indifferent antagonism is equally true with the ergot alkaloids. This partial inhibition would be expected if glycogenolysis was mediated through both α and β adrenotropic receptors. If these α and β adrenergic blockers were given together, then the increase in blood sugar and lactic acid can be abolished.

If the contention is true that epinephrine has a greater glycogenolytic effect than either levarterenol or isoproterenol because it stimulates both α and β receptors, then it is reasonable to assume that the combined blockade of both α and β receptors will completely inhibit glycogenolysis. This has been shown to be the case. The results of this study give only an incomplete insight into the mechanisms involved in glycogenolysis, but do suggest that the receptors involved include both the α and the β adrenotropic receptors.

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Polarography of Diastereoisomeric *N*-Nitrosoephedrines

By LOUIS MALSPEIS and NELL G. M. HUNG

The polarographic reductions of *N*-nitrosoephedrine (*erythro* isomer) and *N*-nitrosopseudoephedrine (*threo* isomer) were examined in the pH range 0.6 to 10. Below pH 3.2, the limiting currents of both compounds are diffusion controlled and identical. In the pH range 3.2 to 8.2, the limiting currents of the diastereoisomers are controlled by diffusion and rate of reaction. At a given pH value in this region, under identical conditions, the limiting current for *N*-nitrosoephedrine is greater than that for *N*-nitrosopseudoephedrine. The kinetic currents are catalyzed both by hydrogen ion and general acids. With Delahay's equation, the heterogeneous rate constants of protonation of the diastereoisomers were calculated. The rate of protonation of *N*-nitrosoephedrine is greater than that of *N*-nitrosopseudoephedrine. These relative rates are interpreted in terms of structural considerations. The rate of protonation is greater with that isomer in which there is a lesser degree of intramolecular hydrogen-bonding. Kinetic currents can be used to distinguish between diastereoisomers.

LIMITING POLAROGRAPHIC currents which are controlled by the rate of a chemical reaction and by diffusion are generally referred to as kinetic currents (1, 2). One type of kinetic current is controlled by the rate of reaction in the electrode reaction layer of an electro inactive species which diffuses to the electrode surface from the bulk of the solution. Since the relative reaction rates of diastereoisomers depend upon the population of the possible conformations (3), kinetic currents could be used to distinguish between diastereoisomers. At a given concentration that diastereoisomer which undergoes reaction at the higher rate should afford the higher kinetic current. The primary object of this investigation is to determine the effect of configurational differences on the kinetic currents of diastereoisomers.

The compounds chosen for study are the *N*-nitroso derivatives of (−)-ephedrine and (+)-pseudoephedrine, diastereoisomers having well established configurations (4–7) (see Scheme I). These compounds satisfy the requirement that a center of asymmetry must not be involved in the reaction at the electrode surface, a requirement which increases the likelihood that the reduction mechanisms of the two compounds under comparable conditions will be the same.

EXPERIMENTAL

Materials.—The diastereoisomeric *N*-nitrosoephed-

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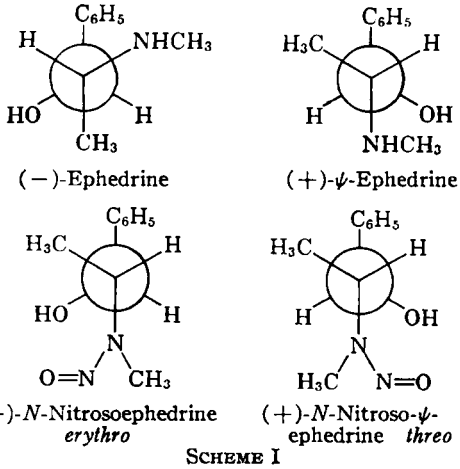


SCHEME I

rines were prepared from a commercial sample of (−)-ephedrine hydrochloride and a research sample of (+)-pseudoephedrine hydrochloride kindly furnished by Burroughs Wellcome and Co., using a modification of the method of Mitchell (8). The rate of nitrosation using 10% hydrochloric acid was extremely slow; the rate was rapid when concentrated hydrochloric acid was used. To the alkaloidal salt (5.0 Gm.) dissolved in 13 ml. water was added a solution of 3.0 Gm. of sodium nitrite in 5 ml. of water. To this mixture was added 2.0 ml. of concentrated hydrochloric acid with constant stirring for 10 minutes. The reaction mixture was stirred for 1 hour longer and the resulting yellow oil was separated and purified according to Mitchell's procedure. There was obtained 2.58 Gm. of *N*-nitrosoephedrine, m.p. 93.8 to 94.2°, $[\alpha]_D^{25}$ + 80.7° (reported (8) m.p. 93°, $[\alpha]_D^{20}$ + 80.5°) and 3.03 Gm. of *N*-nitrosopseudoephedrine, m.p. 88.0 to 88.5°, $[\alpha]_D^{22.5}$ + 126.7° (reported (8) m.p. 86.0°, $[\alpha]_D^{20}$ + 124.5°).

The buffer systems were prepared from reagent grade chemicals which were used without further purification. The composition of the buffers for the pH dependence study are listed in Table I.

Equipment.—Polarograms were recorded with a Leeds and Northrup Electro-Chemograph, type E; the electrolysis vessel was an H-type cell containing a saturated calomel reference electrode separated from the solution compartment by a potassium chloride–agar plug and a sintered-glass disk. The polarographic cell was thermostated at 25 ± 0.1°.

TABLE I.—BUFFER COMPOSITION, IONIC STRENGTH = 0.29

Buffer	pH[a]	Composition, moles/L.		
1		HCl		KCl
	0.60	0.300		...
	1.60	0.030		0.260
	2.61	0.003		0.287
2		H_3Citrate	NaH_2Citrate	KCl
	3.11	0.005	0.00175	0.2882
	3.23	0.005	0.00250	0.2875
	3.39	0.005	0.00345	0.2866
	3.53	0.005	0.00400	0.2860
	3.70	0.005	0.00477	0.2853
3		$HC_2H_3O_2$	$NaC_2H_3O_2$	KCl
	3.94	0.005	0.001	0.289
	4.38	0.005	0.002	0.288
	4.75	0.005	0.005	0.285
	5.11	0.005	0.012	0.278
	5.20	0.005	0.015	0.275
	5.47	0.005	0.020	0.270
	5.70	0.005	0.045	0.235
4		KH_2PO_4	K_2HPO_4	KCl
	5.57	0.005	0.0003	0.2841
	5.82	0.005	0.0006	0.2832
	6.15	0.005	0.0011	0.2817
	6.45	0.005	0.0021	0.2787
	6.65	0.005	0.0033	0.2751
	6.74	0.005	0.0041	0.2727
	6.82	0.005	0.0050	0.2700
	6.89	0.005	0.0061	0.2667
	7.00	0.005	0.0075	0.2625
	7.11	0.005	0.0093	0.2571
	7.21	0.005	0.0116	0.2502
	7.33	0.005	0.0150	0.2400
	7.48	0.005	0.0200	0.2250
	7.62	0.005	0.0283	0.2001
	7.84	0.005	0.0450	0.1500
	7.95	0.005	0.0600	0.1050
	8.22	0.005	0.0950	...
5		NH_4OH	NH_4Cl	KCl
	8.95	0.005	0.0117	0.2783
	9.33	0.005	0.0050	0.2850
	9.61	0.005	0.0021	0.2879
	10.03	0.005	0.0006	0.2894

[a] pH values of the buffer solutions after the addition of 10% by volume of 95% ethanol.

