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Voltammetric determination of *N*,*N*′-dinitrosopiperazine in simulated gastric juice

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Abstract

A voltammetric method has been developed for the determination of *N*,*N*′-dinitrosopiperazine (DNPZ) in simulated gastric juice. The method is based on measuring the differential pulse polarographic peak produced in pH 3 Britton Robinson buffer. A well defined, diffusion-controlled cathodic wave was obtained at −0.77 V versus Ag/AgCl over the range 0.4–24 μg/ml with minimum detectability (S/N = 2) of 0.072 μg/ml (5×10^{-7} M). The proposed method was successfully applied to study the possible in vivo production of the nitroso-derivatives of piperazine under the standard nitrosation reaction conditions recommended by WHO. The method has some distinct advantages over the reported GC methods.

Keywords: Simulated gastric juice (SGJ); Nitrosopiperazine (NPZ); Dinitrosopiperazine (DNPZ); Piperazine; Voltammetry

1. Introduction

N-Nitroso compounds are powerful carcinogens that are formed when amines and amides react with nitrite [1]. The formation has been demonstrated in gastric juice of animals [2,3] and humans [4,5]. Gastric cancer is more common in areas where drinking water contains much nitrate [6], and bacteria [7] which reduce nitrate to nitrite; in addition, increased nitrite concentrations are often found in the hypochlorhydric stomach [8,9]. Drugs with amino or amide groups have been shown to yield genotoxic N-nitroso derivatives [10–13].

Piperazine is widely used as an anthelmintic, in addition to being an active drug in the treatment of gout and urinary lithiasis by increasing uric acid excretion and dissolving urate crystals [14]. It is rapidly nitrosated under simulated gastric conditions to produce the carcinogens *N*-mononitrosopiperazine (MNPZ) and *N*,*N*′-dinitrosopiperazine (DNPZ) [15]. DNPZ — which is the more potent carcinogen [16] — is not immediately formed, however MNPZ is probably, in part, converted by transnitrosation to DNPZ [17]. Long-term, low-grade exposure to piperazine may also involve a considerable risk, not least since the more potent carcinogen DNPZ is more likely to be produced when nitrite is in excess of piperazine [15]. These findings support the views that piperazine drugs should be formulated with a nitrite trap (e.g. ascorbic acid) to slow down the nitrosation reaction.

Several methods have been reported for the determination of *N*-nitrosopiperazine in biological fluids. A spectrophotometric method was reported [18]. Gas liquid chromatographic (GLC) methods, using nitrogen-specific flame ionisation detection [19], or with thermal energy [20], were also reported. Although these 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 still a need for an alternative substitute for the chromatographic methods; voltammetry was a promising substitute regarding sensitivity, time-saving and simplicity.

2. Experimental

2.1. Apparatus

The polarographic study and the DPP measurements were carried out using the Polarecord E 505 Metrohm

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Table 1
Effect of pH on the development of the polarographic waves of *N*,*Ń*-dinitrosopiperazine

pH	$E_{1/2}$ (mV)	$\Delta E_{1/2}/\Delta$pH (mV)	$\alpha\ n_a$ [a,b]	$w_{1/2}$ (mV) [c]
1	−650		0.56	85
2	−730	−80	0.52	85
3	−770	−40	0.72	80
4	−870	−100	0.55	80
5	−1020	−150	0.50	85
6	−1200	−180	0.60	85
7	−1230	−30	0.56	85
8	−1260	−30	0.57	90
9	−1290	−30	0.58	90
10	−1310	−20	0.59	100

[a] α: the transfer coefficient.
[b] n_a: the number of electrons transfered in the rate-determining step.
[c] $w_{1/2}$: is the half-peak width in the DPP mode.

