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Photoinduced alkaline pH-jump on the nanosecond time scale

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Abstract

The triphenylmethane leucohydroxide salt 4-(dimethylamino)-4′-(trimethylammonium) triphenylmethanol iodide has been used as a photoactivatable caged hydroxide to rapidly increase the pH of a neutral aqueous solution on the nanosecond time scale. Photoexcitation of the compound with a nanosecond ultraviolet laser pulse results in the formation of a colored carbocation with a rate greater than 10^8 s^{-1}; recombination occurs with a multiexponential kinetics. The dissociation is completely reversible on time scales of 10^2 s and offers a large time window after the pH-jump, in which the proton transfer reactions can be studied. The effectiveness of the photodissociation process to increase the pH of the solution has been demonstrated by means of the pH indicator bromoxylenol blue.

1. Introduction

There is a general interest in compounds that are able to release an active species under controlled conditions, the so-called 'caged compounds'. In particular, photocleavable caged compounds are a precious tool in the study of biological processes [1–3], especially in time-resolved studies of cellular functions and of macromolecular properties [4–6]. The recent development of time-resolved techniques based on pulsed-lasers has opened the possibility of investigating protein folding reactions on submillisecond time scales [7–12]. We are interested in investigating fast events in protein folding/unfolding triggered by proton transfer reactions. To this purpose, we have recently reported the use of photoactivatable caged protons to induce acid perturbations in solutions containing model polypeptides and proteins [5,6,13]. Proton transfer reactions are among the fastest known reactions and their kinetic characterization has become feasible only after the introduction of photoactivatable caged compounds that release protons or hydroxide ions [4,14]. Light-induced acidification was generally obtained by using aromatic alcohols or *o*-nitrobenzyl derivatives, whereas alkalinization was obtained by means of *N*-etherosubstituted aromatic compounds [3,14,15]. In solution, aromatic alcohols and *N*-etherosubstituted aromatic compounds afford short transient pH pulses, which disappear within a few microseconds after the molecules have relaxed to their electronic

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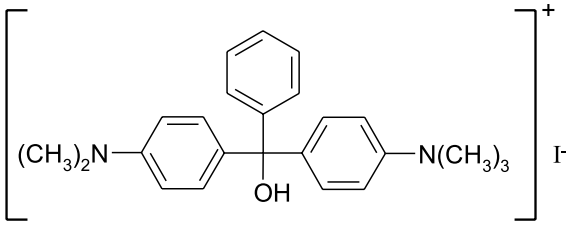
ground states [4,14–16]. On the contrary, compounds as *o*-nitrobenzaldehyde photorelease irreversibly the proton and achieve a step decrease in pH [17–19]. An interesting class of photosensitive compounds are triarylmethanes that, depending on the solvent, undergo either homolytic cleavage to give radicals [20,21] or heterolytic cleavage [22] upon ultraviolet illumination. Solutions of triphenylmethane leucohydroxide derivatives in polar solvents undergo heterolytic cleavage within a few nanoseconds from photolysis, proceeding from the excited singlet state [23,24]. Mostly, the tertiary alcohol is colorless, with a peak absorbance at 254 nm, whereas the carbocation formed upon photolysis is characterized by a green–blue color with absorption maxima at 420 and 620 nm [25]. The hydroxide ion is then reversibly bound via a thermal reaction which is complete in a few minutes [26]. In principle, a rapid increase in pH can be used to refold acid-denatured proteins, refold basic proteins from neutral solutions [27] or unfold neutral solutions of native proteins [28,29]. However, the photodissociation of triarylmethanes can be induced in aqueous solution at pH above 6 [25,26]. Therefore, the use of these compounds to photoinduce the alkalinization of the solution is limited to refolding/unfolding studies starting from neutral solutions. In this work we have performed a preliminary study on the kinetics of the recombination reactions that follow the nanosecond photodissociation of 4-(dimethylamino)-4′-(trimethylammonium) triphenylmethanol iodide ((**1**) in Scheme 1). The utility of the compound in kinetic investigations is shown for the alkaline induced deprotonation of the pH indicator bromoxylenol blue on the submillisecond time scale.



(**1**)

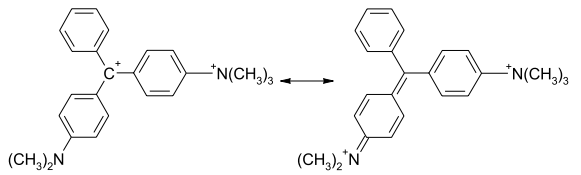
Scheme 1.

