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The interaction of benzoic acid and chloroxylenol with cetomacrogol

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The interaction of benzoic acid with the non-ionic surfactant cetomacrogol 1000 has been examined by means of solubility and pH measurements, potentiometric titration and equilibrium dialysis. Solubility measurements and equilibrium dialysis have also been used to assess the interaction of chloroxylenol with cetomacrogol. Following earlier proposals, the interactions have been expressed both in terms of a distribution coefficient for the partition of preservative between the micelles and aqueous phase, and as a ratio of the total preservative concentration to the amount free in the aqueous phase. It is suggested that a more generally useful method is to express the degree of saturation of the aqueous phase with preservative as a function of the degree of saturation of the total system.

THE inactivation of preservatives in the presence of non-ionic surfactants has been the subject of many investigations and it is established that the inactivation arises from an interaction between molecules of the surfactant and the preservative. Different theories have been advanced to account for the nature and extent of this interaction but it is generally agreed that antimicrobial activity depends on the concentration of unbound or free preservative (Wedderburn, 1964).

Higuchi & Lach (1954) reported the formation of hydrogen bonded complexes between polyethylene glycols and phenols and between polyethylene glycols and organic acids. Since most non-ionic surfactants have polyethylene glycol chains, many authors have attributed both the solvent properties and inactivation of preservatives to complex formation. Evans (1964) has shown that complex formation between surfactant monomer and preservative is unlikely and has suggested that the inactivation arises from solubilisation of preservative within the surfactant micelles. Moreover, Mulley (1964) has collected evidence from a number of sources which indicates that the solubilisation of a wide range of solutes in non-ionic surfactants can be treated as a solution process within the hydrocarbon-like interior of the micelle. He considers the data do not support suggestions that solubilisation is controlled by more specific factors such as complex formation. Kostenbauder (1962) maintains that it is unnecessary to distinguish between the mechanisms of complex formation and micellar solubilisation and considers that solubilisation and micelle formation itself fall within the broad scope of the complex formation described by Higuchi & Lach (1954).

Although the exact nature of the interaction is in doubt, aqueous solutions of a non-ionic surfactant probably provide ideal conditions for association to occur between the preservative and the surfactant. The possibilities exist for hydrogen bonding, both in the monomer and in the micellar states, and also for partitioning into the deeper regions of the micelle.

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Methods used to study the interaction between preservatives and non-ionic surfactants have included partition (Allawala & Riegelman, 1953), solubility (Blaug & Ahsan, 1961; Goodhart & Martin, 1962; Matsumoto & Aoki, 1962), dialysis (Patel & Kostenbauder, 1958; Deluca & Kostenbauder, 1960; Bahal & Kostenbauder 1964; Blaug & Ebersman, 1964; Patel & Foss, 1964; Blaug & Rich, 1965), and potentiometric titration (Evans 1964; Donbrow & Rhodes 1965).

Donbrow & Rhodes (1963) have shown that the pH of acid buffer solutions is increased in the presence of cetomacrogol presumably due to preferential solubilisation of the acid within surfactant micelles. Hence a simple pH measurement should also provide a convenient method of estimating the amount of free solute.

Using benzoic acid and chloroxylenol solubilised in cetomacrogol solutions, various techniques for assessing the interaction between preservatives and non-ionic surfactants have been examined, and methods of expressing this interaction are compared.

Experimental

Materials. Benzoic acid recrystallised from water, m.p. 122°, chloroxylenol recrystallised from light pertoleum, m.p. 115–116°, and cetomacrogol 1000 B.P.C. (Texofor A1P, Glovers Chemicals Ltd.). Cetomacrogol 1000 has the general formula $Me{\cdot}[CH_2]_m\ [O{\cdot}CH_2{\cdot}CH_2]_n{\cdot}OH$ where m may be 15 or 17 and n may be 19 to 23. The molecular weight was taken as 1300. Following the method of Ginn & Church (1959) solutions of cetomacrogol were passed through a column of mixed bed ion-exchange resins to remove alkaline impurities. The refractive index of the effluent was measured at 25° and the concentration of cetomacrogol determined from a calibration curve. All solutions were made using freshly boiled and cooled glass distilled water.

