

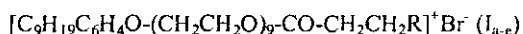
Novel Quaternary Ammonium Surfactants of Potential Biocidal Activity

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FIVE quaternary ammonium compounds were synthesized having the formula :



where I_a: R = triethylamine; I_b: R = triethanolamine; I_c: R = hexamine; I_d: R = N-methyl diethanolamine and I_e: R = pyridine. Critical micelle concentration (CMC), effectiveness (π_{cmc}), efficiency (Pc₂₀) and maximum surface excess (Γ_{max}) of the novel compounds were calculated using equilibrium surface tension profiles at 25°C. The biocidal activity of these compounds were tested on SMC-SRB (stabilized mixed culture of sulphate reducing bacteria isolated from garden soil and enriched using Postgate medium B and E). Minimal inhibitory concentration (mic) for the prepared quaternary ammonium compounds were detected for SMC-SRB using sulphide titrimetric determination method with different concentrations (10-300ppm), using cetyl trimethyl ammonium bromide (CTAB) as a reference. It was found that triethanolamine and N-methyldiethanolamine derivatives showed the highest biocidal effect.

Keywords : Quaternary ammonium compounds, SRB, SMC-SRB, Microbiological induced corrosion (MIC), Biocidal activity, Minimal inhibitory concentration (mic).

Sulphate reducing bacteria (SRB) in water and /or oil systems utilize sulphate mainly as the terminal electron acceptor in the anaerobic oxidation of organic substrates. They produce and accumulate large amounts of sulphide in their natural habitats (Wagner, 1993 and Lee *et al.*, 1995). Chemical control by the use of biocides is probably the most common method of controlling biocorrosion (Brunt, 1987 and Boivin, 1995).

The direct cost of microbiological induced corrosion (MIC) is estimated as \$30-50 billion per year. Also, in the USA industries spend \$1.2 billion annually on biocidal chemicals to fight MIC that, only in the natural gas industry can account for 15 to 30 percent of corrosion related pipeline failures. However, in Egypt the petroleum industries suffers from MIC, it was estimated that one company (Gulf Sues Petroleum Company, GUPCO) spent more than one million \$/year to combat MIC (El-Raghy *et al.*, 1998).

The quaternary ammonium compounds have been in use for many years but have only limited application in the offshore oil business. They are stable over a wide pH range but their surfactant characteristics can lead to be handled but may be toxic to marine life, (Brunt, 1987). The assessment of the application of biocides will be mainly based on their mode of action, which determines the microbiological spectrum.

The hexamine derivative, 1-(3-chloroallyl)-3,5,7-triaza-1-azonia-damantane chloride, has a good spectrum of activity and has been used in the treatment of water systems, (Brunt, 1987). Halogenated biocides, (Keevil *et al.*, 1990; Lechevallier *et al.*, 1990) as well as other types of compounds such as formaldehyde, (Rossmoore, 1988), isothiazolone (Fuller *et al.*, 1985 and Sondossi *et al.*, Collier *et al.*, 1990) and glutaraldehyde, (Eagar *et al.*, 1986) are frequently used.

In several studies, stabilized mixed culture of sulphate reducing bacteria (SRB-SMC) was used as a composed system to determine the actual influence of the biological action of bacteria in industry, (Ghazy, 1997). N-derivatives of quaternary ammonium salts were used as biocides to prevent SRB activity, (Bessemers, 1983).

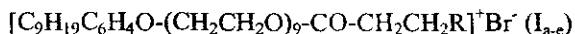
Some quaternary ammonium compounds act as corrosion inhibitor and decreased sulphide production by SRB at low concentration than some biocides of a commercial source. Thus, quaternary ammonium compounds had double purpose, (Ateya *et al.*, 1998). Alkyl substituted amines and quaternary ammonium compounds inhibit many bacteria including *Desulfovibrio desulfuricans* (Davies *et al.*, 1968). Further more, quaternary ammonium compounds were safe to handle, (Lewis, 1991).

