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Synthesis,and Characterization of new Gemini Cationic Surfactants and Its Inhibition Behaviour Study on Cell Line Breast Cancer MCF-7

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Abstract **- On the basis of alkyl alcohol and eipchlorohydrine three novel multi-alkyl quaternary-ammonium salt cationic surfactants have been created. 1H-NMR and FTIR spectrometry researches are used on this investigation. Artificial products structures are confirmed by FTIR and 1H-NMR spectrometries. There is an increase in alkyl group in the order C4 < C6 < C8. This growth is used to pick out the decline in critical micelle concentration (CMC), which discovered that the higher the floor features, the bigger the alkyl group. MTT cytotoxicity assay is used to assess the organic efficacy of Gemini surfactants as well as estimating their toxicity. Compound A3 was discovered to has a high impact on MCF-breast most cancers cell line. It reached the best rate of inhibition of 27.28% - 5.4% with concentration 25 – 400 µg ml-1, in comparison with compounds A1 and A2. The rate of inhibition for A1 is 36.3% - 5.3% with concentration 25 – 400 µg ml-1. The rate of inhibition for A2 is 48.9% - 5.6 % with concentration 25 – 400 µg ml-1.**

***Keywords*— Gemini surfactants, electrical conductivity, critical micelle concentration and MTT cytotoxicity.**

I. INTRODUCTION

The identity of Gemini surfactant is a sort of surfactant that has a couple of unique traits distinguishing it from the others. The foremost traits of it are two, the primary one is its shape and the second is its particular properties. Gemini surfactants shape includes a couple of hydrophilic head group and hydrophilic tail group. These two groups are connected with the aid of using a spacer at or close to the top group. The particular properties that Gemini surfactant has are low critical micelle concentration, proper water solubility and aggregation behavior, excessive performance in decreasing oil/water interfacial tension and finally, exciting rheological residences.

In addition, the eye of the instructional researchers and the sector specialists were attracted by the aid of using the exciting rheological properties that Gemini surfactant had [1-7]. Gemini surfactants are mainly essential and progressive substances in detergents, cosmetics, non-public care products, components for paints and coatings, material science, natural synthesis, and finally, pharmacy because of their unique features with a huge variety of hydrophilic - lipophilic balance (HLB).

Another thrilling part of Gemini surfactant traits has the advantage of numerous interests currently through the specialized communities. This part is the creation of cationic Gemini surfactants. The interest got here because of their particular ability to form a complex with a variety of negatively charged molecules [8]. This complex is used extensively as an antistatic agent [9], softener and oil properly drilling agents [8].

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Furthermore, in our present day a lot of traits to all of the information associated with Gemini surfactant were increasing. One of them is using Gemini amphiphilic aggregates in gene delivery [10,11], antimicrobial activity [12,13], and sooner or later the synthesis of mesoporus material [14,15]. Another issue of those traits is the creation and observe of several cationic Gemini surfactant categories [16-20].

Gemini surfactants that had morpholine moieties as hydrophilic head groups have sure blessings that cause them to unique a number of novel surfactants. An instance of those blessings is their capacity to show a sturdy inhibition due to the presence of nitrogen and oxygen atoms in the Symmetric ring [21].

The opportunity of the usage of cationic morpholine surfactant in numerous packages is a vital issue that investigations bear in mind till now. Morphloine amphiphle having petro amassing and dispersing properties is proved [22]. Synthetization of a sequence of metallo surfactants from the response of fatty acids with morpholine and then investigation of their floor properties and antimicrobial activity are performed [23]. A new Gemini surfactants have been investigated due to the micellization and viscosity homes, the use of floor tension, conductivity, fluorescence and finally, rheology techniques. The type, size and polarity of the cationic head groups they all had effects on the physicochemical traits of the aqueous surfactant system.

