ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1127–1131

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Polarographic determination of prenalterol hydrochloride through treatment with nitrous acid

F.A. Aly, F. Belal*, M.I. Walash

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt

Received for review 3 January 1995

Abstract

A simple and sensitive polarographic method is described for the determination of prenalterol hydrochloride through treatment with nitrous acid. The different experimental parameters affecting the derivatization process, as well as the polarographic analysis were studied. The derivatization product was found to be reducible at the dropping mercury electrode over the whole pH range in Britton–Robinson buffers. At pH 5, a well defined diffusion-controlled cathodic wave was produced. The limiting current vs. the concentration plot was linear over the range 0.015–0.15 mM in the direct current mode, with a minimum detectability of 0.8 μM. The proposed method was applied successfully to the determination of prenalterol hydrochloride either in the pure form or in its dosage forms.

Keywords: Pharmaceutical analysis; Polarography; Prenalterol hydrochloride

1. Introduction

Prenalterol hydrochloride is a sympathomimetic agent with stimulant effects on the beta-adrenoceptors. It has an inotropic action on the heart with relatively little chronotropic effect. It is used in the treatment of heart failure associated with myocardial infarction, open heart surgery and shock [1].

In spite of the clinical importance of prenalterol hydrochloride, few methods have been described for its determination and no official methods for its assay have been reported. A review of the literature revealed that prenalterol hydrochloride content was determined in raw material and in certain dosage forms by colorimetric [2–4], spectrophotometric and fluorimetric methods [2]. In biological fluids, it was determined by gas–liquid chromatography [5] and high-performance liquid chromatography [6–8]. Polarographically, prenalterol was determined by a similar procedure but employing the DPP mode; the concentration range was 2–12 μg ml^{-1} [3].

Functionalization polarography, i.e. the conversion of an electro-inactive compound into an active one, is achieved by the introduction of an electro-active group through chemical reactions. These reactions occur selectively, rapidly and with a yield of nearly 100% [9]. Several pharmaceutical compounds have been assayed through this approach [10–14].

In the present work, prenalterol hydrochloride, which is polarographically inactive, was determined polarographically through treatment with nitrous acid.

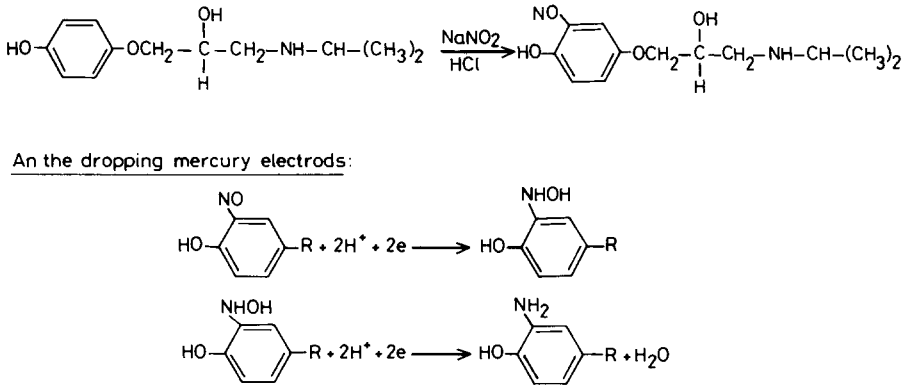
2. Experimental

2.1. Apparatus

The polarographic study was carried out using the Polarecord E 506 and a 663 VA

* Corresponding author.

SSDI 0731-7085(95)01525-6



Scheme 1. Proposal for the polarographic reduction of prenalterol hydrochloride after treatment with nitrous acid.

polarographic stand (Metrohm, Herisau, Switzerland). The polarograms were recorded at a potential scan rate of 100 mV per 10 s. A three-electrode system, composed of a dropping mercury electrode as the working electrode, an Ag°/AgCl reference electrode and a graphite rod as the auxiliary electrode, was used. The dropping mercury electrode had the following characteristics: $m = 1.979$ mg s^{-1}, $t = 2.65$ s per drop. The effect of the mercury height was studied using a Sargent–Welch Polarograph Voltammetric Analyzer (Sargent–Welch Scientific Co., USA), equipped with an Ag°/AgCl reference electrode and a platinum wire as auxiliary electrode.

2.2. Reagent and materials

The following reagents were used: hydrochloric acid (Prolabo, France), 0.1 M solution; sodium hydroxide (Prolabo, France), 0.1 M aqueous solution; sodium nitrite (Merck, Germany), 2% solution; ammonium sulphamate (Fluka, Switzerland), 5% solution; Britton–Robinson buffers 0.08 M, pH 2.09–11.98 [15]; prenalterol hydrochloride (Boehringer Ing., Germany), dosage forms containing prenalterol hydrochloride being purchased from commercial sources.

