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MINIREVIEW

Gemini Alkylammonium Salts as Biodeterioration Inhibitors

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Abstract

To protect materials against biodeterioration, physical, biological or chemical methods can be used. Chemical inhibitors of biodeterioration are the most common and effective. A new class of chemical inhibitors-gemini alkylammonium salts-shows excellent biocidal properties and good ecological profile. These compounds can be applied as biodeterioration inhibitors in a wide variety of materials.

Key words: gemini alkylammonium salts, quaternary ammonium salts, microbial activity in biodeterioration

Introduction

Microorganisms, the first inhabitants of the biosphere, possess the ability to survive and adapt to almost any challenge. This ability must have been laid down in their genomes during their long and successful sojourn on our Earth. In many cases microorganisms are essential for normal metabolic and biotechnology processes. However, they also cause disease and demises. Moreover, the microbial spoilage-biodeterioration – of wood, paper, textiles, paints, stonework, steel, costs many millions of euro each year. To protect hard materials against biodeterioration, biological, physical and chemical methods are used (Allsopp *et al.*, 2004). Physical methods exploit mostly UV or γ radiation, high or low temperature and strong electric or magnetic field. In turn, biological methods use some kind of safe microorganisms like *Bacillus fluorescens* or proteinaceous toxins-bacteriocins-produced by bacteria to inhibit the growth of similar or closely related bacterial strains. The chemical methods are based on microbiocides, *i.e.* chemical compounds with biocidal activity. Microbiocides include some phenols and their derivatives, organic and inorganic halogen compounds, oxidizing substances, quaternary ammonium compounds, alcohols, aldehydes and organic and inorganic acids (Block, 2001; Fraise *et al.*, 2004; Manivannan, 2008; Paulus, 2005; Cross *et al.*, 1994). The most important group of microbiocides are quaternary ammonium compounds (QAC) because of their wide spectrum of biocidal activity, the safety of application and low costs. Quaternary ammonium compounds were introduced as antimicrobial agents by Domagk over seventy years ago (Domagk, 1935). The first generation of QAC was standard benzalkonium chloride, *i.e.* alkylbenzyldimethylammonium chloride, with specific alkyl distribution, namely C_{12}, 40%; C_{14}, 50% and C_{16}, 10% (Fig. 1a) (Block, 2001). The second generation of QAC was obtained by substitution of the aromatic ring in alkylbenzyldimethylammonium chloride by chlorine

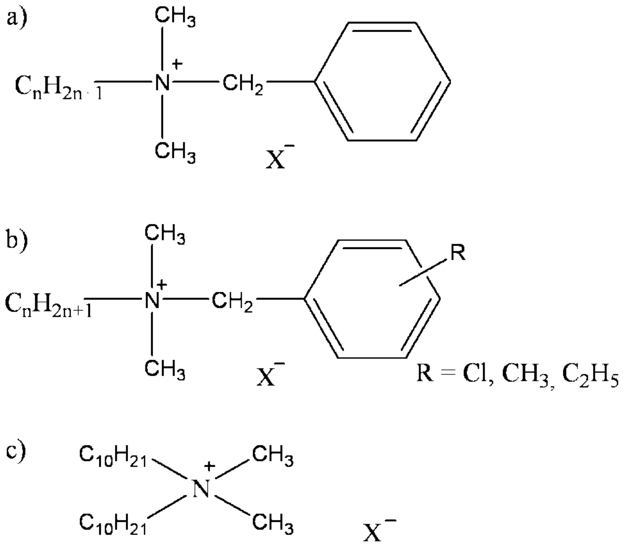

Fig. 1. Structures of quaternary alkylammonium salts (QAC).

