

Oxidation-Reduction of Nitroxyl Radicals : Cyclic Voltammetric Response in Aqueous Media

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Received 9 September 1981; revised and accepted 16 November 1981

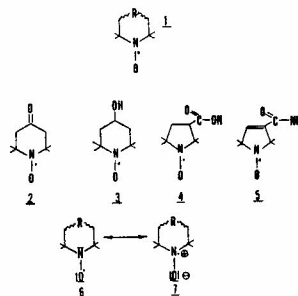
Oxidation-reduction processes of some stable nitroxyl radicals (2-5) of the type (1) having both pyrrolidine and piperidine rings with different substituents have been studied in aqueous solution over the pH range 4-10 using the cyclic voltammetric technique. They show one-electron oxidation and reduction processes of reversible, quasi-reversible and irreversible types depending upon R group as in 1, pH and scan rate conditions. The oxidation and the reduction potentials vary depending on the size of the ring and its substituents. E° for oxidation ranges from 0.55 to 0.72V $\pm$ 0.01V (vs SCE), whereas that for reduction ranges from 0.26 to 0.37V $\pm$ 0.01V (vs SCE). Unlike the oxidation reaction, the reduction process strongly depends on pH of the solution. For one of them, viz. 2, 2, 6, 6-tetramethyl-4-oxopiperidine-1-oxyl (2), an irreversible chemical reaction followed by its oxidation is observed at pH < 4.0 and the rates of this reaction at pH 3.7 and 4.0 are ~ 0.064 and 0.143 sec^{-1} respectively.

STABLE nitroxyl radicals of the type (1) are most often used¹⁻³ as the electron-spin probes for studying structural and conformational properties of biopolymers, viz. proteins, nucleic acids, lipids, etc. The reactions involving the unpaired electron of nitroxides have been described by several authors⁴⁻⁹. The one-electron reduction of a few nitroxyl radicals has been studied polarographically in aqueous solution by Neimann *et al.*⁸ whereas both oxidation and reduction of di-*t*-butyl nitroxide have been studied in acetonitrile at rotating platinum electrode (RPE) and dropping mercury electrode (DME) respectively by Hoffmann *et al.*⁹.

Herein we report a study of the oxidation-reduction processes of nitroxyl radicals (2-5) in aqueous solution over a wide pH range using cyclic voltammetry (CV)¹⁰. The four nitroxyl radical species (2-5) are abbreviated as Tempone, Tempol, Tempacid and Tempamide respectively throughout the text. Cyclic voltammetry of the oxidation of 2 and 3 have been studied in acetonitrile by Summermann *et al.*¹¹.

Materials and Methods

All the chemicals used were of AR grade. Tempol and Tempacid were obtained from Aldrich Chemical Company, USA and Eastman Kodak Company, USA, respectively. Sodium acetate, sodium dihydrogen orthophosphate, disodium hydrogen phosphate, boric acid, sodium chloride, etc. were from BDH, India. Tempone and Tempamide were synthesized following the reported procedures described elsewhere¹². Acetate, phosphate or borate buffers were prepared as described elsewhere¹³.



Physical measurements — Cyclic voltammograms were recorded using PARC Universal Programmer model 175 and PARC polarographic analyser model 174A of Princeton Applied Research Company, USA. Hanging mercury drop electrode (Metrohm model 9323) was used for the reduction studies, whereas a planar platinum electrode (polished surface area $\sim 0.18 \text{ sq. cm.}$) fabricated from a platinum rod (diameter, 5mm) tightly fitted to a teflon cylinder (outer diam, 12mm; inner diam, 5mm) was used to study the oxidation of nitroxyl radicals. Constant potential electrolysis was performed using PARC Potentiostat/ Galvanostat model 173 and a platinum gauze electrode. pH of solutions were recorded using a digital pH meter model DPH 500 (Global Electronics India Ltd). EPR spectra were recorded on a JEOL 3X100 EPR spectrometer, (JEOL Ltd, Japan).

