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FULL LENGTH ARTICLE

Surface parameters, biodegradability and antimicrobial activity of some amide ether carboxylates surfactants

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Abstract In the present investigation, a series of amide ether carboxylates surfactants RCONHCH$_2$CH$_2$O (CH$_2$CH$_2$O)$_6$CH$_2$COONa (AEC), with different alkyl chain lengths from (C_{12} to C_{18}) and ($C_{18=}$, $C_{18==}$) were synthesized.

The surface parameters particularly effectiveness (Π_{cmc}), efficiency (P_{C20}), maximum surface excess (Γ_{max}) and minimum area per molecule (A_{min}) values were investigated. In addition, the standard free energies of micellization (ΔG^0_{mic}) and adsorption (ΔG^0_{ads}) were calculated for the prepared surfactants in aqueous solution. The prepared surfactants were tested for their biodegradability in the water of the River Nile according to the Die-away test method. Their antimicrobial activity against strains of bacteria, yeast and fungi were also investigated.

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1. Introduction

With the recent growing tendency toward products that are safe to the human body, various attempts have been made to relieve the irritation caused by the detergent on the skin which continuously or frequently comes into contact directly with the human body. Amide ether carboxylates, which are known as less irritating surfactants, give no squeaky feeling at use. However they give a serious slippery feeling characteristic of anionic surfactants [1].

Examples of known techniques related to the application of amide ether carboxylates surfactants to detergents include a cosmetic composition containing an amide ether carboxylates [2], a detergent composition wherein an amide ether carboxylates surfactant is used together with a polyoxyethylene alkyl sulphate, an amide ether carboxylic acid obtained from fat and detergent containing the same [3] and a detergent containing a soap as the main component together with an amide ether carboxylic acid and an alkyl ether carboxylic acid salt [4].

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The aim of the present investigation is to prepare a series of amide ether carboxylates type surfactants with different alkyl chain lengths and studying some of their surface parameters and thermodynamic standard free energies (ΔG^0_{mic} and ΔG^0_{ads}). The biodegradability and the correlation with the antimicrobial activity of these compounds were also studied.

2. Experimental procedures

A series of amide ether carboxylates were synthesized with different alkyl chain lengths from C_{12} to C_{18}, $C_{18=}$ and $C_{18==}$, by esterification, amidation, ethoxylation and carboxymethylation reaction steps of the corresponding fatty acids (Lauric, Myristic, Palmitic, Stearic, Oleic, Linolinic) [5].

Surface tensions of the prepared surfactants in aqueous solutions were measured at 25 °C using the platinum ring method (Tensiometer-K6, Krüss Company, Germany).

3. Biodegradability

Biodegradability Die-away test in River Nile water of the anionic surfactant was determined by the surface tension method [6,7]. In this test each surfactant was added at a level of 50 ppm, then the solution was incubated, samples were withdrawn daily, and filtered before measuring their surface tension value. This process was repeated for 7 days. From the surface tension measurements, the biodegradation percent (D%) was calculated as follows:

$$D = (\gamma_t - \gamma_0)/(\gamma_{bt} - \gamma_0) \times 100 \quad (1)$$

where: γ_t = surface tension at time t (day), γ_0 = surface tension at time 0 (initial surface tension), γ_{bt} = surface tension of the blank experiment at time t.

4. Antimicrobial activity

The antimicrobial activity of the synthesized products was measured against a wide range of test organisms.

4.1. Source of microorganisms

The tested organisms were obtained from the unit of Micro Analytical Center, Cairo University, Cairo, Egypt.

4.2. The media

The bacterial species grow on nutrient agar, consist of 3 g/l beef extract, 5 g/l peptone, 3 g/l sodium chloride and 20 g/l agar. Then, the mixture was heated to boil and sterilized in an autoclave.

4.3. Measurement of antimicrobial activity using diffusion disc method for bacteria and fungi

Most of the synthesized compounds were evaluated for their antimicrobial activity using the agar diffusion technique (5 mg/ml solution in dimethyl formamide was used).

A filter paper sterilized disc saturated with a measured quantity of the sample was placed on a plate containing solid bacterial medium (nutrient agar broth) or fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the tested organism after inoculation. The diameter of the clear zone inhibition surrounding the sample was taken as a measure of inhibitory power of the sample against the particular test organism.

