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Synthesis, interfacial properties, and antimicrobial activity of a new cationic gemini surfactant

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Abstract−Tetramethylene-1,4-bis(N,N-dodecylammonium bromide), cationic gemini surfactant, (12-4-12) was first synthesized with an one-step and shortened procedure and its interfacial and antimicrobial properties were compared with a conventional single-chain cationic surfactant, cetyltrimethylammonium bromide (CTAB). The interfacial and thermodynamic properties of both surfactants reveal that critical micelle concentration (CMC) of this novel synthetic cationic dimeric surfactant is lower than that of cationic monomeric surfactant at almost 15 times of its magnitude, which is due to the increase in hydrophobicity of the surfactant molecules by having dual hydrocarbon chains. In comparison with CTAB, the produced compound 12-4-12 yields much better interfacial and thermodynamic properties. The antimicrobial activities of the synthesized gemini surfactant were tested against eight strains of bacteria, as well as two strains of fungi. The results showed that both 12-4-12 compound and CTAB exhibited higher inhibitory effects on the growth of Gram-positive bacteria and fungi than that of Gram-negative bacteria. The minimum inhibitory concentrations in molar of 12-4-12 against all tested Gram-negative bacteria were lower than those of CTAB, which is hypothetically due to the lower HLB together with smaller CMC values of our gemini surfactant.

Keywords: Cationic Gemini Surfactant, Antibacterial, Antifungal, Interfacial Phenomena

INTRODUCTION

Cationic surfactants have been widely employed for their importance in practical applications. Fundamental and applied studies on cationic surfactants have been extensively reported in several aspects of surfactant research [1-3]. They show excellent abilities in their applications in both individual and mixture usages. Cationic surfactants show great synergism and potent compatibility with anionic, nonionic and zwitterionic surfactants depending on their functional group constituent in their molecules [4-6]. They can be used as fabric softeners [7], antistatics [8], herbicides [9], adhesion promoters in asphalt [10], metal corrosion inhibitors [11], toiletries and hair conditioners [12], mineral floatation agents in ores [13], and antimicrobials [14,15].

The development of novel antimicrobial agents has continuously emerged due to the rapid increase of drug resistance of bacteria. Disinfectants, such as alcohols, aldehydes, chlorhexidine, povidone, iodine, and quaternary ammonium compounds, are chemical agents that inhibit or kill microorganisms in or on inanimate surfaces. Quaternary ammonium compounds, widely used to disinfect patient-care supplies or equipment, are cationic surface-active detergents. The bactericidal action of quaternary compounds has been attributed to inactivation of energy-producing enzymes, denaturation of proteins, and disruption of the cell membrane [16]. Although newer quaternary ammonium salts have been reported as better biocidal compounds for pharmaceutical and medical applications, agriculture, and industry [17-20], novel potential antimicrobial agents remain a keen interest of researchers in investigation and fabrication.

Cationic gemini surfactants are good candidates as they are substantially interesting owing their superior surface-active properties, high ability in surface tension reduction, and lower critical micelle concentration (CMC), leading to the use of a smaller amount of surfactants in comparison with commercially conventional single-chain surfactants [21-23]. The excellent interfacial properties of these potent amphiphiles could be due to their designed molecular structure of the dual hydrocarbon chains linked by either flexible or rigid spacer, which typically comprises aliphatic or aromatic molecule near the polar head groups [21].

Previously, synthesis and characterization of several categories of gemini surfactants have been reported in literature. Cationic gemini surfactants show antimicrobial activity at different levels depending on their designed structures and target cells. It has been found that the longer hydrocarbon chains, the greater antimicrobial functionality [15,20]. The symmetrical amphiphiles containing amino acid groups reveal excellent antifungal and antimicrobial properties upon the hydrophilic-lipophilic balance (HLB), the cationic charge density, and the amino acid sequence on the polar head group [24]. While sugar-based cationic gemini surfactants show antimicrobial

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activity on gram-positive and gram-negative bacteria, and antifungal activity equally to commercial available controls but required a smaller quantity of gemini surfactants [25].

Nevertheless, this study offers an experimentally comparative investigation into interfacial and micellar thermodynamic properties as well as antimicrobial ability of a synthetic cationic gemini surfactant of tetramethylene-1,4-bis(N,N-dodecylammonium bromide) and a conventional single-chain cationic surfactant of cetyltrimethylammonium bromide. Hence, our aim was to investigate their physicochemical properties and antimicrobial behavior between mono- and dimeric surfactant structures as they both comprise quaternary ammonium groups.

MATERIALS AND METHODS

1. Chemicals

n-Dodecylamine was purchased from Acros Organics with purity greater than 97%, and 1,4-dibromobutane was purchased from Fluka with purity greater than 98%. They both were used as received to synthesize cationic gemini surfactant, 12-4-12. Acetonitrile (ACS reagent grade) purchased from Carlo Erba was used as a solvent in synthesis and recrystallization to purify the product obtained from the chemical reaction. Cetyltrimethylammonium bromide (CTAB) was purchased from Acros Organics with purity greater than 99% and was used as received without further purification. All bacterial culture media were bought from Hi-media, India. The high purity of deionized water (18.2 MΩ·cm) was used to prepare samples throughout the experiments.

