Synthesis, characterization, surface and antimicrobial activityof some nonionic di-Schiff base surface-active agents

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Abstract

A series of nonionic Schiff base surfactants were prepared by the reaction between polyethylene glycol, glyoxalic acid and phenylene diamine. The prepared compounds were characterized using elemental analysis, FTIR and ^{1}H-NMR spectroscopy. The surface activities of the prepared nonionic surfactants were determined using surface and interfacial tension measurements. The thermodynamic properties of these surfactants in adsorption and micellization were calculated at 25ºC. The results showed that the increase in the polyethylene glycol chain length (nonionic chains) increases their adsorption tendencies at the interface.

Keywords: Schiff base; nonionic surfactants; surface activity; adsorption; micellization.

1. Introduction

Today there is a tendency to reduce the use of certain classes of surfactants polluting the environment and to change them for new ones [1-3]. Accordingly, efforts are being made to develop new classes of surfactants with reduced foaming capacity and milder action, which operate at a lower temperature with synergistic action of their components [4-6]. Such surfactants should be environmentally friendly and cost efficient. Alkyl glycosides [7], sugar based [8] natural product surfactants [9] have very recently been used in the production of detergents, and therefore their properties are little studied. The use of environmentally friendly surfactants for production of detergents shows promise not only from the efficiency standpoint, but also in view of the environmental protection [10]. Environmentally friendly surfactants are characterized by high biodegradability resulting from being manufactured from natural raw materials. At the same time, these are mild, low toxic surfactants providing no skin irritation. In recent years, the cost of surfactants has demonstrated a steady tendency to decrease. In our previous works, we synthesized several types of surfactants which are environmentally friendly [11-14]

2. Materials and Experimental procedures

Instrumentation

Micro elemental analysis: VarioElementar Analyzer; Infrared Spectroscopy: FTIR (Genesis Fourier transformer FTIRTM); H1-NMR Spectroscopy: Varian NMR – 300 model; Mercury 300 MHz; Atomic Absorption Spectroscopy (AAS): Hitachi 180/80 atomic absorption spectrometer; Tensiometer: Tensiometer K6 Krüss, Germany, (Dünoytensiometer with platinum ring).

Synthesis

Synthesis of polyethylene glycol dialdehyde compounds

Glyoxalic acid and polyethylene glycol (Mwt=400, 600, 1000 g/mole) were esterified in 2:1 molar ratio, respectively, in in xylene as a solvent and in the presence of 0.1 wt% of *p*-toluene sulfonic acid as a dehydrating agent. The reaction was stopped after obtaining the required amount of water of the reaction [15]. The obtained dialdehyde esters were washed by petroleum ether to remove the catalyst, and then dried under vacuum at 75 ºC for 24 h to obtain white waxy product, yield: 86%.

Synthesis of nonionic di-Schiff base derivatives

Equimolar amounts of *p*-phenylene diamine and the prepared polyethylene glycol dialdehyde derivatives were refluxed in isopropanol in the presence of dehydrating agent (p-toluene sulfonic acid, 0.1 wt%) [16]. The reaction mixture was refluxed for 6 h. the reaction products were waxy and varied between yellow to brown in yield of 80-85%.

Synthesis of nonionic di-Schiff base surfactants

The nonionic di-Schiff base nonionic surfactants were prepared via the reaction between the prepared nonionic di-Schiff bases (0.1 mole) and oleic and linoleic acids (0.2 mole) in xylene as a solvent and 0.1 wt% of p-toluene sulfonic acid as a dehydrating agent [17]. The reaction was completed after the removal of the calculated amount of water of the reaction (3.6 mL). The reaction products were washed by diethyl ether to remove the unreacted compounds and the used catalyst, to yield 80% of brown waxy products.

Measurements

Surface and interfacial tension measurements of nonionic di-Schiff bases

Surface tension measurements were obtained using De-NöuyTensiometerKrüss K-6 with a platinum ring. Freshly aqueous solutions of the synthesized Schiff base nonionic

surfactants were prepared in double distilled water in a wide concentration range of 10 to 0.01 m mol/L at 25 °C [18]. Apparent surface tension values were measured three times for each sample within 2 min interval between each reading, [19]. Interfacial tension measurements were measured between 10 mL of the amphiphiles solution of concentration (0.1%) and 10 mL of the paraffin oil as nonpolar phase at 25 °C [20].

