# Surface, Biological and Antitumor Activity of some thio- based cationic surfactants

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Abstract: Three thio-based cationic surfactants were synthesized depend on reaction of anthranilic and sulphanilic acids with fatty isothiocyanate having hydrocarbon chain length of $C_{12}$, $C_{16}$ and $C_{18}$ followed byquaternerization with ethyl iodide. Elemental analysis, FTIR spectra, mass spectra and $H^1$ NMR spectra were performed to insure the structure of the prepared compounds. Their anticancer activity was tested by using Ehrlich Ascites Carcinoma (EAC) as a model system of mice cell tumor. The compounds were also tested in vitro on three human tumor cell lines: MCF 7 (breast carcinoma), HEPG 2 (liver carcinoma) and HCT 116 (colon carcinoma), antimicrobial activity against different species of bacteria and fungi using agar well diffusion methods. The surface properties of these surfactants were investigated. The surface properties studied included Critical Micelle Concentration (CMC), maximum surface excess (Γmax), and minimum surface area (A min). Free energy of micellization (ΔGmic) and adsorption (ΔGads) were calculated.
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**Key words**: Surface activity, Biological activity, Antitumor activity, thio-bsed cationic surfactants.

## 1- Introduction

Malignancy is a diverse class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on/and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood).

Cancers are caused by abnormalities in the genetic material of the transformed cells [1]. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may randomly occur through errors in DNA replication, or are inherited, and thus present in all cells from birth. The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome. In vivo studies have revealed that some of anionic, cationic, non-ionic, and zwitter surfactants have antitumor activity [2] and inhibit the development of tumor metastases [3,4]. The action of one halogenic quaternary ammonium compound on the in vitro proliferation of different lines of human cancer cells, indicate that halogenic quaternary ammonium present a potent growth inhibitory activity of different cancer cells lines. The presence of quaternary ammonium group, responsible for some alkylating effect [5] Cationic surfactants (CSs) have been used as antibacterial [6-8] and antifungal [9-10] agents as well as denaturants of protein molecules [11,12] in biomedical and biochemical studies [13-15] and in the design of new functional materials [16,17], drug delivery systems [18–21], and as corrosion inhibitors [22].

Quaternary ammonium salt compounds show germicidal, antibacterial, antifungal and anticancer activities. A series of quaternary ammonium salts with general formula R3N+X, which include hetero nitrogen atom was the hetero atom, enhance the biological action [23].

Among the various classes of surfactants the cationic and more especially the quaternary ammonium salts are the most effective antimicrobial agent which kill or inhibit the growth of both Gram-positive and Gram-negative bacteria. In clinical and industrial environments quaternary ammonium compounds are widely used for controlling bacterial growth. The mode of antimicrobial action of quaternary ammonium compounds involves distortion of outer membrane lipid bilayers through association of the positively charged quaternary ammonium with the polar head groups of acidic phospholipids. The hydrophobic tail partinteracts with the hydrophobic membrane core [24]. The studied surface activity relationship showed that in addition to the positively charged site, a significant hydrophilic component of the surfactant is used for controlling the biological activity of the cationic surfactant [25].

Several works deal with the synthesis of different cationic surfactant compounds and studied the relationship between surface activity and antimicrobial activity against wide strain of pathogenic bacteria, fungi and yeast [26-28]. Some quaternary ammonium compounds with different counterions, such as chloride or sulphate, have been used widely in clinical, food production health care, and domestic environments as the antiseptics preservatives to

eliminate bacterial infections and contaminations. However, since the irritant and cytotoxic effects of these compounds on human cells and tissues, such as keratinocytes, broblasts, cornea, and respiratory mucosa, have previously been shown,[29-32] improvement of Quats is necessary not only for antimicrobial activity but also as far as safety with human cells.

The aim purpose of this work is to report on arelatively simple and practical synthesis of new environmentally friendly thio-based cationic surfactants derived from condensation of anthranilic and sulphanilic acids with amixture of fatty acid chlorides and ammonium thiocyanate (structure and abbreviations shown in Schem 1), as well as to evaluate their Surface, biological and antitumor activities.

## II - Materials& Methods

Fatty acids (lauric, palmitic and stearic), thionyl chloride, ammonium thiocyanate, anthranilic, sulphanilic acids and ethyl iodide were obtained from Merck and were purifiedand acetone and di ethyl ether were all obtained from Merck and used without further purification..

FTIR spectra were recorded on a Perkin Elmer spectrometer in 4,000- 400 cm-1 range using KBr discs. 1H NMR spectra were recorded on A brucker model DRX-300 NMR spectrometer with CDCL3 as solvent and mass spectra were recorded on a Joel JMS-AX 500 (EI and FAB). Elemental analyses (C,H,O,N,I,S) of the synthesized materials were performed using a varian Elemental instrument and are in satisfactory agreement with the calculated values.

### II-1 -Synthesis of Fatty Acid Chlorides

Fatty Acid Chlorides are prepared according to the method [33].

In a four necked round bottom flask equipped with stirrer and thermo pocket charge 0.1mole of fatty Acid at room temperature and heat to 40℃., then add 0.15mole of Thionyl Chloride under Nitrogen atmosphere over a period of 1 hour and heat the reaction mixture to reflux for 1 ½ hour to obtain crude Acid Chloride.

### II-2 - Synthesis of 2 -Carboxy-N- (alkanoyl (Ethyl)Ammonio) Carbonothioyl)-N-Ethylbenzenaminium Iodide and 4 –Sulphonate -N-(Alkanoyl (Ethyl)Ammonio) Carbonothioyl) –Ethyl benzenaminium Iodide (11a-c and 111a-c).

Ammonium thiocyanate (0.0063 mol) was dissolved in 50 mL dry acetone and introduced into a 250-mL round-bottom flask after adding 1.5 mL fatty acid Chloride according to [34]. After heating the solution at 90°C for about 60 min, (0.01mol) anthranilic acid and sulfanilic acid were added and the solution was continuously stirred for about 8–10 h. After stirring, ethyl Iodide was added to the solution and placed aside on reflux for about 6 h. Finally, the product collected on ice and washed with doubly distillated water for the removal of impurities. The crude product was crystallized by diethyl ether. The pure products (IIa-c), (IIIa-c) were prepared and their physicochemical properties investigated as shown in table (1) such as mocular formula, mocular weight, colour, melting point, yield % and elemental analysis (Table 1), (Table 6). The Structure of the prepared compounds were analyzed by IR, 1HNMR, Mass Spectra, The reactions were illustrate in scheme 1:

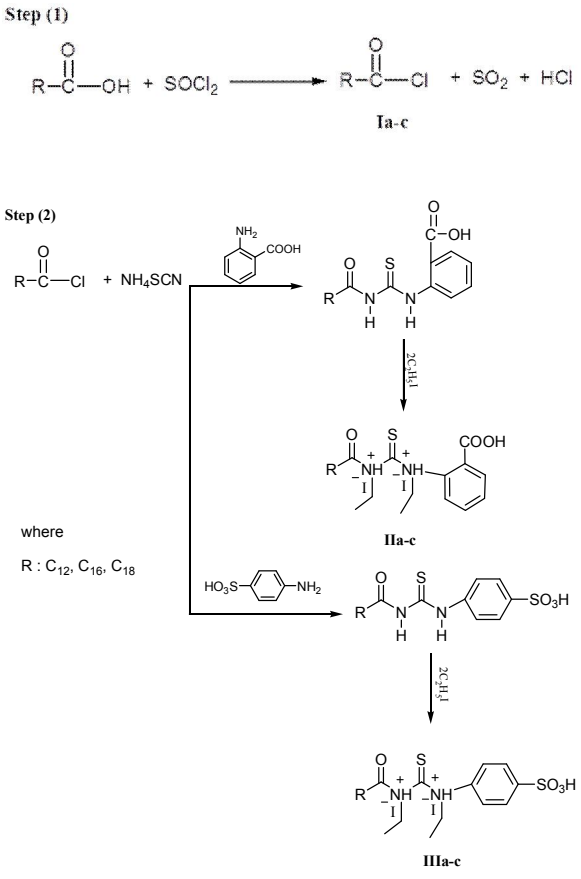

Table 1: *Physicochemical properties of* 2 -Carboxy-N-(Dodecanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide

| R | R | Abbrevations | Mocular formula | Mol.Wt | Colour | M.P | Yield % | Elemental analysis C % | H % | N% | S% | I% | O% Cal./found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dodecyl | Dodecyl | 2a | $C_{25}H_{42}N_2SO_3I_2$ | 704.11 | Yellowish brown | 285-290 | 85 | 42.60<br>42.63 | 6.01<br>6.03 | 3.98<br>3.10 | 4.54<br>4.56 | 36.07<br>36.09 | 6.81<br>6.83 |
| Hexadecyl | Hexa-decyl | 2b | $C_{29}H_{50}N_2SO_3I_2$ | 760.17 | Yellowish red | 300-305 | 87 | 45.78<br>45.80 | 6.63<br>6.66 | 3.68<br>3.70 | 4.20<br>4.22 | 33.39<br>33.42 | 6.31<br>6.33 |
| Octadecyl | Octa-decyl | 2c | $C_{31}H_{54}N_2SO_3I_2$ | 788.20 | Brown | 300-305 | 90 | 47.20<br>47.23 | 6.90<br>6.94 | 3.55<br>3.57 | 4.06<br>4.08 | 32.19<br>32.22 | 6.09<br>6.12 |

**II-3 Biological Activity**

The synthesized cationicamphiphiles were screened for their antimicrobial activity against bacteria and fungi using agar well diffusion methods [35]. Bacteria species used in this study were Pseudomonas aeruginosa (RCMB 010043), Escherichia coli (RCMB 010052) as gram negative bacteria: Streptococcus pneumoniae (RCMB 010010), Bacillissubtilis (RCMB 010067) as gram positive bacteria and Aspergillusfumigatus (RCMB 02568), Syncephalastrumracemosum (RCMB 05922), Geotricum candidum (RCMB 05097) and Candida albicans (RCMB 05036) as fungi.

**II- 4 Antitum or Activity Elhrlich ascites carcinoma (EAC)**

The antitumor activity for the compounds under investigation was carried out at pharmacology unit, National Center Institute, Cairo, University.

The choice of Elhrlich ascites carcinoma (EAC) as a model system of mice cell tumor and it is a suitable tool for studying the biological behavior of malignant tumor and drug action within cells. A set of sterile test tubes used, where 2.5× 10 5 tumor cells per ml were suspended in in phosphate buffer saline. Then 25, 50, 100 µg/ml from drug were added to the suspension, kept at 37C0 for 2 hrs. Typan blue exclusion test was then carried out to calculat the percentage of nonviable cells[36].

**Total number of NVC of cell**

%NVC=number of NVC/total number of cell x100 (7)

**II-5 Measurements of human potential cytotoxicity by SRB assay**

Human Potential cytotoxicity of the compound was tested using the method [37]

The cells were plated in 96-multiwell plate (104 cells/well) for 24 hrs before treatment of cells to the wall of the plate. Different concentrations of the compound under test (0,5,12.5,25,50µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound to be tested for 48hrs at 37°C and in an atmosphere of 5% CO2. After 48hrs, the cells were fixed, washed and stained with Sulfo-Rhodamine -B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. The color intensity is measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line for the specified compound.

**II-6 Surface Tension Measurements:**

Surface tension measurements (ST) were performed for freshly prepared solutions of surfactants in concentration ranges $10^{-1}$ to $10^{-6}$mol / l using Du-Nouytensiometer at 25°C. These values which remained constant for a period of 30 min. were taken as the surface tension of the solution. The results were accurate within ±0.1 mNm-1. The critical micelle concentration (cmc) and the surface tension at the cmc were determined as the values of the break point of surface tension vs. concentration. Also the hydrophilic substituent's have a considerable role in their cmc values [38-39]. Plots of the free energies of adsorption and micellization vs. carbon numbers were illustrated. The maximum surface excess concentration air water interface (Γmax) The packing densities of surfactants at the air – water interface are very important to interpret the surface activity of the surfactants [40] and can be calculated by the following Gibbs adsorption isotherm equation (1) [41, 42].

$$\Gamma_{max} = \frac{1}{2.303RnT}\left(\frac{\delta\gamma}{\delta \log C}\right)T \quad (1)$$

where R = gas constant (8.314 Jmol-1 K-1), T is absolute temperature (298 K), (δγ/δ log C) is the slope of the γ vs Log C plot at 25°C, The surface area occupied by surfactant molecules at the air/ water interface (Amin) is calculated using the equation (2):

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

Where Γmax and $N_A$ are the maximum surface excess and Avogadro's number respectively.

Effectiveness of the synthesised cationic surfactants is determined from the difference between the surface tension of the surfactant solution at the critical micelle concentration ($\gamma_{CMC}$) and the surface tension of the distilled water ($\gamma_o$).

[43-44] as shown in eqn. (3). Larger πcmc value of aqueous surfactants solution indicates its higher surface activity than aqueous surfactant solution with smaller πcmc value [45].

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

Adsorption efficiency, (PC20) of the surfactant concentrations that are capable of suppressing the surface tension of the solution by 20 mN\m and is obtained by using eqn. (4)

$$P_{C20} = - \log C_{20} \quad (4)$$

$C_{20}$ is the minimum cocentration which lead to saturation of the surface adsorption [46]. The surfactant with larger cmc/$C_{20}$ratios has greater tendency to adsorp at the interface than to form micelle in the solution [47- 48 ].

