



Synthesis and Evaluation of Some Amine Oxide Surfactants and Their Antimicrobial Activity

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Abstract

A series of three amine oxide-based surfactants were synthesized by esterification of diethanolamine with different fatty acids (octanoic, dodecanoic and hexadecanoic). The obtained esters of diethanol amine were alkylated with (1, 10) Dibromodecane and the products were oxidized with hydrogen peroxide to produce amine oxide surfactants. The chemical structures of these surfactants were confirmed using FTIR and ¹H-NMR spectra. Also, the surface activity of the synthesized amine oxide surfactants has been studied including critical micelle concentration (CMC), effectiveness, efficiency, maximum surface excess and minimum surface area. The synthesized amine oxide surfactants were also evaluated as antimicrobial agents against different strains of bacteria including Gram-positive, Gram-negative, and fungi.

Keywords: Amphoteric surfactants, Amine oxide surfactants, Surface properties, Critical micelle concentration (CMC), Antimicrobial activity

1. Introduction

One of the most significant classes of amphoteric surfactants is Amine Oxide surfactants. Amine oxides are characterized by a direct combination of the oxygen atom and the nitrogen atom in a tertiary amine molecule as illustrated in **Figure (1)**. Because of the significant dipolar moment between oxygen and nitrogen, which resembles a positive charge on the nitrogen and a negative charge on the oxygen, these nitrogen-containing surfactants exhibit either nonionic or cationic behaviour in an aqueous solution depending on the pH. In an acid solution, the amino group is protonated and becomes in the cationic form while in neutral and alkaline solutions the nonionic form predominates [1- 3].

Their sensitivity to pH implies that their charge can be modified as a function of pH, which enables control over their properties and their aggregation forms [4-8].



Fig. (1) Chemical structure of amine oxide surfactants

Amine oxide surfactants can be easily prepared, and they have superior surface activity properties. Such as high emulsifying capacity, and excellent foaming qualities, particularly when they are combined with cationic and anionic surfactants [9, 10]. They are widely used in pharmaceutical and cosmetic formulations because of these factors [11, 12].

It should be noted that amine oxide surface-active agents attracted considerable attention owing to their extraordinary low toxicity, low skin irritation, ease of degradation, sterilization, and moisturizing properties [13-15]. Owing to these properties they have been widely used in various fields of chemistry and biochemistry [16].

Moreover, in this kind of surfactant, the oxygen atom in the covalent bond is protonated in acidic aqueous solutions giving them bactericidal characteristics that are close to those of quaternary ammonium salt surfactants. They exhibit high antimicrobial activity against a variety of microorganisms [17-19].

Amine oxide-based surfactants are also often called 'soft surfactants' due to the fact that they are biodegradable and environmentally friendly [7, 20, 21]. In this paper we aimed to prepare different amine oxide-based surfactants and study their surface

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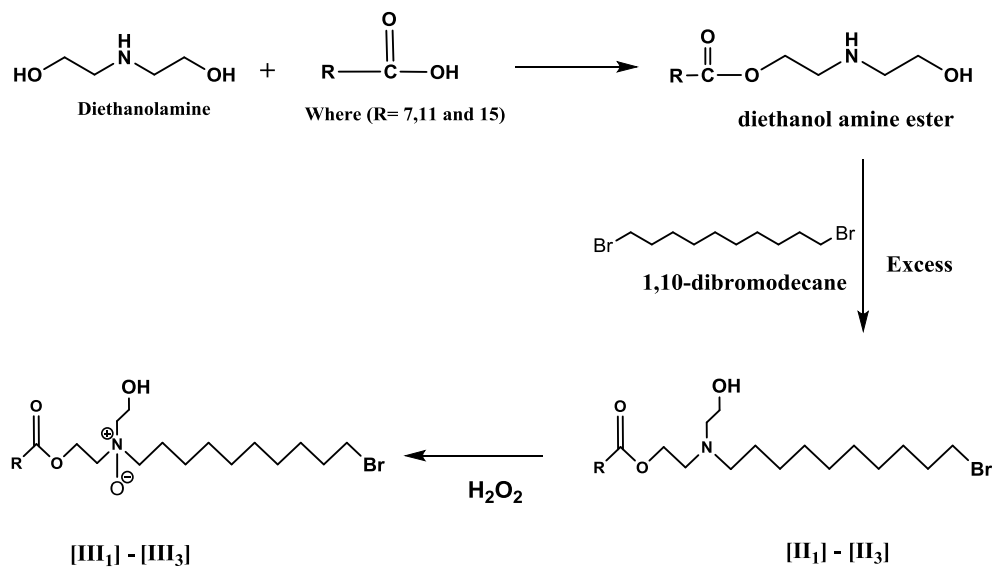
parameters. They were also evaluated as antimicrobial agents against different microorganisms, including fungi, Gram-positive and Gram-negative bacteria.