Results and Discussion

Electron paramagnetic resonance (EPR) spectra of nitroxyl radicals of type(1) indicate that the isotropic coupling constant of the nitrogen atom in the nitroxide group increases by more than 10% in going from hydrophobic to hydrophilic solvents³. Thus, a polar solvent interacts with the lone pairs

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of electrons of the oxygen atom thereby increasing its electron affinity. As a result the three π -electron distribution of the $-\text{NO}$ groups is shifted towards the limiting structure (7). The nitroxyl radical species in aqueous solution available for oxidation or reduction will predominantly have the structure (7).

(a) *Oxidation of nitroxyl radicals* — Let us consider first the oxidation reaction of Tempol, which has been studied at a planar platinum electrode using CV over a wide range of $p\text{H}$ and potential scan rates (v). Some selected data are displayed in Table 1 and Fig. 1. E_{pa} and E_{pc} represent the anodic and cathodic peak potentials whereas ΔE_p and $\bar{E}_p$ stand for the peak potential difference ($E_{pa} - E_{pc}$) and average of the peak potentials [$1/2(E_{pa} + E_{pc})$] respectively. The data in Table 1 indicate that the one-electron (vide infra) oxidation reaction of Tempol is quite reversible at $p\text{H} \sim 4.0$ up to a scan rate of 50 mV sec^{-1} . But it changes to a quasi-irreversible type¹⁰ at $p\text{H} > 5.0$ until it reaches $p\text{H} \sim 9.0$. At and above $p\text{H} 9.0$, the electron transfer process becomes irreversible. The one-electron oxidation reaction of Tempol can be expressed by Eq. (1), the E'_0 ($\approx \bar{E}_p$) of which is $0.57 \pm 0.01 \text{ V}$ (Table 1) (versus SCE).

The constant potential electrolysis of the solution of Tempol in the $p\text{H}$ range 4–8 at a platinum electrode at a potential greater than E_{pa} viz. 0.80 V (vs. SCE) leads to the loss in intensity for EPR signal. The amount of electricity used in this process corresponds to that required for a one-electron process. The oxidised solution when electrolysed further at a potential less than E_{pc} , i.e. 0.00 V (vs SCE) regains the intensity of triplet EPR line corresponding to that of a nitroxyl radical species. The oxidation of a nitroxyl radical to the corresponding nitrosonium ion is favoured by the fact that the removal of the unpaired electron leads to considerable strengthening of the nitrogen-oxygen bond¹⁴.

The general behaviour of the oxidation of the other nitroxyl radicals is similar to that of Tempol (see

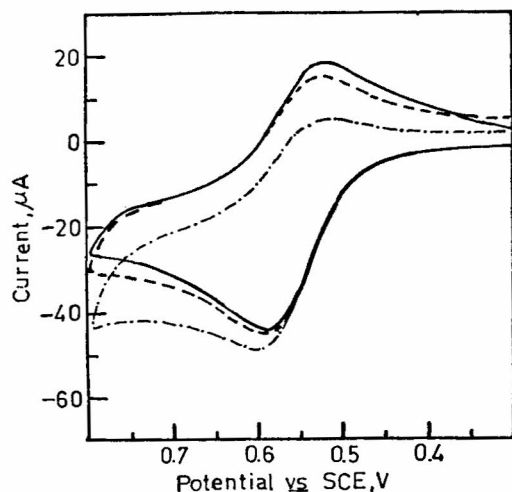


Fig. 1 — Cyclic voltammograms on oxidation of Tempol (conc. $\sim 2 \text{ mM}$) at platinum electrode at $p\text{H}$ 6.22 (—), 7.22 (---) and 8.07 (— · —).

