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The formation and properties of the melatonin radical: a photolysis study of melatonin with 248 nm laser light

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The photolysis of melatonin in aqueous solution has been studied spectrometrically with a 248 nm laser. The formation of hydrated electrons in a monophotonic process has been confirmed in neutral solution with a quantum yield of 0.22. Two main absorption bands at 340 and 460 nm plus an absorption shoulder resulted from the counterpart of the ejected electron, a melatonin radical, in solution. The big difference for the relative intensity of the absorption peaks under various pH conditions reveals that the melatonin radical exists in the solution through an acid–base equilibrium. In support from the pH dependence of the spectrum of the intermediate, the pK_{a_1} for the doubly-protonated melatonin radical against the mono-protonated melatonin cation radical was estimated to be -0.95 and the pK_{a_2} for the mono-protonated melatonin cation and melatonin neutral radical was 4.5 ± 0.5. This work will benefit the basic understanding about melatonin as a UV-light protector, as a light receptor and the antioxidation functions of melatonin.

1 Introduction

Melatonin, *N*-acetyl-5-methoxytryptamine, is an indole-based hormone produced mainly by the pineal gland in brain. The balance of melatonin has been proven to be crucial in many health-related studies and many cancer-related diseases.[1,2] Its physiological functions as a miraculous drug exhibiting antitumor and anti-ageing actions have been well claimed.[3–5] According to the theory of ageing, accumulated free radical damage is one of the main factors responsible for DNA modification and carcinogenic changes in tissues influencing the rate of ageing, so the miraculous physiological functions of melatonin were considered to be associated with its super ability to scavenge oxidation radicals.[6–8] For example, melatonin scavenges hydroxyl radicals rapidly, at near to a diffusion-controlled reaction rate.[9] Therefore, it is of considerable importance to investigate the properties of the possible oxidation reaction product – the melatonin radical.

Another major concern that has been emphasized is the sunscreen effect of melatonin as an ultraviolet (UV) light protector.[10,11] In the past two decades, the occurrence of skin cancers (both non-melanoma and melanoma) have been increasing alarmingly.[12] It has been found that melatonin and its light-degraded products are still able to scavenge free radicals effectively. Even if the melatonin breaks down to various products, these retain the protective properties of melatonin. This opens up another avenue of use for melatonin-sunscreens.[13] Thus, elucidation of the mechanism for the photon-induced reaction of melatonin is significant.

In addition, many investigations have provided convincing evidence that melatonin is a light-sensitive hormone involved in the light response of our immune and nervous systems.[14,15] Studies have shown that the retina can, when stimulated by the proper wavelengths of light, cause the synthesis of melatonin in the pineal gland. The pineal gland reduces the production melatonin from 100% at night to 10% during the day.[16,17] Hence, it is expected that clarification of the direct reaction of melatonin under light radiation can bring a better understanding to our body systems.

However, no details have yet been found on the photolysis of melatonin although there have been many recent publications about melatonin. In the present work, we will focus mainly on mechanism of photoionization of melatonin; as well, we will discuss the properties of the radicals produced with 248 nm light photons, and, for the sake of comparison, a pulse radiolysis study was also conducted.

2 Experimental

Melatonin was purchased from Aldrich and used as received. Unless otherwise indicated, all of the solutions were freshly prepared with distilled water purified through a Millipore device (Milli-Q, Element A-10). The solutions were bubbled with high purity argon, nitrous oxide or oxygen for each purpose over 20 min prior to the photonization. The acidity and basicity of the solution were carefully adjusted with 20 mM phosphates within the pH region from 3 to 11 and self-buffered with perchloric acid or NaOH in the highly acidic or highly basic pH range. The ionic strength was fixed to the expected value with the addition of a calculated amount of a perchlorate stock solution.

Steady-state UV-visible spectra were recorded on a UV-3000 spectrophotometer (Hitachi). The fluorescence of melatonin was measured with a PMA-11 Photonic Multichannel Spectral Analyzer (Hamamatsu Photonics Co.). Transient absorption experiments were carried out with an excimer laser (Compex 102, Lambda Physik, pulse width 20 ns, energy used usually 98 mJ/pulse in this work) with an absorption–spectrometric system (a 150 W pulsed Xenon lamp as the source of analyzing light and a fast oscillograph connected to the computer through GPIB). The relative output energy of the laser, I/I_{max}, measured by a ScienTech SPHD50 Joulemeter, was changed by putting thin Pyrex glass plates before the samples. The sample solution was kept flowing through the quartz cell and was changed completely after each laser pulse by a Teflon pump. The light length of the quartz cell is 1.5 cm. Pulse radiolysis of the melatonin aqueous solutions was performed using the same absorption–spectrometric system and a LINAC accelerator in UTNL, where the pulse duration was adjusted to be 10 ns during determination. Dosimetry was based on the initial yield of dithiocyanate radical anion obtained in N_2O-saturated samples of 10 mM KSCN solution ($\varepsilon = 7950\ M^{-1}\ cm^{-1}$ at 472 nm).[18] Usually, we used 82 Gy for the spectral measurements and 13 Gy for kinetic measurements.

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3 Results and discussion

3.1 The absorption and emission of melatonin

The ground-state absorption spectrum of melatonin in water extends from 200 to 320 nm with a maximum at 278 nm having a molar absorption coefficient of 5.4×10^3 M^{-1} cm^{-1} (as shown in Fig. 1). The spectrum remains roughly unchanged over a wide pH range from 3 to 11. The emission spectrum of melatonin in aqueous solution consists of a broad band with maximum at 350 nm, very similar to the reported spectrum recorded in water–ethanol solution.[19] The intensity of fluorescence remains almost constant at pH 3–11 while the fluorescence becomes very weak in strongly acidic and alkaline conditions. The indolyl ring is the chromophore; the different fluorescence properties of melatonin should result from the change of the structure of the indolyl ring. On the melatonin molecule, the indolyl ring consists of a relatively reactive site N1, which is expected to donate H^+ ions in the highly alkaline solution and to accept H^+ ions in the highly acid solution. Therefore, the neutral melatonin molecule is considered to be the fluorescent species and the fluorescence is quenched when melatonin exists in solution as the N1 deprotonated species and the protonated species. The acid–base equilibrium related to the fluorescence properties is displayed in Scheme 1.

