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# Dilute solution properties of clindamycin 2-palmitate hydrochloride

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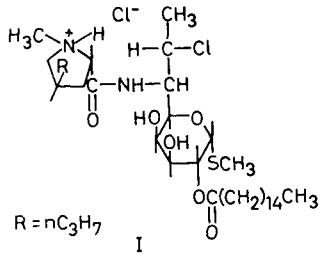
Clindamycin 2-palmitate hydrochloride forms micelles in aqueous solution at concentrations above $2{\cdot}2 \times 10^{-3}$ M. Dilution of micellar solutions of the drug to concentrations less than $2{\cdot}0 \times 10^{-3}$ M produces turbid systems. Turbidity is caused by unionized drug, produced by hydrolysis of the salt, phasing out of solution at concentrations too low for micelles to be formed. Changes in specific conductivity and turbidity associated with the phasing-out process have been measured and explained in terms of the rates of the various processes involved, including demicellization and emulsion stability. The phenomenon of phasing-out, below a critical concentration in aqueous solution, is predicted to be a general one for weakly ionized surfactants where the unionized species possesses low water solubility.

Clindamycin 2-palmitate hydrochloride (I) is an essentially tasteless derivative of the antibiotic clindamycin (Sinkula, Morozowich & Rowe, 1973). The palmitate ester is formulated as flavoured granules for paediatric use.




I

In aqueous solution the compound behaves somewhat as an association colloid, being highly surface active and capable of micellar aggregation (E. L. Rowe, unpublished data). However, some solution properties have been observed which are not usually considered typical of ionic surfactants. For example, on dilution of a more concentrated solution to less than approximately $2{\cdot}0 \times 10^{-3}$ M the solution becomes turbid, the turbidity increasing with time and the second phase being deposited on glass surfaces as a thin film. A continually increasing electrical conductance is found to be associated with this phenomenon.

The physical state of a drug in a formulation is important in determining the chemical and physical stability and its bioavailability. The present work was undertaken to gain a better understanding of the dilute solution properties of this antibiotic. Studies have been made of changes in the conductance and in the turbidity which occur after dilution of a stock solution ($1{\cdot}0 \times 10^{-2}$ M) to a series of concentrations between $3{\cdot}0 \times 10^{-3}$ and $4{\cdot}0 \times 10^{-5}$ M. The findings are thought to apply to certain types of ionic association colloids.

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## MATERIALS AND METHODS

Commercial clindamycin 2-palmitate hydrochloride was further purified by dissolving in hot ethyl acetate and precipitating into cold acetonitrile.

*Conductance measurements.* All conductance measurements were made using a Wayne Kerr B642 autobalance universal bridge, linked to a Servoscribe potentiometer recorder so that conductance was continuously monitored with time.

Experiments were carried out using a cylindrical glass cell fitted with bright platinum electrodes and an inlet for addition of water or drug solution. The cell constant (approximately $0{\cdot}2$ cm$^{-1}$) was measured accurately at several points in the conductance range studied, using potassium chloride solutions of accurately known concentration. For all measurements the cell contained a total of 50 ml of solution, well stirred by magnetic rotation of a glass covered iron pellet inside the cell.

All solutions were made up using redistilled water (specific conductance $< 5 \times 10^{-6}$ ohm$^{-1}$ cm$^{-1}$). The stock solution of clindamycin 2-palmitate hydrochloride was made at least 24 h before use and kept at 25°.

The stability of the drug in the stock solution was considered from the point of view of both the

clindamycin species and the palmitate ester linkage. The stability of clindamycin has been studied in aqueous solution (Oesterling, 1970) and was found to be optimum at pH 4. We have also studied the hydrolysis of the 2-palmitate to clindamycin and palmitate acid in distilled water, and concluded that the overall stability of the drug justified keeping the stock solution at 25° for at least one week. Under these conditions, it is believed that complications in the phenomena described in the present work do not arise from instability of the drug.

The required volume of water was first pipetted into the cell and allowed to reach thermal equilibrium as indicated by constant conductance. The required volume of stock solution was added and the conductance recorded until no further change with time was apparent. Duplicate runs for about twenty dilutions between $3{\cdot}0 \times 10^{-3}$ and $4{\cdot}0 \times 10^{-5}$ M were carried out.