2. Materials and methods

The compound 4,4′-bis(dimethylamino) triphenylmethane leucohydroxide was synthesized by reaction between 4,4′-bis(dimethylamino)benzophenone and phenyl lithium in ether and subsequent hydrolysis with methanol. In order to enhance its solubility in water, the salt 4-(dimethylamino)-4′-(trimethylammonium) triphenylmethanol iodide ((**1**) in Scheme 2) was prepared by treating in toluene the neutral leucohydroxide with freshly distilled methyl iodide [26].

In the pH-jump experiments, the solutions were nitrogen saturated to avoid competition by carbonate buffering. The pH of the solutions was adjusted by addition of concentrated aqueous NaOH or HCl. Concentrations of (**1**) in flash photolysis experiments were kept at about 0.6 mM, affording an absorbance of about 1 (1 cm pathlength) at 308 nm.

The transient absorption experiments were conducted with an experimental setup already described [30]. Photoexcitation at 308 nm was obtained with an excimer laser (EMG50, Lambda Physik, 8 ns FWHM, 25 mJ). The beam was focused to a line with a 0.5 m cylindrical lens. The probe light in the pH-jump experiments and in the determination of the overall time course of the carbocation formation and recombination was a cw HeNe laser (Nec Corporation, 633 nm, 10 mW). The monitoring beam was passed inside a rectangular quartz cuvette (4 mm × 10 mm) along the 10 mm path, at right angle with the pump beam. The cuvette was held in a temperature controlled holder. The holder was flushed with nitrogen to prevent condensation at low temperatures and to minimize CO_2 uptake by the solutions during the kinetic experiments. The transmitted



(**2**) (**3**)

Scheme 2.

intensity of the cw beam was monitored by a large area Si avalanche photodiode (Hamamatsu, S2385) and the output voltage was amplified using two cascaded broadband operational amplifiers (Burr Brown, OPA643, gain ×49). The probe beam was passed through a monochromator (H25, Jobin Yvon) before being focused onto the Si avalanche photodiode to remove the high intensity stray light from the pump laser. The voltage signal was digitized by a digital sampling oscilloscope (LeCroy 9370, 1 GHz, 1 Gs/s). Typically 16 traces were averaged at the repetition rate of 1 shot every 3 min, to get a single signal. Care was taken in order to avoid thermal lensing effects in the measured signal.

Transient spectra were measured using a cw Xe lamp as detection source and removing the cylindrical lens from the pump beam path [31].

3. Results and discussion

3.1. Photodissociation and rebinding of the hydroxide ion

Fig. 1A and B shows the time course of the transient absorbance of an aqueous solution of compound (**1**), measured at 633 nm, following photolysis by a 308 nm laser pulse; the pre-pulse pH was 10.9 and $T = 293$ K. As expected, photolysis of (**1**) at pre-pulse pH above 6 leads to the formation of a colored species with rate constant above the experimental resolution of the setup ($\approx 10^8$ s^{-1}). The measured transient spectrum at the end of the laser pulse (see Fig. 1C) is consistent with the reported spectrum of the carbocation, formed upon heterolytic cleavage of the parent carbinol [24]. The decay of the transient absorbance occurs with a multiphasic behavior, extending from the nanosecond time scale (Fig. 1A) to the tens of seconds (Fig. 1B). We propose (vide infra) that the multiphasic decay of the transient absorbance can be associated with recombination reactions of the carbocation with hydroxide from the solution, characterized by different rate constants. The slowest recombination was known from the original work of Irie [26]. In our experiments we consistently found the presence of ad-

