Effect of Colloidal Association on the Measured Activity of Alkylbenzyldimethylammonium Chlorides against *Pseudomonas aeruginosa*

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The antibacterial activities of a homologous series (C_8–C_{18}) of alkylbenzyldimethylammonium chlorides (ABDAC) against *Pseudomonas aeruginosa* have been measured using both a minimum inhibitory concentration (MIC) procedure and a sterilization kinetics test carried out in deionized water. There was a log–linear relationship between activity measured by kinetics and carbon number. With MIC there was a log–linear relationship up to C_{14}, when there was a turndown in activity. Consideration of the colloidal association of ABDAC in deionized water and in a simple salts growth media leads us to suggest that use of high concentrations of nutrient salts in MIC tests will lower the effective concentration of the surface active agents. This change may be responsible for the turndown in activity observed in MIC tests, and that in such circumstances the MIC test does not give a true reflection of the intrinsic activity of the compounds. Literature reports of parabolic relationships between ABDAC alkyl chain length and antimicrobial activity are reinterpreted on this basis.

During a study[1] to measure the sensitivity of a series of stepwise polymyxin-resistant *Pseudomonas aeruginosa* strains to alkylbenzyldimethylammonium chlorides (ABDAC), we observed that only lower order polymyxin-resistant pseudomonads could be tested for sensitivity using a minimum inhibitory concentration (MIC) test procedure. When higher order resistant pseudomonads were studied using such a method, an increase in ABDAC concentrations up to their aqueous solubility failed to inhibit growth. We have suggested[1] that this could be due in part to the fact that micellar states of the salt are reached at the high concentrations of ABDAC needed to inhibit growth and that this is interfering with their antibacterial action. However, with some of the concentrations used where no end point could be reached, we were below the reported[2,3] aqueous critical micelle concentrations (CMC).

This observation has led us to develop a test procedure sensitive enough to allow lower concentrations of ABDAC to be used, so avoiding the possibility of entering the micellar state.[1] To develop a baseline of activity for the ABDAC, we have examined the effectiveness of a homologous series against a wild type *Pseudomonas aeruginosa* strain.

Our findings lead us to question the normally accepted parabolic relationship reported to exist between the lipophilicity of many quaternary ammonium salts and their antibacterial activities. Many of the original antimicrobial tests described in such reports involved the use of high concentrations of nutrient salts, which would alter the concentration of monomeric quaternary ammonium salt. This may be responsible for some of the observed parabolic relationships, rather than the intrinsic antibacterial activity of the compounds under study.

Alkylbenzyldimethylammonium chlorides have long been accepted as effective agents in aqueous pharmaceutical formulations. Domagk[4] made the original observation that ABDAC's were antibacterial and provided the impetus for the production of a large number of analogues, although the original molecules still retain their position as the most effective and pharmaceutically acceptable. The mode of action of quaternary ammonium compounds against membranes has been reviewed.[5,6] Resistance to them is probably associated with lack of penetration.[7-9] Brown and Norton[10] have summarized much of the earlier work on the use of these molecules as preservatives in ophthalmic formulations (Table I).

Benzalkonium chloride (BKC) is a mixture of predominantly C_{12} and C_{14} homologues. On the basis of Shinoda equation calculations[16] the CMC value of BKC in water will be in the range 0.01–0.001 M. Table I reveals that the majority of the studies were carried out in this concentration range (0.02% ≡ 0.005 M) and above. Undoubtedly mixed monomer/micellar states existed in these test systems. It seems probable that current recommended preservative concentrations in various pharmacopoeiae are based on such studies.

Table I. Summary of Early Studies on Benzalkonium Chloride (BKC)

Concn of BKC used, % w/v	Inoculum size	Test/ comments	Time for effectiveness	Ref
0.02	2×10^6 mL		45 min	11
0.01	2×10^6 mL		9 h	11
0.05	10 mL		30 min	12
0.08	100 mL		30 min	12
0.01	1×10^8 mL	In vivo in saline	Not effective	13
0.01	1×10^8 mL	Nutrient broth subculture	5 min	13
0.05	1×10^8 mL	No adequate inactivation	2 days	14
0.02	24-h broth culture		30 min	15
0.01	24-h broth culture	*a*	30 min	15

[a] Some strains studied were not sterilized until 6 h of contact.