(Herisau, Switzerland). The drop time of 1 s was electronically controlled using a 506 stand of the same company. The polarograms were recorded using a potential scan rate of 10 mV/s. A three-electrode system, the dropping mercury electrode (DME) as a working electrode, a Ag/AgCl reference electrode and a platinum wire auxiliary electrode, was used. Phase selective alternating current (AC_t) polarograms were recorded using the same instrument. The superimposed alternating voltage was 15 mV at a frequency of 75 Hz and a phase angle of 90°.

2.2. Materials and reagents

- Piperazine citrate (Pharco Pharmaceutical Co., Alexandria, Egypt).
- Sodium nitrite (E. Merck).
- Pepsin (Prolabo).
- Britton–Robinson buffers (BRb), 0.08 M, pH range 2–10 [21].

Table 2
Application of the proposed method to the analysis of *N*,*Ń*-dinitrosopiperazine in SGJ

µg added	µg found	% Recovery
0.4	0.408	102.00
0.8	0.800	100.00
1.6	1.608	100.50
2.4	2.384	99.33
4.8	4.784	99.66
8.0	8.024	100.30
16.0	15.984	99.90
24.0	24.064	100.26
Mean ± S.D		100.24 ± 0.80

- Simulated gastric juice (SGJ): prepared according to USP 2000 [22].
- DNPZ was synthesized in our laboratory according to the method described by USP 2000 [22].

2.3. Standard solutions

Standard solution of DNPZ was prepared by dissolving 10.0 mg in the least volume of methanol then completing to 50 ml with SGJ in a measuring flask.

2.4. Polarographic measurements

Aliquots of the standard solution containing DNPZ, within the concentration range cited in Table 2, were transferred into a 25 ml measuring flask and then completed to the mark with pH 3 BRb. The contents of the flask were transferred into the polarographic cell. Nitrogen gas was passed through the cell for 5 min and the DPP polarogram was recorded over the range of −0.4 to −1.2 V versus the Ag/AgCl electrode.

2.5. Conditions of nitrosation

To check the possible formation of DNPZ from the interaction between the drug and the nitrite-rich gastric juice, the nitrosation reactions were carried out under the conditions recommended by WHO [23] (condition A), in physiological like conditions containing a therapeutic dose of the drug (condition B) and near the limit of quantitation of the method (condition C). All the reactions were carried out at 37°C and stirred for 3 h, with checks at 5, 15, 30, 60, 120 and 180 min intervals.

2.5.1. Condition A
In this reaction the following concentrations were used: drug, 10 mmol/l; and sodium nitrite, 40 mmol/l at pH values of 1.2 and 3.5 in distilled water and in SGJ.

2.5.2. Condition B
The following concentrations were used: drug, 2 mmol/l; and sodium nitrite, 2.9 mmol/l at pH values of 1.2 and 3.5 in distilled water and in SGJ.

2.5.3. Condition C
In this reaction the following concentrations were used: drug, 2 mmol/l; and sodium nitrite, 0.2 mmol/l at pH values of 1.2 and 3.5 in distilled water and SGJ.

3. Results and discussion

The DNPZ is reducible at the DME giving a well defined, diffusion controlled cathodic wave at pH 3 BRb in both the DC_t and DPP modes. In simulated gastric juice DNPZ behaved similarly (Fig. 1).

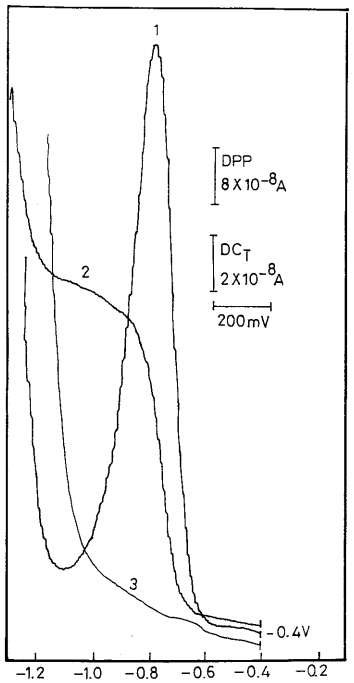


Fig. 1. Typical polarogram of *N*,*N*′-dinitrosopiperazine (0.044 mM) in BRb pH 3.0. 1-DPP 2-DC_t 3-SGJ.