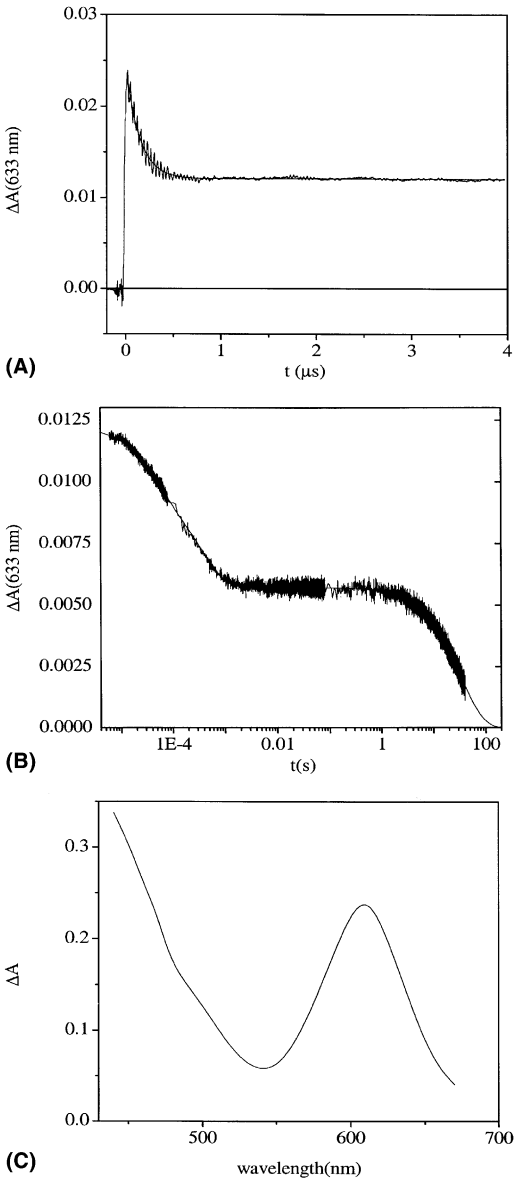


Fig. 1. Transient absorption at 633 nm after photolysis with a 308 nm laser pulse of an aqueous solution of compound (**1**) (0.6 mM) at pre-pulse pH = 10.9. The trace is the average of 16 single shots. (A) The rise in the absorbance occurs below the experimental resolution, whereas the decay on the sub-microsecond time scale can be described by a monoexponential decay function plus an offset (solid line). (B) On longer time scales, the remaining carbocations recombine with hydroxide with a complex kinetics, extending from the microseconds to the tens of seconds. The solid line is the result of a fit to a three exponential decay function. (C) Transient spectrum measured at the end of the laser pulse.

ditional faster reactions, evidenced in Figs. 1A and B.

The slow recombination allows the investigation of proton transfer reactions in a time window extending from about 500 ns to hundreds of milliseconds. The longer accessible time range depends on how tight the photolysis beam is focused [30]. With our setup the largest time window, which is not affected by diffusional motions of the solution, extends to about 500 ms.

The solid line in Fig. 1A is the result of a fit to a single exponential decay (plus an offset). The recovered rate constant $k_1 = 1/\tau_1 = (1.1 \pm 0.1) \times 10^7\ s^{-1}$ (Table 1) is essentially independent of the pre-pulse pH in the interval 6.2–11.4. This finding proves that the recombination rate is not determined by the bulk concentration of free hydroxide: in that case, an increase of the rate k_1 with pH should have been observed.

Increasing the ionic strength of the solution by addition of KCl reduces the rate k_1, as shown in Fig. 2 for two representative concentrations. The data were analyzed according to the equation [32]

$$\log(k_1) = \log\left(k_1^0\right) + 1.02 z_A z_B \sqrt{I}, \qquad (1)$$

where k_1^0 and k_1 are the rates observed in the absence and in the presence of KCl, respectively, I is the ionic strength of the solution, and z_A and z_B are the charges of the reacting ions. A plot of $\log(k_1/k_1^0)$ as a function of the square root of the ionic strength shows a linear behavior (Fig. 3) with a slope of -0.96 ± 0.05, indicating that the reaction being monitored is characterized by charged reactants with an effective charge product $z_A \times z_B \approx -1$. The ionic strength dependence suggests that the reaction being observed is the recombination between the carbocation (charge +1) and the hydroxide ion (charge −1). This process

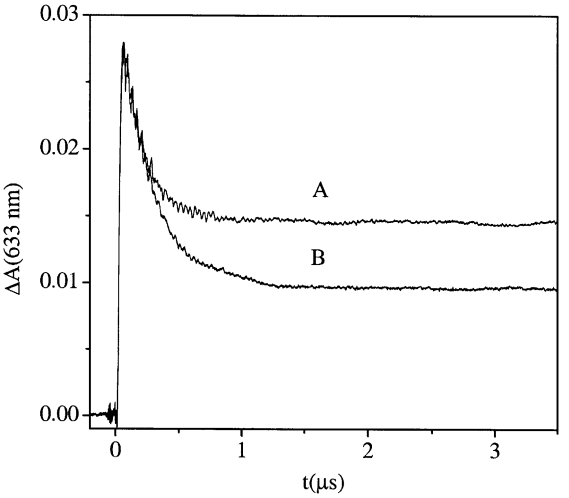


Fig. 2. Transient absorbance at 633 nm following photoexcitation of a solution of (**1**) at pre-pulse pH = 10.9 in the presence of two different ionic strengths. Curve A: [KCl] = 1.5 mM; curve B: [KCl] = 84 mM.

