

# Synthesis, Surface Properties and Antimicrobial Activity of Some Germanium Nonionic Surfactants

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**Abstract:** Esterification reaction between different fatty acid namely; lauric, stearic, oleic and linoleic acids and polyethylene glycol-400 were performed. The produced polyethylene glycol ester were reacted with *p*-amine benzoic acid followed by condensation reaction with germanium dioxide in presence of sodium carbonate to form desired germinate surfactants. The chemical structures of the synthesized surfactants were determined using different spectra tools. The surface parameter including: the critical micelle concentration (CMC), effectiveness ( $\pi_{cmc}$ ), efficiency ( $Pc_{20}$ ), maximum surface excess ( $\Gamma_{max}$ ) and minimum surface area ( $A_{min}$ ), were calculated from the surface tension measurements. The synthesized surfactants showed higher surface activity. The thermodynamic parameters showed that adsorption and micellization processes are spontaneous. It is clear that the synthesized nonionic surfactants showed their tendency towards adsorption at the interfaces and also micellization in the bulk of their solutions. The synthesized surfactants were tested against different strain of bacteria using inhibition zone diameters. The synthesized surfactants showed good antimicrobial activities against the tested microorganisms including Gram positive, Gram negative as well as fungi. The promising inhibition efficiency of these compounds against the sulfate reducing bacteria facilitates them to be applicable as new categories of sulfate reducing bacteria biocides.

**Key words:** germinate surfactants, surface activity, antimicrobial activities, sulfate reducing bacteria

## 1 Introduction

To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of urgency. Many of the crude drugs, which are sources of medicinal preparations, still originate from wild growing material. In this order, developments are done to produce new antibiotics to resist the growth of the different bacterial genera<sup>1-3</sup>.

On the other hand, sulfate-reducing bacteria have been treated as phenotypic group which are found in different phylogenetic lines. Sulfate reducing bacteria get their energy from reducing elemental sulfur to H<sub>2</sub>S. They grow inside septic tank lines and cause rapid deterioration of concrete mains and the reinforcing steel there in. The resistance route against these types of bacteria is based on using efficient biocides. These biocides are either surfactant compounds or metal complexes<sup>4</sup>.

Hence, selection and application of good biocide is needed in petroleum product pipelines. Studies of surfactants as biocides showed a strong relationship between their surface and biological activity. Increasing the surface activity was accompanied in several studies by a strong

biocidal activity, and vice versa. That was referred to action mode of these compounds as biocides towards the different microorganisms. Several works described the action mode of the surfactants as biocides<sup>5,6</sup>.

Metallo micelles were found effective biocide for different types of bacteria including SRB<sup>7</sup>. Germanium and its compounds were used as dietary supplements since 1970s. It also induce erythropoietic, antimicrobial or immunomodulating activities, anti-viral activity, immunity enhancement, oxygenen richment, free radical scavenging, heavy metal detoxification, induction of interferon, macrophages and T-suppressor cells and augmentation of natural killer cell activity<sup>8</sup>.

In this work, a series of germinate nonionic surfactants were synthesized (**Scheme 1**) and evaluated for their potent action against different bacterial strains including pathogenic bacteria, fungi and sulfate reducing bacteria (SRB). The synthesized germanium based surfactants showed high potency against the tested microorganisms. The results were compared with erythromycin/metronidazole drug.

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## 2 Experimental technique

### 2.1 Chemicals

Reagent grade germanium dioxide, *p*-amine benzoic acid and polyethylene glycol-400 were purchased from Alfa Chemical Company. Lauric, stearic, oleic and linoleic acids were analytical grade chemicals were obtained from Aldrich chemical company (Germany). All the reagents were analytical grade and used as received. Organic solvents were used without further purification.

### 2.2 Synthesis

#### 2.2.1 Synthesis of polyethylene glycol ester

lauric, stearic, oleic and linoleic acid (0.1 mol.) and polyethylene glycol-400 (9 U of ethylene glycol per molecule) were esterified individually in xylene (250 mL) as the solvent under reflux conditions at 138°C and 0.01% *p*-toluene sulphonic acid as a catalyst, until the azeotropic amount of water (0.1 mol., 1.8 mL) was removed. Then the solvent was removed using vacuum rotary evaporator. Subsequent purification was done by means of vacuum distillation to remove the excess and residual materials<sup>9</sup>. The product obtained was designated as in step (I) **Scheme 1**.

#### 2.2.2 Synthesis of *p*-amine benzoic acid ester

Polyethylene glycol ester (0.1 mol.) and *p*-amine benzoic acid (0.1 mol.) were esterified individually in xylene (250 mL) as the solvent under reflux conditions at 138°C and 0.01% *p*-toluene sulphonic acid as a catalyst, until the azeotropic amount of water (0.1 mol., 1.8 mL) was removed. Then the solvent was removed using vacuum rotary evaporator. Subsequent purification was done by means of vacuum distillation to remove the excess and residual materials<sup>9</sup>. The product was designated as in step (II) **Scheme 1**.

#### 2.2.3 Synthesis of germanium based nonionic surfactants

Germanium dioxide (0.01 mol.), sodium carbonate (0.01 mol.) and 50 ml of distilled water were mixed, than refluxed to 100-150°C with agitation until complete solubility of germanium dioxide. To the reaction mixture was added (0.02 mol.) of nonionic surfactant (product of step II) with continuous refluxing and stirring for 2 h<sup>10</sup>. Cooling and evaporation of water were than carried out to give crude product, which was crystallized from ethanol to yield: bis(2-(polyethoxy laurate) ethyl) 4,4'-(oxogermanediyl) bis(azanediy) dibenzoate (I), bis(2-(polyethoxy stearate) ethyl) 4,4'-(oxogermanediyl) bis(azanediy) dibenzoate (II), bis(2-(polyethoxy oleate) ethyl) 4,4'-(oxogermanediyl) bis(azanediy) dibenzoate (III), bis(2-(polyethoxy linoleate) ethyl) 4,4'-(oxogermanediyl) bis(azanediy) dibenzoate (IV), **Scheme (1)**.