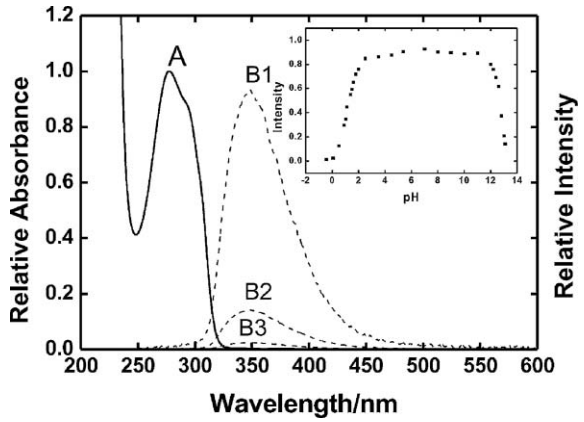


Fig. 1 Absorption spectrum (A) and fluorescence spectrum (B) of melatonin solution at pH 7.00 (B1), at pH 13.01 (B2), and at pH 0.07 (B3). Inset: curve showing pH dependence of the fluorescence intensity of melatonin at 350 nm due to the acid–base equilibrium yielding $pK_a = 12.7 \pm 0.1$ and $pK_b = 1.2 \pm 0.1$.

In this context, we use HMel, H_2^+Mel and Mel^- to represent the neutral melatonin molecule, the protonated species and the deprotonated species, respectively.

The dependence of fluorescence on pH is associated with the acid–base equilibrium of melatonin. From the plot of intensity against pH, we measured the pK_b and pK_a of melatonin as 1.2 ± 0.1 and 12.7 ± 0.1, respectively. Our result coincides well with the reported pK_a value of 12.3 by Mahal *et al.*[20] Because no pK_b values can be found in the literature, we can only compare it with the pK_b value of indole ($pK_b = -2$). Based on *ab initio* calculations with Gaussian 98, the N1 atom on melatonin exhibits a higher electron density than that on indole; this fact means that the N1 atom on melatonin is easier to be protonated, and therefore the pK_b value for melatonin can be accepted as reasonable.

3.2 Transient absorption spectra of melatonin

Excitation of an air-saturated aqueous solution of 0.2 mM melatonin at pH 5.6 by a laser pulse produces a transient absorption consisting of three bands with maxima around 340, 460 and 720 nm at the end of the laser pulse (Fig. 2). The absorption peak at 720 nm disappears within 0.8 μs under the experimental conditions while the peak at 460 nm decays much more slowly with a half-life of over 0.35 ms. The absorption at 340 nm increases in the first 1 μs then decays, lasting with a half-life of longer than 30 μs. The substantial difference between the absorption peaks of melatonin indicates the formation of several intermediate species by the laser photolysis.

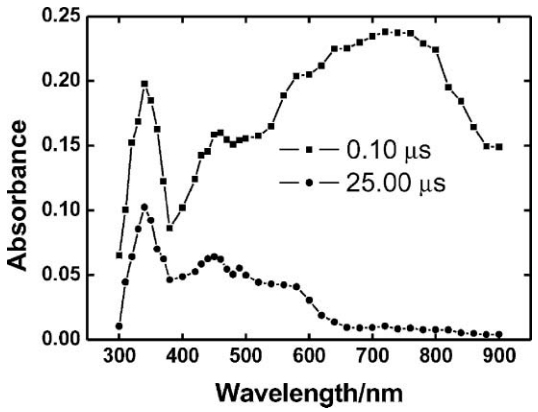


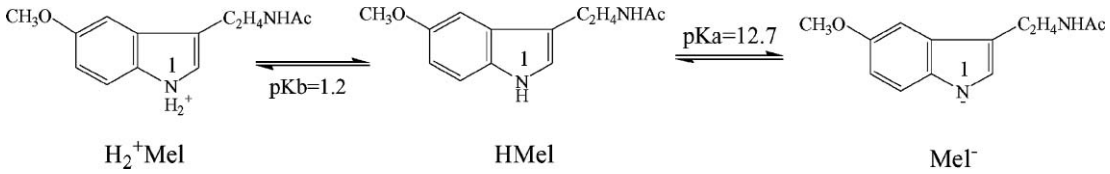
Fig. 2 Transient spectra obtained by laser photolysis of an air-saturated 0.2 mM melatonin solution at pH 5.6.

3.3 Formation of the hydrated electron

The absorption band around 720 nm decays exponentially with a pseudo-first-order rate constant of *ca.* 5.5×10^6 s^{-1} in the air-saturated solution. Its disappearance is greatly speeded up by the saturation with N_2O and pure-O_2 whereas is retarded by argon saturation. Based on this fact and its optical spectrum shape, we attributed the absorption band to the hydrated electron (e^-_{aq}).[21] To confirm the existence of the hydrated electron, several well known electron scavengers were used to measure the scavenging kinetics, and the rate constants accompanied with the reported values are summarized in Table 1. It is clear that our data are consistent with the reported rate constants[22–25] of the reactions between these scavengers and hydrated electrons. Therefore, it can be concluded safely that electron detachment reactions occur in the photolytic process of melatonin.

3.4 Monophotonic formation of the hydrated electron and its quantum yield

Fig. 3(A) shows the result of measurements of the hydrated electron absorbance at 720 nm in an argon-degassed solution of 0.2 mM melatonin as a function of the relative laser energy. Under our experimental conditions, the formation of the hydrated electron was proven to be a monophotonic process by the linear increase of the absorption against the pulse energy. As a reference system, the behavior of an argon-saturated KI solution with an identical ground-state absorbance is also presented in Fig. 3(B). Of course, the detachment of an electron



Scheme 1 Acid–base equilibrium of melatonin.

