Properties of Monolayers of Normal Alkyl Betaines at the Air–Water Interface

N. G. EVANS and N. PILPEL

Abstract ☐ A study has been made of the effects of pH and of temperature on insoluble films of N-octadecyl and N-behenyl NN-dimethyl glycine at the air-water interface. Condensation of the films at pH 4.5–6 is ascribed to the formation of zwitterions but there is no indication at present that an anionic species is produced on alkaline substrates. The two compounds have entropies of spreading of 1.58 and 2.55 cal. deg.$^{-1}$ cm.$^{-2}$, respectively; their enthalpies of spreading are 4.11 and 6.94 kcal. mole^{-1} cm.$^{-2}$. The results suggest that there is intramolecular neutralization of charges between N$^+$ and COO$^-$ groups in each molecule.

Keyphrases ☐ Alkyl betaines, normal—monolayer at air-water interface ☐ pH, temperature effects—normal alkyl betaine films ☐ Force-area characteristics—normal alkyl betaine films ☐ IR spectrophotometry—identity

The properties of insoluble films of pure alkyl betaines (N-alkyl NN-dimethyl glycines) at the air-water interface have not previously been described.

The compounds have the general formula (1, 2)
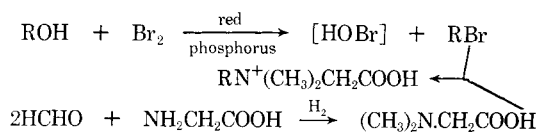
$$RN^+(CH_3)_2CH_2COOH$$
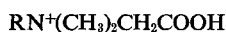
where R is the normal alkyl chain.

The lower homologs which have been prepared in a pure state with R containing up to 16 carbon atoms (3, 4) are soluble in water. They behave as cationic surfactants in acidic media but since, apparently, they do not become anionic in alkaline media, they cannot be regarded as truly ampholytic in nature (1).

The higher homologs with R containing 18 or more carbon atoms are sparingly soluble in water and have to be synthesized by different methods from the lower homologs.

In the present investigation N-octadecyl and N-behenyl NN-dimethyl glycine were prepared by reacting alkyl bromides with NN-dimethyl glycine and recrystallizing the products from nonaqueous solvents.

$$ROH + Br_2 \xrightarrow[\text{phosphorus}]{\text{red}} [HOBr] + RBr$$

$$RN^+(CH_3)_2CH_2COOH \longleftarrow$$

$$2HCHO + NH_2CH_2COOH \xrightarrow{H_2} (CH_3)_2N.CH_2COOH$$

Monomolecular films of the two betaines were spread at the air-water interface and a study made of the effects produced on them by varying the pH and the temperature of the substrate.

Because of the presence of a quaternary nitrogen atom and a carboxylic group, the molecules retain an electrostatic charge over a wide range of values of pH.

A study of the effects of temperature on the π-A characteristics of a monolayer enables one to evaluate the energy functions of spreading (5, 6). Knowing the enthalpy and entropy of spreading of two compounds which differ only in the length of their hydrocarbon
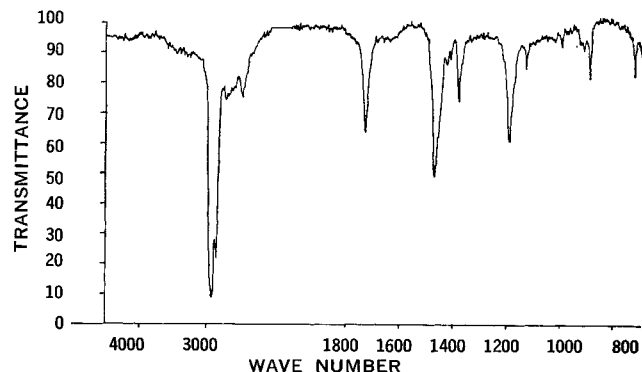

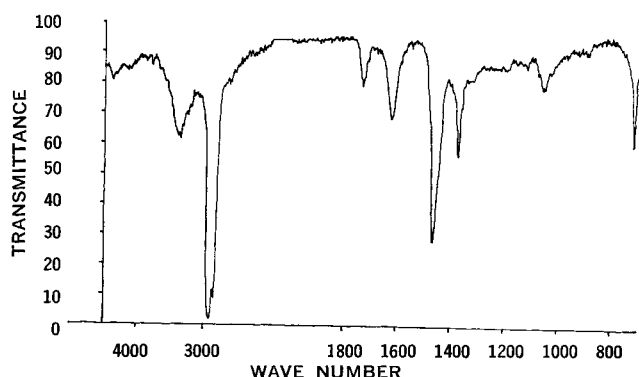Figure 1(A)—*IR spectrum of octadecyl betaine hydrochloride.*

Figure 1(B)—*IR spectrum of behenyl betaine.*

chains, information may be derived concerning the orientation of the molecules in a monolayer and the forces that act between them.