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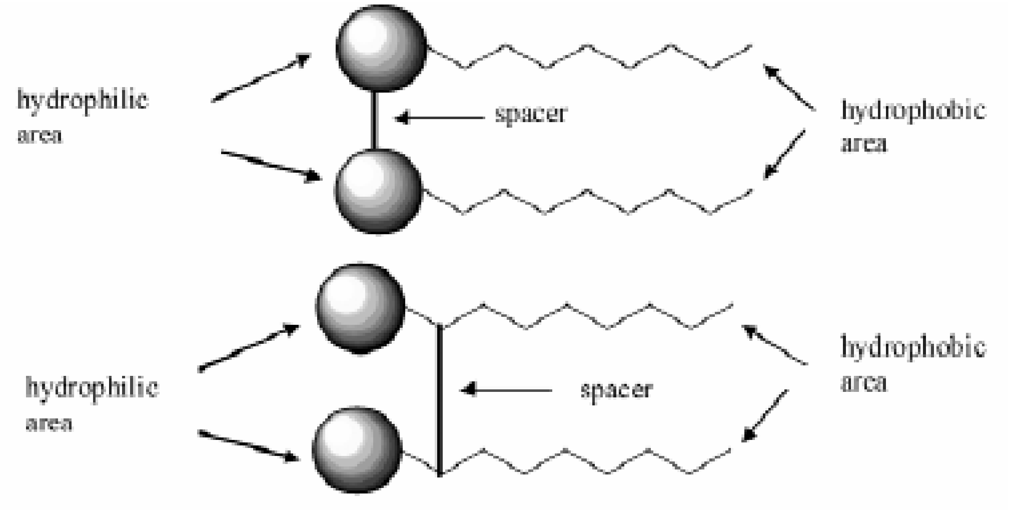


Fig. 2. Schematic representation of gemini alkylammonium salts.

or alkyl group to get a product like alkyldimethylethylbenzylammonium chloride (Fig. 1b) with alkyl distribution C_{12}, 50%; C_{14}, 30%; C_{16}, 17% and C_{18}, 3%. The dual quaternary ammonium salts are the third generation of QAC. This product is a mixture of equal proportions of alkyldimethylbenzylammonium chloride with alkyl distribution C_{12}, 68%; C_{14}, 32% and alkyldimethylethylbenzylammonium chloride with alkyl distribution C_{12}, 50%; C_{14}, 30%; C_{16}, 17% and C_{18}, 3%. The twin chain quaternary ammonium salts, like didecyldimethylammonium chloride, are the fourth generation of QAC (Fig. 1c). The concept of synergistic combination in the dual QAC has been applied to twin chain quaternary ammonium salts. The mixture of dialkyldimethylamoonium chloride (dioctyl, 25%; didecyl, 25%, octyldecyl, 50%) with benzalkonium chloride (C_{12}, 40%; C_{14}, 50%; C_{16}, 10%) is the newest blend of quaternary ammonium salts which represents the fifth generation of QAC's (Block, 2001).

Structures and properties of gemini surfactants. Gemini alkylammonium salts represent a new class of dimeric surfactants made up of two identical or different amphiphilic moieties having the structure of monomeric quaternary alkylammonium salts connected by a spacer group (Zoller, 2009; Menger *et al.*, 2000; Zana *et al.*, 2004) (Fig. 2). This class of quaternary ammonium salts can be considered the sixth generation of QAC.

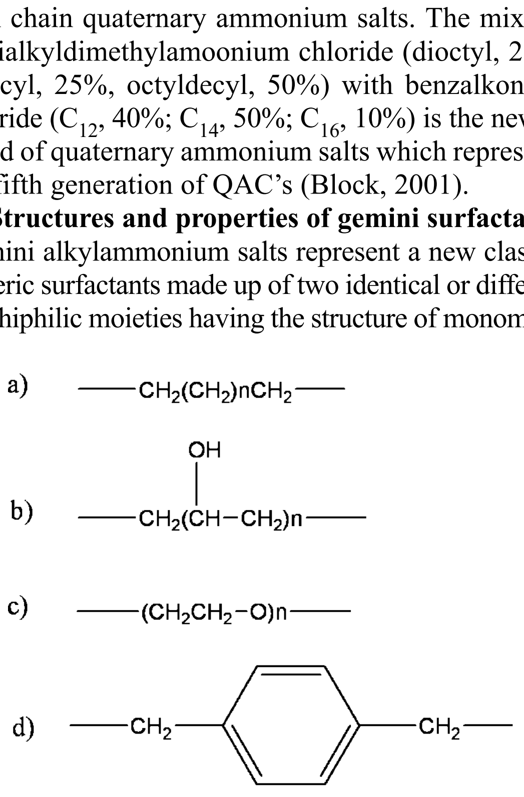


Fig. 3. Types of spacers in gemini surfactants.