Table 1 Rate constants for various scavengers with the hydrated electron

Scavenger	$k_{2(\text{this work})}/\text{M}^{-1}\ \text{s}^{-1}$	$k_{2(\text{reported})}/\text{M}^{-1}\ \text{s}^{-1}$	Ref.
Air- and O_2-saturation	2.0×10^{10}	1.8×10^{10}	22
NO_3^- (1–5 × 10^{-4} M)	1.2×10^{10}	9.7×10^{9}	23
CH_3COCH_3 (0.5–1 × 10^{-3} M)	8.2×10^{9}	7.7×10^{9}	24
Cu^{2+} (1–5 × 10^{-4} M)	6.4×10^{9}	5.8×10^{9}	25
Ag^+ (1–5 × 10^{-5} M)	9.7×10^{10}	4.5×10^{10}	25

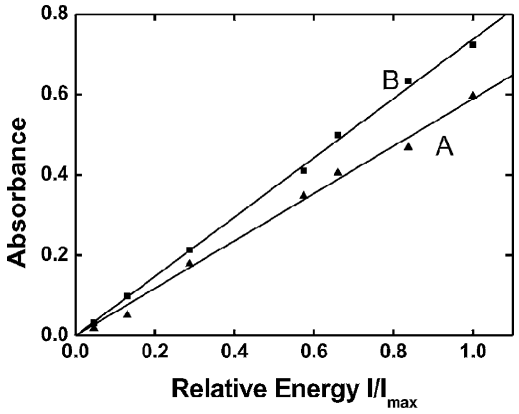


Fig. 3 Dependence of absorbance at 720 nm on laser intensity immediately after laser photolysis of an N_2-saturated solution: (A) 0.2 mM melatonin; (B) KI. The ground-state absorbance of both solutions was adjusted to be identical, *i.e.* Abs = 0.386, and the laser intensity is a relative value.

from KI is also a monophotonic process based on our plot, which has been well established in the literature.[26]

From this figure, we may calculate the quantum yield of the hydrated electron for melatonin from the slope of the two lines:

$$\Phi_{e_{aq}^-}(\text{melatonin}) = \frac{\text{slope(melatonin)}}{\text{slope(KI)}}\Phi_{e_{aq}^-}(\text{KI})$$

Taking the quantum yield of the hydrated electron for KI, $\Phi_{e_{aq}^-}(\text{KI}) = 0.28$ into account,[27] we calculated the quantum yield of the monophotonic formation of hydrated electrons at 248 nm to be 0.22. The result clearly indicates that the photoionization of melatonin in aqueous solution is a quite efficient process.

3.5 Behavior of the hydrated electron

The decay of the hydrated electron was distinctly accelerated by the increase of the ground-state melatonin concentration as displayed in Fig. 4. This fact indicates that some reactions take place between the hydrated electron and melatonin. However, our efforts to fit the disappearance of the hydrated electron to pure exponential kinetics was not successful. Besides

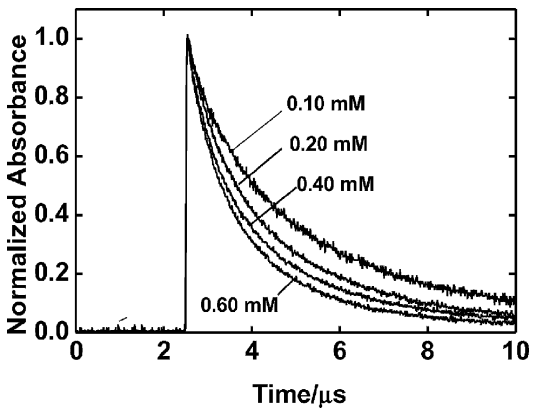


Fig. 4 Time profiles of the absorbance of the hydrated electron at 720 nm observed in the photolysis of various initial concentrations of melatonin.

the attachment reaction of the hydrated electron onto the ground-state melatonin, normally pseudo-first-order, the non-exponential kinetics means that the reactions of electrons with some other transient species are not negligible in this system. In order to determine the kinetics of the hydrated electron with melatonin, we performed an experiment on the pulse radiolysis of a melatonin solution in presence of argon-saturated 0.1 M *tert*-butanol under neutral pH conditions. In such a solution, the hydrated electron was formed while the other oxidizing species were scavenged so that we could obtain the relatively pure reaction kinetics. Kinetic measurements for the decay of the hydrated electron at 720 nm were made from the exponential disappearance of the transient absorption against time for different melatonin concentrations. Based on the pseudo-first-order decay rate against several concentrations of melatonin, the rate constant was calculated to be $k_{e_{aq}^-} = 6.3 \times 10^8\ \text{M}^{-1}\ \text{s}^{-1}$, which is well supported by previously reported values, $7.0 \times 10^8\ \text{M}^{-1}\ \text{s}^{-1}$,[20] and $4.2 \times 10^8\ \text{M}^{-1}\ \text{s}^{-1}$.[28] With this value, we fitted the decay of the hydrated electron in the photolysis experiment with the mixed kinetics of exponential decay and reciprocal decay using the following function equation:

$$-\frac{\text{d}[e_{aq}^-]}{\text{d}t} = k_{e_{aq}^-}[\text{melatonin}]_0[e_{aq}^-] + 2k[e_{aq}^-]^2 \quad (1)$$

In eqn. (1) the terms in square brackets represent the corresponding concentrations. All of the time-resolved data fit the equation very well at each concentration of melatonin in our experiment. The results show that a second-order reaction takes place in the system with a rate constant $k = 1.3 \times 10^{11}\ \text{M}^{-1}\ \text{s}^{-1}$. Because the reaction occurs at such a rapid rate our measurements of the rate constant must consequently include a large error. In the laser flash investigation, it was also found that the hydrated electron from tryptophan, *N*-methyltryptophan and tyrosine disappears more rapidly than predicted by homogeneous reactions. A weakly bound complex between the electron and radical – the so-called 'loose complex' model – was postulated in which the back reaction competes with separation to yield the free radical.[29]

3.6 Formation of the melatonin radical

When melatonin releases an electron, the melatonin radical is expected to be produced as the counterpart of the ejected electron in the photolysis process. In order to eliminate the absorption of the hydrated electron and to obtain a neat spectrum of the melatonin radical, N_2O gas and 0.1 M *tert*-butanol were introduced into the solution as scavengers of hydrated electrons and hydroxyl radicals respectively, based on the reactions

$$e_{aq}^- + N_2O \rightarrow O^{\bullet -} + N_2 \quad (2)$$

$$O^{\bullet -} + H_2O \rightarrow OH^- + OH^{\bullet} \quad (3)$$

where $k = 9 \times 10^9\ \text{M}^{-1}\ \text{s}^{-1}$ (ref. 30), and then the hydroxyl radical converts the into some less reactive species *via*

$$\textit{tert}\text{-BuOH} + OH^{\bullet} \rightarrow {}^{\bullet}CH_2C(CH_3)_2OH + H_2O \quad (4)$$

where $k = 6.0 \times 10^8\ \text{M}^{-1}\ \text{s}^{-1}$ (ref. 31).

