Reactions of nitrate radical with amino acids in acidic aqueous medium: a flash photolysis investigation

B. Venkatachalapathy, P. Ramamurthy *

Department of Inorganic Chemistry, School of Chemistry, University of Madras, Madras 600 025, India

Abstract

The reactions of nitrate radical (photochemically generated by the flash photolysis of a mixture of ceric ammonium nitrate and nitric acid) with amino acids are reported. An electron transfer reaction pathway is proposed for phenylalanine and histidine, whereas a hydrogen abstraction pathway is invoked for the other amino acids, based on the magnitudes of the second- order rate constants and the observation of cation radicals. The reactivity of the nitrate radical is compared with that of other oxidizing radicals, such as $Cl_2^{\cdot-}$, $CO_3^{\cdot-}$ and $SO_4^{\cdot-}$. The nitrate radical is expected to show a higher reactivity than $SO_4^{\cdot-}$ based on their redox couples, but an inverse reactivity relationship is observed and is suggested to be due to the difference in solvation in the transition state.

Keywords: Nitrate radical; Amino acids; Flash photolysis

1. Introduction

Considerable attention has been paid to the investigation of the reactions of nitrate radical with organic substrates in solution [1-5] and in the gas phase [6-9]. The rate constants of reaction of nitrate radical with a variety of molecules in acetonitrile [4,10-12] and in neutral and acidic aqueous media [12,13] have also been reported. In the gas phase, nitrate radical is generated by the reaction of atomic fluorine with nitric acid [14]. In solution, nitrate radical is generated by the following routes: (1) pulse radiolysis [15-17] of aqueous solutions of nitric acid or nitrate ion; (2) photolysis of acidic solutions of ceric ammonium nitrate (CAN) [1,18] or acetonitrile solutions of CAN [19]; (3) reaction of photogenerated sulphate radical with nitrate ions [20]. In addition, we have recently shown [21] that nitrate radical can be generated by the direct photolysis of nitrate ions in acetonitrile medium. In this study, we concentrate on the reactions of nitrate radical with amino acids in aqueous medium.

2. Experimental details

CAN was of AR grade from Merck, India. A mixture of 5×10^{-3} M CAN and 6 M nitric acid (AR grade) was used in the flash photolysis studies. The amino acids valine, β-

phenylalanine, leucine and proline were purchased from Fluka, arginine monochloride, hydroxyproline and histidine from BDH, UK and threonine, 2-aminobutyric acid, glutamic acid and serine from SD's, India. Water was triply distilled over alkaline permanganate.

The flash photolysis experiments were performed using an Applied Photophysics KN-020 flash photolysis spectrometer. The system comprises a 100 W tungsten iodine lamp as monitoring source, a pair of LR-16 xenon flash lamps as excitation source, a Datalab DL-401 transient recorder as storage device and a Scopex oscilloscope as display device. An acetone filter was used to filter excitation light below 300 nm. All of the flash photolysis studies were carried out at 25 ± 1 °C. The rate constants reported are the average of three experiments with fresh solutions for every flash.

3. Results and discussion

3.1. Flash photolysis of CAN

CAN in 6 M HNO_3 shows a strong, broad charge transfer absorption with a maximum at 340 nm. Nitrate radical was generated by the flash photolysis of a mixture of 5×10^{-3} M CAN and 6 M HNO_3 according to the reaction

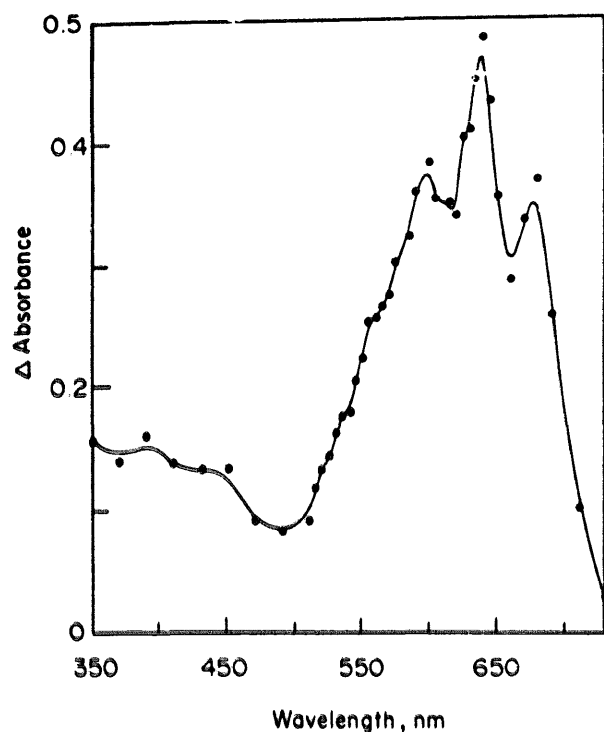$$Ce(IV)---NO_3 \xrightarrow{h\nu} Ce(III) + NO_3^{\cdot}$$

Fig. 1. Transient absorption spectrum of nitrate radical recorded immediately after flash.

