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Deprotonation yields, pK_a, and *aci*-nitro decay rates in some substituted *o*-nitrobenzaldehydes†

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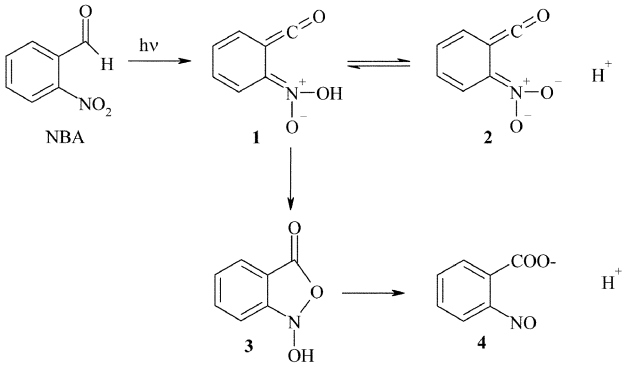
In this paper we report the deprotonation yields, the pK_a, and decay kinetics of the *aci*-nitro intermediates of some substituted 2-nitrobenzaldehydes that can be used as photoactivatable caged proton compounds. The decay of the *aci*-nitro absorbance for 2-nitrobenzaldehyde occurs within a few nanoseconds from photoexcitation. Addition of electron donating methoxy substituents at positions 4 and 5 leads to lower deprotonation yields, higher pK_a, and slower decays of the *aci*-nitro intermediates. On the contrary, the decay rate is accelerated by the introduction of an electron-withdrawing Cl atom at position 4 in the phenyl ring, with little influence on the deprotonation yield and pK_a of the *aci*-nitro intermediate.

Introduction

The photochemistry of nitro compounds has been a topic of continuous interest, also in connection with the development of photoactivatable caged compounds.[1,2] The primary photochemical event of electronically excited *ortho*-nitrotoluenes is hydrogen atom abstraction by the excited nitro group.[3–5] This reaction, proceeding both from the singlet and the triplet states, is responsible for the deexcitation of the chromophore *via* π-electron reorganization to the ground state *aci*-nitro intermediate. At neutral pH, the nitronic acid then rapidly deprotonates to give the nitronate anion. Subsequent reduction of the nitro group leads to the formation of the nitroso product. A well known example of this photochemistry is the photoreaction of *o*-nitrobenzaldehyde (NBA), sketched in Scheme 1, to give *o*-nitrosobenzoic acid (**4**), through the *aci*-forms (**1**, **2**) and the cyclic intermediate (**3**).[6]



Scheme 1

Flash photolysis experiments indicate that the *aci*-species of these compounds in water, at neutral pH, undergo deprotonation with rates in the 10^8 s^{-1} range.[6–9] The high deprotonation rate is mainly due to the low pK_a of the *aci*-nitro intermediate.[10] This property also accounts for the fact that the *aci*-nitro intermediate is completely deprotonated at pH above 3–4, depending on the compound.[7,11] Our previous results have shown that, taking advantage of this property, *o*-nitrobenzaldehyde and its derivatives can effectively be used as photoactivatable caged protons to obtain acidic pH-jumps on the nanosecond time scale.[11–14] The deprotonation rate of the nitronic acid is an important parameter that characterizes the maximum speed at which the concentration of free protons in solutions can be changed. The extent of the pH jump is of course affected by the photolysis yield which, for 2-nitrobenzaldehyde, is approximately 0.45.[6] The pK_a of the *aci*-nitro intermediate is also a key factor determining the lowest achievable pH, after photolysis of the compound. In case photoproducts possess ionizable groups with pK_a higher than the pK_a of the *aci*-nitro intermediate, they may actually further limit the maximum obtainable proton concentration jump. Although the *aci*-nitro intermediates of 2-nitrobenzaldehyde derivatives have a low pK_a, the 2-nitrosobenzoic products actually limit the lowest achievable pH to approximately 3.5. A new compound, 1-(2-nitrophenyl)ethyl sulfate (caged sulphate) has been recently synthesized that promises to be able to achieve larger pH jumps in solution (below 3).[15] The main advantage of this compound over 2-nitrobenzaldehydes is the low pK_a (1.92) of the sulphate product. However, since *o*-nitrobenzaldehyde has proven to be a useful photoactivatable compound to induce rapid and efficient proton release, it is interesting to investigate the possibility of using related compounds with similar, and hopefully improved, deprotonation properties. Among the possible substituents of the benzene ring, methoxy groups are of particular interest. These substituents have been inserted at positions 4 and 5 of the ring to increase the extinction coefficient in the near UV[16–19] and to enhance the two-photon cross section when using near-infrared, femtosecond lasers for cellular applications.[20]

