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Photosensitization by fluorescein dyes and tetracycline drugs at an oil/water interface

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Measurements were made of the changes that occur in the interfacial tension of isopropylbenzene when floating on water containing between 10^{-5} and 10^{-3} M of various fluorescein dyes and tetracycline drugs and irradiated for up to 240 min with ultraviolet light. Singlet oxygen was found to be an active intermediate in the reactions. The results are discussed in relation to adverse photosensitization in-vivo due to fluorescein dyes and tetracycline drugs.

Tetracyclines (I) are broad spectrum bacteriostatic agents and are extensively used in the treatment of urinary tract infections, cholera, gonorrhoea, etc. They have certain structural similarities to the fluorescein dyes (II) which are used in foods and cosmetics. Both have polycyclic configurations with resonating structures which absorb ultraviolet and visible light and both act as photosensitizing agents (Stempel & Stempel 1973). Tetracyclines can cause phototoxic effects in patients when they are subsequently exposed to sunlight. Clinical symptoms associated with their use include oedema, papules, erythema, photo-oncholysis, and demethylchlortetracycline is reported to be more photosensitive than other tetracyclines (Sams 1960). Dyes which have been incorporated in cosmetics may cause adverse effects when these are applied to the skin (Willis 1975). In the presence of light and oxygen dyes can also cause damage to micro-organisms and cells (Spikes & Straight 1967). Various mechanisms have been proposed to explain the action of photosensitizing agents (Stempel & Stempel 1973; Storck 1965) but due to technical difficulties in dealing with living organisms, the processes involved in photosensitization in-vivo are not well understood.

An in-vitro technique is now available for studying the photosensitizing action of drugs and dyes on isopropylbenzene and other organic liquids by measuring the changes produced in the interfacial tensions of their solutions when subjected to ultraviolet irradiation (Sanniez & Pilpel 1980). This technique has been used in the present work. Samples of isopropylbenzene were floated on water containing fluorescein dyes or tetracycline drugs in various amounts and were irradiated for 240 min. Measurements were made of the changes that occurred in the interfacial tensions both in the absence and presence of certain oxygen quenchers namely triethylene diamine (dabco) (Quannes & Wilson 1968), sodium azide (Hasty et al 1972) and L-histidine (Nilsson et al 1972). The objective was to see whether the quencher had any effect on the reactions and thus whether a singlet oxygen mechanism might be involved.

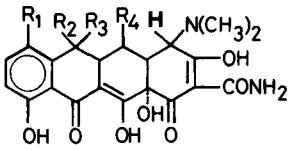
From the results obtained it should be possible to establish the overall kinetics of the reaction and throw some light on the mechanisms by which these compounds produced photosensitization in-vivo.

* Correspondence.

MATERIALS AND METHODS

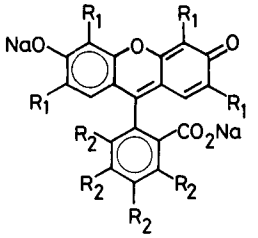
Isopropylbenzene (99% Aldrich) was further purified by passing it five times under slight pressure through a tightly packed bed of Fuller's earth 2·5 cm

(I)



	R_1	R_2	R_3	R_4
Oxytetracycline	H	Me	OH	OH
Dimethylchlortetracycline	Cl	H	OH	H

(II)



	R_1	R_2
Eosine y	Br	H
Erythrosine	I	H
Rose bengal	I	Cl

deep and was recovered after each passage (d^{24}_0 0·864 gm cm^{-3}; surface tension 30·0 mNm^{-1} at 20 °C and interfacial tension 37·8 mNm^{-1} at 20 °C).

The best available grades of rose bengal, erythrosine and eosine Y (Aldrich), oxytetracycline hydrochloride (Pfizer) and demethylchlortetracycline hydrochloride (Lederle) were checked for their purity by ultraviolet spectroscopy and were used without further purification. Dabco (Triethylene diamine), sodium azide and L-histidine were of analytical grade.

Triple distilled water from an all-glass still was used throughout (surface tension 72·5 mNm^{-1} at 20 °C, specific conductivity 1·3 × 10^{-6} ohm^{-1} cm^{-1} at 20 °C and pH 5·4).

