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A model for the influence of emulsion formulation on the activity of phenolic preservatives

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Basic mathematical models are suggested for calculating the concentration of preservatives which is chemically available in the water of emulsions. It is influenced by the total concentration of preservative in the emulsion, the oil : water partition coefficient of the preservative, the oil : water ratio and the concentration of emulgent. The activity of the preservative is related to the concentration free in the water.

The ability of microorganisms to grow in emulsions is well established (Bennett, 1962; de Navarre, 1962; Wedderburn, 1964; Noble & Savin, 1966) but assessments of the ability of preservatives to prevent microbial invasion of emulsified products have depended largely on empirical tests involving inoculation of the finished product and examination during a prolonged period of storage (Wedderburn, 1964). More detailed and less empirical studies of the influence of emulsion components have used simplified models involving a minimum of components. A preservative added to an oil-water mixture partitions between the two phases (Bean, Richards & Thomas, 1962; Hibbott & Monks, 1961), its activity being controlled by the concentration in the aqueous phase which in turn is dependent upon the total concentration of preservative in the mixture, the oil : water partition coefficient of the preservative, the phase : volume ratio and the temperature (Bean, Richards & Thomas, 1962; Bean & Heman-Ackah, 1964; Heman-Ackah & Konning, 1967; Hibbott & Monks, 1961). A third component of even the simplest emulsion is the emulgent which usually is a micelle-forming surface-active agent. Anionic surface-active agents at concentrations below the critical concentration for micelle formation (CMC) increase the activity of phenolic preservatives but at concentrations above the CMC they depress the activity as a result of partitioning of the preservative between the micelles and the water (Alexander & Tomlinson, 1949; Bean & Berry, 1951; Evans & Dunbar, 1965). The CMC of non-ionic surface-active agents is much lower than that of anionics and in practice such solutions of non-ionics are rarely encountered at sub-CMC concentrations.

The precise nature of the interaction between non-ionics and preservatives is uncertain and may be either complex formation (Guttman & Higuchi, 1956; Mulley & Metcalf, 1956; Patel & Kostenbauder, 1958; Ansel, 1965) or solubilization of the preservative within the micelles of the non-ionic (Goodhart & Martin, 1962; Hurwitz, De Luca & Kostenbauder, 1963; Evans, 1964; Mulley, 1964). Irrespective of which theory may be correct, both have been successfully employed to verify the hypothesis that the antimicrobial activity of preservatives in aqueous solutions of non-ionics is, as in aqueous solutions of anionics, directly related to the concentration of free uncombined preservative (Pisano & Kostenbauder, 1959; Blaug & Ahsan, 1961; Mitchell, 1964).

Pisano & Kostenbauder (1959), advocates of the complexation theory, have shown that the concentration of free preservative in aqueous solutions of non-ionics is

$$C_W = \frac{C_A}{R} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where C_W = concentration of preservative free in the water, C_A = total concentration of preservative in the aqueous solution, R = ratio of total : free preservative.

The value of R increases as the concentration of non-ionic is increased (Pisano & Kostenbauder, 1959), and, for any specified value of C_A, the biological activity decreases (Mitchell, 1964; Malcolm, 1967).

Notwithstanding failures of conventional concentrations of preservatives to protect emulsions from microbial contamination (Noble & Savin, 1966), few systematic studies have investigated the activity of preservatives in complete emulsions. The present communication suggests two simple mathematical models to relate changes in the basic formula of an emulsion to the concentration of free preservative and relates this concentration to the activity.

THEORETICAL

In a simple oil-water dispersion a preservative is partitioned between the oil and aqueous phases, according to the following mathematical model (Bean & Heman-Ackah, 1964),

$$C_W = \frac{C(\phi + 1)}{(K_W^o \phi + 1)} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where C = total concentration of preservative in the system, C_W = concentration of preservative in the water, ϕ = oil : water ratio, K_W^o = oil : water partition coefficient of the preservative.

In an emulsion, a small proportion of the total emulgent is at the oil-water interface, but most, particularly if it has negligible oil-solubility, is dispersed throughout the water as micelles where it behaves as an additional phase. Any preservative added to the emulsion is partitioned between the three phases according to the oil : water partition coefficient, micelle : water partitionc oefficient and the volumes of each of the phases.

