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Journal of Photochemistry and Photobiology A: Chemistry 171 (2005) 209–214

Journal of Photochemistry and Photobiology A:Chemistry

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Radiolysis and photolysis studies on active transient species of diethylstilbestrol

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Received 2 April 2004; received in revised form 6 September 2004; accepted 7 October 2004
Available online 8 December 2004

Abstract

Diethylstilbestrol (DES) is one of the most active estrogens in the environment. In this paper pulse radiolysis and laser flash photolysis studies were carried out to investigate mechanisms and dynamic characteristics of the transient species of DES produced by UV light and electron beam irradiations. The double bond between the benzene rings was supposed to be the most sensitive site attacked by hydroxyl radicals under high-energy electron beam irradiations. In laser flash photolysis DES was found to be either photoionized or excited by 248 nm laser to produce deprotonated radical cations and excited triplet states. It was found that DES could be degraded completely in oxygen-saturated aqueous solution under the UV lights. All the active species produced in pulse radiolysis and laser flash photolysis can cause environmental biologic effects. The work on degradation mechanisms of DES can be of help in finding ways to tackle the environmental DES problems.

Keywords: Diethylstilbestrol; Radiolysis; Photolysis

1. Introduction

Diethylstilbestrol (DES, Fig. 1A), the first synthetic non-steroid estrogen drug, has a similar structure to natural steroid estrogen 17β-estrodiol (E2, Fig. 1B), but is more effective (about 2000 times more active than of E2) and less expensive. It had been unfortunately assumed that DES could be of help in protecting pregnancy from miscarriages, and the drug was administered to millions of pregnant women in the United States and Europe for about half a century since 1947 [1].

Today, however, it has become known that DES exposure would yield serious consequences. The relative risk for so-called 'DES daughters' to develop an adenocarcinoma of the vagina and cervix at a young age is estimated at 1 in 1000. The probability is much higher for a DES-administrated woman to develop vaginal adenosis or non-carcinogenic alterations of the genital tract [2]. And it cannot be stated at present yet with certainty whether this group is likely to show any late effects, such as an increased tumor risk after the menopause. On the other hand, 30% of 'DES sons' have showed various types of abnormalities of the genital tract, e.g. hypospadias, cryptorchism and epididymal cysts.

Mountains of evidence revealed that transient excited states and free radicals of estrogen derivatives play an important role in damaging biological tissue as confirmed with xenoestrogens studied in vitro and in vivo. Bisphenol A has been found to cause photoallergy in some chemical plants. ESR and GC/MS studies showed that under UV lights a semiquinone structured product following cleavage of a methyl radical was supposed to be decomposed from bisphenol A [3]. ESR was used to assess the antioxidant efficacy of phytoestrogens such as genistein [4]. In toxicological investigations, Crump et al. found, with a novel approach that combined physiologic and molecular end points, that environmental or subambient levels of octylphenol could alter the expressions of hypothalamic genes and disrupt Leopard Frog tadpoles' growth pattern [5]. QSAR analysis [6] and non-enzyme mechanism of estrogenic activity [7] assume that phenolic estrogens are converted to radicals, by the ROS from fast growing cells, which attack related DNA segments.

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doi:10.1016/j.jphotochem.2004.10.003

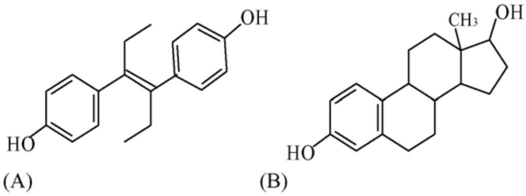

Fig. 1. Structure of diethylstilbestrol (A) and 17β-estrodiol (B).

The time courses of how a binding occurs and how an unbound ligand becomes a protein–ligand complex remains unknown, because of formidable experimental and theoretical challenges. It is anticipated that binding dynamics may take place on multiple time scales, from milliseconds (or longer) to picoseconds, which is inaccessible by conventional methods [8]. Pulse radiolysis and laser flash photolysis are effective for studies on dynamics of transient chemical species [9]. In this work, transient dynamic characteristics of DES were studied with the two methods, in an attempt to get insights into degradation processes of DES and to understand toxicology of DES in natural conditions. In this paper, characteristic transient absorption spectra and dynamics of transient species of DES are reported.

