# Electrochemical Redox Pattern for Nicotinamide Species in Nonaqueous Media

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**Abstract:** Electrochemical investigation of biologically important 1-substituted 3-nicotinamides (1-methylnicotinamide, nicotinamide mononucleotide, nicotinamide adenine dinucleotide ($NAD^+$), deamino-$NAD^+$, and nicotinamide adenine dinucleotide phosphate ($NADP^+$)) in nonaqueous media (acetonitrile and dimethyl sulfoxide) indicates a redox pattern of initial single-electron addition to the molecule to form a neutral free radical, which dimerizes at the 6 position. Further reduction of the radical requires proton participation; *i.e.*, simultaneous addition of an electron and a proton to the ring is involved. The redox pattern for unsubstituted 3-nicotinamide exhibits two successive one-electron additions (potentials separated by *ca.* 0.5 V) with the product of the initial one-electron reduction being a negatively charged free radical which also dimerizes at the 6 position (the rate constant for the dimerization is $3 \times 10^4$ l. mol$^{-1}$ sec$^{-1}$). The rate constants for dimerization of the neutral free radicals derived from the 1-substituted nicotinamides are about $10^6$ l. mol$^{-1}$ sec$^{-1}$. The dimers show a greater stability in nonaqueous media than in aqueous media. The site of dimerization in the nicotinamide sequence agrees well with the predictions made on the basis of quantum mechanical calculations. In the presence of proton donors, the nicotinamides gave a wave pattern similar to that seen in aqueous media.

Although there have been a considerable number of studies of the electrochemical behavior of members of the biologically important sequence of compounds ranging from nicotinamide (3-nicotinamide) to nicotinamide adenine dinucleotide ($NAD^+$) in aqueous media,[2] apparently the only study[3] employing other media is one of $NAD^+$ in 50% dioxane, which indicated $NAD^+$ to be reducible only in the presence of $n$-$Bu_4NI$. This result is in contrast to the readily observed reducibility of $NAD^+$ in aqueous media, employing a variety of background electrolytes.

Work currently in progress in our laboratory, as well as published studies such as those cited, have shown that the nicotinamide sequence of compounds show two one-electron reduction waves in aqueous medium at pH values exceeding 9. The primary product formed in the process producing the initial cathodic wave Ic is a neutral radical which rapidly dimerizes. Oxidation of the dimer at positive potential (−0.04 to −0.32 V for the different compounds in the series) regenerates the original molecule. The origin of cathodic wave IIc is not clear; it has been ascribed to the reduction of the neutral radical formed at wave Ic with probable proton participation

$$R\cdot + H^+ + e \rightleftarrows RH\cdot \qquad (1)$$

where R· represents the neutral radical, and to simultaneous reductions of the dimer and the neutral radical.[2c] There are also reports[4] indicating that wave IIc was not observable.

The need for studying the nicotinamide series of compounds in purely nonaqueous solvents like acetonitrile (AN) and dimethyl sulfoxide (DMSO) arises from the poorer ability of these media, compared with water, for donating a proton in the wave IIc process; it would thus be possible to clarify the reduction mechanism. Furthermore, it was shown that electrolysis at the wave Ic potential of 1-ethyl-4-carbamoylpyridinium species in AN produced a blue pyridinyl radical (1-ethyl-4-nicotinamide radical) suitable for electron spin resonance study;[5] under aqueous conditions, this radical rapidly dimerizes. This result suggests that free radicals of other members of the nicotinamide sequence of compounds would be more stable in nonaqueous than in aqueous media.

The present study involves the electrochemical reduction of nicotinamide, 1-methylnicotinamide (1-methyl-3-carbamoylpyridinium ion; $MCP^+$), nicotinamide mononucleotide ($NMN^+$), nicotinamide adenine dinucleotide ($NAD^+$), deamino nicotinamide adenine dinucleotide (nicotinamide hypoxanthine dinucleotide; deamino-$NAD^+$ or $DNAD^+$), and nicotinamide adenine dinucleotide phosphate ($NADP^+$) in nonaqueous media (AN and DMSO) using polarography at the dme, cyclic voltammetry, alternating current (ac) polarography, and controlled potential electrolysis and coulometry.

## Results and Discussion

Two basically different structures are present in the nicotinamide series studied. Nicotinamide itself is a neutral species with the pyridine nitrogen lone pair of electrons occupying an $sp^2$ hybrid orbital localized in the molecular plane. The 1-substituted 3-nicotinamides (1-methylnicotinamide, $NMN^+$, $NAD^+$, $DNAD^+$, and $NADP^+$) contain a positively charged pyridine ring nitrogen (pyridinium species) due to loss of an electron on salt formation. The results are presented with this difference in mind.

Only nicotinamide and 1-methylnicotinamide are

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(2) (a) J. N. Burnett and A. L. Underwood, *J. Org. Chem.*, **30**, 1154 (1965); (b) J. N. Burnett and A. L. Underwood, *Biochemistry*, **4**, 2060 (1965); (c) D. Thevenot and G. Hammouya, *Experientia, Suppl.*, **18**, 631 (1972); (d) B. Janik and P. J. Elving, *Chem. Rev.*, **68**, 295 (1968); (e) P. J. Elving, J. E. O'Reilly, and C. O. Schmakel in "Methods of Biochemical Analysis," Vol. 21, D. Glick, Ed., Wiley, New York, N. Y., 1973, in press; (f) C. O. Schmakel, K. S. V. Santhanam, and P. J. Elving, work in progress.

(3) C. Carruthers and V. Suntzeff, *Arch. Biochem. Biophys.*, **45**, 140 (1953).

(4) B. Ke, *J. Amer. Chem. Soc.*, **78**, 3649 (1956); R. C. Kaye and H. I. Stonehill, *J. Chem. Soc.*, 3244 (1952).

(5) W. M. Schwartz, E. M. Kosower, and I. Shain, *J. Amer. Chem. Soc.*, **83**, 3164 (1961).

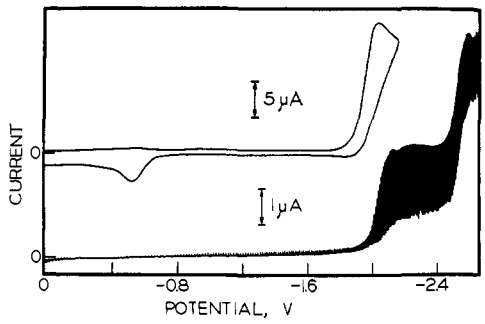



Figure 1. A dc polarogram (lower curve) and a cyclic voltammogram at the hmde (upper curve) of 2.1 m*M* nicotinamide in DMSO (0.1 *M* in tetraethylammonium perchlorate). The scan rate on cyclic voltammetry is 200 mV/sec.

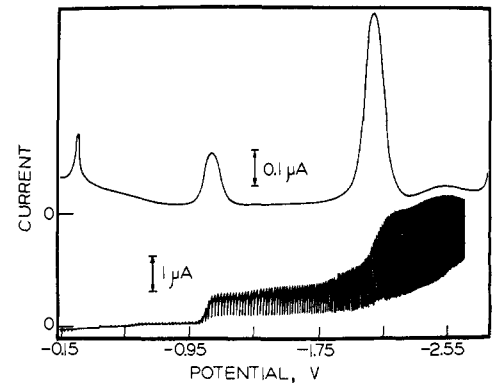



Figure 2. A dc polarogram (lower curve) and an ac polarogram (upper curve) of 0.82 m*M* nicotinamide adenine dinucleotide phosphate ($NADP^+$) in DMSO (0.1 *M* in tetraethylammonium perchlorate). Ac polarography: frequency of 50-Hz and 10-mV peak-to-peak amplitude.

sufficiently soluble in both of the inert ("aprotic") solvents used (AN and DMSO; dielectric constants at 25° are 37.5 and 46.7, respectively; viscosities are 0.342 and 1.996 cP, respectively) for their electrochemical behavior to be investigated in both solvents; the remaining nicotinamides were investigated only in DMSO. Unless otherwise specified, the background electrolyte was 0.1 *M* $Et_4NClO_4$; potentials given are *vs.* the aqueous sce. Measured potentials were corrected for liquid junction effects ($E_j$) on the basis of Rb(I), whose $E_{1/2}$ is −1.94 V in AN and −1.95 V in DMSO; *i.e.*, the $E_j$ correction between the two solvents is only of the order of 10 mV; the need for such corrections is well documented.[6] For convenience, the behavior for the whole sequence of compounds is summarized on the basis of the experimental approach used.

