metabolite Ro 5-2180 is excreted as the intact compound. No measurable amounts of intact diazepam have yet been found to be excreted in human urine, and the 3-hydroxy compound Ro 5-6789 (oxazepam) (13, 14) is a major urinary metabolite of diazepam in man.

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Polarography of Various *N*-Alkyl-*N*-nitrosoureas

By EDWARD R. GARRETT and ANTHONY G. CUSIMANO

The irreversible polarographic reductions of various *N*-alkyl-*N*-nitrosoureas were studied from a pH of 1.1–6.7. Below pH 3.2, the limiting current, $i_{lim.}$, of *N*-methyl-*N*-nitrosourea (NNMU) is controlled by diffusion. Above this pH, adsorption or chemical reaction is the electrode-controlling process and buffer effects are observed. Similar results were obtained for *N*-ethyl-*N*-nitrosourea (NNEU) and 1,3-dimethyl-1-nitrosourea (SRI-1384), except that the buffer effects were greatly diminished for the latter compound. The acetate buffer effects on $i_{lim.}$ have been quantified and have been attributed either to adsorption of the nitrosourea (NNMU) and the buffer components onto the microelectrode, or to a reaction between these species. The polarographic method offers a sensitive (1×10^{-6} *M*) and reliable (0.91 per cent standard error among days) assay procedure for the nitrosoureas. All of the compounds studied in this series were more easily reduced than the parent methyl compound. A correlation between the change in the half-wave potentials and the Taft substituent constants (σ^*) was noted for simple alkyl groups such as methyl, ethyl, butyl, etc. A correlation between the half-wave potentials and the apparent first-order rate constants (k, sec.$^{-1}$) for hydroxyl ion catalyzed solvolysis was noted for several of the compounds studied.

THE ANTILEUKEMIC and antiviral activities of various *N*-nitrosoureas have been reported (1–4). They have a high degree of lipoid solubility, are essentially nonionized, and are not bound to any extent by plasma proteins.

Degradation studies (5) of the broad spectrum antibiotic streptozotocin indicated that it is a substituted *N*-methyl-*N*-nitrosourea, and the maintenance of the biological activity could be related to the stability of the *N*-nitroso group.

A physicochemical parameter which could be correlated with biological activity, chemical structure, or reactivity would be useful in molecular modification to obtain a better drug. Although the mechanism of action of the *N*-alkyl-*N*-nitrosoureas is not known for certain, it is plausible that they may act as alkylating agents since a diazoalkane may be a solvolytic intermediate (6) or that they may act *via* a redox mechanism. The polarographic half-wave potential ($E^1/_2$) is a good electrochemical measure of the ease of reduction of such compounds.

Polarographic studies of *N*-nitrosoureas have been limited (5, 7) and have primarily been used to study the degradation of streptozotocin (5) and *N*-methyl-*N*-nitrosourea (7). This is in contrast to the detailed work on the polarographic reductions of various *N*-nitrosamines which have been studied by many investigators (8–14).

The purposes of this investigation were to characterize polarographically the reduction of a series of *N*-alkyl-*N*-nitrosoureas, to develop analytical methods, and to test for correlations of half-wave potentials with solvolytic rate constants and with substituent constants.

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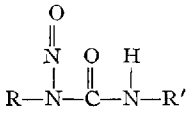
TABLE I.—NAMES AND STRUCTURES OF VARIOUS *N*-ALKYL-*N*-NITROSOUREAS



Compd.	Symbol	R	R′	Mol. Wt.
N-Methyl-*N*-nitrosourea	NNMU[a]	CH_3	H	103.1
N-Ethyl-*N*-nitrosourea	NNEU[a]	CH_3CH_2	H	117.1
N-Butyl-*N*-nitrosourea	NNBU[a]	$CH_3(CH_2)_3$	H	145.1
N-Isobutyl-*N*-nitrosourea	NNisoBU[a]	$CH_3CH(CH_3)CH_2$	H	146.1
N-Allyl-*N*-nitrosourea	NNAU[a]	CH_2=$CHCH_2$	H	129.1
N-Cyclohexyl-*N*-nitrosourea	NNCyU[a]	C_6H_{11}	H	171.2
N-Benzyl-*N*-nitrosourea	NNBcU[a]	$C_6H_5CH_2$	H	179.2
1,3-Dimethyl-1-nitrosourea	SRI-1384[b]	CH_3	CH_3	117.1
1,1′-Trimethylene bis(3-methyl-3-nitrosourea)	SRI-1631[b]	CH_3	$(CH_2)_3NHCON(NO)CH_3$	246.2
1,3-Bis (2-chloroethyl)-1-nitrosourea	SRI-1720[b]	$ClCH_2CH_2$	CH_2CH_2Cl	214.1
3-(*p*-Fluorophenyl)-1-methyl-1-nitrosourea	SRI-1746[b]	CH_3	*p*-F-C_6H_4	197.2
1-Methyl-1-nitroso-3-phenethylurea	SRI-1833[b]	CH_3	$CH_2CH_2C_6H_5$	207.2
1-Nitroso-1-phenethylurea	SRI-1834[b]	$C_6H_5CH_2CH_2$	H	193.2
1-(2-Chloroethyl)-nitroso-3-phenylurea	SRI-1879[b]	$ClCH_2CH_2$	C_6H_5	227.7

[a] Compounds synthesized in these laboratories (6). [b] Code number of the Southern Research Institute, Birmingham, Ala. (3).

EXPERIMENTAL

Materials and Equipment.—The *N*-alkyl-*N*-nitrosoureas (Table I) were synthesized either in these laboratories (6) or by the Southern Research Institute, Birmingham, Ala. (3). All other chemicals used were of analytical reagent grade. The buffer compositions were similar to those used by Malspeis and Hung (14). The pH values of all buffers except hydrochloric acid solutions were measured, before and after each run, with a Beckman expanded scale pH meter, model 76. The pH meter was standardized at 25.0° with Beckman standard buffers of pH 4 and 7. The pH values of hydrochloric acid buffers were calculated using the activity coefficients (15) at 25.0°. The ionic strength (μ) of each solution was adjusted to 0.200 with KCl.

