Bactericidal Properties of Quaternary Ammonium Compounds in Dispersed Systems

G. J. BALEY *, G. E. PECK x, and G. S. BANKER

Abstract □ Alkyltrimethylammonium bromides of selected chain length were synthesized and characterized with respect to their bactericidal properties. A partitioning method was developed, and the aqueous phases of the partitioned systems were evaluated for their antibacterial activity against *Staphylococcus aureus*. The relationships between the quaternary ammonium compound chain length and the aliphatic alcohol chain length were determined. The effect of selected hydrocarbons on the bactericidal effectiveness of the systems was studied. Cream systems corresponding to the partitioned systems were prepared and evaluated against *S. aureus*. The relationship between the aqueous phase concentration of the bactericidal agent was studied, and a correlation was shown between the bactericidal activity of the partitioned systems and cream systems.

Keyphrases □ Quaternary ammonium bromides, various—synthesized, bactericidal activity determined in simple partition systems, compared to cream systems □ Alkyltrimethylammonium bromides, various—synthesized, bactericidal activity determined in simple partition systems, compared to cream systems □ Bactericidal agents—various alkyltrimethylammonium bromides synthesized, activity determined in simple partition systems, compared to cream systems

In earlier studies (1), the partition coefficient of a series of quaternary ammonium compounds in selected aliphatic alcohols and hydrocarbons was evaluated and correlated to the physical stability of cream systems corresponding to the partitioned systems. In this study, the relationship between partitioning properties of selected quaternary ammonium compounds and their bactericidal properties in dispersed systems was evaluated.

The bactericidal effectiveness of a topical cream depends primarily on the inherent bactericidal properties of the bactericidal agent and its concentration in the aqueous phase of the system. The concentration in the aqueous phase depends upon the partition coefficient of the compound between the aqueous phase and the oil phase of the emulsion.

The partition coefficient of a solute is the ratio of the concentration of the agent in the oil phase divided by the concentration of the agent in the aqueous phase. The antimicrobial activities of antibacterials in oil-in-water systems were dependent on their aqueous phase concentration (2, 3), and another study (4) showed that the activity of solubilized iodine preparations was controlled by the concentration of iodine in the aqueous phase. In other reports, partition coefficients were used in the design of pharmaceutical dosage forms (5) and partitioning was related to preservative activity in emulsions (6).

Partitioning data were investigated relative to the preservation of multidose parenterals (7). An attempt was made to correlate partition coefficients, chemical structure, and solvent nature in dosage form design (8). Recent studies considered the availability of antimicrobial agents from emulsion systems and high petrolatum-based ointments (9, 10). Recently, the interaction of quaternary ammonium bactericides with biological materials was studied to understand their use (11, 12).

The purpose of this study was to correlate the partition characteristics of quaternary ammonium compounds with their bactericidal properties in simple partition and cream systems.

Table I—Critical Kill Dilutions of Aqueous Solutions of the Alkyltrimethylammonium Bromide Quaternary Ammonium Compounds

Alkyl Group	Experimental CKD	Literature[a] CKD
Lauryl	1:7,000	1:4,000
Myristyl	1:50,000	1:38,000
Cetyl	1:90,000	1:80,000
Stearyl	1:60,000	1:64,000

[a] Reference 15.

EXPERIMENTAL

The apparatus, materials, partitioned systems, and cream systems, including their method of manufacture, were described previously (1).

Test Culture—The Gram-positive bacteria, *Staphylococcus aureus*[1] (FDA 209, ATCC 6538), was maintained on nutrient agar, grown in nutrient broth, and tested in "Letheen" broth (13).

Preparation of Dilution—A 1% stock solution of the synthesized quaternary ammonium compounds was prepared in sterile distilled water. The final dilutions were made from the stock solution, directly into the medication tubes.

A 1:15 dilution of the aqueous phase from each partition study was prepared, and final dilutions were made directly into the medication tubes.