The free energies of micellization (ΔGmic.) and adsorbtion (ΔGads.) were calculated using the thermodynamic Eqs. (5,6) [49,50,51] as follow:

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

$$\Delta G_{ads} = \Delta G_{mic} - (0.006\ \pi_{CMC} \times A_{min}) \quad (6)$$

**III. Results and discussion:**

This work is report one simple practical synthesis of new environmentally friendly thio-based cationic surfactants derived from condensation of anthranilic and sulphanilic acidswith amixture of fatty acids chlorids and ammonium thiocyanate) to produce compounds IIa-c & IIIa-c as illustrated in schem 1.

**Table 2. Critical micelle Concentrations (CMC), Surface tension at cmc (γcmc), surface max ($\tau_{max}$), Minimum Area\Molecule ($A^2$), Standard Free Energy of Micellizaion (ΔGmic.), Standard Free Energy of Micellizaion per Methylene group (ΔGmic/CH2), Standard Free Energy Of adsorption (ΔGads) and Standard Free Energy of adsorption per methylene group (ΔGads/CH2) of 2 -Carboxy-N-(Dodecanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide at Liquid Air Interface at 25C°.**

| R | Abbrevation | cmc mol/l | $\gamma_{cmc}$ mN\m | $\tau_{max}$ mole/$cm^2$ | Area mole $A^2\times10^2$ $Nm^2$ | $-\Delta G_{mic}$ KJ/mol | $-\Delta G_{mic}/CH_2$ KJ/mol | $-\Delta G_{ads}$ KJ/mol | $-\Delta G_{ads}/CH_2$ KJ/mol |
|---|---|---|---|---|---|---|---|---|---|
| Dodecyl | IIa | 7.1 x $10^{-3}$ | 28.0 | 1.25 | 132.8 | 12.01 | 0.11 | 12.29 | 0.12 |
| Hexadecyl | IIb | 6.68 x $10^{-3}$ | 30.0 | 1.42 | 116.9 | 12.29 | | 12.59 | |
| Octadecyl | IIc | 6.3 x $10^{-3}$ | 32.0 | 1.6 | 103.8 | 12.58 | | 12.90 | |

**Table 3. Surface Tension Reduction (C20), Efficiency ($PC_{20}$), cmc\$C_{20}$ Ratios, Effectiveness ($\pi_{cmc}$) and Effective Number of Carbon Atom ($N_{eff}$) of 2 -Carboxy-N-(Dodecanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide at the liquid /air interface at 25C°**

| R | Abbrevation | $C_{20}$ | $PC_{20}$ | cmc\$C_{20}$ | $\pi_{eff}$ | $\pi_{cmc}$ mN\m | $N_{eff}$ |
|---|---|---|---|---|---|---|---|
| **Dodecyl** | **IIa** | **1.24 x $10^{-3}$** | **2.91** | **5.73** | **26.74** | **44** | **15.5** |
| **Hexadecyl** | **IIb** | **1.12 x $10^{-3}$** | **2.95** | **5.96** | **26.82** | **42** | **19.5** |
| **Octadecyl** | **IIc** | **0.89 x $10^{-3}$** | **3.25** | **7.08** | **28.97** | **40** | **21.5** |

**Table 4: Antimicrobial Activity of the Synthesized Thio-based Cationic Amphiphiles**

| | Mean of Zone diameter (mm) different concetrations | | | |
|---|---|---|---|---|
| | Gram-positive | | Gram-negative | |
| Tested micro organisms / Samples | Streptococcus pneumoniae (RCMB 010010) | Bacillissubtilis (RCMB 010067) | Pseudomonas aeruginosa (RCMB 010043) | Escherichia coli (RCMB 010052) |
| IIa | 21.2±0.58 | 24.2± 1.2 | 16.5± 1.2 | 20.1 ± 1.2 |
| IIb | 21.9±0.72 | 25.5±0.58 | 17.0± 1.2 | 20.4± 0.63 |
| IIc | 23.2±0.63 | 27 ± 1.2 | 17.2± 1.2 | 20.6 ± 1.5 |
| IIIa | 18.6± 1.2 | 20± 1.5 | 14.5± 1.2 | 20 ± 0.63 |
| IIIb | 20.3 ± 1.5 | 21± 0.63 | 15.5 ± 1.2 | 20.3 ± 0.58 |
| IIIc | 21.0± 1.2 | 22 ± 1.2 | 16 ± 1.2 | 21 ± 1.2 |
| Control | 23.8± 0.72 | 32.4 ± 0.63 | 17.3± 0.58 | 19.9 ± 0.63 |

Ampiciline is used as reference for Gram positive bacteria and Gentamicine is used as reference
For Gram negative bacteria. IIa-c is 2 -Carboxy-N-(Alkanoyl (Ethyl)Ammonio)Carbonothioyl)-N-Ethylbenzenaminium Iodide, III a-c is 4 –Sulphonate -N-(fatty alkanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide a= $C_{12}$, b = $C_{16}$, C = $C_{18}$

Confirmation of the structures of the prepared compounds illustrated in figs 1-6 Spectra of 2 - Carboxy-N- (Dodecyl (Ethyl)Ammonio) Carbonothioyl)-N- Ethylbenzenaminium Iodide (IIa).

IR spectra (cm-1, KBr) for compound IIa showed strong absorbtion band at 3444.87, 3417.88 $cm^{-1}$ for (N–H) and 3.037.5 $cm^{-1}$ for (Ar–H, C–H $SP^2$), 2824.09, 2850.79 $cm^{-1}$ for (C–H, $SP^3$) $cm^{-1}$ for 1627.76 $cm^{-1}$ for (CO) and 1720.79 $cm^{-1}$ for (COOH) fig(1).

$^1$HNMR Spectra for compound IIa showed chemical shift (δ, DMSO): 0.87-0.91 (3H, t, CH3), chemical shift (δ) 1.27-1.35, chemical shift (δ) (24H, ($CH_2$, m), chemical shift (δ) 1.61-1.67for (3H, t, $CH_3$), chemical shift (δ) 2.32-2.34 for (4H, $2CH_2$), chemical shift (δ) 7.28 for (2H, d, CH) and chemical shift (δ) 12 (H,S, COOH)n. fig (2).

ESI-MS for for compound IIa showed m/z; (704.0, 7.4 %) and base peak m/z 156.2. fig (3).

Spectra of 4 –Sulphonate -N-((Dodecanoyl (Ethyl)Ammonio) Carbonothioyl)-Ethylbenzenaminium Iodide (IIa).

IR spectra ($cm^{-1}$, KBr) showed strong absorbtion band at 3472.2, 3370.9 $cm^{-1}$ for (N–H), 3195.6 $cm^{-1}$ for (Ar–H, C–H $SP^2$), 2853.9, 2924.7 $cm^{-1}$ for (C–H $SP^3$), 1627.5 $cm^{-1}$ for (CO) and 1160.4 $cm^{-1}$ for ($SO_3H$). fig (4).