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

The reduction process of DNPZ is pH dependent. Fig. 2 and the data in Table 1 show that the wave of DNPZ exhibits cathodic shift upon increasing the pH of the solution. Logarithmic analysis of the wave in the DC_t mode obtained in the 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 using the treatment of Meites and Israel [24] and are listed in Table 1. They were found to increase up to pH 3 then decrease irregularly. It is concluded that the reduction process is $[H^+]$ dependent.

3.2. Study of the wave characteristics

Increasing the mercury height (h) resulted in a corresponding increase in the wave height (w); a plot of w versus $\sqrt{h}$ gave a straight line, also a plot of $\log h$ versus $\log w$ gave a straight line with a slope of ca. 0.5. Changing the buffer concentration in the range 0.016–0.08 M revealed that the wave heights were independent on the buffer concentration. These two facts indicate diffusion controlled reduction process. The alternating current behaviour (AC_t) was studied using a phase selective angle of 90° in BRb of pH values 3, 7 and 10, the summit potentials (Es) were 0.225, 0.555 and 0.635 V more negative than the corresponding $E_{\frac{1}{2}}$ values. Fig. 3 shows that, at pH 3, both the depolarizer and its reduction product are adsorbed to the surface of DME, at pH 7, only the reduction product is adsorbed, while at pH 10, neither the depolarizer nor its reduction product are adsorbed. From this study, it is concluded that the reduction wave is mainly (diffusion) controlled and adsorption phenomenon played a limited role in the reduction process.

The relation between the diffusion current, id (μA), and the concentration C (μg/ml) was found to be rectilinear over the concentration range 0.4–24 μg/ml. Linear regression analysis of the data gave the following equation:

$$id = -0.018 + 0.125\ C \qquad (r = 0.9999)$$

The minimum detection limit (S/N = 2) was found to be 0.072 μg/ml (5×10^{-7} M).

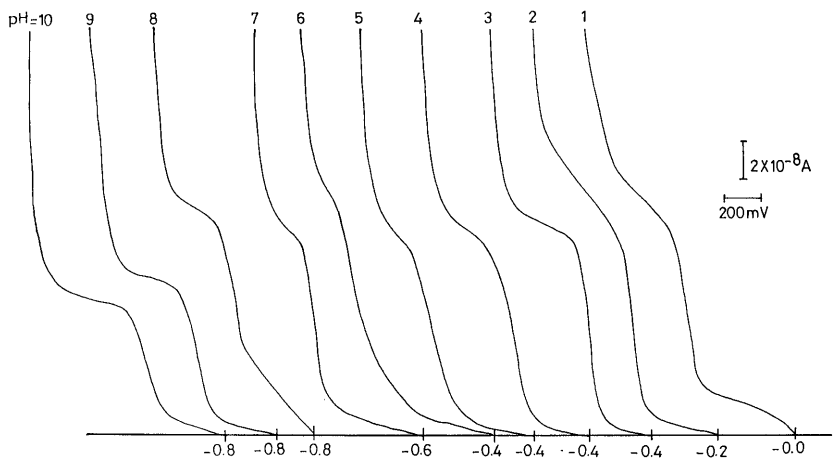


Fig. 2. Effect of pH on the polarographic behaviour of *N*,*N*′-dinitrosopiperazine (0.044 mM).

