14 *Egypt. J. Chem*. **55**, No. 2, pp. 201-211(2012)

Novel Cationic Schiff Base Surfactants: Surface Studies and Biocidal Activities against Bacteria Fungi and Sulfate Reducing Bacteria

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NOVEL series of cationic Schiff base surfactants were synthesized and characterized using microelemental analysis, FTIR and ^{1}H-NMR spectra. The properties of the synthesized surfactant solutions such as critical micelle concentration (CMC), effectiveness (Π_{CMC}), surface excess concentration (Γ_{max}), surface area (A_{min}) and free energy of micellization (ΔG_{mic}) were determined using surface tension measurements. These Schiff base surfactants were evaluated for their antibacterial activity against Gram positive and Gram negative bacteria and fungi. The results of the biocidal activities revealed high potent action of the Schiff bases towards bacteria and fungi. The biocidal activity of the tested compounds against sulfur (sulfate) reducing bacteria (SRB) showed promising results in the area of oil field applications.

Keywords: Cationic surfactants, Schiff-base, Micellization, Adsorption, Surface properties, Biocidal activity and Sulfate reducing bacteria.

Cationic surfactants are classes of compounds having one or more hydrophobic group attached to a positively charged atom[(1)]. Cationic surfactants application are in cosmetics, cleaners and bactericides, oil recovery and micro- and meso-porous structures (zeolites) applications[(2-4)]. Schiff bases are considered good antibacterial compounds due to the easily preparing procedures and their ability to attach to several function groups. Schiff bases have been studied extensively because of their high potential chemical permutation. Antibacterial, antifungal[(5-7)], antitumour, anticancer, and antiviral activities[(8-10)] of Schiff bases have been reported.

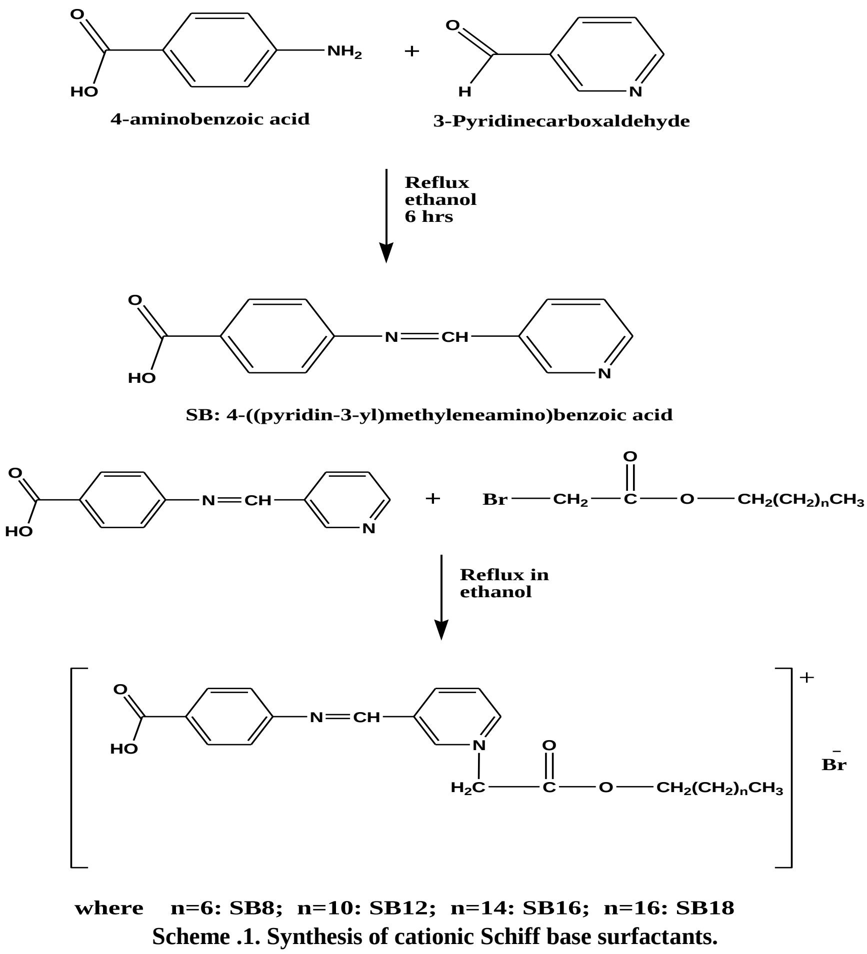
Sulfur reducing bacteria have been treated as phenotypic group which are found in different phylogenetic lines. Sulfur reducing bacteria gain their energy from reduction of elemental sulfur or its components into H_2S. They grow inside septic tank lines and cause rapid deterioration of concrete and reinforcing steel therein. The resistance route against SRB is based on using efficient cationic or metal complex compounds. Examples of biocides used are quaternary ammonium hydroxide[(11)], Schiff base of pyrolidione, pyridone with o-phenylenediamine and their metal complexes[(12)]. Metallomicelles are effective biocide for different types of bacteria[(13)]. In this work, a series of Schiff base cationic surfactants were synthesized and evaluated for their potent action against different bacterial strains including pathogenic bacteria, sulfur reducing bacteria (SRB) and fungi.

