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A note on the solubilization of preservative mixtures by cetomacrogol

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Preliminary data are reported for the solubilization of several pairs of preservatives by the non-ionic surfactant cetomacrogol. In all cases the addition of a second preservative altered the equilibrium solubility of the first to an extent which depended on the particular co-solute and the concentration added.

While the interaction of preservatives with non-ionic surfactants has received much attention, information concerning the solubilization of preservative mixtures is scarce. The behaviour of such systems is of interest because of their frequent use in pharmaceutical and cosmetic products. At present, effective preservative concentrations must be selected using data derived from individual preservatives. However, one preservative may influence the binding of another and significantly alter the total amount of preservative that must be added to maintain an effective free concentration.

METHODS

Cetomacrogol was treated as described previously (Mitchell & Brown, 1966). All preservatives were recrystallized before use.

Solubilities were determined by rotating excess solid preservative, in water or cetomacrogol solutions containing an appropriate concentration of a second or co-solute preservative, at 25 ± 0·1° for 48 h. Equilibrated solutions were filtered and assayed as described below. In surfactant systems containing either excess chloroxylenol or excess of two preservatives, a water-immiscible liquid phase separated. For these systems an equilibrium dialysis technique was employed using Perspex dialysis cells of the design described by Patel & Foss (1964) and a nylon semi-permeable membrane. Sufficient of each solute to saturate both aqueous and surfactant solutions was added to the aqueous compartment. The cells were rotated at 25 ± 0·1° for 7 days after which the surfactant compartment was sampled and assayed for each preservative. Results obtained using this technique for chloroxylenol were in agreement with those obtained using the method of Mulley & Metcalf (1956).

Benzoic acid was estimated by potentiometric titration with sodium hydroxide. Dichloroxylenol was estimated by the 4-amino-antipyrine colorimetric reaction (Brown, Guttman & Anderson, 1969). Methyl paraben, propyl paraben and chloroxylenol were estimated spectrophotometrically at 256, 256 and 285 nm respectively. Solutions containing both methyl and propyl parabens were extracted with three, 5 ml volumes of chloroform. The extracts were pooled, concentrated and adjusted to a suitable volume with sodium-dried ether. Both preservatives were estimated by gas-liquid chromatography using butyl paraben as internal standard, a flame ionization detector and columns packed with 3% cyclohexanedimethanol succinate on Gas-Chrom Q.

In solutions containing benzoic acid and methyl paraben, benzoic acid was determined by potentiometric titration. Methyl paraben in these solutions was estimated spectrophotometrically by correcting the absorbance observed at 256 nm for the absorbance contributed by benzoic acid. Absorbances of methyl paraben and benzoic acid are additive at 256 nm. A similar procedure was used for the estimation of methyl paraben in the presence of dichloroxylenol. In this case dichloroxylenol was first estimated directly by the 4-aminoantipyrine method. Phenols with *p*-alkyl substituents, including methyl paraben are not detected (Emerson & Beegle, 1943). Methyl paraben and chloroxylenol, in solution together, were estimated using the spectrophotometric procedure described by Reilley & Sawyer (1961) for the analysis of a two-component mixture. The absorbances of both compounds were additive at 256 and 285 nm.

RESULTS AND DISCUSSION

The water solubilities of all preservatives were unchanged by the addition of a second preservative. This implies that small molecule/small molecule complexes were not formed in significant concentration in aqueous solution.

The effect of varying concentration of propyl paraben on the solubility of methyl paraben in different cetomacrogol concentrations is shown in Fig. 1A. At low concentrations, the propyl ester produces a sharp increase in solubility which reaches a maximum and then declines. The solubility then increases approximately linearly with propyl paraben concentration. The similarity in shape of the curves for different surfactant concentrations indicates that the solubility change is largely independent of surfactant concentration.

The addition of benzoic acid and dichloroxylenol to cetomacrogol solutions also causes changes in the solubility of methyl paraben (Fig. 1B), although the results for each compound are qualitatively different. Increasing concentrations of benzoic acid produces an apparently linear increase, while with dichloroxylenol, the solubility initially decreases to a minimum and then rises again. The addition of chloroxylenol produces similar effects to those seen with dichloroxylenol (Fig. 2A). Here again it is evident that the change in solubility of the methyl ester shows little dependence on cetomacrogol concentration.

The influence of increasing concentrations of methyl paraben on the solubilization of propyl paraben and chloroxylenol is shown in Fig. 2B. The propyl ester undergoes solubility changes similar to those exhibited by methyl in the presence of propyl. However, beyond the maximum, the concentration of propyl paraben which dissolves is significantly less than the solubility when no co-solute is present. This is in contrast to the net increase in solubility seen with the methyl ester (Fig. 1A).

The solubility of chloroxylenol is dramatically reduced by the addition of methyl paraben (Fig. 2B). Cetomacrogol solutions saturated with methyl paraben will dissolve only 61% of the chloroxylenol that can be solubilized in solutions free from the methyl ester.

The data of Figs 1 and 2 serve to illustrate that solubilities of preservatives solubilized as mixtures may differ substantially from those determined for the compounds individually. Furthermore it is apparent that different co-solute preservatives may have qualitatively different effects, while the magnitude of the increase or decrease in solubility is highly dependent on the concentration of co-solute.