Several mathematical models may be devised to illustrate how the concentration of free preservative in the water is influenced by the magnitude of the afore-mentioned physical parameters, but objections may be raised to some. For example, to a model embodying a term for the volume of micellar phase objection may be made that the volume of emulgent (e.g. Polysorbate 80) added to water to yield a specified volume of aqueous solution, may not represent the volume of the micellar phase since any hydration of the micelles would modify their volume.

Such objections may be avoided by using models which do not include a term denoting the *volume* of the micellar phase but which necessitate reference to experiments relating the value of R (equation 1) to the concentration of non-ionic in the aqueous phase. In this connection it must be recognized that an increase in the concentration of non-ionic increases the total concentration of preservative in the aqueous phase, and the oil : aqueous phase partition coefficient falls. The concentration in the aqueous phase at equilibrium can thus be described by a model similar to that of Bean & Heman-Ackah (1964).

$$C_A = \frac{C(\phi + 1)}{(K\phi + 1)} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where C_A = concentration of preservative in the total aqueous phase, C = total concentration of preservative in the emulsion, ϕ = oil : water ratio, K = oil : total aqueous phase partition coefficient of the preservative.

The concentration of free preservative in the water may be calculated by invoking the model of Pisano & Kostenbauder (eqn 1).

$$C_W = \frac{C(\phi + 1)}{(K\phi + 1)} \ /R$$

or

$$C_W = \frac{C(\phi + 1)}{(K\phi R + R)} \quad .. \quad .. \quad .. \quad (4)$$

Although, with increasing non-ionic concentration the apparent oil : water partition coefficient (K) falls, the true oil:water partition coefficient (K_W^o) remains constant. Part of the additional preservative partitioned into the aqueous phase is complexed with the nonionic and $C_W = C_A/R$ (Pisano & Kostenbauder, 1959) and therefore

$$K_W^o = KR \text{ or } C_W = \frac{C(\phi + 1)}{(K_W^o\phi + R)} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

Experimentally, this is probably a simpler expression to employ than (4).

Thus, the concentration of free preservative in the water of an emulsion (C_W) may be calculated from either (4) or (5) provided that the following parameters are known: (i) total concentration of preservative in the emulsion (C); (ii) oil : water ratio (ϕ); (iii) the ratio of total : free preservative in the aqueous phase (R); and either (iv) the oil : total aqueous phase partition coefficient of the preservative for the concentration of non-ionic emulgent present (K), or (v) the true oil : water partition coefficient for the distribution of the preservation between the oil and the water (K_W^o).

EXPERIMENTAL

Materials

Polysorbate 80 conforming to the monograph of Polysorbate 80 in U.S.P. XVI and B.P.C. 1968. It is virtually insoluble in liquid paraffin. *Phenol* (A.R. quality). p-*Chloro*-m-*cresol* (Laboratory Reagent grade). *Liquid paraffin B.P.C.* 1963. S.G. 0·830–0·870. *Nutrient broth*, Oxoid granules (C.M.1) at a concentration of 1·3% w/v. *Indicator broth*, nutrient broth containing 1% w/v lactose and 0·0016% w/v bromocresol purple. *Nutrient agar*, nutrient broth solidified with 2% w/v Kobé agar ("Oxoid").

Organism

Escherichia coli (NCTC 5933) cultivated and maintained as described by Bean & others (1962). The inoculum was a nephelometrically standardized suspension of the organism stored in sterile water at 4°.

Methods

Preservative-polysorbate 80 *interaction.* Quantitative estimates of the interactions between phenol and polysorbate, and between chlorocresol and polysorbate were obtained using a dialysis technique similar to that used by Patel & Foss (1964). A

solution (20 ml) of preservative and polysorbate was pipetted into one compartment of a Perspex dialysis cell and water (20 ml) into the other compartment. The cell was stoppered and agitated in a water bath at 25 ± 0·1° for 5 days, after which samples from each compartment were assayed spectrophotometrically for the preservative, using the method of Johnson & Savidge (1958). This was repeated for a series of different concentrations of polysorbate.

