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Nicotinamide-NAD Sequence: Electrochemical and Allied Chemical Behavior *

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Summary

Electrochemical redox mechanisms in aqueous and non–aqueous media, including associated chemical reactions, structure and behaviour of intermediate species, and phenomena such as adsorption, have been elucidated for a sequence of compounds ranging from nicotinamide to NAD^+. Dimerization rates of the free radicals, produced on the initial one–electron reduction, have been measured. The electrochemical behaviour has been correlated with structural, solvation, molecular orbital and other pertinent characteristics and parameters.

Introduction

The nicotinamide sequence of compounds is of considerable biological importance, *e.g.*, nicotinamide (3–carbamoylpyridine) (Fig. 1) itself is an essential dietary factor in producing pyridine nucleotides such as NAD^+, which function as coenzymes for the pyridinoproteins which are among the principal components in the electron transport chain in biological redox reactions. A basic understanding of the electrochemical redox behaviour of the sequence should contribute to a better understanding of the electron transport chain, since the site of both biological and electrochemical redox activity in the pyridine nucleotides is the pyridine ring.

Review of the literature on the electrochemical behavior of the nicotinamide sequence in aqueous media [1] indicates that, while certain aspects have been thoroughly treated, others were only partially explained or not considered, *e.g.*, in respect to the roles of adsorption at the interface and of kinetic processes, *e.g.*, dimerization of the initially produced free radicals. Aside from a fragmentary study of NAD^+ in 50 % dioxane, the only study of the sequence in nonaqueous media is one employing acetonitrile (AN) and dimethylsulfoxide (DMSO) [2]; such

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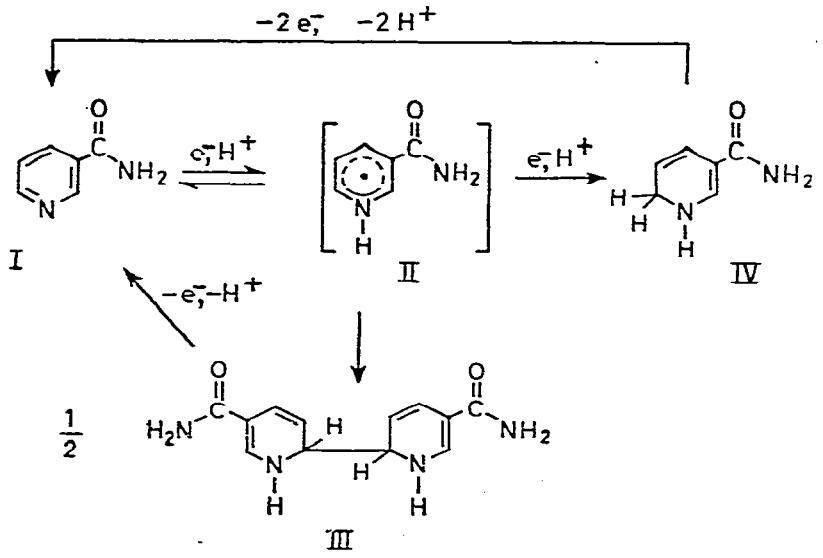

Fig. 1.
Reaction paths for the electrochemical behavior of nicotinamide and its reduction products.

study in aprotic media is especially helpful in clarifying the nature of the observed second reduction step.

The present paper covers the following sequence: nicotinamide, N′–methylnicotinamide, 1–methyl–3–carbamoylpyridinium ion (1–methyl–nicotinamide, MCP+), nicotinamide mononucleotide (NMN+), nicotinamide adenine dinucleotide (NAD+, DPN+, coenzyme I), nicotinamide adenine dinucleotide phosphate (NADP+, TPN+, coensyme II), and deamino nicotinamide adenine dinucleotide (nicotinamide hypoxanthine dinucleotide, deamino–NAD+, DNAD+). Nicotinamide itself differs from 1–substituted nicotinamides such as the nucleotides in having a basic ring nitrogen due to the presence of the lone electron pair; consequently, its redox behavior would be expected to show a pH–dependence differing from those of the nucleotides except in the pH region where the ring nitrogen is protonated. The discussion is presented with this difference in mind.

Electrochemical behaviour patterns and interpretation

The electrochemical behavior patterns of the nicotinamide sequence have been observed by means of *d.c.* polarography and phase–selective *a.c.* polarography at the D.M.E. (dropping mercury electrode), cyclic voltammetry at the H.M.D.E. (hanging mercury drop electrode) and P.G.E. (pyrolytic graphite electrode), controlled electrode potential electrolysis and coulometry at massive mercury electrodes, and thorough examination of partially and completely electrolyzed solutions of the

various compounds. Due to lack of space, only essential features are given, *e.g.*, Table 1. Details and data can be found in the papers summarized in reviews, [1] *e.g.*, studies by the present authors in aqueous media (1*d*, 3) and nonaqueous media. [2] Behavior, unless specifically otherwise noted, is in aqueous media.

