Effect of Nonionic Surfactant on Bactericidal Activity of Cetylpyridinium Chloride

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Abstract □ The interaction of cetylpyridinium chloride with a nonionic surfactant was examined by equilibrium dialysis; and by using the simple aqueous phase saturation model, the effect of the surfactant upon the biological activity of the bactericide was predicted. Determination of the degree of reduction of bactericidal activity showed good agreement with the predicted result over a concentration range of 10–100 p.p.m. of bactericide. Below a concentration of 10 p.p.m., a discrepancy between the predicted and experimental results was possible.

Keyphrases □ Cetylpyridinium chloride—effect of polysorbate 80 on bactericidal activity against *Escherichia coli* □ Polysorbate 80—effect on bactericidal activity of cetylpyridinium chloride against *Escherichia coli* □ Formulation, pharmaceuticals—polysorbate 80–cetylpyridinium chloride interaction, effect on bactericidal activity

The protection of pharmaceutical products from contamination by microorganisms is a subject of considerable importance. It is quite possible that new regulations of control agencies will cause a rapid increase in studies of bactericidal action in medicinal preparations. The complexity of a pharmaceutical preparation can cause problems for the formulator trying to provide protection for his products from fungal and bacterial attack. In particular, interaction with pharmaceutical adjuvants, such as surfactants, can have a profound effect upon the activity of bactericides.

Following the classic work of Hampil (1), others have shown that the presence of a surfactant can have varied effects upon bactericidal activity (2). Bean and Berry (3) studied the bactericidal activity of clorophene (benzylchlorophenol) in surfactant solutions. Their results indicated that the biological activity of a bactericide was dependent upon the concentration of micellar drug.

Later, Allawala and Riegelman (4) proposed that the total quantity of drug in a surfactant system could be termed the "capacity" while the concentration of nonmicellar drug was denoted as the "activity." It was suggested that the bactericidal activity of a drug in such a system would be directly related to the activity. Humphreys *et al.* (5) compared the physicochemical estimates of binding of benzoic acid with microbiological data. Their results indicated that in the systems they examined the simple theory equating antimicrobial activity with nonbound bactericide concentration was a somewhat inadequate oversimplification. However, their results also showed that formulations based upon the simple theory had a more than satisfactory degree of protection.

The present article reports studies on the effect of micellar binding upon antimicrobial activity. Polysorbate 80[1] was selected as the surfactant, and cetylpyridinium chloride was selected as the antimicrobial agent; both substances are in widespread use.

EXPERIMENTAL

Materials—Cetylpyridinium chloride[2] and polysorbate 80[3] were used. The dialysis cell used was as previously described (6).

Micellar Binding—The interaction of various concentrations of cetylpyridinium chloride with 0.1% surfactant was examined by equilibrium dialysis using the method previously described (6). The molar absorptivity of the cetylpyridinium chloride at 259 nm. was found to be 4.02×10^3; the Beer–Lambert law was obeyed.

Bactericidal Activity—The bactericidal activity of cetylpyridinium chloride in water and in surfactant solutions against *Escherichia coli* type I (CN 4407) was assessed by determining the percent survival of cells after exposure in such mixtures for 2 hr. at 30°. Viable counts were performed before and after exposure by diluting with water to give about 100 colonies per roll tube when plated. Five roll tubes were set up from each mixture, and each experiment was repeated several times. On each occasion the bactericidal activity was determined of an aqueous cetylpyridinium chloride solution and of the same concentration of cetylpyridinium chloride in 0.1% surfactant solution. Additionally the bactericidal activity of a 0.001% aqueous cetylpyridinium chloride solution was determined on every occasion to check the reproducibility of sensitivity of the test suspension. Over 10 months, some 150 points were determined, from which Fig. 1 was constructed. Inspection of the data initially suggested that the curves were discontinuous; the figures were drawn by a computer programmed on this basis to produce, by least-squares

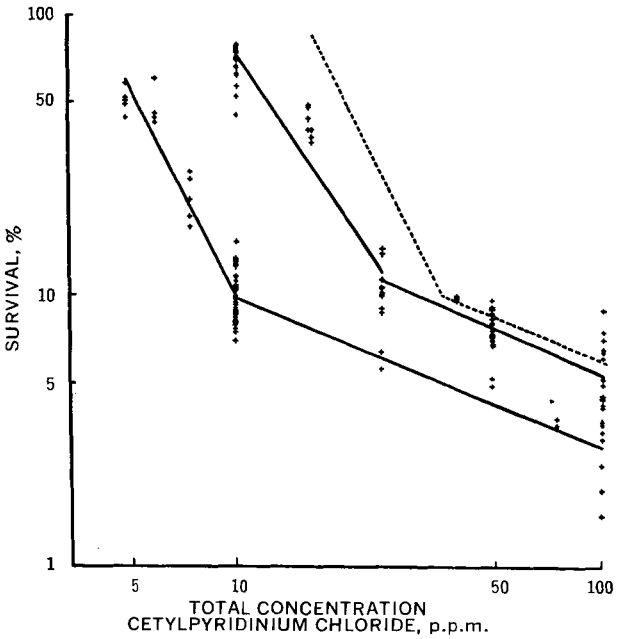

Figure 1—*Bactericidal activity of cetylpyridinium chloride in water and in a solution of polysorbate 80. Key: lower curve, aqueous system; upper curve, system containing surfactant; and dotted curve, predicted activity in surfactant system.*

[1] Tween 80.