2. Materials and methods

2.1. Materials

Diethylstilbestrol (DES) was purchased from Tokyo Chemical Incorporation (TCI) and used without further purification. The DES samples were dissolved in 0.5 mol dm^{-3} aqueous KOH solution and diluted to 1×10^{-3} mol dm^{-3}, followed by neutralization with $HClO_4$ solution of the same molarity. The parent solutions were first saturated with N_2 to prevent any thermal oxidation by O_2 in the air.

Unless additionally indicated, all solutions were freshly made with triply distilled water and protected from light at all times. pH value of the solution was adjusted by adding NaOH, $HClO_4$ or phosphate solution. Just before the pulse radiolysis experiments, the solutions were deaerated with high-purity N_2O, O_2 or N_2 for at least 20 min for different purposes. All experiments were performed at room temperatures.

The reagents, all in AR (analytic-grade reagent), were from Shanghai Reagent Chemical Co. Tert-butanol **(**T-BuOH**)**, in AR, was distilled before use.

2.2. Stable UV absorbance measurement

The DES solutions were irradiated by Xe lamp UV lights to investigate its degradation under the UV irradiation. The 500 W-Xe lamp was produced by Shanghai Film Equipment Works. The UV lights were focused by an optical lens onto a 1 cm quartz cell through a filter. The stable UV spectra of DES solution samples were tested by a TU-1800 UV–vis spectrometer provided by Beijing Puxi General Instrument Co. ZDZ-1 ultraviolet dosimeter **(**Made in Shanghai, China) with response range between 230 and 290 nm, and HI9142 portable dissolved oxygen detector (Made in HANNA, Italy) were used to analyses the UV dose and O_2 concentration.

2.3. Laser flash photolysis experiment

Laser flash photolysis experiments were carried out with a KrF excimer laser, which delivers up to 50 mJ of 248 nm-light in a single pulse of 20 ns. The analysing light beam passed perpendicularly to the 1 cm quartz cell, and entered a monochromator equipped with a Hamamatsu R955 photomultiplier. The signals were collected by an HP54510B 300-MHz transient recorder. The data were processed with a personal computer.

2.4. Pulse radiolysis experiment

Pulse radiolysis experiments were performed on a 10-MeV linear accelerator, with pulsed electron beam with duration of 8 ns. A 2 cm cell was used for the radiolysis experiment. Sample replenishment before each beam pulse was achieved with a flowing system. The dose delivered by an electron beam pulse was determined by thiocyanate dosimeters. The dosimetry parameters are, $G[(CNS)_2^{\bullet-}] = 6.0$ in a 1×10^{-2} mol dm^{-3} KCNS solution, saturated with N_2O by taking $\varepsilon_{480} = 7600$ dm^3 mol^{-1} cm^{-1} for $(CNS)_2^{\bullet-}$. In this work, the dose per electron beam pulse was 8 Gy. Details of the facility can be found in a previous paper [9].

3. Results and discussion

3.1. Laser excitation of diethylstilbestrol

For all the photolysis experiments, absorbance of the tested DES solution was 0.4–1.0 at 248 nm. A 2×10^{-4} mol dm^{-3} DES solution, buffered with 2×10^{-3}mol dm^{-3} phosphate, has an absorbance of 0.55 at 248 nm. This solution was buffered with 2×10^{-3} mol dm^{-3} phosphate and checked with 248 nm laser flash photolysis [9].

As shown in Fig. 2, transient absorption spectra were obtained from photolysis of 2×10^{-4} mol dm^{-3} DES solution samples deaerated by N_2, O_2 and N_2O, respectively, and buffered with 2×10^{-3} mol dm^{-3} phosphate (pH = 7.0) and excited by 248 nm laser beam. In the system of N_2-deaerated solution, two transient species with maximum absorption at 310 and over 650 nm were observed. As the transient species with maximum absorption over 650 nm disappeared, in N_2O-saturated system instead of deoxygen system, it can be supposed that DES was ionized by 248 nm light to produce hydrated electron because only its maximum transient absorption is over 650 nm and can be scavenged by N_2O. There are some active transient species, which cannot be quenched in O_2-saturated system, this case is quite different from 4,4′-bisphenol tested by Mohanty et al. [10].

