Interaction of Preservatives With Macromolecules IV*

Binding of Quaternary Ammonium Compounds by Nonionic Agents

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Equilibrium dialysis studies utilizing a semipermeable nylon membrane indicate a high degree of association and accompanying inhibition of quaternary ammonium germicides such as cetylpyridinium chloride and benzalkonium chloride with nonionic surfactants such as Tween 80. Cetylpyridinium chloride was also found to bind to methylcellulose, but not to PVP or Polyox. Benzalkonium chloride was not bound to methylcellulose, PVP, or Polyox under the conditions of this study.

ALTHOUGH SEVERAL investigators have presented microbiological data indicating that nonionic surface-active agents can interfere with the activity of cationic germicides such as the quaternary ammonium compounds, textbooks and reference works generally do not emphasize the high degree of inactivation which can sometimes occur in these systems. It has been suggested that the observed inactivation is attributable to a preferential association or binding of the cationic agent with the nonionic surfactant (1), although no previous data have been published which would indicate the magnitude of this association. The present work was undertaken to obtain quantitative data for the degree of binding of several cationic agents by some typical nonionics and to compare the degree of binding with the degree of inactivation of the cationics.

Phospholipids such as lecithin have long been popular as neutralizing or inactivating media for quaternary ammonium compounds in germicidal testing (2–7). Quisno, Gibby, and Foter suggested the use of an inactivating medium consisting of lecithin and Tween 80, indicating that the lecithin was the primary inactivator with the Tween acting as a dispersing agent (8). In 1949 Gershenfeld and Stedman (9) reported their observations on the activity of several cationics, including cetylpyridinium chloride and cetyltrimethylammonium bromide, in the presence of varying concentrations of a nonionic surfactant, noting enhancement of activity at low surfactant concentration and inhibition at higher surfactant concentrations. Davies (10) reported that a polyethylene glycol cetostearyl ether was capable of neutralizing the bacteriostatic effect of cetyltrimethylammonium bromide and Ritter (11) indicated that Tween 80 was capable of neutralizing the bactericidal effect of cetylpyridinium chloride on *tubercle bacilli*. Barr and Tice (12) investigated seven quaternary ammonium compounds in the presence of 5% polyoxyethylene 20 sorbitan monostearate and found that only benzalkonium chloride was effective in a concentration of 0.1%. Wedderburn (13) indicated that benzalkonium chloride also was subject to inactivation by nonionics, including sucrose ester surfactants, under the conditions of her studies. Moore and Hardwick (1) presented microbiological data on combinations of quaternary ammonium compounds and nonionic surfactants and devoted considerable discussion to the relative effectiveness of such combinations. Other reports of inactivation are discussed in the recent review by Beckett and Robinson (14).

Studies cited above (1, 9) indicate that in very dilute solutions of the nonionic surfactants there is an enhancement of the effectiveness of the quaternary ammonium compound, but at higher concentrations of the surfactant the activity of the germicide is greatly diminished. This is typical of the behavior of many germicides in the presence of surface-active agents (1, 15, 16).

In the present study two typical quaternary ammonium compounds, cetylpyridinium chloride and benzalkonium chloride, were selected to illustrate the relative magnitude of any interaction which might occur between these agents and nonionics such as Tween 80, methylcellulose, polyvinylpyrrolidone, and high molecular weight ethylene oxide polymers. The particular quaternary ammonium compounds used in this study were selected principally because both agents show absorption in the ultraviolet and thus can readily be determined spectrophotometrically.

EXPERIMENTAL

Reagents.—Cetylpyridinium chloride (1-hexadecylpyridinium chloride);[1] benzalkonium chloride U. S. P. (a mixture of alkyldimethylbenzylammonium chlorides in which the alkyls range from C_8H_{17} to $C_{18}H_{37}$);[2] cetyldimethylbenzylammonium

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chloride;[3] Tween 80,[4] a commercial sample and also a sample passed through an ion exchange column; methylcellulose, 15 c. p. s.;[5] polyvinylpyrrolidone;[6] Polyox WSR-35.[7]

To assure that any observed inactivation of quaternary ammonium compounds by Tween 80 would not be attributable to the presence of unesterified oleic acid in the Tween sample, the Tween solution was passed through a mixed-bed ion exchange column[8] to remove traces of oleic acid which might be present. Quantitative removal of oleic acid was established by adding known amounts of free oleic acid to Tween 80 samples and determining oleic acid content by potentiometric titration with approximately 0.1 *N* NaOH before and after passage through the ion exchange resin. Subsequent studies indicated that the contribution of any traces of oleic acid in the commercial sample of Tween 80 to the inactivation of quaternary ammonium compounds was probably insignificant.