Experimental

Synthesis

Synthesis of Schiff base (SB)

The Schiff base under investigation was synthesized throughout condensation reaction between 0.1 mole of p-aminobenzoic acid and 0.1 mole of 3-pyridinecarboxaldehyde that were refluxed in 100 ml of methanol as a solvent for 8 hr. The reaction mixture was left to cool overnight and then filtered. The product was recrystallized from methanol and finally dried under vacuum at 40 ^{o}C to afford pale yellow crystals of the corresponding pyridine Schiff base (yield 93%, m.p. 60-62 ^{o}C). The chemical structure of the produced Schiff base is represented in Scheme 1.



Scheme .1. Synthesis of cationic Schiff base surfactants.

Synthesis of the fatty alcohol ester of bromoacetic acid (C8-18)

Bromoacetic acid (0.1 mol.) and fatty alcohol including: octanol, dodecanol, hexadecanol and octadecanol (0.1 mol.) were esterified individually in xylene as a solvent and in presence of 0.01% p-toluene sulphonic acid as a catalyst. The mixture was refluxed until the azeotropic amount of water (1.8 ml) was removed. After removal of the solvent, the catalyst was removed from the reaction product by extracting in petroleum ether[(14).] to afford the different fatty alcohol esters of bromoacetic acid. The products were symboled as (C8) for octyl bromoacetate, (C12) for dodecyl bromoacetate, (C16) for hexadecyl bromoacetate and (C18) for octadecyl bromoacetate esters.

Preparation of quaternary cationic surfactants

The prepared Schiff base (SB) was quaternized individually by the prepared C8, C12, C16 and C18 in presence of ethanol as a solvent to produce the desired quaternary ammonium Schiff base surfactants which were designated as SB8 for octyl derivative, SB12 for dodecyl derivative, SB16 for hexadecyl derivative and SB18 for octadecyl derivative [(15)].

Instruments

Elemental analyses were performed using Vario elemental instrument. FTIR spectra were performed using Fourier-transformer infrared spectrophotometer. ^{1}H-NMR spectra were performed using Bruker model DRX-300 NMR spectrometer with TMS as an internal standard.

Surface and interfacial tension measurements (γ)

The surface tension of freshly prepared aqueous solution of SB8, SB12, SB16 and SB18 in concentration range of 0.0025-0.00001 mol L^{-1} at 25 ^{o}C was measured with a Krüss K-6 tensiometer (Hamburg, Germany) using Du Noüy platinum ring detachment method and was calibrated using deionized water at 25^{o}C. The readings were taken in triplicate for each solution to check repeatability and the surface tension values were within an error less than or equal to ±1 mN/m[(16)]. The interfacial tension measurements were obtained between aqueous solution of the synthesized cationic Schiff bases at concentration of 0.1 wt% and light paraffin oil at 25 ^{o}C using identical procedures of the surface tension measurements [(17,18)] .

Antimicrobial studies

The synthesized cationic Schiff base surfactants were screened for their antimicrobial activity against bacteria and fungi using agar well diffusion method [(19)].

