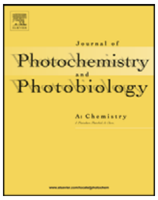
Journal of Photochemistry and Photobiology A: Chemistry 203 (2009) 13–17

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem



Laser flash photolysis of new water-soluble peroxyl radical precursor

Ali El-Agamey*

School of Physical and Geographical Sciences, Keele University, Keele, Staffordshire ST5 5BG, UK

ARTICLE INFO

Article history:
Received 27 August 2008
Received in revised form
13 November 2008
Accepted 1 December 2008
Available online 24 December 2008

Keywords:
Peroxyl radicals
Laser flash photolysis
α-Cleavage
Acetylperoxyl radical
4-Acetyl-4-phenylpiperidine hydrochloride

ABSTRACT

Laser flash photolysis (LFP) of commercially available water-soluble 4-acetyl-4-phenylpiperidine hydrochloride (APP) in the presence of oxygen gives rise to acetylperoxyl radical (ACP). The formation of ACP during LFP of APP has been established by determining their rate constants with $ABTS^{2-}$ in methanolic and aqueous solutions (0.15 ± 0.02 and $2.0 \pm 0.2 \times 10^9\ M^{-1}\ s^{-1}$, respectively). These rate constants are in agreement with those reported in the literature.

The reactivity of ACP was investigated with crocin in aqueous and methanolic solutions. Moreover, the rate constant for the reaction of ACP with nitrite anion (NO_2^-) in aqueous solution was determined to be $4.45 \pm 0.4 \times 10^6\ M^{-1}\ s^{-1}$.Overall, APP can be used as ACP precursor in aqueous and organic solutions using the more accessible LFP technique than the normally used (especially in aqueous solutions) pulse radiolysis technique.

1. Introduction

Peroxyl radicals are important intermediates in chemistry and biology. They are involved in atmospheric chemistry, combustion and many oxidation reactions. Also, they are the main radicals responsible for lipid peroxidation, which can lead to oxidative stress. For example, the hepatotoxicity of CCl_4 has been attributed to the formation of trichloromethylperoxyl radical ($CCl_3O_2^{\bullet}$) which triggers lipid peroxidation processes [1,2].

Pulse radiolysis (PR) and laser flash photolysis (LFP) are two of the most important techniques to investigate fast reactions between peroxyl radicals and different compounds in aqueous and organic solutions [3]. Most of the reported peroxyl radical reactions in aqueous solutions have been investigated using pulse radiolysis. However, this technique is not easily accessible to many laboratories [4]. Peroxyl radical generation using pulse radiolysis has been described previously [4].

Laser flash photolysis is a far more available than pulse radiolysis. However, its use to investigate peroxyl radical reactions in aqueous solution is rare because of the poor solubility of most peroxyl radical precursors in water. Using laser flash photolysis technique, there are few examples for the reactions of peroxyl radicals with different substrates, using commercially unavailable cobalt or nickel complexes as precursors, in aqueous solutions [5–8]. For example [5,6], the generation of acetylperoxyl radicals using organo-cobalt complexes is shown in Scheme 1.

The use of water-soluble azo compounds as peroxyl radical precursors is not possible due to the very low quantum yield of the cleavage process in aqueous solutions [8,9].

In this study, LFP of water-soluble and commercially available 4-acetyl-4-phenylpiperidine hydrochloride (APP), which has an analgesic activity [10], is reported in methanolic and aqueous solutions (see ground state spectra in supplementary material). In air-saturated solution, acetylperoxyl radicals, generated from photolysis of APP, are the main reactive peroxyl radicals. In addition, the reactivity of acetylperoxyl radical toward 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$), crocin and nitrite anion in aqueous solutions is reported (Fig. 1).