Apparatus and procedure

The ultraviolet source was a 125 W Philips MB/U medium pressure arc lamp with a silica filter transmitting at wavelengths between 290 and 360 nm. The light intensity reaching the sample was 1·5 × 10^{-9} einstein s^{-1} cm^{-2} as measured by potassium ferrioxalate actinometry (Hatchard & Parker 1956) and atmospheric oxygen was freely available at the sample surface.

15 cm^3 of isopropylbenzene was floated on 20 cm^3 of an aqueous solution of the drug or dye with or without the addition of a quencher in a 5 cm diameter glass pot forming a layer 0·5 cm deep. Each sample was irradiated for a known length of time while keeping its temperature constant at 25 ± 2 °C and after allowing it to equilibrate for 10 min in darkness, its interfaction tension (IFT) was measured with a du Nouy tensiometer (White Electrical Co. Ltd) using a platinum ring (circumference 4 cm) and employing the equation due to Zuidema & Waters (1941).

$$\gamma = P\left[0{\cdot}725 + \left(\frac{0{\cdot}0145P}{C^2(D-d)}\right)^{1/2}\right] \qquad (1)$$

γ is the interfacial tension, P is the tensiometer reading, C is the circumference of the platinum ring, D and d are the densities of water and the isopropylbenzene at 20 °C respectively. The accuracy of the measurements was estimated at ± 1% and it was confirmed by calibrating the instrument against samples of pure benzene (IFT = 35·0 m Nm^{-1} at 20 °C) and carbon tetrachloride (IFT = 45·0 m Nm^{-1} at 20 °C).

At the end of 240 min of irradiation, the hydroperoxides formed in the oil phase were determined by iodometric titration (Lea 1946) and the percentages of dye and drug decomposed were determined by ultraviolet spectroscopy.

RESULTS

The fluorescein dyes (II) and the tetracycline drugs (I) were more soluble in water than in isopropylbenzene and thus partitioned mainly in the aqueous phase. They also adsorbed at the isopropylbenzene/water interface causing decreases in IFT with increases in concentration. Typical graphs showing this effect are plotted in Fig. 1. The shapes of the

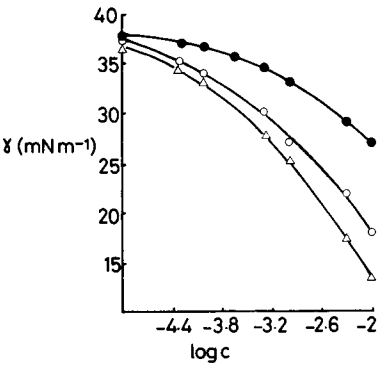


FIG. 1. Interfacial tension versus log c for fluorescein dyes and a tetracycline in isopropylbenzene/water system. Key: ○ Eosine; △ Rose bengal; ● Demethylchlortetracycline.

curves indicated that both the dyes and the drugs were being absorbed according to the Gibb's equation.

$$-d\gamma/d\log c = 2{\cdot}303\,\Gamma RT \qquad (2)$$

where c is the concentraction, Γ is the surface excess, R is the gas constant, γ is the interfacial tension, and T is the absolute temperature. The surface excesses (μ mol m^{-2}) at different concentrations were converted into areas, A, per drug or dye molecule at the interface using the expression

$$A = \frac{1.667}{\Gamma}\ nm^2\ molecule^{-1} \qquad (3)$$

The corresponding surface pressure, π mNm^{-1} was the difference between the interfacial tension of pure isopropylbenzene and that of isopropylbenzene containing the dye or drug. Typical force-area curves are shown in Fig. 2. On this basis all the dyes and drugs were being similarly adsorbed at the isopropylbenzene/water interface to form gaseous films (Pilpel 1956).

The effects of irradiation on the IFT values of representative samples are shown in Figs 3–5. It was observed that in the 240 min of irradiation, the IFT of isopropylbenzene decreased slightly when floating on pure water but more markedly when increasing

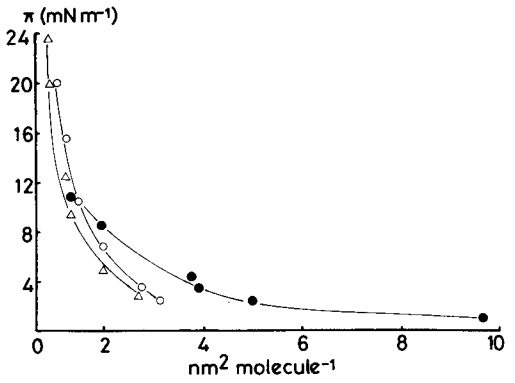


FIG. 2. Force-area curve for fluorescein dyes and demethylchlortetracycline in isopropylbenzene. Key: ○ Eosine; △ Rose bengal; ● Demethylchlortetracycline.

