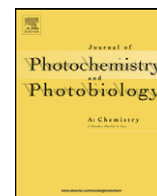




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Photostability of the sunscreensing agent 4-*tert*-butyl-4'-methoxydibenzoylmethane (avobenzone) in solvents of different polarity and proticity

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ABSTRACT

The most widely used UVA absorber in broad-spectrum sunscreens is 4-*tert*-butyl-4'-methoxydibenzoylmethane (avobenzone). However, the photostability of avobenzone is solvent-dependent. The aim of this work was to investigate the photostability of avobenzone in solvents of different polarity and proticity. Four solvents were employed, namely, cyclohexane, ethyl acetate, dimethylsulfoxide and methanol. The cause of the instability of avobenzone in these solvents was determined by means of UV spectroscopy, high performance liquid chromatography–mass spectrometry and nuclear magnetic resonance spectroscopy. The effect of oxygen on the photo-instability was also determined. Avobenzone was found to lose absorption efficacy as a result of photoisomerisation from the enol to the keto form and/or photodegradation to form photoproducts that absorb principally in the UVC region, depending on the solvent. It was found to be essentially photostable in the polar protic solvent methanol but photoisomerised in the polar aprotic solvent dimethylsulfoxide. In the nonpolar solvent cyclohexane, it photodegraded appreciably. Both photoisomerisation and photodegradation occurred to a similar extent in the moderately polar aprotic solvent ethyl acetate. Photoisomerisation occurred only in the presence of oxygen whereas photodegradation occurred irrespective of oxygen. This knowledge is important in order to achieve the correct formulation for sunscreens incorporating avobenzone.

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1. Introduction

Solar ultraviolet (UV) radiation incident on the earth's surface can be divided into two parts: the UVB region (290–320 nm) and the UVA region (320–400 nm). Recent evidence [1] suggests that both wavebands are responsible for the induction of skin cancer in humans. Consequently, photoprotective measures that rely on sunscreensing agents use a combination of absorbers in order to afford broadband protection over the entire solar UV range.

There are few chemical absorbers that provide protection in the UVAI (340–400 nm) range. Of these 4-*tert*-butyl-4'-methoxydibenzoylmethane (tradenames avobenzone, Parsol 1789, Eusolex 9020) is the most widely used. However, a number of sunscreen products containing this filter are not sufficiently photoprotective because of the photoinstability of avobenzone [2].

Avobenzone forms part of the dibenzoylmethane group of sunscreens and has a wavelength of maximum absorption (λ_{\max})

ranging from 350 nm to 365 nm depending on the solvent used. It exists in two tautomeric forms: the enol-tautomer (or enol form) and the keto-tautomer (or keto form). The keto form occurs only in one geometric form whereas the enol form has been postulated to occur in many geometric configurations [3–6]. Since the enol form is asymmetric it has two obvious isomeric forms, namely, two *cis*-enols as reported by Dubois et al. [3], enol form A and enol form B as shown in Fig. 1. Vila et al. [6] have shown that an equilibrium between these two *cis*-enols exists both in the solid and solution phases of avobenzone. These *cis*-enols are stabilised by an intramolecular hydrogen bond ("chelated" enol). Cantrell and McGarvey [4] and Andrae et al. [5] suggested that transient enol forms were also possible isomers of the enol form of avobenzone. These transient species were either produced as a result of rotation about a single bond (between carbon 8 and 9 in enol form A or 7 and 8 in enol form B of Fig. 1) or isomerisation at the double bond of the enol form (between carbon 7 and 8 in enol form A or 8 and 9 in enol form B of Fig. 1) as displayed in Fig. 2 [4]. The different photochemical properties of these many tautomers give rise to the complex photochemistry of avobenzone.

In a sunscreen formulation, avobenzone exists predominantly in the enol form which absorbs in the UVA wavelength range. This

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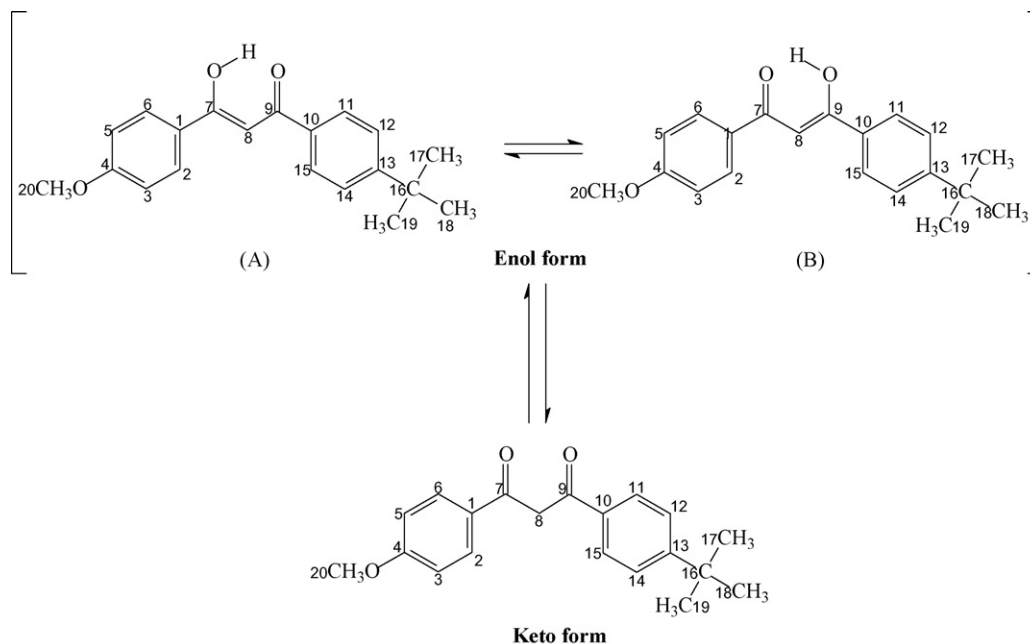


Fig. 1. The keto–enol tautomerism of avobenzene.

absorption band shifts to longer wavelength with an increase in solvent polarity [7,8]. This is indicative of a $\pi \rightarrow \pi^*$ transition in the CO-conjugated ethylene system of the enol form [9,10]. In solution an equilibrium mixture of the enol and keto forms is present. The relative amounts of the two isomers are solvent dependent but the equilibrium always lies in the direction of the enol tautomer. The keto form absorbs in the UVC range from 260 to 280 nm. Upon irradiation the enol form is photoisomerised to the keto form accounting for the large loss in absorption observed in solution [3,5,9,11–15] or in thin films [16,17]. The keto form can thermally revert to the enol form [3,14,15].

Laser excitation of avobenzene at 355 nm produces a transient enol species that absorbs maximally at 300 nm and hence cannot be the keto form [5]. This species recovers to the ground state chelated enol form with a solvent-dependent lifetime of the order of milliseconds [4,5]. Cantrell and McGarvey [4] proposed that this

transient enol species is formed as a result of isomerisation about the double bond, since the transient enol species formed through rotation can easily revert to the enol form through rapid rotation about a single bond. They also proposed that the two transient species are in equilibrium with each other, and the one formed by rotation is in equilibrium with the enol form (see Fig. 2). The kinetics of decay of this transient enol in non-polar solvents is mixed first- and second-order and the second-order component is more pronounced in non-polar solvents (as is the case with the parent structure dibenzoylmethane [18]). The second-order component is thought to arise from the formation of a hydrogen-bonded dimer leading to rapid proton exchange back to the ground-state enol or the keto form. These transient enols are likely to arise from the lowest excited singlet state as proposed for dibenzoylmethane [18].