could be envisioned as a 'geminate' recombination of the hydroxide still in the electrostatic cage of the carbocation, situation indicated as $(C^+ \cdots OH^-)$ (Eq. (2)). The geminate ion pair can either evolve into the ground state carbinol or into the separated ions diffusing freely in solution:

$$COH \underset{k_{-a}}{\overset{k_a}{\rightleftarrows}} (C^+ \cdots OH^-) \underset{k_{-b}}{\overset{k_b}{\rightleftarrows}} C^+ + OH^- \qquad (2)$$

In our picture the forward rate k_a is much smaller than the backward rate k_{-a}, since at pH above neutrality the equilibrium ground state carbocation concentration is negligible. The forward escape rate k_b is likely much larger than k_{-b}, since from our data there is no evidence of pH dependence of the overall recombination rate even at the highest pH investigated (pH = 11.4). The geminate

Table 1
Rate constants and activation parameters for the recombination reactions in aqueous solutions at pre-pulse pH = 10.9:1 is the geminate rebinding, 2 and 3 are the pH dependent second-order processes

	1	2	3
$k_i = 1/\tau_i$ (s^{-1})[a]	$(1.1 \pm 0.1) \times 10^7$	$(2.7 \pm 0.3) \times 10^4$	$(3.0 \pm 0.3) \times 10^3$
E_a (kJ mol^{-1})	14 ± 4	16 ± 5	≈ 0
$\Delta H^{\ddagger}$ (kJ mol^{-1})	12 ± 4	13 ± 5	≈ 0
$\Delta S^{\ddagger}$ (J $K^{-1} mol^{-1}$)	-68 ± 12	-116 ± 17	-181 ± 10

[a] At $T = 293$ K.

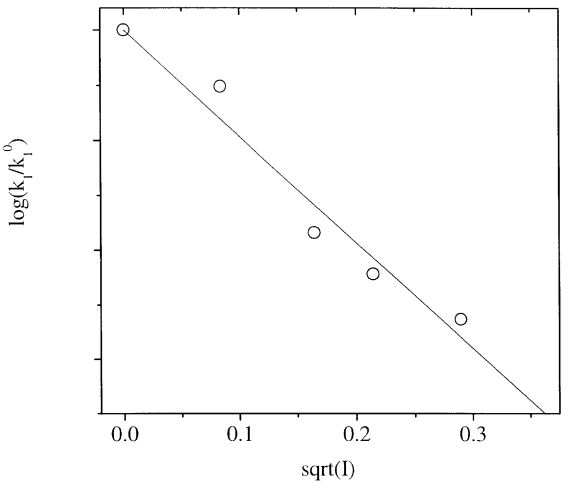


Fig. 3. Plot of $\log(k_1/k_1^0)$ as a function of the square root of the ionic strength. k_1^0 and k_1 are the rates observed with different ionic strengths. The ionic strength was varied by addition of KCl ($T = 293$ K, pre-pulse pH = 10.9).

ion pair disappears through two concomitant, essentially unidirectional, reactions. In terms of the resulting rate constant, the measured rate k_1 can be written as:

$$k_1 \approx k_{-a} + k_b, \tag{3}$$

where the rates k_a and k_{-b} have been neglected.

The possibility that the nanosecond decay is due, at least partly, to a triplet–triplet absorbance cannot be completely ruled out. However, the ionic strength dependence of the rate constant strongly suggests that the ionic reaction dominates this decay. In addition, we did not observe differences in the lifetime of this transient when experiments were conducted either in nitrogen or in air saturated solutions. On the contrary, collisional quenching by molecular oxygen is generally observed for triplet states.

A pH-dependent recombination phase is detected in the long microseconds, as shown in a semi-log plot in Fig. 1B. The transient spectrum at the end of the geminate recombination phase is identical to the spectrum measured at the end of the laser pulse (reported in Fig. 1C), suggesting that the species with longer lifetimes can be identified with the carbocation. This phase is best described by a biexponential decay, with lifetimes τ_2 and τ_3, plus an offset; the corresponding rates, $k_2 = 1/\tau_2$ and $k_3 = 1/\tau_3$, measured at $T = 293$ K and pre-pulse pH = 10.9 are reported in Table 1. These two decays are well separated (approximately one order of magnitude). Both k_2 and k_3 increase with increasing pH suggesting that this biexponential relaxation is associated with the recombination reaction between hydroxide and carbocation. Attempts to fit the data with a second-order reaction scheme [32] did not lead to satisfactory results and therefore we present only a qualitative description of the kinetics of this reaction. Plots of the apparent rates k_2, and k_3, measured at different pre-pulse pH values (not shown), were linear for both parameters. From the slopes we could estimate the bimolecular rate constants $k_2^b = (1.3 \pm 0.5) \times 10^7$ M^{-1} s^{-1}, and $k_3^b = (1.6 \pm 0.5) \times 10^6$ M^{-1} s^{-1}.