A 1:30 or greater dilution of each cream was prepared in sterile distilled water. The tube containing the cream dilution was shaken on a vortex mixer[2] until the contents appeared to be dispersed evenly. The phases were allowed to separate for 1 hr, after which a sample of the lower phase was removed with a 15.2-cm (6-in.), 18-gauge needle attached to a 10-ml syringe[3]. Final dilutions were made directly into the medication tubes.

Antibacterial Test Conditions—The critical kill dilution of each system was determined using the AOAC phenol coefficient method (14). The critical kill dilution is the highest dilution killing the test organism in 10 min but not in 5 min. Tests were conducted at 37° in a water bath, and results were read after 48 hr.

RESULTS AND DISCUSSION

Bactericidal Testing—The critical kill dilution (CKD) of each of the four synthesized quaternary ammonium compounds in distilled water was determined; the results are shown in Table I along with a comparison of reported data (15). In aqueous solution, the cetyl derivative was the most effective bactericidally, followed by stearyl-, myristyl-, and lauryltrimethylammonium bromides, respectively.

Ferguson (16) suggested that the toxicities of substances should be compared on the basis of their chemical potentials in the aqueous phase. Ecanow and Siegel (17) suggested that an extension of this concept may be applied to surfactants using the critical micelle concentration (CMC) as a standard state rather than a saturated solution as suggested by Ferguson. The thermodynamic activity and, therefore, the biological activity should be equal to a constant percent of the CMC of the drug.

The activities shown in Table II were calculated by obtaining the ratio of the test critical kill dilution divided by the CMC for each quaternary ammonium compound. The difference between the activities of the various compounds is relatively small, except for the stearyl derivative

[1] Also known as *Micrococcus pyogenes* var. *aureus*.
[2] Scientific Products, Evanston, Ill.
[3] Multifit (Luer-Lok), Becton Dickinson and Co., Rutherford, N.J.

Table II—Activity of Synthesized Quaternary Ammonium Compounds

Alkyl Group	CKD, *M*	CMC, *M*	Activity, CKD/CMC, *M*
Lauryl	4.63×10^{-7}	1.8×10^{-2}	2.57×10^{-5}
Myristyl	5.96×10^{-8}	3.6×10^{-3}	1.65×10^{-5}
Cetyl	3.03×10^{-8}	1.0×10^{-3}	3.03×10^{-5}
Stearyl	5.95×10^{-8}	3.3×10^{-4}	2.58×10^{-4}

which has an activity approximately 10-fold larger than the other three compounds.

On the other hand, a comparison indicated a 60-fold difference between the largest and smallest CMC values of the four quaternary ammonium compounds. Thus, Ferguson's principle appears to apply to the compounds studied, since the activities are in the same order of magnitude and substantiate the hypothesis that the bactericidal activity is the result of some nonspecific physical activity in contrast to the involvement of a specific chemical mechanism.

The equilibrated aqueous phases from the partitioning systems were evaluated for their critical kill dilutions. In the following experimental data, two critical kill dilutions are listed: an experimental and a corrected value. The experimental value is based on the actual dilution of the aqueous phase that was prepared to meet the critical kill dilution requirement. The corrected critical kill dilution takes into account the weight of the partitioned alcohol phase, which was not removed and evaluated; consequently, this value represents the total initial oil-in-water system. If the total nonaqueous phase was completely inert and acted only to dilute the aqueous phase, the corrected critical kill dilution would correspond to the experimental critical kill dilution for the whole cream formula.

The corrected critical kill dilution was calculated by substituting into the following equation:

$$\text{corrected CKD} = \frac{(\text{experimental CKD}) \times (\text{total weight of system})}{\text{weight of aqueous phase}} \quad \text{(Eq. 1)}$$

The relationships between quaternary ammonium compound chain length and aliphatic alcohol chain length were studied for the selected systems. Various systems were evaluated in which a hydrocarbon was included, and the change in bactericidal effectiveness was noted.