In this work, five novel quaternary ammonium compounds (I_{a-e}) were prepared. Then evaluated as surface active materials, their biocidal role was tested on SMC-SRB using cetyltrimethylammonium bromide as a reference.

Material and Experimental

Synthesis of quaternary ammonium compounds

Equimolar amounts of nonyl phenol ethoxylate (EO-9) and bromo-propionic acid were esterified in toluene as a solvent in presence of conc. HCl as a catalyst till the removal of the water of the reaction to give the corresponding nonyl phenol ethoxylate-(EO-9)-bromopropionate. 0.15mole of triethylamine, triethanolamine, hexamine, N-methyldiethanolamine or pyridine was refluxed with 0.1mole of nonyl phenol ethoxylate-(EO-9)-bromopropionate in methyl alcohol while the reaction with hexamine was performed in chloroform. The oily product was extracted from the reaction mixture using diethyl ether in 60% yield, and then the solvent was evaporated over water bath to produce the quaternary ammonium compounds with the following chemical formula:

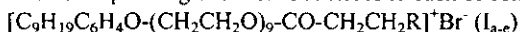


where I_a: R = triethylamine; I_b: R = triethanolamine; I_c: R = hexamine; I_d: R = N-methyl diethanolamine and I_e: R = pyridine. The structures were confirmed via elemental analyses and FTIR (Tables 1, 2).

TABLE 1. Elemental analyses of the prepared quaternary ammonium compounds.

Compound	M.Wt	C%		H%		N%		Br%	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
I _a	852	59.15	59.13	9.15	9.16	1.64	1.63	9.38	9.35
I _b	900	56.00	56.02	8.66	8.68	1.55	1.53	8.88	8.89
I _c	888	56.75	56.73	8.10	8.13	6.30	6.32	9.00	9.03
I _d	870	56.55	56.54	8.73	8.70	1.60	1.61	9.19	9.17
I _e	830	59.27	59.29	8.19	8.16	1.68	1.65	9.63	9.65

The corresponding chemical structures to each of compounds abbreviated:



I_a: R = triethylamine;

I_b: R = triethanolamine;

I_c: R = hexamine;

I_d: R = N-methyl diethanolamine; and

I_e: R = pyridine.

TABLE 2. FTIR spectroscopic analyses of the prepared quaternary ammonium compounds.

Compound	C = O Ester	C - N	N ⁺	C - H	OH
I _a	1725	1350	3050	2872, 2922	--
I _b	1722	1355	Overlap with OH gp.	2931, 2885	3355
I _c	1727	1397	3003	2958, 2870	--
I _d	1724	1390	Overlap with OH gp.	2960, 2885	3375
I _e	1726	1352	3100	2925, 2875	--

For abbreviations see footnote of Table 1.

Surface and interfacial tension

Surface and interfacial tension were measured using Du-Nouy Tensiometer (KRUSS K-6) with platinum ring. Freshly prepared aqueous solutions of quaternary ammonium compounds were measured for different concentrations at 25°C (Harris, 1954 and Findlay, 1963).

Emulsion stability

The emulsifying property was determined by the time required for an aqueous volume separating from the emulsion layer to reach 9ml counting from the moment of the cession of shaking. The sample was shaken 5 times (5sec each), (Negm, 2003).

Foam height

Foam height of a 0.1% solution of the prepared quaternary ammonium compounds I_{a-e} was measured in bidistilled water using Ross-Miles test at 25°C (Negm, 2003).

Measurements of the biocidal activity

Microorganisms

The stabilized mixed culture-sulphate reducing bacteria (SMC-SRB) was isolated from garden soil.

