

The temperature coefficient of some bactericides in oil : water dispersions

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The temperature coefficient of a bactericide in oil : water dispersion changes with the nature of the oil and the phase : volume ratio. The variations in the temperature coefficient are due to temperature effects on the availability of the bactericide in the aqueous phase, and at the oil : water interface of the dispersions. Failures in the preservation of oil : water formulated products encountered during storage in different climates may be due to such underlying phenomena which are not considered in the initial stages of formulation.

THE manner in which temperature influences antibacterial activity is important theoretically and practically. Information is available about temperature coefficients of commonly used antimicrobial agents in aqueous solutions but cannot be used to predict preservative performance in oil-water formulations because of the interaction of many factors (Kabelik, 1947, Galloway, 1952; de Navarre, 1962.)

Some of the factors which control the activity of a bactericide in oil : water dispersions have been reported elsewhere (Bean, Richards & Thomas, 1962; Bean & Heman-Ackah, 1964; Bean, Heman-Ackah & Thomas, 1965a, 1965b). The overall activity is dependent largely on the concentration of the bactericide partitioned to the aqueous phase of the dispersion, but it is enhanced by an effect due to the oil : water interface. A rise in temperature may shift the oil : water partition coefficient (K_w^o) and hence alter the concentration of the bactericide available in the aqueous phase and at the oil : water interface. Consequently, the temperature coefficient of the bactericide in the dispersion (θ_s) differs from that in aqueous solution (θ_A), and it is dependent on the nature of the oil and the ratio of the phases (Bean & Heman-Ackah, 1963).

The present communication reports a correlation between the temperature coefficient of a bactericide in the dispersion (θ_s) and the rate of change of the oil : water partition coefficient with temperature which may be used for the primary evaluation of preservatives in oil : water systems.

Experimental

MATERIALS AND METHODS

The test organism (*Escherichia coli* NCTC 5933), oils, bactericides and experimental techniques, were as described previously (Bean & Heman-Ackah, 1963, 1964). A spectrophotometric method was used in assaying the aqueous phase concentrations of bactericides in oil : water systems when determining K_w^o ; an extinction time method was employed for the evaluation of bactericidal activity.

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LINEAR RELATIONSHIP BETWEEN THE OIL:WATER PARTITION COEFFICIENT (K_w^0) AND TEMPERATURE (T)

The oil:water partition coefficients, K_w^0 , of bactericides were determined at 5, 15, 25, 35 and 45° respectively. Table 1 shows that a rise in temperature may cause an increase in K_w^0 (e.g. phenol in liquid paraffin:water), a slight increase in K_w^0 (e.g. chlorocresol in arachis oil:water) or a decrease

TABLE 1. INFLUENCE OF TEMPERATURE ON THE PARTITION COEFFICIENT (K_w^0) OF BACTERICIDES IN OIL:WATER SYSTEMS

Temperature °C	Phenol in paraffin: water	Phenol in liquid paraffin: water containing 5% propylene glycol in aqueous phase	Phenol in liquid paraffin: water containing 0.9% sodium chloride in aqueous phase	Chlorocresol in liquid: paraffin water	Chlorocresol in arachis oil: water	Phenylmercuric acetate in liquid paraffin: water
5	0.0592	0.0351	0.0389	1.192	113.4	0.1405
15	0.0620	0.0488	0.0586	1.341	116.7	0.1203
25	0.0688	0.0594	0.0992	1.528	116.7	0.0953
35	0.1167	0.0801	0.1360	1.782	116.0	0.0847
45	0.1535	0.1104	0.1787	2.003	117.2	0.0700
K_w^0 at 45°	2.59	3.14	4.59	1.68	1.03	0.50
K_w^0 at 5°						

in K_w^0 (e.g. phenylmercuric acetate in liquid paraffin:water). The presence of 5% propylene glycol in the aqueous phase of liquid paraffin:water decreases K_w^0 for phenol at all the test temperatures. On the other hand, 0.9% sodium chloride in the aqueous phase of liquid paraffin:water increases the value of K_w^0 for phenol at 25–45°, causes no change at 15°, and decreases K_w^0 at 5–15°.

The relationship between $\log K_w^0$ and T is linear for all bactericide:oil:water systems (Fig. 1) and is summarised by the expression:

$$\log K_w^0 = \epsilon T + \text{constant.}$$

ϵ is defined as the *temperature coefficient of the partition coefficient* (Heman-Ackah, 1965) and it is the appropriate increase in K_w^0 per °C rise in temperature. Its numerical value may be positive or negative depending upon whether the K_w^0 increases or decreases with rise in temperature (Table 3).

