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Determination of Amlodipine in Pharmaceutical Formulations by Differential-Pulse Voltammetry with a Glassy Carbon Electrode

A differential-pulse voltammetric method was developed for the determination of amlodipine based on the oxidation of the dihydropyridine group on the surface of glassy carbon electrode under stationary and rotating conditions. The experiments were conducted in a supporting electrolyte consisting of 0.2 M KCl, 0.1 M phosphate buffer, and 10% (*v/v*) methanol during investigation of initial potential and pH effects. No adsorption effect was observed on using an initial potential of 0 mV and the supporting electrolyte solution at pH 5.5 under both stationary and rotating conditions. The factor affecting the voltammetric current was diffusional in the range of 200–1000 rpm for rotating, and 2–40 mV s^{-1} for stationary conditions up to a concentration of 0.04 mg mL^{-1} amlodipine. The limit of detection (LOD) and the limit of quantitative (LOQ) for the rotating and stationary techniques were found to be 0.004 and 0.0072 mg mL^{-1} (for $S/N = 3.3$) and LOQ 0.012 and 0.022 mg mL^{-1} (for $S/N = 10$), respectively. The proposed method was applied to the tablets containing amlodipine and according to the statistical evaluations acceptable results were obtained at the 95% probability level.

Keywords: Amlodipine; Differential pulse voltammetry; Pharmaceutical analysis

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Introduction

Amlodipine {*R,*S-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxy-carbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine} is a potent dihydropyridine calcium channel blocker [1]. The structure of amlodipine differs from that of other dihydropyridines in possessing a basic side-chain attached to the 2-position of the dihydropyridine ring as shown in Figure 1. It has been reported to show the highest oral bioavailability and the longest half-life of elimination in humans among all the drugs of its class [2]. It is clinically used in the treatment of hypertension and angina [3].

Several analytical methods for determination of amlodipine in biological fluids have been reported, e.g. high-performance liquid chromatography (HPLC) with ultraviolet [4–6], electrochemical [7], amperometric [8], and mass spectrometric [9] detection, capillary gas chromatography (GC) with electron capture detection [10, 11], high performance thin-layer chromatography (HPTLC) [12], radioimmunoassay [13], and micellar electrokinetic chromatography [14].

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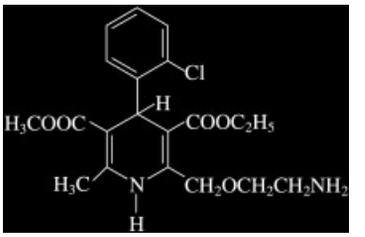

Figure 1. Chemical structure of amlodipine.

A capillary electrophoretic method for chiral separation of amlodipine [15] and spectrophotometric [16–19] and HPTLC [20] methods for the determination of amlodipine in pharmaceutical formulations have been published. Furthermore, some reports were published on the oxidation of 1,2-dihydropyridine derivatives [21, 22].

The aim of this study is to examine the voltammetric behavior of the amlodipine molecule based on the oxidation of the dihydropyridine group on the surface of rotating and stationary glassy carbon electrodes by the differential pulse (DP) technique. All the voltammetric parameters were explored by the DP technique. However, the rate of rotation and rate of potential effects were examined by direct current voltammetry. The validity of the method was tested by comparing the results to those of UV spectrophotometry, which has been accepted as a

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comparison method. All of the results were evaluated with common statistical tests.

Results and discussion

Method efficiency

Several supporting electrolyte solutions were tested for the voltammetric studies of amlodipine using a glassy carbon electrode at the stationary and rotating conditions. Since the solubility of this compound in water is low, various solvents were tested to prepare the most suitable solution. Methanol proved to be the best solvent and therefore the effect of methanol concentration was examined in the supporting electrolyte. It was found that the most suitable mixture was 0.2 M KCl, 0.1 M phosphate buffer, and 10% (*v/v*) of methanol. Since the resting time between application of the potential and scanning is very important in the systems where adsorption occurred, a fixed duration of thirty seconds was applied before the starting potential sweeping. Possible absorption effects were thus avoided with the resting time application.

