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Additional Information

Transient UV-Vis Absorption Spectroscopic Characterisation of 2'-Methoxyacetophenone as a DNA Photosensitiser

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Abstract

2'-Methoxyacetophenone (2M) presents improved UVA absorption as compared with other acetophenone derivatives. On the basis of transient infrared spectroscopy it has been previously claimed that 2M is an interesting photosensitiser for cyclobutane pyrimidine dimers (CPDs) formation. In the present paper, a complete UV-Vis transient absorption spectroscopic characterisation of this compound is provided, including triplet-triplet spectra, triplet lifetimes and rate constants for quenching of 2M by a dimeric thymine derivative. Furthermore, generation of singlet oxygen has been proven by time-resolved near IR phosphorescence measurements. Overall, the obtained results confirm the potential of 2M as a DNA photosensitiser, not only for CPDs formation, but also for oxidative damage.

Key words: DNA damage, nanosecond spectroscopy, singlet oxygen, triplet state

1. Introduction

The behaviour of aromatic ketones upon light absorption has been intensively investigated due to its particular features such as relatively long absorption wavelengths and high intersystem crossing quantum yields.¹ The photoreactivity of these compounds depends on the nature ($n\pi^*$ vs $\pi\pi^*$) of the lowest triplet state; thus, Norrish I/II, hydrogen abstraction or Paterno-Büchi are typical $n\pi^*$ photoreactions. In this context, variations in the aromatic substituents or the solvents have a marked influence on the relative energy of the $n\pi^*/\pi\pi^*$ triplet states and hence on the photoreactivity pattern.² Aromatic ketones such as benzophenones and acetophenones have been often used as triplet photosensitisers;¹ in particular, they have turned out to be very useful tools to investigate the photosensitisation mechanism responsible for the formation of DNA lesions like oxidative damage or cyclobutane pyrimidine dimers (CPDs).³⁻⁹

While solar light is essential for life on Earth, its absorption by DNA is in the origin of a number of lesions that, if not properly repaired, may lead to mutagenicity and even to cancer.¹⁰⁻¹⁵ The most abundant DNA lesions formed upon direct irradiation are CPDs. They can also be generated through photosensitisation by endogenous or exogenous agents and arise from a formal [2+2] photocycloaddition between two pyrimidines located consecutively in the same DNA strand.¹⁶

2'-Methoxyacetophenone (2M) presents enhanced UVA absorption as compared with other acetophenone derivatives.¹⁷ This allows selective excitation of 2M in the presence of pyrimidines, which is mandatory for an appropriate CPDs photosensitiser.¹⁶ Previous transient spectroscopic studies have made use of pico- and nanosecond IR detection to show formation of thymine dimers through a 2M-photosensitised pathway.¹⁷ Although valuable photophysical information was provided like the time constant of triplet formation (in the sub-ns region) and the intersystem crossing quantum yield (Φ_{ISC} , close

to unity) a more detailed investigation on 2M properties would be necessary to fully characterise this ketone as a potential DNA photosensitiser.

With this background, the aim of the present work is to undertake a more complete investigation on 2M using UV-Vis transient absorption spectroscopy with special emphasis on the characterisation of the triplet state and the rate constant of its quenching by a dimeric thymine model, in connection with CPDs formation. Furthermore, the capability of 2M to produce $^1\text{O}_2$, as a key parameter related to oxidative DNA damage, is established by means of time-resolved near IR emission at 1270 nm.

2. Experimental Section

2.1 Materials and solvents

S-Flurbiprofen (S-FB) and 2M were purchased from Sigma-Aldrich. The (HPLC)-grade solvents (ethanol and acetonitrile) were obtained from Scharlau and used without further purification. Water was purified through a Millipore MilliQ system. Solutions of PB 10 mM (pH = 7.6) were prepared by dissolving Na_2HPO_4 and NaH_2PO_4 in deionized water.