### 2.3 Measurements

#### 2.3.1 Surface tension

Surface tension measurements were performed using a Kruss K6 tensiometer by the platinum ring detachment

method ( $\pm 0.5$  mN/m). Freshly prepared aqueous solutions of the synthesized surfactants were used with a concentration range of 0.05–0.00000125 M/L at 25°C<sup>11,12</sup>.

#### 2.3.2 Antimicrobial studies

The synthesized germanium based nonionic surfactants (I, II, III, and IV) were screened for their antimicrobial activity against a wide range of pathogenic bacteria and fungi using well diffusion method<sup>13,14</sup> and erythromycin/metronidazole was taken as a reference<sup>15</sup>.

##### 2.3.2.1 Source of Microorganisms

The different species of tested organisms were obtained from the unit of operation development center, Egyptian Petroleum Research Institute, Cairo, Egypt.

##### 2.3.2.2 The Media

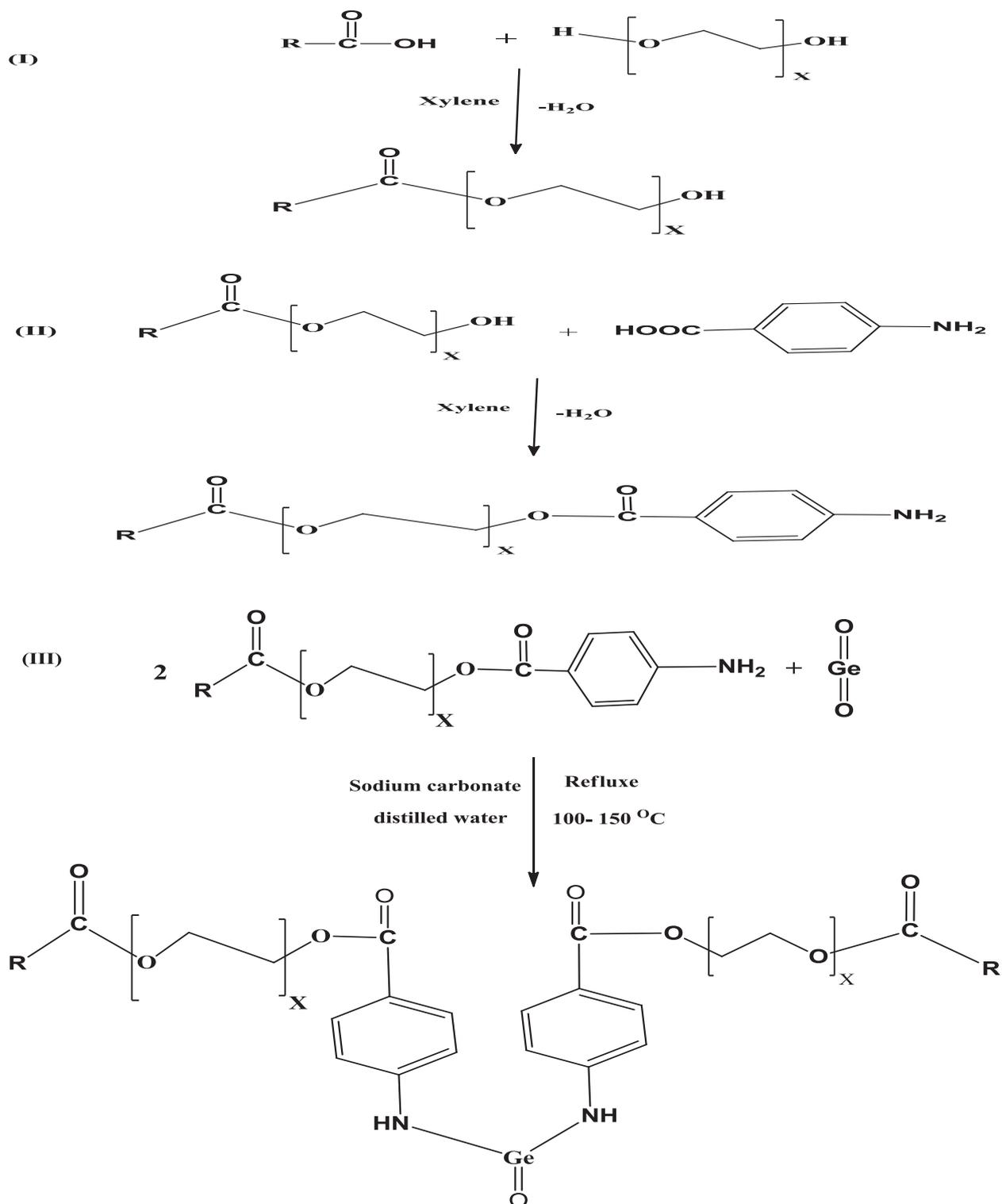
The following media used in the antimicrobial activity of synthesized products, the bacterial species grow on nutrient agar, while fungi mold grow on Czapek's Dox agar. Nutrient agar consists of Beef extract (3.0 g/l); Peptone (5.0 g/l), Sodium chloride (3.0 g/l) and Agar (20.0 g/l), then, completes the volume to one liter, heated the mixture until the boiling, and sterilizes the media by autoclave. While, Czapek's Dox agar consists of Sucrose (20.0 g/l), Sodium nitrate (2.0 g/l), Magnesium sulfate (0.5 g/l), Potassium Chloride (0.5 g/l), Ferrous sulfate (0.01 g/l) and Agar (20.0 g/l), then, complete the volume to one liter, heated the mixture until the boiling, and sterilize the media by autoclave<sup>16</sup>.

##### 2.3.2.3 Growing of microorganisms

An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms, the technique which used is: Filter-paper disc-agar diffusion (Kirby-Bauer)<sup>16</sup>. The bacterial and fungal strains were cultured according to the standards of the National Committee for Clinical Laboratory<sup>14</sup>. The diameters of inhibition zones were measured after 24-48 hours at 35-37°C (for bacteria) and 3-4 days at 25-27°C (for yeast and fungi) of incubation at 28°C, with subsequent filtering to remove mycelia fragments before the solution containing the spores was used for inoculation.

