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**PAPER**

# Laser flash photolysis study on the retinol radical cation in polar solvents†

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Laser flash photolysis (LFP) of retinol in argon-saturated methanol gives rise to a transient at 580 nm (transient A). Formation of transient A is accompanied by a transient growth at 370 nm. The rate of this growth is retinol concentration-dependent. The transient growth at 370 nm was removed in the presence of $N_2O$, which is known to scavenge solvated electrons. These results can be interpreted by formation of retinol$^{\bullet+}$ ($\lambda_{max}$ = 580 nm) and solvated electrons following LFP of retinol. Subsequently, the solvated electrons are rapidly scavenged by retinol to form retinol$^{\bullet-}$ ($\lambda_{max}$ = 370 nm in methanol). On the other hand, transient A is not ascribed to the retinyl cation, as was previously proposed, because the retinyl cation, generated from LFP of retinyl acetate, and transient A show different reactivities towards halide ions (*e.g.* $k_{Br} = 1.7 \times 10^9$ and $1.51 \times 10^{10}$ $M^{-1}$ $s^{-1}$ respectively, in acetonitrile). After demonstrating the identity of transient A as retinol$^{\bullet+}$, its reactions with carotenoids were examined in air-saturated polar solvents. In the presence of carotenoids, an enhancement in the decay of retinol$^{\bullet+}$ was observed and was accompanied by formation of the corresponding carotenoid radical cations *via* electron transfer from carotenoids to retinol$^{\bullet+}$. Furthermore, the reactivity of retinol$^{\bullet+}$ towards pyridine derivatives was investigated in air-saturated polar solvents. It was found that the decay of retinol$^{\bullet+}$ was accelerated with concomitant formation, with the same rate, of a transient at 370 nm. Similar observations were obtained with increasing pH of air-saturated aqueous 2% Triton X-100 of retinol$^{\bullet+}$. The 370 nm (or 380 nm in the case of Triton X-100) transient is attributed to the base adducts or deprotonated neutral radicals. On the basis of these results, the reactivities of the retinyl cation and retinol$^{\bullet+}$ are compared and the consequences of retinol$^{\bullet+}$ formation within biological environments are discussed.

## Introduction

Retinoids have many biological roles such as vision, reproduction and normal cell differentiation.[1] Retinoids also constitute one of the major ingredients in many cosmetic products.[1] However, there are some reports about the phototoxicity and photocarcinogenicity of retinol (vitamin A).[1,2] Thus, it is important to investigate the photochemistry of retinol in order to understand the mechanism behind these deleterious effects.

The first singlet excited state ($S_1$) of retinol (327 kJ $mol^{-1}$ and lifetime ~2.3 ns) is characterized by low quantum yields of fluorescence and intersystem crossing (~0.006 and ~0.003, respectively) in polar solvents.[3,4] Furthermore, the triplet energy of retinol was estimated as 140–150 kJ $mol^{-1}$ in non-polar solvents.[5,6]

Laser flash photolysis (LFP) of retinol in a variety of polar solvents led to the formation of a strong transient ($\lambda_{max}$ = 580–590 nm, transient A), which was attributed to either the retinyl cation[7–11] or a mixture of a retinol radical cation and the retinyl cation.[12] Chattopadhyay *et al.*[9] proposed photoejection of an

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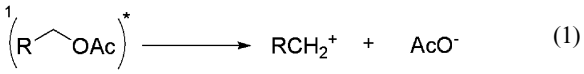
† Electronic supplementary information (ESI) available: Transient spectra of retinol$^{\bullet+}$, $^3$retinol*, retinyl cation, APO$^{\bullet+}$ and their kinetic absorption profiles in polar solvents; transient spectra of retinol$^{\bullet+}$ in the presence of pyridine in polar solvents; kinetic absorption profiles at 580 and 370 nm obtained following LFP (355 nm) of retinol (or retinyl acetate) in the presence of pyridine derivatives (or different carotenoids or tetra-*n*-butylammonium halides) in polar solvents; kinetic absorption profiles at 580 and 380 nm (obtained following LFP (355 nm) of retinol in aqueous TX-100) at pH = 10.5, influence of air, argon and $N_2O$ on kinetic absorption profiles at 370, 400 and 580 nm (obtained following LFP (355 nm) of retinol) in polar solvents; influence of retinol concentration on the rate of secondary growth of the transient at 370 nm (obtained following LFP (355 nm) of retinol) in methanol; dependence of the intensity of retinol$^{\bullet+}$ on laser energy; dependence of the intensity of transient at 380 nm (obtained following LFP (355 nm) of retinol in aqueous TX-100) on pH; dependence of $k_{obs}$ for the decay of retinol$^{\bullet+}$ and retinyl cation on tetra-*n*-butylammonium halide concentration in polar solvents; dependence of $k_{obs}$ for the decay of retinol$^{\bullet+}$ on pyridine derivative concentration (or carotenoid concentration) in polar solvents; scheme for the reaction of solvated electrons with $N_2O$ gas in aqueous solutions; scheme for the possible pathways for the reaction of retinol$^{\bullet+}$ with pyridine in polar solvents and scheme for *cis–trans* isomerization *via* retinyl cation. See DOI: 10.1039/c1ob05814b

electron as the primary step following LFP of retinol in polar solvents. It is difficult to determine the real identity of this transient because of the spectral resemblance of the retinol radical cation and the retinyl cation.[13–15] Many reports, which proposed transient A as the retinyl cation, based their evidence on the absence of any spectral absorbance due to solvated electrons.[8,11]

In order to clarify the identity of transient A, the best approach is to generate either the retinol radical cation or the retinyl cation by alternative methods and compare their reactivities with that of transient A. The retinyl cation ($\lambda_{max}$ = 590 nm in acetonitrile) can be easily produced following LFP of retinyl acetate in polar solvents (eqn (1)).[8,10,11,13,16–18]




(1)

In this manuscript, the well-established photochemistry of retinyl acetate has been used as a reference to investigate the identity of transient A. This has been achieved by comparing the kinetic behaviour of both the transients, transient A and retinyl cation, towards various substances, such as halides. Moreover, the reactions of transient A with carotenoids (Fig. 1) and different pyridine derivatives in methanol and benzonitrile have been investigated to further clarify the reactivity of transient A. The influence of pH on the decay of transient A (identified later as the retinol radical cation) has also been studied in aqueous 2% Triton X-100.