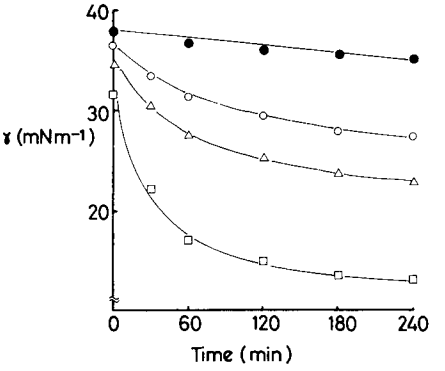


FIG. 5. Plot of interfacial tension versus irradiation time for isopropylbenzene with demethylchlortetracycline. Key: ● zero; ○ $1{\cdot}0 \times 10^{-4}$; △ $5{\cdot}0 \times 10^{-4}$; □ $1{\cdot}0 \times 10^{-3}$ M.

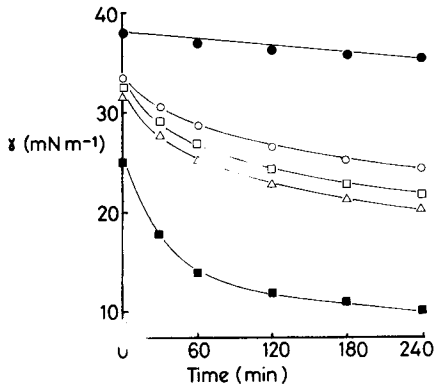


FIG. 3. Plot of interfacial tension versus irradiation time for isopropylbenzene with eosine. Key: ● zero; ○ $1{\cdot}0 \times 10^{-4}$; □ $1{\cdot}5 \times 10^{-4}$; △ $2{\cdot}0 \times 10^{-4}$; ■ $1{\cdot}0 \times 10^{-3}$ M.

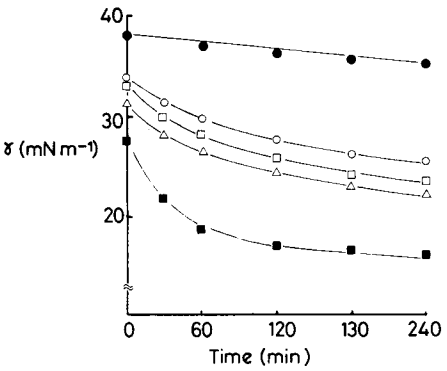


FIG. 4. Plot of interfacial tension versus irradiation time for isopropylbenzene with rose bengal. Key: ● zero; ○ $1{\cdot}0 \times 10^{-4}$; □ $1{\cdot}5 \times 10^{-4}$; △ $2{\cdot}0 \times 10^{-4}$; ■ $1{\cdot}0 \times 10^{-3}$ M.

amounts of the dyes and tetracyclines (10^{-5}–10^{-3} M) were added to the substrate. When the singlet oxygen quenchers Dabco, sodium azide or L-histidine (10^{-2} M) were also included in the substrate there was a smaller decrease in IFT with time. The effects at any particular concentration of the sensitizing dye or drug could be quantitatively expressed in terms of a Photosensitive Index (PI) defined by (cf. with Felmeister & Schaubman 1969)

$$\mathrm{PI} = \frac{(\gamma_1-\gamma_{240})-(\gamma_o-\gamma_f)}{(\gamma_1-\gamma_{240})} \times 100 \qquad (4)$$

where γ_1 = IFT of isopropylbenzene plus dye or drug before irradiation, γ_{240} = IFT of isopropylbenzene plus dye or drug after 240 min irradiation, γ_o = IFT of isopropylbenzene before irradiation, γ_f = IFT of isopropylbenzene after 240 min irradiation.

The PI is a measure of the amounts of surface active products (hydroperoxide, alcohols, etc.) produced by photolysis at the isopropylbenzene/water interface. The PI values both in the absence and the presence of singlet oxygen quenchers are given in Table 1.