Table 1. Pertinent electrochemical characteristics of nicotinamides.

Compound	Solvent (*a*)	$-U_{1/2}$(*b*) V	I μA	$-U_p$(*b*) V	$D \times 10^5$(*c*) cm²/s
Nicotinamide	H_2O	I 1.60(*d*)	3.8	1.32(*e*)	0.98
		II		1.43(*e*)	
	AN	I 2.00			
		II 2.45			
	DMSO	I 2.01	1.2	2.11	0.48
		II 2.50			
N′–Methyl-nicotinamide	H_2O	I 1.60(*d*)	2.1	1.29(*e*)	
MCP^+	H_2O	1.05	2.3	1.14	1.06
	AN	1.04	3.3	1.10	2.1
	DMSO	1.01	1.4	1.03	0.37
NMN^+	H_2O	0.98		1.14	0.45
	DMSO	0.99	1.3		0.31
NAD^+	H_2O	0.91	1.66	1.16	0.55
	DMSO	0.98	1.20	1.05	0.28
$DNAD^+$	H_2O	0.95	1.38	1.16	0.38
	DMSO	1.00	0.65	1.10	0.08
$NADP^+$	H_2O	0.95	1.18	1.16	0.28
	DMSO	1.06	0.91	1.16	0.14

(*a*) Viscosities in centipoises and dielectric constants in debyes at 25 °C are 0.89 and 78.0 for water (H_2O), 0.32 and 35.0 for acetonibrile (AN), and 1.96 and 46.0 for dimethylsulfoxide (DMSO).

(*b*) Potentials are referred to aqueous S.C.E. and are corrected for liquid junction potentials by using Rb(I) as a standard. Roman numbers refer to wave sequence; non-numbered data are for the first or only wave seen.

(*c*) The diffusion coefficient, D, has been calculated from the diffusion current, using the simple form of the Ilkovic equation.

(*d*) $U_{1/2}$ is pH-dependent; the value given is for pH 9 where the two waves seen in acid solution have coalesced.

(*e*) U_p is pH-dependent; values given are for pH 5.

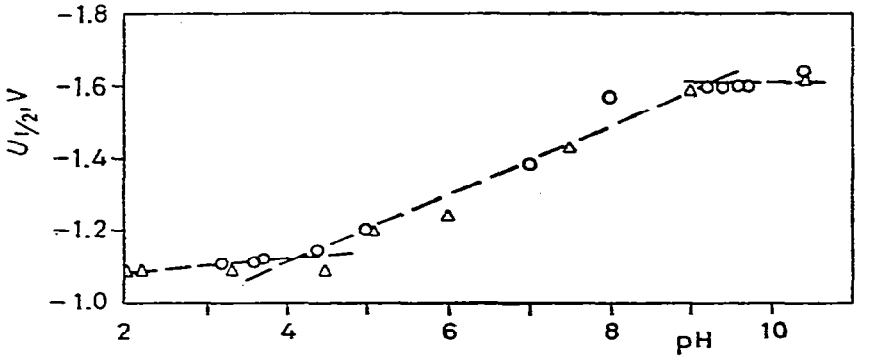

Fig. 2.
Variation with pH of the half-wave potential, $U_{1/2}$, for the first cathodic polarographic wave of nicotinamide (circles) and N′–methylnicotinamide (triangles).

In acid media, nicotinamide shows two adjacent cathodic waves with a closely following catalytic hydrogen discharge wave. $U_{1/2}$ for wave I*c* is nearly pH–independent below pH 4 and above pH 9, and varies linearly with pH between pH 4 and 9 (Fig. 2); N′–methylnicotinamide shows identical behavior. The catalytic wave disappears between pH 7 and 8. Between pH 3 and 7, the two nicotinamide waves are of equal height, result from 1–electron faradaic processes, and shift negatively with a constant *ca.* 0.11 V separation in $U_{1/2}$. The nearly merged 2–electron wave seen above pH 8 shows an inflection about half way up the wave, which disappears with increasing pH; addition of Et_4NCl at pH 9.4 separates the wave into two reversible 1–electron