In order for chemical sunscreen absorbers to impart a high screening efficiency they should be photostable. Any light-induced

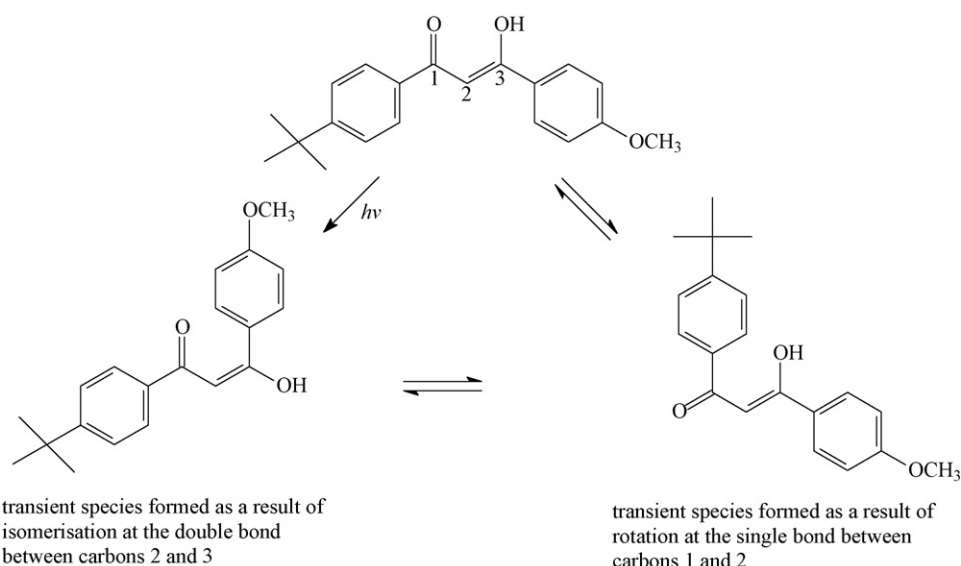


Fig. 2. The proposed equilibrium between the enol form of avobenzene and its transient forms [4].

Table 1
Characteristics of the solvents used to investigate the photostability of avobenzone, the wavelengths of maximum absorption (λ_{\max}) of avobenzone in these solvents and the equilibrium constants for enol–keto tautomerism ($K_e = [\text{enol}]/[\text{keto}]$).

Solvent	μ/D [30]	ϵ (25 °C) [30]	α^a [31]	β^b [31]	π^{*c} [31]	$E_T(30)^d$ /kcal mol ⁻¹ [32]	λ_{\max}/nm	K_e
Methanol	1.70	32.63	0.93	(0.62)	0.60	55.4	358	
DMSO	3.96		0	0.76	1.00	45.1	363	
DMSO- <i>d</i> ₆	3.9 ^e	47.24 ^e						10
Ethyl acetate	1.78	6.02	0	0.45	0.55	38.1	356	
Cyclohexane	0	2.015	0	0	0	30.9	351	
C ₆ D ₁₂	0 ^e	2.28 ^e						46

^a α parameter related to the hydrogen-donating ability of the solvent.

^b β parameter related to the solvent's ability to accept a proton in a solute-solvent hydrogen bond.

^c π^* index of solvent polarity-polarizability.

^d $E_T(30)$ empirical parameter that incorporates both solvent polarity and hydrogen bonding effects.

^e Data from Ref. [33].

decomposition reduces the photoprotective capacity, for example, by forming non-UV-absorbing photoproducts or photosensitising the degradation of other components. The formation of photoproducts of unknown toxicity and their accumulation on the skin of the user of the product could lead to potentially deleterious effects. In addition, the efficacy of the absorber can be dependent on the solvent in which it is dissolved. Therefore the absorbers need to be formulated in the appropriate medium in order to exhibit the desired characteristics.

Irreversible photodegradation of avobenzone has been observed in nonpolar solvents and concentrated solutions [3,11–15]. The photoproducts form from α -cleavage of the photo-excited keto form of avobenzone as they all stem from either a benzoyl or a phenacyl radical. Cantrell and McGarvey [4] excited avobenzone in acetonitrile at 266 nm and observed the formation of the triplet state of the keto form. They also observed that excitation of the keto form caused photodegradation of avobenzone. This is why triplet state quenchers, such as 3-(4-methylbenzylidene) camphor and octocrylene, are able to photostabilise avobenzone [4,19].

Sayre and Dowdy [20] found that upon UVA exposure not only did avobenzone in sunscreen products photodegrade but UVB absorbers, such as EHMC, also photodegraded when in the presence of avobenzone. This was supported by Panday [21] who showed that avobenzone photosensitises the photoisomerisation of *trans*-EHMC. Chatelain and Gabard [19] were able to photostabilise these avobenzone–EHMC combinations by the addition of bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S).

Damiani et al. reported that avobenzone, when illuminated *in vitro*, caused DNA strand breaks [22] and oxidative protein damage [23] as a result of the formation of carbon-centred radicals, which most likely are benzoyl and phenacyl radicals as proposed by Schwack and Rudolph [11]. Indolinonic nitroxide radicals were able to trap these radicals and hence reduce both types of damage. The photodegradation of avobenzone resulted in the absorber only displaying some degree of inhibition to UVA-induced lipid peroxidation [24], and complete failure to stop UVA-induced cytotoxic effects in keratinocytes, such as apoptosis [25]. Tran et al. [26] also reported that avobenzone, although it showed good absorption capacity, failed to protect the biological target.

Not only does avobenzone generate carbon-centred free radicals on photodegradation but it is also able to generate singlet oxygen by oxygen quenching of the triplet state of the keto form [4]. Puccetti and Chaudhuri [27], however, were not able to detect singlet oxygen photogenerated by avobenzone in their experiments but reported that singlet oxygen induced the degradation of avobenzone [27,28]. This is in contrast to the work of de Sola et al. [29] who reported that avobenzone photosensitises the formation of singlet oxygen in methanol and acetonitrile.

It has therefore been shown that in certain media the UVA-filtering effect of avobenzone is strongly reduced. In addition, since sunscreens molecules may penetrate the epidermis and the photodecomposition products are in contact with dermal structures, and avobenzone and its decomposition products can be damaging to cells, it is of importance to understand the photochemistry of avobenzone in different media. Although a number of studies have focussed on the photoinstability of avobenzone in solvents of different polarity, little or no emphasis has been placed on the proticity of the solvent.

This work was aimed at determining the photostability of avobenzone in solvents of different polarity and proticity, namely, methanol, dimethyl sulfoxide (DMSO), ethyl acetate and cyclohexane, and monitored the photochemical interactions by a range of analytical techniques. The polarity and hydrogen bonding characteristics of the solvents investigated are summarised in Table 1. The information derived from this work can be used to improve the manner in which avobenzone-containing sunscreens are formulated.

2. Experimental

2.1. Materials

Avobenzone was kindly donated by BASF Johannesburg. Deionised water was obtained from a Milli-Q⁵⁰ purification system (Millipore). The solvents used were HPLC-grade methanol from BDH, GC-grade DMSO from Merck, HPLC-grade DMSO from Sigma–Aldrich, GC-grade ethyl acetate from Riedel-de Haën and HPLC-grade cyclohexane from Aldrich. Dimethyl sulfoxide-*d*₆ from Acros (99.9 atom%) and cyclohexane-*d*₁₂ from Aldrich (99.6 atom%) were used for NMR analysis.

2.2. Irradiation

All irradiations were conducted by using an Osram HBO 500 W/2 high-pressure mercury lamp. In order to mimic solar UV radiation falling on the earth, a 10-mm-thick Pyrex filter was placed between the lamp and sample to be irradiated. The filter allowed only wavelengths greater than 300 nm to pass through. The spectral output of this combination is shown in Fig. 3. The samples to be irradiated were contained in quartz cuvettes.