After 240 min of ultraviolet irradiation, the oil phases of the samples were found to contain hydroperoxides which were measured by iodometric titration (Lea 1946). Results for the systems containing 10^{-4} M of the dyes and drugs are given in Table 2. Examination of the aqueous phases of the systems containing 10^{-4} M of eosine, erythrosine, oxytetracycline and demethylchlortetracycline showed that their pH values remained constant at 6·0, 5·9, 4·1, 4·0 respectively. The aqueous phase for the 10^{-4} M rose bengal system showed a slight decrease in pH from 6·0 to 5·8. In the same period of 240 min, ultraviolet spectroscopy showed that about 10, 15

Table 1. Effect of singlet oxygen quenchers on PI values.

Sensitizer	Quencher	Quantity (M × 10^2)	PI value
Eosine	None	—	71·0
	Sodium azide	2	15·7
	Dabco	4	20·2
	L-Histidine	1	43·2
Erythrosine	None	—	72·9
	Sodium azide	2	27·0
	Dabco	4	25·0
	L-Histidine	1	47·0
Rose bengal	None	—	73·7
	Sodium azide	2	31·0
	Dabco	4	29·0
	L-Histidine	1	49·0
Demethylchlortetracycline	None	—	76·0
	Sodium azide	2	30·0
	L-Histidine	1	40·0
Oxytetracycline	None	—	59·5
	Sodium azide	2	4·2
	L-Histidine	1	10·5

and 50% of eosine, erythrosine and rose bengal respectively and about 15% of each tetracycline had decomposed, the percentage being smaller at higher initial concentrations. The dyes and the drugs were therefore acting as photosensitizers and not as photocatalysts.

DISCUSSION

Chien (1965) showed that on exposure to ultraviolet light, isopropylbenzene was slowly oxidized to the hydroperoxide which could then decompose further to form acetophenone, methyl and hydroxyl radicals (Emanuel 1965).

$$2\,PhCH(CH_3)_2 \xrightarrow{h\nu} \underset{2\,O_2}{PhC(CH_3)_2\,OOH} + \underset{}{PhC(CH_3)_2 + HO_2}$$

(hydroperoxide)

$$\downarrow$$

$$PhCOCH_3 + CH_3 + HO$$

(acetophenone) (5)

where Ph = phenyl.

The hydroperoxide and ketone are hydrophilic which would account for the decreases observed in the interfacial tensions of the present solutions on irradiation. It is apparent that although isopropylbenzene absorbs within the ultraviolet range 290–360 nm (uv transmission cut off λ max 286 nm), it is relatively stable over a period of 240 min and the changes produced in the interfacial tension are relatively small (Fig. 3).

Table 2. Amount of hydroperoxide in oil phase after 240 min of irradiation.

Sensitizer (10^{-4} M)	Hydroperoxide M × 10^3
None	0·014
Eosine	2·2
Erythrosine	1·6
Rose bengal	1·3
Demethylchlortetracycline	2·4

The dyes and drugs sensitized the photooxidation of isopropylbenzene as measured by PI values (Table 1). It seems likely that sensitization might involve a singlet oxygen mechanism and this has been confirmed by the observation that when singlet oxygen quenchers Dabco, N_3-ions and L-histidine were added to the systems, the PI values decreased indicating that photo oxidation of isopropylbenzene had been retarded by their addition. The singlet oxygen, $^1O_2$ is formed by an energy transfer process due to interaction between the photo-excited dye and ground state molecular oxygen, $^3O_2$ (Gollnick et al 1970).

The fluorescein dyes and the tetracycline drugs probably sensitize the photo-oxidation of isopropylbenzene by the following scheme (Mizuno et al 1981; Wiebe & Moore 1977):

D	$\xrightarrow{h\nu}$	$^1D^*$ absorption
$^1D^*$	$\longrightarrow$	$^3D^*$ intersystem crossing
$^3D^* + {}^3O_2$	$\longrightarrow$	$D + {}^1O_2$ formation of singlet oxgen
$^1O_2$	$\longrightarrow$	$^3O_2$ deactivation
$PhCH(CH_3)_2 + {}^1O_2$ (isopropylbenzene)	$\longrightarrow$	$PhC(CH_3)_2OOH$ formation of hydroperoxide (6)

where D = Dye or drug

Kinetics

The data shown in Figs 3–5 were next analysed to determine the overall kinetic order of the photochemical reactions that had occurred. It was found than when $-\log [d\gamma/dt]$ for the first 60 min of irradiation was plotted against log c where c is the concentration of dye or drug, straight lines were obtained. Typical plots are shown in Fig. 6 from which it follows that

$$\log [d\gamma/dt] = \log K + n \log c$$

or

$$d\gamma/dt = Kc^n \quad (7)$$

where K is a constant for each graph and n is their slope.

