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Effect of Quaternary Ammonium Surfactants of Fatty Acid Derivatives on Microorganisms

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Abstract: *Biological activities for three cationic surfactants named N-(2-(lauroxy) ethyl)-N, N-bis (2-hydroxyethyl) octane-1- ammonium bromide (Surfactant-L), N, N-bis (2-hydroxy ethyl)-N-(2-(myristyloxy) ethyl) octane-1-ammonium bromide (Surfactant-M), N, N-bis (2-hydroxy ethyl)-N-(2-(hexadecanoxy) ethyl) octane-1-ammonium bromide (Surfactant-P) were investigated by Well diffusion and N-broth dilution methods. They demonstrated marked antimicrobial activity against gram positive, gram negative bacterias and fungal. The pathogens antimicrobial activity was increased by Lauric acid derivative to palmitic acid derivative of quaternary ammonium surfactant.*

Key Words: *Cationic Surfactant, Antimicrobial activity, Fatty acids, Well diffusion, N-broth dilution*

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I. Introduction

Surfactants are amphophilic compounds,which decrease the surface tension of solution[1]. The properties of surfactants play a pivotal role in various industries, hospitals and at home as an wetting agents, solubilizing agents, foaming and anti-foaming agents,cleaning detergents, personal care products, etc [2]. Surfactants are one among the constituents among various disinfectants since they have very good antimicrobial properties. Currently cationic surfactants play a pivitol role as antiseptic and sanitizing agents, because they do have the ability to bind and diffuse with cell walls of the microorganisms, followed by the disruption of the membrane [3] . Quaternary ammonium salts are not availed to systemic application as they are toxic to mammalian cells [4], but at the same time it can be used for topical applications like oral antiseptics, mouthwash, antiseptic lotions etc. The commonly used surfactants are benzylalkonium chloride (BAC), hexadecyl trimethylammonium bromide (HDTMA), cetyltrimethylammonium bromide (CTAB). Massive quantities of surfactants are being used in household and industry every day. After use, residual surfactants are discharged into sewage systems or directly into surface waters, and most of them end up dispersed in different environmental compartments such as soil, water or sediment. The toxic effects of surfactants on various aquatic organisms are well known [5-6]. The highest concern is the release of untreated waste water or waste water that has undergone primary treatment alone. The discharge of waste water polluted with massive quantities of surfactants could have serious effect on the ecosystem. Future studies of surfactant toxicities and biodegradation are necessary to withdraw highly toxic and non-biodegradable compounds from commercial use and replace them with more environmentally friendly ones [7]. Developed a new type of cleavable cationic surfactant termed esterquats by introducing an easily cleavable oxycarbonyl group in the lipophilic portion of the molecule. This made the surfactant more readily hydrolysable, thus improving its biodegradability [8]. The antimicrobial activity was evaluated against gram negative and gram positive bacteria and fungi. Salts which possess more than eight carbon atoms in the alkyl chain showed good antimicrobial activity. The efficiency of these salts as antimicrobial agents may be due to increased hydrophobicity. The presence of an ester group would have increased the biological activity against fungi. Therefore attention has been focused on biodegradable cationic surfactants based on quaternary ammonium salts [9]. The objective of the present work is evaluating the antimicrobial activity of the three cationic surfactants again Gram-positive, Gram-negative and fungi by Well diffusion method and N-broth method.

II. Materials and Methods

2.1 Chemicals

Nutrient Agar and potato dextrose agar were purchased from Himedia. Dimethyl sulfoxide purchased from Merck. Surfactant-L, Surfactant-M and Surfactant-P.

2.2 Microorganisms

In this experiment five pathogens were used. This group included Gram-positive bacteria (*Bacillus subtilis* PCM 2021, *Staphylococcus aureus* MTCC 1144), Gram-negative bacteria (*Escherichia coli* MTCC 1687, *Pseudomonas aeruginosa* MTCC 424) and Fungi (*Candida albicans* MTCC 3017)

2.3 Determination of antimicrobial activity

The synthesized cationic surfactants were tested against bacteria (Gram-positive and Gram-negative) and Fungi by employing, Well diffusion and N-broth dilution methods [10].

