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Competitive interaction of preservative mixtures with cetomacrogol

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The binding of various preservatives, alone and in pairs, to cetomacrogol has been investigated. Data for the compounds alone indicate that all preservatives combined with two distinct classes of sites in the cetomacrogol micelle. The data were expressed in the form of Scatchard plots and the association constant and number of sites in each class were calculated. All combinations of preservatives were shown to exhibit substantial competition resulting in diminished binding, however the nature of the competitive process is not the same in all cases. Chloroxylenol and dichloroxylenol do not compete significantly with methyl paraben in the first class of sites, but substantial competition was observed in the second class. In contrast, methyl paraben and propyl paraben compete strongly for the first class of sites. Competition with these compounds also occurred in the second class of sites but this was coincident with a large increase in the number of secondary binding sites which tended to increase binding.

Recently, data were reported which indicated that the apparent solubility of a preservative in cetomacrogol solution may be altered substantially by the addition of a second preservative (Crooks & Brown, 1973). It is likely that these observations also reflect changes which occur in under-saturated systems. However, it is difficult to make such predictions on the basis of solubility data alone since the binding is determined at constant preservative activity. There is evidence to indicate that the fraction of preservative bound to the micelles is frequently not a linear function of the total preservative concentration (Mitchell & Brown, 1966; Brown, 1968; Kazmi & Mitchell, 1971; Brown & Crooks, 1973).

In a study concerned with the use of a dynamic dialysis method for studying preservative surfactant interactions (Brown & Crooks, 1973), the dialysis rate of methyl paraben from cetomacrogol solutions was increased by the addition of chloroxylenol to the dialysis bag. This indicated that chloroxylenol decreased the interaction of methyl paraben with the micelles. These findings merit further investigation. The present work is a study of the interaction of several pairs of commonly used preservatives with the non-ionic surfactant cetomacrogol in under-saturated solutions.

MATERIALS AND METHODS

Materials

Cetomacrogol, methyl *p*-hydroxybenzoate (methyl paraben), n-propyl-*p*-hydroxybenzoate (propyl paraben) and chloroxylenol were as described previously (Brown & Crooks, 1973). 2,4-Dichloro-*m*-xylenol (dichloroxylenol, Cocker Chemicals Ltd.) was recrystallized from light petroleum m.p. 95°–96°.

Methods

Analytical procedures for individual preservatives and preservative mixtures were as described by Crooks & Brown (1973). The interaction of preservatives alone, and in the presence of a competitor preservative, was determined either by equilibrium dialysis or dynamic dialysis according to Brown & Crooks (1973). To ensure that the effect of the competitor preservative remained constant throughout each experiment, the *competitor-free concentration* was maintained constant on both sides of the membrane. This was achieved by determining the bound versus free profile for the competitor and surfactant independently. Thus the free concentration for any particular total competitor concentration was known.

RESULTS AND DISCUSSION

There are numerous ways of representing interaction data. These have been compared by Brown (1968) and Kazmi & Mitchell (1971). The main objectives of most of the approaches are firstly to provide some simple index describing the binding behaviour which can be used to predict the preservative activity in a variety of circumstances. Secondly, methods have been suggested for their potential value in elucidating the mechanism of interaction. A number of these includes the implicit assumption that the concentration bound is a linear function of the concentration free. This assumption is made when interaction data are represented by a single micelle water partition coefficient (McBain & Hutchinson, 1955; Evans, 1964; Evans & Dunbar, 1965; Donbrow & Rhodes, 1963; Mitchell & Brown, 1966; Humphreys & Rhodes, 1968) or by calculating the ratio of total/free preservative concentration as a function of surfactant concentration as suggested by Patel & Kostenbauder (1958). It is also made when predictions about the binding in unsaturated solutions are made using solubility data. If the concentration bound is not a linear function of the free concentration, the above indices have little predictive value.

As an alternative to the two-phase approach, binding to micellar surfactant molecules can be considered to obey the law of mass action, as suggested by Garrett (1966). Binding to a surfactant molecule with n identical and independent binding sites, each having association constant K, may be described by the expression:

$$\bar{V} = \frac{D_b}{S} = \frac{n\,K\,D_f}{1 + K\,D_f} \qquad .. \quad .. \quad .. \qquad (1)$$

where $\bar{V}$ is the number of moles of preservative bound per mole of surfacant; D_b and D_f are the concentrations of preservative bound and free respectively and S is the concentration of surfactant. It is apparent that D_b/D_f is not a constant but varies with D_f. However when D_f is very small or, in the case of weak interactions, where K is small, the denominator of equation (1) approaches unity and D_b/D_f approaches a constant analogous to a simple partitioning process. Thus the mass action approach may be considered to be generally applicable.