The dimeric thymine model (Thy-C₃-Thy) was synthesised and purified following the established procedure in the literature.¹⁸⁻²⁰

2.2 Irradiation procedures

2M-Photosensitised irradiation of Thy-C₃-Thy was performed by preparing a 0.043 mM solution of 2M in PB 10 mM. To 172 mL of this solution, Thy-C₃-Thy (2.2 mg) was added in order to have the same concentration as 2M. The irradiation was performed in quartz cuvettes with lamps emitting in the 310–390 nm range (with a maximum at 350 nm), using a multilamp photoreactor (Luzchem LZC-4V).

2.3 Steady state measurements

UV-Vis absorption spectra were registered with a simple beam spectrophotometer (Varian Cary 50 employing quartz cells of 1 cm path length).

2.4 Laser flash photolysis

A pulsed Nd: YAG SL404G-10 Spectron Laser Systems, using 355 nm as the excitation wavelength and pulses duration of 10 ns x pulse⁻¹, was employed to carry out the laser flash photolysis (LFP) experiments. The energy was set lower than 15 mJ x pulse⁻¹. The apparatus consisted of the pulsed laser, a pulsed Lo255 Oriel Xenon lamp, a 77200 Oriel monochromator, an Oriel photomultiplier tube (PMT) housing, a 70705 PMT power supply and a TDS-640A Tektronix oscilloscope. The output signal from the oscilloscope was transferred to a personal computer. Lifetime of singlet oxygen in ACN was recorded at 1270 nm with a Hamamatsu NIR detector upon excitation with a 355 nm Nd: YAG laser and using acetophenone as a reference compound (absorbance ca. 0.6 at λ_{exc} = 355 nm). Photophysical measurements in solution were recorded at room temperature (22°C), employing 1 cm path length quartz cells with 3 mL capacity and were bubbled during 15 min with N₂, air or O₂ before acquisition.

Transient absorption spectra were obtained from a deaerated solution of 2M in ACN, EtOH and aqueous medium (absorbance between 0.3 and 0.4 at λ_{exc} = 355 nm).

For quenching experiments S-FB (from 0 to 1.2 mM) and Thy-C₃-Thy (from 0 to 1 mM) were added to a solution of 2M in PB 10 mM with an absorbance of 0.4 at 355 nm. Kinetic decays were monitored at 370 and 500 nm for the S-FB quenching and at 500 nm for Thy-C₃-Thy.

3. Results and discussion

The ultraviolet (UV) spectrum of 2M displays two maxima centred ca 250 and 310 nm and a tail extending up to 360 nm (figure 1A). Accordingly, transient absorption spectra were recorded upon excitation at 355 nm in deaerated H₂O, ACN and EtOH, with a Nd: YAG laser (figure 1B). A band with a broad maximum between 400 and 450 nm was found in every solvent immediately after excitation, being more intense and 10 nm red-shifted in aqueous solution.