2.3.1 Well Diffusion Method

Nutrient agar was prepared by dissolving agar in dissolved water and then poured into a sterile perti dish and allowed for few minutes to solidify. Wells of 8 mm diameter were made by using a sterile cork borer. 24 hours cultured (Gram positive bacteria, Gram-negative bacteria and Fungi) were swabbed in the well. Four different concentrations of the surfactant (25 µl, 50 µl, 75 µl and 100 µl) were loaded into the four wells separately and one well is loaded with DMSO (control) which is inert. The plates were subjected to incubation at 37 ^{0}C for 24 hrs. After the incubation the inhibition diameter of the four different concentrations of the surfactant was measured. Same procedure followed for the other two surfactants.

2.3.2 N-broth Dilution Method

Different concentration of the surfactants were added to four different test tubes already loaded with 5 ml of the potato dextrose and 0.1ml of the 24 hours cultured bacteria respectively . These tubes were incubated at 37 ^{0}C for 24 hrs. The optical densities were measured spectrometically at 600 nm.

The percentage of visible cells was calculated using the following formula

$$\% \text{ of inhibition} = \left\{ \frac{\text{Control O.D.} - \text{Test O.D}}{\text{Control O.D}} \right\} \times 100$$

Where O.D is Optical Density

III. Results

3.1 Well Diffusion Method

The antimicrobial activity of the surfactant for Gram-positive, Gram-negative bacterias and fungi are displayed in Figure 3.2, 3.3 and 3.4. From the bar figure it is clear that the antimicrobial activity increasing Surfactant – L to Surfactant – P and this may be due to the increase in carbon chain length. Increase in carbon chain length increases the rate of collision of the surfactant molecule on the cell wall and therefore increase the disruption of the membrane which caused cell death [11].

The antimicrobial activities contributed by the surfactant are due to the dispersed of the surfactant molecule through the cell wall of the micro-organisms followed by interruption of the membrane (Figure 3.1) [12].

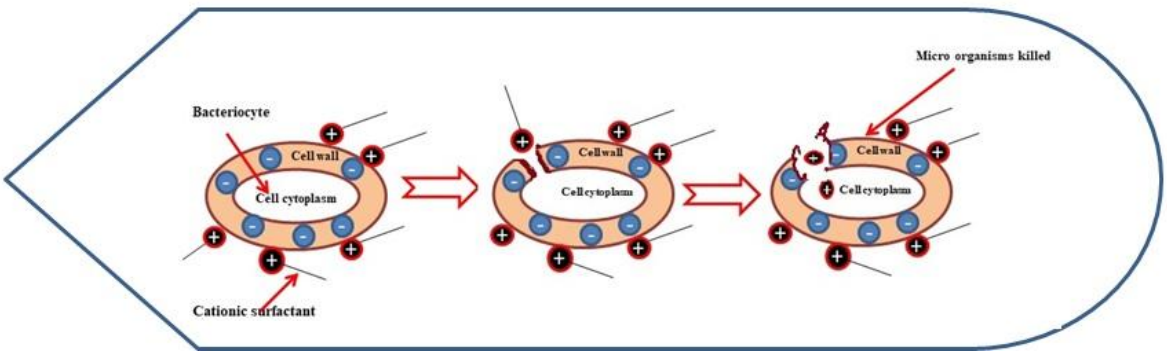
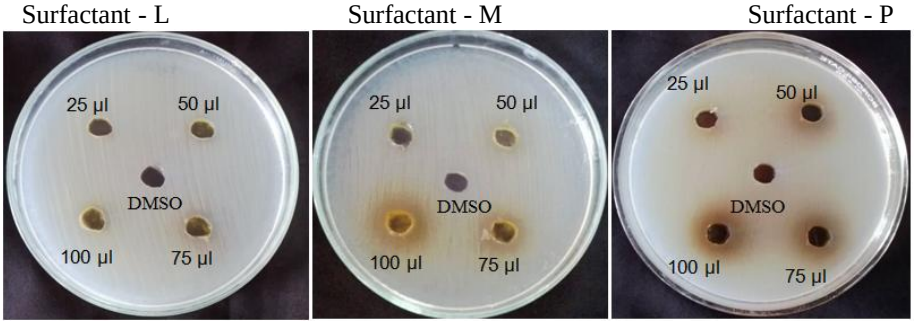


Figure 3.1 schematic diagram for diffusion of the surfactant molecule to the cell wall of the micro-organism