Table 2). The case of Tempone at $p\text{H} > 3.5$ is the case of a one-electron transfer process followed by an irreversible chemical reaction¹⁰ (reaction 2.) Here, the cathodic peak could be located at $p\text{H} \sim 4.0$ only at a higher scan rate (Fig. 2). Further, the subsequent reduction of the oxidised solution of Tempone at $p\text{H} > 4.0$ at 0.00 V (vs SCE) does not generate any free radical. The rate constant (K_f) of the chemical reaction (2) could be measured (Table 3) at $p\text{H}$ 4.0 and 3.7 from the cyclic voltammograms of Tempone at different scan rates using the method of Nicholson and Shain^{10,15}.

An attempt has been made to identify the decomposed products. The UV spectrum of the electroly-

TABLE 1 — CYCLIC VOLTAMMETRIC DATA^a ON OXIDATION OF TEMPOL AT DIFFERENT $p\text{H}$ VALUES

Scan rate (V sec^{-1})	E_{pa} , (V)	E_{pc} , (V)	ΔE_p , (V)	$\bar{E}_p$, (V)
$p\text{H} = 3.94^b$				
0.01	0.595	0.530	0.065	0.56
0.02	0.590	0.530	0.060	0.56
0.05	0.595	0.530	0.065	0.56
0.10	0.610	0.520	0.090	0.57
$p\text{H} = 4.99^b$				
0.01	0.600	0.530	0.070	0.57
0.02	0.600	0.525	0.075	0.57
0.05	0.610	0.520	0.090	0.57
0.10	0.620	0.515	0.105	0.57
$p\text{H} = 6.22^c$				
0.01	0.590	0.520	0.070	0.56
0.02	0.590	0.520	0.070	0.56
0.05	0.600	0.515	0.085	0.56
0.10	0.610	0.510	0.100	0.56
$p\text{H} = 7.22^c$				
0.01	0.590	0.520	0.070	0.56
0.02	0.595	0.520	0.075	0.56
0.05	0.600	0.515	0.085	0.56
0.10	0.610	0.510	0.100	0.56
$p\text{H} = 8.07^e$				
0.01	0.600	0.515	0.085	0.56
0.02	0.600	0.520	0.080	0.56
0.05	0.610	0.520	0.090	0.57
0.10	0.620	0.515	0.105	0.57
$p\text{H} = 7.75^d$				
0.01	0.615	0.535	0.080	0.58
0.02	0.620	0.530	0.090	0.58
0.05	0.630	0.520	0.110	0.58
0.10	0.650	0.520	0.130	0.59
$p\text{H} = 9.09^d$				
0.01	$\sim 0.600^e$	— ^f	—	—
$p\text{H} = 10.3^d$				
0.01	$\sim 0.600^e$	— ^f	—	—

(a) Potentials recorded here are at platinum electrode vs SCE at room temperature (26°C); concentrations of compounds were $\sim 2 \times 10^{-3} \text{ M}$.

(b) Acetate buffer (1M) with 0.1M NaCl

(c) Phosphate buffer (1M) with 0.1M NaCl

(d) Borate buffer (1 M) with 0.1M NaCl.

(e) Broad, peak, difficult to locate precisely.

(f) Peak not observed.

TABLE 2 — CYCLIC VOLTAMMETRIC DATA* ON OXIDATION OF TEMPONE, TEMPACID AND TEMPAMIDE AT SOME SELECTED pH VALUES

pH	E_{pa} , (V)	E_{pc} , (V)	ΔE_p , (V)	E'_{ox} , (V)
TEMPONE				
4.04 ^b	0.685	— ^e	—	—
7.00 ^c	0.620	— ^e	—	—
7.78 ^d	0.670	— ^e	—	—
8.94 ^d	~0.780 ^f	— ^e	—	—
TEMPACID				
4.10 ^b	0.580	0.510	0.070	0.55
7.05 ^c	0.590	0.480	0.110	0.54
8.02 ^c	0.580	0.490	0.090	0.54
8.99 ^d	0.630	0.470	0.160	0.55
9.94 ^d	~0.650 ^f	0.460	~0.190	~0.56
TEMPAMIDE				
5.00 ^b	0.760	0.695	0.065	0.73
7.00 ^c	0.755	0.690	0.065	0.72
7.91 ^d	0.765	0.665	0.100	0.72
8.94 ^d	~0.800 ^f	0.650	~0.150	~0.73

(a) Potentials recorded here are at platinum electrode vs SCE at room temperature (26°C); concentration $\sim 2 \times 10^{-3} M$ and scan rate at 0.01 V sec⁻¹.