The lauryl-, myristyl-, cetyl-, and stearyltrimethylammonium bromides, at initial concentrations of 0.418, 0.452, 0.492, and 0.535%, respectively (corresponding to about 1.0 mEq/75.0 g), were each evaluated after equilibration with lauryl, myristyl, cetyl, and stearyl alcohols (15% each) against *S. aureus*. The relationships among the concentration of the quaternary ammonium compound in the aqueous phase, the quaternary ammonium compound chain length, the chain length of the aliphatic alcohol, and their effect on the critical kill dilution of the system are given in Table III.

The cetyl and stearyl quaternary ammonium compounds were ineffective as bactericidal agents, showing a corrected critical kill dilution of less than 1–13 with each alcohol. The poor killing properties may be related to the low concentration of quaternary ammonium compound present in the aqueous phase. The myristyl derivative was the most effective quaternary ammonium compound in the homologous series, even

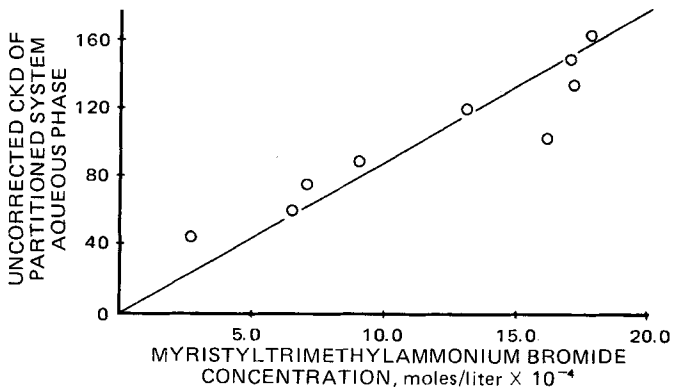

Figure 1—*Relationship between myristyltrimethylammonium bromide aqueous phase concentration and the uncorrected critical kill dilution of the aqueous phase.*

though the lauryl derivative was present in a greater concentration in each system. In the partitioning studies, the bactericidal properties of the quaternary ammonium compounds were a function of both their inherent bactericidal properties and their concentration in the aqueous phase. The myristyl quaternary ammonium compound, as a result of its intrinsic critical kill dilution, possessed the greatest bactericidal effectiveness for the concentration present in the aqueous phase.

Effect of Hydrocarbons—The critical kill dilutions for the lauryl, myristyl, and cetyl quaternary ammonium compounds in cetyl and stearyl alcohols were evaluated (Table IV) in systems that contained no hydrocarbon, 15% mineral oil, or 15% white petrolatum. The critical kill dilutions obtained appeared to be related to the inherent activity and the concentration of quaternary ammonium compound remaining in the aqueous phase of the partitioned systems.

The stearyl alcohol base systems were bactericidally more effective than the corresponding cetyl alcohol systems, due probably to the higher concentration of quaternary ammonium compound present in the aqueous phase. Both mineral oil and white petrolatum enhanced the critical kill dilution by decreasing the equilibrium partition distribution coefficient (1), thus making more quaternary ammonium compound available for bactericidal action.

The myristyltrimethylammonium bromide systems, without exception, were bactericidally more effective than either the lauryl or cetyl systems when combined with the same aliphatic alcohol and hydrocarbon. The greater activity of the myristyl compound was related to both its concentration in the aqueous phase and to its inherent bactericidal properties.

Effect of Percent Hydrocarbon—The effects of the percent hydrocarbon on the critical kill dilution of the myristyl quaternary ammonium compound in 15% cetyl or stearyl alcohol were evaluated (Table V). As the concentration of the hydrocarbon increased, the critical kill dilution of the system against *S. aureus* also increased. The addition of white petrolatum appeared to have a greater influence on increasing the critical kill dilution of a system than the corresponding percent mineral oil. The superiority of white petrolatum at increasing the critical kill dilution appeared to be related to the increase in the concentration of the myristyl compound remaining in the aqueous phase.