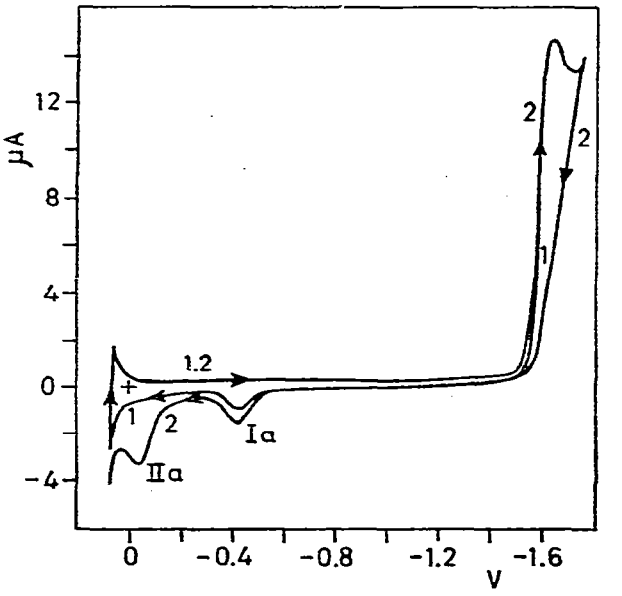

Fig. 3.
Cyclic voltammogram of nicotinamide (1.27 m*M*) in pH 9.4 carbonate buffer at the H.M.D.E. Roman numerals refer to waves; Arabic numerals to sweep. Scan rate is 100 mV/s.

waves ($U_{1/2}$: —1.56 and —1.66 V). Cyclic voltammetry indicates that the wave I*c* product is not further reduced in the wave II*c* process, and that the product of the single cathodic 2-electron peak (U_{pc} = —1.72 V) at pH 12 produces an anodic peak at —0.21 V but that sweep reversal at intermediate pH following the apparently single cathodic peak produces two anodic peaks (Fig. 3), which correspond to oxidation of the 1-electron and 2-electron reduction products. At higher scan rates, *e.g.*, Fig. 4, complementary cathodic–anodic peak I pairs are seen, whose 60-mV separation in peak potential is characteristic of a 1-electron reversible redox couple. The latter presence allows calculation of the dimerization rate of the free radical produced in the initial 1-electron process.

The 1-substituted nicotinamides generally show two well separated 1-electron waves. $U_{1/2}$ for wave I*c* is generally pH-independent, except that NMN+ wave I shifts negatively in the range of pH 5.0 to 7.5 due to its secondary phosphate dissociation. $U_{1/2}$ for wave II*c* may show slight pH-dependence. Typical patterns of NAD^+ in the absence and presence of a surfactant, which would prevent reduction product adsorption, are shown in Fig. 5 and 6.

In nonaqueous media, nicotinamide exhibits two 1-electron waves (potentials separated by *ca.* 0.6 V); the 1-substituted derivatives show a single 1-electron wave. Proton donor addition produces wave patterns similar to those seen in aqueous media, *i.e.*, nicotinamide wave I*c* decreases and shifts to slightly more positive potential, wave II*c* decreases

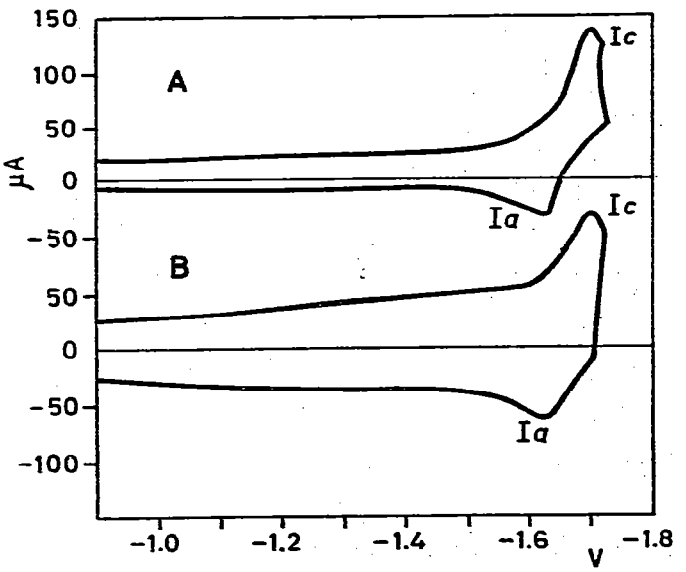

Fig. 4.
Cyclic voltammograms of (*A*) nicotinamide (scan rate = 23 V/s) and (*B*) N'-methylnicotinamide (scan rate = 18 V/s) in pH 9.0 carbonate buffer, showing the reversible cathodic–anodic peak couple for the first electron-transfer process present at high sweep rates.