Determination of water-solubility. Excess benzoic acid was equilibrated with water by rotation in a sealed cylinder in a water-bath thermostatically controlled at 25° ± 0·1°. The amount of benzoic acid in an aliquot of filtrate measured potentiometrically was 0·33 g/100 ml. The water solubility of chloroxylenol at 20° is 0·031 g/100 ml (Mitchell, 1964).

Determination of solubility in solutions of cetomacrogol. The solubility of benzoic acid in varying concentrations of cetomacrogol solution was found using the method given for water-solubility. Excess chloroxylenol separates as a liquid crystalline phase which cannot be separated by filtration. The solubility of chloroxylenol in solutions of cetomacrogol at 20° was therefore determined by the method of Mulley & Metcalf (1956).

Measurement of pH and potentiometric titration. pH measurements were made using a glass-saturated calomel electrode system and a Beckman Research pH meter (relative accuracy ± 0·001 under optimum conditions). The electrode system was standardised using 0·05 M potassium hydrogen phthalate solution and the electrode response was checked on this buffer after each experiment. The pH/e.m.f. relationship

of the electrode was checked periodically using 0·05 M sodium borate. Potentiometric titrations were made using potassium hydroxide freed from carbon dioxide by the method of Albert & Serjeant (1962a). The sample volume was 20 ml and the strength of potassium hydroxide was such that 2 ml was required for neutralisation. pH measurements and potentiometric titrations were made in an atmosphere of nitrogen.

Equilibrium dialysis. Perspex dialysis cells were made to the design of Bahal & Kostenbauder (1964). The dialysis membrane was rubber latex refluxed with several changes of water until the washings were clear. The membrane was permeable to benzoic acid and chloroxylenol but not to cetomacrogol. A solution of preservative in cetomacrogol was placed in one side of the cell and water, or water plus preservative, was placed in the other. The cells were rotated in the water-bath at 25° for benzoic acid and 20° for chloroxylenol until equilibrium was reached. The amount of benzoic acid on each side of the membrane was found by potentiometric titration. The amount of chloroxylenol was assayed spectrophotometrically by the ΔE method of Elvidge & Peutrell (1961) (sample buffer C, blank buffer A, λ_{max} 301 mμ). The differential method avoids probable errors due to the release of absorbing impurities from the rubber membrane and interference from the presence of surfactant.

Results and discussion

It has been shown that the bactericidal activity of chloroxylenol in aqueous solutions of cetomacrogol is related to the degree of saturation of the system with chloroxylenol (Mitchell, 1964). The degree of saturation was given by

$$R = C/C_s \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where R is the saturation ratio, C is the chloroxylenol concentration and C_s its solubility. Bactericidal activity was shown to be due to the concentration of chloroxylenol in the free aqueous phase and not to the total amount present in the system. It was suggested that a better index of bactericidal activity would be the degree of saturation of the free aqueous phase rather than the total solution as defined in (1). Treating solubilisation in surfactant solutions as a distribution phenomenon, R can be subdivided into R_m, the degree of saturation of the micelles and R_w, the degree of saturation of the aqueous phase. Hence,

$$R_m = C_m/C_{sm} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$R_w = C_w/C_{sw} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

and $$C = C_m + C_w \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where C_m and C_w are the concentrations in the micellar and aqueous phases respectively and C_{sm} and C_{sw} are the saturation solubilities of the micellar and aqueous phases respectively. At saturation, $C_s = C_{sm} + C_{sw}$ and R, R_m and $R_w = 1{\cdot}0$.

Following the method of McBain & Hutchinson (1955) it is possible to calculate a distribution coefficient, K_m, for the partition of unionised solute between the micelles and aqueous phase where

$$K_m = \frac{[\text{unionised preservative}]\ \text{micelle}}{[\text{unionised preservative}]\text{water}} = \frac{C'_m/[\text{cetomacrogol}]}{C'_w/[\text{water}]} \quad (5)$$

Solute molecules within the micelle are apparently unionised (Evans 1964; Donbrow & Rhodes 1965), hence $C'_m = C_m$.

