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Bactericidal activity of chloroxylenol in aqueous solutions of cetomacrogol

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The bactericidal activity of chloroxylenol in water and in solutions of the non-ionic surface-active agent cetomacrogol is shown to be related to the degree of saturation of the system, expressed as a Saturation Ratio, which is the ratio of the amount of chloroxylenol present to its solubility. A saturated solution of chloroxylenol in water is shown to have the same bactericidal activity as saturated surfactant solutions containing up to 100 times as much chloroxylenol. It is apparent that bactericidal activity depends on the amount of chloroxylenol in the true aqueous "phase" and not the total amount present. The bactericidal activity of undersaturated solutions of chloroxylenol in cetomacrogol falls markedly as the Saturation Ratio is reduced. This is attributed to a change in the distribution of chloroxylenol in favour of the micelles where it is apparently without bactericidal activity.

THE effects of surface-active agents on the bactericidal properties of phenols and other compounds have been much investigated. Bean & Berry (1951, 1953) using benzylchlorophenol (5-chloro-2-hydroxy-diphenylmethane) and chloroxylenol (4-chloro-3,5-xylenol) solubilised in potassium laurate solutions claimed that bactericidal activity depends on the concentration of phenol in the micelles and that the amount of phenol in water alone makes no significant contribution to the total bactericidal activity even though the water is saturated with phenol. In contrast, Allawala & Riegelman (1953a, b) concluded that the bactericidal action of iodine solubilised in Antarox A-400 (a polyethylene glycol nonyl phenol derivative) is related to the degree of saturation of the water with iodine and not the total concentration of iodine present, i.e., in the water and micelles combined. Further work by the same authors (1954) showed, in accordance with the proposals of Ferguson (1939), that the bactericidal activity of many phenols in water depends on the thermodynamic activity of the solution. Equitoxic solutions were shown to be those in which the thermodynamic activity or degree of saturation and not the actual concentration was the same. The thermodynamic activity of the phenol solution was expressed in terms of the percentage saturation by setting the saturated solution as the standard state of reference.

The bactericidal activity of solutions of chloroxylenol in water and in Cetomacrogol 1,000 B.P.C. (polyethylene glycol 1,000 monocetyl ether) against *Escherichia coli* is now reported. In agreement with the findings of Allawala & Riegelman, bactericidal activity is shown to be related to the degree of saturation of the solution. The degree of saturation is expressed as a Saturation Ratio R, in which

$$R = \frac{c}{c_S} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where c is the concentration and c_S the solubility of chloroxylenol. The degree of saturation is expressed as a ratio rather than as a percentage

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since equation (1) has been used previously to show the dependence of rates of oxidation (Carless & Mitchell, 1962) and rates of hydrolysis (Mitchell, 1963, 1964) of solubilised and emulsified systems on the degree of saturation.

Method

Determination of the solubilities of chloroxylenol in water and in aqueous solutions of cetomacrogol 1,000. The solubilities of chloroxylenol in water and in solutions of cetomacrogol at 20° were determined by the methods described by Mulley & Metcalf (1956).

Determination of the mean death-time of E. coli *in chloroxylenol solutions.* The death-times of *E. coli* in solutions of chloroxylenol in water and in cetomacrogol were estimated by an extinction time method similar to that described by Berry & Bean (1954). The method of cultivating the test organism and sampling the reaction mixture was modified as follows:

(i) *E. coli* from a freeze dried culture was transferred to a slope of peptone agar. Subcultures were made every 24 hr for 4 weeks. From the fourth day slopes were used to prepare suspensions containing $2{,}000 \times 10^6$ organisms per ml in quarter strength Ringer's solution.