A transient spectrum from the photolysis of an N_2O-saturated melatonin solution with 0.1 M *tert*-butanol under neutral pH

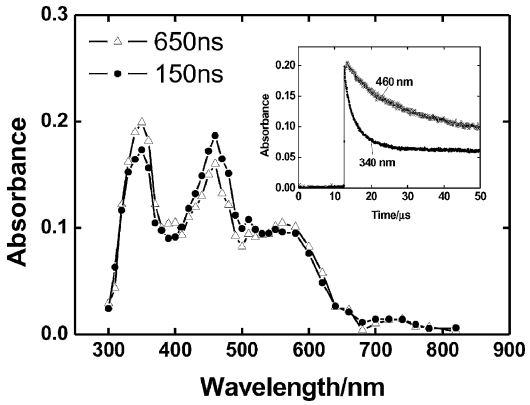


Fig. 5 Transient spectra of melatonin at various times after laser photolysis of a 0.20 mM melatonin solution saturated with N_2O at pH 5.6. Inset: the time-resolved profiles at 340 and 460 nm.

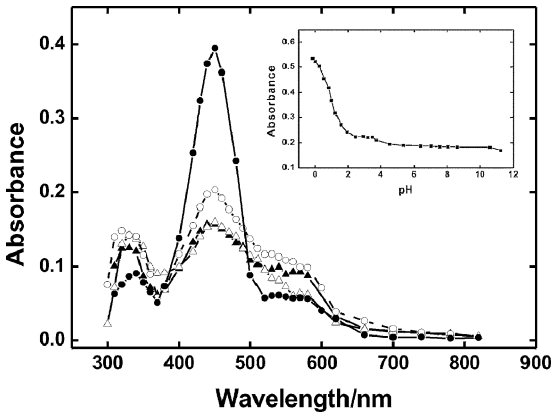


Fig. 6 Photolysis of melatonin aqueous solutions (0.20 mM) under various pH conditions: (–●–) pH 0.82, where the spectrum is the same in presence of allyl alcohol; (–○–) pH 4.86; (–▲–) pH 7.55; (–△–) pH 10.55. The last three spectra were from N_2O-saturated solutions in the presence of 1.0 M *tert*-butanol. Inset: the pH dependence of the absorption at 460 nm.

conditions was recorded (shown in Fig. 5). From this spectrum, we can find that the melatonin radical has two absorption peaks around 340 and 460 nm with an absorption shoulder at 560 nm. In fact, the radicals of indole derivatives are characterized by such absorption spectra,[32,33] and in general, the spectra exhibit one UV and one or two visible absorption bands.

Assuming that the melatonin radicals and e^-_{aq} are formed in stoichiometric amounts, it follows that the melatonin radical and e^-_{aq} are generated with the same concentrations using the laser pulse of the same energy. Thus, the extinction coefficient (ε_{Mel}) of the melatonin radicals can be estimated using e^-_{aq} as an 'internal standard'

$$\varepsilon_{Mel} = \frac{A_{Mel}}{A_{e^-_{aq}}} \times \varepsilon_{e^-_{aq}} \cdot .$$

Here, A_{Mel}, and $A_{e^-_{aq}}$ represent the absorbance of melatonin radical measured in the N_2O-saturated solution and the absorbance of e^-_{aq} at 720 nm measured in the deoxygenated solution. The $\varepsilon_{e^-_{aq}}$ term is the extinction coefficient of e^-_{aq} which was well determined as 1.85×10^4 M^{-1} cm^{-1} at 720 nm in previous reports.[23] So at 460 nm, ε_{Mel} was determined to be 4.3×10^3 M^{-1} cm^{-1} in neutral solution.

When paramagnetic ions, such as 50 mM Mn^{2+}, were added to the melatonin solution as a quencher of the exited triplet state in order to clarify the origin of the radical and electron, the photolysis results showed that, within experimental error, the transient spectrum, photoionization quantum yield, the rate of growth and decay and even the fluorescence are not changed in the presence of the paramagnetic ions. This independence indicates that there is no triplet-state component in the observed transient, or at least that the presence of the triplet state of melatonin can be neglected in the wavelength range studied. Therefore, the photoionization of melatonin with 248 nm photons has no significant contribution from the triplet state.

3.7 The acid–base equilibrium of the melatonin radical

We recorded the transient spectra of melatonin solutions after laser pulses at various very low pH values as displayed in Fig. 6. On one hand, there is some similarity for all of the spectra from various solutions, *i.e.* all of the spectra comprise two absorption bands at 340 (A_{340}) and 460 nm (A_{460}) with an absorption shoulder around 560 nm. The 460 nm band is indicative of localization of positive charge at the electron-rich methoxy group as reported for radical cations of methoxybenzoic acids.[33]

On the other hand, there is a difference between the spectra, for example, the height of the absorption peaks exhibits obvious dependence on the pH value of the solutions.

In the acidic solutions of pH < 3, the absorption peak at 460 nm increases as the acidity of the solutions is increased, and it rises rapidly especiallt at around pH 1.0. The ratio of A_{460} against A_{340} at pH 0.82 is about 5, almost four times the ratio at neutral pH, which is only 1.1. Obviously, in acidic solution, a primary radical, possibly from direct photolysis, dominates the spectrum within the first 2 μs and a secondary radical is derived subsequently from the primary radical. This conclusion is supported by the decay of the absorption peaks with different time-resolved profiles (Fig. 7). For instance, in the solution at pH 0.82, the decay of A_{460} is accompanied by the growth of A_{340} and A_{560} within the first 2 μs and then all three absorption bands decay gradually.