2. Methodology

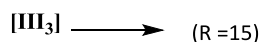
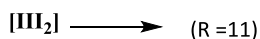
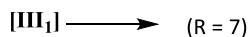
2.1. Materials

Octanoic, dodecanoic and hexadecanoic acids were supplied by Fluka. Diethanolamine was supplied by Acros Organics. P-toluene sulphonic acid supplied by Acros Organics. Xylene from CARLO ERBA Reagents. Sodium Hydroxide and Sodium hydrogen carbonate were purchased from Adwic, Egypt. (1, 10) Dibromodecane was supplied by Alfa Aesar. Hexane and Ethyl Acetate were supplied by CARLO ERBA Reagents. Hydrogen Peroxide was purchased from Acros Organics. Ethanol supplied by Adwic, Egypt. Manganese Dioxide from Al-Nasr Chemicals, Egypt.

2.2. Methods



Where:



Scheme (1) Preparation of amine oxide surfactants

2.2.2. Reaction of the esterified diethanol amine with (1, 10) Dibromodecane:

Diethanol amine esters (5 mmol), sodium hydrogen carbonate (11 mmol), and sodium dodecyl

2.2.1. Esterification of diethanolamine with the fatty (Octanoic, dodecanoic, and hexadecanoic) acids:

Diethanolamine (0.1 mol) was esterified with (0.1 mol) octanoic, dodecanoic, and hexadecanoic acids respectively, the reaction was catalyzed by P-toluene sulphonic acid (0.5 mmol, 1.8 ml) in the presence of solvent (xylene). Reaction mix. was refluxed using continuous stirring until removal of theoretical amount of water, indicating the reaction's completion. Reaction mixture allowed to cool down to room temp., neutralized by addition of (5%) aqueous sodium carbonate solution, followed by repeated washing with distilled water to get rid of the excess sodium carbonate and the catalyst. Separation of the organic layer which contains the esterified diethanol amine was carried out, and the used solvent distilled off. The products of different esterified diethanol amine were obtained as shown in **scheme (1)** [22].

sulfate (20 mg) were mixed with (20 mL) water, the mixture was heated at 80 °C for 5 min., followed by addition of excess dibromodecane to reaction mix. and heating for 12 hours. Reaction mixture left to cool, and then filtered. Filtrate was washed with an

ethyl acetate and hexane mixture to yield the final products N-decyl bromide of diethanol amine esters (**II**₁- **II**₃) as yellowish liquids with yields of (65%, 68% and 70%) respectively [23].

2.2.3. Preparation of amine oxide surfactants:

Drop wise addition of (H₂O₂, 3.9 mmol) to a solution of the prepared (N- decyl bromide of diethanol amine esters (1.6 mmol) in absolute ethanol), was carried out with continuous stirring, then the mixture was heated to reflux. After (4 hrs.), the reaction mixture was allowed to cool, and the extra of H₂O₂ was decomposed with MnO₂. The filtration was used to get rid of the MnO₂, the filtrate was then dissolved in diethyl ether, washed with water then dried, giving the conventional amine oxide-based surfactants [**III**₁], [**III**₂] and [**III**₃] as brownish oils with Yields of (67%, 70% and 72%) respectively as illustrated in **scheme (1)** [24].