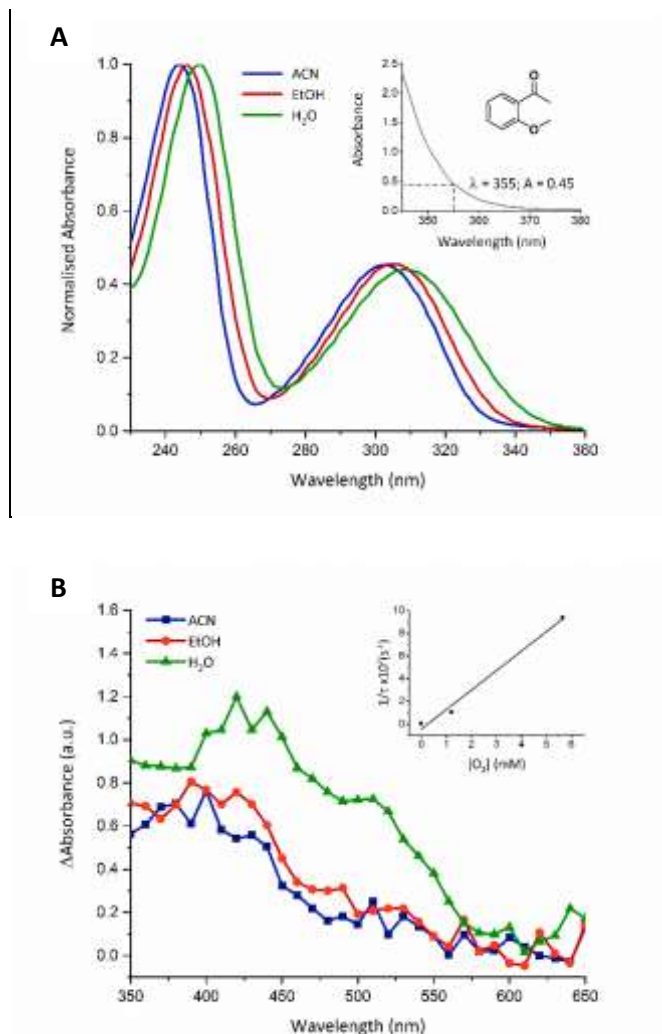
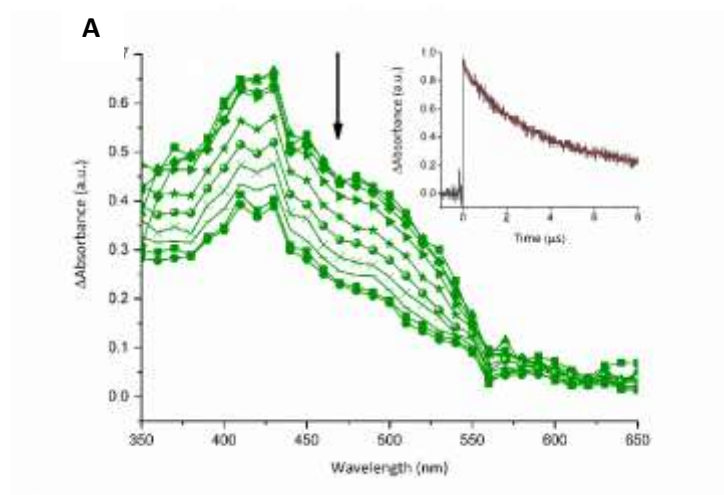


Figure 1. A) 2M UV-Vis spectra in acetonitrile, ethanol and water. **Inset)** Magnification of the long-wavelength absorption region of 2M (6.5 mM) in buffered aqueous solution, used in the transient absorption experiments. **B)** 2M transient absorption spectra in anaerobic ACN, EtOH and H₂O immediately after laser excitation. **Inset)** Reciprocal of ³2M* lifetime in ACN at 430 nm under N₂, air and O₂ against O₂ concentration (Stern-Volmer plot).

The temporal evolution of the observed transient species was monitored at 430 nm, and the lifetime was determined from the decay traces. In buffered aqueous solution the lifetime was 3.8 μ s (figure 2A, Inset). Based on the high ISC of 2M (ca. 1),¹⁷ this transient was tentatively assigned as the triplet excited state. Indeed, this is consistent with the quenching observed under air and O₂ atmospheres (figure 1B, Inset). However, an unambiguous spectroscopic evidence for the nature of this transient species was needed. To confirm the assignment, a triplet-triplet energy transfer (TTET) experiment was carried out, in which the energy of the triplet excited state of 2M was transferred to S-FB, to

form unequivocally its triplet excited state. In the present case, S-FB does not absorb light beyond 300 nm,²¹ allowing for selective excitation of 2M at 355 nm. As a matter of fact, when a 1.2 mM S-FB solution was submitted to the LFP at $\lambda_{\text{exc}} = 355$ nm no signal was observed. Moreover, although the triplet absorption spectrum of S-FB shows some overlap with that of 2M, it is possible to discriminate between the two species based on their differences in molar absorption coefficients and lifetimes.²¹ Thus, it is possible to monitor the increase of the signal corresponding to $^3\text{S-FB}^*$ at the expense of $^3\text{2M}^*$. As shown in figure 2B, the $^3\text{2M}^*$ spectrum displayed immediately upon laser excitation disappeared with time; concomitantly, a new spectrum built up presenting an intense band centred at 370 nm. Two microseconds after laser excitation, the observed spectrum was coincident with that of $^3\text{S-FB}^*$,²¹ indicating that the TTET was complete. Furthermore, the transient absorption at 370 nm increased with increasing concentration of S-FB (figure 2B inset). When $^3\text{2M}^*$ was monitored at 500 nm in the presence of S-FB (figure 2C), a faster decay was progressively observed. Plotting the reciprocal of $^3\text{2M}^*$ lifetime against S-FB concentration, a k_q of $3.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ was obtained (figure 2C Inset).



