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Effects of ultraviolet light on lecithin monolayers in the presence of fluorescein dyes and tetracycline drugs

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A Langmuir trough was employed to measure the effects of u.v. irradiation on the force area characteristics of monolayer films of lecithin spread on aqueous solutions of some dyes and tetracycline drugs both in the absence and presence of Ca^{2+} and Ni^{2+}. Irradiation caused photochemical reactions which resulted in the expansion of the films and the extent of reaction was measured by the Photosensitive Indices. The reaction with tetracyclines was inhibited by adding Cu^{2+} or Ni^{2+} to the substrate. The kinetic orders of the reactions are given.

The fluorescein dyes, which are used in foods and cosmetics, and the tetracycline drugs, which are broad spectrum antibiotics, have certain similarities. Both have polycyclic configurations with resonating structures which absorb ultraviolet (u.v.) and visible light and both act as photosensitizing agents (Stempel & Stempel 1973). Tetracyclines sometimes cause phototoxic effects in patients subsequently exposed to sunlight. The dyes, when used in cosmetics on occasions may cause adverse effects on the skin (Willis 1975).

There are few literature reports on the irradiation of monomolecular films of compounds that can be used as models for biological membranes. A convenient in-vitro method for studying the reaction between dye/drug and a body tissue under the influence of u.v. light is to spread a model membrane, e.g. lecithin as a monolayer on the surface of an aqueous solution of dye/drug, irradiate the system and measure the resulting expansion of the film (Nejmeh & Pilpel 1978). The results are expressed in terms of the Photosensitive Indices of the dyes or drugs employed (Felmeister & Schaubman 1969). In the present work, measurements have been made of the changes that occurred in the force area π-A curves of lecithin spread on various aqueous substrates of the fluorescein dyes and the tetracycline drugs, both in the absence and presence of copper and nickel ions when irradiated for up to 240 min. The effects of the ions on the Photosensitive Indices were measured and the results were analysed to determine the overall kinetic orders of the photochemical reactions between lecithin and the various substrates.

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MATERIALS

Lecithin (L-α-dipalmitoyl phosphatidyl choline) was 99% pure from Sigma Laboratories. The best available grades of rose bengal, erythrosine and eosine Y (Aldrich), oxytetracycline hydrochloride (Pfizer) and demethylchlortetracycline hydrochloride (Lederle) were used without further purification. Spectrograde ethanol and n-hexane 99% were from BDH. Copper sulphate and nickel sulphate were A.R. grades. Triple distilled water (surface tension 72·5 mNm^{-1} at 20 °C, specific conductivity $1{\cdot}3 \times 10^{-6}$ ohm^{-1} cm^{-1} at 20 °C and pH 5·3) was obtained from an all-glass still.

Apparatus and procedure

The u.v. source was a Philips mercury lamp (HPK 125 W) which was mounted horizontally in a reflector, 60 cm above the centre of a thermostatted Langmuir trough with a silica filter transmitting at wavelengths between 290 and 360 nm. The intensity of the light was uniform over the surface of the liquid in the trough, $0{\cdot}8 \times 10^{-9}$ einstein s^{-1} cm^{-2} as measured by potassium ferrioxalate actinometry (Hatchard & Parker 1956).

After all the usual precautions (Ries et al 1975) including temperature control (Gaines 1966), had been taken, the Langmuir trough was lightly waxed, then filled with triple distilled water containing known concentrations of dye or drug (10^{-5}–10^{-4} M). 0·1 ml of a 0·5 mg ml^{-1} solution of lecithin in 90:10 (v/v) hexane–ethanol was spread on its surface from an Agla syringe (Wellcome) and allowed to equilibrate for 5 min. Force area (π–A) curves were then measured at 25 ± 1 °C using a compression rate of 4×10^{-2} nm^2 $molecule^{-1}$ min^{-1} until the film collap-

sed. Experiments were performed to investigate the effects on the π–A curves of the lecithin of:

(a) concentration of dye or drug in the substrate;
(b) length of time up to 240 min for which the film had been irradiated in the uncompressed state;
(c) addition to the substrate of known concentrations of Cu^{2+} or Ni^{2+}.

RESULTS AND DISCUSSION

It was found that adding up to about 5×10^{-4} M of dye or drug to the substrate caused expansion of the lecithin monolayer. This is illustrated typically in Fig. 1 and in Figs 2 and 3. This expansion indicates that the dye or drug had penetrated the known micropore structure of the lecithin monolayer (Ries et al 1975) and formed a mixed film.