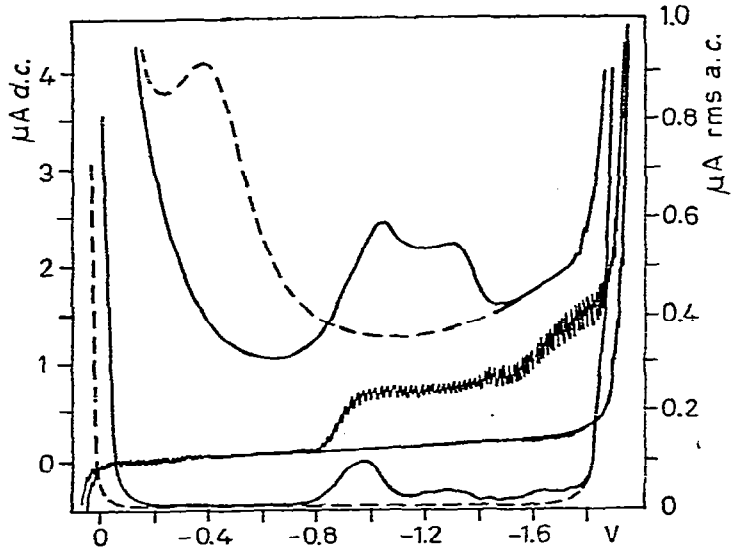

Fig. 5.
d.c. and *a.c.* polarograms of NAD^+ (0.31 m*M*) in KCl–carbonate buffer (pH 9.3.). *d.c.* polarograms shown with and without electroactive species present. *a.c.* polarograms: solid lines represent in-phase (lower set) and quadrature (upper set) components of total *a.c.* current; dashed lines represent corresponding background currents.

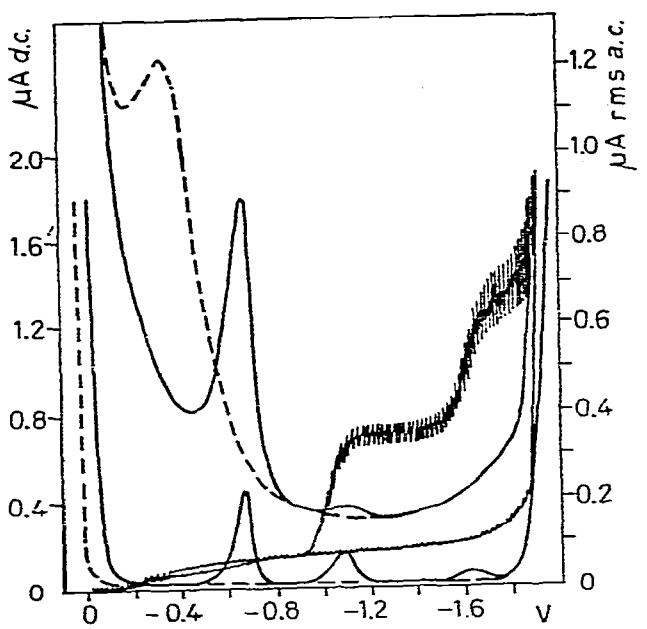

Fig. 6.
d.c. and *a.c.* polarograms of NAD^+ (0.31 m*M*) in Et_4NCl–carbonate buffer (pH 9.6). Curves are identified in caption to Fig. 5.

in height, and a new wave appears very close to wave I*c*, with which it merges; wave I*c* of the 1–substituted nicotinamide is unchanged but a new wave starts to grow at more negative potential, eventually attaining the same height as wave I*c*. The appearance and characteristics of a complementary cathodic–anodic peak couple indicate wave I*c* to arise from a reversible 1–electron reduction followed by rapid dimerization.

Redox mechanistic paths

The reaction scheme outlined in Fig. 1 seems best to fit the combined data for nicotinamide (I) and N′–methylnicotinamide. At pH above the nicotinamide pK_a (3.3), the initial reduction involves uptake of an electron and a proton to form a neutral free radical (II) (source of wave I*c*); this is followed by irreversible dimerization to the 6,6′ dimer (III). At the potential of wave II*c*, nicotinamide is reduced to the 1,6–dihydropyridine (IV) (equivalent to a 1–electron reduction of the free radical); the dimer is not reduced at this potential. At considerably more positive potential than that of wave I*c*, the dimer is oxidized to nicotinamide; at still more positive potential, the 1,6–dihydropyridine species is also oxidized to nicotinamide.

Below pH 3 to 4, wave I*c* shows only slight pH–dependence because most of the nicotinamide will be protonated at N (I) and the unprotonated compound will rapidly protonate as the protonated form is reduced. The essential reactions in the wave I*c* process are, consequently, as follows, where R represents the nicotinamide:

$$R + H^+ \rightleftarrows RH^+ \quad (1)$$

$$RH^+ + e^- \rightleftarrows \dot{R}{\cdot}H \quad (2)$$

$$2\,R{\cdot}H \rightarrow (RH)_2 \quad (3)$$

The wave II process involves reactions 1 and 2 plus reduction of the free radical, which may proceed stepwise:

$$R{\cdot}H + H^+ + e^- \rightarrow RH_2 \quad (4)$$

Above pH 3 to 4, the wave I*c* energy–controlling step involves simultaneous addition of an electron entering the ring electronic system and a proton localizing itself on N(1). In the slightly acidic to slightly alkaline region (Fig. 2), protonation is sufficiently rapid that it is not a limiting factor. The essential steps in the wave I*c* process are, accordingly,

$$R + H^+ + e^- \rightleftarrows R{\cdot}H \quad (5)$$

$$2\,R{\cdot}H \rightarrow (RH)_2 \quad (6)$$

Dimerization of the neutral free radical must be exceedingly rapid since its oxidation is not seen even at high scan rates on cyclic voltammetry. The free radical oxidation seen at pH 9 and above (Fig. 4)

probably involves the negatively charged radical anion, whose dimerization would be slowed down due to electrostatic repulsion. Protonation would be excepted to be slow. The radical, once formed, is more easily reduced than the original electroactive species as indicated by the almost simultaneous addition at pH 12 of two electrons to produce a polarographic wave of slope expected for a reversible 2–electron wave. The following reactions are, accordingly, involved,

$$R + e^- \rightleftarrows R^{\bullet -} \quad (7)$$

$$2\ R^{\bullet -} \rightarrow (R_2)^{2-} \quad (8)$$

$$(R_2)^+ + 2H^+ \rightarrow R_2H_2 \quad (9)$$

$$R^{\bullet -} + e^- \rightarrow (R:)^{2-} \quad (10)$$

$$(R:)^{2-} + 2H^+ \rightarrow RH_2 \quad (11)$$

In the case of the 1–substituted nicotinamides, the reaction scheme outlined in Fig. 7 seems best to fit all available data. The mechanism is in some ways similar to that of nicotinamide itself. However, as might be expected for a positively charged pyridine nucleus (V), the first reduction step is independent of pH and occurs at slightly more positive potential; thus, two well defined 1–electron waves appear in slightly alkaline solution. The initial reversible uptake of an electron to form a neutral free radical (VI) (source of wave I*c*) is followed by irreversible dimerization, largely or entirely to the 6,6′ dimer (VII). At the wave II*c*

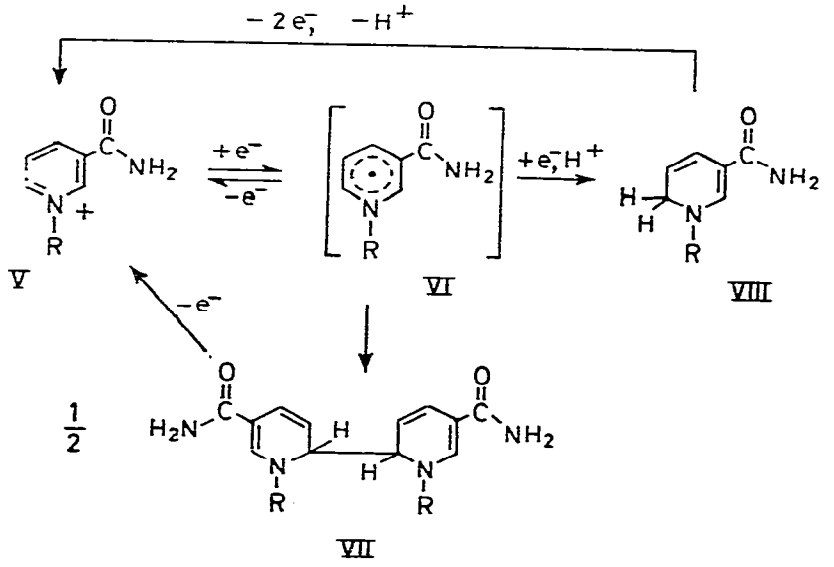

Fig. 7.

Reaction paths for the electrochemical behavior of 1–methyl–3–carbamoylpyridinium ion (MCP^+), the nicotinamide nucleotides, and their reduction products. In the case of the nucleotides, some of the dihydropyridine substituent (VIII) may be present as the 1,4 isomer.

potential, the cation is reduced in a 2–electron process to the 1,6–dihydropyridine (VIII) ; the dimer is not reduced at this potential. The dimer can be oxidized to the original species at a potential considerably more positive than that of wave I*c* ; the 1,6–dihydropyridine is oxidizable at still more positive potential.

The redox mechanism for NAD^+, which is apparently the same for NMN^+, $NADP^+$ and $DNAD^+$, differs slightly in that the wave II*c* product is largely enzymatically active 1,4–NADH but 1,6–NADH is also formed under certain conditions. The dimer is not reduced at potentials on the wave II*c* limiting portion, *i.e.*, it is not an intermediate in the formation of this wave. However, at a potential close to background discharge, NAD dimer appears to be very slowly reduced to a dihydropyridine species ; the mechanism for this reduction is presently unknown.

