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Photophysical characterisation of the plant growth regulator 2-(1-naphthyl) acetamide

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Highlights

A full photophysical characterisation has been carried out on the plant growth regulator 2-(1-naphthyl) acetamide
these shows a marked solvent dependence
the compound acts as a relatively strong excited state oxidant
sensitised singlet oxygen reactions compete with electron transfer in the excited state reactivity
this may be important in the photoreactivity under environmental conditions

Abstract

The photophysical properties of the widely used plant growth regulator 2-(1-naphthyl) acetamide (NAD) were studied in water and representative organic solvents (ethanol, ethylene glycol, acetonitrile, chloroform, 1,4-dioxane) employing steady-state and time-resolved spectroscopy. Quantum yields and lifetimes of fluorescence, phosphorescence and triplet formation and triplet-triplet absorption spectra were obtained. From these, all radiative and radiationless rate constants have been determined, together with singlet and triplet excited state energies (4.00 and 2.69 eV, respectively). The fluorescence quantum yield and lifetime increased on going from water (Φ_F 0.066, τ_F 35.0 ns) to non-hydrogen bonding solvents (Φ_F 0.357, τ_F 51.0 ns in 1,4-dioxane), probably due to decreased internal conversion. Fluorescence was quenched by several anions through an electron transfer process. A limit on the reduction potential of $^1\text{NAD}^*$ of E° 2.1 ± 0.2 V was estimated. The attribution of the transient absorption seen in nanosecond laser flash photolysis to $^3\text{NAD}^*$ was confirmed by energy transfer and oxygen quenching. Quenching of triplet states leads to singlet oxygen formation, with quantum yields varying from 0.097 in water to 0.396 in chloroform. However, these are lower than the triplet state quantum yields, particularly in water (Φ_T 0.424), indicating competing quenching pathways, probably involving electron transfer. The relevance of these results to the photoreactivity of NAD under environmental conditions is discussed.

Keywords: 2-(1-naphthyl) acetamide; Pesticides; Excited states; Solvent polarity; Nanosecond laser flash photolysis; Fluorescence

1. Introduction

Naphthalene derivatives are of environmental and biological interest, and have widespread application in fields such as agriculture, pharmaceuticals and industry. 2-(1-naphthyl) acetamide (NAD) (Fig. 1) is a synthetic plant growth regulator whose behaviour results from its structural similarity to the natural plant growth regulator hormone indole auxin [1]. It has been used for many years in agriculture to promote the growth of numerous fruits, for root cuttings and as a fruit thinning agent [2,3]. However, NAD and its residues have been the object of studies by the European Commission and other regulatory bodies, and a risk assessment has been presented [4]. In addition, analytical methods such as luminescence [5,6] and chromatography [7,8] have been developed for its quantification in food residues and water. According to the U.S.Environmental Protection Agency (EPA) approximately 20,000 lbs (9000 kg) of naphthalene acetate active ingredients such as NAD, 1-naphthyl acetic acid (1-NAA), etc., are applied annually in the USA [9].

Unfortunately, in contrast with the full photophysical characterization available for naphthalene [10], there is a lack of detailed information in the literature on the excited state properties of NAD. Detailed study of photophysical properties of such molecules is of fundamental importance [11], normally requiring information on the effect of solvents on the ground and excited states of molecules [12-14], and, in particular, how such interactions affect the nature and energy of the electronic excited states. This is particularly relevant to environmentally relevant compounds, such as NAD, as their photodegradation may take place both in water and in the more hydrophobic environments occurring in plants and soils. Changing solvent polarity and hydrogen bond ability with these systems has been shown to lead to shifts in electronic absorption and luminescence spectra [15-20]. In addition, although more limited information is available, they may also cause changes in triplet excited state behaviour [21-22].

We have recently reported a detailed study of the photodegradation of NAD, both under UV light and simulated solar irradiation conditions [23], including identification of major degradation products and toxicity studies. The primary photoproducts lead to a significant increase in toxicity, which subsequently decreases upon prolonged irradiation. Mechanistic studies of the effect of oxygen indicate involvement of both triplet and singlet excited states in the photoreactivity of NAD, together with a major role of the radical cation. However, the lack of photophysical data on NAD limits understanding its photoreactivity. To rectify this, we have carried out a detailed study,

involving complete characterisation of excited states, including the effects of solvent properties such as polarity (dielectric constant), hydrogen bonding, refractive index and viscosity on the NAD ground and excited state behaviour. Solvents of three different types were selected: water, ethanol, ethylene glycol and chloroform were used as polar hydrogen-bond donating solvents, acetonitrile was used as polar aprotic non-hydrogen-bond donating solvent and 1,4-dioxane was used as a non-polar and non-hydrogen-bond donating solvent. Detailed results are presented on singlet and triplet state properties and of the overall set of deactivation rate constants: k_F , k_P , k_{ic} and k_{isc} . Quenching studies of NAD excited states by oxygen and of fluorescence by a variety of inorganic anions have also been studied to obtain insights into the relative importance of electron and energy transfer in excited state deactivation.

2. Materials and methods

2.1. Materials

2-(1-naphthyl) acetamide (NAD), biphenyl, benzophenone and phenalenone (97%) were purchased from Sigma Aldrich and naphthalene from J. K. Baker Chemical Co.. All products were used as received. Acetonitrile was purchased from Merck, ethanol, ethylene glycol, deuterium oxide, methylcyclohexane and glycerol from Sigma Aldrich, and 1,4-dioxane, chloroform and cyclohexane from Panreac. All solvents were spectroscopic grade.

An aqueous NAD stock solution was prepared with deionised water (Millipore Milli-Q; resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$), at natural pH. Fresh daily solutions were prepared from dilution of this stock solution. Solutions in organic solvents were prepared daily.

Samples were bubbled for 20 minutes with argon and oxygen to have de-aerated and oxygenated samples, respectively.

2.2. Absorption and fluorescence studies

UV-visible absorption spectra measurements were acquired either on a Cary 3 double-beam (Varian) or on a Shimadzu UV-2010 double-beam spectrometer with 1 cm quartz cuvettes over the range 200-800 nm.

Corrected steady state fluorescence emission and excitation spectra were measured using a 1 cm quartz cuvette on a Horiba-Jobin-Yvon SPEX Fluorolog 3-22 spectrofluorometer equipped with a 300 W xenon lamp. Fluorescence emission and excitation spectra were registered at absorption and emission maxima wavelength,

respectively, with 1 mm slits. Fluorescence quantum yields were measured using naphthalene in ethanol as standard ($\Phi_F = 0.21$) [10], according to equation (1):

$$\Phi_{F,S} = \Phi_{F,R} \frac{I_S A_R n_S^2 f_S}{I_R A_S n_R^2 f_R} \quad (1)$$

where

$$f_S = \frac{I_{N_2}^S}{I_{O_2}^S} \quad \text{and} \quad f_R = \frac{I_{N_2}^R}{I_{O_2}^R}$$

Φ_F represents the fluorescence quantum yield, I is the integrated area under the

respective fluorescence emission spectra, A is the respective absorbance, n is the

refractive index of the solvents used and f represents the de-aerating factor, which is given by the ratio of the integrated area under the respective fluorescence spectra in absence and presence of oxygen, for the sample and the reference. The subscripts S and R refer to the sample and reference, respectively. Both the sample and reference were excited at the same wavelength. The absorbance of the solutions at the excitation wavelength was maintained around 0.1 and 1 mm slits were used.

The energy of singlet state, E_S , was determined from the crossing point of the normalized absorption and fluorescence emission spectra.

Fluorescence lifetimes, τ_F , were measured using the method of time correlated single photon counting (TCSPC) with a Horiba-JI-IBH NanoLED (282 nm) as excitation source, a Philips XP2020Q photomultiplier, and Canberra instruments TAC and MCA. Measurements were performed in de-aerated samples at the maximum emission

wavelength. The fluorescence decays were analysed using the modulating functions method of Striker [24] with automatic correction for the photomultiplier “wavelength shift”. The decay was fitted by using FluoFit Pro version 4.

Fluorescence quenching studies were performed in water by adding different concentrations (range 10^{-4} - 10^{-2} mol L⁻¹) of aqueous KBr, KI, KCl, KSCN, NaN₃ solutions, and in acetonitrile using triethylamine solution in the same solvent, to NAD solution at a constant concentration (1.0×10^{-5} mol L⁻¹). Stern-Volmer plots were constructed from relative integrated fluorescence emission intensities and the Stern-Volmer quenching coefficients, K_{SV} , were obtained by linear regression according to expression (2):

$$\frac{I_0}{I} = 1 + K_{SV} [Q] \quad (2)$$

where I_0 and I are the fluorescence intensities in absence and presence of quencher, respectively, $[Q]$ represents the quencher concentration and the slope of the curve is equal to the Stern-Volmer constant K_{SV} .