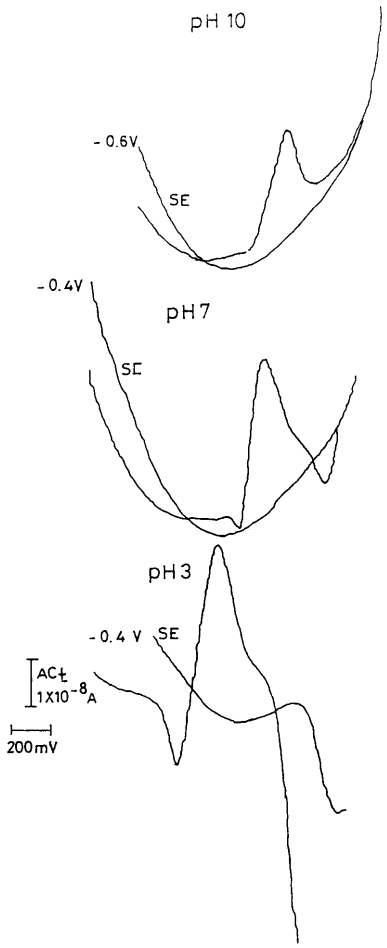


Fig. 3. Alternating current polarogram for *N*,*N*′-dinitrosopiperazine in BRb of different pH values; superimposed alternating voltage 15 mV; frequency 75 Hz; phase angle 90°; SE, supporting electrolyte.

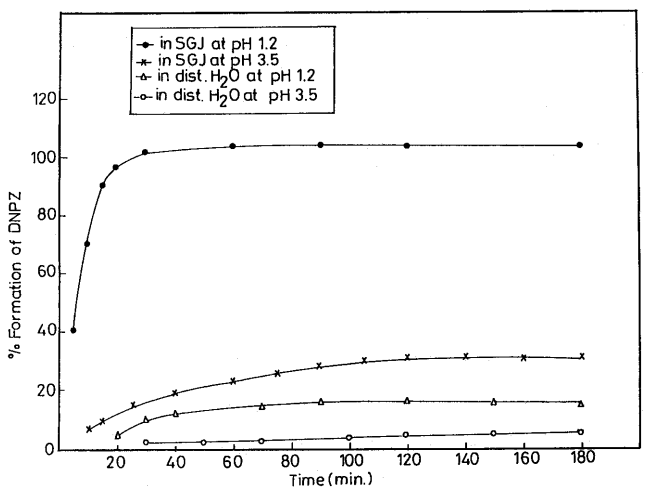
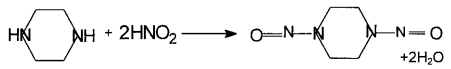


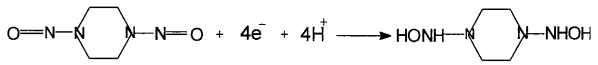
Fig. 4. Formation of DNPZ (under condition A).

3.3. Number of electrons involved in the electrode reaction

By comparing the wave height of DNPZ with that of an equimolar solution of a compound having the same reducible functional group (nitroso) and nearly identical diffusion coefficient, i.e. tauromustin [25] in BRb pH 3, the ratio of the wave heights was 2:1 for DNPZ and tauromustin, respectively. Since the reduction of tauromustin involves two electrons, this indicates that four electrons are transferred during the reduction process of the first wave, from which it is concluded that, the depolarizer contains two nitroso groups and thus the dinitroso derivative is formed. The proposed reactions are as follows:



At the DME :



3.4. Analytical applications

DNPZ gave a well defined cathodic wave in BRb of pH 3. Under these conditions, no interference was noticed from SGJ. Moreover, at this pH, the wave in the DC_t mode has the highest αn_a value and in the DPP has the smallest $w_{\frac{1}{2}}$ value. Therefore BRb pH 3 was chosen during this study. To assess the validity of the proposed method, it was applied to the determination of different amounts of standard DNPZ in SGJ covering the concentration range in Table 2. The results are satisfactorily accurate and precise.