$^1$HNMR for IIIa: showed chemical shift (δ, DMSO) 0.88-0.91 for (3H, t, (CH3), (δ, DMSO) 1.25-1.3 for (24H, m, $(CH2)_{12}$), (δ, DMSO) 1.61-1.65 for (3H, t, CH3), (δ, DMSO) 7.32 for (2H, d, CH) and (δ, DMSO) 8 for (NH, S). fig(5).

ESI-MS forcompound IIIa showed m/z (740.0, 63.97 %) and base peak 641.4. fig (6).

**III.1 Surface tension and Critical Micelle Concentration (CMC)**

Surface tension was measured to determine the Critical Micelle Concentration (CMC). The variation for the surface tension vs. log. concentration of the synthesized surfactants at 25°C are shown in figs (7, 9). In all cases surface tension of the solution decrease rapidly with the intial increase in surfactants cocentration. At the break point, The surface tension began to decrease slowly, indicating the formation of surfactantaggregations. The concentration at the break point is shown as the CMC of the cationic surfactants series. It was found that with hydrophobic chain length increasing, the cmc value gradually decreased for the enhanced hydrophobic interaction between the longer alkyl chains, Tables 2, 7. Derivatives which contain sulfonate groups (IIIa-c) have a higher depression in cmc values than those containing carboxylate groups (IIa-c) and the maximum value for cmc was observed for (IIIa-c); $9.4 \times 10^{-3}$ mmol -1 while the lowest value was observed for (IIc) at $6.3\times 10^{-3}$ mmol -1 at 25 C0.

From surface tension lowering ability γcmc in Tables (2, 7), it can be seen that $\gamma_{CMC}$ of compounds (IIa-c), **(IIIa-c) increases with increasing hydrophobic chain length.**

**III-2 Maximum surface excess ($\Gamma_{max}$) and minimum surface area ($A_{min}$)**

The calculated values of the maximum surface excess showed increasing trend from $C_{12}$ to $C_{18}$ as represented from the slope of pre-CMC region of surface tension profile; figs 7, 9.

The values of ($A_{min}$) listed in tables 2,7 Increasing the maximum surface excess values indicates the increasing of adsorbed molecules at the interface, hence the area available for each molecule will decrease. That causes the compacting of surfactant molecules at the interface to form denser layer.

From Tables (2,7), it can be seen the maximum area occupied at the interface was 147.5 $nm^2$ which obtained for IIIa surfactants, While the lowest area was 103.8 $nm^2$ for IIc surfactants and Amin is greatly influenced by the hydrophobic alkyl chain length. Increasing the hydrophobic alkyl chain length decreases $A_{min}$ values at the interface. Also, 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 to the air/water interface. That can be referred to the change of the hydrophobicity of the molecules under consideration by the type of substituents.

**III- 3 Effectiveness ($\pi_{CMC}$), Effeciency ($PC_{20}$)) and ($CMC/C_{20}$) ratio**

Other important surface active parameters, such as the adsorption effeciency ($P_{C20}$) the effectiveness of surface tension reduction ($\pi_{CMC}$) and ($CMC/C_{20}$) ratio can also be obtained from the surface curve as listed in tables 2,7.

By inspected the data in Tables 2,7, the surface tension lowering ability ($\gamma_{CMC}$) of cationic amphiphiles increase with increasing hydrophobic chain length. This explained the increase of hydrophobicity of surfactants and compounds with dodecyl group in the alkyl chain for two series that showed the maximum surface lowering ability. The effectiveness (πCMC) values showed gradual decrease by increasing the hydrophobic chain length indicating the increasing of accumulated surfacants molecules at the interface. The largest $\pi_{CMC}$ was observed for IIa 44 mN\m.

Adsorption efficiency, ($P_{C20}$) of the surfactant concentrations that are capable of suppressing the surface tension of the solution by 20 mN\m Therefore, $C_{20}$ can be ameasurment for the adsorption efficiency of the surfactant molecule greater the value of $PC_{20}$is, the higher adsorption effeciency of surfactant is. The effeciency values PC20 of the targeted surfactants (Tables 3, 7) showed their good tendency to modify the surface activity of their solutions at considerably low concentrations.

**Table 5: Antifungal Activity of the SynthesisedThio-based Cationic Amphiphiles:**

| Tested microorgaisms / Samples | Mean of Zone diameter (mm) different concetrations | | | |
|---|---|---|---|---|
| | Aspergillusfumigatus (RCMB 02568) | Syncephalastrum racemosum (RCMB 05922) | Geotricumcandidum (RCMB 05097) | Candida albicans (RCMB 05036) |
| **IIa** | 22.3 ± 0.72 | 19.2± 0.58 | 24.2 ± 0.63 | NA |
| **IIb** | 22.9 ± 0.58 | 19.5 ± 0.63 | 22.8 ± 0.72 | NA |
| **IIc** | 23.2 ±0.58 | 19.6± 0.72 | 20± 0.72 | NA |
| **IIIa** | 20.6± 0.63 | 18.4 ± 0.58 | 23 ± 0.72 | NA |
| **IIIb** | 21.6 ± 0.58 | 18.7± 0.63 | 21.5 ± 1.2 | NA |
| **IIIc** | 23.4± 0.58 | 19.5 ± 0.72 | 20.7 ± 0.72 | NA |
| **Control** | 23.7± 1.2 | 19.7 ± 0.72 | 28.7 ± 0.72 | 25.4 ± 0.58 |

Amphotericin B is used as reference for fungi is 2 -Carboxy-N-(Alkanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide, III $_{a-c}$ is 4 –Sulphonate -N-((fatty alkanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide, IIa-c, a= $C_{12}$, b = $C_{16}$, C = $C_{18}$

$N_A$: No activity

**Table 6: Physicochemical properties of 4 –Sulphonate -N-(fatty alkanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide derivatives**

| R | Abbrevation | Mocular formula | Mol.Wt | Colour | M.P | Yield % | Elemental analysis C % H % N% S% I% O% Cal./found | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dodecyl | $III_a$ | $C_{24}H_{42}N_2S_2O_4I_2$ | 740.01 | Reddish brown | 303-306 | 77 | 38.91<br>38.93 | 5.72<br>5.75 | 3.78<br>3.80 | 8.65<br>8.68 | 34.29<br>34.32 | 8.65<br>8.67 | 8.64<br>8.66 |
| Hexadecyl | $III_b$ | $C_{28}H_{50}N_2S_2O_4I_2$ | 796.16 | Orange | 310-315 | 80 | 42.47<br>42.49 | 6.37<br>6.39 | 3.54<br>3.56 | 8.09<br>8.13 | 32.08<br>32.11 | 7.45<br>7.48 | 8.08<br>8.11 |
| Octadecyl | $III_c$ | $C_{30}H_{54}N_2S_2O_4I_2$ | 824.19 | Brown | 315-320 | 85 | 43.68<br>43.70 | 6.60<br>6.63 | 3.40<br>3.43 | 7.77<br>7.79 | 30.79<br>30.82 | 7.76<br>7.78 | 7.76<br>7.78 |

