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Voltammetric determination of isoxsuprine and fenoterol in dosage forms and biological fluids through nitrosation

F. Belal *, H.A. AL-Malaq, A.A. AL-Majed

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O.Box 2457, Riyadh 11451, Saudi Arabia

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Abstract

A simple and highly sensitive voltammetric method was developed for the determination of isoxsuprine HCl (I) and fenoterol HBr (II) in dosage forms and biological fluids. The method is based on treatment of the two compounds with nitrous acid followed by measuring the cathodic current produced by the resulting nitroso derivatives. The voltammetric behavior was studied adopting Direct Current (DC_t), Differential Pulse (DPP) and Alternating Current (AC_t) polarography. Both compounds produced well-defined, diffusion-controlled cathodic waves over the whole pH range in Britton–Robinson buffers (BRb). At pH 11 and pH 9, the values of diffusion-current constants (*Id*), were 9.4 ± 0.3 and 7.7 ± 0.4 for I and II, respectively. The current–concentration plots for I were rectilinear over the range of 0.6–12 μg/ml and 0.1–12 μg/ml in the DC_t and DPP modes, respectively. As for II, the range was 1–20 μg/ml and 0.1–20 μg/ml in the DC_t and DPP modes, respectively. The minimum detectability ($S/N = 2$) were 0.02 μg/ml ($\approx 6 \times 10^{-8}$ M) and 0.01 μg/ml ($\approx 2.6 \times 10^{-8}$ M) for I and II, respectively, adopting the DPP mode. The proposed method was applied to the determination of both compounds in dosage forms and the results obtained were in good agreement with those obtained using reference methods. The proposed method was further applied to the determination of isoxsuprine in spiked human urine and plasma. The percentage recoveries adopting the DPP mode were 98.84 ± 1.18 and 99.26 ± 0.97, respectively.

Keywords: Isoxsuprine; Fenoterol; Voltammetry; Dosage forms; Biological fluids

1. Introduction

Isoxsuprine and fenoterol are two phenolic compounds belonging to two different pharmacological categories. Isoxsuprine, 1-(4-hydroxyphenyl)-2-(1-methyl-2-phenoxy-ethylamino) propan-1-ol, is a vasodilator that produces the effects of β-adrenoceptor stimulation and α-adrenoceptor antagonism, the former effect is the predominant. It is used in the treatment of cerebral and peripheral vascular diseases. It is also used to arrest premature labor [1].

Several analytical methods have been reported for the determination of isoxsuprine in pure form,

* Corresponding author. Tel.: +966-1-4677348; fax: +966-1-4676220/383.
E-mail address: ffbelal@ksu.edu.sa (F. Belal).

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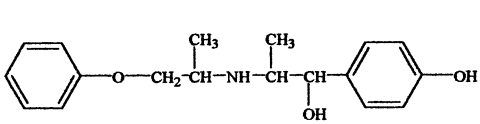
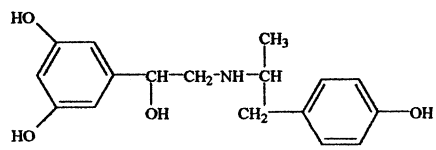
dosage forms and biological fluid. A good guide to the work published up to 1997 is found in the comprehensive review written by Belal et al. [2]. The more recent publications for that drug include spectrophotometry [3], multivariate calibration [4] and HPLC [5].

As for fenoterol, 1-(3,5-dihydroxyphenyl)2-(4-hydroxy-α-methylphenethyl-amino) ethanol, it is a direct-acting sympathomimetic agent with predominantly β-adrenergic activity and a selective action on β_2-receptors. It is used as a bronchodilator, its bronchodilating action is relatively more prominent than its effect on the heart. It is used in the treatment of asthma, exercise-induced asthma and premature labor [1]. Various analytical methods have been applied for its determination in formulations or in biological fluids. These methods include spectrophotometry [6], gas chromatography coupled with mass spectrometry [7,8], fluoro-immunoassay [9,10], radioimmunological assay [11], HPLC [12,13], voltammetry [14], coulometry [15], colorimetric-flow injection [16], isotope-labeling [17], electrophoresis [18,19] and isotachophoresis [20].

In this piece of work, the reaction of isoxsuprine HCl (I) and fenoterol HBr (II) with nitrous acid was studied in an attempt to develop a simple polarographic method for their determination in dosage forms and biological fluids. The results obtained were promising.