For this research a new collection was created. This creation was done by including hydroxyl and ether companies to the lipophilic aspect and an ester organization to the spacer. The final result is a chain of a new ester functionalized cationic Gemini surfactant totally based on morpholine or piperidine. The basic properties and antimicrobial activity were measured and remarked. The measurement indicated that the special cationic head groups have a considerable effect on the react places of the surfactants. Breast cancer is the most frequent malignancy among women worldwide, accounting for 25% of all cancers, with an estimated 2.088 million cases recorded in 2018 [24]. It is also the leading cause of female cancer-related deaths. Although substantial improvement in survival from this disease has been reported in high-resource countries, the risk continues to increase, yielding high mortality rates in middle and low-income countries.

II. EXPERIMENTAL

A. Materials and instruments: -

The following substances have been purchased from Company: epichlorohydrin (98% purity Sigma-Aldrich,germane), piperidine (98% purity, Sigma-Aldrich germane,), butanol (98% purity Sigma-Aldrich, germane), Hexanol (98% purity Sigma-Aldrich, germane), Octanol (98% purity Sigma-Aldrich, germane), 1,6 -di bromo hexane (98% purity, Merk, germane), ethyl acetate (99% purity, Sigma-Aldrich, germane), ethanol (99.8% purity, Merk, germane).

The characterization by^{1}HNMRWere recorded on Bruker AM500 spectrometer. The NMR spectra of the prepared gemini surfactants Were recorded in DMSO and chemical shifts recorded Were internally referenced to TMS(0ppm) and Fourier transform infrared (FTIR) Were recorded on Perkin Elmer tonser27 Bruker verified the structural characters of these new gemini surfactants on a Thermo Electron Corporation Nicolet400 FTIR spectrophotometer . TLC approach was completed on aluminum sheets covered through a homogeneous silica gel sorbent layer of 90 - 120 m thickness, 5-17 Sorbent size (m). The calculating of the surfactant solution's CMC values was completed through the use of electric conductivity and a WTW Inolab cond 740 conductivity meter (Germany).

B. Synthesise

1) Synthesis of Alkyl Glycidyl Ethers ı

The synthesis of Alkyl Glycidyl ethers is going through certain steps. The heating of Alkyl alcohol (0.1 mol) in the reaction flask to 40 ºC, sodium hydroxide (6g, 0.15 mol), TBAB (0.2g, 0.00063 mol) and hexane have been delivered. After that the combination become stirred for 30 min at the same temperature. The second step is the addition of Eipchlorohydrine (18.5g, 0.2 mol). In batches for 30 min the temperature must be maintained between 38-40 ºC. After that the mixture was ascended under reflux distillation for 2-6 hr. In the third step the product is cooled and the organic layer is separated. Finally, The result is a yellow precipitate, yield of (0.78g, 78%). Evaporate the solvent from the organic layer under low pressure and the residue are discarded

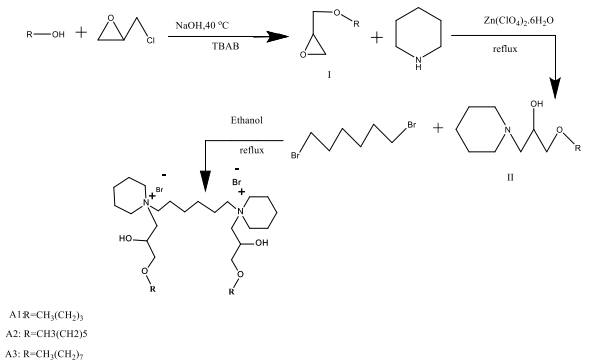
2) Synthesized 1-butoxy-3-(piperidin-1-yl) propan-2-ol ıı

Piperidin (1.5g, 0.0125mol) was added with stirring into a solution of Zn(ClO4)2.6H2O (0.1g) and then the solution was stirred for 20 min at room temperature. Compound ı (1.3g, 0.01mol) was added, while the temperature of the reaction mixture was maintained at 80 ºC for 24 hr. The produced precipitate was collected by filtration, recrystallized from ethanol, and then dried under vacuum to afford ıı as a yellow precipitate, yield (0.80g, 80%), M.P. (202 ºC).

