Differential Pulse Polarographic Determination of *N*-Nitroso-2-pyrrolidinemethanol

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The pulse polarographic behavior of a new *N*-nitroso compound, *N*-nitroso-2-pyrrolidinemethanol, in buffered solutions has been studied. The effects of temperature, mercury pressure, modulation amplitude, surfactant concentration and solvent, and supporting electrolyte have been investigated. The reduction process is complicated and pH-dependent. In acid solutions, *N*-nitroso-2-pyrrolidinemethanol gives one irreversible reduction wave over a wide pH range below 7. The reduction of the *N*-nitroso compound is diffusion-controlled in d.c. and of normal pulse polarographic modes. In basic solutions, the reduction is also diffusion-controlled. The 10^{-7} mol dm^{-3} content of *N*-nitroso-2-pyrrolidinemethanol has been determined.

In recent years, interest in the electrochemical behavior of biologically important compounds such as nitrosamines and derivatives of hydantoin has increased since some of these compounds are carcinogenic, mutagenic, or teratogenic.[1-5] We are interested in the electrochemical behavior of nitrosamine, *N*-nitroso derivatives of pyrrolidine skeleton. Of many analytical techniques,[6-17] differential pulse (DP) polarography provides both qualitative and quantitative information on both non-volatile and volatile *N*-nitroso compounds.[17] The kinetics and mechanism of the electrochemical process have been discussed.[15-19] L-2-Pyrrolidinemethanol is an important inhibitor of protein synthesis in biochemistry and biology.[20] L-2-Pyrrolidinemethanol (prolinol) reacts in the presence of acid with nitrite to form the relatively non-volatile compound, *N*-nitroso-2-pyrrolidinemethanol (NOProlinol). No determination of the product of prolinol nitrosation seems to have been described. Sensitivity and detection limits for this compound were determined.

Experimental

Apparatus. Polarograms were recorded with a Model 174 Polarographic Analyzer with a Model 174/70 Drop Timer. The measurement conditions were the same as those reported.[17] Measurements of pH were made with a Corning Model 110 expanded scale digital pH meter. Except for temperature dependence studies, all the measurements were carried out at room temperature (20±1) °C.

Materials. The chemicals used were of reagent grade and were dissolved in deionized water. NOProlinol was prepared from L-2-pyrrolidinemethanol (Aldrich Chemical Company, Inc.). A stock solution of 1.95×10^{-2} mol dm^{-3} NOProlinol was prepared in 0.1 mol dm^{-3} HCl and kept in a refrigerator. This solution was found to be stable for over 5 months by means of polarography. The pH of the polarographic solutions was adjusted with Britton-Robinson buffer in the pH range 1.82—11.5. Measurements were made in HCl or H_2SO_4 below pH 1 and in NaOH above pH 12.

Results and Discussion

Effects of pH on the NOProlinol Reduction. The effect of pH on the reduction wave is shown in Fig. 1. The results are in line with those on other nitrosamines, supporting the mechanism of reduction of the protonated form in acid solution. Free NOProlinol like *N*-nitrosopyrrolidine is directly reduced in basic solutions since NOProlinol is present in the solution as a neutral molecule. The slopes of the potential *vs.* pH curves indicate involvement of one proton per electron in the rate determining step in the pH range 3—7 (*ca.* −65 mV/pH). The larger slope below pH 3 (*ca.* −116 mV/pH) seems to indicate involvement of two proton per electron. Complications of adsorption and the double layer structure make it difficult to interpret these data and account for values of the slope which differ from integral multiples of 58 mV.

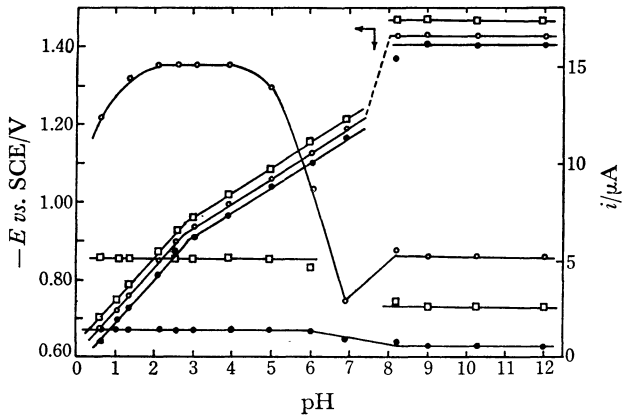

Fig. 1. Effect of pH on the wave height and half-wave or peak potentials for *N*-nitroso-2-pyrrolidinemethanol in 0.1 mol dm^{-3} LiCl containing 50% (volume fraction) B-R buffer.
Concentration: 9.75×10^{-5} mol dm^{-3}; scan rate, v: 2 mV s^{-1}; modulation amplitude, ΔE: −50 mV; ●: d.c. mode; □: NP mode; ○: DP mode.