2.3. Phosphorescence studies

The phosphorescence measurements used the Horiba-Jobin-Ivon SPEX Fluorolog 3-22 spectrofluorometer equipped with a Spex 1934D phosphorimeter accessory having pulsed excitation lamp and appropriate software. Phosphorescence studies of NAD were carried out in ethanol, acetonitrile and ethylene glycol solutions frozen at 77 K in order to avoid quenching by molecular oxygen or other impurities dissolved in these solvents. The NAD containing glass was excited at the maximum absorption wavelength. Slits of 4.0 mm were used for excitation and emission measurements.

The emission and excitation spectra were recorded as well as decay studies in order to determine phosphorescence lifetimes. Phosphorescence quantum yields were determined using benzophenone in ethanol as reference ($\Phi_P = 0.74$) [10], and are calculated using the following equation:

$$\Phi_{P,S} = \Phi_{P,R} \frac{I_S A_R}{I_R A_S} \quad (3)$$

where Φ_P represents the phosphorescence quantum yield, I is the integrated area under the respective phosphorescence spectra, A is the respective absorbance and the subscripts S and R refer to the sample and reference, respectively.

2.4. Transient absorption spectra, triplet absorption coefficient, lifetimes and quantum yields

Transient absorption experiments were carried out on a nanosecond laser flash photolysis spectrometer from Applied Photophysics (LKS 60). Excitation was achieved at 266 nm and/or 355 nm from the Quanta Ray GCR130-1 Nd:YAG laser (pulse width ~ 5 ns). The transient absorbance was monitored by a detection system consisting in a pulsed xenon lamp (150 W), monochromator and a IP28 photomultiplier Housing (Applied Photophysics). The signal from the photomultiplier was digitized by a programmable digital oscilloscope TDS 30528 (Tektronix) and the signal was analyzed with a 32-bit RISC-processor kinetic spectrometer.

Triplet lifetimes, τ_T , were calculated from kinetic analysis of the transient decays. The rate constants for oxygen quenching of the triplet state $k_T^{O_2}$ were determined by measuring the decay of the triplet-triplet absorption at the absorption maximum for NAD in each solvent in the presence and absence of air.

The triplet absorption coefficient, ϵ_T , was determined by the energy transfer method

[25] using benzophenone in acetonitrile ($\epsilon_{520\text{ nm}} = 6500\text{ L mol}^{-1}\text{ s}^{-1}$; $\Phi_T = 1.0$) [10] as triplet energy donor. All solutions were de-aerated by purging with argon, and sealed prior to measurement. The excitation wavelength was of 355 nm. The molar triplet-triplet absorption coefficients [26] were then determined from equation (4):

$$\frac{\epsilon_{TT}^D}{\epsilon_{TT}^A} = \frac{\Delta OD^D}{\Delta OD^A} \quad (4)$$

where ϵ_{TT}^D and ϵ_{TT}^A are the triplet molar absorption coefficients of donor and acceptor, respectively, ΔOD^D is the maximum absorbance from the transient triplet-triplet absorption spectra of the donor in the absence of acceptor; ΔOD^A is the maximum absorbance of the acceptor triplet when the acceptor and donor are present. When the acceptor decay rate constant (k_3) is not negligible, a correction is necessary to calculate the ΔOD^A , given by equation (5):

$$\Delta OD_{obs}^A = \Delta OD^A \exp\left[-\frac{\ln(k_2/k_3)}{k_2/k_3 - 1}\right] \quad (5)$$

where k_2 is the decay rate constant of the donor in the presence of the acceptor and ΔOD_{obs}^A is the maximum obtained in the triplet-singlet difference spectra of the acceptor in the presence of the donor.

The triplet quantum yields of NAD, Φ_T , were obtained by the comparative actinometry method using naphthalene in methylcyclohexane as reference ($\epsilon = 13200 \text{ L mol}^{-1} \text{ cm}^{-1}$; $\Phi_T = 0.75$) [10]. Optically matched samples of NAD and naphthalene around 0.2 at the excitation wavelength were irradiated with the 266 nm laser in the nitrogen-saturated solvent. The quantum yield of triplet formation of NAD is giving according to equation (6):

$$\Phi_T^S = \frac{\epsilon_{TT}^{naphthalene}}{\epsilon_{TT}^S} \frac{\Delta OD_{max}^S}{\Delta OD_{max}^{naphthalene}} \Phi_T^{naphthalene} \quad (6)$$

where S and R refers to the sample and reference, respectively. All measurements were conducted at room temperature.

2.5. Singlet oxygen measurements

Singlet oxygen quantum yield, Φ_Δ , was detected using time-resolved phosphorescence [27] by monitoring room temperature phosphorescence decay centered at 1270 nm using a Hamamatsu R5509-42 photomultiplier, cooled at 193 K in a liquid nitrogen chamber, following laser excitation of aerated NAD solutions in different solvents at 266 nm, with an Applied Photophysics flash kinetic spectrometer. Decays were then extrapolated to time zero and the initial phosphorescence signal studied as a function of laser intensity. This was then compared with a standard, biphenyl in cyclohexane ($\Phi_\Delta = 0.73$) [28], excited at 266 nm. Studies were carried out in optically matched solutions ($abs = 0.2$) in the above solvents. The singlet oxygen quantum yield sensitized by NAD was calculated from the equation (7):

$$\Phi_\Delta^S = \frac{I^S}{I^R} \frac{Abs^R}{Abs^S} \frac{n_S^2}{n_R^2} \Phi_\Delta^R \quad (7)$$

where I is the intensity of the phosphorescence decay measured at 1270 nm, Abs is the absorbance of the solutions at 266 nm, n is the refractive index for each solvent, Φ_Δ is the singlet oxygen quantum yield and S and R represent sample and reference, respectively. We note that the radiative lifetime of singlet oxygen shows a solvent dependence [29]. However, although the use of different solvents for sample and reference in these measurements may increase the uncertainty of the singlet oxygen

yield in water, we do not feel the error is significant, and in the absence of a reliable singlet oxygen standard for measurements in water with excitation at 266 nm prefer to use a well characterized standard.

Photosensitized formation of singlet oxygen, $^1\text{O}_2$, was achieved by using phenalenone as sensitizer in chloroform ($\Phi_{\Delta} = 0.98$) [30]. Aerated solutions of phenalenone with constant concentration ($3.0 \times 10^{-5} \text{ mol L}^{-1}$) were excited with a pulse laser at 355 nm in the presence of NAD concentrations ranging from 1.0×10^{-4} to $1.0 \times 10^{-2} \text{ mol L}^{-1}$. The singlet oxygen phosphorescence decay was measured at 1270 nm for each solution and by studying the pseudo-first-order rate constant as a function of quencher concentration it was possible to obtain the quenching rate constant of $^1\text{O}_2$ by NAD.

3. Results and discussion

To simplify the presentation in this paper, all the figures presented in the text will refer to NAD in water, which is of most interest for environmental considerations. However, results are given for all solvents in the tables.

3.1. Absorption spectroscopy

The UV absorption spectrum of NAD was recorded in water, D_2O , ethanol, ethylene glycol, acetonitrile, chloroform and in 1,4-dioxane. As shown in Fig. 1, the UV absorption spectrum of NAD in aqueous solution presents two main bands: the short wavelength absorption has a maximum around 223 nm while the lowest energy band within the wavelength range 250-320 nm shows vibrational structure with maximum centred at 280 nm. Both of these bands are characteristic of $\pi\text{-}\pi^*$ transitions of the aromatic ring.

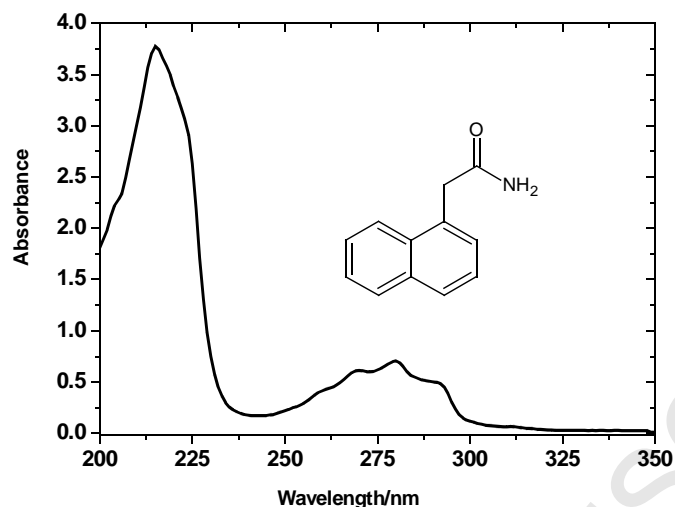


Fig. 1. UV absorption spectrum of aqueous NAD solution (1.0×10^{-4} mol L $^{-1}$) and its chemical structure.

