Reactions of Pyridin-3-ol with Pulse-radiolytically Generated Reducing Species

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Rate constants for reactions of hydrated electron, hydrogen atom, and other reducing radicals such as $(CH_3)_2COH$ and CO_2^- with pyridin-3-ol at different values of pH have been determined using the pulse-radiolysis technique. The absorption spectra of the transient product species and kinetic parameters pertaining to them have been investigated. Of the above reducing species all except e^-_{aq} lead to transient species other than the one-electron reduction product of pyridin-3-ol. $(CH_3)_2\dot{C}OH$ and CO_2^- radicals are found to add on to the pyridin-3-ol ring. Pyridin-3-ol radicals formed by reaction with e^-_{aq} are found to be highly reducing in nature and transfer electrons to thionine, methylene blue, safranine, anthraquinone 2-sulphonate, paraquat, and benzophenone with almost diffusion-controlled rate constants.

Radiation chemical studies on vitamins are of importance in connection with the radiation sterilization of medical products.[1] There are reports on the pulse-radiolysis studies of pyridoxine (vitamin B_6) and other B group vitamins.[2] Pyridoxine is derived from 3-hydroxy pyridine (pyridin-3-ol) by substitution of $-CH_2OH$ groups in the 4 and 5 positions and $-CH_3$ group in the 2 position. Pyridin-3-ol and its derivatives are found to have antihypoxic and antiamnestic activity in mice.[3] In the literature no detailed pulse-radiolysis study of pyridin-3-ol is reported. It is also of interest to know how the reactivity of primary species of water radiolysis varies with substitution in the pyridine ring and hence, we have undertaken a pulse radiolysis study of pyridin-3-ol in aqueous solution. In the present paper reactions of pyridin-3-ol with the reducing species produced by pulse radiolysis of aqueous solutions are reported.

Experimental

Details of the pulse radiolysis experimental set-up used have been given elsewhere.[4] Single pulses of 7 MeV electrons of 25 ns and 2 μs duration were made use of, depending on the nature of information sought. For 25 ns pulses typical doses were of the order of 25 Gy per pulse as measured by KCNS dosimeter ($G\varepsilon$ 21 520 $dm^3\ mol^{-1}\ cm^{-1}$ per 100 eV at 500 nm)[5] and for 2 μs pulses they were 4–5 times higher.

Pyridin-3-ol (Fluka, purum grade) was purified by recrystallization from ethanol–water mixture. All the other chemicals used were of the highest purity available. The solutions were prepared in water having conductivity of less than 0.1 μS, which was obtained by passing distilled water through Barnstead Nanopure™ cartridge filtration systems to remove all ionic and organic impurities. The solutions were purged with Iolar grade (Indian Oxygen Ltd) gases using appropriate pretraps as and when necessary. Solutions were changed after each pulse. The pH of the solutions were adjusted using H_2SO_4, KH_2PO_4, $Na_2HPO_4{\cdot}2H_2O$, $Na_2B_4O_7{\cdot}10H_2O$, and NaOH in appropriate concentrations. For studying the reactions of e^-_{aq} and H atom, t-butyl alcohol was used as the OH radical scavenger. A matrix containing isopropyl alcohol (1 mol dm^{-3}) and acetone (0.2 mol dm^{-3}) was used at all the pH values to study the reactions of isopropyl alcohol radicals which were generated as follows.

$$(CH_3)_2CHOH + OH(H) \longrightarrow (CH_3)_2COH + H_2O(H_2) \quad (1)$$

$$(CH_3)_2CO + e^-_{aq} \xrightarrow{H^+} (CH_3)_2COH \quad (2)$$

Sodium formate was added to the N_2O saturated solutions to generate CO_2^- radicals according to equations (3) and (4)

$$e^-_{aq} + N_2O \longrightarrow OH + OH^- + N_2 \quad (3)$$

$$HCOO^- + OH(H) \longrightarrow CO_2^- + H_2O(H_2) \quad (4)$$

and in acid solutions also by the process, (5).