The spacer may be hydrophobic (aliphatic or aromatic) (Fig. 3a and 3d) or hydrophilic (polyether, hydroxyalkyl) (Fig. 3b and 3c). It can also be rigid (stilbene) or flexible (polymethylene chain). The length of hydrocarbon spacer chain can vary from two methylene groups up to 20 methylene groups. The spacer group must connect the two amphiphilic moieties at the level of, or in close vicinity to, the head group. The symmetric gemini alkylammonium salts can be depicted as [m-s-m], where m is the number of carbon atoms in the hydrophobic chain and s is the number of methylene groups in the spacer (Fig. 4a).

The gemini alkylammonium salts show unique micelle-forming and surface-adsorbing properties in aqueous solution. Critical micelle concentration (cmc) for gemini surfactants is usually two orders lower than for corresponding monomeric surfactants. For example, the cmc value of dodecyltrimethylammonium bromie (DTAB) (Fig. 4b), which is a typical monomeric cationic surfactant, is 1.5×10^{-2} M, whereas the cmc value for trimethylene-1,3-bis-(*N*,*N*-dimethyl-*N*-dodecyloammonium)bromide [12–3–12] is $9.1\text{–}9.6 \times 10^{-4}$ M (Zana *et al.*, 2004). Critical micelle concentrations are very sensitive to the structure of surfactant. For gemini surfactants of [m-s-m] type cmc values decrease with an increase of spacer length (Table I). Moreover, the thermodynamic data for gemini surfactants, enthalpy (ΔH^o) an free energy (ΔG^o), are much lower then those for monomeric alkylammonium salt (Table I) (Zana, 2004). It clearly indicates that stability of gemini alkylammonium salts is much higher in comparison to monomeric alkylammonium salt, like DTAB. Increases stability of gemini surfactants *vs.* monomeric salts is also observed in the solid state.

Table I
Critical micelle concentrations (cmc) and thermodynamic data for DTAB and [12-s-12] gemini surfactants.

Surfactant	cmc (mM) (25°C)	ΔH°_M (kJ/mol) (25°C)	ΔG°_M (kJ/mol) (25°C)
DTAB*	15	–1.7	–19.1
12–2–12	0.84	–22	–47.3
12–4–12	1.17	–9.3	–45.1
12–6–12	1.03	–8.5	–44.6
12–8–12	0.83	–9.0	–44.2
12–10–12	0.63	–11.6	–45.5
12–12–12	0.37	–12.2	–46.8

* DTAB – dodecyltrimethylammonium bromide

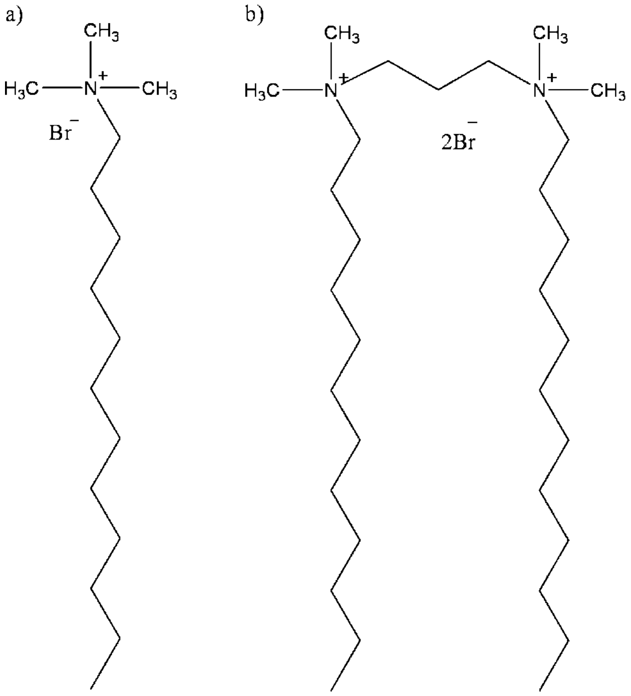


Fig. 4. Structures of trimethylene-1,3-bis-(*N*,*N*-dimethyl-*N*-dodecyloammonium) bromide [12–3–12] (**a**) and trimethyldodecyl ammonium bromide (DTAB) (**b**).