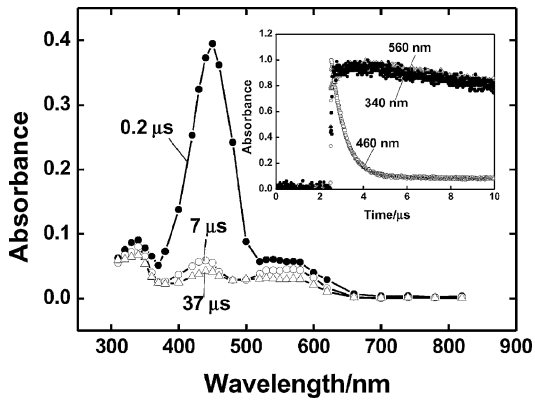


Fig. 7 Transient spectra of melatonin solutions in the presence of 0.1 M allyl alcohol at pH 0.82. Inset: time profiles of the three absorption peaks. The absorbances were normalized with factors 10, 1.6 and 20 for 340, 460 and 560 nm, respectively.

We designate the primary intermediate as a doubly-protonated melatonin radical ($H_2^+Mel^{\bullet+}$) and the secondary species as the singly-protonated melatonin radical ($HMel^{\bullet+}$). The formation of $H_2^+Mel^{\bullet+}$ can be described as the photoionization of protonated melatonin (H_2^+Mel) and $HMel^{\bullet+}$ is originated from $H_2^+Mel^{\bullet+}$ through an acid–base equilibrium process.

$$H_2^+Mel + h\nu \rightarrow H_2^+Mel^{\bullet+} + e^- \quad (5)$$

$$H_2^+Mel^{\bullet+} \overset{k_{a1}}{\rightleftharpoons} HMel^{\bullet+} + H^+ \quad (6)$$

This mechanism is supported by the three aspects that follow.

Firstly, the growth A_{340} and A_{560} is synchronous with the decay of A_{460}, which reveals that the transient species with an absorption band at 340 and 560 nm originates from the A_{460} [eqn. (6)]. There is a reasonable doubt that the increase in A_{340} and A_{560} is the result of the reaction between ground-state melatonin and a H atom because the H atom can be formed in highly acidic solution through an electron reacting with a hydrogen

ion. However, this hypothesis was excluded because of the same spectra and kinetics in the absence and in the presence of a very efficient H atom scavenger, 0.20 M allyl alcohol ($k = 2 \times 10^9$ M^{-1} s^{-1}).[23,34] Therefore, it can be said that the increase of the absorption at 460 nm is not due to the reaction of some radical with a H atom, and that the spectrum after the fast decay does not come from the H-addition product.

Secondly, in acidic solution A_{460} disappears relatively quickly following very good first-order decay kinetics within the first 2 μs, then it deviates from the first-order kinetics outside this time scale. However, even within 2 μs, over 80% of the absorbance is consumed, so we can fit the initial part of the decay to obtain the first-order rate constants. The result shows that the first-order rate constant of A_{460} is dependent upon the acidity of the solution, *i.e.* the decay rates decrease as the acidity of the solution is enhanced (as shown in the inset of Fig. 8).

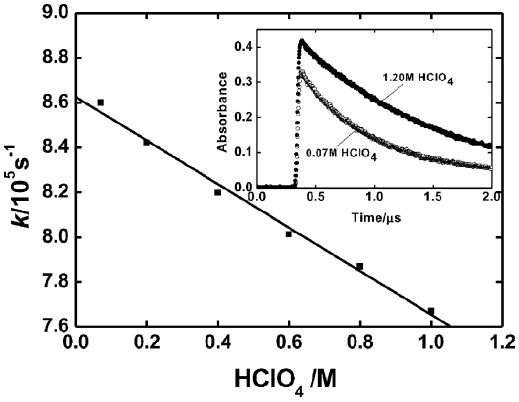


Fig. 8 Effect of acidity on the decay kinetics of the absorption at 460 nm in 0.2 mM melatonin solution with 1.0 M *tert*-butanol, N_2O-saturated. The total ionic strength was fixed to 2.4 M.

The pH dependence can be explained by the forward and reverse reaction in eqn. (6), where the kinetics are expressed by the following:

$$-\frac{d[H_2{}^+Mel^{\bullet+}]}{dt} = k_f[H_2{}^+Mel^{\bullet+}] - k_r[H^+][HMel^{\bullet+}] \qquad (7)$$

Here, k_f and k_r represent the rate constants of the forward reaction and the reverse reaction, respectively.

At the pH region below 0 we measured this equilibrium, and found that almost all of the melatonin exists in solution in the form of the doubly-protonated molecule because the pH is much below the pK_a. Thus the initially-ionized species from the molecule is $H_2{}^+Mel^{\bullet+}$ and the $HMel^{\bullet+}$ radical is formed from $H_2{}^+Mel^{\bullet+}$ after some time. For each run, assuming that the initial concentration of $H_2{}^+Mel^{\bullet+}$ is C_0, then the concentration of $HMel^{\bullet+}$ is equal to $C_0 - H_2{}^+Mel^{\bullet+}$, and replacing the concentration of $HMel^{\bullet+}$ in eqn. (7), we have

$$-\frac{d[H_2{}^+Mel^{\bullet+}]}{dt} = (k_f - k_r[H^+])[H_2{}^+Mel^{\bullet+}] + k_r[H^+]C_0\,. \qquad (8)$$

If the last term of eqn. (8) is kept constant for each run we can neglect this term because of its smallness compared with the first term at the beginning of the decay. Thus, the apparent first-order rate constant for A_{460} at the beginning stage is $k_f - k_r[H^+]$, which is well evidenced by the good linear relationship between the acidity and the apparent rate constant. From the slope and the interception of the line, we obtained $k_f = 8.6 \times 10^5$ s^{-1} and $k_r = 9.7 \times 10^4$ M^{-1} s^{-1}. Because there are two positive charges accumulating on the $H_2{}^+Mel^{\bullet+}$ radical, it is easy to release H^+ rapidly. The higher acidity retards the ionization of the $H_2{}^+Mel^{\bullet+}$ radical because of the reverse reaction.