2. Synthesis of Cationic Gemini Surfactant and Structure Characterization

The 12-4-12 cationic gemini surfactant was synthesized and purified based on the adaptation of previous proposed experimental procedures [26,27] and is depicted in Fig. 1. Synthesis of cationic gemini surfactant firstly starts by dissolving n-dodecylamine

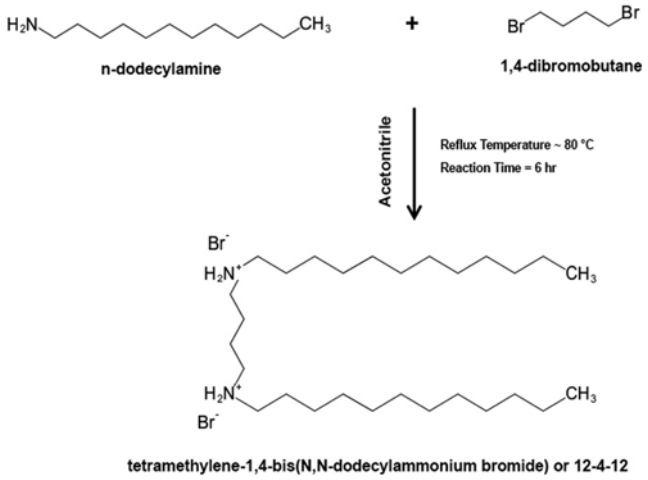

Fig. 1. Schematic representation of proposed chemical reaction for synthesis of tetramethylene-1,4-bis(N,N-dodecylammonium bromide).

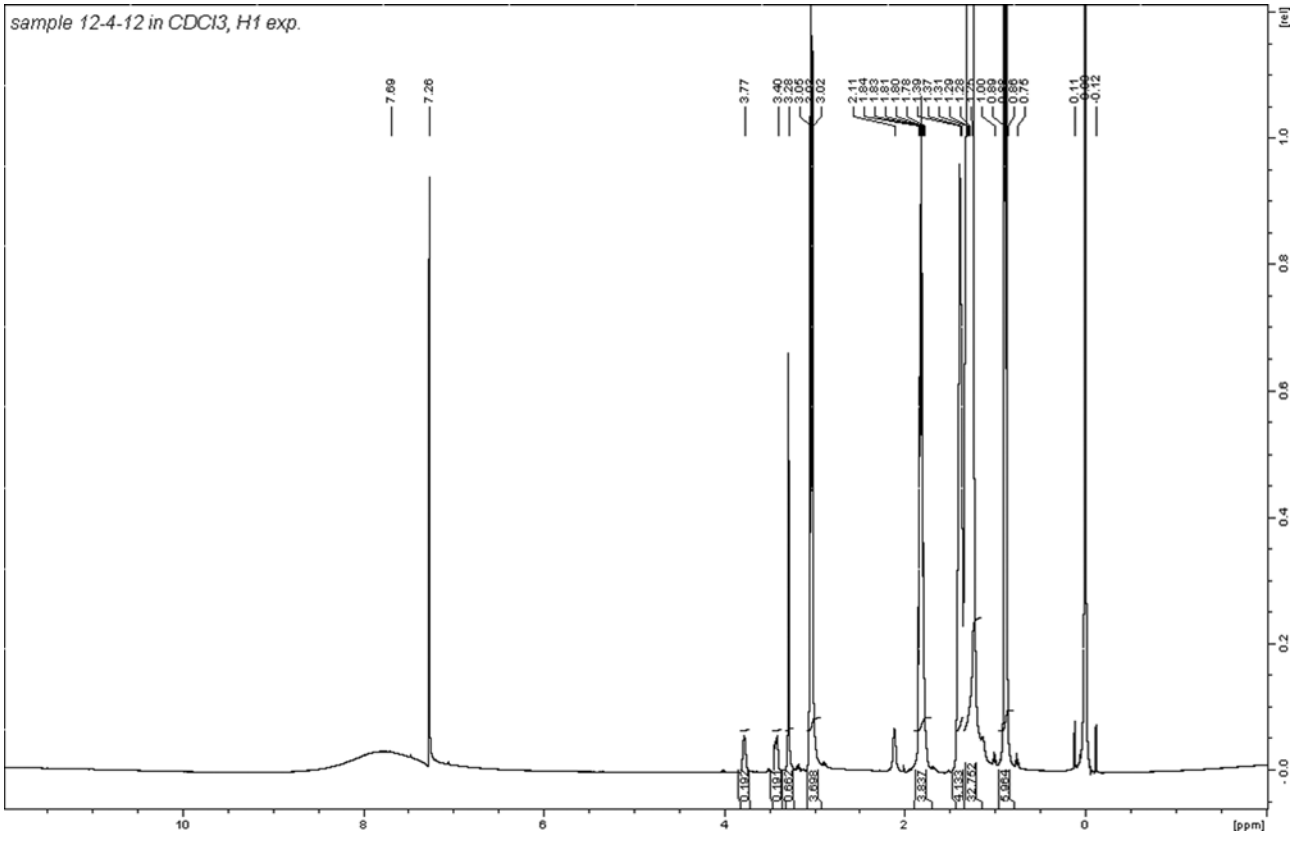

Fig. 2. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of tetramethylene-1,4-bis(N,N-dodecylammonium bromide).

(0.10 mole) in 40 mL acetonitrile. The mixed solution was then added to stirred acetonitrile (20 mL) solution of 0.05 mole of 1,4-dibromobutane. Acetonitrile was used as a solvent because its dielectric constant is higher (higher polar molecule) than methanol or ethanol [26], the reaction time can be shortened to 5-6 hrs in comparison to 24-48 hrs if using ethanol or methanol as a solvent. The reaction was in a stirred reflux reactor for 6 hours at reflux temperature around 80 °C. After the solvent was removed by vacuum evaporation, the crude product obtained was washed and recrystallized using acetonitrile for several times.

The obtained cationic gemini surfactant was collected by filtration and dried in the vacuum oven producing tetramethylene-1,4-bis(N,N-dodecylammonium bromide) as a white crystal flake. The synthetic cationic gemini surfactant was structurally characterized by ^{1}H and ^{13}C NMR using Bruker AVANCE III 500 MHz operating at 400 MHz for ^{1}H and 150 MHz for ^{13}C. Deuterochloroform ($CDCl_3$) was used as a solvent. Chemical shifts are expressed in δ (ppm) using TMS as the internal standard. The mass spectra were recorded on an Agilent GC/MS MSD (7890A/5975C) at 70 eV. Elemental analysis was achieved using a CHNS elemental analyzer (model EA3000; EURO VECTOR Instruments).

