Received: 26 June 2010 Revised: 26 August 2010 Accepted: 26 August 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.200

Voltammetric assay of anti-anginal drug nicorandil in different solvents

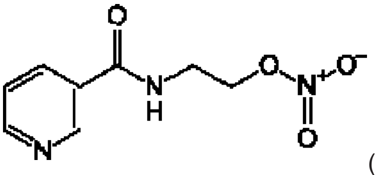
Rajeev Jain* and Rajeev Kumar Yadav

Voltammetric behaviour and assay of nicorandil were investigated using square-wave and cyclic voltammetry. Voltammograms in Britton-Robinson (BR) buffer exhibited one well-defined and two merged reduction peaks. The influence of different buffers, electrolytes, pH, scan rates, and concentration of the drug on cathodic peak current was studied. On the basis of electrochemical behaviour of nicorandil, a direct square-wave voltammetric procedure for quantitation of nicorandil has been developed and validated. The proposed square-wave voltammetric method allows quantitation over the range 12.5–62.5 μg mL^{-1} with correlation coefficient of 0.992. The limit of quantification and limit of detection were 21.95 μg mL^{-1} and 6.58 μg mL^{-1}, respectively. Precision and accuracy were also checked and found within limits. The developed square-wave method has also been successfully applied for the determination of nicorandil in pharmaceutical formulations. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: Voltammetry; Nicorandil; Assay; Glassy Carbon Electrode; Pharmaceutical formulation

Introduction

Nicorandil is an orally efficacious anti-anginal drug (A).[1] It belongs to the class of compounds known as potassium channel activators which are characterized by their arterial vasodilator properties.


(A)

Nicorandil has venodilating properties which are attributable to a nitrate group in its chemical structure.[2] A literature survey reveals that a few methods are reported for assay of nicorandil in bulk forms, dosage forms, and biological matrix. Monographs of drug substance and drug product are not mentioned in US and British pharmacopoeia.[3,4] A spectrophotometric method has been reported for nicorandil determination. The method is based on the reduction of the nitroxy ethyl group of nicorandil into carbonyl compound and nitrite ion by NH_4Cl and Zn dust. The nitrite ion thus formed reacts with potassium iodide and starch in dilute HCl medium to form a blue product, which absorbs at 550 nm.[5] Another spectrophotometric method is based on the reaction of nicorandil with sulphanilic acid reagent in presence of cyanogen bromide solution giving yellow chromogen, which shows maximum absorbance at 460 nm.[6]

A fluorometric method using HPLC postcolumn UV detection for determination of nicorandil in plasma has been reported.[7] Other methods involve solid-phase extraction of the drug from plasma, separation by HPLC, detection with photoconductivity and tandem mass spectrometry have also been reported.[8,9]

Electro-analytical techniques have proven to be useful for selective and sensitive quantitation of many drugs owing to their excellent specificity, sensitivity, speed of analysis and relative low cost and, therefore, have been used for the determination of active pharmaceutical ingredients in bulk, dosage forms, and biological matrices. These features make electro-analytical techniques suitable for the determination of trace determination of environmentally and biologically important compounds.[10–20] Electrochemical behaviour of drugs can also give insights into their metabolic pathway or their *in vivo* redox processes or pharmacological activity.[21]

There appears to be no comprehensive electro-analytical method for the determination of the drug nicorandil in bulk form and pharmaceutical dosage forms. In the present paper, voltammetric behaviour and quantitation of nicorandil at a glassy carbon electrode using cyclic and square-wave voltammetry has been reported.

Experimental

Reagents and materials

Nicorandil standard (98.1% purity) was obtained from local bulk drug supplier. Tablets containing nicorandil (*Zynicor* ®) labeled 5 mg were obtained from commercial source. Ultrapure water obtained from Milli-Q purification system (Millipore Corp., Milford, MA, USA) was used throughout the studies. KCl (0.1 M) solution used as supporting electrolyte. All chemicals used were of analytical reagent grade quality and were employed without further purification.