EXPERIMENTAL

Preparation of Materials—The two betaines were prepared by dissolving the appropriate alkyl bromide and NN-dimethyl glycine in isopropanol at 40°, adding anhydrous sodium carbonate and stirring for 72 hr.

In the case of the behenyl compound, the precipitate was repeatedly recrystallized from methanol-acetone (1:2). The octadecyl compound, however, appeared to be in a complex form (7); it was therefore evaporated to dryness, dissolved in a minimum of methanol, and treated with a slight excess of concentrated HCl (8), the precipitate was washed until free of electrolytes and recrystallized in the form of the betaine hydrochloride from ethanol-acetone (1:2). The hydrochloride hydrolyses completely and therefore exhibits the properties of the free base (3).

The 1-bromo octadecane used was 99% pure (Eastman Kodak, m.p. 27.5°). The 1-bromodocosane (m.p. 37.5°) was synthesized by bromination of pure docosanol (Fluka Limited, m.p. 73°) using red phosphorus and bromine. The NN-dimethyl glycine (m.p. 181.5°) was prepared by hydrogenating a mixture of glycine and formaldehyde using palladized charcoal catalyst (9).

The analyses of the betaines are given in Table I and Fig. 1 (A

and B) shows their IR spectra. These were free from strong absorption bands at 2000 cm.$^{-1}$ due to N—H stretching, showing that the nitrogen atoms had been completely quaternized. The absorption bands at 2900 cm.$^{-1}$ were ascribed in C—H stretching, while the bands at 1700 cm.$^{-1}$ were due to the C=O group. The octadecyl compound had a weak absorption pattern between 2400 and 2700 cm.$^{-1}$. This probably arose from dimerization of the compound
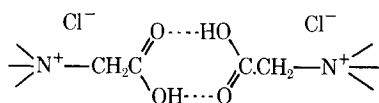
The behenyl betaine, however, being a free base did not dimerize due to residual negative charges on both oxygen atoms.
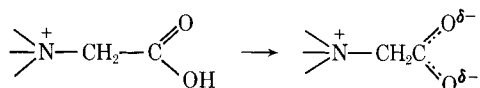
The IR spectra were free from broad absorption bands, indicating absence of residual inorganic reagents and the purity of the compounds was confirmed by the elemental analysis shown in Table I and by the sharpness of the melting point of the octadecyl betaine.

Measurement of Force-Area Characteristics—A thermostated, semi-automatic horizontal film balance, whose design and operation has been described (10), was used for measuring the π-A curves of the betaines on triple-distilled water (surface tension 72.8 dyne cm.$^{-1}$ at 20°, conductivity 1.1 to 1.2 × 10^{-6} Ω^{-1} cm.$^{-1}$, pH 5.6). The apparatus was equipped with a polonium PDC 1 ionizing air electrode system for simultaneously measuring surface potentials.

The pH of the substrate was controlled between 3 and 12 with AR grade HCl or NaOH and its temperature was maintained between 10 and 30° with an accuracy of ± 0.1°.

The films were spread from a 1-mg./ml. solution in redistilled AR grade chloroform to a predetermined area per molecule, allowed to equilibrate for 5 min. and then compressed at the rate of 3 Å^2/molecule/minute. This ensured reproducible π-A curves.