2.3. Confirmation of the prepared amine-oxide based surfactants:

The prepared amine-oxide surfactants were confirmed by:

FTIR spectra were estimated using ATI Mattson genesis and FTIR spectrophotometer, (EPRI, Egypt). ¹H-NMR spectra were evaluated in CDCl₃ using a Jeol ECA 500 MHz NMR spectrometer, at 500 MHz. Measurements performed at Micro analytical Center at Cairo university.

2.4. Surface Tension:

Surface and interfacial tensions of the investigated amine oxides were measured using a (Kruss – type K6) tensiometer equipped with platinum-iridium DuNouy ring at several concentrations of surfactant. Distilled water of a surface tension 72 mN/m at 25°C used in preparation of different concentrations of surfactant solutions.

2.5. Antimicrobial evaluation of the synthesized surfactants:

The antimicrobial activity was evaluated at micro-analytical Center- Faculty of Science- Cairo university against different microorganisms via the modified Kirby-Bauer Disc-Diffusion Method [25].

The tested amine oxide surfactants were tested against, Gram +ve bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556), Gram -ve bacteria (*Escherichia coli* ATCC 23282 and

Pseudomonas aeruginosa ATCC 10145), and Filamentous Fungi (*Aspergillus niger* ATCC 16404) compared to *Kanamycin*, *Ampicillin* and *Amphotericin B* as standard drugs, for Gram-positive, Gram-negative bacteria and fungi, respectively.

3. Results and discussion:

3.1. Characterization of the synthesized amine oxide surfactants:

Chemical structures were verified using the IR spectrum. The three prepared surfactants show absorption band at (1734- 1737 cm⁻¹) arising from the (C=O) group contributing to the ester group. Moreover, (N-O) band corresponding to amine oxide surfactants appears at 933 cm⁻¹. The IR spectrum data for the prepared amine oxide surfactant [**III**₁] represented in **Fig. (2)**

Also, ¹HNMR spectra of the synthesized surfactant (**III**₁) as shown in **Fig. (3)** showed different bands at δ= (0.84) ppm for [**CH**₃] group, δ= (1.23) ppm for the repeated methylene groups [(**CH**₂) n], δ= (2.33) ppm for [**CH**₂-**COO**] group, δ= (4.27) ppm for [**COO-CH**₂] group, δ= (3.52) ppm for [**-COO-CH**₂-**CH**₂-**NO**] group, δ= (3.66) ppm for [**CH**₂-**OH**] group, δ= (3.36) ppm for [**-CH**₂-**CH**₂-**OH**] group, δ= (4.27) ppm for [**OH**] group and δ= (3.50) ppm for [**CH**₂-**Br**] group.

3.2. Surface active parameters for the synthesized amine oxide surfactants:

3.2.1. Surface and interfacial tensions:

The main characteristic of a surfactant is surface activity, which characterized as the capacity and efficiency to decrease surface tension in aqueous solution. Surface and interfacial tension measurements of synthesized amine oxides are presented in **Table (1)**. As illustrated, all the prepared surfactants exhibit excellent surface activity expressed by surface tension ranging from (32 to 38.5 mN/m) at surfactants concentration (0.1%). The surface tension of the three amine oxide surfactants was determined and plotted as a function of concentration as shown in **Fig. (4)**. Also, from data shown in **Table (1)**, It was found that the amine oxide surfactants affect their ability to lower the interfacial tension values. These surfactants can be used as biocides and corrosion inhibitors in a variety of applications due to their lower interfacial tension values [26].