(I) Isoxsuprine



(II) Fenoterol

Functionalization polarography, which means the conversion of a polarographically inactive compound into an active one, is achieved by the introduction of an electroactive group through chemical reactions. These reactions should occur rapidly and with a yield of about 100% [21]. This approach could be successfully applied to the determination of EDTA [22], penicillines [23], prenalterol [24], metyrosin [25] and thioxanthenes [26].

Adopting that technique, isoxsuprine could be accurately determined in biological fluids at the therapeutic level of concentration that follows an oral dose of 20 mg. However, the method could not be applied to Fenoterol because of the very small dose that leads to a mean therapeutic concentration of about 0.3 ng/ml [8]. All the reported methods for the determination of isoxsuprine in biological fluids rely on the use of chromatography [27,28], after a lengthy extraction procedure using organic immiscible solvents. Hence the proposed method — as applied to biological fluids — is an alternative substitute, which is equally sensitive, but more simple and time saving, as no prior extraction step is required before measurement. The results obtained were sufficiently accurate and precise.

2. Experimental

2.1. Apparatus

The polarographic study and the differential pulse 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 dropping-mercury-electrode (DME), a Ag°/AgCl reference electrode and a platinum wire as the auxiliary electrode, was used. Phase selective alternating current (AC_t) polarograms of solutions containing 12 μg/ml of isoxsuprine, were recorded using the same instrument. The superimposed alternating voltage being 15 mV, at a frequency of 75 Hz and a phase angle of 90°.

2.2. Materials and reagents

Isoxsuprine HCl (batch no. 191401) was purchased from Sigma, Saint Louis, MO, USA. Tablets containing isoxsuprine: Duvadilan tablets labeled to contain 20 mg each (batch no 544066) were obtained from commercial sources. Fenoterol HBr (batch no. 271307) was kindly provided by Boehringer, Ingelheim, Germany. Tablets containing fenoterol: Berotec tablets labeled to contain 2.5 mg each (batch no. 801702) and metered aerosols for oral inhalation: Berotec aerosols labeled to contain 200 μg/puff (batch no. 701776), were obtained from commercial sources. Plasma was kindly provided by King Khalid University Hospital, Riyadh, KSA, and urine was obtained from healthy volunteers. The following reagents were also prepared:

1. Sodium nitrite (Merck, Germany): 2 and 0.5% aqueous solutions.
2. Ammonium sulphamate (Fluka, Germany) 5% (w/v) aqueous solution.
3. Britton–Robinson buffers (BRb): 0.08 M solutions covering the pH range 2.1–12 [29].
4. Hydrochloric acid (Prolabo, France): 1 and 2 M solutions.
5. Sodium hydroxide (Winlab, UK): 2 M aqueous solution.
6. Acetonitrile (BDH, Poole, England)

Stock solutions containing 1.0 mg/ml of I and II were prepared in distilled water, and further diluted with the same solvent to get working solutions.

2.3. Procedures

2.3.1. Recommended procedure

Transfer aliquots of the working solution into separate 25-ml volumetric flasks. For I add 1 ± 0.1 ml of 1 M HCl followed by 0.5 ± 0.1 ml of 2% sodium nitrite solution. As for II add 0.5 ± 0.1 ml of 1 M HCl followed by 0.5 ± 0.1 ml of 0.5% sodium nitrite solution. Heat in a water bath for 5 ± 1 min at 100°C. Cool, then add 5 ml of ammonium sulphamate solution and shake well until no more nitrogen gas is evolved. Neutralize the solutions with sodium hydroxide solution then complete to the mark with BRb of pH 11 or pH 9 for I or II, respectively. Transfer the whole contents of the flasks into the polarographic cell, pass nitrogen gas for 5 min then record the polarograms in the DC_t and DPP modes over the potential range -0.2– -1.6 V versus Ag°/AgCl. Plot the current (μA) versus the concentration (μg/ml) to get the calibration graph. Alternatively, derive the corresponding regression equation.

2.3.2. Application of the proposal method to tablets containing I or II

For isoxsuprine (I), weigh and pulverize 20 tablets. To a quantity of the powder containing 20 mg of (I) add 50 ml of 0.1 M HCl and heat on a boiling water bath for 30 min. Cool, dilute to 100.0 ml with 0.1 M HCl then filter.

As for Fenoterol (II), weigh and pulverize 20 tablets and transfer a quantity of the powder equivalent to 5.0 mg of the drug into a 100 ml volumetric flask. Make up to the volume with water, shake for 15 min then filter. Transfer aliquots of the filtrate containing suitable amounts of either drugs over the working range (Table 2) into 25 ml volumetric flask. Complete as under 'recommended procedure'. Determine the nominal content of the tablets either from the calibration graph or using the corresponding regression equation.