Voltammetric behavior of amlodipine

Mode of voltammetric technique

The voltammetric behavior of amlodipine was examined by the differential pulse technique using a glassy carbon electrode under rotating and stationary conditions.

Effect of initial potential

The effect of the initial potential in the range of –200 to +400 mV, employing 1.0×10^{-4} M amlodipine at pH 5.5, was investigated. The plots of current versus initial potential exhibited similar curves under stationary and rotating conditions. The magnitudes of currents decreased from the potential of –200 toward 0 mV and they reached plateau between the potentials of 0 and +400 mV. Adsorptive stripping voltammetry might be achieved using negative potentials more negative than –100 mV. Accordingly, it was concluded that 0 mV potential is the most suitable initial potential for both rotating and stationary electrodes.

Effect of pH on the peak current and peak potential

To investigate the effect of pH on the shape of polarization curves, peak current and peak potential values, DP voltammograms of 0.04 mg mL^{-1} amlodipine were recorded in the pH range of 1.46–12.02. Careful consideration was given to initiating the potential from 0 mV under the same voltammetric and analytical conditions. Well shaped oxidation voltammograms appeared in all the ranges studied. The peak current and peak potential values were plotted against pH (Figure 2a and b).

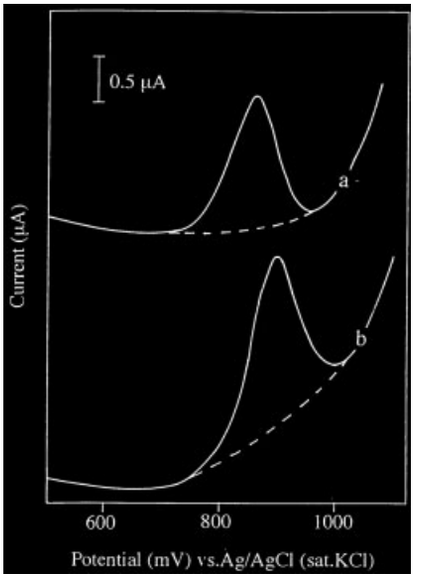

Figure 2. Dependence of peak current (a) and peak potential (b) values of 0.04 mg mL^{-1} amlodipine in 0.1 M buffer, 0.2 M KCl, and 10% (*v/v*) methanol against pH in the rotating (750 rpm) and stationary condition applying an initial potential of 0 mV, a rate of potential of 10 mV s^{-1}, and a pulse height of 50 mV.

The plots of peak current versus pH under both stationary and rotating condition exhibit a plateau between pH 5 and 8. In the examination of the shape of the curves, the most symmetrical voltammograms appeared around pH 5.5 and where peak potentials were +870 mV for stationary and +900 mV for rotating systems. The final results led us to conduct the experiments at this pH.

To elucidate the factor affecting the voltammetric current, the rotation rate and rate of potential effects were investigated by direct current voltammetry. The DC voltammograms of 0.04 mg mL^{-1} amlodipine solution at pH 5.5 were recorded employing the rotating glassy carbon electrode in the range of 200–2000 rpm and starting from 0 mV anodically. The limiting current values were measured and they were plotted against the square-root of the rotation rate ($\omega^{1/2}$) as described elsewhere [23]. A linear relationship corresponding to the equation $i(\mu A) = 0.470\omega^{1/2} + 0.1289$ ($r = 0.9994$) was obtained up to 1000 rpm at this concentration. The result confirms that the factors which influence the voltammetric current are diffusional. However, the voltammetric current gradually turns into adsorptional from diffusional at concentrations higher than 0.04 mg mL^{-1} of amlodipine.

Effect of potential rate

The effect of potential rate (ν) on the current at 0.04 mg mL^{-1} amlodipine solution using stationary

glassy carbon electrode was examined in the range of 2–100 mV s^{-1} by the DC technique. The plots of limiting currents against $v^{1/2}$ showed a linear relationship up to 40 mV s^{-1} that almost passes through origin and is described by the equation [i(μA) = 0.8202$v^{1/2}$ – 0.048; r = 0.9995] at that concentration. The dependence proves that the factor controlling the current is totally diffusional. Such behaviour was observed not only at higher amlodipine concentrations but also at the lower potential rate up to 10 mV s^{-1}.