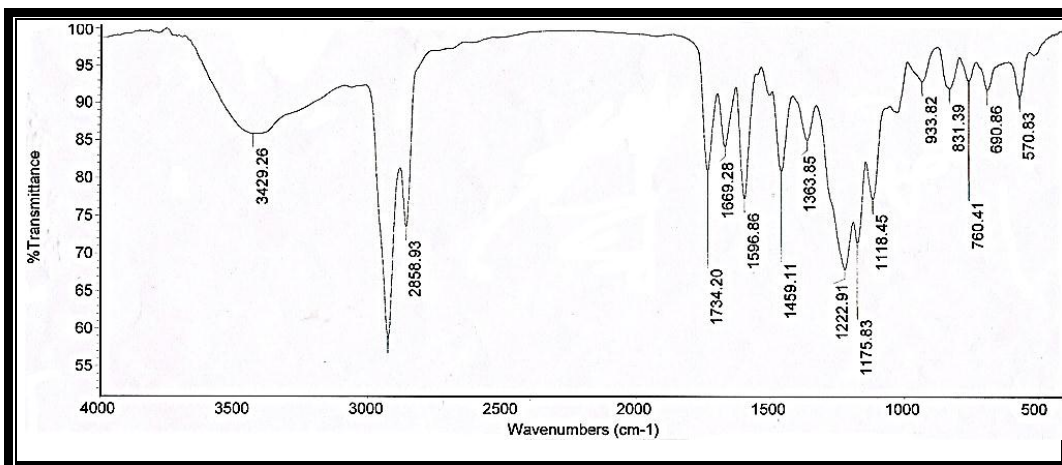
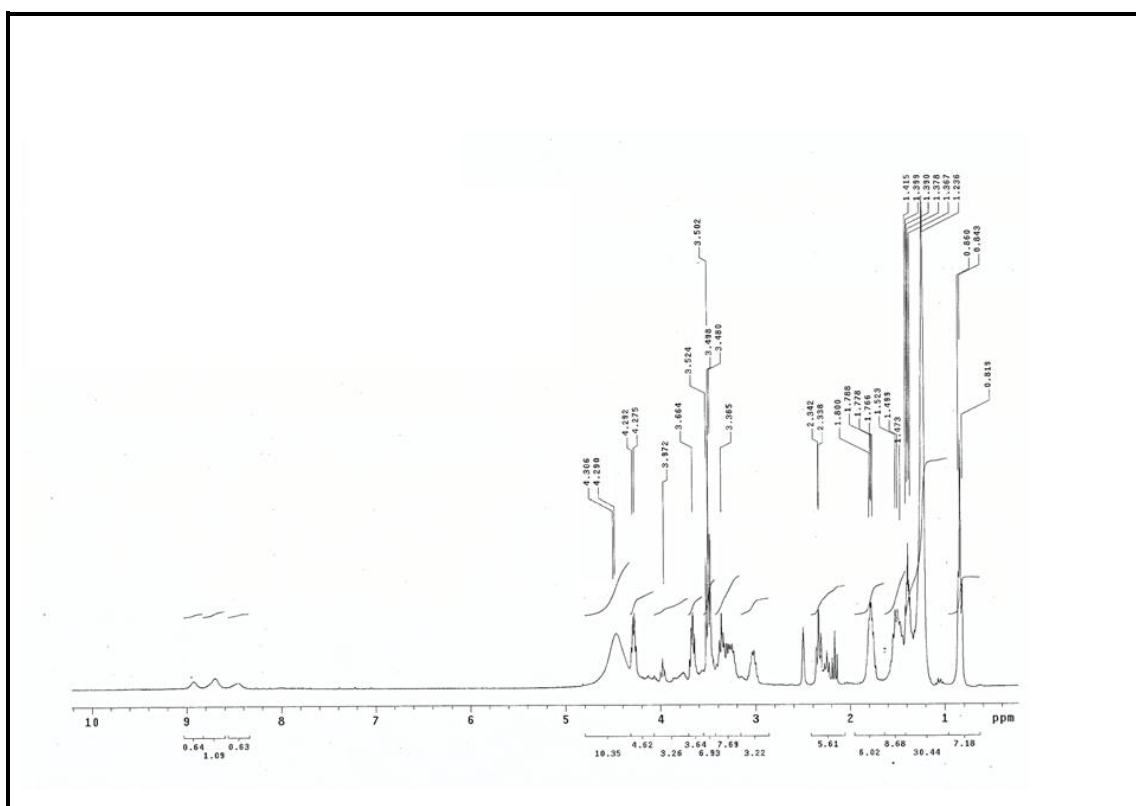
Fig. (2): FTIR-spectrum of (III₁)Fig. (3): ¹H-NMR spectrum of (III₁)