##### 2.3.2.4 Measurements of resistance and susceptibility

For preparation of discs and inoculation, 1.0 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 (6mm in diameter) were cut in the agar plates using proper sterile tubes. Then, filled up to the surface of agar with 0.1 ml solution of the synthesized compounds consists of 1mg surfactants in 1ml of DMF (DMF has negligible influence on the growth of the microorganisms). The plates were left on a leveled surface, incubated for 24 h at 30°C for bacteria and then the diameter of the inhibition zones were measured. The inhibition zone formed by these compounds against the particular test microorganisms determined the biocidal activity of the synthetic compounds. The mean value for



R= Lauric, X= 9: bis(2-(polyethoxy laurate)ethyl) 4,4'-((oxogermanediyl)bis(azanediy))dibenzoate (I)

R= Stearic, X= 9: bis(2-(polyethoxy stearate)ethyl) 4,4'-((oxogermanediyl)bis(azanediy))dibenzoate (II)

R= Oleic, X= 9: bis(2-(polyethoxy oleate)ethyl) 4,4'-((oxogermanediyl)bis(azanediy))dibenzoate (III)

R= Linoleic, X= 9: bis(2-(polyethoxy linoleate)ethyl) 4,4'-((oxogermanediyl)bis(azanediy))dibenzoate (IV)

Scheme 1 Synthesis of germanium based nonionic surfactants.

three replicates was used to calculate the zone of growth inhibition of each sample<sup>17, 18</sup>.

### 2.3.2.5 Microorganism

The used microorganisms were Gram-positive bacteria (*Bacillus Pumilus* and *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas Aeuroginosa* and *E. coli*) and Fungi (*Candida albicans* and *Aspergillus niger*). While the sulfate reducing bacteria was tested against (*Desulfomonas pigra*).

## 3 Results and Discussion

### 3.1 Structure

The chemical structures of the synthesized germinate nonionic surfactants (I, II, III and IV) were confirmed using elemental analysis, FT-IR and <sup>1</sup>H-NMR spectroscopy as follows:

**Elemental analysis:** The elemental analyses were performed for the synthesized germanium nonionic surfactants using Vario Elementar instrument. The purity of all synthesized nonionic surfactants was identified by elemental analyses. The chemical structures of the synthesized surfactants were confirmed using microelemental analyses, which showed good coincidence between the calculated and found values of C, H, N, O and Ge (%), the results were represented in Table 1.

**FT-IR spectra:** The chemical structure of the synthesized germinates nonionic surfactant was recorded by FT-IR spectra in the range 4000–500 cm<sup>-1</sup>. The FT-IR absorption spectra showed an absorption band at the 2922 cm<sup>-1</sup> region was due to the methyl symmetric stretching vibration. The adsorption band at 2849 was due to the methyl asymmetric stretching vibration. The strong adsorption band at 1735 cm<sup>-1</sup> indicating to the formation C=O ester group, whereas the peak at 1106 cm<sup>-1</sup> is due to C-O stretching absorption bands. The adsorption band at 1604 cm<sup>-1</sup> indicating to the formation Ge=O group. Band appears at 1352 cm<sup>-1</sup> due to formation Ge-N group. The adsorption band at 1697 was due to C=C group of aromatic ring whereas the peak at 1424 is due to rocking (CH<sub>2</sub>)<sub>n</sub>. The strong adsorption band appear at 3415 was due to –

NH group. The FT-IR absorption spectra confirmed that the disappearance of OH band of acid (broad band), this confirmed that esterification reaction is occurred, the replacement of H atom in amino benzoic acid by Ge atom to form Ge-N. FT-IR spectra recognized the expected functional groups in the synthesized germanium based nonionic surfactants (Fig. 1).

**<sup>1</sup>H-NMR spectra:** <sup>1</sup>H-NMR spectra of the synthesized bis (2-(polyethoxy laurate) ethyl) 4,4'-(oxogermanediyl) bis (azane diyl) dibenzoate (I) in CDCl<sub>3</sub>, showed signals at: δ = 0.84 ppm (t, 3H, CH<sub>3</sub>), δ = 1.22 ppm (m, nH, (CH<sub>2</sub>)<sub>n</sub>), δ = 1.57 ppm (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), δ = 1.96 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 2.62 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 4.02 ppm (s, 1H, ph NH-Ge), δ = 4.62 ppm (t, 2H, OCH<sub>2</sub>CH<sub>2</sub>O), δ = 7.11 ppm (s, 4H, phenyl ring) (Fig. 2, as representative sample).

**<sup>1</sup>H-NMR spectra:** <sup>1</sup>H-NMR spectra of the synthesized bis (2-(polyethoxy stearate) ethyl) 4,4'-(oxogermanediyl) bis (azaned iyl) dibenzoate (II) in CDCl<sub>3</sub>, showed signals at: δ = 0.85 ppm (t, 3H, CH<sub>3</sub>), δ = 1.23 ppm (m, nH, (CH<sub>2</sub>)<sub>n</sub>), δ = 1.83 ppm (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), δ = 2.08 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 2.60 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 3.15 ppm (s, 1H, ph NH-Ge), δ = 3.69 ppm (t, 2H, OCH<sub>2</sub>CH<sub>2</sub>O), δ = 7.25 ppm (s, 4H, phenyl ring).

**<sup>1</sup>H-NMR spectra:** <sup>1</sup>H-NMR spectra of the synthesized bis (2-(polyethoxy oleate) ethyl) 4,4'-(oxogermanediyl) bis (azan ediy) dibenzoate (III) in CDCl<sub>3</sub>, showed signals at: δ = 0.81 ppm (t, 3H, CH<sub>3</sub>), δ = 1.21 ppm (m, nH, (CH<sub>2</sub>)<sub>n</sub>), δ = 1.62 ppm (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), δ = 2.46 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 3.02 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 3.35 ppm (s, 1H, ph NH-Ge), δ = 3.78 ppm (t, 2H, OCH<sub>2</sub>CH<sub>2</sub>O), δ = 7.31 ppm (s, 4H, phenyl ring).