White power, yield 64.5%. ^{1}H NMR (400 MHz, $CDCl_3$) as shown in Fig. 2: δ=0.86-0.89 (t, 3H, ***CH$_3$***), 1.250-1.39 (m, 20H, CH_3-***(CH$_2$)$_{10}$***-CH_2), 1.78-1.84 (m, 4H, N-CH_2-***CH$_2$***-***CH$_2$***-CH_2-N), 3.02-3.05 (t, 2H, CH_3-$(CH_2)_{10}$-***CH$_2$***-N-***CH$_2$***). ^{13}C NMR (150 MHz, $CDCl_3$) as shown in Fig. 3: δ=14.13 (***CH$_3$***), 26.42-26.64 (N-CH_2-***CH$_2$***-***CH$_2$***-CH_2-N), 22.70-40.21 (CH_3-***(CH$_2$)$_{11}$***-N), 51.38 (CH_3-$(CH_2)_{11}$-N-***CH$_2$***-CH_2-CH_2-CH_2-N). LC-MS: (H_2O), 427.81 $[M-H]^+$. Elemental analysis of the synthesized surfactant is analytical calculation for C=57.33, H=10.65, N=4.78. Found: C=57.35, H=10.67, N=4.80. All the experimental data confirmed the molecular structure and purity of tetramethylene-1,4-bis(N,N-dodecylammonium bromide), the cationic gemini surfactant produced and the synthesized surfactant is completely soluble in water at the range of concentration studied.

3. Interfacial and Micellization Properties

The surface tension of both 12-4-12 and CTAB in aqueous solution was carried out by a Du Noüy ring method at 30 °C using Krüss K100 equipped with micro dispenser, a dosing unit for fully automatic measurements of the critical micelle concentration (CMC). Prior to the micellization, the adsorption of surfactant molecules, the so-called surface excess concentration (Γ_t), the mean molecular area (a_m), and the standard free energy of micellization and adsorption can be determined from the experimental data using the Gibbs adsorption isotherm and thermodynamic equations [4].

4. Determination of Antimicrobial Activities

4-1. Test Pathogens and Culture Condition

Test pathogens used in this study were obtained from Thailand Institute of Scientific and Technological Research (TISTR), including *Staphylococcus aureus* (TISTR1466), *Staphylococcus epidermidis* (TISTR518), *Bacillus cereus* (TISTR687), *Escherichia coli* (TISTR780), *Pseudomonas aeruginosa* (TISTR781), *Pseudomonas aeruginosa* (TISTR1287), *Salmonella typhi* (TISTR292), *Candida albicans* (TISTR5779) and *Aspergillus niger* (TISTR3012). Mueller Hinton agar (MHA), Sabouraud dextrose Agar (SDA): (g/L: peptone 10; dex-