At low concentrations, the Gibb's equation is

$$d\gamma = -2{\cdot}303\,\Gamma RT\, d \log c \quad (8)$$

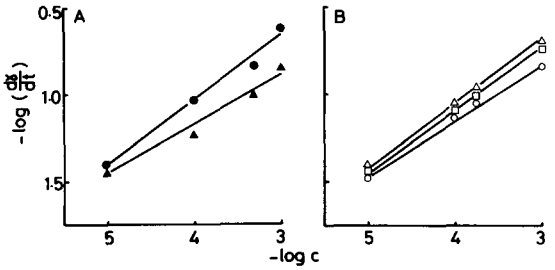


FIG. 6. Plot of −log [dγ/dt] versus log dye or drug concentration in isopropylbenzene after 60 min of irradiation. Key: A: ● Demethylchlortetracycline; ▲ Oxytetracycline. B: ○ Eosine; △ Rose bengal; □ Erythrosine.

Combining equations 7 and 8

$$d \log c/dt = -K c^n/2{\cdot}303\ RT \qquad (9)$$

It is observed that equation 9 is very similar to the general differential kinetic equation (Laidler 1965)

$$dc/dt = -K_A c^{nc} \qquad (10)$$

where c is the reactant concentration, K_A is the reaction rate constant and n_c is the order of reaction with respect to the concentration. Therefore, n in equation 9 can be interpreted as an overall kinetic order with respect to concentration (c) of the photochemical reactions producing surface active products at the oil/water interface. The n values were found to be about 0·3 for all the dyes and the drugs (Table 3).

Table 3. Values of n.

Sensitizer	n
Eosine	0·31
Erythrosine	0·32
Rose bengal	0·36
Oxytetracycline	0·28
Demethylchlortetracycline	0·37

The integration method (Laidler 1965) can be used for determining the kinetic order, n_t with respect to time. This was done by solving equation 11 by a process of trial and error, substituting in values of a and x for the first 60 min of irradiation and different values of n_t until K becomes constant.

$$K = \frac{1}{t(n_t - 1)}\left[\frac{1}{(a - x)^{nt-1}} - \frac{1}{a^{nt-1}}\right] \qquad (11)$$

a is the drop in IFT for 240 min of irradiation, x is the drop in IFT after irradiation time t, and K is the rate constant. The required value of n_t was found to be 0·75 for all the dyes and drugs (Tables 4 and 5). In treating the experimental results in this way it should be appreciated that several simultaneous reactions could be taking place and it would be extremely difficult to analyse each reaction separately. The fact that n_t was found to be greater than n_c indicates that some inhibition was occurring due to the products of the photochemical reactions (Laidler 1965). These, being surface active, would tend to inhibit further

Table 4. Rate constants for photolysis of dyes at isopropylbenzene/water interface with n_t = 0·75.

Sensitizer	Concn (M)	a (mNm^{-1})	t (min)	x (mNm^{-1})	(a − x) (mNm^{-1})	K (mNm^{-1})$^{0·25}$ min^{-1}
Eosine	1·0 × 10^{-5}	3·49	30	1·19	2·30	0·017
			60	2·00	1·49	0·017
Erythrosine	,,	3·62	30	1·20	2·42	0·018
			60	2·07	1·55	0·018
Rose bengal	,,	3·68	30	1·23	2·45	0·018
			60	2·12	1·56	0·018
Eosine	1·0 × 10^{-4}	8·00	30	2·30	5·70	0·018
			60	4·00	4·00	0·018
Erythrosine	,,	8·50	30	2·50	6·00	0·019
			60	5·00	4·00	0·019
Rose bengal	,,	8·75	30	2·65	6·10	0·020
			60	4·60	4·15	0·020
Eosine	2·0 × 10^{-4}	10·20	30	3·20	6·80	0·022
			60	5·50	4·70	0·021
Erythrosine	,,	11·00	30	3·60	7·40	0·023
			60	6·20	4·80	0·023
Rose bengal	,,	11·25	30	3·80	7·40	0·024
			60	6·30	4·90	0·023
Eosine	1·0 × 10^{-3}	11·46	30	5·46	6·00	0·037
			60	8·63	2·85	0·036
Erythrosine	,,	13·90	30	6·60	7·30	0·038
			60	10·40	3·50	0·037
Rose bengal	,,	15·10	30	7·50	7·50	0·041
			60	12·00	3·00	0·043