NP and d.c. current for NOProlinol were constant over a wide range of pH as in the case of *N*-nitrosopyrrolidine.[17] This might be due to the presence of neutral molecules of both *N*-nitroso compounds in basic solu-

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tions. On the other hand, the decrease in the DP current at low pH might be due to a decrease in the reduction rate, whereas the NP and d.c. current remains constant (Fig. 1). When the concentration of the B-R buffer was less than 50%, the peak potential shifted to more negative values with decreasing buffer concentration, DP peak height and NP and d.c. waves remaining constant. Therefore, it is desirable to use at least a 50% B-R buffer.

In acid solutions, the dependence on modulation amplitude of DP peak height and peak potential showed the same tendency as in basic solutions. This dependence is similar to that of *N*-nitrosopyrrolidine. It is advantageous to work at larger pulse amplitudes to get better sensitivity when the electrode process is irreversible.

Reversibility. The NP and d.c. polarographic waves were analyzed logarithmically in order to obtain $n_a\alpha$, where n_a is the number of electron involved in potential-determining step and α transfer coefficient. Typical results for the d.c. waves are given in Table 1. In acid solutions, the $n_a\alpha$ values were 0.75. However, the values in basic solutions were 0.58. In ethanol–water mixtures, the same tendency was also observed (Tables 3 and 4). The irreversible reduction gives the DP current theoretically less than the reversible DP current, the current-concentration relation depending on rate parameters. The peak resolution is measured by the peak width at half height, $W_{1/2}$. The results for $W_{1/2}$ are given in Tables 2, 3, and 4. The values are larger than the theoretical values for reversible reductions, indicating that the reduction is irreversible.

Table 1. Polarographic characteristics of NOProlinol in aqueous solution

pH	Slope of *i-t* value[a]	$n_a\alpha$[b]	*p* of h_{corr} d.c.	Dependence[c] NP
1.12[d]	0.20	0.72	0.45	0.65
2.59	0.19	0.91	0.50	0.70
4.96	0.20	0.77	0.50	0.66
6.06	0.22	0.66	0.48	—
8.16	0.24	0.57	—	0.72
9.03	0.21	0.58	—	—
10.26	0.22	0.57	0.50	0.70
12.04	0.22	0.59	0.47	0.72
13.11[e]	0.22	0.58	0.48	0.70

Supporting electrolyte: 0.1 mol dm^{-3} LiCl containing 50% B-R buffer solution. a) Slope=d lg *i*/d lg *t*. b) $n_a\alpha=(2.303RT/F)\mathrm{d}[\lg(i_d-i)/i]/\mathrm{d}E$. i_d=limiting current. c) $p=\mathrm{d}\lg i_d/\mathrm{d}\lg h_{corr}$. d) 0.1 mol dm^{-3} HCl. e) 0.1 mol dm^{-3} NaOH.

Table 2. Relative temperature coefficients at 20 °C and peak half width ($W_{1/2}$)

pH	Relative temp coeff, (% K^{-1}) d.c.	NP	DP[a]	$W_{1/2}$,[a] (mV)
2.11	1.73	1.49	1.19	110[b]
10.03	1.84	1.66	1.12	145[c]

Supporting electrolyte is 0.1 mol dm^{-3} LiCl containing 50% B-R buffer. a) $\Delta E=-50$ mV. b) Observed in the pH range 1.12—6.06. c) Observed in the pH range. 8.16—13.11.

Table 3. Polarographic characteristics of NOProlinol in ethanol–water mixture (apparent pH 2.10)

Ethanol, % (volume fraction)	Slope of *i-t* value[a]	$n_a\alpha$[b]	$W_{1/2}$[c] (mV)
0	0.19	0.88	110
10	0.20	0.79	105
20	0.19	0.93	100
30	0.19	0.84	100
40	0.18	0.87	100

Supporting electrolyte is 0.1 mol dm^{-3} $NaClO_4$ containing 50% B-R buffer. a) Slope=d lg *i*/d lg *t*. b) $n_a\alpha=(2.303RT/F)\mathrm{d}[\lg(i_d-i)/i]/\mathrm{d}E$. c) $\Delta E=-50$ mV.