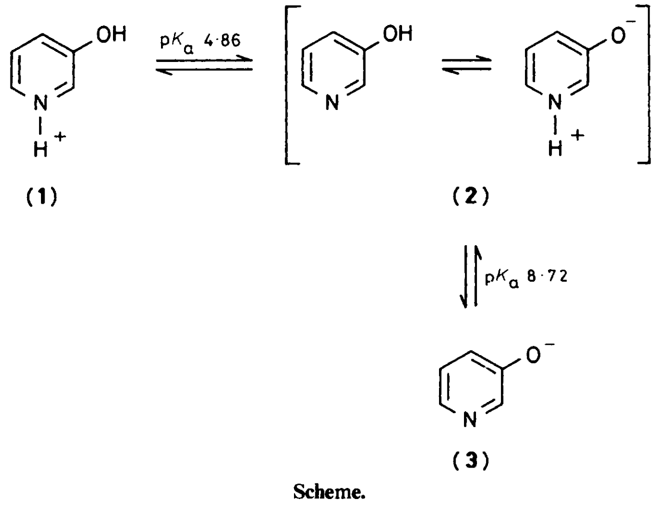
$$HCO_2H + OH(H) \longrightarrow CO_2H + H_2O(H_2) \quad (5)$$

In neutral solutions H atoms were generated by reaction of e^-_{aq} with 1 mol dm^{-3} KH_2PO_4 (ref. 6).

$$H_2PO_4^- + e^-_{aq} \longrightarrow H + HPO_4^{-2} \quad (6)$$

Results

Reactions of e^-_{aq}.—Pyridin-3-ol has pK_a values at 4.86 and 8.72 (ref. 7). The different conjugate acid base forms are shown in the Scheme. Absorption spectral parameters of these forms are: λ_{max}/nm ($\varepsilon_{max}/dm^3\ mol^{-1}\ cm^{-1}$): (**1**) 283 (5 840), 222 (3 730); (**2**). 315 (3 060), 278 (2 320), 246 (5 120); (**3**). 298 (4 960),



Scheme.

Table 1. Spectral and kinetic parameters of the reactions of pyridin-3-ol with various reducing species.

pH	Reducing species	Reaction rate constant/dm^3 mol^{-1} s^{-1}	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}	$(2k/\varepsilon l)$ s^{-1}
6.8	e^-_{aq}	1.4×10^{10}	400–410	1 040	3.5×10^6
10.5	e^-_{aq}	1.5×10^9	390–400	1 245	2.6×10^6
6.8	$(CH_3)_2COH$	1.1×10^7	400	3 895	7.6×10^4
2	$(CH_3)_2COH$	3.8×10^8	400–405	3 570	9.6×10^4
2	H	1.8×10^9	390–400	1 520	6.5×10^5
5.8	H	3.2×10^9	410	2 165	9.3×10^5
6.8	CO_2^-	7.8×10^7	400	—	6.6×10^2
2.6	CO_2^-	4.8×10^8	405		$3.5 \times 10^{5\,a}$

[a] First order.

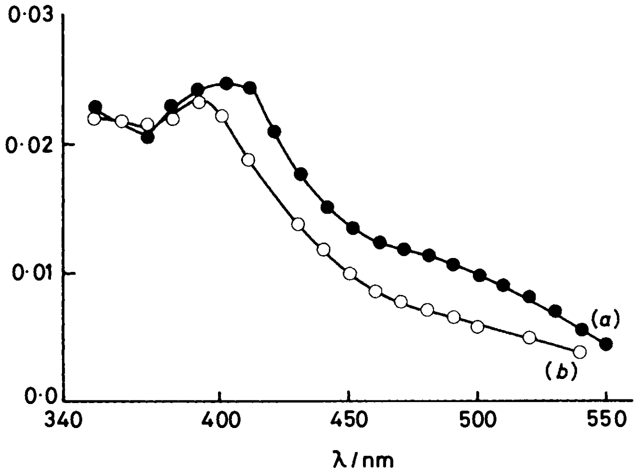


Figure 1. Absorption spectra of the transient species produced by reaction of e^-_{aq} with pyridin-3-ol at (*a*) pH 6.8 (●) and (*b*) pH 10.5 (○).