Emulsification efficiency

10 mL of the synthesized nonionic surfactants (0.1%) were mixed with 10 mL of paraffin oil in graduated quick fit cylinder and shacked vigorously for 5 min and then allowed to separate the two-phase layers in a water bath adjusted at 30 °C. Emulsification efficiencies were expressed in seconds and determined by measuring the time required for separation of 9 mL of pure surfactant solution [21].

Microorganisms

The biocidal activity of the synthesized surfactants was tested against different bacterial strains (ATCC: American Type Culture Collection) as follows: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis*ATCC 55422, *Desulfomonaspigra* ATCC 29098 and *Staphylococcus typhimurium* ATCC 27948.

Growing of microorganisms

The bacterial strains were cultured on nutrient medium, while the fungi strains were cultured on malt medium. For bacteria, the broth media were incubated for 24 h. As for fungus, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile Sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation.

Measurements of resistance and susceptibility

For preparation of discs and inoculation, 1 mL of inocula were added to 50 mL of agar media (40 °C) and mixed. The agar was poured into 120 mm petri dishes and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using proper sterile tubes. Then, holes filled up to the surface of agar with 0.1 mL of the synthesized cationic surfactants dissolved in DMF (1 mg/mL DMF). The plates were left on a leveled surface, incubated for 24 h at 37 °C for bacteria and 48 h for fungi and then the diameters of the inhibition zones were measured.

The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds. The mean value obtained for three individual replicates was used to calculate the zone of growth inhibition of each sample. The results were compared with a similar run of Cetyl trimethyl ammonium bromide (CTAB) [22] as an antibacterial reference and Grisofluvine as an antifungal reference. Both antimicrobial activities were calculated as a mean of three replicates.

Minimum inhibitory concentration

The biocidal activity of the synthesized surfactants against the tested strains was expressed as the minimum inhibitory concentration (MIC) values, defined as the lowest concentration of compounds inhibiting the development of visible growth after 24 h of incubation. The MIC values were determined by dilution method [23]. The compounds tested were dissolved in a mixture of distilled water/alcohol (3/1; v/v) at various concentrations and the 1 mL aliquot of the cationic surfactants solutions was added to the 14 mL agar media. The final concentrations of the tested surfactants in the medium were 300, 200, 100, 40, 20, 10 and 4 µg/mL.
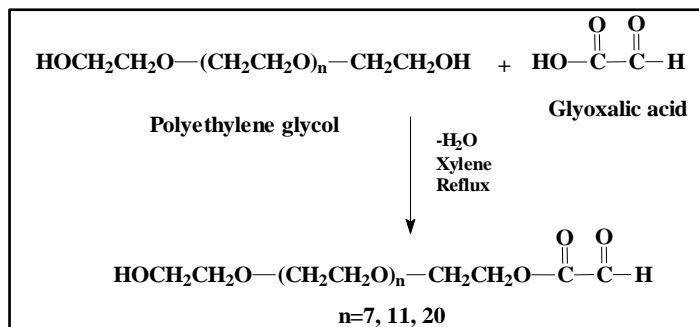
3. Results and Discussion

Structure of the prepared compounds and intermediates

The synthesis of the di-Schiff base nonionic surfactants was preceded through three reactions. The first reaction was the reaction between polyethylene glycol with different molecular weights (Mwt: 400, 600 and 1000 g/mole) and glyoxalic acid in equimolar ratio to obtain the corresponding esters (denoted as E400, E600, E1000), as represented **in Scheme 1.**

The obtained esters were characterized using FTIR and ^{1}H-NMR spectroscopy. FTIR spectra of compound E400 showed the following absorption bands: 3459, 2868, 1748, 1247, and 1086- cm^{-1}, corresponding to the following function groups: O-H terminal hydroxyl groups of polyethylene glycol; C-H stretching vibration of the methylene groups; C=P stretching of the formed ester groups; C-O stretching of the ester groups; and C-O stretching of the ethylene glycol repeated units, respectively, Figure 2.

The second reaction was the reaction of p-phenylene diamine and the prepared esters to obtain the corresponding di-Schiff bases (denoted as SE400, SE600, SE1000), Scheme 2. FTIR spectra of SE400 showed the appearance of sharp absorption band at 1644 cm^{-1} indicating the formation of azomethine group (-CH=N-) of the Schiff base moiety. The other characteristic groups of the starting esters (E400, E600, E1000) were also appeared, Figure 3. That indicates the formation of the desired di-Schiff base compounds as represented in Scheme 2.