The effect of changing the initial spreading area is illustrated in Fig. 2 which shows the π-A curves of the octadecyl compound at
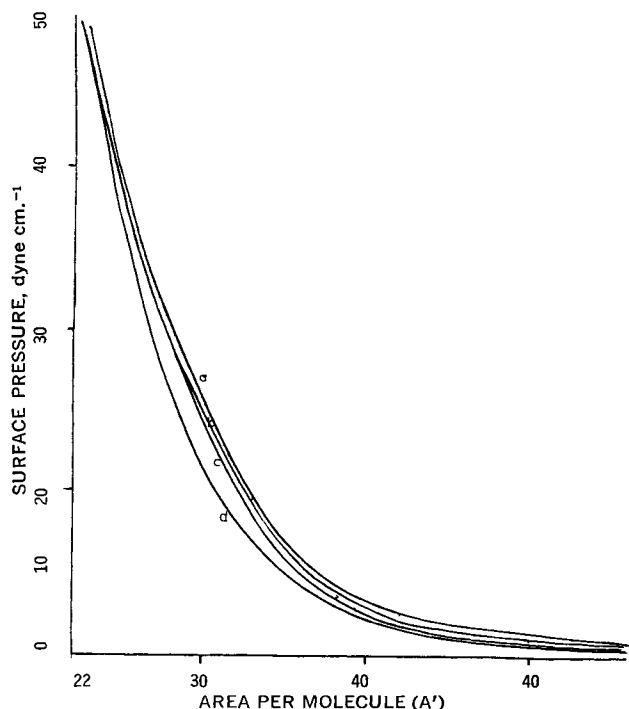
Figure 2—*The effect of initial spreading area on the π-A curves of octadecyl betaine hydrochloride. Key:* a = 90Å^2, b = 80Å^2, c = 70Å^2, d = 100Å^2/molecule.

Table I—Analysis of Betaines

| | Octadecyl Betaine HCl | | Behenyl Betaine | |
	Found, %	Theoretical, %	Found, %	Theoretical, %
Carbon	67.2	67.4	73.58	75.66
Hydrogen	11.4	11.7	13.00	13.18
Oxygen	8.7	8.2	8.24	7.75
Nitrogen	3.7	3.6	3.88	3.39
Chlorine	9.0	9.1	—	—
Mol. wt.	386	391	412	414
M.p.	182.5	—	Unobtainable due to frothing from 150° upwards.	

pH 5.6 and 20°. The curves became increasingly expanded as the material was initially spread at from 70 to 90 Å^2/molecule, but at 100 Å^2/molecule there was slight condensation. This suggests that the betaine films become discontinuous above 90 Å^2/molecule (confirmed by measurement of the surface potential as shown in Fig. 3). At large areas per molecule, the betaine molecules may adopt a variety of orientations in the surface as a result of the weak repulsive forces between their head groups due to partial intramolecular neutralization of their charges.

RESULTS

Figure 4 (A and B) shows the effect of pH on the π-A curves of the two compounds at 20°. The films were substantially more expanded than those of the corresponding long chain fatty acids (11), the octadecyl compound being more expanded than the behenyl compound because of its shorter hydrocarbon chain length.

Both betaines appeared to be in the liquid expanded state over practically the whole range of surface pressure, though the behenyl film became highly viscous (as tested with talc particles) at areas less than 30 Å^2/molecule.

