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Voltammetric determination of *N*-nitrosoderivatives of atenolol and propranolol in simulated gastric juice

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Abstract

A highly sensitive and simple voltammetric method is proposed for the determination of *N*-nitrosoatenolol (NA) and *N*-nitrosopropranolol (NP) in simulated gastric juice. The method is based on measuring the differential-pulse polarographic peak produced by NA and NP in Britton–Robinson buffers of pH 3 and 4 for NA and NP, respectively. Both compounds yielded diffusion-controlled current with diffusion–current constants of 7.23 ± 0.03 and 9.46 ± 0.06 for NA and NP, respectively. The current–concentration plots were rectilinear over the range 0.16–9.6 μg ml^{-1} with minimum detectability ($S/N = 2$) of 0.015 μg ml^{-1} (5×10^{-8} M) for NA; for NP the range was 0.08–8.0 μg ml^{-1} with minimum detectability ($S/N = 2$) of 0.009 μg ml^{-1} (3×10^{-8} M). The proposed method was successfully applied to study the possible in vivo production of the nitroso-derivatives under the standard nitrosation reaction conditions recommended by WHO. The method is characterized by simplicity and higher sensitivity as compared with the reported HPLC method.

Keywords: Simulated gastric juice; Nitrosoatenolol; Nitrosopropranolol; Voltammetry; β-Blockers

1. Introduction

Evidence of intragastric formation of carcinogenic *N*-nitroso compounds from nitrosable amine precursors has been provided by countless experiments on laboratory animals [1–3] and has been directly demonstrated in humans [4–6]. Drugs with amino or amide groups belong to the family of nitrosable molecules, and several of them have been shown to yield genotoxic *N*-nitrosoderivatives [7–9]. Robbiano et al. [10] found that β-adrenergic antagonists such as, atenolol and propranolol, etc. react with sodium nitrite in HCl medium to produce the corresponding *N*-nitrosoderivatives. These derivatives were shown to produce DNA fragmentation in addition to DNA repair synthesis in primary cultures of both rat and human hepatocytes. Moreover, one of them, *N*-nitrosopropranolol (NP) was found to exert mutagenic activity in mammalian cells [10]. Later on, Martelli et al. [5] reported on the genotoxic activity of NP and *N*-nitrosoatenolol (NA).

Therefore, it is useful in the assessment of cancer risk to check the presence of *N*-nitrosoderivatives of these drugs in the gastric juice of patients receiving them, as these drugs are widely used for the treatment of hypertension and cardiac arrhythmia, often in high doses and for long periods [10]. Two HPLC methods were reported for the determination of these compounds [9,10]. Although chromatographic methods offer a high degree of specificity, sample clean-up and the instrument limitations preclude their use in routine clinical studies. Therefore, there is a need for an alternative to the HPLC methods, and voltammetry by virtue of its high sensitivity was a promising substitute. The proposed voltammetric method has some distinct advantages over HPLC methods regarding sensitivity, time-saving and simplicity.

2. Experimental

2.1. Apparatus

The polarographic study and the DPP measurements were carried out using the Polarecord E505 Metrohm

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Table 1
Performance data for the proposed method [a]

Comp.	pH	*Id*/*C*	Concentration range	Detection limit	Regression equation	Correlation coefficient
1. NA	4.0	7.23 ± 0.03	0.16–9.6	0.015 μg ml^{-1} (5×10^{-8} M)	$C = -0.41 + 21.2Id$	0.999
2. NP	3.0	9.46 ± 0.06	0.08–8.0	0.009 μg ml^{-1} (3×10^{-8} M)	$C = -0.39 + 14.5Id$	0.995

[a] Where C = concentration in μg ml^{-1}. Id = the current in μA.

(Herisau, Switzerland). The drop time of 1 s was electronically controlled using a 506 Stand from the same company. The polarograms were recorded using a potential scan of 10 mV s^{-1}. A three-electrode system, the dropping mercury electrode (DME) working electrode, an Ag° | AgCl reference electrode, and a platinum wire auxiliary electrode, were used. Phase selective alternating current (AC_t) polarograms 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

- Atenolol (free base) was obtained from EIPICO (Cairo, Egypt) and Propranolol·HCl from Sigma Chemical Co. (St. Louis, MO, USA). The *N*-nitrosoderivatives were synthesized in our laboratory according to the method described by Mazzel et al. [11].
- Sodium nitrite (E. Merck).
- Pepsin (Prolabo).
- Britton–Robinson buffers (BRb); 0.08 M pH range 2.1–12 [12].
- Simulated gastric juice (SGJ): prepared according to USP 1995 [13].
- Sodium thiocyanate (BDH).