2.3.3. Application of the proposal method to aerosols containing Fenoterol

Shake the aerosol container, then place its mouth-piece under 80 ml of water in a 100-ml beaker and spray 25 metered doses. After mixing, transfer to a 100-ml volumetric flask and make up to volume with water. Shake for about 15 min then filter. Transfer aliquots of the filtrate containing suitable amounts of the drug over the working range (Table 2) into 25-ml volumetric flask. Complete as under 'recommended procedure'. Determine the nominal content of the aerosols either from the calibration graph or using the corresponding regression equation.

2.3.4. Application of the proposed method to the determination of isoxsuprine HCl in spiked human urine

Into each of five centrifugation tubes, transfer 3.0 ml of spiked human urine containing different concentrations of the drug then add 6 ml of acetonitrile to precipitate the proteins and mix well. Centrifuge for 10 min, then transfer 4.5 ml of the clear supernatant into an evaporating dish. Evaporate on a boiling water bath, then dissolve the residue in 1 M HCl. Transfer into 25 ml volumetric flask then wash with 2 ml of water and transfer the washing into the same volumetric flask. Proceed as described under 'recommended procedure' beginning with the words 'followed by 0.5 ml of 2% sodium nitrite'. Determine the nominal content of isoxsuprine from a previously plotted calibration graph or using the corresponding regression equation.

2.3.5. Application of the proposed method to the determination of isoxsuprine HCl in spiked human plasma

Into each of five centrifugation tubes, transfer 3.0 ml of spiked human plasma containing different concentrations of the drug then add 6 ml of acetonitrile to precipitate the proteins and mix well. Centrifuge for 10 min, then transfer 4.5 ml of the clear supernatant into an evaporating dish. Evaporate on a boiling water bath, and dissolve the residue in 1 M HCl. Transfer into 25-ml volumetric flask then wash with 2 ml of water and transfer the washing into the same volumetric flask. Proceed as described under 'recommended procedure' beginning with the words 'followed by 0.5 ml of 2% sodium nitrite'. Determine the nominal content of isoxsuprine from a previously plotted calibration graph or using the corresponding regression equation.

3. Results and discussion

Treatment of both isoxsuprine and fenoterol with nitrous acid was found to produce polarographically active species. Being phenolic compounds, the obtained products are assumed to be the corresponding ortho nitroso derivatives. The two derivatives developed well-defined cathodic waves over the whole pH range in BRb. Typical polarograms of isoxsuprine and its nitroso derivative is shown in Fig. 1. Fenoterol produced a

Table 2
Performance data for the proposed method using both DC_t and DPP modes[a]

No.	Parameter	Isoxsuprine HCl		Fenoterol HBr	
		DC_t	DPP	DC_t	DPP
1.	Concentration range (μg/ml)	1–12	0.1–12	1–20	0.1–20
2.	Minimum detectability (μg/ml)	–	(0.02 6×10^{-8} M)	–	0.01 (2.6×10^{-8} M)
3.	Diffusion current constant (*Id*)	9.4 ± 0.3	–	7.7 ± 0.4	–
4.	Regression equation[a]	$id=0.032C+0.0033$	$id=0.057C+0.0047$	$id=0.022C+0.011$	$id=0.0399C+0.006$
5.	Correlation coefficient	0.998	0.999	0.999	0.999
6.	Standard deviation of the residuals ($S_{y/x}$)	3.25×10^{-3}	1.16×10^{-2}	9.165×10^{-3}	9.47×10^{-3}
7.	Standard deviation of the intercept (S_a)	2.27×10^{-3}	8.55×10^{-3}	5.1×10^{-3}	5.17×10^{-3}
8.	Standard deviation of the slope (S_b)	3.034×10^{-4}	9.62×10^{-3}	5.14×10^{-4}	4.2×10^{-4}
9.	Applications	Dosage forms	Dosage forms & biological fluids	Dosage forms	Dosage forms & biological fluids

[a] *id* is the diffusion current in μA. *C* is the concentration in μg/ml.