Table 1: Amine oxide surfactants properties (surface, interfacial tensions, foam height, and emulsion stability) at 25°C

Surfactant	S. Tension (mN/m)	Interf. Tension (mN/m)	Foam Height (mm)	Emulsion Stab. (sec)
[III ₁]	35	12	50	140
[III ₂]	32	6	35	150
[III ₃]	38.5	3	30	187

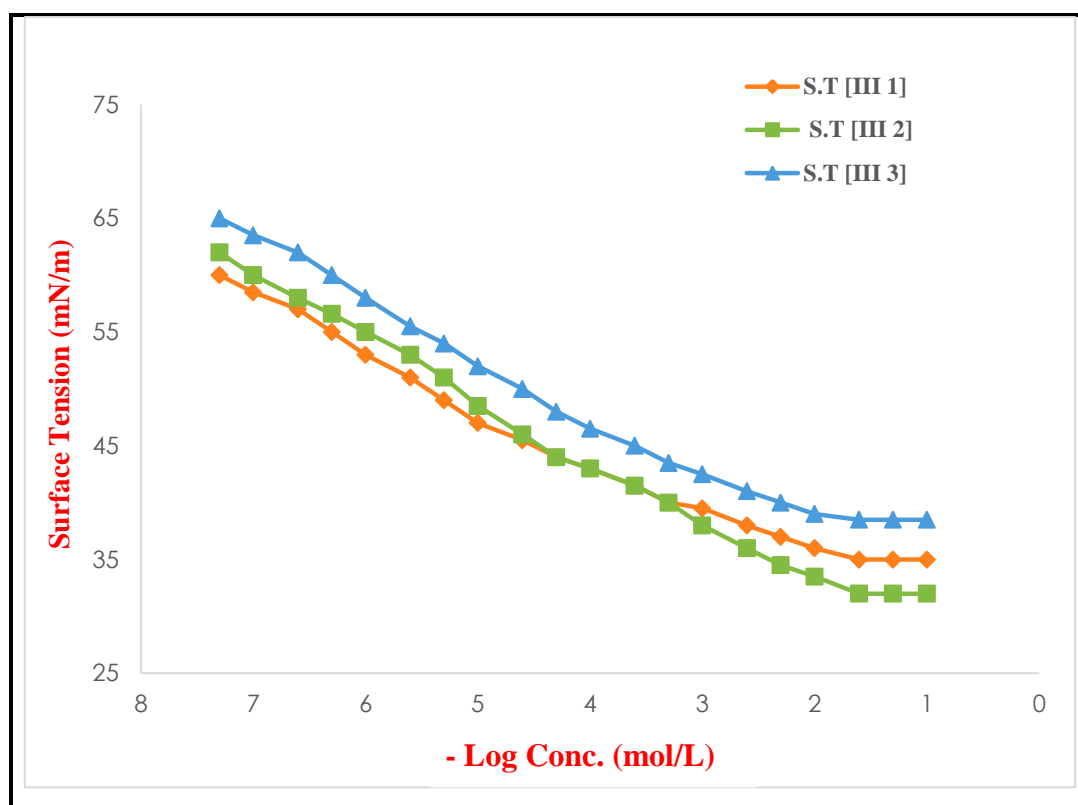


Fig. (4) Variation of surf. tension with log. Conc. for synthesized amine oxides in water at 25°C

3.2.2. Emulsion stability and Foam height:

One of the most crucial characteristics of surfactants is emulsification. **Table (1)** shows the emulsifying powers of the used amine oxide surfactants as a function of time. It is evident that variation in alkyl chain length affects the emulsifying power. According to the data in **Table (1)**, the **[III₃]** surfactant was more effective at emulsion stabilization and might be employed in the textile, cosmetic, and emulsion paint industries.

