Scientia Pharmaceutica (Sci. Pharm.) **72**, 131–142 (2004)

Anodic Polarographic Determination of Isradipine in Pharmaceutical Formulations

F. Belal*; A.A. Al-Majed and S. Julkhuf

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Saudi Arabia. Fax: ++ 966-1-4676200. E-mail: ffbelal@yahoo.com

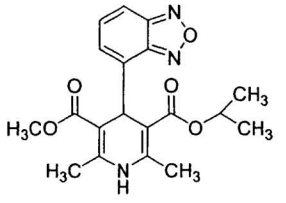
Abstract

The anodic behaviour of isradipine was studied using cyclic voltammetry (CV), direct current (DC_t) and differential-pulse (DPP) polarography. Isradipine exhibited well-defined anodic polarographic waves over the pH range of 4–8 in Britton-Robinson buffer (BRb). At pH 6, the analytical pH, the diffusion-current constant (Id) was 2.12 ± 0.42 (n = 4). The oxidation potential is – 0.10 V vs Ag/AgCl reference electrode with correlation coefficients of 0.9977 and 0.9948 in the DC_t and DPP modes, respectively. The current-concentration plots were rectilinear over the ranges 5-14 μg/ml and 2.4-12 μg/ml using the DC_t and DPP modes, respectively. The lower detection limit was 1.12 μg/ml (3.02×10^{-6} M) adopting the DPP mode. The proposed method was applied to commercial tablets and capsules, the % recoveries were 100.52 ± 1.267 and 99.4 ± 0.87 (n = 6) for the tablets and 101.3 ± 1.57 and 100.7 ± 2.10 (n = 6) for the capsules adopting both DC_t and DPP modes, respectively. The number of electrons involved in the electrode process was accomplished and a proposal of the electrode reaction was presented.

Keywords:

Isradipine, anodic polarography, dosage forms.

Introduction



Scheme 1: Structural formula of Isradipine.

Isradipine, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinecar-

boxylic, methyl, 1-methylethyl ester, is a calcium-channel blocking agent with properties similar to that of nifedipine. It is used in the treatment of hypertension and angina pectoris [1].

Isradipine is the subject of monographs in both the British Pharmacopoeia, BP [2] and the United States Pharmacopoeia, USP [3]. The BP [2] recommends spectrophotometric measurement for the raw material and liquid chromatography for the formulations. The USP [3] on the other hand, described liquid chromatography for both the drug and its formulations.

Reviewing the literature revealed that all the reported methods for isradipine in dosage forms and biological fluids rely on the use of chromatographic techniques such as thin layer chromatography (TLC) [4,5], GC [6-9] and HPLC [4-17]. Although chromatographic methods offer a high degree of specificity, yet, sample clean-up and the instrumentation limitations preclude their use in routine clinical studies. The proposed method was developed as an alternative substitute to the chromatographic methods, and the results obtained were promising. The presence of the oxidizable dihydropyridine ring structure in the molecular formula of isradipine initiated the present study. Recently, a voltammetric method based on the reduction of furazayl ring of isradipine has been proposed for its determination [18].

The proposed method is characterized by being stability-indicating, as it is based on the presence of dihydropyridine ring and the oxidation of the latter is the major pathway for the degradation of this class of compounds [19].

Experimental

Reagents and Materials

- Isradipine was kindly provided by Novartiz Pharma, Cork, Ireland, Batch # 19525 D and was used as received. Tablets and capsules containing isradipine were obtained from commercial sources in the local market. Lomir SRO capsules Batch # B1016 (5 mg of isradipine/capsule). Lomir tablets Batch # T1001 (2.5 mg of isradipine/tablet). Both are products of Novartis Pharma, AG, Basle, Switzerland.

- Britton-Robinson buffers (BRb) 0.08 M covering the pH range 2.1–12 [20].

- Standard solutions: A stock solution of isradipine (1.0 mg ml^{-1}) was prepared in methanol, and was further diluted with the same solvent to give the appropriate working standard solutions. The solutions are stable for one week if kept in the refrigerator.

