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Application of the Ferguson principle to the antibacterial activity of mono- and multi-component solutions of quaternary ammonium surface-active agents

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Critical micelle concentrations (CMC) have been measured by the surface tension method for binary mixtures of dodecyltrimethylammonium bromide with tetradecyltrimethylammonium bromide, binary mixtures of C_{14}/C_{16} homologues, a ternary mixture of the $C_{12}/C_{14}/C_{16}$ compounds and additionally for each component individually. The antibacterial activities of the systems against *Escherichia coli* were determined by a British Standard Method (B.S. 3286: 1960). Thermodynamic activities of the solutions at two survivor levels, calculated from the physical and biological measurements, were sufficiently constant to sustain the Ferguson principle for these micelle-forming antibacterial agents. A theoretical treatment of micelle formation for multi-component solutions of surfactants gave CMC's by calculation in close agreement with the experimentally determined values. The purity and composition of the surfactants was determined by gas liquid chromatography.

Links between the surface and micellar properties of quaternary ammonium compounds and their antibacterial action have been noted in many literature reports and attempts have been made to put these relations on a more fundamental basis by suggesting that the Ferguson principle applied to the antibacterial action of cationic surfactants (Ecanow & Siegel, 1963). In a study on three quaternary ammonium chlorides, that were not members of a homologous series, Weiner, Hart & Zografi (1965) reported a clear relation between the thermodynamic and antibacterial activities could be recognized if the former were expressed as a ratio of the surface concentration produced by a solution and the surface concentration at the critical micelle concentration (CMC), rather than by using bulk solution concentrations.

In practice most quaternary ammonium compounds are mixtures of homologues and it therefore seemed of value to test whether the Ferguson principle extended to multi-component solutions. Further experimental evidence on mono-component systems using a different series of compounds from those investigated by Weiner & others also seemed desirable. This paper describes experiments on both aspects using the widely studied homologous series n-dodecyl, n-tetradecyl and n-hexadecyl trimethylammonium bromides individually, and with binary systems containing C_{12}/C_{14} or C_{14}/C_{16} mixtures over a wide range of mol fractions of each component. Cetrimide B.P., a ternary mixture containing the same three homologues, was also investigated.

EXPERIMENTAL AND RESULTS

Assessment of homologue composition and purity of the surfactants

(a) *n-Alkyltrimethylammonium bromides.* Samples of dodecyltrimethylammonium bromide (DTAB) (C_{12}), tetradecyltrimethylammonium bromide (TTAB) (C_{14}) and hexadecyltrimethylammonium bromide (HTAB) (C_{16}) were kindly provided by Dr. J. E. Adderson. Certain analytical data for TTAB has been given by Adderson & Taylor (1964). The homogeneity of the three surfactants was confirmed by the method of Laycock & Mulley (1966).

(b) *Cetrimide B.P.* This was identical with sample B, Table 1 of our earlier paper. It contained about 27, 62, and 11% respectively of the C_{12}, C_{14}, and C_{16} homologues (mol fractions 0·251, 0·628 and 0·121) and the mean molecular weight calculated from this composition is 332.

Table 1. *Bactericidal concentrations of quaternary ammonium compounds and their mixtures for two survivor levels of* E. coli, *and associated* CMC *and thermodynamic activities*

Compound or mixture (mol fraction)	Bactericidal concentration (mM) at survivor levels of 1·0%	0·01%	CMC (mM)	Thermodynamic activities at survivor levels of 1·0%	0·01%
C_{12}	0·26	0·60	14·7	0·018	0·041
C_{12}/C_{14} (0·766/0·234)	0·11	0·30	6·90	0·016	0·043
C_{12}/C_{14} (0·522/0·478)	0·076	0·175	4·3	0·018	0·041
C_{12}/C_{14} (0·267/0·733)	0·06	0·13	3·2	0·019	0·041
C_{14}	0·047	0·11	3·1	0·015	0·036
C_{14}/C_{16} (0·764/0·236)	0·026	0·068	1·57	0·017	0·043
C_{14}/C_{16} (0·520/0·480)	0·019	0·052	1·12	0·017	0·046
C_{14}/C_{16} (0·265/0·735)	0·015	0·039	0·95	0·016	0·041
C_{16}	0·014	0·036	0·90	0·016	0·040
$C_{12}/C_{14}/C_{16}$ (0·251/0·628/0·121)	0·033	0·087	2·20	0·015	0·040