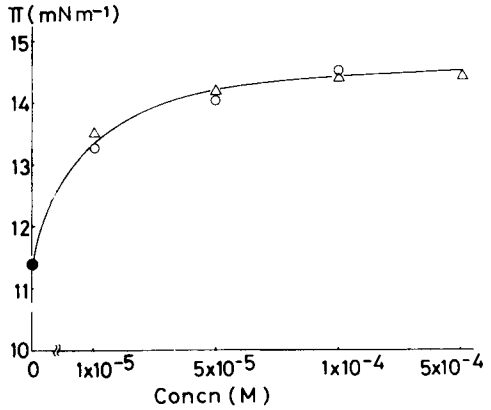

FIG. 1. Plot of π (at 0·56 nm^2) of lecithin monolayer versus concentration of dye or drug in the substrate. Key: ● lecithin; ○ rose bengal and △ demethylchlortetracycline.

The standard free energy of spreading $\Delta G°$ of the lecithin at different pressures π on the different substrates can be calculated from the expression (Harkins 1952)

$$\Delta G° = -\pi\delta A$$

where δA is the change in area per molecule caused by adding the dye or drug. Assuming arbitrary surface areas per molecule of 0·56 nm^2 and 0·77 nm^2, it was found that $\Delta G°$ decreased from about $-3{\cdot}8$ KJ mol^{-1} to $-4{\cdot}6$ KJ mol^{-1} and from $-2{\cdot}5$ KJ mol^{-1} to $-2{\cdot}9$ KJ mol^{-1} respectively, i.e. by between about 8 and 14% when 10^{-5} M of each dye or drug was present in the water, indicating that these additives facilitated the spreading.

The force-area curve of lecithin on pure water was unaffected by u.v. irradiation (see left hand curves on Figs 2 and 3), but, when a dye or drug was present in the substrate, irradiation caused the film to

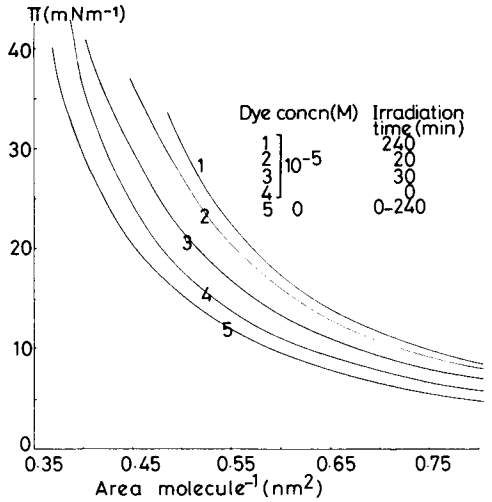

FIG. 2. Effect of irradiation on force area curve of lecithin on aqueous solutions of rose bengal.

expand and it was displaced to the right (Figs 2 and 3). There was no measurable change in the pH of the system which might have otherwise affected the shape of the π–A curve of lecithin (Nejmeh & Pilpel 1978).

In order to compare the expansion produced by irradiation of lecithin monolayers on different substrates, it is convenient to define a Photosensitive Index (cf. Felmeister & Schaubman 1969)

$$PI = \frac{\pi_1 - \pi_{60}}{\pi_0 - \pi_{60}} \times 100$$

π_0 is the surface pressure of the monolayer at an arbitrarily chosen area/molecule of 0·56 nm^2 on water before irradiation. π_1 is its surface pressure at the same area on the particular substrate before irradiation. π_{60} is its surface pressure at the same area on the particular substrate after 60 min irradiation.

Values of PI for lecithin on substrates containing dyes or drugs plus in some cases added metal ions are listed in Table 1. The values for the dyes were between 58 and 81 and for the drugs between 53 and 71. The latter (only) decreased in the presence of divalent metal ions. This is probably due to complexation in which divalent metal ions bind to the tetracycline drugs through oxygen atoms (Baker & Brown 1966).

Kinetics

The various π – A curves were next analysed to determine the overall kinetics of the photochemical reactions that had occurred as a result of irradiation.

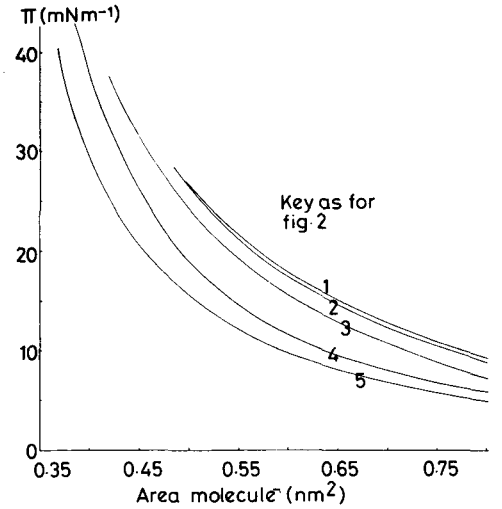

FIG. 3. Effect of irradiation on force area curve of lecithin on aqueous solutions of demethylchlortetracycline.