(ii) 0·2 ml of bacterial suspension was inoculated into 5 ml of chloroxylenol solution at 20°. At pre-determined time intervals, corresponding to 1/6th to 1/10th of the anticipated death-time, samples were taken from the reaction mixture with a platinum loop and transferred to 5 ml of nutrient broth at 37°. At the end of the experiment the tubes were transferred to an incubator at 37° and examined for evidence of growth after 3 days. Each experiment was made in replicate and the mean death-time calculated according to the method of Berry & Bean (1954).

Results and discussion

The solubility of chloroxylenol in water at 20° was 0·031 g/100 ml. The solubilities of chloroxylenol in cetomacrogol solution at 20° agreed with the values of Mulley & Metcalf (1956). The results in Tables 1–3 show that the death-time of *E. coli* in solutions of chloroxylenol in cetomacrogol

TABLE 1. DEPENDENCE OF DEATH-TIME OF *E. coli* IN CHLOROXYLENOL-CETOMACROGOL SOLUTIONS AT 20° ON THE SATURATION RATIO

Saturation Ratio R	Chloroxylenol %		Cetomacrogol moles/litre	Number of replicates	Mean death-time (min)	Standard deviation	Coefficient of variation
	c	cs					
1·00	0·1	0·1	0·0026	16	52	3·596	6·92
1·00	0·3	0·3	0·0098	24	53	4·667	8·81
1·00	1·5	1·5	0·0490	16	49	3·124	6·38
1·00	3·0	3·0	0·0960	19	49	7·431	15·17
0·90	0·1	0·111	0·0030	16	108	7·528	6·84
0·90	0·3	0·333	0·0110	16	104	11·58	11·13
0·90	1·5	1·670	0·0540	16	104	10·20	9·81
0·90	3·0	3·333	0·1070	16	98	11·98	12·48

$$R = c/c_s \quad \ldots \quad (1)$$

depends on the Saturation Ratio (R) as defined in equation (1). For any given value of R the death-times agree closely. When R = 1·0 the death-times in chloroxylenol-cetomacrogol solutions are approximately the same as in a saturated solution of chloroxylenol in water (Table 4). Thus a saturated solution of chloroxylenol in water has the same bactericidal activity against *E. coli* as a saturated solution of chloroxylenol in 0·096 M cetomacrogol containing 100 times as much chloroxylenol.

TABLE 2. DEPENDENCE OF DEATH-TIME OF *E. coli* IN CHLOROXYLENOL-CETOMACROGOL SOLUTIONS AT 20° ON CHLOROXYLENOL CONCENTRATION. (Cetomacrogol concentration 0·096 moles/litre)

Chloroxylenol %	Saturation ratio R	Number of replicates	Mean death-time (min)	Standard deviation	Coefficient of variation
3·00	1·00	19	49	7·431	15·17
2·85	0·95	16	84	5·007	5·96
2·70	0·90	16	96	9·037	9·51
2·55	0·85	5	75–79 hr	—	—

When R = 1·0 both the micelles and the true aqueous "phase" are fully saturated with chloroxylenol. Since the death-times in these solutions are the same as in a saturated solution of chloroxylenol in water it is apparent that the bactericidal activity is due to the fraction of chloroxylenol present in the true aqueous "phase" and not to the amount of chloroxylenol in the micelles nor to the total amount present in the system. These findings are in agreement with the Ferguson principle (Ferguson, 1939) as developed by Allawala & Riegelman (1953a, 1954) who showed that equitoxic solutions, both in water and in surface-active agents, are those having the same thermodynamic activity or degree of saturation and not the same concentration. (The reader is referred to the original papers for a full discussion of these concepts).

