J. Pharm. Pharmacol., 1965, **17**, 217–221 Received October 19, 1964

Applicability of the Ferguson principle to systems of mixed preservatives

W. P. EVANS

Using solubility and distribution data for methyl and propyl *p*-hydroxybenzoates, the degree of saturation of the aqueous phase of an oil-in-water emulsion has been calculated for various oil : water ratios. In emulsions of low oil content the propyl ester gives the higher degree of saturation of the aqueous phase, while in emulsions of high oil content the methyl ester gives the higher degree of saturation. Irrespective of the oil : water ratio, a mixture of the esters cannot give a higher degree of saturation or a higher thermodynamic activity of the aqueous phase, than either ester alone. The finding of many investigators that mixtures of the esters have higher biological activities than a single ester must therefore depend, not only on the degree of saturation, or thermodynamic activity of the aqueous phase, but also on some other unknown factors.

DETERIORATION from mould formation or bacterial action of cosmetic creams during storage occurs mainly in oil-in-water emulsions, and is rare in water-in-oil emulsions. Generally the organisms responsible for deterioration need an aqueous medium for growth, consequently cosmetic products contain preservatives which are effective in preventing growth in the aqueous phase. The most widely used preservatives are esters of *p*-hydroxybenzoic acid, the methyl and propyl esters being generally favoured.

The literature contains conflicting views (Manowitz, 1962) about whether to use these preservatives singly (Atkins, 1950) or in combination (Boehm, 1959); those who advocate using the mixture claim a broader spectrum of microbiological activity than that given by their separate constituents, and also that the mixture exhibits a greater preservative action than would be expected from the individual activity (Boehm, 1959).

It is well known from bacterial studies that the activity of the esters of *p*-hydroxybenzoic acid is related to the length of the alkyl chain; the propyl is more active than the methyl ester but increasing the chain length reduces water solubility and increases oil solubility, i.e. the oil : water distribution coefficients are markedly increased. As deterioration in cosmetic preparations occurs in the water phase, only that proportion of the added preservative which is dissolved in the aqueous phase can inhibit the growth of micro-organisms. But, is the greater bacterial activity of the propyl compared to the methyl ester more than offset by its lower water solubility and its higher oil solubility? Atkins (1950) claims that this is often so and that the methyl ester is then the more reliable preservative; Atkins' data, therefore, do not support the widely held belief that a combination of the two esters is better than either alone.

Since there is no apparent agreement in the published literature about whether a mixture of esters is better than a single ester in emulsion systems, calculations based on solubility and distribution data might throw

From the Unilever Research Laboratory, Port Sunlight, Cheshire.

further light on the problem. Such calculations, previously suggested by Manowitz (1962) would show how the degree of saturation and the thermodynamic activity of the aqueous phase of an emulsion containing a single ester or a mixture of esters varies with the oil content of the emulsion, and with the distribution coefficient of the esters. Such an approach should therefore indicate whether a mixture of esters would, a priori, be expected to have a higher biological activity than a single ester, assuming that biological activity is proportional to the degree of saturation of the aqueous phase (Ferguson's Principle).

BIOLOGICAL ACTIVITY AND DEGREE OF SATURATION

The work of Ferguson (1939) and others has shown for structurally non-specific agents that the biological activity is related to the thermodynamic activity rather than to the concentration of the active agent. This principle has been further substantiated by an examination of the relative effectiveness of a large series of narcotics by Brink & Posternack (1948), and of factors governing the permeability of the insect cuticle by Webb (1949). The thermodynamic activity of a solute will, of course, depend upon the choice of standard state; one convenient method (Allawala & Riegelman, 1954) is to express it in terms of the per cent saturation, or degree of saturation, of the aqueous phase by setting the saturated solution as the standard state of reference. Allawala & Riegelman have shown that equitoxic solutions of phenols of widely different solubilities are those in which the thermodynamic activities or the degrees of saturation, are the same, rather than solutions in which the actual or stoichiometric concentrations are the same. From a study of 23 different phenols Allawala & Riegelman (1954) showed that, while the concentrations (moles/litre) of equitoxic solutions varied by a factor of approximately 10^4, the degrees of saturation of equitoxic solutions of the phenols were all between 18 and 20%.