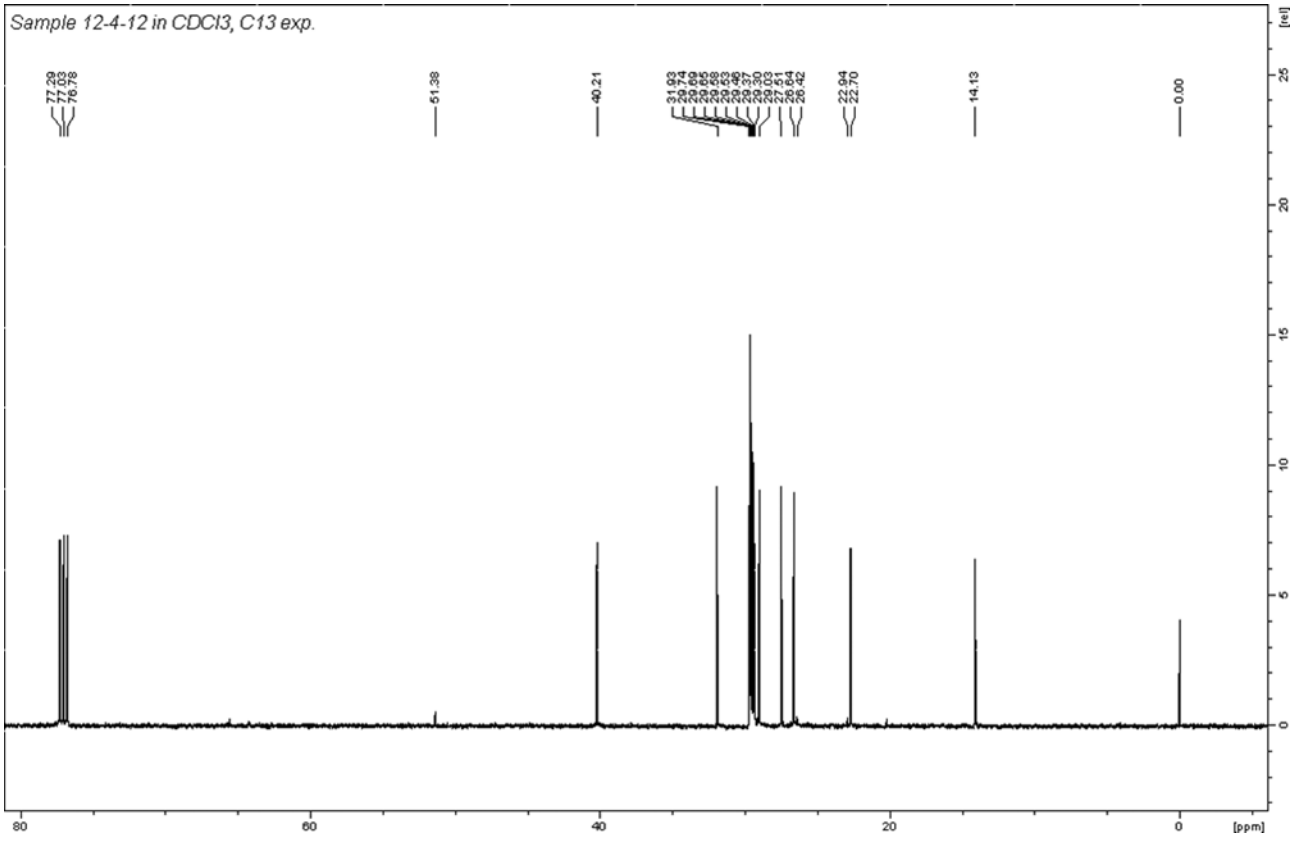

Fig. 3. ^{13}C NMR spectra (150 MHz, $CDCl_3$) of tetramethylene-1,4-bis(N,N-dodecylammonium bromide).

trose 40; agar 15; pH 5.6) and potato dextrose agar (PDA) were used for culturing of bacteria, yeast and fungus, respectively. The cultivation temperature of bacteria and yeast was 37 °C, whereas the incubation temperature for fungus culturing was 30 °C.

4-2. Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) Values

MIC studies of the compound were performed according to the standard reference methods for bacteria [28] and yeast [29] using 96-well microtiter plate in 0.2 ml volumes. The compound was dissolved in sterile water to yield a final concentration of 5 mg/mL. Compound stock solution was serial two-fold diluted from 1,024 μg/mL to 0.5 μg/mL in MHB broth and SDB broth depending on the strains of test pathogens. A standardized inoculum was added into each well to achieve the final inoculum size at 5×10^5 CFU/mL. Inoculum plates were incubated at 37 °C for 16-24 hrs. The compound in media without test pathogens was prepared for blank. Absorbance reading of each well was measured at 625 nm for bacteria and 530 nm for yeast by microplate spectrophotometer (Bio-Rad Laboratories). The MIC value was recorded as the lowest concentration of the compound which no growth (OD_{625} and $OD_{530}\leq$ 0.1) of test microorganisms [30] MBC and MFC was determined by sub-culture of the wells showing no growth on an agar plate without the compound. Three replicates were performed. CTAB was used as positive control for comparison.

4-3. Anti-mycelial Growth Assay of Fungi

Anti-mycelial growth activity of the compound was determined in *A. niger* by fungal growth inhibition assay using PDA medium as described previously [31-33]. PDA medium along with the compound 1,024 μg/mL was two-fold diluted to 1 μg/mL in PDA medium, prior to set solidification. Seven days cultured of *A. niger* on PDA was cut by sterile cork borer (8 mm in diameter) and place onto the center of the petri dish. The plates were then incubated at 30 °C for seven days. Fungal pathogen cultured on the PDA without the compound was used as a control. The fungal growth was measured by the radiant of fungal pathogen and calculated for the percentage of inhibition by the following formula:

$$\text{Inhibition (\%)}=[(Ø_c-Ø_t)/Ø_c]\times100\% \quad (1)$$

where $Ø_c$ and $Ø_t$ are the radial growth of control and radial growth in treatment, respectively.

RESULTS AND DISCUSSION

1. Interfacial and Micellization Properties

The monolayer of 12-4-12 and CTAB molecules at the air/water interface was investigated through the Gibbs adsorption isotherm parameters as tabulated in Table 1. The relationship between surface tension and surfactant concentration shown in Fig. 4 and 5 can be used to determine the surface excess concentration (Γ_{max}), which represents the adsorption capacity at the air/water interface and can be expressed by:

$$\Gamma_{max}=-\frac{1}{2.303nRT}\left(\frac{\partial\gamma}{\partial\log C}\right)_T \quad (2)$$

where the parameter n is the number of moles of the solute species. It can be taken as 3 for dimeric (gemini) surfactant (a divalent surfactant ion and two univalent counterions) in the absence of a swamping electrolyte, and as 2 for monovalent surfactant [20]. R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is the absolute temperature. $(\partial\gamma/\partial\log C)_T$ is the slope in the surface tension isotherm when surfactant concentration is just before the CMC. The efficiency of surfactants in lowering surface tension of water can be measured by effectiveness or surface pressure (π_{CMC}) attained at the CMC:

$$\pi_{CMC}=\gamma_o-\gamma_{CMC} \quad (3)$$

where γ_o is surface tension of pure water and γ_{CMC} is surface tension of the solution at the CMC corresponding to the conditioned temperature. The most potent surfactant is the one which results in the largest reduction of the surface tension at the CMC [34]. The mean molecular area per molecule gives an insight of degree of packing and orientation of the adsorbed surfactant molecules at the air/water interface. It can be expressed by:

$$a_m=\frac{10^{16}}{N\Gamma_{max}} \quad (4)$$

where Γ_{max} is the surface excess concentration (Γ_{max}) in a unit of mol/cm^2 and N is Avogadro's number. For infinite dilution (CMC is 10^{-2} M or less), a standard Gibbs free energy of micellization (ΔG_{mic}) and adsorption (ΔG_{ads}) can be approximated without significant error by [4,35]:

$$\Delta G_{mic}=2.303RT\log(CMC/\omega) \quad (5)$$

$$\Delta G_{ads}=\Delta G_{mic}-6.023\times10^{-1}\times\pi_{CMC}\times a_m \quad (6)$$

