ACTA CHEMICA SCANDINAVICA 11 (1957) 990—996

Electroorganic Preparations III. Polarography and Reduction of N-Nitrosamines

HENNING LUND

Leo Pharmaceutical Products, Copenhagen, Denmark

N-Nitrosamines of secondary aliphatic, heterocyclic, and aromatic amines are investigated polarographically. The formation of N-nitrosamines is used for the polarographical determination of secondary amines. The polarographic reductions of the N-nitrosamines are shown by controlled potential electrolysis to be, in acid solution a four electron reduction to the hydrazine, whereas the reduction in alkaline solution is a two electron reduction to the secondary amine with the formation of nitrous oxide.

Dimethylamine has been determined by polarography of its N-nitroso derivative [1,2], but no polarographic investigation of other N-nitrosamines has been reported. N-Nitroso phenylhydroxylamine has been investigated by Kolthoff and Liberty [3]. As there might be a possibility to determine secondary amines in the presence of primary and tertiary amines by polarography of their N-nitrosamines a polarographic investigation of N-nitrosamines was undertaken. The course of the reduction of the N-nitrosamines at a macro mercury electrode was proved by means of controlled potential electrolysis.

POLAROGRAPHIC INVESTIGATION

Effect of pH on half-wave potentials. In Table 1 are compiled the half-wave potentials and diffusion currents of N-nitroso dimethylamine (I), N,N'-dinitroso piperazine (II), N-nitroso morpholine (III), N-nitroso N-methylaniline (IV), and N-nitroso diphenylamine (V) at different pH-values.

The half-wave potentials of (I), (II) and (III) vary linearly with pH from pH about 0 to pH about 6. The slopes of the $E_{\frac{1}{2}}$ *vs* pH plots in this pH interval were 0.100, 0.097 and 0.098 V per pH-unit for (I), (II) and (III), respectively. At pH-values higher than about 7 the half-wave potentials were independent of pH. The half-wave potentials of (IV) vary from pH 1 to pH 7 linearly with pH with a slope of 0.082 and are independent of pH at higher pH-values. At pH-values lower than 1 the half-wave potentials are less negative than required

Table 1. Half-wave potentials (*vs* S.C.E.) and diffusion currents at different pH-values of N-nitroso dimethylamine (I), N,N'-dinitroso piperazine (II), N-nitroso morpholine (III), N-nitroso N-methylaniline (IV), and N-nitroso diphenylamine (V). Concentration 1.00×10^{-3} M, 0.01 % gelatin added as maximum suppressor.

pH		0.20	0.60	0.95	2.75	3.60	5.05	7.15	10.35	12.5
(I)	$-E_{\frac{1}{2}}$ V	0.87	0.91	0.94	1.12	1.21	×	1.53§	1.54	1.55
	i_d μA	13.5	13.5	13.5	13.4	13.3		8	6.5	6.4
(II)	$-E_{\frac{1}{2}}$ V	0.74	0.78_5	0.85	1.00	1.07	1.24	1.29	1.34	1.35
	i_d μA	26.3	26.3	26.3	26.3	26.2	25.9	25.5	14.1	13.3
(III)	$-E_{\frac{1}{2}}$ V	0.75	0.79	0.83	0.99_5	1.09	1.26_5	1.38	1.40	1.40
	i_d μA	12.8	12.8	12.8	12.7	12.3	11.9	11.7	6.9	6.4
(IV)	$-E_{\frac{1}{2}}$ V	0.58_5	0.64_5	0.68	0.86	0.92	1.02	1.23	1.30	1.31
	i_d μA	12.0	12.0	11.9	11.9	11.8	11.7	11.6	6.0	5.95
(V)	$-E_{\frac{1}{2}}$ V	0.47	0.51	0.56_5	0.71_5	0.76_5	0.85_5	0.98	1.03	1.03
	i_d μA	10.5	10.5	10.5	10.4	10.4	10.4	10.1	5.43	5.20

× No wave § Poorly defined wave

by the straight line in the $E_{\frac{1}{2}}$ *vs* pH plot. The half-wave potentials of (V) vary linearly with pH at pH values lower than 1 with a slope of 0.130 V per pH-unit. From pH about 2 to pH about 7 the slope of the $E_{\frac{1}{2}}$ *vs* pH plot is 0.063 and at pH-values higher than 8 the half-wave potentials are independent of pH.

