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# A study of aqueous systems of purified non-ionic surfactant by membrane osmometry

The application of the membrane osmometry to the determination of number average micellar molecular weights ($\bar{M}_n$) has been reported by Coll (1969, 1970) and Attwood, Elworthy & Kayne (1969, 1970). We report the value of $\bar{M}_n$ of a purified sample of a non-ionic surfactant of pharmaceutical interest, polysorbate 80 and show that the effect of solubilizing nitrofurazone in micelles can be studied by membrane osmometry. Further, osmotic pressure measurements on the purified sample are compared with the commercial sample as well as a sample containing a known amount of polyoxyethylene glycol 600 (PEG 600).

A commercial sample of polysorbate 80 (polyoxyethylene-20-sorbitan monooleate) B.P.C. was purified by partitioning between 5N sodium chloride solution and ethyl acetate (Weibull, 1960). Samples were dried under passage of indifferent gas for 2 h at 30°/15 mm Hg followed by 1 h at 30°/3 mm Hg. Precipitated salts were filtered off through a sintered glass filter (G 3). Removal of contaminating substances was followed by thin-layer chromatography (Thakkar, Kuhn & Hall, 1967; Cerdas, Carlier & others, 1968). The extraction procedure removed polyoxyethylene glycols or polyoxyethylated sorbitans (or both). The saponification values for the purified and non-purified polysorbate 80 were 61·1 and 48·8 respectively; the hydroxyl values were 54·6 and 74·0 respectively. The critical micelle concentration (cmc) for the purified polysorbate 80 at 25° was found by the method of Becher (1962) to be 0·11 g/litre (extrapolated value). Nitrofurazone B.P.C. was found to be solubilized to an extent of $2{\cdot}32 \times 10^{-5}$ mol/g surfactant by purified polysorbate 80 aqueous solutions at 25·0° (±0·05°).

A Melabs CSM-2 recording membrane osmometer was used in these measurements at 25·0° (±0·1°). A cellulose membrane (prepared by drying a film from a 25% solution of cellulose acetate in acetone + dimethylformamide and treating the film for about 5 min in 70° water) was used for all the measurements. The molecular weight of a protein, cytochrome-C (mol. wt. 13 400), was found from the osmometer to be 15 000, thus showing that the membrane was non-permeable to molecules larger than 15 000. The accuracy of osmometer was ±0·1 cm of solvent.

In all experiments, solutions of concentration much larger than the cmc were placed on the solution side, and the solvent side was filled with a solution of 1 g/litre (several times the cmc). The number of monomers in equilibrium with micelles in the above solutions does not significantly increase with concentration (i.e. over the cmc). Hence the osmotic pressure arising from the monomers will be expected to be negligible. Since the membrane was found to be non-permeable to molecules of weight over 15 000, we did not consider any possibility of micelle diffusion ($\bar{M}_n \gg$

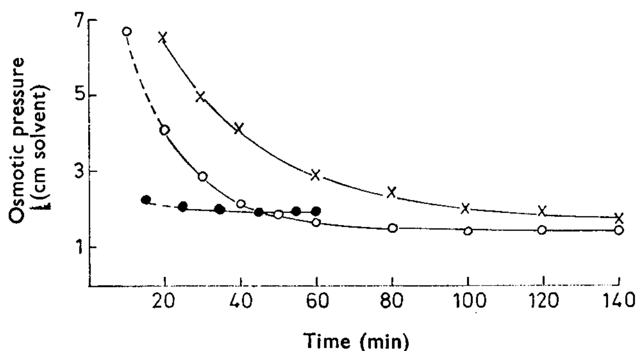


FIG. 1. Variation of osmotic pressure with time at 25° (concentration 9·00 g/litre). ● = purified polysorbate 80. ○ = mixture of purified polysorbate 80 and PEG 600 (4:1 w/w). × = non-purified polysorbate 80.

15 000). In the experiments with nitrofurazone, both the solutions in the solvent and solute side were saturated with nitrofurazone.