Before the polyvinylpyrrolidone was employed in the dialysis studies, the sample was extracted with ether in a Soxhlet extractor for forty-eight hours.

Conductivity Measurements.—To establish approximate critical micelle concentrations for the samples of cetylpyridinium chloride and cetyldimethylbenzylammonium chloride used in this study, conductivity measurements were obtained for aqueous solutions at room temperature (23–25°). An Industrial Instruments model RC M15 conductivity bridge and a dipping type conductivity cell with a cell constant of approximately 1.00 $cm.^{-1}$ were employed. The water used was that used throughout the study and had a specific conductance of approximately 2×10^{-1} ohm^{-1}.

Dialysis Studies.—Dialysis membranes employed in the studies involving Tween 80 were nylon membranes as described previously by Patel and Kostenbauder (18). These membranes were previously shown to be impermeable to Tween 80 (18). For dialysis studies involving methylcellulose, polyvinylpyrrolidone, and Polyox, Visking cellulose casings were employed. The general procedure for these studies consisted of placing inside the dialysis bag 20 ml. of a solution containing the quaternary ammonium compound and, after tightly knotting the open end, placing the bag into a 125-ml. bottle containing 40 ml. of Tween or other polymer solution. A polyethylene film and a screw cap were employed as the closure, and the bottles were rotated at 9 r. p. m. in a constant temperature bath at 30°. As might be expected for the long chain quaternary ammonium compounds, equilibration times of five to seven days were necessary for cetylpyridinium chloride solutions and two to three days for benzalkonium chloride solutions. The presence of Tween 80, however, reduced the equilibration time for cetylpyridinium chloride to one to two days.

After equilibration, aliquots were removed from both sides of the membrane and concentrations of quaternary ammonium compounds were determined spectrophotometrically at a wavelength of 259 $m\mu$ for cetylpyridinium chloride and 261.5 $m\mu$ for benzalkonium chloride, using a Beckman DU spectrophotometer. Any interference due to the macromolecule was eliminated by using the appropriate concentration in the reference cell.

Microbiological Studies.—Observations were made on the influence of several nonionic surface-active agents on the bacteriostatic activity of cetylpyridinium chloride and benzalkonium chloride, using *Aerobacter aerogenes*,[9] a Gram-negative organism. The methods and culture medium previously described by Pisano and Kostenbauder (19) were employed. Approximate inhibitory concentrations for cetylpyridinium chloride and benzalkonium chloride in the presence of Tween 80, Triton X-100,[10] and Pluronic L62,[11] were obtained through visual observation of samples each day for a period of two weeks.

RESULTS

Critical Micelle Concentration for Cationics.—Approximate critical micelle concentrations for cetylpyridinium chloride and cetyldimethylbenzylammonium chloride (CDBAC) were obtained from the conductivity data illustrated in Fig. 1. The critical micelle concentration for cetylpyridinium chloride was found to be approximately 1.0×10^{-3} *M*, while that for the CDBAC was found to be approximately 1.0×10^{-4} *M*.

Tween 80.—Cetylpyridinium chloride was found to interact to an extremely high degree with Tween 80 when the cationic was employed in concentrations comparable to those which might be encountered in product formulations. Figures 2 and 3 illustrate typical adsorption isotherms for this system, and in Fig. 4 these data are presented in a manner which indicates that in an aqueous system containing Tween 80 and cetylpyridinium chloride, the ratio of total to free cetylpyridinium chloride is primarily a function of the concentration of Tween 80. As illustrated in a previous publication (19), the ratio of total to free germicide can often be employed to predict required preservative concentrations in the presence of the Tween. Figure 4 indicates that at a concentration of 1% Tween 80, approximately 95% of the cetylpyridinium chloride present is bound to the Tween and thus inactivated. Even at a concentration of 0.1% Tween, the data suggest that approximately 60% of the total cetylpyridinium chloride would be inactivated. The dialysis studies indicated no significant difference in binding of cetylpyridinium chloride by a commercial sample of Tween 80 and a sample passed through an ion exchange resin to remove any oleic acid present.