CALCULATIONS

The data of Atkins (1950) for the solubility of the methyl and propyl *p*-hydroxybenzoate in water and in an oil (composition not specified) at 16° are: S_w 0·31 and 0·035, S_o 1·0 and 2·0 g/100 ml for the methyl and propyl ester respectively. $K_D = 3{\cdot}2$ and $57{\cdot}1$;

where S_w, S_o, K_D = water solubility, oil solubility, and distribution coefficient respectively.

When the ester distributes itself between the water and the oil phase, the concentration in the water phase can be derived from the equation

$$C_w = \frac{\text{Total weight of ester}}{V_w + K_D V_o} \quad .. \quad .. \quad .. \quad (1)$$

where C_w = concentration of ester in the water phase (g/ml water)

V_w, V_o = Volume of the water and oil phase respectively.

If it is accepted that biological activity is proportional to the thermodynamic activity of the active agent, which in turn is proportional to the per cent saturation, or degree of saturation, of the water phase, it is possible to write:

Biological Activity α Thermodynamic Activity α Degree of Saturation $=$

$$\frac{\text{Total weight ester}}{S_W (V_W + K_D V_O)} \quad .. \quad .. \quad .. \quad (2)$$

where S_W = water solubility of ester (g/ml).

Using equation (2) and K_D calculated from Atkins (1950) data given above, the degree of saturation of the water phase of an emulsion at various total weights of the esters, and two different oil/water ratios have been calculated and are given in Table 1.

TABLE 1. VARIATION OF THE DEGREE OF SATURATION OF THE AQUEOUS PHASE OF AN OIL : WATER EMULSION WITH DIFFERENT AMOUNTS OF METHYL AND PROPYL *p*-HYDROXYBENZOATES AND WITH VARYING OIL WATER RATIOS
(Calculated from solubility data of Atkins (1950)

	Water : oil 95 : 5% v/v		Water : oil 60 : 40% v/v	
	Degree of saturation		Degree of saturation	
Total wt. ester (g)	Methyl ester	Propyl ester	Methyl ester	Propyl ester
0·01	0·029	0·076	0·017	0·012
0·03	0·087	0·23	0·051	0·036
0·05	0·14	0·38	0·03	0·061
0·06	0·17	0·46	0·10	0·073
0·10	0·29	0·76	0·17	0·12
0·13	0·38	1·0	0·22	0·16
0·20	0·58	>1·0	0·34	0·24

Discussion

It is clear from Table 1 that, in the system containing water : oil 95 : 5%, the propyl ester at any given total weight of the esters, gives a higher degree of saturation of the water phase than the methyl ester; consequently if biological activity is indeed proportional only to the degree of saturation, the propyl ester should be the more active in this system. However, in the system water : oil 60 : 40%, for any given weight of the ester the methyl ester gives the higher degree of saturation in the water phase implying, as before, that in this system, the methyl ester should have a higher biological activity.

Generally, for any given weight of the esters, in systems of high water : low oil content, the propyl ester will give the higher degree of saturation of the water phase; as the oil content of the system increases a certain oil : water ratio is reached at which, for any given weight of the esters, the degree of saturation will be the same for both esters. Increasing the oil content of the system above this critical level—the "cross-over" point, gives systems in which, for any given weight of the esters, the methyl ester will always give the higher degree of saturation of the water phase.

The oil : water ratio of the system at the "cross-over" point i.e., where both esters give the same degree of saturation, depends only on the values of the distribution constants, K_D and the water solubilities S_w; it can be easily shown from equation (2), at any given total weight of the esters, that the two esters will give solutions of the same degree of saturation (and hence same biological activity) when:

$$V_o = \left(\frac{S_w^{Me} - S_w^{Pr}}{S_w^{Pr} K_D^{Pr} - S_w^{Me} K_D^{Me}} \right) V_w \qquad (3)$$

where S_W^{Me}, S_W^{Pr} = Water solubility of the methyl and propyl ester respectively.

K_D^{Me}, K_D^{Pr} = Distribution coefficients of the methyl and propyl ester respectively.

For systems where the volume of the oil phase, V_o, is less than the value given by equation (3), then the propyl ester, for any given weight, will give a higher degree of saturation of the water phase than the methyl ester; similarly when V_o is greater than the value given by equation (3) then the methyl ester will give the higher degree of saturation of the water phase. It is clear that a higher degree of saturation is obtained either with one or the other of the esters depending on the oil : water ratio; under no conditions does a mixture of esters give a higher degree of saturation than either ester singly.