In Table 2 are given the half-wave potentials and diffusion currents at different pH-values for *p*-nitroso N,N-dimethylaniline. The half-wave potentials vary linearly with pH in the examined pH interval with a slope of 0.068 V per pH-unit.

Effect of pH on diffusion current. The diffusion currents are for the N-nitrosamines fairly constant from pH 0 to pH about 5. In an interval around pH 7 the diffusion currents drop to a value which is half the value in acid solution. At pH-values higher than about 9 the diffusion currents again are fairly independent of pH. As shown in Fig. 1 the plot of the diffusion current *vs* pH has its highest slope at the same pH as that, at which the half-wave potentials start to be independent of pH. The diffusion currents of *p*-nitroso N,N-dimethylaniline are fairly constant in the examined pH-interval and have

Table 2. Half-wave potentials (*vs* S.C.E.) and diffusion currents at different pH-values of *p*-nitroso N,N-dimethylaniline. Concentration 1.0×10^{-3} M, 0.01 % gelatin added as maximum suppressor.

pH	4.0	5.0	6.5	8.0	10.0	12.5
$-E_{\frac{1}{2}}$ V	-0.01_5	0.05	0.16	0.26	0.38	0.55
i_d μA	12.1	12.1	11.9_5	11.8	11.7	11.6

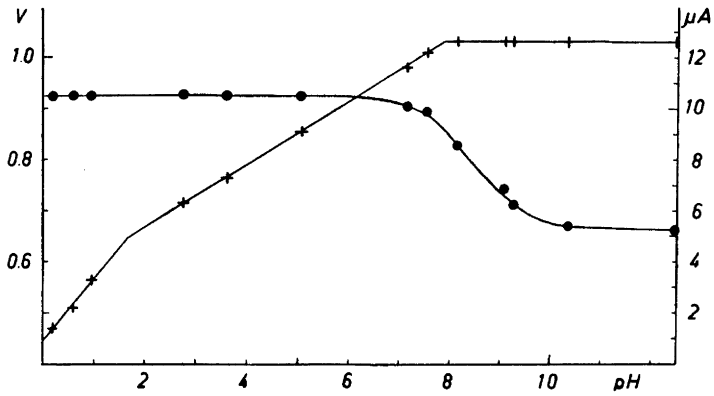

Fig. 1. Half-wave potentials +(V *vs* S.C.E.) and diffusion currents ● (μA) of N-nitroso diphenylamine plotted against pH. Concentration 10^{-3} M, 0.01 % gelatin added as maximum suppressor.

a value nearly equal to the value of the diffusion current of N-nitroso N-methylaniline in acid solution.

Effect of mercury height on limiting current. The data presented in Table 3 for limiting currents of N-nitroso dimethylamine and N-nitroso diphenylamine at different mercury column heights indicate that the limiting currents both in acid and alkaline solution are diffusion controlled. A diffusion controlled limiting current is nearly proportional to the square root of the corrected mercury column height and it is seen in Table 3 that $i_d/h^{1/2}$ is fairly constant.

Variation of diffusion currents with concentration. The variation of diffusion currents with concentration is substantially linear for the compounds between 1.5×10^{-4} and 2×10^{-3} M. At higher concentrations there is a slight negative deviation.

Wave form. The waves of N-nitroso dimethylamine, N,N'-dinitroso piperazine, and N-nitroso morpholine are somewhat drawn out whereas those of

Table 3. Dependence of limiting currents on mercury column heights for N-nitroso dimethylamine and N-nitroso diphenylamine in acid and alkaline solution. Concentration 10^{-3} M, 0.01 % gelatin added as maximum suppressor.

pH	N-nitroso dimethylamine				N-nitroso diphenylamine			
	1.0		12.5		1.0		12.5	
h cm	i_dμA	$i_d/h^{\frac{1}{2}}$	i_dμA	$i_d/h^{\frac{1}{2}}$	i_dμA	$i_d/h^{\frac{1}{2}}$	i_dμA	$i_d/h^{\frac{1}{2}}$
28.5	10.4	1.95	4.87	0.914	7.80	1.46	3.90	0.732
38.5	12.1	1.95	5.55	0.895	9.30	1.50	4.59	0.740
48.5	13.5	1.94	6.40	0.919	10.50	1.51	5.20	0.747
58.5	15.2	1.99	6.92	0.907	11.55	1.51	5.64	0.738