Neither the shape of the low energy absorption band nor its vibronic structure was influenced by change in solvent. However, a slight red shift was observed in the absorption maximum wavelength when passing from water to a non-polar organic solvent, as can be seen from the results presented in Table 1. This is in agreement with the polarizing effect of the solvent medium on the π - π^* transition [20,31,32]. The molar absorption coefficients of NAD, ϵ , were determined for each solvent at the maximum absorption wavelength band, and the results are reported in Table 1. The ϵ_{280} value in water is in good agreement with previous reports (6747 L mol $^{-1}$ cm $^{-1}$) [4], and is a typical value for a π - π^* transition. A slight increase in ϵ with decrease of solvent polarity was observed for all solvents except chloroform. The low value obtained in this solvent, 4740 L mol $^{-1}$ cm $^{-1}$, may well be associated with contact charge transfer contributions in this medium [33].

Table 1. Maximum absorption wavelength (λ_{\max}) and molar absorption coefficient (ϵ), for NAD singlet ground state as function of solvent polarity. The refractive index (n_D),

[10] dielectric constant (ϵ) [10] and viscosity (η) [10] of solvents are given for better

clarity of discussion.

Solvent	Abs. (λ_{\max}/nm)	ϵ^a ($\text{L mol}^{-1} \text{cm}^{-1}$)	n_D	ϵ	η ($\times 10^{-3} \text{ Pa s}$)
Water	280	6540	1.333	80.1	1.00
D ₂ O	280	-	1.328	-	-
Ethylene glycol	283	6443	1.431	37.7	19.9
Ethanol	282	7477	1.361	24.5	1.20
Acetonitrile	282	7090	1.344	35.9	0.34
Chloroform	283	4740	1.445	4.81	0.58
1,4-Dioxane	283	7250	1.422	2.20	1.43

^a Estimated errors $\pm 5\%$

3.2. Fluorescence spectra, quantum yields, lifetimes and singlet excited state energy

NAD fluorescence emission and excitation spectra were measured in water, D₂O and in the studied organic solvents. Fig. 2 shows the fluorescence emission ($\lambda_{\text{ex}} = 280 \text{ nm}$) and

excitation spectra ($\lambda_{\text{em}} = 324 \text{ nm}$) of aqueous NAD solution ($1.0 \times 10^{-5} \text{ mol L}^{-1}$) while

Table 2 summarizes the results in all solvents. The fluorescence emission spectrum shows a broad band with vibronic structure extending from 300 to 400 nm. In aqueous solution, two maxima at 324 and 338 nm were observed together with two shoulders at

higher wavelengths. This is consistent with previous reports on the fluorescence spectra of NAD [34]. For all the solvents, the emission spectra were not affected when excitation was made at different wavelengths, confirming its origin from a single emitting species and that NAD does not contain any fluorescent impurities. No effect was observed on the shape of the emission or excitation spectra on going from H₂O to the other solvents, although, as seen in the results presented in Table 2, there is a slight red shift in the emission maximum wavelength with solvent polarity. In all solvents, NAD excitation and absorption spectra were identical, and were mirror images of the emission spectrum indicating that there is no significant change in the structure of NAD chromophore between the ground and excited state.

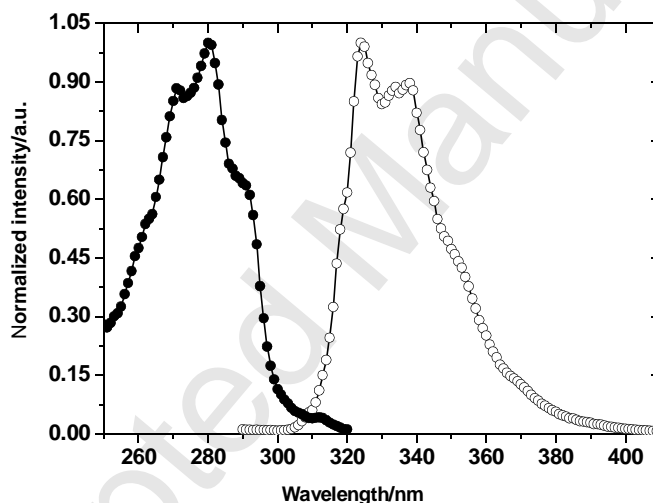


Fig. 2. Normalized excitation (●) and emission (○) spectra of aqueous NAD solution (1.0×10^{-5} mol L⁻¹).

The Stokes shift data, given by the difference between the maximum peak of normalized absorption and emission spectra, and the energy of the lowest singlet excited state, E_S , which is estimated from the intersection of the normalized absorption and emission spectra, are also included in Table 2. No significant changes are observed on the Stokes shifts when replacing water by organic solvents. Moreover, E_S is independent on solvent, with an energy of 4.00 eV.

The fluorescence quantum yields, Φ_F , as well as the singlet lifetimes, τ_F , were determined in de-aerated solutions (see Table 2). As will be discussed later, molecular oxygen quenches the singlet excited state. The results indicate that NAD fluoresces with

reasonable quantum yields (≤ 0.357), with Φ_F increasing as one goes to non-hydrogen bonding solvents. These values are similar to those reported in literature for naphthalene derivatives. For instance, Tamaki *et al.* reported a quantum yield of 0.14 for naphthyl acetic acid derivatives in acetonitrile [35]. The fact that the fluorescence quantum yields are less than unity indicates the relevance of radiationless pathways in the deactivation of NAD singlet excited state, $^1\text{NAD}^*$. The low value obtained in water, 0.066, may be associated with hydrogen bonding effects involving the stretching modes of the hydroxyl group, which could facilitate the radiationless decay to the ground state, and is consistent with quantum yields of other aromatics, such as benzene [36], that are markedly smaller in water than in other common solvents. Although acetonitrile and ethylene glycol have comparable dielectric constants ($\epsilon \cong 36$), the fluorescence quantum yield in acetonitrile is twice than that in ethylene glycol. As will be discussed shortly, comparison of the photophysical behavior in the two solvents is complicated by the observation of bi-exponential fluorescence decays in ethylene glycol. However, the results are consistent with hydrogen bonding with ethylene glycol enhancing radiationless deactivation and decreasing the fluorescence quantum yield. The lowest value of fluorescence quantum yield is presented in chloroform, which can be explained by contact charge transfer [33] as was suggested above from the absorption spectra. Moreover, and as can be seen by the results in Table 2, the replacement of H_2O by D_2O had no effect on NAD emission properties. With certain systems, notably lanthanides, [37,38] different lifetimes are observed in D_2O compared with H_2O due to the effect of vibronic coupling on the non-radiative decay. However, the extent of coupling decreases with increasing separation between the electronic states, and the fact that no differences are observed between the fluorescence lifetimes of NAD in the two solvents indicates that this effect is insignificant here.

Table 2. Maximum emission wavelength (λ_{max}), Stokes shift, singlet excited state energy (E_S), determined fluorescence quantum yields (Φ_F) and measured lifetimes (τ_F), chi squared factor (χ^2) and calculated radiative rate constant ($k_R = \Phi_F/\tau_F$) for the emission

decay of NAD in water, D_2O and organic solvents.

Solvent	Em. (λ_{\max}/nm)	Stokes shift (nm)	E_S (eV)	Φ_F^a	τ_F/ns	χ^2	$k_R \times 10^6/\text{s}^{-1}$
Water	324	44.0	3.99	0.066	35.0	1.09	1.89
D ₂ O	324	44.0	3.99	0.068	34.1	1.05	1.99
Ethylene glycol	325	42.0	3.99	0.096	52.9	0.98	1.81
Ethanol	325	43.0	4.09	0.096	49.0	1.05	1.96
Acetonitrile	325	43.0	4.00	0.186	54.0	1.08	3.44
Chloroform	325	42.0	4.09	0.022	6.77	1.10	3.25
1,4-Dioxane	326	43.0	3.95	0.357	51.0	1.01	7.00

^a Estimated errors $\pm 10\%$

The fluorescence lifetimes, τ_F , in water, D₂O and organic solvents were measured with nanosecond time resolution, with excitation at 282 nm and observation at the maximum emission wavelength. Fig. 3 shows the decay curve of NAD fluorescence in water while the results for all systems are summarized in Table 2. The fluorescence decay of NAD could be fitted by a single exponential for all the solvents, except ethylene glycol and ethanol, revealing a single emitting species. In those two solvents a better fit was obtained by a bi-exponential decay. To attempt to clarify the bi-exponential behavior seen in ethylene glycol and ethanol, NAD fluorescence decays were studied in three other alcohols, 1-propanol, 2-propanol and methanol. In methanol, the fluorescence decay of NAD followed a mono-exponential fit with a lifetime of 49.5 ns while in 1-propanol and 2-propanol the decays were well fitted using a bi-exponential, with the major components having lifetimes of 48.0 and 48.4 ns, respectively. In all the studied alcohols, except methanol, a small contribution was observed from a short lifetime component (≈ 4.5 ns). Although we have not yet managed to attribute this, its contribution (5.0 %) is always small, and the lifetimes presented in Table 2 for ethanol and ethylene glycol are those for the major component in the decay.