The collapse pressures—about 40 dyne cm.$^{-1}$ for the octadecyl and about 50 dyne cm.$^{-1}$ for the behenyl compound—were practically independent of pH. Thus even on strongly alkaline substrates they remained insoluble [unlike the long-chain fatty acids (11)].
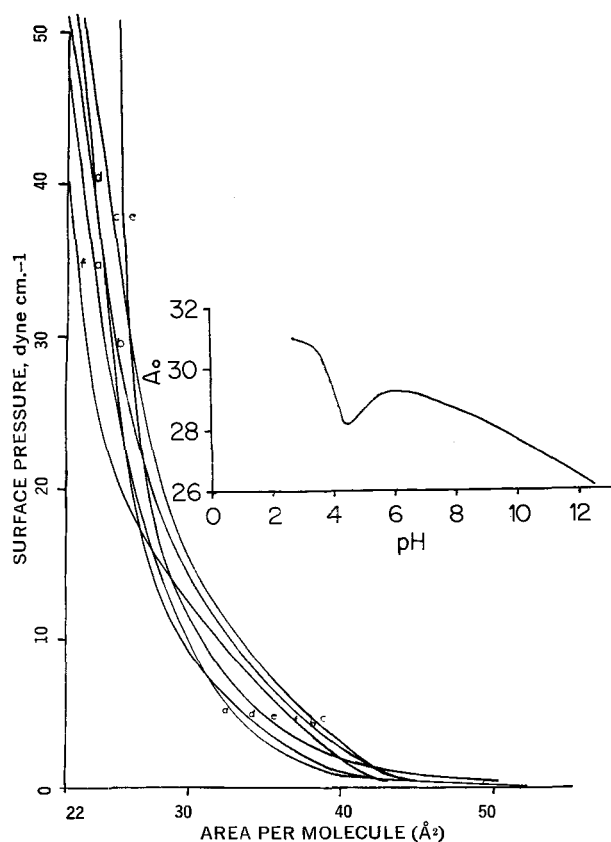
Figure 4 also shows plots of A_0, the extrapolated area per molecule at zero pressure, *versus* pH. Both betaine films showed a minimum which was ascribed to the ionization of the monolayer with the formation of zwitterions. The different positions of the minima for the compounds was due to the presence of chloride
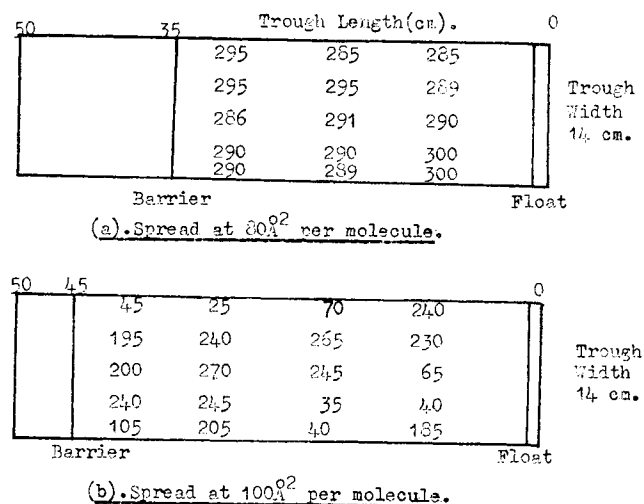
Figure 3—*Surface potential distribution of octadecyl betaine HCl on triple-distilled water, pH 5.6, temperature 25° (in millivolts).*
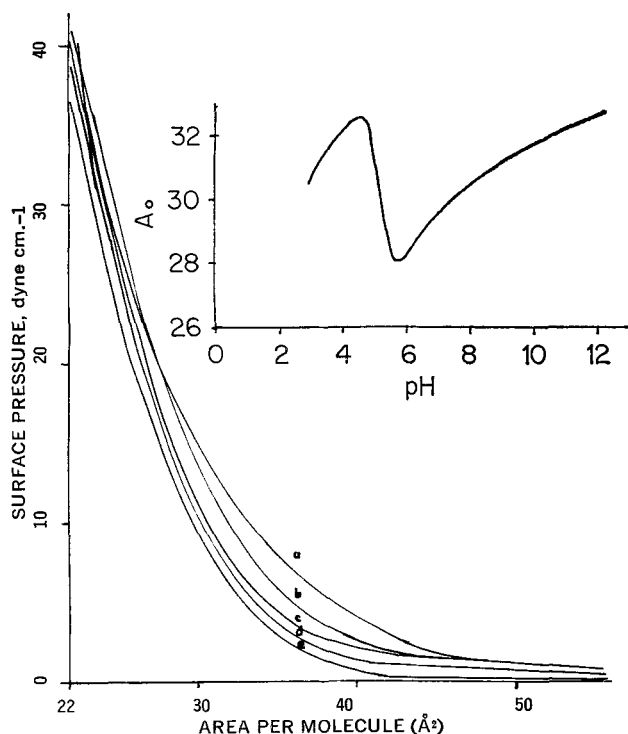
Figure 4(A)—*The effect of pH upon the π-A curves of octadecyl betaine hydrochloride.* Key: a = pH 5.6, b = pH 11.95, c = pH 11.00, d = pH 3.4, e = pH 3.0.

ions in the substrate affecting the ionization of the betaine hydrochloride.

Although it is sometimes possible to make an estimate of the extent of such ionization by assuming the validity of the Boltzman and Gouy equations (11, 12), this procedure would be unreliable with the betaines because of their high negative surface potentials (varying from −300 to −450 mv.). It leads to the conclusion that the pH in the surface film could be 5 or 6 units lower than that in the bulk of the substrate, which appears very unlikely.