N-nitroso N-methylaniline and N-nitroso diphenylamine more approach the form of a reversible wave. None of them are, however, reversible. The plots of log $(i/i_d—i)$ *vs* the electrode potential at pH about 1 yield straight lines for N-nitroso N-methylaniline and N-nitroso diphenylamine with the slopes of 0.068 and 0.047, respectively. For N-nitroso dimethylamine, N,N'-dinitroso piperazine, and N-nitroso morpholine the first part of the waves yield a straight line with the slopes of 0.080, 0.125 and 0.115, respectively, whereas the second part of the waves yield a curve which deviates from the straight line corresponding to a drawn out wave. As a reversible one-electron reduction would yield a straight line with the slope of 0.059 the slope of N-nitroso diphenylamine 0.047 suggests that more than one electron is involved in the potential determining step.

Discussion. The half-wave potentials depend upon the structure in a manner which was to be expected. The introduction of a phenyl group lowers the half-wave potential, but at the same time both the form of the wave and the dependence of the half-wave potential upon pH approach the behaviour of a reversible reduction. This is probably due to the increased resonance possibilities of the primarily formed radical present when a phenyl group participates in the resonance system.

The polarographic analysis shows that the electrode reaction in acid solution involves twice as many electrons as the electrode reaction in alkaline solution as the limiting currents are twice as high in acid as in alkaline solution and both of them are diffusion controlled. This would be in accordance with the assumption that the reduction in acid solution yielded the hydrazine and in alkaline solution either the tetrazene or the amine and nitrous oxide. It seems reasonable to assume that in acid solution it is the protonated nitrosamine $RR'N—NOH^+$ which is reduced whereas the free nitrosamine is reduced in alkaline solution. This is consistent with the fact that the half-wave potentials in alkaline solution are independent of pH, whereas they in acid solution have a linear dependence upon pH. It seems necessary to assume the reduction of a doubly protonated molecule $(C_6H_5)_2NH^+—N=OH^+$ in order to explain the behaviour of N-nitroso diphenylamine in strongly acid solution, where the half-wave potentials are dependent upon the square of the hydrogen ion concentration. A similar pH dependence was found [3] for N-nitroso phenylhydroxylamine. The electrode reaction of N-nitroso diphenylamine in strong acid solution is a four electron reduction.

The reduction of *p*-nitroso N,N-dimethylaniline requires the same number of electrons in acid and alkaline solution, whereas the reduction of N-nitroso phenylhydroxylamine in acid solution involves six electrons and in alkaline solution four electrons [3]. The polarographic behaviour of N-nitrosamines, N-nitroso phenylhydroxylamine, and *p*-nitroso N,N-dimethylaniline is thus different.

ANALYTICAL INVESTIGATION

English [2] found that methylamine on reaction with nitrous acid in strong acetic acid yielded some nitromethane which interfered with the determination of dimethylamine. Trimethylamine in high concentration yielded some N-nitroso dimethylamine under the same conditions.

Using the procedure described below only negligible side reactions took place for the following primary amines: methylamine, ethylamine, and *cyclo*-hexylamine. Trimethylamine was nitrosated to some extent, whereas triethylamine was only slightly attacked and N-ethylpiperidine reacted only to a slight degree with nitrous acid when the concentration of the tertiary amine was less than 5×10^{-3} M in the final solution.

Primary aromatic amines form diazonium compounds on reaction with nitrous acid and these [4] or their coupling products are reducible at potentials in the neighbourhood of those of secondary nitrosamines. Tertiary aromatic amines form *p*-nitroso compounds on reaction with nitrous acid and these are reducible at potentials lower than those of N-nitrosamines. High concentrations of primary and tertiary aromatic amines will thus make a determination of secondary amines difficult.

In a mixture containing secondary aromatic and aliphatic amines it is possible to determine both kinds of amines as there is a difference of more than 0.2 volt between the half-wave potentials of the nitrosamines of secondary aromatic and aliphatic amines.

Nitrosation of amines. Attempts to nitrosate amines in dilute hydrochloric acid with excess of sodium nitrite were successful for amines as diphenylamine and N-methylaniline, whereas diethylamine and piperidine did not yield the nitrosamines under these conditions. Morpholine and diethanolamine yielded some nitrosamine but the yield was not quantitative. As it apparently was the weak bases which reacted with nitrous acid under acid conditions it was tried to nitrosate the strong amines at higher pH-values. It was found that in an acetate buffer, pH about 4, all the amines tried gave quantitative yields of the nitrosamines.