*Turbidity measurements.* Turbidity develops rapidly in all solutions of concentration lower than approximately $2{\cdot}0 \times 10^{-3}$ M. Trial experiments with a solution of $1{\cdot}8 \times 10^{-3}$ M concentration showed that changes in turbidity with time could be followed conveniently at a wavelength of 350 nm using 4 cm cells, thermostated at 25°. The required volume of drug solution was measured into the cell, followed by the required volume of water previously thermostated at 25° and 'absorbance' recorded for approximately 20 min after dilution.

## RESULTS

*Conductance measurements.* Data obtained from conductance measurements are given in Table 1. Three different types of change of conductance with time after dilution may be distinguished. These are illustrated in Fig. 1 by curves (a), where the dilution is to a concentration greater than $2{\cdot}0$–$2{\cdot}2 \times 10^{-3}$ M, (b) where the dilution is to a concentration in the range $1{\cdot}8 \times 10^{-3}$–$1{\cdot}0 \times 10^{-3}$ M and (c) where the dilution is to a concentration less than $1{\cdot}0 \times 10^{-3}$ M.

In type (a) the conductance reached a steady value almost immediately (mixing time) and remained at that value. In type (b) the conductance reached almost immediately did not remain at a steady value but increased continuously with time; the increase was very slow over an initial period, then rapid before moderating to a slow upward drift. The initial time lapse, $T_L$, before the rapid rise in conductance, became shorter as the dilution was increased in this range. In type (c) the conductance reached almost immediately continued to rise with no lapse as in type (b).

Table 1. *Results of conductance measurements following dilution of a stock solution of clindamycin 2-palmitate hydrochloride (concentration $1{\cdot}0 \times 10^{-2}$ M).*

| C† (M) | Ko $\mu\Omega^{-1}$ cm$^{-1}$ | $T_L$ (min) | $K_F$ $\mu\Omega^{-1}$ cm$^{-1}$ | R $\mu\Omega^{-1}$ cm$^{-1}$ min$^{-1}$ |
|---|---|---|---|---|
| $3{\cdot}0 \times 10^{-3}$ | 263·2 | — | 263·2* | 0 |
| 2·5 | 237·2 | — | 237·2* | 0 |
| 2·2 | 225·7 | — | ** | — |
| 2·0 | 207·7 | — | ** | — |
| 1·8 | 195·6 | 3·0 | 207·4 | 2·36 |
| 1·6 | 180·9 | 1·0 | 203·4 | 4·50 |
| 1·4 | 169·9 | 0·2 | 194·0 | 4·82 |
| 1·2 | 153·1 | 0·1 | 179·5 | 5·28 |
| 1·0 | 138·1 | 0 | 165·1 | 5·40 |
| 0·8 | 119·0 | 0 | 146·2 | 5·44 |
| 0·6 | 95·7 | 0 | 120·9 | 5·04 |
| 0·5 | 85·2 | 0 | 110·7 | 5·10 |
| 0·4 | 70·4 | 0 | 96·8 | 5·28 |
| 0·3 | 58·4 | 0 | 80·1 | 4·34 |
| 0·25 | 50·1 | 0 | 67·7 | 3·52 |
| 0·2 | 40·0 | 0 | 53·3 | 2·66 |
| 0·1 | 18·3 | 0 | 23·2 | 0·98 |
| 0·08 | 17·2 | 0 | 21·6 | 0·88 |
| 0·06 | 12·7 | 0 | 15·4 | 0·53 |
| 0·04 | 6·5 | 0 | 7·8 | 0·26 |

* Specific conductance unchanged after 60 min.
** Specific conductance increasing very slowly.
† = concentration after dilution. Ko = immediate specific conductance. $T_L$ = time lapse. $K_F$ = specific conductance at $T_L$ + 5 min. R = average rate of change of conductance during 5 min after $T_L$.

At concentrations of $2{\cdot}0$ and $2{\cdot}2 \times 10^{-3}$ M, after dilution, the conductance-time effects were just detectable. It was not possible, however, to ascribe unequivocal values either to a time lapse or to a subsequent rate of change of conductance in these cases; the indications are clear that this is because the time lapse is long and the subsequent rate of change of conductance small.

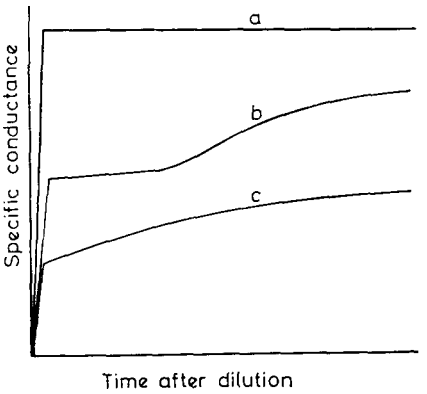


FIG. 1. Profiles of change of specific conductance with time after dilution of clindamycin 2-palmitate hydrochloride to concentrations. (a) above $2{\cdot}2 \times 10^{-3}$ M. (b) between $1{\cdot}0 \times 10^{-3}$ and $1{\cdot}8 \times 10^{-3}$ M. (c) below $1{\cdot}0 \times 10^{-3}$ M.