3) Synthesized 1,1-(hexane -1,6-diyl)bis(1-(3-butoxy-2-hydroxypropyl)piperidine-1-ium)bromide A1

Compound ıı (2.1g,0.01mol) was dissolved in ethanol when the solution became clear.1,6-dibromohexan (0.75g,0.005 mol) was added to the solution and reacted for 48 h at 60 ºC. The reaction progress was monitored by TLC (eluent=0.5:2.5: 10 KNO3: H2O): CH3CN). The produced precipitate was filtrated, recrystallized from ethanol, and then dried under vacuum to afford A1 as a yellow precipitate, yield (0.76g, 76%) M.P.(155ºC).

Compounds A2 and A3 were prepared in the same way.



Scheme 1: syntheses of cationic gemini surfactants

C. *Calculate the critical colloidal concentration using conductivity*

A series of concentrations (0.1-1×10-4) molar of the prepared surfactants were prepared, and the electrical conductivity G was recorded for the prepared solutions at a temperature of 25 m^0, where the number of readings was 10 at a reading rate per two minutes for all the prepared materials. It was converted into a specific conductivity L, and the CMC value of the prepared materials was calculated from the graphs of the molar concentration values against
The spesific conductivity .

III. RESULTS AND DISCUSSION

A. *FTIR spectra*

FTIR Spectrum of organized compound of A1 Figure 1 changed into characterized with the aid of using the presence of essential absorption bands at 3382.18 cm-1 is the vibration height of –OH. The adsorption peak at 2944.32cm−1 may be attributed to the uneven vibration of the CH_2 group; 1469.23cm−1 is the flexural vibration height of C-H; C–O stretching band1184.96 cm-1, C-N at 11279.31 cm-1. According to the analysis, it could be concluded that the synthesized product is the goal product [25].

The infrared spectrum of compound A2 is given the vibration height of –OH at 3217.90cm-1. The adsorption height at 2956.94cm−1 may be attributed to the uneven vibration of the CH_2 group. However, they have been found at 1387.83 cm−1 for symmetric bending (CH_3), at 1454.21 cm−1 for symmetric bending (CH_2), and at 768.85 cm−1 for –(CH_2) n– rock; C–O stretching band1163.88 cm−1, C–N at1284.67 cm−1 [25] as proven in Figure 2.

The FT-IR Spectra Figure 3 of compound A3 confirmed bands at 2858.47 and 2959.34 cm−1 for the alkyl element for uneven and symmetric stretching (CH), respectively. However, they have been found at 1319.37 cm−1 for symmetric bending (CH_3), at 1451.93 cm−1 for symmetric bending (CH_2), and at 735.91 cm−1 for –(CH_2) n– rock. And look bands at 1094.34 and1199.89 cm−1due to stretching C–O, C–N+ at 1234.56 cm−1, 3216.72 cm−1 changed into because of stretching OH. FTIR spectra confirmed the predicted practical agencies in the synthesized compound [26].