2.3. UV spectroscopy

Solutions of avobenzone were prepared from 1×10^{-3} M stock solutions made up by mass in the different solvents. These solutions were irradiated for set time intervals and their UV spectra obtained after each irradiation period. For those experiments aimed at deter-

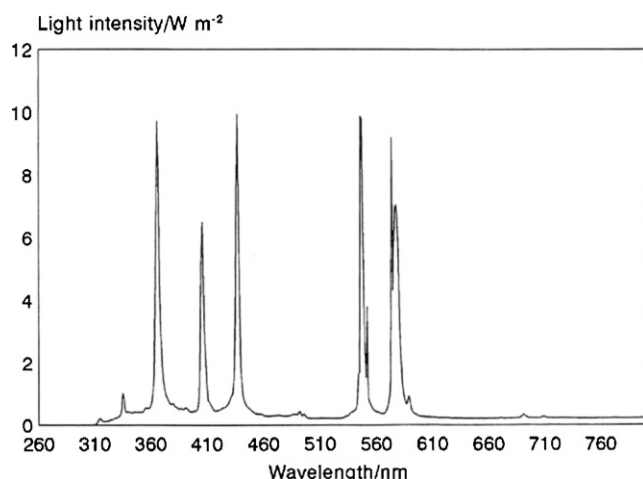


Fig. 3. Spectral output of the Osram HBO 500W/2 high-pressure mercury lamp in conjunction with the 10-mm-thick Pyrex filter used for all the irradiations in this work.

mining the effect of oxygen, the samples were first de-aerated by bubbling nitrogen (that had been pre-saturated with the solvent) through them and the cuvette was then stoppered. All absorption spectra were acquired with a PerkinElmer Lambda 35 double-beam UV-vis spectrophotometer.

2.4. Gas chromatography/mass spectrometry studies

A 1×10^{-2} M solution of avobenzone dissolved in each of DMSO, ethyl acetate and cyclohexane was analysed both before and after 18 h of UV-irradiation. The GC-MS instrument consisted of a Hewlett Packard 6890 series GC system, an injector (with a $10 \mu\text{l}$ syringe) and mass selective detector. The computer system for analysing and producing mass spectral data utilised the Hewlett Packard ChemStation software (Version B.01.00) coupled with the Wiley275 mass-spectral library.

The GC-MS analyses were performed on an HP-5MS column with an MS detection procedure scanning compounds of mass range 50–550 amu. Only splitless injection was employed since avobenzone concentrations were of the order of 10^{-2} M, which were sufficiently low to avoid column overloading. The total ion chromatogram and MS spectra were gathered 3 min from sample injection in order to allow the solvent to elute.

The following temperature program was found to be adequate for eluting all the photoproducts that resulted from the photodegradation of avobenzone. The temperature program was: 80°C held for 1 min, an increase of $20^\circ\text{C}/\text{min}$ to 250°C , 250°C held for 1 min, an increase of $6^\circ\text{C}/\text{min}$ to 280°C , 280°C held for 2 min, an increase of $10^\circ\text{C}/\text{min}$ to 290°C , and 290°C maintained for 4 min. The injector temperature was set at 280°C and the detector was kept at 310°C . Helium gas served as the mobile phase with a flow rate of 1 ml min^{-1} .

2.5. HPLC studies

The HPLC system was composed of a PerkinElmer series 200 autosampler, a Waters 600 multisolvent delivery system, and a Waters 996 photodiode array (PDA) detector linked to a De'Mark Pentium II computer where the results were analysed by Waters Millennium Version 4.00 software.

Separations were effected on a Nucleosil 100 C18 column of 250-mm length, 4.6-mm internal diameter and $5\text{-}\mu\text{m}$ particle size. A solvent composition of 85:15% (v/v) methanol:water was used

for the isocratic elution of avobenzone photoproducts obtained after irradiation in different solvents. All samples were filtered through $0.45\text{-}\mu\text{m}$ Millex syringe filters and solvents were filtered through $0.45\text{-}\mu\text{m}$ Durapore membrane filters before being used in the HPLC. Helium gas was used to sparge the mobile phase prior to use. The solvent flow rate was 1 ml min^{-1} . Ten-microliter aliquots of the samples were injected by means of the autosampler onto the column. The sample passed through a Waters Guard-Pak μ -Bondapak C18 column before entering the analytical column. The chromatograms were monitored at a wavelength of 272 nm.

The avobenzone solutions in methanol and ethyl acetate were injected directly through the Nucleosil 100 C18 column of the HPLC instrument for these photostability investigations. On the other hand, solutions of avobenzone in DMSO and cyclohexane required sample preparation before HPLC analysis. The cyclohexane solutions required solvent evaporation and re-dissolution of the left-over solid extract in the same volume of methanol prior to HPLC analysis. Avobenzone solutions in DMSO were diluted in methanol, since the two solvents are miscible, before being injected into the HPLC instrument.

2.6. NMR studies

The NMR spectra were recorded on either a 300-MHz Varian Gemini spectrometer or a 400-MHz Varian Unity Inova NMR spectrometer.

Samples for NMR analysis were prepared by weighing about 20 mg of avobenzone and dissolving it in the NMR solvent of interest. NMR spectra were acquired before and after UV-irradiation of the samples. For these analyses the samples were UV-irradiated whilst contained in the NMR tube.

In order to illustrate avobenzone degradation, the NMR peaks were set at the same vertical and integral scale (width and height, respectively) before and after irradiation. The ^1H NMR spectra were obtained at 300 MHz and 400 MHz for the 300 MHz Varian Gemini and 400 MHz Varian Unity Inova spectrometers, respectively. The ^{13}C NMR spectra were, on the other hand, obtained at the following radiofrequencies: 75 MHz (for the 300 MHz Varian Gemini NMR) and 100 MHz (for the 400 MHz Varian Unity Inova NMR). The NMR spectra obtained for avobenzone in DMSO and cyclohexane, before and after irradiation, included ^1H NMR and DEPT spectra.

3. Results

3.1. General observations

The efficacy of a substance used as a chemical absorber in a sunscreen can be determined by assessing how well it retains its absorption capacity upon irradiation with wavelengths such as those that fall on the earth's surface.

The wavelengths of maximum absorption (λ_{max}) of avobenzone in cyclohexane, ethyl acetate, methanol and DMSO are listed in Table 1. These results show that the λ_{max} of avobenzone in polar solvents (such as methanol and DMSO) undergoes a bathochromic shift relative to the hypsochromic shift observed in non-polar solvents, namely, ethyl acetate and cyclohexane. This feature is in accordance with most sunscreens [8]. These λ_{max} values also substantiate that avobenzone is a UVA absorber, and in particular a UVAI absorber.

HPLC analysis confirmed that before irradiation in all the four solvents investigated avobenzone exists as an equilibrium mixture of the enol and keto tautomers with the equilibrium lying predominantly towards the enol form. The keto form (with a λ_{max} of 269 nm) eluted with a retention time between 5 and 7 min and the enol form

