

RESEARCH PAPERS

THE BACTERICIDAL ACTIVITY OF PHENOLS IN AQUEOUS SOLUTIONS OF SOAP

PART I.—THE SOLUBILITY OF A WATER-INSOLUBLE PHENOL IN AQUEOUS SOLUTIONS OF SOAP

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INTRODUCTION

THE investigation described in the present and subsequent papers is concerned with the bactericidal activity of phenols when dissolved in aqueous solutions of soap. It shows that the solubility of sparingly water-soluble phenols in aqueous solutions of potassium laurate is related to the formation of micelles in the solutions, and that the mechanism of solubilisation is the same as that quoted by Lawrence¹, McBain and Johnson² and Hartley³ for the solubilisation of water-insoluble dyes by potassium laurate and other soaps. It seeks to prove that the bactericidal activity of water-insoluble phenols solubilised by aqueous solutions of soap is a function of the concentration of the phenol within the micelles of the soap solution, rather than the concentration of the phenol in the solution as a whole. A number of attempts have been made previously to ascertain the effect of soap and other paraffin-chain surface-active compounds on the activity of phenolic bactericides. The results of the earlier attempts appear to be contradictory, but they can be reconciled with the present knowledge of the properties of soap in aqueous solution.

Hamilton⁴ showed that a solution containing 1.3 per cent. of phenol and 1.3 per cent. of soap had only the same activity as a 1.2 per cent. solution of phenol alone, while Lange⁵ found that the activity of aqueous solutions of cresol is improved by the addition of soap. Hampil⁶ found that the addition of 0.2 to 0.5 per cent. of sodium oleate to solutions of phenol, *m*-cresol or hexylresorcinol had a marked depressant effect on the bactericidal activity. The author suggested that the observed depression might be due (1) to the occurrence of a true chemical reaction between the soap and the phenol, resulting in a non-active substance, (2) a protective colloidal action by the soap on the bacteria, (3) the ability of the soap to remove the phenolic material from the solution in a definite partition coefficient ratio or (4) a combination of physico-chemical factors which may or may not have been expressed in the previous statements. Frobisher⁷ reported that the effect of sodium oleate on the bactericidal activity of aqueous solutions of phenol depended upon its concentration therein. The addition of up to 0.05 per cent. of sodium oleate to a 1 per cent. solution of phenol increased the activity of the latter, but the addition of 0.1 per cent. decreased it. A similar

effect was observed by Cade⁸ who noted that a 0.05 per cent. solution of phenol exhibited maximal activity when it contained 0.25 per cent. of sodium ricinoleate. Further experiments by Cade also established that the concentration of soap that must be added to a solution of a phenol to produce the maximal bactericidal activity varies according to the constitution of the phenol.

Studies by Alexander and Trim⁹ on the essentially similar problem of the anthelmintic activity of aqueous solutions of hexylresorcinol and soap, showed that the biological activity of such solutions can be related to the interfacial tension between the solution and liquid paraffin. The interfacial tension is lowest and the anthelmintic activity highest when the soap concentration corresponds with the "critical concentration for the formation of micelles." At the critical concentration, which is a characteristic of each soap, the relation between a number of physical properties and the soap concentration changes abruptly. These changes have been described elsewhere¹⁰. Immediate interest lies in the fact that at the "critical concentration" of an aqueous solution of a soap, the paraffin-chain ions of the soap agglomerate to form groups of ions or "micelles." The micelles are liquid structures and hydrocarbon in nature. They increase in size with increase in the concentration of the soap solution until the upper critical concentration or "critical concentration for completion of micelle formation" is reached. The micelles have been shown to be centres of solubilisation of water-insoluble materials^{1,2,3,11,12,13,14,15,16,17}. The latter are insoluble in aqueous solutions of soap which are below their critical concentration. At the critical concentration these substances become soluble and their solubility per molecule of soap increases rapidly as the soap concentration is increased up to the critical concentration for completion of micelle formation, after which there is no further increase. The present paper describes the solubility of a sparingly water-soluble phenol in aqueous solutions of potassium laurate, and shows that the mechanism of solubilisation is similar to that described by other workers for the solubilisation of fixed and volatile oils and water-insoluble dyes.

EXPERIMENTAL

I. CHOICE OF MATERIALS

(a) *The Phenol.* The phenol selected for study was 5-chloro-2-hydroxy-diphenylmethane (benzylchlorophenol), which has a solubility in water of about 1 in 15,000. Because of its low water-solubility it provides the simplest type of system, and, when dissolved in soap solutions, does not affect materially the proportions of the micelles³. Another important factor contributing to the choice of this compound is that even a saturated aqueous solution of it is shown to possess no appreciable bactericidal action. In a later paper, the properties of 2-chloro-5-hydroxy-1:3-dimethylbenzene (chloroxylenol) in similar circumstances will be described. This phenol is slightly more soluble in water (1 in 3,000).