At air/ water interface. So the Increasing the hydrophobic chain length of the studied amphiphiles results adecrease in the surface tension of the surfactant solution indicating the high tendency of the longer hydrophobic molecules to adsorb at the interface. The lowest $PC_{20}$ value was observed to 3a 2.85. The value of $CMC/C_{20}$ ratio is to determine structural factors in the adsorption and micellization process. From Tables 2, 7, it can be seen that The $CMC/C_{20}$ ratios increase with the increase of hydrophobic chain length for series IIa-c, IIIa-c. This result suggests that cationic surfactants with long hydrocarbonchains adsorp at the air/ water interface in preference to forming micelles, wherease cationic surfactants with short hydrocarbon chains do not adsorp efficiently at the interface. The affinity of aparticular surfactant to reduce surface tension of solvent depend upon $CMC/C_{20}$ ratio, greater the observed value, greater is the tendency of amphiphile to reduce surface tension of the system. It can found that the $CMC/C_{20}$ values of cationic surfactants containing sulphonate group and longer chain length are higher than those of cationic surfactants containing carboxylate group of the same chain length. the $CMC/C_{20}$ values (7.08) for IIc (carboxyate derivatives) and (8.43) for IIIc (sulphonate derivatives).

**III-4 The interfacial activity (I act.)**

The interfacial activity ($I_{act.}$) which expressed physicochemical parameter.

$\Delta G_{ads}/A_{min}$ where (ΔGads) is astandared free energy of adsorption of the surfactant at the air/ solution interface and ($A_{min}$) is the minimum surface area of the surfactant as shown in Tables 2,6. It is clear that I act decreased by increasing alkyl chain length and high depression were observed for compound IIa.

3.2 Thermodynamic Parameters; Free energy of micellization and adsorption ($\Delta G_{mic.}$ and $\Delta G_{ads}$).

Thermodynamic functions describe the energetic parameters of adsorption and micellization process of surfactants molecules either at the interface or in bulk of their solutions.

Micellization and adsorption free energies are in negative values, Which indicate theire spontaneous occurrence in the solution, Whill large negative value indicate the majorty of one process than the other. The standard free energies for adsorption and micellization of the synthesised surfactants are negative. The value of adsorption free energies are mor negative than those of micellization process. That indicate the high tendency of of the synthesised surfactants towards adsorption at the interface than micellization and the tendency towards adsorption referred to the interaction between the aqueous phases and the hydrophobic chains which pumps the surfactants molecules to the interface. The presence of these surfactants at the interface decrease the different in phases interactions. The maximum depression in $\Delta G_{ads}$ was observed at -12.9 KJ / mol for IIc. This result showed the

applicability of these surfactants in several applications including: emulsification, detergency and corrosion inhibition application[52].

**IV- Biological Activity**

Quaternary nitrogen compounds act as amphiphilic cations in aqueous solution so many cationic surfactants are used as pesticides and antimicrobial against wide spectrum of organisms as well as for killing sulfate reducing bacteria which can exist in petroleum field and cause many problems such as block out pipe line, produce H2S toxic gas, form iron sulfide hard scale and cause microbial corrosion [53-55].

Tables (4, 5) show the antibacteria and anti fungal activities of the prepared compounds IIa-c, IIIa-c. Ampicillin and gentamicin were taken as the refrence drugs for antibacterial activity and amphotericin B as antifungi. By analyzing the data in table 4,5, it is clear that the difference in activity depend on the prepared cationic surfactants. Increasing the hydrophobic chain length increase the antibacterial activity, these results are in consistence with the adsorption tendency of these amphiphiles at the interface. The octadecyl derivatives (C18) in the two series showed the maximum antimicrobial activities against the tested bacterial and fungi strains. Overall, it was observed that the synthesized cationic surfactants exhibited higher antibacterial activity against Gram – positive bacteria than Gram negative bacteria. This can be explained by different cell membrane structures of the two bacterial types. On the other hand, the most effective factor is the carbon chain length; it was found that, as the carbon chain length (hydrophobic part) increases, the compound efficiency also increases. That can be related to their ability of adsorption at the interface. Increasing the absorbability[56-62] increases their action on the cell membrane. The results of the antifungal activity of the synthesized quaternary ammonium salts showed that negative effect against the most pathogenic fungal strain,. That may be ascribed to the resistivity of the fungi strain to the aggressive environmental components due to the rigidity of their cellular membranes, so that these fungi have high resistance against synthesized cationic surfactants and best results for compounds (IIIa-c) for all fungi species except Candida albicans (RCMB 05036) as illustrated in Table 5.

**V- Antitumor Activity using EAC:**

**Table 7: Critical micelle Concentrations (CMC), Surface tension at cmc ($\gamma_{cmc}$), surface max ($\tau_{max}$), Minimum Area\Molecule ($A^2$), Standard Free Energy of Micellizaion ($\Delta G_{mic.}$), Standard Free Energy of Micellizaion per methylene group ($\Delta G_{mic}/CH_2$), Standard Free Energy Of adsorption ($\Delta G_{ads}$) and Standard Free Energy Of adsorption per methylene group$\Delta Gads/CH_2$of 4 –Sulphonate -N-(fatty alkanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide at Liquid Air Interface at 25C°**

| R | Abbrevations | CMC mol/l | $\gamma_{cmc}$ mN/m | $\tau_{max}$ X $^{-10}$ mole/cm$^{-2}$ | Area mole $A^2 \times 10^2$ nm$^2$ | $-\Delta G_{mic.}$ KJ/mol | $-\Delta G_{mic}/CH_2$ KJ/mol | $-\Delta G_{ads}$ KJ/mol | $-\Delta Gads/CH_2$ KJ/mol |
|---|---|---|---|---|---|---|---|---|---|
| Dodecyl | IIIa | 9.40 x $10^{-3}$ | 29.00 | 1.13 | 147.5 | 11.56 | 0.124 | 11.97 | 0.111 |
| Hexadecyl | IIIb | 8.90 x $10^{-3}$ | 32.00 | 1.14 | 145.6 | 11.69 | | 12.26 | |
| Octadecyl | IIIc | 7.50 x 10-3 | 34.00 | 1.17 | 141.3 | 12.12 | | 12.56 | |

From Table 9, it is clear that the cytotoxic effects of these compounds were dose dependent, i.e. by increasing the concentration on these compounds in the culture media; the percentage of non-viable cells (EAC) is increased. Also from data of Table 9 one observes that upon testing the compounds which contains more than one benzene ring, the antitumor activity dramatically increases from 23 % of non-viable EAC cells at 25 mg/ml to 95% of these cells at 100 mg/ml and also NVC% increases by increasing the chain length of the hydrophobic radical. To achieve the maximum therapeutic damage of tumor cells using the minimum concentration of drugs, the tumor cells lines used in this study for 3a which causes the death of 95% of EAC cell are liver carcinoma (HEPG2), breast carcinoma (MCF 7) and colon carcinoma (HCT 116), Tables 10 and 11. The 50% growth inhibitory concentration (IC 50) values for 3a-c at different concentration ranged from 5 to 50 mg/ml is recorded (18.7,16.4,14.2) whill (IC 50) for 2a-c (12.5, 10.6, 8.7). Data showed that 3a was found to exhibit high activity in vitro system on the tumor cell lines investigated and the highest cytotoxic effect on HEPG2, HCT116 and MCF7, respectively.

