J. Pharm. Pharmac., 1978, 30, 75–80 Received September 26, 1977

The effect of drug concentration on the thermal (dark) degradation of promethazine hydrochloride in aqueous solution

B. J. MEAKIN*, JOHN STEVENS** AND D. J. G. DAVIES

Pharmaceutics Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 2AY, U.K.

The thermal (dark) degradation of promethazine hydrochloride in aqueous solution presents a complex kinetic picture. The process is oxygen dependent and is modified by EDTA. In citrate buffer, pH 4·0, ionic strength 0·5M, containing 0·1% EDTA, the thermal degradation at 90° can be fitted to first order rate plots at drug concentrations up to $1·56 \times 10^{-2}$M (0·5%) and to zero order rate plots at drug concentrations greater than $9·35 \times 10^{-2}$M (3·0%). At intermediate concentrations no simple equation can describe the data. These effects have been correlated with the formation of drug micelles and the rate data have been interpreted on the basis of a first order monomer process and a half order micellar process occurring simultaneously.

Like other phenothiazine drugs, promethazine undergoes thermal and photochemical degradation which is oxidative in character, aqueous formulations being particularly susceptible (Nakugawa, Kubota & Miyazaki, 1957; Waaler, 1960; Yamamoto & Fujisawa, 1964; Fujisawa, Kawamura & Kawabata, 1966; Maghoub, 1968; Bornschein, Wuenschmann & Pfeifer, 1972). However, satisfactory quantitative kinetic information is not available as most published work on the drug relates largely to the identification of break down products and the elucidation of reaction pathways and has involved the use of high stress conditions such as ultraviolet irradiation and the addition of chemical oxidizing agents which bear little relation to stresses occurring under normal storage conditions. Where the conditions have been more pertinent the stability data for the drug are suspect because the assay techniques were not specific for the drug in the presence of its breakdown products (Ryan, 1959; Floderer & Horvathy, 1965; Maghoub, 1968).

The development of a g.l.c. assay that is valid for both thermally and photolytically degraded solutions (Meakin, Davies & others, 1976) has enabled a comprehensive study of the factors that affect the stability of the drug to be undertaken. Since the effect of light cannot be separated from thermal effects, it is necessary to evaluate the thermal decomposition of the drug in the dark before any photolytic study. Like many antihistamines, promethazine forms aggregates or micelles (Attwood, Florence & Gillan, 1974) and the rate of degradation of molecules, which can aggregate, differs according to whether the compound is in its monomeric or micellar states (Motsavage & Kostenbauder, 1963; Anderson & Polack, 1968). This paper reports investigations into the effect of drug concentration on the thermal, dark stability of aqueous solutions of promethazine hydrochloride.

* Correspondence.
** Present address: The Boots Company Ltd., Formulation Research Group, R4, Pennyfoot Street, Nottingham.

MATERIALS AND METHODS

Materials. Promethazine hydrochloride B.P., a gift from May and Baker Ltd, was used as received. Oxygen and 'high purity' nitrogen were supplied by Air Products Ltd. Buffer salts were AR and all other chemicals were reagent grade or better. Water for kinetic experiments was freshly distilled from an all glass still; for critical micelle (cmc) determinations, water was double distilled. Sørensen's citrate buffer (Documenta Geigy, 6th edn), was used because of its low temperature coefficient (Bates, 1973).

pH Measurements were made using a Radiometer type 27 pH meter fitted with a pH A630P scale expander in conjunction with a Pye-Ingold 405 combined glass-silver chloride electrode. All pH measurements were made on solutions equilibrated to 25° ± 0·1° after standardizing the meter against two appropriate standard buffers (Bates, 1973).