A Sargent model XV polarograph was used. The electrolysis cell was a Sargent H-type which was immersed in a constant-temperature bath at 25.0° ± 0.1°.

Polarographic Procedure.—A master solution of the *N*-nitrosourea in distilled water was prepared at least every other day and was refrigerated when not in use. Degradation under these conditions was negligible. In those experiments where all of the compounds were studied, 8% alcohol by volume was used in preparing the master solution since some of the nitrosoureas, in particular the disubstituted derivatives, were difficultly soluble in water alone. An aliquot of the master solution was diluted with the appropriate buffer which was previously purged with nitrogen. No more than 1% alcohol by volume was present in the polarographed solutions. Alcohol did not have an effect on the polarograms.

The sample solutions were purged with nitrogen for 10 min., and their polarograms run over that of the appropriate blank solutions. Due to the instability of the nitrosoureas in phosphate buffers, this purging was limited to 3 min. The buffers were purged for 15–30 min. prior to the dilution of aliquots of the master solution.

Each polarogram was recorded at a constant mercury head and at a constant temperature of 25.0° ± 0.1°. The indicator electrode used was the dropping mercury electrode (DME), whereas a saturated calomel electrode (SCE) was the reference electrode. The DME had values of $m^{2/3}t^{1/6}$ of 2.780 mg.$^{2/3}$ sec.$^{-1/2}$ open circuit and 2.766 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.0 v. *versus* SCE in HCl–KCl buffers, 2.799 mg.$^{2/3}$ sec.$^{-1/2}$ open circuit and 2.786 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.0 v. *versus* SCE in tartrate buffers, 2.753 mg.$^{2/3}$ sec.$^{-1/2}$ open circuit and 2.741 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.1 v. *versus* SCE in acetate buffers, and 2.741 mg.$^{2/3}$ sec.$^{-1/2}$ open circuit and 2.695 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.4 v. *versus* SCE in phosphate buffers. These values were obtained at a column height of 62.2 cm.

Measurement of Half-Wave Potential and Limiting Current.—An arbitrary procedure was chosen to measure the half-wave potentials. The limiting currents of the polarographed solutions of the *N*-nitrosoureas were not exactly parallel to the currents of the blank solutions at the same applied potentials.

A line was drawn through the polarogram of the blank at the midpoints of the oscillations (curve A in Fig. 1). Another line was drawn through the midpoints of the oscillations for the sample polarogram in the region where the wave height halted its precipitous rise and before the hydrogen discharge wave (curve B in Fig. 1). The distances between these two lines were halved (curve C in Fig. 1). This third line intersected the polarogram at a potential which was designated the half-wave potential ($E_{1/2}$). No correction for resistance across the cell was necessary since the resistance was always less than 500 ohms.

The limiting currents, $i_{lim.}$ (Fig. 1), were estimated

from the differences between the currents for the sample solutions and the blank solutions at potentials 0.35 v. more negative than the half-wave potential. This was a reasonable estimate of the midpoint of the linear portion of the polarogram between the wave for the reduction of the *N*-nitrosourea and the hydrogen discharge wave.

PROCEDURES

Calibration Curve for NNMU.—A 10.3-mg. sample of NNMU is accurately weighed into a 25-ml. volumetric flask. It is dissolved and brought to volume with distilled water to give a 4×10^{-3} *M* master solution. This solution is refrigerated when not in use and is prepared freshly each day. Into several 25-ml. volumetric flasks are placed aliquots of the master solution ranging from 0.025–2.00 ml. Enough nitrogen purged distilled water is added to each flask to make 2.00 ml. and acetate buffer is added to volume. The acetate buffer is prepared so that the final solution is: $[HC_2H_3O_2]$ = 0.005 *M*, $[NaC_2H_3O_2]$ = 0.003 *M*, and [KCl] = 0.197 *M*. The polarogram of each standard solution is then run.

Assay Method.—The polarographic procedure is the same as that previously described. Each sample is purged with nitrogen for 10 min. and the polarogram is run over that of a blank solution. Samples of unknown concentrations of NNMU are prepared and run in a similar manner. The limiting currents are measured at −1.3 v. *versus* SCE.

The method may be modified somewhat to study the fast solvolysis of these compounds in alkaline or strong acid media at higher temperatures. In this case the degrading solution is kept in the electrolysis cell during the entire run, and the polarogram is obtained at specified time intervals.

RESULTS AND DISCUSSION

Wave Characteristics.—The *N*-alkyl-*N*-nitrosoureas (Table I) exhibited well-defined polarographic waves throughout the entire pH range. The polarographic parameters, $E_{1/2}$ and $i_{lim.}$, were easily measured and no maxima were observed. Typical polarograms of NNMU at various pH values and in various buffers are shown in Fig. 1. The curves are drawn through the midpoints of the oscillations. The half-wave potential shifted to more negative values with increased pH of the solution. Since the reduction of most organic compounds involves hydrogen ions, this shift is in accordance with the Nernst equation (16).

The irreversible nature of the reduction was evident from the results of the logarithmic analyses (16) of representative polarograms of NNMU.

Reversibility is characterized by a single straight line for the plot of the applied potential, *E versus* $\log[i/(i_{lim.} - i)]$, where *i* is the current at a specified potential, *E*, and $i_{lim.}$ is the limiting current. For a reversible electrode process the number of electrons (*n*) involved in the reduction can be directly obtained from the slopes of such plots (16). Reversible polarographic waves are symmetrically S-shaped.