## Results and discussion

### (A) Identification of transient A (at 580 nm)

**(1) LFP of retinol in air and $N_2O$-saturated solutions.** LFP (355 nm) of an argon-saturated methanolic solution of retinol leads to the formation of two transients at 580 and 400 nm (Fig. 2). The transient at 400 nm was previously identified as triplet retinol ($^3$retinol*: * denotes an excited state).[3,9,11,19] This has been confirmed by the disappearance of this transient in an air-saturated solution. In addition, similar to previous reports,[9,12] LFP of retinol in an argon-saturated methanolic solution does not show any absorption, due to the solvated electrons. However, there are other examples of photoionization processes in which solvated electrons were not observed in the literature.[9,20] Many reasons were given in order to explain this phenomenon.[9,12]

One possibility, which has not been examined before with retinol, is the fast reaction between solvated electrons and retinol to form the retinol radical anion (retinol$^{\bullet-}$), which has a $\lambda_{max}$ at 370 nm and decays slowly (on a millisecond time scale) in methanol.[23,24a] Therefore, if retinol$^{\bullet-}$ is formed, different kinetic behaviours will be observed within 360–420 nm range due to the spectral overlap between $^3$retinol* and retinol$^{\bullet-}$ in this range. Fig. 3 confirms the formation of more than one transient within this range. Moreover, there is a transient growth at 370 nm, which is retinol concentration-dependent, at shorter time scales (Fig. 4). By varying retinol concentrations, the rate constant ($k_{retinol}$) was determined to be 1.5 ± 0.5 × $10^{10}$ $M^{-1}$ $s^{-1}$ (measured using two different concentrations of retinol; see Fig. S2†), which is in agreement with the reported rate constant for the reaction of solvated electrons with retinol in methanol ($k = 1.3 \times 10^{10}$ $M^{-1}$ $s^{-1}$)[24a] In addition, this growth is removed after saturation of the solution

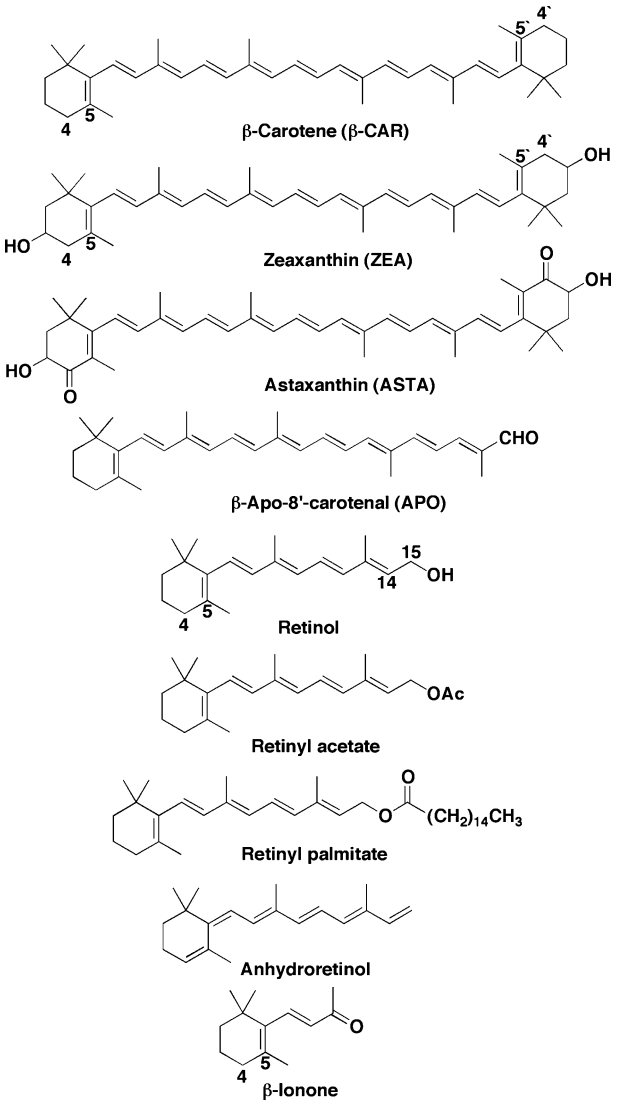



**Fig. 1** Structures of retinoids and carotenoids used in this study.

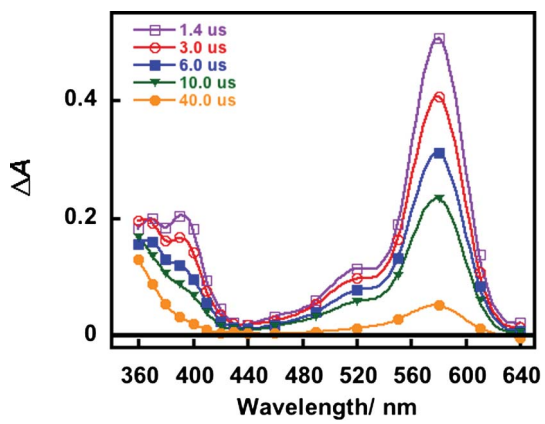



**Fig. 2** Transient spectra obtained following LFP (355 nm) of retinol (~1 × $10^{-4}$ M) in argon-saturated methanol (laser energy: ~15 mJ).[21]

with nitrous oxide gas,[23] which is known to be an electron trap (Fig. 4 and Scheme 1). Furthermore, in an air-saturated solution, the signal intensities of the transient profiles, within the range of

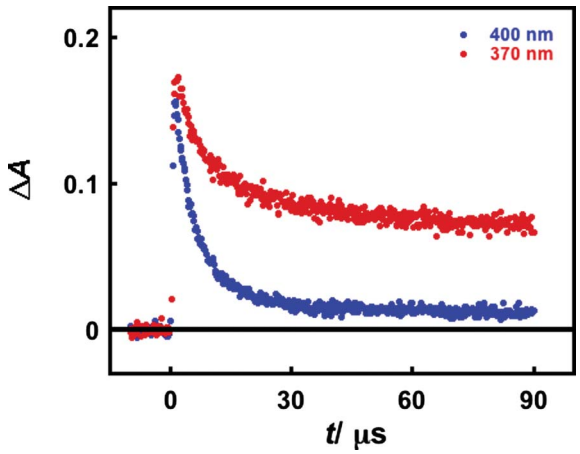



**Fig. 3** Transient profiles, obtained following LFP (355 nm) of retinol (~1 × $10^{-4}$ M), at 370 and 400 nm in argon-saturated methanol (laser energy: ~15 mJ).