Flash photolysis of the above mixture at the charge transfer absorption band results in the formation of transients in the wavelength range 350–750 nm. The transient absorption spectrum recorded immediately after the flash shows three absorption maxima at 600, 640 and 680 nm, as reported in the literature, characteristic of nitrate radical. The strongest absorption is at 640 nm (Fig. 1) and this wavelength has been used to study the dynamics of the reactions of amino acids with nitrate radical.

3.2. Kinetics of the reaction with amino acids

Nitrate radical has a lifetime of 2 ms in the absence of amino acids. A decrease in the lifetime of nitrate radical is observed with increasing concentrations of amino acids. The decay of nitrate radical has been observed at different concentrations of amino acids, and all these plots obey first-order kinetics (Figs. 2 and 3). Oxygen present in the medium does not perturb the dynamics of the decay. The second-order rate constants were obtained from plots of k_{obs} vs. [amino acid] (Fig. 4) and the data for selected amino acids are given in Table 1. It is obvious from Table 1 that phenylalanine is the most reactive of the amino acids investigated. The formation of a new transient is observed for phenylalanine at higher concentrations, but no transient is observed for the other amino acids. Other amino acids, e.g. cysteine, methionine, glutathione, tryptophan and tyrosine, were considered for the investigation, but could not be explored due to their thermal reaction with Ce(IV).
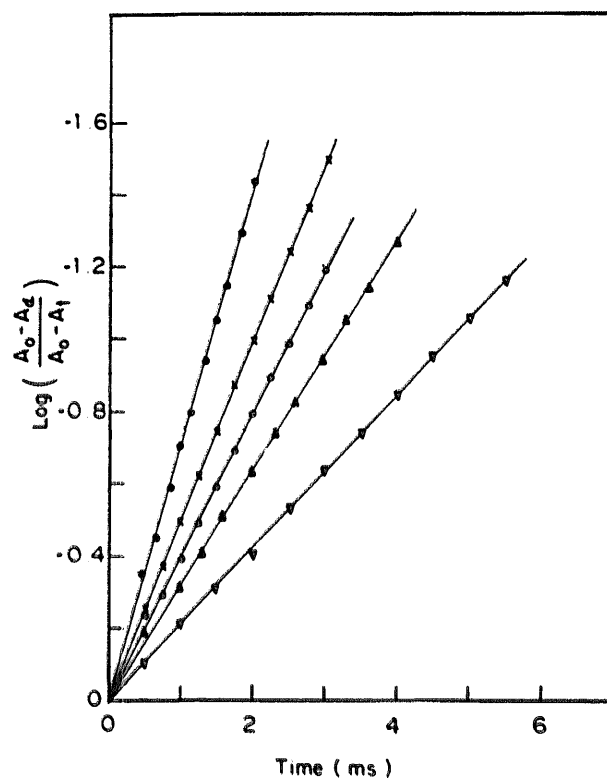

Fig. 2. First-order plot of the reaction between nitrate radical and valine at different concentrations of valine: ∇, absence of valine; $\blacktriangle$, 3.5×10^{-3} M; $\bigcirc$, 7.5×10^{-3} M; $\times$, 1.25×10^{-2} M; $\bullet$, 2.25×10^{-2} M.
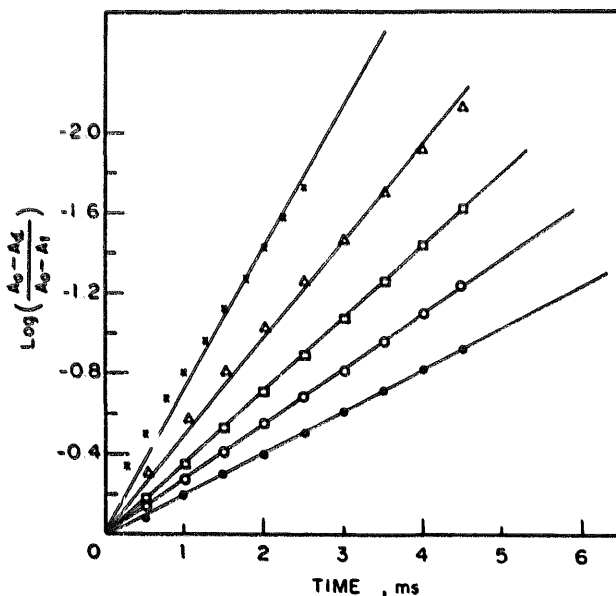

