J. Pharm. Pharmac., 1968, **20**, *Suppl.*, 4S–7S Received April 1, 1968

The effect of a non-ionic surfactant upon the antifungal activity of benzoic acid

K. J. HUMPHREYS*, G. RICHARDSON AND C. T. RHODES

The effect of a non-ionic polyoxyethylene surfactant upon the fungicidal activity of benzoic acid has been investigated by a viable count method. The results show that theories equating antimicrobial activity to the concentration of non-micellar preservative are inadequate. The significance of these findings is discussed and some possible additional factors involved in the fungicidal process indicated.

PRESERVATION of creams from microbial spoilage has attracted considerable attention; Bean, Heman-Ackah & Thomas (1965) have emphasized that both the physical and microbiological properties of the system must be considered.

In any L_1 isotropic, aqueous liquid, system containing a surfactant and drug there exists an equilibrium between the micellar and non-micellar species of cosolute.

$$[D_w] \overset{K}{\rightleftharpoons} [D_m] \qquad (1)$$

where $[D_w]$ represents the concentration of free, unbound drug and $[D_m]$ the concentration of micellar drug.

It has been suggested (Allawala & Riegelman, 1953) that in solubilized systems the preservative activity is a function of $[D_w]$, $[D_m]$ acting as an inert reserve of drug. This concept has been extended by a number of workers, for example Mitchell (1964) and Mitchell & Brown (1966) who postulated that the activity of a drug in a solubilized system was governed by an R value or Saturation Ratio:

$$R = C/C_s \qquad (2)$$

where C is the drug concentration and C_s its saturation solubility.

By means of equilibrium dialysis and solubility techniques Humphreys & Rhodes (1968) have shown that the solubilization of benzoic acid by a series of n-alkyl polyoxyethylene surfactants is governed by a form of the Distribution Law (3) and the distribution constant, K_d, has been evaluated for these systems.

$$K_d = [D_m^o]/[D_w^o] \qquad (3)$$

where $[D_m^o]$ is the saturation solubility of drug in a hypothetical micellar bulk phase and $[D_w^o]$ is the saturation solubility of drug in the aqueous phase.

From the data collected by Humphreys & Rhodes it is possible to estimate the $[D_w]$ and $[D_m]$ values in the surfactant systems studied within the temperature range, 18–45°. Thus it is possible to test quantitatively the validity of the theory outlined above concerning the biological activity of cosolute in the presence of surfactant. This theory suggests

From: School of Pharmacy, Portsmouth College of Technology, Portsmouth, Hampshire, England.

* Present address: School of Pharmacy, Sunderland Technical College, Sunderland, Co. Durham.

that the antimicrobial activity of benzoic acid in systems having the same $[D_w]$ value should be identical regardless of the $[D_m]$ value or the total concentration of the drug in the system $[D_t]$.

In the present paper we report studies of the correlation between $[D_w]$ and the fungicidal activity of benzoic acid in a number of systems with and without surfactant.

Experimental

Materials. Analar benzoic acid; a commercial sample of an n-alkyl-polyoxyethylene surfactant (Texofor, Glover) of mean molecular formula $Me[CH_2]_{15}\cdot O[CH_2\cdot CH_2\cdot O]_{30}\cdot OH$, characterized according to Rhodes (1967).

The Test organism was a 24 hr culture of *Schizosaccharomyces pombé* washed from agar slopes and resuspended in water.

Method of count. Samples of reaction mixture (1 ml), appropriately diluted, were plated in roll tubes, each containing 4 ml of Wort Agar (Oxoid), and these were incubated inverted (bungs removed) for four days at 27°.

Experimental design. Controls were used to establish (a) The acid tolerance of the organism, (b) The absence of fungicidal activity of the surfactant, (c) The absence of activity from "carry-over" of reaction mixture into the roll tubes.

Fungicidal studies. The fungicidal activity of benzoic acid was determined in the presence and absence of 2% w/v Texofor. Comparison was made between the activity of (A) solutions of benzoic acid, (B) solutions of benzoic acid in 2% surfactant of the same total benzoic acid content and (C) solutions of benzoic acid, of concentration equivalent to that of the free acid in the surfactant solution. All solutions were adjusted to pH 3·0 with hydrochloric acid.

TABLE 1. COMPARISON OF FUNGICIDAL ACTIVITY OF EQUAL AMOUNTS OF BENZOIC ACID IN THE PRESENCE AND ABSENCE OF SURFACTANT

Reaction mixture	% survival in replicate determinations				
	Exposure time (hr)	1½	1¾	1¾	3½
A Benzoic acid alone	$[D_t] = [D_w]$	15·0 mM	26·0 mM	26·0 mM	26·0 mM
		2·4	0·1	0·014	0
		2·9	0·03	0·000	0
		3·0	0·2	0·007	0
		2·9	0·1	0·2	0
				0·007	0
	Mean	2·8	0·1	0·01	<0·0001
B Benzoic acid + surfactant	[Dt]	15·0 mM	26·0 mM	26·0 mM	26·0 mM
	[Dw]	7·5 mM	13·5 mM	13·5 mM	13·5 mM
		105	3·2	12·8	0·8
		96	3·2	12·7	0·7
		109	4·6	13·1	0·8
		97	3·5	11·2	0·9
		87		15·3	
	Mean	99	3·6	13·0	0·8

Saturation solubility, C_S (benzoic acid) in *N*/1000 HCl = 26·5 mM. C_S (benzoic acid) in 2% w/v surfactant = 51·0 mM.