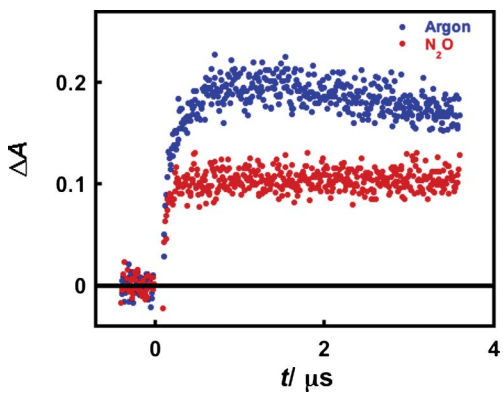



**Fig. 4** Transient profiles, obtained following LFP (355 nm) of retinol (~1 × $10^{-4}$ M), at 370 nm in argon or $N_2O$-saturated methanol (laser energy: ~15 mJ).

$$e^-_{MeOH} + N_2O \longrightarrow N_2 + O^-$$

$$O^- + CH_3OH \longrightarrow {}^-OH + {}^\bullet CH_2OH$$

**Scheme 1**

360 and 420 nm, are greatly reduced due to the scavenging of the transients formed by oxygen (Fig. S3†).[24,25] Also, a similar trend has been observed following LFP of retinol in aqueous 2% (w/v) Triton X-100 (See Fig. S5 and S6†).[26,27]

The results described above clearly demonstrate the formation of retinol$^{\bullet-}$ following LFP of retinol in methanol and aqueous 2% TX-100 and this can be taken as a direct evidence for the formation of retinol$^{\bullet+}$ *via* a photoionization process (Scheme 2).

$$\text{Retinol} \xrightarrow{h\nu} \text{Retinol}^{\bullet+} + e^-_{Solv}$$

$$e^-_{Solv} + \text{Retinol} \longrightarrow \text{Retinol}^{\bullet-}$$

**Scheme 2**

A similar observation was reported for the photoionization of biphenyl in alcohols.[30]

**(2) Reactivities toward halides.** LFP of retinol and retinyl acetate in air-saturated methanol and acetonitrile gives rise to a strong transient at 580 nm (transient A and the retinyl cation respectively) and a very weak transient in the range of 360–400 nm (eqn (1) and Fig. S7–S10†). This weak transient can be attributed to a ketyl radical (~390 nm)[9,31,32] and anhydroretinol (350–420 nm)[33] formed following the deprotonation of retinol$^{\bullet+}$ and the retinyl cation, respectively (Scheme 3 and Fig. 1).

In the absence of any additives, the transients (at 580 nm) formed following LFP of retinol and retinyl acetate in methanol and acetonitrile show different kinetic behaviour (Fig. 5 and S11†). Moreover, in the presence of bromide ions, which are used as tetra-*n*-butylammonium bromide, the rates of decay of both the retinyl cation and transient A are enhanced and lead to the formation of strong transients at ~370 nm. The rate of decay of the 580 nm transient matches the rate of formation of the transient at 370 nm (Fig. 6 and S12†). The transient (at 370 nm) can be attributed to addition products formed from the reactions of bromide ions with these transients (eqn (2)).[34–38]

$$\text{Retinol}^{\bullet+} + Br^- \rightarrow \text{Br–Retinol}^{\bullet} \quad (2)$$

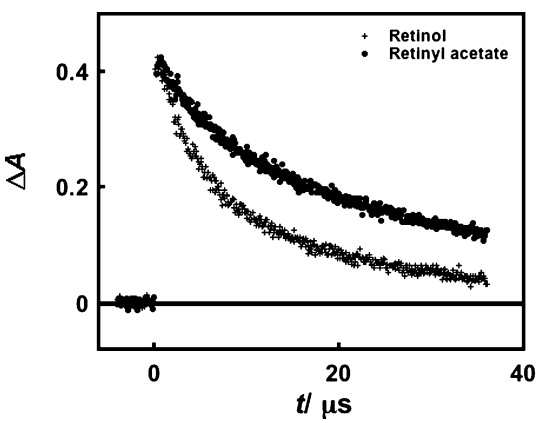
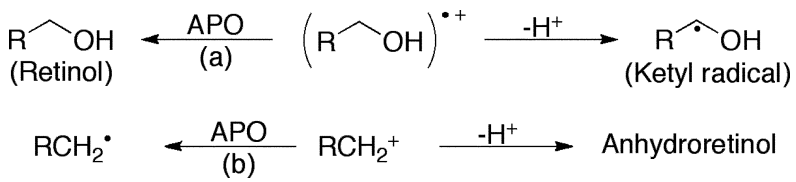



**Fig. 5** Normalized kinetic absorption profiles at 580 nm obtained following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) or retinyl acetate (Abs. at 355 nm ~0.8 in a 1 cm cell) in air-saturated methanol (laser energy: ~15 mJ).

The rate constants ($k_X$) for the reactions of halide ions with the transients formed following LFP of retinol and retinyl acetate in




**Scheme 3**

**Table 1** Rate constants ($k_X$) for the reactions of 580 nm transients, formed following LFP of retinol or retinyl acetate, with halide ions in various solvents

| | $k_X/10^{10}$ M$^{-1}$ s$^{-1}$ | | | |
|---|---|---|---|---|
| | Retinol | | Retinyl acetate | |
| $X^-$ | Acetonitrile | Benzonitrile | Acetonitrile | Benzonitrile |
| $Br^-$ | 1.51 ± 0.10 | 1.33 ± 0.06 | 0.17 ± 0.01 | 0.92 ± 0.04 |
| $Cl^-$ | 1.33 ± 0.10 | 1.19 ± 0.05 | 0.12 ± 0.03 | 0.82 ± 0.05 |

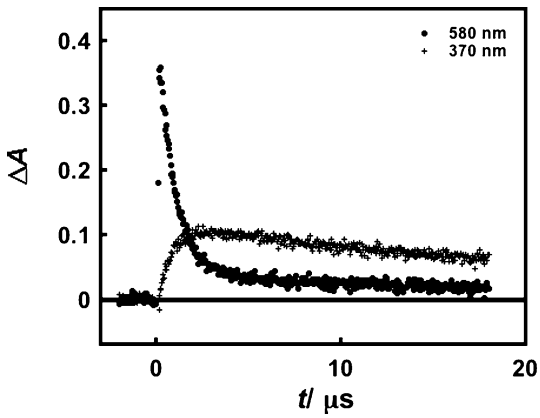



**Fig. 6** Kinetic absorption profiles at 580 and 370 nm obtained following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) and tetra-*n*-butylammonium bromide ($5.0 \times 10^{-5}$ M) in air-saturated acetonitrile (laser energy: ~15 mJ).