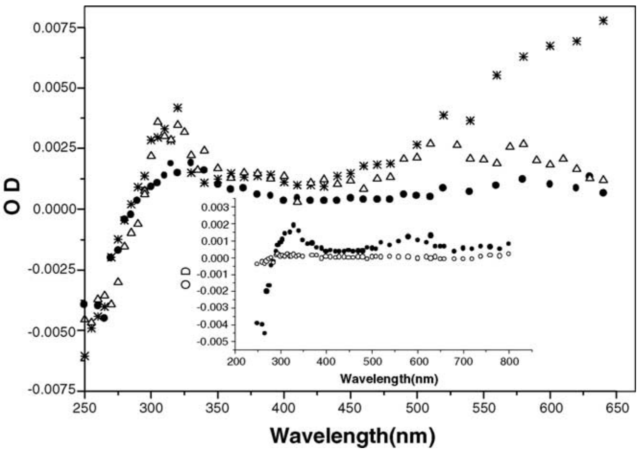

Fig. 2. Transient spectra obtained from 248 nm laser flash photolysis of aqueous DES solutions of 2×10^{-4} mol dm^{-3} observed at 1 μs after the laser pulse. The data correspond to DES solutions of (i) N_2-saturated (*), (ii) O_2-saturated (△), and (iii) N_2O-saturated (●). Inset: transient absorption spectra of the N_2O-saturated system observed at 1 μs (●) and 33 μs (○).

With the transient species at about 310 nm, it can be assumed that radical cation or deprotonated radical cation was created via the following reactions.

$$\text{DES} + h\nu \rightarrow \text{DES}^{\bullet +} + e_{aq}{}^{-} \quad (1)$$

$$\text{DES}^{\bullet +} \rightarrow \text{DES}^{\bullet} + \text{H}^{+} \quad (2)$$

$$e_{aq}{}^{-} + \text{N}_2\text{O} \rightarrow \text{OH}^{-} + {}^{\bullet}\text{OH} + \text{N}_2 \quad (3)$$

In fact, phenolic compound can be ionized by 248 nm light especially in basic aqueous solution [10] and as a DES molecule has a conjugated double bond between two phenols, it can be deduced that DES can be easily ionized by 248 nm light even in neutral aqueous solution.

3.2. Contrast of kinetic patterns under different atmosphere

Take a further step to analyse the time-resolved absorption trace recorded at 310 nm by radical cation or deprotonated radical cation of DES, and 270 nm by a bleaching process of grand state of DES, as shown in Fig. 3, one finds that the radical cations and bleaching of grand state of DES occurred right after the laser pulse. The former has a growing process within a few microseconds after the starting point, followed with decay process within a few tens of microseconds. The growing process may assign to the deprotonation of DES radical cation as described by the following reactions.

$$\text{DES}^{\bullet +} \rightarrow \text{DES}^{\bullet} + \text{H}^{+} \quad (4)$$

$$\text{DES}^{\bullet} + \text{O}_2 \rightarrow \text{DESOO}^{\bullet} \quad (5)$$

Considering that the deprotonated radical cation of DES may react with O_2, the concentration of radical cation (including deprotonated radical cation) of DES created in O_2-saturated system should be lower than in systems without oxygen, and the growing process of deprotonated radical cation in O_2-saturated system is slower, too, as shown in Fig. 3.

