-selective angle of 90° in BRb of pH values of 5, 7 and 10. The summit potentials (E_s) were shifted 140, 120 and 10 mV more negative than the corresponding $E_{1/2}$, respectively. Fig. 3 demonstrates that, at pH 5, 7 and 10, both the drug and its reduction product are adsorbed to the mercury surface. However, addition of methanol (30% v/v) to the electrolysed solution decreases the effect of the adsorption process. The diffusion-coefficient (D) of nilvadipine was determined in BRb of pH 5 according to Ilkovich equation [16] and was found to be 4.8×10^6 cm^2 s^{-1}. This small value may be attributed to the bulky nature of the molecule.

3.3. Number of electrons involved in the reduction process

The number of electrons transferred during the reduction process was accomplished through comparing the waveheight of nilvadipine with that obtained from an equimolar solution of an earlier studied compound having the same functional group (nitro group) and of nearly identical value of diffusion-coefficient, i.e. nicardipine [17]. In BRb of pH 5, both compounds gave two waves. The first wave of the two compounds were equal in height, corresponding to a 4-electrons transfer process, and consequently 2-electrons for the second one. It is evident from the experimental results that, a slow electron-transfer reaction is involved in the reduction of nilvadipine. Logarithmic analysis of the waves established that two electrons are involved in the rate-determining step of the first wave, and the shift in $E_{1/2}$ potentials with increasing pH indicates that two hydrogen atoms are consumed in this step. Based on these facts, and by analogy to the earlier reported mechanisms for nitro compounds [18], the following pathway may be postulated for the first wave:

$$R\text{–}NO_2 + 2H^+ + 2e \overset{\text{slow}}{\rightarrow} R\text{–}NO + H_2O$$

$$R\text{–}NO + 2H^+ + 2e \overset{\text{first}}{\rightarrow} R\text{–}NH\text{–}HO$$

The second wave involves two electrons and is due to a further reduction of the hydroxy–amino group to the primary amine:

$$RNH \cdot OH + 2H^+ + 2e \rightarrow R\text{–}NH_2 + H_2O$$

3.4. Analytical applications

Polarograms of nilvadipine in BRb of pH 5 exhibit a very well-defined cathodic wave. No polarographic maxima was developed, therefore, no maximum suppressor was needed. The current is diffusion-controlled and proportional to the