It is evident from these considerations that to characterize such interaction processes it is necessary to use techniques which permit the binding to be determined at a number of preservative concentrations. The results may then be usefully expressed in the form of a Scatchard plot.

Scatchard plots of the interaction of methyl paraben with cetomacrogol in the presence and absence of chloroxylenol are shown in Fig. 1A. As reported previously

(Kazmi & Mitchell, 1971; Brown & Crooks, 1973) the plots are curved. This implies that the binding sites are not identical and suggests the existence of more than one class of sites. In this case the binding to 'i' classes of sites may be represented by the following:

$$\bar{V} = \sum_{i=o}^{i=i} \frac{nKD_f}{1 + KD_f} \qquad (2)$$

Curved Scatchard plots may be resolved using a modification of the method of Hart (1965) as described by Brown & Crooks (1973) to evaluate the binding constants. It is evident that chloroxylenol reduces the binding of methyl paraben considerably. This effect increases with increasing chloroxylenol concentration. The data suggest that chloroxylenol competes with methyl paraben for micellar binding sites causing a decrease in the degree of binding. Addition of varying concentrations of dichloroxylenol and propyl paraben also caused substantial modifications to the degree of interaction of methyl paraben. The binding data can be adequately represented as an interaction with two classes of sites and the binding parameters are summarized in Table 1. The lines joining the experimental points in Fig. 1A and B have been generated from the appropriate set of binding parameters. These demonstrate the

Table 1. *Binding parameters for methyl paraben in the presence of varying concentrations of competitor preservatives.*

Preservative	Competitor	Competitor free concentration $\times 10^4$M	n_1	K_1 M^{-1}	n_2	K_2 M^{-1}
Methyl paraben	Nil	—	0·16	588	3·2	30·4
	Propyl paraben	4·13	0·15	254	77·1	1·7
	Chloroxylenol	3·20	0·16	688	18·1	2·9
		6·40	0·17	619	23·7	1·9
	Dichloroxylenol	0·52	0·16	648	3·8	15·7
		1·04	0·16	623	5·9	10·1

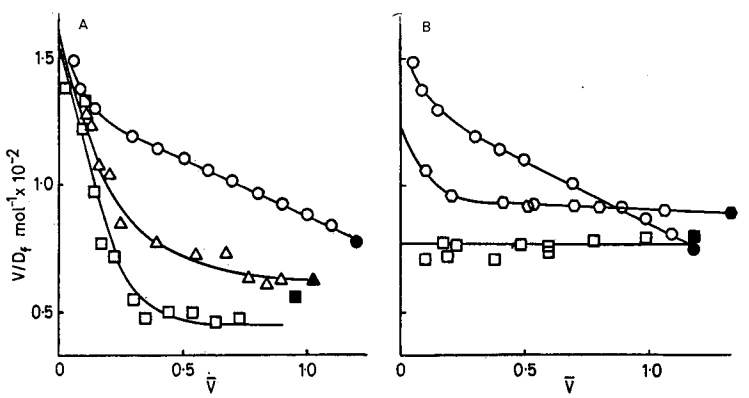

FIG. 1A. The influence of chloroxylenol on the binding of methyl paraben to 0·019M cetomacrogol at 25°. Chloroxylenol, free concentrations ($\times 10^4$M) ○ 0·0; △ 3·2; □ 6·4. Shaded symbols represent solubility points.

B. The influence of propyl paraben on binding of methyl paraben to 0·019M cetomacrogol at 25°. Propyl paraben, free concentrations ($\times 10^4$M) ○ 0·0; hexagon 4·13; □ 18·75.

agreement between the experimental data and the theoretical model. Methyl paraben, as well as other preservatives, appears to interact with two regions in the micelle. The primary class of sites has a low binding capacity (low n_1 value) but a high affinity (K_1) for the ester while the second class of sites has a much lower affinity and a greater capacity as indicated by the binding parameters (Table 1). This model of two classes of sites is in good agreement with nmr data (Corby & Elworthy, 1971) which suggested that *p*-hydroxybenzoates are solubilized in both the oxyethylene region and the core of the cetomacrogol micelle.