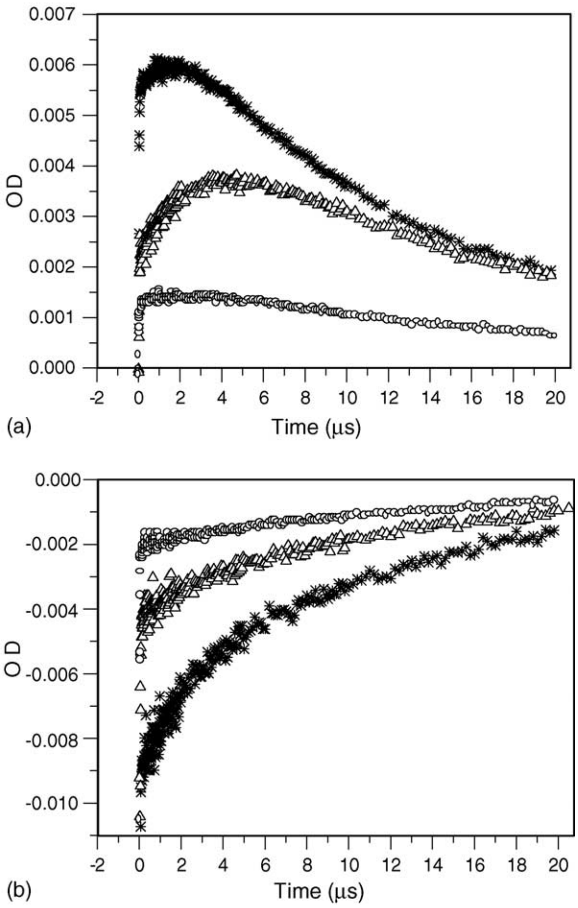

Fig. 3. The absorption-time profiles observed at (a) 310 nm and (b) 270 nm obtained from (i) N_2-saturated solution (*), (ii) O_2-saturated solution (△), (iii) N_2O-saturated solution (○) with 2×10^{-4}mol dm^{-3} DES aqueous solution buffered with phosphate at pH 7.

On the other hand, the total yield of bleaching grand state of DES must correspond with all transient products of DES produced from laser flash photolysis, in the O_2-saturated system the absorption value of bleaching is less than that determined in the deoxygen system. So it can be inferred that in addition to DES ionization upon 248 nm light, there might be another transient absorption from the excited states of DES, which could be quenched by oxygen. It means that the total yield of bleaching in the de-oxygen system should include both the ionized and excited DES. Accordingly, excited process should be involved in 248 nm laser flash photolysis of DES. However, no more characteristic transient signals were observed in the wavelength region. This can lead to the deduction that excited states of DES might have characteristic transient absorption in the same way as the radical cations. By determining the OD values of bleaching in both de-oxygen system and O_2-saturated system at zero time after laser pulse, we were able to calculate the ratio of ionization to excitation of DES in the 248 nm laser flash photolysis. It was 0.51.

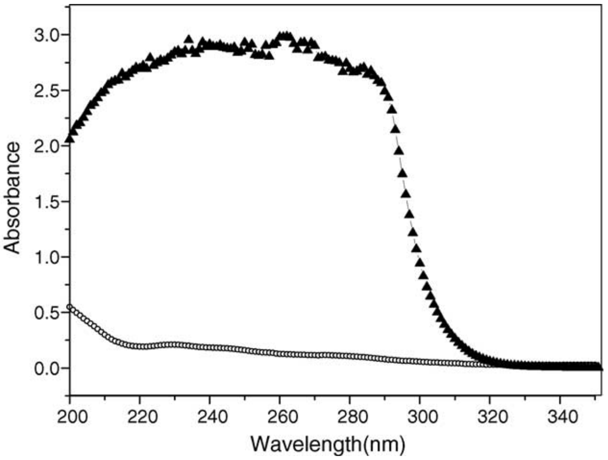

Fig. 4. Before (▲) and after (○) 2 h irradiation with Xe lamp UV beam, UV absorbance analysis of oxygen-saturated DES solution.

In the oxygen-saturated solution, the excited states of DES could transfer its excited energy to oxygen to produce singlet oxygen (reaction 6), which could in turn oxidize DES effectively and resulted in degradation of the molecule (reactions 6–8). This has been confirmed by a Xe lamp UV absorbance analysis, in which stable degradation products of DES were recorded under the UV lights, as shown in Fig. 4. The absorbance of 5×10^{-4} mol dm^{-3} DES with 70 nm-bandwidth was depressed drastically, and from this one can suppose that DES had been degraded with UV light in oxygen-saturated system. There was 10.03–12.19 mg dm^{-3} O_2 (pure oxygen bubbles, 15 °C room temperature) in the water solution. UV-B intensity was detected to be 370.3–402.9 μW cm^{-2} on the surface of quartz cell.

