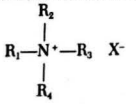
combination in the dual quats has been applied to twin chain quats dialkyldimethyl ammonium chlorides (dioctyl—25%, didecyl—25%, octyldecyl—50%) was combined with benzalkonium chlorides (R=C_{12}—40%, C_{14}—50%, C_{16}—10%). According to the work of Schaeufele (1984), a 60/40 blend of the above quaternaries proved to be superior to the individual components tested via the AOAC Use-Dilution Test (Official Methods of Analysis of the AOAC, 1984). This newest blend of quaternaries represents the fifth generation of quaternary ammonium compounds. The blend remained active under the most hostile conditions, was less toxic and less costly, and provided more convenient disinfectants.

In the 1980s the toxicity of quaternaries underwent scrutiny by the EPA and other U.S. regulatory agencies. The safety of the biocides in general received top priority over their efficacy. A new class of biocides, the polymeric quaternaries, has emerged, which are less toxic than the standard benzalkonium chlorides and less powerful than the dual quats or twin chain quats. The polymeric quaternaries are milder and have found applications in pharmaceuticals as preservatives (Stark, 1985; Merianos, 1988). The polymeric quaternary ammoniums are polyelectrolytes, representing the sixth generation of quaternary ammonium compounds. They are milder and safer than all other classes based on LD_{50} and cytotoxicity (Stark, 1985). More recently another synergistic combination, which may be considered the seventh generation of quaternary blends of bis-quats and polymeric quaternary (Polyionenes), offer excellent antimicrobial activity against oral flora bacteria, namely *Bacteroids gingivalis* 318, A7A128, *Actinomyces viscosus*, and *Streptococcus mutans* in the level of 1 to 5 ppm in a pharmaceutically accepted polymer formulation (MerPan Chemical Consultants Brochures, MerPanocide L. and MerPanocide LHD) (Merianos, 1988). Reduction of toxicity in the bis-quats without compromising efficacy is not an easy task. These preliminary results required further testing to confirm safety and true synergism in this series of polymeric quaternaries with bis-quats blends (Merianos, 1986).

CHEMISTRY

The quaternary ammonium compounds are the products of a nucleophilic substitution reaction of alkyl halides with tertiary amines. Chemically, they have four carbon atoms linked directly to the nitrogen atom through covalent bonds, while the anion in the original alkylating agent becomes linked to the nitrogen by an electrovalent bond. The general formula for the quaternary ammonium compounds is represented as follows:



R_1, R_2, R_3, and R_4 are alkyl groups that may be alike or different, substituted or unsubstituted, saturated or unsaturated, branched or unbranched, and cyclic or acyclic, and that may contain ether or ester or amide linkages; they may be aromatic or substituted aromatic groups. The nitrogen atom plus the attached alkyl groups forms the positively-charged cation portion, which is the functional part of the molecule. The portion attached to the nitrogen by an electrovalent bond may be any anion, but is usually chloride or bromide to form the salt.

Depending on the nature of the R groups, the anion, and the number of quaternary nitrogen atoms present, the antimicrobial quaternary ammonium compounds may be classified as follows.

Monoalkyltrimethyl Ammonium Salts

In this instance, one R group is a long-chain alkyl group, and the remaining R groups are short-chain alkyl groups, such as methyl or ethyl groups. All of the quaternary compounds in this group are prepared from the reaction of a tertiary amine with an alkyl halide (Domagk, 1935; Shelton et al., 1946; Hartmann, 1929). The tertiary amine may be the long-chain alkyldimethylamine or the short-chain trimethylamine, which react with methyl halide or with the long-chain alkyl halide, respectively. Examples of commercially available products in this group are cetyltrimethylammonium bromide as CTAB, alkyltrimethyl ammonium chloride as Arquad 16, alkylaryltrimethyl ammonium chloride as Gloquat C, cetyldimethyl, ethylammonium bromide as Cycloton D256B, Ammonyx DME, and Bretol.

Monoalkyldimethylbenzyl Ammonium Salts

In this group, one R is a long-chain alkyl group, a second R is a benzyl radical, and the two remaining R groups are short-chain alkyl groups, such as methyl or ethyl groups. These compounds are prepared by the reaction of a long-chain alkyldimethylamine with the benzyl halide (Wakeman and Tesoro, 1954; Domagk, 1938; Dunn, 1936).

Examples of commercially available products in this group are alkyldimethylbenzyl ammonium chlorides, as BTC 824, Hyamine 3500, Cyncal Type 14, and Catigene.

In addition, there are substituted benzyl quaternary ammonium compounds such as dodecyldimethyl-3,4-dichlorobenzyl ammonium chloride, sold under the trade name of Riseptin. There are also mixtures of alkyldimethylbenzyl and alkyldimethyl substituted benzyl (ethylbenzyl) ammonium chlorides, such as BTC 2125M, Barquat 4250 (Stepan; Lonza Brochures).

Dialkyldimethyl Ammonium Salts

In this instance, two R groups are long-chain alkyl groups, and the remaining R groups are short-chain alkyl groups, such as methyl groups. These compounds are prepared by the reaction of the long-chain alkyldimethylamine with a long-chain alkyl halide or dialkylmethylamine with methyl halide (Kirby and Frick, 1963; Tonaka, 1944; Kuhn et al., 1940).

Examples of commercially available products in this

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group are didecyldimethyl ammonium halides, such as Deciquam 222 and Bardac 22, and octyldodecyldimethyl ammonium chloride, such as BTC 812 (Stepan; Lonza Brochures).

Heteroaromatic Ammonium Salts.

In this group, one R chain is a long alkyl group, and the remaining three R groups are provided by some aromatic system. Thus, the quaternary nitrogen to which these three R groups are attached is part of an aromatic system such as pyridine, quinoline, or isoquinoline.

These compounds are prepared by reaction of the aromatic amine with a long-chain alkyl halide (Browning et al., 1922; Shelton et al., 1946; Mosher and Howard, 1948). Examples of commercially available products in this group are cetylpyridinium halide (CPC and Ceepryn), reaction product of hexamethylenetetramine with 1,3-dichloropropene to give cis-isomer 1-[3-chloroallyl]-3,5,7-triaza-1-azoniaadamantane (Dowicil 200), alkylisoquinolinium bromide (Isothan Q), and alkyldimethylnaphthylmethyl ammonium chloride (BTC 1100).

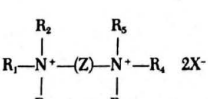
Polysubstituted Quaternary Ammonium Salts

In this group, the cation portion of the molecule is the same as that described for any of the aforementioned groups. However, the anion portion is not a small inorganic ion as previously described, but a large, high-molecular-weight organic ion. These compounds are prepared by reaction of the quaternary ammonium halides with the sodium, potassium, or calcium salt of a high-molecular-weight organic moiety, so that an exchange of the anions is effected (Wakeman et al., 1971; Shibe et al., 1955).

Examples of commercially available products in this group are alkyldimethylbenzyl ammonium saccharinate (Onyxide 3300 and Loroquat QA 100), and alkyldimethylethylbenzyl ammonium cyclohexylsulfamate (Onyxide 172).