**<sup>1</sup>H-NMR spectra:** <sup>1</sup>H-NMR spectra of the synthesized bis (2-(polyethoxy linoleate) ethyl) 4,4'-(oxogerm anediyl) bis (azanediy) dibenzoate (IV) in CDCl<sub>3</sub>, showed signals at: δ = 0.81 ppm (t, 3H, CH<sub>3</sub>), δ = 1.21 ppm (m, nH, (CH<sub>2</sub>)<sub>n</sub>), δ = 1.96 ppm (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), δ = 2.25 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 2.28 ppm (m, 2H, CH<sub>2</sub>CH<sub>2</sub>COO), δ = 3.59 ppm (s, 1H, ph NH-Ge), δ = 4.15 ppm (t, 2H, OCH<sub>2</sub>CH<sub>2</sub>O), δ = 7.15 ppm (s, 4H, phenyl ring).

**Table 1** Microelemental analysis of the synthesized surfactants.

Comp.	M. Wt. (gm/mole)	Microelemental analysis									
		% C		% H		% N		% O		Ge	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
I	1413.75	62.88	62.44	6.85	6.80	1.98	1.97	28.30	28.10	5.14	5.10
II	1581.89	65.30	64.84	7.65	7.59	1.77	1.76	25.28	25.11	4.59	4.50
III	1577.86	65.46	65.01	7.41	7.36	1.78	1.76	25.35	25.17	4.60	4.57
IV	1573.83	65.63	65.17	7.17	7.12	1.78	1.77	25.41	25.24	4.62	4.58

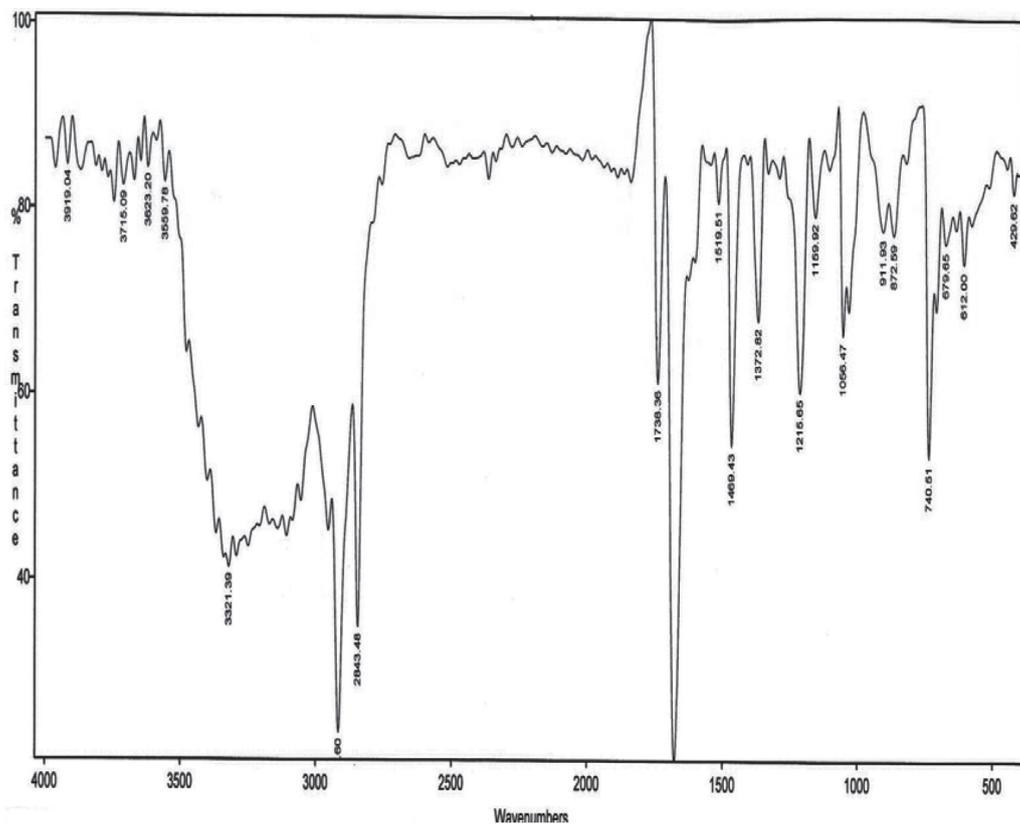


Fig. 1 FT-IR Spectrum of the synthesized germinates nonionic surfactant.