Figure 5 shows the effects of temperature on the π-A curves of the two betaines at pH 5.6, and Fig. 6 gives their surface pressure/ temperature relationships at an arbitrary area of 30 Å²/molecule.

DISCUSSION

From these results one can calculate the energy functions of spreading of the betaine films on the assumption that the free energy change for spreading dG_s is (5)
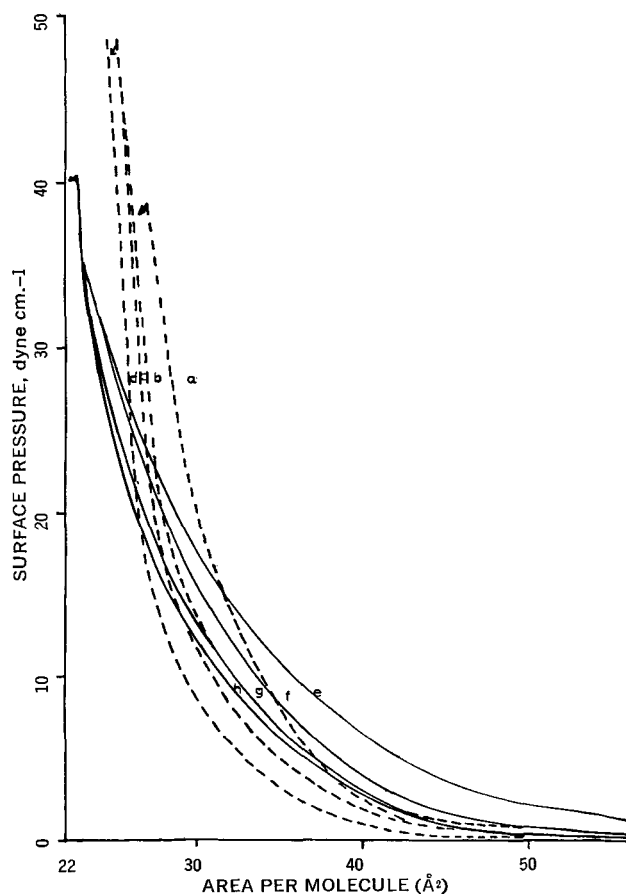
$$dG_s = -(\gamma_w - \gamma_m)dA_m = -\pi dA_m \qquad \text{(Eq. 1)}$$

where γ_w is the free surface energy of water in erg cm.$^{-2}$; γ_m is the free surface energy of the monolayer; dA_m is the increase in area occupied by the monolayer cm.^{2}; π is the surface pressure in dyne cm.$^{-1}$.

The increase in entropy S_s which accompanies spreading of the film by 1 cm.^{2} is given by the slopes of the lines in Fig. 6 and q_s the heat absorbed as the film expands by 1 cm.^{2} is obtained from

$$S_s = q_s/T \text{ erg cm.}^{-2} \text{ deg.}^{-1} \qquad \text{(Eq 2)}$$

where T is temperature in °K.

Figure 4(B)—*The effect of pH upon the π-A curves of behenyl betaine.* Key: a = pH 2.7, b = pH 3.45, c = pH 3.8, d = pH 4.5, e = pH 5.6, f = pH 11.4.

Figure 5—*The effect of temperature on the π-A curves of octadecyl betaine hydrochloride and behenyl betaine.* Key: a = 30°, b = 20°, c = 15°, d = 11.5°, for octadecyl betaine HCl; e = 28.5°, f = 20°, g = 16°, h = 13°, for behenyl betaine.

Temp. °C.	Temp. °K.	Entropy of Spreading S_s erg deg.$^{-1}$ cm.$^{-2}$	Heat Absorbed on Spreading q_s erg cm.$^{-2}$	Surface Pressure, π erg cm.$^{-2}$	Enthalpy of Spreading h_s erg cm.$^{-2}$
		Octadecyl Betaine			
13	286	0.367	104.9	11.5	93.5
16	289	0.367	106.1	13.0	93.1
20	293	0.367	107.5	15.6	91.9
28.5	301.5	0.367	110.6	17.8	92.8
		Mean 0.367 = 1.58 cal.deg.$^{-1}$ cm.$^{-2}$			Mean 92.8 = 4.11 kcal. mole^{-1} cm.$^{-2}$
		Behenyl Betaine			
11.4	284	0.590	167.8	8.5	159.3
15	288	0.590	169.9	11.5	158.4
20	293	0.590	172.9	13.3	159.6
25	298	0.590	175.8	16.8	159.0
30	303	0.590	178.8	19.3	159.5
		Mean 0.590 = 2.55 cal. deg.$^{-1}$ cm.$^{-2}$			Mean 159.1 = 6.94 kcal. mole^{-1} cm.$^{-2}$

The total increase in heat content is

$$h_s = q_s - \pi \text{ erg cm.}^{-2} \qquad \text{(Eq. 3)}$$

Values of the energy functions of spreading are given in Table II.