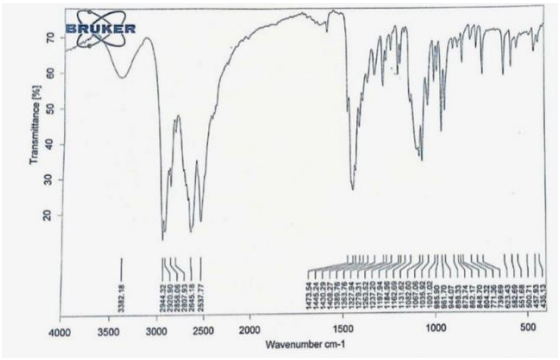


Figure 1: FT-IR Spectrum of cationic Gemini surfactant A1

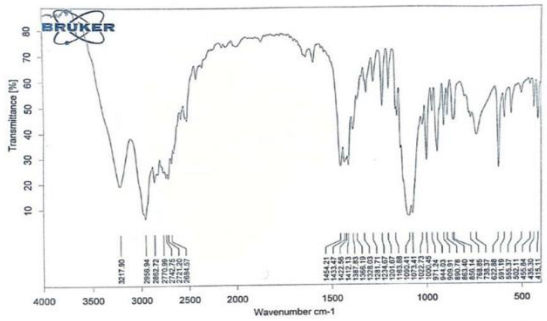


Figure 2: FT-IR spectrum of cationic gemini surfactant A2

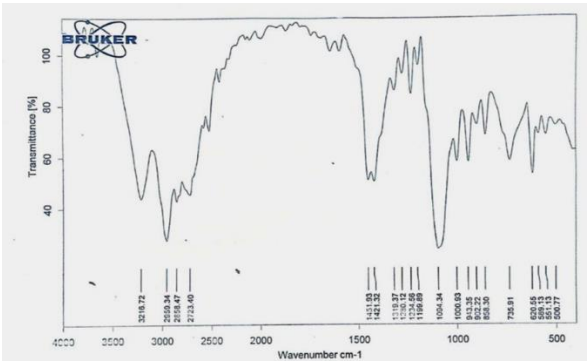


Figure 3: FT-IR spectrum of cationic gemini surfactant A3

B. *^{1}H-NMR spectra*

^{1}H-NMR Spectrum of compound A1,figure 4 showed multiple signals The specific analysis is as follows: 1H NMR(DMSO) δ 0.88–0.94 (t, 6H, $(CH_3)_2$), 1.43–1.70 (m, 16H, $(CH_2)10$), 3.34 -3.46 (t, 4H, $(O\text{-}CH_2)_2$), 3.29, 3.32 (t, 4H, CH_2CH_2), 4.07 (s, 2H, $(OH)_2$), 1.31 (m ,4H, CH_2CH_2) 1.76 (d,4H, CH_2 CH_2), 3.15 – 3.20 (m, 4H, $(CH_2CH_3)_2$) [27].

Figure 5 and figure 6 ^{1}H-NMR Spectrum of compound A2, in the presence and absence of the solvent confirmed more than one signals. The particular evaluation is as follows: ^{1}H NMR(DMSO) δ1.43 (t, 6H, $(CH_3)_2$), 1.71–1.84 (m, 24H, $(CH_2)_{12}$), 3.0-3.07 (t, 4H, $(O\text{-}CH_2)_2$), 3.29, 3.32 (t, 4H, CH_2CH_2), 4.5 (s, 2H, $(OH)_2$), 1.31 (m, 4H, CH_2CH_2) 1.43 (m, 2H, (CH OH)$_2$), 1.55(m ,4H,

$(CH_2CH_2CH_3)_2$), 3.53 (m, 4H, $(CH_2CH_3)_2$) [28].8-9 in figure 5impurities due to the presence of the solvent.

Figure 7 and figure 8 ^{1}H-NMR Spectrum of compound A3, in the presence and absence of the solvent confirmed more than one signals. The particular evaluation is as follows:^{1}H NMR(DMSO) δ1.25 (t, 6H, $(CH_3)_2$), 1.63-1.89 (m, 32H, (CH_2)16), 3.44-3.54 (t, 4H, $(O\text{-}CH_2)_2$), 3.29, 3.32 (t, 4H, CH_2CH_2), 4.5 (s, 2H, $(OH)_2$), 1.31 (m ,4H, CH_2CH_2), 3.11 (m ,2H, $(CH\ OH)_2$), 1.43 (m ,4H, $(CH_2CH_2CH_3)_2$), 3.20-3.26 (m, 4H, $(CH_2CH_3)_2$) [28]. 8-9 in figure 7 impurities due to the presence of the solvent.