Procedure. For the quantitative determination of secondary amines the following procedure was found suitable. To 1.00 ml of an amine solution containing about 1 mg of the amine is added 1 ml of acetate buffer (10 ml glacial acetic acid + 10 ml 2 N NaOH + 80 ml water) and 1 ml 20 % aqueous sodium nitrite (prepared daily). The mixture is warmed for about 15 min in a water bath at 80°. After cooling there is added 1 ml 4 N HCl, 5 ml saturated potassium chloride solution, 5 ml ethanol and 1 ml of an aqueous 5 % ammonium sulfamate solution and the volume is adjusted to 25.0 ml. This is quantitatively transferred to the polarographic cell and purified nitrogen is bubbled through for 10 min. At the end of that time the last traces of nitrite are destroyed by adding 1.00 ml 5 % ammonium sulfamate solution and the solution is polarographed.

By using this procedure a linear connection between wave height and concentration (from about 2×10^{-4} to about 4×10^{-3} M) was found for the following amines: diphenylamine, N-methylaniline, diethylamine, piperidine, piperazine, morpholine and diethanol amine.

As mentioned above high concentrations of aromatic amines will invalidate the results. The same is the case with ethylene diamine. The reason is probably that ethylene diamine forms ethylene oxide on treatment with nitrous acid [5]. The ethylene oxide reacts with excess of nitrous acid [6] or nitrogen dioxide [7] with the formation of nitroethanol [6] or nitroethanol nitrate [7] which is reduced at about the same potential as aliphatic nitrosamines.

PREPARATIVE REDUCTIONS

Preparative reductions at controlled potential at a macro mercury electrode have been used to prove the course of the polarographic reduction. It was found that the N-nitrosamines at pH 1 and 5 yielded the corresponding unsymmetric hydrazine by a four electron reduction in isolated yields of about 80 %. No other reduction products were isolated.

$$RR'N-N=OH^+ + 4\,e^- + 4\,H^+ \rightarrow RR'N-NH_3^+ + 2\,H_2O$$

In alkaline solution the reduction consumed two electrons per molecule and the secondary amine was isolated in high yield. Besides nitrous acid could be isolated.

$$2\,RR'N-N=O + 4\,e^- + 3\,H_2O \rightarrow 2\,RR'NH + N_2O + 4\,OH^-$$

The course of the reductions is not surprising in view of the facts that the nitrosamines in acid solution by chemical reduction mostly yield the hydrazines and that as found by Schueler and Hanna [8] N-nitroso diphenylamine by reduction with $LiAlH_4$ yielded diphenylamine as the main product.

EXPERIMENTAL

The polarograph was a recording polarograph Radiometer Type PO 3a. The capillary delivered 2.346 mg of mercury per second at a corrected mercury column height of 48.5 cm; the drop time t = 3.12 sec (20 % alcohol, 1 N KCl, open circuit); the capillary constant $m^{2/3}t^{1/6}$ = 2.14 $mg^{2/3}\,sec^{-\frac{1}{2}}$.

The reference electrode was a silver/silver chloride electrode in aqueous saturated KCl solution separated from the test solution with an agar plug and a fritted glass diaphragm. The reference electrode was measured against the saturated calomel electrode (S.C.E.).

The cell and circuit used for the controlled potential reductions was the same as used in part I[9]. The coulometer was an oxygen-hydrogen coulometer adapted from Lingane [10].

To the polarographic investigation was used a medium consisting of 1 M KCl in 20 % aqueous ethanol. The buffer solutions were the same as used in part I. The nitrosamines were prepared according to wellknown procedures. The compounds were distilled or recrystallized before use. The solvents and reagents were analytical grade.