Instrumentation

All voltammetric measurements were performed with Micro auto lab type III (Eco-Chemie BV, Utrecht, the Netherlands)

---

* *Correspondence to: Rajeev Jain, School of Studies in Chemistry, Jiwaji University, Gwalior-474 011, India. E-mail: rajeevjain54@yahoo.co.in*

School of Studies in Chemistry, Jiwaji University, Gwalior-474011, India

potentiostat-galvanostat with 757VA computrace software. The three-electrode system consisted of a glassy carbon working electrode, Ag/AgCl (3M KCl) reference electrode and a graphite rod as auxiliary electrode. 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. All voltammetric measurements were carried out at ambient temperature.

Preparation of standard and test solutions

Stock solutions of nicorandil standard and test (1000 µg mL^{-1}) were prepared in different solvents and surfactants. Ten tablets were ground to fine powder; a sufficient amount of powder for preparing a stock solution of 1000 µg mL^{-1} was weighed, transferred into 10 mL volumetric flask and made up to the volume. Solution was sonicated for approximately 10 min and than centrifuged. Clear supernatant liquid was withdrawn. Stock solutions were protected from light and stored at 1–10 °C. All voltammograms were recorded by mixing BR buffer, 0.1 M KCl and stock solution.

Results and Discussion

Nicorandil yielded a cathodic peak at – 1.82 V which is accompanied by two merged smaller peaks at lower potentials (−1.22 and −1.42 V). The peak at −1.82 was developed, optimized, and found suitable for analytical purposes. Instrumental variables for quantitative determination of nicorandil were examined and square-wave voltammetric method was found to have higher sensitivity in comparison to other electro-analytical techniques.

Optimization of various operational parameters

To establish the optimum conditions for the quantitation of nicorandil various experimental variables like pH, solvents, frequencies, supporting electrolytes, etc., were also examined.

Effect of pH

The pH of the solution had a significant influence on the cathodic peak current response and peak potential of nicorandil. Peak potential shifted towards negative potential with rise in pH. It could be explained as a consequence of protonation involved in the reduction process which is facilitated at lower pH values. The maximum peak current was observed at pH 2.5 in BR buffer but stable and well defined peak could be observed at pH 12.0 (Figure 1). Therefore pH 12 was chosen for the electro-analytical assay of the drug.

Selection of appropriate solvent for stock solution of nicorandil

Solvent for stock solution was selected on the basis of current response, stability, and shape of nicorandil peak. Results depicted in Figure 2 clearly indicate the significant increment in response