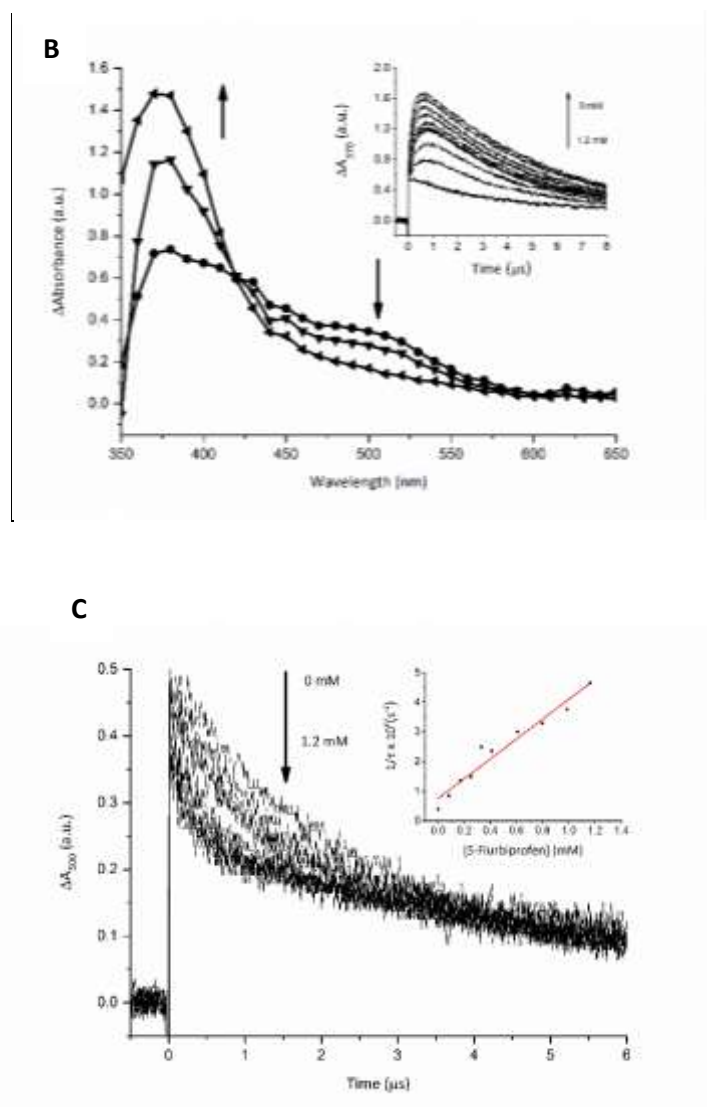


Figure 2. **A)** Transient absorption spectra of 2M in neutral PB under deaerated conditions at different times after 355 nm laser excitation. **Inset)** Kinetic decay of 2M at 430 nm in PB under deaerated conditions. **B)** Transient absorption spectra of 2M in the presence of *S*-flurbiprofen (1.2 mM) immediately after the laser pulse, 0.1 and 1 μ s after laser excitation (355 nm). **Inset)** Kinetic trace for the *S*-FB band at 370 nm. **C)** Kinetic decay of 2M at 500 nm after increasing amounts of *S*-flurbiprofen (from 0 to 1.2 mM) were added. **Inset)** Stern-Volmer quenching of 2M triplet by *S*-flurbiprofen.