The tested organisms were Gram-negative bacteria (*Escherichia coli*, NCTC10416), and (*Pseudomonas aeruginosa*, NCIB-9016), and Gram-positive bacteria (*Bacillus subtilis*, NCIB3610, *Staphylococcus aureus*, NCTC7447). The fungi (*Aspergillus niger*, Ferm-BAMC-21) and unicellular fungi as (*Candida albicans*). The bacteria and fungi were maintained on nutrient agar medium. After 24 h of incubation at 30 °C for bacteria and 48 h of incubation at 28 °C for fungi, the diameter of the inhibition zone in mm was measured.

4.4. SRB (sulphate reducing bacteria) Desulfomonas pigra

The tube dilution technique was used for SRB

1. Account of the number of live microorganisms in blank solution.
2. In this procedure, the biocide in test tubes was diluted out in the growth medium in a dilution series.
3. All the tubes then were incubated with the organism in test tubes.

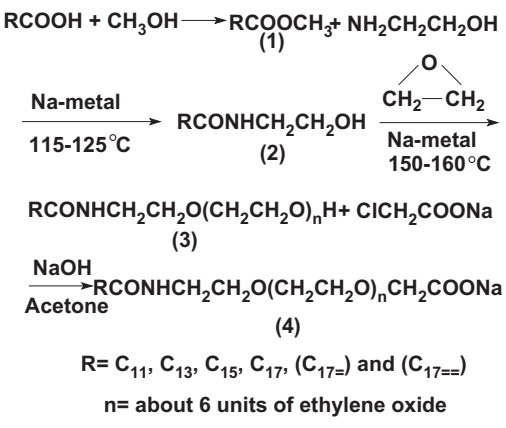
5. Results and discussion

In a previous article [5], the synthesis of a series of amide ether carboxylates with different alkyl chain lengths from C_{12}, C_{18}, $C_{18=}$ and $C_{18==}$ was done through the following reaction steps (Scheme 1).

5.1. Surface parameters

5.1.1. Surface tension (γ) and critical micelle concentration (CMC)

The surface active parameters, such as critical micelle concentration, effectiveness of adsorption (P_{C20}), minimum surface area (A_{min}) and maximum surface excess (Γ_{max}) were determined from the data at 25 °C Table 1. The plots of surface tension (γ) versus ($\log c$) (logarithmic values of surfactant



Scheme 1 The preparation of amide ether carboxylate surfactants (AEC).

Table 1 The critical micelle concentration (CMC) and surface parameters of the prepared surfactants from surface tension measurements at 25 °C.

Surfactant	CMC × 10^2 M	γ_{CMC} mN m^{-1}	Π_{CMC} mol dm^{-3}	$\Gamma_{max} \times 10^{10}$ mol cm^{-2}	$P_{C20} \times 10^{-5}$	A_{min} nm^2
Lauric AEC (c_{12})	0.018	34	38	2.643	3.7	0.628
Myristic AEC (c_{14})	0.047	35	37	2.479	5	0.760
Palmitic AEC (c_{16})	0.104	46	26	1.456	3	1.141
Stearic AEC (C_{18})	0.075	47	25	1.342	4.3	1.238
Oleic AEC ($C_{18=}$)	0.103	40	32	1.505	8.8	1.104
Linoleic AEC ($C_{18==}$)	0.133	35	37	1.576	4.7	1.053

concentration) for all surfactants I_{a-f} are shown in Figs. 1 and 2.

Sharp decrease of the surface tension was observed as the concentration increases, then the curves break rather rapidly at still relatively low concentration and continue to decrease slowly as the concentration increases. From the intersection points in these figures the critical micelle concentrations (cmc) were determined for the prepared compounds as listed in Table 1. The obtained cmc of the synthesized surfactants show an increasing trend with increase in the chain length of the alkyl group, and increasing with double bond. Inclusion of the increase in cmc values can be attributed to an increase in the solubility of the surfactant molecules i.e., the presence of polar atoms as oxygen or nitrogen in the hydrophobic chain (but not associated with a head group), results in an increase in the cmc [8–10].

5.1.2. Effectiveness (Π_{CMC})

The surface tension (γ) is not affected by the change in concentration above cmc, and hence γ values at the cmc are used to calculate the surface pressure (effectiveness) values

$$\pi_{cmc} = \gamma_0 - \gamma_{cmc} \quad (2)$$

where; γ_0 and γ_{cmc} are the surface tension of pure water and surface tension at cmc, respectively [11].