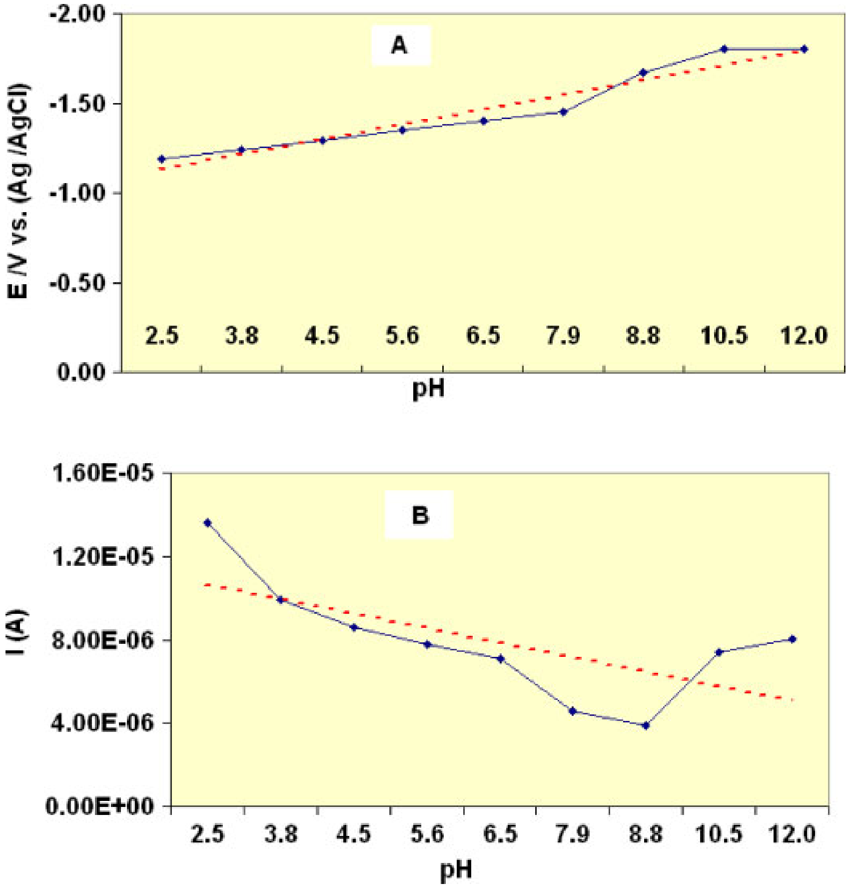


Figure 1. (A) Influence of pH on the reduction of nicorandil (50 µg mL^{-1}) in BR buffer (pH 2.5–12) after 10 s equilibration time; frequency (f) = 60 Hz. (B) Influence of pH on peak current I (A) of nicorandil (50 µg mL^{-1}) in BR buffer (pH 2.5–12) after 10 s equilibration time; frequency (f) = 60 Hz.

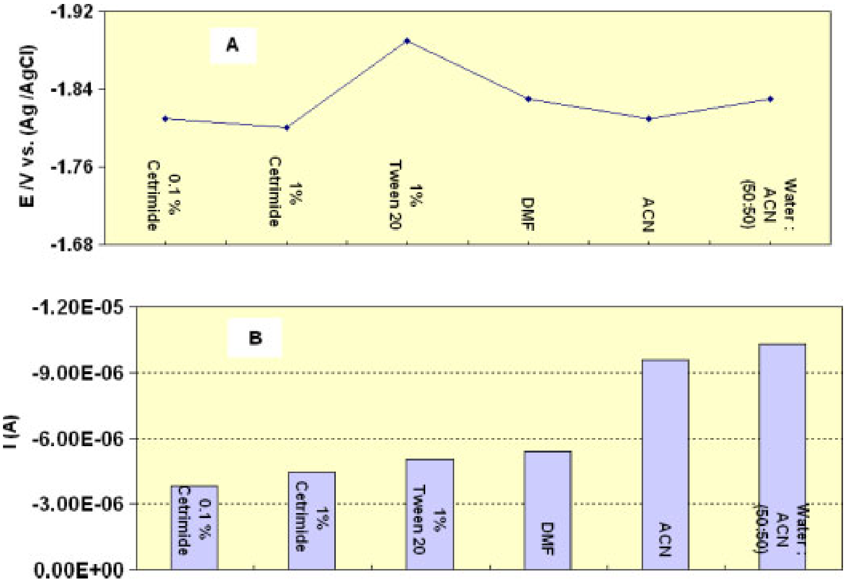


Figure 2. (A) Peak potential of nicorandil (50 µg mL^{-1}) in different solvents and surfactants (0.1% Cetrimide, 1% Cetrimide, 1% Tween 20, DMF, ACN and water: ACN (50 : 50). (B) Peak current I (A) of nicorandil (50 µgmL^{-1}) in different solvents and surfactants (0.1% Cetrimide, 1% Cetrimide, 1% Tween 20, DMF, ACN and Water: ACN (50 : 50).

Table 1. Operational parameters of proposed square-wave voltammetric procedure

Parameters	Value
Buffer	BR buffer
pH	12.0
Purge time (s)	30
Stirring rate (rpm)	2000
Equilibration time (s)	10
Frequency (Hz)	60

of nicorandil in water: acetonitrile (50 : 50) mixture. On comparing the response of nicorandil in organic solvents and in the presence of surfactants (0.1% Cetrimide, 1% Cetrimide and 1% Tween 20), it is observed that nicorandil shows substantial decrease in peak current in all other solvents tested, and in the presence of surfactants (Figure 2).

Operational parameters of proposed square-wave voltammetric methodology have been optimized and tabulated (Table 1).