In this work we use nanosecond transient absorption and time resolved photoacoustics to compare the yields, decay rates,

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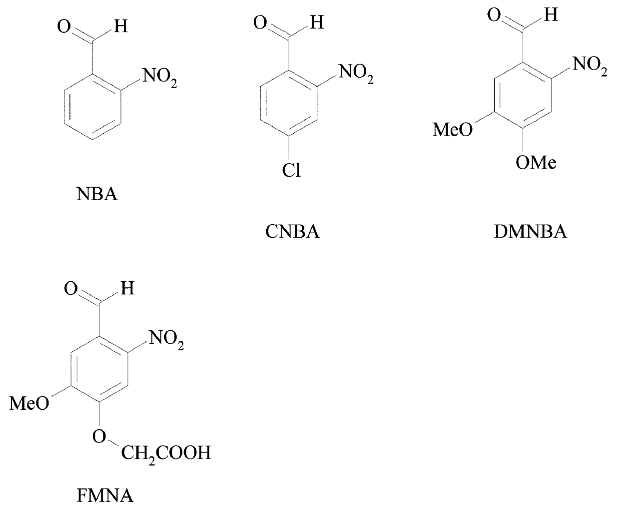
† Dedicated to Professor Silvia Braslavsky, to mark her great contribution to photochemistry and photobiology particularly in the field of photothermal methods.

and pK_a of *aci*-nitro intermediates for NBA, 4-chloro-2-nitrobenzaldehyde (CNBA), 4,5-methoxy-2-nitrobenzaldehyde (DMNBA) and 4-formyl-6-methoxy-3-nitrophenoxyacetic acid (FMNA) (Scheme 2).



Scheme 2

Experimental

Materials

2-Nitrobenzaldehyde, 4-chloro-2-nitrobenzaldehyde, and 4,5-methoxy-2-nitrobenzaldehyde were from Sigma-Aldrich. 4-formyl-6-methoxy-3-nitrophenoxyacetic acid was synthesized according to the literature.[21] Stock solutions were prepared in deionized water and filtered to remove undissolved compound. Solutions were freshly prepared before use by dilution of the stocks and saturated with nitrogen to remove dissolved CO_2. The pre-pulse pH was adjusted to the desired value by addition of concentrated NaOH or HCl aqueous solutions. Absorption spectra were measured with a computer-interfaced Jasco 7850 spectrophotometer. Spectra of the photoproducts were obtained by irradiation of the samples with 355 nm light until the spectrum, recorded at regular time intervals, did not show any changes upon irradiation. Photoacoustic experiments at acidic pH were performed in the presence of 0.1 M NaCl.

The absorbance of the compounds at the excitation wavelength (355 nm) was kept at about 2 cm^{-1} in the transient absorbance experiments and at 0.4 cm^{-1} in the photoacoustics experiments. Bromocresol purple (BP) (Kodak) or Brilliant Black BN (BBBN) (Aldrich) in water were used as photocalorimetric references in the photoacoustic experiments.[22] The pH of the reference solution was adjusted to 9 for BP and to 6 for BBBN in order to avoid instability in optical absorption at the excitation wavelength.