Effect of pulse height

The effect of pulse height of 0.04 mg mL^{-1} M amlodipine solution on the peak current was examined in the range of 5–100 mV. The dependence of peak height versus pulse amplitude remained unchanged. Therefore, a 50 mV pulse height was used throughout the study.

Concentration effect

The variation of peak current with amlodipine concentration over the range of 0.0079 mg mL^{-1} to 0.004 mg mL^{-1} in the aqueous supporting electrolyte consisting of 0.2 M KCl, 0.1 M phosphate buffer, and 10% (*v/v*) methanol at pH 5.5 at a constant potential rate of 10 mV s^{-1} and a pulse height of 50 mV was examined by the DP technique for both rotating and stationary glassy carbon electrodes. The rate of rotation used during these studies was 500 rpm. Straight lines which fit the equations [i(μA) = 15337.4C(M) + 0.05; r = 0.9999] for the rotating system and [i(μA) = 9969.3C(M) – 0.03; r = 0.9997] for the stationary system were obtained where C(M) is the molar concentration. The results show that the proposed method, being sensitive and accurate, can be used for the determination of amlodipine.

The voltammograms of amlodipine (0.004 mg mL^{-1}) in the optimum rotating and stationary conditions employing the DP technique are given in Figure 3.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the data of 0.022 mg mL^{-1} M of amlodipine solution according to the validation procedures [24]. The LOD and LOQ for rotating and stationary glassy carbon electrodes were 0.004 and 0.0072 mg mL^{-1} (for S/N = 3.3) and LOQ 0.012 and 0.022 mg mL^{-1}, respectively.

Application of the method to pharmaceutical tablets

The proposed method was applied in the case of Norvasc® tablets containing 5 mg of amlodipine. The tablets

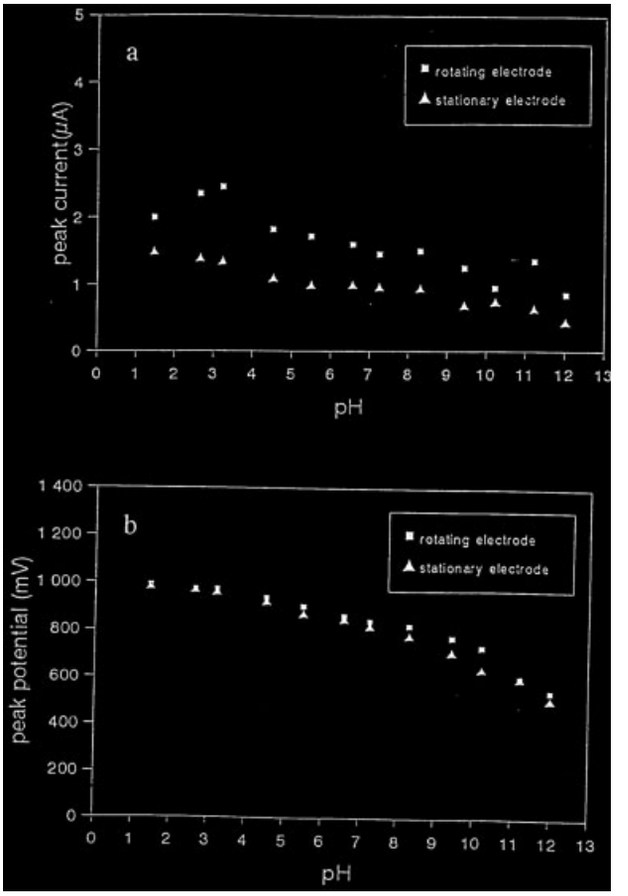

Figure 3. DP voltammograms of 0.04 mg mL^{-1} amlodipine at pH 5.5 in supporting electrolytes consisting of 0.1 M acetate buffer, 0.2 M KCl, and 10% (*v/v*) methanol, with initial potential of 0 mV, pulse height of 50 mV, potential rate of 10 mV s^{-1} and rotation rate of 500 rpm, (a) stationary, (b) rotating glassy carbon electrodes.

were processed as described in the Experimental section and the optimum voltammetric conditions were employed for the analysis. No interference was observed from the excipients and coating materials of the tablets.