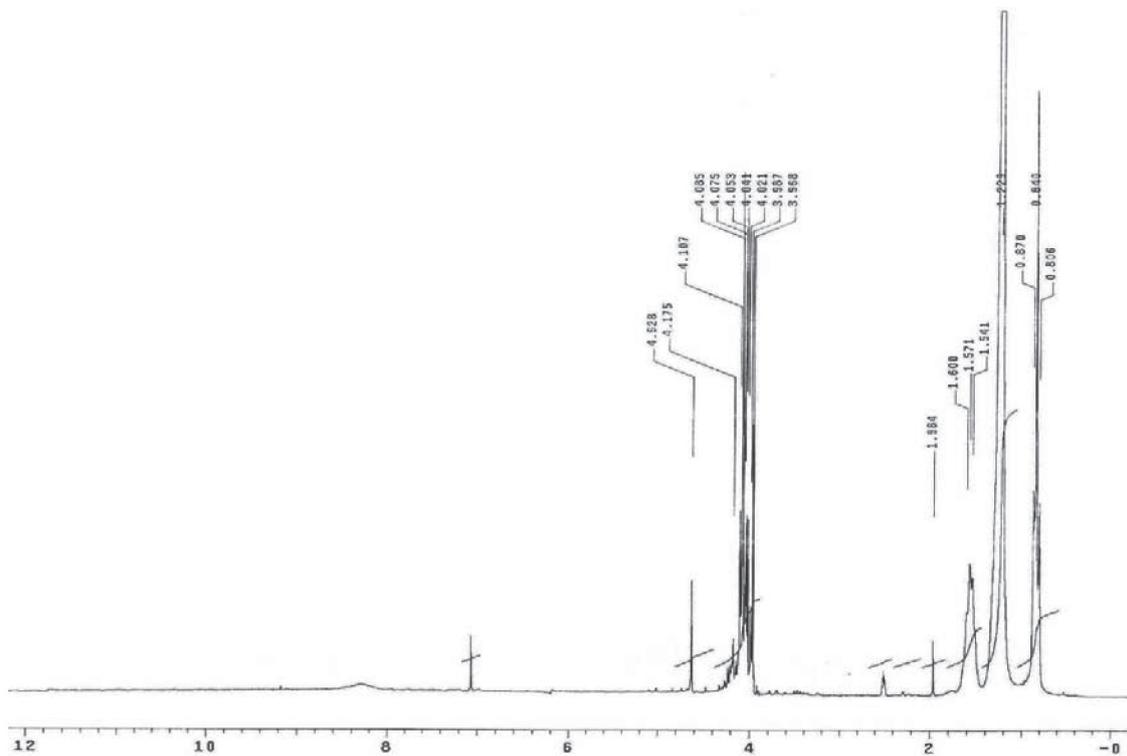


Fig. 2 <sup>1</sup>H-NMR Spectrum of the synthesized germinates nonionic surfactant.

### 3.2 Surface activity

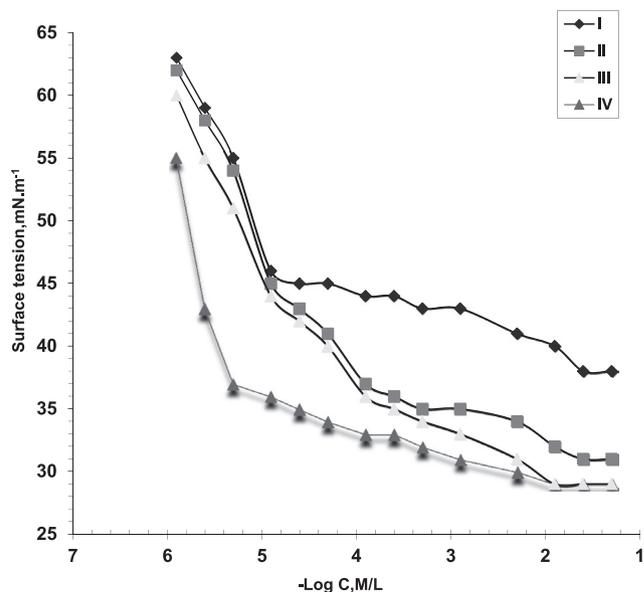
The surface tension values are gradually decreased by increasing the surfactant concentration. That indicates the accumulation of the surfactant molecules at the air/water interface by increasing the concentration.

There are two distinguishable regions in the surface tension- concentration profile. The intercept between these two characteristic regions on the profile produces a so called critical micelle concentration. This value is characteristic for all type of surfactants and completely dependent on the chemical structure of the surfactant used.

The repulsion force came from the polar phase on the hydrophobic chains and the attraction force between the water molecules and the partially charged poly ethylene glycol nonionic chains. The former force suppresses the molecules adsorbed at the air–water interface to decrease the repulsion and consequently decreases the high energy situation.

Figure 3 represents the surface tension vs.  $-\log C$  relationship of the synthesized nonionic surfactants (I, II, III, and IV) at 25°C.

From the surface tension data the micelle formation is the most vital point of view in the surfactant fundamentals. The surfactant molecules when dispersed in water tend to be adsorbed at the interface, leading to decrease the surface tension of the solutions at low concentration ( $1.25 \times 10^{-6} - 5 \times 10^{-3}$  M/L) and surface tension values ranged 30–63 mN/m for synthesized compounds. Further increase in the concentration is followed by a gradual reduction of the surface tension until the surface of the solution becomes completely occupied by the surfactant molecules, after which the excess molecules tend to self-aggregate in the bulk of the solution, thus forming micelles at high concentration ( $1.25 \times 10^{-2}$  M/L) and surface tension values equal 40, 32, 29, 29 mN/m for I, II, III, IV, respectively. This concentration is called the critical micelle concentration, abbreviated as CMC. There is equilibrium between the single states adsorbed molecules and the aggregated structure. The shape of the micelles formed depends on several factors and produces different aggregative structures including spherical (a), rod (b), bilayer (c), vessels (d) and finally reversed (e) at concentration,  $2.5 \times 10^{-2} - 5 \times 10^{-2}$



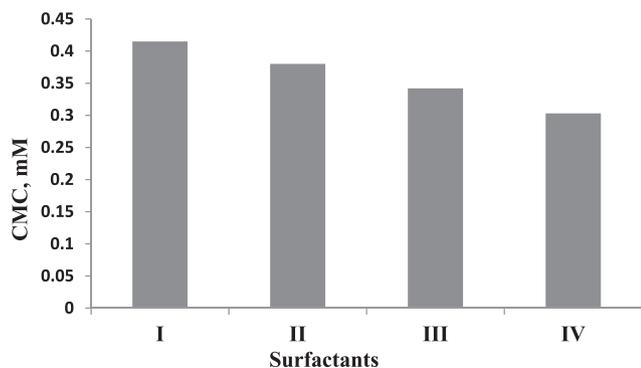
**Fig. 3** Surface tension vs.  $-\log$  concentration of germinates nonionic surfactant (I) at different temperature.

M/L and surface tension values are 38, 31, 29, 29 mN/m for compounds I, II, III, IV, respectively.