2.3. Standard solutions

Standard solutions of either NA or NP were prepared by dissolving 10 mg in the least volume of methanol and completing to 50 ml with water in a measuring flask.

2.4. Polarographic measurements

Transfer an aliquot containing NA or NP within the concentration range cited in Table 1, into a 25-ml measuring flask. Complete to the mark with BRb of the appropriate pH (Table 1). Transfer the entire contents of the flask into the polarographic cell. Pass nitrogen gas for 5 min. Record the DPP polarogram over the range 0 to −0.8 V for NP and −0.6 to −1.4 V for NA, using a pulse amplitude of 70 mV.

2.5. Nitrosation following the conditions recommended by WHO

To check the possible formation of NA and NP from the interaction between the drugs and the nitrite-rich gastric juice, the nitrosation reactions were carried out under the conditions recommended by WHO [14]. All the reactions were carried out at 37°C for 3 h and checked at 15, 30, 60, 120 and 180 min. The following concentrations were used: drug: 10 mM l^{-1}, and sodium nitrite: 40 mM l^{-1} at pH values of 1.5 and 3.5.

3. Results and discussion

The *N*-nitrosoderivatives of atenolol and propranolol are reducible at the DME giving well-defined cathodic waves. Fig. 1 shows typical polarograms of both NA and NP in the DC_t and DPP modes in BRb of pH 3 and 4, respectively; SGJ does not interfere with any of them.

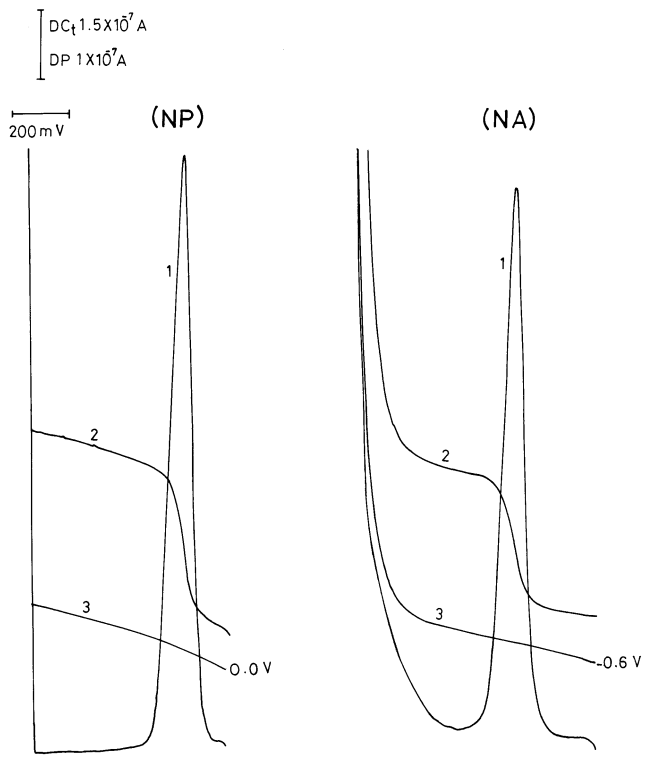

Fig. 1. Typical polarograms of NA and NP in BRb of pH 3 and 4, respectively. 1: DPP peak. 2: DC_t wave. 3: DC_t wave of SGJ.

Table 2
Effect of pH on the development of the polarographic wave of NA

pH	$-E_{1/2}$ (mV)	ΔpH	$\Delta E_{1/2}$	$\Delta E_{1/2}/\Delta$pH	Id/C	αn_a	$w_{1/2}$
0.0	610	1.0	80	80	5.96	0.66	1.2
1.0	690	1.0	80	80	6.80	0.54	1.15
2.1	770	1.1	80	73	6.95	0.64	1.1
3.0	860	0.9	90	100	6.95	0.69	1.2
4.0	950	1.0	80	80	7.32	0.87	1.3
5.0	1020	1.0	70	70	7.12	0.82	1.2
6.0	1095	1.0	75	75	6.95	0.85	1.3
7.0	1160	1.0	65	65	6.75	0.60	1.4
8.0	1200	1.0	40	40	6.63	0.72	1.6
9.0	1245	1.0	45	45	6.21	0.75	1.6
10.0	1290	1.0	45	45	5.72	0.85	1.6
11.0	1335	1.0	45	45	5.20	0.69	1.8
12.0	1380	1.0	45	45	5.0	0.65	1.9