Such logarithmic analyses yielded two straight line segments for pH 1.10 solutions. Single straight lines were obtained for solutions in the pH range

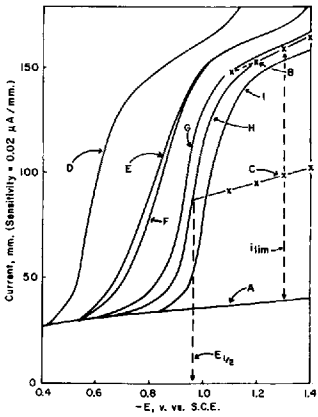


Fig. 1.—Typical polarograms of NNMU (1.1×10^{-4} *M*) in various buffers at 25.0° and at column height of 62.2 cm. Key: A, polarogram of blank solution; B, polarogram of sample solution; C, line through half-values of limiting current intersecting wave at half-wave potential ($E_{1/2}$); D, HCl–KCl buffer, pH 1.10; E, HCl–KCl buffer, pH 2.33; F, tartrate buffer, pH 2.52; G, acetate buffer, pH 3.70; H, acetate buffer, pH 4.81; I, phosphate buffer, pH 6.28.

2.0–4.8. However, the calculated values of *n* were less than unity. Typical values of *n* were 0.35–0.37 in HCl–KCl buffer (pH 2.33) and tartrate buffer (pH 2.78), and 0.54–0.59 in acetate buffers from pH 3.5–4.8.

The skew S-shaped curves (Fig. 1) are also representative of irreversibility where the electron transfer process is either slower or of the same order of magnitude as the electrode controlling process, *e.g.*, diffusion or adsorption.

Effect of pH on Half-Wave Potential and Limiting Current.—Typical data for the half-wave potentials and the limiting currents for NNMU at various pH values are presented in Table II. The half-wave potential-pH profile is shown in Fig. 2. The latter has two straight line segments. The intersection of these segments at a pH of 3.2 indicates a change in either the mechanism of the reduction reaction or in the electrode controlling process. The equations which describe the dependence of the half-wave potential on pH for NNMU are

$$-E_{1/2} = 0.165\ \text{pH} + 0.384 \quad \text{(pH 1.0 to 3.2)} \quad \text{(Eq. 1)}$$

$$-E_{1/2} = 0.030\ \text{pH} + 0.824 \quad \text{(pH 3.2 to 6.8)} \quad \text{(Eq. 2)}$$

Large changes in pH produced only small changes in the limiting current throughout the entire pH range (Table II). Malspeis and Hung (14) have reported that the wave height produced by the reduction of *N*-nitrosoephedrine decreased with pH. This was attributed to reduction controlled by both reaction rate and diffusion to the microelectrode. An electrode process in which the limiting current is essentially independent of pH is said to be controlled either by diffusion of the compound to the microelectrode or by adsorption onto the electrode surface (17). Malspeis and Hung (14) ascribed their results to a polarographic pKa with

TABLE II.—EFFECT OF pH ON LIMITING CURRENT ($i_{lim.}$) AND HALF-WAVE POTENTIAL ($E_{1/2}$) FOR REDUCTION OF NNMU AT 25.0°

Buffer	pH	$i_{lim.}$, μA [a]	$-E_{1/2}$, v. vs. SCE [b]
HCl–KCl	1.10	2.02	0.570
	1.38	2.00	0.605
	2.04	2.03	0.719
	2.33	2.05	0.781
Tartrate	2.51	2.05	0.815
	2.56	2.03	0.815
	2.67	2.04	0.828
	2.70	2.05	0.835
	2.80	2.02	0.841
Lactate	3.00	...	0.876
	3.18	...	0.906
	3.40	...	0.916
	3.77	...	0.934
	4.00	...	0.949
Acetate	3.50	2.04	0.925
	3.65	2.03	0.932
	3.72	...	0.942
	3.87	2.07	...
	3.94	2.05	0.948
	4.10	2.05	0.955
	4.53	...	0.958
	4.81	2.04	...
	5.10	2.03	0.974
Phosphate	5.52	2.02	0.996
	5.80	2.03	1.002
	6.28	2.01	1.010
	6.50	2.02	1.016
	6.70	...	1.020

[a] Limiting currents were measured at a potential 0.35 v. more negative than the half-wave potential. Each result is the average of three determinations. The deviations from the means were ±0.03. [NNMU] was 1×10^{-4} *M*. Height of mercury column was 58.0 cm. [b] Each result is the average of three determinations. The deviations from the means were ±0.003. [NNMU] was 1.1×10^{-4} *M*. Height of mercury column was 62.2 cm.

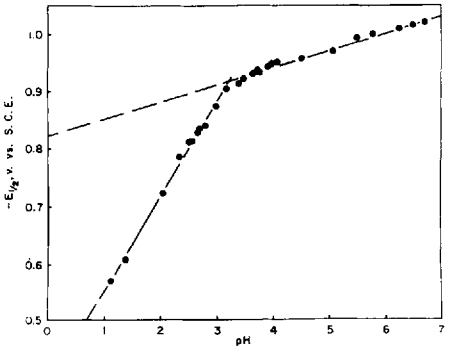


Fig. 2.—Effect of pH on half-wave potential ($E_{1/2}$) for reduction of NNMU (1.1×10^{-4} *M*) at 25.0° and at column height of 62.2 cm.

nitrosamine protonated at the microelectrode. Zahradnik (12) reported that the pKa values of *N*-nitroso derivatives of secondary aliphatic and heterocyclic amines ranged from −1.28 to −1.99 in 18.4% sulfuric acid at 22°. The absence of a spectrophotometric pKa was shown by no significant change in the absorbance and wavelength of maximum absorption of NNMU with pH at values above 1.10. There was no titratable pKa above pH 2.00 (6). There is no indication of a polarographic pKa for NNMU from a pH of 1.10–6.50.