The most efficient surfactant is the one which gives the largest reduction of the surface tension at the critical micelle concentration (cmc). Values π_{cmc} at 25 °C are given in Table 1 and

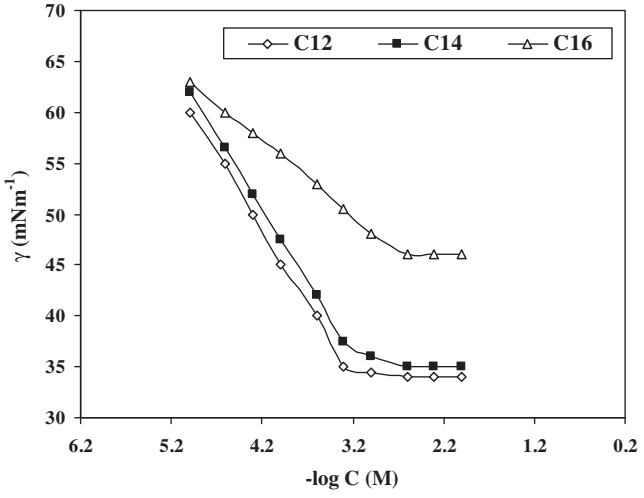


Figure 1 Variation of the surface tension with logarithm of concentrations for synthesized C_{12}, C_{14}, and C_{16} in water at 25 °C.

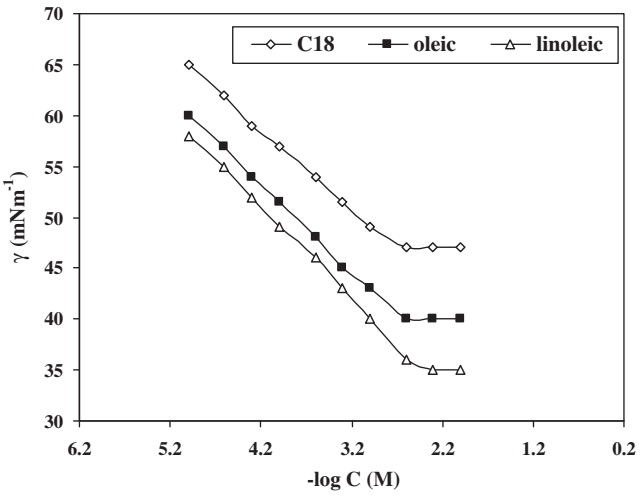


Figure 2 Variation of the surface tension with logarithm of concentrations for synthesized C_{18}, $C_{18=}$, $C_{18==}$ in water at 25 °C.

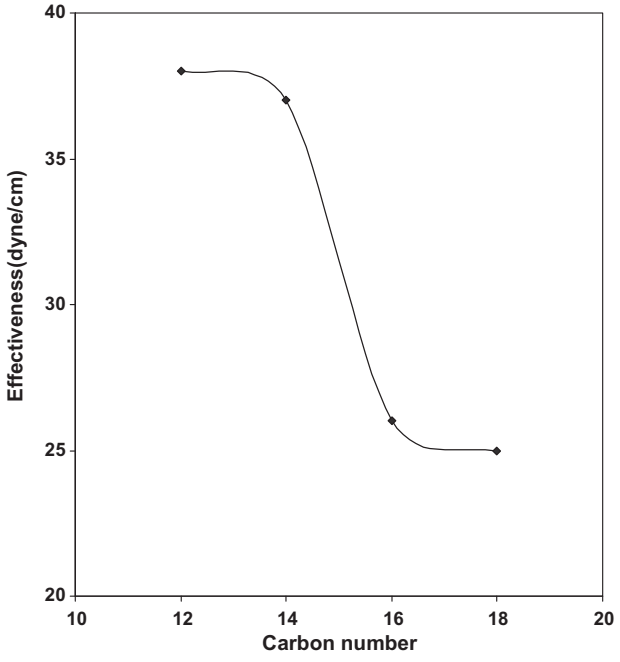


Figure 3 Relation between the carbon number and effectiveness of amide ether carboxylates surfactants.

plotted against the total carbon number of alkyl chains of the prepared surfactants Fig. 3. The effectiveness of the prepared surfactants decreases with increase in the alkyl chain length and then increases with the presence of double bond. According to the results obtained, C_{12}, C_{14} and $C_{18==}$ were found to be the most efficient as shown in Figs. 4 and 5.