2. Materials and methods

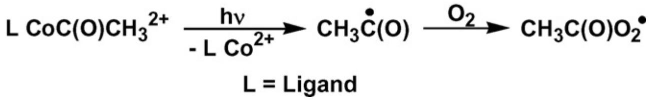
2.1. Materials

Methanol (Fisher Scientific, HPLC grade), sodium nitrite (Fisher Scientific), 4-acetyl-4-phenylpiperidine hydrochloride (Aldrich), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$) (Sigma) were used as received. Purified crocin was kindly supplied by Dr. V. Partali and Dr. H.R. Sliwka (NTNU, Inst. Kjemi, Trondheim, N-7491 Norway) and was used as received. Argon was supplied by the British Oxygen Company (BOC). For aqueous solutions, ultra pure water (conductivity <0.067 μS/cm, Elga UK) was used.

---

* Permanent address: Chemistry Department, Faculty of Science, New Damietta, Damietta, Egypt. Tel.: +20 57 2410575; fax: +20 57 2403868.
E-mail address: a_el_agamey@yahoo.co.uk.

1010-6030/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2008.12.015



$$\mathrm{L\,CoC(O)CH_3^{2+}} \xrightarrow[-\mathrm{L\,Co^{2+}}]{h\nu} \mathrm{CH_3\dot{C}(O)} \xrightarrow{O_2} \mathrm{CH_3C(O)O_2^{\bullet}}$$

$$\mathrm{L = Ligand}$$

Scheme 1.

2.2. Experimental methods

The LFP system was constructed in-house at Keele University using a variety of components from various suppliers, including Applied Photophysics.

The fourth harmonic (266 nm) of a Spectron Q-switched Nd:YAG laser was used to excite the samples in this work. Changes in absorbance of the sample are monitored using a 275 W Xenon arc Lamp (Kratos) at 90° to the laser path. For UV–vis measurements, an Applied Photophysics *f*/3.4 grating monochromator (260–820 nm) coupled to a Hamamatsu R928 photomultiplier tube (PMT) was used. For the detection of short-lived transients (100’s ns timescale), a time delay pulse generator and lamp pulsing unit were used.

The PMT analog output is converted into the digital form using a Tektronix TDS 320 digital oscilloscope. The data recorded consists of 512 data points and the vertical resolution is 8-bit. Finally, the digital signal is transferred to an IBM-compatible PC via a GPIB interface where it can be stored and analyzed. The time resolution of the Laser flash photolysis system is 50 ns.

All transients are recorded from single irradiation except those at short timescales ($\leq$1 μs/division), which were averaged four times to improve S/N ratio. Oxygen-free solutions were prepared by saturating the solutions with argon and this was examined by measuring the triplet lifetime of benzophenone and it was in agreement with the literature. In addition, flow system was used for argon-saturated samples to avoid any interference due to sample degradation.

All the experiments were carried out at ~20 °C. More detailed LFP description is included in supplementary material.

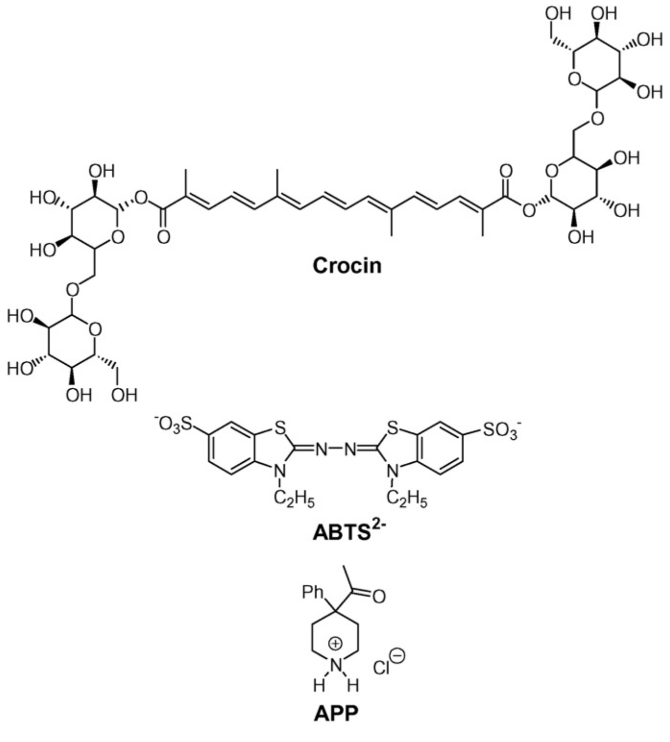


Fig. 1. Structures of crocin, ABTS^{2-} and APP.