Similar results were obtained for the reduction of *N*-ethyl-*N*-nitrosourea (NNEU) and 1,3-dimethyl-1-nitrosourea (SRI-1384). The equations describing the effect of pH on the half-wave potential for these compounds are for NNEU

$$-E_{1/2} = 0.153\ \text{pH} + 0.367 \quad \text{(pH 1.0 to 3.7)} \quad \text{(Eq. 3)}$$

$$-E_{1/2} = 0.027\ \text{pH} + 0.827 \quad \text{(pH 3.7 to 5.0)} \quad \text{(Eq. 4)}$$

and for SRI-1384

$$-E_{1/2} = 0.158\ \text{pH} + 0.330 \quad \text{(pH 1.0 to 4.3)} \quad \text{(Eq. 5)}$$

$$-E_{1/2} = 0.034\ \text{pH} + 0.808 \quad \text{(pH 4.3 to 7.0)} \quad \text{(Eq. 6)}$$

The similarities in the coefficients of the pH terms indicate that the mechanism of the reduction reaction or the electrode controlling process is the same for the three compounds and probably for all simple alkyl substituted nitrosoureas.

Effect of Height of Mercury Column on Limiting Current.—The dependence of the limiting current on the height of the mercury column is shown in

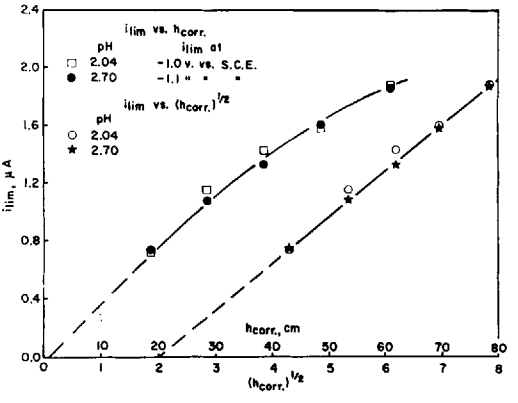


Fig. 3.—Effect of height of mercury column on limiting current for reduction of NNMU (9×10^{-5} *M*) in HCl–KCl (pH 2.04) and tartrate buffers (pH 2.70) at 25.0°.

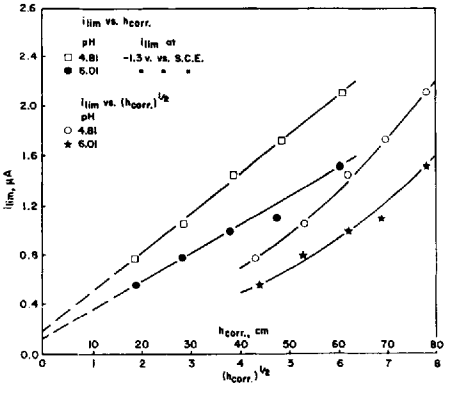


Fig. 4.—Effect of height of mercury column on limiting current for reduction of NNMU in acetate (pH 4.81, [NNMU] 9×10^{-5} *M*) and phosphate (pH 6.01, [NNMU] 8×10^{-5} *M*) buffers at 25.0°.

Figs. 3 and 4 for NNMU. In hydrochloric acid and tartrate buffers the limiting current was directly proportional to the square root of the corrected height of the mercury column (Fig. 3). Therefore, the control of the electrode process is consistent with diffusion of the nitrosourea to the microelectrode (17) in these buffers and at these pH values.

In acetate and phosphate buffers (Fig. 4) the limiting current was proportional to the corrected height of the mercury column and not to its square root. Thus, the control of the electrode process is consistent with adsorption of the nitrosourea onto the dropping mercury electrode surface (17) in these buffers and at these pH values.

These relations are consistent with the change in linearity in the half-wave potential-pH profile of NNMU (Fig. 2). Below a pH of 3.2, the electrode process is diffusion controlled, and above this pH adsorption may be the controlling process.

Effect of Buffer Concentration on Polarographic Parameters.—In tartrate buffers (0.001–0.01 *M* in each component) there was no significant change in the limiting current and half-wave potential of NNMU with buffer concentration at a particular pH. In acetate and phosphate buffers (0.001–0.01 *M* in each component) some decreases were observed in the limiting current and some positive shifts in the half-wave potential. A similar effect had been observed previously (5).

The parameters derived from polarograms in acetate buffers (0.010–0.200 *M* in each component) at several pH values are given in Table III. A typical plot of the limiting current against the concentration of acetic acid for various pH values is given in Fig. 5. Similar plots of varying slopes were obtained for the limiting current *versus* concentration of sodium acetate. It is apparent that the limiting current is dependent upon the concentrations of acetic acid, sodium acetate, and hydrogen ion in the acetate buffer region.

This functional dependence of the limiting current may be quantified in a manner similar to the procedure established for general acid-base catalysis (18). Therefore, we have

$$i_{lim.} = i_0 + a_{H^+}[H^+] + b_{HA}[HA] + c_{Ac^-}[Ac^-] \quad \text{(Eq. 7)}$$

where a_{H^+}, b_{HA}, and c_{Ac^-} are coefficients for the effect of a particular buffer species, i_0 is the limiting current in the absence of buffer species, and $[H^+]$, $[HA]$, and $[Ac^-]$ are the molar concentrations of hydrogen ion, acetic acid, and sodium acetate, respectively.

At constant pH, Eq. 7 reduces to

$$i_{lim.} = i_0 + a_{H^+}[H^+] + \{b_{HA} + c_{Ac^-}([Ac^-]/[HA])\}[HA] \quad \text{(Eq. 8)}$$

A plot of the limiting current *versus* the concentration of acetic acid is a straight line (Fig. 5) at each pH with

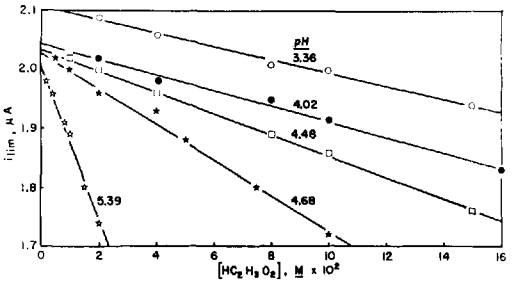


Fig. 5.—Effect of concentration of acetic acid on limiting current for reduction of NNMU (9×10^{-5} *M*) at higher buffer concentrations at 25.0° and at column height of 60.0 cm.