**Polarography.** In DMSO, nicotinamide gives two well-formed, diffusion-controlled (*h* variation) cathodic waves of equal height ($E_{1/2}$ = −2.01 and −2.50 V) (Table I and Figure 1). The diffusion current constants, *I*, and wave slopes ($E_{1/4} - E_{3/4}$) suggest one-electron faradaic processes consisting of a reversible electron transfer followed by irreversible dimerization.[7] Other compounds in the series (Table I) show only one wave due to reduction of the nicotinamide moiety with the exception of $NMN^+$, which shows a small prewave ($E_{1/2}$ = −0.38 V) due to adsorption ($i_l$ is linearly proportional to *h*).

Values of *I* for 1-methylnicotinamide in AN are about 2.3 times those in DMSO, reflecting the difference in viscosity of the two solvents, which affects the diffusion coefficient, *D*. The reciprocal of the ratio of the square roots of the viscosities, $\eta$, is 2.4, which is in good agreement with the experimental *I* and, consequently, $D^{1/2}$ ratios; *i.e.*, the $\eta D$ products (× $10^5$) are 0.69 in AN and 0.71 in DMSO.

The diffusion coefficient values, calculated from the polarographic *I* values (Table I), decrease with increasing size of the molecule except for $DNAD^+$ whose *D* is much smaller than would be expected on the basis of the Stokes–Einstein model. This anomaly may be due to greater solvation and/or association.[8]

Solutions of $DNAD^+$ and $NADP^+$ in DMSO exhibit a second reduction wave ($E_{1/2}$ = −1.96 V), whose height is about 2.2 times that of the first wave (Figure 2). This wave is due to reduction of Na(I), introduced by the use of sodium salts. The reduction of Na(I) derived from $NaClO_4$ occurs in DMSO[9] with $E_{1/2}$ = −2.0 V (the small potential difference probably arises from the uncompensated *iR* drop in the earlier report). The higher diffusion current for Na(I) is to be expected due to its much larger *D* value compared with $DNAD^+$ or $NADP^+$; $D_{Na^+}$ = 6.7

**Table I.** Polarographic Reduction of Nicotinamide Sequence in Nonaqueous Media

| Compound | Solvent[a] | $-E_{1/2}$[b] | Wave slope,[c] mV | $I$[d] | $D^e \times 10^5$ cm²/sec | $X$[f] |
|---|---|---|---|---|---|---|
| Nicotinamide | AN | I 2.00<br>II 2.45 | 46 | | | |
| | DMSO | I 2.01<br>II 2.50 | 48–50 | 1.23 | 0.48 | 0.50 |
| 1-Methylnicotinamide | AN | 1.04 | 46 | 3.3 | 2.1 | 0.50 |
| | DMSO | 1.01 | 45 | 1.4 | 0.37 | 0.50 |
| $NMN^+$ | DMSO | 0.99 | 45 | 1.25 | 0.31 | |
| $NAD^+$ | DMSO | 0.98 | 46 | 1.20 | 0.28 | 0.55 |
| $DNAD^+$ | DMSO | 1.00 | 50 | 0.65 | 0.08 | |
| $NADP^+$ | DMSO | 1.06 | 48 | 0.91 | 0.14 | 0.55 |

[a] AN = acetonitrile; DMSO = dimethyl sulfoxide. [b] Potentials are referred to aqueous sce and are corrected for liquid junction potentials by using Rb(I) as a standard. Roman numbers refer to wave sequence. [c] Wave slope = $E_{1/4} - E_{3/4}$. [d] Diffusion current constant, $I$, = $i_d/Cm^{2/3}t^{1/6}$. [e] Diffusion coefficient calculated from experimental diffusion current constant. [f] $X$ is a measure of the dependence of the limiting current on the dme mercury height (*h*): $\log i_l = X \log h$.

(6) (a) I. M. Kolthoff and J. F. Coetzee, *J. Amer. Chem. Soc.*, **79**, 870 (1957); (b) J. Broadhead and P. J. Elving, *J. Electrochem. Soc.*, **118**, 63 (1971).

(7) (a) Bonnaterre and Cauquis[7b] have derived the following expression for a one-electron reversible process followed by irreversible dimerization

$$E = E_c^\circ - \frac{RT}{F} \ln \frac{i^{2/3}}{i_d - i} - \frac{RT}{3F} \ln \frac{1.5Fm}{\lambda_f^2 C}$$

where $m = D/\delta$, $\lambda_f^2 = \delta^2 k_f/D$, $\delta$ = thickness of the diffusion layer, and $k_f$ = dimerization rate constant. Substitution of the appropriate current values leads to a value of 46 mV for $E_{1/4} - E_{3/4}$. (b) R. Bonnaterre and G. Cauquis, *J. Electroanal. Chem.*, **32**, 199 (1971).

(8) Since coulometry of $DNAD^+$ gives an *n* of 1.0, purity of the $DNAD^+$ is not a factor. However, $DNAD^+$ differs markedly from the other $NAD^+$ species in having a hypoxanthine moiety in place of the adenine moiety. The presence of the hydroxyl group on the hypoxanthine may favor association.

(9) J. N. Butler, *J. Electroanal. Chem.*, **14**, 89 (1967).

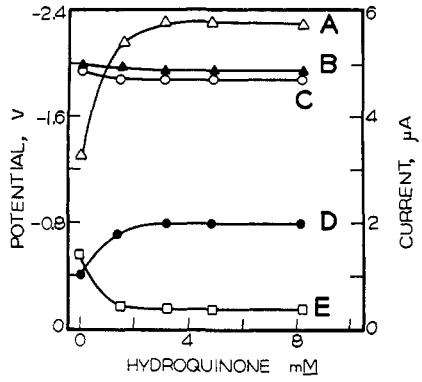



Figure 3. The variation of electrochemical characteristics of nicotinamide (1.01 m$M$) in DMSO (0.1 $M$ in $Et_4NClO_4$) with the concentration of the proton donor (hydroquinone). Polarographic wave Ic: (C) half-wave potential; (D) diffusion current. Cyclic voltammetric peak Ic; (B) peak potential; (A) peak current. Cyclic voltammetric anodic peak: (E) peak potential. Cyclic voltammetric scan rate: 0.1 V/sec. Due to the merging of wave Ic with the more positive wave IIc, which grows with increasing proton donor concentration (*cf.* text), the data represent the combined wave at higher hydroquinone concentration.

$\times 10^{-6}$ cm$^2$/sec (ref 9; the value is verified in the present study).

Similarly, in both AN and DMSO, 1-methylnicotinamide gives an anodic wave ($E_{1/2}$ = −0.20 V in AN and −0.18 V in DMSO) due to oxidation of mercury in the presence of chloride ion formed on dissociation of the 1-methylnicotinamide salt used.