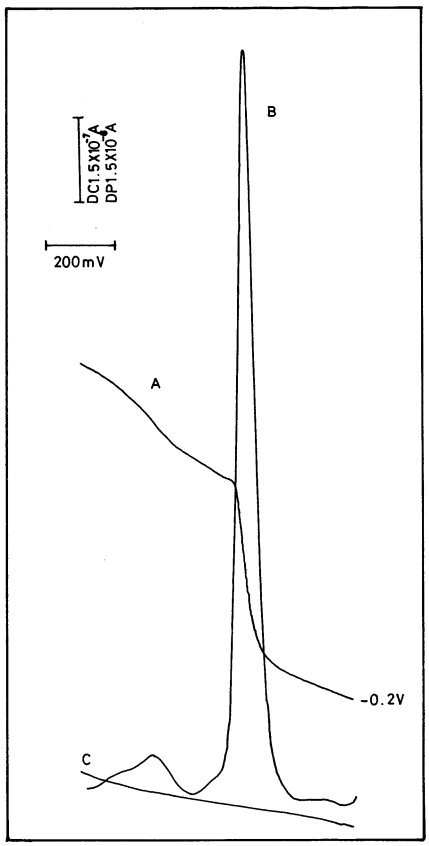


Fig. 1. Typical polarogram of the reaction product of isoxsuprine HCl (12 μg/ml) in BRb pH 11. A:DPP mode. B:DC_t mode. C:DC_t polarogram of isoxsuprine HCl (12 μg/ml).

similar polarogram. The half wave potentials ($E_{1/2}$) were shifted towards more negative values upon increasing the pH of the medium; this behavior may be due to the decrease in $[H^+]$. Table 1 and Fig. 2 demonstrate the cathodic shift of $E_{1/2}$ values as a function of pH. Logarithmic analysis of the reduction waves of the nitrosoderivatives of I and II obtained in BRb of different pH values adopting DC_t mode resulted in straight lines. 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 quasi reversible for I and completely irreversible for II. The values of αn_a were calculated using the treatment of Meites and Israel [30] and are listed in Table 1. The number of protons, Z, consumed in the rate-determining step of the electrode reaction is given by the following formula [31].

$$\Delta E_{1/2}/\Delta pH = -0.059Z/\alpha n_a$$

The results obtained at different pH values are shown in Table 1.

3.1. 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 straight lines, the slopes of the two plots were 0.69 and 0.75 for I and II, respectively. Changing the buffer concentration over the range of 0.016–0.074 M for I and 0.016–0.059 M for II, produced a negligible effect on the waveheights of both compounds. The temperature coefficients calculated according to Meites [32] over the range 20–65°C were 1.38 and 2.17%/°C for I and II, respectively. The above three characteristics point out to a diffusion-controlled reduction process partially affected by adsorption phenomenon. The alternating current behavior of I and II was studied in BRb of different pH values at phase angle of 90°. In BRb of pH values of 5, 7 and 11, the summit potentials (E_s) of I were shifted to more negative values of 260, 140 and 120 mV than the corresponding $E_{1/2}$ values, respectively. Fig. 3 shows the AC_t behavior of I, from which it is concluded that, only the depolarizer is adsorbed to the surface of mercury, while its reduction product is not. Compound II behaves similarly.

3.2. The derivatization process

The derivatization procedure was optimized for each compound separately. Each variable was changed while the other variables were kept constant, and the corresponding limiting current was measured. The experimental parameters in the experimental part gave the highest values of limiting current.

3.3. Stability of the nitroso-derivatives

The stability of the produced nitroso-deriva-

tives at their analytical pH values was evaluated by measuring the waveheights at room temperature after increasing time intervals. Fig. 4 shows that, for both compounds, the nitroso-derivatives were stable for at least 2 h.

Table 1
Effect of pH on the development of the polarographic waves of isoxsuprine and Fenoterol produced upon treatment with nitrous acid[a]

Compound	pH	$-E_{1/2}$ (mV)	$-E_{1/2}/\Delta$ pH	id/c	$W_{1/2}$(mV)	an_a	Z (H^+)
Isoxsuprine HCl	1	100	9.1	5.3	Broad	1.75	0.27
	2.1	110	44	4.9	Broad	1.49	1.12
	3	150	40	4.1	100	1.53	1.03
	4	190	70	4.4	100	1.21	1.43
	5	260	40	4.7	105	1.10	0.74
	6	300	80	4.1	110	1.24	1.69
	7	380	60	5.1	110	1.21	1.23
	8	440	30	6.2	100	1.31	0.67
	9	470	40	5.4	80	1.27	0.86
	10	510	10	5.5	80	1.43	0.24
	11	520	40	6.3	75	1.54	1.04
	12	560		4.2	85	1.57	-
Fenoterol HBr	3	120	40	8.2	80	0.92	0.63
	4	160	80	9.6	150	0.74	1.01
	5	240	30	9.6	130	0.63	0.32
	6	270	70	9.8	175	0.63	0.74
	7	340	90	9.8	145	0.61	0.96
	8	430	30	10.1	110	0.63	0.32
	9	460	25	10.6	100	0.65	0.28
	10	485	40	9.6	100	0.59	0.40
	11	525	5.0	9.8	90	0.62	0.05
	12	530		8.9	95	0.77	-

[a] $W_{1/2}$, is the half-peak width in DPP mode. $\alpha n_{a,}$ is the number of electrons transferred in the rate-determining step. a is the transfer coefficient.