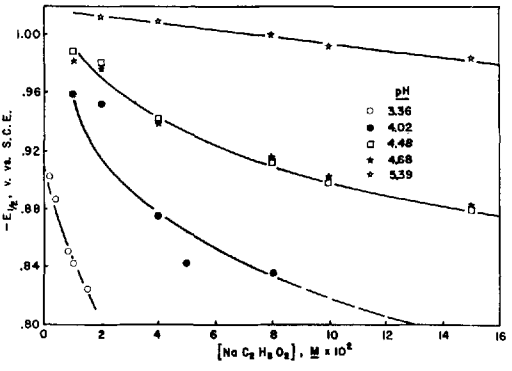


Fig. 6.—Effect of concentration of sodium acetate on half-wave potential for reduction of NNMU (9×10^{-5} *M*) at higher buffer concentrations at 25.0° and at column height of 60.0 cm.

Table III.—Effect of Buffer Concentration on Limiting Current and Half-Wave Potential for Reduction of NNMU (9×10^{-5} *M*) at Higher Concentrations of Acetic Acid and Sodium Acetate at 25.0° and at Column Height of 60.0 cm.

pH	$[HC_2H_3O_2]$, *M*	$[NaC_2H_3O_2]$, *M*	$i_{lim.}$, μA[a]	$-E_{1/2}$, v. *vs.* SCE[a]
4.48	0.010	0.010	2.02	0.989
	0.020	0.020	2.00	0.981
	0.040	0.040	1.96	0.943
	0.080	0.080	1.89	0.913
	0.100	0.100	1.86	0.898
	0.150	0.150	1.76	0.880
4.68	0.005	0.010	2.02	0.982
	0.010	0.020	2.00	0.977
	0.020	0.040	1.96	0.940
	0.040	0.080	1.93	0.916
	0.050	0.100	1.88	0.903
	0.075	0.150	1.80	0.882
	0.100	0.200	1.72	0.860
4.02	0.020	0.010	2.02	0.960
	0.040	0.020	1.98	0.953
	0.080	0.040	1.95	0.875
	0.100	0.050	1.92	0.843
	0.160	0.080	1.83	0.837
5.39	0.002	0.020	1.98	1.012
	0.004	0.040	1.96	1.010
	0.008	0.080	1.91	1.000
	0.010	0.100	1.89	0.992
	0.015	0.150	1.80	0.985
	0.020	0.200	1.74	0.970
3.36	0.020	0.002	2.09	0.903
	0.040	0.004	2.06	0.887
	0.080	0.008	2.01	0.850
	0.100	0.010	2.00	0.842
	0.150	0.015	1.94	0.825

[a] Each result is the average of three determinations. Limiting currents were measured at a potential 0.35 v. more negative than the half-wave potential.

$$\text{slope} = b_{HA} + c_{Ac^-}([Ac^-]/[HA]) \quad \text{(Eq. 9)}$$

and

$$\text{intercept} = i_0 + a_{H^+}[H^+] \quad \text{(Eq. 10)}$$

The values of the slopes of Eq. 8, when plotted against the buffer ratio in accordance with Eq. 9, gave a straight line with an intercept, b_{HA}, and a slope, c_{Ac^-}. Similarly, the values of the intercepts of Eq. 8, when plotted against the hydrogen ion concentration, gave a straight line with an intercept, i_0 and a slope, a_{H^+}. The same constants were evaluated similarly from a plot of the limiting current against the concentration of sodium acetate in accordance with an expression analogous to Eq. 8.

The resultant quantifiable expression for the limiting current in acetate buffers, [NNMU] = 9×10^{-5} M, is

$$i_{lim.}, \mu A = 2.02 + 210\,[H^+] - 0.740\,[HA] - 1.13\,[Ac^-] \quad \text{(Eq. 11)}$$

This expression is valid for acetate buffers up to 0.300 M in total buffer and where the concentration of NNMU is less than 0.16 mM. The expression is not valid below pH 3.2 for NNMU since buffer effects were not observed and would not be expected for a diffusion controlled process (17). Similar effects were found in phosphate buffers. It must be realized that the i_0 values will depend on the concentration.

The effect of concentration of sodium acetate on the half-wave potential of NNMU is shown in Fig. 6. The half-wave potential at a specific pH shifted nonlinearly in a positive direction as the buffer concentration increased. Similar buffer effects were observed for the antibiotic streptozotocin (5). It had been suggested that this effect might be due to complexation of the antibiotic with the buffer components in the region of the microelectrode since the half-wave potential of a complexed species depends upon the concentration of the complexing agent (16).

Mechanisms for Effects of Buffers on Polarographic Parameters.—Adsorption may be the electrode-controlling process (17, 19) for reduction in acetate and phosphate buffers. The shift in the half-wave potential to more positive values with increased buffer concentration (Table III) implies that such adsorption could be enhanced by the components of acetate buffers. Competition for adsorption sites may exist between the nonreducible buffer components and the nitrosourea. This would limit the numbers of molecules available for reduction and result in the observed decrease in limiting current with increased buffer concentration. Consistent with the hypothesis is the negative deviation from linearity of the plot of limiting current *versus* NNMU concentration at high acetate buffer concentrations (Fig. 7). The Ilkovic equation holds at low buffer concentrations for the same NNMU concentration range and the calibration curve is linear to 0.320 mM in NNMU for acetate buffers less than 0.020 M in total buffer.