TABLE 3. DEPENDENCE OF DEATH-TIME OF *E. coli* IN CHLOROXYLENOL-CETOMACROGOL SOLUTIONS AT 20° ON CETOMACROGOL CONCENTRATION. (Chloroxylenol concentration 1·5%)

Cetomacrogol (moles/litre)	Saturation ratio R	Number of replicates	Mean death-time (min)	Standard deviation	Coefficient of variation
0·049	1·00	16	49	3·124	6·38
0·051	0·95	16	88	7·192	8·08
0·054	0·90	16	104	10·20	9·81
0·057	0·85	5	75–79 hr	—	—

In solutions of chloroxylenol in water the bactericidal activity decreases as the concentration of chloroxylenol, and thereby the Saturation Ratio is reduced (Table 4). In chloroxylenol-cetomacrogol solutions the Saturation Ratio can be reduced either by decreasing the concentration of chloroxylenol (Table 2) or by increasing the concentration of cetomacrogol (Table 3). The resulting increase in death-time is more marked than in corresponding chloroxylenol-water solutions and when R = 0·85 the death-time becomes nearly the same as in water or cetomacrogol solutions without chloroxylenol (Table 4). Such results accord with the numerous reports that non-ionic surface-active agents inactivate preservatives (Beckett & Robinson, 1958). The difference in death-time between solutions of chloroxylenol in water and in cetomacrogol when $R < 1 \cdot 0$ may be attributed to the change in distribution of chloroxylenol in favour of the micelles in a solution of surface-active agent on reducing the Saturation Ratio. Presumably when $R = 0 \cdot 85$ there is insufficient chloroxylenol in the true aqueous "phase" to exert a bactericidal action. From solubility studies and the evidence of ultra-violet absorption spectra Mulley & Metcalf (1956) suggested that incorporation of chloroxylenol into micelles of cetomacrogol is governed by the hydrogen bonding which occurs between the phenolic hydroxyl group of chloroxylenol and the ether chain of the non-ionic surface-active agent. This hydrogen-bonded complex is presumably inactive.

TABLE 4. DEATH-TIME OF *E. coli* IN SOLUTIONS OF CHLOROXYLENOL IN WATER AND IN SOLUTIONS OF CETOMACROGOL AT 20°

Chloroxylenol %	Cetomacrogol (moles/litre)	Saturation ratio	Replicates	Mean death-time (min)
0·031	0·000	1·00	16	47
0·029	0·000	0·95	16	74
0·028	0·000	0·90	16	85
0·026	0·000	0·85	16	121
0·000	0·000	—	5	79–84 hr
0·000	0·096	—	5	79–84 hr
0·000	0·120	—	5	79–84 hr

In simple solutions of chloroxylenol in water, changes in concentration are related directly to the thermodynamic activity of phenol and so to its bacterial activity. The bactericidal activity of chloroxylenol in the solubilised state, however, is due to its concentration in the true aqueous "phase" and not the total concentration. Hence a better index of bactericidal activity would be the degree of saturation of the true aqueous "phase" rather than the total solution as defined in equation (1). This would require a knowledge of the distribution of chloroxylenol between the micelles and the true aqueous "phase". Nevertheless the concept of Saturation Ratio provides a simple and convenient means of defining the degree of saturation of the solution as a whole. Moreover, the death-times in undersaturated solutions in cetomacrogol at a given value of R are in close agreement indicating that the degree of saturation of the true aqueous "phase" with chloroxylenol is the same. Hence the degree of saturation as expressed by the Saturation Ratio, can be used as an index of the bactericidal activity of chloroxylenol-cetomacrogol solutions.

It is of interest to note that in solutions of surface-active agents, phenomena as different as bactericidal activity, rates of oxidation (Carless & Mitchell, 1962) and rates of hydrolysis (Mitchell, 1963, 1964) are dependent on the degree of saturation of the system. This is to be expected since the degree of saturation of a given solvent system is a measure of the thermodynamic activity of the system and therefore of its chemical potential. Solutions of surface-active agents differ from simple solvents in that rates of reaction in the former are apparently controlled by the distribution of reactant between the micelles and the true aqueous "phase". With bactericidal activity and hydrolysis the rate of reaction appears to depend mainly on the amount of reactant in the true aqueous "phase", whereas in oxidation it is controlled mainly by the amount in the micelles.

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