$$\mathrm{DES} + h\nu \rightarrow {}^{1}\mathrm{DES}^{*} \rightarrow {}^{3}\mathrm{DES}^{*} \qquad (6)$$

$$ {}^{3}\mathrm{DES}^{*} + \mathrm{O_2} \rightarrow \mathrm{DES} + {}^{1}\mathrm{O_2}^{*} \qquad (7)$$

$$\mathrm{DES} + {}^{1}\mathrm{O_2}^{*} \rightarrow \mathrm{degradationproducts} \qquad (8)$$

In the N_2O saturated system, however, hydrated electrons produced from DES ionization could be transferred into OH radicals, which would attack DES to produce longer life DES radicals. With saturation of N_2O the hydrated electron produced from ionization of DES was transferred into OH radical, which would attack DES to produce DES radical with a long lifetime.

3.3. Pulse radiolysis of DES in a flow system

In radiolysis of aqueous solution, primary radical species generated included hydroxyl radical $^{\bullet}$OH, hydrated electron e_{aq}^{-} and hydrogen atom H. (reaction 9), with G($^{\bullet}$OH) = G(e_{aq}^{-}) = 2.8 and G(H$^{\bullet}$) = 0.6. In the N_2O-saturated solution, hydrated electrons were rapidly converted into hydroxyl radical (reaction 10). T-BuOH, $(CH_3)_3COH$ is an effective scavenger of $^{\bullet}$OH (reaction 11).

$$\mathrm{H_2O(radiolysis)} \rightarrow \mathrm{H}^{\bullet}, {}^{\bullet}\mathrm{OH}, e_{aq}^{-}, \mathrm{etc} \qquad (9)$$

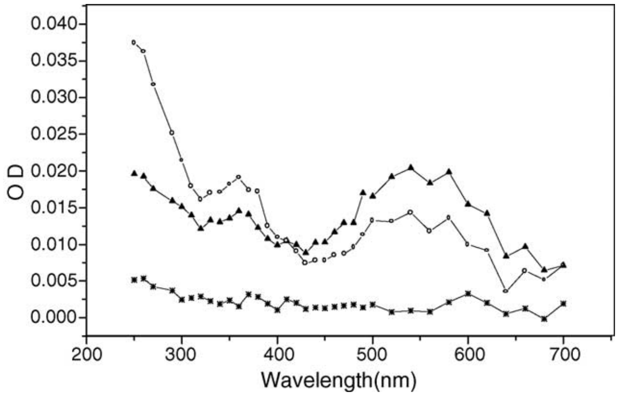

Fig. 5. Transient absorption spectra obtained from pulse radiolysis of N_2O-saturated aqueous solution containing 2×10^{-4} mol dm^{-3} DES and 2×10^{-3} mol dm^{-3} phosphate buffered at pH 7 recorded at 1 μs (■), 20 μs(▲) and 200 μs (○).

$$e_{aq}^{-} + \mathrm{N_2O(H_2O)} \rightarrow \mathrm{OH}^{-} + {}^{\bullet}\mathrm{OH} + \mathrm{N_2} \qquad (10)$$

$$ {}^{\bullet}\mathrm{OH} + \mathrm{(CH_3)_3COH} \rightarrow {}^{\bullet}\mathrm{CH_2(CH_3)_2COH} + \mathrm{H_2O} \qquad (11)$$

3.4. Transient absorption spectra of DES attacked by hydroxyl radical at pH 7

Hydroxyl radical causes damage of the phenolic compounds through electron transfer; hydrogen abstraction and $^{\bullet}$OH adduct [11]. In Fig. 5 are the transient absorption spectra with maximum absorption at about 250, 360 and 520 nm recorded, respectively, at 1, 20 and 200 μs after electron beam pulse with the N_2O-saturated aqueous solution containing 2×10^{-4} mol dm^{-3} DES at pH 7.

From the growing and decaying kinetics at 360 and 520 nm, one finds that the transient species with maximum absorption at 520 nm might be assigned to the phenoxyl radical via reaction (12) and the species with maximum absorption at 360 nm may be assigned to the dimmer of rearranged phenoxyl radical of DES via reaction (13), as shown in Fig. 6.