To further characterise 2M as a DNA photosensitiser, its capability to induce oxidative photodamage through a type II mechanism was evaluated by measuring $^1\text{O}_2$ production. Thus, time-resolved phosphorescence emission at 1270 nm upon 355 nm laser excitation of a 2M solution was monitored using acetonitrile as solvent and the parent acetophenone as a reference (figure 3). The decay traces were nearly superimposable, which indicates that the singlet oxygen quantum yield (Φ_Δ) of 2M equals that of

acetophenone ($\Phi_{\Delta} = 0.52$).²² This indicates that 2M can indeed promote DNA oxidation via a type II photosensitisation.

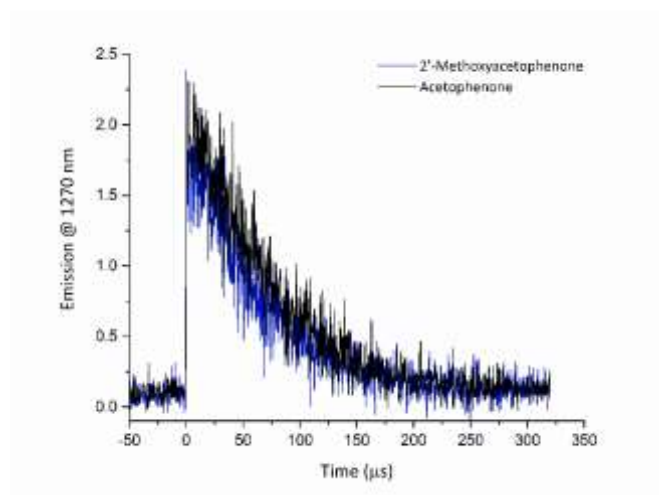
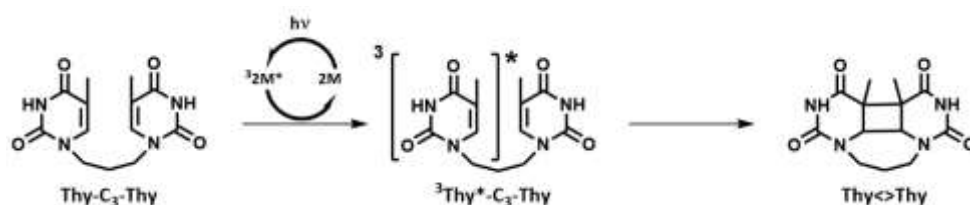


Figure 3. Time-resolved detection of singlet oxygen emission at 1270 nm for acetonitrile solutions of 2M and acetophenone.

At this point, a more complete picture of 2M as a potential DNA photosensitiser was available. Since the singlet oxygen reactivity towards DNA is well known, attention was focused on triplet photosensitisation. In this pathway, thymine (Thy) should be involved in the process as it is the canonical DNA base with the lowest triplet energy.

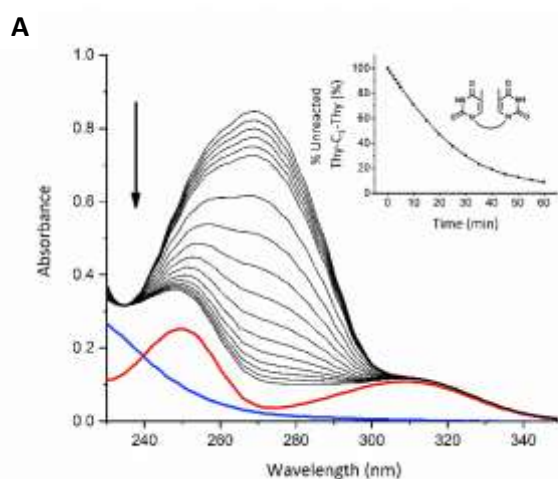
Irradiation of 2M in the presence of the dimeric thymine derivative Thy-C₃-Thy was performed and monitored through UV-Vis spectroscopy in order to test the disappearance of the enone chromophore associated with CPDs formation.