The dropping mercury electrode had values of $m^{2/3}t^{1/6}$ of 2.165 mg.$^{2/3}$ sec.$^{-1/2}$ open circuit and 2.092 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.35 v. *versus* S.C.E. in orthophosphate buffer, 2.165 mg.$^{2/3}$ sec.$^{-1/2}$ open circuit and 2.084 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.35 v. *versus* S.C.E. in acetate buffer, 2.140 mg.$^{2/3}$ sec.$^{-1/2}$ open circuit and 2.045 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.70 v. *versus* S.C.E. in ammonia buffer, and 2.170 mg.$^{2/3}$sec.$^{-1/2}$ at pH 3.70 and 2.144 mg.$^{2/3}$ sec.$^{-1/2}$ at pH 3.11 open circuit in citrate buffer. The values of $m^{2/3}t^{1/6}$ are at a corrected mercury head of 80.9 cm., except in the experiments concerned with the variation of average limiting current with the head of mercury.

Applied voltages were measured with a Rubicon model 2730 potentiometer and the *i*R drop in the circuit was measured with an Industrial Instruments model RC conductivity bridge. A Beckman model G pH meter was used.

Polarographic Procedure.—A stock solution of the nitrosamine was prepared daily by dissolving a weighed amount of the compound in 95% ethanol. The sample solution was made by the dilution of 5.00 ml. of the stock solution to 50.00 ml. with the appropriate buffer solution. The polarographic cell was rinsed with the sample solution and then filled. The solution was deaerated in the cell by bubbling nitrogen gas through the solution in accordance with standard practice, and the polarographic wave was recorded at a constant head of mercury (80.9 cm. corr.). The initial potential of the polarogram was measured and the voltage span of the polarograms was calibrated with the Rubicon potentiometer. The reported half-wave potentials are corrected for ohmic drop; the reported limiting currents are corrected for the residual current. Damping "2" was used throughout the study. The reported limiting currents are average currents.

The ritual used for determining half-wave potentials involved bisecting the distance between the residual current and the tangent to the wave and constructing a parallel to the residual current through this point; the voltage corresponding to the point of intersection of the rising wave with the parallel was taken as the half-wave potential.

RESULTS

Wave Characteristics.—Both nitrosamines gave a single wave in acidic solutions up to pH 3.7. A second wave was observed in the pH range 3.9 to 6.9. In alkaline solution, pH 8.95 to 10.03, only

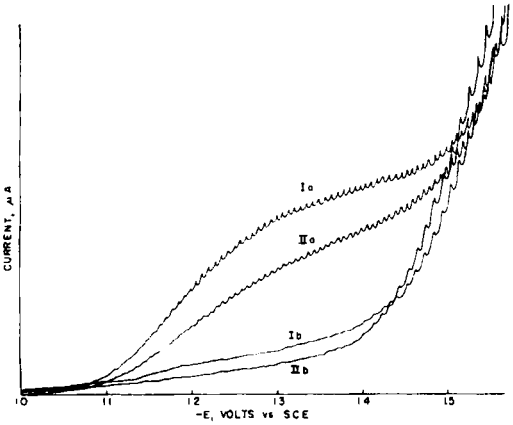


Fig. 1.—Polarograms of *N*-nitrosoephedrine (I) and *N*-nitrosopseudoephedrine (II) in 0.41 *M* acetate buffer (*a*) at pH 5.69 and in 0.0045 *M* phosphate buffer (*b*) at pH 7.48. Depolarizer concentration 4×10^{-4} *M*.

TABLE II.—WAVE HEIGHTS AND HALF-WAVE POTENTIALS IN 4×10^{-4} *M* SOLUTIONS OF THE DIASTEREOISOMERIC *N*-NITROSOEPHEDRINES AT VARYING pH AT 25°

pH	*N*-Nitrosoephedrine $-E^{1/2}$, v. *vs.* S.C.E.	*N*-Nitrosoephedrine Wave Ht.,[a] μA.	*N*-Nitrosopseudoephedrine $-E^{1/2}$, v. *vs.* S.C.E.	*N*-Nitrosopseudoephedrine Wave Ht.,[a] μA.
0.60	0.730	5.51	0.728	5.51
1.60	0.843	5.51	0.837	5.51
2.61	0.958	5.51	0.952	5.51
3.11	1.005	5.51	0.996	5.51
3.23	1.018	5.51	1.010	5.51
3.39	1.033	5.51	1.027	5.47
3.53	1.052	5.47	1.046	5.39
3.70	1.060	5.43	1.042	5.35
3.94	1.084	5.24	1.081	4.96
4.38	1.108	4.80	1.115	4.57
4.75	1.125	4.61	1.136	4.13
5.11	1.146	4.09	1.156	3.62
5.20	1.151	3.94	1.163	3.39
5.47	1.166	3.31	1.178	2.60
5.70[b]	1.175	2.80	1.184	2.21
5.57	1.165	1.52	1.163	1.22
5.82	1.174	1.35	1.174	1.03
6.15	1.178	1.06	1.177	0.79
6.45	1.181	0.77	1.189	0.57
6.65	1.184	0.60	[c]	0.47
6.74	1.186	0.53	[c]	0.41
6.82	1.190	0.47	[c]	0.35
6.89	[c]	0.43	[c]	0.33
7.00	[c]	0.41	[c]	0.27
7.11	[c]	0.41	[c]	0.26
7.21	[c]	0.33	[c]	0.25
7.33	[c]	0.40	[c]	0.22
7.48	[c]	0.27	[c]	0.19
7.62	[c]	0.22	[c]	0.17
7.84	[c]	0.21	[c]	0.18
7.95	[c]	0.20	[c]	0.13
8.22	[c]	0.18	[c]	0.12
8.95	1.522	2.87	1.524	2.48
9.33	1.524	2.70	1.532	2.30
9.61	1.532	2.52	1.531	2.22
10.03	1.540	2.30	1.542	2.09

[a] Data were secured at −1.35 v. *vs.* S.C.E. in the pH range 0.60 to 8.22 and at −1.70 v. *vs.* S.C.E. in the pH range 8.95 to 10.03. Currents in the pH range 0.60 to 3.70 are extrapolated values. [b] Buffer 3. [c] Poorly defined wave.