Results and Discussion

Analyses

Microelemental analyses of the synthesized Schiff bases showed their purity ≈ 98% (Table1). IR spectra showed strong and broad band at (2500-3500) cm^{-1} due to the OH group of the acid, absorption band at 850–890 cm^{-1} corresponding to the benzene nucleus. The presence of absorption band at 1718 cm^{-1} indicates the formation of C=O ester group. The disappearance of the two bands at 1735 and 3315 cm^{-1} due to the carbonyl υ(C=O) and υ (NH2) stretching vibrations and the appearance of a strong new band at 1692 cm^{-1} determines the formation of azomethene υ (HC=N) linkage. The absorption bands at 2858 and 2926 cm^{-1} correspond to the stretching of the two groups CH_2 and CH_3 in the fatty chains.

^{1}H-NMR spectra: 0.96 ppm (S, 6H, CH_3); 1.29 ppm (m, nH, CH_2) (where n = 16H decanoate, 28H hexadecanoate, 32H octadecanoate) and 4.08 ppm (T, 2H,-OCH_2); 2.4ppm (s, 2H,CH_2-CO) and 8.7 ppm; 9.5 ppm; 9.7 ppm and 9.9 ppm for pyridinium peaks; 11 ppm (S, 1H, OH-carboxylic)

TABLE 1. Elemental analysis of the synthesized Schiff base cationic surfactants.

Comp.	Microelemental analysis							
	% C		% H		% N		% Br	
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
SB8 (477.39)	57.87	57.46	6.12	6.08	5.87	5.83	16.74	16.62
SB12 (533.50)	60.79	60.36	6.99	6.94	5.25	5.21	14.98	14.87
SB16 (589.61)	63.51	62.71	7.69	7.64	4.75	4.72	13.55	13.46
SB18 (617.66)	64.17	63.72	8.00	7.94	4.54	4.50	12.94	12.85

Surface activity

Figure1 represents the variation of the surface tension against log conc. of SB8, SB12, SB16 and SB18 at 25 ◦C. The profile showed sharp break points corresponding to the critical micelle concentration values of the different surfactants[20]. The values of the surface tension appeared relatively high compared to the conventional cationic surfactants, That may be due to the presence of the aromatic rings in the molecules. Also, it could be observed that increasing the number of methylene groups along the hydrophobic chains from 12 to 18 units increases the critical micelle concentrations considerably (Fig. 2). That can be explained according to the previous work [21] due to the assumption of repulsion between the hydrophobic chains and the water phase, which forces the surfactant molecules to micellize in the bulk of their solutions in order to decrease that repulsion. On the other hand, increasing the hydrophobic chain length from 8 to 12 methyl groups (–CH_2–) improves the surface activities as the C8 is not considered as surfactant[22] .

The highest CMC values were observed at 3.98 mM and 3.16 mM for SB8 and SB18, respectively while the lowest value was observed for SB12 at 1.26 mM at 25 ◦C, which referred to the above reasons. The efficiency values (Pc_{20})

of the synthesized surfactants are defined as the concentration of the amphiphiles solutions that decreases the surface tension to 51 mNm^{-1} calculated using the data in Fig.1. The Pc_{20} values describe the ability of surfactant molecules to adsorb at the interface and also indicate the accumulation extent of the surfactant molecules at the interface. The increase in the hydrophobic chain length of the studied amphiphiles, results in fast decrease in the surface tension of the surfactant solution indicating the high tendency of the longer hydrophobic molecules to adsorb at the interface. The lowest Pc_{20} value is observed to SB12.

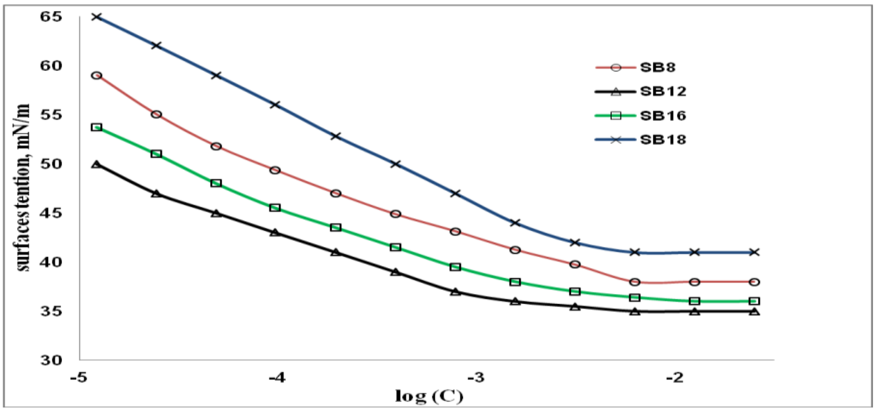


Fig.1. The relation between the surface tension and log concentration of the synthesized cationic Schiff base amphiphiles at 25 ◦C