acetonitrile and benzonitrile are estimated from the plot of the observed pseudo-first-order rate constant ($k_{obs}$) for the decay of either transient at 580 nm *versus* halide ion concentration using eqn (3), where $k_0$ (intercept) is the apparent rate constant for the decay of either transient in the absence of halide ion (See Table 1 and Fig. 7 and S13–S15†).

$$k_{obs} = k_0 + k_X [X^-] \quad (3)$$

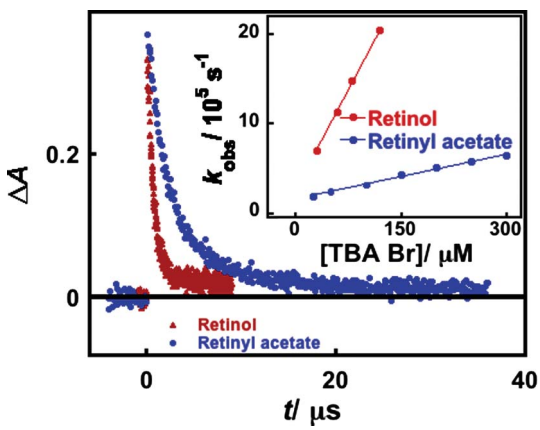
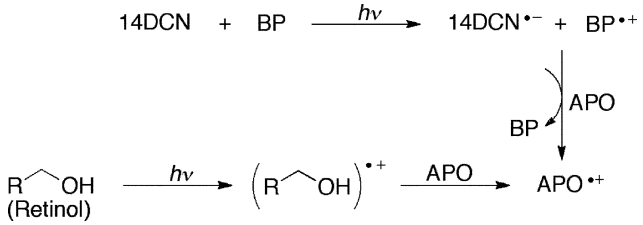



**Fig. 7** The influence of tetra-*n*-butylammonium bromide ($1 \times 10^{-4}$ M) on the transient profiles at 580 nm formed following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) or retinyl acetate (Abs. at 355 nm ~0.8 in a 1 cm cell) in air-saturated acetonitrile (laser energy: ~15 mJ). The inset shows plots of pseudo-first-order rate constants ($k_{obs}$) for the decay of the transient profiles at 580 nm *versus* tetra-*n*-butylammonium bromide concentration.

The rate constants for the reactions of halide ions with the retinyl cation in acetonitrile are in agreement with the reported rate constants for the same reactions ($k_{Cl}$ and $k_{Br}$ in acetonitrile are 2.6 and $1.4 \times 10^9$ M$^{-1}$ s$^{-1}$, respectively).[12,13] Also, the rate constants for the reactions of halide ions with transient A are within the same order of magnitude as the previously studied reaction, using a pulse radiolysis technique, of bromide ion with retinol$^{\bullet+}$ in acetone ($k_{Br} = 4.1 \times 10^{10}$ M$^{-1}$ s$^{-1}$).[34,39]

The one order of magnitude difference between the rate constants for the reactions of halide ions with the retinyl cation and transient A in acetonitrile (Table 1 and Fig. 7) as well as the different kinetic reactivity toward halides in benzonitrile (Table 1 and Fig. S13 and S14B†) and methanol (Fig. S17†)[40] clearly indicate that transient A, formed following LFP of retinol in acetonitrile, benzonitrile and methanol, is not the retinyl cation.

**(3) Reactivities toward β-apo-8′-carotenal (APO).** After demonstrating the significant difference in the reactivity of transient A from that of the retinyl cation, it is important to confirm that transient A is indeed retinol$^{\bullet+}$. For this purpose, 1,4-dicyanonaphthalene (14DCN) with biphenyl (BP) as a cosensitizer[41–43] have been employed to generate the transient spectra of the β-apo-8′-carotenal radical cation (APO$^{\bullet+}$) in methanol ($\lambda_{max} = 820$ nm)[44] and benzonitrile (Scheme 4). Similar spectra have been obtained following LFP of retinol in the presence of APO in the same solvents (Fig. 8 and S18†). These results support the formation of retinol$^{\bullet+}$, following LFP of retinol in methanol and benzonitrile, as a precursor for APO$^{\bullet+}$ (Scheme 4).




**Scheme 4**

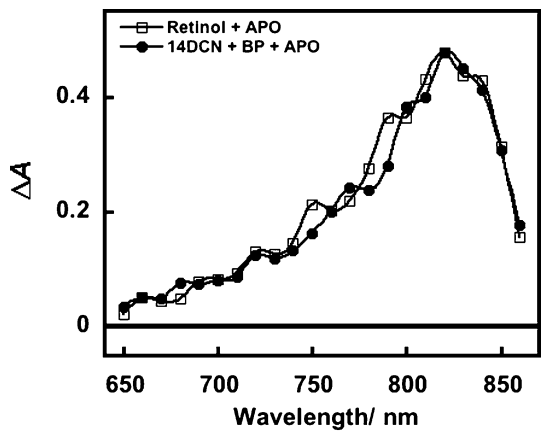



**Fig. 8** Normalized transient spectra of APO$^{\bullet+}$ obtained following LFP (355 nm) of (1) retinol ($4.5 \times 10^{-5}$ M) and APO ($1.0 \times 10^{-4}$ M) after 5 μs (laser energy: ~20 mJ) or (2) 1,4-dicyanonaphthalene ($5.0 \times 10^{-3}$ M), biphenyl (0.3 M) and APO ($1.0 \times 10^{-4}$ M) after 3 μs (laser energy: ~10 mJ) in air-saturated methanol.

Moreover, LFP of retinol and APO (~$1 \times 10^{-4}$ M) in air-saturated methanol leads to the disappearance of the weak transient ketyl radical (360–400 nm) due to the new competitive pathway (a) available for retinol$^{\bullet+}$ (Scheme 3 and Fig. 9 and S7†). This also supports the identification of transient A as retinol$^{\bullet+}$. On the other hand, LFP of retinyl acetate in the presence of APO (~$1 \times 10^{-4}$ M) gives rise to transient profiles (Fig. 9 and S9†) within the same spectral range due to the formation of retinyl radical (pathway b), which absorbs within the same spectral range (370–400 nm)[34] as anhydroretinol (Scheme 3).