Blois and Swarbrick[6] reviewed the studies of many workers relating various colloidal properties of quaternary ammonium salts to their antibacterial action. Similarly other workers have applied the Ferguson principle to the antibacterial action of cationic surface active agents[17] in which they defined the thermodynamic activity as the ratio of the CMC to a minimum antibacterial effective concentration. In a study on three quaternaries of equivalent alkyl chain length but having different polar head groupings, other workers[18] have shown that a definite relationship exists between thermodynamic activity and antibacterial activity, the physical index being expressed as the ratio of the Gibbs surface concentration produced by a solution and the surface concentration at the CMC (i.e., the Gibbs surface excess), rather than using bulk solution concentrations. Laycock and Mulley[19] have extended this concept to mixed surfactant systems.

As early as 1953 it was reported[20] that for a homologous series of cationic surface active agents, a "parabolic" relationship could exist between their antibacterial properties and their hydrophobic character. That is, there existed

a linear relationship between activity and alkyl chain length with increased carbon number up to a maximum of between 12 and 14, at which region there could be observed a decrease in activity. The turn down point depended upon the particular series being studied. The most commonly quoted series of experiments specifically on the ABDAC's was carried out a decade ago.[2] These showed that with broth dilution tests a peak activity against various microorganisms could be observed at an alkyl chain length of 14 carbon atoms. These findings have been analyzed in terms of a regression equation of the linear free energy type, showing a relationship between antibacterial activity and computed partition coefficients.[21] They have been further analyzed by Blois and Swarbrick[22] who suggested that the turn down in activity is probably related to more than one physical property of the compounds: (a) the length of the hydrocarbon chain, such that the longer the chain the greater the tendency for the molecule to be adsorbed at the surface of a bacterium, and (b) the reduction in aqueous solubility of the molecule as the carbon number is increased. These workers failed to point out, however, that in all systems studied in the original work the maximum aqueous solubilities of the compounds were several log cycles higher. Also they did not discuss the relevance of micellization to the thermodynamic state of the ABDAC molecules in the test system.

Experimental Section

To compare the results found using our newly developed test procedure with literature reports we repeated the earlier minimum inhibitory concentration (MIC) studies using simple salts, chemically defined media (CDM).

In addition to the antimicrobial studies, physical measurements on the colloidal properties of the surface active agents have been performed. The appreciation that chemically defined media could affect these measured properties has led us to study the effect of CDM on the measured CMC values using conductimetric and surface tension methods.

Materials. Alkylbenzyldimethylammonium chlorides: the monohydrates of an homologous series of alkylbenzyldimethylammonium chlorides (C_8–C_{18}) (kindly donated by Dr. Royal A. Cutler, Sterling-Winthrop Research Institute, Rensselaer, N.Y.). Their physical properties having been reported earlier,[2] their high purity was confirmed by surface tension measurement of the CMC.

Chemically defined media (CDM): $MgCl_2$ (1.6×10^{-4} M); NaCl (5.0×10^{-4} M); KCl (5.0×10^{-4} M); $(NH_4)_2HPO_4$ (2.0×10^{-2} M); $(NH_4)H_2PO_4$ (2.0×10^{-2} M); NH_4SO_4 (1.0×10^{-4} M); NH_4FeSO_4 (1.8×10^{-6} M); glucose (2.0×10^{-2} M). This composition caused *Pseudomonas aeruginosa* cultures to enter a stationary phase as a result of oxygen depletion.

Organism: *Pseudomonas aeruginosa* (ATCC 9027), originally reconstituted from a freeze-dried culture using nutrient broth and then grown in CDM and subsequently stored on CDM–agar slopes at 10 °C.

Methods. Conductimetric Titration. To determine the critical micelle concentrations of the quaternary ammonium salts a previously described automated conductimetric titrimeter[3] was used. For measurements in the presence of CDM an external standard technique was used. The protocol was to deliver a concentrated aqueous solution of the surface active agent either to 50-mL double distilled deionized water or to equal volumes of CDM solutions at 25 °C.

Surface Tension Studies. These were carried out using a De Nouy tensiometer at room temperature (22–25 °C), using 20-mL volumes of solution under test.

Microbiological Studies. For the MIC test procedure 0.1 mL of a dilution of an overnight, oxygen depleted culture of the organism, in CDM, was added to 4.9 mL of CDM media containing varying concentrations of ABDAC. Dilutions were chosen so that a nominal 10^4 cell number was the inoculum size as determined by optical density measurements. Inoculated solutions were incubated at 37 °C for 7 days. After this time turbid or clear solutions were taken to imply growth or no growth of the organism, respectively. Findings are reported in this paper as those minimal concentrations of surface active agent used for which no growth could be observed after 7 days. We have reported a sterilization kinetics test system in which there can be no significant alteration of the thermodynamic activity of the quaternary ammonium compounds by the nutrient salts.[1] Essentially it consisted of suspending the bacteria in water and making colony counts after exposure to drug. The concentration necessary to reduce the colony count to 10% of the original after either 30 min or 2 h contact was determined from the log–linear survival curves.