- Methanol, AR grade (Aldrich, USA).
- Acetone (Winlab, U.K.).
- Mercury: AnalaR (BDH, Poole, UK).
- Nitrogen, 99.999% (Saudi Industrial Gas Co., Riyadh, Saudi Arabia).

Apparatus

The cyclic voltamograph (757 VA computrace) was obtained from Metrohm (Herisau, Switzerland). It consists of a hanging mercury drop electrode in the DPP mode, a saturated Ag/AgCl reference electrode and a platinum wire as the auxiliary electrode. Nitrogen gas was used for deoxygenation. Scan rate was 2 mV/sec and mercury drop size was 0.2 mm^2. Current range was 0–30 nA and potential range was –0.7 to –1.35 V. A computer driven HP Printer was used.

The polarographic study and the differential pulse polarographic (DPP) measurements (pulse amplitude 50 mV was applied) were carried out using the Polarecord E 506 Metrohm (Herisau, Switzerland). The drop-time of 1 second was electronically-controlled using a 505 Stand from the same company. The polarograms were recorded using a potential scan-rate of 10 $mV.sec^{-1}$. A three-electrode system composed of a Dropping Mercury Electrode (DME) as the working electrode, Ag/AgCl reference electrode, and a platinum wire as the auxiliary electrode, was used. The solutions were purged with pure nitrogen gas for 5 min before being polarographed at room temperature.

Procedures

Recommended Procedure

Transfer aliquots of isradipine working standard solution into a set of 25-ml volumetric flasks, so that, the final concentration is in the range cited in Tab. 2. Add sufficient methanol so that its content should be always 20% (v/v). Complete to volume (25 ml) with BRb of pH 6. Pass nitrogen gas for 5 min. Record the current in the DC_t and DPP modes within the range of –0.4 to + 0.4 V. Plot the produced current (in μA) in both the DC_t and DPP modes versus the final concentration of the drug (in μg/ml) to get the calibration graph. Alternatively, derive the corresponding regression equation.

Analysis of Commercial Tablets and Capsules

Empty the contents of 10 capsules (average weight 410 mg) or weigh and pulverize 20 tablets (average weight 86 mg), then mix the powder. Weigh accurately a quantity of the powder equivalent to 25 mg of isradipine and transfer into a 50-ml volumetric flask. Add about 40 ml of methanol (in case of the tablets) or acetone (in case of the capsules) and sonicate for half an hour. Filter into a 50-ml volumetric flask. Wash the residue and flask with the same solvent and transfer the washing into the same volumetric flask and complete to the mark with the same solvent. Transfer aliquot volumes containing isradipine over the concentration range in Tab. 2, 3–10 μg/ml and 5–12 μg/ml in the DPP mode and DC_t mode, respectively into a 25-ml volumetric flask. Adjust the volume to 5 ml with methanol. Complete to the mark with BRb of pH 6. Proceed as described under Recommended Procedure. Determine the nominal content of the tablets or capsules using either the calibration graph or the corresponding regression equation adopting both DC_t and DPP modes.

Results and Discussion

The cyclic voltammogram of isradipine (1×10^{-3}M) in BRb of pH 6 using a scan rate of 2.0 mV/sec. [Fig. 1]. The volammogram exhibited both cathodic and

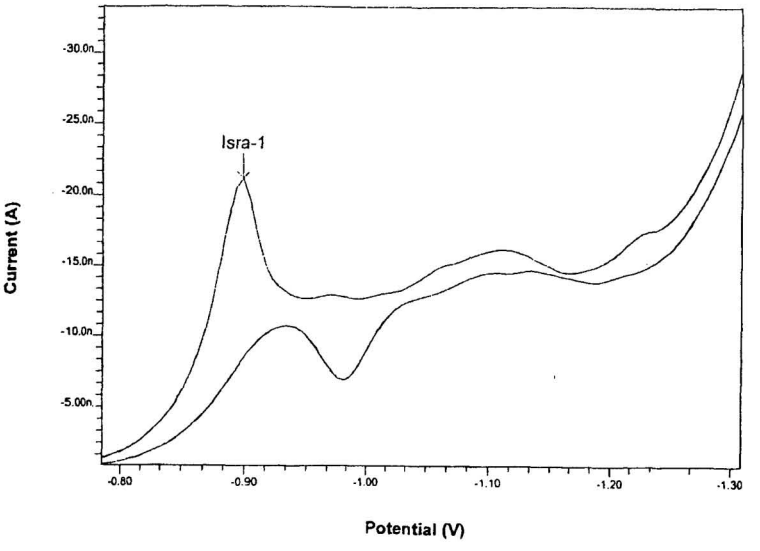


Fig. 1. Cyclic voltammogram of isradipine (1×10^{-3} M) in BRb of pH 6. Scan rate of 2.0 mV/sec.