5.1.3. Efficiency (P_{C20})

The efficiency (P_{C20}) is determined by the concentration (mol/L) of the surfactant solutions that are capable of suppressing the surface tension by 20 dyne/cm.

The values of the efficiency of the prepared surfactants are shown in Table 1. It is obvious that the efficiency of these surfactants increases with increasing the alkyl chain length and decreases with the presence of double bonds. Further, the values of efficiency of adsorption, P_{C20} are useful in comparing the efficiency of adsorption of the surfactant on air/water interface. The larger the P_{C20} value, the more efficiently the surfactant is adsorbed at the interface and the more efficiently it reduces surface tension.

5.1.4. Maximum surface excess (Γ_{max})

The maximum surface excess (Γ_{max}) is defined as the effectiveness of adsorption at an interface. The maximum surface excess concentration of surfactant ions, Γ_{max}, were calculated from the slope of the straight line in the surface tension plot ($d\gamma/d\ln C$) (Figs. 1 and 2) below CMC, using the appropriate form of Gibbs adsorption equation:

$$\Gamma_{max} = -(\delta\gamma/\delta log c)_T/2.30RT \quad (3)$$

where Γ_{max} is the maximum surface excess concentration of surfactant ions, R is the gas constant, T is the absolute temperature, C is the concentration of surfactant, γ is the surface tension at given concentration and n is the number of species ions in solution. Pumping of surfactant molecules to the boundary surfaces between phases to form an adsorbed layer is one of the most objective applications of surfactants as a vital branch of chemistry in several applications [12,13]. The values of maximum surface excess concentration were calculated and listed in Table 1. It was found that the maximum surface excess concentration decreased by increasing the carbon chain length and increased with double bond due to hydrophobic effect of the carbon chain.

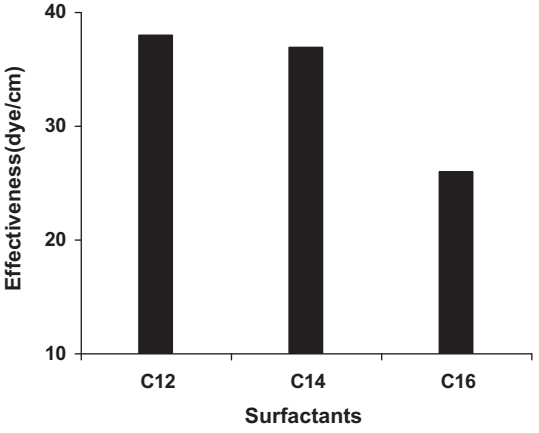


Figure 4 The effectiveness of the surfactants (AEC) (C_{12}–C_{16}).

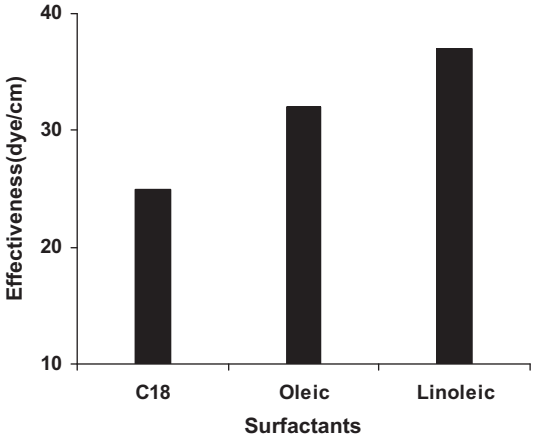


Figure 5 The effectiveness of the surfactants (AEC) (C_{18}, $C_{18=}$, $C_{18==}$).

5.1.5. Minimum area per molecule (A_{min})

The minimum surface area per adsorbed molecule, A_{min}, can be obtained as follows:

$$A_{min} = 10^{16}/N_A\Gamma_{max} \quad (4)$$

where N_A is the Avogadro's number and Γ_{max} (mol m^{-2}) is the maximum surface excess of adsorbed surfactant molecules at the interface.

The values of area per molecule for the prepared surfactants were calculated and listed in Table 1. It was found that the surface excess Γ_{max} and the area per molecule A_{min} vary with the molecular structure, showing a large area per molecule with the increase of alkyl chain length which indicates that the molecules are less tightly packed at the air/water interface for the flexible, longer alkyl chain surfactants [14].