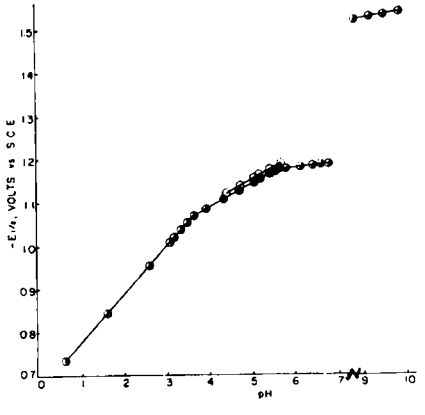


Fig. 2.—Variation of half-wave potential, $E_{1/2}$, with pH for ● *N*-nitrosoephedine and ○ *N*-nitrosopseudoephedrine. Identical half-wave potentials for both diastereoisomers are denoted by ◑.

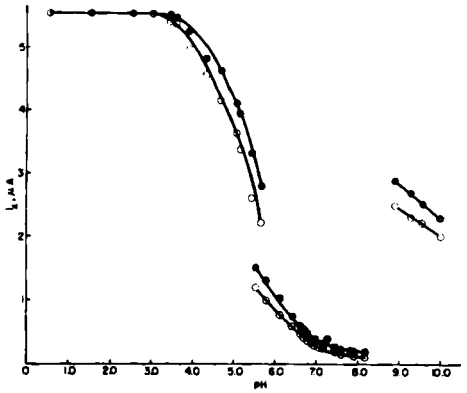


Fig. 3.—Variation of limiting current with pH at 25°C. and ionic strength 0.261. Key: ●, 4×10^{-4} *M* *N*-nitrosoephedrine; ○, 4×10^{-4} *M* *N*-nitrosopseudoephedrine. pH range 0.60 to 8.22, −1.35 v. *vs.* S.C.E.; pH range 8.95 to 10.03, −1.70 v. *vs.* S.C.E. Currents in the pH range 0.60 to 3.70 are extrapolated values. Identical currents for both diastereoisomers are denoted by ◑. pH 0.60 to 2.60, buffer 1; pH 3.10 to 3.65, buffer 2; pH 3.94 to 5.70, buffer 3; pH 5.57 to 8.22, buffer 4; pH 8.95 to 10.03, buffer 5.

one wave was seen. The reported values of potential and current refer to the first wave in the pH range where two waves occur.

The second wave possessed a prominent maximum. The total height of the first and second waves remained constant in both the acetate (16.4 μA.) and orthophosphate buffer systems (13.5 μA.). Accurate values of the half-wave potentials could not be determined because of the maximum. Within the experimental error, the half-wave potentials of the second wave and *total* wave heights for both diastereoisomers appeared to be the same in both buffer regions.

The first wave in strongly acidic solution is well defined, and up to pH 3.23 the waves appear to be identical for both isomers. With increasing pH, the characteristic wave shape of the compounds becomes increasingly drawn out. Figure 1 shows that the current-potential curve of the first wave for *N*-nitrosopseudoephedrine is more irreversible than the curve for *N*-nitrosoephedrine at pH 5.69 in acetate buffer. At pH 6.65 for the *threo* isomer and pH 6.89 for the *erythro* isomer, the waves become poorly defined. Typical poorly defined waves at pH 7.48 are given in Fig. 1.

The irreversible character of the reduction was demonstrated by the results of the logarithmic analysis of the first wave. At pH 0.6 and 1.6, the plot of log $(i/i_d - i)$ *versus* E exhibited two straight line segments. At pH 2.6, the log plot yielded a single straight line with a slope of 11.8 $v.^{-1}$ for both diastereoisomers. At pH 3.1, straight line log plots with slopes of 12.0 $v.^{-1}$ for *N*-nitrosoephedrine and 11.0 $v.^{-1}$ for *N*-nitrosopseudoephedrine were obtained. At higher pH values, the log plots showed two and three straight-line segments and occasionally single straight lines. Similar results have been reported for the reduction of aromatic *N*-nitrosohydroxylamines (9).

pH Dependence.—The half-wave potentials and limiting currents of the diastereoisomeric nitrosamines at different pH values are listed in Table II.

The half-wave potentials of both compounds shift to more negative values with increase in pH. It is evident from Fig. 2 that the linear variation of half-wave potential with pH in the acid region (pH 0.60 to 6.82) is divided into three pH ranges. In the pH range 0.60 to 3.53, the half-wave potentials of the isomers are approximately the same. The half-wave potential varies with pH according to the expression $E_{1/2}(E) = -0.668 - 0.110$ pH for *N*-nitrosoephedrine and $E_{1/2}(\psi) = -0.663 - 0.110$ pH for *N*-nitrosopseudoephedrine, where the intercepts are obtained by extrapolation. The half-wave potential of the *erythro* isomer is clearly more positive than that of the *threo* isomer in the pH range 3.70 to 5.70 (acetate buffers); however, the slopes of the $E_{1/2}$ *versus* pH plots are identical, so that $E_{1/2}(E) = -0.863 - 0.055$ pH and $E_{1/2}(\psi) = -0.873 - 0.055$ pH. The half-wave potentials of both isomers are virtually identical in the pH range 5.57 to 6.82 (phosphate buffers), and the linear variation of $E_{1/2}$ with pH is slight; the expression $E_{1/2} = -1.073 - 0.016$ pH applies to both diastereoisomers.

In the alkaline region, only the pH range 8.95 to 10.03 (ammonia buffers) was examined in the present study. The half-wave potentials of both isomers are identical in this region, and there is also a

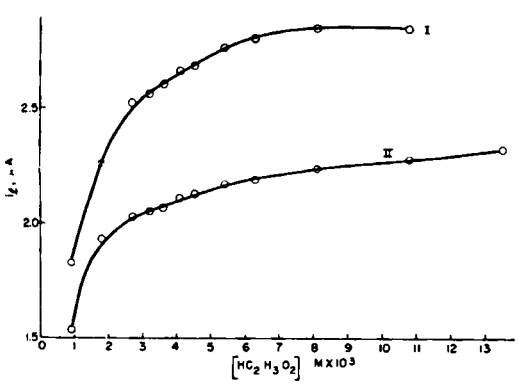


Fig. 4.—Variation of limiting current with concentration of $HC_2H_3O_2$ (−1.35 v. *vs.* S.C.E.). Acetate buffer pH 5.65; ionic strength, 0.261. Curve I: 4×10^{-4} *M* *N*-nitrosoephedrine; curve II: 4×10^{-4} *M* *N*-nitrosopseudoephedrine.

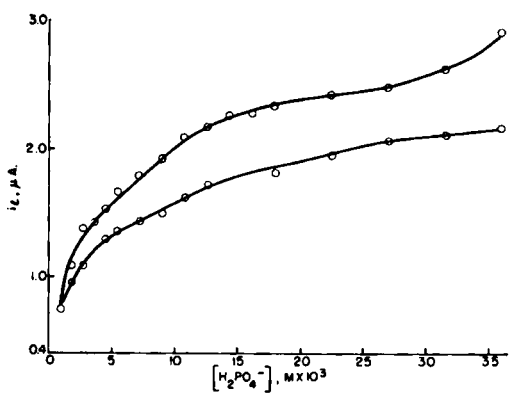


Fig. 5.—Variation of limiting current with concentration of $H_2PO_4^-$ (−1.35 v. *vs.* S.C.E.). Phosphate buffer pH 5.59; ionic strength 0.261. Upper curve, 4×10^{-4} *M* *N*-nitrosoephedrine; lower curve, 4×10^{-4} *M* *N*-nitrosopseudoephedrine.

linear shift of $E_{1/2}$ with pH to more negative values as the pH is increased. The pH-dependence of the half-wave potentials in this region is given by $E_{1/2} = -1.380 - 0.021$ pH.