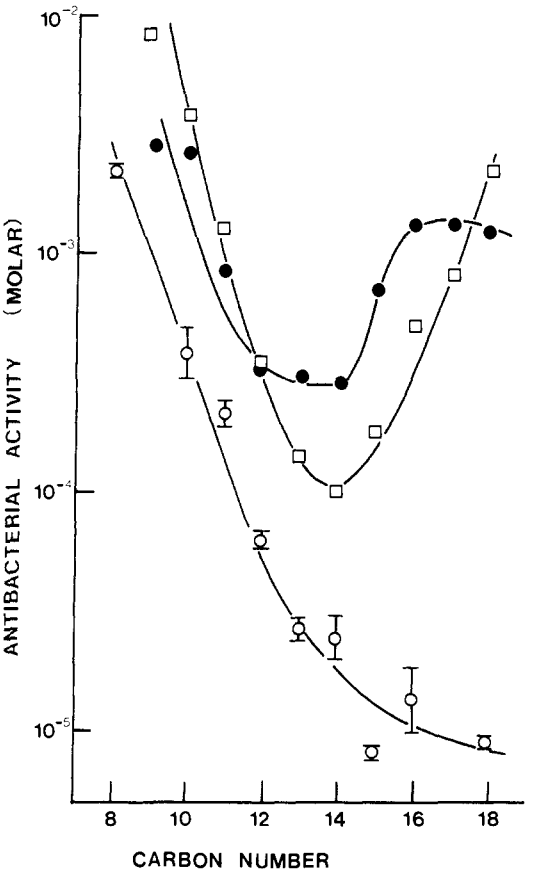

Figure 1. Effect of alkyl chain length on the activity of ABDAC's against *Pseudomonas aeruginosa*: (⊖) MIC + standard errors for this study; (●) MIC against Sterling Winthrop strain 211 (calculated from ref 2); (□) concentration killing in ⩾10 min and ⩽5 min (similarly calculated from ref 2).

Results and Discussion

Figure 1 relates the activity of the ABDAC's against *Pseudomonas aeruginosa* (ATCC 9027), as measured in the MIC test, and the alkyl chain carbon number. Also shown in this figure are similar relationships calculated from previously reported measurements.[2] The data are plotted in molar concentration form and show two end points of activity: a broth dilution bacteriostatic concentration and a minimum concentration of ABDAC producing sterility in not less than 5 min but not more than 10 min. Our results are different to those reported previously, in that for all the homologues studied lower concentrations are required to inhibit the growth of the organism used. Although we obtained a similar relationship between antibacterial activity and chain length up to carbon number 14, the subsequent change in activity above this number is not as marked as those reported previously.

The relationship between the MIC end point values and a measured colloidal property of the ABDAC is given by Figure 2, in which the critical micelle concentration values

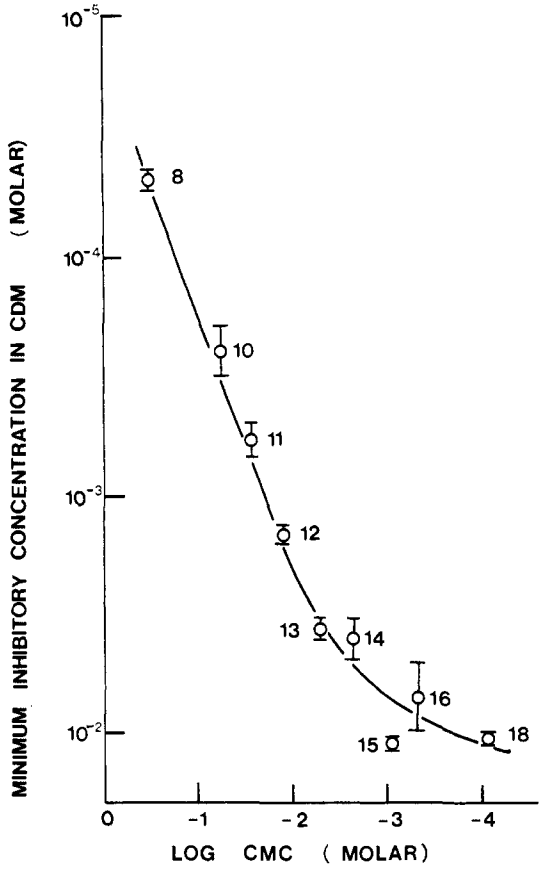

Figure 2. Relationship between MIC of a homologous series of ABDAC's against *Pseudomonas aeruginosa* and the critical micelle concentration measured in deionized distilled water. The carbon number of the alkyl chain appears beside each datum point.

for these surface active agents,[3] as obtained in double distilled deionized water, are related to the MIC values found in a CDM environment. Again there exists a change which corresponded to the C_{15} homologue.