This was done at an arbitrary surface pressure of 13·4 mNm^{-1} by applying the differential method for the order of reaction (Nejmeh & Pilpel 1978)

$$\frac{dc}{dt} = -K_A c^{nc}$$

where c is the dye or drug concentration; K_A is the reaction rate constant; t is the irradiation time; and n_c the order with respect to concentration or true order. Assuming that the change in area of the monolayer is proportional to the change in concentration of the dye or drug then the order of reaction n_c is given by the slope of the plot of $-\log dA/dt$ vs $-\log c$ (see Fig. 5), dA/dt being the average rate of expansion of the film during the first 60 min of irradiation (see Fig. 4). The values of n_c were found to be 0·5 and 0·7 for rose bengal and demethylchlortetracycline respectively (Fig. 5).

The kinetic orders of reaction, n_t with respect to time were obtained by the integration method

Table 1. PI values on different substrates.

Dye or drug (10^{-5} M)	Metal ion (10^{-5} M)	PI
Eosine	—	58
Eosine	Ni^{2+}	58
Erythrosine	—	81
Rose bengal	—	80
Rose bengal	Cu^{2+}	80
Demethylchlortetracycline	—	71
Demethylchlortetracycline	Cu^{2+}	20
Demethylchlortetracycline	Ni^{2+}	30
Oxytetracycline	—	53
Oxytetracycline	Cu^{2+}	20

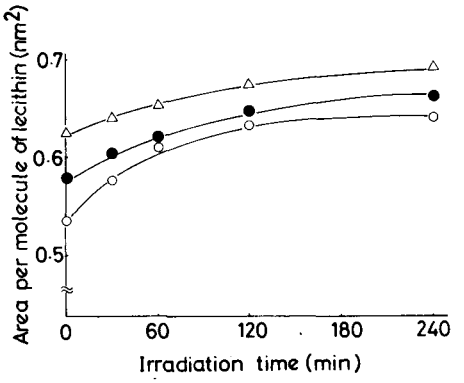

FIG. 4. Effect of irradiation on the area per molecule of lecithin (at 13·4 mNm^{-1}) on rose bengal substrates. Key (M): ○ 1 × 10^{-5}; ● 5 × 10^{-5} and △ 1 × 10^{-4}.

(Laidler 1965). This was done by solving the equation

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

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 became constant. K is the reaction rate constant, x and a are the increases in area/molecule (at 13·4 mNm^{-1}) after time t and after 240 min of irradiation respectively. For all the dyes and drugs the value of n_t was found to be 0·75.

Applying exactly the same analysis to the $\pi - A$ curves of lecithin on substrates containing the dyes and drugs plus Cu^{2+} or Ni^{2+}, it was found that the value of n_t for the former was unchanged but for the latter decreased to zero. This again suggests the formation of complexes between the tetracyclines and heavy metals (Albert & Rees 1956) which appear to be stable towards u.v. light.

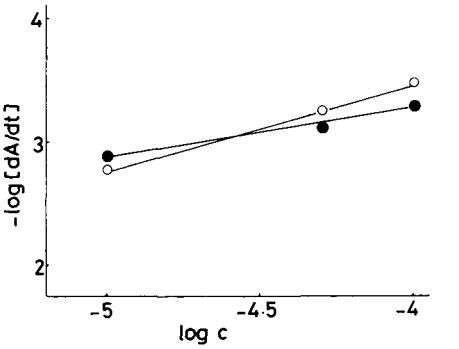

FIG. 5. -log initial rate of expansion of lecithin film on irradiation versus log concentration of dye or drug. Key: ● rose bengal n_c = 0·5 and ○ demethylchlortetracycline n_c = 0·7.

Lecithin is the principal phospholipid in the epidermal cells (Montagna & Lobitz 1964). It seems possible that the expansion that occurs in a lecithin monolayer on irradiation in the presence of tetracyclines might be the cause of the increase in permeability of cell membranes leading to oedema and erythema which occurs in (some) patients taking these drugs (Stempel & Stempel 1973). Since it has been found in the present work that the expansion is inhibited by the presence of Cu^{2+} and Ni^{2+} it might be that by adding these to tetracycline dosages at appropriate concentrations, the photosensitizing action of these drugs might be reduced. On the other hand the dose of the drug would then have to be increased since it is known that divalent metal ions, e.g. Fe^{2+} cause a reduction in the serum levels of tetracyclines (Neuvonen 1970).

Conclusions

(i) Fluorescein dyes and tetracycline drugs caused expansion of a lecithin monolayer due to the formation of a mixed film.

(ii) The films are further expanded by irradiation with u.v. light but the expansion is inhibited in the tetracycline systems by the presence of Cu^{2+} or Ni^{2+}.

(iii) The kinetic orders of the photochemical reactions are between 0·5 and 0·7 but decrease to zero in the tetracycline systems when Cu^{2+} or Ni^{2+} are present.

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