Thirdly, a sigmoidal curve displays the dependence of the absorption at 460 nm on the acidity of the solutions. From the sigmoid curve, we can observe an inflection point at around pH 1.0 ± 0.1, which appears at almost the same pH condition as the pK_b for the equilibrium $H_2{}^+Mel$ and HMel. It is known that the ground-state species of melatonin have different distributions in aqueous solution through an acid–base equilibrium. From pH 3 to lower pH values, the distribution of protonated melatonin increases as the acidity is increased. The equilibrium constant for reaction can be estimated from the bi-directional rate constants, $pK_{a_1} = k_f / k_r = -0.95$. This p$K_a$ value means that the acidity of $H_2{}^+Mel^{\bullet+}$ is much stronger than $H_2{}^+Mel$. The reason for the enhancement of the acidity is due to the elimination of an electron from the protonated melatonin which results in a decrease in the electron density, so the doubly-positively-charged radical releases a proton easily.

The residual spectrum after the time scale of the first-order decay is due to the $HMel^{\bullet+}$ radical, and its three peaks disappear rather slowly. Its decay was found to obey a reciprocal kinetics expression at 560 nm with $k/\varepsilon = 5.0 \pm 0.1 \times 10^5$ M^{-1} s^{-1}. This reveals a recombination reaction for the radical as follows:

$$HMel^{\bullet+} + HMel^{\bullet+} \rightarrow \text{Products} \qquad (9)$$

The transient spectrum obtained from the pulse radiolysis of 1.0 mM melatonin solutions in the presence of 0.1 M *tert*-butanol under high acidity conditions (pH 0.82) is precisely the same as the spectrum of the secondary radical from photolysis (as shown in Fig. 9). The normalized spectra can overlap each other. In addition, the decay kinetics of the absorptions around 460 and 560 nm from pulse radiolysis were also found to be second-order with $k/\varepsilon = 4.9 \pm 0.1 \times 10^5$ M^{-1} s^{-1} (as shown in Fig. 10). The decay below 400 nm seems much more complex, maybe because the recombination products in reaction 9 always exhibit strong absorption in the shorter wavelength range. Hence, the radical in the pulse radiolysis is the same species as the secondary radical in photolysis.

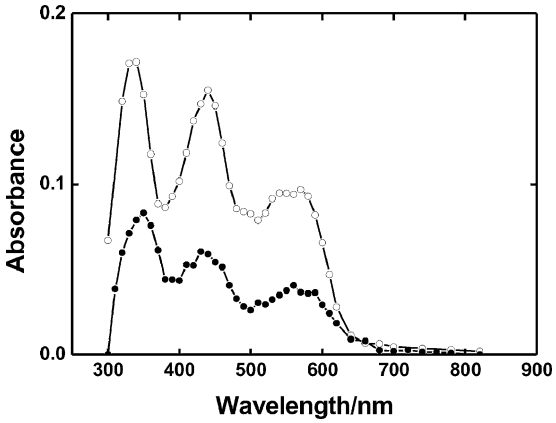


Fig. 9 Absorption spectra of melatonin solution at pH 0.82 obtained by photolysis (–○–) and radiolysis (–●–) at 7.00 μs after the pulse.

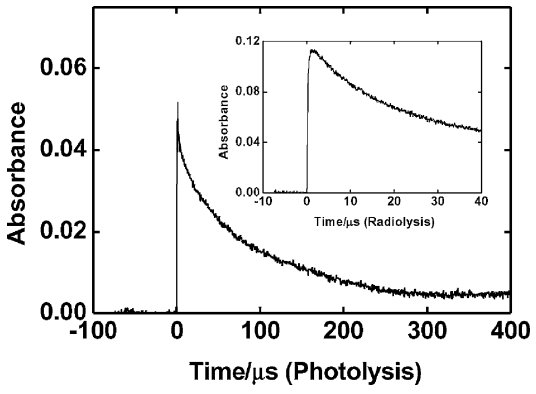
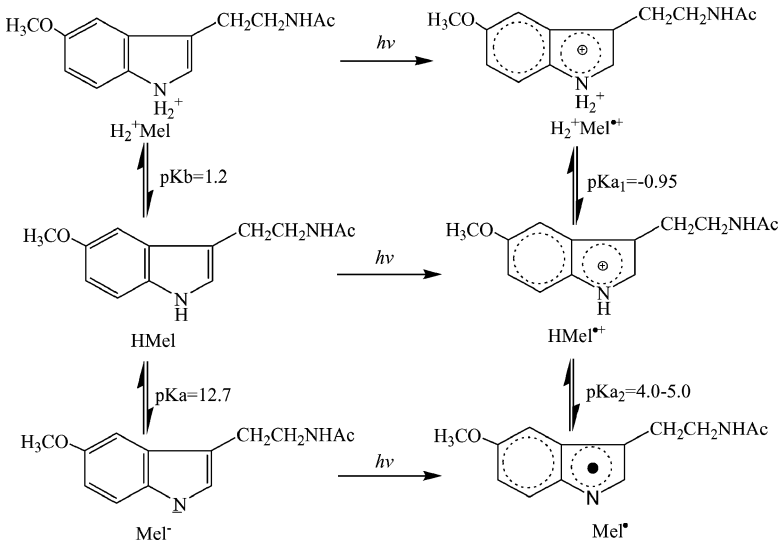


Fig. 10 Because of decay of the absorption at 460 nm, the decay kinetics for photolysis were measured on a longer time scale (500 μs).



Scheme 2 Acid–base equilibrium of melatonin and its radicals.

In the very acidic solutions in the presence of *tert*-butanol, the H atom is the main primary product from the pulse of radiation so that the absorption profiles are the spectra for the product from H atom reacting with melatonin. Mahal *et al.*[20] and Roberts *et al.*[28] also observed the same absorption spectra and they attributed the spectra to an H-addition reaction of melatonin. However, their proposal can not explain the same products, $HMel^{\bullet+}$, from both pulse radiolysis and laser photolysis. It seems that the H atom reacting with melatonin in acid solution through a hydrogen abstraction is more reasonable:

$$H_2{}^+Mel + H^\bullet \rightarrow HMel^{\bullet+} + H_2 \qquad (10)$$

In some cases, in highly acidic solution, the H atom effectively behaves as an oxidant and it reacts with organic compounds by abstracting H. In this respect it resembles the hydroxyl radical, although the latter is more reactive and less selective in abstraction reactions.[35]

The extinction coefficient of $HMel^{\bullet+}$ at 560 nm is estimated from the pulse radiolysis experiment as 2.9×10^3 M^{-1} cm^{-1}. Hence, the rate constant for the recombination of $HMel^{\bullet+}$ is 1.5×10^9 M^{-1} s^{-1}.