Values of the distribution coefficient calculated from the solubility curve (Table 1) are reasonably constant for different concentrations of

TABLE 1. DISTRIBUTION COEFFICIENTS FOR THE PARTITION OF UNIONISED BENZOIC ACID AND CHLOROXYLENOL BETWEEN THE MICELLES AND AQUEOUS PHASE OF CETOMACROGOL SOLUTIONS CALCULATED FROM SOLUBILITY CURVE

Benzoic acid (25°)			Chloroxylenol (20°)		
Cetomacrogol	C_s	$K_{sm} \times 10^{-3}$	Cetomacrogol	C_s	$K_{sm} \times 10^{-3}$
0·000	0·0273		0·000	0·00197	
0·010	0·0437	3·5	0·005	0·0106	49
0·040	0·0929	3·5	0·010	0·0196	50
0·060	0·1257	3·5	0·049	0·0958	54
0·100	0·1913	3·5	0·096	0·1916	55

C_s = Solubility of preservative.

K_{sm} = Distribution coefficient of unionised preservative in saturated surfactant solutions.
$= \frac{C_{sm}/[\text{cetomacrogol}]}{C'_{sw}/[\text{water}]}$

C_{sm} = Solubility of preservative in micelles $= C_s - C_{sw}$

C_{sw} = Solubility of preservative in aqueous phase. (Assumed to be the same as the water-solubility.

C'_{sw} = Concentration of unionised preservative in aqueous phase at saturation, $= C_{sw} - C_{\bar{s}w}$

$C_{\bar{s}w}$ = Concentration ionised preservative in aqueous phase at saturation calculated from the Henderson equation $pH = pKa + \log [C_{\bar{s}w}/C_{sw}]$

For benzoic acid solutions pH at saturation = 2·910, pKa at 25° = 4·18, $C_{sw} = 0·0273$.

For chloroxylenol solutions, $C'_{sw} = C_{sw} = 0·00197$.

All concentrations in moles/litre total solution.

cetomacrogol, but the solubility method is in effect a 'one-point' method of estimating the degree of interaction. In each case a saturated solution is being considered and it cannot be assumed that the extent of interaction will be the same in unsaturated solutions (cf. chloroxylenol). Moreover in the calculation of K_m, it is assumed that solubility in the aqueous phase at saturation, C_{sw}, is the same as the water-solubility. This assumption is open to question in certain cases (Evans, 1964) but seems valid for benzoic acid and chloroxylenol in cetomacrogol, since the solubilities are directly proportional to the concentration of cetomacrogol over the concentration range studied when corrections have been made for the solubilities in water.

The amount of benzoic acid in the aqueous phase was also determined potentiometrically as described by Evans (1964) and some of the calculated distribution coefficients are given in Table 2. Values of K_m increased slightly up to half-neutralisation and thence more markedly with further additions of base. Values of K_m also depended upon the initial concentration of acid and the degree of saturation. In the potentiometric method,

TABLE 2. DISTRIBUTION COEFFICIENTS FOR THE PARTITION OF UNIONISED BENZOIC ACID BETWEEN THE MICELLES AND AQUEOUS PHASE OF CETOMACROGOL SOLUTIONS AT 25° CALCULATED FROM POTENTIOMETRIC TITRATION

Initial R	C	Cetomacrogol	pH	C_w^-	$K_m \times 10^{-3}$
0·910	0·1000	0·0500			
	0·0990	0·0495	3·84	0·00990	3·5
	0·0952	0·0476	4·80	0·0476	3·7
0·523	0·1000	0·1000			
	0·0990	0·0990	4·09	0·00990	3·5
	0·0981	0·0981	4·44	0·0196	3·6
	0·0971	0·0971	4·67	0·0291	3·6
	0·0961	0·0961	4·87	0·0385	3·7
	0·0952	0·0952	5·07	0·0476	4·0
	0·0944	0·0944	5·26	0·0566	4·1
	0·0935	0·0935	5·46	0·0654	4·3
0·455	0·0500	0·0500			
	0·0495	0·0495	3·90	0·00495	4·1
	0·0476	0·0476	4·84	0·0238	4·2
0·266	0·0500	0·1000			
	0·0495	0·0991	4·20	0·00495	4·7
	0·0476	0·0952	5·14	0·0239	4·7
0·232	0·01000	0·01000			
	0·00971	0·00971	4·08	0·00291	4·4
	0·00952	0·00952	4·43	0·00477	4·6