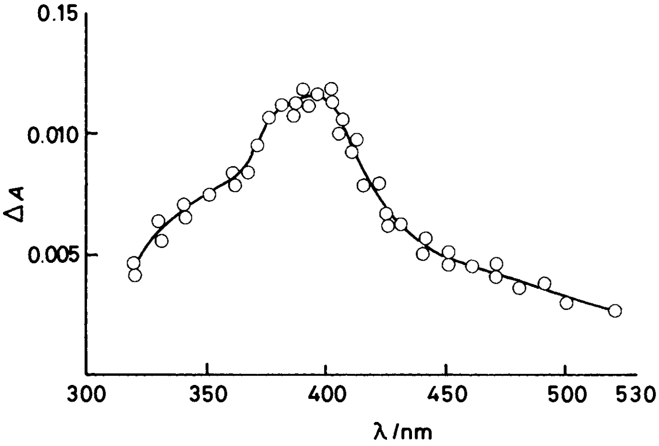


Figure 2. Absorption spectrum of the transient species formed in e^- beam pulsed deoxygenated solutions containing 2×10^{-4} mol dm^{-3} pyridin-3-ol, 0.02 mol dm^{-3} H_2SO_4 (pH 1.7) and 0.1 mol dm^{-3} t-butyl alcohol.

236 (11 000). The reaction of form (**2**) with hydrated electron was found to be very fast at pH 6.8. The rate constant as determined by following the pseudo-first-order decay of the hydrated electron absorption at 720 nm was found to be 1.4×10^{10} dm^3 mol^{-1} s^{-1}. The reaction product was found to absorb in the 340–600 nm region [Figure 1(*a*)]. However, the reaction with the deprotonated form (**3**) present at pH 10.5 was an order of magnitude slower, $k = 1.5 \times 10^9$ dm^3 mol^{-1} s^{-1}. The absorption spectrum of the reaction product at pH 10.5 is more or less similar to the one at pH 6.8 [Figure 1(*b*)] with a shift in the λ_{max} of about 10–15 nm. At both pH values the product species decayed by good second-order kinetics. Their spectral and kinetic parameters are given in Table 1. To ascertain whether the species formed by reaction of e^- with pyridin-3-ol reacts with t-butyl alcohol radicals, formed in OH scavenging (also reported in the case of other pyridine radicals[8] and heterocyclic compounds[9]), experiments were carried out in the presence of glucose as an OH scavenger. In the presence of t-butyl alcohol as an OH scavenger the transient absorption signals at 400 nm decays to a definite small final level because of the formation of a product species, but in the presence of glucose it decays to zero level in a lower time scale. In the presence of glucose the decay of the transient was clearly second order with almost the same $2k/\varepsilon l$ value as found in the presence of t-butyl alcohol. From this it would appear that t-butyl alcohol radicals react to lesser extent with the pyridin-3-ol radicals in competition with disproportionation of the latter. In any case initial transient absorption is due to the e^-_{aq} reaction product only, as it is formed in the time scale in which e^-_{aq} decays completely at 720 nm and hence the extinction coefficient corresponds to that of the one-electron reduced species of pyridin-3-ol.

Reaction with Hydrogen Atoms.—H-atom was found to react with the protonated form of pyridin-3-ol (**1**) (pH 2) with a rate constant of 1.8×10^8 dm^3 mol^{-1} s^{-1} as determined by following the pseudo-first-order formation of the product transient species. The absorption spectrum of the latter (Figure 2) exhibited λ_{max} at 395 nm. It was found to decay by good second-order kinetics with $2k = 9.7 \times 10^8$ dm^3 mol^{-1} s^{-1}. Taking G_H 3.3 and assuming that all the H atoms react to give only one transient species, its extinction coefficient at 400 nm has been evaluated to be 1 935 dm^3 mol^{-1} cm^{-1}.