Relationship between Quaternary Ammonium Compound Concentration and Critical Kill Dilution—A relationship between the

Table III—Critical Kill Dilutions of Equilibrated Quaternary Ammonium Compounds in 15% Aliphatic Alcohol–Water Systems against *S. aureus*

	Lauryl Form			Myristyl Form			Cetyl Form			Stearyl Form		
Aliphatic Alcohol (15%)	Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$	Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$	Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$	Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$
Lauryl	30	25	7.05	45	40	2.19	<15	<13	0.95	<15	<13	0.77
Myristyl	30	25	7.51	45	40	2.35	<15	<13	1.17	<15	<13	1.14
Cetyl	30	25	9.02	45	40	3.05	<15	<13	1.53	<15	<13	1.42
Stearyl	30	25	10.05	75	65	4.58	<15	<13	2.68	<15	<13	1.58

Table IV—Critical Kill Dilutions of Equilibrated Quaternary Ammonium Compounds in Aliphatic Alcohol Combinations with and without 15% Hydrocarbon against *S. aureus*

Aliphatic Alcohol (15%)	Alkyl Group	No Hydrocarbon			15% Mineral Oil			15% White Petrolatum		
		Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$	Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$	Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$
Cetyl	Lauryl	20	15	9.40	30	20	14.56	30	20	16.52
	Myristyl	45	40	3.11	90	65	11.45	105	75	16.19
	Cetyl	<15	<13	1.40	30	20	8.52	45	30	14.25
Stearyl	Lauryl	30	25	10.29	30	20	15.81	45	30	17.42
	Myristyl	75	60	4.45	120	85	13.08	135	95	17.17
	Cetyl	<15	<13	2.84	30	20	9.80	45	30	15.25

Table V—Critical Kill Dilutions of Equilibrated Myristyltrimethylammonium Bromide in 15% Aliphatic Alcohol with Increasing Concentrations of Hydrocarbon against *S. aureus*

Aliphatic Alcohol (15%)	Hydrocarbon, %	Mineral Oil			White Petrolatum		
		Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$	Experimental CKD	Corrected CKD	Concentration of Active Compound in Aqueous Phase, moles/liter $\times 10^{-3}$
Cetyl	0	45	40	3.11	45	40	3.11
	5	60	45	4.45	60	45	8.15
	10	60	45	7.23	75	55	12.24
	15	90	65	11.45	105	75	16.19
	20	150	95	17.07	—	—	—
Stearyl	0	75	65	4.45	75	65	4.45
	5	90	70	6.59	90	70	6.57
	10	90	70	9.45	120	90	13.08
	15	120	85	13.08	135	95	17.17
	20	165	105	17.82	—	—	—

antibacterial agent concentration in the aqueous phase of an emulsion and the critical kill dilution of the emulsion has been suggested (2–8). It was observed throughout the study that the critical kill dilution of the aqueous phase was related to the aqueous phase concentration of quaternary ammonium compound determined in the partitioning studies. The concentration of the myristyl quaternary ammonium compound derivative in each partition study for which critical kill dilution data were available was averaged for each experimental critical kill dilution value, irrespective of the alcohol or additive present (Fig. 1). The average aqueous phase myristyl quaternary ammonium concentration was plotted against the uncorrected critical kill dilution of the systems. The uncorrected critical kill dilutions were employed because only the relationship between the critical kill dilution and concentration was being observed. This plot substantiates the hypothesis that the critical kill dilution is related to the concentration of quaternary ammonium compound in the aqueous phase.

Critical Kill Dilution Evaluation of Creams—The critical kill dilutions of creams prepared with the lauryl, myristyl, and cetyl quaternary ammonium compounds in cetyl and stearyl alcohols with 15% hydrocarbon were evaluated (Table VI). In general, the same critical kill dilution pattern was observed for the creams as for the corresponding partitioned systems (Table IV). The stearyl alcohol base creams with each quaternary ammonium compound were more effective than creams prepared with cetyl alcohol, except for the cetyl quaternary ammonium compound containing cream with 15% mineral oil. The increased effectiveness of the stearyl alcohol base creams may again be related to larger concentrations of quaternary ammonium compound in the aqueous phase, as was suggested in the partitioning studies.