In the N_2O-saturated melatonin solution in the presence of 1.0 M *tert*-butanol with pH > 4.0, the electron is scavenged by N_2O and is then converted into an inactive species by *tert*-butanol; consequently, the transient spectra result from of the neat melatonin radical. Unlike the dramatic pH dependence in very acidic solution, the neat spectra are slightly dependent on the acidity of the solutions with pH values over 4.0; shortly, the absorption peaks at 460 and 560 nm decrease with the increasing pH value. Here, we could only give out a rough range for the inflection point at pH 4.0–5.0 on the sigmoid curve of the absorptions at 560 nm *versus* pH because of the small changes.

Unequivocal demonstration that the change in the spectrum is associated with deprotonation from N was obtained from comparative experiments with indoles and their *N*-methyl derivatives.[36,37] The spectrum obtained from one-electron oxidation of the former changes with pH on going from acid to neutral solution. With the *N*-methyl substituted compounds, the spectra were found to be pH-independent over a wide pH range. This is clearly due to the absence of proton at N, implying that with other indoles, deprotonation of the radical cation takes place from this position.

The decay for the radical in the pH range 4–6 and >6 seems so complicated that we cannot fit the kinetics into any simple form, not even for a mixture of first order and second order. It means that several reactions are involved in the weakly acidic solution and in the basic solution. The possible reason is that the recombination reaction and the hydrolysis reaction become comparable so that none of them can dominate the decay expression. Most importantly, the hydrolysis reaction products are hydroxyl addition species and their absorption bands overlap with primary radicals and with each other. Therefore, it is difficult to obtain a clear idea of the kinetics for the melatonin radical in non-highly-acidic solution.

Based on our results, we can find that melatonin is a light-sensitive compound and that it can be decomposed efficiently under UV-light exposure. The sunscreen effect of melatonin in cosmetics is mainly based on its scavenger ability and its pharmacological function; this is different from the widely-applied sunscreens, titanium dioxide and zinc oxide,[38,39] whose role as sunscreens is mainly due to their stability and reflecting ability of UV-light. And maybe, melatonin and its photolyzed products can scavenge the primary free radicals produced by UV-light in the skin cells and melatonin can cause skin cells to proliferate in small amounts, these two points are the main reason for its sunscreen effect.

4 Conclusions

The photolysis process of melatonin near neutral pH takes place monophotonically with a quantum yield of 0.22. The photoionization of melatonin can result in the formation of a melatonin radical, accompanied by the ejection of an electron. It is possible for the released electron to be converted into various species according to its environment; for example, a hydrated electron in neutral solution, a H atom in acid solution, and hydroxyl radical in N_2O-saturated solution. The melatonin radical in solution exhibits three characteristic absorption bands at 340, 460 and 560 nm and the radical exists in the solution as various species based on the acid–base equilibrium (Scheme 2). From the equilibrium, we can deduce that if the oxidation of melatonin occurs *in vivo*, the neutral melatonin radical is the possible intermediate under the pH conditions of the human body.

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References

1 A. A. Abd-Allah, E. M. El-Sayed, M. H. Abdel-Wahab and F. M. A. Hamada, Effect of melatonin on estrogen and progesterone receptors in relation to uterine contraction in rats, *Pharm. Res.*, 2003, **47**, 349–354.