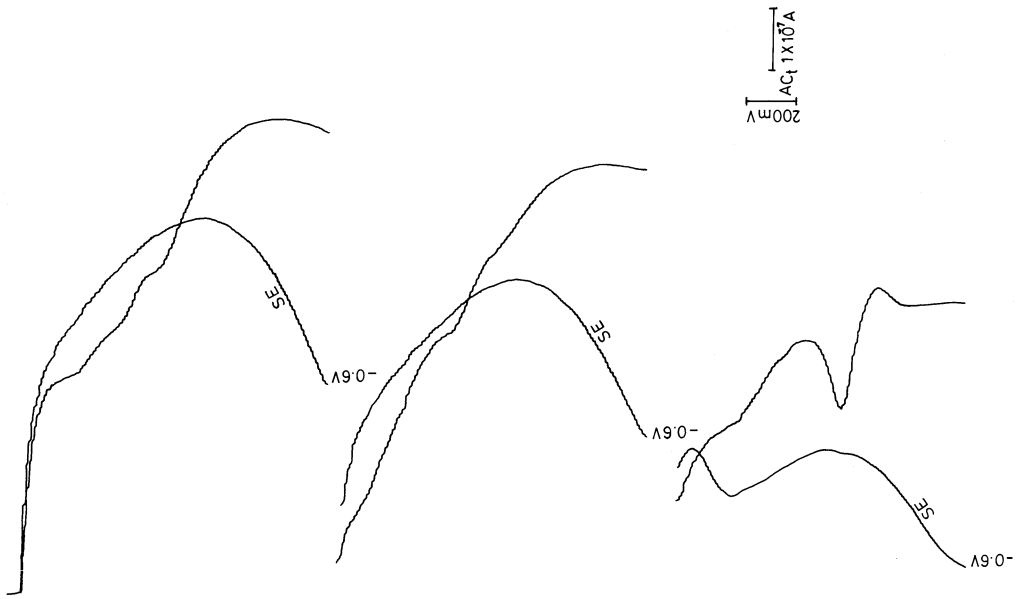

Fig. 2. Alternating current polarogram of NA (4.8 μg ml^{-1}) in BRb of different pH values superimposed alternating voltage: 15 mV, frequency 75 Hz, phase angle 90°, SE: supporting electrolyte.

3.1. Effect of pH on the development of the polarographic waves

The reduction process of both NA and NP is pH-dependent. The data in Table 2 show that the waves of NA — as a model example — exhibit cathodic shift upon increasing the pH value of the solution. Logarithmic analysis of the waves of NA obtained in BRb of different pH values 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 completely irreversible. The αn_a values were calculated according to the treatment of Meites and Israel [15] and are listed in Table 2. It is noticed that the degree of reversibility increases up to pH 4.0 ($\alpha n_a = 0.87$), then decreases and increases again at pH 10. NP showed similar behavior upon changing the pH of the solution.

3.2. Study of the wave characteristics

Increasing mercury height (h) resulted in a corresponding increase in waveheight (w); a plot of $\sqrt{h}$ versus w gave a straight line; also, a plot of $\log h$ versus $\log w$ gave a straight line with a slope of about 0.5. Changing the buffer concentration over the range 0.01–0.08 M resulted in a negligible increase in waveheight. These two characteristics point out to a diffusion-controlled process for both NA and NP. The alternating current behavior (AC_t) of both NA and NP was studied using a phase selective angle of 90° in BRb with pH

Table 3
Application of the proposed method to the analysis of NA and NP in SGJ containing NaSCN

Comp.	Added (μg)	Found (μg)	% Recovery
NA	0.16	0.156	97.5
	0.32	0.315	98.44
	0.64	0.633	98.91
	1.28	1.27	91.22
	2.4	2.37	98.75
	3.2	3.15	98.44
	6.4	6.32	98.75
	8.0	7.85	98.15
$\bar{X} \pm$ SD			98.52 ± 0.53
NP	0.08	0.077	96.25
	0.16	0.156	97.50
	0.32	0.314	98.13
	0.96	0.95	98.96
	2.4	2.36	98.33
	3.2	3.14	98.13
	6.4	6.34	98.59
	8.0	7.85	98.13
$\bar{X} \pm$ SD			98.00 ± 0.82

values of 4, 7 and 10. The summit potentials (E_s) of NA — as a model example — were 150, 25 and 25 mV more negative than the corresponding $E_{\frac{1}{2}}$ values. Fig. 2 shows that at pH 4, neither the depolarizer nor its reduction products are adsorbed to the mercury surface, while at pH 7 and 10, only the reduction product is adsorbed.