The effect of pH on the limiting currents is shown in Fig. 3. Since the present study is primarily concerned with kinetic currents in the pH range 3.53 to 8.22, comparisons of limiting currents in the acid region were made at −1.35 v. *versus* S.C.E. Accordingly, it was necessary to extrapolate currents in the pH range 0.60 to 3.70 to −1.35 v. *versus* S.C.E. Comparisons of limiting currents in the alkaline region are at −1.70 v. *versus* S.C.E. The limiting currents for both isomers were identical and constant in the pH range 0.60 to 3.23. With increasing pH, the height of the waves decreased in the manner characteristic of polarographic currents controlled by reaction rate and by diffusion. The striking result is that at a given pH in the range 3.39 to 8.22 under identical conditions, the limiting current for *N*-nitrosoephedrine is invariably greater than the current for *N*-nitrosopseudoephedrine. For each set of buffers in the acid region of the pH-limiting current profile (Fig. 3), the concentration of the buffer acid was maintained constant (Table I).

TABLE III.—DEPENDENCE OF WAVE HEIGHT ON ACETIC ACID CONCENTRATION (−1.35 V. *vs.* S.C.E.) IN ACETATE BUFFERS AT pH 5.65 AND IONIC STRENGTH 0.261

$[HC_2H_3O_2]$ $M \times 10^3$	Wave Ht., μA. *N*-Nitrosoephedrine[a]	Wave Ht., μA. *N*-Nitrosopseudoephedrine[a]
13.5		2.32
10.8	2.84	2.28
8.1	2.84	2.24
6.3	2.80	2.19
5.4	2.76	2.17
4.5	2.68	2.13
4.1	2.66	2.11
3.6	2.60	2.07
3.2	2.56	2.05
2.7	2.52	2.03
1.8	2.26	1.93
0.9	1.83	1.54

[a] Depolarizer concentration 4×10^{-4} *M*.

TABLE IV.—DEPENDENCE OF WAVE HEIGHT ON DIHYDROGEN PHOSPHATE CONCENTRATION (−1.35 V. *vs.* S.C.E.) IN ORTHOPHOSPHATE BUFFERS AT pH 5.59 AND IONIC STRENGTH 0.261

$[H_2PO_4^-]$ $M \times 10^3$	Wave Ht., μA. *N*-Nitrosoephedrine[a]	Wave Ht., μA. *N*-Nitrosopseudoephedrine[a]
36.0	2.93	2.18
31.5	2.64	2.13
27.0	2.50	2.08
22.5	2.44	1.97
18.0	2.35	1.83
16.2	2.30	...
14.4	2.28	...
12.6	2.19	1.73
10.8	2.11	1.63
9.0	1.93	1.51
7.2	1.80	1.44
5.4	1.70	1.36
4.5	1.54	1.30
3.6	1.43	...
2.7	1.38	1.08
1.8	1.09	0.96
0.9	0.77	0.75

[a] Depolarizer concentration 4×10^{-4} *M*.

TABLE V.—DEPENDENCE OF WAVE HEIGHT ON DEPOLARIZER CONCENTRATION (−1.35 V. *vs.* S.C.E.) IN ORTHOPHOSPHATE BUFFER AT pH 7.45 AND IONIC STRENGTH 0.261

Concn., $M \times 10^4$	Wave Ht., μA. *N*-Nitrosoephedrine	Wave Ht., μA. *N*-Nitrosopseudoephedrine
20	0.768	0.673
16	0.626	0.532
12	0.496	0.413
8	0.366	0.307
4	0.248	0.189

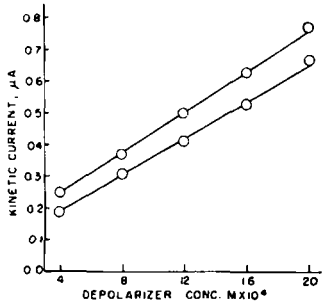


Fig. 6.—The kinetic current in phosphate buffer, pH 7.45, ionic strength 0.261 as a function of *N*-nitrosoephedrine (upper curve) and *N*-nitrosopseudoephedrine (lower curve) concentrations (−1.35 v. *vs.* S.C.E.).

The discontinuity in the pH profile at about pH 5.6 occurs where there is a change from acetate buffers to orthophosphate buffers. It is evident that the limiting current depends on the concentration and type of the buffer acid as well as the hydronium ion concentration.

In the alkaline region, pH 8.95 to 10.03, a linear decrease of limiting current with increasing pH was observed for both isomers. The ammonia concentration was maintained constant in this experiment. It is significant that at a given pH under identical conditions the limiting current of the *erythro* isomer is greater than that of the *threo* isomer.

Variation of Limiting Current with General Acid and Depolarizer Concentration.—The dependence of the limiting current on the concentrations of acetic acid at pH 5.65 and dihydrogen phosphate ion at pH 5.59 are summarized in Figs. 4 and 5 and Tables III and IV. In these experiments, the buffer ratio was maintained constant; constancy of pH was checked throughout the experiment. In each case, at very low concentrations of general acid the limiting currents were very low and increased rapidly with an increase in the general acid concentration. Then followed a range of concentrations of the general acid over which the limiting current proved to be a linear function of the buffer acid concentration. At higher concentrations of the general acid, the rate of change of wave height with concentration of the buffer acid decreased, and the limiting currents appeared to approach a constant value. For a substrate concentration of 4×10^{-4} *M*, it was observed that the limiting current varies linearly with $[HC_2H_3O_2] = 2.7 \times 10^{-3}$ to 5.4×10^{-3} *M* at pH 5.65 and that the limiting current varies linearly with $[H_2PO_4^-] = 4.5 \times 10^{-3}$ to 10.8×10^{-3} *M* at pH 5.59. It is apparent in both cases that the slope of the

TABLE VI.—VARIATION OF AVERAGE LIMITING CURRENT[a] WITH HEAD OF MERCURY FOR *N*-NITROSOEPHEDRINE AT pH 5.59, 7.45, 8.22, AND 9.33 AND IONIC STRENGTH 0.261