where the CMC is expressed in mol/L and ω is the molar concentra-

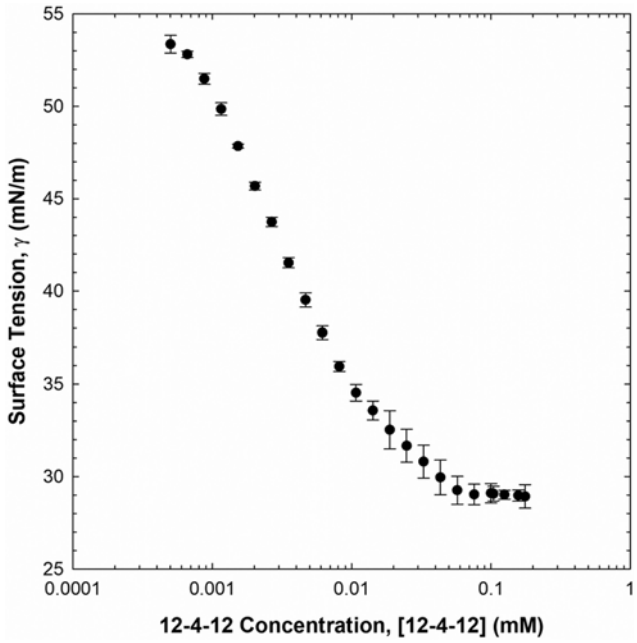

Fig. 4. Surface tension (γ) as a function of tetramethylene-1,4-bis(N,N-dodecylammonium bromide) concentration in aqueous solution at 30 °C.

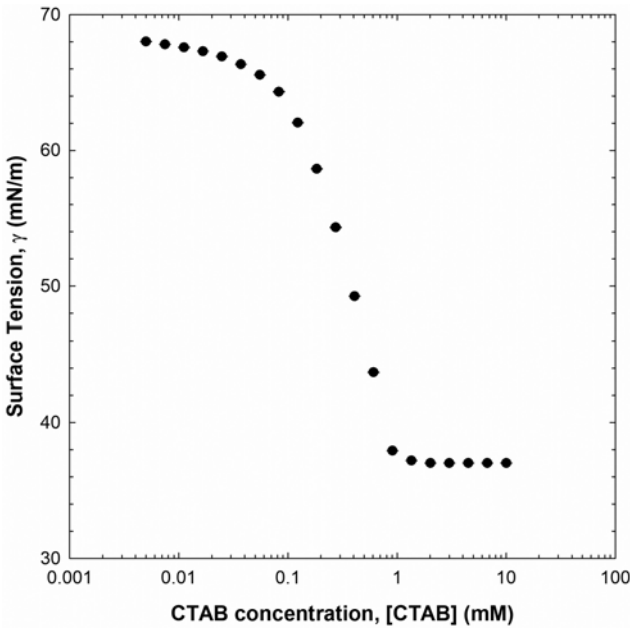

Fig. 5. Surface tension (γ) as a function of cetyltrimethylammonium bromide concentration in aqueous solution at 30 °C.

tion of water at that absolute temperature (55.269 mol/L at 303.15 K). From the experimental results shown in Fig. 4-5 and Table 1, these two different surfactants have different effect on the surface activity and micellization in their solution.

As can be seen, the critical micelle concentration of cationic monomeric surfactant is higher than that of synthetic cationic dimeric surfactant at almost 15 times of its magnitude. The CMC of synthetic 12-4-12 is approximately 0.0649 mM, while the experimental CMC of CTAB is 0.9878 mM, which in good agreement with other previous data investigated through surface tension measurement technique at the same temperature [4,15]. Analyzing the obtained CMCs shows that the attribution to CMC reduction in 12-4-12 solution is according to the increase in hydrophobicity of the surfactant molecules by having dual hydrocarbon chains. The gradual increase in number of hydrocarbon atoms in the surfactant structure decreases the CMC values monotonically [4,15]. The adsorption trend at the air/water interface of surfactant molecules is increased, which decreases their CMC when hydrophobicity increases [25], and this is the main reason for micellization at an ultralow concentration [15]. However, even for conventional monomeric surfactants, when the number of methylene groups is increased, there is also a monotonic increase in the surface activity of the surfactant [36].

Moreover, the synthetic cationic gemini surfactant, 12-4-14, shows much higher efficiency by giving the larger reduction of the surface tension at the CMC in comparison with CTAB, as it shows higher effectiveness or surface pressure (π_{CMC}) attained at the CMC. On the other hand, it requires less amount of surfactant molecules to fully adsorb at the air/water interface as seen in the lower value of surface excess concentration (Γ_{max}). In addition, the dual hydrocarbon chain increases the higher repulsion between surfactant molecules and the aqueous phase. As a result, it forces the surfactant molecules to the interface more effortlessly [15]. The values of mean molecular area per molecule of 12-4-12 and CTAB are also obtained from the surface tension measurement. The higher value of mean surface area occupied by each surfactant molecule at the interface indicates the better packing and orientation of molecules [4,37]. The hydrophilic-lipophilic balance (HLB) of 12-4-12 was computed based on Davies method [38]. The HLB value of 12-4-12 is approximately 12.5, which is lower than of CTAB of 21.4 [39]. The lower HLB value indicates that 12-4-12 is favored to solubilize in lipid phase and it could probably be a good dispersant.

The calculated standard Gibbs free energies of micellization and adsorption for 12-4-12 and CTAB show negatively values, indicating that these two processes occur spontaneously in aqueous solution. Thus, the spontaneity of micellization and adsorption of surfactant molecules are exothermic processes due to the repulsion between the hydrophobic moieties in surfactant molecules and polar solvent [40]. Also, both Gibbs free energies of micellization and adsorption for 12-4-12 are more negative values than of CTAB. Since the standard Gibbs free energies were determined at constant temperature of 30 °C, the entropy and enthalpy changes of micellization and adsorption were not able to be estimated in this study. However, the entropy of micellization is expected to be more positive, indicating that micellization process is entropy driven [4]. The adsorption and micellization processes are generally endothermic, but some studies on novel gemini surfactants observed both small negative (endothermic) and positive values (exothermic) of enthalpy change, which is dependent on hydrocarbon chain length, functional head groups, and spacers in molecular structure of gemini surfactant, counterions and solvent [41-44].