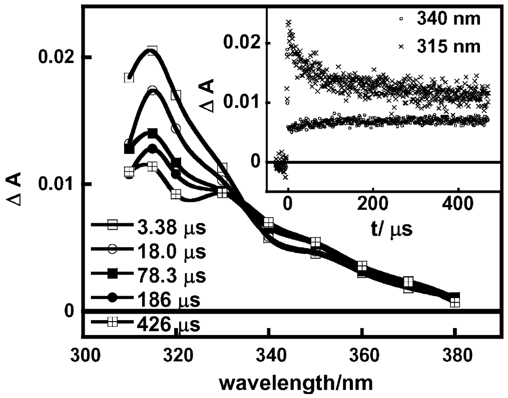


Fig. 2. Transient absorption spectra obtained following LFP (266 nm) of APP (1.5×10^{-3} M) in argon-saturated aqueous solution (Laser energy = 12.8 mJ/pulse). The inset shows kinetic traces at 315 and 340 nm.

3. Results and discussion

3.1. Laser (266 nm) flash photolysis of 4-acetyl-4-phenylpiperidine hydrochloride (APP)

Laser flash photolysis of APP in oxygen-free aqueous solution gives a transient spectrum with λ_{max} = 315 nm (Fig. 2).

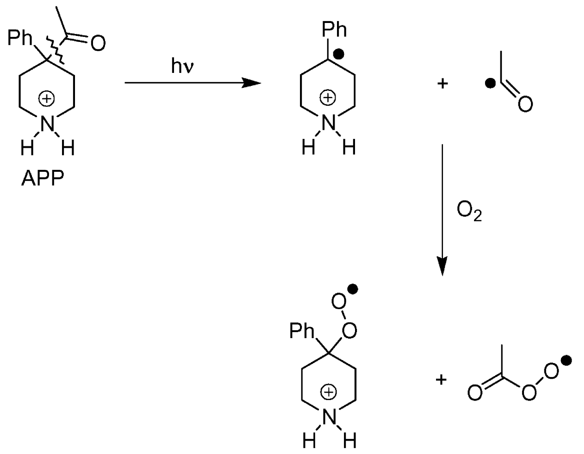
The second-order decay of the 315 nm transient is accompanied by a transient growth at 340 nm. In addition, no other transient was observed at longer wavelengths (between 400 and 650 nm) in oxygen-free aqueous solutions. Moreover, no information about the triplet absorption spectrum and triplet lifetime of APP was previously reported. However, the very short triplet lifetime of some structurally related ketones (e.g. τ_T (pinacolone) <1 ns [11]), compared to the lifetime of the transient observed at 315 nm, excludes the possibility of attributing the transient spectrum (Fig. 2) to the triplet APP (^{3}APP). In addition, at short timescales (1–2.5 μs), the formation of the transient is instantaneous (see Fig. S2 in supplementary material), which indicates that the lifetime of the excited state responsible for transient formation is less than 100 ns.

APP has an alkyl-substituted benzyl moiety in the α-position of carbonyl group. Moreover, the transient spectrum (Fig. 2) is similar to the benzyl radical spectrum reported by Maouf et al. [12] and its λ_{max} is close to literature values (314–318 nm) [12–16]. Therefore, from the structure of APP and the observation of a transient spectrum similar to that of benzyl radical, it can be suggested that APP undergoes α-cleavage from a very short-lived triplet state (or excited singlet state) to give acetyl radical and a substituted benzyl radical. In the presence of oxygen, both radicals form the corresponding acetylperoxyl radical (ACP) and benzylperoxyl radical derivative, respectively (Scheme 2). As a further evidence, in air-saturated aqueous solution, there is a rapid decay ($k = 1.0 \pm 0.2 \times 10^9$ M^{-1} s^{-1}) for the transient at 320 nm, which is in support for its identification as carbon-centered radical (substituted benzyl radical).