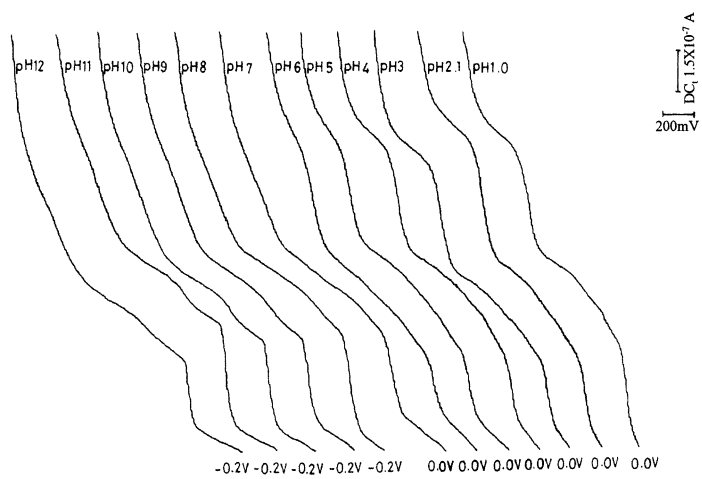


Fig. 2. Effect of pH on the development of the polarographic waves of isoxsuprine HCl (12 μg/ml) in the DC_t mode.

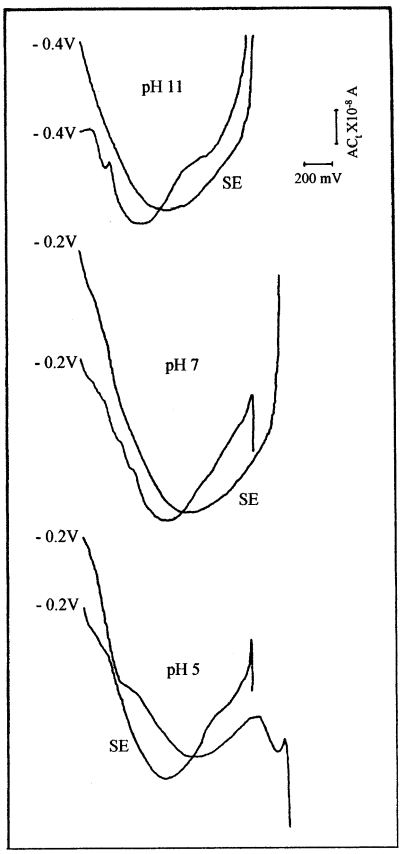


Fig. 3. Alternating current behavior of isoxsuprine HCl (12 μg/ml) in BRb of different pH values. Superimposed alternating voltage: 15 mV, frequency 75 Hz and phase angle 90°. (SE: supporting electrolyte).

3.4. Analytical applications

Polarograms of the reaction products exhibited very well-defined cathodic waves and steep peaks at the chosen pH values, as denoted by the high values of αn_a and small values of $W_{1/2}$. For both compounds, no polarographic maxima was developed, hence no maximum suppressor was needed. The plots representing the relationship between the concentration of each of the studied drugs and the diffusion current gave straight lines over the ranges cited in Table 2. Linear regression analysis of the data gave the corresponding regression equations shown in the same table. The minimum detectability was accomplished adopting the DPP mode by determining the concentration which gave a signal double that of the noise ($S/N = 2$). The figures obtained are listed in Table 2. Statistical evaluation [33] of the data through determination of the standard deviation of the residuals ($S_{x/y}$), standard deviation of the intercept (S_a) and standard deviation of the slope (S_b) for both DC_t and DPP modes, are shown in the same table. The small figures obtained refer to the high precision of the method.

To assess the validity of the proposed method, it was applied to the determination of authentic samples of the studied drugs. Different concentrations over the working concentration range were analyzed three times each using DC_t and DPP techniques. The results obtained (Table 3) were statistically compared with those given using reference methods [34]. No significant difference was noticed between the two methods regarding accuracy and precision as revealed by the t-test and F-test, respectively, [33].