The melting points of ethylene-1,2-bis-(*N*,*N*-dimethyl-*N*-alkyloammonium)iodides [12–2–12] increase with increased length of hydrocarbon chain, what indicates strong hydrophobic interactions between hydrocarbon chains and better packaging in the crystals (Fig. 5) (Brycki *et al.*, 2010). In the contrary, melting points of monomeric alkylammonium salts decrease as the length of hydrocarbon chain increase, this being in accordance with an increase of conformational freedom as hydrocarbon chain become longer. One of the most important parameters of surface activity is the ability to decrease the surface tension of water, what strongly depend on the area of surfactant at the air/water interface (Broze, 1999; Lai, 1999; Holmberg *et al.*, 2003). The area per molecule in a saturated monolayer at the water-air interface, made by gemini surfactant is bigger than that for the corresponding monomeric surfactants. For ethylene-1,2-bis-(*N*,*N*-dimethyl-*N*-dodecyloammonim)bromide [12–2–12] the area is 0.72 nm^2 whereas for DTAB this area is 0.49 nm^2 per molecule (Zana *et al.*, 2004). The efficiency of decreasing of the surface tension of water is often characterized by the concentration C_{20}, *i.e.*, the surfactant concentration required for lowering the surface tension of water by 0.02N/m (Holmberg *et al.*, 2003). For [12–2–12] and DTAB these values are 0.0083 and 0.21 wt.%, respectively (Zana *et al.*, 2004). It means that gemini surfactant [12–2–12] is over 25 times more effective then DTAB to decrease the surface tension of water.

Antimicrobial activity of gemini surfactants. The mechanism of biocidal activity of quaternary ammonium

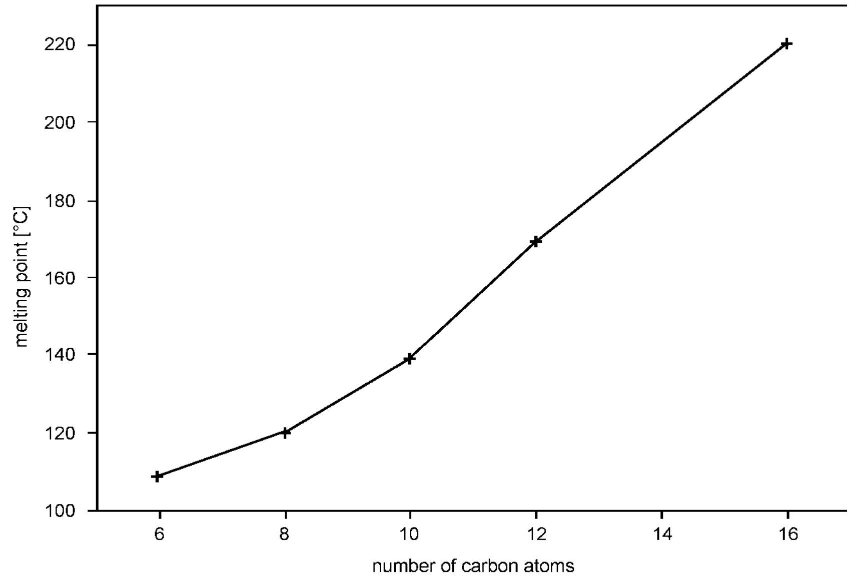


Fig. 5. Relationship of melting points of trimethylene-1,3-bis-[(*N*,*N*-dimethyl-*N*-alkylammonium)iodide] [m-3-m] *vs.* number of carbons in hydrocarbon chain.

Table II
MIC (µg/ml) for gemini alkylammonium surfactants [12-s-12] (Laatiris, 2008).

Microorganisms	MIC (µg/mL)		
	12–2–12	12–3–2	12–4–12
Staphylococcus aureus (ATCC 9144)	6	6	1.5
Pseudomonas aeruginosa (ATCC 27857)	200	200	200
Escherichia coli (ATCC 9637)	50	50	50

Table III
MIC (µg/ml) of geminis (Pérez, 1996).