(b) *The Soap.* The soap selected for the investigation was potassium laurate. It is the lowest member of the homologous series of potassium fatty acid soaps exhibiting advanced micellar structure¹⁸, and therefore

the lowest member possessing marked solubilising action. Soaps of the higher members of the series have a greater solubilising action per molecule², but their water-solubility at 20°C. is too low for them to be of any practical value as solvents. The physical properties of aqueous solutions of potassium laurate have been the subject of several investigations, and those properties which are affected by micelle formation are well plotted. In particular, the critical concentration of potassium laurate has been determined by various methods and is, therefore, known with some certainty. The experimentally obtained value depends on the physical property considered¹¹⁻¹⁹, and is given as being between 0.008M²⁰ and 0.076M²¹ but most frequently as about 0.025M^{22,23}.

2. PREPARATION OF THE POTASSIUM LAURATE

The potassium laurate was prepared from lauric acid with the following characteristics: acid value, 278.0, iodine value, 0.04, m.pt. 39.2°C., collected at 160° to 163°C. A molar solution of potassium laurate was prepared by adding the calculated volume of 2N potassium hydroxide solution to 400.4 g. of grated lauric acid and warming the mixture to about 50°C. When the reaction was complete the solution was cooled to 20°C. and sufficient freshly boiled and cooled distilled water added to produce 2.0 litres. The soap solution thus prepared was stored under nitrogen in glass-stoppered bottles of 250 ml. capacity. For use the molar solution was diluted to 0.1M with distilled water that had been freshly boiled and cooled under soda lime. Each ml. of the 0.1M solution contained 0.02383 g. of potassium laurate.

3. THE DETERMINATION OF THE SOLUBILITY OF BENZYLCHLOROPHENOL IN AQUEOUS SOLUTIONS OF POTASSIUM LAURATE

The experiments of McBain, Merrill and Vinograd¹⁶ show that the solubility of water-insoluble compounds in aqueous solutions of soap is independent of whether saturation is reached from under-saturation or over-saturation. The latter method was used in our determinations of the solubility of benzylchlorophenol in aqueous solutions of potassium laurate, because it is easier to dilute a concentrated solution until precipitation of the benzylchlorophenol occurs, than to saturate a solution of potassium laurate by the gradual addition of weighed amounts of the phenol. The method consisted essentially of preparing a series of solutions of benzylchlorophenol in potassium laurate such that each solution contained a smaller proportion of benzylchlorophenol/potassium laurate than the preceding one, and diluting each solution with freshly boiled and cooled distilled water until precipitation of the benzylchlorophenol occurred. The concentrations of potassium laurate necessary to keep in solution the various proportions of benzylchlorophenol/potassium laurate were calculated, and a solubility curve was constructed to show the number of molecules of benzylchlorophenol that can be solubilised per molecule of potassium laurate at different concentrations of the latter.

(a) *Preparation of a "Stock" Solution.* For convenience, a concentrated solution of potassium laurate almost saturated with benzylchlorophenol was prepared. The concentrated reference or "stock" solution

contained : benzylchlorophenol, 25.5 g., M potassium laurate solution, 250.0 ml., distilled water (freshly boiled and cooled), to 1,000.0 ml.

The benzylchlorophenol was dissolved in the potassium laurate solution with the aid of gentle heat. When solution was complete, it was cooled to 20°C. and the volume adjusted to 1,000.0 ml. with freshly boiled and cooled distilled water. The solution was stored under nitrogen in glass-stoppered bottles of 250 ml. capacity, each bottle being sealed with a "viscose" cap.

(b) *Preparation of the "Working" Solution.* Preliminary trials showed that a dilution of 2 parts by volume of the "stock" solution with 3 parts by volume of freshly boiled and cooled distilled water, produced a more convenient "working" solution, each millilitre of which contained : benzylchlorophenol, 0.0102 g., potassium laurate, 0.0238 g.