Using the sterilization kinetics procedure, we have obtained death–time curves for all of the homologues. As before[1] the relationship between viable count and time was exponential. That is

$$V_t/V_0 = e^{-kt} \quad (1)$$

where V_0 and V_t refer to the number of viable bacteria in the reaction vessel at time = zero and time = t, respectively, and k is a constant for the system depending upon the sensitivity of the organism and the concentration of quaternary salt used. At some of these concentrations an initial slight lag time before bactericidal action could be observed was noted. In these instances, which occurred when low concentrations of the C_{16} and C_{18} homologues were studied, the initial data points were excluded from subsequent analysis.

The viable count–time data has been analyzed via least-squares regression, by computing the rates of change of the logarithms of the viable counts with respect to time. Once these relationships had been derived the results were further analyzed by deriving the relationships between the rates of kill and the quaternary concentration used, i.e.

$$\log (\mathrm{d} \log V/\mathrm{d}t) = a \log (\text{drug concentration}) + b \quad (2)$$

Table II gives the regression and correlation coefficients derived for this expression. All the homologues studied gave rectilinear relationships as illustrated by Figure 3. To produce usable end points for comparing this test procedure with the MIC test, we have arbitarily chosen those concentrations of quaternary producing a reduction in viable count to 10% of the original in (a) 30 min and (b) 2 h. Tables III and IV give these computed concentrations and their standard errors. These two new antibacterial end points, showing activity of the quaternary ammonium salts in a low ionic strength environment, have been correlated with the carbon number of the alkyl chain (Figure 4). This reveals that using the 2-h end point there

Table II. Regression and Correlation Coefficients[a] Derived for the Expression, Log (d Log $V/\mathrm{d}T$) = a Log (Concentration) + b, Where (d Log $V/\mathrm{d}T$) Refers to the Rate of Change of Colony Count with Time for Different ABDAC Chain Lengths

n	a	b	N	r
8	1.014	2.051	6	0.989
10	2.479	8.785	5	0.992
11	2.129	7.963	6	0.976
12	2.411	9.704	6	0.987
13	1.725	7.105	6	0.881
14	2.448	11.244	5	0.989
15	1.715	7.730	5	0.995
16	0.986	4.046	4	0.919
18	0.786	2.964	6	0.933

[a] n is the carbon number of the alkyl chain of the ABDAC, a and b are the regression coefficients for the expression, and N and r are the number of data points used and the correlation coefficient, respectively.

Table III. Concentrations and Standard Errors at 95% Confidence Limits for ABDAC to Reduce the Colony Count of *Pseudomonas aeruginosa* to 10% in 30 min

Alkyl chain length	Concn,[a] M	Standard error, M
8	3.300×10^{-4}	3.961×10^{-4}-2.840×10^{-4}
10	7.242×10^{-5}	7.499×10^{-5}-7.001×10^{-5}
11	3.672×10^{-5}	4.489×10^{-5}-2.939×10^{-5}
12	2.306×10^{-5}	2.592×10^{-5}-2.051×10^{-5}
13	1.061×10^{-5}	1.593×10^{-5}-7.065×10^{-6}
14	6.386×10^{-6}	7.270×10^{-6}-5.609×10^{-6}
15	4.265×10^{-6}	4.658×10^{-6}-3.905×10^{-6}
16	2.523×10^{-6}	3.611×10^{-6}-1.304×10^{-6}
18	2.254×10^{-6}	4.008×10^{-6}-1.267×10^{-6}

[a] The concentrations necessary to reduce the colony count to 10% were computed from the log linear time-survival curve.