The prepared di-Schiff base nonionic surfactants were obtained from the reaction between the obtained di-Schiff bases and oleic acid in 1:2 molar ratio, Scheme 3. The obtained nonionic surfactants were characterized by FTIR and ^{1}H-NMR spectroscopy, Figures 4, 5.

$$HOCH_2CH_2O—(CH_2CH_2O)_n—CH_2CH_2OH \quad + \quad HO—\overset{O}{\underset{}{C}}—\overset{O}{\underset{}{C}}—H$$

Polyethylene glycol   **Glyoxalic acid**

-H$_2$O
Xylene
Reflux

$$HOCH_2CH_2O—(CH_2CH_2O)_n—CH_2CH_2O—\overset{O}{\underset{}{C}}—\overset{O}{\underset{}{C}}—H$$

n=7, 11, 20

Scheme 1: Preparation of polyethylene glycol mono glyoxalate esters.
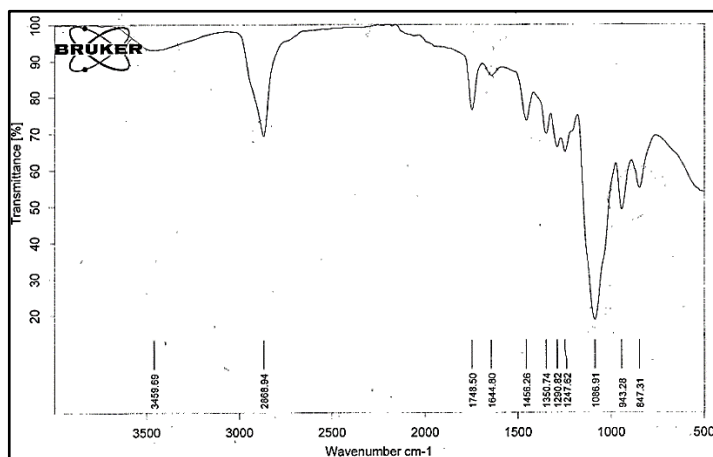
Fig (2): IR spectra of polyethylene glycol mono glyoxalate esters.

$$HOCH_2CH_2O—(CH_2CH_2O)_n—CH_2CH_2O—\overset{O}{\underset{}{C}}—\overset{O}{\underset{}{C}}—H \quad + \quad H_2N—\bigcirc—NH_2$$