The second-order transient decay at 315 nm, ascribed to benzyl radical derivative, can be attributed to radical–radical reaction. Moreover, the formation of stable transient at 340 nm can be ascribed to products formed from dimerization and/or disproportionation reactions of the radicals generated from α-cleavage of APP (Fig. 2). Also, the initial fast step of 340 nm transient is due to the absorption of benzyl radical derivative, generated from α-cleavage of APP, at this wavelength (see Fig. 2).

LFP of APP in methanolic solution gives similar transient spectra (see Fig. S3 in supplementary material).

The system was further examined by studying the reactions of the peroxyl radicals, formed from the α-cleavage of APP in



Scheme 2.

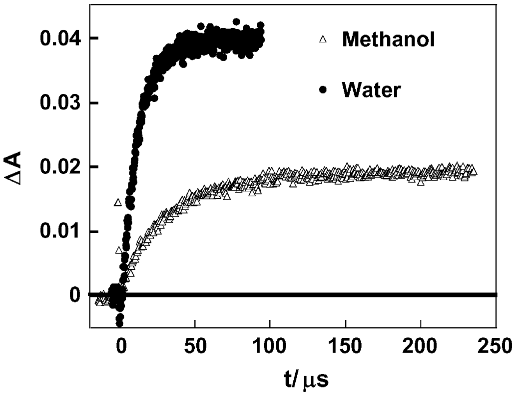


Fig. 3. Transient profiles, at 415 nm, obtained following LFP (266 nm) of APP (4×10^{-3} M) and $ABTS^{2-}$ (5×10^{-5} M) in air-saturated methanolic and aqueous solutions (Laser energy = 1.5 mJ/pulse).

air-saturated solution, with $ABTS^{2-}$, which is a good electron-donor. Following LFP (266 nm) of APP in air-saturated methanolic or aqueous solutions containing $ABTS^{2-}$ (5×10^{-5} M), a strong visible absorption at 415 nm, characteristic of $ABTS^{\bullet-}$, is observed (Fig. 3).

The $ABTS^{\bullet-}$ formation ($\varepsilon = 34700\,M^{-1}\,cm^{-1}$ at 417 nm) [17–19] arises from an electron transfer reaction between the peroxyl radicals and $ABTS^{2-}$ (Eq. (1) and Figs. 3 and 4). Since benzylperoxyl radicals are much less reactive than acylperoxyl radicals [20], the observed reaction is mainly due to the reaction of ACP with $ABTS^{2-}$

$$RO_2^{\bullet} + ABTS^{2-} \xrightarrow{k_q} RO_2^{-} + ABTS^{\bullet-} \qquad (1)$$

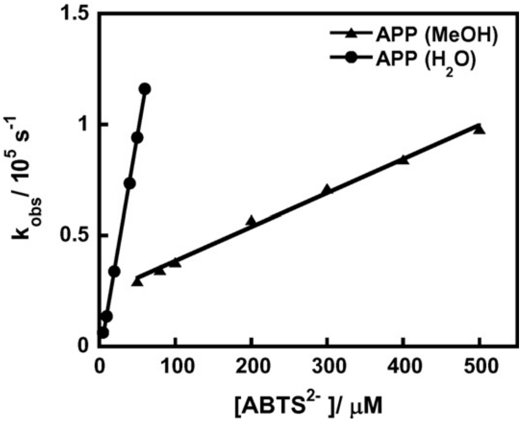


Fig. 4. Plots of pseudo-first order rate constants (k_{obs}) for formation of $ABTS^{\bullet-}$, at 415 nm, from the reaction of acetylperoxyl radicals with $ABTS^{2-}$, in air-saturated methanolic and aqueous solutions versus the concentration of $ABTS^{2-}$ (Laser energy = 1.5 mJ/pulse).