Laser flash photolysis

The laser flash photolysis apparatus for transient absorbance detection was described previously.[23] The photolysis laser is a frequency tripled (355 nm, 160 mJ) nanosecond Nd:YAG laser (Surelite II-10, Continuum). Monitoring at 458 nm was achieved by means of an Argon laser (JDS Uniphase). The cuvette was held in a temperature-controlled sample holder (FLASH 100, Quantum Northwest, Inc.). The transmitted intensity of the cw beam was monitored by a preamplified (Avtech AV149) avalanche silicon photodiode (Hamamatsu, S2382). A 0.25-m monochromator (H25, Jobin Yvon) was placed before the photodiode in order to remove stray light from the pump laser. The voltage signal was digitized by a digital sampling oscilloscope (LeCroy 9370, 1GHz, 1GS/s or LeCroy LT374, 500 MHz, 4 Gs/s). The sample was changed after each flash. In order to increase the signal-to-noise ratio, the pulse traces from 4 to 9 samples were averaged. Samples were changed after each flash.

Time resolved photoacoustics

The experimental setup and the photoacoustics data analysis have been described previously.[24,25] Photoexcitation at 355 nm was achieved by the same laser used in the laser flash photolysis setup. The pressure wave induced in solution was detected by a PZT piezoelectric transducer (Panametrics V-103). The signal was then amplified (60 db) and recorded by a digitizing oscilloscope (LeCroy 9450A) operated at 2.5 ns/channel. A quartz cuvette was mounted inside a temperature controlled sample holder (Quantum Northwest, Inc. TASC 300) and degassed with nitrogen. Volume changes were measured with a two-temperature method.[26] Data acquisition and deconvolution analysis[27] were performed by means of dedicated software (Sound Acquisition and Sound Analysis, Quantum Northwest, Inc.). The number of laser shots averaged to generate each sample waveform was 9, whereas 100 laser shots were averaged to generate each reference waveform. The sample was not changed after each flash since at the low power density used in the photoacoustics experiments there was no appreciable accumulation of photoproducts during the experiment. In any case, the solution was stirred in between laser shots to avoid accumulation of photoproducts in the illuminated volume.

The sample waveform is assumed to be convolution of a reference waveform (determined with a compound releasing all of the absorbed energy as heat within a few nanoseconds) and a sum of exponential decay functions:[28]

$$H(t) = \sum_i \frac{\varphi_i}{\tau_i}\exp(-t/\tau_i)$$

where φ_i is the preexponential factor of the transient with lifetime τ_i. The values of φ_i and τ_i are the results of the deconvolution analysis.[27] We have determined the structural volume changes using a two-temperature method.[26,29] The sample waveform was acquired at $T_{\beta=0}$ (3.9 °C[30] in water and 2.7 °C in aqueous solutions with 0.1 M NaCl) and was compared to a reference waveform acquired at a slightly higher temperature, $T_{\beta\neq0} = 6.0$ °C. At $T_{\beta=0}$ the thermal expansion coefficient, β, is zero and the signals of thermal origin vanish. The sample waveforms measured at $T_{\beta=0}$ originate purely from structural changes in the solution and include no enthalpic contribution. The extent of the observed structural volume change for each decay component, ΔV_i, is calculated from φ_i as:

$$\Delta V_i = \varphi_i E_\lambda \left(\frac{\beta}{C_p\rho}\right)$$

where E_λ is the molar energy content of the laser pulse, C_p the specific heat, and ρ the density. The parameter $\beta/C_p\rho$ is calculated at $T_{\beta\neq0}$. For dilute aqueous solutions and for pH values above 4 the thermoelastic parameter $\beta/C_p\rho$ has essentially the same value it has for water at pH = 7 and can be determined from literature values,[30] while for solutions with NaCl 0.1 M it must be determined experimentally using a comparative method.[31] In case the reaction yield Φ_i is known, the reaction volumes $\Delta V_{R,i}$ can be determined as: $\Delta V_{R,i} = \Delta V_i/\Phi_i$.