-H$_2$O
Xylene
Reflux

$$HOCH_2CH_2O—(CH_2CH_2O)_n—CH_2CH_2O—\overset{O}{\underset{}{C}}—CH=N$$

$$HOCH_2CH_2O—(CH_2CH_2O)_n—CH_2CH_2O—\overset{}{\underset{O}{C}}—CH=N$$
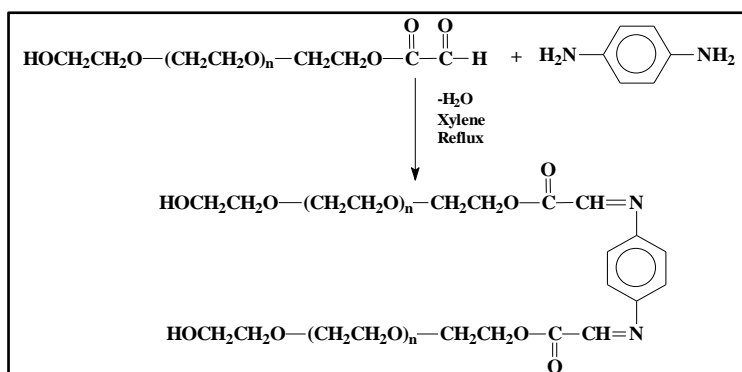
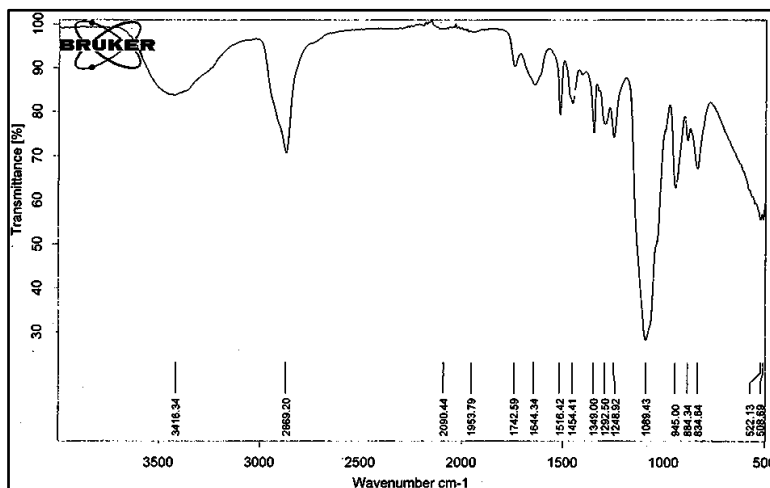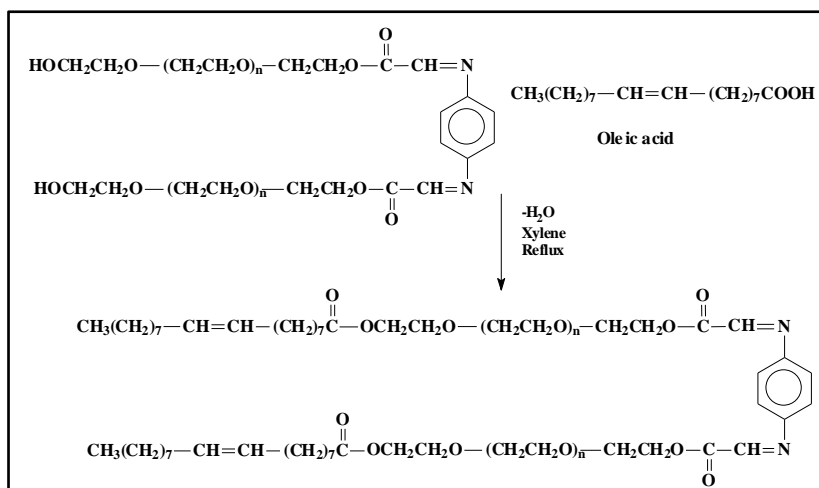Scheme 2: Preparation of the nonionic di-Schiff bases.

Fig (3): IR spectra of the prepared nonionic di-Schiff base.

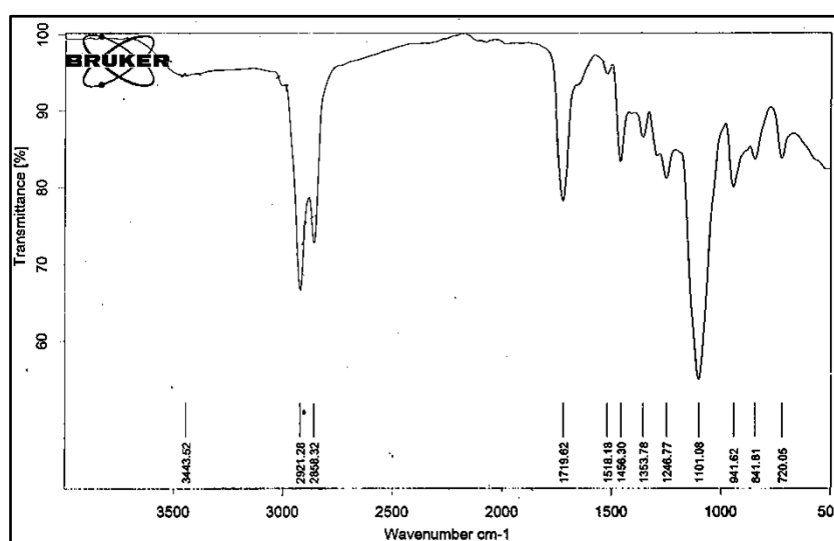Scheme 3: Preparation of the nonionic di-Schiff base surfactants.