(b) Acetate buffer (1M) with 0.1M NaCl.

(c) Phosphate buffer (1M) with 0.1M NaCl.

(d) Borate buffer (1M) with 0.1M NaCl.

(e) Peak not observed.

(f) Very broad peaks, difficult to locate precisely.

 TABLE 3 — DATA^{a,b} ON THE RATE CONSTANT OF AN IRREVERSIBLE REACTION FOLLOWING THE OXIDATION OF TEMPONE

pH	Scan rate (V sec ⁻¹)	τ , S	i_p^c/i_p^a	k_{tr}	k_t (sec ⁻¹)
pH = 3.70					
3.70	0.02	12.65	0.574	0.71	0.056
	0.05	4.90	0.732	0.35	0.071
4.04	0.05	4.90	0.576	0.71	0.145
	0.10	2.40	0.734	0.34	0.142

(a) τ is the time in seconds taken to sweep from $\bar{E}_p$ to the switching potential.

(b) i_p^c/i_p^a is the ratio of the cathodic peak current and the anodic peak current, which is measured from the experimental curves using the method described in ref. 9, p. 542.

sate of Tempone after oxidation at a potential greater than its E_{pa} shows the absence of intense band ($\pi \rightarrow \pi^*$) at 235 nm due to $>N-O$ moiety¹⁶ and the appearance of an equally intense band at 263 nm. This is possibly due to the removal of an electron from the bonding NO π -orbital and the formation of a $-N=O$ moiety after the leavage of the piperidyl ring of the corresponding nitrosonium ion. It was not possible at this stage to purify the decomposed product for further analysis.

It has been postulated¹² that the nitrosonium salts are unstable in aqueous solution because of the reaction(3). However, such a reaction is not observed in the cyclic voltammetric time scale (Tables 1 and 2 and Fig. 1). Thus this reaction, if at all it occurs should be certainly very slow (< 0.02 sec⁻¹) compared to the electron transfer rate. Interestingly the solu-

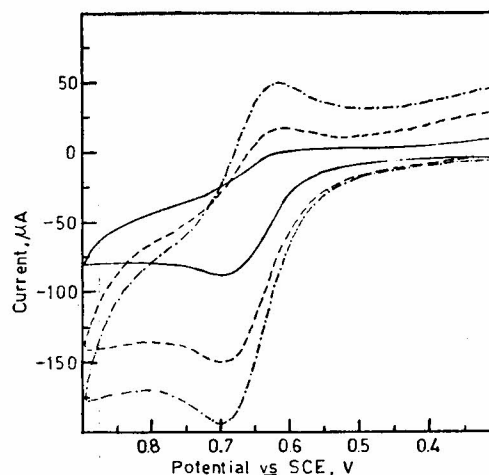
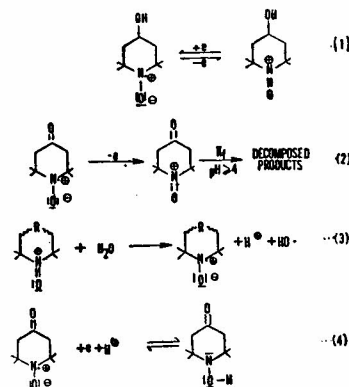


Fig. 2 — Cyclic voltammograms on oxidation of Tempone (conc. $\sim 2mM$) at platinum electrode at pH 4.04 with scan rates; 0.02 (—), 0.05 (---) and 0.1 V sec⁻¹ (— · —)



tions of Tempacid or Tempamide after oxidation $\sim 1.0V$ (vs SCE), if stored for a few hours at room temperature, regains the EPR signal intensity which has been lost during the oxidation process. This process of oxidation and self-reduction could be repeated several times only in the case of these two compounds without appreciable decomposition of them.