2.3. General procedure

A 1.5×10^{-3} M solution of prenalterol hydrochloride was prepared in water. Portions (0.25–2.5 ml), accurately measured, were transferred into separate 25 ml volumetric flasks. One millilitre of 0.1 M hydrochloric acid followed by 1 ml of 2% sodium nitrite solution were added, and the mixture shaken well for 2 min. Two millilitres of ammonium sulphamate solution were then added and shaken well until no more nitrogen was evolved. One millilitre of 0.1 M sodium hydroxide was added and the reaction mixture diluted to the mark with buffer solution pH 5.0. The whole solution was transferred into the polarographic cell. Nitrogen gas was passed through the solution for 5 min. The polarograms were recorded in the potential range from 0.0 to −1.4 V vs. Ag°/AgCl electrode.

2.4. Procedure for tablets

Twenty tablets were weighed and powdered. An accurately weighed amount of the powder equivalent to 20 mg of prenalterol hydrochloride was transferred into a small conical flask. Extraction with 3×30 ml portions of water was performed. The contents were then transferred into a 100 ml standard flask and diluted to the mark with water. Two millilitres of this solution were transferred into a 25 ml standard flask and the above procedure then followed. The nominal content of the tablets was calculated either from a previously plotted calibration graph or using the regression equation. Alternatively, the concentration was determined by a comparison with the waveheight obtained from a simultaneously prepared standard.

2.5. Procedure for ampoules

The contents of 20 ampoules were mixed. Twenty millilitres of the mixed solution were transferred into a 100 ml calibrated flask and made up to the mark with water. Two millilitres of this solution were then transferred into a 25 ml standard flask and the above-described procedure followed. The nominal content of

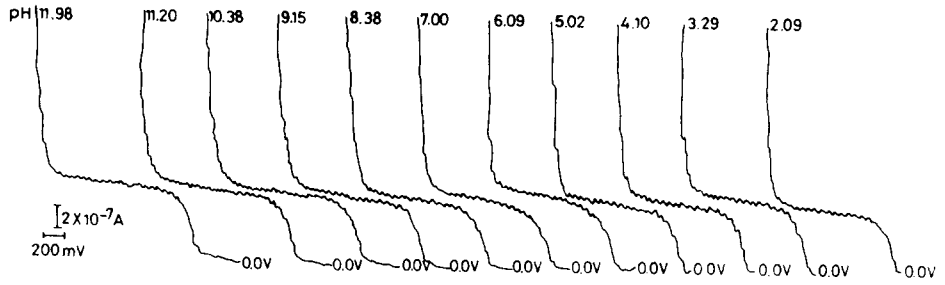


Fig. 1. Effect of pH on the development of the polarographic waves of derivatized prenalterol hydrochloride (6×10^{-5} M).

the ampoules was calculated either from a previously plotted calibration graph or using the regression equation. Alternatively, the concentration was determined from a comparison with the waveheight obtained from a simultaneously prepared standard.

3. Results and discussion

Treatment of prenalterol hydrochloride with nitrous acid was found to yield a polarographically active derivative. The derivative was obtained rapidly at room temperature within 2 min using 1 ml of 0.1 M hydrochloric acid and 1 ml of 2% sodium nitrite. The derivative was assumed to be the corresponding nitroso-derivative (Scheme 1).

The resulting derivative exhibits a well-defined cathodic wave over the entire pH range in Britton–Robinson buffers, as shown in Fig. 1. The $E_{1/2}$ potentials were shifted towards more negative values upon increasing the pH. A plot of $E_{1/2}$ vs. pH (Fig. 2) shows regions of linearity with two breaks at pH 7.3 and 9.5, corresponding to the pK_a of phenolic OH and the ammonium ion, respectively. It was reported that prenalterol hydrochloride has a pK_a of 9.5 (ammonium ion) and 10 (phenolic OH) [16]. Upon derivatization, the pK_a of phenolic OH is shifted to 7.3 owing to the presence of the O-nitroso group.

Linear regression analysis between $E_{1/2}$ and pH produced the following equations:

$$E_{1/2}(\mathrm{V}) = -0.0199 - 0.0366\,\mathrm{pH} \quad (1)$$

$$E_{1/2}(\mathrm{V}) = -0.2701 - 0.0624\,\mathrm{pH} \quad (2)$$

Logarithmic analysis of the resulting waves at different pH values resulted in straight lines. If the rate-determining step involves the transfer of two electrons (a free radical one-electron transfer is not likely to occur), the slopes of the plots suggest that the reduction is highly irreversible. The αn_α values were calculated according to Meites and Israel [17], and ranged from 0.4 to 0.66. These values point out the irreversible character of the reduction process.