anodic peaks. The principal anodic peak (with peak potential of –0.98 V) is accompanied with a cathodic peak (with peak potential of –0.90 V). The difference between the peak potentials is 80 mV. As the oxidation of the dihydropyridine nucleus involves the transfer of 2 electrons, it is clear that, the oxidation process is completely irreversible.

Influence of pH on the Anodic Waves

Fig. 2 shows typical DC_t and DPP polarograms of isradipine in BRb of pH 6. The effect of pH on the development of the anodic waves is shown in Fig. 3. Well-defined anodic waves were obtained over the pH range 4-8. Below pH 4 and above pH 8 no waves were developed. The waves were positively shifted upon increasing the pH of the medium. The relation between the half-wave potentials ($E_{1/2}$) of the anodic waves and pH is expressed by the following regression equation:

$$E_{1/2}\ (mV) = 191.8 - 18.1\ pH \quad .. \quad .. \quad .. \quad (R = 0.9955)$$

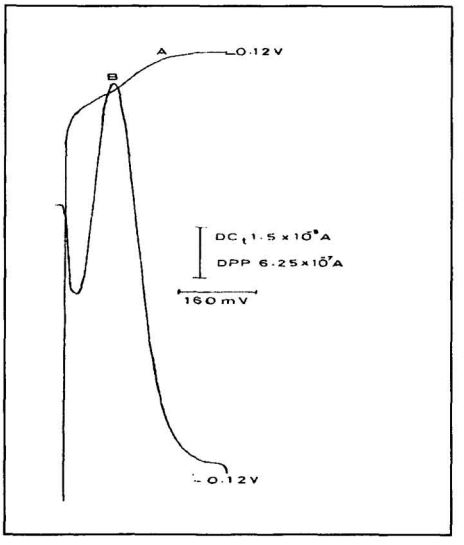


Fig. 2. Typical DC_t (A) and DPP (B) polarograms of isradipine (14 μg/ml) in BRb of pH 6. Potential scan rate 10 mV/sec and drop time: 1 sec.

Logarithmic analysis of the anodic waves 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 oxidation process is irreversible in nature. The αn_a values were calculated according to the treatment of Meites and Israel [21] and are listed in Tab. 1. The number of protons (Z_{H+}) involved in the rate-determining step of the reduction process was also calculated according to the following formula [22].

$$\Delta E_{1/2} / \Delta pH = - 0.059\, Z_{(H+)} / \alpha n_a$$

where α is the transfer coefficient and n_α is the number of electrons transferred in the rate-determining step. The small figures obtained for $Z_{(H^+)}$ (Tab. 1) point out to the irreversibility of the electrode process.

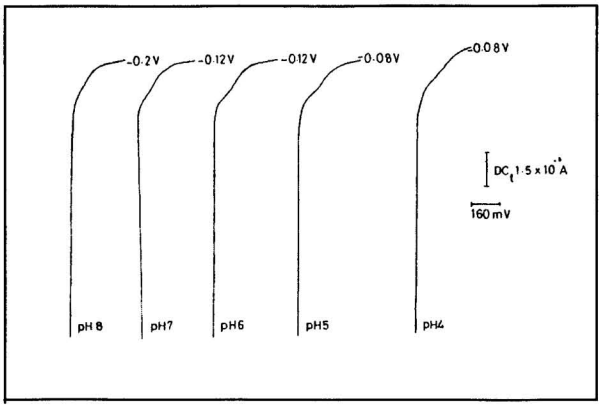


Fig. 3. Effect of pH on the development of the anodic weaves of isradipine (14 μg/ml) in BRb. Potential scan rate 10 mV/sec and drop time: 1 sec.