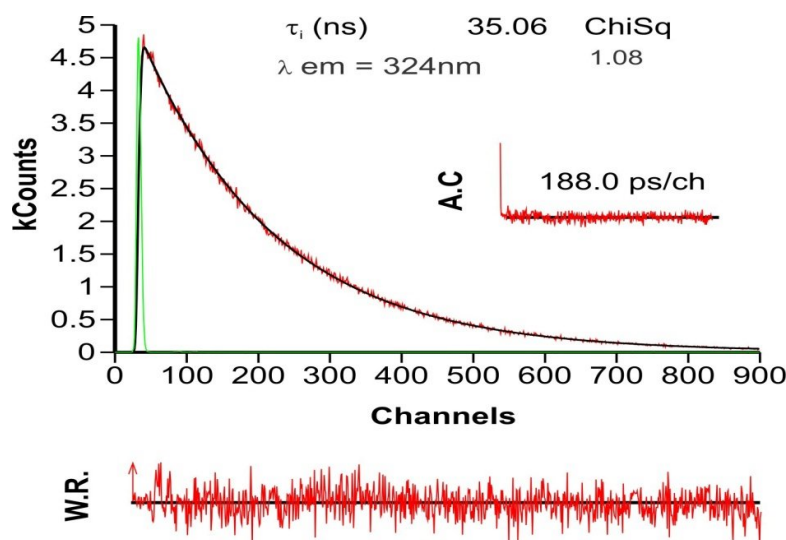


Fig. 3. Fluorescence decay of de-aerated aqueous NAD solution (1.0×10^{-5} mol L⁻¹) obtained with $\lambda_{\text{ex.}} = 282$ nm at room temperature. The autocorrelation function (A.C.), weighted residual (W.R.) and chi-squared value (χ^2) are presented as insets. The green line in the decay is the pulse instrumental response.

τ_F is higher in organic solvents (around 50 ns) than in water (35 ns, *ca.* 70% less than those in organic solvents). As with the fluorescence quantum yields this may reflect a strong contribution of internal conversion in the decay of the singlet excited state in water. In general, although the values obtained for τ_F are close to those obtained for other naphthalene derivatives [10], they are somewhat smaller, probably due to the effect of the substituent groups. The low value of τ_F observed in chloroform, 6.77 ns, suggests another pathway may be involved in the deactivation, and is in agreement with the suggestion of contact charge transfer quenching of the singlet excited state by the solvent. Additionally, the replacement of water by deuterium oxide had no effect on the fluorescence lifetime.

3.3. Fluorescence quenching by inorganic anions

Under environmental conditions, various additional species may contribute to the photochemical degradation of NAD. Many studies have been published concerning the involvement of electron transfer as a quenching mechanism of fluorescence for aromatic molecules such as naphthalene, anthracene, biphenyl, etc [39-42]. In addition, in our studies of NAD photodegradation, we observed an effect of azide ion [23]. Although

this is commonly used to identify mechanisms involving singlet oxygen, we suggested in our case that the effect involves quenching of NAD excited states. Therefore, we studied the quenching of NAD fluorescence with some halide ions, thiocyanate, azide, and also with the known electron-transfer quencher triethylamine (TEA), to obtain insights on mechanisms that may be involved in its excited state deactivation. Aqueous solutions of KBr, KI, KCl, KSCN, NaN_3 and a triethylamine solution in acetonitrile with different concentrations were added to a NAD solution at constant concentration of $1.0 \times 10^{-5} \text{ mol L}^{-1}$. In each case, a Stern-Volmer plot of I/I_0 vs $[Q]$ was constructed, where $[Q]$ represents the quencher concentration and the slope of the curve is equal to the Stern-Volmer constant K_{SV} . Fig. 4 shows, as one example, the effect of the quencher KSCN in NAD fluorescence. As can be seen in the figure, the addition of KSCN did not cause any change in the shape of emission spectra or formation of new bands, but only led to the decrease in fluorescence intensity due to the quenching process. Similar behaviour was observed with the other quenchers. Moreover, the excitation spectra in the presence of the quenchers resemble the excitation and absorption spectra in absence of the quenchers, confirming the lack of any ground state complexation.

Good straight lines were observed in the Stern-Volmer plots within the concentration range 10^{-4} - $10^{-2} \text{ mol L}^{-1}$, as can be seen by the inset plot in Fig. 4, strongly supporting a dynamic quenching mechanism. From the K_{SV} values reported in Table 3, it is possible to calculate the fluorescence quenching rate constant, k_q , for dynamic quenching, from the relationship $K_{SV} = k_q \tau_F$, where τ_F values are taken from Table 2. These results are also included in Table 3.

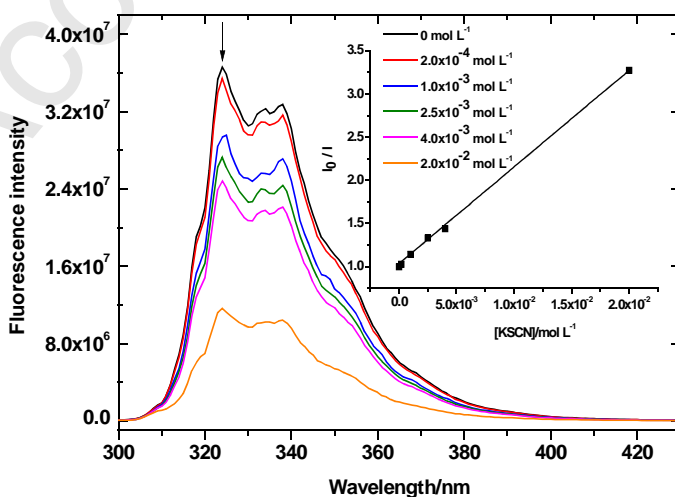


Fig. 4. Quenching of NAD fluorescence (1.0×10^{-5} mol L⁻¹) by KSCN in aqueous solution. The inset represents the Stern-Volmer plot.

Table 3. Stern-Volmer constants (K_{SV}) and fluorescence quenching rate constants (k_q) for NAD singlet excited state by anions (in water) and triethylamine (in acetonitrile). The redox potentials of the quenchers are also given for better discussion of results.

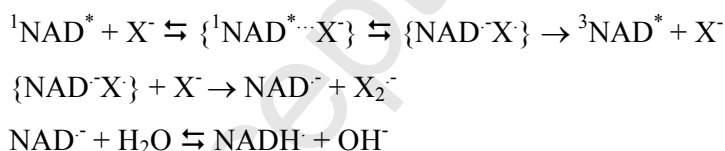
Quencher	$K_{SV}/$ L mol ⁻¹	$k_q/$ L mol ⁻¹ s ⁻¹	Reduction potential ^a /V
Cl ⁻	≤0.0038	≤ 1.08×10^5	2.41 ^[43]
Br ⁻	18.1	5.18×10^8	1.92 ^[43]
I ⁻	180	5.15×10^9	1.33 ^[43]
SCN ⁻	123	3.52×10^9	1.63 ^[43]
N ₃ ⁻	102	2.91×10^9	1.33 ^[43]
TEA	79.0	1.46×10^9	0.96 ^[10]

^a E° values for X[•]/X⁻ vs. the normal hydrogen electrode (NHE).

In aqueous solution, k_q varies in the range 5.18×10^8 - 5.15×10^9 L mol⁻¹ s⁻¹, indicating that there is a marked effect of the type of anion in the quenching of the fluorescence. From Table 3 we observe that this is related to the reduction potential of the X[•]/X⁻ couple, with I⁻ having the highest k_q , 5.15×10^9 L mol⁻¹ s⁻¹, and Cl⁻ the lowest. The low k_q value observed with Cl⁻ is a limit since the fluorescence intensity of NAD was almost constant with increasing concentrations of KCl, showing that Cl⁻ is not effective in quenching NAD fluorescence. Furthermore, N₃⁻ has a very similar efficiency in quenching the fluorescence of NAD to SCN⁻, but is less effective than I⁻. Both electron transfer and heavy atom effects may be responsible for the quenching. For electron transfer, the k_q values can be related to the free energy of reaction through the Rehm-Weller relationship [44]. The free energy depends on the electrochemical data, and a reasonable linear relationship between log k_q vs redox potential can be drawn for the anions I⁻, Br⁻ and SCN⁻ ($r=0.856$), with slope 1.6. A similar semilogarithmic relationship between the quenching rate and electrochemical data has been reported for the fluorescence quenching of naphthalene derivatives by halide ions [39,40], and suggests that the mechanism responsible for the quenching involves electron (or charge) transfer from the

anion to NAD singlet excited state, $^1\text{NAD}^*$. We note that in both ref. 39 and our case the quenching efficiencies of N_3^- and SCN^- are very similar, although their redox potentials are different. However, the recommended value of 1.33 V for the reduction potential of the azide/azidyl couple is for buffered neutral solution [43]. Hydrazoic acid (HN_3) is a weak acid (pK_a 4.65 at zero ionic strength) [45] and it is likely that the reduction potential for $\text{N}_3^\bullet/\text{N}_3^-$ in our unbuffered solution will differ from this recommended value. Additional support for an electron transfer mechanism comes from the fluorescence quenching of NAD in acetonitrile by the electron donor TEA.