[3] Sample obtained through the courtesy of Dr. George D. Wessinger, Sterling-Winthrop Research Institute, Rensselaer, N. Y.
[4] Tween 80 is polyoxyethylene 20 sorbitan monooleate, Atlas Powder Co., Wilmington, Del.
[5] Methocel, 15 c. p. s., the Dow Chemical Co., Midland, Mich.
[6] Flasdone, Antara Chemicals Division of General Aniline and Film Corp., New York, N. Y.
[7] Polyox WSR-35 is a poly(ethylene oxide) of exceptionally high molecular weight (17), Union Carbide Chemicals Co., New York, N. Y.

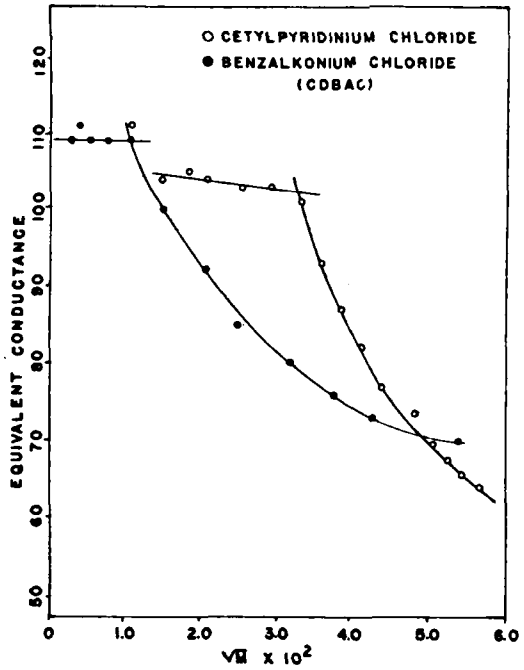


Fig. 1.—Determination of critical micelle concentration for cetylpyridinium chloride and cetyldimethylbenzylammonium chloride through conductivity measurements, 23–25°. Critical micelle concentrations were approximately 1.0×10^{-3} *M* for cetylpyridinium chloride and 1.0×10^{-4} *M* for cetyldimethylbenzylammonium chloride.

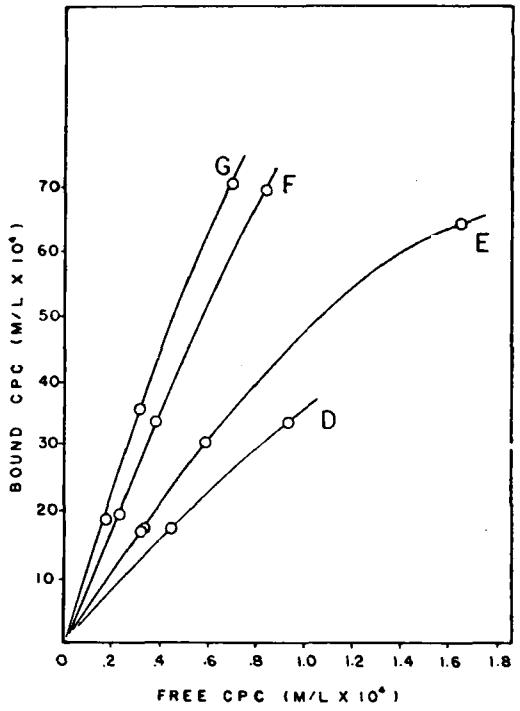
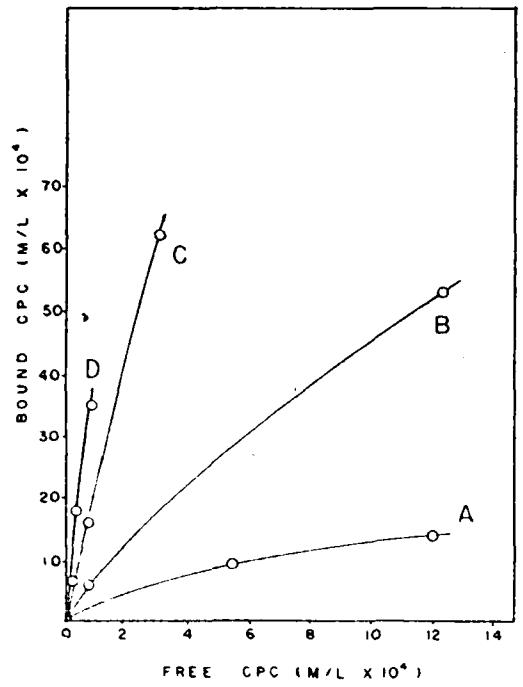