Reduction of N-nitroso diphenylamine in acetate buffer. 1.0 g of N-nitroso diphenylamine was dissolved in 120 ml methanolic 2 N LiCl + 10 ml glacial acetic acid + 15 ml aqueous 2 N LiOH. The nitrosamine was reduced at a cathode potential of −1.05 V (*vs.* S.C.E.) with an initial current of 0.4 A. The reduction was followed polarographically. In the coulometer were evolved 385 ml (22°, 760 mm) corresponding to a consumption of 4 electrons per molecule. After completion of the reduction the reaction mixture was diluted with water, made alkaline and extracted several times with ether which was dried over potassium carbonate. To the ether was added alcoholic hydrogen chloride and the precipitate (980 mg) was recrystallized from alcohol-ether (870 mg 78 %). (Found: C 65.25; H 6.00; N 12.55; Cl 15.99. Calc. for $C_{12}H_{13}N_2Cl$: C 65.31; H 5.93; N 12.70; Cl 16.06). The compound was identified as unsymmetric diphenylhydrazine hydrochloride by its analysis.

Reduction of N-nitroso diphenylamine in alkaline solution. 4.0 g of N-nitroso diphenylamine was dissolved in 140 ml methanolic 2 N LiCl + 15 ml methanolic 1 N LiOH and reduced at −1.15 V *vs* S.C.E. The initial current was 0.3 A, 540 ml $O_2 + H_2$ were evolved (23°, 756 mm) corresponding to a consumption of 2 electrons per molecule. When the reduction was completed the reaction medium was diluted with water and the precipitate filtered off. The mother liquor was extracted with ether, which was dried and evaporated.

The precipitate and the residue (3.15 g) were recrystallized from dilute ethanol yielding 2.80 g (82 %) of a compound which was identified as diphenylamine by its m.p. 50–51° (53°), its analysis (Found: C 85.08; H 6.78; N 8.09. Calc. for $C_{12}H_{11}N$: C 85.17; H 6.55; N 8.28) and the analysis of the hydrochloride (Found: C 70.13; H 5.86; N 6.80; Cl 17.26. Calc. for $C_{12}H_{12}NCl$: C 70.14; H 5.89; N 6.82; Cl 17.25).

Reduction of N-nitroso morpholine. 7.0 g of N-nitroso morpholine was dissolved in 150 ml aqueous 2 N potassium chloride and 10 ml 2 N sodium hydroxide. The cathode chamber was deaerated with purified nitrogen and a slow current of nitrogen was passed through the solution during the reduction. After bubbling through the catholyte the nitrogen passed through a wash bottle immersed in an acetone-carbon dioxide cooling bath, then through a cold finger immersed in boiling nitrogen and finally through a solution of ferrous sulfate. The reduction was conducted at a cathode potential of −1.30 V with an initial current of 1.2 A. After completion of the reduction the cooling of the cold finger with boiling nitrogen was stopped. The cold finger contained a white solid which disappeared rapidly when not cooled in boiling nitrogen. No colour was detected in the ferrous sulfate solution. The white solid was identified as nitrous oxide by the following facts: Its m.p. and b.p. were between −80° and −200° (N_2O has b.p. −88° and m.p. −102°), it was colourless and did not react with ferrous sulfate.

The reaction mixture was saturated with potassium hydroxide and repeatedly extracted with ether. The ether was dried with potassium carbonate. On addition of alcoholic hydrogen chloride a precipitate, 3.9 g, was obtained. It was identified as morpholine hydrochloride by its analysis. (Found: C 39.09; H 8.30; N 11.24; Cl 28.69. Calc. for $C_4H_{10}NOCl$: C 38.87; H 8.16; N 11.33; Cl 28.68).

REFERENCES

1. Smales, A. A. and Wilson, H. N. *J. Soc. Chem. Ind.* **67** (1948) 210.
2. English, F. L. *Anal. Chem.* **23** (1951) 344.
3. Kolthoff, I. M. and Liberti, A. *J. Am. Chem. Soc.* **70** (1948) 1885.
4. Elofson, R. M., Edsberg, R. L. and Mecherly, P. A. *J. Electrochem. Soc.* **97** (1950) 166.
5. Hofmann, A. W. *Jahresber. Fortschr. Chem.* **1859** 386.
6. Japan 156256 (1943); *Chem. Abstr.* **44** (1950) 2008.
7. Darzens, G. *Compt. rend.* **229** (1949) 1148.
8. Schueler, F. W. and Hanna, C. *J. Am. Chem. Soc.* **73** (1951) 4996.
9. Lund, H. *Acta Chem. Scand.* **11** (1957) 283.
10. Lingane, J. J. *Electroanalytical Chemistry.* Interscience Publishers, New York, London 1953, p. 349.

Received March 20, 1957.