Also, from data listed in **Table (1)**, The prepared amine oxide surfactant **[III₁]** has the best foaming qualities making it suitable for use in the production of detergents.

3.2.3. Critical micelle concentration (CMC) and (γ_{CMC}):

CMC values of amine oxide surfactants **[III₁, III₂, and III₃]** were calculated by determining the break-point of the surf. tension against – Log concentration curves. **Figure 4** shows two distinctive regions, in which surface tension varies significantly at low concentrations, but it is relatively weak at higher concentrations. The CMC is indicated by the intercept of these two regions [27].

In our investigation of the prepared amine oxide surfactants, we found that increasing the hydrophobic

alkyl chain lengths (R), where R = (7, 11 and 15), exhibits lower CMC values as shown in **Table (2)**. This indicates that the long chain of hydrocarbons is hydrophobic in nature and less soluble in aqueous media, in other words, the hydrophobic effect is the principal factor behind the micellization process. Hence the CMC value of **[III₃]** is lower than that of **[III₁]**.

Also, values of surf. tension at CMC (γ_{CMC}) for the synthesized amine oxide surfactants were observed in **Table (2)**. Obtained results show that (γ_{CMC}) for both **[III₁]** and **[III₂]** were so close, but in case of **[III₃]** surfactant the (γ_{CMC}) value was higher than them.

3.2.4. Effectiveness (π_{cmc}):

Values of π_{cmc} were calculated using surface tension values at CMC [28]. **Table (2)** showed that the π_{cmc} values for **[III₁, III₂ and III₃]** were equal to (33.05, 34.58 and 29.26) mN/m, respectively, indicating that **[III₁ & III₂]** are more effective in reducing (g_{CMC}) than **[III₃]**.

3.2.5. The Efficiency (Pc_{20}):

Pc_{20} is an appropriate measure of the surfactant's efficiency in reducing surface tension [29]. As listed in **Table (2)**, **[III₂]** surfactant has the highest value of the Pc_{20} along the synthesized surfactants series.

3.2.6. The Surface Excess (Γ_{max}):

(Γ_{max}) measures how many surfactant molecules are present at the air-water interface at the CMC at

25°C [30]. (Γ_{max}) of the prepared surfactants is calculated according to Gibbs adsorption equation:

$$\Gamma_{max} = - (\delta\gamma / \delta \log c)_T / 2.303 n RT$$

where ($d\gamma / d \log c$) is the slope of (g vs.-log C) plot at room temp., T is absolute temperature and R= (8.314 mol⁻¹). Table (2) shows the variation of Γ_{max} values along the prepared series, which can be attributed to the developed repulsive forces between the hydrophobic molecules and the aqueous phase, leading the molecules to migrate towards the interface, where the surfactant molecules with the longer hydrophobic chains pack more tightly at the air–water interface.

3.2.7. The minimum surface area (A_{min}):

A_{min} occupied by a molecule adsorbed at interface can be calculated using:

$$A_{min} = 10^{16} / N_A \cdot G_{max}$$

where $N_A = 6.023 \times 10^{23}$ (Avogadro's number) [31, 32]. Table (2) showed that the consequent increase of (G_{max}) causes crowding at the interface, which reduces A_{min} . We can therefore deduce from the increased Γ_{max} that the increase in the hydrophobic character causes the formation of a tightly packed monolayer film of the long-chain analogues at the air/aqueous solution interface (and thereby, the decreased A_{min}). We can conclude that the investigated surfactants have a high tendency to adsorb at the different interfaces which boosts their ability to be used as corrosion inhibitors and emulsifiers [33].