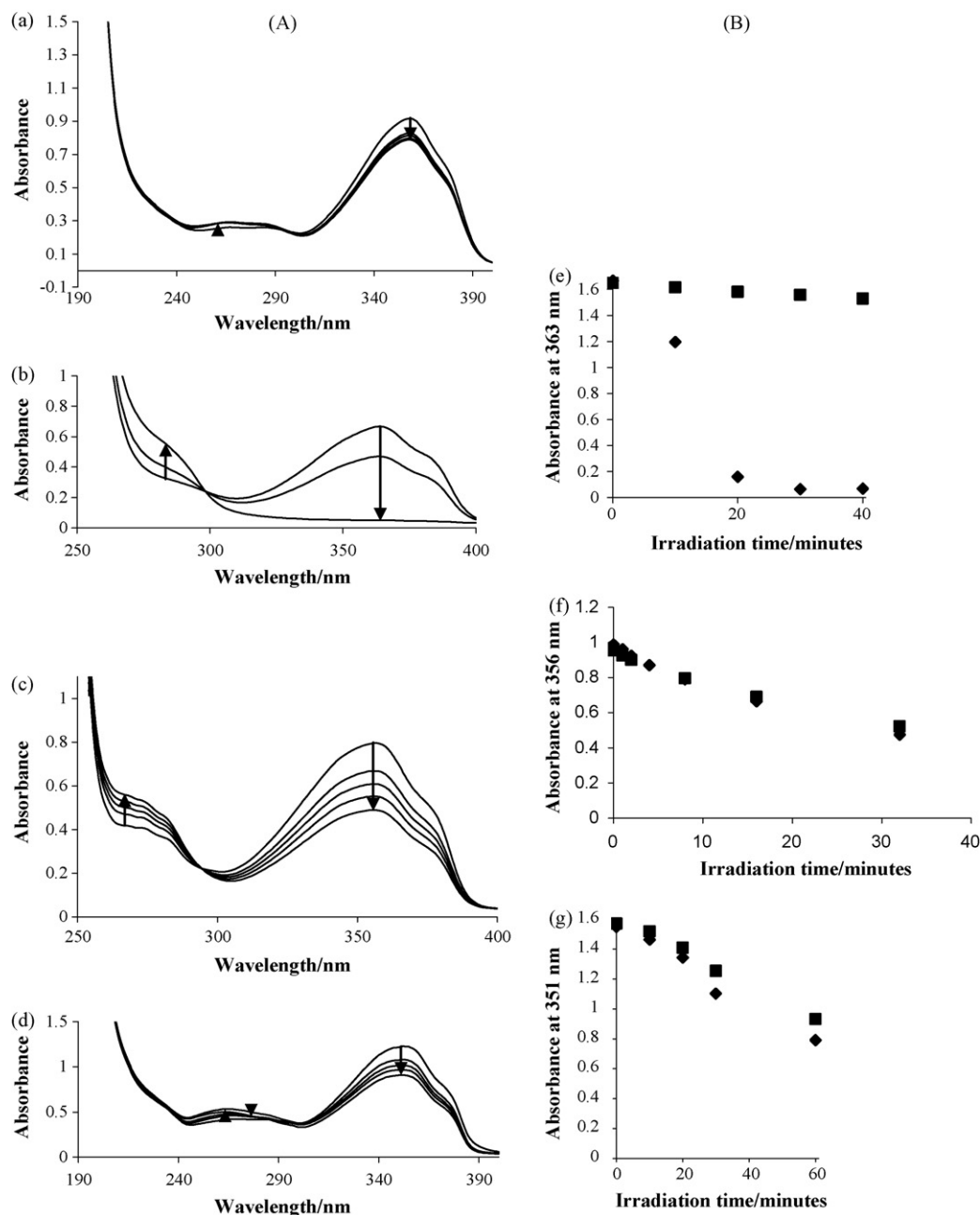


Fig. 4. Panel A: Spectral changes observed upon irradiation of avobenzone with wavelengths of light greater than 300 nm. (a) 2.6×10^{-5} M avobenzone dissolved in methanol after time intervals of 30 min, (b) 1.5×10^{-5} M avobenzone dissolved in DMSO after time intervals of 5 min, (c) 1.8×10^{-5} M avobenzone dissolved in ethyl acetate after time intervals of 1 min, and (d) 2.0×10^{-5} M avobenzone dissolved in cyclohexane after time intervals of 30 min. Panel B: Changes in absorbance with increasing irradiation time for (e) 5.0×10^{-5} M avobenzone in DMSO, (f) 2.4×10^{-5} M avobenzone in ethyl acetate and (g) 1.0×10^{-5} M avobenzone in cyclohexane, in the presence (◆) and absence (■) of oxygen.

(with a λ_{\max} of 360 nm) between 19 and 24 min depending on the solvent of dissolution.

The predominance of the enol form in solution was also ascertained by NMR spectroscopy in deuterated methanol, DMSO and cyclohexane. The relative amounts of the two tautomers were calculated from the integral ratios of the vinylic and methylene protons and used to calculate the equilibrium constant $K_e = [\text{enol}]/[\text{keto}]$ shown in Table 1. This constant could not be determined in deuterated methanol since the presence of the ketonic methylene protons was not evident in this solvent. The K_e values indicate that the equilibrium shifts towards the more polar keto form as the solvent polarity increases.

The efficacy of avobenzone as a sunscreen absorber was investigated in the different solvents by irradiating dilute air-equilibrated solutions of avobenzone for varying time intervals with wavelengths greater than 300 nm and monitoring the resulting absorption spectra. The latter are shown in Fig. 4. In DMSO, ethyl acetate and cyclohexane a marked decrease in absorbance at the λ_{\max} is observed with a concomitant increase in absorbance at shorter wavelengths in the UVC range. In methanol a much smaller loss in absorption occurs at the λ_{\max} than for the other solvents investigated. In this case, apart from the initial loss in absorbance during the first 30 min, the absorber appears relatively photostable thereafter. In DMSO and ethyl acetate a clear isosbestic point is

Table 2

GC retention times of chemical substances present in UV-irradiated (for 18 h) solutions of avobenzone dissolved in cyclohexane, ethyl acetate and DMSO and their identities as determined from the fragmentation patterns reported in the literature [11] and the Wiley275 library.

Retention times of substances in irradiated avobenzone solutions/min			Fragmentation pattern ^a	Compound identified
Cyclohexane	Ethyl acetate	DMSO		
5.5	–	–	162 (M ⁺ , 12), 147 (100), 119 (45), 103 (10), 91 (94), 77 (17), 65 (11), 57 (7)	4- <i>t</i> -Butyl benzaldehyde
5.7	–	–	Wiley275 library	Dicyclohexyl ether
9.1	–	–	Wiley275 library	4-Methoxy benzoic acid, cyclohexyl ester
9.5	–	–	Unknown	Unknown
12.8	12.8	–	296 (M ⁺ , 2), 161 (26), 146 (6), 135 (100), 118 (9), 107 (6), 92 (13), 91 (6), 77 (16)	4- <i>t</i> -Butyl-4'-methoxy benzil
13.1	13.1	–	161 (M ⁺ , 100), 146 (29), 133 (3), 131 (6), 118 (32), 105 (17), 91 (33), 76 (8), 57 (15)	4- <i>t</i> -Butyl phenylglyoxal
15.1	15.1	15.1	310 (M ⁺)	Avobenzone
15.4	–	–	Wiley275 library	1,4-Bis(4-methoxy phenyl)butane-1,4-dione
15.9	–	–	324 (M ⁺ , 6), 161 (32), 146 (5), 135 (100), 118 (7), 92 (11), 77 (17)	4- <i>t</i> -Butyl-4'-methoxy dibenzoyl ethane
16.6	–	–	161 (M ⁺ , 100), 146 (8), 118 (17), 105 (6), 91 (11), 77 (4)	4,4'-Di- <i>t</i> -butyl benzil ^b

^a Fragmentation pattern given as *m/z* value with its percentage abundance in parentheses.

^b Tentative assignment.

seen indicative of the presence of at least two species in equilibrium. In the last 30 min of irradiation in cyclohexane, there were losses in absorbance both at 351 nm (the λ_{\max}) and at 263 nm and the spectrum no longer passes through the isosbestic point indicating the presence of other photoproducts. The loss in absorption at 263 nm may be due to the formation of intermediate photoproducts which also absorb above 300 nm. Consequently, these photoproducts further photodegrade upon subsequent irradiation with light of wavelengths greater than 300 nm.

In all three solvents in which significant photo-instability was observed the photoreaction was thermally reversible in the dark. The rate of recovery was very fast in ethyl acetate (20 min for complete recovery with a first-order rate constant, k_{-1} , of $1.8 \times 10^{-3} \text{ s}^{-1}$) and DMSO (40 min, $k_{-1} = 8.6 \times 10^{-4} \text{ s}^{-1}$) but much slower and incomplete in the case of cyclohexane ($k_{-1} = 7.0 \times 10^{-5} \text{ s}^{-1}$). The latter again indicates the presence of other photoproducts, besides the two tautomers, as mentioned above.