2. Antimicrobial Activities

One of the most common applications of quaternary ammonium compounds is the use as disinfectant due to their effective antimicrobial activities. It has been reported that the antimicrobial actions of these quaternary ammonium surfactants depend on several factors, such as the length of alkyl chain and molecular structure [45]. In the present study, antibacterial and antifungal activities of the synthetic compound 12-4-12 are demonstrated as shown in Table 2 and Fig. 6. When compared to CTAB by the MICs in molar concentration, 12-4-12 exhibited low activity against the tested Gram-positive bacteria and fungi, but had higher efficiency in tested Gram-negative bacteria. The reported efficacy of CTAB against each bac-

Table 1. Surface adsorption isotherm and thermodynamic parameters of tetramethylene-1,4-bis(N,N-dodecylammonium bromide) (12-4-12) and cetyltrimethylammonium bromide (CTAB) in aqueous solution at 30 °C

Surfactant	CMC (mM)	γ_{CMC} (mN/m)	π_{CMC} (mN/m)	$\Gamma_{max}\times10^{10}$ (mol/cm^2)	a_m (Å^2)	ΔG_{mic} (kJ/mol)	ΔG_{ads} (kJ/mol)
12-4-12	0.0649±0.005	29.0	41.0	0.193	857.8	−34.418	−34.417
CTAB	0.9878±0.002	37.0	33.0	1.122	148.1	−27.56	−27.57

Table 2. MICs and MBCs of tetramethylene-1,4-bis(N,N-dodecylammonium bromide) (12-4-12) and cetyltrimethylammonium bromide (CTAB)

Test pathogens	CTAB		12-4-12	
	MIC (μg/mL [μM])	MBC (μg/mL [μM])	MIC (μg/mL [μM])	MBC (μg/mL [μM])
Gram-positive bacteria				
S. aureus TISTR1466	2 [5.5]	4 [11]	16 [27]	16 [27]
S. epidermidis TISTR518	1 [2.7]	8 [22]	8 [14]	16 [27]
B. cereus TISTR687	8 [22]	8 [22]	32 [55]	32 [55]
Gram-negative bacteria				
E. coli TISTR780	32 [89]	32 [88]	32 [55]	32 [55]
P. aeruginosa TISTR781	128 [351]	256 [702]	64 [109]	64 [109]
P. aeruginosa TISTR1287	256 [702]	256 [702]	128 [218]	128 [218]
S. typhi TIRTS292	64 [176]	64 [176]	32 [55]	64 [109]
Fungus				
C. albicans TISTR5779	8 [22]	16 [44]	32 [55]	128 [220]

ND: Not determined

terial species varied depending on both the methods used [46] and the strains of tested bacteria [47].

The MICs and MBCs of CTAB obtained from this study were in the range of those previous published data, which are 1-512 μg/mL (2-700 μM). Based on the differences in cell wall structure, which are the heart of Gram stain reaction, bacteria can be divided into two major groups: Gram-positive and Gram-negative. Gram-negative bacteria are in general more resistant to a large number of antibiotics and chemotherapeutic agents than are Gram-positive bacteria [48]. It has been proposed that the killing action on Gram-positive bacteria of cationic gemini surfactants depends on the hydrophobicity and amphiphilic properties of surfactant because of the necessity of both electrostatic interaction with bacterial cell wall and hydrophobic interaction with cell membrane [49]. Whereas, in Gram-negative bacteria, lipopolysaccharide composition of their outer membrane may prevent the entrance of some hydrophobic-hydrophilic disinfectants, requiring higher concentration to inhibit their growth. As expected, more susceptibility to disinfectant in Gram-positive bacteria was seen in the present study similar to many published documents [46,49-52].

In accordance with the report of the same class of synthetic gemini surfactants by Kuaperkar and coworkers [52], the antimicrobial potency of our compound showed the same order of activity: Gram-positive bacteria>fungi>Gram-negative bacteria. In terms of antifungal activity, it has been proposed that fungal membranes are composed of glucosamine and chitin, which makes cells more rigid and harder to kill [49]. As shown in Fig. 6, the inhibitory effect of 12-4-12 on mycelial growth of *A. niger* was comparable to CTAB in the low range of concentration, but less effect was seen in the higher concentration. Although the compound 12-4-12 obtained from the present study showed relatively less efficacy to inhibit and kill Gram-positive bacteria and fungi, the MICs in molar concentration of 12-4-12 against all of tested Gram-negative bacteria were lower than those of CTAB. Because of the high content of lipids in the outer membrane, the cell wall of Gram-negative bacteria is relatively resistant to dissociation by disinfectants and antiseptics [53]. The outer membrane of Gram-negative bacteria is also resistant to

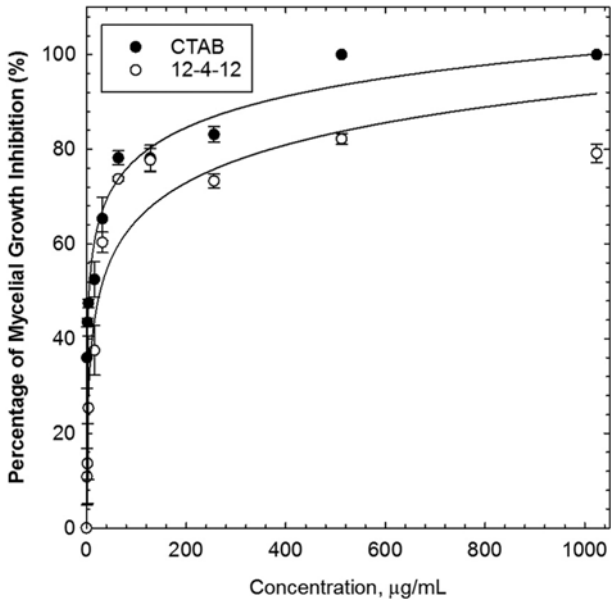

Fig. 6. Mycelial growth inhibition by tetramethylene-1,4-bis(N,N-dodecylammonium bromide) (12-4-12) and cetyltrimethylammonium bromide (CTAB).

detergents, especially anionic and neutral detergents.