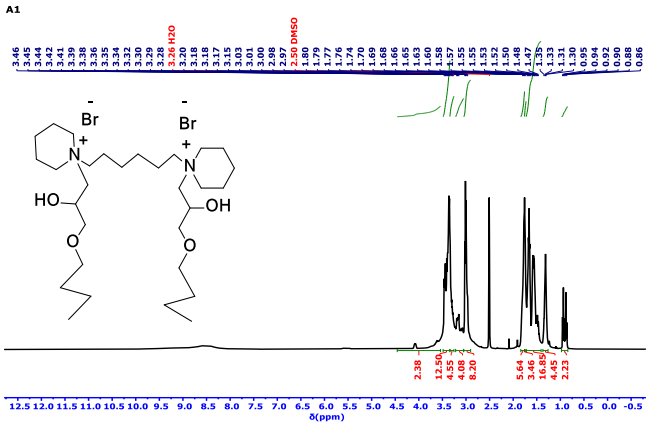


Figure 4: ^{1}H-NMR of cationic gemini surfactant A1

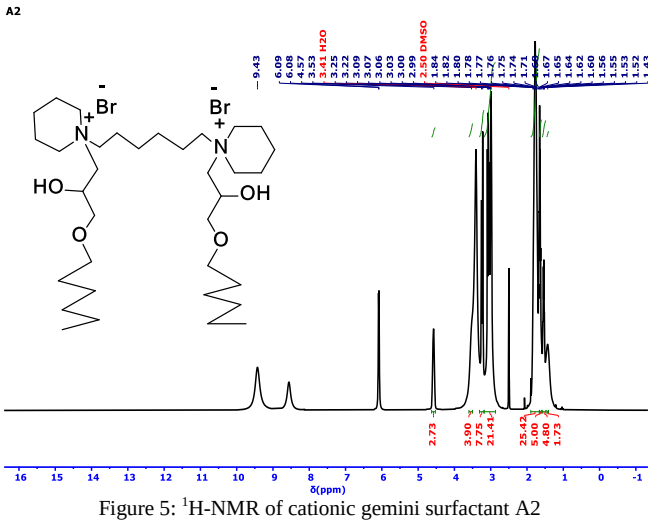


Figure 5: ^{1}H-NMR of cationic gemini surfactant A2

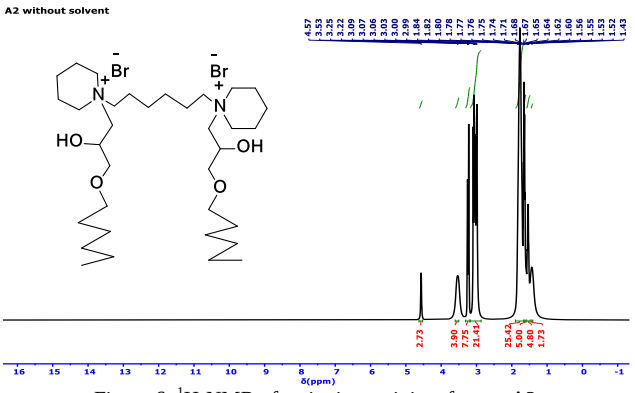


Figure 6: ^{1}H-NMR of cationic gemini surfactant A2

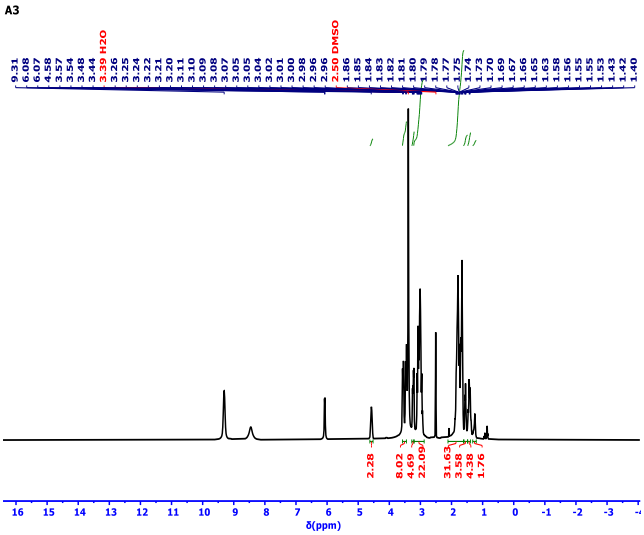


Figure 7: ^{1}H-NMR of cationic gemini surfactant A3

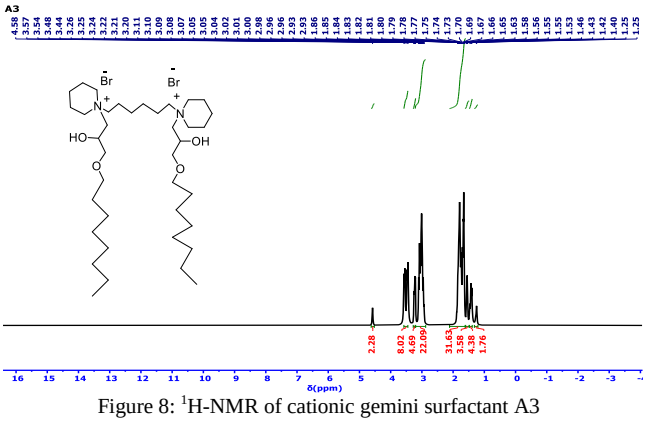


Figure 8: ^{1}H-NMR of cationic gemini surfactant A3

C. Determination of CMC by conductivity method

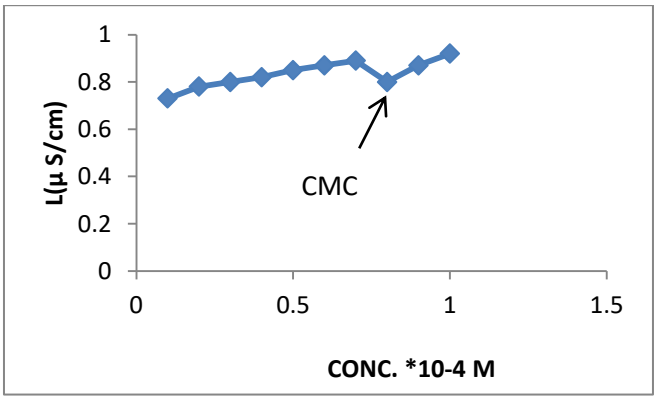
Many diluted concentrations of the gemini surfactant in water (0.1×10^{-4} M) to (1×10^{-4}M) have been used and the values have been recorded through electric conductivity (G) for organized solutions at 25º C. The values were transformed into particular conductivity L as showed in Figure 1. They are also plotted with the change in concentration and CMC extraction from plot 4. CMC decreases with the increase chain alkyl and reduces with the increase polarity of the compounds [29].

L=GA ……..1

Where,

L =specific conductivity, A =cell constant, and G=electrical conductivity.

Observed the conductivity was increase linearly with concentration due to an increase in the number of released amphiphilic molecules in the solution to reach a critical micelles concentration point CMC. The big increase in the conductivity because increasing of free ions released in solution.