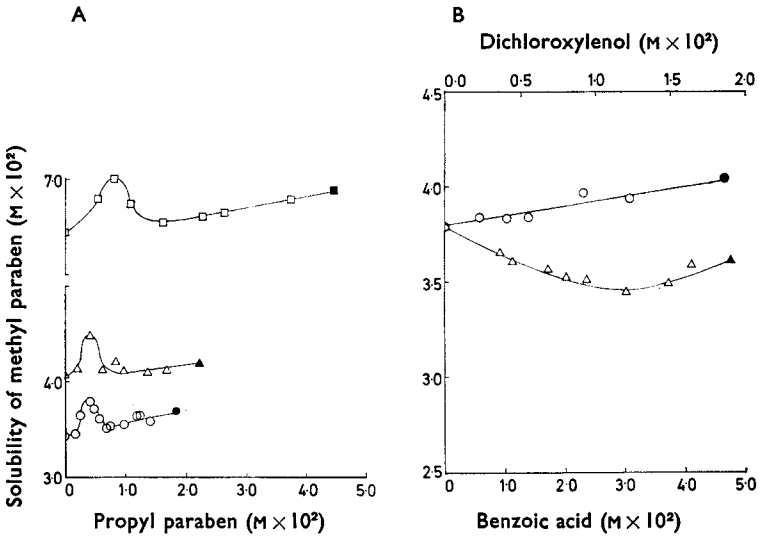

FIG. 1. A. The solubility of methyl paraben as a function of concentration of propyl paraben and cetomacrogol at 25°. Cetomacrogol concentrations: □—□ 0·042M, △—△ 0·021M, ○—○ 0·019M. Solid points indicate solutions saturated with respect to both methyl and propyl esters.

B. The solubility of methyl paraben in 0·019M cetomacrogol solutions at 25° as a function of △—△ dichloroxylenol and ○—○ benzoic acid concentration. Solid points indicate solutions saturated with respect to both methyl paraben and co-solute.

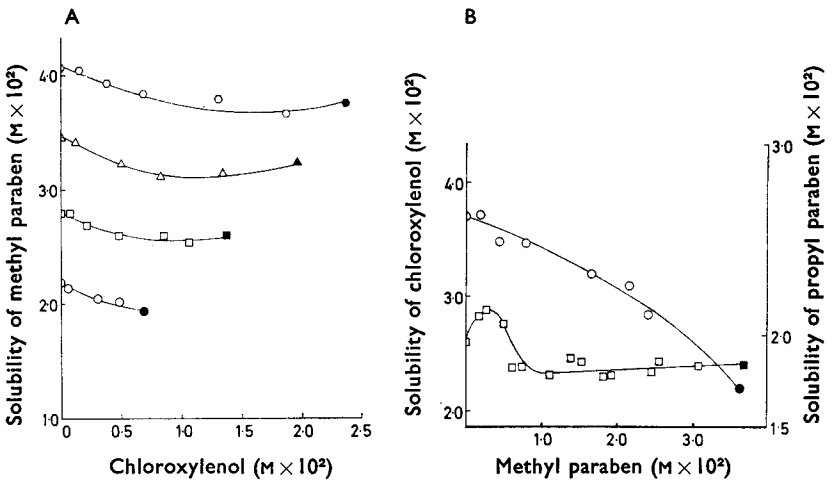

FIG. 2. A. The influence of varying concentration of chloroxylenol on the solubility of methyl paraben at 25° in solutions containing cetomacrogol of concentration hexagons 0·0208M, △—△ 0·0156M, □—□ 0·0104M, ○—○ 0·0052M. Shaded points indicate solutions saturated with respect to both methyl paraben and chloroxylenol.

B. The Influence of varying concentration of methyl paraben on the solubility of □—□ propyl paraben and ○—○ chloroxylenol in solutions containing 0·019M cetomacrogol at 25°. Shaded points indicate systems saturated with respect to both solute and co-solute.

The effect of one preservative on the solubilization of another might be expected to depend on the respective solubilization mechanisms of the two compounds. If the compounds were solubilized by association with specific sites in the micelle it is likely that molecules with similar binding mechanisms would compete for the binding sites. This would lead to a diminished solubility of each. In addition, there is the possibility of a co-solubilization effect where one solubilizate causes structural alterations in the micelle enhancing its capacity for another (Kolthoff & Graydon, 1951). The simultaneous operation of two such mutually antagonistic processes would explain the occurrence of maxima and minima in the solubility plots.

Although the reasons for this behaviour are not clear, these findings may have considerable practical significance. For example, reduction in the degree of interaction of one or both components of a preservative mixture may result in an increased free concentration of preservative, for a given concentration added, and consequently in enhanced antimicrobial activity. The net result would be an apparent synergism between the two preservatives. On the other hand if the extent of binding of one or both compounds is increased the free concentration would then be reduced. Consequently the preservative activity would be less than that anticipated from binding studies of the individual component.

REFERENCES

BROWN, K. F., GUTTMAN, D. E. & ANDERSON, A. S. (1969). *J. pharm. Sci.*, **58**, 1393–1398.
EMERSON, E. I. & BEEGLE, L. C. (1943). *J. org. Chem.*, **8**, 433–437.
KOLTHOFF, I. M. & GRAYDON, W. F. (1951). *J. Phys. Colloid Chem.*, **55**, 699–712.
MITCHELL, A. G. & BROWN, K. F. (1966). *J. Pharm. Pharmac.*, **18**, 115–125.
MULLEY, B. A. & METCALF, A. D. (1956). *Ibid.*, **8**, 774–779.
PATEL, N. K. & FOSS, N. E. (1964). *J. pharm. Sci.*, **53**, 94–97.
REILLEY, C. N. & SAWYER, D. T. (1961). *Experiments for Instrumental Methods*, p. 122. McGraw-Hill: New York.