Also, it is clear that the surface tension values decreased by increasing the hydrophobic chain length to reach the minimum value for the linoleic derivative (IV). Increasing the hydrophobic chain length of the surfactant molecules increases their surface activity. Increase the hydrophobicity of the synthesized surfactant with increase the carbon chain length and unsaturation sites lead to pumps the surfactant molecules to the surface which leads to the depression of their surface tension values. The increasing of the side chain length of the synthesized surfactants and unsaturation sites (I, II, III and IV) decreases their critical micelle concentration (CMC) values significantly (Table 2). The lowest CMC value obtained was 0.30 mmol/L for linoleic derivative (IV) while the highest was 0.42 mmol/l for lauric derivative (I) at 25°C (Fig. 4). On the other hand, the effectiveness values ( $\pi_{cmc}$ ) (the depression of the surface tension at the CMC) were increased gradually by increasing the hy-

**Table 2** Critical micelle concentration (CMC), effectiveness ( $\pi_{cmc}$ ), efficiency ( $Pc_{20}$ ), maximum surface excess ( $\Gamma_{max}$ ) and minimum surface area ( $A_{min}$ ) of the synthesized germanium based- nonionic surfactants at 25°C.

Compound	CMC, mM	( $\pi_{cmc}$ )	( $Pc_{20}$ )	$\Gamma_{max} \times 10^{-11}$ , mol.cm <sup>-2</sup>	$A_{min}$ , nm <sup>2</sup> molecule <sup>-1</sup>
I	0.42	30.0	5.2	1.695E-10	97.97
II	0.38	32.0	5.0	1.29E-10	128.70
III	0.34	37.5	4.9	5.77E-11	287.75
IV	0.30	40.0	4.5	4.143E-11	400.79



**Fig. 4** The CMC values of the synthesized surfactants at 25°C.

drophobic chain lengths of the synthesized surfactants. The highest depression in the surface tension at CMC was observed 40 mN/m for the linoleic derivative (Table 2). Efficiency values are used to differentiate between two surfactants each one belongs to a different surfactant series. This parameter can differentiate between two types of surfactants, cationic/cationic, anionic/anionic or cationic / anionic, etc. The higher  $Pc_{20}$  values indicate more surface active surfactants, and vice versa. Within same surfactant series, this fact is also found. Comparing the obtained  $Pc_{20}$  values of the synthesized germanium nonionic surfactants ( $Pc_{20}$  for synthesized compound **I** = 5.2, **II** = 5, **III** = 4.9 and **IV** = 4.5 mM) and the traditional nonionic surfactants as reported by Negm *et al.*<sup>19</sup> in synthesis and surface properties of some nonionic Schiff base surfactants ( $Pc_{20}$  for traditional nonionic surfactants, SBG-10 = 1.37, SBG-12 = 1.60, SBG-16 = 0.60, SBG-18 = 0.52 and SBG-olaete = 0.21 mM). Also, efficiency of other nonionic surfactants studied by several works as reference<sup>20, 21</sup>, it can be concluded that the synthesized surfactants are more surface active than the common nonionic surfactants at 25°C.

The maximum surface excess,  $\Gamma_{max}$ , describes the accumulation of surfactant molecules at the air-water interface, and can be calculated according to the reference of<sup>22</sup> using  $(\partial g / \partial \log C)$  term.

$$\Gamma_{max} = 1/2.303 nRT (\partial \gamma / \partial \log C) \quad (1)$$

Where  $R$  = gas constant (8.314) and  $T = t + 273$  (°K), the value of  $n$  in the Gibbs equation is the number of ionic species whose concentration at the interface varies with the surfactant concentration in the solution.

Increasing the maximum surface excess (the accumulation of surfactant molecules at the air/water interface,  $\Gamma_{max}$ ) indicates the surfactant/water repulsion in the bulk of the solution which pumps the surfactant molecules to the air/water interface. Longer hydrophobic chain lengths showed lower  $\Gamma_{max}$  values (Table 2).

The maximum surface excess values were used to calculate the average area occupied by single surfactant mole-

**Table 3** Thermodynamic parameters of adsorption and micellization for the synthesized germanium based- non-ionic surfactants at 25°C.

Compound	$\Delta G_{ads}$ , kJ/mol	$\Delta G_{mic}$ , kJ/mol
I	-21.0603	-19.2969
II	-21.9863	-19.5152
III	-26.2479	-19.7734
IV	-29.6953	-20.0764

cule at water-air interface ( $A_{min}$ ) using equation (2)<sup>23</sup>.

$$A_{min} = 10^{14} / N_A \Gamma_{max} \quad (2)$$

where  $N$  is Avogadro's number.

The calculated area occupied by surfactant molecule at the interface ( $A_{min}$ ) is greatly influenced by the hydrophobic chain length. Increasing the hydrophobic chain length increases  $A_{min}$  values at the interface (Table 2).

### 3.3 Thermodynamics of Adsorption and Micellization

The thermodynamic data of adsorption and micellization of the synthesized surfactants in their solution were calculated using the following equations<sup>24</sup>, and data are summarized in Table 3:

$$\Delta G_{mic}^0 = 2.303RT \log CMC \quad (3)$$

$$\Delta G_{ads}^0 = \Delta G_{mic}^0 - (0.06 \pi_{cmc} A_{min}) \quad (4)$$

where  $R$  is the universal gas constant (= 8.314 J molK<sup>-1</sup>),  $T$  the absolute temperature,  $\pi_{cmc}$  is the effectiveness and  $A_{min}$  is the minimum surface area.

It is clear that for synthesized nonionic surfactant (I, II, III, and IV) the free energies of micellization and adsorption ( $\Delta G_{mic}$  and  $\Delta G_{ads}$ ) have negative sign indicating that the two processes are spontaneous. Comparing the two values showed slight increase of  $\Delta G_{ads}$  than  $\Delta G_{mic}$ . The more negativity of  $\Delta G_{ads}$  values indicates the adsorptive tendency of these surfactants than micellization tendency due to the preference of adsorption is governed by the thermodynamic stability of the molecules at the air-water interface.

The micellization free energies of the synthesized surfactants in their aqueous media showed a negative sign, indicating a spontaneously micellization process. The driving force of micelle formation is the repulsion occurring between the hydrophobic chains and the polar medium. The stabilization of the formed micelles occurred due to the hydrogen bonds which were formed between the polyethylene glycol groups and the water molecules<sup>25</sup>.