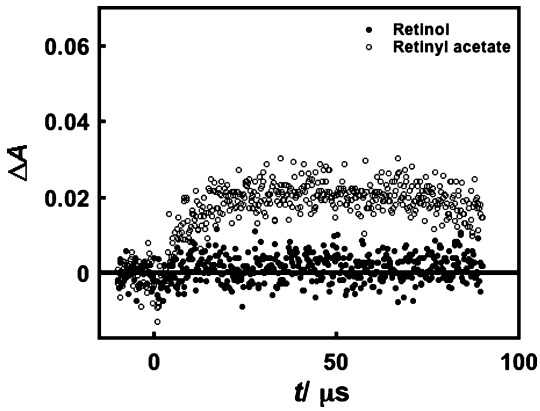



**Fig. 9** Kinetic absorption profiles at 370 nm obtained following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) or retinyl acetate (Abs. at 355 nm ~0.8 in a 1 cm cell) in the presence of APO ($1.0 \times 10^{-4}$ M) in air-saturated methanol (laser energy: ~15 mJ).

It is important to note that the formation of anhydroretinol is confirmed by its detection following photoirradiation of either retinyl acetate or retinyl palmitate in polar solvents.[2,18,45–48] Therefore, formation of anhydroretinol can be taken as a probe for the retinyl cation formation. In the photoirradiation of retinol in ethanol, anhydroretinol was not detected to any extent.[48] This again supports the formation of retinol$^{\bullet+}$, instead of the retinyl cation, following LFP of retinol.

### (B) Influence of laser energy on photoionization

Generally, photoionization is a biphotonic process, however, there are many examples of monophotonic photoionization processes.[30,49,50] In the literature, both monophotonic and biphotonic processes were reported for photolysis of retinol in polar solvents.[9,11,12] Under our experimental conditions, the plots of the yield of the 580 nm transient *versus* laser energy are linear in methanol, benzonitrile and aqueous 2% Triton X-100 (Fig. S19†). This suggests (but does not demonstrate) that the photoionization of retinol is a monophotonic process.[51]

### (C) Reactions with carotenoids

The reactivity of retinol$^{\bullet+}$ towards various carotenoids (β-carotene (β-CAR), zeaxanthin (ZEA), astaxanthin (ASTA) and APO in Fig. 1) has been investigated in methanol and benzonitrile. For example, in the presence of ASTA, the rate of decay of retinol$^{\bullet+}$ is accelerated and a transient growth of the ASTA$^{\bullet+}$ is observed (Fig. 10 and eqn (4)). Similar trends have been observed with other carotenoids (Fig. S20–S22†).[52]

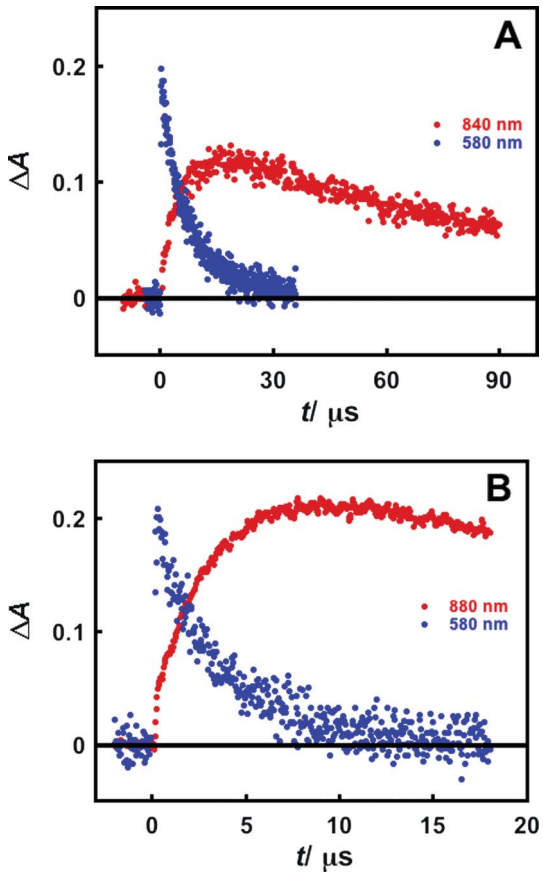



**Fig. 10** Kinetic absorption profiles at (A) 580 and 840 nm obtained following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) in the presence of ASTA (~$2 \times 10^{-5}$ M) in air-saturated methanol (laser energy: ~10 mJ) and (B) 580 and 880 nm obtained following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) in the presence of ASTA (~$8 \times 10^{-5}$ M) in air-saturated benzonitrile (laser energy: ~25 mJ).

$$\text{Retinol}^{\bullet+} + \text{ASTA} \rightarrow \text{Retinol} + \text{ASTA}^{\bullet+} \quad (4)$$

By varying the carotenoid concentration, the rate constants ($k_{CAR}$) for the reactions of retinol$^{\bullet+}$ with carotenoids were determined from the plot of the observed pseudo-first-order rate constant ($k_{obs}$) for the decay of retinol$^{\bullet+}$ at 580 nm *versus* carotenoid concentration using eqn (5) (See Table 2 and Fig. S23–S26†).

$$k_{obs} = k_0 + k_{CAR}\,[\text{CAR}] \quad (5)$$

The variance of the rate constants in Table 2 reflects the differences in the electron-donor ability of the carotenoids. Therefore, rate constants for the reactions of retinol$^{\bullet+}$ with carotenoids can be taken as a guide for the relative reduction potentials of carotenoid radical cations (See Scheme 5). According to Scheme 5, β-CAR$^{\bullet+}$ has the lowest reduction potential whereas retinol$^{\bullet+}$ has the highest reduction potential *i.e.* β-carotene is able to reduce zeaxanthin$^{\bullet+}$, astaxanthin$^{\bullet+}$, APO$^{\bullet+}$ and retinol$^{\bullet+}$. This conclusion is in broad agreement with the results of previous reports.[53–55]

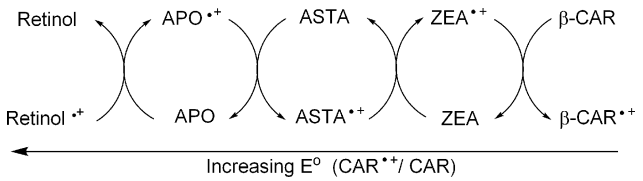
### (D) Reactions of retinol$^{\bullet+}$ with pyridine derivatives in polar solvents and the influence of pH on retinol$^{\bullet+}$ in aqueous 2% Triton X-100