The investigation was also performed on de-aerated solutions to establish whether the absence of oxygen has an effect on the loss in absorbance observed in UV-irradiated solutions of avobenzone dissolved in DMSO, ethyl acetate and cyclohexane. Our previous work [21] showed that oxygen has no effect when avobenzone is dissolved in methanol. The results of these experiments are depicted in Fig. 4. The photostability of avobenzone increased in the order ethyl acetate, cyclohexane and DMSO, in the absence of oxygen. These results indicate that avobenzone is more photostable in all solvents in the absence of oxygen but this photostabilisation is only significant in DMSO. In the absence of oxygen only a 7% loss in absorbance of avobenzone in DMSO was observed compared with the 96% loss in the presence of oxygen for the same irradiation period of 40 min.

Therefore avobenzone was observed to be photo-unstable in DMSO, ethyl acetate and cyclohexane but relatively photostable in methanol. The photo-instability was significantly diminished in the absence of oxygen in DMSO. In order to ascertain whether the observed instability was due to photoisomerisation from the enol to the keto form, or due to photodegradation, or both, irradiated solutions of avobenzone were characterised by chromatography and NMR spectroscopy in order to detect and monitor the photoproducts formed. Evidence will be provided below to show that in DMSO the photoinstability is the result of photoisomerisation. On the other hand, in cyclohexane the main cause is photodegradation and both processes occur to a similar extent in ethyl acetate.

3.2. Irradiation of avobenzone in DMSO

A $1 \times 10^{-2} \text{ M}$ solution of avobenzone dissolved in DMSO was analysed by GC–MS prior to and after 18 h of irradiation. The fragmentation of each photoproduct was compared with those of the electron impact fragmentations in the Wiley275 library, as well as other literature [11], and is summarised in Table 2. No avobenzone photoproducts were detected and the avobenzone peak height remained the same before and after irradiation. The most likely explanation for this is that avobenzone photoisomerises to the keto form which would therefore elute together with the enol form through the HP-5MS column and hence no change is observed.

In order to determine whether photoisomerisation had occurred, a different chromatographic technique was required. Hence HPLC analyses were performed with a PDA detector. The high performance liquid chromatogram of $7.61 \times 10^{-3} \text{ M}$ avobenzone after 15 h of irradiation in DMSO is displayed in Fig. 5. The peak area of the enol form of avobenzone has decreased by 75% after irradiation. The major peak is of the keto form of avobenzone which elutes at 5.4 min ($\lambda_{\max} = 269 \text{ nm}$), and four other photoproducts appear at 2.5 min ($\lambda_{\max} = 267 \text{ nm}$), 3.8 min ($\lambda_{\max} = 231 \text{ nm}$), 4.5 min ($\lambda_{\max} = 267 \text{ nm}$) and 9.1 min ($\lambda_{\max} = 363 \text{ nm}$) in the chromatogram of the irradiated sample but their concentrations are small. Although no photoproducts other than the keto-isomer were detected by GC–MS analyses their formation observed here helps to explain the observation that in the absence of oxygen some loss in absorbance was observed. If the absorbance loss in aerated solutions was solely due to photoisomerisation one would expect the absorbance in the de-aerated solution to remain close to the initial value throughout. We can therefore conclude that the main cause for the loss in absorbance exhibited by avobenzone in DMSO is photoisomerisation.

The latter statement was confirmed by NMR analyses of a $1 \times 10^{-2} \text{ M}$ solution of avobenzone in deuterated DMSO before and after UV-irradiation. The chemical shifts observed in the ^1H and DEPT NMR spectra of avobenzone dissolved in deuterated DMSO are listed in Table 3. The greater integral value of the vinylic proton at 7.23 ppm than the methylene protons at 4.75 ppm and the absence of any CH_2 peak in the DEPT spectrum confirms the predominance of the enol form of avobenzone in DMSO as stated earlier. Andrae et al. [9] and Dubois et al. [3] showed by ^1H NMR that avobenzone also exists in the enol form in acetonitrile (also a polar aprotic solvent).

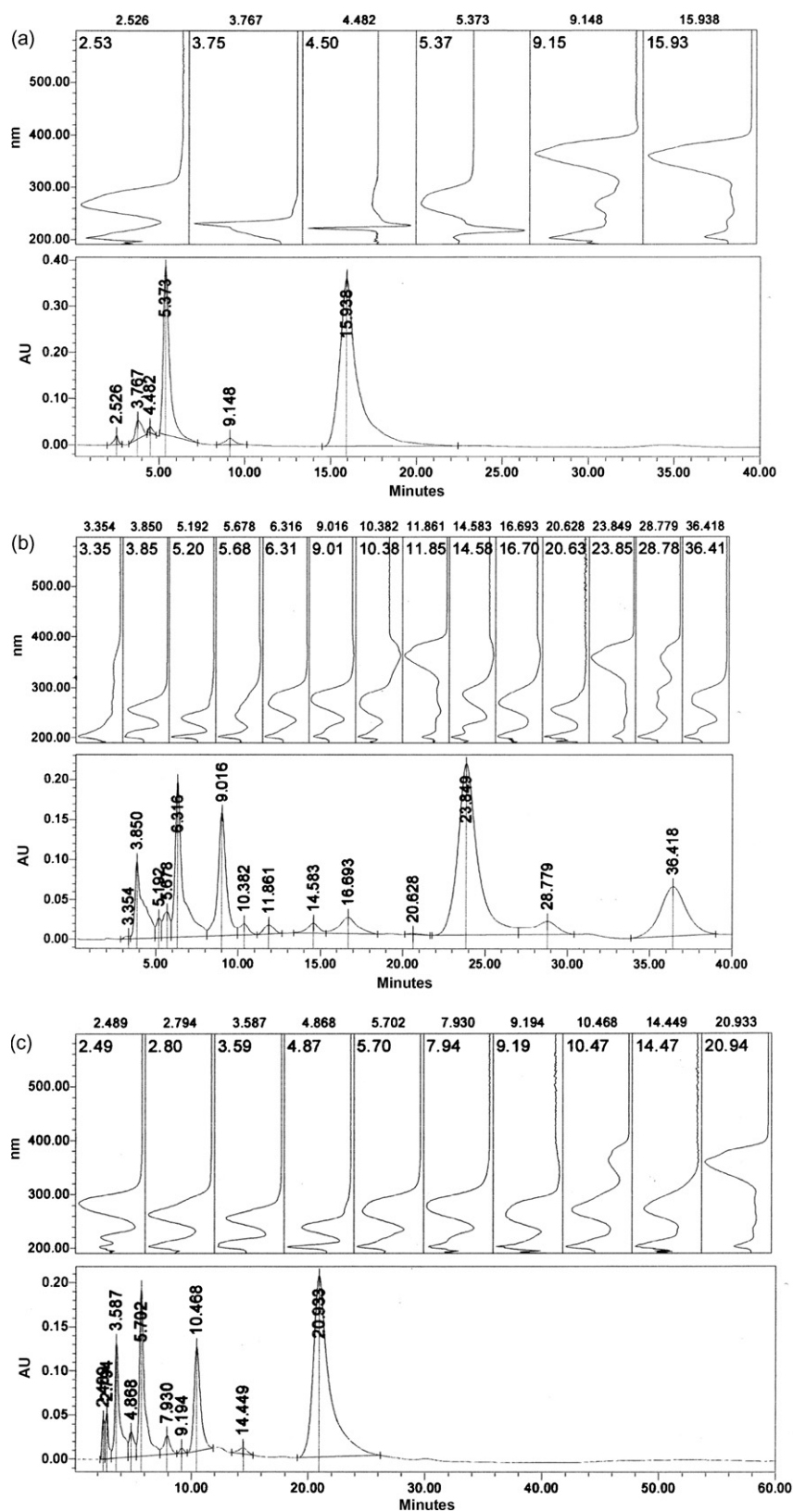


Fig. 5. High performance liquid chromatograms eluted with a mobile phase composition of 85:15 (v/v) methanol:water and detected at 272 nm of (a) 7.61×10^{-3} M avobenzone irradiated for 15 h in DMSO (the keto form elutes at 5.4 min and the enol form at 16 min), (b) 5.70×10^{-3} M avobenzone dissolved in cyclohexane after 18 h of irradiation (the keto form elutes at 6.3 min and the enol form at 24 min; the rest of the peaks are photoproducts) and (c) 5.31×10^{-3} M avobenzone irradiated for 15 h in ethyl acetate (the keto form elutes at 5.7 min and the enol form at 21 min; the rest of the peaks are photoproducts).