R = Saturation ratio.
C = Total concentration benzoic acid.
$K_m = \frac{C_m/[\text{cetomacrogol}]}{C'_w/[\text{water}]}$
C_m = Concentration benzoic acid in micelles = $C - C_w$
C_w = Total concentration benzoic acid in aqueous phase = $C_w^- + C'_w$
C_w^- = Concentration ionised benzoic acid in aqueous phase calculated from the amount of added base.
C'_w = Concentration unionised benzoic acid in aqueous phase calculated from the Henderson equation (pKa benzoic acid at 25° is 4·18). All concentrations in moles/litres total solution.

free acid is titrated to salt and throughout a titration the ionic strength will vary continuously. Moreover there will be large variations in ionic strength in titrations where the initial concentration of acid is different. Donbrow & Rhodes (1965) have criticised Evans' method of calculation on the grounds that the Henderson equation is used without correction for ionic strength. Therefore activity coefficients were estimated and amounts of unionised acid in the aqueous phase were recalculated using the Henderson equation in the form given by Albert & Serjeant (1962b) where pK_a^T for benzoic acid at 25° was taken as 4·18 (Ives, 1933). The recalculated values of K_m however, still increased throughout an individual titration and as the degree of saturation of the initial solution with benzoic acid was reduced, despite corrections for the change in ionic strength. Hence the variations in distribution coefficients are probably due to the effect of the change in salt concentration on micelle formation, solubilisation and the distribution coefficients of semipolar solutes. Donbrow & Rhodes (1965) recommend back-titration of the acid from the salt form since the ionic strength of the solution and hence the value of the activity coefficient correction will remain relatively constant provided the volume of titrant is small. Compared with other methods of studying the interaction between solute and surfactant however, the potentiometric method suffers from the disadvantage that the titration itself disturbs the system.

The potentiometric method depends on pH measurements and the calculations are based on the assumption that the unionised acid is

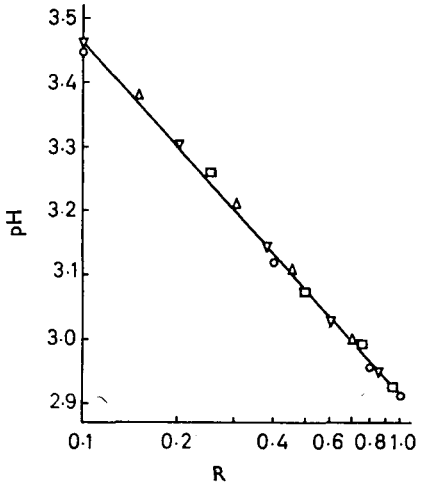

FIG. 1. Variation of pH with log saturation ratio (R) for solutions of benzoic acid in water and in cetomacrogol at 25°. ○, water; □, 0·05 M benzoic acid; ▽, 0·01 M cetomacrogol; △, 0·06 M cetomacrogol.

partitioned between the micelles and the free aqueous phase. Hence it should be possible to estimate this partition by a simple pH measurement without disturbing the system by adding base. Readings of pH at various degrees of saturation for solutions of benzoic acid are shown in Fig. 1. The pH values both in water and cetomacrogol are in close agreement for any value of R, irrespective of the actual amounts of benozic acid or cetomacrogol. This shows that the pH of the solution depends only on the concentration of benzoic acid in the aqueous phase. Values of K_m calculated from pH measurements are given in Table 3.

TABLE 3. DISTRIBUTION COEFFICIENTS FOR THE PARTITION OF UNIONISED BENZOIC ACID BETWEEN THE MICELLES AND AQUEOUS PHASE OF CETOMACROGOL SOLUTIONS AT 25° CALCULATED FROM pH MEASUREMENTS

R	Cetomacrogol	C	pH	$K_m \times 10^{-3}$
0·95	0·0152	0·0500	2·922	3·6
0·85	0·0100	0·0372	2·948	3·5
0·70	0·0600	0·0880	2·995	3·6
0·50	0·0446	0·0500	3·076	3·6
0·30	0·0600	0·0377	3·200	3·7
0·10	0·0100	0·00437	3·467	4·0

R = Saturation ratio.
C = Total concentration benzoic acid.
$K_m = \frac{C_m/[\text{cetomacrogol}]}{C'_w/[\text{water}]}$
C_m = Concentration benzoic acid in micelles = $C - C_w$
C_w = Total concentration benzoic acid in aqueous phase, from Fig. 1 and equn. 3
C'_w = Concentration unionised benzoic acid in aqueous phase = $C_w - \bar{C_w}$
$\bar{C_w}$ = Concentration ionised benzoic acid in aqueous phase calculated from the Henderson equation.
All concentrations in moles/litre total solution.