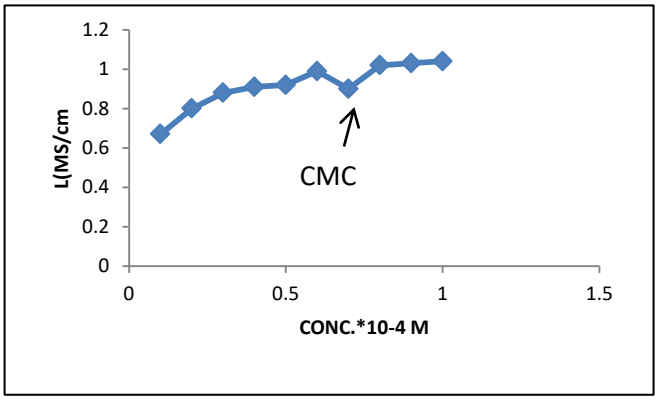


Figure 8: CMC of (A1) surfactant

Figure 9: CMC of (A2) surfactant

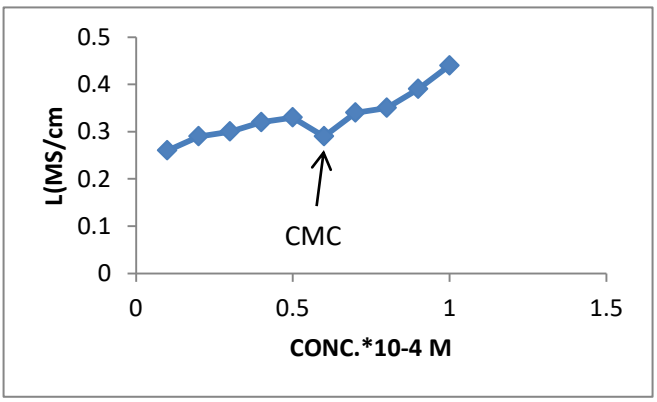


Figure 10: CMC of (A3) surfactant

D. The effect of gemini cationic surfactants on cell line MCF-7

Three surfactant compounds have been examined using the MTT cytotoxicity assay to decide the toxic impact of the organized compounds on MCF-7 breast cancer cells. Then the cell suspension became located at an attention of (1×104 -1×105) hope cell in a Plate with 96 holes to a very last length of 200 micrometers of entire subculture medium for every layer. The plates have been blanketed with an opaque cover, shaken gently-stirred, and incubated in an incubator with 5% CO2 at a temperature of 37 ºC for 24 hours [30].

Cytotoxicity Assay: using MTT cytotoxicity assay to decide the toxic impact of piperidin compounds on breast cancer

cells MCF-7. The outcomes of the check of A1 impact compound at the breast cancer cell line MCF-7 confirmed an interest with an inhibition rate of 58.2%, 49.1%, 36.3%, 23.7%, 15.7% and with concentration 400, 200, 100, 50, 25 µg ml-1 respectively.

Moreover, when testing the effect of A1 compound on a normal cell line WRL68, the results showed an inhibition rate that ranged between 36.3% -5.3% at concentrations from 25 -400µg ml-1 as shown in Table 1.

Table 1: The effect of compound A1 on breast cancer cell line MCF -7 and normal cell line WRL68 using the MTT test

Concentrationµg mL^{-1}	Mean viability (%) ± SD	
	WRL68	MCF-7
400	63.7±3.30	41.78±3.68
200	72.53±1.12	50.9±3.32
100	83.64±3.28	63.7±4.29
50	94.63±1.51	76.31±3.13
25	94.63±1.35	84.37±2.7

And while calculating the IC50, the results confirmed widespread variations p≤ 0.0001 while dealt with A1 compound of MCF -7 most cancers cells 107.1µgml-1 and regular WRL68 138.7µg ml-1 as proven in Figure 11.

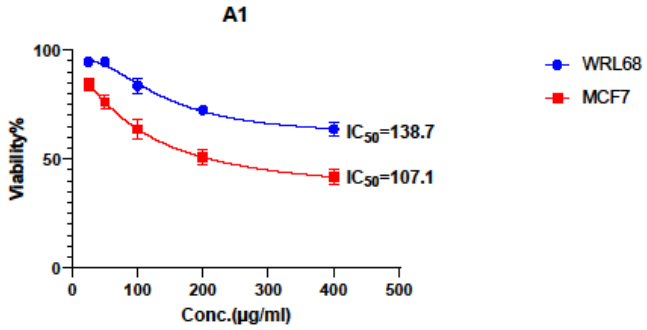


Figure 11: Effect of A1 compound on the cell line MCF -7and WRL68 using the MTT test

The results of the test of A2 compound at the breast most cancers cell line MCF-7 confirmed an interest with an inhibition rate of 46.4%, 38.5%, 26.2%, 15.5%, 6.1% and with concentration 400, 200, 100, 50, 25 µg ml-1 respectively.

And when testing the effect of A2 compound on a normal cell line WRL68, it showed an inhibition rate that ranged between 48.9% -5.6% at concentrations from 25 -400µg ml-1 as shown in Table 2.