CMC *measurements.* The surface tension method was adopted using the Du Nouy ring apparatus (Cambridge Instrument Co.). Solutions of the surfactants or surfactant mixtures were prepared in water which had been distilled from an all-glass apparatus, and the surface-tensions measured at 21° ± 1°. Plots of surface-tension against the logarithm of the surfactant concentration were linear, giving two straight lines the intersect of which was taken as the CMC. Surface ageing (Padday, 1960), observed at the lower concentrations, was not significant near the CMC. Although the Harkins & Jordan (1930) corrections altered the surface-tension values in the expected way, the CMC values did not change by more than 1–2% i.e. CMC obtained from corrected and uncorrected surface-tension values for DTAB, TTAB and HTAB were 14·7 and 15·0, 3·10 and 3·15, and 0·9 and 0·9 mmol litre^{-1} respectively. These agree well with the more reliable published results (for a detailed discussion see Laycock, 1969). Results for binary mixtures of the three surfactants and the ternary cetrimide sample were obtained, therefore, using uncorrected surface-tension data and are shown in Fig. 1.

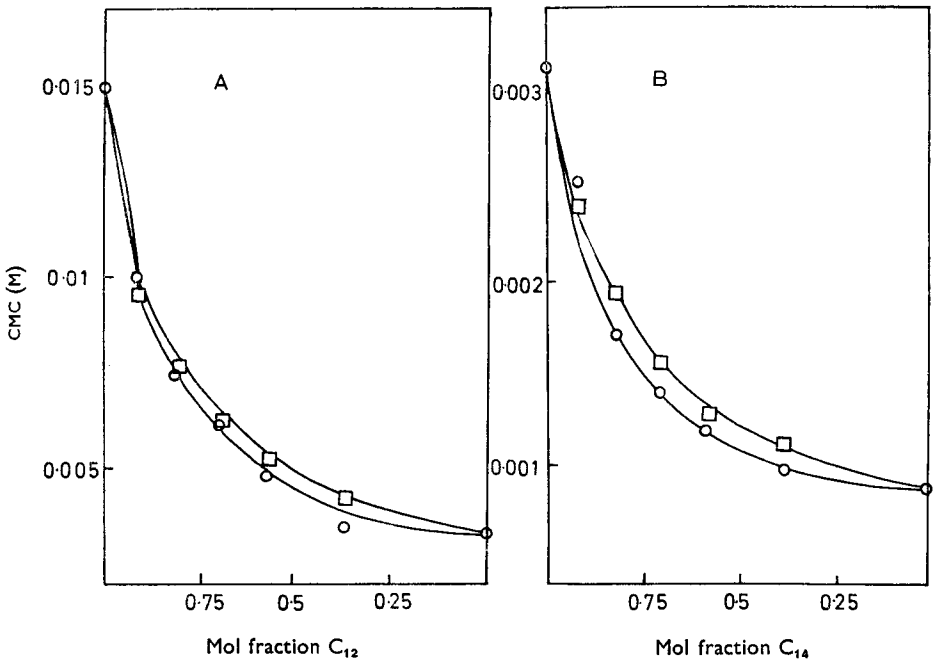

FIG. 1. The CMC at 21° of binary mixtures of cationic surfactants determined by the surface-tension method (circles) compared with calculated values (squares). A. C_{12}/C_{14} mixtures. B. C_{14}/C_{16} mixtures.

The CMC of cetrimide was 2·2 mmol litre^{-1}. The CMC values of the mixtures were always between those of the individual components, although, small amounts of the component with the lower CMC produced a disproportionately large reduction (Klevens, 1948; Lange, 1953 & Shinoda, 1954). The last two authors have examined the CMC values of surfactant mixtures which may be calculated from the values of the individual components and the equation used by Shinoda is:

$$\frac{C_{m1}^{1+Kg}\, x'}{x' + (1 - x') \exp\left[(m_2 - m_1)\, \omega/kT\right]} + \frac{C_{m2}^{1+Kg}\,(1 - x') \exp\left[(m_2 - m_1)\, \omega/kT\right]}{x' + (1 - x') \exp\left[(m_2 - m_1)\, \omega/kT\right]} = C_{mix}^{1+Kg}$$

Where C_{m1}, C_{m2} and C_{mix} are the CMC of the two surfactants and the mixture respectively; x' and $(1 - x')$ are the respective mol fractions; m_1 and m_2 are the numbers of carbon atoms in the paraffin chains; ω is the surface energy change per methylene group passing from the aqueous environment into the micelle and is equal to 1·08 kT; K_g is a constant relating the CMC and the concentration of gegenions. A value of 0·56 for K_g was used (Shinoda, 1954).