Dialysis studies were made using benzoic acid and chloroxylenol in cetomacrogol solutions. At equilibrium, the activity of the preservatives is identical on both sides of the membrane and, for the solutions used, it was assumed that the concentration of free preservative on both sides of the membrane is equal. By analysis of each of these solutions the concentration of preservative in the free aqueous phase and micelles is readily found, and the distribution coefficient calculated (Fig. 2).

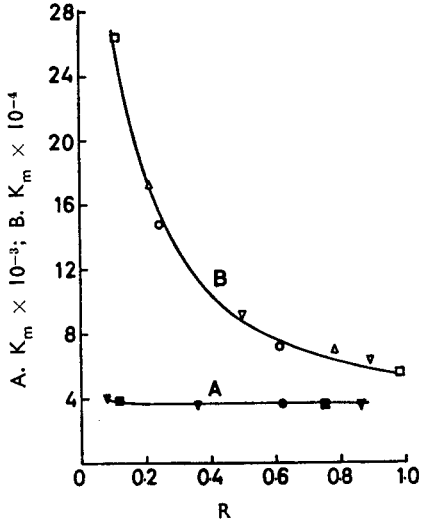

FIG. 2. Variation of distribution coefficient with saturation ratio (R) for the partition of benzoic acid and chloroxylenol between the micelles and aqueous phase of cetomacrogol solutions calculated from equilibrium dialysis. A. Benzoic acid: cetomacrogol solutions at 25°; cetomacrogol concentration (moles/litre); ●, 0·01; ■, 0·04; ▼, 0·10. B. Chloroxylenol: cetomacrogol solutions at 20°; cetomacrogol concentration (moles/litre); △, 0·005; ○, 0·01; □, 0·049; ▽, 0·096.

For solutions of benzoic acid in cetomacrogol there is close agreement between values of K_m determined by solubility, pH measurement and dialysis. K_m is constant over a wide range of benzoic acid and cetomacrogol concentrations but increases slightly at low degrees of saturation. In contrast, K_m for chloroxylenol-cetomacrogol solutions increases markedly as the saturation ratio is reduced, and is constant for different concentrations of solute and surfactant only at a given value of R (Fig. 2). Under these circumstances a distribution coefficient has little meaning.

Many authors (e.g. Higuchi & Lach, 1954; Patel & Kostenbauder, 1958; Blaug & Ahsan, 1961; Storz, DeKay & Banker, 1965) have expressed the ratio of the total solute concentration, C, to the concentration of the free form, C_w, as a function of the macromolecule concentration. Fig. 3 shows results for benzoic acid and chloroxylenol plotted in this manner. The ratio C/C_w is a function of cetomacrogol concentration for all concentrations of benzoic acid but for chloroxylenol it is a function