The experimentally measured osmotic pressure, P, between a sample solution of concentration c g/litre and a solvent of concentration c′ g/litre is given by (Coll, 1970; Attwood & others, 1970)

$$P = RT (c - c')/\bar{M}_n + RTB [(c - c')^2 + 2(c - c') (c' - cmc)]$$

where B is the second virial coefficient, RT has the usual values. The plots of $P/(c - c')$ versus $(c - c')$ were extrapolated to $c=c'$, and $\bar{M}_n$ was calculated, as given below:

$$[P/(c - c')]_{c=c'} = RT/\bar{M}_n + 2RTB(c' - cmc)$$

B had a value of $0{\cdot}5 \times 10^{-4}$ ml mol $g^{-2}$, and the correction term $2RTB(c' - cmc)$ gave a small correction of about 1% on $\bar{M}_n$.

The values of $\bar{M}_n$ for purified polysorbate 80 with and without nitrofurazone are 123 and 121 $\times 10^3$ respectively (at 25°). Thus the $\bar{M}_n$ does not change due to solubilization of nitrofurazone, within the experimental error (maximum 5%). This is to be expected, since only 2–3 molecules of nitrofurazone are solubilized per micelle.

The following systems were studied in order to determine the effect of impurities on the osmotic pressure: (*a*) purified polysorbate 80; (*b*) mixture of polysorbate 80 and PEG 600 (4:1 w/w); (*c*) non-purified polysorbate 80. The osmotic pressure curves against time are given in Fig. 1.

In the osmometer used, the rapid fall due to instrumental factors occurs within the first 5–10 min. Thereafter, any slow decrease is attributable to the diffusion of the solute. It can thus be concluded from Fig. 1, that the impurities in the sample are not completely solubilized in the micelles, thus giving rise to the initial decrease in osmotic pressure, when comparing the curves for purified and non-purified samples. Further, the curve for polysorbate 80 + PEG 600 mixture shows that PEG 600 gives rise to osmotic pressure and is not completely solubilized by the micelles, as expected from its hydrophilic nature.

These studies indicate that membrane osmometry of micellar systems can be useful in the determination of the effect of solubilization on the micellar molecular weight. Further it is possible to determine whether impurities are completely solubilized by the micelles.

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# Further evidence on the question of polymorphism in aspirin

Several reports have recently appeared describing the preparation and properties of polymorphic forms of aspirin (Tawashi, 1968; Summers, Carless & Enever, 1970). Other reports have however suggested that the evidence for polymorphism so far presented is inconclusive and that the differences which exist between the various forms could be due to differences in crystal size and habit (Pfeiffer, 1971) or to crystal defects (Mitchell, Milaire & others, 1971). We wish to report some observations we have made during an investigation into the biopharmacy of salicylates which may help in resolving the conflict between these various viewpoints.

Preliminary studies using the procedures of Tawashi (1968) and Summers & others (1970) suggested that some salicylic acid was formed on dissolving aspirin in non-aqueous solvents. These observations were further investigated by equilibrating excess dry aspirin at 20° in sodium dried n-hexane and n-octane. Samples of solution were removed at various times and their absorbance measured over the range 270–320 nm using an SP 500 spectrophotometer. After 400 h 40 $\mu$g/ml of aspirin and 20 $\mu$g/ml of salicylic acid were found in the n-hexane solution and 30 $\mu$g/ml of aspirin and 10·5 $\mu$g/ml of salicylic acid in the n-octane using the Extinction Ratio method.

In view of the low solubility of aspirin in n-hexane at 20° it was necessary, in order to obtain an adequate yield, to reflux excess dry aspirin with n-hexane at 68° for 1½–2 h. After filtration, analysis of the hot solution showed the presence of approximately 600 $\mu$g/ml of aspirin and 60 $\mu$g/ml of salicylic acid. Portions of the hot solutions were allowed to stand at 0° or 20° for 14 h and the resulting crystals collected by filtration. The filtrates were then allowed to evaporate at the same temperatures to approximately a quarter of their original volume. After 200–350 h further samples of crystals were collected by filtration. The properties of the crystals obtained from these experiments are shown in Table 1 together with those of samples prepared by recrystallization from 96% ethanol and by sublimation at 118° onto a cold surface at 17° under various pressures.

The amount of salicylic acid present in the samples was determined both by the Extinction Ratio method in absolute ethanol and by a modification of the B.P.