Bis-Quaternary Ammonium Salts

In this group of compounds, there are two symmetric quaternary ammonium moieties arranged in the general formula:



Here the R groups are as described for any of the aforementioned groups, Z is a carbon-hydrogen chain, and an anion is attached to each quaternary nitrogen via an electrovalent bond. These compounds are prepared by reaction of a bis-tertiary amine with alkyl halide or of a di-halo compound with a tertiary amine (Hwa, 1963; DeBenneville and Bock, 1950; Babbs et al., 1956).

An example of a commercially available product in this group is 1,10-bis(2-methyl-4-aminoquinolinium chloride)-decane, sold under the trade name of Dequadin or Sorot. Another example of bis-quat is 1,6-Bis [1-methyl-3-(2,2,6-trimethyl cyclohexyl)-propyldimethylammonium chloride] hexane or triclobisonium chloride sold by trade name Triburon. Another commercially available bis-quat is CDQ (Buckman Brochures), used for industrial water treatment for controlling sulfate-reducing bacteria (*Desulfovibrio* sp.). The CDQ is prepared by reaction of alkyl[C_{12}—40%, C_{14}—50%, C_{16}—10%] dimethylamine with dichloroethyl ether. Also, reaction of 1,4-dichloro-2-butene with two moles of alkyldimethylamines or hexamethylenetetramine offer another example of bis-quats with broad spectrums of biocidal activity.

Polymeric Quaternary Ammonium Salts

Many different types of polymeric quaternary ammonium salts have been reported to have antimicrobial activity (Ghosh, 1988, 1986; Ikeda and Tazuke, 1983, 1984, 1985; Samour, 1978; Rembaum, 1973, 1975, 1977). The methods of preparation of these polymers are also many, from free radical polymerization of monomers containing quaternized nitrogen, to cationic, anionic polymerization, polycondensation of diamines with dihalides, or polycondensation of haloamines. The last method was used by Rembaum for the preparation of ionenes, which are polyelectrolytes with positively-charged nitrogen atoms located in the backbone of polymeric chain. This type of polycation was first reported in 1941 and is formed by the Menchutkin reaction from ditertiary amines and dihalides (Rembaum, 1973). Although a number of patents were published since 1941 concerning the applications of ionene polymers (Fush and Stamberger, 1965; Boyer and Santiago, 1974; Walker and Cambre, 1970), little information was available on the mechanism and kinetics of their formation, or their solution properties. The scope of this polycondensation reaction, the effect of concentration and solvent on rates and molecular weight of ionenes, and a proposed mechanism were first reported by Rembaum in 1968, when it was realized that under well defined conditions relatively high molecular weight (3,3 ionene chloride 65,000) ionenes were obtainable. The polymerization conditions for the formation of ionenes involves a total concentration of 3.0 mol [1.5 mol of each monomer] in a mixture of DMF/MEOH solvent [1:1 or 4:1] and 5 to 7 days of reaction time at room temperature to give polyionenes with molecular weight of about 65,000. In condensation polymerization the purity of the condensing species and stoichiometry are critical in obtaining high-molecular-weight polymer. Rembaum (1973) reported that polyionenes exhibit the following biologic effects: (1) *Bactericidal action*, (2) *Formation of insoluble complexes with DNA and heparin*, (3) *Neuromuscular blocking action*, (4) *Cell lysis and aggregation*, and (5) *Cell adhesion*. The antimicrobial and antifungal properties of ionenes were studied by the zone of inhibition method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida sporogenes*, *S. typhimurium*, *M. smegmatis*, *Pseudomonas mirabilis*, *Pseudomonas vulgaris*,

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Table 13–5. *Minimum Inhibitory Concentration of Polyionenes*

Polyionene	MIC (ppm)	
	S. aureus	*E. coli*
1. 3, 3-ionene bromide	>128	>128
2. 6, 6-ionene bromide	16	16
3. 6, 10-ionene bromide	4	4
4. 2, 10-ionene bromide	4	8
5. 6, 16-ionene bromide	4	32

Candida globosum, *M. verrucaris*, *F. oxysporum* and *Alternaria sp.* The MIC for five of the most active ionenes bromides against *S. aureus* and *E. coli* are listed in Table 13–5.

The 6,10 ionene bromide is the most active in these series, and at concentrations up to 4 ppm stimulated the growth of normal cells while this range showed inhibition and death of the transformed human cells W138, possibly by electrostatic cytotoxic interaction (Rembaum et al., 1975). It has been suggested that malignant cells are more electronegative than normal cells. If so, malignant cells should demonstrate a greater affinity for the electropositive ionene polymer. This hypothesis has been supported by experimental work. However, this preliminary study appears to warrant more extensive studies to evaluate this class of polymers as chemotherapeutic agents. Rembaum concluded that polyionenes and their low-molecular-weight analogues constitute a unique model system for a molecular probe of the living cell machinery, because their structure, their positive charge densities, their counterions, and their molecular weights can be varied systematically. These considerations apply not only to the study of toxicity or antimicrobial activity, but also to the understanding of the interaction of the polyionenes with DNA or as molecular probes to elucidate the properties of cell membranes.

The high charge density of polyionenes is responsible for their bactericidal and fungicidal activities, for the prolonged duration of the curarizing action, and for the formation of complexes with DNA and heparin. The differences in the biologic properties or stability of the complexes may be explained on the basis of electrostatic association between the negative and the positive moieties of the interacting molecules.

Because the normal and neoplastic cells show an array of different surface properties, including an increase in anodic mobility after transformation, some polyionenes (e.g., 6,10 ionene bromide) may preferentially bind to cancer cells and inactivate them. This differential binding and toxicity to transformed cells conforms with topologic changes of membrane binding sites in the transformed cells. All four products in Table 13–6 are ionenes containing the quaternary nitrogen atom on the backbone of the polymer chain. The main difference is the molecular weight, which is due to reactivity of the alkylating monomer, reaction time, solvent, temperature, and method of preparation. The ionene bromides (MW 65,000) reported by Rembaum, however, are not commercially available, despite their many biologic effects and potential applications, perhaps due to poor yields, incomplete reaction (65 to 75% conversion), long reaction time, and toxicity. The other three products listed in Table 13–6 are items of commerce. The WSCP or Busan 77 from Buckman Laboratories, which is chemically identified as poly[oxyethylene(dimethyliminio)-ethylene (dimethyliminio)-ethylenedichloride] is made by condensation of N,N″ tetramethylethylenediamine with dichloroethyl ether in water to give molecular weight 2000 to 3000. The molecular weight was measured by Gel Permeation Chromatography (GPC) using polyethyleneglycol standard according to Levy and Dubin's reported method (Merianos, 1986).

The manufacturer has provided brochures and data sheets describing minimum inhibitory concentration (MIC of 10 to 50 ppm) levels indicating a broad spectrum of antimicrobial activity, with dual functionality as microbicide and polymer clarifier for swimming pools. The material is registered with the Environmental Protection Agency as a swimming-pool algicide, with 2 ppm of active product used as a maintenance dose, and 5 to 8 ppm of active product used to rid pools of heavy, objectionable algae growth. The presence of WSCP at only 2 ppm can reduce by 80% or more the chlorine needed to kill the bacteria. In general, WSCP enhances the activity of all oxidizers used in the swimming pools. In addition, the material is registered with the Environmental Protection Agency as a cooling water treatment biocide at 20 to 40 ppm of active product. WSCP microbicide formulations can be used to control the growth of algae, bacteria, and fungi on cooling towers and other parts of commercial and industrial recirculating cooling water systems.