Recently it has been reported[6] that reaction of e^-_{aq} with $H_2PO_4^-$ can be used as a source of H atoms for pulse-radiolysis studies in neutral solutions. Accordingly, H atom reactions with pyridin-3-ol at neutral pH were studied in solutions containing 1 mol dm^{-3} KH_2PO_4 and 0.25 mol dm^{-3} Na_2HPO_4 (pH 5.8). Under these conditions H atoms were found to react with pyridin-3-ol (**2**) with a rate constant of 3.2×10^9 dm^3 mol^{-1} s^{-1}. The reaction product is found to absorb in the 330–600 nm region (Figure 3) with λ_{max} at 410 nm. It was found to decay following good second-order kinetics with $2k/\varepsilon l$ value of 9.3×10^5 s^{-1} at 410 nm.

Reactions with Isopropyl Alcohol Ketyl Radicals.—Isopropyl alcohol radical [$(CH_3)_2COH$] was found to react rather slowly with the neutral form of pyridin-3-ol (**2**) at pH 6.8 (k = 1.1×10^7 dm^3 mol^{-1} s^{-1}). The absorption spectrum of the

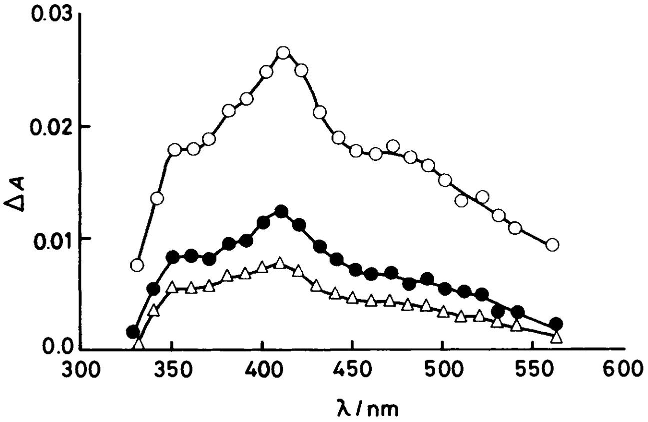


Figure 3. Time-resolved absorption spectrum of the transient species formed in e^- beam pulsed deoxygenated solutions containing 5×10^{-5} mol dm^{-3} pyridin-3-ol, 1 mol dm^{-3} KH_2PO_4, 0.25 mol dm^{-3} Na_2HPO_4 and 0.1 mol dm^{-3} t-butyl alcohol (pH 5.8) at 3 μs (○), 53 μs (●) and 103 μs (△) after the electron pulse.

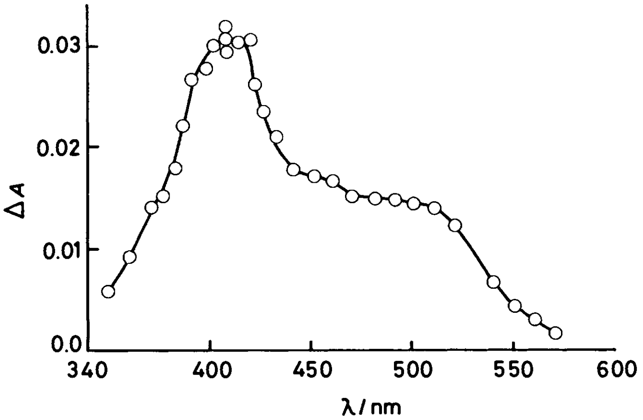


Figure 4. Absorption spectrum of the transient species formed by reaction of $(CH_3)_2COH$ radicals with pyridin-3-ol in solutions containing 2×10^{-3} mol dm^{-3} pyridin-3-ol, 1 mol dm^{-3} isopropyl alcohol and 0.2 mol dm^{-3} acetone at pH 6.8.