Fig. 3. First-order plot of the reaction between nitrate radical and phenylalanine at different concentrations of phenylalanine: $\bullet$, absence of phenylalanine; $\bigcirc$, 5.56×10^{-6} M; $\square$, 1.11×10^{-5} M; $\triangle$, 1.67×10^{-5} M; $\times$, 2.80×10^{-5} M.

3.3. Hydrogen abstraction vs. electron transfer

The rate of decay of nitrate radical increases with increasing concentration of amino acids. For phenylalanine at higher
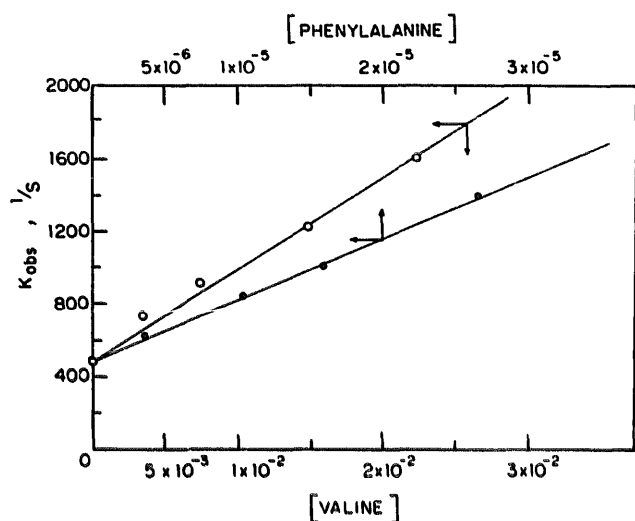
Fig. 4. Plot of k_{obs} vs. [valine] (O) and [phenylalanine] (●).

Table 1

Rate constants for the reaction of amino acids with nitrate radical

Amino acid	Second-order rate constant ($M^{-1} s^{-1}$)
β-Phenylalanine	$(3.1 \pm 0.1) \times 10^7$
Histidine	$(1.5 \pm 0.2) \times 10^7$
Arginine	$(6.5 \pm 0.3) \times 10^6$
Glutamine	$(2.2 \pm 0.1) \times 10^5$
Leucine	$(2.1 \pm 0.4) \times 10^5$
Proline	$(1.6 \pm 0.2) \times 10^5$
Threonine	$(1.1 \pm 0.1) \times 10^5$
Serine	$(5.8 \pm 0.3) \times 10^4$
Valine	$(5.2 \pm 0.5) \times 10^4$
Hydroxyproline	$(5.1 \pm 0.4) \times 10^4$
Aspartic acid	$(3.5 \pm 0.2) \times 10^3$
2-Aminobutyric acid	$(3.4 \pm 0.3) \times 10^3$
Alanine	$(1.7 \pm 0.1) \times 10^3$
Glycine	$(1.5 \pm 0.2) \times 10^3$

concentrations, the growth of a new transient is observed in a few milliseconds which decays in a few hundreds of milliseconds (Fig. 5). Both the transient growth and decay obey first-order kinetics with rate constants of $(2.47 \pm 0.03) \times 10^2$ s^{-1} and 1.82 ± 0.02 s^{-1} respectively. The dynamics and intensity of absorption are not perturbed by the oxygen dissolved in the medium. The transient absorption spectrum recorded immediately after the flash has a maximum at 550 nm (Fig. 6) and a weak absorption at 350 nm.