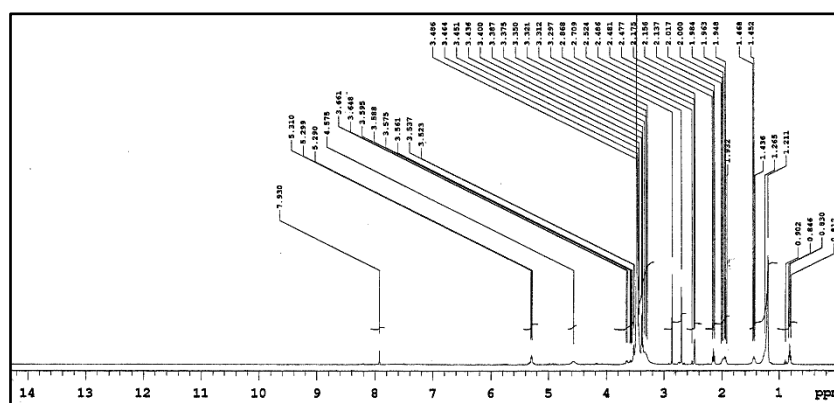Fig (4): IR spectra of the nonionic di-Schiff base surfactant DSB-400.

Fig (5): ^{1}H-NMR spectra of nonionic di-Schiff base surfactant DSB-400

Surface activity

Figure 1 represents the relation between the surface tension and *log* concentration of the synthesized nonionic di-Schiff base nonionic surfactants at 25°C. It is clear that the surface tension profile has the characteristics of the nonionic surfactants including the relatively higher surface tension values. It is clear that the surface tension-concentration relations are characterized by distinguishable two regions. The first region at lower concentration range and characterized by a fast decrease in the surface tension values. The second region located at higher concentrations at which the surface tension values remains almost constant.

The depression in the surface tension of the surfactants solutions by dissolving the surfactants in the aqueous medium in the first region is related to the amphipathic structures of the surfactant molecules. Surfactant

molecules have organic and polar parts. The interaction between the aqueous medium and these two parts leads to locate the surfactant molecules at the air/aqueous interface, and consequently decreases the surface tension of the solution. Increasing the surfactant concentration increases the adsorbed molecules at the interface and further increase forced the molecules to locate in the bulk of the solution, which accompanied by stability in the surface tension (the second region). The intercept between the two regions indicates the critical micelle concentration values (CMC) of each surfactant [24].

It is clear from Figure 1 that the decreasing in the number of the polyethylene glycol units attached to the surfactant molecules from 9 units (PEG-400) to 23 units (PEG-1000) decreases the surface tension values.Decreasingthe number of polyethylene glycol units in the molecule decreases their tendencies toward dissolving in the aqueous medium, which increases their adsorption at the interface. The adsorption of surfactant molecules with short chain PEG at the interface decreases the surface tension of the solutions considerably. The adsorption of the surfactant molecules at the interface is decreased by decreasing the PEG chains due to the decrease in the number of hydroxyl groups in the chains [24]. As it obvious from Figure 1 that DSB-400 has the lowest surface tension values than DSB-600 and DSB-1000 surfactants in their solutions [25].

The critical micelle concentration values (CMC) of the prepared nonionic surfactants were determined by the intercept of the pre- and post-micellar regions in Figure 1. These values are listed in Table 1. As a general observation, CMC values depend on the number of polyethylene glycol units attached to the different molecules. The gradual increase of the polyethylene glycol units in the surfactant molecules increases the CMC values considerably from 0.372 to 0.794 mM at 25 °C.

The effectiveness of the surface-active agent (π_{cmc}) is defined as the difference between the surface tension value of the surfactant solution at the CMC and that of the bidistilled water at constant temperature, and can be calculated from the relation:

$$\gamma_{cmc} = \gamma_o - \gamma_{cmc}$$

where, γ_o is the surface tension of the bidistilled water, γ_{cmc} is the surface tension at CMC.

Increasing the effectiveness value indicates the high surface activity of the surfactant. It is clear from Table 1 that DSB-400 has the highest surface activity among the prepared surfactants.

The efficiency values (Pc_{20}) is defined as the concentration of the surfactant solution that decreases the surface tension to 51 mN/m and were calculated for the prepared nonionic surfactants using the data in Figure 6. The Pc_{20} values describe the ability of these molecules to adsorb at the interface and also indicate the accumulation extent of the surfactant molecules at the interface. Data in Table 1 revealed that the increase in the polyethylene glycol chain length from 9 to 23 units decreases the adsorption of the nonionic surfactant molecules at the interface. The lowest Pc_{20} value was observed for DSB-

400 indicating their higher surface activity than DSB-600 and DSB-1000 nonionic surfactants.