[2] Koch Light Laboratories Ltd., Colnbrook, England.

[3] Honeywill-Atlas, Carshalton, England.

Table I—Potentiation Effect of Surfactant upon Cetylpyridinium Chloride (All Systems Contained 0.001% Cetylpyridinium Chloride)

Concentration of Surfactant, % w/v	Percent Survival of *E. coli* at 2 hr. at 30°
0.1	68.4
0.01	19.0
0.001	8.8
0.0001	2.5
0.00001	2.5
0	9.6

regression analysis, the four straight lines shown in Fig. 1. Reaction mixtures contained initially about 10^6 viable cells $cm.^{-3}$. Controls were performed to establish the stability of the suspension during the experimental period and also that the degree of dilution in the viable count was sufficient to inactivate any cetylpyridinium chloride "carryover" into the roll tubes.

RESULTS AND DISCUSSION

The results of the dialysis study showed that the interaction between cetylpyridinium chloride and 0.1% polysorbate was governed by a form of the partition law; a similar finding was made in an investigation of the interaction of benzoic acid and a series of *n*-alkyl polyoxyethylene surfactants (6). From the dialysis results, it was possible to estimate the concentrations of free and micellar-bound drug in the surfactant systems. It was thus possible, using the simple aqueous phase saturation theory, to predict the bactericidal activity of such systems relative to purely aqueous ones.

The results of the viable count studies of the bactericidal activity of cetylpyridinium chloride in aqueous systems and those containing 0.1% polysorbate 80 are shown in Fig. 1. The log plot of survival *versus* concentration is expected to be linear (7), and the finding that both curves showed distinct inflection at about 10 p.p.m. free cetylpyridinium chloride (*i.e.*, 10 p.p.m. total cetylpyridinium chloride for the system without surfactant and approximately 27 p.p.m. total cetylpyridinium chloride for the surfactant system) was unexpected. This was not attributable to experimental design because all points were collected in a random manner. So far, no explanation of this finding is forthcoming, although molecular aggregation of the bactericide is a possible reason. Nevertheless, the experiment itself is internally consistent since both test and control systems behaved in a similar manner with respect to this finding; therefore, valid comparisons can be made between expected and actual activities for both parties of the graph.

The dotted line in Fig. 1 indicates the bactericidal activity predicted from the simple aqueous phase saturation theory in surfactant systems. It is apparent that in concentrations above 10 p.p.m. free cetylpyridinium chloride, there is, within experimental error, excellent agreement between the physicochemical estimate of binding (by equilibrium dialysis, 73%) and the biological estimate (potency ratio estimate from Fig. 1, 70%).

Below a 10 p.p.m. free cetylpyridinium chloride concentration, the situation is less clear. There is some difficulty in deciding which points should be used in the regression analysis of low mortality data since ultimately the curves must show convergence at very low concentrations to a common intercept of 100% survival.

The lines as drawn from the lower concentrations are nonparallel, so a single biological estimate of binding cannot be made. Between a 6- and 10-p.p.m. free cetylpyridinium chloride concentration, the best biological estimate of binding is 63%; this is probably not significantly different from the physicochemical estimate of 73%. However, any tendency for departure from expectation is in the direction of greater, rather than less, activity. Such a trend is not surprising because the effect of the surfactant is twofold. On the one hand, micellar binding results in inactivation of bactericide; on the other, some potentiation of activity, possibly through surface effects, occurs (8). When this latter point was examined, we found that a fourfold increase in activity of bactericide (*i.e.*, reduction in percentage survival from 9.6 to 2.5) is caused by a very low surfactant concentration of 0.00001% (Table I). This effect is not observed if cells pretreated with surfactant are washed before exposure to the bactericide. It is possible that this potentiating effect is due to the surfactant temporarily modifying the permeability of the bacterial membrane. It seems likely that this potentiating effect would be more readily observed in systems of low bactericidal activity. This is not inconsistent with the results at low concentrations. Unfortunately, even with the high degree of replication involved and the use of a variable response experimental design, biological variation imposes limits on the useful comparisons that can be made between actual and predicted activity.

If a formulation containing surfactant was prepared on the basis of the simple aqueous phase saturation theory, it appears from our results that the degree of protection conferred would always be at least adequate. The systems containing surfactant were never less active than predicted.

Clearly, the effects of surfactants upon bactericidal activity are complex. In view of the increasing emphasis being placed upon microbiological aspects of standards for pharmaceutical formulation, this field merits further attention from pharmaceutical scientists.

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