It may be seen that the values of q_s increase with temperature but that S_s and h_s remain practically constant.

Since the two betaines differed only in the lengths of their hydrocarbon chains, the enthalpy of a single CH$_2$ group can be calculated from the difference in enthalpy of the two compounds. The enthalpy of a CH$_2$ group is:

$$\Delta h = \frac{6.94 - 4.11}{4} = 700 \text{ cal. mole}^{-1} \text{cm.}^{-2} \qquad \text{(Eq. 4)}$$

and the entropy:

$$\Delta S = \frac{2.55 - 1.58}{4} = 0.24 \text{ cal. deg.}^{-1} \text{cm.}^{-2} \qquad \text{(Eq. 5)}$$

The agreement between the figure for ΔS and the figure of 0.20 cal. deg.$^{-1}$ cm.$^{-2}$ obtained from results on the C$_{16}$ and C$_{18}$ carboxylic

Figure 6—*The effect of temperature on the surface pressure at 30Å^2 per molecule. Key: A = octadecyl betaine HCl; B = behenyl betaine.*

acids (5, 6), spread under similar conditions, shows that the energy required to overcome the van der Waals' forces of attraction between the molecules in the monolayer is a function only of their hydrocarbon chain length and is practically independent of the nature of their head groups.

For a single CH$_2$ group, the free energy change ΔG is given by:

$$\Delta G = \Delta h - T\Delta S \qquad \text{(Eq. 6)}$$

substituting in the values of h and S

$$\Delta G = 602 \text{ cal./CH}_2 \text{ group}$$

i.e., a positive quantity. Thus spreading of a betaine is not a spontaneous process but takes place only if heat is absorbed from the substrate.

Two types of interaction might occur between the head groups of molecules in a betaine monolayer: (a) intermolecular attraction between $\overset{+}{N}$ and the COO$^-$ groups of neighboring molecules, leading to a partial neutralization of their charge, or (b) internal (intramolecular) neutralization of the charge on each molecule by interaction between its $\overset{+}{N}$ and COO$^-$ group.

Although exclusively the first type of interaction has been proposed to explain why the critical micelle concentrations of the short-chain, water-soluble betaines are lower than those of the corresponding anionic and cationic surfactants (3), if only this type of interaction occurred in a betaine monolayer, then the film would be more condensed and the spreading energy higher than that of the corresponding fatty acid film.

This does not accord with the present experimental results, which are better explained by assuming that the total charge on each betaine head group is reduced by intramolecular neutralization, reducing the electrostatic repulsion between neighboring head groups and permitting the films to remain coherent at areas per molecule substantially greater than their areas at closest packing.

This assumption of intramolecular neutralization would also satisfactorily account for the lower CMC of the soluble homologs which arises as a result of balance between electrostatic repulsive forces of the charged heads and van der Waals' attractive forces of hydrocarbon chains.

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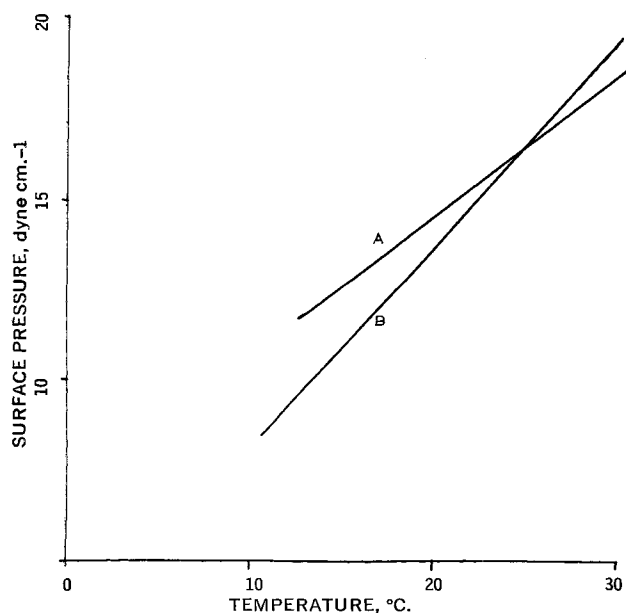
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Influence of Ultraviolet Irradiation on the Surface Activity of Phenothiazine Drugs