Bacillus subtilis



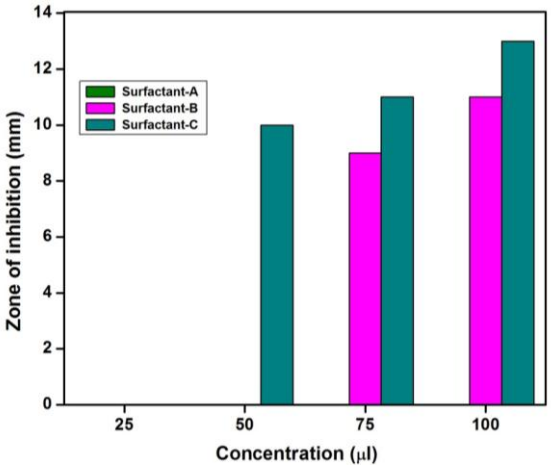


Figure 3.2 Antimicrobial activity against *Bacillus subtilis*

Pseudomonas aeruginosa

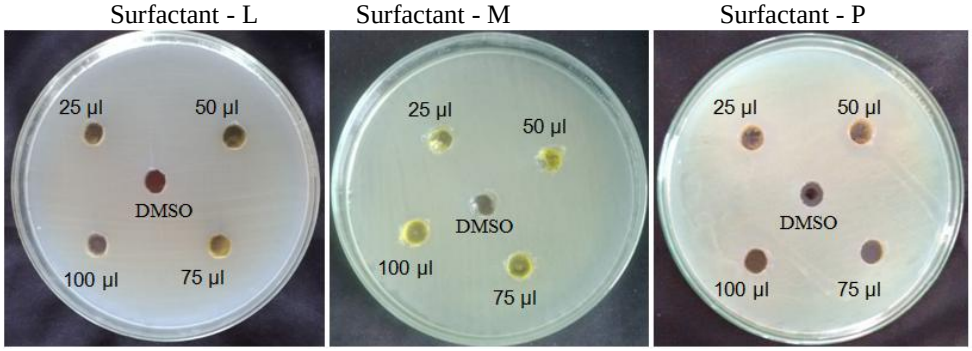


Figure 3.3 Antimicrobial activity against *Pseudomonas aeruginosa*

Candida albicans

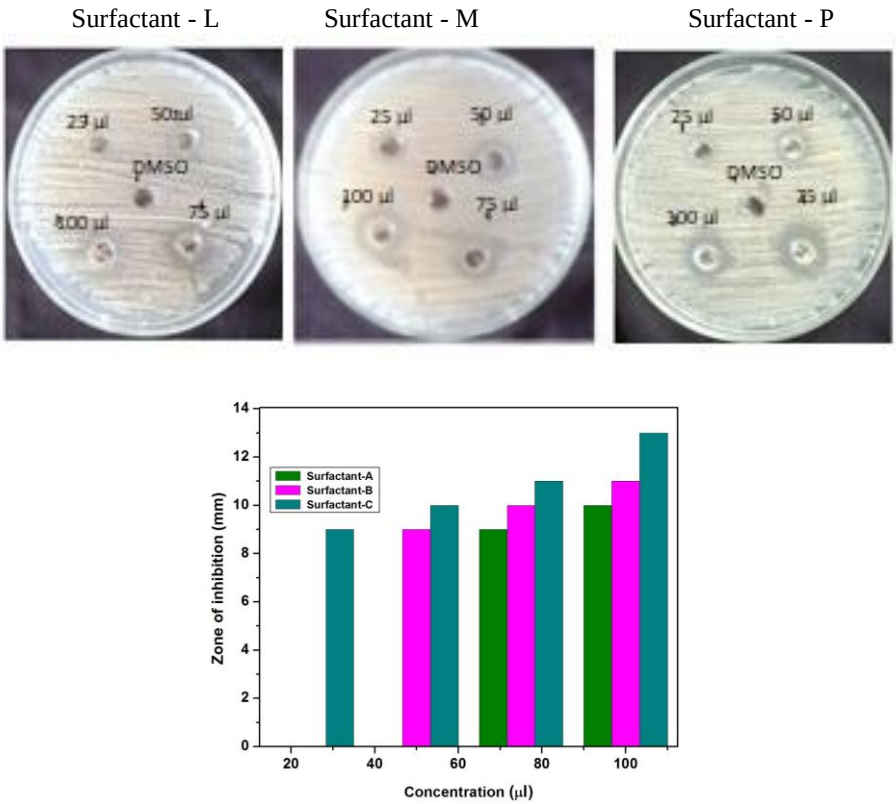


Figure 3.4 Antimicrobial activity against *Candida albicans*

3.2 N-broth Method

Minimum Inhibition Concentration (MIC) and 50 % Inhibition Concentration (IC_{50}) values were determined for the three cationic surfactants and summarized below. From the table 3.1 the IC_{50} values for the Surfactant - L with respect to *B.Subtilis* (Gram positive) arises above 100 ppm and for Surfactant - M the IC_{50} values arise at 80 ppm. IC_{50} value for Surfactant - P was found to be 60ppm. From the above data it is very clear that the inhibition property of the surfactant increases with increase in chain length (Surfactant - L to Surfactant - P).

Table 3.1 Minimum inhibition concentration value of surfactants on *B.Subtilis*

Concentration (ppm)	*B.Subtils* (OD %)		
	Surfactant - L	Surfactant -M	Surfactant - P
10	0	0	25.84
20	0	22.51	31.13
30	0	27.08	39.17
40	8.56	32.02	43.52
50	12.43	35.09	49.3
60	18.49	39.99	52.45
70	22.75	45.16	59.45
80	29.46	51.08	63.44
90	33.05	54.66	68.08
100	40.88	59.78	73.55

In case of Surfactant - M, the minimum inhibition concentration starts at 20 ppm of the surfactant concentration indicating a value of the optical density value 8.56 % and increases further with increase in concentration. The Surfactant - P is more effective since the minimum inhibition concentration starts at 10 ppm of the surfactant. It is clear from the above data that the Surfactant - P is more effective in the inhibition of Gram-positive bacteria followed by Surfactant - M and Surfactant - L. This can be attributed to the alkyl chain length and the ester group in the alkyl chain.