The myristyl quaternary ammonium compound was the most effective compound in the series, while the lauryl compound showed one-fifth to two-thirds the effectiveness of myristyltrimethylammonium bromide. The reason the cetyl derivative was slightly less effective than the myristyl compound was probably related again to the lower concentration in the aqueous phase of the cream system—not to inherent bactericidal properties.

The inclusion of either 15% mineral oil or 15% white petrolatum increased the critical kill dilution of the cream, which again appeared to correlate with the increased aqueous phase concentration observed in the partitioning studies. Even more significant was the fact that the addition of white petrolatum to the formula permitted the preparation of systems exhibiting *both* acceptable bactericidal effectiveness and satis-

Table VI—Critical Kill Dilution Evaluation of Creams Prepared with and without 15% Hydrocarbon against *S. aureus*

Aliphatic Alcohol (15%)	Alkyl Group	No Hydrocarbon	15% Mineral Oil	15% White Petrolatum
Cetyl	Lauryl	45	30	60
	Myristyl	60	90	150
	Cetyl	<30	60	30
Stearyl	Lauryl	60	30	60
	Myristyl	120	150	180
	Cetyl	30	30	60

Table VII—Critical Kill Dilution Evaluation of Creams Prepared with and without Various Percent Hydrocarbons against *S. aureus*

Aliphatic Alcohol (15%)	Hydrocarbon, %	Mineral Oil	White Petrolatum
Cetyl	0	60	60
	5	75	120
	10	75	135
	15	90	150
	20	105	—
Stearyl	0	120	120
	5	135	150
	10	135	180
	15	150	180
	20	165	—

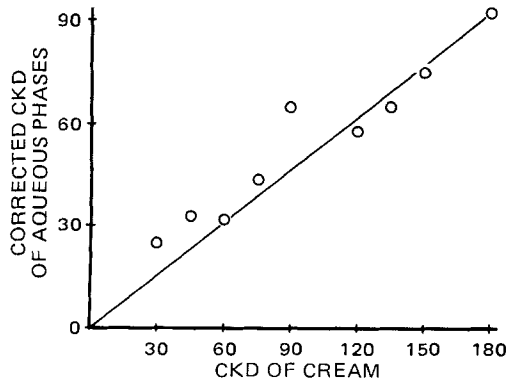

Figure 2—*Relationship between the critical kill dilutions of cream systems and the corrected critical kill dilutions of the partitioned aqueous phases.*

factory emulsion stability. A satisfactory combination of these two properties was not possible in the cream systems containing no hydrocarbon or 15% mineral oil.

Effect of Percent Hydrocarbon—The effect of the concentration of hydrocarbon on the critical kill dilution of creams emulsified with the myristyl quaternary ammonium compound and containing either cetyl or stearyl alcohol was evaluated (Table VII). The critical kill dilutions of the creams were similar in rank order to those observed in the critical kill dilution evaluation of the partitioned systems. The critical kill dilutions increased with increasing hydrocarbon concentration, with the stearyl alcohol creams being more effective than the corresponding cetyl alcohol base creams.

The white petrolatum creams showed a larger increase in critical kill dilution compared to the creams containing mineral oil of the same concentration. The increase may again be attributed to the greater concentration of quaternary ammonium compound present in the aqueous phase of the white petrolatum creams.

The relationship between the critical kill dilution of a cream and its corresponding aqueous phase was observed throughout the previous study. Figure 2 compares the relationship by pairing the corrected critical kill dilutions for the partitioned aqueous phases with the corresponding cream formula. The critical kill dilutions of the creams were ranked, and the corresponding corrected critical kill dilutions of the corresponding aqueous phases were averaged. From the data obtained, it appeared that on the average a cream was about twice as effective as the corresponding partitioned aqueous phase. The correlation technique appears to offer a method of correlating the bactericidal effectiveness of a cream and *in vitro* conditions, such as in the partitioning studies. The result also indicates that the nonaqueous phase also possesses antibacterial activity; otherwise, the slope of the plot would equal unity.