Another polymeric quaternary ammonium compound is the product Mirapol A-15 from the Miranol Chemical Company, which is chemically identified as poly[N-3-dimethylammonio)propyl]N-[3-ethyleneoxyethylenedimethylammonio)propyl]urea dichloride] or by the CTFA name Polyquaternium 2. This product is made by reacting a symmetrically substituted urea ditertiary amine with dichloroethylether in water to give MW 2000 to 3000.

The manufacturer, who is primarily concerned with hair care products, reports a static level of 80 ppm and a destruction level of 100 ppm against *Pityrosporum ovale*. Additional examination of this structure demonstrates inhibitory levels at 100 ppm against both *Staphylococcus aureus* and *Escherichia coli*. By a time/kill water treatment screening procedure, the product demonstrates a high-percentage kill of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* at 10, 15, and 20 ppm of active product following a 30-minute contact period. Although unpublished reports by Petrocci have demonstrated antimicrobial activity for this product, attempts to repeat these results with current production of this product failed to confirm biocidal activity. This product

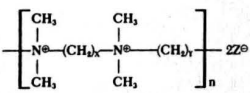
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is not sold as an antimicrobial but as a hair conditioning agent for shampoos.

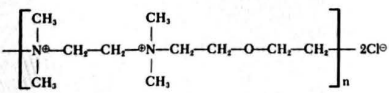
The last structure on Table 13–6 of the polymeric quaternary ammonium compound, from Onyx (Stepan) Chemical Company, is chemically identified as α-4-[1-tris(2-hydroxyethyl)ammonium chloride-2-butenyl] poly[1-dimethyl ammonium chloride-2-butenyl]-W-tris(2-hydroxyethyl)ammonium chloride, or Onamer M or Polyquat or with the CTFA name Polyquaternium 1. The Onamer M is made by reacting 1,4-bis[dimethylamino]-2-butene (0.9 mol), triethanolamine (0.2 mol), and 1,4-dichloro-2-butene (1.0 mol) in water to give an average molecular weight of 5000 to 10,000 (Green et al., 1975, 1977, 1978, 1982; Good et al., 1987). The purpose of the triethanolamine in the preparation was to randomly terminate the polymeric chain, so that we obtained a low-molecular-weight product by design. The 1,4-dichloro-2-butene alkylating agent is very reactive and gave us a 98% conversion of organic chloride to ionic within 6 to 8 hours in water. Polyquaternium 1 was originally intended for hair conditioning applications (Merianos et al., 1977); however, with the good antimicrobial activity combined with excellent toxicologic data, it emerges as an excellent preservative candidate for ophthalmic preparations (Stark, 1985). Today this product is registered with FDA as a preservative for contact lens solutions (Opti-Tears, Opti-Clean, Opti-Soft, Opti-Free, Polyflex Tears-Naturale II—all are trade names of Alcon Labs, Fort Worth, TX 76134). Table 13–7 lists the comparative cytotoxic response of Onamer M at various concentrations and other solutions by in-vitro testing of Mouse L929 cells (Stark, 1985). The most common ophthalmic preservatives are thimerosal, benzalkonium chloride, and chlorhexidine. These compounds are toxic to the eye and may cause corneal erosion and corneal ulceration resulting in pain. This problem is particularly severe with quaternary ammonium compounds that are concentrated more than 400 times by hydrophilic lenses. Chlorhexidine is concentrated as much as 100-fold by hydrophilic contact lenses, which results in the potential for injury to the eye. The comparative cellular toxicity of soft contact lenses soaked in Onamer M at various concentrations and other solutions was determined by in-vitro testing. Mouse L929 cells were grown

Table 13–6. *Polymeric Polyquaternary Ammonium Compounds*

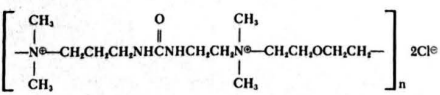
A. Ionenes A. Rembaum Applied Polymer Symposium No. 22 299-317 (1973)



B. Poly[oxyethylene(dimethyliminio)ethylene(dimethyliminio)ethylene dichloride] (WSCP or Busan 77)

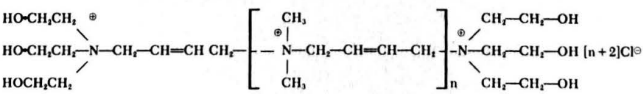


C. Polyquaternium 2 CTFA-adapted name (Mirapol-A15)



n = 6 (average)

D. Polyquaternium 1 CTFA-adapted name (Onamer M)



plus triethanolamine hydrochloride

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Table 13–7. *Comparative Cytotoxicity of Soft Contact Lenses Soaked in Preserved Solutions on Mouse L929 Cells*

Lens Soaked	Cytotoxic Response: Cell Lysis	Cytotoxic Response: Zone of Cell Death (mm)	Cytotoxicity Conclusion†
Saline	–	0	None
0.001% Onamer M	–	0	None
0.01% Onamer M	–	0	None
0.01% Onamer M	–	0	None
0.01% Onamer M	–	0	None
0.1% Onamer M	–	0	None
0.3% Onamer M	–	0	None
1.0% Onamer M	+	31	Moderate
Alkyltriethanol ammonium*			
Chloride [0.03%] + thimerosal [0.002%]*	+	16	Minimal
Chlorhexidine [0.005%] + thimerosal [0.001%]*	+	25	Moderate
Thimerosal [0.001%]*	+	10	Minimal
Sorbic acid [0.1%]*	+	16	Minimal
0.01% Benzalkonium chloride	+	48	Severe
0.01% Benzalkonium chloride	+	64	Severe

*Commercially available marketed solutions.

†Cytotoxicity was rated as follows: Minimal zone of decoloration was 20 mm; Moderate zone of decoloration was 20–40 mm; Severe zone of decoloration was 40 mm.

on a basal salts medium using standard tissue culture techniques. The cells were grown until confluent growth was obtained. HEMA soft contact lenses were cycled through seven 8-hour cycles of fresh solution prior to exposure to the mouse cells. After soaking, the lenses were rinsed in water. The mouse cells were then exposed to each respective lens for 24 hours, whereupon the cell growth was examined microscopically and with staining procedures. The cytotoxic response cell lysis and zone of cell death was reported as in Table 13–7. The results from Table 13–7 conclusively show that Onamer M is not cytotoxic to Mouse L929 cells. This cytotoxic response may be related to the acute oral LD_{50} of these compounds listed on Table 13–8. Additional work is needed to prove if there is a correlation of cytotoxicity and acute oral LD_{50} of the biocides within the same class or different ones. This seems to be the case, if one compares Onamer M with the benzalkonium chloride and the other products. All are more toxic than Onamer M, as can be seen from LD_{50} reported in the literature. Petrocci et al. in 1979 examined this compound and determined that the product was bacteriostatic against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus faecalis* at 50 ppm of active product.