UV spectrophotometry served as a comparison method. The calibration studies were performed in the amlodipine concentration range of 0.0047 mg mL^{-1} to 0.024 mg mL^{-1} in the presence of 10% (*v/v*) methanol. The measurements were recorded at 240 nm. A calibration graph was drawn and no deviation was observed. The equation was computed to be [A = 12664.6 C(M) + 0.01; r = 0.9997].

The results of the methods were compared to each other at the 95% probability level. The results of the statistical evaluations are given in Table 1. According to the results

Table 1. The statistical evaluations of assay results of amlodipine in Norvasc® tablet by the voltammetric method employing rotating and stationary conditions and by UV spectrophotometry.

Modes	DP Voltammetry (rotating electrode)	DP Voltammetry (stationary electrode)	UV Spectrophotometry
Mean (n = 8)	4.99 mg	5.04 mg	4.91 mg
Standard deviation	0.10	0.10	0.09
RSD	2.09	1.98	1.94
Confidence limit	±0.09	±0.09	±0.08
t-tests of significance	0.67	1.23	Table $t_{0.05}$ = 2.37
F-tests of significance	1.23	1.35	Table $F_{0.05}$ = 3.79

of *t*- and *F*-tests, insignificant differences appeared between the two methods. Furthermore, the content of the tablet satisfied the official requirements [24].
In conclusion, the method proposed in this study is practical, sensitive, and accurate for the analysis of amlodipine preparations in quality control laboratories.

Experimental

Apparatus

The voltammetric system comprised a Polaropulse Model PRG-5 with dual-function EGMA type cell for polarography and voltammetry, 3.00-mm diameter glassy carbon disc as working electrode (BAS West Lafayette, IN, USA), platinum wire as auxiliary electrode, and Ag/AgCl; saturated KCl as reference electrode (Tacussel, Lyon, France). The voltammograms were recorded on a Model SE 790 x-y recorder (BBC Goertz Metrawatt, Vienna, Austria). A Model M 822 pH-meter (Electromag, Istanbul, Turkey) with glass electrode was employed for measuring and adjusting the pH of the solution. Spectrophotometric studies were accomplished using a Model UV-2401PC double beam spectrophotometer (Shimadzu, Kyoto, Japan) in quartz cells.

Chemicals

Standard amlodipine (99.97%) and Norvasc® tablets were kindly supplied by Pfizer Ilaç Sanayi A.S. (Istanbul, Turkey) and were used without further purification. All the other chemicals used in the experiments were products of Merck Co. (Darmstadt, Germany) and they were all of analytical grade. Double-distilled water was used for preparation of the solutions. The commercial preparations of amlodipine (Norvasc® tablets containing 5 mg of active material) were obtained locally.

Supporting electrolyte and voltammetric procedure

A supporting electrolyte consisting of 0.1 M phosphate buffer, 0.2 M KCl, and 10% (*v/v*) methanol was employed to conduct the experiments and the pH of the solutions was adjusted with 0.1 M HCl and 0.1 M NaOH. The determination of amlodipine was achieved in the same supporting electrolyte at an apparent pH value of 5.5.

Stock solution of amlodipine was prepared in methanol. Dilutions were made from this solution to the final concentrations of the related components as given above. For voltammetric studies, 10 mL of supporting electrolyte containing amlodipine was placed in the cell. Approximately 0.04 mg mL^{-1} amlodipine was employed for the investigation of the effect of pH on the limiting current and the other voltammetric parameters. The electrode was polished with 0.3 µm particle size alumina; then it was rinsed with distilled water and dried with a non-abrasive tissue paper before each experiment.