Biological measurements

The British Standard method (B.S. 3286: 1960) for "Laboratory Evaluation of Disinfectant Activity of Quaternary Ammonium Compounds by Suspension Test

Procedure" was used for measuring the antibacterial activity of the surfactants and their mixtures. In the particular form of the test adopted, a suspension of a test organism (*Escherichia coli*, NCTC 86) was treated at 21° with a range of concentrations of the surfactants and their mixtures for a fixed time of 15 min so that at least three and preferably four solutions gave survivors in the range 10 to 0·0001%.

Of the two inactivators recommended in the British Standard, 2% lecithin mixed with 3% "Lubrol W", and 10% polysorbate 80, only the polysorbate was satisfactory. The lecithin–Lubrol W inactivator was sometimes lethal. Suspensions of the test organism from subcultures between the 4th and 14th, containing about 100×10^6 organisms per ml were used in the tests and the numbers surviving after treatment with the surfactants were counted by the plate method using a nutrient agar medium (Oxoid CM3). Duplicate plates were used in all counts and an average taken from the results. Plots of log concentration of surfactant against log % survivors gave straight lines (visual estimation) Fig. 2. The error of repeatability lies within ±30%.

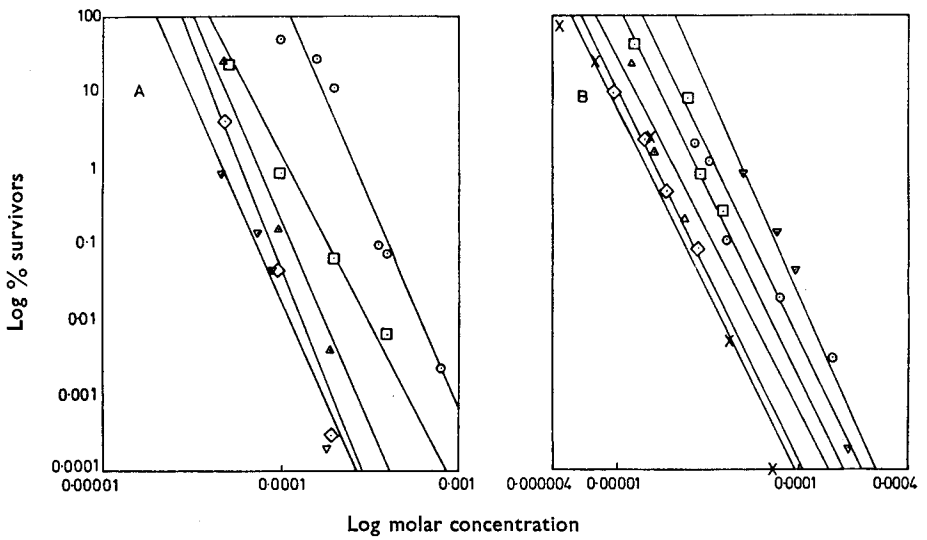

FIG. 2. Effect of concentration of quaternary ammonium compounds on *E. coli*. A, C_{12}/C_{14} mixtures. B, C_{14}/C_{16} mixtures and a ternary mixture.
A. ○ = C_{12}; □ = C_{12}/C_{14}, 0·766/0·234; △ = C_{12}/C_{14}, 0·522/0·478; ◇ = C_{12}/C_{14}, 0·267/0·733; ▽ = C_{14}. B. ▽ = C_{14}; ○ = $C_{12}/C_{14}/C_{16}$, 0·251/0·628/0·121; □ = C_{14}/C_{16}, 0·764/0·236; △ = C_{14}/C_{16}, 0·520/0·480; ◇ = C_{14}/C_{16}, 0·265/0·735; × = C_{16}.

DISCUSSION

One of the ways in which Ferguson's principle may be stated in relation to the toxic effects of materials on bacteria, is that solutions of compounds having the same thermodynamic activity will have equal antibacterial action. For sparingly soluble materials the thermodynamic activity of a solution is given with reasonable accuracy by the ratio of the solution concentration and the concentration of a saturated solution at the same temperature. With surfactants it is convenient to treat the CMC as a solubility limit for the monomolecularly dispersed species (Hartley, 1936), especially when the aggregation number of the micelles is high (Elworthy, Florence & Mac-