Table 13–8. *Acute Oral LD_{50}: Commercial Antimicrobial Quaternary Ammonium Compounds*

Compounds	LD_{50}
1. Cetylpyridinium chloride	0.20 g/kg
2. Benzalkonium chloride	0.30 g/kg
3. Domiphen bromide	0.32 g/kg
4. Methylbenzethonium chloride	0.35 g/kg
5. Benzethonium chloride	0.42 g/kg
6. Didecyldimethylammonium chloride	0.53 g/kg
7. Octyldodecyldimethylammonium chloride	0.72 g/kg
8. BTC 2125M	0.75 g/kg
9. Dowicil 200	2.20 g/kg
10. 6,10-ionene bromide	1.00 g/kg
11. WSCP or Bussan 77	2.77 g/kg
12. Mirapol A-15 (not sold as antimicrobial)	2.85 g/kg
13. Onamer M or Polyquat or Polyquaternium-1	4.47 g/kg

Chem. Pharm. Bull., *34*, 4215–4224.

In addition, Onamer-M, when tested by the EPA-required Use-Dilution method, demonstrated efficacy as a hard surface disinfectant at 300 ppm of active product against *Salmonella choleraesuis*, at 350 ppm of active product against *Staphylococcus aureus*, and at 800 ppm of active product against *Pseudomonas aeruginosa*. The product demonstrated excellent activity by a time/test at 10 and 15 ppm of active product against *Pseudomonas aeruginosa* and *Enterobacter aerogenes* following a 30-minute contact period.

At least two characteristics are common to the WSCP and Onamer-M polymeric quaternaries, making them uniquely different from the ordinary biocidal quaternary ammonium compound. One is the absence of foaming, even at high aqueous concentrations. The other is the remarkably low toxicity shared by these polymeric quaternary products. As an example, for Onamer-M at 30% active material, primary abraded and intact skin irritation scores were zero for all observation periods. Draize eye irritation studies recorded a mild conjunctival irritation with scores of 2 or 4 in each rabbit, which cleared on the second day of observation. The acute oral LD_{50} in rats was determined to be 4470 mg/kg. The product was described as nonmutagenic by the mouse lymphoma forward mutation assay and by the sex-linked recessive lethal test in *Drosphila melanogaster*. In addition, it was not considered carcinogenic by the in-vitro transformation of Balb/3T3 cells assay.

For reasons of structure, size, foaming, and toxicity, these polymeric quaternary compounds must be consid-

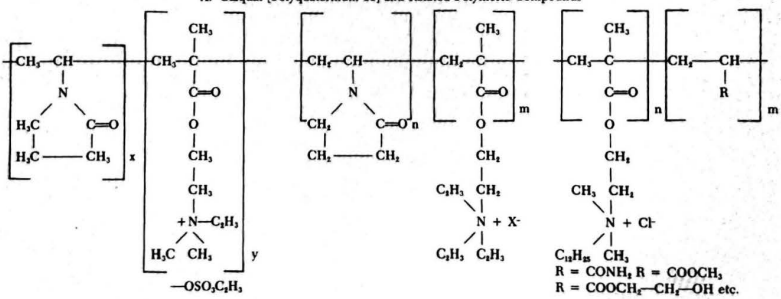
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ered uniquely different from the biocidal quaternary ammonium compounds heretofore utilized. The results obtained to date should encourage synthesis within this structure group to produce additional compounds for consideration. Besides the polyionenes quaternary polymers there are other classes of antimicrobial polymers in which the quaternary nitrogen is pendant away from the backbone chain of the polymer (Table 13–9). Samour and coworkers (1978) reported a number of quaternary monomers, homopolymers, and copolymers of N-vinylpyrrolidone and 2-methacryloxyethyl-N,N,N-triethyl ammonium bromide and iodide with antimicrobial activity. This activity increases as the content of quaternary ammonium moiety increases. The quaternary nitrogen is pendant away from backbone chain and requires the proper lipophilic groups and proper charge density of the quaternary nitrogen for biocidal activity. In Table 13–9 are listed the most common polymers made by free radical polymerization. None of these polymers is used as an antimicrobial, but they are commercially available as hair conditioning agents for shampoos and other cosmetic products. Examples are Gafquats (Polyquaternium 11) and Merquats (Polyquaternium 7). These products are made by the free radical polymerization of diallyl quaternary monomer, and their molecular weight is over 100,000 (Butler et al., 1966—U.S. Patent No. 3,288,770).

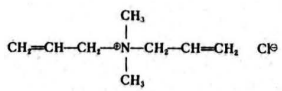
However, more recently Andrews et al. (1981—U.S. Patent No. 4,304,894) reported related Terpolymer, with molecular weight 10,000 to 20,000, to have surprisingly effective sterilizing activity against *Candida albicans* at 0.1%, when it was used as a preservative for soft contact lenses without accumulation into the lenses.

Table 13–9. *Free Radical Polymeric Quaternary Ammonium Compounds*

A. Gafquat [Polyquaternium 11] and Related Polymeric Compounds



B. Merquat (Polyquaternium 7)



Diallyl Dimethyl Ammonium Chloride



Poly(Dimethyl Diallyl Ammonium Chloride) (DMDAAC)

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Ikeda and Tazuke (1983) reported that antimicrobial activities (MIC) of poly[trialkyl(vinylbenzyl)ammonium chloride] type of polycations against bacteria and fungi were more active than the corresponding monomers; however, the absolute activity is low compared to commercially used quaternaries. The antibacterial assessment was based only on the conventional spread plate method, which has been widely used to evaluate the antibacterial activity of ordinary antibiotics and disinfectants. However, the antibacterial activity of the polycations can not be determined precisely by this method on account of the adsorption of the polycations by some constituents in the culture media. The activity must be evaluated in the absence of any interfering material. More recently, Ikeda and Tazuke (1984, 1985) obtained peculiar results for poly[dodecyldimethyl{vinylbenzyl}ammonium chloride] and its monomer using the conventional spread plate method for evaluation. The MIC of the monomer was 10 to 100 ppm against *Escherichia coli*, *Aerobacter aerogenes* and *Pseudomonas aeruginosa*, whereas the MIC of the polymer product was greater than 1000 ppm against the same organisms.

The poly[dodecyldimethyl{vinylbenzyl}ammonium chloride interacted with the negatively charged species (such as sodium caseinate of the agar plate) and produced an insoluble complex, leading to inactivation of the polymer. In order to evaluate the antibacterial activity of such disinfectants correctly, it is preferred that the products come in contact with bacterial cells in sterile water or saline, and then that the surviving cells be counted after being cultivated on agar plates. Using this viable counting method the antibacterial activity of poly-[dodecyldimethyl{vinylbenzyl}ammonium chloride] was determined against *Staphylococcus aureus*, and at a concentration of 0.5 ppm all the bacterial cells were killed within 30 minutes of contact, whereas the corresponding monomer was inactive. Compounds with the longest alkyl chain studied (dodecyl) were found to exhibit high activity, and this was ascribed to the contribution of the increased hydrophobicity of the compounds to the activity.