The rate constants (k_q), for the reactions in methanolic and aqueous solutions, were determined as 0.15 ± 0.02 and $2.0 \pm 0.2 \times 10^9\,M^{-1}\,s^{-1}$, respectively from the plot of the observed pseudo-first order rate constant (k_{obs}) for the growth of $ABTS^{\bullet-}$ at 415 nm versus [$ABTS^{2-}$] using Eq. (2), where k_1 (intercept) is rate constant for the decay of ACP in the absence of $ABTS^{2-}$ (Fig. 4)

$$k_{obs} = k_1 + k_q[ABTS^{2-}] \qquad (2)$$

These values are in good agreement with those reported for the reactions of ACP, generated from different precursors, with $ABTS^{2-}$ in methanolic and aqueous solutions, respectively (0.18 ± 0.02 and $1.8 \times 10^9\,M^{-1}\,s^{-1}$, respectively) [20,21]. The higher reactivity of peroxyl radicals in aqueous solution, compared with organic solvents, has been reported previously and is ascribed to the stabilization of the transition state induced by the rise of solvent polarity [22,23]. In Fig. 3, the size of $ABTS^{\bullet-}$ signal in water is greater than that in methanol because the rate constant for the reaction in water is much faster than that in methanol.

Following laser (266 nm) photolysis of APP in argon-saturated methanolic or aqueous solutions containing $ABTS^{2-}$ (5×10^{-5} M), no $ABTS^{\bullet-}$ absorption was observed, suggesting that the reactions of $ABTS^{2-}$ with acetyl or benzyl radical derivative are very slow. These observations are consistent with previous reports, which showed that acetyl [21] and benzyl radicals [20] are unreactive towards $ABTS^{2-}$.

Moreover, the reactions of acetylperoxyl radicals, generated from α-cleavage of APP in the presence of oxygen, with crocin and nitrite anion have been investigated as further examples for the use of APP as peroxyl radical precursor.

3.2. Crocin

Following LFP (266 nm) of APP and crocin in air-saturated aqueous solution, two transients are observed at 590 and 680 nm, respectively (Fig. 5). The pseudo first order (or first order) rate constants for the formation of these transients are $7.9 \pm 0.8 \times 10^4\,s^{-1}$ and $6.3 \pm 0.7 \times 10^4\,s^{-1}$, respectively.

In a previous study for the reaction of triplet 1-nitronaphthalene (^{3}NN) with crocin in aqueous solution, competitive triplet energy transfer and electron-transfer reactions have been observed (Scheme 3) [26].

The electron-transfer reaction generates $crocin^{\bullet+}$, which has a transient spectrum (λ_{max} = 680 nm) [26] similar to that observed in the reaction of ACP with crocin. Therefore, this intermediate can be attributed to $crocin^{\bullet+}$

$$Crocin + ROO^{\bullet} \rightarrow Crocin^{\bullet+} + ROO^{-} \qquad (3)$$

In addition, according to previous reports, the transient at 590 nm can be attributed to either an ion-pair comprising $crocin^{\bullet+}$ and the peroxide anion [$crocin^{\bullet+} \ldots {}^{-}ACP$] or, alternatively, an isomer of $crocin^{\bullet+}$ [27–29].

LFP of air-saturated methanolic solution of APP and crocin gives no transients at 680 or 590 nm. However, there is little bleaching at 490 nm (see Fig. S4 in supplementary material). The absence of electron-transfer transients can be attributed to the greater energy barrier for the reaction in methanol. This barrier is reduced in aqueous solution due to the extra stabilization of the polar transition state in more polar solvent (water). The presence of little bleaching indicates the presence of inefficient reaction, as reflected by the degree of the bleaching in methanol versus that in water (see Fig. S4), between ACP and crocin (e.g. addition reaction).