REFERENCES

(1) G. Baley, Ph.D. dissertation, Purdue University, West Lafayette, Ind., 1968.

(2) H. S. Bean, J. P. Richards, and J. Thomas, Proceedings of the 21st International Congress of Pharmaceutical Sciences, Pisa, Italy, 1961, p. 174.

(3) H. S. Bean and S. H. Heman-Ackah, *J. Pharm. Pharmacol.*, **16**, 58T (1964).

(4) N. A. Allawala and S. Riegelman, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 267 (1953).

(5) E. R. Garrett and O. R. Woods, *ibid.*, **42**, 736 (1953).

(6) H. W. Hibbott and J. Monks, *J. Soc. Cosmet. Chem.*, **12**, 2 (1961).

(7) L. Lachman, T. Urbanyi, and S. Weinstein, *J. Pharm. Sci.*, **52**, 244 (1963).

(8) R. Collander, *Acta Chem. Scand.*, **4**, 1085 (1950).

(9) S. Tannenbaum, *TGA Cosmet. J.*, **2** (1), 24 (1970).

(10) K. Tsuji, E. Stapert, J. Robertson, and P. Waiyaki, *Appl. Microbiol.*, **20**, 798 (1970).

(11) D. Blois and J. Swarbrick, *J. Pharm. Sci.*, **61**, 390 (1972).

(12) *Ibid.*, **61**, 393 (1972).

(13) G. F. Reddish, "Antiseptics, Disinfectants, Fungicides, and Sterilization," Lea & Febiger, Philadelphia, Pa., 1954, p. 112.

(14) "Official and Tentative Methods of Analysis," 7th ed., Association of Official Agricultural Chemists, Washington, D.C., 1950, p. 88

(15) R. S. Shelton, M. G. VanCampen, C. H. Tilford, H. C. Lang, L. Nisonger, F. J. Bandelin, and H. L. Rubenkoenig, *J. Am. Chem. Soc.*, **68**, 753 (1946).

(16) J. Ferguson, *Proc. Roy. Soc.*, **B127**, 387 (1939).

(17) B. Ecanow and F. P. Siegel, *J. Pharm. Sci.*, **52**, 812 (1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 28, 1973, from the *Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.*

Accepted for publication July 7, 1976.

Presented at the Industrial Pharmaceutical Technology Section, APhA Academy of Pharmaceutical Sciences, San Diego meeting, November 1973.

Supported by Miles Laboratories, Elkhart, IN 46514.

* Present address: Pharmaceutical Manufacturing Development, The Upjohn Co., Kalamazoo, MI 49001.

[x] To whom inquiries should be directed.

Determination of Solasodine in Fruits of *Solanum* Species

LEHEL TELEK

Abstract □ A potentiometric nonaqueous titration procedure was developed for the quantitative determination of solasodine in fruits of the *Solanum* species. The steroid glycoalkaloids were extracted from freshly harvested fruits with 2% acetic acid and methanol. After hydrolysis, the common aglycone solasodine was extracted with benzene. An aliquot was mixed with an equal volume of acetone and titrated potentiometrically with 0.005 *N* perchloric acid in dioxane, using glass and silver electrodes for the determination.

Keyphrases □ Solasodine—potentiometric analysis in fruits of *Solanum* species □ *Solanum* species—potentiometric analysis of solasodine in fruits □ Potentiometry—analysis, solasodine in fruits of *Solanum* species □ Steroids—solasodine, potentiometric analysis in fruits of *Solanum* species

The consumption of pharmacologically active steroids is increasing significantly (1). The use of steroidal contraceptives will spread with the pressing need for birth control. In the growing demand for raw materials, solasodine is the most competitive in supplementing diosgenin in the partial synthesis of steroid hormones (2). The special