Fig. 3.—Adsorption isotherms for binding of cetylpyridinium chloride by Tween 80 in aqueous solution at 30°. *D*, 2.5%; *E*, 5.0%; *F*, 7.5%; *G*, 10.0% TW 80.



The marked scatter of points in Fig. 4 is partly a result of magnification of experimental error due to the pronounced binding which occurs. Calculation of *r*, the ratio of total to free preservative, in some cases involves dividing the total concentration by a number smaller by a factor of approximately 100; thus, a small error in either total or free concentration is greatly magnified in calculation of the *r* value. Despite the inherent inaccuracy of such treatment of data, the convenience of a graph such as that presented in Fig. 4 justifies its presentation.

Data for the binding of benzalkonium by Tween 80 are illustrated in Fig. 5. While the binding of benzalkonium chloride is considerably less than that exhibited by cetylpyridinium chloride, it nevertheless is sufficient to cause inactivation. At a concentration of 1% Tween 80, approximately 50% of the benzalkonium chloride present is bound to the Tween. There appeared to be no significant difference in the degree of binding exhibited by benzalkonium chloride U. S. P., which is described as consisting of alkyls in the range $C_8 - C_{18}$ (20), and the binding shown by a pure sample with a C_{16} chain, i. e., CDBAC.

Figure 6 represents adsorption isotherms for the interaction of cetylpyridinium chloride and CDBAC with a very dilute solution of Tween 80. This study was included to permit observations on the degree of binding in the presence of free quaternary

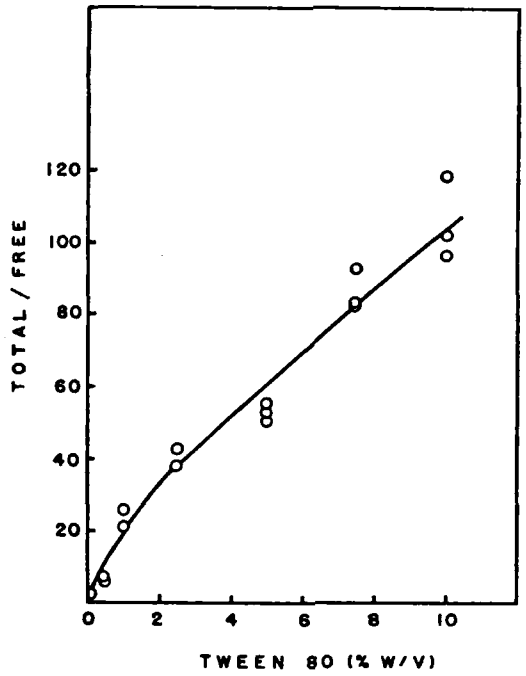


Fig. 4.—The data illustrated in Figs. 2 and 3 plotted to give the ratio, r, of total to free cetylpyridinium chloride as a function of the concentration of Tween 80.