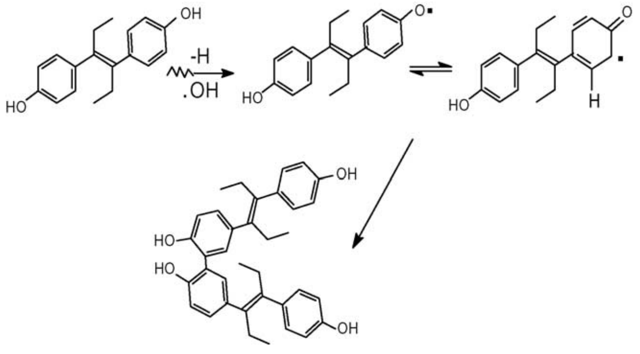

Fig. 6. Supposed deproton reactions of DES.

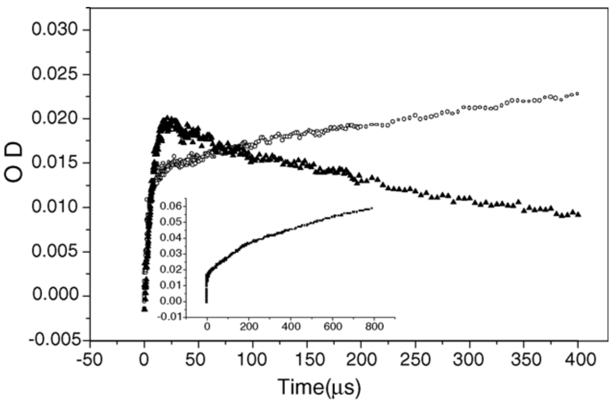

Fig. 7. Transient absorption decay traces observed at 520 nm (▲) and growth traces observed at 360 nm (○) Inset: T–T absorption curve of DES observed at 260 nm (■).

$$\mathrm{DES} + {}^{\bullet}\mathrm{OH} \rightarrow \mathrm{DES}^{\bullet} + \mathrm{H_2O} \tag{12}$$

$$2\mathrm{DES}^{\bullet} \rightarrow \mathrm{DES{-}DESdimmer} \tag{13}$$

As shown in the Fig. 7, the formation of dimmer observed at 360 nm is very well synchronous with the decay of phenoxyl radical observed at 520 nm.With addition of T-BuOH as ${}^{\bullet}$OH scavenger, the transient absorption with maximum absorption at 360 and 520 nm disappeared, which can be further used to confirm reaction mechanisms of ${}^{\bullet}$OH radicals with DES.

3.5. *Effects of pH on products of DES attacked by ${}^{\bullet}$OH radical*

PH value of the solution can drastically affect the transient absorption spectra of DES attacked by ${}^{\bullet}$OH, as shown in Fig. 8a. A broad and strong absorption band recorded could be seen in the spectra at pH 11 of the DES solution, whereas with the solutions at pH 3 and pH 7 the absorptions were far less. In basic solution of pH 11, both OH residuals of DES molecule were disassociated to phenoxyl anion, therefore the ${}^{\bullet}$OH radicals, being not to abstract hydrogen from OH residuals of DES, could attack benzene ring or α-C of ethyl group of DES. This is supposed to have occurred before degradation and mineralization of DES especially under oxygen-saturated condition.

Inspecting the dynamic characters observed at 520 nm with pH 11 shown in Fig. 8b the growing and decaying process of the transient species is very different from that of dimmer recorded at pH 7. The species is produced at once after beam pulse and then decay with longer time than that of phenoxyl radical as shown in Fig. 7.

Comparing the kinetics determined at 360 and 520 nm at pH 11 in Fig. 8c, it can be seen that the transient species with maximum absorption at 360 and 520 nm were not the same. And the species with maximum absorption at 520 nm might be assigned to ${}^{\bullet}$OH adduct of DES at the benzene ring, which could involve another growing process, whereas the species with maximum absorption at 360 nm might be assigned to ${}^{\bullet}$OH adduct of DES at α-C of ethyl group. Further studies, however, will be conducted to identify the two transient species and TEQ value of their stable products.