To obtain evidence for the fact that electron transfer is involved in the excited singlet state quenching, aerated and de-aerated solutions of NAD in the presence of KI and KSCN, respectively, were studied by laser flash photolysis with excitation of NAD at 266 nm. The transient bands corresponding to the formation of the radical anions $\text{I}_2^{\bullet-}$ at 380 nm and of $(\text{SCN})_2^{\bullet-}$ at 480 nm [46] were observed as well as the formation of NAD triplet state, confirming parallel processes of electron transfer and intersystem crossing (*isc*). With the study by Shizuka *et al.* in 50% ethanol-water [40], there was no evidence for formation of the inorganic anion radical. However, this requires a second step in which the initially formed radical pair reacts with halide ions [47], which may be inhibited in the mixed solvent medium used by Shizuka *et al.* [40]. A possible mechanism is:



where X represents the halide anion. The fact that weak quenching was observed with bromide ion and no significant quenching with chloride ion places a limit on the reduction potential of $^1\text{NAD}^*$ of $E^\circ 2.1 \pm 0.2$ V.

3.4. Phosphorescence spectroscopy and triplet state characterisation

The phosphorescence emission spectra, quantum yields, Φ_P , and lifetimes, τ_P , of NAD were measured at 77 K in ethanol, acetonitrile and ethylene glycol glasses. As can be seen in Fig. 5, NAD presents well-resolved emission spectrum in acetonitrile with three main vibronic bands, which are comparable to those obtained in fluorescence spectrum.

Similar trends are observed in ethylene glycol and ethanol glasses, although the spectra are not so well resolved due to problems with light scattering. The lowest wavelength band obtained in acetonitrile is assigned to the 0-0 transition between the lowest triplet state (T_1) and the ground state (S_0), and gives the energy of the triplet excited state (E_T), 2.69 eV. This value is close to the literature value for naphthalene (2.66 eV) [10].

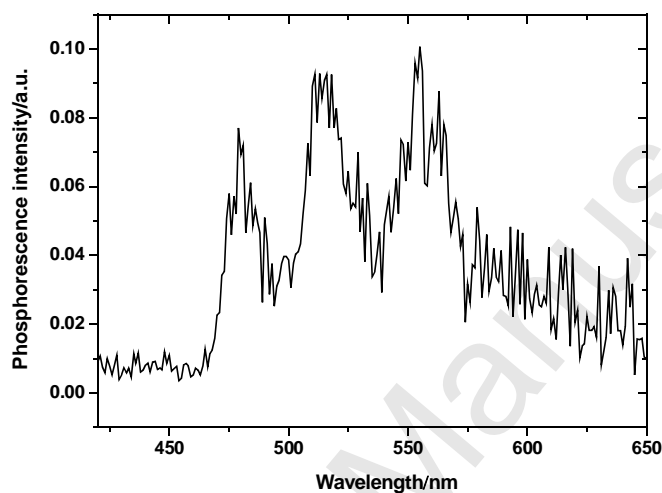


Fig. 5. Phosphorescence spectrum of NAD (1.0×10^{-5} mol L $^{-1}$) in acetonitrile at 77 K.

The Φ_P , calculated in ethanol, ethylene glycol and acetonitrile at 77 K using benzophenone in ethanol at 77 K as reference (0.73) [10] gave similar values (Table 4).

Table 4. Phosphorescence properties of NAD in ethanol, ethylene glycol and acetonitrile glasses at 77 K.

Solvent	Φ_P^a	τ_P/ms	k_R/s^{-1}	Φ_{isc}^b (T_1-S_0)
Ethylene glycol	0.043	210	0.22	0.57
Ethanol	0.052	64	0.79	0.50
Acetonitrile	0.043	0.12	400	0.65

^a Estimated errors $\pm 10\%$

^b Calculated using equation(8)

The phosphorescence lifetime, τ_P , was determined in the above glasses as the average of at least 10 measurements, with a relatively low measurement error. The results are also presented in Table 4. The fact that the phosphorescence lifetime in ethylene glycol is in the hundreds of milliseconds time range is in agreement with the triplet excited state, corresponding to a (π - π^*) transition, as expected from other reports for naphthalene and its derivatives [10]. A comparison of τ_P in polar protic solvents (ethylene glycol and ethanol) and in the polar aprotic solvent (acetonitrile) supports the role played by the hydrogen bond on the decay lifetime. The explanation for the decrease in lifetime on going from ethylene glycol to acetonitrile is not clear. However, this is completely reproducible. The facts that similar quantum yields are observed with these two solvents while the lifetime is much shorter in CH₃CN, indicates that the magnitude of the effects involved must be similar with both radiative and non-radiative processes.

The lowest NAD triplet excited state, $^3\text{NAD}^*$ (T_1) may be deactivated to the singlet ground state S_0 by radiative (phosphorescence) or radiationless processes (*isc*). We have calculated the rate constant for phosphorescence decay k_p ($= \Phi_p/\tau_P$) as well as the radiationless quantum yield Φ_{isc} (given in Table 4) for the deactivation of $^3\text{NAD}^*$ state, according to the expression (8):

$$\Phi_{isc}(S_1 - T_1) = \Phi_p + \Phi_{isc}(T_1 - S_0) \quad (8)$$

The results given in Table 4 show that the deactivation of $^3\text{NAD}^*$ state occurs preferentially by *isc* rather than by the radiative process of phosphorescence.

3.5. Triplet-triplet absorption spectra, absorption coefficients, lifetimes and triplet state quantum yields

Nanosecond laser flash photolysis studies were undertaken to obtain more detailed information on the reactive species that may be formed upon NAD photoexcitation. Excitation in the lowest energy absorption band of NAD (250-320 nm) was accomplished with frequency quadrupled (266 nm) pulses from a Nd/YAG laser. The transient absorption spectra were acquired on de-aerated NAD solutions in water, D₂O, ethylene glycol, ethanol, acetonitrile, chloroform and 1,4-dioxane, at different delays times after laser excitation. Fig. 6 shows the trends obtained for a de-aerated aqueous NAD solution. Inset is shown NAD decay at 420 and 330 nm. Three main transient absorption bands arising from NAD were observed: a negative band at 280 nm corresponding to the bleaching of the ground state, an intense band with maxima at 420

and 390 nm, and a third band with maximum at 330 nm. An additional band with maximum absorption at 720 nm attributed to the solvated electron was also observed [23]. At the laser energies used (<18 mJ) the hydrated electron is clearly formed through a monophotonic process.

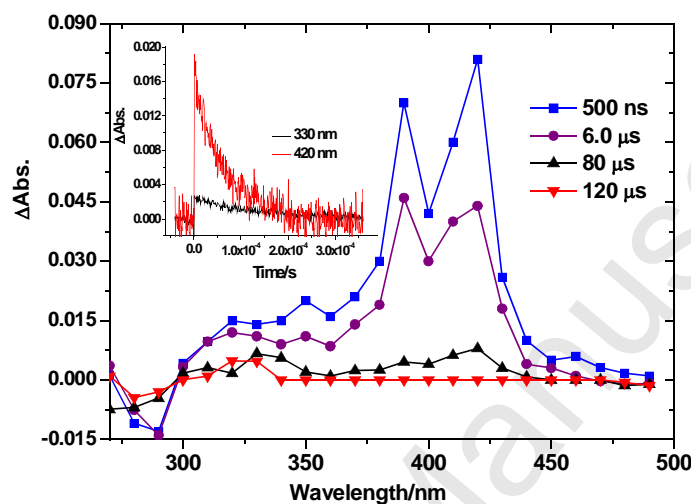


Fig. 6. Transient absorption spectra of de-aerated aqueous NAD solution (3.5×10^{-5} mol L^{-1}) obtained by nanosecond laser flash photolysis with excitation at 266 nm, recorded at 500 ns, 6.0, 80, and 120 μs after pulse. The kinetic decay profiles at 420 and 330 nm are displayed as insets.

The transient absorption band at 330 nm shows a decay profile different from the band at 420 nm (inset Fig. 6), with a rate constant of 8.5×10^3 s^{-1} and may be attributed to its radical cation, as has previously been seen with naphthalene [48]. When water was replaced by organic solvents, a 5 nm red shift of the main band at 420 nm occurs in 1,4-dioxane and in chloroform (see Table 5). The band at 330 nm was not observed in any of the organic solvents, within the time scale studied.

