Influence of Alkyl Chain Length on Micellization and Antibacterial Efficacy of Cationic Surfactants in the Presence of 3-Chloro-4-Fluoroaniline

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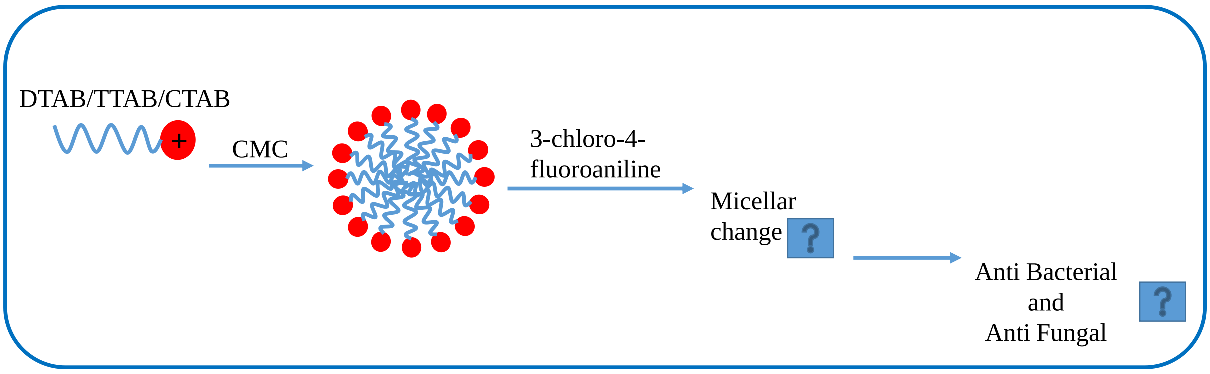
Graphical Abstract



Abstract:

Cationic surfactants, specifically Dodecyltrimethylammonium bromide (DTAB), Tetradecyltrimethylammonium bromide (TTAB), and Cetyltrimethylammonium bromide (CTAB), exhibit varying micellization behavior influenced by their hydrophobic chain length and the presence of aromatic substituents. This study explored the physicochemical and bactericidal properties of these surfactants in the presence of 3-Chloro-4-Fluoroaniline using methods such as conductometry, viscosity measurements, spectroscopy, and dynamic light scattering (DLS). The interactions between the cationic surfactants' head groups and the aromatic amine resulted in notable changes in critical micelle concentration (CMC), micellar dimensions, and aggregation behavior, with CMC values decreasing in the order DTAB < TTAB < CTAB, suggesting enhanced hydrophobic interactions with longer alkyl chains.

The inclusion of 3-chloro-4-fluoroaniline altered micellization characteristics, leading to a more favorable interaction, characterized by a lower CMC and modifications in micellar shape. Thermodynamic analyses showed that micelle formation in mixed systems was spontaneous and driven by increases in entropy.

Furthermore, the antibacterial effectiveness of the surfactant-additive systems was evaluated against various Gram-positive and Gram-negative bacteria and fungi. These systems demonstrated greater antibacterial efficacy than the individual components, attributed to enhanced membrane disruption from coupled micelles. Notably, CTAB combined with 3-chloro-4-fluoroaniline exhibited the highest antibacterial activity due to its lower CMC and more stable micelle structure.

In conclusion, the research establishes a detailed link between physicochemical interactions and biological functionality, emphasizing the promise of surfactant-aromatic amine complexes as adjustable antibacterial agents with improved effectiveness.