After 60 min under the employed irradiation conditions Thy-C₃-Thy practically disappeared, (figure 4A and inset). The key steps involved in the overall process following irradiation of 2M are energy transfer from ³2M* to a thymine unit leading to ³Thy* and the subsequent reaction of the excited base with another Thy in its ground state giving rise to the final CPD through a formal [2+2] photocycloaddition (Scheme 1).



Scheme 1. CPDs formation process through 2M photosensitisation.

Under the employed conditions 2M was found to be photostable, as demonstrated by the fact that its absorption band with maximum at 310 nm remained unaltered along the irradiation. Conversely, a progressive decrease corresponding to the unaltered Thy unit was observed. It is noteworthy that as the photoreaction proceeds, the absorption displayed by the reaction mixture resembles that of 2M as the CPDs formed do not absorb significantly above 240 nm (the absorption spectrum of the isolated CPD is shown in figure 4A, blue line). It is also noticeable that, in this experiment, 2M did not give rise to oxetane byproducts as a result of a Paterno-Büchi reaction, as it happens with benzophenone, widely used in pyrimidine photosensitisation experiments.⁵



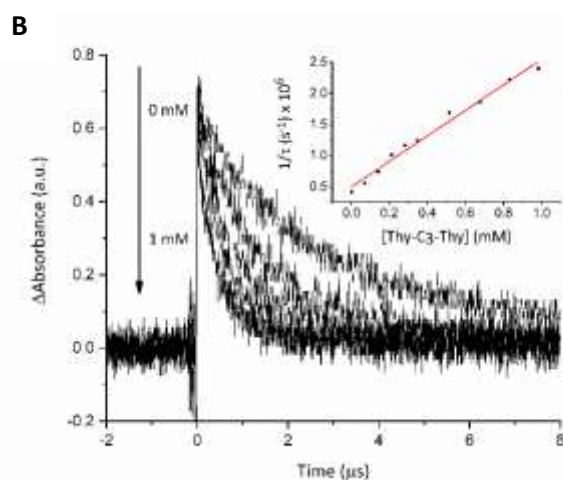


Figure 4. A) 2'-Methoxyacetophenone photosensitised irradiation of Thy-C₃-Thy in PB, under deaerated conditions. Spectra of 2M (red) and Thy-C₃-Thy CPDs (blue) at the same concentration are shown for better comparison. **Inset)** Unreacted Thy-C₃-Thy (%) monitored at 270 nm. **B)** Kinetic decays of 2M in deaerated PB at 500 nm upon addition of increasing amounts of Thy-C₃-Thy (from 0 M to 9.8×10^{-4} M). **Inset)** Stern-Volmer quenching of 2M triplet by Thy-C₃-Thy.

After 60 min under the employed irradiation conditions Thy-C₃-Thy practically disappeared, (figure 4A and inset).

To confirm that an excited state reaction was actually taking place, quenching of ³2M* by Thy-C₃-Thy was examined. Indeed, addition of increasing concentrations of Thy-C₃-Thy (figure 4B) resulted in a consistent shortening of the 2M triplet lifetime. From the slope of the Stern-Volmer linear plot (figure 4B, inset) a k_q value of 2.1×10^9 was obtained.

In summary, the photophysical parameters determined for 2M as a potential DNA photosensitiser are given in table 1.

Table 1. Triplet characterisation of 2'-methoxyacetophenone by transient UV-Vis transient absorption spectroscopy.

k_{O_2}	$1.7 \times 10^7 \text{ mM}^{-1} \text{ s}^{-1}$
τ_T	3.8 μs
Φ_Δ	0.5
k_Q (S-FB)	$3.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$
k_Q (THY-C ₃ -THY)	$2.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$

4. Conclusions

Overall, a complete UV-Vis transient spectroscopic characterisation of 2'-methoxyacetophenone has been performed. The triplet state reactivity of this photosensitizer towards a Thy model has been determined based on the obtained photophysical parameters and on the rate of CPDs formation revealed by UV-Vis monitoring of the steady-state irradiation. These results confirm the potential of 2M as a convenient DNA photosensitizer for mechanistic studies.

Conflicts of interest

There are no conflicts to declare.

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