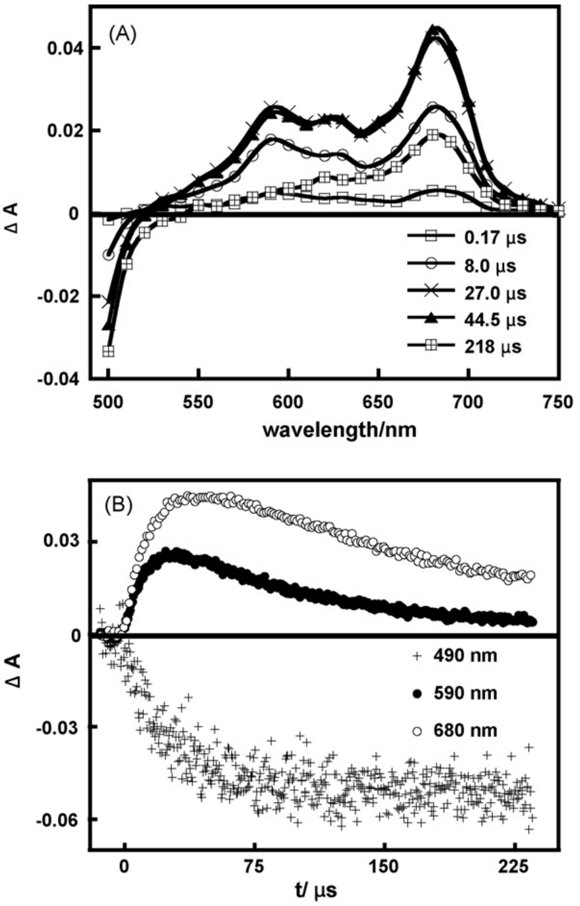


Fig. 5. (A) Transient absorption spectra obtained following LFP (266 nm) of APP (5.1 × 10–3 M) and crocin (1.1 × 10-5 M) [24,25] in aqueous air-saturated solution (Laser energy = 4.5 mJ/ pulse). (B) Kinetic traces at 490, 590 and 680 nm.

In this example, the solubility of APP in aqueous and organic solutions has been used to monitor the influence of solvent polarity on the electron-transfer reaction feasibility. This advantage can be extended to other unexplored reactions.

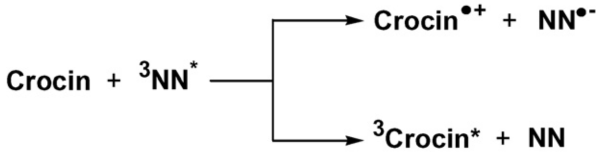
3.3. *Nitrite*

The reaction of ACP with nitrite anion has been investigated in aqueous solution. This reaction has not been studied before and it can be used as a source for $\mathrm{NO_2}^\bullet$

$$\mathrm{RO_2}^\bullet + \mathrm{NO_2}^- \xrightarrow{k_3} \mathrm{RO_2}^- + \mathrm{NO_2}^\bullet \tag{4}$$

Since ACP [30–33], nitrite anion and $\mathrm{NO_2}^\bullet$ are weakly absorbing in the UV region, it is difficult to monitor the reaction of acetylperoxyl radical with nitrite anion. However, the rate constant for this reaction (k_3) can be measured by competition with a reaction of acetylperoxyl radical with ABTS^{2-} since the product of this reaction ($\mathrm{ABTS}^{\bullet-}$) can be observed optically. The growth of $\mathrm{ABTS}^{\bullet-}$ is a pseudo-first order reaction and the observed rate constant (k_{obs}) is given by the following equation

$$k_{\mathrm{obs}} = k_1 + k_{\mathrm{q}}[\mathrm{ABTS}^{2-}] + k_3[\mathrm{NO_2}^-] \tag{5}$$



Scheme 3.