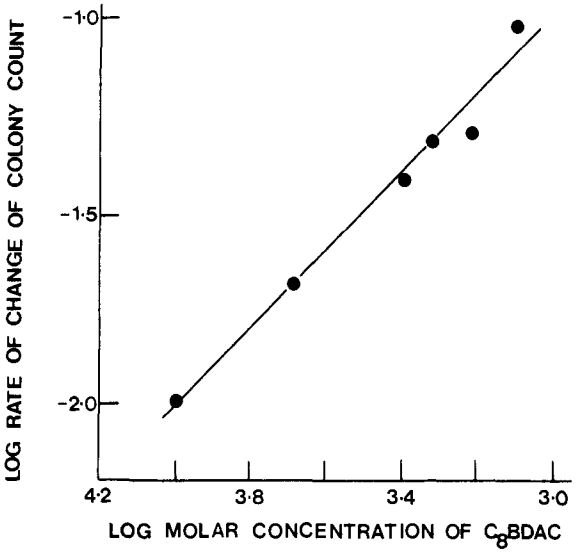

Figure 3. Effect of concentrations of octylbenzyldimethylammonium chloride on the rate of change of colony count of *Pseudomonas aeruginosa*.

Table IV. Concentrations and Standard Errors at 95% Confidence Limits for ABDAC to Reduce the Colony Count of *Pseudomonas aeruginosa* to 10% in 2 h

Alkyl chain length	Concn,[a] M	Standard error, M
8	8.452×10^{-5}	1.027×10^{-4}-6.965×10^{-5}
10	4.139×10^{-5}	4.487×10^{-5}-3.819×10^{-5}
11	1.915×10^{-5}	2.309×10^{-5}-1.584×10^{-5}
12	1.298×10^{-5}	1.451×10^{-5}-1.162×10^{-5}
13	7.616×10^{-6}	1.061×10^{-5}-5.463×10^{-6}
14	3.625×10^{-6}	4.094×10^{-6}-3.210×10^{-6}
15	1.900×10^{-6}	2.077×10^{-6}-1.738×10^{-6}
16	6.194×10^{-7}	9.491×10^{-7}-4.032×10^{-7}
18	3.861×10^{-7}	5.889×10^{-7}-2.530×10^{-7}

[a] See footnote *a*, Table III.

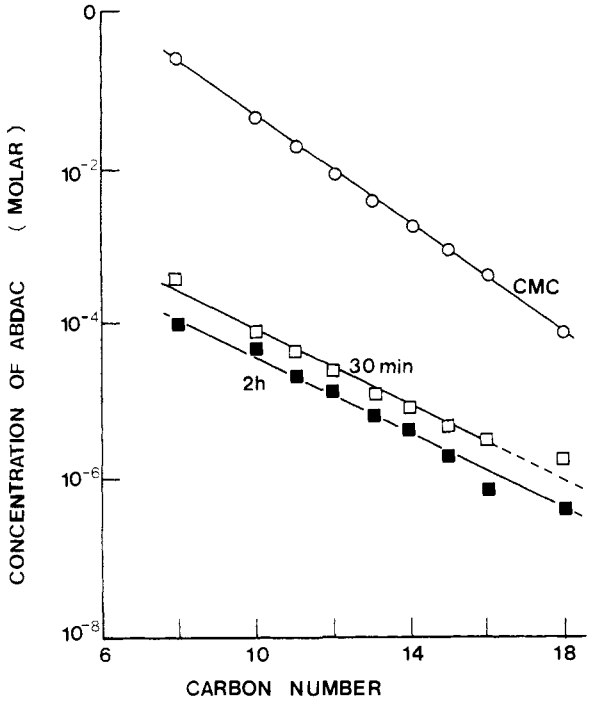

Figure 4. The effect of carbon number of the alkyl chain of the ABDAC's on their antibacterial activity in the sterilization kinetics test and on their CMC values in deionized distilled water: (□) 30-min end point; (■) 2-h end point; (○) CMC.

is a rectilinear relationship between activity and carbon number. With the 30-min end point, in which higher concentrations are required, there was possibly an indication of a deviation from linearity at and above carbon 15. Also given in this figure are the CMC values as measured in a similar deionized water environment.

We stress that although this is a commonly reported type of correlation, purporting to show a significant relationship between colloidal association (or surface activity) and antibacterial activity, it is only an inference. Further evidence would be required to demonstrate conclusively that the same properties causing self-association of the alkyl homologues contribute also to the antibacterial action of the molecules.