The absorption band with maximum around 420 nm can be assigned to the NAD triplet-triplet absorption, and is in good agreement to that seen for triplet states of naphthalene and derivatives [10,21,48]. To confirm this assumption, and since it is well known that molecular oxygen is a strong quencher of triplet excited states of aromatic molecules, solutions of NAD in all solvents were excited at 266 nm as function of oxygen concentration. The decay rate constant of NAD absorption band at the maximum wavelength was measured in each solvent, and the triplet lifetimes, τ_T , calculated as the

inverse of this rate constant. These results are summarized in Table 5. A marked effect of oxygen was observed in the decay of the transient band at 420-425 nm, as shown for aerated and de-aerated aqueous NAD solution (Fig. 7), where the decay of $^3\text{NAD}^*$ is faster in the presence of oxygen. This behaviour was observed for all the solvents, supporting the idea that this band corresponds to $^3\text{NAD}^*$.

In aerated and oxygenated solutions, the decay of the triplet state fitted a mono-exponential rate law. In contrast, in some cases, in de-aerated solutions, a better fit was achieved by a bi-exponential decay, probably due to triplet-triplet annihilation as suggested by τ_T decreasing with an increase of laser energy. In water, τ_T values of 1.4, 67.7 and 0.454 μs were found in aerated, de-aerated and oxygenated solution, respectively. The replacement of H_2O by D_2O did not cause any significant changes on the triplet lifetimes, as can be seen by the results given in Table 5.

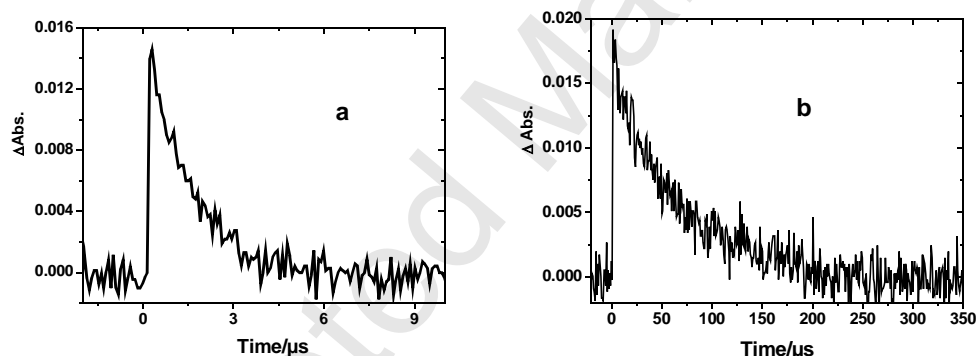


Fig. 7. Kinetic decay of aqueous NAD triplet state monitored at 420 nm in aerated (a) and de-aerated (b) conditions.

Table 5. Photophysical parameters for the NAD excited triplet state (lifetimes and quantum yield) as function of solvent polarity.

Solvent	Abs. ($\lambda_{\text{max}}/\text{nm}$)	$\tau_T^{\text{Argon}}/\mu\text{s}^a$	$\tau_T^{\text{Air}}/\mu\text{s}^a$	$\tau_T^{\text{O}_2}/\mu\text{s}^a$	Φ_T^a
Water	420	68	1.4	0.45	0.424
D_2O	420	65	1.5	0.39	0.414
Ethylene glycol	420	338	3.7	0.93	0.610
Ethanol	420	125	0.30	0.10	0.547
Acetonitrile	420	57	0.25	0.086	0.691
Chloroform	425	42	0.41	-	0.522

1,4-Dioxane	425	67	0.42	-	0.427
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^a Estimated errors $\pm 15\%$

Further confirmation of the triplet nature of the 420-425 nm transient species came from sensitization experiments using benzophenone (BP) as a high quantum yield triplet donor ($\Phi_T = 1.0$, $E_T = 287 \text{ kJ mol}^{-1}$, 2.98 eV) [10]. Fig. 8 shows the transient absorption spectra obtained at different times after excitation by 355 nm laser pulse of a de-aerated solution of BP in acetonitrile ($2.0 \times 10^{-3} \text{ mol L}^{-1}$) containing $4.0 \times 10^{-4} \text{ mol L}^{-1}$ of NAD. Under these conditions the laser light is totally absorbed by BP to form its triplet excited state.

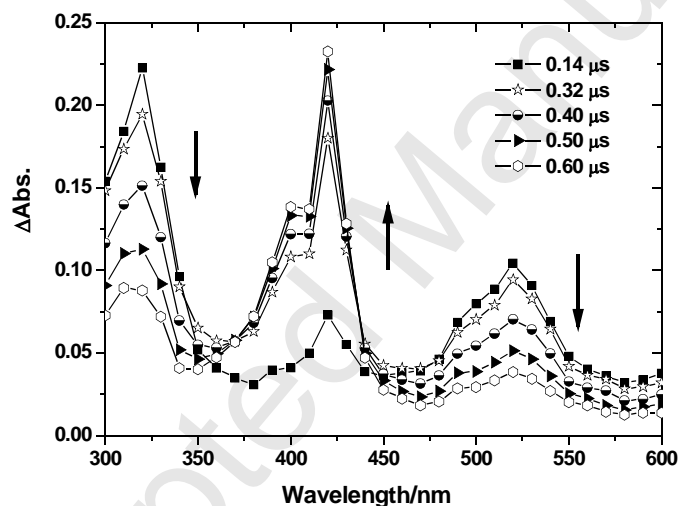
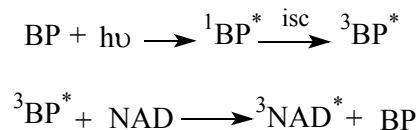


Fig. 8. Transient spectra observed at various times after subjecting a de-aerated solution of benzophenone ($2.0 \times 10^{-3} \text{ mol L}^{-1}$) containing NAD ($4.0 \times 10^{-4} \text{ mol L}^{-1}$) in acetonitrile with laser excitation at 355 nm.

The spectra show three bands with maximum absorption at 320, 420 and 520 nm. The bands at 320 and 520 nm are in good agreement with the formation of benzophenone triplet excited state (${}^3\text{BP}^*$) in acetonitrile [10], while the band at 420 nm corresponds to ${}^3\text{NAD}^*$. This band is identical to that obtained upon excitation of de-aerated NAD solution with a 266 nm laser pulse (Fig.6). The spectra at successive times show a decrease of the absorption band at 520 nm and a consequent increase of the band at 420 nm. Additionally, the decay rate of the ${}^3\text{BP}^*$ donor at 520 nm ($3.65 \times 10^6 \text{ s}^{-1}$) is slower than the apparent acceptor ${}^3\text{NAD}^*$ build up rate at 420 nm ($6.42 \times 10^6 \text{ s}^{-1}$) due to the

simultaneous acceptor triplet decay. These results indicate that energy transfer occurs from ${}^3\text{BP}^*$ to NAD with formation of ${}^3\text{NAD}^*$ according to:



This experiment was repeated with various concentrations of NAD (3.5×10^{-5} – 4.0×10^{-4} mol L $^{-1}$) maintaining constant BP concentration (2.0×10^{-3} mol L $^{-1}$). The decay rate at 520 nm of ${}^3\text{BP}^*$ was found to be linear with NAD concentration. A plot of the observed decay rates as function of NAD concentration allows the calculation of the quenching rate constant for the above reaction of 1.67×10^{10} L mol $^{-1}$ s $^{-1}$, corresponding to diffusion control in a collisional process. From consideration of energetic effects on the rate [49], this indicates that the triplet energy of NAD must be significantly less than that of benzophenone (2.98 eV), which is in agreement with results from phosphorescence in this solvent which gives a ${}^3\text{NAD}^*$ energy of 2.69 eV.

We also used this experiment to calculate the triplet state molar absorption coefficient, ϵ_T , of NAD through the energy transfer method ($\epsilon_{520 \text{ nm BP}} = 6500$ L mol $^{-1}$ cm $^{-1}$, $\Phi_T = 1.0$) [25]. A molar absorption coefficient of 13820 L mol $^{-1}$ cm $^{-1}$ was obtained for ${}^3\text{NAD}^*$ in acetonitrile at 420 nm. Assuming that this coefficient is constant and independent of solvent, we have used this value to calculate the intersystem crossing quantum yield (or triplet quantum yield), Φ_T , of NAD in the different solvents by the comparative method [26] using naphthalene in methylcyclohexane ($\epsilon = 13200$ L mol $^{-1}$ cm $^{-1}$, $\Phi_T = 0.75$) [10]. The results obtained of Φ_T for NAD in water, D $_2$ O, ethylene glycol, ethanol, acetonitrile, chloroform and 1,4-dioxane are given in Table 5. The Φ_T is dependent on solvent polarity, ranging from 0.42 in water to 0.69 in acetonitrile, indicating that the formation of ${}^3\text{NAD}^*$ by intersystem crossing is an important decay pathway of ${}^1\text{NAD}^*$ and may be relevant to the photoreactivity. Changing water to deuterated water did not affect the triplet state properties, as can be seen by the results in Table 5.