Finally, the concentration of the carbocation vanishes within a few minutes in a further recombination phase, that is coupled with diffusional processes mixing the solution (Fig. 1B). This phase is consistent with the slow recombination previously reported [26]. There is no residual absorbance change at very long times, indicating that the carbocations have completely reacted with the hydroxide to regenerate compound (**1**). This last phase cannot be properly described in terms of rate constants, since the measured apparent rate is mixed with the contributions from diffusional motions of the solution through the illuminated volume.

In conclusion, the overall recombination process of the freely diffusing ions can be schematically summarized as

$$C^+ + OH^- \rightleftarrows COH$$

(Heterogeneous recombination) (4)

The observed multiple exponential rebinding shows that a heterogeneous kinetics characterizes the reaction between hydroxide and the carbocation. The source of this heterogeneity is not clear at the moment, but the well-known presence of mesomeric forms of the carbocation ((**2**) and (**3**) in Scheme 2) could be implicated. The kinetics of OH^- rebinding could be modulated by the different electrophilic centers of the di-cation: the central

carbon (**2**), the dimethylamino nitrogen (**3**) or the trimethylammonium group ((**2**) or (**3**)). Further experiments will be necessary to understand the complete mechanism underlying the reported data.

The temperature dependence of the rebinding kinetics was measured in order to estimate the activation parameters of the processes. Table 1 reports the activation parameters determined experimentally for the first three decays through the Arrhenius (Eq. (5)) or the Eyring (Eq. (6)) relations:

$$\ln(k_i) = \ln\left(k_i^0\right) - \frac{E_a}{RT} \tag{5}$$

$$\ln\left(\frac{k_i h}{k_B T}\right) = \frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{RT} \tag{6}$$

In these equations $k_i = 1/\tau_i$ is the rate constant of process i ($i = 1, 2$ or 3), h is Planck's constant, k_B is Boltzmann's constant, R is the gas constant and T is the temperature (K). When using Eq. (5), the activation energy (E_a) was obtained from the slope of the linear plot of $\ln(k_i)$ vs $1/T$ (not shown). When the Eyring relation was used, the activation entropy and enthalpy ($\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$) were obtained from the intercept and the slope of the linear plot of $\ln(k_i h/k_B T)$ vs $1/T$ (not shown). The enthalpic barriers are relatively small (processes 1 and 2) or even negligible (process 3) for all of the processes. On the contrary, the entropic barriers are relevant and all negative, indicating that a substantial entropic barrier must be crossed to restore compound (**1**).

3.2. Deprotonation of the pH indicator bromoxylenol blue

The photoinduced release of hydroxide ions can be used to induce deprotonation of a weak acid with suitable pK_a. To show that this reaction can be effectively performed, a neutral aqueous solution of the caged hydroxide (**1**) was photodissociated in the presence of the pH indicator bromoxylenol blue ($pK_a = 7.2$). Transient absorption was monitored at 633 nm, where bromoxylenol blue exhibits an absorption band characteristic of the deprotonated form. The acid form has a very low absorbance at this wavelength. Thus, upon deprotonation of the indicator, an increase in the absorbance at 633 nm is expected to occur. Fig. 4 reports the transient absorbance at 633 nm for a solution of the caged hydroxide (**1**) alone (A) and in the presence of bromoxylenol blue (B) at pre-pulse pH = 7.2. When the solution is flashed with the UV laser, the hydroxide release increases the pH. This increase in $[OH^-]$ can be estimated from the absorbance at $t = 1$ μs by using the carbocation molar extinction coefficient $\varepsilon(633\text{ nm}) = 215\ M^{-1}\ cm^{-1}$. The absorbance, for trace A, represents the change in carbocation concentration at that time. When the indicator is present, the increase in $[OH^-]$ shifts the equilibrium of bromoxylenol blue towards the deprotonated form (In^-) according to the kinetic scheme:

$$OH^- + H^+ \underset{k_{-c}}{\overset{k_c}{\rightleftarrows}} H_2O \tag{7}$$

$$HIn \underset{k_{-d}}{\overset{k_d}{\rightleftarrows}} H^+ + In^- \tag{8}$$

$$HIn + OH^- \underset{k_{-e}}{\overset{k_e}{\rightleftarrows}} H_2O + In^- \tag{9}$$

