

## COMMUNICATIONS

### Solubilization in a non-aqueous solvent

The ability which solutions of surfactants in organic solvents, have to solubilize large quantities of water, is well known (Shinoda, 1967). In particular, the anionic surfactant di-(2-ethylhexyl) sodium sulphosuccinate (Aerosol OT, Manoxol OT) has been widely used in such studies. Recently, Frank & Zografi (1969), have demonstrated the phase changes which occur on the addition of water to Aerosol OT in various hydrocarbon solvents. Initial quantities of water are solubilized giving clear solutions. Continued addition of water gives rise to first a turbid region followed by a blue translucent region and a subsequent second turbid region. The amount of water incorporated before the first turbid region is observed, is quite high (e.g. 20 mol H<sub>2</sub>O mol<sup>-1</sup> Aerosol OT in octane, Frank & Zografi, 1969).

The properties of water are often profoundly affected by the addition of other polar molecules. We have therefore investigated the effect which the addition of such molecules to water might have on the solubilization of water in non-aqueous solvents by examining the solubilization of water, and of water-formamide, and water-*N*-methyl formamide (NMF) mixtures, by Manoxol OT (British Drug Houses) in heptane.

Solubilization, at 293K ± 0.5, was by adding H<sub>2</sub>O-amide mixtures from a micro-meter syringe to solutions of the surfactant, until the first signs of turbidity appeared. The solutions were equilibrated overnight, and final adjustments to the amounts of solubilize required to saturate the system were made, if necessary.

Fig. 1 shows a linear dependence between mol H<sub>2</sub>O solubilized mol<sup>-1</sup> Manoxol OT and concentration of Manoxol OT for various H<sub>2</sub>O-NMF mixtures. It can be seen that the amounts of H<sub>2</sub>O incorporated rise initially, for low concentrations of NMF in the mixture, whilst further increases in the amide concentration result in decreases in the amount of H<sub>2</sub>O solubilized. Similar behaviour was observed for H<sub>2</sub>O-formamide mixtures. In Fig. 2 the ratio of mol water solubilized mol<sup>-1</sup> Manoxol OT is plotted against mol fractions of amide for both H<sub>2</sub>O-formamide and H<sub>2</sub>O-NMF mixtures, in

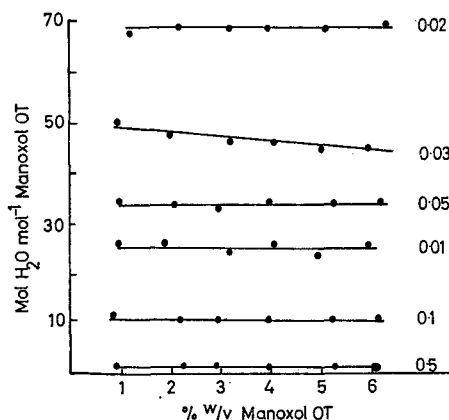


Fig. 1. Mol H<sub>2</sub>O solubilized per mol Manoxol OT in heptane, as a function of the concentration of Manoxol. Individual lines represent mol fractions of NMF in the H<sub>2</sub>O - NMF solubilizates.

a 5% w/v Manoxol OT solution. For H<sub>2</sub>O–NMF systems the total amount solubilized reaches a peak between NMF mol fractions of 0.015 and 0.02, where approximately 4.5 times more H<sub>2</sub>O is solubilized than in the absence of NMF. For H<sub>2</sub>O–formamide systems the peak occurs between mol fractions 0.030 and 0.035, and the amount of H<sub>2</sub>O solubilized is increased by approximately 4.1 times.

Confirmation of the solubilization results was obtained by light scattering. Measurements were made with a Fica 42000 photogonio-diffusometer at a wavelength of 546 nm. Solutions were clarified by filtration through 0.1  $\mu$ m Millipore filters and the refractive index increments were measured at 546 nm using a differential refractometer. The micellar weight of Manoxol OT in heptane was found to be  $1.19 \times 10^4$ . Kon-No & Kitahara (1971) obtained a value of approximately  $9.30 \times 10^3$  using a number-average molecular weight technique, which might be expected to give lower values than light-scattering. For H<sub>2</sub>O–NMF systems, preliminary measurements showed that the micellar size reached a maximum at a similar H<sub>2</sub>O–NMF ratio as the maximum in the amount solubilized.

It is assumed, generally, that the H<sub>2</sub>O molecules are solubilized in the centre of the Manoxol OT micelles due to an ion-dipole interaction between H<sub>2</sub>O and the Na<sup>+</sup> of the anionic surfactant (Kitahara & Kon-No, 1969). Since the amounts solubilized at low concentrations of formamide and NMF in the H<sub>2</sub>O–amide mixtures are very much higher than for H<sub>2</sub>O alone, it may be concluded that the interactions between the mixed systems and the Na<sup>+</sup> are greater. This may not be surprising since both formamide and NMF have very high dielectric constants and might be expected to react readily with ions.