The proposed method was further applied to the determination of the two compounds in their dosage forms (tablets and aerosols). The results obtained (Table 4) were in good agreement with those given adopting reference methods [6,34]. Common tablet excepients, such as talc, magnesium stearte, starch, lactose and avisil, did not interfere with the assay. The determination of isoxsuprine in spiked human urine and plasma could be achieved adopting the DPP mode. Isoxsuprine is administered orally in a dose of 20 mg. It is well absorbed from the gastro-intestinal tract. The maximum concentration in the circulation occurs about 1 h after administration by mouth, and is maintained for about 3 h. A plasma half-life of 1.5 h has been reported. Isoxsuprine is in part conjugated to the blood and is excreted in the urine [1]. It is, therefore, anticipated that, the blood level concentration will be about 0.5 μg/ml, which is higher than the lower working range in the DPP mode. The proposed method was successfully applied to spiked samples of human urine and plasma. Acetonitrile was added to precipitate the proteins and the clear supernatant was used for the analysis. The method was optimized and justified by the sufficiently accurate and precise percentage recoveries (Table 5).

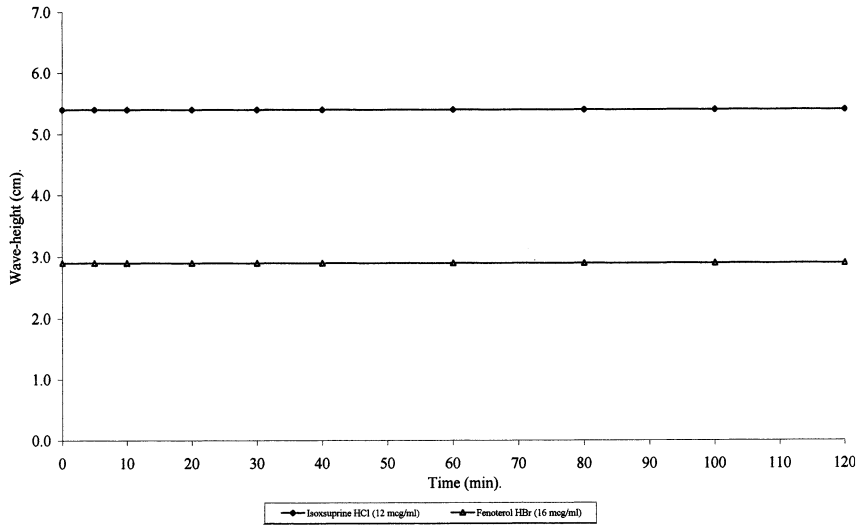


Fig. 4. Effect of time on the stability of the nitroso-derivatives, measured by DC_t.

Table 3
Application of the proposed method to the determination of isoxsuprine and fenoterol in pure form

Compound	Proposed method						Reference method [34]
	DPP			DC_t			
	Amount taken (μg/ml)	Found (μg/ml)	% Found	Amount taken (μg/ml)	Found (μg/ml)	% Found	
Isoxsuprine HCl	0.4	0.395	98.50	0.8	0.802	100.25	
	0.8	0.794	99.25	1.0	1.004	100.40	
	2.0	1.977	98.85	2.0	1.994	99.70	
	4.0	4.020	100.5	4.0	3.995	99.88	
	8.0	8.020	100.25	8.0	8.000	100.00	
Mean ± SD			99.47 ± 0.87			100.05 ± 0.28	100.55 ± 0.65
t	1.979 (2.262)[a]			0.274 (2.262)[a]			
F	1.280 (5.19)[a]			5.263 (6.39)[a]			
Fenoterol HBr	0.6	0.590	98.56	2	2.015	100.74	
	0.8	0.786	98.28	4	3.931	98.28	
	1.0	0.995	99.53	6	6.019	100.33	
	2.0	1.997	99.84	8	7.961	99.51	
	4.0	4.069	101.72	10	10.074	100.74	
	8.0	7.094	99.22	12	11.794	98.28	
Mean ± SD			99.53 ± 1.22			99.65 ± 1.15	99.44 ± 2.41
t	0.079 (2.306)[a]			0.188 (2.306)[a]			
F	3.88 (5.41)[a]			4.39 (5.41)[a]			

[a] The figures in parenthesis are the tabulated values of *t* and *F* at 95% confidence level.