	N-Nitrosoephedrine[b]											
h corr.,[c] cm.	pH 5.59[d]		pH 5.59[e]		pH 5.59[f]		pH 7.45[f]		pH 8.22[f]		pH 9.33[g]	
	i_l, μA.	$i_l/h^{1/2}$	i_l, μA.	$i_l/h^{1/2}$	i_l, μA.	$i_l/h^{1/2}$	i_l, μA.	$i_l/h^{1/2}$	i_l, μA.	$i_l/h^{1/2}$	i_l, μA.	$i_l/h^{1/2}$
80.9	2.62	0.291	1.93	0.216	1.55	0.172	0.236	0.0262	0.165	0.0184	2.80	0.311
65.3	2.50	0.309	1.81	0.224	1.44	0.178	0.213	0.0263	0.154	0.0190	2.56	0.317
49.3	2.32	0.330	1.69	0.241	1.38	0.196	0.189	0.0269	0.136	0.0194	2.30	0.328
31.2	2.08	0.371	1.50	0.267	1.26	0.225	0.159	0.0286	0.112	0.0201	1.95	0.349

[a] Data were secured at −1.35 v. *vs.* S.C.E. at pH 5.59, 7.45, and 8.22 and at −1.70 v. *vs.* S.C.E. at pH 9.33. [b] Depolarizer concentration 4×10^{-4} *M*. [c] Mercury height corrected for back pressure; $h_{soln.}$ = 2.5 cm. [d] $[H_2PO_4^-] = 0.036$ *M*. [e] $[H_2PO_4^-] = 0.009$ *M*. [f] $[H_2PO_4^-] = 0.0045$ *M*. [g] $[NH_4OH] = 0.0045$ *M*.

linear plot for the *erythro* isomer is greater than that for the *threo* isomer.

Preliminary experiments were performed to arrive at an estimate of the rate constants of protonation due to water, hydronium ions, and the $H_2PO_4^-$ ion. Several studies of the above type using a constant buffer ratio $[H_2PO_4^-]/[HPO_4^=]$ were made in the narrow pH range 5.59 to 5.91. The slopes of the plot of limiting current against $[H_2PO_4^-]$ were "almost" parallel; slight deviations are undoubtedly due to protonation of the nitrosamines by $HPO_4^=$ ion.

The variation of the limiting currents with the concentration of the substrate was examined at pH 7.45, where the limiting current is largely kinetic current. The limiting current varied linearly with concentration for both diastereoisomers between 4×10^{-4} *M* and 2×10^{-3} *M* (Table V, Fig. 6).

Variation of Average Limiting Current with Head of Mercury.—Wave heights of *N*-nitrosoephedrine were determined at pH values 5.59, 7.45, 8.22, and 9.33 at different mercury column heights from 31.2 to 80.9 cm. (Table VI). The average limiting currents were corrected for variations of residual current with mercury column height, and the mercury head was corrected for back pressure. The experiments in the pH range 5.59 to 8.22 were carried out in phosphate buffers; those at pH 9.33 were done in ammonia buffers. In each experiment a decrease in limiting current and an increase in the ratio $i_l/h^{1/2}$ resulted from a decrease in the height of the mercury column. At pH 5.59, the concentration of dihydrogen phosphate ion was varied from 4.5×10^{-3} to 3.6×10^{-2} *M* to determine the effect of the buffer acid concentration on the variation of the average limiting current with the mercury head. It is seen that with increasing concentration of the buffer acid, the increase in the ratio $i_l/h^{1/2}$ with the decrease in the mercury head becomes more pronounced.

DISCUSSION

Over-all Reactions.—Systematic studies of the polarographic reduction of *N*-nitrosamines of secondary aliphatic and heterocyclic amines were reported by Lund (10) and by Zahradník, Svátek, and Chvapil (11). Based upon controlled potential electrolysis at a macro mercury electrode, Lund proposed that a four-electron reduction yielding a hydrazine derivative occurs in the acid region and that a two-electron reduction yielding the secondary amine occurs in alkaline solution.

$$RR'N{-}N{=}O + 4H^+ + 4e^- \rightarrow RR'N{-}NH_3^+ + 2H_2O$$

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

The same course of the reduction in the acidic region was proposed by Zahradník and co-workers based upon their analysis of the polarographic waves. Analogous to the reaction scheme postulated by Kolthoff and Liberti (12) for the reduction of *N*-nitrosophenylhydroxylamine, Zahradník, *et al.*, suggested that the reduction proceeds in two stages: a two-electron reduction of the nitrosamine to the aminohydroxylamine, followed by a two-electron reduction of the aminohydroxylamine to the hydrazine derivative. In the present study the acid region is considered to extend to pH 8.22. The assumption is made that the hydrazine derivative is the product of the electrochemical reduction.

pH Dependence of Half-Wave Potentials.—The fact that the slopes of the plots of $E_{1/2}$ *versus* pH are the same for both *N*-nitrosoephedrine and *N*-nitrosopseudoephedrine indicates that the number of hydrogen ions which react with the substrate for each electron which is transferred is the same for both diastereoisomers in each of the three pH ranges of the acid region (Fig. 2). It is noted that changes of slope occur with changes of buffer system in the pH region where the current is not entirely diffusion controlled. The first change of slope occurs at about pH 3.5; at this pH the limiting current changes its character from diffusion controlled current to diffusion and kinetic-controlled current. The second change of slope is at about pH 5.7, where the limiting current becomes largely kinetic controlled. At a given pH value in both acetate and orthophosphate buffers, the half-wave potentials were not dependent upon the concentration of the buffer acid, although a slight shift to more negative potentials was observed at high orthophosphate buffer concentrations.

According to Elving (9), no satisfactory interpretation of the magnitudes of the slopes of the $E_{1/2}$ *versus* pH plots is known for the aromatic *N*-nitrosohydroxylamines. The same problem is present in the reduction of the *N*-nitrosamines. The different slopes in the acid region suggest that the reaction mechanisms at the electrode surface change and that the buffer components participate in the reactions.

Current Controlled by Reaction Rate and Diffusion.—It appears from the shape of the pH-limiting current profile (Fig. 3) that the nitrosamine is protonated prior to reduction (11). The unprotonated nitrosamine is either not reduced at the electrode surface, or more likely, it is reduced at greater negative potentials than the protonated compound. The second wave may be due to reduction of the unprotonated compound.

Unlike the kinetic currents of most weak acids which have been studied (13), where the weak acid

and its conjugate base are present in solution, the *N*-nitroso derivatives of aliphatic amines are so weakly basic that the protonated nitrosamine is not present in aqueous solution at pH 0.5 or greater. The ultraviolet absorption spectra of *N*-nitrosoephedrine and *N*-nitrosopseudoephedrine did not change over the pH range 0.60 to 8.22. Hazeldine and Martinson (14) observed that the ultraviolet spectrum of diethylnitrosamine is the same in aqueous neutral, acidic, or alkaline solutions. Zahradník, *et al.* (11), reported that the pK values at 22° of *N*-nitroso derivatives of secondary aliphatic and heterocyclic amines determined in 18.4% sulfuric acid (H_o = −0.80) range from −1.28 to −1.99. Thus, it is concluded that the nitrosamine exists only as the unprotonated species in the bulk of the solution, and that it is only in the field of the electrode that protonation occurs.