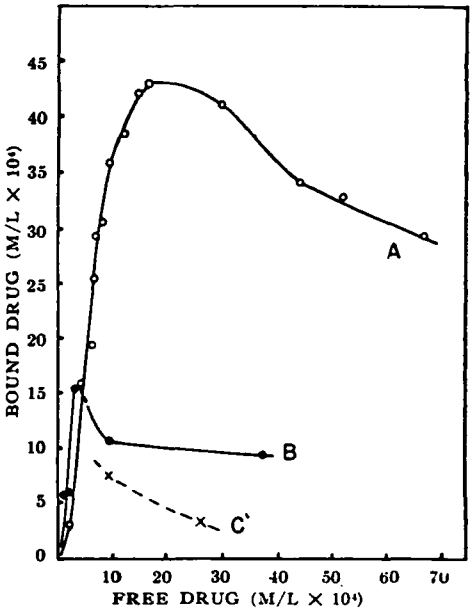


Fig. 6.—Adsorption isotherms for binding of cetylpyridinium chloride and cetyldimethylbenzylammonium chloride in 0.2% Tween 80 at 30°. These curves illustrate the degree of binding occurring at concentrations of the quaternary ammonium compounds both above and below their normal critical micelle concentrations. *A*, cetylpyridinium Cl; *B*, benzalkonium Cl(CDBAC); *C*, cetylpyridinium Cl, 0.05 *M* NaCl. Curve *C* illustrates the influence of electrolyte on the degree of interaction.

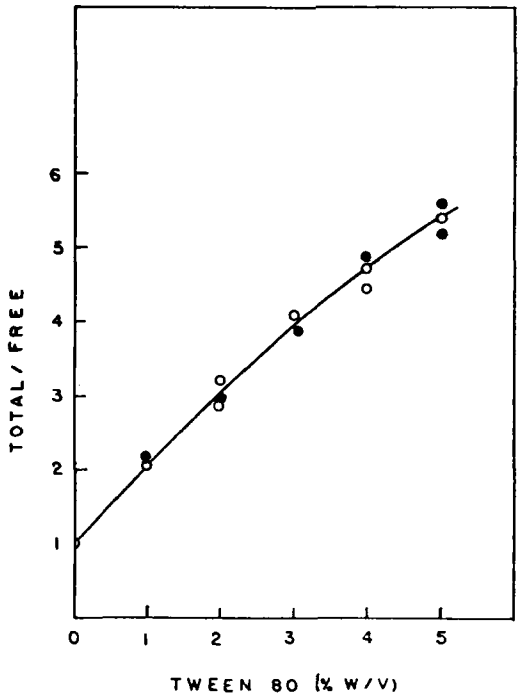


Fig. 5.—The ratio, r, of total to free benzalkonium

80, showing a maximum in the adsorption isotherm in the region of the critical micelle concentration.

Methylcellulose.—Figure 7 shows the interaction of cetylpyridinium chloride with methylcellulose. The binding is highly dependent on the cetylpyridinium chloride concentration, passing through a maximum in the region of the normal critical micelle concentration for cetylpyridinium chloride. Benzalkonium chloride was found to exhibit no detectable binding to methylcellulose up to a concentration of 2% methylcellulose and 0.125% cationic.

Figure 8 shows the influence of pH and electrolyte on the interaction of cetylpyridinium chloride with methylcellulose. The binding appears to be increased somewhat in the presence of strong base, but is suppressed by 0.05 *M* NaCl and almost eliminated by 0.05 *M* HCl.

Polyvinylpyrrolidone.—Neither cetylpyridinium chloride nor benzalkonium chloride was found to interact significantly with polyvinylpyrrolidone at a concentration of 2% polyvinylpyrrolidone and 0.08% quaternary ammonium compounds.

Polyox.—Neither cetylpyridinium chloride nor benzalkonium chloride was found to interact significantly with this polymer at concentrations of 0.2% Polyox and 0.07% quaternary ammonium compound.

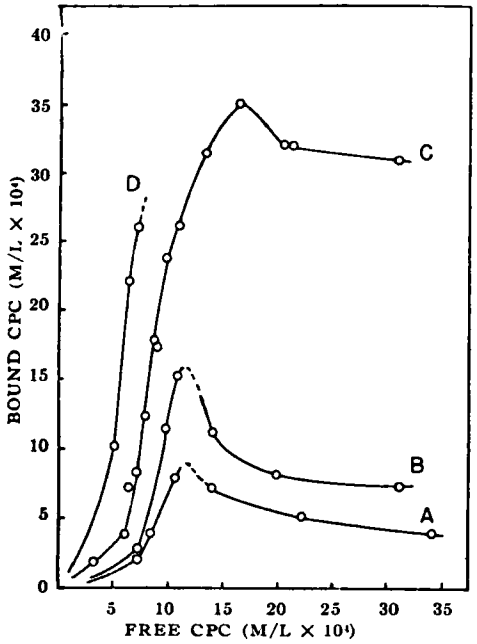


Fig. 7.—Binding of cetylpyridinium chloride by methylcellulose at 30°. *A*, 0.05%; *B*, 0.1%; *C*, 0.5%; *D*, 1.0% methylcellulose.