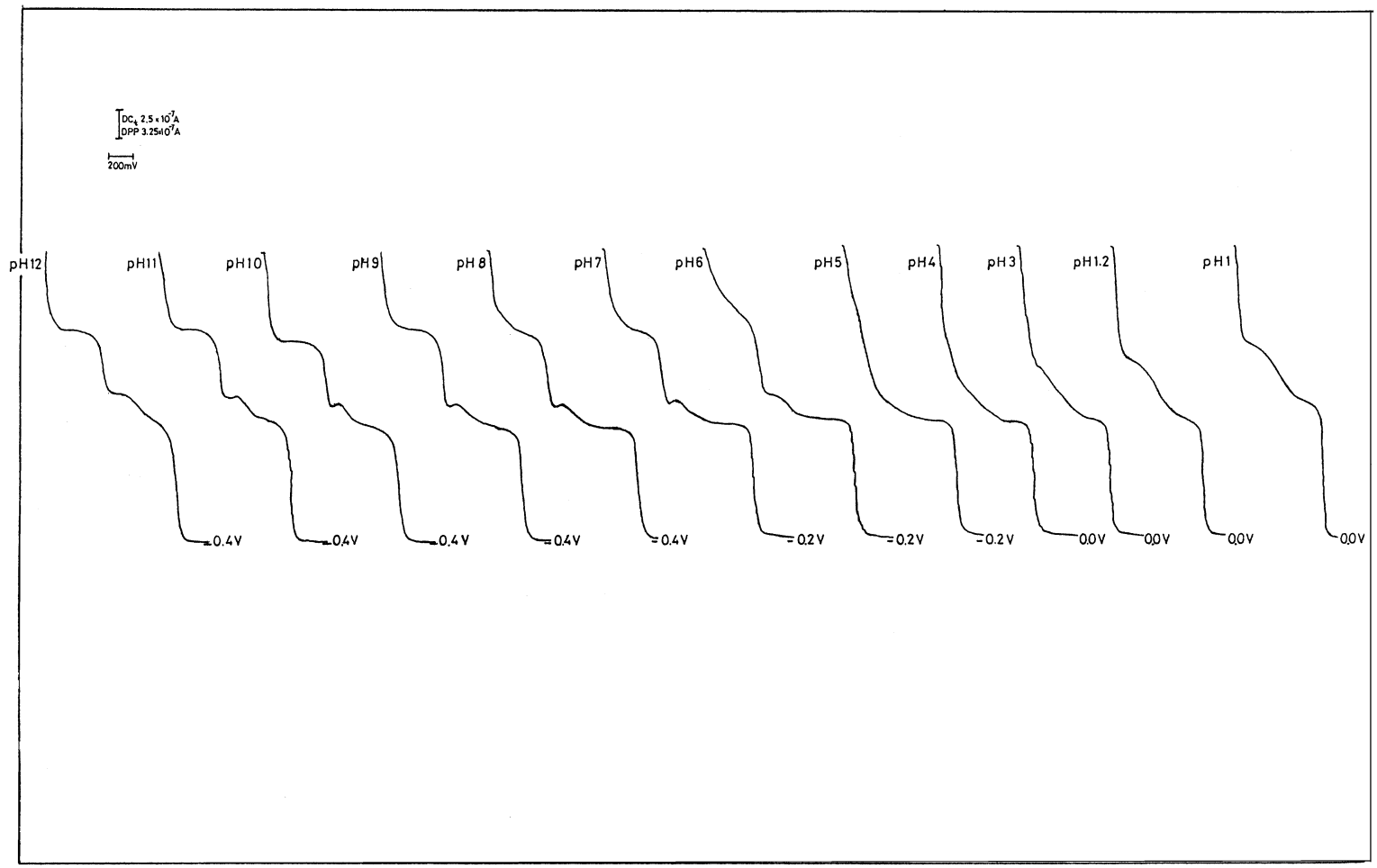

Fig. 2. Effect of pH on the development of the polarographic waves of nilvadipine (1×10^{-4} M). Scan rate 10 mV/s, drop time 1 s.

concentration over a convenient range. At that pH value, the DC_t wave was the steepest and the peak had the least half-peak width, $w_{1/2}$ (Table 1).

Solutions of nilvadipine in methanol were found to be stable for 3 days if kept in the refrigerator. In BRb of pH 5 (the analytical buffer) the solutions were found to be stable for at least 3 h.

The relation between the limiting diffusion current (id) in the DC_t mode and the peak current (ip) in μA, and the concentration of nilvadipine is rectilinear over the ranges cited in Table 2. The analytical performance data of the proposed method (regression equations, correlation coefficients, minimum detectability and diffusion-current constant Id) are compiled in the same table.

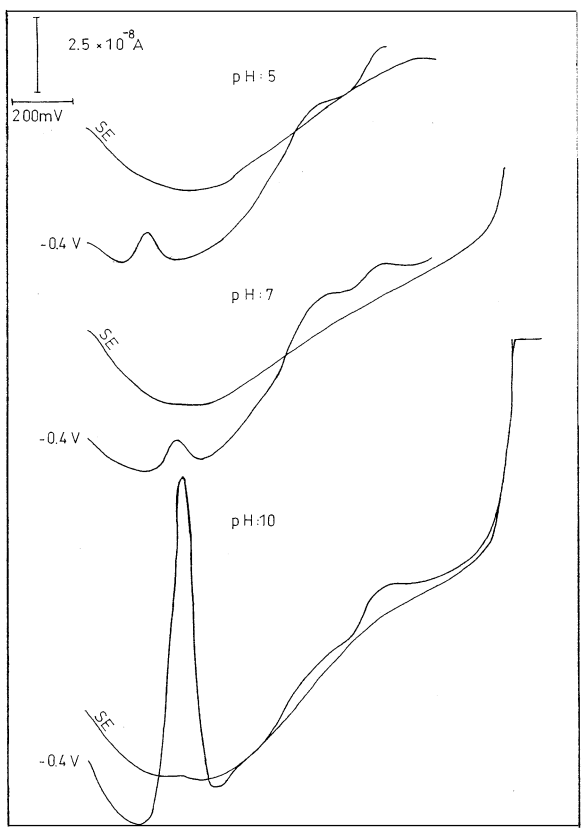

Fig. 3. Alternating current behaviour of nilvadipine (1×10^{-4} M) in BRb of different pH values. Superimposed alternating voltage 15 mV; frequency 75 Hz; phase angle 90°, SE: supporting electrolyte.