Table 2: The effect of compound A2 on breast cancer cell line MCF -7 and normal cell line WRL68 using the MTT test

Concentrationµg mL^{-1}	Mean viability (%) ± SD	
	WRL68	MCF-7
400	51.11±4.7	53.62±4.3
200	60.49±1.45	61.5±1.51
100	71.60±1.3	73.80±1.47
50	84.5±2.3	84.52±2.6
25	94.4±0.8	93.90±0.92

And while calculating the IC50, the effects confirmed massive variations p≤ 0.0001 while handled with A2 compound of MCF -7 most cancers cells (87.26µgml-1) and ordinary WRL68 (95.7µg ml-1) as proven in Figure (12). Figure 12: Effect of A2 compound on the cell line MCF -7and WRL68 using the MTT test.

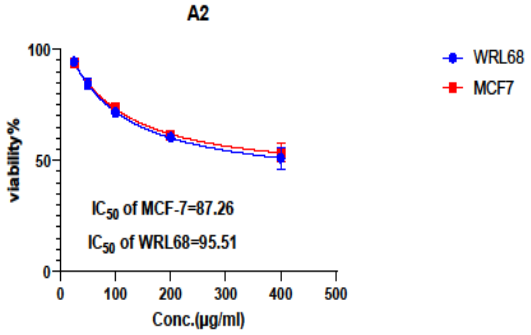


Figure 12: Effect of A2 compound on the cell line MCF -7and WRL68 using the MTT test

Results of the test of A3 compound at the breast most cancers cell line MCF-7 confirmed an interest with an inhibition rate of 60.2%, 44.8%, 38.1%,27%, 14.4% and with concentration 400, 200, 100, 50, 25 µg ml-1 respectively. And while checking out the impact of A3 compound on an ordinary cell line WRL68 it confirmed an inhibition rate that ranged between (27.28% -5.4%) at concentrations from (25 -400µg ml-1) as proven in Table 3.

Table 3: The effect of compound A3 on breast cancer cell line MCF -7 and normal cell line WRL68 using the MTT test

Concentrationµg mL^{-1}	Mean viability (%) ± SD	
	WRL68	MCF-7
400	72.72±2.7	39.81±2.50
200	83.64±1.45	55.20±10.4
100	76.23±3.7	61.95±0.46
50	85.6±2.17	73.07±2.8
25	94.52±0.4	85.53±1.79

And while calculating the IC50, the consequences confirmed massive variations p ≤ 0.0001 while handled with A3 compound of MCF -7 most cancers cells (49.7µgml-1) and regular WRL68 (313.0µg ml-1) as proven in Figure 13.

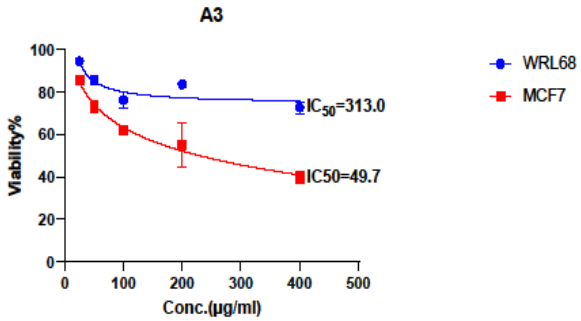


Figure 13: Effect of A3 compound on the cell line MCF -7and WRL68 using the MTT test

IV. CONCLUSIONS

The possibility of preparing cationic Gemini surfactants with heterocyclic shape and the usage of piperidin as a raw material in its composition through measuring FTIR and1H-NMR. Getting of Gemini surfactants effective high biological. The study showed that increasing the length of the hydrophobic (lipophilic) terminal chain due to the decrease in the CMC value of it. The biological effectiveness of the compound(A3) is the highest in comparison (A1) and(A2).we notice that with increasing the length of the alkyl chain ,the effectiveness of the inhibition of gemini surfactants on breast cancer cell line increases, while we notice that the longer the alkyl chain increases, the concentration of CMC decreases, and therefore the relationship is inverse between the effectiveness of the compounds on breast cancer and the CMCconcentration.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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