Chloroxylenol has a relatively small effect on binding of the methyl ester to the first class of sites (Fig. 1A), however in the second class of sites the secondary association constant, K_2, shows an apparent decrease of 10- to 15-fold, indicating strong competition. A concomitant large increase occurs in the number of sites available in the second class, n_2.

The fact that chloroxylenol selectively modifies binding in the secondary sites and has little effect on the primary sites supports the concept of the existence of 2 classes of binding sites. Although the affinity of sites in the second class is reduced, the number available for binding increases and hence the binding capacity increases. Thus there appear to be two mutually antagonistic processes operating in mixed preservative systems, one tending to increase binding, the other tending to diminish it. Dichloroxylenol (Table 1) produces effects similar to those seen with chloroxylenol. Binding of methyl paraben to the first class of sites is changed very little while smaller but qualitatively similar changes occur in the second class with a two- to threefold decrease in the association constant, K_2, and a slight, but significant, increase in the number of sites.

In contrast, the closely related compound, propyl paraben, competes strongly with methyl paraben in both classes of sites (Fig. 1B). A free concentration of $4{\cdot}13 \times 10^{-4}$M propyl ester causes substantial, apparent reductions in both K_1 and K_2, however, there is a very large increase in n_2 (Table 1). In the presence of a higher concentration of the propyl ester ($18{\cdot}75 \times 10^{-4}$M) binding to the first class of sites is completely suppressed and the Scatchard plot becomes virtually horizontal.

Inspection of the simplest form of the Scatchard equation:

$$\frac{\bar{V}}{D_f} = nK - K\bar{V} \qquad (3)$$

indicates that, as the slope approaches zero, K approaches zero and n approaches infinity. In this case the process involves a weak or non-specific interaction of enormous binding capacity which is analogous to a distribution phenomenon. Binding parameters were not calculated in this case because the errors become greatly magnified. However, the fraction of methyl paraben bound is largely independent of its concentration and corresponds to an apparent micelle/water partition coefficient of 75. This example illustrates that although $\bar{V}/D_f$ may change very little with variations of total concentration, this does not necessarily indicate that the interaction process is a partition phenomenon. It may be a special case of binding according to the law of mass action.

Propyl paraben competes more effectively with methyl paraben than either chloroxylenol or dichloroxylenol. This might be expected because of the structural similarity of the esters. It further suggests that competition for the first class of sites requires a greater structural specificity than the competition in the second class.

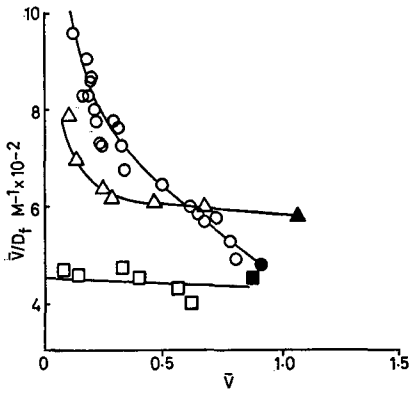

FIG. 2. The influence of methyl paraben on binding of propyl paraben to 0·019M cetomacrogol at 25°. Methyl paraben, free concentrations (×10²M) ○ 0·0; △ 0·51; □ 1·54.

Fig. 2 shows Scatchard plots of equilibrium dialysis data for the interaction of propyl paraben with cetomacrogol in the presence of the methyl ester. As expected there is evidence of competition in both classes of sites. This is reflected by the binding parameters (Table 2). In solutions containing a free concentration of 0·51 × 10^{-2}M methyl paraben, the primary association constant is decreased about three times and the secondary association constant fivefold. At a higher methyl paraben free concentration (1·54 × 10^{-2}M), interaction with the primary sites is entirely suppressed, as seen previously. Although there is considerable scatter in the data, they serve to illustrate that the binding approaches a non-specific interaction analogous to a distribution phenomenon similar to that discussed previously for the reverse combination.

The influence of methyl paraben on the binding of chloroxylenol is also that expected from the reverse combination. Relatively little change occurs in the binding constants of the first class of sites but substantial competition occurs in the second class (Table 2).

Table 2. *Binding parameters for various preservatives in the presence of methyl paraben.*

Preservative	Methyl paraben free concentration ×10²M	n_1	K_1 M^{-1} × 10^{-3}	n_2	K_2 M^{-1}
Propyl paraben	0·00	0·08	14·250	2·8	245·0
	0·51	0·07	4·729	10·7	55·3
Chloroxylenol	0·00	0·68	4·357	41·5	17·0
	0·16	0·63	3·651	46·4	12·0
	0·32	0·64	3·907	58·7	7·5

From the foregoing data there appear to be at least two classes of binding sites in the cetomacrogol micelles for each of the compounds studied. In the first class of sites competition was observed only with the *p*-hydroxybenzoates which are structurally very similar. Combinations of methyl paraben with chloroxylenol or with dichloroxylenol did not compete.