At equilibrium the concentration of preservative in the cell compartment containing no surface-active agent was equal to the concentration of free preservative in the compartment containing surface-active agent. If the total amount of preservative in the cell is known, then the ratio of total to free preservative (R) may be calculated.

Oil-water partition coefficients. Oil-water-preservative mixtures each containing a known concentration of preservative were equilibrated at 25 ± 0·1°. After separation the aqueous phase was assayed spectrophotometrically for the preservative, using the method of Johnson & Savidge (1958) and the partition coefficients calculated from the assay results.

Determination of extinction times. Extinction times were determined by the method of Bean & Heman-Ackah (1964). Reaction mixtures of the required composition, containing 20 × 10^6 viable *E. coli*/ml in the aqueous phase, were placed in glass-stoppered tubes and maintained at 25 ± 0·1°. At intervals corresponding to approximately 1/7th of the expected extinction time, 1 ml samples were transferred to 24 ml of nutrient broth and the mixture was shaken vigorously; 1 ml of the broth dilution was then transferred to 24 ml of indicator broth providing a ×625 dilution of the phenol in the original sample; the final dilution was incubated at 37° for 48 h. Bacterial growth produced a change in the colour of the indicator from purple to yellow thereby distinguishing turbidity due to growth, from turbidity due to oil droplets in the broth. Five replicate determinations were made for each extinction time.

RESULTS

Preservative—polysorbate 80 *interaction*

The ratio total: free preservative (R) was linearly related to the concentration of polysorbate for both phenol and chlorocresol (Fig. 1). The slopes of these regressions were 0·27 and 4·7 for phenol and chlorocresol respectively and the value of 0·27 for phenol compares with that of 0·26 found by Patel & Foss (1964).

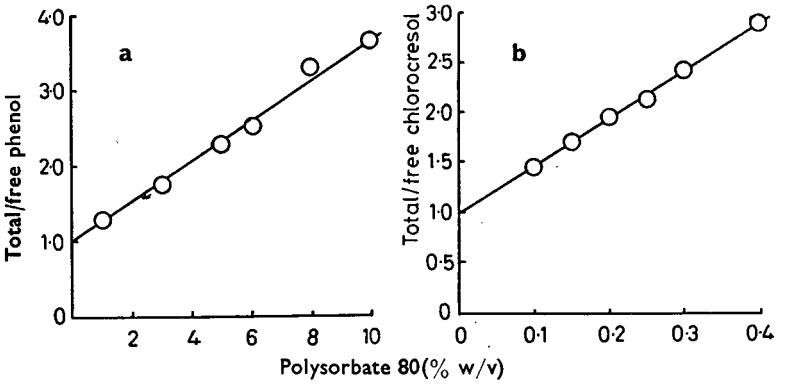

FIG. 1. The association of phenols and polysorbate 80. a. Phenol. b. Chlorocresol.

The influence of polysorbate 80 *on the apparent oil : water partition coefficients of phenol and chlorocresol*

The inclusion of polysorbate in a liquid paraffin-water dispersion reduces the proportion of phenol or chlorocresol remaining in the oil phase at equilibrium, and increases the proportion in the immiscible aqueous phase. That is, the oil : aqueous phase partition coefficient falls (Fig. 2) although the true oil : water partition coefficient

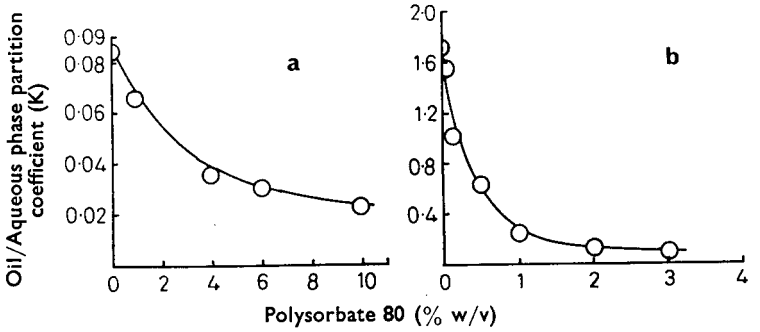

FIG. 2. Effect of polysorbate 80 on oil : aqueous phase partition coefficients of phenols between liquid paraffin and water. a. Phenol. b. Chlorocresol.

must remain unchanged on addition of polysorbate. If the oil : aqueous phase partition coefficient is known for any given polysorbate concentration, and the ratio of total : free phenols in the aqueous phase is known, the concentration of free preservative in the water may be calculated using equation (4).