**Effect of Proton Donor.** The two one-electron waves of nicotinamide and the one-electron wave observed for the 1-substituted nicotinamides arise from the reduction of the pyridine ring in these compounds. Nicotinamide is initially reduced (wave Ic) to a free radical anion and the 1-substituted compounds to a neutral free radical; this difference is evident in the reactivities to added proton donors. Thus, nicotinamide wave IIc in DMSO or AN decreases in height on adding benzoic acid or hydroquinone, and a new wave appears very close to the original wave Ic, whose $E_{1/2}$ in the presence of a proton donor is shifted more positively by 10 mV to reach a value closer to $E_{1/2}$ of nicotinamide in an aqueous medium ($E_{1/2}$ at pH 12.0 = −1.70 V). With increasing proton donor concentration and accompanying growth of the new wave IIc, the latter begins to merge with wave Ic; the combined wave reaches a limiting value of about twice the height of the original wave Ic at molar ratios exceeding 2 proton donor to 1 nicotinamide (Figure 3). On the basis of the p$K_a$ of 22.0 obtained for benzoic acid in AN by Kolthoff, *et al.*,[10] the [H$^+$] required for the appearance of a fully developed new nicotinamide wave II is $5 \times 10^{-13}$ $M$ (*cf.* subsequent discussion). The pattern observed in the organic solvent containing the proton donor is thus similar to that seen in an aqueous medium;[2f] *i.e.*, the two one-electron cathodic waves almost merge but an inflection is visible.

In the presence of a proton donor, 1-methylnicotinamide (Table II) and NAD$^+$ show markedly different behavior. The wave I height is almost unchanged but a new wave appears ($E_{1/2}$ = −1.79 V for 1-methylnicotinamide (Figure 4) and −1.99 V for NAD$^+$). The wave II height increases with increasing proton donor concentration[11] to reach a limiting value equal to the wave I height at a molar ratio of acid to nicotinamide of about 8. The [H$^+$] required for the appearance of a fully developed 1-methylnicotinamide wave II is about $6 \times 10^{-13}$ $M$. This behavior is quite different from that of aromatic hydrocarbons in AN on proton addition.

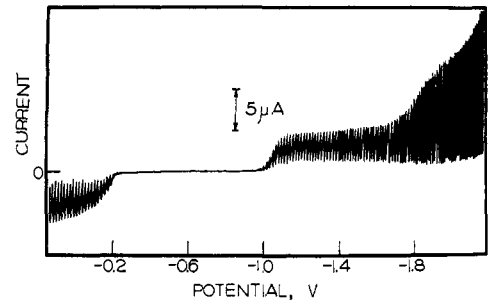



Figure 4. A dc polarogram of 0.39 m$M$ 1-methylnicotinamide in acetonitrile (0.1 $M$ in tetraethylammonium perchlorate and 1.68 m$M$ in benzoic acid).

**Table II.** Polarographic Behavior of 1-Methylnicotinamide (Effect of a Proton Donor)[a]

| Donor concn, m$M$ | Concn,[b] H$^+$ × $10^{13}$, $M$ | —Wave Ic— $-E_{1/2}$, V | —Wave Ic— $i_d$, μA | —Wave IIc— $-E_{1/2}$, V | —Wave IIc— $i_d$, μA |
|---|---|---|---|---|---|
| 0.00 | | 1.04 | 2.6 | nw[c] | nw |
| 0.79 | 2.8 | 1.04 | 2.7 | 1.79 | 0.8 |
| 1.44 | 3.8 | 1.04 | 2.8 | 1.79 | 1.1 |
| 2.74 | 5.2 | 1.04 | 2.8 | 1.79 | 1.7 |
| 3.96 | 6.3 | 1.04 | 2.6 | 1.79 | 2.8 |
| 5.42 | 7.3 | 1.04 | 2.6 | 1.79 | 2.8 |

[a] Solvent, acetonitrile; proton donor, benzoic acid, concentration of 1-methylnicotinamide, 0.54 m$M$; background electrolyte, 0.1$M$ $Et_4NClO_4$. Data are for cathodic waves. [b] Calculated on the basis of the dissociation constant of benzoic acid in AN being $10^{-22.10}$ [c] nw = no wave appears.

The electrochemical reduction of aromatic hydrocarbons generally proceeds through two successive one-electron additions with a potential separation not exceeding 0.6 V. Proton addition increases the first wave height with a corresponding decrease in the second wave height. In the presence of a large excess of proton donor, most of the aromatic hydrocarbons show one fully developed wave. The mechanism is represented as being

$$R + e \rightleftharpoons R^- \quad (2)$$

$$R^- + H^+ \longrightarrow RH\cdot \quad (3)$$

$$RH\cdot + e \longrightarrow RH^- \quad (4)$$

where R is the aromatic hydrocarbon. The protonated species RH· has a higher electron affinity than R itself and, consequently, is reduced at the same potential as R.

The nicotinamides studied have a pattern similar to the one described except that on reduction the 1-substituted nicotinamides form a neutral free radical as opposed to a negatively charged free radical; the

(10) I. M. Kolthoff, M. K. Chantooni, Jr., and S. Bhowsnik, *J. Amer. Chem. Soc.*, **90**, 23 (1968).

(11) At high benzoic acid concentration (50 m$M$), H(I) ion discharge sets in at about the same potential as wave II, complicating measurement of this wave.

neutral radical has little tendency to react with the proton donor. Thus, the wave Ic height of all of the 1-substituted compounds is unaffected by added proton donor. The appearance of a new wave at a more negative potential is due to reduction of the free radical in a process involving addition of both an electron and a proton.

The low proton activities, at which wave II for the 1-substituted nicotinamides is fully developed (or wave II for nicotinamide has completely shifted to a more positive potential), suggest either that the undissociated proton donor is involved in the protonation process or else that rapid protonation of the nicotinamide radical is accompanied by an equally rapid shift in the equilibrium

$$HA \rightleftharpoons H^+ + A^- \qquad (5)$$

Support for protonation involving the undissociated acid is indicated by inappreciable dissociation of hydroquinone in DMSO.[12]

**Cyclic Voltammetry.** Cyclic voltammetric data, except where specifically otherwise indicated, were obtained at a hanging mercury drop electrode (hmde).

**Table III.** Cyclic Voltammetry of Nicotinamides at HMDE in DMSO (0.1 *M* $Et_4NClO_4$)[a]

| Scan rate, $v$, V/sec | Cathodic peak Ic: $-E_{pc}$, V | $i_{pc}$, $\mu$A | $i_{pc}/v^{1/2}$ | Anodic peak I: $-E_{pa}$, V | $i_{pa}$, $\mu$A | $i_{pa}/v^{1/2}$ |
|---|---|---|---|---|---|---|
| | | | Nicotinamide | | | |
| 0.10 | 2.10 | 7.5 | 23.7 | 0.58 | 0.75 | 2.37 |
| 0.15 | 2.10 | 9.0 | 23.2 | 0.58 | 0.85 | 2.2 |
| 0.20 | 2.11 | 10.0 | 22.4 | 0.58 | 1.50 | 3.3 |
| 0.48 | 2.11 | 16.0 | 23.2 | 0.58 | 4.0 | 5.8 |
| 0.80 | 2.11 | 20.0 | 22.4 | 0.58 | 6.0 | 6.7 |
| 1.00 | 2.11 | 22.0 | 22.0 | 0.58 | 8.0 | 8.0 |
| | | | 1-Methylnicotinamide | | | |
| 0.10 | 1.09 | 4.5 | 14.2 | 0.29 | 1.1 | 3.5 |
| 0.15 | 1.09 | 5.0 | 13.0 | 0.29 | 1.5 | 3.9 |
| 0.20 | 1.09 | 5.7 | 12.9 | 0.29 | 2.0 | 4.4 |
| 0.48 | 1.10 | 10.0 | 14.5 | 0.29 | 5.0 | 7.2 |
| 0.80 | 1.10 | 12.0 | 13.4 | 0.29 | 6.0 | 6.7 |
| 1.00 | 1.10 | 15.0 | 15.0 | 0.29 | 7.5 | 7.5 |
| 2.50 | 1.10 | 24.0 | 15.2 | | | |
| | | | $NAD^+$ | | | |
| 0.10 | 1.03 | 4.1 | 12.9 | 0.23 | 1.25 | 3.9 |
| 0.15 | 1.05 | 4.8 | 12.4 | 0.23 | 1.70 | 4.4 |
| 0.20 | 1.05 | 5.5 | 12.2 | 0.23 | 1.90 | 4.3 |
| 0.48 | 1.05 | 9.0 | 13.0 | 0.23 | 3.0 | 4.4 |
| 1.00 | 1.05 | 12.0 | 12.0 | 0.23 | 7.0 | 7.0 |
| 2.50 | 1.06 | 19.0 | 12.0 | 0.23 | 11.0 | 7.0 |
| | | | $DNAD^+$ | | | |
| 0.10 | 1.10 | 1.4 | 4.4 | 0.22 | 0.6 | 1.9 |
| 0.15 | 1.10 | 1.6 | 4.1 | 0.22 | 0.95 | 2.4 |
| 0.20 | 1.10 | 1.9 | 4.3 | 0.22 | 0.95 | 2.4 |
| | | | $NADP^+$ | | | |
| 0.10 | 1.16 | 0.85 | 2.8 | 0.28 | 0.40 | 1.26 |
| 0.15 | 1.16 | 0.95 | 2.5 | 0.28 | 0.60 | 1.55 |
| 0.20 | 1.16 | 1.10 | 2.5 | 0.28 | 0.60 | 1.34 |