The limiting current in the reduction of the protonated nitrosamine is determined by the diffusion of the nitrosamine to the electrode and by the rate of protonation at the electrode surface. In the pH range 0.60 to 3.39 for *N*-nitrosoephedrine and 0.60 to 3.23 for *N*-nitrosopseudoephedrine, the limiting current is invariant with pH change (Fig. 3). The concentration of the hydrogen ion in the electrode double layer is high in this region, and the current is diffusion controlled. It is apparent that the diffusion currents of both diastereoisomers are the same, and it follows that the diffusion coefficients of their conjugate acids are the same. Assuming that four electrons are involved in the reduction, the diffusion coefficient D, was calculated from the classical Ilkovic equation to be 7.36×10^{-6} cm.^{2} sec.$^{-1}$.

With increasing pH, the limiting currents decrease as they are controlled by both diffusion and the rate of protonation. Since the diffusion coefficients of both diastereoisomers are the same, the higher limiting current for *N*-nitrosoephedrine at a given pH value in the range 3.39 to 8.22 under identical conditions indicates that the rate of protonation at the electrode surface of *N*-nitrosoephedrine is greater than that of *N*-nitrosopseudoephedrine.

The fact that the limiting current for the reduction of secondary aliphatic and heterocyclic *N*-nitrosamines in strongly acidic and strongly alkaline solutions is diffusion controlled was established by both Lund (10) and Zahradník, *et al.* (11), who found that the limiting current varied linearly with the square root of the corrected mercury column height. The present study is primarily concerned with the reduction of aliphatic *N*-nitrosamines in aqueous buffers where the current is both diffusion and kinetic controlled. In Table VI, it is seen that the average limiting current decreases and the ratio $i_l/h^{1/2}$ increases as the head of mercury is decreased at pH 5.59, 7.45, 8.22, and 9.33. Clearly, the current is not diffusion controlled in these buffers. The decrease in limiting current with a decrease in the head of mercury is characteristic of currents controlled both by reaction rate and diffusion (15). Unexpectedly, the currents at pH 7.45 and pH 8.22 do not appear to be pure kinetic currents, for the magnitude of the currents are dependent on the mercury head.

At about pH 5.6, there is a discontinuity in the pH-limiting current profile, which indicates that the buffer acids also protonate the nitrosamines at the electrode surface. Since protonation occurs in the potential field of the electrode, the kinetic current due to protonation of the substrate by acetic acid must be larger than the kinetic current, due to protonation by dihydrogen phosphate ion because of the repulsion of the negatively charged ion by the electrode. Consequently, the limiting currents at pH 5.57 in acetate buffers are higher than the limiting currents in orthophosphate buffers at the same concentration of buffer acid.

There are three concentration regions of interest in the plots of limiting currents *versus* general acid concentration (Figs. 4 and 5). At very low concentrations of the general acid, the concentration of the general acid in the electrode double layer is less than that in the bulk of the solution as the electrolysis proceeds. The limiting current in this first region is very low since the catalyst molecule must diffuse to the electrode double layer prior to protonation of the substrate. As the concentration of the buffer acid is increased, the change of the buffer acid concentration in the electrode double layer is relatively small as the electrolysis proceeds, and the concentration of the buffer acid in the electrode double layer can be assumed to be the same as that in the bulk of the solution. The kinetic current in this region varies linearly with the concentration of buffer acid. In the third region, the concentrations of the buffer acid are high, and the limiting current tends to become independent of the buffer acid concentration. The attainment of a constant limiting current is the consequence of reaching a limiting rate of protonation at high catalyst concentrations since the catalyst concentrations are much larger than the substrate concentrations in these systems.

The fact that the slopes of the linear variation of limiting current with the concentrations of acetic acid or dihydrogen phosphate ion are greater for *N*-nitrosoephedrine than for *N*-nitrosopseudoephedrine suggests that the former compound is protonated at a faster rate by these acids.

It is of interest to note that Zahradník and coworkers (11) also observed dependence of the limiting current on phosphate buffer concentration in the polarographic reduction of *N*-nitrosopyrrolidine. However, the reported pH dependency of the wave height showed no discontinuity upon change of the buffer system. In this laboratory, it was observed that the limiting current in the reduction of *N*-nitrosopyrrolidine at pH 5.57 is greater in acetate buffer than in orthophosphate buffer having the same concentration. It is probable that the concentrations of both acetate and phosphate buffers which were used by Zahradník, *et al.* (11), were very high,

TABLE VII—HETEROGENEOUS PSEUDO FIRST-ORDER RATE CONSTANTS OF PROTONATION (−1.35 v. *vs.* S.C.E.) IN ORTHOPHOSPHATE BUFFERS AT pH 5.91 AND IONIC STRENGTH 0.261

$[H_2PO_4^-]$, $M \times 10^3$	*N*-Nitrosoephedrine[c] $(k_r)_h$, cm./sec.$^{-1}$ Exptl.[a]	Calcd.[b]	*N*-Nitrosopseudoephedrine[c] $(k_r)_h$, cm./sec.$^{-1}$ Exptl.[a]	Calcd.[b]
9.0	0.398	0.436	0.306	0.309
7.2	0.388	0.395	0.282	0.280
5.4	0.347	0.353	0.249	0.251
4.5	0.328	0.332	0.238	0.237
3.6	0.309	0.312	0.225	0.223

[a] Experimental values determined by the graphic method from Delahay's equation (15). [b] Calculated values are from the heterogeneous rate constants of protonation of Table VIII. [c] Depolarizer concentration 4×10^{-4} *M*.

TABLE VIII.—ORDERS OF MAGNITUDE OF RATE CONSTANTS OF PROTONATION OF DIASTEREOISOMERIC *N*-NITROSOEPHEDRINES (−1.35 v. *vs.* S.C.E.)

Compd.	$(k_0)_h$, cm. sec.$^{-1}$	$(k_{H^+})_h$, L. cm. moles^{-1} sec.$^{-1}$	$(k_{H_2PO_4^-})_h$, L. cm. moles^{-1} sec.$^{-1}$
N-Nitroso-ephedrine	0.171	4.73×10^4	23.0
N-Nitrosopseudo-ephedrine	0.115	4.07×10^4	15.9

or that the acetate buffer concentration was low and the phosphate buffer concentration was very high.

Orders of Magnitude of Rate Constants of Protonation.—The rate constants of protonation of the nitrosamines can be calculated from the kinetic currents (16), providing the ionization constants of the conjugate acids are known. However, the concept of the ionization constant of the protonated nitrosamines in dilute aqueous solution is meaningless because these compounds are strong acids and are formed only at the electrode surface. Nevertheless, Zahradník, *et al.* (11), using the pK value determined in 18.4% sulfuric acid, calculated the reaction rate constant of protonation of *N*-nitrosopyrrolidine to be 5.0×10^4 L./mole^{-1}/sec.$^{-1}$, and the rate constant of dissociation to be 9.6×10^{15} L./mole^{-1}/sec.$^{-1}$ with Koutecky's equation.