3.2.8. The standard free energies of micellization (ΔG_{mic}) and adsorption (ΔG_{ads}):

Table 2

The Critical micelle concentration (CMC) and the surface parameters of synthesized amine Oxide surfactants at 25 °C

Surfactant	CMC x10 ² (mol / l) l)	γ_{CMC} (mN/m)	π_{cmc} (mN/m)	(Γ_{max} x10 ¹¹ (mol/cm ²)	A_{min} (nm ²)	Pc_{20} (mol/L)	ΔG_{ads}° kJ/mol o	ΔG_{mic}° kJ/mol
[III ₁]	0.075	38.95	33.05	4.91	3.38	4.6	-34.75	-34.69
[III ₂]	0.049	37.42	34.58	5.58	2.98	5.6	-38.50	-38.43
[III ₃]	0.036	42.74	29.26	4.33	3.84	4.3	-38.74	-38.68

Table 3

Data in Table (2) showed the spontaneity of ΔG_{mic} and ΔG_{ads} of the prepared amine oxide surfactants as a result of their negative values. The two processes represent phase transformations of the molecules, which can exist freely in solution, or adsorb at interface or present in solution as micelles.

3.3. Antimicrobial activity for the synthesized amine oxide surfactants:

As we previously stated that there are a lot of applications for this type of surfactant according to its unique structure which contains both positive and negative charges in the same molecule. One of their most important applications is being used as biocides. The role of these compounds can be summed up in increasing the cell membranes permeability of microorganisms which causes damages of their biological balance (within cytoplasm), causing death [34].

According to data recorded in Table (3), we can conclude that the synthesized amine oxides inhibited the growth of all examined bacteria and fungi. The results can be summarized as follows:

With respect to **Gram-positive bacteria** (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556), it was found that [III₁, III₂ and III₃] exhibit moderate to good effect towards the tested species compared with the used standard (Kanamycin). And in case of **Gram-negative bacteria** (*Escherichia coli* ATCC 23282 and *Pseudomonas aeruginosa* ATCC 10145) compared with the used standard (Ampicillin), the prepared compounds show good biocidal activity. Also, comparing the synthesized surfactants with (**Amphotericin B**) for Filamentous Fungi (*Aspergillus niger* ATCC 16404), it was found that both [III₂] and [III₃] surfactants have higher efficiency than the standard (**Amphotericin B**) itself.

The antimicrobial activity of the synthesized amine oxides Against different pathogenic bacteria and fungi

Co Surfactant	Test organism	G ⁺ G ⁺		G ⁻ G ⁻		Fungi
		<i>Bacillus subtilis</i>	<i>Staph. Aureus</i>	<i>Escherichia coli</i>	<i>Pseud. aeruginosa</i>	<i>Aspergillus niger</i>
Standard	Kanamycin (G ⁺)	27	25	--	--	--
	Ampicillin (G ⁻)	--	--	25	26	--
	Amphotericin B (Antifungal agent)	--	--	--	--	17
[III ₁]	14	16	16	17	-ve	
[III ₂]	13	13	13	13	19	
[III ₃]	16	20	15	13	20	

4. Conclusions:

- Three amine oxide-based surfactants were synthesized, and their surface-active characteristics were discussed according to the length of their hydrophobic alkyl chain. It was obvious that with increasing the hydrophobic alkyl chain lengths exhibit lowering in their CMC values.
- Increasing the alkyl chain lengths of the prepared surfactants has an impact towards both (Γ_{max}) and (A_{min}) values.
- By investigating antimicrobial activity for the amine oxide prepared surfactants, they show moderate to good effects towards the tested species compared with the used standards.

5. Conflict of interest:

There are no conflicts to declare.

6. Acknowledgments:

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7. Reference:

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