pH	$-E_{1/2}$ (mV)	$\Delta E_{1/2}/\Delta pH$	id/C	$W_{1/2}$ (mV)	αn_a	$Z_{(H^+)}$
4	120		2.44	92	0.67	
		20				0.23
5	100		2.48	88	0.61	
		14				0.14
6	86		2.57	76	0.77	
		22				0.29
7	64		2.52	80	0.73	

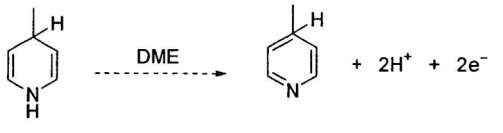
Tab. 1. Effect of pH on the development of anodic waves of Isradipine.

Study of the Wave Characteristics

Increasing the mercury reservoir height (h) resulted in a corresponding increase in waveheight (W); a plot of W vs $\sqrt{h}$ gave a straight line. A plot of Log W vs Log h gave a straight line with a slope of 0.69. Changing the buffer concentration over the range 0.01–0.06 M (final concentration) resulted in a negligible increase in waveheight. These two characteristics point out to a diffusion-controlled process partially affected by adsorption phenomenon as indicated by the slightly high value of the slope (0.69). However, addition of methanol (20%, v/v) to the electrolysed solution decreases the effect of the adsorption process. The diffusion coefficient (D) of isradipine was determined in BRb of pH 6 according to Ilkovic equation [23] and was found to be 1.33×10^{-5} cm^2/sec. This small value may be attributed to the bulky nature of the molecule.

Number of Electrons Involved in the Oxidation Process

The number of electrons transferred during the electro-oxidation process was estimated through comparing the waveheight of isradipine with that obtained from an equimolar solution of an earlier studied compound of the same chemical group (1,4-dihydropyridine) and of nearly identical value of diffusion coefficient, that is nifedipine [24]. In BRb of pH 6, both compounds gave one wave of the same height corresponding to the consumption of 2 electrons (nifedipine gave an additional ill-defined anodically-shifted wave). The first wave of nifedipine corresponds to the consumption of 2 electrons. It is evident from the experimental results for isradipine that a slow electron-transfer reaction is involved in the oxidation process. Logarithmic analysis of the waves established that two electrons are involved in the rate-determining step of the anodic wave, and the shift in $E_{1/2}$ potentials with increasing pH indicates that two protons are consumed in this step. Based on these facts, and depending on the presence of the dihydropyridine ring structure, the following pathway for the electrode reaction may be postulated (Scheme 2):



Scheme 2: Proposed pathway of the electrode oxidation reaction.

Analytical Applications

DC_t polarograms of isradipine in BRb of pH 6 exhibit well-defined anodic waves. No polarographic maxima were developed; therefore, no maximum

suppressor was needed. The current is mainly diffusion-controlled and proportional to the concentration of the analyte over the concentration range 5-14 μg/ml and 2.4-12 μg/ml for DC_t and DPP modes, respectively. At that pH value, the DC_t wave was the steepest and the peak (in the DPP mode) had the least half-peak width ($W_{1/2}$) as shown in Tab. 1.

Solutions of isradipine in methanol were found to be stable for at least one week if kept away from light in the refrigerator. In BRb of pH 6 (the analytical pH) the solutions were found to be stable for at least three hours. The relation between each of the limiting diffusion-current (id) in the DC_t mode and the peak current (ip) in the DPP mode, and the concentration of isradipine (μg/ml) is rectilinear over the ranges indicated in Tab. 2. Data on the analytical performance of the proposed method (parameters of the regression equations, correlation coefficients, minimum detectability (taking S/N = 2), and diffusion-current constant, Id) are compiled in the same table.