Nitrate radical is a powerful oxidizing agent with a redox potential [22] of 2.5 V for the couple $NO_3^{\bullet}/NO_3^{-}$. In addition, nitrate radical undergoes hydrogen abstraction reactions. For phenylalanine and histidine, the rate constants are higher than those reported in Ref. [12] for hydrogen abstraction by nitrate radical from other substrates. Based on the magnitudes of the rate constants for phenylalanine and histidine, electron transfer from these substrates to the nitrate radical is proposed. The observation of a new transient absorption for phenylalanine is attributed to the formation of the phenylalanine cation radical (the transient absorption spectrum recorded is very similar to those reported for amino acid cation radicals in Ref. [23]). The observation of the phenylalanine cation radical suggests that the mechanism of reaction is electron transfer from phenylalanine to the nitrate radical. The histidine cation radical absorbs around 400 nm, where CAN exhibits a strong charge transfer absorption; this could be the reason why we do not observe the absorption due to the histidine cation radical.
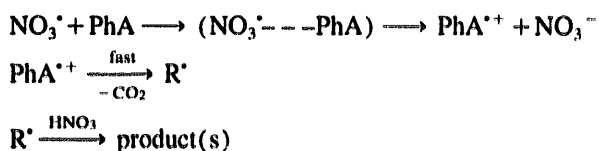
The growth and decay of the transient formed at 550 nm are independent of the concentration of phenylalanine. This reveals that the formation of the phenylalanine cation radical does not occur directly from the reaction between nitrate radical and phenylalanine, but through an intermediate. In order to explain the concentration-independent nature of the transient, the formation of a π-complex intermediate between the nitrate radical and phenylalanine is invoked. The formation of a π-complex between electron deficient Cl$^{\bullet}$ and aro-

matic donors is known [24,25]. Moreover, these types of complexes have been proposed [5] in the one-electron oxidation of alkyl benzenes. Since the plot of $1/k_{obs}$ vs. $1/[PhA]$ is not linear, the presence of an equilibrium between the reactants (nitrate radical and phenylalanine (PhA)) and the π-complex can be ruled out. It is reported in Ref. [26] that amino acid cation radicals are more stable in acidic conditions, and we have observed a long-lived phenylalanine cation radical in acidic medium. The decay of the cation radical may be due to the loss of CO_2, leading to the formation of radical species, and the radicals thus formed may also be oxidized in the presence of excess nitric acid in the medium. The above discussion is depicted in the following scheme.

$$NO_3^{\bullet} + PhA \longrightarrow (NO_3^{-} - - -PhA) \longrightarrow PhA^{\bullet +} + NO_3^{-}$$

$$PhA^{\bullet +} \xrightarrow[-CO_2]{fast} R^{\bullet}$$

$$R^{\bullet} \xrightarrow{HNO_3} product(s)$$

From these observations and the magnitudes of the rate constants, it is suggested that all the amino acids studied, except phenylalanine and histidine, should undergo hydrogen abstraction

$$NO_3^{\bullet} + AH \longrightarrow HNO_3 + A^{\bullet}$$

Nitrate radicals also undergo nitration reactions. The rate constant of the addition reaction of benzene with nitrate radical is $1 \times 10^6 M^{-1} s^{-1}$ [27]. This rate constant is 1/30th of the observed rate constant for phenylalanine and hence nitrate radical addition to the phenyl ring is unimportant.