3.3. Analytical applications

Both NA and NP gave well-defined cathodic waves in BRb of pH 3 and 4, respectively. Under these conditions, no interference was noticed from SGJ. In addition, at these pH values, the peaks (in the DPP mode) were the steepest (highest αn_a values and lowest $w_{1/2}$ values) and highest Id/C value (Table 1). To assess the validity of the proposed method, it was applied to the determination of different amounts of both NA and NP in SGJ containing NaSCN covering the concentration range in Table 1. The results abridged in Table 3 are satisfactorily accurate and precise.

The proposed method was further applied to follow-up the nitrosation reaction of both atenolol and propranolol in SGJ containing NaSCN at pH values of 1.5 and 3.5 under the conditions recommended by WHO. The yields of NA and NP were determined after 15, 30, 45, 60, 90, 120, 150, and 180 min, so that the time course of the nitrosation reaction was examined at an interval which is longer than the normal emptying times of the stomach, taking into account some pharmacological and pathological conditions that may delay the actual stomach emptying.

From Fig. 3, it is evident that, both NA and NP are formed immediately after the addition of sodium nitrite to the drug in SGJ. The amount of nitroso derivative increased during the first 90 min, then either decreased (NP) or continued to increase (NA), or remained constant, according to the pH of the medium.

4. Conclusions

A simple, accurate and highly sensitive method is proposed for the determination of the *N*-nitrosoderivatives of atenolol and propranolol. The method was developed as a promising substitute to the reported HPLC methods. However, the suggested method has

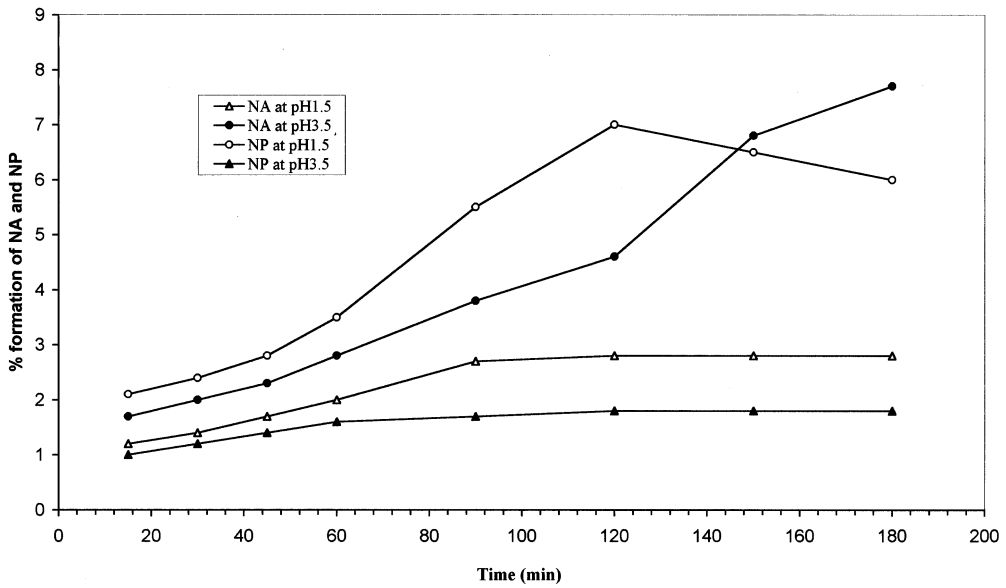

Fig. 3. Formation of NA and NP in SGJ at pH 1.5 and 3.5 under the conditions recommended by WHO.

some distinct advantages regarding sensitivity (lower concentration range 0.08 μg ml^{-1} compared with 0.4 μg ml^{-1} of NP in HPLC). In addition, the proposed method is simple, time-saving (2 min run-time) while the HPLC method must be performed under strict conditions, the pH must be less than 3 and molarity of the mobile phase must be < 0.03 M, otherwise interference of SGJ with the determination will occur.

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