The explanation is that the outer membrane of Gram-negative bacteria has negative charges [54] and is impermeable to hydrophilic macromolecules. It allows only restricted diffusion of hydrophobic compounds through its lipopolysaccharide-rich surface membrane [55]. In addition to the charges and molecular sizes, lipid solubility seems to be important to the antibacterial activity of detergent against Gram-negative bacteria. Not surprisingly, it has been demonstrated that more hydrophobic surfactants possess higher antimicrobial potency [52]. It has also been shown that there is a correlation between the CMC value of surfactants and their antibacterial effects: the lower CMC, the more efficacy of antibacterial activity [52,56].

CONCLUSIONS

The novel synthetic compound 12-4-12 of cationic gemini surfactant shows much better interfacial and thermodynamic properties, such as having lower critical micelle concentration, giving a larger reduction of the surface tension, showing higher surface pressure, requiring smaller amount of surfactant molecules to fully adsorb at the air/water interface, having higher value of mean surface area occupied by each surfactant molecule at the interface, and having lower hydrophilic-lipophilic balance (HLB) value. The experimental result also reveals the antimicrobial activities of the synthesized cationic gemini surfactant tested against eight strains of bacteria and two strains of fungi. The experimental results showed that both cationic gemini surfactant and conventional cationic surfactant exhibited higher inhibitory effects on the growth of Gram-positive bacteria and fungi than that of Gram-negative bacteria. The minimum inhibitory concentrations in molar concentration of gemini surfactant against all tested Gram-negative bacteria were lower than those of a conventional cationic surfactant. It could be attributed to the lower HLB and CMC values which indicate the higher lipid solubility and better surface-active properties, respectively. Further toxicity investigation of this compound is needed for its disinfectant or antiseptic applications. In conclusion, the cationic gemini surfactant which was synthesized from this study by a novel, one-step, and short procedure without catalyst could be promising surface active and antimicrobial agents.

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SYMBOLS

a_m : mean molecular area
$Ø_c$: radial growth of control
$Ø_t$: radial growth in treatment
Γ_{max} : the surface excess concentration
ΔG_{ads} : standard Gibbs free energy of adsorption
ΔG_{mic} : standard Gibbs free energy of micellization
γ : surface tension
γ_o : surface tension of pure water
γ_{CMC} : surface tension at the critical micelle concentration
π_{CMC} : effectiveness or surface pressure attained at the CMC

REFERENCES

1. A. Colomer, A. Pinazo, M. A. Manresa, M. P. Vinardell, M. Mitjans, M. R. Infante and L. Pérez, *J. Med. Chem.*, **54**, 989 (2011).
2. D. N. Rubingh and P. M. Holland, Cationic surfactant: Physical chemistry; Surfactant Science Series 37, Marcel Dekker, New York (1991).
3. T. Geng, C. Zhang, Y. Jiang, H. Ju and Y. Wang, *J. Mol. Liq.*, **232**, 36 (2017).
4. M. J. Rosen and J. T. Kunjappu, *Surfactants and interfacial phenomena*, Wiley, New Jersey (2012).
5. V. Pradines, C. Bijani, J. L. Stigliani, M. Blanzat, I. Rico-Lattes and G. Pratviel, *ChemPhysChem*, **16**, 3877 (2015).
6. M. Lechuga, M. Fernández-Serrano, E. Jurado, J. Núñez-Olea and F. Ríos, *Ecotoxicol. Environ. Saf.*, **125**, 1 (2016).
7. M. G. Mondal and A. P. Pratap, *J. Oleo Sci.*, **65**, 663 (2016).
8. L. Zhi, Q. Li, Y. Sun and S. Yao, *J. Surfactants Deterg.*, **19**, 337 (2016).
9. M. C. Galán-Jiménez, E. Gómez-Pantoja, E. Morillo and T. Undabeytia, *Sci. Total Environ.*, **538**, 262 (2015).
10. M. Ronald and F. P. Luis, *Constr. Build. Mater.*, **123**, 162 (2016).
11. M. A. Hegazy, A. Y. El-Etre, M. El-Shafaie and K. M. Berry, *J. Mol. Liq.*, **214**, 347 (2016).
12. J. Falbe, *Surfactants in consumer products: theory, technology and application*, Springer-Verlag, Heidelberg (1986).
13. Z. Huang, H. Zhong, S. Wang, L. Xia, W. Zou and G. Liu, *Chem. Eng. J.*, **257**, 218 (2014).
14. A. Donauerová, J. Bujdák, M. Smolinská and H. Bujdáková, *J. Photochem. Photobiol. B*, **151**, 135 (2015).
15. M. A. Migahed, N. A. Negm, M. M. Shaban, T. A. Ali and A. A. Fadda, *J. Surfactants Deterg.*, **19**, 119 (2016).
16. D. H. Deck and L. G. Winston, in Basic and clinical pharmacology, B. G. Katzung, S. B. Masters and A. J. Trevor, Eds., Lange Medical Books (2012).
17. Z. Jia and W. Xu, *Carbohydr. Res.*, **333**, 1 (2001).
18. Y. Liu, K. Ma, R. Li, X. Ren and T. S. Huang, *Cellulose*, **20**, 3123 (2013).
19. G. C. Daniels, E. B. Iezzi and P. A. Fulmer, *Prog. Org. Coat.*, **95**, 91 (2016).
20. K. Kuperkar, J. Modi and K. Patel, *J. Surfactants Deterg.*, **15**, 107 (2012).
21. F. M. Menger and C. A. Littau, *J. Am. Chem. Soc.*, **115**, 10083 (1993).
22. S. M. Tawfik, *J. Ind. Eng. Chem.*, **28**, 171 (2015).
23. I. Aiad, S. M. Shaban, H. Y. Moustafa and A. Hamed, *Prot. Met. Phys. Chem. Surf.*, **54**, 135 (2018).
24. A. Pinazo, M. A. Manresa, A. M. Marques, M. Bustelo, M. J. Espuny and L. Pérez, *Adv. Colloid Interface Sci.*, **228**, 17 (2016).
25. N. A. Negm and A. S. Mohamed, *J. Surfactants Deterg.*, **11**, 215 (2008).
26. B. Brycki, K. Kowalczyk and A. Kozirog, *Molecules*, **16**, 319 (2011).
27. Y. Wang, Y. Han, X. Huang, M. Cao and Y. Wang, *J. Colloid Interface Sci.*, **319**, 534 (2008).
28. F. R. Cockerill, M. A. Wikler, J. Alder, M. N. Dudley, G. M. Eliopoulos, M. J. Ferraro, D. J. Hardy, D. W. Hecht, J. A. Hindler, J. B. Patel, M. Powell, J. M. Swenson, R. B. Thomson, M. M. Traczewski, J. D. Turnidge, M. P. Weinstein and B. L. Zimmer, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard - 9th Ed., Clinical and Laboratory Standards Institute, Pennsylvania (2012).
29. J. L. Rodríguez-Tudela, M. Cuenca-Estrella, T. M. Díaz-Guerra and E. Mellado, *J. Clin. Microbiol.*, **39**, 2513 (2001).
30. J. H. Oh, Y. J. Jeong, H. J. Koo, D. W. Park, S. C. Kang, H. V. Khoa, L. B. Le, J. H. Cho and J. Y. Lee, *Molecules*, **19**, 3607 (2014).