The second class of sites, however, appears to interact less specifically. All combinations exhibited competition to some degree as shown by the apparent decrease in K_2 with increasing competitor concentration. Associated with the competition, however, was an increase in the number of sites available (n_2) with increasing competitor concentration. This increase implies that the competitor causes some reorganisation of the micelles which leads to a greater binding capacity. Thus, rather than producing a diminution of binding, the degree of interaction tends to increase. This is consistent with earlier findings (Crooks & Brown, 1973) that, under certain conditions, the addition of a second preservative may increase the solubility of the first. This is perhaps similar to the co-solubilization effect reported by Kolthoff & Graydon (1951), where the addition of one solubilizate alters the solubilizing capacity of the micelle for another. Related work by Klevens (1949, 1950) showed that the inclusion of long chain alcohols markedly enhances the solubilizing capacity of potassium myristate solutions.

The Scatchard plot is useful in providing information about the nature of the binding process. It provides information regarding the sites of interaction, their number and the strength of association. However without experience it is difficult to derive practical information regarding the concentration which will be bound in a particular surfactant solution at a specified free concentration. Kazmi & Mitchell (1971) have proposed that this can be achieved by plotting the curve over a wide range of $\bar{V}$ and D_f and determining n and K values from the slope in the region of interest. Presumably these values are then to be used to recalculate $\bar{V}$ and D_f.

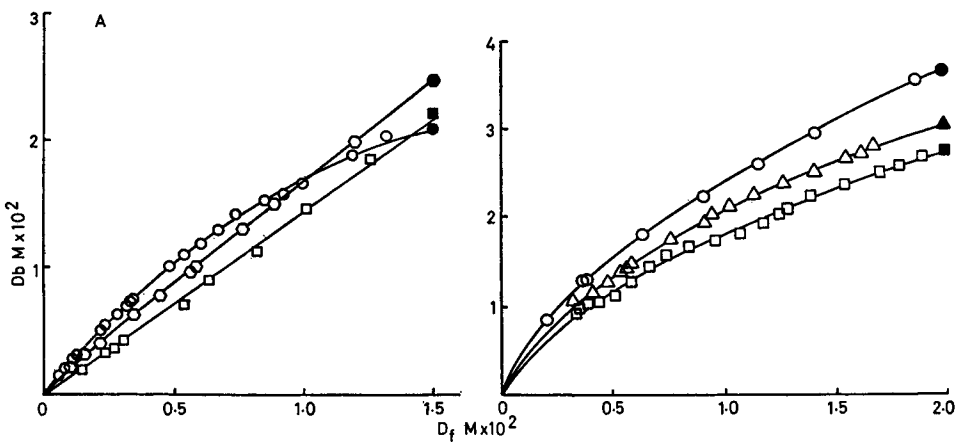

FIG. 3A. Binding isotherms for methyl paraben in 0·019M cetomacrogol at 25° in the presence of propyl paraben, free concentrations ($10^4 \times$M) ○ 0·0; hexagon 4·13; □ 18·75.

B. Binding isotherms for chloroxylenol in 0·019M cetomacrogol at 25° in the presence of methyl paraben, free concentrations ($\times 10^2$M) ○ 0·0; △ 0·16; □ 0·32.

Fig. 3(A and B) shows plots of D_b versus D_f for two representative systems. These binding isotherms have practical value in that they clearly illustrate the effect of the competitors on the extent of binding of the preservatives. The concentration of preservative bound may be read directly from the graph for any specified free concentration and it is a simple matter to calculate the total concentration needed.

It is interesting to note that the curve for binding of methyl paraben in the presence of $4{\cdot}3 \times 10^{-4}$M propyl paraben intersects that for methyl paraben alone (Fig. 3A).

Table 3. *Binding of preservatives individually and as mixtures in cetomacrogol solution* (0·019M).

Preservative combination	Concentration %	% Free singly	% Free mixed
Methyl paraben	0·15	31·2	46·4
Chloroxylenol	0·29	3·5	5·2
Methyl paraben	0·15	31·2	38·4
Chloroxylenol	0·20	2·8	4·0
Methyl paraben	0·24	32·8	36·4
Propyl paraben	0·11	13·0	21·4

The effect of the propyl ester is to cause a net decrease in binding at low concentrations of the methyl ester, however, as the concentration of methyl ester increases the situation becomes reversed and the binding becomes greater in the presence of propyl than in its absence. This illustrates the difficulty of predicting the degree of binding from solubility data. Although less obvious, the higher concentration of propyl produces a similar situation.