Media

Postgate medium B, (Postgate, 1984) composed of (g/l) KH₂PO₄, 0.5; NH₄Cl, 1.0; CaSO₄, 1.0; MgSO₄.7H₂O, 2.0; sodium lactate, 3.5; yeast extract, 1.0; ascorbic acid, 0.1; thioglycollic acid, 0.1; FeSO₄.7H₂O, 0.5; tap water, 1L. The pH value was adjusted to 7-7.5 by 12N NaOH. This medium always contains a precipitate. It was used for enrichment of SRB.

Postgate medium E, (Postgate, 1984) composed of (g/l): KH₂PO₄, 0.5; NH₄Cl, 1.0; Na₂SO₄, 1.0; CaCl₂.6H₂O, 1.0; MgCl₂.7H₂O, 2.0; sodium lactate, 3.5; yeast extract, 1.0; ascorbic acid, 0.1; thioglycollic acid, 0.1; FeSO₄.7H₂O, 0.5; agar, 15.0; tap water, 1L. The pH value was adjusted at 7.6 by adding 12N NaOH. While, reference biocide was cetyltrimethyl-ammonium bromide (CTAB).

Collection of stabilized mixed culture sulphate reducing bacteria (SMC-SRB)

A garden soil sample was collected at a depth of 20-25cm and stored in sterile container. The soil sample was shaken in sterile distilled water to loosen the soil particles and the soil suspension was allowed to settle for 30min. An appropriate volume was taken from the supernatant and inoculated into sterile bottle contained 35ml of Postgate medium B. the vials were incubated at 35°C for 4days. Blacking meant the presence of SMC-SRB.

Isolation and enrichment of the SMC-SRB

Stabilization of SMC-SRB was prerequisite for reproducibility of the Deep agar method technique using postgate medium E, (Postgate, 1984).

Determination of the minimal inhibitory concentrations (mic) of (CTAB) and the prepared quaternary ammonium compounds I_{a-e}

Minimal inhibitory concentration (mic) of I_{a-e} was determined by monitoring the sulphide production using Postgate medium B at concentration (10-300ppm) as biocide. The incubation was done by adding 1ml of 4days enriched old culture of SMC-SRB into the sterile capped bottles containing the previous concentrations of each biocide on a amended media. Incubation was done at 30°C for 7 days. The sulphide concentrations were determined iodometrically, (APHA, 1975) as total sulfides. Total sulfides including dissolved H₂S and HS⁻ as well acid-soluble metallic sulfides present in suspended matter. On the other hand, the biocidal efficiencies were determined according to the following equation:

$$P = \frac{(I_0 - I)}{I_0} \times 100$$

where: P = Biocidal efficiency, I₀, I = uninhibited and inhibited sulfide formation
 Note, this method was not probed with media without SRB.

Results and Discussion

Surface properties

Critical micelle concentration (CMC), interfacial tension, emulsion stability and foaming power

Figure 1 represents the variation of surface tension against concentration of the synthesized quaternary ammonium salts I_{a-e} at 25°C. It is evident that the increase in concentration of the compound solution decreases the surface tension gradually till certain concentration (CMC) after which the surface tension remains almost constant when the concentration increases.