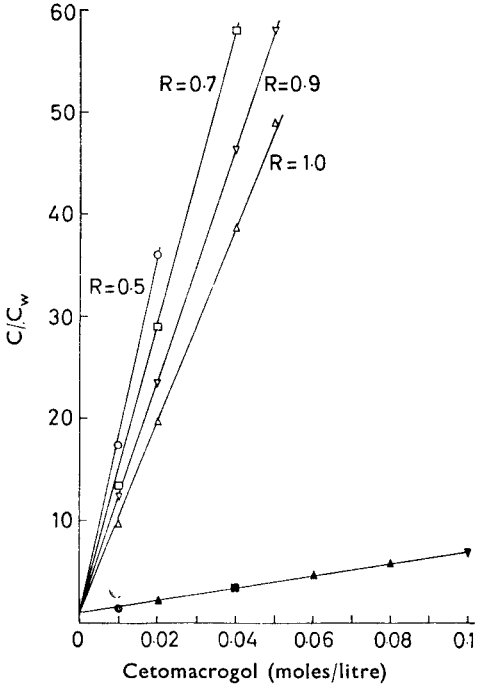

FIG. 3. Ratio of total preservative to free preservative as a function of cetomacrogol concentration. Closed symbols: benzoic acid (25°). Concentration of free benzoic acid (moles/litre): ●, 0·0164; ■, 0·0200; ▼, 0·0234; ▲, 0·0273. Open symbols: chloroxylenol (20°). Concentration of free chloroxylenol (moles/litre): ○, $5{\cdot}5 \times 10^{-4}$; □, $9{\cdot}9 \times 10^{-4}$; ▽, $15{\cdot}3 \times 10^{-4}$; △, $19{\cdot}7 \times 10^{-4}$.

both of cetomacrogol concentration and of chloroxylenol concentration and depends on the saturation ratio.

The solubility method has been used by a number of workers either to estimate K_m or the ratio C/C_w. It is apparent from Figs 2 and 3 that while this method may be satisfactory for certain preservatives, with others such as chloroxylenol it will lead to erroneous conclusions for unsaturated systems.

A more useful method of presenting the results is to plot a partition isotherm showing the % saturation of the micelles (or R_m) as a function of the % saturation of the aqueous phase (or R_w). Alternatively the % saturation of the aqueous phase (or R_w) may be plotted as a function of the % saturation of the total system (or R). The latter is more appropriate in the present study since the important consideration is the amount of preservative available in the aqueous phase as a function of the total amount in the system (Fig. 4). For benzoic acid the plot of R_w against R has a slope of 1·0 indicating that benzoic acid is partitioned between the aqueous phase and the micelles according to simple distribution theory. For chloroxylenol the plot of R_w as a function of R shows a marked

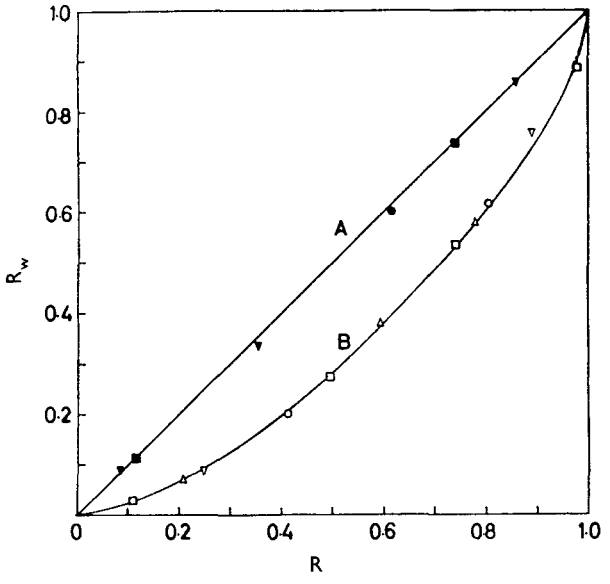

FIG. 4. Dependence of the degree of saturation of the aqueous phase (R_w) on the degree of saturation of the total solution (R) for solutions of benzoic acid and chloroxylenol in cetomacrogol. A. Benzoic acid: cetomacrogol solutions at 25°; cetomacrogol concentration (moles/litre): ●, 0·01; ■, 0·04; ▼, 0·1. B. Chloroxylenol: cetomacrogol solutions at 20°; cetomacrogol concentration (moles/litre): △, 0·005; ○, 0·01; □, 0·049; ▽, 0·096.