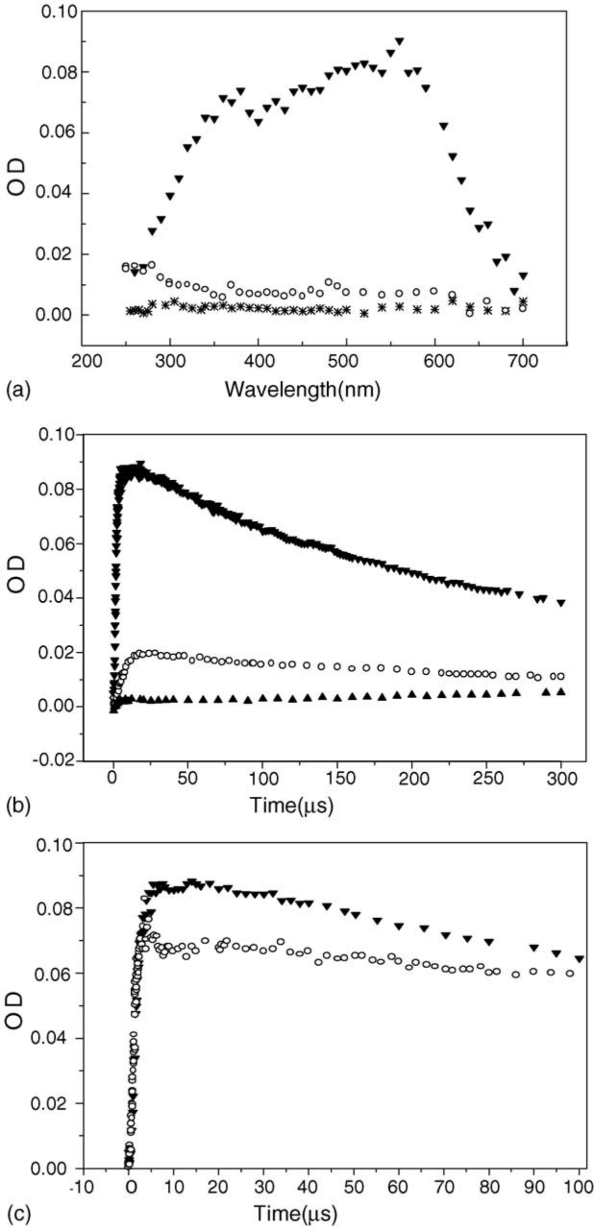

Fig. 8. (a) Transient absorption spectra obtained at 4 μs after the beam pulse from DES solutions of pH 11 (▼), pH 7 (○) and pH 3 (▲), (b) time-resolved absorption curves recorded at 520 nm from DES solutions of pH 11 (▼), pH 7 (○) and pH 3 (▲) and (c) time-resolved absorption curves recorded at 360 (○) and 520 nm(▼) from the pH 11 DES solution.

4. Conclusion

As a synthetic non-steroid estrogen drug, DES has been studied by laser flash photolysis and pulse radiolysis. It has been found that DES can be photoionized by 248 nm light to produce radical cations, which may be deprotonated to transfer neutral radicals and DESOO$^\bullet$ when the system is saturated by oxygen. The 248 nm light excited DES can produce excited states, too, which may transfer the excited energy to oxygen and singlet oxygen can be created. All these cause degradation of DES, as confirmed by analysis of stable DES products after UV light irradiation. In addition, it has been found that DES is very sensitive to $^\bullet$OH radical especially for α-C of ethyl group. $^\bullet$OH can abstract hydrogen from the hydroxyl residue of DES below pH 9.5 to produce phenoxyl radicals, which may be combined each other to produce dimmer. In basic solution of DES, however, the molecule can be dissociated and transfer into phenoxyl anions, and the DES molecules can go degradation by attacking $^\bullet$OH radicals on the benzene ring or α-C of ethyl group of DES.

The natural environments provide endless source of the UV light and $^\bullet$OH oxidants, therefore, DES molecules are continuously excited and transferred into different radicals, and active oxygen is produced along with the process. All these can result in serious biologic effects. However, if DES is in an acid or neutral aqueous solution, its degradation risk to human heath may be alleviated as in this case the degradation products are mostly safer and more stable dimmers.

Acknowledgements

One of the authors, Da-Ying Dou would thank Prof. Wen-Feng Wang and Prof. Wen-Long Zhang for their help in radiolysis experiments.

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