Gas - liquid chromatographic assay. A Pye-Unicam series 104 g.l.c. with dual flame ionization detectors

linked to a Vitatron UR406 linear integrating recorder and fitted with a column containing 5% S.E.30 on silanized Chromosorb W was used. Operating conditions: column temperature 220°; carrier gas argon, flow rate 45 ml min^{-1}; flame gases; hydrogen 20, air 20 lb in^{-2} (140 KN m^{-2}); attenuator range 2000; recorder setting, selector 1, range 6; chart speed 2 cm min^{-1}. Promethazine hydrochloride solutions were diluted to contain between 2·80–9·35 × 10^{-3} M (0·09–0·30%) drug and 0·1% dibutyl phthalate in 50% ethanol and 3 μl samples injected in triplicate directly on to a previously calibrated column; for full details see Stevens (1973) and Meakin & others, (1976).

Kinetic studies in the presence of oxygen. These were carried out under controlled conditions of pH (Sørensen's citrate buffer), ionic strength (adjusted with potassium chloride) and temperature (±0·1°). The standard procedure involved introducing 95 ml of appropriate vehicle into an externally blackened reaction apparatus consisting of a 250 ml conical flask fitted with a condenser and gas diffusor and held in a thermostatted water bath. Gas was delivered to the diffusor via an humidifyer and flow meter at rates up to 20 ml min^{-1}. Gassing does not affect drug concentration at these flow rates over 24 h (Stevens, 1973). After preequilibrating the vehicle for 30 min, 5 ml of vehicle, containing 20 times the required concentration of promethazine hydrochloride was introduced and after rapid and thorough mixing a sample was removed for assay initially and then others at appropriate times, degradation being followed to at least 50%.

Kinetic studies in anoxia. A bulk drug solution in appropriate vehicle was gassed with high purity nitrogen in the dark for 24 h before filling anoxically into ampoules (Tansey, 1969). The ampoules were then heated for varying times before being assayed.

Treatment of kinetic data. Where visually appropriate, the percentage residual drug concentration—time data were fitted to zero or first order rate equations by computerized linear regression analysis which gave the relevant rate constant and its standard deviation, together with the correlation coefficient.

Determination of critical micelle concentration (cmc). Cmcs were determined over the temperature range 25–70° by conductivity and refractometry measurements on drug solutions in citrate buffer, pH 4·0, containing 0·1% EDTA and sufficient potassium chloride to give an ionic strength of 0·5 M in the presence of 1·56 × 10^{-2} M (0·5%) promethazine hydrochloride. Conductivity measurements were made using a Wayne-Kerr Autobalance Universal Bridge type B641 in conjunction with a special high cell constant conductivity cell (Winterborn, 1972). Drug solutions were pre-equilibrated in darkened flasks to the required temperature (±0·01°). The cell was rinsed three times with the appropriate solution before the introduction of the sample for measurement, the conductivity of which was recorded after it had reached a steady reading; determinations were made in duplicate.

Differential refractive index measurements (Δn) were made with a Rayleigh-Haber-Löwe liquid interferometer (Hilger and Watts) according to Bauer & Lewin (1960) using incident light of 546 nm. The instrument was fitted with a 10 mm pathlength two compartment cell mounted in a heated cell jacket which maintained the temperature at ±0·05°. Samples were introduced into the cell under the same protocol as for conductivity measurements.

Treatment of conductivity and refractometry data. Average conductance or Δn values were plotted against percentage drug concentration. The break in the plot corresponding to the cmc, was determined visually and the data divided into two groups, above and below the cmc, excluding any points at or very close to the estimated value. The two groups of data were separately subjected to linear least squares regression analysis and the cmc was taken from the calculated intersection of the two lines.

RESULTS AND DISCUSSION

Preliminary kinetic investigations

Fig. 1 shows degradation rate plots for 1·56 × 10^{-2} M (0·5%) promethazine hydrochloride at 90° in citrate buffer at pH 3·0, adjusted to 0·5 M ionic strength. Under nitrogen, degradation is about 3% after 25 days which is negligible compared with that for solutions stored under air. Continuous oxygenation at 10 ml min^{-1} approximately doubles the rate relative to that under air although in neither case are simple kinetics followed. Raising the pH of the oxygenated system to 4·0 apparently slows the rate initially as well as changing the shape of the rate curve. The decreased rate of oxidation at lower pH possibly results from either trace metal ions in the buffer components or buffer catalysis; both would vary with pH and hence buffer composition.