Results and discussion

Aci-nitro decay

Fig. 1 reports the absorbance spectra of an aqueous solution of 2-nitrobenzaldehyde at pH = 6 and of its photoproduct (**4**); the inset to Fig. 1 shows a transient absorbance signal at 458 nm for

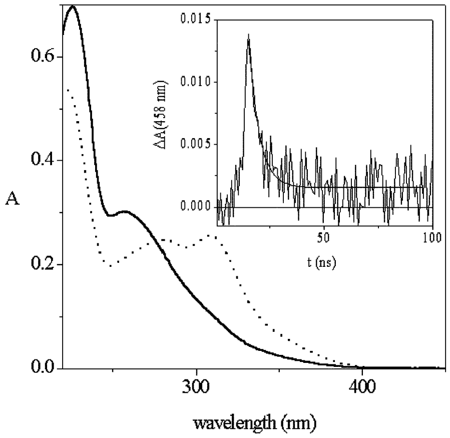


Fig. 1 Absorbance spectra of a 70 µM neutral aqueous solution of NBA (solid line) and its photoproduct (dotted line). Inset: *aci*-nitro transient absorbance at 458 nm of a 3 mM aqueous solution of NBA, pH = 6. The best fit with a monoexponential decay function was obtained with a lifetime $\tau = 6 \pm 2$ ns and is shown as the noiseless line in the plot.

the same sample. The fast rise in absorbance corresponds to the formation of the *aci*-nitro intermediate.[3–6] Fitting of the experimental trace in Fig. 1 gave a lifetime of 6 ± 2 ns for the decay of the *aci*-nitro intermediate, a value which is at the very limit of the time resolution of our setup and is in agreement with our previous determinations.[23] We could not detect longer lived intermediates with transient absorbance. The plateau region of Fig. 1 (inset), from 50 ns onward, is accordingly assigned to the absorbance of the photoproduct, 2-nitrosobenzoate. The transient absorbance signals did not show any significant pH dependence between pH = 3 and 11.

Fig. 2 and Fig. 3 (inset) show the transient absorbance signals at 458 nm for FMNA, and DMNBA, respectively. Fig. 3 shows the absorbance spectrum of a neutral aqueous solution of DMNBA and its photoproduct, which have similar features to the spectra previously reported for FMNA.[21] It is noteworthy that the addition of the electron-donating methoxy functionalities leads to the increase in absorbance in the near UV. The first striking feature emerging from the *aci*-nitro decays of both systems is their much slower decay rate in comparison with NBA. In addition, the *aci*-nitro decay is best described by a double exponential decay function with lifetimes $\tau_1 = 54 \pm 1$ ns (52%) and $\tau_2 = 760 \pm 3$ ns (48%) for FMNA, and $\tau_1 = 205 \pm 5$ ns (20%) and $\tau_2 = 1.05 \pm 0.02$ µs (80%) for DMNBA. The lifetime of the *aci*-nitro then increases when electron donating methoxy-substituents are inserted on the benzene ring, showing that they increase the energetic barrier the *aci*-nitro anion (or the cyclic intermediate) must overcome to proceed to the final product. The biexponential relaxations observed for FMNA and DMNBA, as well as the lower deprotonation yield (*vide infra*), may suggest the presence of different photochemical pathways for the compounds, but we have no satisfactory explanation available as yet. It is possible that the biexponential relaxation arises from the different absorbance spectra of **1** and **2**, or the different isomers of species **1**.[9] The formation of the final product in neutral aqueous solutions is supposed to proceed from the nitrosobenzoic anion *via* a cyclization intermediate. It is difficult to determine whether the rate limiting step is the formation of the cyclic intermediate **3** from **1** or the nitrosobenzoic acid **4** from **3**. Some hints come from a recent theoretical work[32] suggesting that the effects of the substituents on the *aci*-nitro decay rate may be located at the level of the formation of the cyclic intermediate. Our observations show that, while electron-withdrawing substituents accelerate the disappearance of the *aci*-nitro anion **2**, electron donating substituents decrease the decay rate. Contrary to what observed for NBA, the decay rates of the *aci*-nitro intermediates of DMNBA and FMNA show a slight decrease (≈20%) on lowering the pH from 10.2 to 2.6. The lack of pH dependence of the *aci*-nitro decay rates for *o*-nitrobenzaldehydes could be related to the more pronounced acid character of the *aci*-nitro intermediates which, at the investigated pH, are deprotonated. As reported for 2-nitrotoluene,[9] substantial acid catalysis for the *aci*-nitro decay is observed when the pH of the solution is close to or lower than the pK_a of the *aci*-nitro intermediate, a range which was not explored in the present investigation.