In our research, we found that 2a surfactant affects tumor tissue at very low concentrations at values lower than their cmc values which means that there is a strong relationship between very small values of cmc of this compound and its ability to reach IC50 values under very low concentration. This is due to the fact that increasing the concentration of surfactants causes an increase in the adsorption process on cells membranes till the cmc is reached; after this adsorption slowly decreases and then stops due to the formation of micelles, which prevent mobility and suppresses antitumor activity. increasing their doses. A significant number of studies have demonstrated successful control of the particle size,

morphology and surface functionalization of the surfactants for diverse applications [64–68].

Antitumor activity of these surfactants was evaluated against three human tumor cells such as HEPG2 (liver), HCF7 (breast) and HCT116 (colon). The cytotoxic effects of these compounds were dose dependent, i.e. by increasing the concentration on these compounds in the culture media; the percentage of non-viable cells (EAC) was increased. Also, data showed that (IIIa) was found to exhibit higher activity than (IIa) in vitro system on the tumor cell lines investigated and the highest cytotoxic effect on HEPG2, HCT116 and MCF7, respectively.

**Table 8: Surface Tension Reduction ($C_{20}$), Efficiency($PC_{20}$), cmc/$C_{20}$ Ratios, $\pi_{eff}$ Effectiveness ($\pi_{cmc}$) and Effective Number of Can atom ($N_{eff}$) of 4 – (Ethyl)Ammonio)CarbonothioylSulphonate -N-(fatty alkanoyl)- N-Ethylbenzenaminium Iodide derivatives at liquid air interface at 25C°.**

| R | Abbrevations | $C_{20}$ | $PC_{20}$ | CMC\\$C_{20}$ | $\pi_{eff}$ | $\Pi_{CMC}$ mN/m | $N_{eff}$ |
|---|---|---|---|---|---|---|---|
| Dodecyl | IIIa | $1.4 \times 10^{-3}$ | 2.85 | 6.71 | 25.2 | 43 | 18.0 |
| Hexadecyl | IIIb | $1.12 \times 10^{-3}$ | 2.95 | 7.95 | 25.8 | 40 | 22.0 |
| Octadecyl | IIIc | $0.89 \times 10^{-3}$ | 3.05 | 8.43 | 26.1 | 38 | 24.0 |

**Table 9: Antitumor activities of thio based cationic surfactants Derivatives Using (E.A.C.)**

| Samples name | Sulphanilic IIIa-c | | | | Anthranilic IIa-c | | | |
|---|---|---|---|---|---|---|---|---|
| | % Inhibition of cell viability | | | | | | | |
| | | | Ug/ml | | | | | |
| Concentrations Abbrevations | | 25% | 50% | 100% | Concetrations abbrevations | 25% | 50% | 100% |
| Dodecyl | IIIa | 23% | 47% | 95% | IIa | 13% | 32% | 90% |
| Hexadecyl | IIIb | 22% | 45% | 90% | IIb | 15% | 35% | 88% |
| Octadecyl | IIIc | 20% | 40% | 87% | IIc | 18% | 37% | 80% |

**Table 10: Inhibitory effect of 2- Carboxy N-(lauroyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide**

| Compound(IIa) | Cell lines | | |
|---|---|---|---|
| | HEPG2 | MCF7 | HCT116 |
| 2- CarboxyN-(lauroyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide | $IC_{50}$ | $IC_{50}$ | $IC_{50}$ |
| | 12.5µg | 8.7µg | 10.6µg |

**Table 11: Inhibitory effect of 4- Sulphonate N- (lauroyl (Ethyl)Ammonio)Carbonothioyl)-Ethylbenzenaminium Iodide**

| Compound(IIIa) | Cell lines | | |
|---|---|---|---|
| | HEPG2 | MCF7 | HCT116 |
| 4- Sulphonate N- (lauroyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide | $IC_{50}$ | $IC_{50}$ | $IC_{50}$ |
| | 18.7µg | 14.2µg | 16..4 µg |

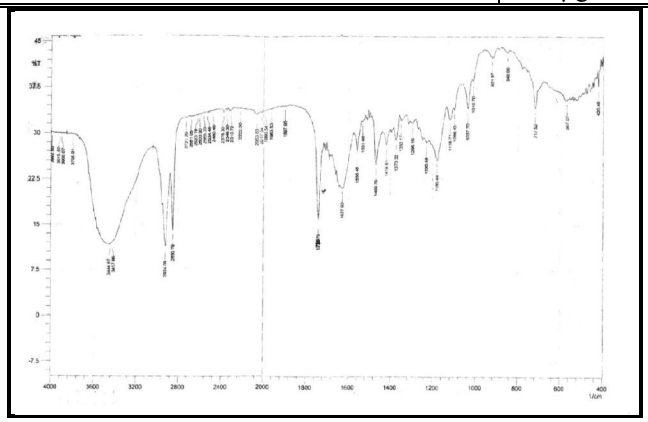

Figure (1) FITR spectra of 2-carboxy- (Dodecanoyl (Ethyl ammonio) carbonothioyl–N–Ethyl benzenaminium Iodide

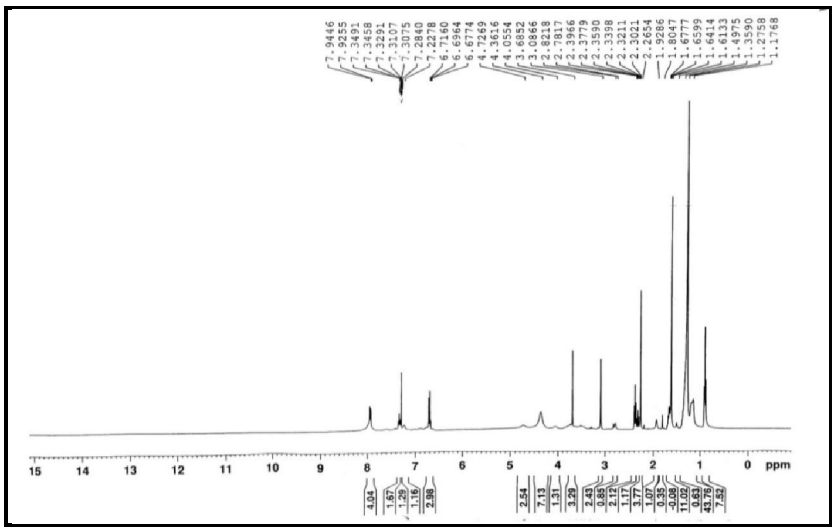


Figure. (2) $H^1$NMR spectra of 2-carboxy-(Dodecanoyl(Ethyl ammonio) carbonothioyl–N–Ethyl benzenaminium Iodide