In conclusion, the most significant finding was that the polymers are more active than the corresponding monomers, particularly against gram-positive bacteria. The higher activity of the polymers was interpreted as due to favored adsorption onto the bacterial cell surface and the cytoplasmic membrane with subsequent disruption of its integrity, although they have the disadvantage of diffusing through the cell wall, especially with the gram-negative bacteria.

Sheldon et al. (1985—U.S. Patent No. 4,532,128) reported that polymeric quaternary ammonium compounds having repeat vinylbenzylammonium units were antimicrobial and particularly useful for preserving ophthalmic solutions.

Ikeda and Tazuke in 1985 reported an optimal MW region of 14,300 for the 6,6-ionene bromide with antibacterial activity minimum bactericidal concentrations (MBC) of 6.6 ppm to 10 ppm against *Staphylococcus aureus*. The same workers reported that polymeric biguanides of MW 11,900 and poly{alkyl[C_8–C_{12}]-dimethyl[vinylbenzyl]ammonium chloride} of similar MW exhibit better bactericidal action against gram-positive than gram-negative bacteria. The poly{dodecyldimethyl[vinylbenzyl]ammonium chloride} was the most active with 0.5 ppm against *Staphylococcus aureus*, which suggests that hydrophobicity plays an important role in bactericidal action.

In conclusion, in all three types of polymers reported by Ikeda and Tazuke (1985), there is a clear MW dependence of the biocidal activity—that is, polymers with low MW as well as high MW exhibit lower bactericidal activity—and there exists an optimal MW region for the biocidal action. This MW dependence was confirmed in my work with Onamer M analogues (Merianos, 1986). It is my belief that a fine balance exists in the polymeric biocides between MW, biocidal activity (cytotoxicity), and (toxicity) acute oral LD_{50} as seen in Tables 13–8 and 13–7.

ANALYSIS

The chemical structure of quaternary ammonium compounds permits a variety of quantitative procedures to determine their concentration. Recently various analytical methods have been reported for the determination of benzalkonium chlorides by high-performance liquid chromatography (HPLC) (Marsh et al., 1983; Sato et al., 1984) and gas chromatography (GC) (Suzuki et al., 1989; Cybulski, 1984; Ng et al., 1986). Chemical ionization mass spectroscopy (MS) has been used to identify and determine the proportions of various alkyl chain lengths in commercial mixtures of benzalkonium chlorides (Dauod et al., 1983). The diphasic or the dye transfer method or the Epton method (1947) cannot be used for the determination of polymeric quaternary ammonium compounds. Recently, two methods for determination of low concentration of Onamer-M or polyquaternium 1 antimicrobial preservative in ophthalmic solutions (Good et al., 1987; Stevens and Eckardt, 1987) were reported. A direct titration technique for the accurate, quantitative determination of cationic and anionic polyelectrolytes has been reported by Wang and Shuster (1975). The technique is based on a direct neutralization reaction between cationic and anionic forms of the polymers. Modification of poly[vinylsulfuric acid] potassium salt (PVSAK) method has been used for determination of high concentration of Onamer-M (Merianos et al., 1977). The cationic polyelectrolytes show a light blue color in the presence of toluidine blue O dye, and the blue color turns to bluish purple when the titration end point is reached. With the many methods available, the method of choice is usually dictated by the level of quaternary ammonium compound anticipated in the sample. For solutions estimated to contain 0.5% or more quaternary ammonium compound, the chemist may employ a diphasic or direct titration procedure.

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STRUCTURE-ACTIVITY RELATIONSHIPS

Jacobs and Heidelberger in 1916 were the first to report correlation between chemical structure and antimicrobial activity of benzyl and substituted benzyl hexamethylenetetrammonium salts. The results obtained with some 40 substances upon *Bacillus typhosus* demonstrated the existence of direct relationships between chemical constitution and bactericidal action within the series. The degree of the bactericidal action, however, is determined by the position, character, and number of groups substituted in the benzene nucleus. By introduction of the methyl, chlorine, bromine, iodine, cyano, and nitro groups into the benzene nucleus of the parent benzylhexamethylenetetrammonium salt, the bactericidal power of this compound was notably enhanced. The substitution of these groups in the ortho position almost invariably resulted in substances that were more active than their meta or para isomers; the introduction of the methoxy group was without marked effect.

Several substances in which two hexamethylenetetrammonium groups on the same aromatic nucleus (namely bis-quats 1,2-xylylenebis[hexamethylenetetrammonium chloride and mesitylenebis[hexamethylenetetrammonium chloride]) were found to be the most active of the substances of this series when tested against *Bacillus typhosus*. Comparative tests with other bacterial types demonstrated that these compounds possessed a marked degree of specificity for *Bacillus typhosus*. The bactericidal character is directly attributable to the presence of the hexamethylenetetramine nucleus.

The same general principle of increased antimicrobial activity was observed with benzalkonium and substituted benzalkonium chlorides, as illustrated in Table 13–10. The AOAC germicidal and detergent sanitizers test was used, and the hard water tolerance in ppm as $CaCO_3$ was measured. The test organism was *Escherichia coli* ATCC No. 11229; a 99.999% reduction in 30 seconds is required with quaternary level of 200 ppm to pass.

A synergistic blend of alkyl[C_{12}/C_{14} 70/30] 2,4,6-trimethylbenzyldimethyl ammonium chloride [C] has the highest hard water tolerance (HWT) of 1300 ppm, with the 2,4,5 isomer [D] having the next best HWT of 1200 ppm, which is twice as high as the corresponding benzalkonium chloride [A]. The mesitylenyl and pseudocuminyl quaternary compounds are highly crystalline nonhygroscopic materials with excellent microbiocidal properties; however, economics favors alkyl[C_{12}/C_{14} 70/30] ethyl[15% ortho, 85% para]benzyldimethylammonium chloride[B]. The HWT of the alkyl[C_{12} and C_{14}] ethylbenzyldimethylammonium chloride is reported in U.S. Patent Nos. 3,472,939 and 3,525,793.

Synergistic blends of microbiocidal quaternary ammonium compounds with dodecyl to tetradecyl proportion 85/15 to 55/45 are listed in Table 13–11.

Kourai and coworkers (1985) have reported on quantitative structure-activity relationships of antimicrobial N-laurylpyridinium iodides (Table 13–12). The antimicrobial activities of N-laurylpyridinium iodides (19 compounds) having various substituents (methyl, ethyl, propyl, amino, carboxyl, and carbomoyl groups) on the pyridine nuclei were studied against *Escherichia coli* K12, *Bacillus subtilis var. niger*, *Aspergillus niger*, and *Candida utilis* with respect to their chemical structures. The N-lauryl-2,4,6-trimethylpyridinium iodide had the highest antimicrobial activity, whereas N-lauryl-2-carboxylpyridinium iodide had the lowest activity.

In general it can be seen from Table 13–12 that electron-releasing groups on the pyridine nucleus such as amino or methyl group markedly enhanced the activities, and the electron-attracting groups such as carboxyl or carbamoyl group highly reduced them. There was a clearly linear relationship between the antimicrobial activities and the acidic dissociation constant (pK_a) for the corresponding pyridines, and the same relationships exist between the pK_a and both bacteriolytic and bactericidal activities.