3.4. Comparison of nitrate radical with other oxidizing radicals

From the redox couple data [22], it is obvious that the $NO_3^{\bullet}$ radical is a stronger oxidizing agent than $SO_4^{\bullet -}$, $CO_3^{\bullet -}$ and $Cl_2^{\bullet -}$ radicals. The redox couples [23] and rate constants [13] are given in Table 2. Based on the redox couple data,
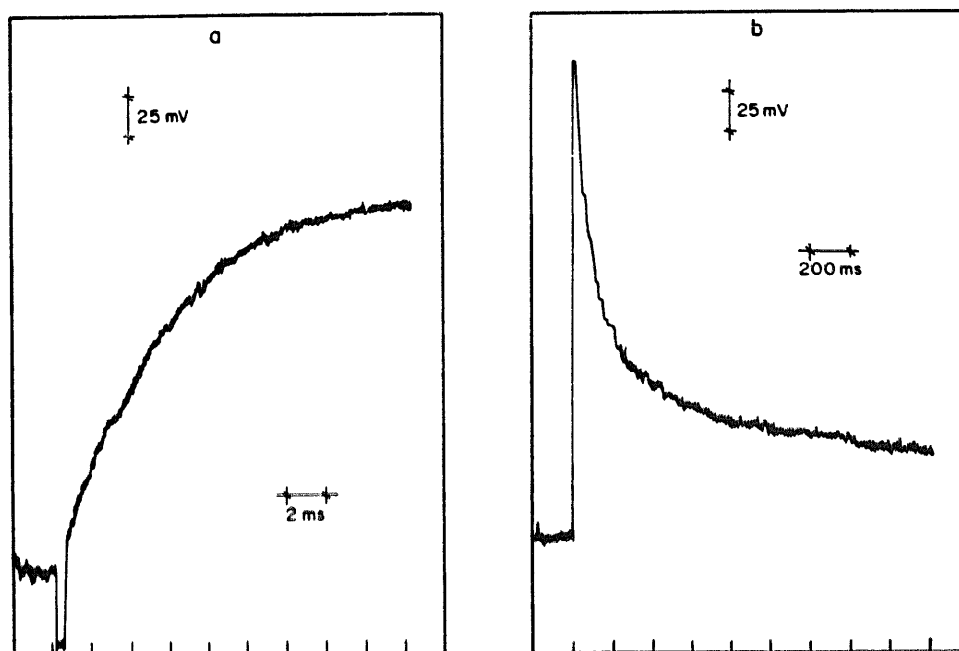
Fig. 5. (a) Transient absorption growth of phenylalanine cation radical at 550 nm; [PhA] = 2.0 × 10⁻⁴ M. (b) Transient absorption decay of phenylalanine cation radical at 550 nm; [PhA] = 2 0 × 10⁻³ M.
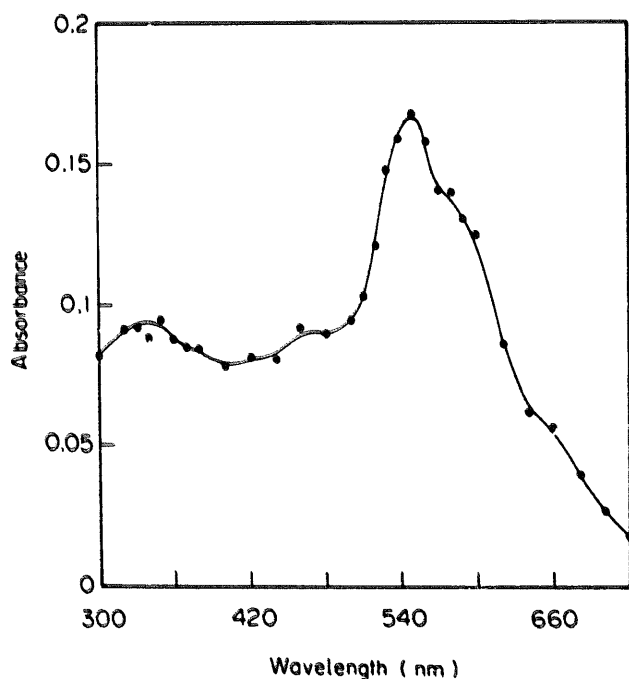

Fig. 6. Transient absorption spectrum of phenylalanine cation radical recorded 18 ms after the flash.

the order of reactivity of the radicals can be arranged as $NO_3^• > SO_4^{•-} > Cl_2^{•-} > CO_3^{•-}$. The reactions of amino acids with these radicals reveal that the order of reactivity is $SO_4^{•-} > NO_3^• > Cl_2^{•-} > CO_3^{•-}$. The opposite trends of reactivity of the nitrate and sulphate radicals may arise from differences in solvation in the transition state. Solvation may yield an additional driving force in the case of $SO_4^{•-}$. The difference in driving force may arise from the different hydrogen bonding interaction in the transition state. Due to the charged, unsymmetrical nature of the $SO_4^{•-}$ radical, it should be more polar than $NO_3^•$, which should influence the hydrogen bonding interaction. It should also be noted that this type of opposite trend has been observed for alcohols [2]. For amino acids, the rate constants available from the literature are for either neutral or weakly acidic conditions, but not for the strongly acidic conditions used in our experiments.

The order of reactivity [23] of the amino acids with the $Cl_2^{•-}$ radical is tyrosine > tryptophan > histidine > phenylalanine. It is reported in the literature [23] that the amino acids glycine, alanine, valine, leucine, isoleucine, proline, serine, threonine, arginine, asparginine, cysteine, aspartic acid and lysine are unreactive with $Cl_2^{•-}$, but this is not the case for the nitrate radical. The nitrate radical reacts with phenylalanine at a higher rate than histidine, and this addi-

Table 2
Comparison of radical reactions with amino acids

Amino acid	$CO_3^{•-}$ $(E_{1/2}^{red} = 1.5)$	$Cl_2^{•-}$ $(E_{1/2}^{red} = 2.09)$	$SO_4^{•-}$ $(E_{1/2}^{red} = 2.43)$	$NO_3^•$ $(E_{1/2}^{red} = 2.5)$
Histidine	8 × 10⁶	1.4 × 10⁷	2.5 × 10⁹	1.5 × 10⁷
β-Phenylalanine	5 × 10⁴	6.0 × 10⁶		3.1 × 10⁷
Serine		1.2 × 10⁵	2.3 × 10⁷	5.8 × 10⁴

tional driving force for the reaction may be due to the formation of a π-type complex with the aromatic moiety in the case of phenylalanine.