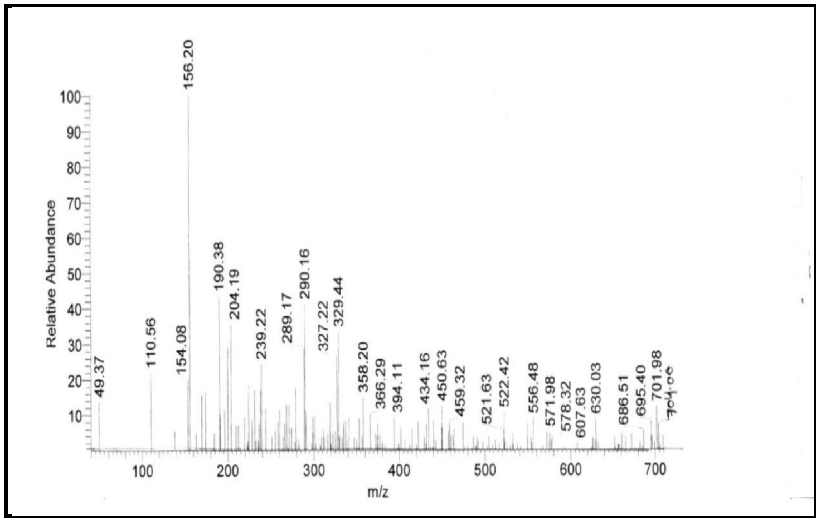


Figure. (3) Mass spectra of 2-carboxy-(Dodecanoyl(Ethyl ammonio) carbonothioyl–N–Ethyl benzenaminium Iodide

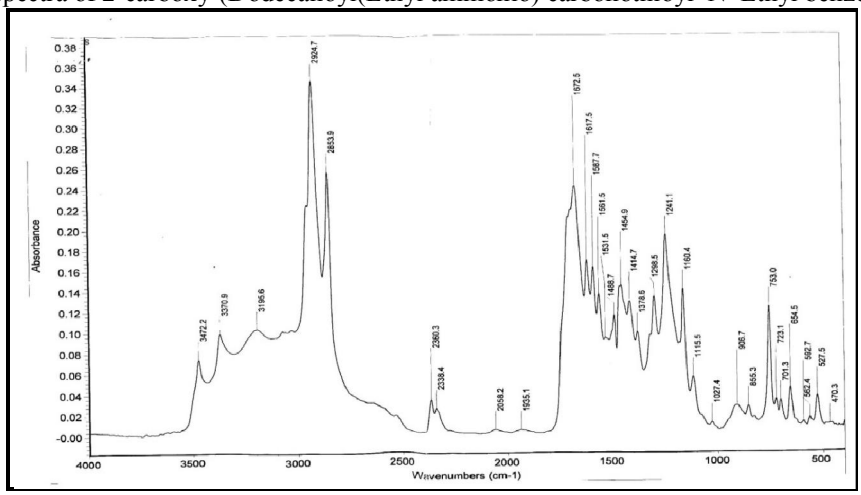


Figure. (4) FITR spectra of 4-sulphonate-N-(Dodecanoyl ethyl ammonio)carbonothioyl–N–Ethyl benzenaminium Iodide

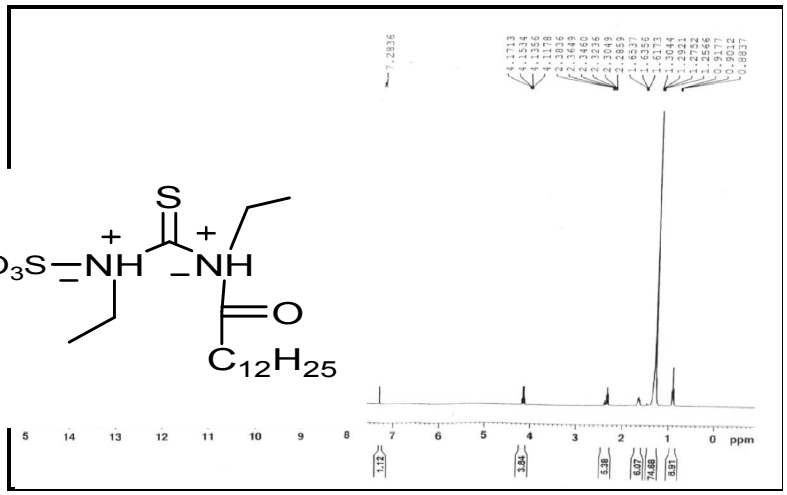


Figure (5): $H^1$NMR Spectra of 4-sulphonate-N-(Dodecanoyl ethyl ammonio) carbonothioyl–N–Ethyl benzenaminium Iodide

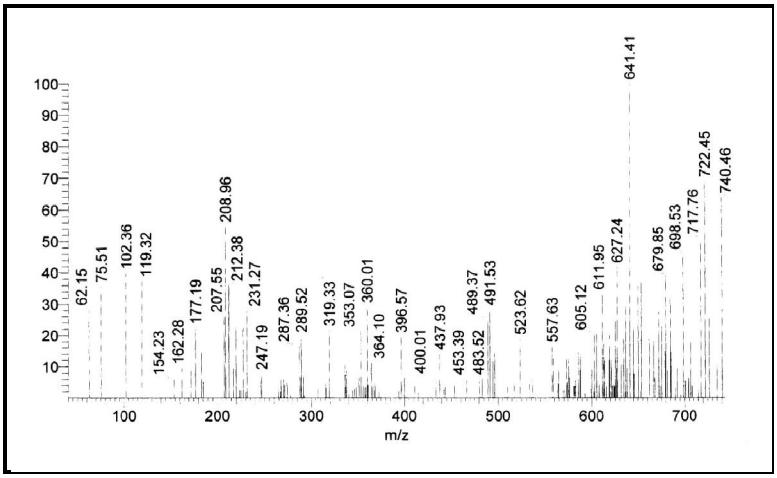


Figure. (6) Mass spectra of 4-sulphonate-N-(Dodecanoyl ethyl ammonio) carbonothioyl–N–Ethyl benzenaminium Iodide