Increasing hydrophobic chain length increases the negativity of the  $\Delta G_{ads}$  values and consequently their adsorption tendency at interface.

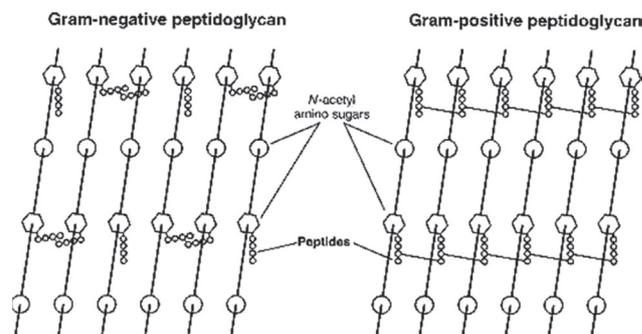
The closeness between the two values ( $\Delta G_{mic}$  and  $\Delta G_{ads}$ )

reveals the equilibrium between the two phases of the surfactant molecules (adsorbed and micellized phase). Also, the slight increase of  $\Delta G_{\text{ads}}$  values showed that these molecules tend to adsorb at the air–water interface till complete surface coverage is reached, then, the micelles formed. Hence, the micellization and adsorption processes are governed by the thermodynamic aspects. The chemical structure of these molecules is the main factor influencing their thermodynamic aspects. The equivalence between adsorption and micellization tendencies qualifies these surfactants as being applicable in the interfacial applications including emulsification, corrosion inhibition, and biocidal application<sup>26)</sup>.

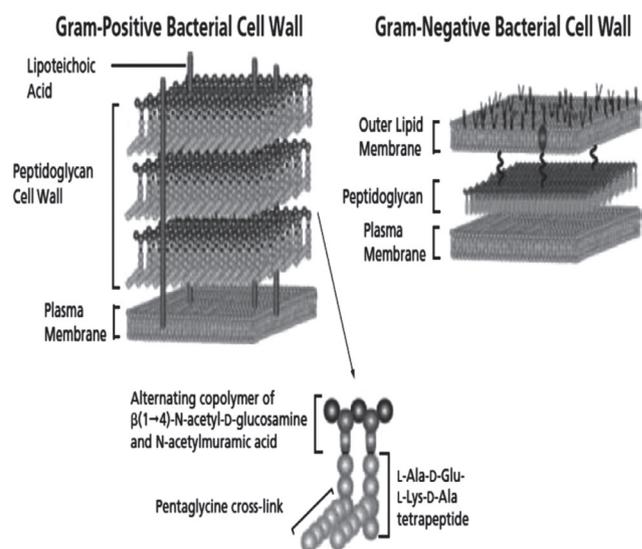
### 3.4 Evaluation of the synthesized surfactants as antibacterial and antifungal agents

Phospholipids and specific amino acids considered the main combination of the bacterial cell membrane. The functionality of the cellular membrane is mainly the diffusion of the material necessary to the biological reactions and excretion of the produced wastes from them. The selective permeability is determined by controlling of the two processes. The controlling factor of the selectivity of the polar species to enter or get out from the cell is the charged amino acids (teichoic acid) and the controlling of the nonpolar materials is the phospholipids and peptidoglycans (Fig. 5). When the selective permeability of the cellular membrane is disturbed for any reason, the biological reactions and activities in the cell is disturbed which leads to the death of the microorganism Gram positive and Gram negative (Fig. 6). The role of the biocides of bacteria and fungi is to disturb and/or destroy the selective permeability of these membranes in order to kill these microorganisms<sup>27, 28)</sup>.

The antimicrobial activities of the synthesized germinates nonionic surfactants (I, II, III and IV) was screened against pathogenic Gram-positive bacteria (*Bacillus Pumilus* and *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas Aeuroginosa* and *E. coli*) and Fungi (*Candida albicans* and *Aspergillusniger*). While the sulfate reducing bacteria was tested against (*Desulfomonas pigra*) using the values of the inhibition zone diameter tests. Indicating that the synthesized compounds have antimicrobial activities, and the difference in their activities depends on the length of hydrophobic chains of prepared surfactants. The optimal length of alkyl chain has been noted to be twelve carbon atoms, which exhibit the maximum inhibition zone. these results are in agreement with results obtained previously, which indicates that the optimal activity toward a variety of bacterial species for numerous structural variations of water soluble surfactants occurs with an alkyl chain between ten and fourteen carbon atoms. These results are in agreement with results reported elsewhere<sup>29–33)</sup>. The presence of the alkyl hydrophobic chains in the biocide structure increases the ad-



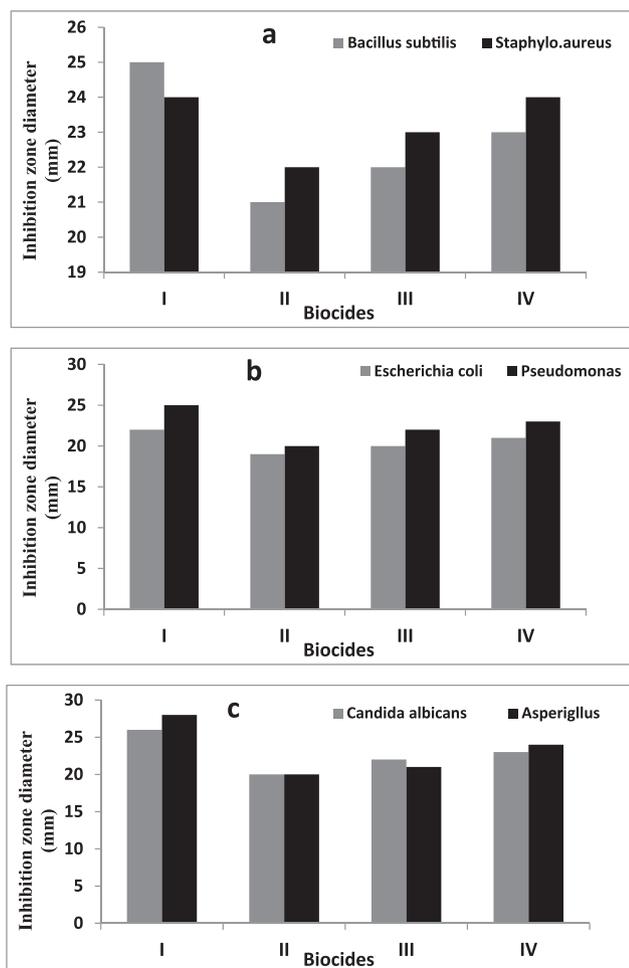
**Fig. 5** The structure of the peptidoglycan in the different bacterial genera.