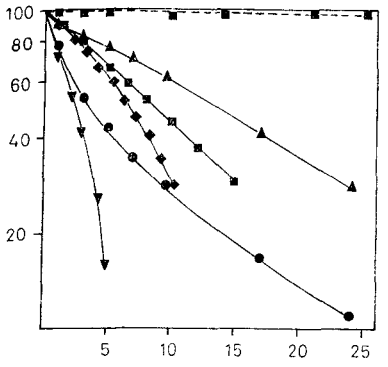


FIG. 1. The effect of oxygen, copper ions and EDTA on the degradation of $1{\cdot}56 \times 10^{-2}$M promethazine hydrochloride in citrate buffer, ionic strength 0·5 M at 90°. ■--■ anoxia, pH 3·0; ▲ stoppered flask under air pH 3·0; ● oxygen flow rate 10 ml min^{-1}, pH 3·0; ◆ oxygen flow rate 10 ml min^{-1}, pH 4·0; ■—■ oxygen flow rate, 10 ml min^{-1}, pH 4·0 with 0·1% EDTA; ▼ oxygen flow rate, 10 ml min^{-1}, pH 4·0 with 10^{-4} M copper sulphate. Ordinate: Percentage residual concentration. Abscissa: Time (h for solutions under air or oxygen, days for anoxic solutions).

That trace metals can affect the degradation rate is shown by the increased rate in the presence of 10^{-4} M copper sulphate, this effect reaching a maximum limiting value at a metal ion concentration of 10^{-3} M. The addition of 0·1% EDTA to the buffered drug solution retarded the rate with the half lives increasing from 3·6 to 13·8 h and 6·5 to 8·8 h at pH 3 and 4 respectively and also straightened the rate curve such that first order kinetics were apparently followed up to at least 70% breakdown. This was shown to be reproducible, five replicate determinations leading to a first order rate constant of $2{\cdot}42 \times 10^{-5}$ s^{-1} (coefficient of variation 0·77%). Further experiments indicated that variation of EDTA concentration within the range 0·05–0·20%, oxygen flow rate within the range 5–20 ml min^{-1} and ionic strength adjusted with potassium chloride to be within the range 0·15–1·00 M had no significant effect on the rate constant (Stevens, 1973).

Effect of drug concentration

Variation in the initial concentration of promethazine hydrochloride from $3{\cdot}11 \times 10^{-3}$ – 0·31 M (0·1 to 10%) affected both the rate and apparent order of the process (Table 1). The order of a reaction (n) can be evaluated from equation (1) where $t_{\frac{1}{2}}$ is the half life, C_0 is the initial drug concentration and k is the rate constant; the order

$$\log t_{\frac{1}{2}} = \log \left[\frac{2^{n-1} - 1}{k\,(n-1)} \right] + (1 - n) \log C_0 \quad . . \quad (1)$$

Table 1. *Effect of promethazine hydrochloride concentration on rate data for degradation in citrate buffer, pH 4·0, ionic strength 0·5 M containing 0·1% EDTA under oxygen at 90°.*

Init. conc. M	Init. rate mol $litre^{-1}$ s^{-1} ($\times 10^7$)	First order rate constant s^{-1} ($\times 10^5$)	Zero order rate constant mol $litre^{-1}$ s^{-1} ($\times 10^7$)	S.d. rate constant ($\times 10^7$)	Half life (h)
$3{\cdot}12 \times 10^{-3}$	−0·90	2·93		5·28	6·57
$6{\cdot}23 \times 10^{-3}$	−1·87	3·03		6·40	6·35
$9{\cdot}35 \times 10^{-3}$	−2·74	2·93		7·36	6·57
$1{\cdot}56 \times 10^{-2}$	−3·77	2·42		1·98	7·95
$3{\cdot}12 \times 10^{-2}$	−4·78				10·84
$6{\cdot}23 \times 10^{-2}$	−5·42			($\times 10^9$)	15·28
$9{\cdot}35 \times 10^{-2}$	−5·83		5·83	2·09	22·28
$1{\cdot}56 \times 10^{-1}$	−6·39		6·39	1·75	33·88
$3{\cdot}12 \times 10^{-1}$	−7·98		7·98	2·93	54·25