**(1) Reactions of retinol$^{\bullet+}$ with pyridine derivatives in polar solvents.** There are very few time-resolved studies about the

**Table 2** Rate constants ($k_{CAR}$) for the reactions of retinol$^{\bullet+}$ with carotenoids in various solvents

| CAR | $n_{db}$[a] | $\varepsilon_{MeOH}$[b] ($\lambda_{max}$)/$10^4$ $M^{-1}$ $cm^{-1}$ | $\varepsilon_{BZN}$[c] ($\lambda_{max}$)/$10^4$ $M^{-1}$ $cm^{-1}$ | $k_X/10^9$ $M^{-1}$ $s^{-1}$ | |
|---|---|---|---|---|---|
| | | | | Methanol ($\lambda_{max}$) | Benzonitrile |
| APO | 9 | 4.3 (465) | 3.86 (477) | 2.38 ± 0.20 (820)[d,e] | 1.37 ± 0.12 |
| ASTA | 11 | 12.1 (478)[d] | 8.41 (495) | 4.54 ± 0.42 (840)[d,e] | 2.40 ± 0.22 |
| ZEA | 11 | 14.3 (452)[d] | 10.25 (468) | 5.76 ± 0.50 (910)[d,e] | 2.50 ± 0.20 |
| β-CAR | 11 | 12.8 (449)[d] | 11.64 (470) | ND[f] (900)[g] | 3.71 ± 0.25 |

[a] $n_{db}$: Number of conjugated double bond. [b] $\varepsilon_{MeOH}$: Molar absorption coefficient of carotenoid in methanol. [c] $\varepsilon_{BZN}$: Molar absorption coefficient of carotenoid in benzonitrile. [d] See ref. 44. [e] See ref. 54. [f] ND: The rate constant is not determined because of the poor solubility of β-CAR in methanol. [g] See ref. 29.




**Scheme 5**

reactions of retinoid$^{\bullet+}$ with bases.[34,56] In these studies, it was shown that the rate constants for the reactions of various retinoid$^{\bullet+}$ with triethylamine depend on the length of the conjugated polyene chain and the functional groups (*e.g.* HO, COOH, CHO) attached to it.[34,56]

LFP (355 nm) of methanolic solution of retinol ($4.5 \times 10^{-5}$ M) in the presence of pyridine (1.0 M) is shown in Fig. 11 and S27†. In the presence of pyridine, the decay of retinol$^{\bullet+}$ ($\lambda_{max}$ = 580 nm) is enhanced and another transient ($\lambda_{max}$ = 370 nm) is generated with a similar rate (Fig. 11). By varying the concentration of pyridine and maintaining it higher than that of the generated retinol$^{\bullet+}$, the rate constant ($k_{base}$) for the reaction of retinol$^{\bullet+}$ with pyridine was determined as $7.05 \times 10^5$ $M^{-1}$ $s^{-1}$ from the plot of the observed pseudo-first-order rate constant ($k_{obs}$) for the decay of retinol$^{\bullet+}$ at 580 nm *versus* pyridine concentration using eqn (6), where $k_0$ (intercept) is the apparent rate constant for the decay of retinol$^{\bullet+}$ in the absence of pyridine (Table 3 and Fig. S28†).[57,58]

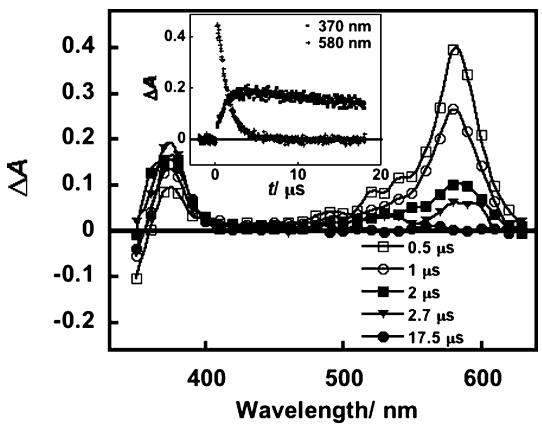



**Fig. 11** Transient spectra obtained following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) and pyridine (1.0 M) in air-saturated methanol (laser energy: ~25 mJ). The inset shows kinetic absorption profiles at 370 and 580 nm.

$$k_{obs} = k_o + k_{Base}\,[Base] \quad (6)$$

**Table 3** Rate constants ($k_{Base}$) for the reactions of retinol$^{\bullet+}$ with pyridine derivatives in methanol and benzonitrile

| Pyridine derivative | p$K_{aH}$[a] | $k_{Base}/10^5$ $M^{-1}$ $s^{-1}$ | |
|---|---|---|---|
| | | Methanol | Benzonitrile |
| Pyridine | 5.29 | 7.05 ± 0.30 | 32.20 ± 1.50 |
| 2-Aminopyridine | 6.82 | 21.22 ± 1.00 | 57.20 ± 5.00 |
| 4-Aminopyridine | 9.17 | 39.20 ± 2.70 | 482.00 ± 25.00 |
| 2,6-Dimethylpyridine | 6.75 | 0.56 ± 0.06 | 1.64 ± 0.15 |

[a] p$K_{aH}$ values are taken from reference 61

Similarly, the rate constants for the reaction of retinol$^{\bullet+}$ with various pyridine derivatives were estimated in methanol and benzonitrile (See Table 3 and Fig. S28† and S31–S34†). In Table 3, it is clear that the rate constants are directly correlated with the basicities of pyridine derivatives. For 2,6-dimethylpyridine (p$K_{aH}$ = 6.75), the rate constant is smaller than expected due to the steric effect of the two methyl groups (Table 3 and Fig. S32†).[42,59]

It can also be seen that the rate constants in benzonitrile are larger than those in methanol (Table 3). This can be attributed to hydrogen bonding between pyridine derivatives and methanol which will decrease the reactivities of pyridine derivatives.[60] Moreover, there is little influence on the decay of retinol$^{\bullet+}$ in the presence of one molar of 2-cyanopyridine (p$K_{aH}$ = 1.12) due to its lower basicities (Fig. S35†).[61]

The previous results clearly indicate that the retinol$^{\bullet+}$ reactivity towards pyridine derivatives depends on the basicity of the pyridine derivative, the characteristics of the solvent medium and steric hindrance.