product transient species exhibited λ_{max} at 400 nm with a shoulder in the 450–500 nm region (Figure 4). The entire spectrum is due to a single species as inferred from the identical formation and decay characteristics at different wavelengths. However, this spectrum is different from that of e^-_{aq} adduct at the same pH and also the $2k/\varepsilon l$) value at 400 nm is different in the two cases. This would suggest that isopropyl alcohol radical does not bring about one electron reduction. It has been reported that isopropyl alcohol radical can undergo an addition reaction and also abstract a hydrogen atom.[10] In the present case it is likely that it adds onto the pyridine ring since the product absorption spectrum (Figure 4) is more or less similar to the one obtained in the case of reaction of OH radicals at this pH.[11] The decay rate of the isopropyl alcohol radical adduct was slower than that of OH reaction product; this may be due to steric hindrance by the bulkier isopropyl alcohol moiety. At pH 2, however, the reaction of isopropyl alcohol radical with pyridin-3-ol (**1**) is faster ($k = 3.8 \times 10^8$ dm^3 mol^{-1} s^{-1}) but the absorption spectrum is similar to the one obtained at pH 6.8.

Reaction with CO_2^- *Radicals*.—At pH 6.8, CO_2^- radical was found to react with the neutral form of pyridin-3-ol (**2**) rather slowly. Direct determination of the rate constant for this reaction by following product formation kinetics or the decay of CO_2^- absorption was not possible because of the overlapping CO_2^- absorptions and the product transient species. For this reason the rate constant for this reaction was determined by competition kinetics using methylene blue and was found to be 7.8×10^7 dm^3 mol^{-1} s^{-1}. (Taking the rate constant for CO_2^- + methylene blue to be 8.5×10^9 dm^3 mol^{-1} s^{-1} which was determined by following the pseudo-first-order formation of semimethylene blue absorbance at 395 nm.) The spectrum of the product transient species is given in Figure 5. The spectrum at 3 μs after the pulse is due to tail absorption of CO_2^-. It is seen that the spectrum observed after 100 μs resembles that obtained in the case of the adduct species formed by $(CH_3)_2COH$ radical reaction. The product decays very slowly by first-order kinetics with a rate constant of 6.6×10^2 s^{-1}. Good first-order decay without any bimolecular component suggests that it may be decaying by rearrangement, although an absorption spectrum attributable to rearranged species could not be observed due to poor signal to-noise-ratio in the spectral region of interest. It is clearly seen that the spectrum (Figure 1) of species formed by reaction of e^-_{aq} with pyridin-3-ol is different from those of species formed by reaction with other reducing radicals (Figures 4 and 5). This shows that $(CH_3)_2COH$ and CO_2^- do not bring about one-electron reduction of pyridin-3-ol.

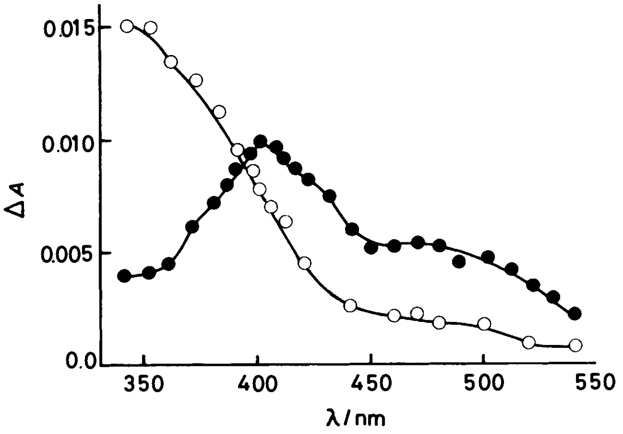


Figure 5. Absorption spectrum of the transient species formed by reaction of CO_2^- with pyridin-3-ol in N_2O-saturated solutions containing 5×10^{-4} mol dm^{-3} pyridin-3-ol and 0.1 mol dm^{-3} HCO_2Na (pH 6.8) at 3 μs (○) and 100 μs (●) after the electron beam pulse.