is thus obtained from a plot of log $t_{\frac{1}{2}}$ against log C_0 as shown in Fig. 2. For the special case of a first order process such a plot has zero slope. Below $9{\cdot}35 \times 10^{-3}$ M (0·3%) initial concentration, the slope of the plot tends to zero indicating that first order kinetics are obeyed; the first order rate constants are not significantly different ($\chi^2_{calc} = 1{\cdot}63, \chi^2_{tab} = 5{\cdot}99, P = 0{\cdot}05$). Above concentrations of $9{\cdot}35 \times 10^{-3}$ M the half lives increase and the slope becomes progressively steeper up to about $9{\cdot}35 \times 10^{-2}$ M when it becomes constant at a value of 0·78, indicating fractional order compliance. Fitting the experimental data to standard rate equations thus produces some misleading results at initial concentrations greater than $9{\cdot}35 \times 10^{-3}$ M. At $1{\cdot}56 \times 10^{-2}$ M the degradation apparently

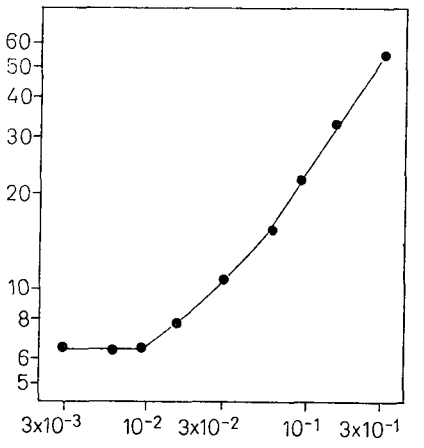


FIG. 2. The effect of drug concentration on the degradation half life of promethazine hydrochloride in citrate buffer, pH 4·0, ionic strength 0·5 M, containing 0·1% EDTA under oxygen, flow rate 10 ml min^{-1}, at 90°. Ordinate: Half life (h). Abscissa: Initial concentration (M).

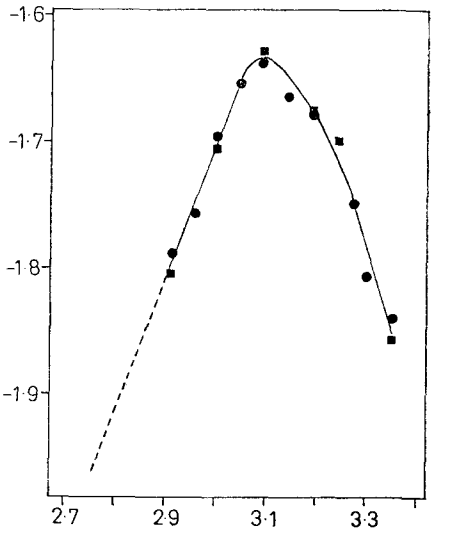
follows first order kinetics (Fig. 1, Table 1) down to 30% residual drug, whereas at concentrations of $9{\cdot}35 \times 10^{-2}$, 0·16 and 0·31 M the degradation data apparently fit zero order plots albeit the rate is still concentration dependent (Table 1). Only the results for $3{\cdot}12$ and $6{\cdot}23 \times 10^{-2}$ M drug concentration did not apparently give a simple kinetic pattern.

The fairly abrupt change in order which occurs between $9{\cdot}35 \times 10^{-3}$ and $1{\cdot}56 \times 10^{-2}$ M drug suggests that some concentration-dependent change in the system is occurring. The phenothiazines are known to be surface active (Florence, 1968) and promethazine has been shown to form small stacked aggregates or micelles in water and normal saline (Florence & Parfitt, 1970, 1971; Attwood & others, 1974). No information is available on the aggregation behaviour at temperatures greater than 34° nor is the effect of buffer salts known. The nature of counter ions influences the aggregation behaviour of cationic surfactants (Jones & Reed, 1968) and promethazine interacts with citrate ions at concentrations below the cmc (Zografi & Zarenda, 1966).