[a] Concentrations of the nicotinamides ranged from 0.3 to 1.2 m*M*. Similar results are obtained for nicotinamide and 1-methylnicotinamide in AN.

(12) (a) Kolthoff and Reddy[12b] failed to see in DMSO the oxidation wave of hydroquinone, whose ionization is necessary before oxidation can be observed; (b) I. M. Kolthoff and T. B. Reddy, *J. Electrochem. Soc.*, **108**, 980 (1961).

Typical data for DMSO solutions are given in Table III.

An initial voltage sweep toward negative potential shows two cathodic peaks for nicotinamide in DMSO ($E_p$ = −2.10 and −2.60 V) (Figure 1). Reversal of the scan 100 mV past peak Ic reveals no complementary anodic peak at slow to moderate scan rates ($v$ = 0.1 to 1.0 V/sec); an anodic peak of $E_{pa}$ = −0.58 V is due to oxidation of the product of the peak Ic process (*cf.* below). The current function, $i_p/v^{1/2}$, for peak Ic is almost constant with the scan rate (Table III). A generally similar pattern is also observed for the 1-substituted nicotinamides (Table III); *e.g.*, a well defined cathodic peak Ic (−1.09 V) on the forward sweep and an oxidation peak Ia (−0.29 V) on the return sweep are observed for 1-methylnicotinamide. For all of the molecules studied, the cathodic current function, $i_p/v^{1/2}$, is almost constant, but the anodic function shows a tendency to increase with increasing $v$. The constancy of the cathodic current function with increasing $v$ suggests that the overall irreversibility of the electrochemical process is caused, as will be shown later, by the rapid chemical reaction involving the primary product of the initial electron transfer reaction. The increasing anodic current function is likely to be due to the decreased loss of cathodic product as a result of diffusion away from the electrode.

Methylnicotinamide peak Ia can be readily shown to be due to oxidation of a product of the peak Ic reduction process. If the potential is held at a value corresponding to nicotinamide reduction, *e.g.*, 100 mV past peak Ic, and the scan then resumed, higher peak Ia currents are observed. For example, on holding an AN solution of 1-methylnicotinamide at −1.08 V for increasing periods of time, $i_{pa}$ seen on the return sweep increases and levels off at longer duration (Table IV). The highest $i_{pa}$ value obtained is about 0.7 of $i_{pc}$, which seems to indicate that the molecule involved in oxidation is lost by diffusion and/or by chemical reaction. However, the important factor is that the Table IV data support the anodic peak as due to a product of the reduction at −1.08 V and not to a species previously present in solution. Parallel results were obtained with $NAD^+$ and the other compounds.

**Table IV.** Effect of Duration of Reduction of 1-Methylnicotinamide on Magnitude of Anodic Peak[a]

| Time, sec | $i_{pa}$, $\mu$A |
|---|---|
| 5.0 | 4.75 |
| 10.0 | 8.75 |
| 20.0 | 10.50 |
| 40.0 | 14.75 |
| 60.0 | 14.00 |
| 80.0 | 14.00 |

[a] Medium, acetonitrile (0.1 *M* $Et_4NClO_4$); scan rate, 0.2 V/sec; experiment, sweep was stopped at −1.08 V (which is $E_{pc}$), held at that potential for the time indicated, and then resumed with reversal at −1.08 V; normal $i_{pc}$ value at 0.2 V/sec, 21.75 $\mu$A.

In the case of $DNAD^+$ and $NADP^+$, a peak due to Na(I) ($E_{pc}$ = −1.96 V) also appears. Sweep reversal past −2.20 V produces an anodic peak ($E_{pa}$ = −1.90 V). The peak potential difference of 60 mV confirms

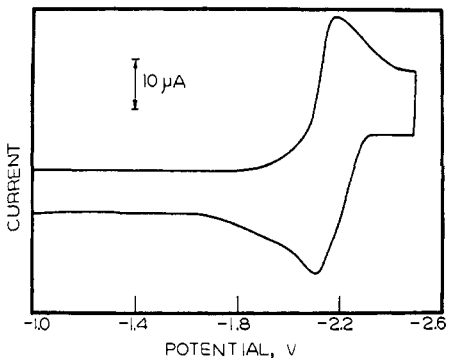



Figure 5. A cyclic voltammogram at the hmde of 2.1 m*M* nicotinamide in DMSO (0.1 *M* in tetraethylammonium perchlorate). Scan rate = 6.0 V/sec.

the reversible one-electron reduction process expected for Na(I). Cyclic voltammetric patterns for $NAD^+$ at slow scan rate (0.1 to 2.5 V/sec) also show a small hump at −0.33 V. $NMN^+$ also shows a small peak ($E_{pc}$ = −0.48 V) corresponding to the dme prewave and a peak ($E_{pc}$ = −1.96 V) due to Na(I). Sweep reversal past −1.0 V produces a well defined anodic peak ($E_{pa}$ = −0.23 V).

**1. Reversibility of Electron Transfer Process.** Electrochemical reversibility is observed for the nicotinamide reduction at scan rates above 6 V/sec (Figure 5) by the appearance of an anodic peak ($E_{pa}$ = −2.10 V), corresponding to reduction peak Ic ($E_{pc}$ = −2.16 V). The difference in peak potentials, $E_{pc}$ and $E_{pa}$, of 60 mV indicates that the reduction process is reversible and involves one electron. Reversible anodic peaks for the corresponding cathodic peaks Ic have been observed for all of the compounds listed in Table III at scan rates ranging from 10 to 80 V/sec.

**2. Effect of Proton Donor.** Cyclic voltammetric patterns for the nicotinamide series in the presence of benzoic acid as the proton donor corroborate the polarographic patterns previously discussed. Cathodic peak Ic of nicotinamide increases with increasing acid concentration, leveling off at an acid/nicotinamide molar concentration of about 3. Peak Ic of all of the 1-substituted nicotinamides is almost unaffected by the presence of the acid (the observed 5% variation in peak height may be due to uncertainty in reproducing the drop area), but a new peak IIc appears; in the case of $NAD^+$ (1.03 m*M*), the height of peak IIc ($E_{pc}$ = −1.99 V) is about three times that of peak Ic at 2–3 m*M* acid. Except for $DNAD^+$ and $NADP^+$, where the $Na^+$ reduction peak at −1.96 V obscures measurement of peak IIc, the peak IIc/Ic ratio is approximately 3 for the other compounds. (The expected peak height ratio for a two-electron process to a one-electron process is 2.8.) Peak IIc is irreversible both in respect to its slope ($E_p - E_{p/2}$) and failure to obtain a complementary anodic peak.