INFLUENCE OF TEMPERATURE ON THE ACTIVITY OF BACTERIDES IN OIL:WATER DISPERSIONS

Temperature influences the activity of bactericides in oil:water dispersions in a complex manner. Temperatures above 50° cause the death of vegetative cells by protein coagulation, enzyme inactivation or both. There are additionally, the indirect effects of temperature on: (i) the distribution of the bactericide between oil and water phases (Table 1) which produces differences in the availability of the bactericide in the

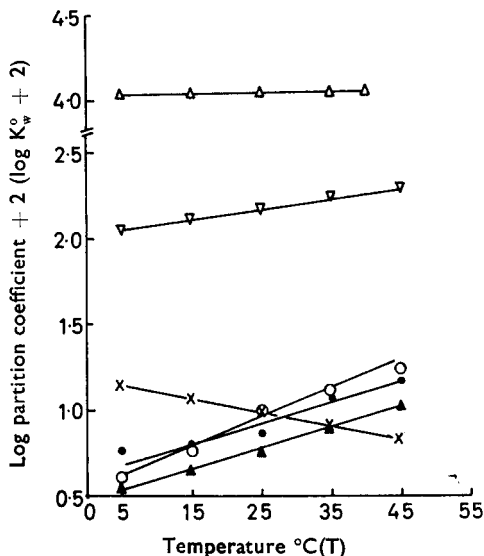


FIG. 1. The linear relationship between partition coefficient and temperature. ● = Phenol:liquid paraffin:water. ▲ = phenol:liquid paraffin:water containing 5% propylene glycol in aqueous phase. ○ = Phenol:liquid paraffin:water containing 0.9% sodium chloride in aqueous phase. ▽ = chlorocresol in liquid paraffin:water. △ = chlorocresol in arachis oil:water. × = Phenylmercuric acetate in liquid paraffin:water.

aqueous phase of the dispersion, and hence in the activity (Bean & Heman-Ackah, 1963); (ii) the oil:water interfacial activity which diminishes with rise in temperature (Heman-Ackah, 1965); (iii) the velocity of the bactericidal action which increases with rise in temperature (Madsen & Nyman, 1907; Chick, 1908; Phelps, 1911).

Table 2 shows the variation of the log of temperature coefficient (θ) of bactericides with the oil:water ratio (ϕ) of the dispersions. One of two phenomena may occur.

1. K_w^o increases with rise in temperature. The normal effect of temperature on the bactericidal activity is offset by an effect due to a progressive depletion of the bactericidal content of the aqueous phase and hence of the oil:water interface. In this instance, the temperature coefficient of the bactericide in the dispersion (θ_s) is less than that in aqueous solution (θ_A) containing the same overall concentration of the bactericide (i.e. the aqueous reference solution), and it decreases with increase in the oil:water ratio (ϕ). A previously reported relationship (Bean & Heman-Ackah, 1963; Heman-Ackah, 1965) applies:

$$\frac{\log \theta_A - \log \theta_s}{\phi} = k \text{ (where } k = \text{constant)}$$

At a unique oil:water ratio, the activity of the dispersion becomes independent of temperature (i.e. $\theta_s = 1$). Under such a condition, the appropriate increase in the bactericidal activity per °C rise in temperature

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is offset by a parallel depletion of the bactericidal content of the aqueous phase and by a smaller effect of the interface on the activity.

2. K_w^o decreases with rise in temperature. The normal effect of temperature on the bactericidal activity is enhanced by an effect due to increased concentration of the bactericide in the aqueous phase and at the oil: water interface. Consequently, the temperature coefficient of the bactericide in the dispersion (θ_s) is greater than that of the aqueous reference solution (θ_A). The following relationship applies (Heman-Ackah, 1965)

$$\frac{\log \theta_A - \log \theta_s}{\sqrt{\phi}} = k' \text{ (where } k' = \text{constant).}$$

Therefore there cannot be a dispersion in which the activity is independent of temperature.

The constant k (or k') is a measure of the change in activity of a bactericide in the dispersion per °C rise in temperature as compared with that of an aqueous reference solution. The concentration of the bactericide

TABLE 2. LOG TEMPERATURE COEFFICIENT OF BACTERICIDES IN AQUEOUS SOLUTIONS AND IN OIL/WATER DISPERSIONS (5–45°)

Oil: water ratio (ϕ)	K_w^o increase with rise in temperature					K_w^o decrease with rise in temperature
	0.5% Phenol in liquid paraffin: water	0.5% Phenol in liquid paraffin: containing 5% propylene glycol in aqueous phase	0.5% Phenol in liquid paraffin: water containing 0.9% sodium chloride in aqueous phase	0.075% Chloro- cresol in liquid paraffin: water	4.0% Chloro- cresol in arachis oil: water	
0 (Aqueous reference solution)	0.0588	0.0557	0.0680	0.0396	—*	0.0219
0.2	0.0547	0.0508	0.0646	0.0374	—	0.0246
0.5	—	—	—	—	0.1019	—
0.6	—	—	—	—	0.0873	—
0.7	—	—	—	—	0.0837	—
0.8	—	—	—	—	0.0698	—
1.0	0.0402	0.0309	0.0503	0.0283	0.0656	0.0285
1.5	0.0266	0.0156	0.0419	—	—	—
2.0	0.0166	—	0.0278	—	—	0.0322
3.0	—	—	—	—	—	0.0351
3.5	—	—	—	0.0030	—	—
5.0	—	—	—	—	—	0.0367
$k = \frac{\log \theta_A - \log \theta_s}{\phi}$	+0.020	+0.025	+0.018	+0.012	—	—
$k' = \frac{\log \theta_A - \log \theta_s}{\sqrt{\phi}}$	—	—	—	—	—	-0.007

* Impracticable to determine

in the aqueous phase of a dispersion changes with rise in temperature because the K_w^o does not remain constant, whereas the concentration of the bactericide in the aqueous reference solution is maintained the same over the range of temperature studied. Consequently, the constant k (or k') reflects the availability of the bactericide in the aqueous phase and at the oil:water interface, and therefore, reflects also the rate of change of the oil:water partition coefficient with temperature.