At concentrations below $1{\cdot}0 \times 10^{-3}$ M, after dilution, the time lapse reduced to zero and values of the average rate of change of conductance, given in Table 1, then refer to the first 5 min following the mixing time. In Fig. 2, the rate of increase of conductance is shown plotted against concentration after dilution.

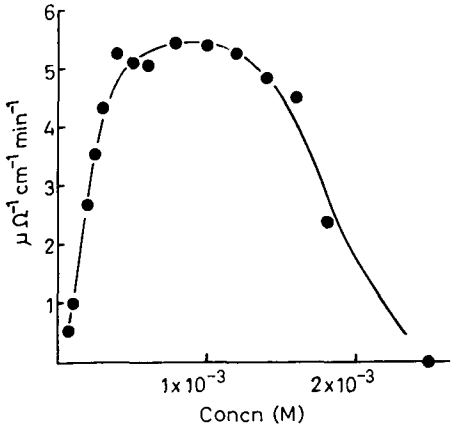


FIG. 2. The rate of change of specific conductance ($\mu\Omega^{-1}\,cm^{-1}\,min^{-1}$) of clindamycin 2-palmitate hydrochloride solutions after dilution.

*Turbidity measurements.* An initial period, $T_o$, during which no change occurred in the absorbance, was determined for concentrations between $2{\cdot}2 \times 10^{-3}$ and $1{\cdot}0 \times 10^{-3}$ M after dilution. The data are given in Table 2. Turbidity developed immediately on dilution to concentrations lower than $1{\cdot}0 \times 10^{-3}$ M. In Fig. 3, the rate of increase in turbidity is shown plotted against concentration after dilution.

Table 2. *Results of turbidity measurements following dilution of a stock solution of clindamycin 2-palmitate hydrochloride (concentration $1{\cdot}0 \times 10^{-2}$ M).*

| Concn after dilution C (M) | Time before turbidity detected $T_o$ (min) | Average rate of change of 'absorbance' during 5 min after $T_o$ min$^{-1}$ × 100 |
|---|---|---|
| $2{\cdot}5 \times 10^{-3}$ | ≃15 | <0·2 |
| 2·2 | 11·0 | 0·4 |
| 2·0 | 7·0 | 1·2 |
| 1·8 | 3·2 | 1·7 |
| 1·6 | 1·4 | 1·9 |
| 1·4 | 1·2 | 2·2 |
| 1·2 | 1·0 | 1·7 |
| 1·0 | 0·8 | 1·1 |

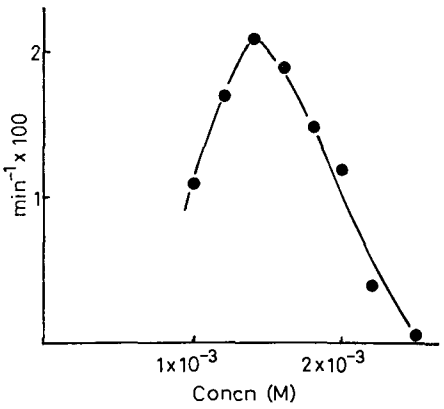


FIG. 3. The rate of change of 'absorbance' at 350 nm ($min^{-1} \times 100$) of clindamycin 2-palmitate hydrochloride solutions after dilution.

## DISCUSSION

Clindamycin 2-palmitate is a weak base (pKa ≃7·6) and therefore the hydrochloride salt undergoes hydrolysis in aqueous solution:

$$RNH^+Cl^- + H_2O \underset{K}{\leftrightharpoons} RN + H_3O^+ + Cl^-$$

where $RNH^+$ and RN represent the protonated and unprotonated (free base) species respectively. The degree of hydrolysis is determined by the equilibrium constant, K, where