Direct estimation of the cmc at 90° under the standard kinetic conditions (excluding oxygen) was not possible because the Wayne Kerr bridge had to be operated at maximum sensitivity and steady readings were unobtainable. Refractometry proved no better; above 70° the interference bands were bent and tended to shift. This was attributed to thermal convection in spite of the temperature holding within ±0·05°. Consequently the temperature dependence of the cmc was evaluated between 25° and 70° with a view to extrapolating the data to 90° using a Van't Hoff type relation, equation 2,

$$\log \text{cmc} = BT^{-1} + C \quad .. \quad .. \quad (2)$$

where B and C are constants involving enthalpies and entropies of micellization and T is the absolute temperature. This plot is shown in Fig. 3 which exhibits a maximum at about 50°. Such behaviour contrasts with that normally observed with ionic surfactants where a minimum in the cmc - temperature relation is frequently obtained (Shinoda, Nakagawa & others, 1963; Anacker, 1970). Standard theories, involving the relative magnitude of the electrostatic repulsion between the polar head groups and the entropic attractive forces resulting from water structuring, cannot explain the temperature dependency of the promethazine association found under these conditions. One possible explanation for the reduction in cmc above 50° may be



FIG, 3. The effect of temperature on the critical micelle concentration of promethazine hydrochloride in citrate buffer, pH 4·0, ionic strength 0·5 M, containing 0·1% EDTA. ●—Conductivity determination. ■—Refractometry determination. Ordinate: Log critical micelle concentration (M). Abscissa: Reciprocal absolute temperature ($K^{-1} \times 10^3$).

associated with alternate stacking of the molecules in the micelle as proposed by Florence & Parfitt (1971). Such an arrangement would reduce the electrostatic interaction between the ionic side chains and Florence & Parfitt have concluded from nmr studies that the drug does become 'less ionic' on micellization. Increase in temperature will also promote attractive interaction between the heterocyclic sulphur and nitrogen atoms, which would become adjacent in an alternating molecular stack, through decreasing the dielectric constant effects. The increase in cmc with rise in temperature between 25° and 50° may well be associated with a charge interaction between citrate anions and promethazine cations (Stevens, 1973) or some kind of 'salting in' effect. The limited temperature data of Florence & Parfitt (1971) also show such an effect, with the cmc for promethazine in normal saline increasing from $1{\cdot}62 \times 10^{-2}$ M at 20° to $2{\cdot}40 \times 10^{-2}$ M at 34°. Fig. 3 shows that extrapolation of the cmc - temperature data to 90° according to equation 2 gives a cmc value of $1{\cdot}09 \times 10^{-2}$ M under the kinetic conditions used which is similar to the concentration of about $9{\cdot}35 \times 10^{-3}$ M at which the break in the rate order plot (Fig. 2) occurs. Thus at concentrations above the cmc it is likely that the overall rate is the sum of a micellar rate and a bulk phase monomer rate, which could

well lead to the apparent overall fractional order. The monomer order and rate constant can be evaluated from the data obtained at initial drug concentrations of $3{\cdot}12$–$9{\cdot}35 \times 10^{-3}$ M. Table 1 shows that neither the half lives nor the calculated first order rate constants at these concentrations are significantly different. Consequently it may be concluded that the monomer rate is first order with a rate constant of $2{\cdot}96 \times 10^{-5}$ s^{-1}. This allows the micellar rate constant and order to be evaluated from equation 3 assuming that the micelle structure remains constant over the concentration range involved, that the breakdown products do not interfere with the micelle structure, the monomer concentration does not increase above the cmc and that the micelles act as a reservoir for degraded monomers.