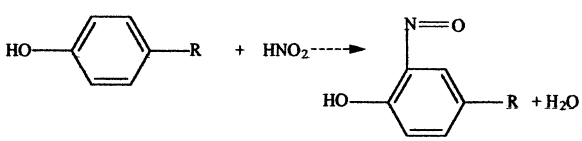
Table 4
Application of the proposed method to the determination of the studied compounds in their pharmaceutical preparations[a]

Preparation	Average recovery%		
	DC_t	DPP	Reference methods ([6,34])
Duvadilan® tablets (Isoxsuprine HCl, 20 mg/tablet)	99.82	99.60	99.89
±SD	0.483	0.839	0.409
t	0.274	0.702	(2.262)*
F	1.388	4.191	(6.26)*
Berotec® tablets (Fenoterol HBr,2.5 mg/tablet)	99.83	99.52	98.40
±SD	1.56	1.44	2.97
t	1.03	0.491	(2.262)*
F	3.59	4.215	(5.19)*
Berotec® aerosols. (Fenoterol HBr, 200 μg/puff)	100.00	100.31	99.25
±SD	1.024	1.149	2.22
t	0.736	1.006	(2.306)*
F	4.69	3.73	5.41)*

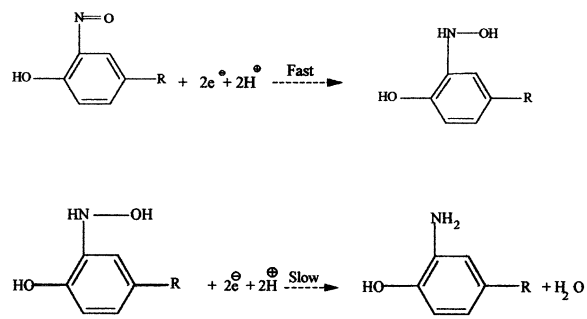
[a] 1- Each result is the average of four separate determinations.
* 2- The figures in parentheses are the tabulated values of *t* and *F* at 95% confidence level.

3.5. Mechanism of the electrode reaction

Both isoxsuprine and fenoterol contain phenolic functional group. They are proposed to react with nitrous acid producing the polarographically active ortho–nitroso derivatives as shown below.



The number of electrons involved in the reduction process could be accomplished through comparing the waveheight of the reaction product with that obtained from an equimolar solution of a previously studied compound having the same functional group, and nearly identical value of diffusion coefficient under the same experimental conditions, that is tauromustine [35]. The waveheights of both compounds were identical, hence it is concluded that, only one nitroso group is introduced into each molecule in spite of the presence of more than one possible site for nitrosation. The electrode reaction is proposed to proceed as follows:



It is evident from the experimental results that, a slow electron-transfer reaction is involved in the reduction of the produced derivatives. Logarithmic analysis of the waves established that two electrons are involved in the rate-determining step, and the shift in $E_{1/2}$ potentials with increasing pH indicates that two hydrogen atoms are consumed in this step. However, comparison with the reduction of tauromustine revealed that four electrons are consumed in the reduction of the nitroso group, two of them are involved in the reduction into hydroxylamine while the other pair is used in the reduction of the later into primary amine. The last reduction step is highly irre-

Table 5
Application of the proposed method to the determination of isoxsuprine HCl in spiked human urine and spiked plasma using DPP mode

Sample	% Recovery
Urine	99.55
	98.52
	100.57
	98.11
	99.55
Mean	99.26
±SD	0.97
Plasma	100.55
	99.33
	98.1
	98.7
	97.5
Mean	98.836
±SD	1.18

versible and the polarographic waves overlap with those of the solvent.

4. Conclusion

A highly sensitive polarographic method is described for the determination of isoxsuprine and fenoterol in their dosage forms and biological fluids. The most striking advantage of the method as applied to biological fluids is that no prior extraction step is required before measurement. The value of minimum detectability ($\approx 6 \times 10^{-8}$ M) is comparable to those reported using chromatographic methods. The method can be readily adopted to routine bioanalytical analysis. It can be also used for routine quality control and content uniformity tests. Moreover, the proposed method can be considered as a stability–indicating assay for both compounds. Oxidation is the main pathway of degradation of fenoterol [13] and isoxsuprine [36]. Oxidation involves the phenolic group, and consequently no nitroso derivative will be formed. Compared with other voltammetric method reported for Fenoterol [14], the proposed method is more simple and time saving, yet, has a comparable detection limit (2.6×10^{-8} M compared with 2.5×10^{-8} M for the reported method).