4. Conclusions

Of the three major reactions of nitrate radical, (1) electron transfer, (2) hydrogen abstraction and (3) nitration, two are observed with amino acids depending on their nature. The electron transfer reaction between phenylalanine and nitrate radical takes place through an intermediate π-complex. A lower reactivity of nitrate radicals compared with sulphate radicals is suggested to be due to the additional driving force for reaction with sulphate radicals as a result of solvation.

Acknowledgements

References

[1] L. Dogliotti and E. Hayon, J. Phys. Chem., 71 (1967) 3802.
[2] Z.B. Alfassi, S. Padmaja, P. Neta and R.E. Huie, J. Phys. Chem., 97 (1993) 3780.
[3] E. Baciocchi, T. Del Giacco, S.M. Murgia and G.V. Sebastiani, Tetrahedron Lett., 44 (1988) 6651.
[4] O. Ito, S. Akiho and M. Iino, J. Org. Chem., 54 (1989) 2436.
[5] T. Del Giacco, E. Baciocchi and S. Steenken, J. Phys. Chem., 97 (1993) 5451.
[6] C.E. Canosa-Mas, P.S. Monks and R.P. Wayne, J. Chem. Soc., Faraday Trans., 88 (1992) 11.
[7] I. Bastian, V. Basiane, K.H. Becker and Z. Tong, J. Phys. Chem., 94 (1990) 2413.
[8] E.J. Dlugokencky and C.J. Howard, J. Phys. Chem., 92 (1988) 1188.
[9] R. Atkinson, J. Phys. Chem. Ref. Data, 20 (1991) 459, and references cited therein.
[10] O. Ito, S. Akiho and M. Iino, Bull. Chem. Soc. Jpn., 62 (1989) 1606.
[11] O. Ito, S. Akiho and M. Iino, J. Phys. Chem., 93 (1989) 4079.
[12] P. Neta and R.E. Huie, J. Phys. Chem., 90 (1986) 4644.
[13] P. Neta, R.E. Huie and A.B. Ross, J. Phys. Chem. Ref. Data, 17 (1988) 1027, and references cited therein.
[14] A. Mellouki, G. Le Bras and G. Poulet, J. Phys. Chem., 91 (1987) 5760.
[15] M. Daniels, J. Phys. Chem., 73 (1969) 3710.
[16] R.K. Broszkiewicz, E. Kozlowska-Milner and A. Blum, J. Phys. Chem., 85 (1981) 2258.
[17] Y. Katsumura, P.Y. Jiang, R. Nagaishi, T. Oishi, K. Ishigure and Y. Yoshida, J. Phys. Chem., 95 (1991) 4435.
[18] P.H. Wine, R.L. Mauldin and R.P. Thorn, J. Phys. Chem., 92 (1988) 1156.
[19] E. Baciocchi, T. Del Giacco, S.M. Murgia and G.V. Sebastiani, J. Chem. Soc., Chem. Commun., (1987) 1246.
[20] M. Exner, H. Herrmann and R. Zellner, Ber. Bunsenges. Phys. Chem., 96 (1992) 470.
[21] N. Meera and P. Ramamurthy, Chem. Phys. Lett., 153 (1988) 13.
[22] D.M. Stanbury, Adv. Inorg. Chem., 33 (1989) 69.
[23] G.E. Adams, J.E. Aldrich, R.H. Bisby, R.B. Cundall, J.L. Redpath and R.L. Willson, Radiat. Res., 49 (1972) 278.
[24] N.J. Bunce, K.U. Ingold, J.P. Landers, J. Lusztyk and J.C. Scaiano, J. Am. Chem. Soc., 107 (1985) 5464.
[25] K.D. Raner, J. Lusztyk and K.U. Ingold, J. Am. Chem. Soc., 111 (1989) 3652.
[26] D.V. Bent and E. Hayon, J. Am. Chem. Soc., 97 (1975) 2612.
[27] R. Atkinson, C.N. Plum, W.P. Carter, A.M. Winer and J. Pitts, Jr., J. Phys. Chem., 88 (1984) 1210.