There is an obvious variance in the relationship between activity and carbon number (Figures 1 and 4), dependent upon the type of test procedure used. This discrepancy between results has led us to reexamine the assumption that in the MIC test the ABDAC molecules were solely in the monomeric form and the corollary that possibly nutrient salts are interfering with the physicochemical properties of the quaternary ammonium salts. We thus have attempted to measure the CMC of the alkyl-

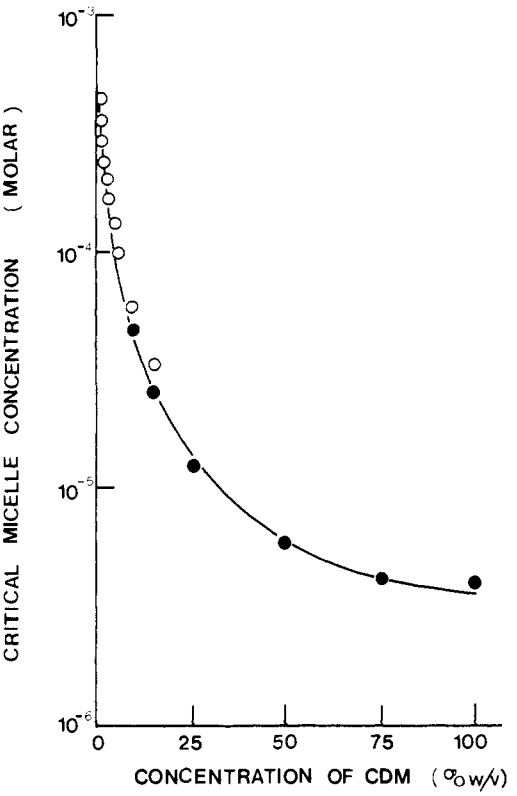

Figure 5. Effect of CDM on the critical micelle concentration of hexadecylbenzyldimethylammonium chloride: (○) determined conductimetrically; (●) determined by surface tension.

benzyldimethylammonium chlorides in a CDM environment. This was performed initially at 25 °C using the external standard technique of the automated conductimetric titration procedure.[3] At 15% v/v CDM the high salt concentrations used caused a nonlinearity in the specific conductivity–concentration relationship for the CDM, and further measurement above this concentration was not possible. By examining the surface tension–concentration profile for hexadecylbenzyldimethylammonium chloride in the presence and absence of CDM at ambient temperature, it has been possible to complete this portion of the study. Results obtained by both methods are given in Figure 5. Salt additives are known to increase the tendency of ionic surface active agents to micellize in water;[23] in some instances the micellar molecular weights can increase indicating a change in micellar type. It is possible that the presence of CDM will act (i) by suppressing the micelle dissociation and (ii) by screening the initial monomer head group repulsion.

Examining first the results obtained with the sterilization kinetics procedure, Figure 4 shows that there is a rectilinear relationship between the two antibacterial end points and alkyl chain length of the quaternary ammonium chlorides. The CMC's of the molecules are some orders of magnitude higher, indicating that only the monomeric form can be responsible for the activity. This large difference between activity concentrations and critical micelle concentrations thus provides a sensitive test to measure the activity of the ABDAC's against a series of resistant pseudomonads.[1] For the MIC results the contrasting observation (Figure 1) is that above carbon 14 and 15, there is a change in activity comparable but not equal to other reported values.[2] Many reported studies have observed such a change in activity with other surface active agents and microbial systems. Hansch and Clayton[21] in their recent compilation of evidence to show parabolic relationships between biological activity and the hydrophobic