2 P. A. Witt-Enderby, J. Bennett, M. J. Jarzynka, S Firestine and M. A. Melan, Melatonin receptors and their regulation: biochemical and structural mechanisms, *Life Sci.*, 2003, **72**, 2183–2198.
3 R. J. Reiter, Melatonin: it's not just for the endocrine system anymore: melatonin in the promotion of health, *Trends Endocrinol. Metab.*, 1999, **10**, 295–296.
4 S. M. Webb and M. P. Domingo, Role of melatonin in health and disease, *Clin. Endocrinol.*, 1995, **42**, 221–234.
5 H. K. Biesalski, Free radical theory of ageing, *Curr. Opin. Clin. Nutr.*, 2002, **5**, 5–10.
6 R. J. Reiter, Melatonin in the context of the free radical theory of ageing, *Ann. N. Y. Acad. Sci.*, 1996, **786**, 362–378.
7 R. J. Reiter, Functional aspects of the pineal hormone melatonin in combating cell and tissue damage induced by free radicals, *Eur. J. Endocrinol.*, 1996, **143**, 421–420.
8 D. X. Tan, L. C. Manchester, R. J. Reiter, B. F. Plummer, J. Limson, S. T. Weintraub and W. B. Qi, Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation, *Free Radical Biol. Med.*, 2000, **29**, 1177–1185.
9 J. A. Horstman, M. Z. Wrona and G. Dryhurst, Further insights into the reaction of melatonin with hydroxyl radical, *Biol. Chem*, 2002, **30**, 71–382.
10 Y. W. Ryoo, S. I. Suh, K. C. Mun, B. C. Kim and K. S. Lee, The effects of the melatonin on ultraviolet-B irradiated cultured dermal fibroblasts, *J. Dermatol. Sci.*, 2001, **27**, 162–169.
11 M. L. Cagnoni, A. Pignone, A. Lombardi and T. Lotti, Melatonin in the treatment of cutaneous sarcoidosis, *J. Eur. Acad. Dermatol. Vener.*, 1995, **5**, S163.
12 D. L. Miller and M. A. Weinstock, Nonmelanoma skin cancer in the United States: incidence, *J. Am. Acad. Dermatol.*, 1994, **30**, 774–778.
13 F. Dreher, B. Gabard, D. A. Schwindt and H. I. Maibach, Topical melatonin in combination with vitamins E and C protects skin from ultraviolet-induced erythema: a human study *in vivo*, *Br. J. Dermatol.*, 1998, **139**, 332–339.
14 T. N. Ivanova and P. M. Iuvone, Circadian rhythm and photic control of cAMP level in chick retinal cell cultures: a mechanism for coupling the circadian oscillator to the melatonin-synthesizing enzyme, arylalkylamine *N*-acetyltransferase, in photoreceptor cells, *Brain Res.*, 2003, **991**, 96–103.
15 T. N. Ivanova and P. M. Iuvone, Melatonin synthesis in retina: circadian regulation of arylalkylamine *N*-acetyltransferase activity in cultured photoreceptor cells of embryonic chicken retina, *Brain Res.*, 2003, **973**, 56–63.
16 H. J. Burgess, K. M. Sharkey and C. I. Eastman, Bright light, dark and melatonin can promote circadian adaptation in night shift workers, *Sleep Med. Rev.*, 2002, **6**, 407–420.
17 A. J. Lewy, B. P. Hasler, J. S. Emens and R. L. Sack, Pretreatment circadian period in free-running blind people may predict the phase angle of entrainment to melatonin, *Neuroendocrinol. Lett.*, 2001, **313**, 158–160.
18 G. V. Buxton and C. R. Stuart, Re-evaluation of the thiocyanate dosimeter for pulse radiolysis, *J. Chem. Soc., Faraday Trans.*, 1995, **91**, 279.
19 V. Pucci, A. Ferranti, R. Mandrioli and M. Raggi, A determination of melatonin in commercial preparations by micellar electrokinetic chromatography and spectrofluorimetry, *Anal. Chim. Acta*, 2003, **488**, 97–105.
20 H. S. Mahal, H. S. Sharma and T. Mukherjee, Antioxidant properties of melatonin: a pulse radiolysis study, *Free Radical Biol. Med.*, 1999, **26**, 557–565.
21 F. Y. Jou and G. R. Freeman, Shapes of optical spectra of solvated electrons. Effect of pressure, *J. Phys. Chem.*, 1977, **81**, 909–915.
22 A. J. Elliot, D. R. Mccracken, G. V. Buxton and N. D. Wood, Estimation of rate constants for near-diffusion-controlled reactions in water at high-temperatures, *J. Chem. Soc., Faraday Trans.*, 1990, **86**, 1539–1547.
23 G. V. Buxton, C. L. Greenstock, W. P. Helman and A. B. Ross, Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals, *J. Phys. Chem. Ref. Data*, 1988, **17**, 513–886.
24 C. C. Lai and G. R. Freeman, Solvent effects on the reactivity of solvated electrons with organic solutes in methanol/water and ethanol/water mixed solvents, *J. Phys. Chem.*, 1990, **94**, 302–308.
25 R. Chen, Y. Avotinsh and G. R. Freeman, Solvent effects on the reactivity of solvated electrons with ions in iso-butanol/water mixed solvents, *Can. J. Chem.*, 1994, **72**, 1083–1093.
26 D. F. Bryant, R. Santus and L. I. Grossweiner, Laser flash photolysis of aqueous tryptophan, *J. Phys. Chem.*, 1975, **79**, 2711–2716.
27 M. C. Sauer, J. R. A Crowell and I. A. Shkrob, Photodetachment from aqueous anions. 1. Quantum yields for generation of hydrated electron by 193 and 248 nm laser photoexcitation of miscellaneous inorganic anions., *J. Phys. Chem. A*, 2004, **108**, 5490–5502.
28 J. E. Roberts, D. N. Hu and J. F. Wishart, Pulse radiolysis studies of melatonin and chloromelatonin, *J. Photochem. Photobiol., B*, 1998, **42**, 125–132.
29 J. F. Baugher and L. I. Grossweiner, Photolysis mechanism of aqueous tryptophan, *J. Phys. Chem.*, 1977, **81**, 1349–1354.
30 A. J. Elliot, A pulse radiolysis study of the temperature dependence of reactions involving H, OH, and e^-_{aq} in aqueous solution, *Radiat. Phys. Chem.*, 1989, **34**, 753–758.
31 B. S. Wolfenden and R. L. Willson, Radical cations as reference chromogens in kinetic studies of one-electron transfer reactions: pulse radiolysis studies of ABTS, *J. Chem. Soc., Perkin Trans. 2*, 1982, 805–812.
32 S. V. Jovanoic and S. Steenken, Substituent effects on the spectral, acid–base and redox properties of indolyl radicals: a pulse radiolysis study, *J. Phys. Chem.*, 1992, **96**, 6674–6679.
33 S. Steenken, P. O'Neoll and D. Schulte-Frohlinde, Formation of radical zwitterions from methoxylated benzoic acids. 1. One-electron oxidation by Ti^{2+}, Ag^{2+}, and SO_4^-, *J. Phys. Chem.*, 1977, **81**, 26–30.
34 W. A. Volkert and R. R. Kuntz, Reactions of hydrogen atoms in aqueous solutions. Some amino acids, *J. Phys. Chem.*, 1968, **72**, 3394–3400.
35 G. G. Jayson, J. P. Keene, D. A. Sterling and A. J. Swallow, Pulse-radiolysis study of some unstable complexes of iron, *Trans. Faraday Soc.*, 1969, **65**, 2453.
36 A. T. Kazwinin, P. O'Neill, G. E. Adams, R. B. Cundall, B. Jacquet, G. Lang and A Junino, One-electron oxidation of methoxylated and hydroxylated indoles by azide. 1. Characterization of the primary indolic radicals, *J. Phys. Chem.*, 1992, **94**, 6666–6670.
37 S. Solar, N. Getoff, P. S. Surdhar, D. A. Armstrong and A. Singh, Oxidation of tryptophan and *N*-methylindole by N_3, $Br_2^{\bullet-}$, and $(SCN)_2^{\bullet-}$ radicals in light- and heavy-water solutions: a pulse radiolysis study, *J. Phys. Chem.*, 1991, **95**, 3639–3643.
38 J. Lademann, H. J. Weigmann, H. Schafer, G. Muller and W. Sterry, Investigation of the stability of coated titanium microparticles used in sunscreens, *Skin Pharmacol. Appl.*, 2000, **13**, 258–264.
39 S. C. Rastogi, UV filters in sunscreen products – a survey, *Contact Dermatitis*, 2002, **46**, 348–351.