The primary difference between reaction paths in aqueous and nonaqueous media is due to the low proton activity in the latter. The wave I*c* process for the 1–substituted nicotinamides is the same. A second wave is seen in nonaqueous media only on proton addition. Since protonation of a free radical generally makes its electrochemical reduction easier than that of the parent molecule, [4] the wave II*c* process, which occurs at more negative potential than that for reduction of the parent cation, R^+, likely involves simultaneous addition of a proton and an electron to $\dot{R}$:

$$R^{\cdot} + H^+ + e^- \rightarrow RH \qquad (12)$$

The nicotinamide redox pattern in nonaqueous media differs slightly from that for the 1–substituted nicotinamides ; further reduction of the free radical is observed (appearance of wave II*c*) even in the absence of proton donor. Based on analogy with the mechanisms postulated for azabenzenes in AN [5] and aromatic hydrocarbons, [6] the mechanism can be summarized as follows :

$$R + e^- \rightleftarrows R^- \qquad (13)$$

$$2\ R^- \rightarrow R_2^{2-} \qquad (14)$$

$$R^- + e^- \rightleftarrows R^{2-} \qquad (15)$$

$$R^{2-} + 2\ H^+ \rightarrow RH_2 \qquad (16)$$

where R represents the neutral nicotinamide molecule. The negatively charged dimer R_2^{2-} is oxidized back to R at less positive potential ($U_{1/2}$: —0.65 V) than the neutral dimers produced from the cationic nicotinamides (—0.16 to —0.33 V). On proton addition, nicotinamide wave I*c* grows to twice its height as a result of protonation of the negatively charged free radical to produce a species reducible at the potential of its formation.

Nature and behavior of individual species

Important aspects of the comparative behaviour of the individual compounds and their intermediates and products are summarized in the following subsections.

1. *Nicotinamide species.* Reversibility of the initial 1–electron step and dimerization of the free radical produced are indicated by the *d.c.* polarographic wave slope, the U_p–$U_{p/2}$ differences and complementary cathodic–anodic peak pairs on cyclic voltammetry, the products of controlled potential electrolysis, and other factors.

Adsorption of nicotinamide, N′–methylnicotinamide, and their reduction products at the interface is negligible. While MCP^+ is also negligibly adsorbed, its dimeric and dihydropyridine products are strongly and moderately adsorbed, respectively; NMN^+ and its reduction products are negligibly adsorbed. These differences in adsorption reflect the presence of a hydrophobic substituent on N (1) of MCP^+ and of a hydrophilic substituent on N (1) of NMN^+, and the difference in hydrophobic nature of the two MCP^+ products.

The behaviour of NAD^+ is, as expected from its structure, more complicated. The *d.c.* waves that appear in addition to the two 1–electron reduction steps, con be explained, largely on the basis of *a.c.* polarography (Fig. 5 and 6), in terms of adsorption phenomena. For example, in Et_4NCl carbonate buffer, the *d.c.* prewave at *ca.* —0.7 V is identified by the corresponding *a.c.* tensammetric peak as a capacitive step; before the step, NAD^+ is adsorbed but, after the step, Et_4N^+ is preferentially adsorbed.

In KCl–carbonate buffer, the capacity current is depressed over the entire potential region. An anomalous *d.c*, capacitive wave or maximum at *ca.* —1.3 V is similarly identified as due to adsorption of the dimer; with increasingly negative potential, K^+ displaces the dimer because of increasing coulombic attraction.

2. *Free radical species.* Rate constants for the free radical dimerizations have been calculated from the cyclic voltammetric complementary cathodic–anodic peak I pairs, using the graphical peak currents ratio and numerical equation methods (Table 2).[2,7]

3. *Dimer and dihydronicotinamide species.* The dimeric products of the initial 1–electron reduction and the dihydropyridine products of the 2–electron reduction were characterized by spectrophotometric, electrochemical, enzymatic and chemical examination of the solutions obtained on controlled potential electrolysis, *e.g.*, identities and amounts of the reduction products could be determined from the anodic waves observed in the electrolyzed solution (Table 3) and from reverse controlled potential anodic coulometry.

Postulation of the 6,6′ dimer and the 1,6–dihydropyridine species is based on the absorption bands observed in the 240–400 nm region (*cf.* differences observed for NAD^+). Before reduction, 1–substituted

Table 2. Dimerization rate constants for free radicals formed from nicotinamide–related compounds.