The maximum surface excess of the surfactant solution (Γ_{max}) is defined as the concentration of the surfactant molecules at the interface near the critical micelle concentration. The maximum surface excess values of the synthesized di-Schiff base nonionic surfactants were calculated using Gibb's-Duhem treatment [26], Table 1:

$$\Gamma_{max} = [d\gamma/dlogC] / [2x2.303RT]$$

where, $d\gamma/dlogC$ is the slope of the surface tension profile in the steeper region, R is the universal gas constant and T is the absolute temperature.

While, the average area occupied by each surfactant molecule at the air/water interface (A_{min})was calculated according to the formula [26]:

$$A_{min} = 1 / [N_A.\Gamma_{max}]$$

where, N_A is Avogadro's number and Γ_{max} is the maximum surface excess at constant temperature.

Increasing the maximum surface excess values indicates the increasing number of adsorbed molecules at the interface per cm^2; hence the area available for each molecule will decrease. That causes the compacting of surfactant molecules at the interface to form denser layer. The maximum surface excess values of the prepared nonionic surfactants were decreased by decreasing the nonionic chain length of the polyethylene glycols. The increase of the maximum surface excess indicates the small area available for each molecule at the interface as represented in Table 1. The larger area was occupied by DSB-400, which indicated the expanding geometry of the molecules. DSB-1000 has the lowest area at the interface which indicated the compactness of its adsorbed molecules at the air/waterinterface.

Thermodynamics of Adsorption and Micellization

The chemical structure of surfactant molecules is characterized by the presence of a charged head group (one, two or more) and long alkyl chains. That structure is called amphipathic structure. This combination acquires the molecule some special characteristics. Which are the ability to presence in the bulk of the solution (throughout micelles formation), and the ability to adsorb at the air/water interface to form arranged monolayer. Hence, it is expected that these two processes of adsorption and micellization occurred in the same time. The dominance of the two processes is decided by the free energy changes. Hence, the free energies of adsorption (ΔG_{ads}) and micellization (ΔG_{mic}) were calculated using the surface activity data in Table 1, according to Rosen's methodology [27]:

$$\Delta G_{mic} = -2.303RTlog (CMC)$$
$$\Delta G_{ads} = \Delta G_{mic} - (0.6023 X \pi_{cmc} X A_{min}).$$

where, R is the universal gas constant, T is the absolute temperature, π_{cmc} is the effectiveness, and A_{min} is the minimum surface area.

The free energy changes of micellization and adsorption showed negative sign indicating the spontaneously of the two processes at 25°C. Also, ΔG_{mic} decreased gradually by decreasing the polyethylene glycol chain lengths. But, ΔG_{ads} has slight increase in negativity than ΔG_{mic}. The

maximum depression in ΔG_{mic} and ΔG_{ads} was observed at -19.57 and -24.68Kj/mole for DSB-400. That showed the higher tendency of the prepared nonionic surfactants towards adsorption rather than micellization. The tendency towards adsorption is referred to the interaction between the aqueous phase and the organic parts in the surfactant molecule, which pumps these molecules to the interface.

Table (1): Surface and thermodynamic parameters of the prepared nonionic surfactants in their solutions at 25 °C

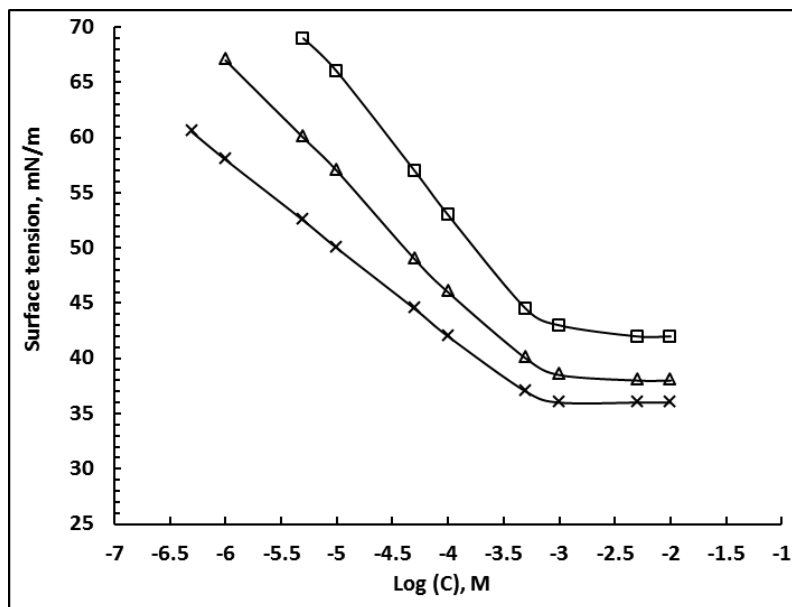| Surfactant	CMC, mM	π_{cmc}, mN/m	Pc$_{20}$, M	Γ_{max} (10^{10}), Molec./cm^2	A$_{min}$, A^2	ΔG_{mic}, kJ/mole	ΔG_{ads}, kJ/mole
DSB-1000	0.794	28.6	0.148	10.88	152	-17.15	-19.77
DSB-600	0.531	33.3	0.033	8.96	185	-18.69	-22.40
DSB-400	0.372	35.5	0.007	6.92	239	-19.57	-24.68