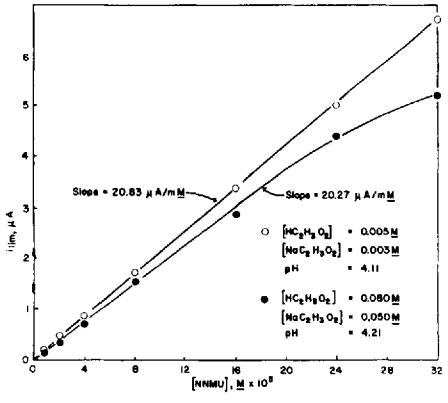
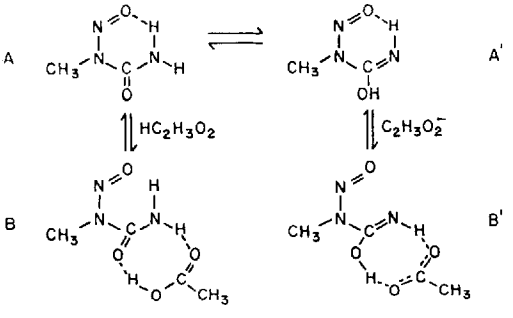


Fig. 7.—Effect of concentration of NNMU on limiting current at low and high acetate buffer concentrations at 25.0° and at column height of 60.0 cm.

In hydrochloric acid and tartrate buffers the limiting current was proportional to the concentration of NNMU to 0.320 mM.

Additional evidence for this competition with buffer, or other substance present in solution, for adsorption sites can be found in the literature. The reduction of nitromethane and nitroethane is easier in citric acid–disodium phosphate and acetate buffers than in either benzoate or phthalate buffers at the same pH (20). This is thought to be due to adsorption phenomena at the microelectrode. In the reduction of cystine (19) the wave was shifted to more negative potentials by the addition of thymol or camphor which prevents or counteracts the stereospecific adsorption of cystine necessary for its reduction.

An alternative explanation may be offered to account for these buffer effects. A catalytic current can range from an independent to a linear function of the height of the mercury column (17). Acetic acid or acetate ion may react with the nitrosourea to form a hydrogen bonded species, B or B′, respectively, in Scheme I. The possibility of



Scheme I

an equilibrium between NNMU and an intramolecularly hydrogen bonded species, A or A′, in Scheme I is also present.

On the basis of this hypothesis of complexation through hydrogen bonding of the buffer species with the nitrosourea (NNMU), it was predicted that the limiting current for the reduction of 1,3-dimethyl-1-nitrosourea (SRI-1384) would show greatly lessened dependence on concentration of the acetate buffer components. The reasoning was that this compound would have half the probability of forming B from A with acetic acid since a nonbonding methyl is substituted for one of the hydrogens on the urea. Also, the possibility of forming an analogous B′ from A′ with acetate ion would not exist since an

TABLE IV.—ANALYSIS OF VARIANCE FOR EFFECT OF CONCENTRATION OF NNMU ON LIMITING CURRENT IN ACETATE BUFFER[a] EVALUATED ON SEVERAL DAYS AT 25.0° AND AT COLUMN HEIGHT OF 60.0 cm.

Source of Variation	Degrees of Freedom	Sum of Sq.	Mean Sq.	F
Concn.	7	0.80162929	0.11451846	...
Days, factor A	2	0.00009963	0.000049815	0.842[b]
Error (*a*)	14	0.00082792	0.000059137	...
Replications, factor B	2	0.00000344	0.00000172	0.0007[c]
Interaction, AB	4	0.00123088	0.00030772	0.121[d]
Error (*b*)	42	0.10701813	0.00254805	...
Total	71	0.91080920	...	...

[a] $[HC_2H_3O_2] = 0.005\ M$, $[NaC_2H_3O_2] = 0.003\ M$, $[KCl] = 0.197\ M$. [b] 5% F = 3.74 for 2 and 14 degrees of freedom. 5% F = 3.23 for 2 and 42 degrees of freedom. [d] 5% F = 2.61 for 4 and 42 degrees of freedom.

TABLE V.—EFFECT OF SUBSTITUENTS ON POLAROGRAPHIC PARAMETERS FOR REDUCTION OF VARIOUS *N*-ALKYL-*N*-NITROSOUREAS[a] IN TARTRATE BUFFERS AT 25.0° AND AT COLUMN HEIGHT OF 60.0 cm.

Compd.	$-E_{1/2}$, v. *vs.* SCE pH = 2.51[e]	$-E_{1/2}$, v. *vs.* SCE pH = 2.80[f]	$i_{lim.}$, μA pH = 2.80[f]	Substituent Constant, σ^{*g}	$-\log(k, \text{sec.}^{-1})^h$
NNMU[b]	0.809	0.846	1.98	0.000	4.36
NNEU[b]	0.741	0.804	1.83	−0.100	4.38
NNBU[b]	0.631	0.646	1.56	−0.130	4.31
NNisoBU[b]	0.651	0.680	1.31	−0.125	4.31
NNAU[b]	0.682	0.692	1.40	...	4.10
NNBeU[b]	0.558	0.610	1.32	+0.215	3.97
NNCyU[b]	0.603	0.624	1.31	−0.150	2.70
SRI-1834[b]	0.530	0.570	1.20	+0.080	4.22
SRI-1384[b]	...	0.730	1.88	...	...
SRI-1631[b]	...	0.586	2.45	...	...
SRI-1720[b]	...	0.580	1.40	...	...
SRI-1746[b]	...	0.542	1.33	...	...
SRI-1833[c]	...	0.598	1.41	...	...
SRI-1879[d]	...	0.478 (1) 0.930 (2)	0.564 (1) 0.912 (2)	...	...

[a] All compounds were initially dissolved in 8% alcohol since some were difficultly soluble in water alone. Each result is the average of three determinations. Limiting currents were measured at a potential 0.35 v. more negative than the half-wave potential. [b] Polarogram was a single, well-defined wave. [c] Polarogram was a single wave, not well-defined. [d] Polarogram was a double, well-defined wave. Master solution became darkened after about 1 hr. at room temperature. [e] Concentration of *N*-nitrosourea was $8 \times 10^{-5}\ M$. [f] Concentration of *N*-nitrosourea was $9 \times 10^{-5}\ M$. [g] From *Reference 24*. At 35.0° and pH = 5.95 (6).

unsubstituted urea is demanded in the formation of B′.