In the case of *S.aureus* all the surfactants shows minimum inhibition concentration from 10 ppm but the optical density % increase from Surfactant - L to Surfactant -M . The IC_{50} value for Surfactant - L was found to be above 100 ppm. In case of Surfactant - M and Surfactant - P, the IC_{50} values were found to be 90 ppm and 70 ppm respectively (Table 3.2).

Table 3.2 Minimum inhibition concentration value of surfactants on *S.aureus*

Concentration (ppm)	*S.aureus* (OD %)		
	Surfactant - L	Surfactant -M	Surfactant - P
10	9.14	13.31	19.32
20	12.2	15.84	21.24
30	16.5	21.09	28.17
40	21.84	27.08	31.6
50	26.05	31.01	39.55
60	29.81	35.08	44.44
70	34.26	41.55	50.08
80	37.08	48	57.34
90	41.21	51.03	63.10
100	46.34	54.61	69.43

IC_{50} values for *E.coli* with respect to Surfactant - L and Surfactant - M start above 100 ppm. For surfactant, the IC_{50} values start at 80 ppm with a optical density % value of 55.31%. The minimum inhibition concentration of Surfactant - L, M and P are 40 ppm, 20 ppm and 10 ppm respectively (Table 3.3).

Table 3.3 Minimum inhibition concentration value of surfactants on *E.coli*

Concentration (ppm)	*E.coli* (OD %)		
	Surfactant - L	Surfactant -M	Surfactant - P
10	0	0	18.9
20	0	14.61	23.14
30	0	18.06	26.19
40	4.56	21.25	30.13
50	12.33	25.88	36.74
60	16.57	29.46	41.44
70	20.53	33.5	48.21
80	23.07	36.08	52.56
90	27.81	41.05	55.31
100	31.09	48.72	58.36

For the bacteria *P-arugenosa* , the IC_{50} values for the Surfactant - L and Surfactant - M are above 100 ppm. Surfactant - P shows IC_{50} value of 100 ppm with optical density (%) value of 51.03%. The minimum inhibition concentration of Surfactant - L, M and P are 50 ppm, 40 ppm and 10 ppm respectively (Table 3.4).