In conclusion the findings obtained suggest that the antimicrobial activities of the N-laurylpyridinium iodides are linearly dependent on the electron density of the ammonium moiety. It needs to be mentioned here, however, that the authors did not report on the stability of the iodide salts. It is well known that iodides under acidic conditions in the presence of oxygen and light can be oxidized to iodine, which is a well known biocide (Merianos, 1988). The only commercially used pyridinium quaternary is cetylpyridinium chloride, as the active ingredient in mouthwash (Cepacol), and in preservatives in pharmaceutical preparations. Economic considerations have prevented the commercialization of the substituted N-laurylpyridinium iodide quaternaries.

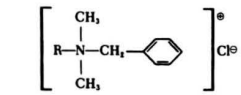
Kourai and coworkers (1989) reported on the relationship between hydrophobicity of bacterial cell surface and drug susceptibility to alkylpyridinium iodides. The value of antimicrobial activity (log 1/MIC) of quaternary iodides having long alkyl chains (hexyl, octyl, decyl, dodecyl, tetradecyl, hexadecyl, octadecyl) against each bacterium was regarded as the drug susceptibility. The partition coefficient of bacterial cells between n-hexadecane and physiologic saline was regarded as representing their hydrophobicity of the cell surface.

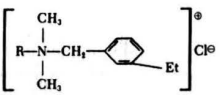
The logarithm of the coefficient was employed as a measure of the hydrophobicity at 37°C. The hydrophobicity of the gram-negative bacteria was always higher than that of the gram-positive bacteria. The susceptibility of the bacteria to each of the alkylpyridinium iodides was analyzed by use of the hydrophobicity, and a quantitative relationship between the susceptibility and their hydrophobicity was found. These findings eventually lead to a conclusion that the first step of the antimicrobial action of the iodides is a hydrophobic interaction between the cell surface and the iodide, and that the magnitude of the drug-susceptibility of the bacteria depends upon the hydrophobicity of the cell surface. Daoud et al. (1983) investigated the relationships between physicochemical properties and the antimicrobial activity of a homologous series of alkyldimethylbenzyl ammonium chlorides

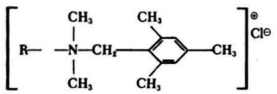
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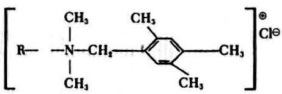
Table 13–10. *AOAC Germicidal and Detergent Sanitizers Test Method* Escherichia Coli *ATCC No. 11229 200 ppm Active Quat to Give 99.999% Reduction of* E. Coli *in 30 Seconds*

	Hard Water Tolerance (ppm) $CaCO_3$			
Alkyl	*A*	*B*	*C*	*D*
R_{12}	100	400	900	1250
R_{14}	700	700	1000	700
R_{16}	400	400	600	500
R_{18}	100	300	550	350
$R_{12\text{-}70}$ $_{14\ (30)}$	650	1000	1300	1200

(A) 

(B) 

(C) 

(D) 

U.S. Patents 3,525,793, 3,472,939

against a variety of microorganisms, and considered whether benzalkonium chloride mixtures could be improved as antimicrobial agents through a more rational choice of alkyl chain length composition. The minimum growth inhibitory concentrations (MIC) of various members of the homologous series of alkyldimethylbenzyl ammonium chlorides were determined against 12 strains of microorganisms, representative of gram-positive and gram-negative bacteria, yeast, and fungi.

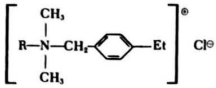
The critical micelle concentrations and octanol-water partition coefficient (P) were measured for alkyldimethylbenzyl ammonium chlorides. The partition coefficients were calculated as the ratio of the concentration in the aqueous and oil (alcohol) phases expressed as the means of the replicate determination. Physicochemical data such as log P and log 1/MIC were subjected to multiple regression analysis to produce quadratic equations of the general form:

$$\text{Log } 1/\text{MIC} = a + b \log P + C [\log P]^2$$

The log P values were plotted against biologic activity for a series of compounds, and a parabolic relationship was obtained. Log P_o would in this instance be the value of log P for compounds with optimal biologic activity. This value of log P_o will vary between different types of organisms. Many such parabolic relationships have been demonstrated, including several for quaternary ammonium compounds (Hansch and Clayton, 1973; Hansch and Fujita, 1964). The levels of antimicrobial activity were observed to be parabolically related to the alkyl chain length, and thereby to log P. The chain lengths with optimal activity varied from organism to organism, reflecting differences in their cell-wall structures. The lower chain lengths [C_{10} to C_{12}] were more active against yeast and fungi, whereas gram-negative organisms were most susceptible towards the more lipophilic C_{16} compounds. This was probably a consequence of the lipophilic nature of the gram-negative cell wall and of the difficulties often encountered by hydrophilic molecules to traversing it. As result gram-negative microorganisms were the least sensitive towards all of the homologues, MIC values being approximately 10 times greater than those towards the gram-positive organisms and the fungi. The possibility of dual binding sites has experimental support from the observations of Salt and Wiseman (1968) working with cetyltrimethyl ammonium bromide, which binds in two stages, representing high- and low-affinity binding sites for the drug. Such a mechanism implies potential synergy in combination of benzalkonium chlorides and might influence the choice of alkyl chain components for preservative mixture. Hansch and Fujita (1964) and Lien et al. (1968) reported that the differences in the biologic activity of chemically related antimicrobials, acting at similar sites within the cell, would reflect their relative ease of penetration through the various liquid barriers of the cells and their ability to react at that site. The outer components of the cells were considered as a series of aqueous and lipophilic layers. Sub-

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Table 13–11. *AOAC Germicidal and Detergent Sanitizers Test* Escherichia Coli *ATCC No. 11229 200 PPM Active Quat to Give 99.999% Reduction of* E. Coli *in 30 Seconds*



U.S. Patent 3,525,793

R		Hard Water Tolerance PPM	
C_{12}	C_{14}	Calculated	Determined
100	0	400	400
95	5	415	500
90	10	430	750
85	15	445	1000
82.5	17.5	453	1050
80	20	460	1110
77.5	22.5	468	1000
75	25	475	1050
70	30	490	1000
65	35	505	1000
60	40	520	900
55	45	535	1000
50	50	550	900
45	55	565	1000
40	60	580	900
35	65	595	800
30	70	610	750
25	75	625	800
20	80	640	800
10	90	670	800
5	95	685	600
0	100	700	700

stances of low water solubility would be unable to penetrate these aqueous layers and would accumulate within the lipid regions; similarly, those with low oil solubility would be unable to cross the lipophilic barriers. Compounds between these two extremes must exist that possess the optimum balance between hydrophilicity and lipophilicity for traversing the cell barriers.

In summary, antimicrobial activity of the compounds was found to be a parabolic function of their lipophilicity and maximized with n-alkyl chain length of between C_{12} and C_{16}. Generally yeast and fungi were most sensitive towards C_{12}, gram-positive bacteria towards C_{14}, and gram-negative bacteria towards C_{16}. The gram-negative cells were the most resistant towards all the compounds, and gram-positive cells the least.