$$\frac{dC_t}{dt} = -k_{monomer}[C_{monomer}] - k_{micelle}[C_{micelle}]^{n_{micelle}} \quad \ldots \quad (3)$$

dC_t/dt is the overall reaction rate obtained by drawing tangents to the zero order plot at $t = 0$, $k_{monomer}$ and $k_{micelle}$ are the rate constants for bulk phase and micellar reactions respectively, $C_{monomer}$ and $C_{micelle}$ represent the drug concentrations, expressed as monomer initially in the aqueous and micellar phases and $n_{micelle}$ is the order of the micellar reaction. Rearrangement of equation 3, and consideration of the data in Table 1, shows that a plot of $\log\left[\frac{dC_t}{dt} + k_{monomer}C_{monomer}\right]$ against $\log C_{micelle}$ should be linear with a slope of $n_{micelle}$ and an intercept of $\log k_{micelle}$. Such a plot is shown in Fig. 4 using the data from Table 1. In view of the assumptions made regarding the aggregation behaviour of the drug, and the inaccuracies associated with drawing tangents, Fig. 4 shows there is a reasonably good fit of the data to equation 3 ($r_{calc} = 0{\cdot}978$, $r_{tab} = 0{\cdot}974$, $P = 0{\cdot}001$) which would suggest half order compliance for the micellar process (slope 0·48, standard deviation 0·05) and a micellar rate constant of $1{\cdot}64 \times 10^{-7}$ M litre^{-1} s^{-1}; aggregation thus protects the drug against degradation. Calculations based on equation 3, explain the absence of any break in the first order plot for $1{\cdot}56 \times 10^{-2}$ M promethazine at 30% degradation when only drug monomers should be present in solution. These show that such a plot is insensitive to the change from a combined fractional order micellar degradation and first order monomer breakdown to a reaction involving only the latter process. Similar arguments involving the arithmetical nature of the degradation can be advanced for drug concentrations above $9{\cdot}35 \times 10^{-2}$ M where fit to zero order rather than first order kinetics occurs. At $3{\cdot}12$ and $6{\cdot}23 \times 10^{-2}$ M drug, the relative magnitudes of the two terms in equation 3 account for the apparent lack of compliance with either of these simple order processes.

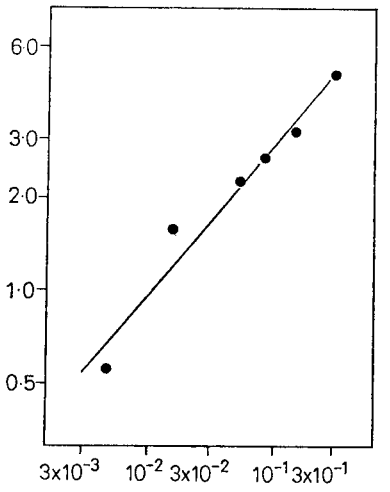


FIG. 4. Log-log plot of micellar rate against micellar concentration according to equation 3, for the degradation of promethazine hydrochloride in citrate buffer, pH 4·0, ionic strength 0·5 M containing 0·1% EDTA under oxygen, flow rate 10 ml min^{-1}, at 90°. Ordinate: Micellar rate (M litre^{-1} s^{-1} × 10^5). Abscissa: Molar concentration of promethazine as micellar.

Waaler (1960) has proposed that the mechanism for the thermal dark degradation of promethazine involves the formation of a reactive complex with oxygen which then undergoes attack by hydroxyl ions to give 10-methyl phenothiazine, acetaldehyde and dimethylamine. Evidence for molecular complexes between oxygen and phenothiazines is also given by the E.P.R. studies of Iwaoka, Kokubun & Koizumi (1971).

The stacked orientation of the molecules in the micelles would be likely to inhibit such a reaction mechanism sterically by making the access of oxygen to the initial complexation site more difficult and certainly the fractional order of the micellar process with respect to drug might suggest a second reactant is rate limiting. The reduction in the cationic character of the molecules present in the micelles suggested by Florence & Parfitt (1970; 1971) and further evidenced by the physical properties of promethazine solutions above the cmc (Meakin, Stevens & Davies, unpublished) would

also retard the attack by hydroxyl ions on the activated oxygen complex.