Effect of oil and polysorbate 80 *concentration on the concentration of free preservative in the water*

For a fixed overall concentration of phenol and a fixed aqueous phase concentration of polysorbate, an increase in oil : water ratio produced an increase in the concentration of free phenol (Fig. 3), irrespective of the concentration of polysorbate.

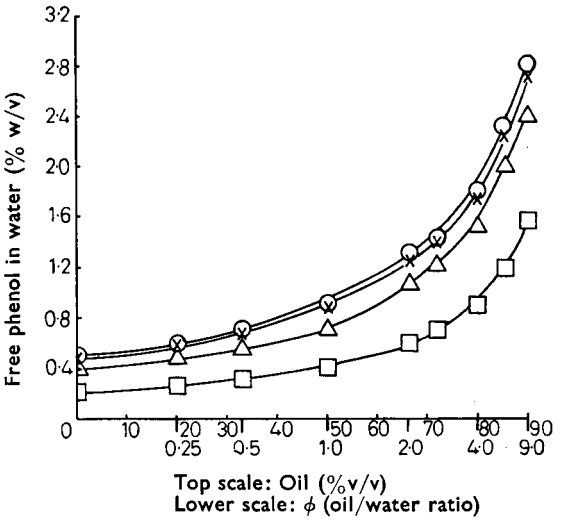

FIG. 3. Effect of proportion of oil in emulsions on concentration of free phenol in water when the total phenol concentration is 0·5% w/v. ○, Polysorbate concentration 0·0% w/v; ×, 0·04% w/v; △, 1·0% w/v and □, 5·5% w/v.

The curvilinear relations in Fig. 3 may be transformed to slightly sigmoidal near-linear relations with a positive slope by transforming the abscissa to $\sqrt{\text{oil : water ratio}}$.

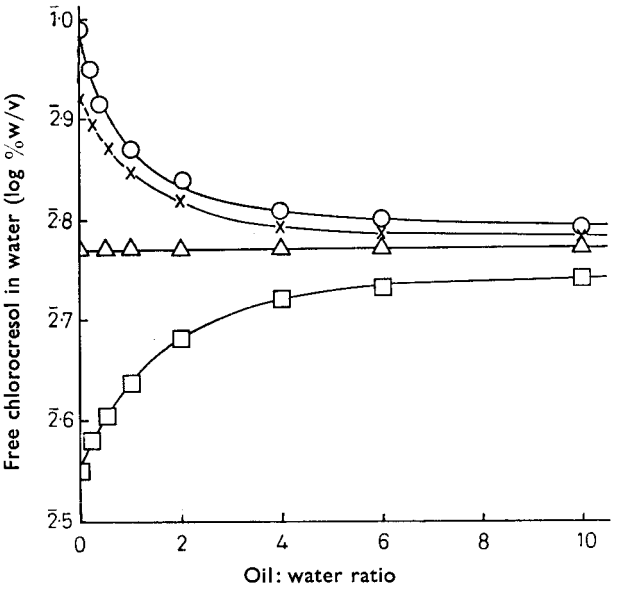

FIG. 4. Effect of oil : water ratio of emulsions on concentration of free chlorocresol in water when total chlorocresol concentration is 0·1% w/v. ○, Polysorbate concentration of 0·0% w/v; ×, 0·04% w/v; △, 0·15% w/v and □, 0·40% w/v.