Compound	Solvent (*b*)	Temp. °C.	Rate constant (*a*)			E_{act} (*c*) kcal mol^{-1}
			k_d l mol^{-1}s^{-1}	*s*	*n*	
Nicotinamide	H_2O (9)	30	1.8×10^6	1.1	5	24
	DMSO	40	3.5×10^4	0.3	8	5
N′–Methyl-nicotinamide	H_2O (9)	30	4.9×10^6	1.2	6	14
MCP^+	H_2O (5 to 9)	30	6.1×10^7	1.1	15	4.1
	AN	40	1×10^6			
NMN^+	H_2O (5 to 9)	30	1.5×10^6	0.1	3	3.6
NAD^+	H_2O (5)	30	2.2×10^6	0.6	11	9.0
	H_2O (9)	30	2.4×10^6	2.1	3	
	DMSO	40	9×10^5			
$DNAD^+$	H_2O (9)	25	1.7×10^6	3.5	3	
	DMSO	40	1×10^6			
$NADP^+$	H_2O (5)	30	4.3×10^6	0.8	3	9.0
	H_2O (9)	30	1.6×10^6	2.6	3	
	DMSO	40	5×10^6			

(*a*) Standard deviation is *s* for *n* measurements of the rate constant under the conditions indicated; generally, the scan rate was varied.

(*b*) The pH for aqueous solutions is indicated in parentheses; AN = acetonitrile; DMSO = dimethylsulfoxide.

(*c*) Activation energies are based on Arrhenius plots of lg k_d *vs.* T^{-1}, generally between 10 and 50 °C.

3–carbamoylpyridinium species generally show a single absorption band at about 265 nm; the dihydropyridine reduction products show single bands at about 400 nm for the 1,2 species and 340 for the 1,4 species, and two bands at about 270 and 350 nm for the 1,6 product; similarly, the 4,4′ dimers produced on reduction absorb at about 340 nm and the 6,6′ dimers at about 260 and 345 nm. Correspondingly, nicotinamide has an absorption band at about 261 nm; its dimeric and dihydropyridine reduction products each absorb at about 265 and 345 nm.

Results for solutions electrolyzed at potentials on NAD^+ wave IIc strongly indicate formation of both 1,4 and 1,6 isomers, plus a small percentage of dimeric 1–electron products, *e.g.*, a typical experiment gave 54 % 1,4–NADH, 35 % 1,6–NADH and 11 % dimer.

The two reduction products undergo acid–catalyzed decomposition involving hydrolysis of the 4,5 double bond even in slightly alkaline

Table 3. Half-wave potentials for oxidation of dimers derived from nicotinamides.

Parent compound	$-U_{1/2}$ (*a*)	$-U_{1/2\,DMSO}$ (*b*)	$\Delta U_{1/2}$
Nicotinamide	0.45	0.65	0.20
MCP^+	0.45	0.36	—0.09
NMN^+	0.28	0.16	—0.12
NAD^+	0.28	0.31	0.05
$NADP^+$	0.28	0.33	0.05

(*a*) Values determined in aqueous solutions of pH 9 to 10.

(*b*) Values determined in DMSO containing $(C_2H_5)_4NClO_4$ as supporting electrolyte.

solutions containing proton donors such as $H_2PO_4^-$. With decreasing pH, the rate increases; at any given pH, the dimer is less stable than the dihydropyridine species; the products of the nucleotides are more stable than those of nicotinamide and MCP^+, emphasizing the effect of the N (1) substituent on reactivity.

The ultimate product of the initial reduction in DMSO and AN is the 6,6′ dimer, whose stability is indicated by the inappreciable variation over 24 h of the spectrophotometric absorption peak and anodic polarographic wave. The greater dimer stability in nonaqueous media may obviously be explained as due to the low proton activity of these media.

Correlation of electrochemical behavior

Reaction medium

With increasing viscosity, η, the diffusion coefficients, based on the polarographic I values, of nicotinamide and MCP^+ become smaller, but the product ηD remains fairly constant (Table I).

The observed two orders of magnitude difference in the dimerization rate constant for the nicotinamide free radical in H_2O and DMSO (Table 2) may be attributed to differences in dielectric constant of the media, as predicted by a double sphere model for the dimerization of like charges.

$U_{1/2}$ for wave Ic of the 1-substituted nicotinamides is not significantly affected by the medium (Table 1), indicating the absence of significant ion-pairing of the positively charged molecules. $U_{1/2}$ for dimer

oxidation also shows little variation with the medium (Table 3); the large difference for nicotinamide dimer may be attributed to the difference in nature of the dimer in the two media: neutral in H_2O and anionic in DMSO.

The sensitivity of the mechanistic pathways to proton availability has been indicated, as has been, for example, the greater stability of the dimers in nonaqueous media.

Dimerization site. The tendency to dimerization (radical attack) of the nicotinamide free radicals is indicated by the free valences in the pyridine ring. The greatest free valences in 1–substituted nicotinamide correspond to the *ortho* carbon atoms at positions 2 (0.658) and 6 (0.666), which should, consequently, be preferentially attacked by free radicals. Experimentally, the 1–substituted 3–nicotinamides do give 6,6′ dimers due to free radical attack. The favoring of the 6 position 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.