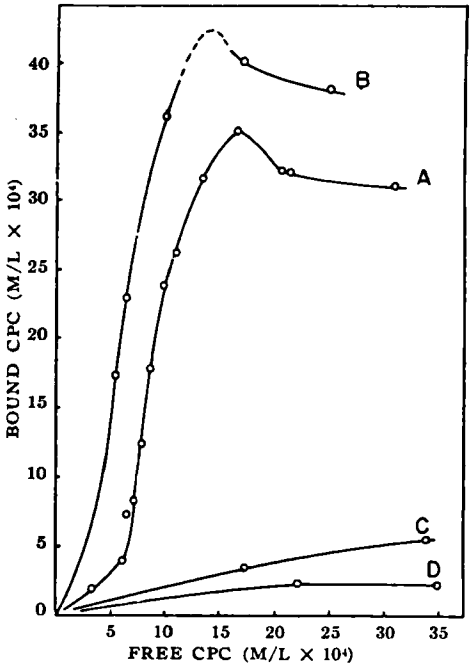


Fig. 8.—Influence of pH and electrolyte on the interaction of cetylpyridinium chloride with 0.5% methylcellulose in aqueous solution at 30°. *A*, distilled water; *B*, 0.003 *M* NaOH; *C*, 0.05 *M* NaCl; *D*, 0.05 *M* HCl.

presented in Table I. These studies were made with polymer solutions which were passed through ion exchange resins to remove any fatty acid or electrolyte present and also with some untreated samples of Tween 80. No attempt was made to determine precise inhibitory concentrations, but the increased concentrations of quaternary ammonium compound indicate a somewhat higher degree of inactivation than would be predicted from a consideration of Figs. 4 and 5.

As would be predicted from the binding studies, benzalkonium chloride was found to be inhibited to a lesser degree than was the cetylpyridinium chloride. This observation is in agreement with that of Barr and Tice (12), who found that in the presence of Tween 60 benzalkonium chloride was effective in a concentration of 0.1% while cetylpyridinium chloride was not

The results obtained with Pluronic L62 and Triton X-100 indicate that these agents show an ability to inactivate the quaternary ammonium compounds quite similar to that exhibited by Tween 80.

Table I.—Influence of Several Nonionic Surfactants on Concentrations of Cationic Required to Inhibit *Aerobacter aerogenes*

Nonionic	Inhibitory Concentration—Cetylpyridinium Cl	Inhibitory Concentration—Benzalkonium Cl
0	1–100,000 to 1–250,000	No growth at 1–100,000
0.5% Tween 80[a]	1–2,500 to 1–5,000	....
2.0% Tween 80[a]	1–250 to 1–500	....
3.0% Tween 80[b]	1–100 to 1–250	1–500 to 1–1,000
3.0% Triton X-100[b]	1–100 to 1–250	....
3.0% Pluronic L62[b]	1–500 to 1–1,000	....

[a] Commercial sample. [b] Treated with ion exchange resin before use.

DISCUSSION

Tween 80.—The formation of mixed micelles of quaternary ammonium compound and nonionic surfactant has been suggested as a possible mechanism for the association with molecules such as Tween 80 (1). For such an interaction, the degree of binding would be expected to increase with increasing length of the hydrocarbon chain of the cationic, at least so long as the concentration of the free cationic did not exceed the normal critical micelle concentration. The critical micelle concentration of the

concentrations of Tween below 0.5% were free cetylpyridinium concentrations of this magnitude approached, although in all cases the concentration of free benzalkonium was in excess of the critical micelle concentration. It was at first suspected that the marked difference in degree of binding of cetylpyridinium chloride and benzalkonium chloride by the Tween might be a result of heterogeneity in the composition of the alkyl of the benzalkonium chloride, since the U. S. P. specifies C_8-C_{18} for this compound. However, subsequent binding studies

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