The limiting currents for 1,3-dimethyl-1-nitrosourea ($8 \times 10^{-5}\ M$) in acetate buffers (0.010 M − 0.200 M in each component) were in order of increasing buffer concentration: 1.59, 1.60, 1.58, 1.51 μA at pH 4.27; 1.55, 1.52, 1.58, 1.57, 1.51 μA at pH 4.85; and 1.54, 1.52, 1.53, 1.52 μA at pH 5.50. This represents less than a 5% decrease in the limiting current with the same increasing concentrations of acetate buffer components that caused large (more than 10%) and systematic decreases in the limiting current of NNMU (Fig. 5, Table III).

In the same concentration ranges of acetate buffers as used for NNMU, the $-E_{1/2}$ values of 1,3-dimethyl-1-nitrosourea were 0.855, 0.826, 0.806, 0.800 v. *versus* SCE at pH 4.27; 0.902, 0.860, 0.837, 0.830, 0.823 v. *versus* SCE at pH 4.85; and 0.960, 0.946, 0.940, 0.938 v. *versus* SCE at pH 5.50. The shift in $E_{1/2}$ to more positive values with increased buffer concentration is similar but to a lesser extent than for NNMU (Table III).

The compound 1,3-dimethyl-1-nitrosourea was found to be more easily reduced than NNMU in acetate buffers at a particular pH. This is also consistent with the mechanism proposed in Scheme I. The presence of a 3-methyl group may inhibit the intramolecular hydrogen bonding of A by a statistical factor of 0.5 and permit more readily the reduction of the *N*-nitroso group.

A plausible mechanism for the reduction of the *N*-nitroso group in NNMU is through the hydroxylamino to an amino group. This route has been well-established for the reduction of *N*-nitrosamines (8–14).

Reliability of Polarographic Assay Method.—The effect of concentration of NNMU on the limiting current in acetate buffer ($[HC_2H_3O_2] = 0.005\ M$; $[NaC_2H_3O_2] = 0.003\ M$; $[KCl] = 0.197\ M$) was evaluated on 3 days at eight concentrations ranging from 0.004 mM in NNMU to 0.320 mM. Three replications were run at each concentration on each day. An analysis of variance (21) of these data was performed in the following manner. The regression coefficients were determined for each of the nine sets of data. From each regression equation a theoretical value of y ($i_{lim.}$, μA) was calculated for each fixed value of x (concentration, mM). The effect of concentration on the values of the limiting current was removed by taking the ratio $y_{exptl.}/y_{theoret.}$. The analysis of variance was then performed on the values obtained for this ratio for each concentration on each day. The results of the analysis of variance are shown in Table IV. The determinations of the limiting current did not vary significantly from day to day. The standard error

TABLE VI.—EFFECT OF SUBSTITUENTS ON POLAROGRAPHIC PARAMETERS FOR REDUCTION OF VARIOUS *N*-ALKYL-*N*-NITROSOUREAS[a] (6×10^{-5} *M*) IN PHOSPHATE BUFFER (pH = 6.03) AT 25.0° AND AT COLUMN HEIGHT OF 60.2 cm.

Compd.	$-E_{1/2}$, v. *vs.* SCE	$i_{lim.}$, μA	Max. Biological[e] Effectiveness (% ILS)
NNMU[b]	0.985	1.29	86
NNEU[b]	0.980	1.22	<40
NNBU[b]	0.963	1.16	<40
NNisoBU[b]	0.982	1.02	...
NNAU[b]	0.984	1.08	<40
NNBeU[c]	0.742 (1)	0.500 (1)	<25
	1.068 (2)	0.632 (2)	...
SRI-1384[b]	0.998	1.23	61
SRI-1631[c]	0.818 (1)	1.50 (1)	100
	1.083 (2)	0.660 (2)	...
SRI-1834[c]	0.754 (1)	0.534 (1)	...
	1.027 (2)	0.680 (2)	...
SRI-1720[c]	0.743 (1)	0.576 (1)	184
	1.050 (2)	0.706 (2)	...
SRI-1746[c]	0.730 (1)	0.690 (1)	<40
	1.068 (2)	0.536 (2)	...
SRI-1833[c]	0.785 (1)	0.808 (1)	...
	1.063 (2)	0.604 (2)	...
SRI-1879[c,d]	0.180 (1)	0.296 (1)	...
	0.953 (2)	0.724 (2)	...

[a] All compounds were initially dissolved in 8% alcohol since some were difficultly soluble in water alone. Each result is the average of three determinations. Limiting currents were measured at a potential 0.35 v. more negative than the half-wave potential. [b] Polarogram was a single, well-defined wave. [c] Polarogram was a double, well-defined wave. [d] Master solution became darkened after about 1 hr. at room temperature. [e] Reported as per cent increase in life span of mice treated with leukemia L1210 over that of controls (2).

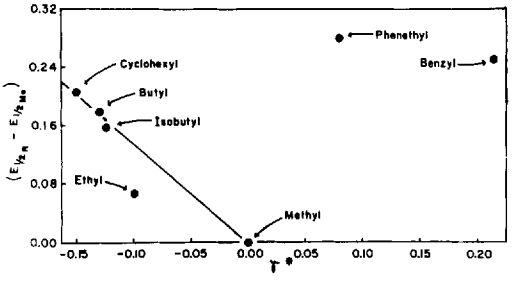


Fig. 8.—Effect of substituents on ease of reduction of various *N*-alkyl-*N*-nitrosoureas (8×10^{-5} *M*) in tartrate buffer (pH 2.51) at 25.0° and at column height of 60.0 cm.

for the difference between two-day means was 0.91%. The standard error for the difference between two concentration means was 1.31%. The regression equation obtained from the average values of the regression coefficients is

$$i_{lim.}, \mu A = 20.85\ [\text{NNMU}],\ M \times 10^3 + 0.062 \quad \text{(Eq. 12)}$$

The intercept is not significantly greater than zero.