The values of the minimum surface area increase with increasing the length of the hydrocarbon chains. Accordingly to the cross sectional area of an aliphatic chain oriented perpendicular to the interface is about 20 Å while values of A_{min} of the prepared surfactants are ranging between 0.628 and 1.238 nm^2 indicating that these molecules are located in tail position on the surface.

5.1.6. Thermodynamic parameters

Adsorption and micellization processes of the surfactant molecules are considered as phase transformation either from singly state molecule in the solution into adsorbed molecules at the interface (adsorption) or into the well aggregated molecules in the form of micelles (micellization).

The functions were calculated using Gibbs adsorption rules [15] as follows:

For micellization:

$$\Delta G^0_{mic} = RTln\text{CMC} \quad (5)$$

where R is the gas constant, T is the absolute temperature and cmc is expressed in the molarity of the surfactant.

For adsorption:

$$\Delta G^0_{ads} = \Delta G^0_m - 6.023 \times 10^{-1} \times \Pi_{CMC} \times A_{min} \quad (6)$$

Standard free energies of micellization and adsorption for the prepared surfactants were calculated at 25 °C according to Gibbs equations of thermodynamics and their values are listed in Table 2.

Table 2 Thermodynamic parameters of the prepared surfactants from surface tension measurements at 25 °C.

Surfactant	ΔG_{mic}, kj/mol	ΔG_{ads}, kj/mol
Lauric AEC (C_{12})	−36.720	−36.735
Myristic AEC (C_{14})	−35.547	−35.562
Palmitic AEC (C_{16})	−31.013	−31.031
Stearic AEC (C_{18})	−30.759	−30.777
Oleic AEC ($c_{18=}$)	−30.287	−30.308
Linoleic AEC ($C_{18==}$)	−27.849	−27.873

Negative values of the standard free energies of both micellization and adsorption for the prepared surfactants indicate that micellization and adsorption are spontaneous processes.

The spontaneity of the process is attributed to the repulsion between the different hydrophobic moieties and the polar solvent.

An increase in $-\Delta G^0_{ads}$ values supports the idea of micellization over adsorption on the solution surface to overcome the repulsion forces occurring at the water/hydrophobe interface as shown in Table 2.

5.2. Biodegradability

Surface tension was used for following the biodegradation of the prepared surfactants. Since all the prepared surfactants under investigation have the same hydrophilic part, hence, hydrophobic chain length is the only factor affecting this process. The results of biodegradation Die-away test in the River Nile water reflected the fact that, lowering of the surface tension is a reverse function of biodegradation. It is clear from the data in Table 3 and Fig. 6 that the biodegradation ratio of all of the prepared compounds ranged from 5% to 67% for the 21th day. In addition, it is clear that there is a direct relationship between the attached alkyl chain length and the rate and percent of biodegradation. As the alkyl chain length increases, the rate of biodegradation decreases.

5.3. Antimicrobial activity of the prepared surfactants against sulphate reducing bacteria (SRB)

Generally, biocides exert their bacteriostatic effect on sensitive organisms by:

1. Inhibition of cell wall permeability.
2. Injuring the cytoplasmic membrane.
3. Inhibition of the protein biosynthesis.
4. Inhibition of the nucleic acid synthesis.

The results of the antimicrobial activity of synthesized amide ether carboxylates (AEC) surfactants against SRB were determined by dilution method and listed in Table 4. The results indicate that the synthesized amide ether carboxylates (AEC) surfactants have antimicrobial activity against the tested microorganisms (SRB) and their activities depend on their chemical structures (mainly the hydrophobic chain length).

Table 3 Biodegradability, %*D*, of amide ether carboxylates.

Compd.	Biodegradability (%/day)																				
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21th
Lauric AEC (C_{12})	5	7.5	13	16	23	26	30	33	36	40	41	42	45	46	48	58	63	64	64	67	67
Myristic AEC (C_{14})	5	10	14	15	18	19	21	26	31	32	36	38	44	44	44	49	49	54	55	56	57
Palmitic AEC (C_{16})	3	7	9	16	18	21	21	24	24	26	26	26	29	29	29	32	38	41	41	44	50
Stearic AEC (C_{18})	3	9	13	13	19	27	30	30	30	31	31	34	41	42	47	48	53	53	56	58	59
Oleic AEC ($C_{18=}$)	2	3	9	9	16	17	19	22	23	23	25	25	34	41	44	45	50	52	52	53	56
Linoleic AEC ($C_{18==}$)	2	2	6	13	13	19	22	25	25	28	28	34	38	44	47	48	50	50	53	56	61