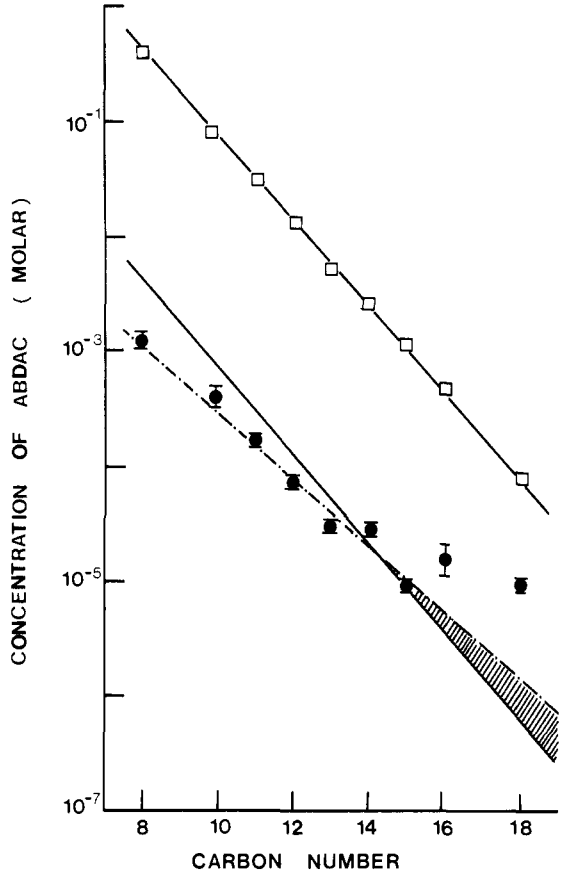

Figure 6. Illustration of hypothesis that colloidal association interferes with the measurement of the antibacterial activity of ABDAC's in a MIC test: (□) CMC measured in deionized distilled water; (⊕) MIC values in CDM and the standard deviations; (—) limiting solubility of the monomeric form of the quaternaries in CDM (i.e., critical micelle concentration in CDM); (·---·) extrapolated line of the C_8–C_{14} activity data of the ABDAC's. Shaded portion represents the amount of monomer required to be released from the micellar state before an effective equilibrium concentration is reached.

character of bioactive molecules gave 103 statistically preferred correlations; 53% of these involved the use of anionic or cationic surface active agents studied in various biological test systems. Of this total, 40 were microbiological studies using MIC or minimum killing concentration end points in nutrient rich systems, in 34 of which ionic surface active molecules had been used. In such systems the influence of the nutrients upon the effective monomeric concentration may have been profound. We explain the variation in the activity–carbon number relationships with the different end points as follows.

Figure 6 is a composite diagram in which we present our proposal. The MIC data determined in CDM are shown together with the limiting solubility line for the monomeric form of the ABDAC, as influenced by CDM. From C_8 to C_{14} it is seen that a rectilinear relationship exists between carbon number and MIC, with each methylene group increment producing a regular increase in activity. It is seen that at about C_{14}–C_{15} the limiting monomer solubility line intersects the MIC data points. It is assumed that the monomeric ABDAC is responsible for inhibiting growth. Considering *Pseudomonas aeruginosa* as a rod with hemispherical ends of dimensions 600 nm (diameter) × 3000 nm (length) and C_{14} ABDAC to have a two-dimensional area of approximately 0.45 nm^2,[24] it can be calculated that about 1.5×10^7 molecules of C_{14} are required to achieve monolayer coverage of each cell. Inocula in the MIC test of 1×10^4 cells and in the kinetics test of 7.5×10^4 cells[1] require approximately 1.5×10^{11} molecules and 1.1×10^{12} molecules to achieve cell monolayer coverage. Figure 6 shows that in a CDM environment the lowest possible monomer concentration is for the C_{18} homologue and is $\simeq 7 \times 10^{-7}$ M, equivalent in a MIC test volume to 2.1×10^{15} molecules and in a kinetics test volume to 1×10^{16} molecules. Thus for the extreme case there is at least a 10^4-fold excess of monomer necessary to produce a monolayer coverage of all cells. For C_8–C_{14} this suggests that an equilibrium between monomer in solution and on the cell exists, i.e.

$$[\text{monomer}]_{\text{solution}} \rightleftharpoons [\text{monomer}]_{\text{cell}}$$

the $[\text{monomer}]_{\text{cell}}$ concentration is that required to produce inhibition and is determined by the relative hydrophobicity of the ABDAC homologues. Considering the C_{15}–C_{18} data, Figure 6 shows that if the C_8–C_{14} MIC data are linearly extrapolated then this line is intersected by the limiting solubility line for the monomers in a simple salts medium. A new equilibrium now exists, i.e.

$$[\text{micelle}] \rightleftharpoons [\text{monomer}]_{\text{solution}} \rightleftharpoons [\text{monomer}]_{\text{cell}}$$

The competing effect of the micelle will remove monomer from solution, so altering the solution ⇌ cell equilibrium and reducing the amount directly available to inhibit cell growth. However, cell inhibition does occur, though at higher concentrations than expected. The MIC test is carried out over a 7-day period and it is suggested that initially some inhibition is possible by ABDAC monomer molecules adsorbing onto the cells from solution. This would cause a shift in the micelle–monomer equilibrium resulting in more monomers being released. These would then have a further inhibitory effect, with a balance being set up between the tendency of the monomer to form micelles or to transfer to the bacterium surface.