3.6. Quenching of ${}^1\text{NAD}^*$ and ${}^3\text{NAD}^*$ by molecular oxygen

Molecular oxygen is an important quencher of both singlet and triplet excited states of molecules [10,11,50,51], producing photophysical consequences such as fluorescence and triplet quenching, enhanced intersystem crossing and production of singlet oxygen,

$^1\text{O}_2$ ($^1\Delta_g$). Hence, we have determined the rate constants for quenching of $^1\text{NAD}^*$ by molecular oxygen, $k_S^{\text{O}_2}$, in the various solvents in oxygenated, aerated and de-aerated solutions, through a Stern-Volmer plot of the fluorescence intensity ratios for aerated and de-aerated solutions vs oxygen concentration. A linear fit was obtained, with the slope giving the Stern-Volmer coefficient K_{SV} ($=k_q\tau_F$). Since the fluorescence lifetimes of NAD are known (Table 2), values of $k_S^{\text{O}_2}$ were then calculated (Table 6). In addition, the rate constants for oxygen quenching of NAD triplet excited state, $k_T^{\text{O}_2}$, were calculated from the following equation (9):

$$k_{obs} = k_0 + k_T^{\text{O}_2} [\text{O}_2] \quad (9)$$

where k_{obs} and k_0 are the observed first-order rate constants for the decay of NAD triplet excited state at the absorption maximum for each solvent in the presence and absence of oxygen, respectively, and $[\text{O}_2]$ is the oxygen concentration in each solvent [10]. The plot of the observed triplet rate constants k_{obs} vs the oxygen concentration gives a linear fit where the slope is equal to $k_T^{\text{O}_2}$ for all solvents. Table 6 lists the quenching rate constants obtained for NAD singlet and triplet excited states by molecular oxygen for all the solvents studied.

Table 6. Quenching rate constants of NAD singlet and triplet excited states, $k_S^{\text{O}_2}$ and $k_T^{\text{O}_2}$, respectively, by molecular oxygen, as function of solvent polarity.

Solvent	$k_S^{\text{O}_2}/$ $\text{L mol}^{-1} \text{s}^{-1}$	$k_T^{\text{O}_2}/$ $\text{L mol}^{-1} \text{s}^{-1}$
Water	1.08×10^{10}	1.51×10^9
Ethylene glycol	6.50×10^8	1.82×10^9
Ethanol	8.55×10^9	9.46×10^8
Acetonitrile	1.81×10^{10}	1.21×10^9
Chloroform	1.47×10^{10}	1.00×10^9
1,4-Dioxane	1.18×10^{10}	1.82×10^9

^a Estimated errors $\pm 10\%$

The results show that the bimolecular quenching rate constants of NAD excited states vary over a wide range (10^8 - 10^{10} $\text{L mol}^{-1} \text{s}^{-1}$), in agreement with literature data concerning aromatic molecules such as naphthalene and naphthalene derivatives [10].

The lowest value of $k_S^{O_2}$, presented in ethylene glycol, is probably due to its high viscosity. Surprisingly, the rate for quenching NAD triplet excited state in this solvent is slightly higher than for the singlet excited state. Although we do not, at present, have any explanation for this, the measurement was repeated and the value is fully reproducible. In general, the quenching rate constants of $^1\text{NAD}^*$ by molecular oxygen, $k_S^{O_2}$, approach the diffusion controlled limiting rate constant, k_{diff} , and present higher values than those obtained for the deactivation of ^3NAD , $k_T^{O_2}$. This difference is related with the spin statistical factor that in triplet excited state is $(1/9) \times k_{diff}$ while for singlet excited states it is 1. For example, in water, the quenching process of $^1\text{NAD}^*$ by molecular oxygen has a rate of $1.08 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ while for $^3\text{NAD}^*$ it is $1.51 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$, about $1/9^{\text{th}}$ that of $^1\text{NAD}^*$, as frequently observed in aromatic systems [52]. Although the details of the quenching mechanism are still not clear, there is general agreement that the mechanism depends on the nature of the system, and involves energy and/or charge transfer leading to the formation of singlet oxygen $^1\text{O}_2$ [11,53,54], a very reactive species, and possibly superoxide anions. Since no other transient species were detected experimentally by laser flash photolysis, we can presume that energy transfer between $^3\text{NAD}^*$ and molecular oxygen leading to $^1\text{O}_2$ formation is highly favorable. This idea is supported by the results presented in the next section concerning the sensitization of singlet oxygen and also by previous studies on quenching of triplet state of naphthalene derivatives and formation of singlet oxygen [55].

3.7. Singlet oxygen measurements

Previous studies on naphthalene derivatives demonstrate that this type of compounds is able to generate singlet oxygen, $^1\text{O}_2$ ($^1\Delta_g$), with relatively good yields [56-59]. We have used time resolved phosphorescence with emission monitored at 1270 nm to determine the singlet oxygen formation quantum yields (Φ_Δ). In addition, photosensitization has been used to study the rate constant for singlet oxygen reacting with NAD.

For the determination of Φ_Δ , optically matched aerated solutions of NAD in various solvents were irradiated with a laser pulse at 266 nm using as reference biphenyl solution in cyclohexane ($\Phi_\Delta = 0.73$) [28]. This was chosen rather than the more commonly used phenalenone standard because of its stronger absorption at the excitation wavelength within the accessible concentration range. The time-resolved phosphorescence emission intensity of these samples at 1270 nm was measured at

different laser pulse energies and extrapolated to the start of the decay. Since all solutions have the same absorbance at the excitation wavelength, for any given laser energy, the number of photons absorbed by any solution will be the same. Individual singlet oxygen luminescence traces were signal averaged and fitted using a single exponential function to yield the luminescence intensity. The phosphorescence intensity was then plotted against the laser energy [27], which gave straight lines whose slopes were compared with that obtained from the reference biphenyl, yielding relative singlet oxygen quantum yields, Φ_A .

The relative Φ_A values obtained for NAD (Table 7) show a dependence on solvent and vary between 0.097 and 0.396, in water and chloroform, respectively. Although these

values are not high, we can say that they are within the range of values already reported for other naphthalene derivatives [60]. Though there may be technical difficulties with measurements in water due to the short singlet oxygen lifetime in this solvent (*ca.* 4 μ s) the effect of this is small since the substitution of water by deuterated water had no effect on the Φ_A value although the lifetime in deuterated water is 20 times higher than in H₂O [61]. A comparison of the Φ_A for NAD in the various solvents and the corresponding Φ_T values (see Table 5) indicates that the former are much lower than the Φ_T values, providing evidence of a relatively low efficiency of energy transfer from ³NAD* to molecular oxygen to produce ¹O₂. The case of water (Φ_T 0.424, Φ_A 0.097) is particularly striking. Therefore, it is likely that quenching of NAD triplet excited state by molecular oxygen in polar solvents also involves other pathways, such as electron transfer to produce the superoxide anion radical and NAD radical cation, both of which are implicated in the photodegradation of this compound in water [23].

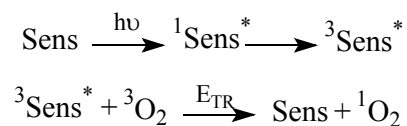
Table 7. Singlet oxygen quantum yield (Φ_A) formation as function of solvent polarity.

Solvent	Φ_A^a
Water	0.097
D ₂ O	0.105
Ethylene glycol	0.261

Ethanol	0.394
Acetonitrile	0.269
Chloroform	0.396
1,4-Dioxane	0.346

^a Estimated errors $\pm 10\%$

To test the reactivity of NAD with $^1\text{O}_2$, this species was prepared by photosensitization [62,63] through energy transfer (E_{TR}) from the triplet excited state of a sensitizer (Sens) to molecular oxygen ($^3\text{O}_2$):



Phenalenone was chosen as sensitizer due to the high quantum yields of $^1\text{O}_2$ formation ($\Phi_A = 0.98$) [29], and chloroform was chosen as a solvent since it gives a sufficiently long singlet oxygen lifetime ($\tau_A = 2.5 \times 10^{-4}$ s) [64]. Aerated solutions containing a constant concentration of phenalenone (3.0×10^{-5} mol L⁻¹) and concentrations of NAD varying within the range 1.0×10^{-4} - 1.0×10^{-2} mol L⁻¹ were excited at 355 nm and the $^1\text{O}_2$ phosphorescence decay was measured at 1270 nm. Rate constants of $^1\text{O}_2$ decay were obtained from the observed phosphorescence lifetimes ($k_{\text{obs}} = 1/\tau_A$). A plot of the observed rate constants k_{obs} vs NAD concentration gave a linear plot (Fig. 9) indicating the overall kinetic equation (10):

$$k_{\text{obs}} = k_0 + k_q [\text{NAD}] \quad (10)$$

where k_0 is the rate constant in absence of NAD and k_q is the quenching rate constant of $^1\text{O}_2$ by NAD.