deviation from a slope of 1·0 showing that, for unsaturated solutions, chloroxylenol is preferentially soluble in the micelles. Allawala & Riegelman (1953) using solutions of iodine in Antarox A-400 (nonylphenol/10 moles ethylene oxide) reported similar results to those found for chloroxylenol in cetomacrogol but also found that the partition in favour of the micelles increased with surfactant concentration. From such plots it is possible to determine the amount of preservative free in the aqueous phase for any concentration of solute and surfactant, or alternatively if the amount of preservative needed in the aqueous phase to prevent microbial growth is known, the amount required in the system as a whole to achieve this concentration can be determined. It has been shown previously (Mitchell 1964) that when $R = 1{\cdot}0$, solutions of chloroxylenol in cetomacrogol have the same antibacterial activity as a saturated solution of chloroxylenol in water. However, when $R < 1{\cdot}0$, chloroxylenol-cetomacrogol solutions were less effective than corresponding chloroxylenol-water solutions. This was attributed to a change in distribution of chloroxylenol in favour of the micelles on reducing R. This is confirmed by the results in Figs 2 and 4. Thus from Fig. 4, in cetomacrogol solutions 50% saturated with chloroxylenol ($R = 0{\cdot}50$), the aqueous phase is only 28% saturated ($R_w = 0{\cdot}28$).

At a given value of R, chloroxylenol-cetomacrogol solutions were found to be equitoxic over a wide concentration range of chloroxylenol.

Fig. 4 shows that for any value of R the degree of saturation of the aqueous phase, R_w, is a constant independent of the concentrations of chloroxylenol or cetomacrogol except in so far as these control R. These findings support the views of other workers that preservative activity depends on the degree of saturation, or thermodynamic activity of the aqueous phase, and show that this is controlled by the degree of saturation of the system as a whole.

Fig. 5 shows equilibrium dialysis data for other preservatives in non-ionic surfactants plotted as R_w against R. Methyl *p*-hydroxybenzoate (Patel & Kostenbauder, 1958) and hexachlorophane (Morgan, 1965), like chloroxylenol, show a deviation from a slope of 1, while chlorbutol (Slade, 1965) behaves in the same manner as benzoic acid. It is apparent that the interaction of different preservatives with non-ionic surfactants does not take place by the same mechanism. However, the results indicate that in each case it is possible to express the interaction in terms of partition between an aqueous and a micellar phase.

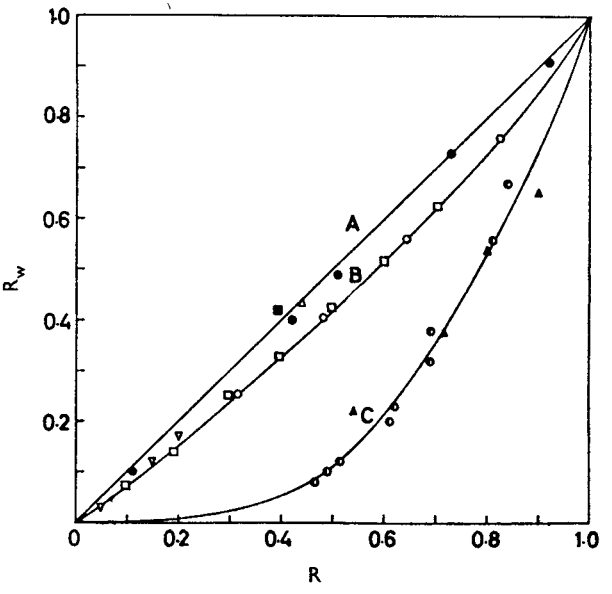

FIG. 5. Dependence of the degree of saturation of the aqueous phase (R_w) on the degree of saturation of the total solution (R) for solutions of various preservatives in non-ionic surfactants. A. Chlorbutol: Brij 35 solutions at 25° (Slade, 1965); Brij 35 concentration (%): △, 0·5; ●, 1·0; ■, 1·5. B. Methyl *p*-hydroxybenzoate: polysorbate 80 solutions at 30° (Patel & Kostenbauder, 1958); polysorbate 80 concentration (%): ○, 3·0; □, 5·0; ▽, 10·0. C. Hexachlorophane: Brij 35 solutions at 25° (Morgan, 1965); Brij 35 concentration (%): ▲, 0·1; ◐, 1·0.

In complex disperse systems the presence of an oil phase, pH and the presence of other materials will all influence preservative effectiveness, but a knowledge of the extent of interaction for a preservative: non-ionic combination will indicate whether the preservative is likely to be of value.

Where a plot of R_w against R shows a marked deviation from a slope of 1·0 then a relatively large concentration of preservative will be required in the total system to ensure an adequate concentration in the aqueous phase. From the viewpoint of overall concentration relative to the concentration in the aqueous phase a suitable preservative should have a slope approaching 1·0.

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