The reproducibility of the assay method from day to day is good and the procedure is quite sensitive. A concentration of NNMU of 1×10^{-6} *M* could be detected. The polarographic method of assay is equally or more sensitive than other procedures for the assay of nitrosoureas (5, 6, 22, 23). Unlike the colorimetric method (5, 22, 23), it may be used in a pH region where the compounds are most stable, *i.e.*, between pH 3.0 and 5.0 (5, 22), and no corrections for drug instability are required. The polarographic method is less time consuming than the colorimetric method which requires a 45-min. incubation period at 50° (23). Although definite buffer effects due to adsorption or chemical reaction have been shown to occur in acetate buffers, a linear calibration curve is obtained up to concentrations of 0.320 m*M* in NNMU as long as the concentration of one of the buffer components does not exceed 0.010 *M*. Therefore, it is suggested that routine assays of these compounds be run in appropriate acetate buffers at 25.0°.

Effect of Substituents on Ease of Reduction of Various N-Nitrosoureas.—The effect of substituents on the ease of reduction of various alkyl substituted nitrosoureas is shown in Tables V and VI for several buffer systems. The data indicate that all of the compounds are more easily reduced than the parent methyl compound with more positive half-wave potentials. A reasonable correlation (Fig. 8) between the change in the half-wave potentials and the Taft (24) substituent constants (σ^*) is noted for the simple alkyl groups, *i.e.*, methyl, ethyl, butyl, etc., in tartrate buffer. Substituents containing an aromatic ring, *i.e.*, benzyl and phenethyl, did not follow this relationship. Thus, the ease of reduction of the nitrosourea due to substitution of another simple alkyl group for methyl seems to depend primarily on an inductive effect. However, it should be noted that the reduction reaction is facilitated by both electron donating groups (ethyl, butyl, etc.) and by electron withdrawing groups (benzyl and phenethyl). It is possible that steric factors may also be involved in the case of the last two compounds.

The most active compound from a biological standpoint is 1,3-bis-(2-chloroethyl)-1-nitrosourea (SRI-1720), which is one of the easiest to reduce.

In the diffusion controlled region (tartrate buffer) the limiting current decreased in approximate order of increasing molecular weights for the simple

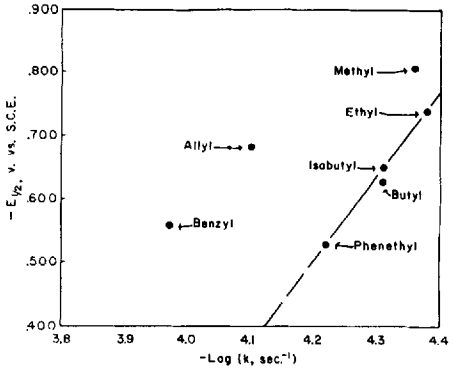


Fig. 9.—Relationship between half-wave potentials (pH 2.51) of various *N*-alkyl-*N*-nitrosoureas (8×10^{-5} *M*) at 25.0° and rate constants (k, sec.$^{-1}$ at 35.0° and pH 5.95) for hydroxyl ion catalyzed solvolysis.

alkyl substituted nitrosoureas, *i.e.*, NNMU, NNEU, NNBU, etc. Compounds with large groups in the R and R′ positions (Table V), *i.e.*, the disubstituted nitrosoureas, show deviations in this respect and imply that steric factors may affect the reduction reaction. Apparently both nitroso groups in SRI-1631 are equivalent and are reduced simultaneously, producing a current about twice that expected for a molecule of this size.

In the adsorption controlled region (phosphate buffer) the changes in the half-wave potentials and the limiting currents due to different substituents are not so pronounced as in the diffusion controlled region. However, many of the nitrosoureas, *i.e.*, NNBeU, SRI-1834, and those disubstituted derivatives with large groups in the R or R′ positions, exhibit a double wave in phosphate buffer (Table VI), indicating that the adsorbed species may be reduced more easily than the free form. Again steric factors may assume a role in the reduction of these particular compounds.

The relationship between the half-wave potentials and the apparent first-order rate constants (k, sec.$^{-1}$) for the solvolysis of various *N*-alkyl-*N*-nitrosoureas (6) in the neutral pH region is shown in Table V and in Fig. 9. In this case large discrepancies are noted when the substituents contain a double bond close to the electroactive site, *i.e.*, allyl and benzyl.

No correlation (Table VI) could be demonstrated between the half-wave potentials of the *N*-nitrosoureas and the available biological activity data (2). However, the biological data are limited and those which are available may not be entirely reliable due to the instability of these compounds in the neutral pH region.

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Rheological Study of Selected Pharmaceutical Semisolids

By JAMES C. BOYLAN

Rheograms were obtained with a Ferranti-Shirley cone and plate viscometer at 20, 25, 30, and 35° for 13 pharmaceutical semisolids. As the temperature was raised, all the products studied showed a decrease in viscosity, thixotropy, and yield value. For many of the semisolids there appears to be a straight line relationship between thixotropic area and temperature. For ointments whose base is predominately white ointment, the viscosity is reduced by a factor of 0.5 for every 5° rise in temperature. At 35° many of the products studied showed similar values for thixotropy and viscosity.

A GREAT VARIETY of test procedures have been utilized over the years for the evaluation of the spreading and flow characteristics of pharmaceutical semisolids. Today, even widely used test equipment, such as the cone penetrometer, leave much to be desired as to the amount and type of data obtained. At the present time no single instrument can provide all the information required for complete product evaluation. However, this situation can be improved by the use of a viscometer capable of obtaining the complete hysteresis profile of non-Newtonian pharmaceutical semisolids.

Schulte and Kassem (1–6) have recently published an excellent series of papers dealing with the flow properties of several semisolid systems including silicone gels, triglyceride gels, polyethylene gels, polyethylene glycol gels, various vaselines, and carbohydrate gels. In addition,

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