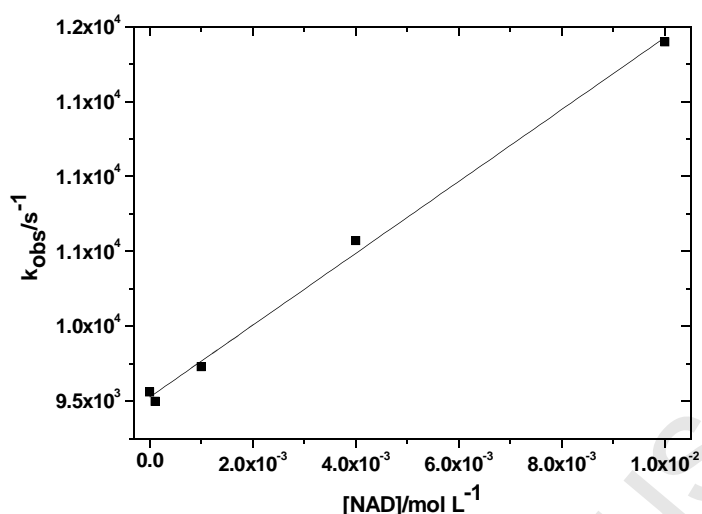


Fig. 9. Stern-Volmer plot of rate constant versus NAD concentration.

From this, a second-order rate constant of $2.4 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1}$ was obtained for the quenching reaction between $^1\text{O}_2$ and NAD in chloroform. Although this value of k_q is small, it lies between the values already reported for compounds within the same family such as 2-naphthol in chloroform ($7.1 \times 10^4 \text{ L mol}^{-1} \text{ s}^{-1}$), dimethyl-naphthalenes (range from 10^4 - $10^5 \text{ L mol}^{-1} \text{ s}^{-1}$) and various other hydroxynaphthalenes (range from 10^6 - $10^7 \text{ mol}^{-1} \text{ L s}^{-1}$) [60,65].

3.8. Deactivation pathways of NAD excited states

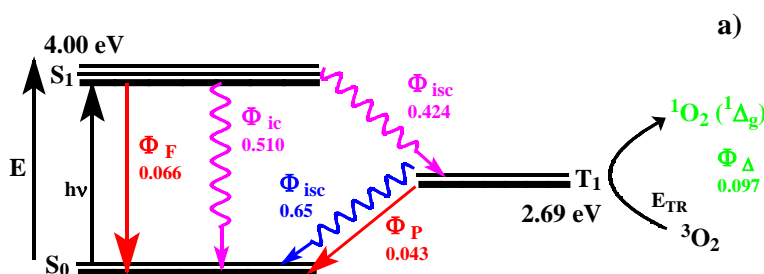
From all above data, it has been possible to fully characterize the photophysical deactivation routes of NAD excited states. These include the radiative processes of fluorescence (S_1 - S_0) and phosphorescence (T_1 - S_0), and the radiationless processes of internal conversion (*ic*) (S_1 - S_0), and intersystem crossing (*isc*) (S_1 - T_1) and (T_1 - S_0). Using NAD fluorescence quantum yields and the measured fluorescence lifetimes (Table 2), the natural radiative rate constant $k_R (= \Phi_F/\tau_F)$ and radiationless rate constants $k_{NR} (= 1-\Phi_F/\Phi_F)$ were determined for NAD in each solvent as shown in Table 8. From these, the natural radiative lifetimes ($\tau_{nat}^0 = 1/k_R$) were calculated, which were found to vary in the range of 290 to 530 ns and are considerable greater than the experimental fluorescence lifetimes, τ_F , showing the major role that radiationless process plays in deactivation of $^1\text{NAD}^*$. Furthermore, the quantum yield of internal conversion, Φ_{ic} , can be calculated from the relationship $\Phi_F + \Phi_T + \Phi_{ic} = 1$, which assumes that only these three processes jointly deactivate $^1\text{NAD}^*$ and also the rate constants for the radiationless

processes, k_{ic} ($= \Phi_{ic}/\tau_F$) and k_{isc} ($= \Phi_T/\tau_F$), since the values of Φ_F and of Φ_T are known (see Table 2 and 5). The photophysical parameters calculated for the deactivation of $^1\text{NAD}^*$ in the different solvents are presented in Table 8. It can be noted that the Φ_{ic} in water, 0.510, is greater than in the other solvents, showing that this is the preferred deactivation pathway in this solvent. From comparison of Φ_F , Φ_{ic} and Φ_{isc} values (see Table 2, 5 and 8) for the other solvents, Φ_{ic} is greater than Φ_F (except in acetonitrile), but the dominant pathway of $^1\text{NAD}^*$ deactivation involves intersystem crossing.

Table 8. Radiative rate constants decay (k_R), natural radiative lifetimes (τ_{nat}^0), radiationless rate constants decay (k_{NR}), radiationless rate constants decay for internal conversion (k_{ic}), radiationless rate constants decay for intersystem crossing (k_{isc}), and quantum yield of internal conversion (Φ_{ic}) of NAD in water, D_2O and in organic solvents.

Solvent	k_R/s^{-1}	τ_{nat}^0/ns	k_{NR}/s^{-1}	Φ_{ic}	k_{ic}/s^{-1}	k_{isc}/s^{-1}
Water	1.88×10^6	532	2.67×10^7	0.510	1.46×10^7	1.21×10^7
D_2O	1.94×10^6	515	2.66×10^7	0.518	1.48×10^7	1.18×10^7
Ethylene glycol	1.81×10^6	552	1.70×10^7	0.294	5.54×10^6	1.15×10^7
Ethanol	1.96×10^6	510	1.84×10^7	0.357	7.28×10^6	1.11×10^7
Acetonitrile	3.44×10^6	291	1.51×10^7	0.123	2.29×10^6	1.28×10^7
Chloroform	3.25×10^6	307	1.44×10^8	0.456	6.73×10^7	7.71×10^7
1,4-Dioxane	6.94×10^6	144	1.25×10^7	0.216	4.20×10^6	8.30×10^6

A Jablonski diagram, summarizing all the processes that occur for NAD in water and in an organic solvent (acetonitrile) is presented in Fig. 10.



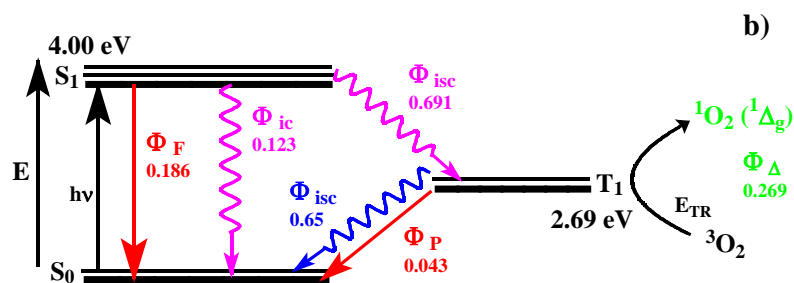


Fig. 10. Jablonski diagram for NAD de-aerated solutions in (a) water and in (b) acetonitrile.

4. Conclusion

A comprehensive study has been accomplished on NAD photophysics in various solvents. From the detailed results obtained along this study, some important conclusions could be drawn: (1) the main deactivation pathway for $^1\text{NAD}^*$ depends on the solvent; in water, the non radiative $S_1 \rightarrow S_0$ internal conversion process is predominant while in organic solvents the radiationless $S_1 \rightarrow T_1$ intersystem crossing becomes the major process; (2) the radiative process of fluorescence $S_1 \rightarrow S_0$ is less important in all solvents when compared to the non radiative ones; (3) $^3\text{NAD}^*$ deactivates mainly by the radiationless $T_1 \rightarrow S_0$ intersystem crossing and the radiative conversion is very low in all solvents; (4) $^1\text{NAD}^*$ is quenched more efficiently by molecular oxygen than $^3\text{NAD}^*$, as expected from spin-statistical factors; (5) NAD excitation leads to $^1\text{O}_2$ formation through triplet sensitization, particularly in non-aqueous solvents. However, singlet oxygen reaction does not appear to be the main route for NAD photodegradation in water [23], and there are indications that in this solvent oxygen quenching may partially occur through electron transfer. This is likely to lead to formation of NAD radical cation. Radical cation formation is also observed upon flash photolysis of degassed aqueous solutions, and this is suggested to be an important intermediate in NAD photodegradation [23]. Finally, as with many other aromatic compounds, it has been shown that NAD singlet excited is also a relatively strong photooxidant.

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