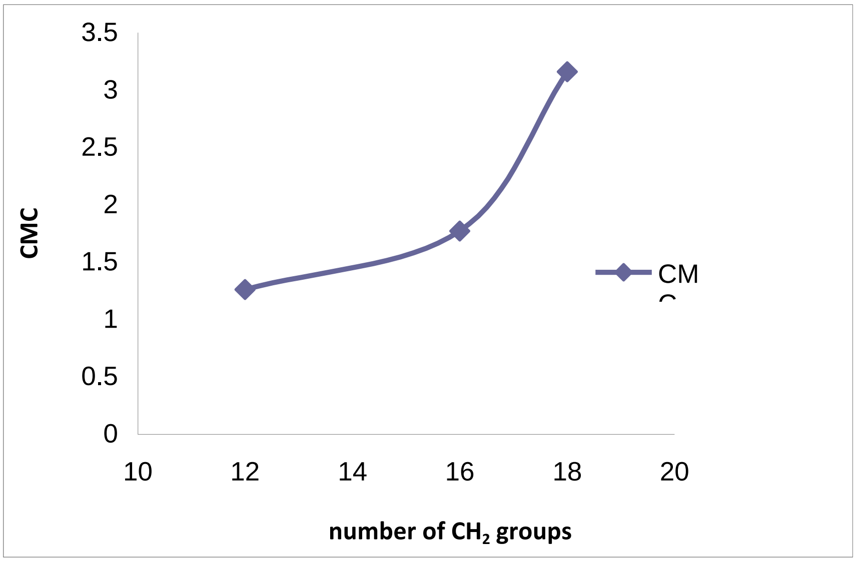


Fig.2. Effect of hydrophobic chain length on the CMC.

Values of the minimum surface area occupied by the cationic Schiff-base amphiphiles at the interface (A_{min}) were calculated according to the equation:

$$A_{min} = 10^{16} / (N_{AV} \cdot \Gamma_{max})$$

where, Γ_{max} and N_{AV} are the maximum surface excess and Avogadro's number, respectively.

The minimum surface area is the area occupied by each molecule at the interface and it depends on the chemical structure of the surfactant molecules hence when the hydrophobic chain length increases (the number of the methylene groups increase) the area of occupied gby each molecule at air-water interface increases. The minimum surface area of the Schiff base cationic surfactants are listed in Table 2.

TABLE 2. Surface properties of the synthesized cationic Schiff bases at 25 ◦C.

Compound	**CMC[a] (mmol/l)**	**Pi cmc[a] (mN/m)**	**Pc_{20} (mmol/l)**	**Γ_{max}X10^{-10} ($molK^{-1}m^{-1}$)**	**A_{min} (nm^2)**	**ΔG_{mic}**	**ΔG_{ads}**
SB8	3.98	33.4	0.0891	3.12	53.2	-13.4	-14.47
SB12	1.26	36.6	0.00794	2.68	61.9	-16.2	-17.55
SB16	1.77	35.6	0.0245	2.83	58.6	-15.36	-16.61
SB18	3.16	31.2	0.281	3.92	42.28	-13.96	-14.75

$\Gamma_{max} = (-\ d\gamma/d\ \log C)/\ (RT)$;

$\Delta G_{mic} = [2.303RT \log (CMC)] - [\log (55.5)]$;

$\Delta G_{ads} = \Delta G_{mic} - \ (0.6023\times10^{-2} \times \Pi_{CMC} \times A_{min})$.