Tomlinson and coworkers in 1977 reported on the effect of colloidal association on the measured activity of alkyldimethyl benzylammonium chlorides (ADBAC) homologous series C_8 to C_{18} against *Pseudomonas aeruginosa* (ATCC 9027). The critical micelle concentrations (CMC) is an important factor in the study of antimicrobial activity of cationics; at high concentrations of ADBAC, formation of a micellar state of the salt is interfering with the cationics' antibacterial action. Tomlinson et al. (1977) questioned the normally accepted parabolic relationship reported to exist between the lipophilicity of many quaternary ammonium salts and their antibacterial activities. The high concentration of nutrient salts, which is used in screening, would alter the concentration of monomeric quaternary ammonium salt. This may be responsible for some of the parabolic relationships, rather than the intrinsic antibacterial activity of the compounds. As early as 1953 it was reported that for the homologous series of cationic surface active agents, a parabolic relationship could exist between their antibacterial properties and their hydrophobic character. Thus, there is a linear relationship between activity and alkyl chain length with increased carbon number up to a maximum of between C_{12} and C_{14}, at which region there is observed a decrease in activity. In Table 13–3, the work of Cutler et al. (1967) showed that with broth dilution tests a peak activity against various microorganisms could be observed at an alkyl chain length of 14 carbon atoms. The turndown in activity is probably related to more than one physical property of the compounds: (a) The longer the chain the greater the tendency for the molecule to be adsorbed at the surface of a bacterium; (b) There is a reduction of aqueous solubility of the molecule as the carbon number is increased. These workers failed to discuss the relevance of micellization to the thermodynamic state of the ADBAC molecule in the test system. Salt additives are known to increase the tendency of ionic surface active agents to micellize in water. In some instances the micellar molecular weights can increase, indicating a change in micellar type.

In conclusion, Tomlinson et al. (1977) have measured the antibacterial activities of ADBAC homologous by the minimum inhibitory concentration procedure and by a sterilization kinetics test carried out in deionized water. There was a log-linear relationship between activity measured by kinetics test and carbon number. With the MIC method there was a log-linear relationship up to C_{14}, then there was a turndown in activity. So consideration of the colloidal association of ADBAC in deionized water and in a simple salts growth media led these authors 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.

Jono and coworkers (1986) investigated the effect of alkyl chain length of benzalkonium chloride (BAC) on the bactericidal activity and binding to organic materials during a short contact time (10 s, 30 s, 1 min, 10 min) by counting survivors. Table 13–13 lists the results. The effect of human serum and dried yeast on the bactericidal activity of ADBAC standard blend OSN Alkyl[C_{12}—59

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Table 13–12. *Minimum Inhibitory Concentration of N-Laurylpyridinium Iodides Against* Escherichia Coli *KI2*, Bacillus Subtilis *Var.* Niger, Candida Utilis, *and* Aspergillus Niger

No.	2	3	4	5	6	Abbreviation	MICs(M)* E. coli KI2	B. subtilis var. niger	C. utilis	A. niger
1.	H	H	H	H	H	Py	53	5.6	61	85
2.	CH_3	H	H	H	H	2-Me-Py	21	3.6	26	26
3.	H	CH_3	H	H	H	3-Me-Py	51	4.9	59	82
4.	H	H	CH_3	H	H	4-Me-Py	31	4.6	45	72
5.	CH_3	H	CH_3	H	H	2,4-Me-Py	20	4.0	32	35
6.	CH_3	H	H	H	CH_3	2,6-Me-Py	20	5.0	30	40
7.	H	CH_3	CH_3	H	H	3,4-Me-Py	40	2.7	20	35
8.	H	CH_3	H	CH_3	H	3,5-Me-Py	74	5.5	17	69
9.	CH_3	H	CH_3	H	CH_3	2,4,6-Me-Py	2.6	0.39	0.49	29
10.	H	$CONH_2$	H	H	H	3-$CONH_2$-Py	71	20	210	67
11.	H	H	$CONH_2$	H	H	4-$CONH_2$-Py	84	24	220	77
12.	COOH	H	H	H	H	2-COOH-Py	1100	380	640	460
13.	H	COOH	H	H	H	3-COOH-Py	780	280	430	400
14.	H	H	COOH	H	H	4-COOH-Py	1000	350	700	460
15.	NH_2	H	H	H	H	2-NH_2-PY	44	10	13	23
16.	H	NH_2	H	H	H	3-NH_2-PY	29	1.8	3.8	35
17.	H	H	NH_2	H	H	4-NH_2-PY	10	1.6	7.0	23
18.	C_2H_5	H	H	H	H	2-C_2H_5-Py	45	12	160	130
19.	C_3H_7	H	H	H	H	2-C_3H_7-Py	24	6.3	53	120

*MICs against bacteria and yeast were measured by a Hitachi Recording Incubator, and MICs against mold by the test-tube dilution method.

to 63%, C_{14}—29 to 34%, C_{16}—6.8 to 7.2%]dimethylbenzyl ammonium chloride and pure C_{12}BAC, C_{14}BAC, C_{16}BAC was compared against 13 strains of bacteria.

It was concluded that from the practical point of view C_{12} benzalkonium chloride was the most effective component of the homologues series in the presence of organic materials. The bactericidal activity of BAC was inhibited by both dried yeast and human serum. The inhibition by 2.5% dried yeast was stronger than that by 10% human serum. As the carbon chain was increased, the inhibition increased. The C_{12}BAC was bactericidal against *Pseudomonas cepacia, Pseudomonas aeruginosa, Achromobacter guttatis, Alcaligenes faecalis,* and *Serratia marcescens* at 5000 ppm in a suspension of 2.5% dried yeast and at 2500 ppm in a 10% solution human serum at 1 min of contact, but C_{14}BAC and C_{16}BAC could not kill them at 10,000 ppm at 10 min. Many factors might be affected by the alkyl carbon length of BAC, producing changes in the bactericidal activity; for example, (1) aqueous solubility; (2) aqueous critical micelle concentration; (3) lipophilicity; and (4) the cell surface characteristics of the microorganisms used. The C_{12}BAC and C_{14}BAC were effectively bactericidal with all bacteria tested, even at a short contact time (30 s to 1 min). The inhibition of bactericidal activity by organic materials was least for the C_{12}BAC (Table 13–13). To clarify the relationship between inhibition of bactericidal activity of BAC by organic materials and binding of BAC to them, the authors assayed unbound C_{12}BAC and C_{14}BAC in a solution of bovine serum albumin by HPLC. As the carbon chain was lengthened, aqueous solubility decreased.