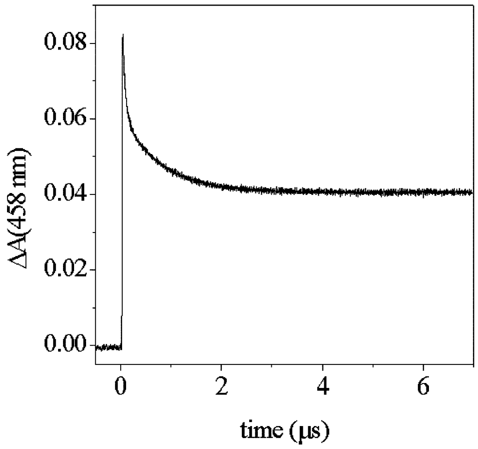


Fig. 2 *Aci*-nitro transient absorbance at 458 nm of an aqueous solution of FMNA at pH = 7. The best fit with a double exponential decay function was obtained with lifetimes $\tau_1 = 54 \pm 1$ ns (52%) and $\tau_2 = 760 \pm 3$ ns (48%).

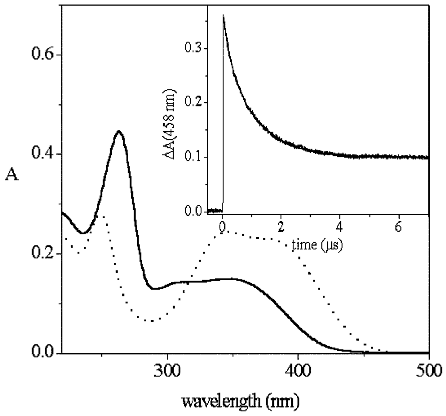


Fig. 3 Absorbance spectra of a 90 µM neutral aqueous solution of DMNBA (solid line) and its photoproduct (dotted line). Inset: *aci*-nitro transient absorbance at 458 nm of a 600 µM aqueous solution of DMNBA at pH = 7. The best fit with a double exponential decay function was obtained with lifetimes $\tau_1 = 205 \pm 5$ ns (20%) and $\tau_2 = 1.05 \pm 0.02$ µs (80%).

No detectable transient was observable for CNBA on our experimental time scale, indicating that the introduction of an electron withdrawing Cl atom on the ring decreases the lifetime of the *aci*-nitro decay below a few nanoseconds. No significant changes in the absorbance spectrum are observed with respect to NBA.

Deprotonation yield and pK_a

Time resolved photoacoustics data have been used to characterize the time frame and the yield of the proton release by the nitronic acid intermediates (Table 1). The main advantage of time resolved photoacoustics over flash photolysis is that it directly provides data on the volume changes associated with the proton transfer reactions with no need of a pH indicator to sense them. In addition, the volume change associated with the reaction of photoreleased protons with hydroxide at alkaline pH gives an internal calibration for the determination of the deprotonation quantum yield.[12] On the contrary, flash photo-

Table 1 pK_a, deprotonation yield ($\Phi_H{}^+$), and *aci*-nitro decay lifetimes for the investigated compounds

	pK_a	$\Phi_H{}^+$	τ_1/ns	τ_2/ns
NBA	2.1 ± 0.1	0.45 ± 0.08	7 ± 2	–
CNBA	2.0 ± 0.2	0.46 ± 0.09	–	–
DMNBA	2.6 ± 0.1	0.096 ± 0.005	205 ± 5	1050 ± 20
FMNA	2.57 ± 0.06	0.15 ± 0.03	54 ± 1	760 ± 3

lysis data require more sophisticated analyses[14] and generally must relay on a reference compound to estimate deprotonation yields. Photoexcitation of neutral aqueous solutions of 2-nitrobenzaldehyde derivatives is accompanied by fast, subresolution (*i.e.* with lifetime below 10 ns) contractions of the solutions. An example of these fast contractions is shown in the inset to Fig. 4 as a negative going waveform (trace B) with the same temporal profile of the reference compound (trace A). These transients are present at all pH values above the pK_a of the *aci*-nitro intermediates.[11–13] The observed contractions arise from the formation of two net charges from the neutral parent compounds, and are due to electrostrictive effects as well as to specific interactions with the solvent.[33–35] No additional transients are observed in neutral or acidic solution of the compounds. The decay of the *aci*-nitro intermediate, which for FMNA and DMNBA occurs with lifetimes detectable by the photoacoustic instrumentation, does not seem to lead to appreciable volume changes of the solution. Reaction of the nitroso-products with protons from the bulk occurs at rates too low to be appreciable by the photoacoustics instrumentation and are accordingly not sensed.