Table 3

Comparison of the ^1H and DEPT NMR chemical shifts (in ppm) of avobenzone prior to UV-irradiation.

Proton/carbon	^1H		DEPT	
	$\text{C}_6\text{D}_6^{\text{a}}$	$\text{DMSO-}d_6^{\text{b}}$	C_6D_6	$\text{DMSO-}d_6$
CH_3 17 18 19	1.33 (s) 9H	1.31 (s) 9H	32 (s)	31 (s)
CH_3 20	3.72 (s) 3H	3.86 (s) 3H	55 (s)	56 (s)
CH_2 8	5.10 keto (very small)	4.75 2H keto		
CH 8	6.65 (s) 1H enol	7.23 (s) 1H enol	92 (s) enol	92 (s) enol
CH 3 5	6.8 (d) 2H	7.10 (d) 2H	114 (s)	114 (s)
CH 12 14	7.4 (d) 2H	7.54 (d) 2H	126 (s)	126 (s)
CH 11 15	7.8 (d) 2H	8.06 (d) 2H	128 (s)	127 (s)
CH 2 6	7.9 (d) 2H	8.14 (d) 2H	130 (s)	130 (s)

^a All peaks are referenced to the solvent peak at 1.39 ppm.

^b All peaks are referenced to the DMSO peak at 2.5 ppm.

The ^1H NMR spectrum of avobenzone dissolved in DMSO after 21 h of irradiation shows only one major difference from that of unirradiated avobenzone and that is the increase in the methylene proton peak at 4.75 ppm. This suggests irradiation solely results in photoisomerisation of the enol form to the keto form. The ratio [enol]/[keto] which was 10 before irradiation has decreased to a value of 4.1. The ratio has decreased by more than half thereby indicating a significant amount of keto form is created upon irradiation. The DEPT NMR spectrum obtained after irradiation did not show any detectable difference in the avobenzone structure.

All three techniques therefore provide evidence that the primary loss of avobenzone absorption in DMSO is due to enol–keto isomerisation.

3.3. Irradiation of avobenzone in cyclohexane

The GC–MS analysis performed in DMSO was repeated for the same concentration of avobenzone dissolved in cyclohexane.

Schwack and Rudolph [11] have also investigated the photostability of avobenzone in cyclohexane. They identified the major photoproducts to be benzaldehydes, benzoic acids, phenylglyoxals, acetophenones, benzils, dibenzoylmethane and a dibenzoyl ethane. They proposed that these photoproducts were formed as a result of α -cleavage of the keto form of avobenzone followed by either recombination or oxidation.

In this study the photoproducts formed in cyclohexane (see Table 2) were also mainly the result of α -cleavage of the keto form of avobenzone (as shown in Fig. 6) to form benzils, a phenylglyoxal and a benzaldehyde. These radicals may have recombined to form the compounds dibenzoyl ethane and 1,4-bis(4-methoxyphenyl)butane-1,4-dione. The ester that eluted at a retention time of 9.1 min was the combined product from the photoproducts of avobenzone and cyclohexane. Dicyclohexyl ether was also formed and arises from the solvent, cyclohexane. The compound eluting at 9.5 min could not be categorised by either the Wiley275 library or the MS fragmentation reported by Schwack and Rudolph [11]. Therefore GC–MS analyses showed that avobenzone had photodegraded from the keto form.

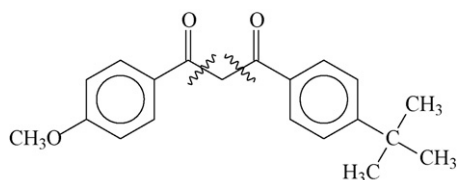


Fig. 6. Schematic diagram showing α -cleavage of avobenzone when irradiated with UV light of wavelengths greater than 300 nm.

Fig. 5 shows the HPLC chromatogram of avobenzone after 18 h of irradiation in cyclohexane. The peak due to the enol form of avobenzone is reduced by 56% following irradiation. Avobenzone has disintegrated into various photoproducts of much shorter λ_{max} than that of the enol form of avobenzone ($\lambda_{\text{max}} = 360$ nm) responsible for absorbing UVA radiation. The avobenzone isomers elute at 23.8 min (enol form) and at 6.3 min (keto form). Irradiation of avobenzone in cyclohexane also causes photoisomerisation since the peak at 6.3 min has increased. Most photoproducts formed after irradiation absorb maximally at short wavelengths, mostly in the UVC range. These photoproducts eluted at 3.9 min ($\lambda_{\text{max}} = 256$ nm), 5.2 min ($\lambda_{\text{max}} = 239$ nm), 5.7 min ($\lambda_{\text{max}} = 241$ nm), 9.0 min ($\lambda_{\text{max}} = 276$ nm), 10.4 min ($\lambda_{\text{max}} = 268$ nm), 11.9 min ($\lambda_{\text{max}} = 363$ nm), 14.6 min ($\lambda_{\text{max}} = 287$ nm), 16.7 min ($\lambda_{\text{max}} = 269$ nm), 28.8 min ($\lambda_{\text{max}} = 360$ nm) and 36.4 min ($\lambda_{\text{max}} = 273$ nm).

The ^1H NMR spectrum (see Table 3) also confirms that avobenzone occurs as the enol form in deuterated cyclohexane. The small peak at 5.10 ppm that represents the two protons attached to the carbon at C-8 of the keto form of avobenzone, based on the results obtained by Andrae et al. [9] and Dubois et al. [3], shows that the keto form of avobenzone exists in minute amounts in solution. This keto form was easily seen from HPLC analyses when the chromatograms were monitored at wavelengths (i.e. 272 nm) where it absorbs maximally. The DEPT NMR spectrum of avobenzone (tabulated in Table 3) in deuterated cyclohexane conforms with the ^1H NMR spectrum.

Avobenzone was irradiated in deuterated cyclohexane for 25 h. The ^1H NMR spectrum obtained after irradiation shows more peaks than the equivalent spectrum for unirradiated avobenzone. This indicates that avobenzone has been modified during irradiation from the predominantly enol form. The single peak due to the nine methyl protons has divided to form three singlet peaks all between 1.0 ppm and 1.4 ppm. The methoxy peak has also broken up to form three major singlet peaks and the same applies to the doublets due to the aromatic protons. This suggests the existence of three major photoproducts of avobenzone arising from photodegradation as was observed with GC–MS and HPLC investigations. The methylene proton peak due to the keto form of avobenzone shows a slight increase. The vinylic proton peak is engulfed by other peaks making it impossible to relate the two forms of avobenzone.

The DEPT NMR spectrum of irradiated avobenzone in deuterated cyclohexane shows five methyl peaks; three due to the nine methyl protons from the *tert*-butyl group and two from the methoxy methyl group. The spectrum shows seventeen CH groups inclusive of the vinylic proton of the enol form of avobenzone. Only the vinylic proton peak remained intact in the irradiated DEPT spectrum, all the other aromatic CH protons have been split up to almost triple the number they were initially. The ^1H NMR and the DEPT spectra show a possible break at the C-8 carbon of avobenzone, hence the reason there is lack of multiple carbon-8 peaks whereas the other peaks re-occur. There must be two major photoproducts in solution that have arisen through a break at the C-8 position, making three major compounds in solution (including avobenzone). The presence of three compounds justifies the existence of three sets of each peak.

A solution of 1×10^{-2} M avobenzone in deuterated cyclohexane was continuously monitored during irradiation with the 300 MHz Varian NMR spectrometer, so as to compare the methylene proton peak with that of the vinylic proton peak. The decrease or increase of these peaks upon irradiation would directly correspond to the photostability of their respective isomers. The change with irradiation of these peaks was determined by measuring their integrated areas. These integrated areas of the methylene protons and vinylic proton peaks were plotted against irradiation time as shown in Fig. 7.