Statistical analysis of the two regression equations was presented in terms of the standard deviation of the residuals ($S_{x/y}$), standard deviation of the slope (S_b), and standard deviation of the intercept (S_a) gave the values cited in Tab. 2. The small values of all these parameters point out to the high precision of the method [25]. The good linearity of the calibration graph and the negligible scatter of the experimental points are clearly evident by the correlation coefficients (close to 1 in both cases). Toestablish the reproducibility of the electrode response, six replicate concentrations were tested at isradipine concentrations of 6, 8 and 10 μg/ml adopting the DC_t mode. Mean current values of 0.1844 ± 0.004; 0.2000 ± 0.0042; and 0.2678 ± 0.0089 μA, respectively were obtained. The precision of these

Parameter	DC_t mode	DPP mode
Concentration range (μg/ml)	5.0–14	2.4–12
Regression equation	id = 0.1767 + 0.0092 C	ip = 0.0286 + 0.01 C
Correlation coefficient	0.9977	0.9948
Diffusion-current constant (Id)	2.12 + 0.42	4.37 ± 0.63
Lower limit of detection	—	1.12 μg/ml (3.02×10^{-6} M)
$S_{y/x}$	2.0×10^{-3}	3.67×10^{-3}
S_a	2.43×10^{-3}	2.73×10^{-3}
S_b	2.58×10^{-4}	4.23×10^{-4}

Tab. 2. Analytical performance data of the proposed anodic polarographic methods.

measurements is expressed by the relative standard deviations (RSD) of 2.17; 2.10, and 3.32, respectively. These small values indicate a highly precise electrode response.

Both DC_t and DPP modes were successfully applied to the determination of isradipine in commercial tablets and capsules. The percentage recoveries based on 6 separate determinations are abridged in Tab. 3. The results are in good agreement with the label claim. Both DC_t and DPP modes proved to be equally useful; however, the DPP mode is more convenient.

The proposed method can be considered as a stability-indicating assay for isradipine as it is based on the presence of the dihydropyridine ring structure, the latter is subjected to atmospheric oxidation and loss of activity [19].

Conclusion

A simple and reliable stability-indicating polarographic method was developed for the determination of isradipine in dosage forms. The lower detection limit (3.02×10^{-6} M) is comparable to that reported by chromatographic methods. As applied to tablets or capsules, the method is very simple and time saving. The proposed method can be considered as stability-indicating assay of the drug.

Preparation	% Recovery ± SD**		Reference method (18)	
	DC_t	**DPP**	**DC_t**	**DPP**
1. Lomir tablets (2.5 mg isradipine/table)	100.52 ± 1.267 t = 0.293 F = 3.920	99.4 ± 0.87 t = 0.488 F = 1.999	100.35 ± 0.66	99.65 ± 1.21
2. Lomir SRO capsules (5 mg isradipine/capsule)	101.3 ± 1.57 t = 1.716 F = 1.389	100.7 ± 2.10 t = 0.034 F = 4.239	99.63 ± 1.85	100.77 ± 0.99

Tab. 3. Application of the proposed polarographic method to the analysis of commercial tablets and capsules.

*Both tablets and capsules are products of Novartis Pharma, AG, Basle, Switzerland.

**Each result is the average of 6 separate determinations.

The theoretical values of t- and F-test at 95.0% confidence limit are 2.20 and 5.05, respectively.

References

[1] Parfitt K. (Ed.).
Martindale, The Complete Drug Reference 33rd Ed.
The Pharmaceutical Press, Massachusetts 2002, 916–18.

[2] The British Pharmacopoeia.
The Pharmaceutical Press, London 2000, 768–44.

[3] The United States Pharmacopoeia.
The United States Pharmacopeial Convention, Rockville, MD 2001, 941–47.

[4] Mieclarek J.
Normal-phase TLC separation of enantiomers of 1,4-dihydropyridine derivatives.
Drug Dev. Ind. Pharm. 2001; 27: 175–79.

[5] Abd-Elghany M.F., Abd-Elkawy M., El-Zeany B., Stewart J.T.
A stability-indicating high performance liquid chromatographic quantitation of isradipine in the presence of its degradation products.
J. Planar Chromatogr. Mod. TLC 1996; 9: 290–92.

[6] Maurer H.M., Arit J.W.
Screening procedure for detection of dihydropyridine calcium channel blockers metabolism in urine as part of a systemic toxicological analysis procedure for acidic compounds by gas-chromatography (GC-MS) after extractive methylation.
J. Anal. Toxicol. 1999; 23: 73–80.