Electrochemical behaviour of nicorandil

Nicorandil gave a cathodic peak at −1.75 V in BR buffer (pH 12.0) accompanied with a shoulder at lower potential. Absence of peak on reverse scan indicates irreversibility of the electrode process. Sweep rate was varied from 0.1 to 0.9 mV/s and a linear increment pattern was found between sweep rate and current (Figure 3).

Method validation

Quantitative evaluation is based on the linear correlation between the peak current and concentration of analyte in square-wave voltammetry. Validation of the procedure for determination of nicorandil in tablets was performed as per ICH guidelines.[22]

Specificity

For the specificity test, voltammograms of solution of excipients such as colloidal silicon dioxide, lactose anhydrous, and stearic acid were recorded at optimized conditions. No interference was observed at reduction potential of nicorandil. Further, the response of the standard solution (50 µg mL^{-1}) with and without excipients was compared. No significant change was observed. Therefore, excipients as majority compound did not interfere with the quantitation of nicorandil in commercial tablet samples.

Linearity and range

Linearity was performed by standard addition method. Linearity of response for nicorandil was determined in the range of 12.5–62.5 µg mL^{-1}. The calibration curve was plotted using peak current versus concentration and found to be linear over the concentration range. Figure 4A indicates that the response is linear over the range. Extended linearity (linearity at higher concentration) experiment was also performed that show the significant dilution of the sample before the measurements can also play an important role in improvement of linear behaviour. On the other hand, higher concentration of nicorandil shows non linear behaviour (Figure 4B).

Sensitivity/detection limit

The limit of detection (LOD) was calculated by Eqn (1):

$$\mathrm{LOD} = 3S_a/b \tag{1}$$

where S_a is the standard deviation of intercept and b is the slope of the regression line. The calculated limit of detection of square-wave voltammetric method is 6.58 µg mL^{-1}. The peak could not be distinguished from the noise at concentration lower than LOD.

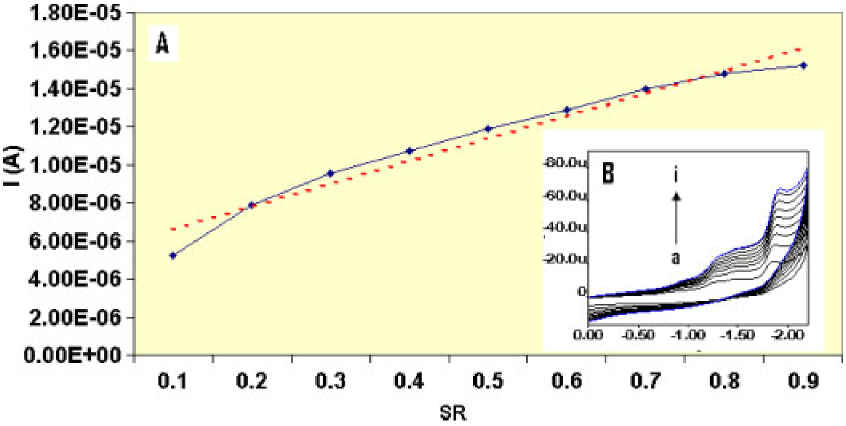


Figure 3. (A) Linear increment in response of nicorandil (300 µg mL^{-1}) at different sweep rate in cyclic voltammogram. (B) Cyclic voltammograms of standard solution containing nicorandil (300 µg mL^{-1}) at different sweep rate (a-0.1, b-0.2, c-0.3, d-0.4, e-0.5, f-0.6, g-0.7, h-0.8 and i-0.9).