Table 4. Polarographic characteristics of NOProlinol in ethanol–water mixture (apparent pH 9.53)

Ethanol, % (volume fraction)	Slope of *i-t* value[a]	$n_a\alpha$[b]	$W_{1/2}$[c] (mV)
0	0.20	0.58	145
10	0.20	0.58	145
20	0.22	0.59	135
30	0.22	0.53	130
40	0.19	0.52	125

Supporting electrolyte is 0.1 mol dm^{-3} $NaClO_4$ containing 50% B-R buffer. a) Slope=d lg *i*/d lg *t*. b) $n_a\alpha=(2.303RT/F)\mathrm{d}[\lg(i_d-i)/i]/\mathrm{d}E$. c) $\Delta E=-50$ mV.

Diffusion Control. For diffusion-controlled waves, the wave height in d.c. mode is approximately proportional to $h_{corr}^{1/2}$ when the natural drop time is used, and that in NP mode is approximately proportional to $h_{corr}^{2/3}$ with a mechanically drop time; *h* presents the height of the mercury reservoir. The values of *p*, the power of h_{corr}, obtained by logarithmic plots (Table 1), indicate that the limiting currents are diffusion-controlled. The relative standard deviation of these values is 0.019, lying in the range of reported values.[21] The results in ethanol–water or methanol–water mixtures are almost the same as those for aqueous solutions. The diffusion-controlled character was confirmed according to the criteria of the time dependence of the limiting currents of the first drops and the agreement of the ratio of limiting currents in d.c. and NP modes with the theoretical value. The diffusion coefficients of NOProlinol were estimated by using the d.c. polarographic data and the Ilkovič equation and checked by the value of the diffusion coefficient of thallium(I) ions measured under the same conditions; the latter was in agreement with the reported value 2.00×10^{-5} cm^2 s^{-1}.[21] The diffusion coefficient of NOProlinol in 0.1 mol dm^{-3} LiCl containing 50% B-R buffer solution was $(7.90\pm0.35)\times10^{-6}$ cm^2 s^{-1} at pH 2.59, and that in the same supporting electrolytes was $(7.55\pm0.30)\times10^{-6}$ cm^2 s^{-1} at pH 9.03 with a 99% probability.

Temperature Dependence. Half-wave potentials of reversible processes are known to be nearly independent of temperature. Although NOProlinol is reduced

irreversibly, its half-wave potential, $E_{1/2}$ and peak potential, E_p, observed in the mixtures containing 40% (volume fraction) ethanol (apparent pH 2.10) were independent of temperature within experimental error as those in mixtures containing 40% (volume fraction) ethanol (apparent pH 9.13). $E_{1/2}$ or E_p of NOProlinol in aqueous solutions was also temperature independent. This result supports the view that the reduction rate depends on adsorption of reactants.[17,22]

Some of the relative temperature coefficients of the current for NOProlinol reduction in the temperature range 1—45 °C are given in Table 2. The results correspond to activation energies of diffusion. The relative temperature coefficients of the limiting current in ethanol–water mixture also lie in the range 1.0—2.0% K^{-1}.

Effect of Organic Solvents. If miscible organic solvents are present in the polarographic solution without specific chemical effects, the limiting current should decrease due to changes in the drop time and the diffusion coefficient with changing ionic strength and the viscosity of the medium. Figure 2 shows the effect of ethanol on the limiting current and $E_{1/2}$ or E_p for the reduction of NOProlinol in the polarographic solutions of apparent pH 9.13. A similar behavior was observed in a solution of apparent pH 2.10 as well as in a methanol–water mixture. Current change in the DP mode becomes more pronounced than in the d.c. or NP mode. However, the reduction of NOProlinol is also diffusion-controlled in ethanol– or methanol–water mixtures (Tables 2 and 3). This is not surprising, since the rate determining step for the reduction involves reactant adsorption,[17] *i.e.*, the alcohol molecules compete with the reactant for adsorption sites easier than water molecules do. Therefore, the decrease in the reaction rate would decrease the DP peak current.

Presence of Surfactants and i-t Curve. The effects of peptone (an enzyme of digest of proteins), Triton X-100, and gelatin were studied. These compounds are reasonable model surfactants, and important matrices to protein synthesis. None of them affected the DP peak current at concentrations less than 10^{-3}%, the slopes of $\lg i$-$\lg t$ being in the range 0.19—0.20. At higher concentrations than 10^{-3}%, they were strongly adsorbed at the dropping mercury electrode, DME, the reaction mechanism being affected.