At pH 2.6 the CO_2^- radical formed in the system containing HCO_2H (1 mol dm^{-3}) and HCO_2Na (0.18 mol dm^{-3}) reacts with the protonated form of pyridin-3-ol (**1**) with a rate constant of 4.8×10^8 dm^3 mol^{-1} s^{-1} as determined directly by monitoring of the growth of product absorption at 405 and 425 nm. The time resolved absorption spectrum of the product species is given in Figure 6. It can be seen that the initial spectrum agrees very well with that obtained in the case of the $(CH_3)_2COH$ radical reaction at this pH, thus showing that CO_2^- radical also adds to the molecule at this pH. The product species decays by good second-order kinetics ($2k/\varepsilon l = 3.5 \times 10^5$ s^{-1}).

Reducing Properties of e^-_{aq} *Reaction Product with Pyridin-*3-*ol at pH* 6.8.—At pH 6.8 the reaction product formed by e^-_{aq} reaction with pyridin-3-ol (**2**) was found to be reducing in nature. It was observed that it transfers electrons to thionine (E_0 −0.04 V),[12] safranine (E_0 −0.185 V),[13] anthraquinone 2-sulphonate (E_0 0.385 V),[14] paraquat (E_0 −0.450 V),[14] and benzophenone with almost diffusion-controlled rate constants (Table 2). Confirmation of electron transfer was done by comparing the absorption spectra obtained by this process with that obtained directly by reaction of the above acceptor

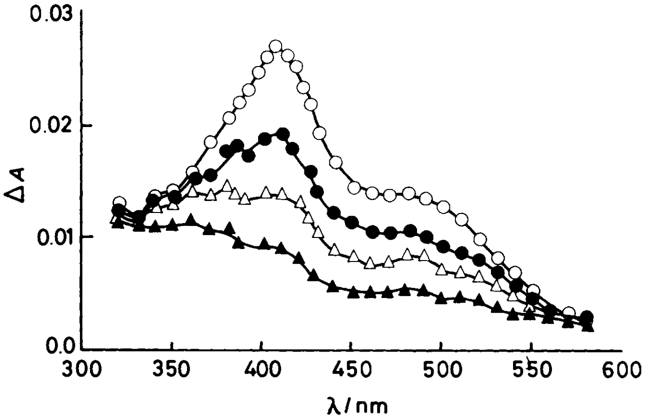


Figure 6. Time-resolved absorption spectrum of the transient species formed in N_2O-saturated solutions containing 1 × 10^{-4} mol dm^{-3} pyridin-3-ol, 1 mol dm^{-3} HCO_2H and 0.18 mol dm^{-3} HCO_2Na at 30 μs (○), 80 μs (●), 130 μs (△), and 230 μs (▲) after the electron beam pulse.

Table 2. Rate constants for electron transfer from e^-_{aq} reaction product of pyridin-3-ol at pH 6.8 to various electron acceptors.

Electron acceptor	Rate constant/dm^3 mol^{-1} s^{-1}
Thionine	4.9 × 10^9
Methylene blue	5.1 × 10^9
Safranine	3.1 × 10^9
Anthraquinone sulphonate	5.0 × 10^9
Paraquat	4.3 × 10^9
Benzophenone	4.9 × 10^9

molecules with e^-_{aq} under identical conditions. The above rate constants were measured by following the product absorption build up. No equilibrium could be established with benzophenone also, which shows that the one-electron reduction potential of pyridin-3-ol at pH 6.8 is much more negative than − 1.0 V. This may be the reason why $(CH_3)_2COH$ (E_0 − 1.05 V) and CO_2^- (E_0 − 2.0 V) radicals add on to the molecule rather than bring about a one-electron reduction.