Thermodynamics of adsorption and micellization

The micellization and adsorption processes of the amphiphiles molecules occurred instantly, but commonly one process may be predominating than the other. The predominance of any of the two processes is governed by the thermodynamic variables of this process. In the studied surfactants, both adsorption and micellization thermodynamic functions are calculated based on the methodology of Rosen(23) and using the surface activity data in Table 2. The free energy changes of micellization and adsorption showed negative sign, indicating the spontaneously of the two processes at 25◦C. Also, it is clear that when the hydrophobic chain length of the synthesized Schiff base surfactants increases, ΔG_{mic} and ΔG_{ads} increase. Also, the slight increase of ΔG_{ads} values may be ascribed to the tendency of the molecules to adsorb at the air-water interface until complete surface coverage and the slight increase of ΔG_{mic} values indicates the tendency of the surfactant molecules to migrate to the bulk of solution to form micelle. Also, the negativity values of ΔG_{ads} are greater than the corresponding ΔG_{mic}. This indicates the tendency of surfactant molecules to be adsorbed at the air-water interface rather than escape to the bulk of their solutions to form micelles. The tendency towards adsorption is refereed to the interaction between the aqueous phase and the hydrophobic chains which pumps the amphiphile molecules to the interface. The presence of these amphiphiles at the interface decreases the phase interaction.

Interfacial tension (γ_{int})

The interfacial tension values of the cationic Schiff base surfactants against paraffin oil at 25 ^{o}C are represented in Fig. 3 which indicates that the interfacial tension value of SB8 is 3.0 mN/m and it increases with increasing the hydrophobic chain length to reach 6.0 mN/m for SB18 surfactant.

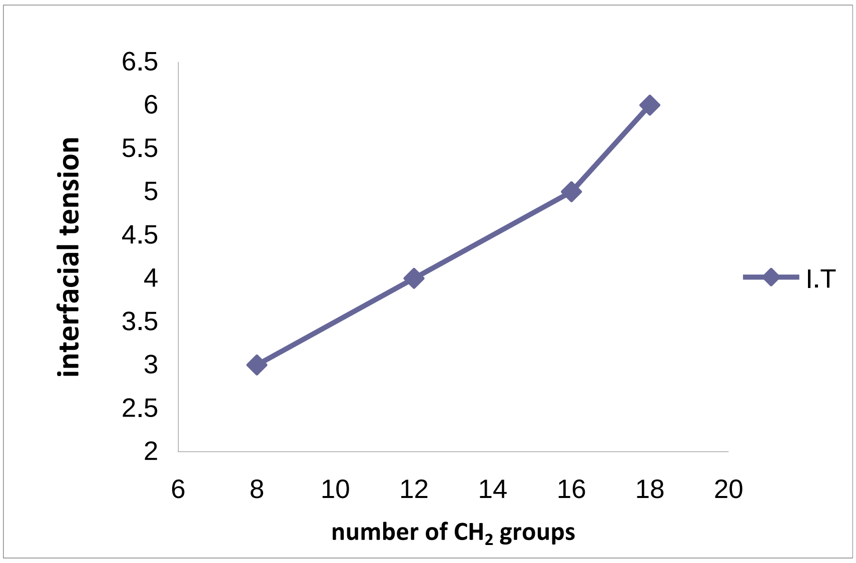


Fig. 3. Relation between hydrophobic chain length and the interfacial tension.

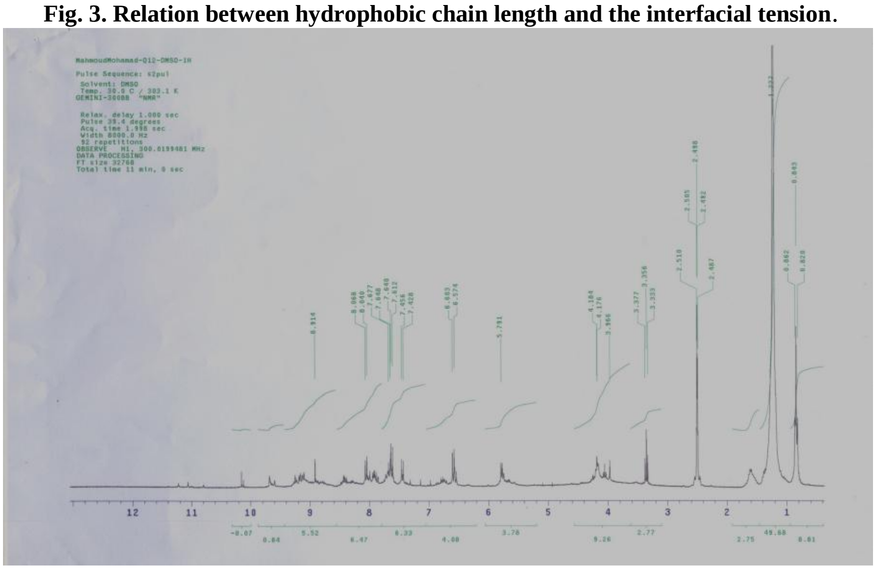


Fig. 4. H NMR spectra of cationic surfactant.