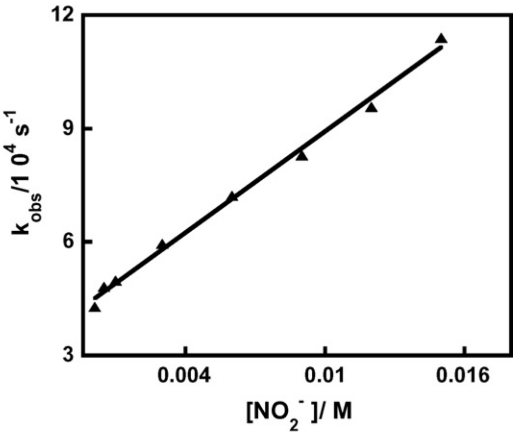


Fig. 6. Plots of pseudo-first order rate constants (k_{obs}) for formation of $\mathrm{ABTS}^{\bullet-}$ (at 415 nm), from the reaction of acetylperoxyl radicals with ABTS^{2-} (2×10^{-5} M) in air-saturated aqueous solution, versus the concentration of $\mathrm{NO_2}^-$ (Laser energy = 1.5 mJ/pulse).

By using a constant concentration of ABTS^{2-} and ensuring pseudo-first order conditions, a plot k_{obs} versus nitrite concentration yields a straight line with a slope equal to k_3 and intercept equal to $k_1 + k_{\mathrm{q}}$ [ABTS^{2-}] (Eq. (5) and Fig. 6).

Using this method (competitive kinetics) [20,34,35], the rate constant for the reaction of ACP with nitrite was determined to be $4.45 \pm 0.4 \times 10^6\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. The reduction potentials of $\mathrm{NO_2}^\bullet$ and acylperoxyl radicals (0.87–1.04 and ~1.1 V versus NHE, respectively) confirm the feasibility of the reaction [36,37].

4. Conclusions

In summary, this first time-resolved study of APP shows that this peroxyl radical precursor can easily be used to investigate fast peroxyl radical reactions in aqueous solutions using laser flash photolysis technique, which is more accessible than pulse radiolysis technique. This will facilitate the investigation of reactive peroxyl radical reactions with different substrates in aqueous solutions. In addition, by virtue of APP solubility in organic and aqueous solutions, it can be used to study the influence of solvent polarity on peroxyl radical reactions (see reactions with ABTS^{2-} and crocin in aqueous and methanolic solutions). For future work, the effect of a peroxyl radical precursor location in the micro-heterogeneous media, such as micelles and microemulsion, will be investigated by studying APP as water-soluble precursor versus the organic-soluble ones.

Acknowledgments

The author is grateful to Leverhulme Trust (Research Grant F/00130F) for financial support and to Dr. David J. McGarvey and T. George Truscott (School of Physical and Geographical Sciences, Keele University, Keele, Staffordshire ST5 5BG, UK) for their valuable comments on the manuscript and to Dr. V. Partali and Dr. H.R. Sliwka (NTNU, Inst. Kjemi, Trondheim, N-7491 Norway) for kindly providing the purified crocin.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2008.12.015.