These systems may also be compared with water–oil microemulsions. Both formamide and NMF have been shown to reduce interfacial energy at the hydrocarbon–water interface (McDonald, unpublished results). Their role in the formation of the large micelles may resemble that of the amphiphilic compounds, such as alcohols, which are used in the formation of microemulsions. Such amphiphiles distribute themselves between the aqueous interior of the microemulsion droplets and their interfacial surfactant layer, causing liquefaction of the interfacial layer and facilitating the incorporation of large amounts of solubilize. Excess of the amphiphile normally leads to decreased solubilization, as was observed in this investigation. The interfacial tension decrease caused by a given molar concentration of NMF is greater than that for an equivalent concentration of formamide which may explain differences in the peak positions noted in Fig. 2.

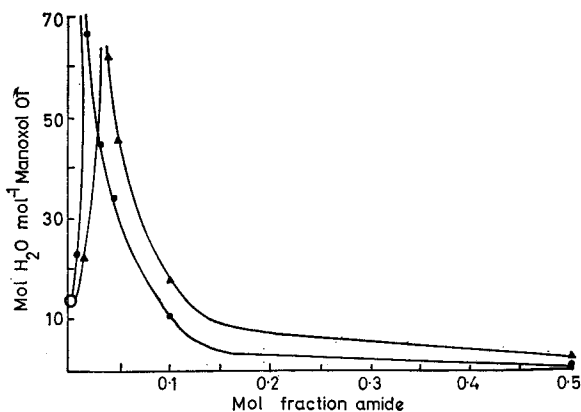


Fig. 2 Mol H<sub>2</sub>O solubilized per mol Manoxol OT as a function of the mol fraction of ▲ formamide and ● NMF in the H<sub>2</sub>O–amide solubilizates. Concentration of Manoxol in heptane is 5% w/v for all systems.

Department of Pharmacy,  
The University,  
Manchester H13 9PL, U.K.

D. ATTWOOD  
C. McDONALD  
SYLVIA C. PERRY

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## Antimicrobial actions of hexachlorophene: iron salts do not reverse inhibition

We have studied the actions of hexachlorophene [2,2'-methylenebis (3,4,6 trichlorophenol); HCP] on cells of *Bacillus megaterium*. HCP produces detectable holes in protoplast membrane (Silvernale, Joswick & others, 1971), promotes leakage of intracellular components except DNA (Joswick, Corner & others, 1971) and causes lysis and fixation of protoplasts (Corner, Joswick & others, 1971). All these effects result from HCP doses greater than the minimum lethal dose. More recently, Frederick, Corner & Gerhardt (1974) found that the minimum lethal dose correlates only with respiratory inhibition and, furthermore, that the site or sites of maximum sensitivity lie within the electron transport chain. We were interested by the report of Adams & Hobbs (1958) that exogenous iron sources cause a reversal of HCP action on *Staphylococcus aureus*. We have examined this putative reversal in *B. megaterium* and also in *S. aureus* but have been unable to confirm their findings.

The possibility of iron reversal was examined in two ways. First, the qualitative filter paper assay of Adams & Hobbs (1958) was used for both an attenuated coagulase positive strain of *S. aureus* and the asporogenous KM strain of *B. megaterium*. All methods, including cell culture techniques, were exactly as described by Adams & Hobbs (1958) for *S. aureus* and, except for the substitution of a different primary growth medium (2% Oxoid peptone), were also used for *B. megaterium*. Second, iron salts were added to cells in the Warburg respirometer (Umbreit, Burris & Stauffer, 1964) to determine whether or not the salts could reverse HCP inhibition of endogenous oxygen uptake. *B. megaterium* was grown according to Frederick & others (1974) and *S. aureus* was grown in nutrient broth as described by Adams & Hobbs (1958).

The contention that iron salts can reverse HCP-mediated growth inhibition was not supported by our results with the qualitative filter paper assays. With *B. megaterium*, concentrations of HCP from 0.2 to 10  $\mu\text{g ml}^{-1}$  were incorporated in the media. In terms of biomass, these concentrations would have represented from about 27 to 1140  $\mu\text{g mg}^{-1}$  of cells (dry weight) provided they were exposed to all the HCP in the solid medium. Proteins and amino acids interact with HCP (Gould, Bosniak & others, 1953; Haque & Buhler, 1972) so that there is uncertainty about the amounts