Table 3.4 Minimum inhibition concentration value of surfactants on *P.arugenosa*

Concentration (ppm)	*P.arugenosa* (OD %)		
	Surfactant - L	Surfactant -M	Surfactant - P
10	0	0	9.64
20	0	0	15.53
30	0	0	21.47
40	0	19.84	26.82
50	11.13	25.06	29.39
60	16.57	29.44	33.04
70	22.58	34.16	39.77
80	27.9	37.88	42.54
90	33.42	42.56	45.69
100	37.99	49.06	51.03

The antifungal activity of the Surfactant - L does not show significant activity where as Surfactant - M and Surfactant -P were found to be good. The increasing order of activity from surfactant - M to Surfactant – P (Table 3.5).

Table 3.5 Minimum inhibition concentration value of surfactants on *C.albicans*

Concentration (ppm)	*C.albicans* (OD %)		
	Surfactant - L	Surfactant -M	Surfactant - P
10	0	14.08	18.48
20	0	19.85	23.83
30	4.16	23.5	28.45
40	8.97	27.18	34.16
50	12.46	31.58	37.54
60	18.09	36.97	43.08
70	22.75	40.17	49.5
80	27.06	46.44	53.4
90	32.54	50.12	58.09
100	38.67	54.26	63.51

IV. Discussion

All the three cationic surfactants showed good antimicrobial activity agent gram positive, gram negative bacterias and fungi. The mechanism of cationic surfactants on microorganism is originally focused on their adsorption tendency on the cellular membranes [13].Gram positive bacteria have a large peptidoglycan structure. It shows a positive result in the Gram stain test and it take up the crystal violet stain used in the test, and then appear to be purple-coloured when seen through a microscope. This is because the thick peptidoglycan layer in the bacterial cell wall retains the stain after it is washed away from the rest of the sample, in the decolorization stage of the test. *Staphylococcus aureus. Bacillus subtilis* are the best characterized gram positive bacteria, which causes serious infections of the skin, soft tissues, bone, lung, heart, brain and blood [14]. The cell wall of Gram-negative bacteria has a more complex multilayered structure than that of Gram-positive bacteria. The peptidoglycan layers are much thinner and lies between the inner and the outer membrane. This outer membrane consists of a phospholipid bilayer connected with an external leaflet of lipopolysaccharides (LPS). LPS renders the Gram-negative bacteria highly hydrophilic with a net negative charge. When decolourising the peptidoglycan layer does not retain the dye and the outer layer becomes pink coloured due to addition of safranin stain. *Escherichia coli* was initially considered as non-harmful bacteria but now it has been found that it is associated with a wide range of diseases and infections including meningeal, gastrointestinal, urinary tract, wound and bacteremia infections in all age groups [15]. Other infections caused by *Escherichia coli* include peritonitis, cholecystitis, septic wounds and bedsores. They may also infect the lower respiratory passages or cause bacteraemia and endotoxic shock especially in surgical or debilitated patients [16]. A fungus is a group of eukaryotic organisms containing chitin in cell walls unlike bacteria, plants and other microorganisms which has cellulose in their cell wall. They can be symbiotic as well as pathogenic in nature, which cause disease in plants and animals. Fungi are diverse and widespread. Humans have cultivated fungi for centuries to consume it as a food, to produce antibiotics and other drugs, to make bread rice, ferment beer and wine. They play an important role in decomposition of organic materials and participate in nutrient cycle. This decomposition recycles vital chemical elements back to the environment in the form which other organisms can assimilate. Most of the plants depend on mutualistic fungi to help their roots absorb minerals and water from the soil. Fungi play ecological diverse roles - they are decomposers (saprobes), parasites, and mutualistic symbiosis [17].

Candida tropicalis is one of the most common *Candida* causing human disease in tropical countries. *C. tropicalis* is particularly virulent in neutropenia hosts commonly with hematogenous seeding to peripheral organs [18]. *Candida.albicans* caused by gentital region in women , mouth and throat area[19].

V. Conclusion

The obtained results by both method of antimicrobial activity for this three cationic surfactant good significant effect in Gram positive and negative bacterias and fungi. We can conclude based on results antimicrobial efficiency increasing order Surfactant – L < Surfactant – M < Surfactant – P by reason of the surfactant - P easily form micelle than other two surfactants, therefore decrease critical micelle concentration value of the surfactant – P.

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