**(2) Influence of pH on retinol$^{\bullet+}$ in aqueous 2% Triton X-100.** In order to study the influence of pH change on the decay of retinol$^{\bullet+}$, retinol was solubilized in 2% Triton X-100 and the effect of pH change (range: 6.0–13) on the yield of transients formed on microsecond time scales was investigated. With increasing pH, the retinol$^{\bullet+}$ decay is enhanced with concomitant formation of a transient at 380 nm (Fig. 12 and S36†). The plot of pH *versus* the yield of the transient at 380 nm on a microsecond time scale is given in Fig. 13. The rate constant for the reaction of retinol$^{\bullet+}$ with $^-$OH was determined to be $4.57 \times 10^8$ $M^{-1}$ $s^{-1}$ from the plot of the observed pseudo-first-order rate constant ($k_{obs}$) for the decay of retinol$^{\bullet+}$ at 580 nm *versus* $^-$OH concentration using eqn (6) (Fig. 14).

In general, addition and deprotonation pathways are proposed for the reactions of radical cations with bases.[62–68]

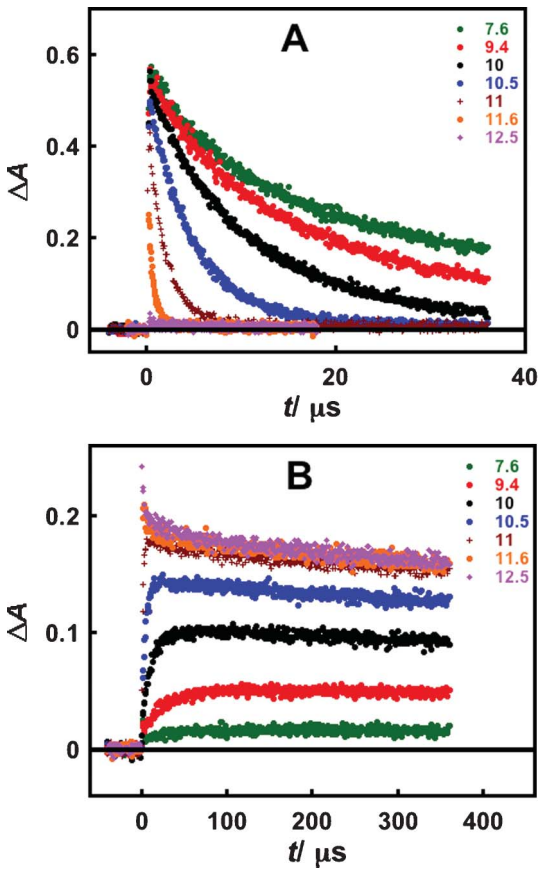



**Fig. 12** The influence of pH on the transient profiles at (A) 580 nm and (B) 380 nm following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) in air-saturated aqueous 2% Triton X-100 (laser energy: ~25 mJ).

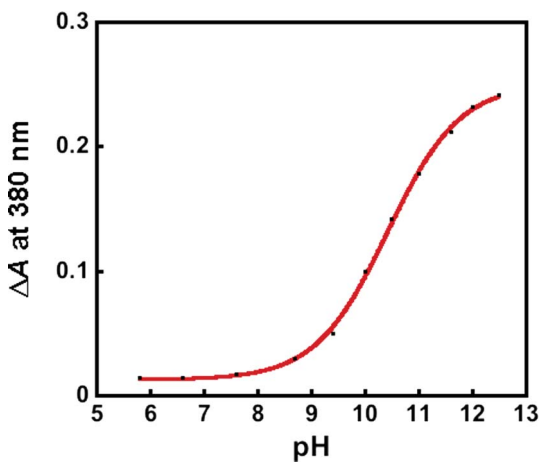



**Fig. 13** Plot of $\Delta A$ *versus* pH for absorbance at 380 nm obtained following LFP (355 nm) of retinol ($4.5 \times 10^{-5}$ M) in air-saturated aqueous 2% Triton X-100 (laser energy ~25 mJ). If deprotonation reaction is the main pathway for the reaction of retinol$^{\bullet+}$ with $^{-}$OH, the p$K_a$ was calculated as 10.4 using a Boltzmann fit.

For deprotonation reactions, many theoretical studies have evaluated the stabilities of different carotenoid neutral radicals generated by proton loss from different positions.[69–74] For example, the most energetically favorable positions for proton loss from the zeaxanthin radical cation, to form the deprotonated zeaxanthin

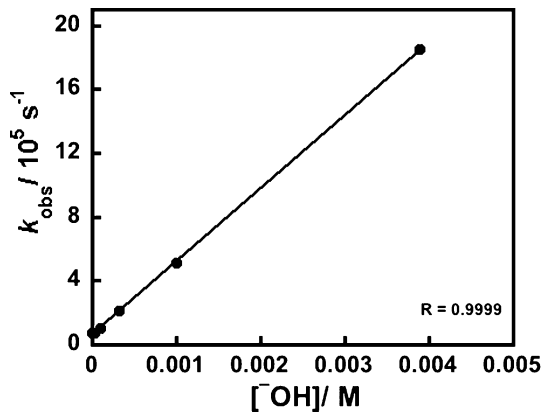



**Fig. 14** Plot of the pseudo-first-order rate constants ($k_{obs}$) for the decay of retinol$^{\bullet+}$ at 580 nm *versus* $^{-}$OH concentration in air-saturated aqueous 2% Triton X-100 (laser energy: ~25 mJ).

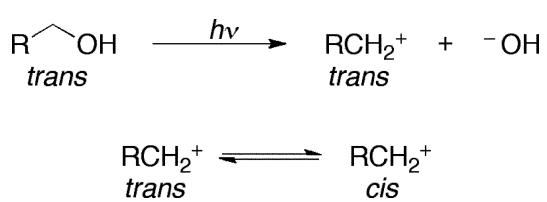
neutral radical ($^{\#}$ZEA$^{\bullet}$), are 4, 4′ and methyl groups at the 5 and 5′ positions (Fig. 1 and eqn (7)).[71]

$$\text{ZEA}^{\bullet+} \rightarrow {}^{\#}\text{ZEA}^{\bullet} + \text{H}^{+} \quad (7)$$

Also, the 4 position and the methyl group at the 5 position are the favorable positions for proton loss from β-ionone radical cation (Fig. 1).[68] Based on the results of these studies, the most probable sites for proton loss will be 4, 15 and the methyl group at the 5 position in retinol$^{\bullet+}$ (Fig. 1).[68]

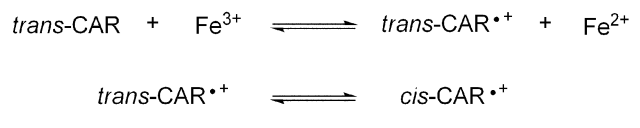
For addition reactions, the positions forming the longest possible delocalized neutral radicals, *i.e.* the most stable radicals, upon nucleophilic addition are the most probable sites for addition reactions. Therefore, it can be concluded that the 5 and 14 positions in retinol$^{\bullet+}$ are the most probable sites for the addition of $^{-}$OH (or pyridine derivatives) to retinol$^{\bullet+}$ (Fig. 1).[35] The addition and deprotonation pathways are outlined for the reaction of retinol$^{\bullet+}$ with pyridine in Scheme S2†.