Ross and Kwartler[20] have presented data for some preservative–bacteria systems showing a similar intersection between activity and colloidal association concentrations but did not discuss such effects. An alteration in the physicochemical properties of antibacterial agents related to a changed activity has been suggested recently by Koelzer and Buechi,[25] who concluded that a turndown in the antimicrobial effectiveness of a C_4–C_{18} series of straight chain aliphatic amines against 14 bacterial species could be due to micelle formation of the amines being promoted by the components of the growth media used. Other workers[26] suggested that amino acids in a nutrient broth may have altered the thermodynamic activity of quinacrine against *Escherichia coli* via a complexation mechanism. In addition, the problems of limiting solubility of an homologous series of alkyl benzoates in relation to their effect on goldfish overturn time have been presented[27] in terms similar to those discussed in this paper.

We further analyze our results in terms of the Ferguson principle which has been defined[18] by the statement that solutions of antibacterial agents having the same thermodynamic activity will have equal antibacterial activity. Like other analyses[19] we have calculated the thermodynamic activity of solutions of the ABDAC's below the CMC as the ratio of the solution concentration and the CMC. We have calculated this for (i) the 30-min and 2-h end points from the sterilization kinetics procedure and (ii) the MIC end point (Table V). For the sterilization kinetics end points we see that the thermodynamic activity increases regularly with chain length. For the MIC test, however, the thermodynamic activities determined in the CDM environments show an equivalent thermodynamic activity up to C_{15}, followed by a large increase in the values

Table V. Thermodynamic Activities of ABDAC against *Pseudomonas aeruginosa* Calculated from MIC, Sterilization Kinetics, and CMC Data

Alkyl chain length	MIC / CMC[a]	MIC / CMC[b]	30 min[c] / CMC	2 h[c] / CMC
8	0.0040	0.3800	0.0011	0.0003
10	0.0068	0.6400	0.0012	0.0007
11	0.0068	0.7171	0.0014	0.0007
12	0.0057	0.5763	0.0019	0.0011
13	0.0057	0.5489	0.0022	0.0016
14	0.0126	1.1431	0.0032	0.0018
15	0.0098	0.8800	0.0047	0.0021
16	0.0330	3.4090	0.0055	0.0014
18	0.1220	11.9650	0.0289	0.0050

[a] CMC values measured in deionized distilled water. [b] CMC values obtained from extrapolation of the hexadecylbenzyldimethylammonium chloride measurements in chemically defined media. [c] 30 min and 2 h refer to concentrations necessary to reduce colony count to 10% in these time intervals.

of the MIC–CMC ratio. We see this as supporting evidence for our earlier arguments.

An alternative explanation for our results can be considered as follows. The existence of parabolic relationships is generally regarded[21] as being due to the highly lipophilic members of a series binding strongly to the first lipid barrier, hindering movement to the active site. Assuming that this theory is plausible how could it explain the nonparabolic profile obtained with the kinetics test? It is assumed that lipophilicity of the quaternary ammonium salts is constant for any molecule from test to test. But it is possible that in the simple salts system an ion pair (simple salt–ABDAC) is formed. Such a species will have a much higher lipophilicity[28,29] due to charge neutrality, i.e., the effective lipophilicity of the quaternary ammonium ion has been extended, and the ion pair would bind strongly to the first lipoidal barrier. In the absence of simple salts the intrinsic lipophilicity of the ABDAC molecules would not be high enough for parabolic relationships to occur. Although this lipophilicity extension model is an attractive one, consideration of the simple salts reveals little likelihood of ion-pair formation occurring except perhaps with the phosphate ions.

Gram-negative organisms have an outer membrane and an inner cytoplasmic membrane, and the linear correlation between carbon chain number and activity may suggest that damage of the outer membrane alone is responsible for kill.

Two further points need comment. First, there is the discrepancy between our MIC results and those reported earlier (Figure 1). These may be interpreted as the strain of organism used being more resistant to the quaternaries than the one used in the present study in CDM. Second, the CMC values used in this study were determined at 25 °C, though all the microbiological tests were at 37 °C. Relative to biological activity concentrations the variation of the CMC with temperature for ionic surfactants is not great.[3] The probable effect would be to alter slightly the limiting monomer solubility line given in Figure 6.

Conclusions

With regard to formulation of solutions with ionic surface active agents like the ABDAC molecules, it appears desirable to create a system free from the complications of micellar states interfering with antimicrobial action. For benzalkonium chloride the presence of *N*-alkyl-substituted ureas, for example, would greatly increase the CMC.

The data presented in this paper show that observed parabolic relationships between biological activity and lipophilicity, found for surface active agents with bacterial systems, may be explained by colloidal association phenomena and that inferences previously drawn from such observations should be treated with caution.

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