The above argument holds only when $K_D^{Pr} S_w^{Pr} > K_D^{Me} S_w^{Me}$; if as is possible with some oils, $K_D^{Pr} S_w^{Pr} < K_D^{Me} S_w^{Me}$ then the propyl ester will always give a higher degree of saturation of the aqueous phase, i.e., no "cross-over" point, irrespective of the oil : water ratio. The essential point is that, whatever the oil : water ratio, a mixture of esters does not give a higher degree of saturation and hence a higher thermodynamic activity of the water phase, than either ester alone.

Atkins (1950) gives no information on the relative phase volumes in the emulsions used in his study, but merely states that the propyl ester was ineffective as a preservative, whereas the methyl ester was completely effective. From equation (3) and the solubility data given by Atkins, it can be calculated that the same weight of the two esters in the emulsions studied by Atkins would give the same degree of saturation when $V_o = 0{\cdot}275\, V_w$. If biological activity is governed solely by the degree of saturation of the aqueous phase then the methyl ester would be the better preservative in the emulsions studied by Atkins provided the emulsions contained >22% of the oil phase.

The same considerations will apply when, in addition to, or instead of the oil phase, the water phase contains a surfactant which solubilises the esters; in this instance K_D in equation (3) will be the distribution coefficient of the ester between the water phase and the micellar phase, and V_o will be replaced by V_m, the micellar volume.

Such systems have been studied by Aoki, Kamata, Yoshioka & Matsuzaki (1956) whose data show the "cross-over" point clearly; these data

show that in water and in low concentrations of polysorbate (Tween) 20, the order of biological activity of the esters is butyl > propyl > ethyl > methyl, while in high concentrations of polysorbate 20, the order of biological activity is exactly the reverse—as predicted in equation 3; the "cross-over" points depend entirely on the values of K_D and S_W of the various esters and could be calculated easily from solubility data (McBain & Hutchinson, 1955) using equation (3). The data of Atkins (1950), and also those of Aoki & others (1956) are in complete agreement with equation (3), i.e., in systems of low oil or micellar content, the higher esters are more effective than the lower esters in giving higher degrees of saturation of the aqueous phase, while the reverse is true in solutions of high oil or high micellar content.

It must therefore be concluded that, for emulsion systems where a mixture of esters is claimed to be more effective than a single ester (Boehm, 1959), the biological activities of such systems are governed not only by the degree of saturation of the aqueous phase but also by some other unknown factors. Whilst it is impossible to specify what these unknown factors are, Bean (1964) and Bean & Heman-Ackah (1964) have established that, in some two-phase systems, the activity of antibacterial agents is governed, not only by the degree of saturation of the aqueous phase, but also by the concentration of the antibacterial agent at the oil-water interface. This factor may also be important in systems in which a mixture of the *p*-hydroxybenzoates have been found to be more effective than a single ester, but further work is required to establish this point.

References

Allawala, N. A. & Riegelman, S. (1954). *J. Amer. pharm. Ass., Sci. Ed.*, **43**, 93–97.

Aoki, M., Kamata, A., Yoshioka, I. & Matsuzaki, T. (1956). *J. pharm. Soc. Japan*, **76**, 939–943.

Atkins, F. (1950). *Mfg Chem.*, **21**, 51–54.

Bean, H. S. (1964). "*The Activity of Antibacterials in Two Phase Systems*". Paper given at the Symposium "Preservative and Antioxidants", Nov., 1964 (London).

Bean, H. S. & Heman-Ackah, S. M. (1964). *J. Pharm. Pharmacol.*, **16**, *Suppl.*, 58*T*–67*T*.

Boehm, E. (1959). *Amer. Perf.*, **73**, 37–38.

Brink, F. & Posternack, S. M. (1948). *J. Cell. Comp. Phys.*, **32**, 211–233.

Ferguson, J. (1939). *Proc. Roy. Soc.*, B **127**, 387–404.

McBain, M. E. L. & Hutchinson, E. (1955). *Solubilisation and Related Phenomena*, p. 75. New York: Academic Press.

Manowitz (1962). *Development in Industrial Microbiology*, New York: Plenums Press, **3**, 295.

Webb, J. E. (1949). *Symp. Soc. exp. Biol.*, **3**, 143–163.