***cis–trans* Photoisomerization.** In contrary to our conclusion about the formation of retinol$^{\bullet+}$, instead of the retinyl cation after the photoexcitation of retinol, Rosenfeld *et al.*[10] proposed that the *cis–trans* photoisomerization of retinol in polar solvents proceeded *via* the formation of the retinyl cation (Scheme 6).[10,75] However, there are many examples in the literature,[76] which involve radical cations as intermediates in the *cis–trans* isomerization reactions (*e.g.* carotenoid radical cations and stilbene radical cations (Scheme 7)). Therefore the *cis–trans* photoisomerization of retinol in polar solvents can be interpreted *via* the formation of retinol$^{\bullet+}$.




**Scheme 6**

**Radical cations *versus* cations.** Since retinol$^{\bullet+}$ and the retinyl cation are closely related species, they can be used as an approximate model to compare the reactivities of radical cations and



**Scheme 7**

cations towards nucleophiles under similar reaction conditions.[77,78] Our experimental results indicate that retinol$^{\bullet+}$ is more reactive towards halide ions than the retinyl cation and their reactivities are solvent-dependent (Table 1). Moreover, retinol$^{\bullet+}$ and the retinyl cation show similar reactivities towards pyridine (Fig. S37†), which is in agreement with the results by Johnston and Schepp,[36] who reported that the rate constants for nucleophilic addition to the styrene radical cation are similar in magnitude to those observed for the related arylmethyl, diarylmethyl and vinyl cations. Thus, depending on the nature of the nucleophile and solvent used, the reactivities of radical cations are either higher than or similar to those of closely related cations.[79]

**Formation of retinol$^{\bullet+}$ in biological environments.** The consequences of retinol$^{\bullet+}$ formation in biological environments, as a result of photoexcitation or free radical oxidation of retinol, depends on its reactivity. Since retinol$^{\bullet+}$ is able to oxidize APO (Fig. 8 and S18†), it can be considered as a strong oxidant.[81] Furthermore, the reactions of neutral radicals, formed following deprotonation or addition reactions of retinol$^{\bullet+}$, with oxygen to form peroxyl radicals can induce pro-oxidant effects. Therefore, formation of retinol$^{\bullet+}$ in biological environments is expected to have harmful effects. To shed light on these harmful effects, the reactivity of retinol$^{\bullet+}$ towards various biological molecules is under investigation.

## Conclusions

The conclusions that arise from the work reported in this paper are as follows:

(i) LFP of retinol in polar solvents leads to the formation of retinol$^{\bullet+}$.

(ii) The reactivity of retinol$^{\bullet+}$ towards pyridine derivatives and halide ions is dependent on the characteristics (polarity and hydrogen bonding ability) of the solvent.

(iii) Depending on the nature of the nucleophile and solvent used, the reactivity of retinol$^{\bullet+}$ is either higher than or similar to that of the retinyl cation.

(iv) Addition or deprotonation reactions are proposed for the reactions of pyridine derivatives (or $^{-}$OH) with retinol$^{\bullet+}$.

(v) Using retinol$^{\bullet+}$, relative reduction potentials of CAR$^{\bullet+}$ were determined in different solvents.

## Experimental section

### Materials

Retinol (≥99%), retinyl acetate, Triton X-100, pyridine (≥99.9%), biphenyl (99.5%) and β-apo-8′-carotenal (≥96%) were purchased from Sigma. Methanol (99.8%), acetonitrile (99.8%), acetone (99.7%), naphthalene (98%), tetra-*n*-butylammonium bromide and 2-cyanopyridine (99%) were purchased from Nacalai Tesque. Tetra-*n*-butylammonium chloride, 1,4-dicyanonaphthalene (98%), 4-aminopyridine and 2,6-dimethylpyridine were purchased from Tokyo Chemical Industry. β-Carotene (Fluka, ≥97%), astaxanthin (Alexis Biochemicals, ≥97%), 2-aminopyridine (Aldrich, 99%), benzonitrile (Wako, 98%), and distilled water (Wako) were used as received. Zeaxanthin was kindly supplied by David J. McGarvey (School of Physical and Geographical Sciences, Keele University, Keele, Staffordshire ST5 5BG, UK) and was used as received. Argon (99.99%) was supplied by Yamazaki Sangyo and nitrous oxide (99%) was supplied by Sumitomo Seika Chemicals, Japan.

### Laser flash photolysis experiments

For the nanosecond laser flash photolysis experiments, solutions were excited by a Nd:YAG laser (Continuum, SLII-10, 4–6 ns fwhm) at 355 nm. Time courses of the transient absorption spectra were measured using a continuous Xe-lamp (150 W) and an In GaAs-PIN photodiode (Hamamatsu 2949) as a probe light and a detector, respectively. The output from the photodiodes and a photomultiplier tube was recorded with a digitizing oscilloscope (Tektronix, TDS3032, 300 MHz). The transient spectra were recorded using fresh solutions following each exposure to the laser. Quartz sample cells (5 mm excitation pathlength × 10 mm monitoring pathlength) were employed for the laser flash photolysis measurements. Argon and $N_2O$-saturated solutions were obtained by argon and $N_2O$ purging respectively, for 10 min prior to use. All experiments were performed at 298 K. The standard errors in the measurements are ±10% unless otherwise stated.

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40 The retinyl cation and transient A show different reactivities toward tetra-*n*-butylammonium chloride (0.12 M) in methanol (Fig. S17†). The low reactivities of these transients towards chloride ions in methanol can be attributed to hydrogen bonding between methanol and chloride ions. A similar trend was observed for the reaction of the retinyl cation with chloride ions in acetonitrile and acetonitrile containing 1.0 M water ($k_{Cl}$ = 2.6 and $0.033 \times 10^9$ $M^{-1}s^{-1}$, respectively)[13] and for the reactions of styrene radical cation derivatives with different halide ions in acetonitrile and water : acetonitrile (4 : 1)[36].
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