RELATIONSHIP BETWEEN THE CONSTANT k (OR k') AND THE TEMPERATURE COEFFICIENT OF THE PARTITION CONSTANT, ϵ

Table 3 shows the relationship between the constant, k (or k') and ϵ the temperature coefficient of the partition constant. For systems where k_w^o increases with temperature rise, k is approximately twice the value of ϵ (Mean Ratio $k/\epsilon = 2.0$) whereas for systems where K_w^o decreases with temperature rise k' is about equal to the value of ϵ (Ratio $k'/\epsilon = 0.9$).

TABLE 3. RELATIONSHIP BETWEEN THE CONSTANT k (OR k') AND THE TEMPERATURE COEFFICIENT OF THE PARTITION CONSTANT (ϵ)

System (K_w^o increase with rise in temp.)	$k = \frac{\log\theta_A - \log\theta_s}{\phi}$	$\epsilon = \frac{\Delta\log K_w^o}{\Delta T}$	Ratio $\frac{k}{\epsilon}$
Phenol in liquid paraffin: water	+ 0.020	+ 0.011	1.8
Phenol in liquid paraffin: water containing 5% propylene glycol in aqueous phase	+ 0.026	+ 0.012	2.2
Phenol in liquid paraffin: water containing 0.9% sodium chloride in aqueous phase	+ 0.018	+ 0.010	1.8
Chlorocresol in liquid paraffin: water	+ 0.012	+ 0.005	2.4
Chlorocresol in arachis oil/water	—*	+ 0.0003	—
System (K_w^o decreases with rise in temp.)	$k' = \frac{\log\theta_A - \log\theta_s}{\sqrt{\phi}}$	$\epsilon = \frac{\Delta\log K_w^o}{\Delta T}$	Ratio $\frac{k'}{\epsilon}$
Phenylmercuric acetate in liquid paraffin: water	— 0.007	— 0.0076	0.9

* $\log\theta_A$ for 4.0% chlorocresol in aqueous solution was impracticable to determine.

There is, therefore, a direct correlation between the temperature coefficient of the bactericide in the dispersion and the rate of change of the oil:water partition coefficient with temperature. This indicates that the toxic concentration of the bactericide in the dispersion is the available concentration in the aqueous phase and at the oil:water interface. It is inferred that the variation of the temperature coefficient of the bactericide with the oil:water ratio is purely a physical phenomenon and that the fundamental bactericidal action remains unaltered.

Discussion

The numerical value of the temperature coefficient (θ) is dependent on the disinfectant and test organism but it varies with (i) the range of temperature used for the determination (Tilley, 1942; Ames & Smith, 1944; Jordan & Jacobs, 1946); (ii) the concentration of disinfectant employed

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for the determination (Chick, 1908; Jordan & Jacobs, 1944; Berry & Michaels, 1950).

In oil: water dispersions it is also shown that θ varies with the oil: water ratio even for the same overall concentration of bactericide, test organism, and range of temperature employed for the experiments. This fact, though inherent in published data, has not been previously commented upon.

Beléhrádek (1935) has said that "when one and the same biological process gives two or more different temperature coefficients under various conditions, three different explanations are possible: (i) the formula used does not adequately express the relationship between temperature and reaction velocity in that particular case, and another type of formula should be tested; (ii) if the formula holds good, then the value of the temperature coefficient is modified by the conditions at which the reaction takes place; (iii) that there are two or more distinct reactions of a catenary series on which the whole process is based under varying conditions." The explanation given in (ii) would account for the variations in the temperature coefficient of bactericides in oil: water dispersions, the modifying conditions being the changes in the concentration of the available bactericide in the aqueous phase and at the oil: water interface, both of which are controlled by the oil: water ratio and temperature.

Failures in preservation have been encountered in oil: water formulated products stored under tropical conditions and it has been claimed that these are readily explained on grounds of chance contamination by different organisms "insensitive" to the preservative (de Navarre, 1962). It is more likely that the underlying phenomenon is the variation in the temperature coefficient of the preservatives in the oil: water systems (θ_s). This means that when a rise in temperature takes place, preservative may pass from the aqueous to the oily phase where it is no longer available to exert its activity. The present work has shown that for any given overall concentration of a bactericide in the dispersion, the activity can be estimated, from a knowledge of the rate of change of K_w^o with temperature and the temperature coefficient of the bactericide in aqueous solution (θ_A). No bacteriological evaluation is necessary.

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