Microorganisms	$C_4(CA)_2$	$C_2(LA)_2$	$C_3(LA)_2$
Gram-negative			
Alcaligenes faecalis ATCC 8750	64	128	64
Streptococcus faecalis ATCC 1054	4	32	16
Escherichia coli ATCC 1054	128	64	>128
Pseudomonas aeruginosa ATCC 9721	32	128	64
Gram-positive			
Bacillus cereus var. mycoides ATCC 11778	64	64	64
Bacillus subtilis ATCC 6633	64	64	32
Staphylococcus aureus ATCC 2518	4	32	16
Staphylococcus epidermidis ATCC 155–1	4	32	16
Micrococcus luteus ATCC 9341	32	64	64

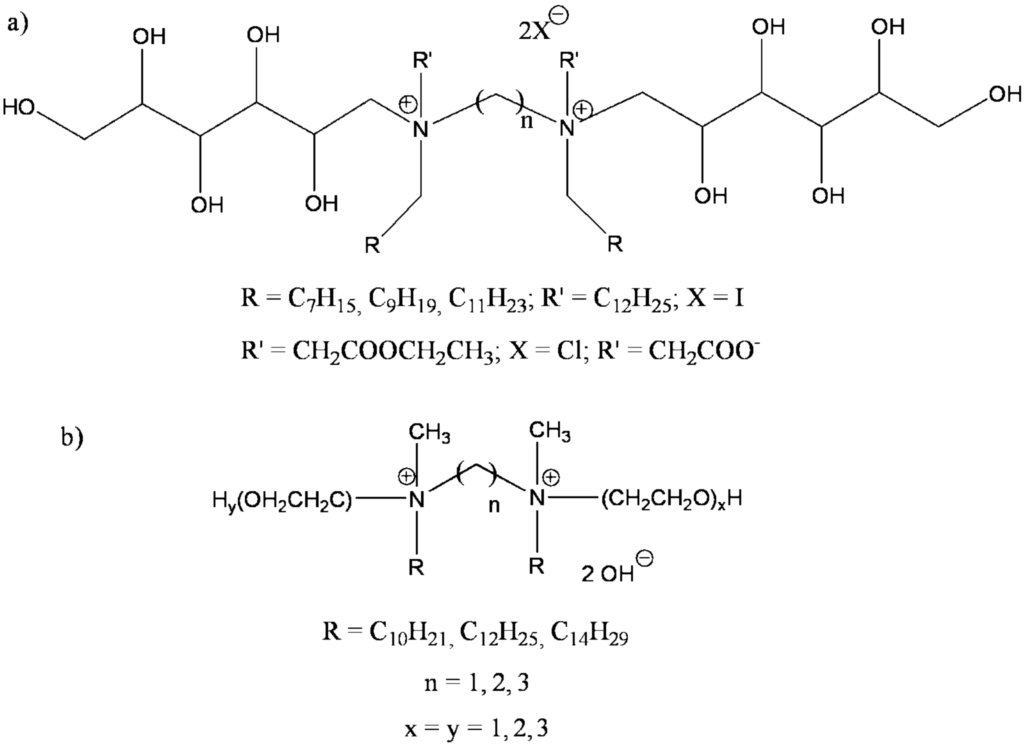
$C_4(CA)_2$: N^{α}, N^{ω}-bis (N^{α}-caprylarginine)-1,2-diaminebutylamide
$C_2(LA)_2$: N^{α}, N^{ω}-bis (N^{α}-laurylarginine)-1,2-diamineethylamide
$C_3(LA)_2$: N^{α}, N^{ω}-bis (N^{α}-laurylarginine)-1,3-diaminepropylamide

salts is based on the adsorption of compound on the bacterial cell surface, diffusion through the cell wall and then binding and disruption of cytoplasmic membrane (Block S., 2001). Damage to the membrane results in the release of potassium ions and other cytoplasmic constituents, precipitation of cell contents and finally the death of the cells. The antibacterial activity (MIC) of quaternary ammonium salts strongly depends on their hydrophilic-lipophilic balance (HLB), according to the equation:

$$\mathrm{Log1/MIC} = a + b\log P + C[\log P]^2$$

where P is an octanol-water partition coefficient, which characterizes HLB of the molecule. The levels of antimicrobial activity are parabolically related to the alkyl chain length, and thereby to logP (Hansch *et al.*, 1973; Hansch *et al.*, 1964). The lower chain lengths, C_{10}-C_{12}, are more active against yeast and fungi, whereas Gram-negative organisms are most susceptible toward the more lipophilic C_{16} compounds. This is probable a consequence of the lipophilic nature of the Gram-negative cell wall and of the difficulties often encountered by hydrophilic molecules to traversing it. The bacterial cell surfaces are usually negatively charged and that adsorption of QAC onto surface is expected to be facilitated by polyammonium cations (Block, 2001). Gemini alkylammonium salts, due to their structures, possess not only double positive charge on two nitrogen atoms but also have higher lipophilic character. Therefore, gemini surfactants in some cases show even hundreds times higher biocidal activity in comparison to monomeric quaternary alkylammonium salts (Zana *et al.*, 2004). This means that the same biocidal effect can be reached using much smaller amounts of biocide, what is of fundamental importance from toxicological and ecological point of view (Zoller, 2004). Symmetrical gemini alkylammonium surfactants [12-s-12] show very good antibacterial activity against both Gram-positive and Gram-negative bacteria (Table II) (Laatiris, 2008). An average minimal inhibitory concentration of [12-s-12] for Gram-positive bacteria is 6 µg/mL and decrease to 1.5 µg/mL for longer spacer. The MIC for Gram-negative bacteria, *Pseudomonas aeruginosa*, is 100 µg/mL. The higher concentration of microbiocide necessary to destroy *Pseudomonas aeruginosa* is a typical feature for almost all kind of microbiocides (Laatiris, 2008).

Applications of new biodeterioration inhibitors depend on several variables. The most important is an antimicrobial efficacy and ecological profile, including biodegradability, bioconcentration and bioaccumulation factors. In addition, biodeterioration inhibitors have to be safe for hard surfaces. From this point of view special interest is focused on gemini alkylammonium salts based on amino acid and sugar derivatives. This group of biodeterioration inhibitors show not only excellent antimicrobial activity against Gram-positive and Gram-negative bacteria but also easily undergoes biodegradation in dilute solutions (Table III) (Pérez, 1996). The efficacy of gemini alkylammonium salts as biodeterioration inhibitors is additionally enhanced by hydrophobisation of surfaces making the settlement of microorganism on this surface difficult. The bigger the gemini surfactant, the higher the degree of hydrophobisation observed. For gemini alkylammonium salts of type [m-s-m], which also prevent corrosion, the most effective is [14–2–14] and then [12–2–12] and [10–2–10] (El Achouri, 2001). The best biocidal activity and effect of hydrophobisation of surface can be reached for gemini surfactants with optimized hydrophilic-lipophilic balance. HLB can be modified by introducing sugar or oxyethylene derivatives to alkylammonium molecules (Fig. 6) These compounds have not only a very good antimicrobial activity, but also can be exploited as micellar hydrosolubilizers for other microbiocides. In case of 2,4,4'-trichloro-2-hydroxydiphenyl ether (triclosan), which is in water practically insoluble, micellar hydrosolubilization can enhanced its solubility over 10 000 times (van Doren *et al.*, 2000; Chiapetta *et al.*, 2008).



$R = C_7H_{15}, C_9H_{19}, C_{11}H_{23}; R' = C_{12}H_{25}; X = I$

$R' = CH_2COOCH_2CH_3; X = Cl; R' = CH_2COO^-$

$R = C_{10}H_{21}, C_{12}H_{25}, C_{14}H_{29}$

$n = 1, 2, 3$

$x = y = 1, 2, 3$

Fig. 6. Polyoxoethylene (a) and glucityl (b) derivatives of gemini alkyl ammonium salts.

Gemini alkylammonium salts are new group of biodeterioration inhibitors. These compounds show excellent antimicrobial activity and possess not only the ability to hydrophobize surfaces but also can act as micellar hydrosolubilizers. The properties of gemini alkylammonium salts make them very competitive compared to other microbiocides.

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