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Abstract ☐ The effect of drugs on the permeability of cell membranes has been related to their surface activity. It was of interest, therefore, to determine the influence of UV irradiation on the surface activity of a series of potential photosensitizers. Solutions of five phenothiazine drugs (1×10^{-4} M) were prepared in an acetate buffer. The solutions were exposed to UV irradiation and the surface pressure determined at fixed intervals by the Wilhelmy plate method. Chlorpromazine and prochlorperazine showed an increase in surface pressure during irradiation. Promazine showed no surface activity either before or after irradiation. Triflupromazine and trifluoperazine exhibited a decrease in surface pressure during irradiation. Both chlorpromazine and prochlorperazine have been clearly established as photosensitizers. The other drugs studied, except for a few isolated instances, have not been reported as photosensitizers. Thus, there appears to be a relationship between an increase in surface activity induced by irradiating a drug and the drug's *in vivo* photosensitizing properties.

Keyphrases ☐ Phenothiazines, surface activity—UV irradiation effect ☐ Surface pressure, phenothiazines—UV irradiation effect ☐ Photosensitization, phenothiazines—surface activity relationship

In previous studies it was established that UV irradiation modified the interaction of some phenothiazine drugs with a lecithin monomolecular film. The chlorine substituted derivatives (chlorpromazine and prochlorperazine) showed a marked increase in reactivity toward the film as the result of irradiation. In contrast, trifluoperazine exhibited a decrease and triflupromazine and promazine no change in reactivity following irradiation (1).

Of the group of drugs studied, only chlorpromazine and prochlorperazine have been reported to be significantly phototoxic. It was postulated, that the ability of UV-irradiated phenothiazine drugs to interact with a lecithin monolayer may be a measure of their *in vivo* membrane-penetrating and phototoxic properties (1).

It was of interest, therefore, to determine whether these irradiation-induced changes were related to changes in surface activity of the drugs themselves. This paper reports the effect of UV irradiation on the surface pressure of solutions of a series of phenothiazine drugs and several known photo-oxidation products of one of these drugs (chlorpromazine).

EXPERIMENTAL

Materials—The following phenothiazine derivatives were used without further purification: chlorpromazine hydrochloride, chlorpromazine sulfoxide hydrochloride, prochlorperazine, and trifluoperazine dihydrochloride[1]; promazine hydrochloride[2]; triflupromazine hydrochloride[3]; chlorpromazine *N*-oxide, and 8-hydroxychlorpromazine.[4] The water used was prepared by distillation of deionized water from all glass equipment. All other chemicals were reagent grade.

Apparatus and General Methods—A 0.1 M sodium acetate-acetic acid buffer, adjusted to pH 5.9 was used as the solvent in all experiments. The solutions to be irradiated were placed in a trough irradiated either from above or below the surface. In the former case, an UV lamp was secured about 50 mm. above the surface of the solution. In the latter, an UV lamp was positioned under a quartz window set into the bottom of the trough. In both cases the lamps were fitted with filters which screened out radiation below 280 mμ. The temperature of the trough and solution was maintained at 25 ± 0.1° by use of a constant temperature water circulator. Surface pressure, π, (the difference between the surface tension of the buffer solution and that of the drug-buffer solution) was measured by the Wilhelmy plate method (2) using a thin, roughened platinum blade attached to a torsion balance.[5]

The drug solutions were irradiated for periods ranging from 15–25 min., during which time surface pressure was determined periodically. Surface pressure was also determined for varying periods after the irradiation was stopped.

RESULTS AND DISCUSSION

In the first phase of the study 1×10^{-4} M solutions of the drugs were irradiated from above the surface for 25-min. periods. Surface pressure was determined at convenient intervals during the irradiation and for 25 min. after the irradiation was stopped. Under these conditions, both chlorpromazine and prochlorperazine exhibited a