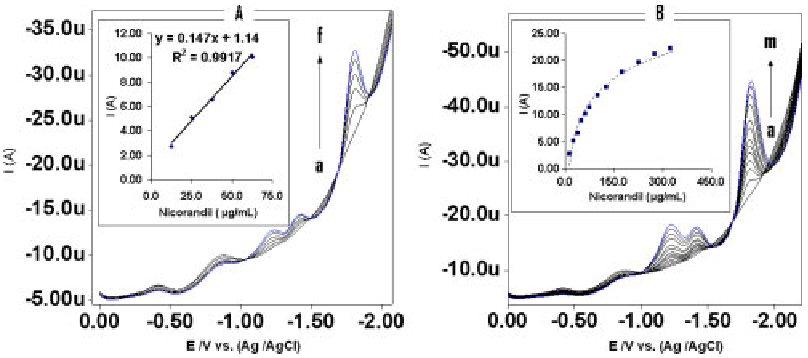


Figure 4. (A) Square-wave voltammograms of nicorandil at different concentrations (a) Blank solution (b) 12.5 µg mL^{-1} (c) 25.0 µg mL^{-1}(d) 37.5 µg mL^{-1}(e) 50.0 µg mL^{-1}(f) 62.5 µg mL^{-1}. Inset picture represents linearity curve. (B) Extended linearity experiment of nicorandil at different concentrations (a) Blank solution (b) 12.5 µg mL^{-1} (c) 25.0 µg mL^{-1}(d) 37.5 µg mL^{-1}(e) 50.0 µg mL^{-1}(f) 62.0 µg mL^{-1} (j) 75.0 µg mL^{-1} (h) 100.0 µg mL^{-1} (i) 125.0 µg mL^{-1} (j) 175.0 µg mL^{-1} (k) 225.0 µg mL^{-1} (l) 275.0 µg mL^{-1} (m) 325.0 µg mL^{-1}. Inset picture represents non linear behaviour at higher concentration.

The limit of quantitation (LOQ) was estimated by Eqn (2):

$$\mathrm{LOQ} = 10\,S_a/b \qquad (2)$$

where S_a is the standard deviation of intercept and b is the slope of the regression line. The lower limit of quantitation of square-wave voltammetric method is 21.95 µg mL^{-1}.

Precision

System precision. Six replicate square-wave voltammetric readings of solution of nicorandil were taken. % RSD of six replicate readings was calculated. Data in Table 2 show an acceptable system precision for the procedure.

Method precision. Six samples of single batch of nicorandil tablets of 5 mg strength were prepared and analyzed as per proposed method. The % RSD value indicates that this procedure has an acceptable method precision (Table 2).

Accuracy

Different amounts of various excipients were taken and spiked with a known amount of nicorandil standard at three different concentration levels each in duplicate. Solutions were prepared and analyzed as per procedure. Mean % recoveries were calculated. Results indicate that the method has acceptable level of accuracy (Table 2).

Stability of nicorandil in analytical solution

Standard and test solutions were prepared and kept at room temperature as well as 1–10 °C. The standard and sample solutions were analyzed initially and at different time points. Cumulative %RSD of 5 time points up to 5 h shows that the solutions are stable at 1–10 °C up to 5 h (Table 2).

Robustness

The robustness was examined by evaluating the influence of small change of some of the important method parameters including strength of KCl, nitrogen purging, equilibration time, pH of BR buffer, etc. Test solution was prepared, analyzed under each condition and assay of nicorandil determined. The obtained results show the reliability of the proposed procedure for the assay of nicorandil and hence it can be considered robust (Table 2).

Analytical application of proposed square-wave voltammetric method

The optimized electro-analytical method was successfully applied for determination of nicorandil in pharmaceutical formulation.

Table 2. Analytical validation parameters

Parameters	Value
Linearity	
Slope	*0.147*
Standard deviation	*0.008*
Intercept	*1.140*
Standard deviation	*0.323*
Correlation coefficient	*0.992*
Standard error of estimation	*0.308*
Sum of squares of regression	*33.782*
Sum of squares of residuals	*0.284*
Accuracy	
Recovery Level-1	*94.21 (% Recovery)*
Recovery Level-2	*103.31 (% Recovery)*
Recovery Level-3	*108.30 (% Recovery)*
Precision	
System Precision	*2.76 (%RSD)*
Method Precision	*4.83 (%RSD)*
Stability in Analytical Solution	
Standard Solution	*1.97 (Up to 5 HRS)*
Sample Solution	*2.09 (Up to 5 HRS)*
Robustness	*4.79 (Overall %RSD)*

Table 3. Determination of nicorandil in pharmaceutical formulation

Labeled amount (mg per tablet)	Found amount (mg per tablet)*	% RSD**
5	4.90	2.03

* Mean of three determinations.
** Relative standard deviation of three determinations.