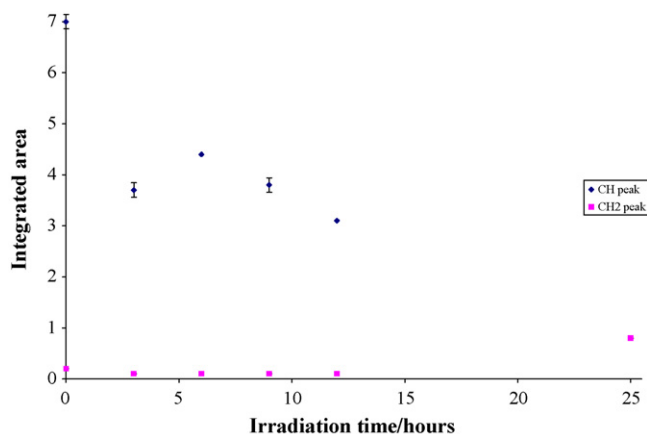


Fig. 7. The variation of the ^1H NMR spectrum integrated areas for the methylene and vinylic proton peaks of a 1×10^{-2} M avobenzone solution in deuterated cyclohexane with increasing periods of UV-irradiation.

After the first 3 h of irradiation, the integrated areas for both peaks were halved, suggesting that both isomers were consumed during irradiation. Subsequent irradiations decreased the integrated area of the vinylic proton peak whereas that of the methylene protons remained constant. The vinylic proton peak was reduced from an integrated area of 7.0 to 3.1 after 12 h of irradiation. After 25 h of irradiation multiple peaks were observed in the NMR spectrum, masking the vinylic proton peak and as a result its integrated area could not be determined. However, after 25 h of irradiation, the methylene proton peak increased in integrated area to about eight times that observed at 12 h irradiation. The initial decrease in the integrated area for both the vinylic and methylene protons is possibly the result of photoisomerisation from the enol to the keto form and the subsequent photodegradation of the keto form. After 25 h of irradiation, the keto form had increased and this is possibly the result of photoisomerisation occurring at a faster rate than photodegradation.

We can therefore conclude that although avobenzone photoisomerises from the enol to the keto form in cyclohexane, the keto concentration does not build up but photodegrades to produce products that absorb in the UVC region and are therefore not photoprotective. Photodegradation accounts for most of the loss in absorbance observed.

3.4. Irradiation of avobenzone in ethyl acetate

GC–MS analyses showed that avobenzone photoproducts were formed in large quantity and diversity in cyclohexane but they were greatly reduced in ethyl acetate (see Table 2). UV spectroscopy investigations show avobenzone losing a significant amount of its UVA absorption capacity after only 1 min of irradiation in ethyl acetate, whereas in cyclohexane the photo-instability was only observed following a 30-min irradiation interval. However, the number of photoproducts identified by GC–MS following the same irradiation interval is smaller in ethyl acetate than in cyclohexane. This could mean that either avobenzone is photostable at high concentrations in ethyl acetate or that it photodegrades and also photoisomerises in this solvent.

The high performance liquid chromatogram of avobenzone dissolved in ethyl acetate after 15 h of irradiation is shown in Fig. 5. After irradiation, the peak due to the enol form of avobenzone, which elutes at 21 min, had decreased by 33% and the peak at approximately 5.7 min with a λ_{max} of 269 nm had increased. Therefore, photoisomerisation has occurred. The other peaks seen in the chromatogram are photoproducts resulting from the pho-

Table 4

Variation on UV-irradiation of the enol and keto forms of avobenzone dissolved in ethyl acetate as determined by HPLC.

Irradiation time/min	HPLC peak area/ 10^5		Concentration of the enol form/ 10^{-4} M
	Enol form detected at 350 nm	Keto form detected at 270 nm	
0	209.0	3.646	11.50
5	191.3	4.410	10.52
20	163.3	26.55	8.982
60	138.6	37.07	7.623

todegradation of avobenzone. The photoproducts detected eluted at 2.5 min (λ_{max} of 285 nm), 2.8 min (λ_{max} of 259 nm), 3.6 min (λ_{max} of 250 nm), 4.9 min (λ_{max} of 240 nm), 7.9 min (λ_{max} of 280 nm), 9.2 min (λ_{max} of 263 nm), 10 min (λ_{max} of 270 nm) and 14 min (λ_{max} of 273 nm). The number of photoproducts formed here is more than in DMSO but less than in cyclohexane in keeping with the GC–MS results.

In order to confirm that photoisomerisation was indeed taking place a 1×10^{-3} M avobenzone solution in ethyl acetate was further investigated by HPLC analysis, following irradiations of 5, 20 and 60 min intervals with wavelengths of light greater than 300 nm. The peak area for the enol form of avobenzone was determined from the peak eluting at 21 min through the Nucleosil 100 C18 column at a detection wavelength of 350 nm. The peak area for the keto form was measured from the peak eluting at 6 min at a detection wavelength of 270 nm. Table 4 shows the variation in the peak areas for the keto and enol forms with irradiation time and the corresponding concentrations of the enol form. The decrease in the enol form is accompanied by an increase in the keto form of avobenzone with irradiation time, indicating that photoisomerisation of the absorber is taking place.

Therefore chromatographic analyses show that in ethyl acetate avobenzone both photoisomerises and photodegrades to similar extents.

3.5. Irradiation of avobenzone in methanol

The high performance liquid chromatogram of avobenzone dissolved in methanol after 15 h of irradiation showed that both the enol and keto forms of avobenzone had decreased minimally (about 7% each) and some new components of very small concentration that absorb below 300 nm had appeared. This was the only analytical technique that showed the presence of any photoproducts. NMR analyses showed no difference in the spectra before and after 25 h of UV-irradiation. Consequently, avobenzone is essentially photostable in methanol.

4. Discussion

From the preceding results it is evident that when avobenzone dissolved in DMSO is irradiated the major photoproduct detected is the keto isomer and its formation is strongly oxygen-dependent. This indicates that photoisomerisation possibly occurs through a triplet excited state. Hence a possible explanation for the UV-stabilisation of avobenzone with triplet-state quenchers. Cantrell and McGarvey [4] reported that laser excitation of avobenzone at 266 nm results in the formation of the triplet state of the keto form which is quenched by oxygen (with a rate constant of $5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) to produce singlet oxygen, $^1\text{O}_2$. The fact that Pucetti and Chaudhuri [27] found that $^1\text{O}_2$ was not generated by avobenzone in aqueous ethanol is now apparent since in that polar protic solvent the formation of the keto form is minimal.