The dimer oxidation peak (Ia) for nicotinamide is shifted about 0.4 V more positive in the presence of 2 m*M* hydroquinone as the proton donor (Figure 3). However, the dimer oxidation peak potentials for the 1-substituted nicotinamides are almost unchanged by the presence of the proton donor. This difference can be associated with the negatively charged nature of the nicotinamide one-electron product and the uncharged nature of the other one-electron products.

**3. Evidence for Dimerization.** The chemical reaction mentioned in connection with the wave of peak Ic process can be identified as dimerization from analysis of cyclic voltammetric curves, polarographic waves, and spectrophotometry, *e.g.*, examination of the solution obtained after controlled potential electrolysis (*cf.* section on latter).

Cyclic voltammetric peak Ic for 1-methylnicotinamide, $NMN^+$, $NAD^+$, and $NADP^+$ has slope ($E_p - E_{p/2}$) values of about 45 mV (total of 25 measurements); the slope is not affected by the presence of a proton donor. The slope of peak IIc, which appears fully developed in the presence of 3–5 m*M* benzoic acid for 1.0 m*M* depolarizer, ranges from 56 to 68 mV. Comparison of these results with one proposed diagnostic criterion[13,14] for reversible electron transfer followed by irreversible dimerization, which predicts an $E_p - E_{p/2}$ value of $39/n$ mV, suggests a second-order dimerization following a one-electron transfer for the wave Ic process. The expected slope for a simple reversible electron transfer is $59/n$ mV.

Evidence for dimerization, based on the $E_p$ shift with depolarizer concentration, which should theoretically be 20 mV for a tenfold concentration change,[13,14] could not be obtained due to the limited solubility of the nicotinamides in AN and DMSO. For most of the compounds studied, the maximum solubility in DMSO corresponds to about 2 m*M*.

Polarographic wave slopes ($E_{1/4} - E_{3/4}$) for dimerization following reversible electron transfer have recently been shown to equal $46/n$ mV.[7b] Such wave slopes ranged from 46 to 50 mV for *ca.* 0.3 m*M* 1-methylnicotinamide, $NAD^+$, $NADP^+$, and $DNAD^+$.

**4. Cyclic Voltammetry at Pge.** In order to avoid any effect, which might arise from the frequently proposed reaction between nicotinamides and mercury, the compounds were examined at the pyrolytic graphite electrode (pge), which has been shown[15] to be well suited for study of electrochemical processes in both aqueous and nonaqueous media. Earlier work with nicotinamide in aqueous media[16] has shown the capabilities of this electrode for electrolytic reduction after an initial surface treatment. The pge can be used in AN and DMSO without any surface treatment. Typically, 1-methylnicotinamide in AN gives a well defined cathodic peak Ic ($E_{pc}$ = −1.08 V) at scan rates from 0.1 to 2.5 V/sec (Figure 6). On reversal of the sweep at −1.3 V, an anodic peak appears ($E_{pa}$ = −0.05 V). The ratio of $i_p/v^{1/2}$ for peak Ic is almost independent of $v$. No complementary (reversible) cathodic peak is observed for the oxidation peak at −0.05 V. These results for the reduction agree with the observations at the hmde.

**Ac Polarography.** Solutions of 1-methylnicotinamide in AN and of $NAD^+$ in DMSO exhibit a well defined ac polarographic peak ($E_s$ = −1.17 and −1.02 V, respectively); no faradaic peaks appear at more negative potential. In the presence of a proton donor (benzoic acid), peak IIc appears ($E_s$ = −2.15 V); at a

(13) R. S. Nicholson, *Anal. Chem.*, 37, 667 (1965).
(14) M. L. Olmstead, R. G. Hamilton, and R. S. Nicholson, *Anal. Chem.*, **41**, 260 (1969).
(15) W. R. Turner and P. J. Elving, *Anal. Chem.*, 37, 467 (1965); G. Dryhurst and P. J. Elving, *Talanta*, **16**, 855 (1969); R. E. Panzer and P. J. Elving, *J. Electrochem. Soc.*, **119**, 864 (1972).
(16) C. O. Schmakel, Ph.D. Thesis, University of Michigan, 1971.

**Table V.** Electrolysis Data for Nicotinamides in Nonaqueous Media

| Compound | Conditions[a,b] | Solvent | Polarography: Wave Ic $-E_{1/2}$, V | Polarography: Wave Ic $i_d$, μA | Polarography: Wave Ia $-E_{1/2}$, V | Polarography: Wave Ia $i_d$, μA | Cyclic voltammetry[c]: Peak Ic $-E_{pc}$, V | Cyclic voltammetry[c]: Peak Ic $i_{pc}$, μA | Cyclic voltammetry[c]: Peak Ia $-E_{pa}$, V | Cyclic voltammetry[c]: Peak Ia $i_{pa}$, μA |
|---|---|---|---|---|---|---|---|---|---|---|
| Nicotinamide | 1 | DMSO | 2.01 | 2.90 | | | 2.10 | 9.0 | 0.58 | 0.75 |
| | 2 | | | | 0.65 | 2.8 | | | 0.46 | 7.5 |
| 1-Methylnicotinamide | 1 | AN | 1.04 | 5.5 | | | 1.08 | 13.0 | 0.40 | 5.0 |
| | 2 | | | | 0.44 | 5.5 | | | 0.40 | 13.0 |
| | 1[d] | AN | 1.04 | 3.2 | | | 1.08 | 5.0 | 0.40 | 1.5 |
| | 2 | | | | 0.44 | 3.2 | | | 0.40 | 5.0 |
| | 1 | DMSO | 1.01 | 1.0 | | | | | | |
| | 2 | | | | 0.36 | 1.0 | | | | |
| $NMN^+$ | 1 | DMSO | 0.99 | 1.4 | | | 1.02 | 2.1 | 0.13 | 0.5 |
| | 2 | | | | 0.16 | 1.4 | | | 0.13 | 0.5 |
| $NAD^+$ | 1 | DMSO | 0.98 | 1.2 | | | 1.03 | 4.3 | 0.19 | 0.7 |
| | 2 | | | | 0.31 | 0.2 | | | 0.19 | 0.7 |
| $NADP^+$ | 1 | DMSO | 1.06 | 1.0 | | | 1.16 | 3.7 | 0.28 | 1.5 |
| | 2 | | | | 0.33 | 0.3 | | | 0.28 | 2.0 |

[a] Concentrations employed ranged from 0.5 to 1.5 m*M*. [b] (1) Before exhaustive electrolysis; (2) after exhaustive electrolysis at a potential on the plateau of the polarographic wave, *i.e.*, 200 mV past $E_{1/2}$ for reduction shown in the table. [c] Cyclic voltammetry at hmde with $v$ = 150 mV/sec. [d] Solution contained 5 m*M* benzoic acid.

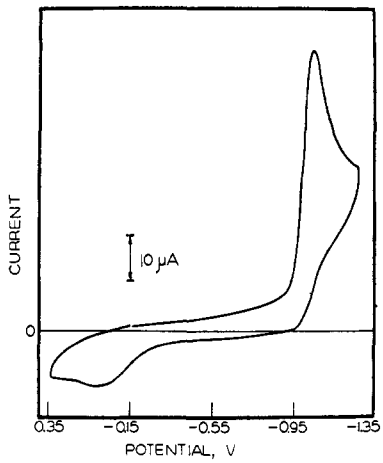



Figure 6. A cyclic voltammogram at a pyrolytic graphite electrode of 0.39 *M* 1-methylnicotinamide in acetonitrile (0.1 *M* in tetraethylammonium perchlorate). Scan rate = 150 mV/sec.