Biological activity

Table 3 represents the antibacterial and antifungal activities of the prepared cationic Schiff bases using well diffusion method against Gram-positive, Gram-negative bacteria and fungi. Cetyl trimethyl ammonium bromide and Grisofluvine were taken as the reference drugs for antibacterial and antifungal activities, respectively. The synthesized cationic Schiff- base amphiphiles showed good biocidal activity against Micrococcus, Sarcina sp. and Pencillium. These results are in good agreements with the results of several investigators dealt with the cationic surfactants(24,25). The hydrophobic chain length has a remarkable influence on the biological activities of the targeted compounds. Increasing the hydrophobic chain length increases the antifungal activity to great extent while, the antibacterial activities are increased gradually.

Antimicrobial activity of prepared cationic surfactants against sulfate-reducing bacteria (SRB)

The results of antimicrobial activity of the synthesized cationic surfactant biocides against SRB were determined and listed in Table 4. The sulfur reducing bacteria (as a potent type of Gram negative bacteria) are characterized by a thick cellular membrane, so that these microorganisms have high resistance against bacterial biocides. Consequently, the creation of efficient antimicrobial agents against the Gram negative bacteria is considered a promising step in the biocidal field. The synthesized surfactants showed good biocidal activity against D. pigra strain and the maximum inhibition efficiency was reported to compound (SB8) which produces inhibition zone diameter of 20 mm per mg inhibitor. The biocidal activities of the synthesized cationic Schiff base surfactants against SRB are arranged in the following arrangement: SB8 > SB12 > SB16 = SB18.

TABLE 3. Biological activity of the synthesized cationic Schiff bases.

Compounds	**Inhibition zone diameter (mm)**					
	Bacteria				**Yeast**	**Fungi**
	Gram positive		**Gram negative**			
	Bacillus pumilus	***Micrococcus sp.***	***Pseudomonas aeruginosa***	***Sarcina sp.***	***Candida albicans***	**Pencillium**
SB8	14	21	15	15	---	21
SB12	---	21	14	17.6	---	16
SB16	16	16	13	18	16	17
SB18	---	17	---	19	17	18
blank	14	13	13	14	15	16

TABLE 4. Antimicrobial activity of prepared cationic surfactants against desulfomonas pigra (SRB).

Compound	Desulfomonas pigra (mm/mg)
SB8	20
SB12	15
SB16	11
SB18	11
Stander: tetracycline	26

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(*Received* 1/7/ 2012;
accepted 30/7/2012)

الخواص السطحية و النشاط البيولوجى لمركبات ذات نشاط سطحى كاتيونية لقاعدة شيف مستحدثه واستخدامها ضد البكتريا والفطريات والبكتريا المختزلة للكبريتات

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تم استحداث مركبات المواد ذات النشاط السطحى الكاتيونية لقاعدة شيف ، وتشخيصها باستخدام طيف الاشعة تحت الحمراء و الرنين النووى المغناطيسى لذرات الهيدروجين. وبقياس التوتر السطحى للمكبات المحضرة ، تم تحديد الخواص السطحية مثل التركيز الميسيلى الحرجل ، الكفاءة السطحية ، التشبع السطحى ، أقل مساحة سطحية للجزيء و الطاقة الحرة لتكوين الميسيل. تقييم المواد المحضرة كمبيدات وموانع لأنواع بكتريا موجبة الجرام وسالبة الجرام والفطريات. وقد أظهرت النتائج قدرة هذه المركبات على مقاومة نمو البكتريا المختلفة و الفطريات و المركبات التي تم تحضيرها نتائج مرغوبة كمبيدات لنمو البكتيريا المختزلة للكبريتات فى مجال البترول.