31. W. H. Himratul-Aznita, N. Mohd-Al-Faisal and A. R. Fathilah, *J. Med. Plants Res.*, **5**, 878 (2011).
32. B. Senhaji, D. Ben Hmamou, R. Salghi, A. Zarrouk, B Chebli, H. Zarrok, I. Warad, B. Hammouti and S. S. Al-Deyab, *Int. J. Electrochem. Sci.*, **8**, 6033 (2013).
33. M. Balouiri, M Sadiki and S. K. Ibnsouda, *J. Pharm. Anal.*, **6**, 71 (2016).
34. M. M. El-Sukkary, N. O. Shaker, D. A. Ismail, S. M. Ahmed, M. F. Zaki and A. I. Awad, *Egypt. J. Pet.*, **21**, 37 (2012).
35. T. F. Tadros, *Surfactants in agrochemicals*, Marcel Dekker, New York (1994).
36. M. J. Rosen and D. J.Tracy, *J. Surfactants Deterg.*, **1**, 547 (1998).
37. A. Maneedaeng and A. E. Flood, *J. Surfactants Deterg.*, **20**, 263 (2017).
38. J. T. Davies in Proceedings of 2nd International Congress Surface Activity Vol. I: Gas/Liquid and Liquid/Liquid Interface, J. H. Schulman Ed., Butterworths (1957).
39. D. B. Miraglia, J. L. Rodríguez, R. M. Minardi and P. C. Schulz, *J. Surfactants Deterg.*, **14**, 401 (2011).
40. D. Mańko, A. Zdziennicka and B. Jańczuk, *Colloids Surf., B.*, **114**, 170 (2014).
41. T. Lu, Y. Lan, C. Liu, J. Huang and Y. Wang, *J. Colloid Interface Sci.*, **377**, 222 (2012).
42. X. Wang, J. Wang, Y. Wang, J. Ye, H. Yan and R. K. Thomas, *J. Phys. Chem. B*, **107**, 11428 (2003).
43. M. S. Borse and S. Devi, *Adv. Colloid Interface Sci.*, **123**, 387 (2006).
44. K. Ghosh and P. Quagliotto, *Indian J. Chem., Sect. A: Inorg., Bioinorg., Phys., Theor. Anal. Chem.*, **48**, 1522 (2009).
45. T. Tsuchido, A. Svarachorn, H. Soga and M. Takano, *Antimicrob. Agents Chemother.*, **34**, 78 (1990).
46. G. Wu, Q. Yang, M. Long, L. Guo, B. Li, Y. Meng, A. Zhang, H. Wang, S. Liu and L. Zou, *J. Antibiot.*, **68**, 661 (2015).
47. S. Ishikawa, Y. Matsumura, F. Yoshizako and T. Tsuchido, *J. Appl. Microbiol.*, **92**, 261 (2002).
48. M. Vaara, *Antimicrob. Agents Chemother.*, **37**, 2255 (1993).
49. N. Fatma, M. Panda, Kabir-ud-Din and M. Beg, *J. Mol. Liq.*, **222**, 390 (2016).
50. M. A. Migahed, N. A. Negm, M. M. Shaban, T. A. Ali and A. A. Fadda, *J. Surfactants Deterg.*, **19**, 119 (2015).
51. A. Cornellas, L. Perez, F. Comelles, I. Ribosa, A. Manresa and M. T. Garcia, *J. Colloid Interface Sci.*, **355**, 164 (2011).
52. K. Kuperkar, J. Modi and K. Patel, *J. Surfactants Deterg.*, **15**, 107 (2011).
53. G. McDonnell and A. D. Russell, *Clin. Microbiol. Rev.*, **12**, 147 (1999).
54. G. Harkes, H. Van der Mei, P. Rouxhet, J. Dankert, H. Busscher and J. Feijen, *Cell. Biochem. Biophys.*, **20**, 17 (1992).
55. M. Vaara, *Microbiol. Rev.*, **56**, 395 (1992).
56. M. Diz, A. Manresa, A. Pinazo, P. Erra and M. Infante, *J. Chem. Soc. Perkin Trans.*, **2**, 1871 (1994).