The reduction is diffusion-controlled, as shown by the linear relation between the limiting current and the concentration and square root of the height of the mercury head, and the negligible effect of the buffer concentration on the limiting current.

The number of electrons transferred in the electrode reaction was determined through correlation analysis, i.e. by comparison of the waveheight with that of an equimolar solution of nitrobenzene in Britton–Robinson buffers at pH 5.0. Nitrobenzene at pH 5.0 gives two waves, the first corresponding to a four-electron transfer process and the second to a two-electron transfer process [18]. The derivatized prenalterol at the same pH gives only one wave with the same height as that of the first wave of nitrobenzene. Based on the above observation, it is concluded that prenalterol hydrochloride is converted into the corresponding nitroso-derivative upon reaction with sodium nitrite in acidic medium.

3.1. Mechanism of the electrode reaction

The nitroso-derivative is reduced at the dropping mercury electrode in two steps [18]: the first is the formation of the hydroxylamine, with the consumption of two electrons; the second is the reduction of the hydroxylamino group to the primary amine, with the consumption of two more electrons (Scheme 1).

3.2. Analytical application

The polarograms of the derivatized prenalterol hydrochloride in Britton–Robinson buffers pH 5 exhibit a well-defined cathodic wave, which can be exploited for analytical

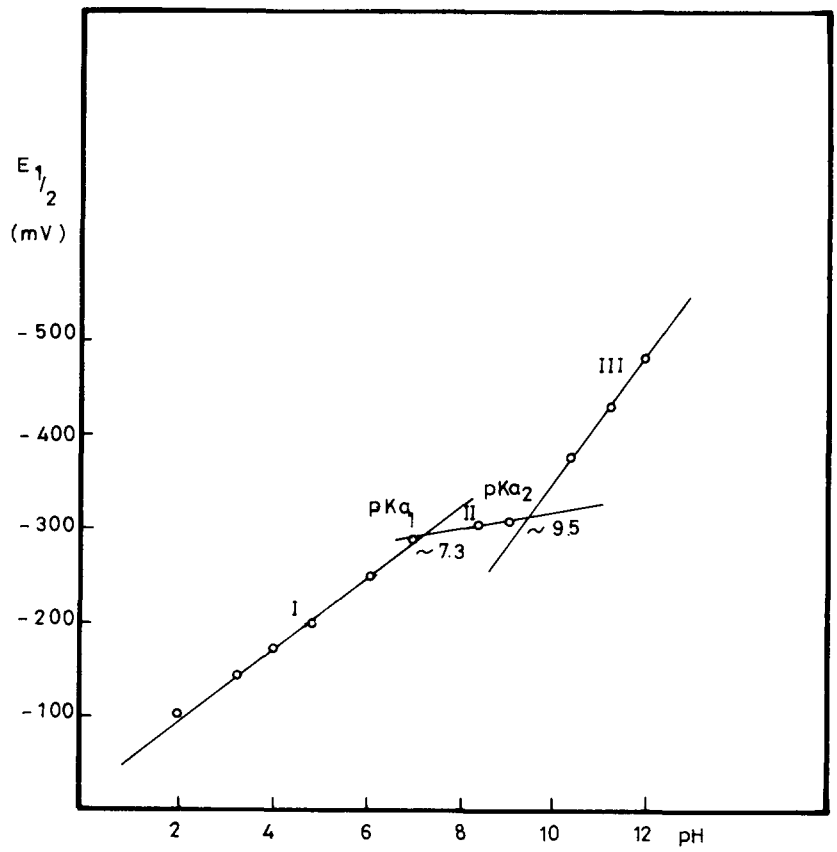


Fig. 2. Effect of pH on the $E_{1/2}$ of derivatized prenalterol hydrocholoride (6×10^{-5} M).

measurements. The limiting current is diffusion-controlled and is a linear function of the concentration over the range 0.015–0.15 mM, with a minimum detectability of 0.8 μM. Regression analysis of the results gave the following equation:

$$C(\text{mM}) = 0.0017 + 0.1343 i_d \qquad (r = 0.9997)$$

At pH 5, the derivative was found to be stable for more than 1 h.

The diffusion-current constant, I_d, was calculated from the waveheight (i_d) according to the Ilkovic equation for various concentrations of prenalterol hydrochloride, and was found to be 4.15 ± 0.11.

The proposed method was successfully applied to the determination of prenalterol hydrochloride in certain dosage forms, and the results obtained are in agreement with those obtained using the difference spectrophotometric method [2] (Table 1).