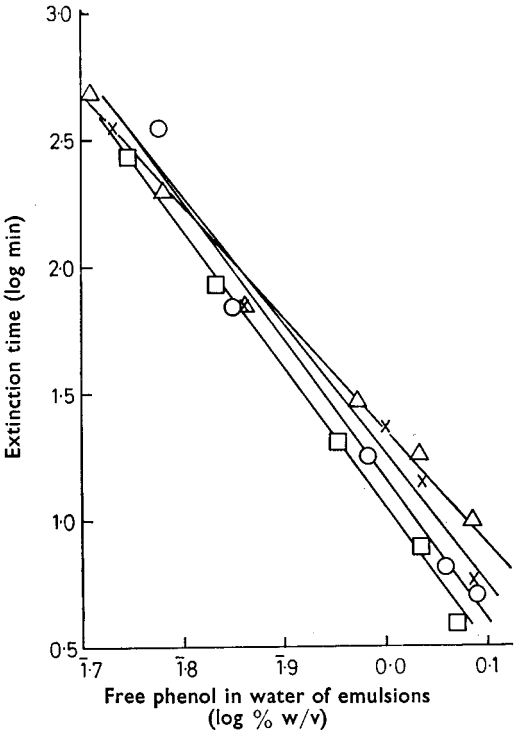

FIG. 5. Relationship between free phenol in emulsions and extinction time for *E. coli*. ○, Polysorbate concentration of 0·0% w/v; □, 0·04% w/v; ×, 1·0% w/v and △, 5·0% w/v.

With chlorocresol, the direction of change in concentration of the free preservative was influenced by both the oil:water ratio and the concentration of polysorbate (Fig. 4). At concentrations of polysorbate less than 0·15% w/v an increase in oil: water ratio produced a decrease in the concentration of free chlorocresol. When polysorbate was in excess of 0·15% w/v, an increase in the oil:water ratio produced an increase in the concentration of free chlorocresol, whilst when the polysorbate concentration was 0·15% w/v, the concentration of free chlorocresol was independent of the oil:water ratio.

The activity of the preservatives in the emulsions

Phenol. The bactericidal activity of phenol in emulsions containing 0–5·0% polysorbate is shown in Fig. 5 for oil:water ratios 0–9·0 (90% oil) where a linear relation is demonstrated between the free phenol in the emulsions and log extinction time.

Chlorocresol. For a fixed overall concentration of 0·1% w/v chlorocresol and an oil:water ratio of less than 2, the direction of change in activity with increasing oil: water ratio was dependent upon the concentration of polysorbate (Fig. 6). Increasing the oil:water ratio above 2, increased activity at all concentrations of polysorbate. For a fixed oil:water ratio, an increase in the concentration of polysorbate decreased the activity of the preservative in the emulsion.

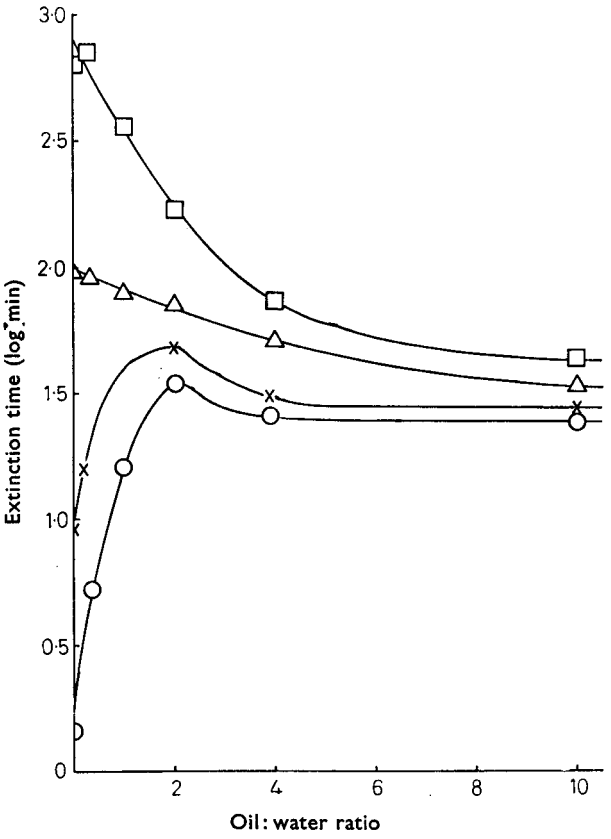

FIG. 6. Effect of oil : water ratio on extinction time for *E. coli* of emulsions containing 0·1% w/v total concentration of chlorocresol. ○, Polysorbate concentration of 0·0% w/v; ×, 0·04% w/v; △, 0·15% w/v and □, 0·40% w/v.