No tedious extraction or filtration procedures have been applied during sample preparation. Only dilution of aliquot from the supernatant layer with the supporting electrolyte (BR buffer pH 12) is required before measurement. Results are tabulated in Table 3.

Conclusion

The electrochemical behaviour of nicorandil was studied first time at glassy carbon electrode by square-wave and cyclic voltammetry. A new square-wave voltammetric method for determination of nicorandil based on its reduction process is developed and validated. The results show that it is a useful technique for the determination of nicorandil in formulations with adequate precision and accuracy. The method is rapid, less expensive, and saves time. Consequently, the proposed method has the potential to be a good analytical alternative for determination of nicorandil in pharmaceutical formulation.

References

[1] M. Kinoshita, K. Sakai, *Cardiovasc. Ther.* **1990**, *4*, 1075.
[2] J. Frampton, M. M. Buckley, A. Fitton, *Drugs* **1992**, *44*, 625.
[3] *United States Pharmacopoeia USP24*, 12601 Twinbrook Parkway: Rockville, MD, USA, **1999**.
[4] *European Pharmacopoeia, 6th edn.* (Suppl. 6.2), Council of Europe: Strasbourg, France, **2007**.
[5] N. Rahman, Y. Ahmad, N. H. A. Syed, S. A. J. Sulaiman, *J. Chin. Chem. Soc.* **2008**, *55*, 367.
[6] C. N. Patel, S. A. Patel, M. M. Patel, *Indian J. Pharm. Sci.* **2005**, *67*, 103.
[7] M. Ken-ichi, N. Yuki, S. Rieko, S. Sigemi, I. Fumio, W. Mitsuo, *J. Chromatogr. B* **1996**, *679*, 155.
[8] F. J. Schwende, R. C. Lewis, *J. Chromatogr.* **1990**, *525*, 151.
[9] B. Jignesh, J. Arvind, S. Bhavin, S. Raghavendra, K. Sandeep, S. Gunta, S. Sadhana, *Biomed. Chromatogr.* **2006**, *20*, 864.
[10] A. J. Bard, L. R Faulker, *Electrochemical Methods: fundamentals and applications*, John Wiley & Sons Inc.: New York, **2002**.
[11] H. Lund, M. M. Baizer, *Organic Electrochemistry: An Introduction and Guide*, John Wiley & Sons Inc.: New York, **2002**.
[12] F. Ibrahim, N. El-Enany, *J. Pharm. Biomed. Anal,* **2003**, *32*, 353.
[13] O.A. Razak, *J. Pharm. Biomed. Anal.* **2004**, *34*, 433.
[14] R. Jain, N. Jadon, K. Radhapyari, *J. Coll. Inter. Sci.* **2007**, *314*, 572.
[15] R. N. Goyal, V. K. Gupta, S. Chatterjee, *Talanta* **2008**, *76*, 663.
[16] R. N. Goyal, V. K. Gupta, N. Bachheti, R. A. Sharma, *Electroanalysis* **2008**, *20*, 757.
[17] S. Issac, K. G. Kumar, *Drug Test. Analysis* **2009**, *1*, 350.
[18] R. Jain, A. Dwivedi, R. Mishra, *Langmuir* **2009**, *25*, 10364.
[19] R. Joseph, K. G. Kumar, *Drug Test. Analysis* **2010**, *2*, 278.
[20] R. Jain, R. K. Yadav, A. Dwivedi, *Colloids Surf. A* **2010**, *359*, 25.
[21] J. M. Kauffmann, J. C. Vire, *Anal. Chim. Acta* **1993**, *273*, 329.
[22] ICH topic Q2(R), Validation of analytical procedures: methodology. ICH Harmonized Tripartite Guidelines, November 6, 1996, CPMP/ICH/281/95, November, **2005**.