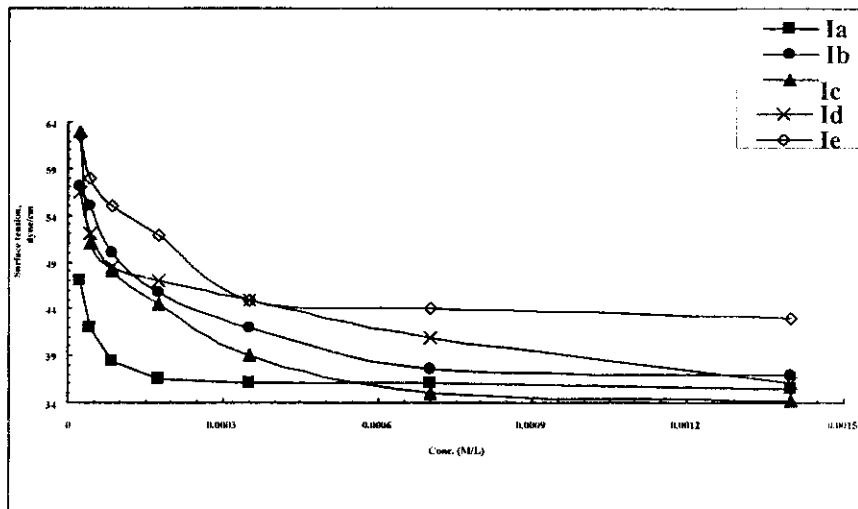


Fig.1. Surface tension vs. concentration of the synthesized cationic derivative.

The interfacial tension values (Table 3) of I_{a-e} surfactant solution/paraffin oil systems showed higher values than that corresponded to the trialkyl-ammonium chloride derivatives, (Rosen, M. J., 1982). The presence of nonionic part within the molecules increased their interfacial tension. That is due to increasing the hydrophilicity of the molecules, then the molecules tend to locate in the bulk of their solutions. Hence, the interfacial tension increased. The hydrophobic moiety in the surfactant molecules has ineffective role on depression of their interfacial tension. That behaviour could also be seen in similar compounds investigated in some recent studies (Ismail, 2001 and Negm, 2003).

The emulsifying power of the synthesized quaternary ammonium surfactants showed different behaviors towards oil/aqueous solution systems, (Table 3). The emulsification powers of these solutions are relatively small. I_a surfactant showed the maximum emulsification efficiency (expressed in seconds) at 210sec. I_d surfactant solution showed the lower one at 32 seconds. It is clear that increasing the polar groups within the surfactants molecules decreases the emulsification power that can be referred to the ability of the polar groups to migrate into the solutions rather than the presence at the interfaces, which decrease their role in the emulsification process. On the other hand, nonpolar groups facilitate the surfactant molecules to be adsorbed at the interface and hence increase their role in the emulsification process.

The synthesized surfactants showed low foaming power, which varied between 15-30ml. While, the foam stability is comparatively lower than the nonionic surfactants.

TABLE 3. Surface properties of the prepared quaternary ammonium compounds I_{a-e} at 25°C .

Compound	Surface tension, Dyne/cm	Interfacial tension Dyne/cm	Emulsion stability, sec.	Foam height, cm
I _a	35.5	10.0	210	3.0
I _b	37.0	13.5	75.0	2.0
I _c	38.0	8.5	115	3.0
I _d	41.0	22.5	32.0	2.0
I _e	43.0	24.0	75.0	1.5

For abbreviations see footnote of Table 1.

Surface parameters

Effectiveness (π_{cmc})

The effectiveness is the difference between the surface tension of the pure water (γ_0) and the surface tension of the surfactant solution (γ) at their critical micelle concentration, and expressed as:

$$\pi_{cmc} = \gamma_0 - \gamma_{cmc}$$

The effectiveness could determine which surfactant is more efficient at its CMC in the same homologous series. The most efficient surfactant is that which gives the highest depression in the surface tension at the critical micelle concentration. It is clear from Table 4 that the triethylammonium bromide derivative and hexammonium bromide derivative (I_{a,e}) owned the highest effectiveness values (33.3, 34.8 dyne/cm), respectively at their CMC (Table 4).

Efficiency (Pc_{20})

The efficiency (Pc_{20}) is determined by the concentration (M/L) of the surfactant solutions capable to superss the surface tension 20dyne/cm. The

values of $P_{C_{20}}$ for the synthesized quaternary ammonium compounds I_{a-e} were found in the range of 0.00001–0.00005M/L indicating the good surface properties of these compounds (Table 4).