Choice of Supporting Electrolyte. Cation adsorption would lower the reaction rate by competition with reactant adsorption and by reduction of the proton activity in the reaction layer. Several supporting

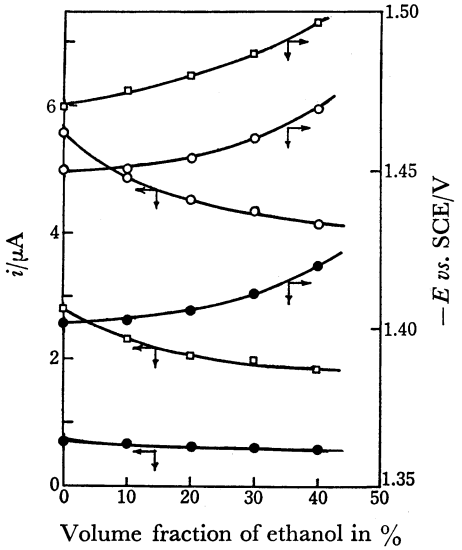

Fig. 2. Effect of the volume fraction of ethanol on the wave or peak height and half-wave or peak potentials for NOProlinol. Apparent pH=9.13; other conditions and symbols same as Fig. 1.

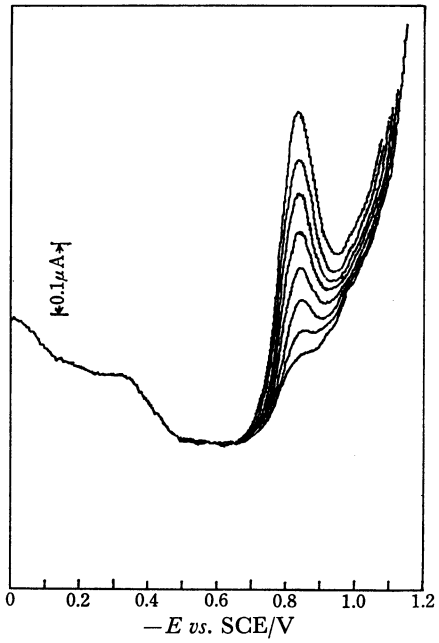

Fig. 3. Differential pulse polarograms of *N*-nitroso-2-pyrrolidinemethanol in 0.1 mol dm^{-3} $NaHSO_4$–Na_2SO_4 (pH 2.06).
Concentration=0, 1.00, 3.00, 5.00, 7.00, 9.00, 11.00, and 13.00×10^{-7} mol dm^{-3} NOProlinol; v: 2 mV s^{-1}; ΔE: −100 mV.

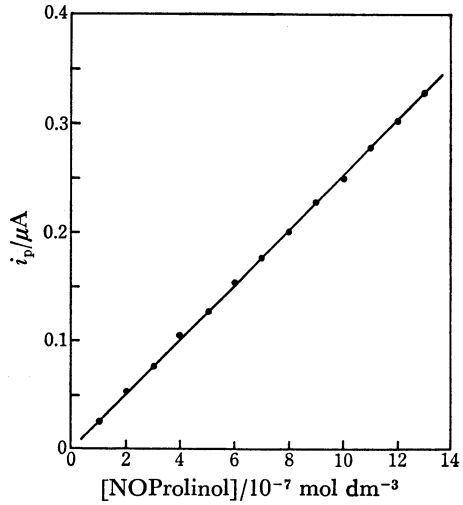

Fig. 4. Calibration curve of *N*-nitroso-2-pyrrolidinemethanol by DP mode.
Conditions same as Fig. 3.

electrolytes were studied in the pH range 2—4, there being no change in current with pH (Fig. 1). Sodium hydrogensulfate–sodium sulfate (0.1 mol dm^{-3}) is suitable for the determination of NOProlinol (Figs. 3 and 4). Potassium chloride (0.1 mol dm^{-3})–hydrochloric acid medium is also suited as supporting electrolyte.

Calibration Curve and Detection Limit. Figure 3 shows typical DP polarograms at the 10^{-7} mol dm^{-3} content of NOProlinol in 0.1 mol dm^{-3} sodium hydrogensulfate and 0.1 mol dm^{-3} sodium sulfate. The DP peak height is proportional to the concentration of NOProlinol between 8×10^{-8} and 1.4×10^{-6} mol dm^{-3} (Fig. 4). The detection limit for NOProlinol under the same conditions shown in Fig. 4 was 6×10^{-8} mol dm^{-3}, as estimated statistically.[23] The detection limit for NOProlinol is somewhat lower than that for the other carcinogenic nitrosamines.

In a sample containing NOProlinol and *N*-nitrosoproline 10^{-7} mol dm^{-3} (pH 4.0), we can determine NOProlinol quantitatively because the difference of E_p of NOProlinol from that of *N*-nitrosoproline is *ca.* 120 mV at $\Delta E=-50$ mV. In the mixture of NOProlinol and *N*-nitrosopyrrolidine, these nitrosamines could not be determined separately since both the compounds show a similar behavior in both acid and basic solutions, and the difference between the two E_p is insignificant.

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