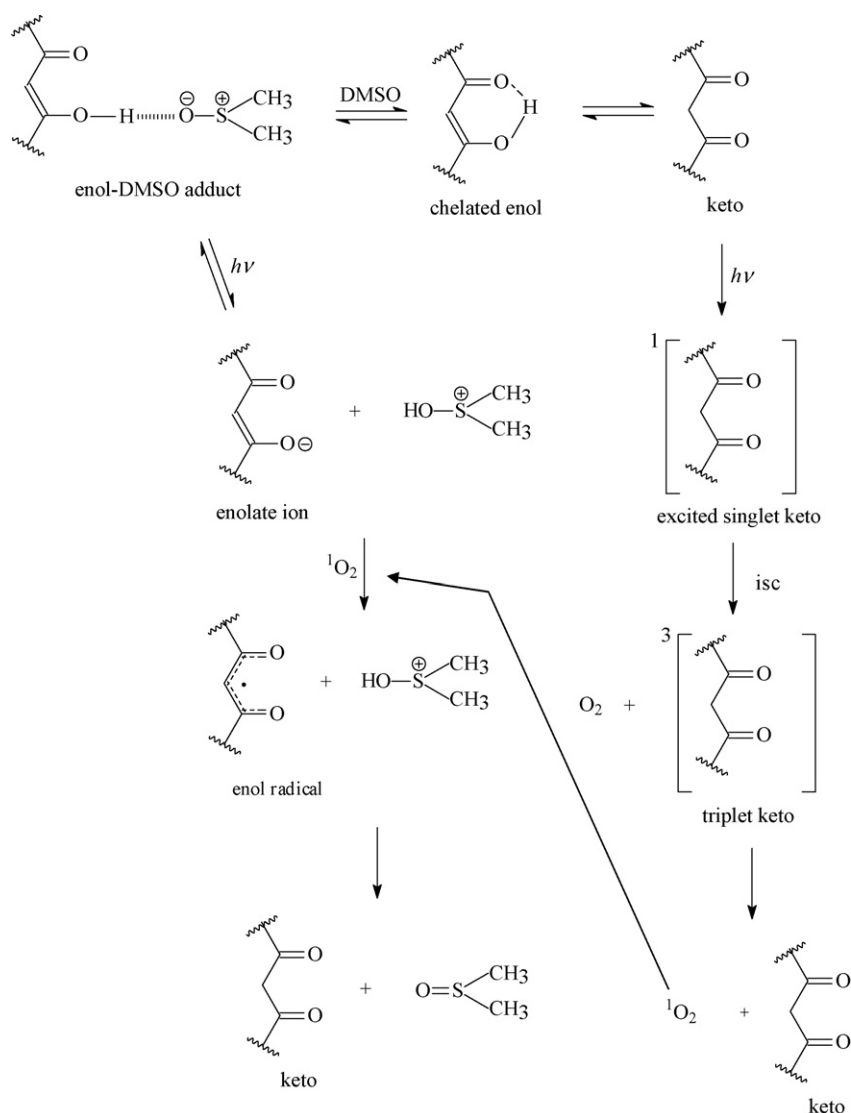


Fig. 8. Possible mechanism for the formation of the keto isomer in DMSO when an equilibrated solution of avobenzone is irradiated in the presence of oxygen.

A photoisomerisation mechanism in DMSO based on a mechanism reported by McGarry et al. [34] that involves the formation of an intermolecular hydrogen bond between the substrate and DMSO is proposed here (see Fig. 8). DMSO has a strong hydrogen bonding accepting affinity ($\beta=0.76$, see Table 1) hence it can easily break the intramolecular hydrogen bond that exists in the ground state “chelated” enol. Consequently, the enol form becomes intermolecularly hydrogen bonded to DMSO in solution prior to irradiation. Upon irradiation the enol–DMSO complex splits to form the enolate anion and a protonated DMSO molecule. Since in an unirradiated solution of avobenzone dissolved in DMSO the proportion of keto form is greater than in a nonpolar solvent, some of the light will be absorbed by this form and after intersystem crossing will produce the triplet keto form. (The keto form has a long absorption tail in the UVB and UVA regions [4].) This triplet state of the keto form of avobenzone can be quenched by ground state oxygen (through triplet–triplet energy transfer) to form the ground state keto and singlet oxygen, 1O_2 [4]. The enolate anion is then oxidised by singlet oxygen to form the neutral avobenzone radical. (This radical has been observed in the electrochemical oxidation of the enolate of dibenzoylmethane [35] and has an oxidation potential of +0.52 V versus SCE. If a similar oxidation potential applies for the enolate

of avobenzone then this oxidation should be possible with singlet oxygen which has an estimated reduction potential of 0.14 V versus SCE [36].) This radical rapidly extracts a hydrogen back from the protonated DMSO to form keto avobenzone. The relatively polar keto form is subsequently stabilised in the polar DMSO solvent. In the absence of oxygen, the enolate anion re-captures the proton from the protonated DMSO molecule and reverts back to the enol form. (Tobita et al. [18] have reported evidence of the enolate ion of dibenzoylmethane and its recovery to the ground state enol form.) The triplet keto form will relax to ground state keto and the equilibrium ratio of the two forms will be unaltered. Consequently, the absence of oxygen minimises photoisomerisation.

The fairly rapid thermal reversion of the keto form to the enol form is likely to involve a solvent-assisted pathway as has been proposed for the simplest β -diketone, malonaldehyde [37,38]. The nucleophilic oxygen in DMSO is likely to perform the same function as the nucleophilic oxygen in the water tetramer proposed there, and thereby facilitates proton transfer via a low activation energy route.

Photoisomerisation of avobenzone also occurs in ethyl acetate. A similar mechanism to that proposed above is likely to apply. Although ethyl acetate is a relatively non-polar solvent, it is likely

that it can hydrogen bond with the enol form of avobenzone to form a complex. (It has been proposed that the transient enol of dibenzoylmethane can hydrogen bond to diethyl ether [18].) Ethyl acetate also possesses proton-donating properties [39], which could recon-vert the enol anion back to the enol form in the absence of oxygen.

In cyclohexane, the solvent cannot hydrogen bond with the enol form and as a result photoisomerisation cannot occur through this pathway. It is also slower and marked by a very slow thermal reversal. The non-chelated enol formed on excitation is likely to revert to the “chelated” enol and keto forms via the pre-equilibria previously proposed [4].

Photodegradation of avobenzone occurred through α -cleavage when solutions of the absorber were irradiated in cyclohexane and in ethyl acetate. The structures of the photoproducts suggest that they either originated from a benzoyl radical or a phenacyl radical. Therefore, the photoproducts identified were characteristic of photodegradation occurring from the keto state, as has been observed by others when avobenzone is irradiated in cyclohexane [11,12,21]. Although the keto form absorbs maximally in the UVC region, which was excluded from our experiments, it does have a long absorption tail into the UVB range. Hence photodegradation could possibly occur from either the singlet or the triplet state of the keto form. Cantrell and McGarvey [4] reported that the triplet state of the keto form absorbed broadly from 300 to 500 nm and had a lifetime of ~500 ns. They also proposed that photodegradation of avobenzone in acetonitrile occurred through the triplet keto form. The triplet excited state of the keto form can therefore photodegrade to various photoproducts or it may generate singlet oxygen. From the spectrophotometric studies performed in the absence of oxygen it is evident that lack of oxygen does not preclude photodegradation hence the singlet keto form may be the dominant source of photodegradation.

Minimal photo-instability of avobenzone in methanol was observed. The work of Tobita et al. [18] with the parent molecule, dibenzoylmethane, suggests that in the presence of alcoholic solvents the transient enol, formed from excitation of the ground state enol, is involved in mutual hydrogen exchange via intermolecular hydrogen bonding with the solvent which accelerates the re-formation of ground-state enol. This process follows fast pseudo-first-order kinetics. This is likely to be the case for avobenzone as well and hence photoisomerisation is minimal. The very slight degradation observed is most likely to arise from the singlet keto form.

5. Conclusion

This research investigated the photostability of avobenzone in four solvents. Avobenzone was found to be photostable in methanol but not in DMSO, ethyl acetate or cyclohexane.

UV, GC-MS, HPLC and NMR studies showed that avobenzone predominantly photoisomerises in DMSO but principally photodegrades in cyclohexane, whereas both processes occur in ethyl acetate. The photoisomerisation of avobenzone in DMSO was found to depend on the presence of oxygen. This suggests that photoisomerisation occurs via a triplet state precursor. The less striking influence of oxygen in cyclohexane suggests that photodegradation, which occurs predominantly through α -cleavage of the diketofrom, must occur from a singlet keto form precursor.

Avobenzone is one of the few approved UVA absorbers; consequently, it is used in most sunscreen formulations. Avobenzone has been shown to be photostable in polar protic solvents. It loses absorbance in both polar and non-polar aprotic solvents by photodegradation and photoisomerisation, either simultaneously or separately.

This work has shown that the statement that avobenzone is photostable in polar solvents and photo-unstable in non-polar solvents is too simplistic. The proton donating ability of the solvent must be taken into account. Hence effective means of photostabilising avobenzone can now be devised.

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