$$K = \frac{[RN]\,[H_3O^+]}{[RNH^+]\,[H_2O]}$$

The possibility must be considered that the increasing conductance after dilution is caused by the hydrolysis of the drug salt thereby releasing mobile hydrogen ions. However, pH measurements with a glass electrode, although difficult because the oily second phase attaches to the electrode membrane, affecting its response, indicated no decrease in pH. If the equivalent ion conductance of the hydrogen ion is taken as 350 $ohm^{-1}\,cm^2$, an increase in hydrogen ion concentration of approximately $10^{-4}$ equivalents $litre^{-1}$ would be required to account for the difference in values of $K_F$ and $K_O$ recorded in Table 1 for the results referred to as type (b), i.e. for concentration after dilution in the range $1{\cdot}8 \times 10^{-3}$ to $1{\cdot}0 \times 10^{-3}$ M. The pH of these solutions is near to 4 and if the conductance rise were due to release of hydrogen ions, the resultant pH change—even in the first 5 min after the time lapse $T_L$—would be several tenths of one pH unit. Such a change would be readily detected and it follows that hydrolysis of the salt, although doubtless

occurring, does not account for the observed conductivity effects. Furthermore, the attainment of the hydrolysis equilibrium would not be expected to be so slow. In addition to hydrolysis, micellar breakdown must be considered. Suppose the micelle is formed from m units of the entity $RNH^+$ and entraps n chloride counter ions:

$$\{(RNH^+)_mCl^-_n\}^{(m-n)+} \underset{K_m}{\rightleftharpoons} mRNH^+ + nCl^-$$

The dissociation constant for the micellar equilibrium is

$$K_m = \frac{[RNH^+]^m\,[Cl^-]^n}{[\{(RNH^+)_mCl_n^-\}^{(m-n)+}]}$$

In a micellar solution, free base produced by hydrolysis would be solubilized in the micelles, so that hydrolysis would continue in the aqueous phase. On dilution of a more concentrated micellar solution to a less concentrated one, no particular noteworthy effects would be expected. Free base produced by the hydrolytic reaction would still be solubilized in the micelles. It is suggested that this is the case in the present experiments where dilution was to some concentration above about $2{\cdot}0 \times 10^{-3}$ M and that a critical micelle concentration is close to this value.

However, on dilution of the concentrated micellar solution to a concentration below $2{\cdot}0 \times 10^{-3}$ M, micellar breakdown would follow with release of solubilized free base, of ionized clindamycin 2-palmitate and of entrapped counter ions. If the free base released exceeded the solubility in the bulk aqueous phase an emulsion would be formed. Conductivity changes would be caused by the released ionized drug, $RNH^+$ and chloride ions; an additional contribution to the conductance would probably arise from the free base microdroplets, charged by adsorption of the various ionic species present. It is suggested that such processes account for the results in the present experiments where dilution was to concentrations in the range $2 \times 10^{-3}$ to $4 \times 10^{-4}$ M. Correlation between the phasing out of free base and the conductance-time effects may be considered from Figs 2 and 3. Both rate of turbidity increase and rate of conductance increase change with the extent of dilution in the same way and reach a maximum in the region of dilution to $1{\cdot}2$–$1{\cdot}4 \times 10^{-3}$ M. The actual characteristics of the experimental conductivity-time and turbidity-time profiles, after any particular degree of dilution, reflect the complex balance of the rates of the various processes occurring, including that of the actual demicellization and of droplet growth and emulsion stability.

Finally, if the degree of dilution is sufficient to allow an increasing proportion of free base to remain in solution in the bulk aqueous phase, then again it would be expected that conductance-time effects, associated with phasing out of the free base, would diminish. This explains the decreasing values of R in Table 1, for dilutions to less than about $4 \times 10^{-4}$ M.

At very low concentrations, all the free base present may remain in solution in the bulk aqueous phase and no conductance-time effects may be observed. The results indicate that this occurs on dilution to less than about $2{\cdot}5 \times 10^{-5}$ M. However, effects of the hydrolysis equilibrium on the conductance values will be expected at lower concentrations.

## CONCLUSIONS

Conductance-time effects associated with the phasing-out process make it difficult to determine a critical micelle concentration from conductivity *vs* concentration plots.

The data do suggest, however, that micelles break down at concentrations below approximately $2 \times 10^{-3}$ M.

We propose that the phasing-out process exhibited by clindamycin 2-palmitate hydrochloride in dilute solution is not specific to this compound but is general for certain types of ionic association colloids. Surfactants possessing weakly ionizable head groups, acidic or basic, would be expected to show similar phasing-out phenomena. These will arise at concentrations below the critical micelle concentration if the concentration of the unionized species, produced by hydrolysis of the salt, exceeds the solubility in the aqueous phase.

## REFERENCES

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