farlane, 1968). Light scattering measurements give an aggregation number of 50 for DTAB and higher values for the longer-chain homologues (Debye, 1949). It therefore seems reasonable to calculate the thermodynamic activity of solutions of quaternary ammonium surfactants below the CMC as the ratio of the solution concentration and the CMC. In Table 1 the concentrations of the quaternary ammonium compounds and certain binary and ternary mixtures which kill 99·0 and 99·99% of *E. coli* cells in 15 min at 21° are listed together with related CMC. The latter were derived from the data in Fig. 1. The last two columns of Table 1 compare the thermodynamic activities at the two survivor levels. In almost every case (TTAB values appear slightly low) the thermodynamic activity producing an equivalent biological response is sufficiently close to a constant, bearing in mind the limited accuracy of the antibacterial test, to sustain the Ferguson principle for the systems studied. Thus the antibacterial activity of individual members of the n-alkyl trimethylammonium bromides is linked to their thermodynamic activities and the relation also extends to binary and ternary mixtures.

In comparing biological activity with the surface properties of the antibacterial solutions it is essential to maintain equivalent physical conditions in both sets of measurements. This is relatively easy to do with the physical study but the presence of the cells in the biological measurements almost certainly introduces a variation. Some of the surfactant will be taken up on the cell surfaces and the cells may also release materials which could affect the physical activity of the antibacterial agent by complex formation, precipitation reactions or other means. Reduction in physical activity by adsorption will be less serious if the cell concentration is low and the comparisons are made at low survivor levels where the solution concentrations are high. The results do not provide any indication that the aforesaid factors have a significant effect even for the HTAB system where the solutions are very dilute.

The use of surface concentrations rather than bulk solution concentrations, Weiner & others (1965), is attractive since the mode of action of quaternary ammonium compounds may well involve the cell surface and be analogous to adsorption at the air-water interface. But surface concentrations are difficult to measure and for theoretical reasons the measurements are normally done in the presence of high concentrations of salt (Weiner & Zografi, 1965), which may itself affect the cells. Weiner & others (1965) reported results using bulk concentrations which show a variation in thermodynamic activities of the equitoxic solutions wider than we have found. Their results based on surface concentrations show exceptionally constant thermodynamic activities. However, whether based on bulk or on surface concentrations the thermodynamic activity producing a level of antibacterial activity must always be derived from a ratio involving the latter, and some variation must be accepted. In the present results this is not greater than that expected from the limitations of the biological measurements.

Calculation of the CMC of multi-component systems from data for the individual components is useful since it enables levels of antibacterial activity to be forecast. For example the concentration of a C_{12}/C_{14} mixture (mol fraction C_{12}, 0·522) which gives a 99·99% kill is 0·175mM (Table 1), based on the experimentally determined CMC. The concentration required by calculation of the CMC of this mixture is 0·21mM. The theory also shows that the CMC of multi-component mixtures will always lie between that of the individual components. Since it is linked to antibacterial action, the same will be true of the biological effect of mixtures. Standards for homologue composition of quaternary ammonium surfactants could be set on this basis.

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REFERENCES

ADDERSON, J. E. & TAYLOR, H. (1964). *Proc. 4th Int. Congr. Surf. Activ.*, **2**, 613–619, Brussels.
DEBYE, P. (1949). *J. phys. Colloid Chem.*, **53**, 1–8.
ECANOW, B. & SIEGEL, F. P. (1963). *J. pharm. Sci.*, **52**, 812–813.
ELWORTHY, P. H., FLORENCE, A. T. & MACFARLANE, C. B. (1968). Solubilization by Surface-active Agents, p. 49, London: Chapman and Hall.
HARKINS, W. D. & JORDAN, H. F. (1930). *J. Am. chem. Soc.*, **52**, 1751–1772.
HARTLEY, G. S. (1936). *Aqueous Solutions of Paraffin-Chain Salts*, Paris: Hermann and Cie.
KLEVENS, H. B. (1948). *J. phys. Colloid Chem.*, **52**, 130–148.
LANGE, H. (1953). *Kolloid-Z.*, **131**, 96–103.
LAYCOCK, H. H. (1969). *M. Pharm. thesis*, Univ. of Bradford.
LAYCOCK, H. H. & MULLEY, B. A. (1966). *J. Pharm. Pharmac.*, **18**, *Suppl.*, 9S–11S.
PADDAY, J. F. (1960). *Proc. 3rd Int. Congr. Surf. Activ.*, **1**, 233–238, Cologne.
SHINODA, K. (1954). *J. phys. Chem., Ithaca*, **58**, 541–544.
WEINER, N. D., HART, F. & ZOGRAFI, G. (1965). *J. Pharm. Pharmac.*, **17**, 350–355.
WEINER, N. D. & ZOGRAFI, G. (1965). *J. pharm. Sci.*, **54**, 436–442.