Maximum surface excess (Γ_{max})

The values of the maximum surface excess were calculated at 25°C for the prepared surfactant (I_{a-e}) at their CMC according to Gibbs equations:

$$\Gamma_{max} = 1/RT (-\partial\gamma/\partial\log C)$$

where $(-\partial\gamma/\partial\ln C)$ is the surface activity (slope of surface tension versus $-\log C$ plots at constant absolute temperature, T and $R = 8.314\text{mol}^{-1}\text{K}^{-1}$).

Maximum surface excess values (Γ_{max}) increase with increasing the polarity of these quaternary surfactants. Hence, N-methyldiethanol ammonium bromide I_d and triethanol ammonium bromide I_b derivatives had the highest maximum surface excess due to the presence of the hydroxyl groups in their structures rather than the others ($I_{a, c, e}$) at the interface. Hexamine and pyridine derivatives showed the lowest Γ_{max} values due to the symmetry around the nitrogen atom. The higher population of surfactant molecules at air/water interface resulting in high depression in their surface tension, which could explained their higher values of effectiveness (Table 4).

TABLE 4. Surface parameters of the prepared quaternary ammonium compounds I_{a-e} at 25°C.

Compound	CMC, M/L	π_{CMC} dyne/cm	$P_{C_{20}}$	$\Gamma_{max} \times 10^{-10}$ mol/K.cm ²
I_a	0.00010	33.3	0.000013	1.24
I_b	0.000012	26.3	0.00010	2.16
I_c	0.000355	34.8	0.000016	1.26
I_d	0.000081	24.3	0.000032	2.68
I_e	0.000436	27.8	0.000158	1.20

For abbreviations see footnote of Table 1.

Minimal inhibitory concentration (mic)

The reproducible stabilized mixed culture was called SMC-SRB indicating that it contained the sulphate reducing bacteria, in addition to the most biofilm aerobic coexisting bacteria, which caused pitting corrosion of mild steel, (Edyvean *et al.*, 1986). Several investigations showed that, up to date no evidence exists that any one of SRB strains is more destructive than another. On the other hand, the diversity of SRB in any ecosystem makes it difficult to represent them by any single strain or even a single genus, (Tatnal, 1981). The successive enrichment and isolation of separate black colonies gave the SMC of SRB. The results in Table 5 showed that the prepared tested biocides have biocidal activities against of SMC-SRB. The CTAB had the lowest inhibitory effect with mic (30ppm) while the mic of the prepared quaternary ammonium

compounds I_{a-e} were ranged between 10 and 60ppm. Due to the economic losses as well as environmental health and safety hazards caused by the activity of SMC-SRB in many industrial sectors such as the oil and gas industry, it was important to minimize the risks resulting from SRB activity. However, it ought to be emphasize that SRB varies in their susceptibility to biocide, (Postgate, 1984).

TABLE 5. Effect of different concentrations of pure CTAB and I_{a-e} in mg/L on sulphide production of SMC-SRB after 7 days incubation at 30°C .

Biocide concentration, ppm.	CTAB	I_a	I_b	I_c	I_d	I_e
0	114	114	114	114	114	114
10	106	78	24	53	23	49
30	91	21	26	43	16	45
60	40	13	21	13	16	25
90	40	13	21	9.1	16	17
120	40	8	24	6.8	19	13
150	34	10	21	6.9	15	15
200	29	8.1	23	6.8	15	16
250	21	3.2	19	5	2.2	9.1
300	15	4.3	17	3	2.2	7

For abbreviations see footnote of Table 1.

The synthesized quaternary ammonium compounds and CTAB were tested to evaluate their effects on SMC-SRB. Since, sulphide was the final product of sulphate reduction by SMC-SRB, the sulphide produced was taken as an indicator for the inhibition activity of I_{a-e} towards SMC-SRB. Obviously, the synthesized quaternaries I_{a-e} showed a relatively higher inhibiting efficiency towards SMC-SRB rather than the reference (CTAB). The higher biocidal activity of the synthesized compounds could be explained due to their positively charged (N^+) attached to the cell membrane. The efficiency of these compounds increased upon increasing the charge density on the cell membrane, which could be correlated to the number of molecules adsorbed at the interface (water/cell membrane). The maximum surface excess (Γ_{max}) describes that influence; thus increasing (Γ_{max}) increases the biocidal activity efficiency of the tested compounds.

