Solubilisation of preservatives by non-ionic detergents

SIR,—Donbrow & Rhodes (1965) suggest that my potentiometric method of determining solubilisation constants (Evans, 1959; 1964) differs from their method (Donbrow & Rhodes, 1963; Rhodes, 1964) in some important respects that involve fundamental points of theory and interpretation. On the contrary both methods are based on the same principle that changes in hydrogen ion concentrations which occur with mixtures of weak acids (or bases) and surfactants are due to the solubilisation of the unionised species by the detergent micelles. Assuming this to be so, the main difference lies in the method of calculating the distribution constants from the observed changes in hydrogen ion concentration, which for the sake of brevity was not given in the original papers (Evans, 1959; 1964).

From measured pH, $$-\log (H^+) = pH - 0{\cdot}5\sqrt{I} \quad .. \quad .. \quad (1)$$

From electroneutrality, $$(A^-) = (H^+) + (Na^+) \quad .. \quad .. \quad (2)$$

From dissociation Constant of acid K_a, $$\log (HA)_w = \frac{\log (H^+)(A^-)}{K_a} - \sqrt{I} \quad .. \quad (3)$$

By difference, $$(HA)_m = (HA)_T - (HA)_w - (A^-) \quad .. \quad (4$$

where I is the ionic strength, and $(HA)_T$, $(HA)_w$, $(HA)_m$ and (A^-) are total acid concentration, the concentration of unionised acid in the water phase, the concentration of unionised acid in the micellar phase, and the concentration of ionised acid in the water phase respectively (moles/litre). Thus although the ionic strength varies during the titration, it is low and is allowed for in the calculations. As an alternative procedure, the free acid can be titrated (Evans & Dunbar, 1964) either in the presence of a swamping concentration of electrolyte (e.g. 0·1M sodium chloride) to keep ionic strength constant, or in some other base medium which simulates conditions in a product or conditions in biological tests.

Since Evans & Dunbar (1964) have shown that data from their recent biological studies are in good agreement with those calculated from the potentiometric method, it would seem that the potentiometric method gives results of sufficient accuracy for bactericidal or pharmacodynamical studies. Furthermore the distribution constants obtained by my method have been used to calculate the concentration of "free" methyl *p*-hydroxybenzoate in polysorbate (Tween) 80 systems and have been shown by Evans & Dunbar (1964) to be in excellent agreement with those obtained by dialysis (Pisano & Kostenbauder, 1959).

This potentiometric method measures the activity of the acid in the water phase, and by difference the concentration of the acid in the micellar phase can be obtained. It is realised of course that the pseudophase model is an approximation, and arguments for and against the model have been given by Hutchinson, Inaba & Bailey (1955), Pethica (1960), Mukerjee (1962) and Elworthy & Macfarlane (1965). In view of the constancy of the calculated distribution constants under the conditions studied (Evans, 1964) the results do not conflict with the pseudophase model—that is, the micelles behave as a separate solvent phase and the solute distribution can be treated quantitatively. That the presence of micelles as a separate phase in the more concentrated solutions (Evans, 1964) should decrease the pH of hydrochloric acid by 0·1 unit (Donbrow & Rhodes, 1965) is debatable, but if accepted, the decrease in the most concentrated solution studied (Evans, 1964) should be no more than 0·04 pH unit. Since in the original work (Evans, 1959; 1964) the pH value was estimated in the second decimal place, the quoted pH values are accurate to $\pm 0{\cdot}03$ pH units. A decrease of less

than 0·04 pH units in 0·01M hydrochloric acid in the presence of a detergent would therefore barely be detectable, and would certainly be insignificant compared to the changes of up to 2 pH units observed with acids which are solubilised.

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The effect of monoamine oxidase inhibition on guanethidine-induced noradrenaline release and sympathetic blockade

SIR,—We have measured the noradrenaline content of the hearts of rats injected with iproniazid alone or followed by guanethidine and have related the noradrenaline depletion to the monoamine oxidase inhibition.

The noradrenaline in individual rat hearts was measured after butanol extraction by fluorimetry (Fielden & Green, 1965). Monoamine oxidase activity was assayed by the dinitrophenylhydrazine method (Green & Haughton, 1961). The hearts from groups of 4 rats were homogenised in 5 or 6 volumes of 0·1 M phosphate buffer (pH 7·4) and 3·2 ml samples of the homogenates were shaken in air at 25° with 0·125 M semicarbazide (0·4 ml) and 0·1 M tyramine (0·4 ml). After 30 min., the reaction was terminated with 0·5 N acetic acid (1 ml); the remaining steps in the assay were then as previously described.

Table 1 summarises the results of experiments in which various doses of iproniazid phosphate were injected subcutaneously into rats 20 hr before subcutaneous injection of guanethidine sulphate (10 mg/kg). The rats were killed after a further 4 hr for assay of the noradrenaline and monoamine oxidase in their hearts. Sympathetic blockade at this time was estimated from the extent of ptosis, which was recorded on a 0–8 scale (Rubin, Malone, Waugh & Burke, 1957). The 20 hr interval was chosen to minimise interference by shorter-lasting effects of iproniazid unconnected with monoamine oxidase inhibition, but very similar results were obtained in a few experiments in which the iproniazid was given only 2 hr instead of 20 hr before the guanethidine, as was done by Gessa, Cuenca & Costa (1963). It is clear from Table 1 that no significant protection would be afforded against guanethidine-induced noradrenaline