[7] Jean C., Laplanche R.
Assay of isradipine and of its major metabolites in biological fluids by capillary gas chromatography and chemical-ionisation mass spectrometry.
J. Chromatogr. B. 1988; 72: 61–69.

[8] Christensen H.R., Angelo H.R., Skajaa K.
Determination of isradipine and its metabolite in serum by capillary column gas chromatography with nitrogen selective detection.
J. Chromatogr. B. 1992; 112: 161–65.

[9] Rask H.S., Angelo H.R., Christensen H.R.
Enantioselective determination of isradipine in human serum using chiral stationary phase liquid chromatography and gas chromatography with nitrogen selective detection.
Chirality, 1998; 10: 808–812.

[10] Tesarova E., Gilar M., Hobza P., Kabelac M., Deyl Z., Smolkova-Keulemanosova E.
Correlation between structure of dihydropyridine calcium antagonists and their retention behaviour and enantioseparation on the β-cyclodextrin stationary phase in HPLC.
J. High Reslut. Chromatogr. 1995; 18: 597–601.

[11] Okamoto Y., Aburatani R., Hata K., Honda M., Inotsume N., Nakano M.

Optical resolution of dihyropyridine enantiomers by high performance liquid chromatography using phenylcarbamates as a chiral stationary phase.
J. Chromatogr. 1990; 513: 375–78.

[12] Takamura K., Kusus F., Abdel-Wadood H., El-Rabbat N., Saleh G., Refaat I.
Redox properties of isradipine and its electrochemical detection in the HPLC determination of the compound in human serum.
Biomed. Chromatogr. 2000; 14: 453–58.

[13] Bartlett M.G., Spell J.C, Mathis P.S., Abdel-Ghany M.F., El-Zeany B.E., Abdel-Kawy M., Stewart J.T.
Determination of degradation products from calcium-channel blocker isradipine.
J. Pharm. Biomed. Anal. 1998; 18: 335–45.

[14] Abdel-Ghany M.F., El-Zeany B.E., Abdel-Kawy M., Stewart J.T.
A stability-indicatng high performance liquid chromatographic assay of isradipine in pharmaceutical preparations.
Anal. Lett. 1996; 29: 1157–1165.

[15] Kuesters E., Dosenbach C., Geber G.
Enantiomeric separation of isradipine and related compounds by liquid chromatography with a chiral stationary phase.
J. High Resolut. Chromatogr. 1991; 14: 769–771.

[16] Boutagy J., Ramble F., Dunagan F.
Determination of isradipine and the oxidative pyridine metabolite in human plasma by high performance liquid chromatography.
J. Chromatogr. 1989; 79: 483–88.

[17] Al-Suwyeh S.A.,
Quick, simple and sensitive HPLC method for the determination of isradipine in plasma and its application in pharmacokinetic studies.
Anal. Lett. 2002; 35: 1205–1213.

[18] Belal F., Al-Majed A., Julkhuf S.
Voltammetric determination of isradipine in dosage forms and spiked human urine and plasma.
J. Pharm. Biomed. Anal. 2003; 31: 989–98.

[19] Albini A. and Fasani E., editor,
Drugs Photochemistry and Photostability.
The Royal Society of Chemistry, Cambridge 1998, 21–23.

[20] Heyrovsky J., Zuman P.
Practical Polarography.
Academic Press, New York, 1965, 163.

[21] Meites L., Israel Y.
The calculation of electrochemical kinetic parameters from polarographic current-potential curves.
J. Am. Chem. Soc. 1961; 83: 4903–4907.

[22] Proszt J., Cieleszky V., Györbiro K.
Polarographie.

Akademiai Kiado, Budapest 1967, 385–91.
[23] Heyrovsky J., Kuta J.
Principles of Polarography.
Academic Press, New York, 1968, 163–65.
[24] Squella J.A., Barnafi E., Perna S., Nunez-Vergara L.J.
Nifepidine: differential pulse polarography and photodecomposition.
Talanta, 1989; 36: 363–367.
[25] Miller J.C., Miller J.N.
Statistics for Analytical Chemistry.
John Wiley and Sons, New York 1983, 83–87.

Received May 18th, 2003
Accepted February 2nd, 2004