Fig. 4(A, B) illustrates the effects of a competitor on the percentage of free preservative as a function of total concentration. In general, large increases in the percentage of free or available preservative occur with increasing concentrations of a second preservative. It is interesting to note that, for the methyl paraben/chloroxylenol systems (Fig. 4A), the greatest increase in % free is seen at higher concentrations of

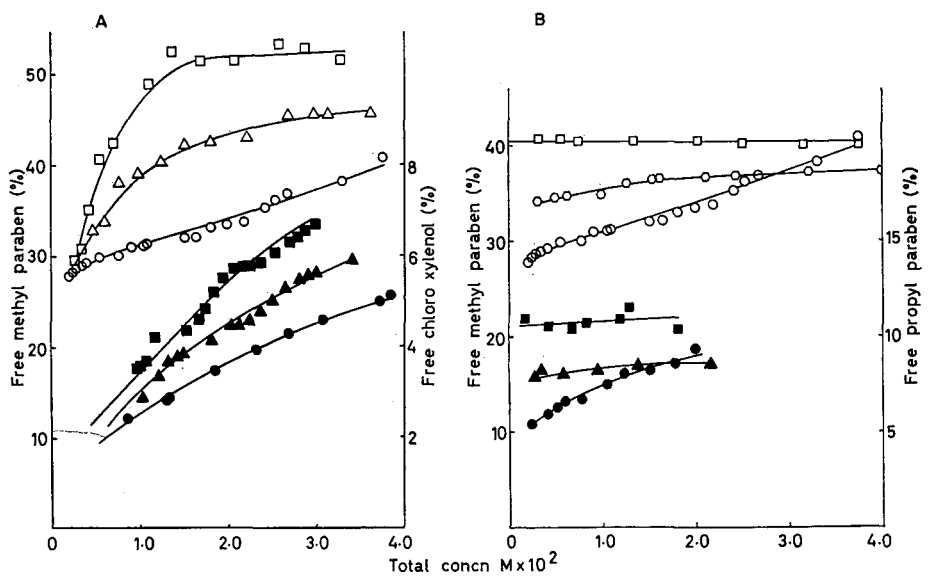

FIG. 4A. Plot of % free preservative against total concentration in 0·019M cetomacrogol for methyl paraben in the presence of chloroxylenol, free concentrations ($\times 10^4$M) ○ 0·0; △ 3·2; □ 6·4, and chloroxylenol in the presence of methyl paraben, free concentration ($\times 10^2$M) ● 0·0; ▲ 0·16; ■ 0·32.

B. Plot of % free preservative against total concentration in 0·019M cetomacrogol for methyl paraben in the presence of propyl paraben, free concentrations (10^4M) ○ 0·0; hexagon 4·13; □ 18·75, and propyl paraben in the presence of methyl paraben, free concentration ($\times 10^2$M) ● 0·0; ▲ 0·51; ■ 1·54.

preservative. This occurs because these compounds compete only for the second class of sites. Thus displacement is largest at the higher concentrations where these sites make the greatest contribution to the overall binding. Relatively little increase in % free is seen at low concentrations. The reverse situation is seen with combinations of methyl and propyl parabens (Fig. 4B). In these systems competition is greatest for the first class of binding sites, thus the largest increase in % free occurs at low concentrations of preservative. Competition also occurs for the second class of sites but the large increase in the number of secondary binding sites leads to an increase in binding and compensates for this. Thus the degree of displacement is much less at higher preservative concentrations.

In addition, the more strongly bound preservatives such as propyl paraben and chloroxylenol show the smallest absolute change in % free. However the relative change is much greater than for less strongly bound compounds such as methyl paraben. Greater preservative activity may thus be derived by choosing those mixtures where displacement of the more strongly bound preservative is optimal.

There have been reports of the possible synergistic action of preservatives in mixtures (Boehm, 1968). The present findings would indicate that preservative mixtures used in non-ionic surfactant systems may show an apparent synergism as a result of mutual competitive displacement from binding sites. Table 3 shows representative values of % free for various preservatives, singly and as mixtures. With further information of this type it should be possible to develop preservative systems of substantially greater activity for formulations containing non-ionic surfactants.

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