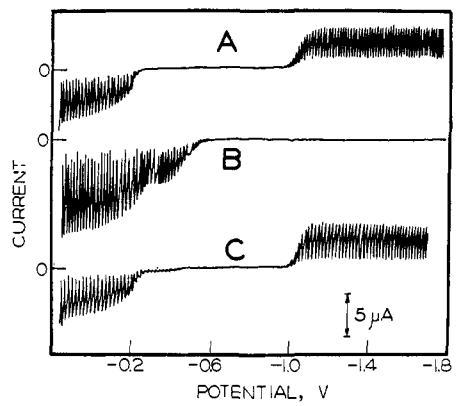



Figure 7. Dc polarograms of 0.39 m*M* 1-methylnicotinamide in acetonitrile (0.1 *M* in $Et_4NClO_4$): (A) before controlled potential electrolysis; (B) after electrolysis at −1.30 V; (C) after electrolysis at −0.32 V of the solution which gave polarogram B.

molar ratio of proton donor to nicotinamide of 2.5, the ac peak heights are about equal.

$NADP^+$ (Figure 2) and $DNAD^+$ show a second ac faradaic peak at −1.96 V (in the absence of a proton source) due to the reversible reduction of $Na^+$ present as a result of the use of sodium salts.

Ac polarographic results suggest reversible one-electron transfer for peak Ic; *e.g.*, the half-width of the peak is about 88 mV for all of the nicotinamides studied (90 mV is expected for a reversible one-electron transfer process). However, the ac faradaic currents for peak Ic are considerably smaller than would be expected for a simple reversible electron transfer process.

**Controlled Potential Electrolysis.** In all experiments, electrolysis of millimolar solutions at a potential on the limiting current plateau of the wave (wave Ic in the case of nicotinamide) gave a faradaic $n$ of 1. Polarographic and cyclic voltammetric data for the solutions examined, before and after electrolysis, are given in Table V.

Electrolysis of nicotinamide in DMSO at −2.20 V resulted in a deep yellow solution which, on exposure to air, rapidly turned colorless; the electrolysis current decayed smoothly to the background value. The electrolyzed solution showed an anodic wave Ia ($E_{1/2}$ = −0.65 V), on whose raising portion a huge maximum is apparent and whose height equals the wave Ic height before reduction, and absorption maxima at 262 and 340 nm, which agree with those reported for the 6,6′ dimer of reduced nicotinamide.[17] Oxidation at −0.50 V produced the original compound ($E_{1/2}$ = −2.01 V). On addition of a proton donor (3 m*M* hydroquinone) to the electrolyzed solution, anodic wave Ia disappears and a new anodic wave ($E_{1/2}$ = −0.20 V) appears.

Electrolysis of 1-methylnicotinamide solutions in AN or DMSO (Figure 7) produced a yellow solution, which remained yellow over a period of 24 hr. The electrolysis took about 90 min. Polarography of the electrolyzed solution produced only an anodic wave ($E_{1/2}$ = −0.44 V in AN), whose current generally equals that of the original polarographic reduction wave (the wave due to mercury chloride oxidation is still present). Electrolytic oxidation of the electrolyzed solution at −0.32 V regenerated all of the original material ($E_{1/2}$ = −1.04 V) and consumed the same number of coulombs as required for the reduction.

The electrolyzed 1-methylnicotinamide solution showed spectrophotometric maxima at 260 and 320

(17) S. Chaykin, *Annu. Rev. Biochem.*, **36**, 161 (1967).

nm, which agree with the reported absorption spectrum of the 6,6′ dimer.[17,18] The solution before reduction showed only one absorption maximum at 256 nm.

On exhaustive electrolysis at −1.30 V, $NMN^+$ solutions turned light yellow; the electrolysis current decayed smoothly to the background value. The electrolyzed solution showed an anodic wave ($E_{1/2}$ = −0.16 V), whose height equals that of the original cathodic wave.

Electrolyzed solutions of $NAD^+$, $NADP^+$, and $DNAD^+$ in DMSO were light yellow and showed only an anodic wave ($E_{1/2}$ of about −0.32 V), whose height was about $^1/_6$ that of wave Ic, and absorption spectra corresponding to formation of the 6,6′ dimer with maxima at 260 and 340 nm.[18] In electrolyzed solutions of $NADP^+$ and $DNAD^+$, the Na(I) wave was unchanged. The smaller anodic waves observed with these compounds appear to be due to low solubility of the dimer rather than to its decomposition, as the solutions after 24 hr still show the absorption maxima at 260 and 340 nm. The anodic wave height is also independent of the starting material concentration.

### Mechanistic Pattern

The electrochemical redox pattern in nonaqueous media (AN and DMSO) for all of the cationic 1-substituted nicotinamides studied (1-methylnicotinamide, $NMN^+$, $NAD^+$, $DNAD^+$, and $NADP^+$) involves an initial one-electron uptake, which produces the first cathodic wave (wave Ic) and is followed by rapid dimerization of the free-radical product

$$R^+ + e \rightleftharpoons R\cdot \quad (6)$$

$$2R\cdot \longrightarrow R\text{–}R \quad (7)$$

where $R^+$ represents the 1-substituted nicotinamide, $R\cdot$ the neutral free radical, and R–R the corresponding dimer. The dimer is oxidized at considerably more positive potential to the original electroactive compound

$$R\text{–}R \longrightarrow 2R^+ + 2e \quad (8)$$

No reduction of dimer or of free radical occurs in nonaqueous media within the available potential range.[19]

It is appropriate to compare these results with the inferences drawn for the nicotinamide analogs in aqueous medium. Under aqueous conditions, further electrochemical reduction of the free radical $R\cdot$ to a negatively charged species

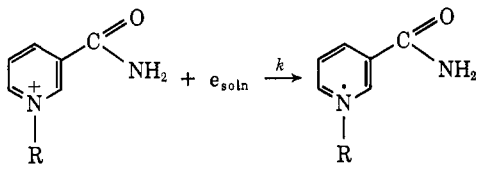
$$R\cdot + e \longrightarrow R^- \quad (9)$$

occurs in solutions of pH exceeding 9.6. The negatively charged species $R^-$ rapidly protonates to form the dihydronicotinamide. This primary difference between behavior in aqueous and nonaqueous solvents is due to the low proton activity in AN and DMSO. In the presence of added proton donor, a second reduction wave of $R^+$ is seen in AN and DMSO (in aqueous media, $E_{1/2}$ for 1-methylnicotinamide wave II is at −1.62 V). Two mechanisms may be formulated for the appearance of the second wave in a nonaqueous protonated medium. Either the protonated radical, $\dot{R}H^+$, is being reduced or reduction of the neutral radical, $R\cdot$, is facilitated by the proton presence. Since the protonation of a radical generally makes its electrochemical reduction easier than that of the parent molecule,[20] we do not believe that the protonated radical is involved in the wave II reduction process, which occurs at a more negative potential than that for the reduction of R. It is, therefore, likely that simultaneous addition of a proton and an electron to $R\cdot$ is responsible for wave II in nonaqueous aprotic solvents, *i.e.*

$$R\cdot + H^+ + e \longrightarrow RH \quad (10)$$

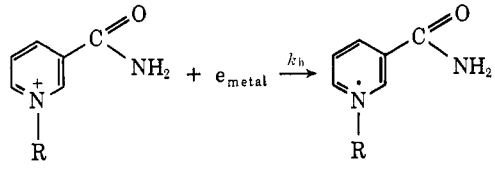
Wave II in aqueous medium has been identified as due to simultaneous reduction of dimer and neutral free radical.[21] However, the dimer is not reducible in AN and DMSO in the available potential range (−2.6 V); this would seem to cast some doubt on the preceding reports of dimer reduction.