Compounds I_b and I_d showed higher (Γ_{max}) (2.16×10^{-10} and 2.68×10^{-10}), hence, their biocidal inhibiting efficiency reaches the maximum (80-88%) at concentration (10ppm) higher than the reference (CTAB) which reaches 7% at the same concentration. The low maximum surface excess (Γ_{max}) showed low biocidal inhibiting efficiency, which observed in compounds I_a , I_c and I_e . Tables 5 & 6 indicate the results of the minimal inhibiting concentration for the prepared quaternary ammonium compounds I_{a-e} in mg/l and their efficiencies.

TABLE 6. Biocide efficiencies % on sulphide production of SMC-SRB after 7 days incubation at 30°C .

Biocide concentration, ppm.	CTAB	I _a	I _b	I _c	I _d	I _e
0	0	0	0	0	0	0
10	7	32	80	54	88	57
30	20	82	77	62	86	61
60	65	89	82	89	86	78
90	65	89	82	92	86	85
120	65	93	80	94	83	89
150	70	91	80	92	87	87
200	75	93	83	94	87	86
250	82	97	83	96	98	92
300	87	96	85	97	98	94

For abbreviations see footnote of Table 1.

The bactericidal power of the quaternaries is exceptionally high against Gram-positive bacteria, and they are also quite active against Gram-negative bacteria. Varieties of damaging effects of quaternaries upon microorganisms have been observed which include denaturation of proteins. Experimental evidence suggests that the most likely site of the damage to the cell is the cytoplasmic membrane and disturbing its semi-permeable character, (Pelezar and Micheal, 1986).

On conclusion, the synthesized surfactants I_{a-e} showed relatively higher inhibiting efficiency towards SMC-SRB rather than the reference (CTAB).

Moreover, increasing the maximum surface excess increased the biocidal inhibiting efficiency as shown in compounds I_{b,d}.

The biocide efficiencies (%) on sulfide production of SMC-SRB after 7 days incubation at 30°C were arranged in the following arrangement :

$$I_d > I_c > I_a > I_e > I_b$$

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الخواص البيولوجية المثبطة لأنواع مستخدمة من المواد ذات نشاط سطحي محتوية على نيتروجين رباعي التكافؤ

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فى هذا البحث تم تحضير خمسة مركبات محتوية على نيتروجين رباعي التكافؤ وشحنته موجبة باستخدام التراى ايثيل امين والتراى ايثانول امين والهكسامين و ن-ميثيل ثنائى ايثانول الامين والبريدين . تم حساب التركيز الميثيلى الحرج لهذه المركبات وباقى الصفات السطحية لهذه المركبات مثل الكفاءة السطحية والتركيز السطحي للجزيئات والمساحة السطحية لها على المحاليل باستخدام علاقة التوتر السطحي - التركيز عند درجة حرارة ٢٥° م .

وكذلك تم استخدام هذه المركبات لمقاومة مزرعة مختلطة للبكتريا المختزلة للكبريتات والمعزولة والمنشطة من تربة الحديقة باستخدام بيانات كوسط غذائى [Postgate B,E] .

تم تحديد أقل تركيز مثبط من هذه المركبات ضد المزرعة المختلطة للبكتريا المختزلة للكبريتات باستخدام المعايير للكبريتيد بطريقة اليود وذلك من خلال تركيزات مختلفة من ١٠-٣٠٠ جزء فى المليون وهذا من خلال المقارنة بمركب مرجعى هو الستيل تراى ميثيل أمونيوم بروميد.