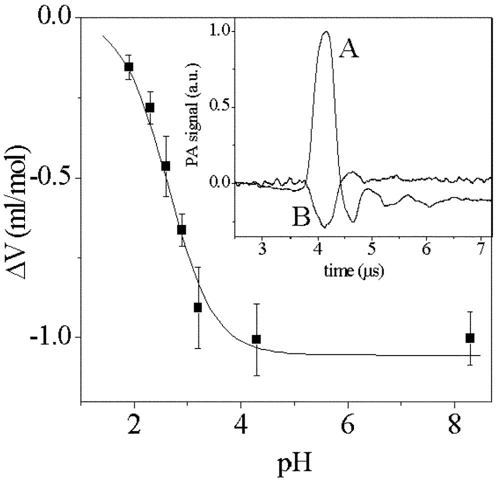


Fig. 4 pH dependence of the fast contraction measured for DMNBA at different pH values. The solid curve is the fit to the data using a simple protonation equilibrium. The inset shows the photoacoustic signal taken at T = 2.7 °C in 0.1 M NaCl solution, pH = 3.2 (curve B). The reference waveform (curve A) was acquired at T = 5.9 °C in the same solvent.

The volume change for deprotonation of CNBA and DMNBA was determined from the fast contraction following photoexcitation. This volume change was essentially independent of the pH of the solution between pH = 4 and 10, as previously observed for NBA and FMNA. The measured values were $\Delta V_1 = -1.0 \pm 0.2$ ml mol^{-1} for DMNBA, and $\Delta V_1 = -3.6 \pm 0.1$ ml mol^{-1} for CNBA.

As the pH is increased above neutrality, protons react with hydroxide in a diffusion mediated, bimolecular reaction, resulting in an expansion of the solution (data not shown).[12] This reaction occurs with a bimolecular reaction rate of 4.9×10^{10} M^{-1} s^{-1} in water, leading to apparent lifetimes in the 10^{-7} s range at pH above 9, thus detectable by the photoacoustic instrumentation. Increasing the pH above 9 speeds up this reaction. When the pH is raised above 11 the reaction occurs with an apparent lifetime below the experimental resolution (≈20 ns). In the presence of ionic strength, the electrostatic screening reduces the bimolecular reaction rate with hydroxide (1.5×10^{10} M^{-1} s^{-1} in 0.1 M NaCl) and the pH can be raised to higher values before the apparent lifetime falls below the experimental resolution.[11] These findings are similar for all investigated compounds. Deprotonation of the nitronic acid does not constitute a rate limiting step for the following reaction of photodetached protons with hydroxide, whose apparent rate can be made at least as fast as 10^8 s^{-1}. The proton release rate for NBA, CNBA, FMNA and DMNBA is therefore 10^8 s^{-1} or larger.

The deprotonation quantum yield, $\Phi_H{}^+$, for CNBA and DMNBA has been determined with time resolved photoacoustics following the procedure we have previously established with NBA and FMNA.[11–13] Briefly, the photodetached protons react with hydroxide to form water molecules with a concomitant volume increase $\Delta V_{R,2} \equiv \Delta V_W = 24.5$ ml mol^{-1}.[33,34] Using the measured volume change ΔV_2, $\Phi_H{}^+$ is obtained as the ratio $\Delta V_2/\Delta V_W$. For CNBA and DMNBA we have $\Phi_H{}^+ = 0.46 \pm 0.09$ and $\Phi_H{}^+ = 0.096 \pm 0.005$, respectively. These yields can be compared with the previous determinations we made for NBA (0.45 ± 0.08) and for FMNA (0.15 ± 0.03).[11]

Using the deprotonation yields calculated above, we can also obtain the molar volume change for the deprotonation reactions, namely $\Delta V_{R,1} = -10.4 \pm 0.5$ ml mol^{-1} for DMNBA, and $\Delta V_{R,1} = -7.8 \pm 0.2$ ml mol^{-1} for CNBA. These volume changes are associated with the solvation of the newly formed charges upon deprotonation of the *aci*-nitro intermediates. For comparison, the volume change for the analogous reactions for FMNA and for NBA are $\Delta V_{R,1} = -11.1 \pm 0.3$ ml mol^{-1}, and -7.1 ± 0.2 ml mol^{-1}, respectively.