(c) *Experimental Details.* A known volume of the "working" solution was run from a micro-burette into each of a series of screw-capped, wide-mouthed bottles of 1 fl. oz. capacity. From a second micro-burette, a measured volume of 0.1M potassium laurate solution was run into each of the bottles, and from a third burette a gradually increasing volume of distilled water was added to each successive bottle of the series. A sufficient number of experiments were set up so that towards the dilute end of the series were systems to which sufficient distilled water had been added to produce turbidity due to benzylchlorophenol coming out of solution. The bottles were closed and incubated for 24 hours at 20°C. After incubation they were examined for the presence of benzylchlorophenol crystals. Further series of experiments were set up so that in each successive series, the proportion by weight of benzylchlorophenol to potassium laurate was less, i.e. a greater volume of potassium laurate solution was added per unit volume of "working" solution. A sufficient number of series of experiments were set up so that a series was eventually reached in which no crystals of benzylchlorophenol developed on incubation for 24 hours. The last experiment in each series in which the benzylchlorophenol remained in solution was taken as the end-point. The weight of benzylchlorophenol which can be dissolved in a solution of potassium laurate of known concentration was calculated from a knowledge of the volumes of "working" solution, 0.1M potassium laurate and distilled water added to the bottle corresponding with the end-point. The amount of benzylchlorophenol which can be dissolved in potassium laurate solutions of different concentrations is calculated in Table I. Figure 1 shows the number of molecules of benzylchlorophenol which can be solubilised per molecule of potassium laurate at different concentrations of the latter. Reference to Figure 1 will show that the solubility of benzylchlorophenol remains at a constant low level for all concentrations of potassium laurate below about 0.015M. Its solubility over this range is about 1 in 2,650 which is in excess of the solubility in water. At about 0.015M potassium laurate the solubility of the benzylchlorophenol per molecule of potassium laurate increases sharply with increases in the concentration of the potassium laurate solution. This increase continues up to about 0.04M potassium laurate, in excess of which the

TABLE I
THE SOLUBILITY OF BENZYLCHLOROPHENOL IN SOLUTIONS OF POTASSIUM LAURATE

Working Solution	Weight of Benzylchlorophenol in Working Solution	Weight of Potassium Laurate in Working Solution	0.1M Potassium Laurate Added	Weight of Potassium Laurate Added	Total Weight of Potassium Laurate	Mols. Benzylchlorophenol / Mol. Soap	Maximum Volume of Water that may be added	Total Volume
1.0 ml.	0.0102 g	0.02383 g	0.05 ml.	0.001191 g	0.02502 g	0.44459	1.0 ml.	2.05 ml.
1.0 ml.	0.0102 g	0.02383 g	0.10 ml.	0.002383 g	0.02621 g	0.42440	1.5 ml.	2.60 ml.
1.0 ml.	0.0102 g	0.02383 g	0.20 ml.	0.004766 g	0.02860 g	0.38893	2.0 ml.	3.20 ml.
1.0 ml.	0.0102 g	0.02383 g	0.30 ml.	0.007149 g	0.03098 g	0.35904	3.0 ml.	4.30 ml.
1.0 ml.	0.0102 g	0.02383 g	0.40 ml.	0.009532 g	0.03346 g	0.33244	3.5 ml.	4.90 ml.
1.0 ml.	0.0102 g	0.02383 g	0.50 ml.	0.011955 g	0.03574 g	0.31123	4.0 ml.	5.50 ml.
1.0 ml.	0.0102 g	0.02383 g	0.60 ml.	0.014298 g	0.03813 g	0.29173	4.5 ml.	6.10 ml.
1.0 ml.	0.0102 g	0.02383 g	0.70 ml.	0.016681 g	0.04051 g	0.27495	5.0 ml.	6.70 ml.
1.0 ml.	0.0102 g	0.02383 g	0.80 ml.	0.019064 g	0.04289 g	0.25935	6.0 ml.	7.80 ml.
1.0 ml.	0.0102 g	0.02383 g	1.00 ml.	0.02383 g	0.04766 g	0.23340	7.0 ml.	9.00 ml.
1.0 ml.	0.0102 g	0.02383 g	1.20 ml.	0.028596 g	0.05243 g	0.21216	8.0 ml.	10.20 ml.
1.0 ml.	0.0102 g	0.02383 g	1.40 ml.	0.033362 g	0.05719 g	0.19450	9.0 ml.	11.40 ml.
1.0 ml.	0.0102 g	0.02383 g	1.60 ml.	0.038128 g	0.06196 g	0.17952	11.0 ml.	13.60 ml.
1.0 ml.	0.0102 g	0.02383 g	1.80 ml.	0.04289 g	0.06672 g	0.16672	12.0 ml.	14.80 ml.
1.0 ml.	0.0102 g	0.02383 g	2.00 ml.	0.04766 g	0.07149 g	0.15560	14.0 ml.	17.00 ml.
1.0 ml.	0.0102 g	0.02383 g	2.20 ml.	0.052426 g	0.07626 g	0.14587	15.0 ml.	18.20 ml.
1.0 ml.	0.0102 g	0.02383 g	2.50 ml.	0.05959 g	0.08342 g	0.13335	18.0 ml.	21.50 ml.
1.0 ml.	0.0102 g	0.02383 g	2.75 ml.	0.065527 g	0.08936 g	0.12448	21.0 ml.	24.75 ml.
1.0 ml.	0.0102 g	0.02383 g	3.00 ml.	0.07149 g	0.09532 g	0.11670	23.0 ml.	27.00 ml.
2.0 ml.	0.0204 g	0.04766 g	0.025 ml.	0.00059 g	0.04825 g	0.46095	1.5 ml.	3.52 ml.