Once the conditions of the proposed method were established, it was applied to follow-up the nitrosation reaction of piperazine in SGJ at pH values of 1.2, 3.5. This was performed under the conditions recommended by WHO, in physiological like conditions containing a therapeutic dose of the drug and near the limit of quantitation for the method. The yields of DNPZ were determined after 5, 10, 15, 30, 60, 120 and 180 min, so that the time course of the nitrosation reaction was examined at intervals which are longer than the normal emptying times of the stomach. Thus, taking into account some pharmacological and pathological conditions which may delay actual stomach emptying. The formation of DNPZ began immediately after the addition of sodium nitrite to the drug in SGJ and the amount of *N*,*N*′-dinitroso derivative increased quickly during the first 20 min at pH 1.2 then became constant up to the third hour. As for pH 3.5 it takes 2 h and then becomes constant as shown in Figs. 4–6. The

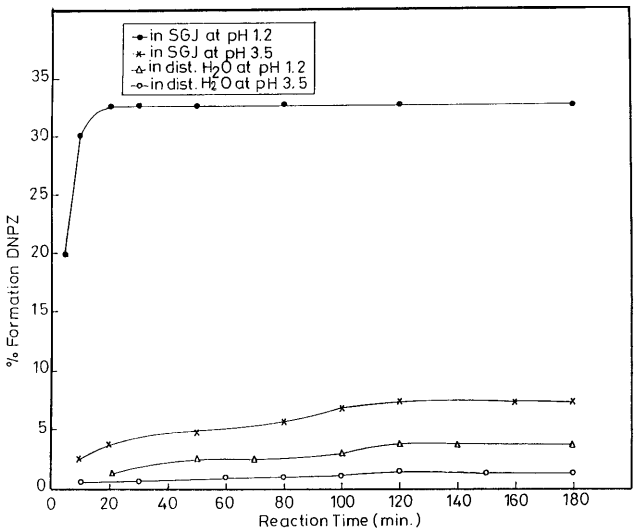


Fig. 5. Formation of DNPZ (under condition B).

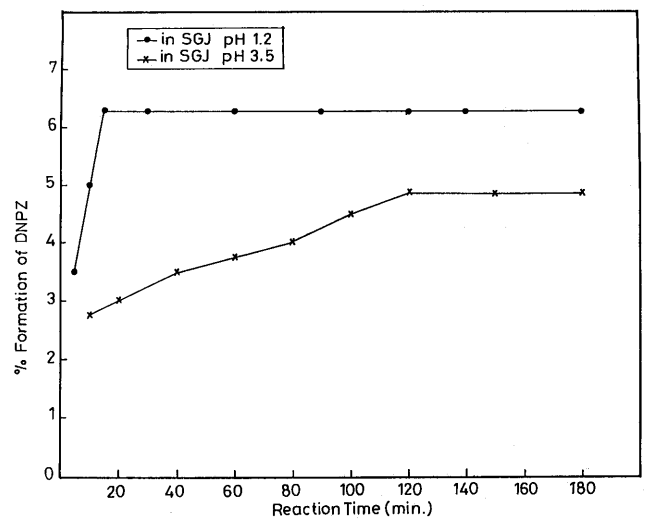


Fig. 6. Formation of DNPZ (under condition C).

Table 3
Percentage of *N*,*N*́-dinitrosopiperazine after 2 h of nitrosation reaction in SGJ

Conditions	Medium	pH 1.2	pH 3.5
A	in distilled H_2O	16.72	5.25
A	in SGJ	104.2	31.52
B	in distilled H_2O	4.26	1.63
B	in SGJ	32.81	7.43
C	in distilled H_2O		
C	in SGJ	6.25	4.87

DNPZ yields obtained in condition A, B and C are reported in Table 3. The DNPZ yield was higher at pH 1.2 than at 3.5 and higher in SGJ than in distilled water, in all conditions. The component responsible for this enhancement was pepsin [26]. When the nitrite concentrations were decreased to 0.2 mM (condition C), which is a condition very close to that of the human stomach, DNPZ formation was still detectable.

4. Conclusions

A simple, accurate and sensitive method is proposed for the determination of DNPZ in SGJ. The method was developed as a promising substitute to the reported GC method. However, the suggested method has some distinct advantages regarding simplicity and time saving.

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