**Fig. 6** Structure of the bacterial cell walls.

sorption of these biocides to the cellular membranes of the microorganism due to the similarity between these chains and the alkyl chains in bacterial lipid layers. Lipids are the class of large biological molecules that does not include polymers. They are grouped together because they have one important chemical property; they have little or no affinity for water. The hydrophobic behavior of lipids is based on their molecular structure. Also, it is believed that interaction with the surface of the microorganism is a function of the hydrocarbon tail subsequently becomes integrated with the lipid bilayer of the cell membrane. This integration causes a disruption in the membrane and inevitably causes leakage of the cell contents. The length of the alkyl chain of the surfactants is thought to contribute to the extent of this membrane disruption, because the higher chain lengths may be incorporated into the lipid bilayers of the plasma membrane. The increased hydrophobic effect of these longer chain tails may aid in this disruption<sup>34, 35)</sup>.

The efficiency of the biocides under investigation can be attributed to the interaction between the hydrophobic chains and the external cellular membrane of the bacteria



**Fig. 7** Bactericidal activity of the synthesized inhibitors against: (a) *Staphylococcus aureus*, *Bacillus subtilis*; (b) *Pseudomonas seruginosa*, *E. Coli*; and (c) *Candida albicans*, *Aspergillus niger*.

or fungi. The interaction is increased by increasing the adsorption tendency of these biocide molecules, which is occurred as a result of the chain length presence. In addition to, the presence of germanium metal in the chemical struc-

tures of the synthesized nonionic surfactant enhances the penetration into lipid membranes and blocking of metal binding sites on the enzymes of the microorganism and consequently, the biocidal activity increased.

Figure 7a, b represents the antibacterial activities of the four germanium nonionic surfactant against the tested bacterial strains (*Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*). For *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, the dodecyl derivative (I) showed the maximum inhibition zone diameter of 24, 22, 25 and 25 mm. The high antibacterial activities of the synthesized germanium nonionic surfactants can be referred to several factors including chemical structural factors and surface factors. The structural factors include the aliphatic hydrocarbon chains side, the benzene ring nucleus, germanium oxide groups and polyethylene oxide head groups. The surface factors include the effectiveness, efficiency, surface pressure and surface area. Furthermore, the high surface activities of these inhibitors ease the interaction between the environmental components and the cellular membrane throughout depressing their selective permeability. Hence, the biological action and the metabolism pathways are disturbed in the microorganisms which lead to their death. The most compatible hydrocarbon chain length with bacterial lipids is the dodecyl chain length

Figure 7c represents the antifungal activities of the synthesized germanium nonionic compounds against two potent fungal strains *Aspergillus niger* and *Candida albicans*. The values of the inhibition zones are ranged between 20 and 28mm that indicates the cytotoxic efficacy of these germinate surfactants against the studied fungi (Table 4). The inhibition diameter values showed that the cytotoxic efficacies of these compounds are strongly related to their surface properties.

### 3.5 Biocidal activity against sulfate reducing bacteria (SRB)

The synthesized surfactants (I, II, III and IV) were evaluated for their cytotoxic action against sulfate reducing bac-

**Table 4** Antimicrobial activity of the germanium based- nonionic surfactants against pathogenic bacteria and fungi.

Compounds	Inhibition zone diameter (mm/mg sample)					
	<i>Bacillus subtilis</i>	<i>Staphylo. aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeuroginosa</i>	<i>Candida albicans</i>	<i>Asperigillus niger</i>
Erythromycin/metronidazole	34/-	42/-	30/-	30/-	-/25	-/27
I	25	24	22	25	26	28
II	21	22	19	20	20	20
III	22	23	20	22	22	21
IV	23	24	21	23	23	24

**Table 5** Antimicrobial activity of the germanium based- nonionic surfactants against sulfate reducing bacteria.

Compounds	( <i>Desulfomonas pigra</i> ) (SRB) Inhibition zone diameter (mm/mg sample)
Control (DMSO)	0
I	13
II	11
III	12
IV	12

terial (*Desulfomonas pigra*, Table 5). Comparison between the inhibition zone diameters of the tested Gram positive, Gram negative and *D. pigra* (Gram-negative) regarding the antibacterial activities of the synthesized biocides, their efficacies against sulfate reducing bacteria were found in considerably high values. But, these values are still in lower values than the other tested bacterial strains (Table 4). That may be ascribed to the resistivity of the SRB bacterial strain to the aggressive environmental components due to the rigidity of their cellular membranes the sulfate 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 considered a promising step in the biocidal field<sup>36</sup>.

The cytotoxic efficacy of the synthesized surfactants was represented good inhibition tendency towards the different bacterial genera and fungi. The inhibition tendency can be ascribed to the unique chemical structure of these compounds due to the presence of the hydrophobic chain length and the ethylene oxide groups. The presence of these groups increases the adsorption of the molecules at the microorganism's cellular membrane and the presence of the germanium metal penetrate easily thorough these membranes. Thence the biological reaction in the cells strongly affected and the fast death for these microorganisms is occurred.

#### 4 Conclusion

The main conclusions of the study may be summarized in the following points:

1. The synthesized surfactants showed higher surface activity.
2. The thermodynamic parameters of micellization and adsorption indicating that the two processes are spontaneous.
3. The synthesized surfactants were tested against different strain of bacteria using inhibition zone diameters and showed good antimicrobial activities against the tested microorganisms.

4. The growth of sulfate reducing bacteria is inhibited in presence of the synthesized biocides.

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