Reduction of nitroxyl radicals — The reduction of the nitroxyl radicals (2-5) have been studied at a hanging mercury drop electrode (HMDE). Unlike the results of Neimann *et al.*[†] the cyclic voltammetric data at HMDE displayed in Table 4 evidently show that below pH 7.0, the one-electron reduction of Tempone hardly depends on pH. The reoxidation peak lies above 0.0V (vs SCE) and is not identifiable due to mercury oxidation. No cyclic voltammetric peaks are observed when the reduction is carried out at platinum electrode instead of mercury. The non-dependence on pH is not clearly

[†]According to Neimann *et al.*¹⁸, the $E_{1/2}$ (vs SCE) at DME for Tempone is constant between pH 6 and 8 at a value of $\sim -150mV$ in citrate-phosphate-borate buffer. Outside this pH region, there is a strong dependence of $E_{1/2}$ on pH, i.e. $\Delta E_{1/2}/\Delta pH \sim 60$ mV.

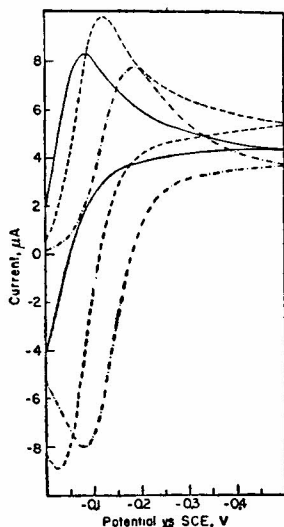


Fig. 3 — Cyclic voltammograms on reduction of Tempone at HMDE at pH 6.06 (—; conc. 3.5mM), 7.07 (---; conc. 4.1mM and 8.05(— · — · —; conc. 3.4mM)

understood since the desired product is expected to be a hydroxyl amine (reaction 4) as described by Neimann *et al.*⁸. This can not be due to any electrode reaction with the compound since no EPR signal is lost by keeping the Tempone solution over mercury. Similar behaviour is shown by other nitroxyl radicals (vide infra).

However, at $pH \geq 7.0$ the reduction strongly depends on pH and both the peaks are visible. The reduction potentials also exhibit some dependence on the anions present in the buffer. The data at $pH \geq 7.0$ show that the reduction of Tempone is quasi-reversible and coupled one-electron-one-protontransfer process¹⁶ since $\Delta E_p / \Delta pH$ is close to 60 mV (Table 4 and Fig. 3). Thus the reduction of Tempone can be expressed by Eq. (4), the reduction potential (E'_0) of which has been calculated¹⁷ to be $0.34 \pm 0.01V$ (vs SCE) (Table 4) using the expression $E'_0 = E_p + 0.059 (m/n) pH$ (at $25^\circ C$) where m and n stand for the number of protons and electrons involved respectively. The reduction of the other three nitroxyl radicals (Table 5) is very similar to that of Tempone except the fact that the reduction of Tempacid is irreversible even at higher pH . Constant potential electrolysis of these nitroxyl radicals at a potential (say at $-0.5V$, vs SCE) less than their respective E_{pc} values, show the loss of EPR signal.