Analysis of pharmaceutical dosage form

For the voltammetric and spectrophotometric assay, twenty Norvasc® tablets, each containing 5 mg amlodipine, were weighed. The average weight of one tablet was calculated and then they were finely powdered in a mortar. A sufficient amount of tablet powder equivalent to the average weight of one tablet was accurately weighed, transferred to a 10-mL flask, and methanol was added to dissolve the active material. The solution was magnetically stirred for 10 min and made up to the final volume with methanol. The solution was then filtered and was diluted with the relevant solutions to accomplish the assay either voltammetrically or spectrophotometrically. The final methanol concentration was 10% (*v/v*) in the spectrophotometric studies and also in the solution used as a blank.

References

[1] R. A. Burges, D. G. Gardiner, M. Gwilt, A. J. Higgins, K. J. Blackburn, S. F. Campbell, P. E. Cross, J. K. Stubbs, *J. Cardiovasc. Pharmacol.* **1987**, *9*, 110.

[2] P. A. Meredith, H. L. Elliott, *Clin. Pharmacokinet.* **1992**, *22*, 22.

[3] D. Murdoch, R. C. Heel, *Drugs* **1991**, *41*, 478.

[4] P. K. F. Yeung, S. J. Mosher, P. T. Pollak, *J. Pharm. Biomed. Anal.* **1991**, *9*, 565.

[5] F. Barbato, B. Cappello, L. Grumetto, P. Morrica, *Farmaco* **1993**, *48*, 417.

[6] S. R. Sankar, M. J. Nanjan, M. Vasudevan, N. Shaat, B. Suresh, *Indian J. Pharm. Sci.* **1997**, *59*, 171.

[7] M. Josefsson, B. Norlander, *J. Pharm. Biomed. Anal.* **1996**, *15*, 267.

[8] M. Josefsson, A.-L. Zackrisson, B. Norlander, *J. Chromatogr. B: Biomed. Appl.* **1995**, *672*, 310.

[9] T. Yasuda, M. Tanaka, K. Iba, *J. Mass Spectrom.* **1996**, *31*, 879.

[10] P. Beresford, P. V. Macrae, D. A. Stopher, B. A. Wood, *J. Chromatogr.* **1987**, *420*, 178.

[11] F. Scharpf, K.-D. Riedel, H. Laufen, M. Leitold, *J. Chromatogr. B: Biomed. Appl.* **1994**, *655*, 225.

[12] K. K. Pandya, M. Satia, T. P. Gandhi, I. A. Modi, R. I. Modi, B. K. Chakravarthy, *J. Chromatogr. B: Biomed. Appl.* **1995**, *667*, 315.

[13] P. Beresford, D. McGibney, M. J. Humphrey, P. V. Macrae, D. A Stopher, *Xenobiotica* **1988**, *18*, 245.

[14] E. Bretnall, G. S. Clarke, *J. Chromatogr. A* **1995**, *700*, 173.

[15] T. S. Small, A. F. Fell, M. W. Coleman, J. C. Berridge, *Chirality* **1995**, *7*, 226.

[16] G. Çetin, S. Sungur, *Sci. Pharm.* **1995**, *63*, 93.

[17] M. N. Reddy, G. T. Rani, K. V. S. P. Rao, D. G. Sankar, K. Sreedhar, *Indian J. Pharm. Sci.* **1997**, *59*, 188.

[18] K. Sridhar, C. S. P. Sastry, M. N. Reddy, D. G. Sankar, K. R. Srinivas, *Anal. Lett.* **1997**, *30*, 121.

[19] B. Avadhanulu, J. S. Srinivas, Y. Anjaneyulu, *Indian Drugs* **1996**, *33*, 36.

[20] G. Chandrashekhar, P. S. N. Rao, K. Smirita, S. K. Vyas, C. Dutt, *J. Planar Chromatogr. -Mod. TLC* **1994**, *7*, 458.

[21] J. Stradins, J. Ogle, V. Kadysh, L. Baumane, R. Gavars, G. Duburs, *J. Electroanal. Chem.* **1987**, *226*, 103.

[22] J. Ludvik, J. Volke, J. Kilima, *Electrochimica Acta* **1987**, *32*, 1071.

[23] V. G. Levich, *Physicochemical Hydrodynamics.* Prentice Hall, Englewood Cliffs, NJ, **1962**.

[24] *The United States Pharmacopoeia,* XXII, Mack Printing Company, Easton, PA, **1990**, p. 1696.