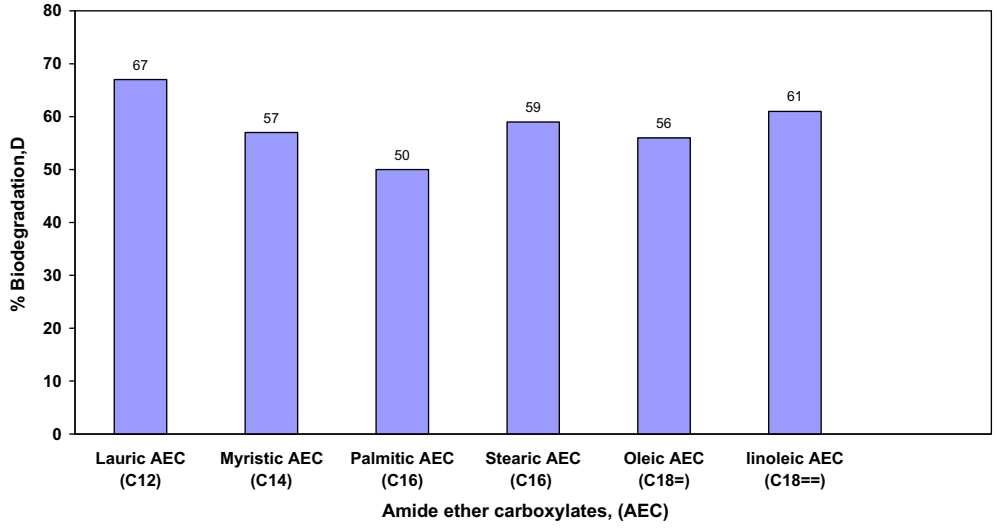


Figure 6 Biodegradability, %*D*, of amide ether carboxylates after (21th days).

Table 4 Antimicrobial activity of synthesized surfactants against sulphate reducing bacteria (SRB) by dilution method.

Inhibitor name	Bacteria count (colony/ml sample) (*Desulfomonas pigra*)					
	Control	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
Lauric AEC (C_{12})	7.38×10^3	6.50×10^3	3.84×10^3	39	0.0	0.0
Myristic AEC (C_{14})	7.38×10^3	6.59×10^3	4.11×10^3	1.31×10^3	0.0	0.0
Palmitic AEC (C_{16})	7.38×10^3	7.01×10^3	6.81×10^3	6.63×10^3	5.96×10^3	3.19×10^3
Stearic AEC (C_{18})	7.38×10^3	7.31×10^3	6.93×10^3	6.28×10^3	5.83×10^3	3.24×10^3
Oleic AEC ($C_{18=}$)	7.38×10^3	7.19×10^3	6.48×10^3	5.93×10^3	5.66×10^3	2.94×10^3
Linoleic AEC ($C_{18==}$)	7.38×10^3	6.89×10^3	4.46×10^3	3.98×10^3	228	16

Table 5 Antibacterial activity of the synthesized surfactant gram-positive, gram-negative bacteria and fungi. Diameter of inhibition zone (mm).

Sample	Diffusion agar technique (5 mg/ml sample)					
	Escherichia coli (G^-)	*Pseudomonas aeruginosa* (G^-)	*Staphylococcus aureus* (G^+)	*Bacillus subtillus* (G^+)	*Candida albicans* (Fungus)	*Aspergillus niger* (Fungus)
Water (control)	0.0	0.0	0.0	0.0	0.0	0.0
Lauric AEC (C_{12})	22	23	5	21	23	17
Myristic AEC (C_{14})	15	13	0	20	14	25
Palmitic AEC (C_{16})	14	14	12	0	13	0
Stearic AEC (C_{18})	13	0	0	0	12	18
Oleic AEC ($C_{18=}$)	0	0	0	13	15	12
Linoleic AEC ($C_{18==}$)	25	19	24	17	16	0

These results are in good agreement with the results of several investigators who dealt with the (AEC) surfactants [16,17].

The action mode of such amide ether carboxylates (AEC) surfactants biocides on the bacterial strain is explained as an electrostatic interaction and physical disruption. The electrostatic interaction occurs between the oppositely charged centers on the cellular membrane and the positively charged head groups of the biocide molecules. While, the physical disruption results are from the penetration of the hydrophobic chains into the cellular membrane due to the similarity in the chemical nature. The interaction between biocide molecules and cellular membrane causes, a strong damage of the selective permeability of these membranes which disturbs the metabolic pathway within the cytoplasm [18].