The electrochemical results in terms of free radical formation and subsequent dimerization are in general agreement with those for pulse radiolysis of 1-methylnicotinamide and $NAD^+$, where dimerization of the intermediate free radical occurs.[22] However, a significant difference in the pulse radiolysis and electrochemical approaches is in the method of initial electron addition to the molecule. Thus, in pulse radiolysis, a solvated electron reacts with the nicotinamide molecule to generate the free radical



(11)

The electron addition rate constant, $k$, in ethanol solution is $4.1 \times 10^{10}$ $mol^{-1}$ $sec^{-1}$, when R is $CH_3$, and $2.5 \times 10^{10}$ $mol^{-1}$ $sec^{-1}$, when R is sugar–phosphate–adenine (as in $NAD^+$);[22] the dimerization rates of the free radicals are less by at least three orders of magnitude. The electrochemical generation of the free radical is a heterogeneous reaction with an electron added to the nicotinamide molecule at the electrode surface



(12)

Unfortunately, rate constants for heterogeneous charge transfer reactions of eq 6 and 13 could not be determined even by ac polarography due to the major reduction in magnitude of the expected ac polarographic current magnitudes in the present situation by the rapid follow-up dimerization reaction even at high frequency.

**Electrochemical Redox Pattern for Nicotinamide.**

(18) K. Wallenfels and H. Schuly, *Justus Liebigs Ann. Chem.*, **621**, 106 (1959).

(19) (a) For example, only one wave was observed for 1-benzyl-3-nicotinamide in *N,N′*-dimethylformamide.[19b] (b) S. Kato, J. Nakaya, and E. Imoto, *Rev. Polarog.*, **18**, 29 (1972).

(20) G. J. Hoytink, *Advan. Electrochem. Electrochem. Eng.*, **7**, 221 (1970); M. E. Peover in "Electroanalytical Chemistry," Vol. II, A. J. Bard, Ed., Marcel Dekker, New York, N. Y., 1967, pp 1–51.

(21) D. J. McClemens, A. K. Garrison, and A. L. Underwood, *J. Org. Chem.*, **34**, 1867 (1969); D. Thevenot and R. Buvet, *J. Electroanal. Chem.*, **39**, 447 (1972).

(22) E. J. Land and A. J. Swallow, *Biochem. Biophys. Acta*, **162**, 327 (1968).

The electrochemical redox pattern for nicotinamide differs slightly from that for the 1-substituted nicotinamides; further reduction of the free radical is observed in the form of the appearance of wave II even in the absence of the proton donor. Based on analogy with the mechanisms postulated for the azabenzenes in AN[23] and for aromatic hydrocarbons,[24] the mechanism for electrolytic reduction of nicotinamide can be summarized as follows

$$R + e \rightleftarrows R^- \quad \text{(wave I)} \tag{13}$$

$$2R^- \longrightarrow R_2^{2-} \tag{14}$$

$$R^- + e \rightleftarrows R^{2-} \quad \text{(wave II)} \tag{15}$$

$$R^{2-} + 2H^+ \longrightarrow RH_2 \tag{16}$$

where R represents the neutral nicotinamide molecule. The nicotinamide free radical is unstable and dimerizes rapidly on the basis of the normal time scales (1 to 4 sec) of the electrochemical experiments used; the free radical half-life is *ca.* 0.02 sec.

The negatively charged dimer $R_2^{2-}$ is oxidized to the original molecule R at less positive potential than the neutral dimers produced from the cationic nicotinamides ($E_{1/2}$ of −0.65 V for nicotinamide *vs.* −0.16 to −0.33 V for the others).

The mechanism proposed for nicotinamide is consistent with that postulated for aqueous media,[2f] where the negatively charged dimer is protonated to give the neutral dimer.

In the presence of a proton donor, the wave I height increases to a maximum value, which is twice its height in the absence of a proton donor and which results from protonation of the negatively charged free radical producing a species which is reducible at the potential of its formation.

**Dimerization Rate Constants.** Rate constants for the dimerization of the free radicals produced from the nicotinamides studied have been calculated using both (a) Nicholson's peak currents ratio method,[14] in which the ratio $i_{pa}/i_{pc}$ is related to the kinetic parameter $kC\tau$ ($k$ represents the rate constant, $\tau$ the switching time involved, and $C$ the bulk concentration), and (b) Saveant's numerical expression[25]

$$i_{pa}/i_{pd} = (1.5\pi k\tau)^{-1/2} \tag{17}$$

in which $i_{pd}$ is the diffusion current expected in the absence of dimerization and is assumed to equal $i_{pc}$. The rate constants thus obtained for nicotinamide in DMSO at 40°, which is close to biological temperature (36.8°), are given in Table VI. The best value of the rate constant for nicotinamide free radicals in DMSO at 40°, based on the data in Table VI and replication of some of the experiments there listed, is considered to be $3.5 \times 10^4$ l. mol$^{-1}$ sec$^{-1}$; variation of the rate constant with temperature (20 to 60°) gives an activation energy of about 5 kcal/mol.

Values of the rate constant for the 1-substituted nicotinamides in liter mole$^{-1}$ second$^{-1}$ (DMSO is the solvent except where indicated) at 40° is $1 \times 10^6$ for 1-methylnicotinamide (AN), $9 \times 10^5$ for NAD$^+$, $1 \times 10^6$ for DNAD$^+$, and $5 \times 10^6$ for NADP$^+$. The latter values may have to be regarded as approximate due to the large charging current contributions during fast cycling processes. The difference in magnitude between the rate constants for nicotinamide and the 1-substituted nicotinamides is in agreement between the charged and uncharged natures of the respective free radicals produced.

**Table VI.** Dimerization Rates for Nicotinamide Free Radicals[a]

| Scan rate, V/sec | $i_{pa}/i_{pc}$ | $\tau$,[b] msec | $k \times 10^{-4}$, l. mol$^{-1}$ sec$^{-1}$ NGM[c] | SNM[d] |
|---|---|---|---|---|
| 6.25 | 0.35 | 64 | 4.1 | 4.0 |
| 6.25 | 0.35 | 64 | 4.1 | 4.0 |
| 7.50 | 0.37 | 66 | 3.5 | 3.2 |
| 7.50 | 0.38 | 66 | 3.5 | 3.0 |
| 8.33 | 0.40 | 48 | 3.6 | 3.6 |
| 10.71 | 0.45 | 37 | 3.5 | 3.3 |
| 13.63 | 0.47 | 29 | 3.8 | 3.7 |
| 15.00 | 0.45 | 33 | 3.9 | 3.7 |

[a] Measurements made by cyclic voltammetry in DMSO at 40°. [b] Switching time. [c] Nicholson's graphical method.[14] [d] Saveant's numerical method.[24]

**Reactivity Sites and Rates.** As previously discussed, the initial electrochemical reduction of the nicotinamides involves reversible formation of a free radical intermediate, which can dimerize if sufficient time is available before it is oxidized. Chemical reductions of similar nicotinamides by, for example, dithionite and boron hydride tend to proceed by a different mechanism as the product of chemical reduction is invariably a dihydropyridine species.

**1. Dimerization Site.** Pullman and Pullman[26] have postulated, on the basis of quantum mechanical calculations, that reactions, which proceed through an ionic mechanism, would involve attack of the 1-substituted 3-nicotinamide at the para position of the pyridine ring and that reagents which react to generate a nicotinamide free radical would attack the ortho positions of the pyridine ring. They considered that, in general, the free valence index reflects the tendency to dimerization (radical attack). The greatest free valences correspond to the two ortho carbon atoms (positions 2 (0.658) and 6 (0.666) in the pyridine ring), which should, consequently, be preferentially attacked by free radicals. Experimentally, the 1-substituted 3-nicotinamides do give in nonaqueous media 6,6′ dimers due to free radical attack at the 6 position of the nicotinamide. The fact that the 6 position appears to be experimentally more favorable is explicable on the basis of the 2 position being less suitable for free radical attack due to molecular crowding resulting from the amide group at position 3.