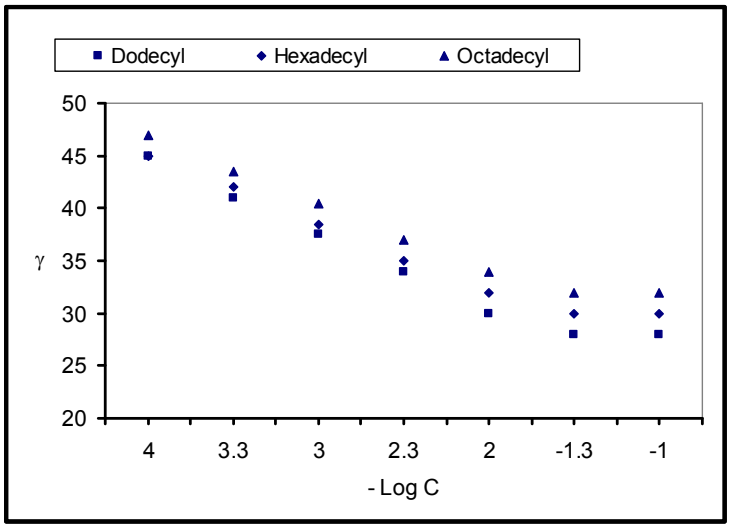


Figure (7): Represents surface tension (γ) vs-log C of 2 -Carboxy-N-((Dodecanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide in distilled water

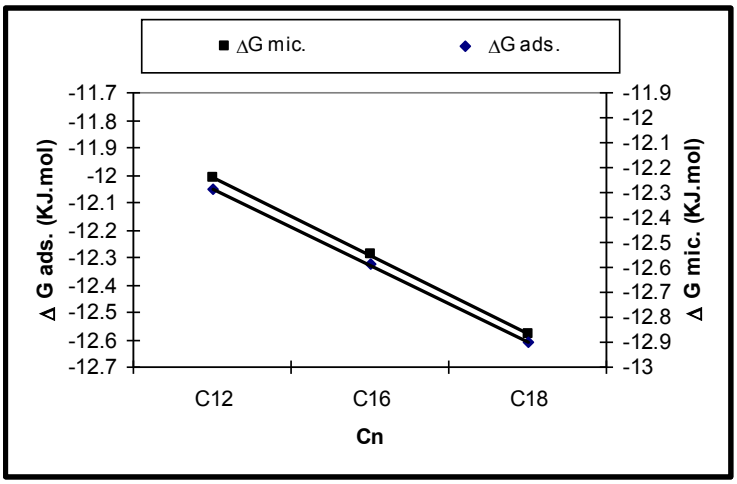


Figure. (8) Represents Standard Free Energy of Micellization (ΔGmic) and Standard Free Energy of Adsorption (ΔGads) vs Carb. no. of 2 -Carboxy-N-)Dodecanoyl (Ethyl)Ammonio) Carbonothioyl)- N-Ethylbenzenaminium Iodide in distilled water

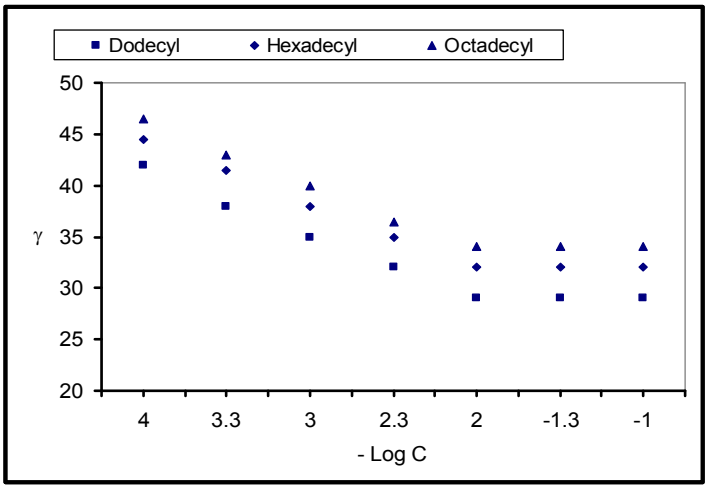


Figure. (9): Represents surface tension (γ) vs-log C of 4–Sulphonate-N-(fatty alkanoyl(Ethyl)Ammonio)Carbonothioyl)-N-Ethylbenzenaminium Iodide in distilled water

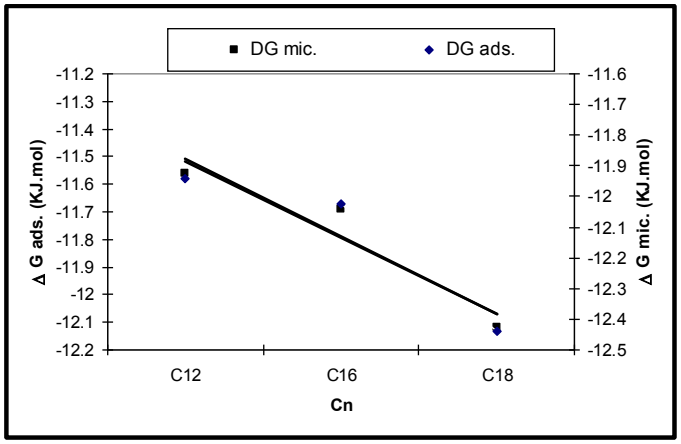


Figure. (10) Represents Standard Free Energy of Micellization (ΔGmic) and Standard Free Energy of Adsorption (ΔGads) vs Carb. no. of 4–Sulphonate-N-(fatty alkanoyl (Ethyl)Ammonio)Carbonothioyl)- N-Ethylbenzenaminium Iodide in distilled water

**6 Conclusion:**

The synthesized surfactants showed higher surface activity and their activity was influenced by the chemical structures and hydrophobic chain length. The temperature of the solutioncauses a significant effect on the thermodynamic parameters of adsorption and micellization. Also, the antimicrobial activity of quaternary salts toward bacteria and fungi were high and depended on the chemical structures of the biocide these surfactants in their solutions were also calculated. Biocidal activity of prepared amphiphiles was investigated against bacterial and fungi species which showed that our surfactants are in general capable of inhibiting the growth of bacteria and fungi to a moderate extent. Also, it is clear that the biological activities of the synthesized surfactants against the tested microorganism increase by increasing their doses. Antitumor activity of these surfactants was evaluated against Ehrlich ascites carcinoma (EAC) and against three human tumor cells such as HEPG2 (liver), HCF7 (breast) and HCT116 (colon). The cytotoxic effects of these compounds were dose dependent, i.e. by increasing the concentration on these compounds in the culture media; the percentage of non-viable cells (EAC) was increased. Also, data showed that surfactant compound (IIIa) was found to exhibit higher activity than surfactant compound(IIa) in vitro system on the tumor cell lines investigated and the highest cytotoxic effect on HEPG2, HCT116 and MCF7, respectively.

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