The reaction volumes are the result of the overall reaction leading to solvation of the photodetached proton and the *aci*-nitro anion:

$$\Delta V_{R,1} = V^0(H^+) + V^0(RO^-) - V^0(ROH)$$

The partial molar volume of the solvated proton has been recently determined as $V^0(H^+) = (-5.5 \pm 0.8)$ ml mol^{-1}[36] and gives a contribution to the observed contractions which is independent of the compound. This is the most relevant contribution to the volume changes observed for NBA and CNBA, but is only one half of the observed $\Delta V_{R,1}$ for DMNBA, and FMNA. The major differences between the observed volume changes are likely to be found in the different specific interactions with the solvent the *o*-nitro compounds have. Photoacoustics data can be used to extract also the pK_a of the nitronic acid intermediates.[11] Experiments are performed in aqueous solutions containing 0.1 M NaCl. The amplitude $\Delta V_{R,1}$ of the fast contraction observed upon photolysis decreases on lowering the pH of the solution and vanishes around pH = 1. From the plot of $\Delta V_{R,1}$ *vs.* pH it is possible to extract the pK_a of the *aci*-nitro intermediate. In Fig. 4 we report the pH dependence of the structural volume change $\Delta V_{R,1}$ for DMNBA. The inset shows a photoacoustic signal taken at $T_{\beta=0} = 2.7$ °C (at this temperature the thermal expansion coefficient of the solution vanishes and the signals have no thermal contribution) and pH = 3.2, together with the reference signal measured at $T_{\beta \neq 0} = 5.9$ °C in the same solvent. The best fit is obtained with a pK_a = 2.6 ± 0.1. For comparison, the pK_a for the *aci*-nitro intermediates of NBA and FMNA are 2.1 ± 0.1 and 2.57 ± 0.06, respectively.[11] The pK_a for CNBA was found to be 2.0 ± 0.2, similar to the pK_a of NBA. The photoacoustics data altogether indicate that the deprotonation reaction of the nitronic acid is affected by substituents at positions 4 and 5 on the ring, but on the nanosecond time scale this influence appears only as a change in the deprotonation yield. Even though the deprotonation rate certainly depends on the nature of the substituents, it is not possible to quantitate these changes with our nanosecond

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investigations. Since for proton transfer reactions an empirical correlation exists between pK_a and deprotonation rate,[10] it is expected that the deprotonation reaction is slower for DMNBA and FMNA because of their higher pK_a.

Conclusions

Our data show that all the investigated compounds allow to achieve the laser induced acidic pH-jump within a few nanoseconds. The addition of methoxy substituents at positions 4 and 5 on 2-nitrobenzaldehyde has interesting effects on the absorbance spectrum with an enhancement of the molar extinction coefficient in the near UV. However, the deprotonation quantum yield is decreased by this substitution and the *aci*-nitro intermediate appears to be stabilized, with a resulting slower decay rate. In our case, it is difficult to establish the specific mechanism leading to the observed effects. Sub-nanosecond transient absorption may prove useful in giving some hints on the specific excited state mechanisms leading to lower deprotonation yields on addition of methoxy substituents.[5] Although the addition of methoxy groups at positions 4 and 5 enhances the molar extinction coefficient (ε) in the near UV at around 350 nm, the overall photosentivity ($\varepsilon\Phi_{H}^{+}$) may be essentially unchanged as a result of the decrease in reaction yield for these chromophores. The addition of an electron withdrawing atom at position 4 has little effect on the deprotonation yield and speeds up the decay of the aci-nitro.

Acknowledgements

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