Table 5. Rate constants for photolysis of drugs at isopropylbenzene/water interface with $n_t = 0{\cdot}75$.

Sensitizer	Concn (M)	a (mNm^{-1})	t (min)	x (mNm^{-1})	(a − x) (mNm^{-1})	K $(mNm^{-1})^{0{\cdot}25}\ min^{-1}$
OTC	$1{\cdot}0 \times 10^{-5}$	3·37	30	1·10	2·27	0·016
			60	1·97	1·40	0·017
DMCTC	,,	4·00	30	1·40	2·60	0·018
			60	2·40	1·60	0·018
OTC	$1{\cdot}0 \times 10^{-4}$	5·70	30	2·00	3·70	0·021
			60	3·40	2·30	0·020
DMCTC	,,	9·10	30	3·10	6·00	0·023
			60	5·00	4·10	0·021
OTC	$5{\cdot}0 \times 10^{-4}$	9·40	30	3·40	6·00	0·024
			60	5·40	6·00	0·022
DMCTC	,,	11·50	30	4·30	7·20	0·027
			60	7·50	4·00	0·028
OTC	$1{\cdot}0 \times 10^{-3}$	13·00	30	6·10	6·90	0·036
			60	9·40	3·60	0·034
DMCTC	,,	18·37	30	9·37	9·00	0·045
			60	14·42	3·95	0·044

OTC = Oxytetracycline. DMCTC = Demethylchlortetracycline.

migration of the dye or drug to the interface and thus continuously reduce the rate of oxidation of the isopropylbenzene.

Relevance to photosensitization in-vivo

It is difficult to investigate photosensitization reactions in-vivo because of analytical and experimental problems. But if it is assumed that a simple oil/water interface is a model for a lipid/water interface in a cell membrane then changes occurring in the in-vitro system might help to explain how photosensitization reactions occur in-vivo.

The present work has shown that the fluorescein dyes and tetracyclines under investigation adsorb at an isopropylbenzene/water interface (Figs 1, 2) and photochemical reactions occur by a mechanism involving singlet oxygen (Table 1). It seems reasonable to suggest that singlet oxygen may be involved in in-vivo photosensitization by these dyes and drugs leading to oxidative damage to cellular materials. Foote (1976) showed that singlet oxygen is an active intermediate in the photosensitized oxidation by these dyes of amino acids, proteins, lipids and other cellular constituents. Singlet oxygen is also involved in actual biological systems; for example in the photodynamic inactivation of *Escherichia coli* by rose bengal (Belzman et al 1978) and in patients suffering from erythropoietic protoporphyria (Goldstein & Harber 1972; Lamola et al 1973). Patients with this disorder are subject to swelling, erythema, and lesions on exposure to light. Their red blood cells contain large amounts of free protoporphyrin which acts as an active sensitizer for the production of singlet oxygen leading to oxidative damage and hemolysis of cells.

All the dyes and drugs now investigated produced species which were surface active at the oil/water interface and this activity can be related to adverse side effects by means of Photosensitive Indices (Felmeister & Schaubman 1969). It is to be expected that surface active photoproducts will be active at the cell wall and may produce changes in the permeability of cell membranes. In fact Allison et al (1966) have shown that photosensitization leads to increased permeability of lysosomes and cell membrane towards liquids and inorganic ions and there may possibly be some connection between these changes and the accumulation of fluid (oedema) that occurs in certain cases.

Conclusions

(i) Fluorescein dyes and tetracycline drugs form gaseous films at the isopropylbenzene/water interface.

(ii) They act as photosensitizers for the oxidation of isopropylbenzene by producing singlet oxygen which acts as a reactive intermediate.

(iii) The kinetic orders of the photochemical reactions are about 0·3 and 0·7 with respect to concentration and time respectively.

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