Discussion and Conclusions

It is interesting to compare the reactivities of pyridin-3-ol, pyridine, phenol, benzene, and pyridoxine with e^-_{aq}. At pH 6.8 where all these molecules are in their neutral forms, the presence of OH group on the benzene ring (giving phenol) increases the e^-_{aq} reaction rate constant from 7 × 10^6 dm^3 mol^{-1} s^{-1} (ref. 15) to 1.8 × 10^7 dm^3 mol^{-1} s^{-1} (ref. 16). On the other hand, the presence of the nitrogen heteroatom within the molecule (as in pyridine) increases the rate constant to a value of 1 × 10^9 dm^3 mol^{-1} s^{-1} (ref. 15). From our experiments it can be seen that the presence of OH group on the pyridine ring further increases reactivity with the e^-_{aq} the rate constant being as high as 1.4 × 10^{10} dm^3 mol^{-1} s^{-1}. This rate constant value is comparable to that for pyridoxine [2.2 × 10^{10} dm^3 mol^{-1} s^{-1} (ref. 2)] where additional substituents are present on the pyridine ring. It is reported that isopropyl alcohol radical does not react with pyridoxine, whereas it does react with pyridin-3-ol with a rate constant of 1.1 × 10^7 dm^3 mol^{-1} s^{-1} and adds on to the molecule. CO_2^- also reacts with pyridin-3-ol at this pH rather slowly (k = 7.8 × 10^7 dm^3 mol^{-1} s^{-1}) but does not bring about one-electron reduction. The reason for this type of behaviour may be that in the pyridoxine molecule apart from OH group in the 3 position of the pyridine ring there are $-CH_2OH$ groups in the 4 and 5 positions and $-CH_3$ in the 2 position which exert steric hindrance to the addition reaction by these radicals. For pyridine it is reported that OH radicals add to the least electron deficient position *viz.* 3 or 5 (ref. 17). It is expected that nucleophilic radicals such as CO_2^- and $(CH_3)_2COH$ will add on to the more electron deficient sites *viz.* 4, 2, or 6 positions. In nicotinic acid (pyridine-3-carboxylic acid) e^-_{aq} adds to the 4 position[18] which may also be the site for the addition of the above radicals to pyridin-3-ol. This is supported by the fact that in pyridoxine in 4 position $-CH_2OH$ group is present and hence no reaction with these radicals is observed. The rate constant for reaction of isopropyl alcohol radical with form (**1**) of pyridin-3-ol (pH 2) is 3.8 × 10^8 dm^3 mol^{-1} s^{-1} at pH 2. For an electrophilic reaction it has been reported that rate constant decreases with protonation of nitrogen.[2] Accordingly, it could be expected that the protonation of nitrogen in pyridin-3-ol increases its reactivity towards a nucleophile such as the $(CH_3)_2COH$ radical. Again at pH 10.5 where pyridin-3-ol is in the deprotonated form (**3**) the reaction is immeasurably slow. The same trend is observed in the case of reactions of CO_2^- radical with pyridin-3-ol at different pH values as can be seen from the value of rate constants given in Table 1. The difficulty in reducing pyridin-3-ol is well illustrated by the ease with which its reduced form transfers electrons to different electron acceptors like thionine, methylene blue, safranine, anthraquinone 2-sulphonate, paraquat, and benzophenone at neutral pH. These observations suggest that the one-electron reduction potential of pyridin-3-ol is more negative than − 1.0 V. The reduction potential of pyridoxine is − 1.52 V. Our observations show that pyridin-3-ol is not getting reduced by $(CH_3)_2COH$ and CO_2^- radicals at pH 6.8, which indicate that reduction potential of pyridin-3-ol (**2**) must be more negative than − 1.0 V. At pH 6.8, the pyridin-3-ol radical formed by e^-_{aq} reaction decays to a final product having low absorption in the 340–360 nm region. As the transient absorption is overlapping with ground state absorption, bleaching recovery could not be observed. But on gamma-radiolysis of pyridin-3-ol in N_2-saturated matrix containing 0.2 mol dm^{-3} t-butyl alcohol, it was found that G(pyridin-3-ol) is roughly half the value of $G(e^-_{aq})$ which suggests that the pyridin-3-ol radical formed disproportionates to pyridin-3-ol and the fully reduced product. This fully reduced form may be absorbing in the far UV region.

Our observations clearly highlight the effect of substituents and also protonation equilibria on the reactivities of a solute molecule with different reducing radicals generated by pulse radiolysis. $(CH_3)_2COH$ and CO_2^- radicals are found to add on to the pyridin-3-ol ring. Pyridin-3-ol radicals formed by reaction with e^-_{aq} disproportionate to the parent pyridin-3-ol and a fully reduced form. Also these radicals are found to be highly reducing in nature.

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