References

[1] J.A. Brent, B.H. Rumack, Clin. Toxicol. 31 (1993) 173–196.
[2] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, third ed., Oxford University Press, Oxford, 1999, pp. 547–552.
[3] R.V. Bensasson, E.J. Land, T.G. Truscott, Excited States and Free Radicals in Biology and Medicine, Oxford University Press, Oxford, 1993.
[4] P. Neta, R.E. Huie, A.B. Ross, J. Phys. Chem. Ref. Data 19 (1990) 413–513.
[5] A. Bakac, J. Am. Chem. Soc. 124 (2002) 3816–3817.
[6] A. Bakac, J. Am. Chem. Soc. 124 (2002) 9136–9144.
[7] D.G. Kelley, A. Marchaj, A. Bakac, J.H. Espenson, J. Am. Chem. Soc. 113 (1991) 7583–7587.
[8] A. Bakac, J.H. Espenson, Inorg. Chem. 28 (1989) 3901–3904.
[9] P.S. Engel, Chem. Rev. 80 (1980) 99–150.
[10] Z.S. Saify, K.M. Khan, S.M. Haider, S.S.T.A. Zeeshan, M. Saeed, M.S. Shekhani, W. Voelter, Z. Naturforsch. B 54 (1999) 1327–1336.
[11] N.J. Turro, Modern Molecular Photochemistry, University Science Books, Mill Valley, CA USA, 1991, pp. 528–532.
[12] A. Maouf, H. Lemmetyinen, J. Koskikallio, Acta Chem. Scand. 44 (1990) 336–338.
[13] R.F.C. Claridge, H. Fischer, J. Phys. Chem. 87 (1983) 1960–1967.
[14] K. Tokumura, T. Ozaki, H. Nosaka, Y. Saigusa, M. Itoh, J. Am. Chem. Soc. 113 (1991) 4974–4980.
[15] H.C. Christensen, K. Sehested, E.J. Hart, J. Phys. Chem. 77 (1973) 983–987.
[16] T.O. Meiggs, L.I. Grossweiner, S.I. Miller, J. Am. Chem. Soc. 94 (1972) 7986–7991.
[17] S.L. Scott, W.-J. Chen, A. Bakac, J.H. Espenson, J. Phys. Chem. 97 (1993) 6710–6714.
[18] P. Maruthamuthu, L. Venkatasubramanian, P. Dharmaljngam, Bull. Chem. Soc. Jap. 60 (1987) 1113–1117.
[19] B.S. Wolfenden, R.L. Willson, J. Chem. Soc. Perkin Trans. II (1982) 805–812.
[20] A. El-Agamey, D.J. McGarvey, Phys. Chem. Chem. Phys. 4 (2002) 1611–1617.
[21] M.N. Schuchmann, C. von Sonntag, J. Am. Chem. Soc. 110 (1988) 5698–5701.
[22] P. Neta, R.E. Huie, P. Maruthamuthu, S. Steenken, J. Phys. Chem. 93 (1989) 7654–7659.
[23] Z.B. Alfassi, R.E. Huie, M. Kumar, P. Neta, J. Phys. Chem. 96 (1992) 767–770.
[24] The concentration of crocin in water was calculated using ε_{443} nm = 89 000 M^{-1} cm^{-1}; see reference [25].
[25] J. Escribano, G.L. Alonso, M. CocaPrados, J.A. Fernandez, Cancer Lett. 100 (1996) 23–30.
[26] B.J. Foss, Synthesis and physical properties of hydrophilic carotenoid derivatives [PhD Thesis], Norwegian University of Science and Technology, 2005.
[27] A. El-Agamey, D.J. McGarvey, J. Am. Chem. Soc. 125 (2003) 3330–3340.
[28] A. El-Agamey, G.M. Lowe, D.J. McGarvey, A. Mortensen, D.M. Phillip, T.G. Truscott, A.J. Young, Arch. Biochem. Biophys. 430 (2004) 37–48.
[29] A. El-Agamey, D.J. McGarvey, in: G. Britton, S. Liaaen-Jensen, H. Pfander (Eds.), Carotenoids Volume 4: Natural Functions, Birkhäuser, Basel, 2008, pp. 119–154.
[30] G. Moortgat, B. Veyret, R. Lesclaux, J. Phys. Chem. 93 (1989) 2362–2368.
[31] M.M. Maricq, J.J. Szente, J. Phys. Chem. 100 (1996) 4507–4513.
[32] C.M. Roehl, D. Bauer, G.K. Moortgat, J. Phys. Chem. 100 (1996) 4038–4047.
[33] M.M. Maricq, J.J. Szente, J. Phys. Chem. A 104 (2000) 7239–7245.
[34] R.D. Small Jr., J.C. Scaiano, J. Am. Chem. Soc. 100 (1978) 296–298.
[35] Z.B. Alfassi, Peroxyl Radicals, John Wiley & Sons, Chichester, 1997.
[36] P. Wardman, J. Phys. Chem. Ref. Data 18 (1989) 1637–1755.
[37] G. Merényi, J. Lind, L. Engman, J. Chem. Soc. Perkin Trans. 2 (1994) 2551–2553.