The results indicate that, the synthesized amide ether carboxylates (AEC) surfactants showed weak to good antimicrobial activity against the tested sulphate reducing bacteria (SRB).

5.4. Evaluation of the synthesized surfactants as antibacterial agent

The antimicrobial activities of the synthesized compounds were evaluated by using the modified Kirby–Bauer disc diffusion method [19–22].

The biocidal activity of the prepared surfactants towards microorganism is found to be dependent on the nature of the target organisms. Gram-positive and Gram-negative bacteria were affected extremely by the synthesized surfactants. The behaviours of the synthesized surfactants at the interface play a vital role in their antimicrobial activity. The surface properties and hydrophilicity of these surfactants showed a tendency towards adsorption at the interface which facilities their adsorption at the bacterial cell membrane. It has been suggested that surfactants inhibit the growth of organisms by the formation of an electrostatic bond with the cell wall and this affects the permeability of protein formation, by cross-linking outer proteins of the cell. The cell walls of all living organisms contain free amine groups that serve as the reactive sites of attack. The cross-links are formed on the cell surface and as essential cellular functions are disrupted, the cell dies [23].

The Gram-positive bacteria cell wall is composed of a peptidoglycan chain of polysaccharide, teichonic acid and phosphated sugar. Teichonic acid gave the Gram-positive bacterial cell wall a negative charge, which may be important in determining the types of substances attracted to the cell membrane [24,25].

In general, the antimicrobial activity of the surfactants depends on the alkyl chain length; however the correlation between the alkyl chain length and the microbial activity is not linear. It is clear from the data that, the antibacterial activities of the compounds decreased with increasing the chain length. This can be seen in the dodecyl (C_{12}), tetrdecyl (C_{14}) and the hexadecyl ones (C_{16}), which may be attributed to a large increase of the lipophilicity of the molecules resulting from the presence of hydrophobic chain, leading them to take more time for crossing the cell membrane, and hence the activity decreases.

For the long chain C_{18} and the unsaturated $C_{18=}$, the chain length does not affect the antimicrobial properties. The only difference is that the C_{18} presents activity against *E. coli* where as the Oleic has activity against *Bacillus subtillus*. On the other hand, Gram-negative bacteria except (*E. coli*) and Gram-positive except (*Bacillus*) are resistant to these surfactants (C_{18}, Oleic) at the tested concentration. By contrast, C_{12} and $C_{18==}$ surfactants present almost similar activity against both Gram-positive and Gram-negative bacteria.

The Linoleic activity attributed to unsaturated long chain fatty acid. Kabara [26] has found that the addition of second ethylenic bond increases the biological activity.

Other C_{16} and C_{14} also show a similar activity against Gram-positive and Gram-negative bacteria. Gram-positive bacteria are more resistant to these compounds than the Gram negative bacteria. This can be explained by the different cell membrane structures of the two bacterial types.

Amphiphilic molecules, which exhibit a larger or lesser tendency both to water and nonpolar phases, have the special ability to dwell in the water medium of the living organism and to interact with the lipid layer of cell membranes of the organism; that is why such substances are called membrane active substances [27]. Each molecule that enters a living organism is always in contact with its cell membranes. That contact may result in a change in the membrane itself, or may have a toxic effect on the membrane which could suffer damage or even destruction, leading to the death of the cell [28].

The results in Table 5 show that the biological activity of the prepared compounds is dependent on both the character of the polar heads (size, electric charge distribution) and the hydrocarbon chain (length). The length of the alkyl chain of the amphiphilic substances incorporated in the membranes affects the biological activity. From the previous results, it can be concluded that, the major role for antibacterial activity is played by the compound (C_{12}), which contains 12 carbon atoms in its alkyl chain. This is in agreement with the results of several other investigators [29–31] that C_{12} had much more significant biocidal activity than the other two derivatives (C_{14} and C_{16}), this may be due to their adsorption on the surface of the bacterial cell, since each molecule of the surfactant which enters a living organism is always in contact with its cell membranes. That contact may result in a change in the membrane itself, or may have a toxic effect on the membrane which could suffer damage or even destruction, leading to the death of the cell [32].

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