These results have demonstrated that the degradation kinetics of promethazine hydrochloride are modified by the physical state of the drug molecules in solution. This effect is likely to be observed with all drug systems that are prone to aggregation and it is thus necessary to carry out both physical and kinetic studies under identical conditions before interpretation of accelerated stability data obtained with such systems can be properly evaluated.

Acknowledgements

This work was supported by an SRC studentship (J.S.). We would also like to thank May and Baker Ltd for the gift of promethazine hydrochloride.

REFERENCES

ANACKER, E. W. (1970). In: *Cationic Surfactants*, Chap. 7. Editor: Jungermann, E. New York: Marcel Dekker Inc.

ANDERSON, R. A., POLACK, A. E. (1968). *J. Pharm. Pharmac.*, **20**, 249–254.

ATTWOOD, D., FLORENCE, A. T. & GILLAN, J. M. (1974). *J. pharm. Sci.*, **63**, 988–993.

BATES, R. G. (1973). *Determination of pH*, Second Edn. New York and London: Wiley.

BAUER, N. & LEWIN, S. Z. (1960). In: *Physical methods of organic Chemistry*, vol. 1, pt II, chap XVIII, 3rd Edn. Editor: Weissberger, A. New York and London: Interscience.

BORNSCHEIN, L., WUENSCHMANN, H. & PFEIFER, S. (1972). *Die Pharmazie*, **27**, 188–189.

FLODERER, I. & HORVATHY, V. (1965). *Acta pharm. hung.*, **35**, 98–103.

FLORENCE, A. T. (1968). *Adv. Colloid Interface Sci.*, **2**, 115–159.

FLORENCE, A. T. & PARFITT, R. T. (1970). *J. Pharm. Pharmac.*, **22**, *Suppl.*, 121S–125S.

FLORENCE, A. T. & PARFITT, R. T. (1971). *J. phys. Chem.*, **75**, 3554–3560.

FUJISAWA, S., KAWAMURA, M. & KAWABATA, S. (1966). *Yakugaku Zasshi*, **86**, 708–713.

IWAOKA, T., KOKUBUN, H. & KOIZUMI, M. (1971). *Bull. Chem. Soc. Jap.*, **44**, 341–347.

JONES, M. N. & REED, D. A. (1968). *Proc. 5th Int. Cong. Surface Activity*, **2** (2), 1081–1089. Barcelona: Ediciones Unidas (1969).

MAGHOUB, A. (1968). M. Phil. Thesis, University of London, U.K.

MEAKIN, B. J., DAVIES, D. J. G., COX, N. & STEVENS, J. (1976). *Analyst*, **101**, 720–727.

MOTSAVAGE, V. A. & KOSTENBAUDER, H. B. (1963). *J. Colloid Sci.*, **18**, 603–615.

NAKUGAWA, T., KUBOTA, T. & MIYAZAKI, H. (1957). *Ann. Rep. Shionogi Res. Lab.*, **7**, 319–329.

RYAN, J. A. (1959). *J. Am. pharm. Ass.* (*Sci. Edn.*), **48**, 240–243.

SHINODA, K., NAKAGAWA, T., TAMAMUSHI, B. & ISEMURA, T. (1963). *Colloidal Surfactants*, New York and London: Academic Press.

STEVENS, J. (1973). Ph.D. Thesis, University of Bath, U.K.

TANSEY, I. P. (1969). M.Sc. Thesis, University of Bath, U.K.

WAALER, T. (1960). *Pharm. Acta Helv.*, **35**, 168–172.

WINTERBORN, I. K. (1972). Ph.D. Thesis, University of Bath, U.K.

YAMAMOTO, R. & FUJISAWA, S. (1964). *Rep. Kongr., Pharm. Wiss. Vortr. Originalmitt*, **23**, Muenster (Westfalen), Ger., 509–514.

ZOGRAFI, G. & ZARENDA, I. (1966). *Biochem. Pharmac.*, **15**, 591–598.