DISCUSSION

As a preliminary to the evaluation of preservative activity in emulsions, simplified models comprising only two of the three essential components of an emulsion have been studied. Pisano & Kostenbauder (1959) examined systems containing water and emulgent and concluded that the activity of preservatives in such systems was related to the concentration of uncomplexed preservative . Similarly the activity of preservatives in oil-water mixtures is dependent primarily upon the concentration in the water, this being influenced by the oil : water partition coefficient of the preservative and the phase-volume ratio (Bean, Richards & Thomas, 1962; Bean & Heman-Ackah, 1964). In both cases mathematical models have proved invaluable in relating preservative activity to parameters of the preservatives and changes in the composition of the systems. Such models may be either, simple, such as those referred to, or complex like the integrated models of Garrett (1966) which incorporate terms to quantify all identifiable factors influencing preservative availability. Not all factors are identifiable and some are difficult to quantify. Experience has shown, however, that simplified models, though admittedly incomplete, are sufficiently accurate for formulation purposes. The more complex a formula the greater the number of factors influencing preservative availability and the greater the number of terms necessary in any operational model to relate changes in preservative availability to formulation changes. The two models suggested in this communication (eqns 4 and 5) are only slightly more complex than the earlier model (Bean & Heman-Ackah, 1964) for computing preservative availability in oil-water mixtures. Their validity has been confirmed by assay of the preservative in the aqueous phase of the emulsions.

For preservatives having an oil : aqueous phase partition coefficient of less than 1·0, an increase in the proportion of oil in the emulsion increases the concentration of free preservative in the water (Fig. 3), thus resembling the effect in simple oil-water mixtures. In fact, calculations show that an increase in the percentage of oil in a liquid paraffin emulsion simultaneously increases the concentration of phenol in the water, aqueous phase, oil-phase and micelles of the polysorbate emulgent.

The changes are more complex when chlorocresol is the preservative and are best understood by reference to Fig. 4. Increasing the proportion of oil reduces the free chlorocresol concentration in the water when the polysorbate concentration is below 0·15% and increases it when it is greater than 0·15%. This is because at these polysorbate concentrations the oil : total aqueous phase partition coefficient (K) is respectively above and below 1·0. When the emulsion contains 0·15% polysorbate the oil : aqueous phase partition coefficient is 1·0 and the concentration of free chlorocresol is independent of the oil : water ratio. The direction of concentration change is always the same for all phases (cf. Bean & others, 1962).

A plot of free phenol in the water of the emulsions against extinction time of *E. coli*, shows a linear relation (Fig. 5). This indicates that the activity of phenol in the emulsions is dependent upon the concentration free in the water, the latter being dependent in turn upon the total concentration of phenol in the emulsion, the oil : water ratio, and the concentration of non-ionic emulgent (eqns 4 and 5).

The relation between the activity of chlorocresol and the proportion of oil or emulgent in the emulsion is much more complex (Fig. 6) and reflects the complex changes in the concentration of free chlorocresol produced by altering the oil : water ratio or concentration of emulgent. To indicate the comparatively large changes in preservative activity which can result from comparatively small changes in the

composition of an emulsion, the chlorocresol results have not been transcribed to relate activity to free chlorocresol. But once again it can be shown that the activity is dependent upon the free chlorocresol in the water. It may be noted that for oil-water mixtures containing 25% v/v liquid parafin and 0·1% w/v total chlorocresol, the extinction time is about 5 min whereas the addition of 0·4% v/v polysorbate, a far lower concentration than is likely to be encountered in practical emulsions, reduces activity one hundred-fold—to about 550 min. On the other hand, if the proportion of liquid paraffin in an emulsion containing 0·4% polysorbate is increased from 25 to 80%, the activity is *increased* about 10-fold.

It is quite meaningless to add an arbitrary concentration of a preservative to an emulsion. The only rational and safe procedure is to decide upon the activity required of a preservative in an emulsion and then to calculate the total quantity required from a knowledge of the parameters discussed in this communication. The calculation may often reveal the unsuitability of the preservative selected.

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