**2. Free Radical Stability.** The stability of the free radicals of 1-substituted 3-nicotinamides studied in the present investigation is strikingly different from that of the free radicals derived from 1-substituted 4-nicotinamides.[5,27] The free radicals of 1-methyl-4-nicotinamide[27] and 1-ethyl-4-nicotinamide are more stable in AN than in an aqueous medium, in which they rapidly dimerize; *e.g.*, stable esr signals have been obtained in AN. The instability of the free radicals of 1-substituted 3-nicotinamides in AN and DMSO can be

(23) J. E. O'Reilly and P. J. Elving, *J. Amer. Chem. Soc.*, **94**, 7941 (1972).
(24) M. E. Peover in "Electroanalytical Chemistry," Vol. II, A. J. Bard, Ed., Marcel Dekker, New York, N. Y., 1967, pp 1–51.
(25) J. M. Saveant, *Electrochim. Acta*, **12**, 999 (1967).
(26) B. Pullman and A. Pullman, *Proc. Nat. Acad. Sci. U. S.*, **45**, 136 (1959).
(27) M. Itoh and S. Nagakura, *Tetrahedron Lett.*, **8**, 417 (1965).

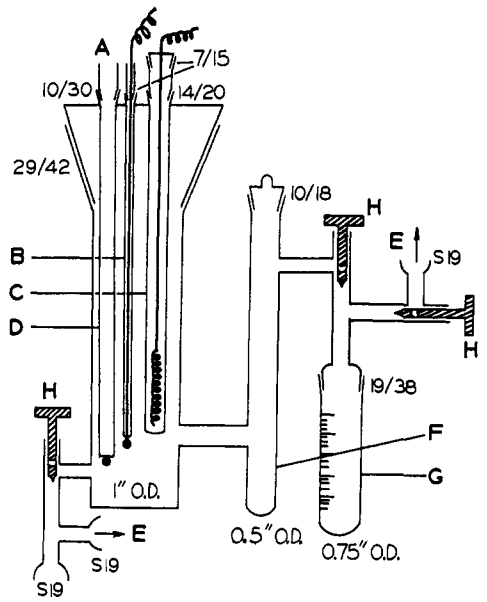



Figure 8. An electrolytic cell for nonaqueous media studies and vacuum line utilization: (A) to the mercury reservoir; (B) hanging mercury drop electrode; (C) counter electrode compartment; (D) dropping mercury electrode (B, C, and D are immersed in the working electrode compartment); (E) to the vacuum line; (F) reference electrode compartment; (G) sample tube or plain tube with appropriate volume marking; (H) Teflon needle valves.

correlated with the 4 position in the pyridine ring being the most reactive site; its availability, *i.e.*, lack of substitution, would enable dimerization to occur readily. Thus, esr signals due to the free radicals of 4-substituted pyridines have been obtained[28] as a result of their stability, *i.e.*, slowness of dimerization of the free radicals.

**Stability of the Dimers.** The ultimate product of the initial electrochemical reduction of the nicotinamide series in DMSO and AN is the 6,6′ dimer, based on the spectral evidence. The stability of these dimers is indicated by the fact that, over a period of 24 hr, the spectrophotometric absorption peak and the anodic polarographic wave did not show any appreciable differences in magnitude. This stability is in contrast to that seen in aqueous media; at pH 9.6, the dimer of 1-methylnicotinamide decomposes at the rate of 6%/hr. Due to this decomposition, the anodic wave height observed for an aqueous electrolyzed 1-methylnicotinamide solution is appreciably less than that of the original wave Ic and is dependent on the duration of the electrolysis.

Similarly, dimers derived from $NAD^+$, $DNAD^+$, and $NADP^+$ are more stable in nonaqueous media.

The cause of the dimer instability in aqueous media appears to be acid catalyzed decomposition, which proceeds even at pH exceeding 9. Formation of the decomposition product is accompanied by appearance of an absorption peak at 296 nm. Burton and Kaplan[29] postulated the absorption maximum at 296 nm in pyridine-derived species to be due to a chromophore of the type

$$-NH-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}=O$$

with the 3,4 double bond of the pyridinium ring removed by proton addition. The greater stability of the dimers in nonaqueous media may then be explained as due to the low proton activity of these media. In an electrolysis of 1-methylnicotinamide in AN at a potential on the plateau of wave Ic in the presence of 50 m*M* benzoic acid, a 100% yield of the dimer (based on coulometric reversal oxidation) was obtained. The dimer yield is obviously not altered by the presence of a weak proton donor ($pK_a$ of benzoic acid in AN = 22.0).

## Experimental Section

**Chemicals.** Nicotinamide, 1-methylnicotinamide, nicotinamide adenine dinucleotide, deamino nicotinamide adenine dinucleotide (nicotinamide hypoxanthine dinucleotide), and nicotinamide adenine dinucleotide phosphate (Sigma Chemical) and nicotinamide mononucleotide (P. L. Biochemicals) were used as received. Tetraethylammonium perchlorate (Eastman White Label) was recrystallized three times and dried before use. Acetonitrile (Eastman Yellow Label) was dried overnight over $CaH_2$ and distilled under vacuum; purified acetonitrile was stored under vacuum over molecular sieves. Dimethyl sulfoxide (Baker analyzed reagent) was used as received or was distilled and dried over molecular sieves.

**Apparatus.** Electrochemical measurements were made with a multipurpose instrument, based on solid state operational amplifiers,[2f,16,30] X–Y recorders, and cathode-ray oscilloscopes.

A vacuum line, similar to the one described by Santhanam and Bard,[31] was used with the following modifications: (a) use of a cell (Figure 8), which enables solutions of known concentration to be prepared in a separate compartment and which allows the cell to be detached from the main manifold and the solution to be transferred into the working and counter electrode compartments (the whole transfer operation is carried out under vacuum); and (b) the use of a two-stage mercury diffusion pump.

Capillary constants of the dme at a mercury height of 70 cm (uncorrected for back pressure) in deoxygenated 0.1 *M* $Et_4NClO_4$ at open circuit were $m$ = 1.0 mg/sec and $t$ = 4.0 sec in AN solution and 1.02 mg/sec and 5.0 sec in DMSO solution, respectively. The hanging mercury drop electrode (hmde) consisted of a platinum wire sealed into the end of a piece of glass tubing, filed flush with the end of the tubing, and then plated with mercury from a mercuric nitrate bath.

All potentials were measured against an aqueous saturated calomel electrode, which was separated from the main compartment by a sintered glass disk and an agar gel. The relative difference in liquid junction potentials between AN and DMSO can be related to the $E_{1/2}$ values for Rb(I) reduction *vs.* aqueous sce, which are −1.94 V in AN, −1.95 V in DMSO, and −2.13 V in water;[8b] the first two values were measured in the present investigations.

**Procedures.** The solvent is distilled into the graduated side arm of the cell containing the background electrolyte and the electroactive material. The cell is disconnected from the vacuum line and the solution is transferred into the electrolysis compartments by opening the appropriate needle valve and suitably tilting the cell. The cell is reconnected to the vacuum line and pumped to obtain the desired vacuum; the diffusion pump and the fore pump are then disconnected by turning the appropriate stopcocks. Prepurified nitrogen is passed into the cell to release the vacuum. The electrodes are introduced into the cell and the electrochemical measurements made.

For exhaustive electrolysis, a mercury pool is placed at the bottom of the cell and the solution stirred by means of a magnetic bar. After electrolysis, the solution is transferred to the side arm by opening the corresponding needle valve. This procedure ensures minimum contact of air with the solution; the arrangement would be excellent for taking samples for spectrophotometric and esr examination.

Ac polarographic measurements were made with a superimposed 50-Hz 10-mV peak-to-peak (3.5 mV rms) perturbation unless otherwise specified. The current reported is the in-phase component of the total current.

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