When the carbon chain is longer than C_{14}, the solubility and the critical micelle concentration of BAC are extremely low. The binding to bovine serum albumin is increased with increase in the carbon chain; the C_{14}BAC bound to bovine serum albumin 2.5 to 3.7 times more than C_{12}BAC did. The bactericidal activity of BAC in a solution of bovine serum albumin depended on the amount of unbound BAC. The results in Table 13–13 show that other factors in human serum also inhibited the activity. Human serum tended to stimulate bactericidal activity against *Staphylococcus aureus* and *Pseudomonas cepacia* when the contact time was short. Human serum may make the surface of some bacteria more sensitive to BAC. These results suggest that C_{12}BAC is the most effective component of the homologous series of BAC in the presence of organic materials. The greater the content of C_{12}BAC in the blends, the more effective the mixture will be as a sanitizer from the practical point of view.

ANTIMICROBIAL ACTIVITY

The assessment of the antimicrobial activity of the quaternary ammonium compounds has, in some recent instances, demonstrated a lack of scientific objectivity. Those statements about antimicrobial quaternary ammonium compound testing that, by the selection of the

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Table 13–13. *Effect of Human Serum and Dried Yeast on Bactericidal Activity of Homologues of the Long-Chain Alkyl Benzalkonium Chlorides*

Organism		Bactericidal Concentration ($\times 10^3$ μg/ml)[a]															
		OSN				C_{12}-BAC				C_{14}-BAC				C_{16}-BAC			
		10s	30s	1 min	10 min	10s	30s	1 min	10 min	10s	30s	1 min	10 min	10s	30s	1 min	10 min
S. aureus	DW	0.5	0.5	0.2	0.05	5	1	0.5	0.1	1	0.4	0.1	0.025	0.4	0.2	0.025	0.01
FDA 209P	HS	1	0.5	0.5	0.5	1	1	0.5	0.5	5	1	0.5	0.5	0.5	0.5	0.5	0.5
	DY	5	5	2.5	2.5	5	5	2.5	2.5	5	5	2.5	2.5	>10	2.5	2.5	2.5
S. epidermidis	DW	0.2	0.1	0.0125	0.006	1	0.4	0.4	0.025	0.8	0.025	0.025	0.006	0.05	0.006	< 0.006	< 0.006
IFO 3762	HS	0.5	0.5	0.5	0.5	2.5	1	0.5	0.5	0.5	0.5	0.5	0.5	2.5	0.5	0.5	0.5
	DY	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	1
P. cepacia	DW	5	1	1	0.4	1	0.8	0.5	0.5	>10	0.5	0.2	0.1	>10	>10	0.2	0.05
ATCC 17774	HS	2.5	0.5	0.5	0.5	2.5	1	0.5	0.5	>10	5	2.5	0.5	>10	>10	>10	2.5
	DY	10	5	5	2.5	5	5	5	2.5	>10	>10	10	5	>10	>10	>10	>10
P. cepacia	DW	5	5	0.2	0.1	5	5	1	0.5	10	5	5	0.2	>10	>10	>10	2.5
H130	HS	5	1	0.5	0.5	5	1	1	0.5	5	5	2.5	1	>10	>10	>10	5
	DY	>10	5	5	2.5	10	5	5	5	>10	>10	>10	10.5	>10	>10	>10	>10
P. aeruginosa	DW	0.05	0.05	0.025	0.01	0.1	0.1	0.05	0.025	0.05	0.5	0.025	0.001	0.1	0.025	0.025	0.01
IFO 13736	HS	2.5	1	0.5	0.5	10	5	0.5	0.5	10	5	2.5	1	>10	>10	5	1
	DY	10	10	5	5	10	5	5	5	>10	>10	10	5	>10	>10	10	5
P. aeruginosa	DW	0.1	0.1	0.025	0.01	0.2	0.1	0.1	0.025	0.2	0.2	0.025	0.01	10	0.1	0.05	0.01
82-2-32	HS	2.5	2.5	0.5	0.5	5	5	2.5	1	10	5	2.5	1	5	5	0.5	0.5
	DY	>10	10	10	10	10	5	5	5	>10	>10	10	10	>10	>10	>10	>10
P. mirabilis	DW	0.2	0.2	0.05	0.05	0.4	0.2	0.01	0.01	0.2	0.05	0.05	0.025	0.1	0.05	0.01	0.01
ATCC 21100	HS	5	2.5	0.5	0.5	5	1	1	0.5	10	2.5	2.5	1	>10	5	5	1
	DY	>10	5	5	2.5	10	5	5	2.5	>10	10	5	5	>10	>10	>10	10
P. mirabilis	DW	5	0.2	0.1	0.01	1	0.4	0.2	0.05	1	0.1	0.05	0.05	>10	0.2	0.05	0.025
82-1-4	HS	5	1	0.5	0.5	5	2.5	1	1	10	2.5	2.5	0.1	>10	>10	2.5	0.5
	DY	>10	5	5	5	10	10	5	5	>10	>10	5	5	>10	>10	>10	10
P. morganii	DW	0.5	0.2	0.1	0.05	0.2	0.2	0.05	0.05	1	0.05	0.01	0.01	1	0.05	0.05	0.025
82-2-11	HS	2.5	1	0.5	0.5	10	5	0.5	0.5	10	5	1	1	>10	>10	5	1
	DY	5	5	5	5	10	5	5	5	10	10	5	5	>10	>10	>10	10
S. marcescens	DW	5	0.4	0.2	0.05	>10	0.4	0.4	0.025	>10	0.4	0.2	0.025	>10	>10	0.025	0.025
82-2-52	HS	5	2.5	1	0.5	10	5	1	0.5	>10	>10	5	2.5	>10	>10	>10	2.5
	DY	>10	>10	5	5	>10	5	5	5	>10	>10	10	5	>10	>10	>10	10
Flavobacterium	DW	0.1	0.05	0.05	0.025	0.2	0.05	0.05	0.025	0.2	0.05	0.025	0.025	0.2	0.1	0.025	0.01
sp. 82-1-98	HS	2.5	0.5	0.5	0.5	5	1	0.5	0.5	5	5	1	0.5	10	5	2.5	0.1
	DY	2.5	2.5	2.5	2.5	5	2.5	2.5	1	10	10	5	1	10	5	5	2.5
A. guttatis	DW	5	5	1	0.2	>10	>10	1	1	>10	>10	10	1	>10	>10	>10	0.5
A-39	HS	5	5	1	0.5	5	5	1	1	>10	>10	>10	>10	>10	>10	>10	>10
	DY	>10	>10	>10	2.5	>10	>10	5	5	>10	>10	>10	>10	>10	>10	>10	>10
A. faecalis	DW	1	1	0.2	0.1	1	0.4	0.2	0.2	>10	0.2	0.2	0.2	>10	>10	>10	0.2
572	HS	10	2.5	1	0.5	10	5	2.5	0.5	>10	10	10	1	>10	>10	>10	10
	DY	>10	>10	10	5	>10	10	5	5	>10	>10	>10	5	>10	>10	>10	>10

[a]Killed from 10^6 to <10 cfu/ml at 25 C. DW, deionized water; HS, 10% human serum; DY, 2.5% dried yeast. OSN, alkyl(59–63% $C_{12}H_{25}$–, 29–34% $C_{14}H_{29}$–, 6.8–7.2% $C_{16}H_{33}$–) dimethylbenzylammonium chloride.

Joyo, K., et al. 1986. Chem. Pharm. Bull., *34*, 4215–4224.

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