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rate of increase begins to fall. In excess of approximately 0.06M potassium laurate, there is negligible increase in the solubility of the benzylchlorophenol per molecule of potassium laurate.

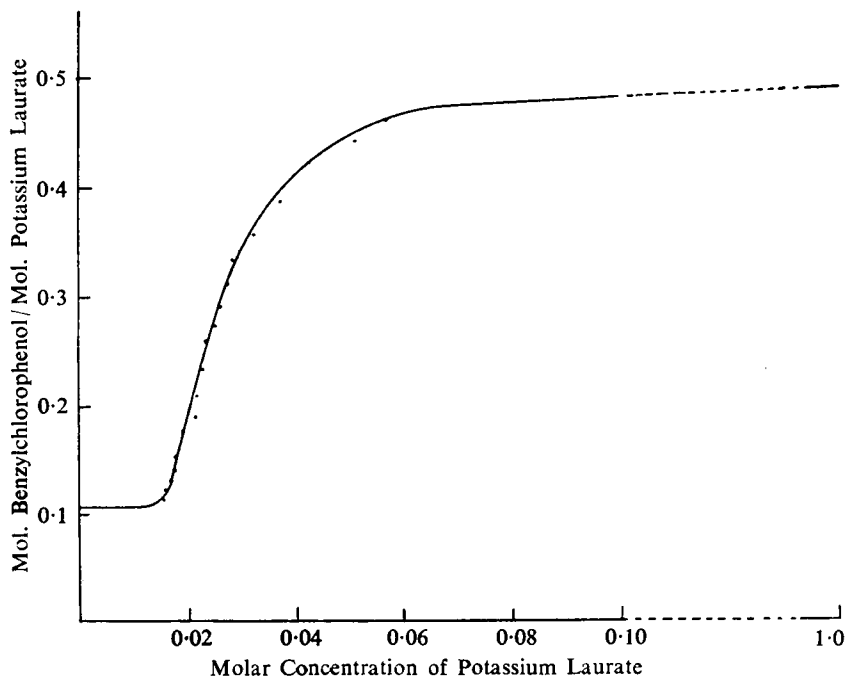


FIG. 1. The Solubility of Benzylchlorophenol in Potassium Laurate Solution.

DISCUSSION

The general form of the solubility curve shown in Figure 1 corresponds very closely to those published by Hartley³, McBain, Merrill and Vinograd¹⁶, McBain and Johnson² and others for the solubility of water-insoluble dyes in aqueous solutions of soap. Correlation of the solubility curves for the dyes and the equivalent conductivity curves of the soaps used as solvents, provided ample evidence that the soap micelles are responsible for the solubilisation. The similarity in shape of the curves for the solubility of benzylchlorophenol in potassium laurate, and for the dyes in potassium laurate and other soaps in solution, together with the fact that the marked increase in the solubility of benzylchlorophenol per molecule of potassium laurate occurs within the limits quoted as the critical concentration for potassium laurate^{22,25}, can again be accepted as sufficient evidence that the soap micelles are responsible for the solubilisation of the benzylchlorophenol.

The constant low level of solubility of the benzylchlorophenol in all concentrations of potassium laurate up to about 0.015M is accounted for by the non-micellar nature of these solutions. The solubility of the phenol in these solutions is of the order of 1 in 2,650. This is somewhat greater than can be accounted for by its water-solubility. A similar

phenomenon was reported by McBain¹⁴ and Corrin, Klevens and Harkins²⁴ who proposed the existence of some pre-micellar particles on which water-insoluble material might be adsorbed.

The sudden increase in solubility at about 0.015M potassium laurate is obviously due to the formation of micelles at this concentration. The increase in the solubility of the benzylchlorophenol per molecule of potassium laurate continues until the concentration of the latter is about 0.06M, at which concentration the micelles presumably reach maximal size. When they have reached their maximal size they are unable to solubilise any further benzylchlorophenol. That is, the solubility of benzylchlorophenol per molecule of potassium laurate reaches a maximum when the micelles are completely formed.

Thus, it is possible to have a micellar solution of potassium laurate in which the micelles (1) contain no benzylchlorophenol, (2) are saturated with benzylchlorophenol or (3) contain some intermediate proportion. The authors have previously suggested that the bactericidal activity of benzylchlorophenol in aqueous solutions of potassium laurate is a function of the concentration of the benzylchlorophenol in the micelles of the potassium laurate²⁵. Subsequent papers will present the evidence on which this statement was based.

SUMMARY

The solubility of benzylchlorophenol in aqueous solutions of potassium laurate has been plotted, and is shown to be dependent on the presence of micelles in the soap solution.

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