TABLE 2. COMPARISON OF FUNGICIDAL ACTIVITY OF BENZOIC ACID SYSTEMS, WITH AND WITHOUT SURFACTANT, OF THE SAME [D_w] CONTENT

Reaction mixture	% survival in replicate determinations					
	Exposure time (hr)	1¾	3½	3½	1½	1¾
	[Dt]	26·0 mM	26·0 mM	26·0 mM	29·0 mM	29·0 mM
	[D_w]	13·5 mM	13·5 mM	13·5 mM	15·0 mM	15·0 mM
B Benzoic acid + surfactant		12·8	0·8	0·1	0·04	0·09
		12·7	0·7	0·1	0·07	0·02
		13·1	0·8	0·2	0·00	0·1
		11·2	0·9	0·1	0·03	0·09
		15·3			0·00	0·07
	Mean	13·0	0·8	0·1	0·03	0·07
	[Dt] = [D_w]	13·5 mM	13·5 mM	13·5 mM	15·0 mM	15·0 mM
C Benzoic acid alone		24·9	5·3	0·2	0·6	2·8
		23·4	7·5	0·2	0·9	3·2
		27·2	6·3	0·2	0·4	3·0
		26·1	7·5		0·4	2·5
			3·8		0·5	2·8
	Mean	25·4	6·1	0·2	0·6	2·9

Saturation solubility, C_B (benzoic acid) in N/1000 HCl = 26·5 mM. C_B (benzoic acid) in 2% w/v surfactant 51·0 mM.

Results

At pH 3·0, no lethal effect upon the organism was detected over a period of 21 hr. Change in pH from 2·0 to 3·0 was found to have little effect (less than 2%) upon the ionization of the unbound acid or the micellar solubilization. The controls showed that solutions of surfactant up to 5% w/v possessed no intrinsic antifungal activity and confirmed that the degree of preparing dilution was sufficient to inactivate reaction mixtures carried over into the counting medium.

The effects of the surfactant upon viable counts are summarized in Tables 1 and 2.

Discussion

Table 1 shows that the fungicidal activity of benzoic acid is reduced by the presence of surfactant. Reference to the values, in each vertical column, all determined under the same experimental conditions, shows that there is a significant increase in the percentage survival when surfactant is added.

Table 2 shows the results of several sets of comparisons of fungicidal activity of a series of benzoic acid systems of the same [D_w] value but differing in [D_t] because of the presence of surfactant. Comparison, within each vertical column, of data obtained under the same experimental conditions, clearly shows significant increases of percentage survival in those systems containing surfactant. From the results it must be concluded that the simple theory as outlined in the introduction, is insufficient to rationalize the fungicidal activity of benzoic acid in a system containing surfactant. From the data presented in Tables 1 and 2 the R, saturation ratio, values may also be calculated and it can be seen that fungicidal activity is not a simple function of this term as has been previously

suggested (Mitchell, 1964; Mitchell & Brown, 1966). There must, therefore, be additional factors controlling the extent of fungicidal activity.

The first possibility is that the non-micellar surfactant may exert a synergistic effect upon the fungicidal activity of $[D_w]$. Since it has been shown by Gershenfeld & Stedman (1949) that synergism occurs with submicellar concentrations of non-ionic surfactants, this is a likely explanation of the results.

Secondly, the micelles may act as a reservoir of drug so that if $[D_w]$ becomes significantly depleted some of the bound drug is released to restore the equilibrium shown in (1). Experiments with solubilized chloroxylenol by Bean & Berry (1951, 1953) suggest the possibility of this mechanism.

A third explanation is that some form of "mixed micelle" is formed between the organism, the surfactant molecules and the benzoic acid, so that the organism is brought into intimate contact with the cosolute. This postulated mechanism appears analogous to that suggested by Bean & Dempsey (1967), who obtained results indicating that phenols exhibited greater antibacterial activity if first adsorbed on to small quantities of carbon. It seems that the organism was also adsorbed on to the treated carbon so that the effective concentration of phenol adjacent to the organism was increased.

The results presented in this paper indicate that, if formulation of L_1 surfactant systems with bactericides is based upon the simple two phase theory, a more than adequate degree of protection will be conferred.

References

Allawala, N. A. & Riegelman, S. (1953). *J. Am. pharm. Ass. Sci. Edn.*, **42**, 267–274.
Bean, H. S. & Berry, H. (1951). *J. Pharm. Pharmac.*, **3**, 639–655.
Bean, H. S. & Berry, H. (1953). *Ibid.*, **5**, 632–639.
Bean, H. S. & Dempsey, G. (1967). *Ibid.*, **19**, Suppl. 197S–202S.
Bean, H. S., Heman-Ackah, S. M. & Thomas, J. (1965). *J. Soc. cosmetic Chem.*, **16**, 15–30.
Gershenfeld, L. & Stedman, R. L. (1949). *Am. J. Pharm. Sci. Ed.*, **121**, 249–266.
Humphreys, K. J. & Rhodes, C. T. (1968). *J. pharm. Sci.*, **57**, 79–83.
Mitchell, A. G. (1964). *J. Pharm. Pharmac.*, **16**, 533–537.
Mitchell, A. G. & Brown, K. F. (1966). *Ibid.*, **18**, 115–125.
Rhodes, C. T. (1967). *Canad. J. pharm. Sci.*, **2**, 16–19.