A meaningful estimation of the rate constants of protonation of the diastereoisomeric *N*-nitrosoephedrines can be obtained using the method of Delahay (15), in which heterogeneous rate constants are calculated from the kinetic currents. In this treatment of kinetic polarographic currents, Delahay assumed that the protonation reaction occurs in a monolayer at the electrode surface. The boundary conditions cited apply to the present situation. Delahay's equation for the average current controlled by reaction rate and diffusion is $\bar{\imath} = 1255\beta n m^{2/3} \tau^{2/3} C_0 (k_r)_h$ in which $\bar{\imath}$ is the average current in microamperes, n is the number of electrons involved in the reduction, m is the rate of mercury flow in mg./sec.$^{-1}$, τ is the drop time in second, C_o is the bulk concentration of the substrate in millimoles per liter, $(k_r)_h$ is the heterogeneous rate constant in cm./sec.$^{-1}$, and β is a function $\gamma[1 - \phi\{(k_r)_h\sqrt{\tau/D}\}]$ $\exp[(k_r^2)_h\tau]/D$ in which D is the diffusion coefficient in cm.^{2}/sec.$^{-1}$, where γ is the average current to maximum current ratio, and $\phi\{(k_r)_h\sqrt{\tau/D}\}$ is the error integral $2/\sqrt{\pi} \int_0^{(\tau)kh\sqrt{\tau/D}} e^{-z^2} dz$. With the graphic method (15), rate constants have been calculated for linear segments of the limiting current *versus* $[H_2PO_4^-]$ plots at constant buffer ratios $[H_2PO_4^-]/[HPO_4^=]$ in the pH range 5.59 to 5.91. A typical set of rate constants is presented in Table VII. The rate constant $(k_r)_h$ is the heterogeneous rate constant for a pseudo first-order reaction. For a reaction catalyzed by both hydrogen ions and general acids in orthophosphate buffer $(k_r)_h = (k_o)_h + (k_{H^+})_h[H^+] + (k_{H_2PO_4^-})_h[H_2PO_4^-] + (k_{HPO_4^=})_h[HPO_4^=]$. The slope of the plot of $(k_r)_h$ *versus* $[H_2PO_4^-]$ in the linear region is given by $\{(k_{H_2PO_4^-})_h + (k_{HPO_4^=})_h[HPO_4^=]/[H_2PO_4^-]\}$, and the intercept is given by $\{(k_o)_h + (k_{H^+})_h[H^+]\}$. The pH range 5.59 to 5.91 was selected to yield the best approximation of the rate constant of protonation due to hydrogen ions.

The limiting current in this pH range, while controlled by both diffusion and rate, is largely rate controlled. Delahay (15) determined that when $(k_r)_h\ D^{-1/2}$ is smaller than 0.05 sec.$^{-1/2}$, the current is completely rate controlled, and for $(k_r)_h\ D^{-1/2}$ values between 0.05 and 5 sec.$^{-1/2}$ the current is controlled by both diffusion and rate. The current in these experiments is substantially kinetic current, since the values of $(k_r)_h\ D^{-1/2}$ ranged from 0.083 to 0.187 sec.$^{-1/2}$. It may also be noted that on the pH-limiting current profile (Fig. 3), the current is entirely kinetic current in the pH range 6.65 to 8.22.

The value of $(k_{HPO_4^=})_h$ certainly must be small as a consequence of the repulsion of the doubly charged $HPO_4^=$ ion by the electrode. Nevertheless, the slight deviations from parallel slopes observed in plots of $(k_r)_h$ *versus* $[H_2PO_4^-]$ in the linear region at constant buffer ratios in the pH range 5.59 to 5.91 for both *N*-nitrosoephedrine and *N*-nitrosopseudoephedrine are undoubtedly due to catalysis by $HPO_4^=$ ions. However, the concentrations of $HPO_4^=$ in this pH range are so small that for the purposes of the present approximate calculations, it will be assumed that $(k_{HPO_4^=})_h[HPO_4^=] = 0$ and that $(k_{H_2PO_4^-})_h$ will be taken as an average value of the slopes. From the slopes and intercepts of the $(k_r)_h$ *versus* $[H_2PO_4^-]$ plots, second-order heterogeneous rate constants of protonation were calculated (Table VIII). A further assumption in these calculations is that the reaction at the electrode surface is first order in both hydrogen and $H_2PO_4^-$ ions.

The agreement between experimental and calculated heterogeneous first-order rate constants is satisfactory (Table VII), considering that the average values of the slopes were used to calculate $(k_{H_2PO_4^-})_h$. It can be shown that above pH 5.6, protonation by H_2O molecules represents a major contribution of the kinetic current.

It is of interest to estimate the order of magnitude of the rate constants of protonation of the nitrosamines in terms of "conventional" rather than "heterogeneous" rate constants for comparison purposes. An approximation of the ionization constant K of the protonated nitrosamines ($K = k_d/k_r$, in which k_d and k_r are the rate constants of dissociation and protonation, respectively) is required for the calculation. However, the rate of protonation of the nitrosamine at the electrode surface is quite different from the rate in the bulk of the solution. Accordingly, the requisite ionization constant is the value at the surface of the electrode determined under conditions that the concentrations of hydrogen ions and nitrosamine at the electrode surface are the same as the concentrations in the bulk of the solution. Based upon this interpretation, the value of K to be used in the calculation is the polarographic value; that is, the pK is the pH at which the limiting current is one-half the diffusion current. It should be noted that fluctuation of the hydrogen ion concentration in the vicinity of the electrode surface (15) permits the determination of only an approximate value of the pK.

The polarographic pK could be determined directly from the pH-limiting current profile (Fig. 3) if the reduction were specific acid-catalyzed. How-

TABLE IX.—ORDERS OF MAGNITUDE OF RATE CONSTANTS OF PROTONATION OF DIASTEREOISOMERIC *N*-NITROSOEPHEDRINES (−1.35 V. *vs.* S.C.E.)

Compd.	Polarographic pK	k_o sec.$^{-1}$	k_{H^+} L. mole^{-1} sec.$^{-1}$	$k_{H_2PO_4^-}$ L. mole^{-1} sec.$^{-1}$
N-Nitrosoephedrine	4.67	8.49×10^{-2}	6.50×10^{9}	1.54×10^{3}
N-Nitrosopseudoephedrine	4.61	4.41×10^{-2}	5.52×10^{9}	0.854×10^{3}

ever, the reductions above pH 3.23 are also catalyzed by the buffer acids. In this case, the polarographic pK can be determined from a constructed pH-limiting current profile in which the limiting current at a given pH is the value extrapolated to zero general acid concentration. An alternate procedure is to calculate the pK from the heterogeneous catalytic constants due to hydrogen ion, as described by Delahay (15). According to Delahay, the pH of the solution in which the limiting current is one-half the diffusion current is given by $(pH)_{1/2} = \log (k_{H^+})_h D^{-1/2} - \log [(k_r)_h D^{-1/2}]_{1/2} - 3$, where $(k_{H^+})_h$ is the heterogeneous catalytic constant for H^+, and $[(k_r)_h]_{1/2}$ is the heterogeneous pseudo first-order rate constant for a limiting current equal to one-half the diffusion current. The values of pK, calculated by this latter procedure, were 4.67 and 4.61 for *N*-nitrosoephedrine and *N*-nitrosopseudoephedrine, respectively.