The kinetics of the deprotonation reaction is reflected in the rise of the absorbance in trace B. The

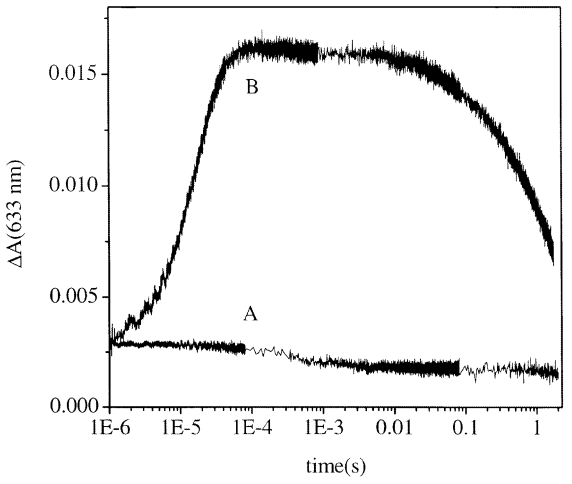


Fig. 4. Transient absorbance at 633 nm following photoexcitation at 308 nm of a solution containing compound (**1**) at pre-pulse pH = 7.2 (trace A). Trace B reports the signal observed when bromoxylenol blue is present.

increase in absorbance is characterized by a single exponential relaxation and the measured lifetime is $\tau = 8.6 \pm 0.2 \times 10^{-6}$ s, resulting in an apparent rate constant $k = 1.16 \pm 0.03 \times 10^5$ s^{-1}. Equilibrium (7), with rate constants $k_c = 1.4 \times 10^{11}$ M^{-1} s^{-1}, and $k_{-c} = k_c \times 10^{(-15.76)}$ plays a minor role in the observed relaxation. It is known that the rate constants for the equilibrium (8) are $k_d = 2.7 \times 10^3$ s^{-1} and $k_{-d} = 4.3 \times 10^{10}$ M^{-1} s^{-1} [30]; relaxation of equilibrium (9) occurs with rates $k_e = 8.9 \times 10^9$ M^{-1} s^{-1} and $k_{-e} = k_e \times 10^{(pKa-15.76)}$ [14,30]. The measured kinetics is not compatible with the simple relaxation of equilibrium (8), since the resulting deprotonation rate would be much smaller than the measured one. At the present $[OH^-]$ ($\approx$13 μM), the dominant reaction is due to equilibrium (9). A simple estimate of the bimolecular rate constant using the kinetic scheme outlined above gives a value of $k_e = 8.9 \times 10^9$ M^{-1} s^{-1}, in perfect accordance with the previously reported data [30].

On time scales longer than 100 μs the recombination of the carbocation with hydroxide leads to the protonation of bromoxylenol blue and to the observed decrease in absorbance.

The main kinetic feature in trace (A) of Fig. 4 is the pH dependent recombination phase (transients with lifetimes τ_2 and τ_3) occurring between 100 μs and a few milliseconds. This phase is much less evident, if at all, in trace (B). The decrease in absorbance at 633 nm for bromoxylenol blue (trace (B) in Fig. 4) is the result of the relaxation of the coupled chemical equilibria described in Eqs. (4),(7)–(9). The coupling between reactions makes the carbocation-OH^- recombination rate much smaller than in the absence of other competitive processes [4,30,33]. In particular, at alkaline pH, the rate of formation of HIn is mostly determined by the low concentration of H^+, reacting with In^-. Under the present experimental conditions this implies apparent lifetimes in the tens of milliseconds [30].

4. Conclusions

The compound 4-(dimethylamino)-4′-(trimethylammonium) triphenylmethanol iodide can be used to photoinduce the release of OH^- ions on submicrosecond time scale. The reaction is reversible on long time scales with no apparent fatigue for the compound. This peculiarity allows the use of the compound in experiments where several laser shots must be used to reduce the noise level. The relaxation kinetics of proton transfer reactions after the pH-jump can be followed in a time window extending from about 500 ns to hundreds of milliseconds.

Acknowledgements

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