Statistical analysis [19] of the results obtained by both methods using Student's t-test and the variance ratio F-test showed no significant difference between the performance of the two methods with regard to accuracy and precision. The proposed method is simple, rapid, accurate and sensitive, and can be readily adopted for use in the control laboratory. In addition, it can be considered as stability indicating, since prenalterol, like other phenolic compounds, is prone to oxidation of the phenolic group to give the corresponding quinone.

A comparison of the proposed method with other reported methods shows them all to be equally accurate and precise. Although the proposed method might be less sensitive than some earlier methods [3,4,6–8], it has a few merits such as simplicity and time efficiency. Moreover, the proposed method is more sensitive than some spectrophotometric methods [2].

Interference from drugs, likely to be co-administered with prenalterol, was studied. No interference was observed from atropine. As for other ketosteroids, the $E_{1/2}$ of fludrocortisone lies far below -1.4 V vs. SCE [20], which is sufficiently far from the $E_{1/2}$ of derivatized prenalterol.

Table 1
Polarographic determination of prenalterol hydrochloride in dosage forms

Preparation	Recovery (%)	
	Proposed method	Spectrophotometric method [2]
Prenalterol hydrochloride tablets (prepared tablets containing 10 mg per tablet)	99.4 101.2 100.3 100.7	100.4 101.5 100.8 100.5
Mean ± SD	100.4 ± 0.76	100.8 ± 0.50
Student's t-test [a]	0.878 (2.447)	
Variance ratio [b]	2.348 (9.28)	
Varbian ampoules [c] (1 mg prenalterol hydrochloride per 1 ml)	101.1 100.9 100.7 99.3	101.7 100.2 101.0 100.6
Mean ± SD	100.5 ± 0.82	100.9 ± 0.64
Student's t-test	0.771 (2.447)	
Variance ratio	1.638 (9.28)	

[a] Tabulated t-value (at $P = 0.05$).
[b] Tabulated F-value (at $P = 0.05$).
[c] Product of Ciba, UK.

References

[1] Martindale, in J.E.F. Reynolds (Ed.), The Extra Pharmacopoeia, 30th edn., The Pharmaceutical Press, London, 1993.
[2] A.M. Wahbi, M.E. Mohamed, E.A.G. Kariem and H.Y. Aboul-Enein, Analyst, 108 (1983) 886–889.
[3] M.E. Mohamed, A.M. Wahbi and E.A.G. Kariem, Anal. Lett., 16 (1983) 1545–1553.
[4] F.A. Aly, F. Belal and M.I. Walash, J. Pharm. Biomed. Anal., 12 (1994) 955–958.
[5] P.H. Degen and M. Ervik, J. Chromatogr., 222 (1981) 437–444.
[6] C.J. Oddie, G.P. Jackman and A. Bobik, J. Chromatogr., 231 (1982) 473–477.
[7] M.R. Gregg, Chromatographia, 20 (1985) 129–133.
[8] G. Musch, Y. Buelens and D.L. Massart, J. Pharm. Biomed. Anal., 7 (1989) 483–497.
[9] H. Oelschlager, in Topics in Pharmaceutical Sciences, Elsevier, North-Holland, Biomedical Press, Amsterdam, 1981, p. 357.
[10] M.I. Walash, M. Risk, F. Belal and A. El-Brashy, Microchem. J., 38 (1988) 300–306.
[11] H. Oelschlager and M. Muller, Pharmazie, 36 (1981) 807–809.
[12] M. Rizk, M.I. Walash, A.A. Abou-Ouf and F. Belal, Pharm. Weekbl. Sci. Ed., 6 (1984) 114–117.
[13] M.M. Ayad and M. Yousef, Analyst, 110 (1985) 963–965.
[14] F. Belal, F.A. Ibrahim, S.M. Hassan and F.A. Aly, Mikrochim. Acta, 107 (1991) 61–69.
[15] J. Heyrovsky and P. Zuman, in Practical Polarography, Academic Press, London, 1968, p. 179.
[16] A.C. Moffat, in Clark's Isolation and Identification of Drugs, 2nd edn., The Pharmaceutical Press, London, 1986, p. 919.
[17] L. Meites and Y. Israel, J. Am. Chem. Soc., 83 (1961) 4903–4906.
[18] B. Kastening, in P. Zuman, L. Meites and I.M. Kolthoff (Eds.), Progress in Polarography, Vol. III, Wiley-Interscience, New York, 1972, p. 259.
[19] A. Petrie, in Lecture Notes on Medical Statistics, 1st edn., Blackwell Scientific, London, 1978.
[20] L.G. Chatten, R.N. Yadav and D.K. Madan, Pharm. Acta Helv., 51 (1976) 381–383.