Present study of nitroxyl radicals (2-5) using the cyclic voltammetric technique conclusively illustrates that their oxidation or reduction potential in aqueous solution has definite dependence on the R group as in 1, even though it has no conjugation with the NO group. It is interesting to note that the oxidation of a nitroxyl radical with R having an amide group substituent in a five membered ring (as in 5) is difficult (E'_0 is 170 mV higher) compared to a situation with 'R' having a carboxyl group substituent (as in 4) (vide Table 2). While the oxidation process of a

TABLE 4 — CYCLIC VOLTAMMETRIC DATA^a ON REDUCTION OF TEMPONE AT DIFFERENT pH VALUES

pH	E_{pa} (V)	E_{pc} (V)	ΔE_p (V)	$\bar{E}_p$ (V)	E'_0 (V)
4.10 ^b	— ^e	(—)0.095	—	—	—
5.05 ^b	— ^e	(—)0.110	—	—	—
6.06 ^c	— ^e	(—)0.085	—	—	—
7.08 ^c	(—)0.025	(—)0.120	0.095	(—)0.073	0.34
8.05 ^c	(—)0.080	(—)0.185	0.105	(—)0.133	0.34
7.96 ^d	(—)0.025	(—)0.220	0.195	(—)0.123	0.35
8.96 ^d	(—)0.085	(—)0.270	0.185	(—)0.178	0.35
9.86 ^d	(—)0.150	(—)0.330	0.180	(—)0.240	0.34

(a) Potentials recorded here are at HMDE vs SCE at room temperature ($26^\circ C$); concentration $\sim 4 \times 10^{-3}M$; scan rate at $0.1 V sec^{-1}$.

(b) Acetate buffer (1M) with 0.1M NaCl.

(c) Phosphate buffer (1M) with 0.1M NaCl.

(d) Borate buffer (1M) with 0.1M NaCl.

(e) Peak not observed.

TABLE 5 — CYCLIC VOLTAMMETRIC DATA^a ON REDUCTION OF TEMPOL, TEMPAMIDE AND TEMPACID AT SELECTED pH VALUES

pH	E_{pa} (V)	E_{pc} (V)	ΔE_p (V)	$\bar{E}_p$ (V)	E'_0 (V)
TEMPOL					
4.99 ^b	— ^e	(—)0.080	—	—	—
7.22 ^c	— ^e	(—)0.100	—	—	—
9.09 ^d	(—)0.120	(—)0.220	0.100	(—)0.170	0.37
10.30 ^d	(—)0.195	(—)0.275	0.080	(—)0.235	0.37
TEMPAMIDE					
5.01 ^b	— ^e	(—)0.190	—	—	—
7.09 ^c	(—)0.100	(—)0.225	0.125	(—)0.163	0.26
7.96 ^d	(—)0.120	(—)0.290	0.170	(—)0.205	0.26
9.87 ^d	(—)0.270	(—)0.385	0.115	(—)0.330	0.25
TEMPACID					
5.04 ^b	— ^e	(—)0.270	—	—	—
7.05 ^c	— ^e	(—)0.360	—	—	—
8.01 ^d	— ^e	(—)0.490 ^f	—	—	—

(a) Potentials recorded here are at HMDE vs SCE at room temperature ($26^\circ C$); concentration $\sim 4 \times 10^{-3}M$; scan rate at $0.1 V sec^{-1}$.

(b) Acetate buffer (1M) with 0.1M NaCl.

(c) Phosphate buffer (1M) with 0.1M NaCl.

(d) Borate buffer (1M) with 0.1M NaCl.

(e) Peak not observed.

(f) This peak is observed only at scan rate of $0.1 V sec^{-1}$.

nitroxyl radical remains invariant with pH , its reduction has strong dependence in the pH of its solution especially at and above $pH 7.0$ (Tables 4 and 5). It is thus clear to visualize that the higher the pH , the more stable the nitroxyl radical (E_{pc} is more negative) towards its reduction by any external agents. This observation might be useful for nitroxyl radicals present in a biological system as in a spin labelling study.

Acknowledgement

The authors gratefully acknowledge the University Grants Commission and Department of Science and Technology, New Delhi, for providing fellowship for one of them (G.T.) to pursue this study.

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