References

[1] J.E.F. Reynolds, Martindale, The Extra Pharmacopoeia, 31st ed., Pharmaceutical Press, London, 1996, pp. 1577–1580.
[2] F. Belal, A.A. Al-Badr, A.A. Al-Majed, H.I. El-Subbagh, in: G.H. Britain (Ed.), A Monograph in Analytical Profile of drug Substances and Excepients, vol. 26, Academic Press, New York, 1999, pp. 359–393.
[3] M.N. Reddy, D.G. Sankar, K.V.P. Rao, Indian Drugs 35 (1998) 163–164.
[4] C. Demir, R.G. Brereton, Analyst 123 (1998) 181–189.
[5] G. Brambilla, M. Fiori, I. Curiel, L. Serpe, P. Gallo, Analyst 123 (1998) 2693–2696.
[6] M.A. Abounassif, E.A. Abdel-Moety, Acta Pharm. Jugosl. 39 (1989) 359.
[7] F.J. Couper, O.H. Drummer, J. Chromatogr. B 685 (1996) 265.
[8] J.G. Leferink, J. Dankers, R.A.A. Maes, J. Chromatogr. B 229 (1982) 217.
[9] C.T. Elliott, A. Baxter, W. Haasnoot, A. Lommen, W.J. McCaughey, Food. Agric. Immunol. 8 (1996) 219.
[10] W. Haasnoot, P. Stouten, A. Lommen, G. Cazemier, D. Hooijerink, R. Schilt, Analyst 119 (1994) 2675.
[11] K.L. Rominger, A. Mentrup, M. Stiasni, Drug Res. 40 (1990) 887.
[12] A. Polettini, M. Montagna, E.A. Hogendoorn, E. Dijkman, P. van-Zoonen, L.A. van-Ginkel, J. Chromatogr. 695 (1995) 19.
[13] G.A. Jacobson, G.M. Peterson, J. Pharm. Biomed. Anal. 12 (1994) 825.
[14] D. Boyd, J.R.B. Rodriguez, A.J.M. Ordieres, P.T. Blanco, M.R. Smyth, Analyst 119 (1994) 1979.
[15] K. Nikolic, L. Arsenijevec, M. Bogavac, J. Pharm. Biomed. Anal. 11 (1993) 207.
[16] S. Tanabe, T. Togawa, K. Kawanabe, Anal. Sci. 5 (1989) 513.
[17] D. Kohler, W. Fleischer, H. Matthys, Respiration 53 (1988) 65.
[18] M. Mazereeuw, A.J.P. Hofte, U.R. Tjaden, J. van-Der-Greef, Rapid. Commun. Mass. Spectrom. 11 (1997) 981.
[19] T. Wachs, R.L. Sheppard, J. Henion, J. Chromatogr. B 685 (1996) 335.
[20] M.H. Lamoree, N.J. Reinhoud, U.R. Tjaden, W.M.A. Niessen, J. van-Der-Greef, Biol. Mass. Spectrom. 23 (1994) 339.
[21] H. Oelschlager, in: P.D. Breimer, P. Speiser (Eds.), Topics in Pharmaceutical Sciences, Elsevier, Amsterdam, 1981, p. 357.
[22] F. Belal, F.A. Aly, M.I. Walash, A.M. Osman, J. Pharm. Biomed. Anal. 17 (1998) 1249.

[23] F. Belal, M.S. Rizk, M. Eid, J. Pharm. Biomed. Anal. 17 (1998) 275.
[24] F.A. Aly, F. Belal, M.I. Walash, J. Pharm. Biomed. Anal. 13 (1995) 1127.
[25] F.A. Aly, F. Belal, A. El-Brashy, Pharm. World Sci. 15 (1993) 208.
[26] F. Belal, S.M. El-Ashry, I. Shehata, M. El-Sherbeny. Mikrochimica Acta. (in press).
[27] A. Hashem, B. Lubczyk, J. Chromatogr. B 563 (1991) 216.
[28] D. Cova, R. Colombo, G. Cellini, Pharmacology 27 (1983) 117.
[29] J. Heyrovsky, P. Zuman, Practical Polarography, Academic Press, London, 1968, p. 179.
[30] L. Meites, Y. Israel, J. Am. Chem. Soc. 83 (1961) 4903.
[31] J. Proszt, V. Cieleszky, K. Gyorbiro, Polarography, Textbook, Akademiai Kiado, Budapest, 1967, p. 385.
[32] L. Meites, Polarographic Techniques, second ed., Wiley Interscience, New York, 1965, p. 140.
[33] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Wiley, New York, 1984.
[34] British Pharmacopoeia, Her Majesty Stationary Office (HMSO), London, 1993, 274.
[35] F. Belal, Electroanalyst 5 (1993) 605.
[36] S. Sevgi, T. Guneri, Sci. Pharm. 60 (1992) 273.