Fig (6): Surface tension vs. log concentration of the prepared nonionic di-Schiff base surfactants: □:DSB-1000, Δ: DSB-600, ×: DSB-400, at 25 °C.

Biological activity

Tables 2-3 show the antibacterial and antifungal activities of the prepared nonionic di-Schiff basesurfactants, which were evaluated by a well diffusion method against Gram-positive, Gram-negative bacteria and fungi. Cetyl trimethyl ammonium bromide (CTABr) and Grisofluvine were taken as the reference drugs for antibacterial and antifungal activities. The biological activities of the reference compounds gave 11.6 and 12.3 mm well diameter for fungi and bacteria, respectively. The synthesized nonionic di-Schiff base surfactants showed higher antifungal and antibacterial activities. The nonionic polyethylene glycol chain length has a remarkable influence on the biological activities of the targeted compounds. Decreasing the nonionic chain length increases the antifungal activity to great extent, while the antibacterial activities were increased gradually. These results are in consistence with the adsorption tendency of these surfactants at the interfaces, Table 1. The highest antibacterial and antifungal activities were observed for DSB-400 surfactant.

Several researchers were dealt with the mode of action of that type of compounds on different microorganisms [28-29]. The most preferable explanation is the surface adsorption mechanism. That is the adsorption of amphiphile molecules on the outer cellular membrane of the microorganism due to their amphipathic characteristics. In addition, the similarity between the hydrophobic chains and the lipid layers and the building units of the cell membranes and the alkyl chains in these compounds [30] facilitates that adsorption. The adsorption of these molecules at the cellular membranes reaches its maximum extent at higher dose (5mg/mL) leading to complete coverage of the membranes by the surfactant molecules. At the complete coverage, the molecules penetrate through it. Accordingly, the selective permeability which characterizes the outer cellular membrane is completely loosed [31]. Hence, the vital transportation of essential components for cell bioreactions and activities is disturbed. That causes death for these microorganisms [32]. The data in Table 1 describes the adsorption tendency of the synthesized nonionic di-Schiff base surfactants at the interfaces, which could be correlated to the adsorption of these derivatives on the cell membranes. Data in Table 2-3 showed higher bactericidal activities of the synthesized surfactants than that for the used standards. That indicates their higher efficiency than one of the most widely used antimicrobial drug in the pharmaceutical market.

Table (2): Bactericidal activity of the synthesized nonionic di-Schiff base surfactants at 5mg/mL

Compound	Inhibition zone diameter, mm *			
	E-coli	Salmonella typhi.	Staphylococcus aur.	Bacillus sub.
DSB-400	27.9	14.2	13.5	26.2
DSB-600	17.5	14.0	13.0	13.7
DSB-1000	13.0	13.5	13.0	13.0
CTABr	12.3	12.3	12.3	12.3

Table (3): Fungicidal activity of the synthesized nonionic di-Schiff base surfactants at 5mg/mL

Compound	Inhibition zone diameter, mm *	
	Aspergillus niger.	Aspergillusflauv.
DSB-400	24.5	25.0
DSB-600	14.5	18.0
DSB-1000	11.2	18.0
Grisofulvine	11.6	11.6

* The indicated inhibition zone diameters are average for three replicates

3. Conclusions

The study succeeded in preparation of three nonionic di-Schiff base surfactants with good surface activity. These surfactants can be used in several applications including antimicrobial field. The antimicrobial studied showed high activity towards the prevention of the microbial growth. The activity was increased by decreasing the nonionic chain length of the polyethylene glycol chains.

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