Assuming that the calculated polarographic *K* values are the ionization constants of the protonated nitrosamines at the electrode surface, it is possible to calculate the orders of magnitude of the conventional rate constants of protonation from the heterogeneous rate constants using the relationship (17) $k_r = K/D\ (k_r)_h^2$ in which k_r is in L. mole^{-1} sec.$^{-1}$, when $(k_r)_h$ is in L. cm. mole^{-1} sec.$^{-1}$, and k_r is in sec.$^{-1}$ when $(k_r)_h$ is in cm. sec.$^{-1}$. The conventional rate constants of protonation calculated in this way are listed in Table IX.

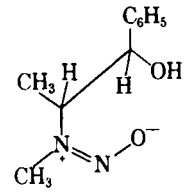
Currents in Alkaline Solution.—Neither Lund (10) nor Zahradník, *et al.* (11), reported currents controlled by reaction rate and diffusion for the reduction of *N*-nitrosamines in alkaline solution. Lund reported that above pH 9, diffusion currents were independent of pH while Zahradník, *et al.*, observed that limiting currents decreased slightly with increasing pH.

A decrease in wave height with increasing pH in the pH range 8.95 to 10.03 was observed in the reduction of both diastereoisomeric *N*-nitrosoephedrines (Fig. 3). Variation of the limiting current with mercury column height (Table VI) indicates that the current in this region is both kinetic and diffusion controlled. The kinetic current for *N*-nitrosoephedrine is greater than that for *N*-nitrosopseudoephedrine. Thus, the diastereoisomers can be distinguished by comparing the limiting currents obtained under identical conditions in alkaline and acidic solutions.

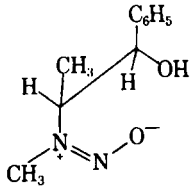
Structural Considerations

While the configurations of (−)-ephedrine and (+)-pseudoephedrine are firmly established, the conformational population of the ground states is still questionable. It is well agreed that the most stable conformation of (+)-pseudoephedrine is the one in which the NH and OH functional groups are gauche. This conclusion is based on crystallographic data (18), infrared (19) and N.M.R. spectra (20), and the results of reaction product studies (21–24). On the other hand, differing views are held regarding the most stable conformation of (−)-ephedrine. The view most widely held, based upon reaction product studies (21–24) and a study of hydrogen bonding in the diastereoisomeric ephedrines by N.M.R. (20), is that the NH and OH groups are in a preferred *trans* conformation. The alternate view, based upon crystallographic data (18) and evidence from infrared spectra that intramolecular hydrogen bonding occurs in ephedrine (19), is that in the most stable conformation of (−)-ephedrine, the NH and OH groups are gauche. The pKa values in 80% cellosolve have been determined to be 9.14 for (−)-ephedrine and 9.22 for (+)-pseudoephedrine; these results have been interpreted in terms of a favored NH, OH *trans* conformation for (−)-ephedrine and a favored NH, OH gauche conformation for (+)-pseudoephedrine (25). However, the very small difference between the pK values suggests that the NH and OH groups are gauche in both diastereoisomers with a greater degree of hydrogen bonding in (+)-pseudoephedrine.

The conformational population of the ground states of the diastereoisomeric *N*-nitrosoephedrines should be the same as that of the diastereoisomeric ephedrines since the conformations are governed by the same nonbonded interactions. Intramolecular hydrogen bonding occurred in both *N*-nitrosoephedrines, suggesting that the N—N = O and OH groups are gauche in both isomers (26), with a higher degree of intramolecular hydrogen bonding in *N*-nitrosopseudoephedrine.



N-Nitrosopseudoephedrine



N-Nitrosoephedrine

It is evident from the structures, that the nonbonded interactions are greater in *N*-nitrosoephedrine. The canonical forms of the *N*-nitrosamines shown above depict the barrier to rotation about the N—N bond. The large contribution of these canonical forms to the structures of the *N*-nitrosamines is indicated by the nonequivalence of the two methyl groups of dimethylnitrosamine, which exhibit two seperate resonances in the room temperature N.M.R. spectrum (27). Intramolecular OH · · · O hydrogen bonding, although involving a seven-membered ring, should stabilize these resonance structures.

Based upon these structural considerations, it is expected that the protonation preceding electron transfer in the electrochemical reduction will take place at the nitroso-oxygen. The compound in which there is the greater degree of hydrogen bonding will be protonated at the slower rate. Accord-

ingly, the kinetic current is expected to be greater for *N*-nitrosoephedrine than that for *N*-nitrosopseudoephedrine. The experimental results confirm this view. In addition, these results suggest that intramolecular hydrogen bonding occurs in both diastereoisomers in dilute aqueous solution, and that the NNO, OH gauche conformation is important in both compounds.

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Coating Pharmaceuticals by Coacervation

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The objectives of this study were to investigate the phenomenon of coacervation as a new means of coating pharmaceuticals and to develop procedures of operation for coating some commonly used drugs. Factors affecting coacervate drop size, some methods of regulating coating thickness, and techniques of controlling the volume of the coacervate produced were determined. Using sodium sulfate solutions and gelatin solutions as the coacervating liquids, five solids and two liquids were coated and processed to produce dry powders.

THE OBJECTIVES of this project were to investigate the phenomenon of coacervation as it is related to the coating of pharmaceuticals and to originate and develop procedures for coating pharmaceuticals by coacervation.

Coacervation is a phenomenon associated with colloidal solutions. A colloidal system is commonly defined as a two-phase system in which one phase is a continuous liquid, while the other phase is a solid which is highly dispersed throughout the liquid as particles ranging in size from 0.001 to 0.5 μ. This definition has been extended to include any (micro) homogeneous system containing colloidal particles or structures derived from them, so that systems containing kinetic units smaller than 0.001 μ may still be considered colloids (1).

In a true solution of macromolecules, that is, a system containing kinetic units smaller than 0.5 μ, certain changes—such as changes of temperature or pH or the addition of substances—can produce a reduction in solubility that will cause a large part of the macromolecules to separate into a new phase. This colloid-rich phase may exist either in a high or low state of dispersion. When a system contains a colloid-rich phase in a low state of dispersion, it is possible to distinguish microscopically and macroscopically between crystallization and coacervation, which is the formation of amorphous liquid drops. These liquid drops constitute the coacervate and under favorable conditions will coalesce in a matter of hours into one clear, homogeneous liquid layer known as a coacervate layer (1).

Coacervates will, in general, accept and surround drops of organic liquids which are immiscible with water, as well as insoluble solid particles, when they are offered in the equilibrium liquid (1). It is this ability of coacervates to coat insoluble materials present in the equilibrium liquid that makes it a method of microencapsulation. "Simple" coacervation as a coating method has been shown to be economically feasible by the National Cash Register Co., Dayton, Ohio,

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