Dimerization rates. Electrochemical techniques employing cyclic voltammetry or *a.c.* polarography generally cannot measure dimerization rates for which k exceeds 10^7 liter mol^{-1} s^{-1}, a limit approached by the rate constants for the nicotinamides (Table 2). The agreement of the electrochemically determined k of 6.1×10^7 with the pulse radiolysis value (6.9×10^7) (8) for MCP^+ is consequently of special interest since it would indicate that the dimerization rate of the neutral free radical is not appreciably affected by the occurrence of the corresponding chemical reaction at the solution electrode interface in the electrical double layer region. However, the electrochemically determined rate constants for NAD^+ and $NADP^+$ are lower by an order of magnitude. [8]

The difference in rate constant magnitude for nicotinamide and the 1–substituted nicotinamides in nonaqueous media is in agreement with the charged and uncharged natures of the respective free radicals produced.

MO correlations

Half–wave potentials of aromatic hydrocarbons and heterocyclic compounds have been satisfactorily correlated with calculated molecular orbital energy levels, *e.g.*, for a 1–electron reduction,

$$U_{1/2} = \mathrm{EA} + \Delta E_{\mathrm{solv}} - \gamma \qquad (17)$$

where EA represents the electron affinity of the molecule (related to energy of lowest empty MO), ΔE_{solv} is the difference in solvation energy of the molecule before and after reduction, and γ is the corrrection term for reference electrode used. LEMO coefficients have been used in place of EA.

Table 4. Experimental half-wave reduction potentials and HÜCKEL molecular orbital data for pyridines and nicotinamides (*a*).

Molecule	$-U_{1/2}$ V	$-U^0$ (*c*) V	$-M_m+1$	$-\Delta(\Delta F_{solv})$ (*d*) eV	$-\Delta(\Delta F_{solv})$ (*e*) eV
Pyridine	2.78		0.841		
1–Methylpyridine	1.27		0.359	0.54	
2–Methylpyridine	2.80		0.837	—0.028	
4–Methylpyridine	2.86		0.832	—0.098	
3–Nicotinamide	2.00	2.16	0.762	0.622	0.46
1–protonated–3–Nicotinamide	1.06	1.18	0.353	0.744	0.62
1–Methyl–3–nicotinamide (MCP^+)	1.04	1.16	0.353	0.764	0.64
NMN^+	0.99	1.07	0.353	0.814	0.73
NAD^+	0.98	1.10	0.355	0.824	0.704
$NADP^+$	1.06	1.16	0.355	0.744	0.644

(*a*) MO calculations were done on an IBM 360 computer with standard coulomb and resonance integral. Because of the positive charge on nitrogen in 1–substituted compounds, a small electronegativity was attributed to the *ortho* carbon atoms and their coulomb integrals have been modified to $\alpha_{C\ ortho} = \alpha_C + 0.3\,\beta$.

(*b*) B. PULLMAN and A. PULLMAN, *Quantum Biochemistry*, Interscience, New York (1963), pp. 106–110.

(*c*) Calculated from the cyclic voltammetric peak potentials after correction for the following chemical reaction.

(*d*) The values are relative to pyridine, $\Delta(\Delta F_{solv}) = \Delta E_{1/2} - (\Delta m)\beta$, where $\Delta E_{1/2}$ is the difference in half-wave potentials between pyridine and the compound of interest in the same solvent, and Δm is the difference in the HMO coefficients for the lowest unoccupied level of the same two molecules. β is taken as equal to —2.0.

(*e*) Calculated from the U^0_c listed, using the approach of footnote *d*.

MO and related solvation energy differences have been calculated for the nicotinamide series and, for completeness, for related pyridines (table 4).

Introduction of an amide group in the 3 position of the pyridine ring reduces $U_{1/2}$ by 0.8 V due to increased contribution from π electrons of the amide group. Addition of a methyl group in the 1 position of pyridine or nicotinamide greatly facilitates reduction due to increased conjugation and the resulting electropositive character of the nitrogen.

Substitution by a methyl group elsewhere on the ring makes reduction slightly harder.

The calculated differences in solvation energies for the two types of processes,

$$R + e^- \rightleftarrows R^- \quad (18)$$

and

$$R^+ + e^- \rightleftarrows R\cdot \quad (19)$$

where R and R^+ represent parent molecule, and R^- and $R\cdot$ are negatively charged and neutral radicals, indicate that each type of process has a fairly characteristic value. The higher difference for the cationic species may result from the positively charged N (1) effectively ordering the solvent dipoles.

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