Table 2
Analytical performance data of the proposed methods

Parameter	DC_t	DPP
Range	1.5–20 μg/ml	0.2–10 μg/ml
Regression equation	$C = -1.25 + 80$ id	$C = -0.45 + 28.4$ ip
Correlation coefficient	0.998	0.996
Diffusion-current-constant (Id/C)	4.78	12.4
Minimum detectability	0.2 μg/ml (5.2×10^{-7} M)	0.05 μg/ml (1.3×10^{-7} M)
$S_{y/x}$	7.32×10^{-3}	8.21×10^{-3}
S_a	4.27×10^{-3}	6.68×10^{-3}
S_b	3.59×10^{-4}	7.5×10^{-4}
Applications	Dosage forms	Dosage forms and biological fluids

Statistical analysis of the regression equations regarding the standard deviation (S.D.) of the residuals $S_{x/y}$, S.D. of the slope S_b and S.D. of the intercept S_a, gave small values of these parameters indicating the high precision of the proposed method [19].

To estimate the reproducibility of the electrode response six replicate concentrations were tested at nilvadipine concentrations of 4, 8, 12, and 16 μg/ml adopting the DC_t mode. Mean current values of 0.066 ± 0.0003; 0.131 ± 0.005; 0.198 ± 0.0009 and 0.265 ± 0.0013 μA, respectively, were obtained. The precision of these measurements is expressed by the relative standard deviations (R.S.D.) of 0.45, 0.38; 0.45 and 0.49, respectively. These small figures point out to the high precision of the electrode response.

Both DC_t and DPP modes were applied to the determination of nilvadipine in commercial capsules. The percentage recoveries based on four separate determinations are abridged in Table 3. The results obtained were compared with those given by an HPLC method recommended by the manufacturer (Merck, Darmstadt, Germany). The method involves the use of acetonitrile/diammonium hydrogen phosphate (0.25% w/v) of pH 7 (6:4) as the mobile phase and C_{18} Nucleosil (250×4.6 mm, i.d.) column. Detection was affected at 254 nm. Statistical analysis of the results obtained by either DC_t or DPP with those given

Table 3
Application of the proposed method to the determination of nilvadipine in commercial capsules

Preparation	% Recovery		Reference method
	DC_t	DPP	
Escor capsules 8 mg nilvadipine per capsule	102.05 ± 0.2	102.5 ± 0.3	100.0 ± 0.5
Escor capsules 16 mg nilvadipine per capsule	100.7 ± 0.325	101.2 ± 0.35	100.63 ± 0.4

Each result is the average of four separate determinations.

by the reference method, reveals no significant difference between the performance of the two methods regarding accuracy and precision, as revealed by *t*-test and *F*-test, respectively [19]. Both DC_t and DPP proved to be equally useful. However, the DPP mode is more convenient.

The high sensitivity of the method allows the determination of nilvadipine in spiked human urine. Nilvadipine is orally administered in a dose of 16 mg daily. The anticipated concentration in urine will be about 0.32 μg/ml, which is within the working concentration range. Linear regression analysis of the concentration of spiked nilvadipine in human urine and the current gave the following equation:

$$C = 0.089 + 39\ \text{ip}$$

where C is the concentration in μg/ml. ip is the current in μA.

The method was applied to spiked human urine. The results are shown in Table 4. The results are satisfactorily accurate and precise.

The major advantage of the proposed method over the reported chromatographic methods is that it does not require a prior extraction step, thus, it is simpler and time saving and moreover no sophisticated instrumentation is needed.

Table 4
Application of the proposed method to the determination of nilvadipine in spiked human urine adopting the DPP mode

Added (μg/ml)	Found (μg/ml)	% Recovery
0.24	0.229	95.42
0.47	0.459	97.87
0.73	0.674	92.33
0.86	0.830	96.51
$\bar{X}$		95.53
± S.D.		1.668

The proposed method can be considered as a stability-indicating assay. It has been reported that this class of compounds (dihydropyridine derivatives) that contain nitro-functional group are light-sensitive. Under the influence of visible and ultraviolet light, these compounds in solution are converted into the nitroso derivatives with consequent loss of activity [13,20]. The polarographic behaviour of the nitrosoderivative is different from that of the nitro group. As the proposed method is based on the presence of nitro-group, the proposed method is regarded as a stability-indicating one.

4. Conclusion

A simple and sensitive reliable method was developed for the determination of nilvadipine in dosage forms and spiked human urine. As applied to urine, the method has the advantage that no prior extraction or clean-up procedure is required. The detection limit (using the DPP mode is comparable to that reported by chromatographic methods. As applied to capsules, the method is very simple, time saving, analysis of one sample takes about 3 min. The method is also stability-indicating, as its is based on the presence of nitro-functional group, and reduction of the nitro group to the nitroso is the major route of its degradation.

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