Introduction:

Cationic surfactants, including Dodecyltrimethylammonium bromide (DTAB), Tetradecyltrimethylammonium bromide (TTAB), and Cetyltrimethylammonium bromide (CTAB), are significant amphiphilic compounds that self-assemble into micelles in aqueous solutions upon reaching their critical micelle concentration (CMC). The length of the alkyl chain significantly affects micellar characteristics: an increase in chain length generally reduces the critical micelle concentration (CMC) and improves micellar stability due to intensified hydrophobic interactions among alkyl tails (Gaynanova, Vasileva, Romanova, Zakharova, & Sinyashin, 2025; Sanchez-Fernandez et al., 2017). Moreover, extensive research on cationic surfactant self-assembly and solvent influences has demonstrated that micelle dimensions, morphology, and aggregation number are acutely responsive to the molecular milieu and additives (Manasi, King, & Edler, 2024; R. Singh, Gurung, Anjudikkal, Narayanan, & Pulikkal, 2025). DTAB, TTAB, and CTAB demonstrate regular variations in micellar structure in deep eutectic solvents and other media, which are associated with chain length and counterion effects (Manasi et al., 2024; R. Singh et al., 2025).

The incorporation of aromatic organic solutes, such as substituted anilines like 3-chloro-4-fluoroaniline, can markedly affect micellization by partitioning into surfactant aggregates and modifying interfacial packing and aggregation thermodynamics. Aromatic amines can integrate into the palisade layer of micelles, potentially engaging via hydrophobic interactions, hydrogen bonding, or π–stacking, thereby influencing the critical micelle concentration (CMC) and micelle development. While limited research has focused on 3-chloro-4-fluoroaniline in DTAB/TTAB/CTAB micelles, the propensity of aromatic additives to influence micelles is extensively documented in micelle–drug and micelle–solute systems across various temperatures and compositions (Hossain et al., 2023). Investigations into drug–surfactant systems with structurally similar amphiphiles, such as ciprofloxacin with CTAB/TTAB, validate the noted trend of CMC modification and micellar stability upon the introduction of an additive (Hossain et al., 2023).

Cationic surfactants possess antibacterial capabilities due to their strong electrostatic attraction to the negatively charged surfaces of microbial cells. This interaction compromises lipid bilayers and destabilizes membranes, resulting in the leakage of cellular constituents and cell death (Lourdes Pérez et al., 2022; Zhou & Wang, 2020). The structural attributes of these surfactants, such as alkyl chain length and headgroup charge density, directly influence these interactions and, hence, the level of antimicrobial efficacy (Pérez, García, & Pinazo, 2022; Lourdes Pérez et al., 2022). Recent studies on cationic surfactants and their biological uses confirm that longer alkyl chains and refined headgroup structures improve binding affinity with microbial membranes. Nonetheless, these alterations may also intensify cytotoxicity [7]. Recent investigations in the 2020s have focused on novel surfactants, such as ester-based and Gemini amphiphiles, which exhibit improved biodegradability and antibacterial properties, highlighting ongoing interest in biologically functional cationic systems (Kowalczyk, Szulc, Koziróg, Komasa, & Brycki, 2025).

Aromatic amines, such as 3-chloro-4-fluoroaniline, are widely used as intermediates in pharmaceuticals and agrochemicals. They exhibit hydrophobic and polar functional groups that may interact with membranes and proteins, potentially influencing biological activity (Liu, 2020). Although direct investigations of 3-chloro-4-fluoroaniline's interaction with micellar systems are scarce in the literature, its designation as a moderately hydrophobic aromatic amine suggests that it may affect solubilization and biological availability in micellar solutions, as other hydrophobic pharmaceuticals do within surfactant systems (Becquart, 2023). The integration of cationic surfactants with aromatic bioactive compounds presents prospects to improve antibacterial efficacy via micelle-mediated transport and membrane targeting.

Experimental

Materials

TTAB, CTAB, and 3-Chloro-4-fluoroaniline were purchased from Loba Chemie, whereas DTAB was purchased from Sigma-Aldrich. Samples were utilized in their original state without any alterations. Deuterated water (D_2O) was used to prepare sample solutions for small-angle neutron scattering (SANS) experiments and was obtained from Sigma-Aldrich. In contrast, Millipore-grade distilled water was used to prepare solutions for subsequent testing.

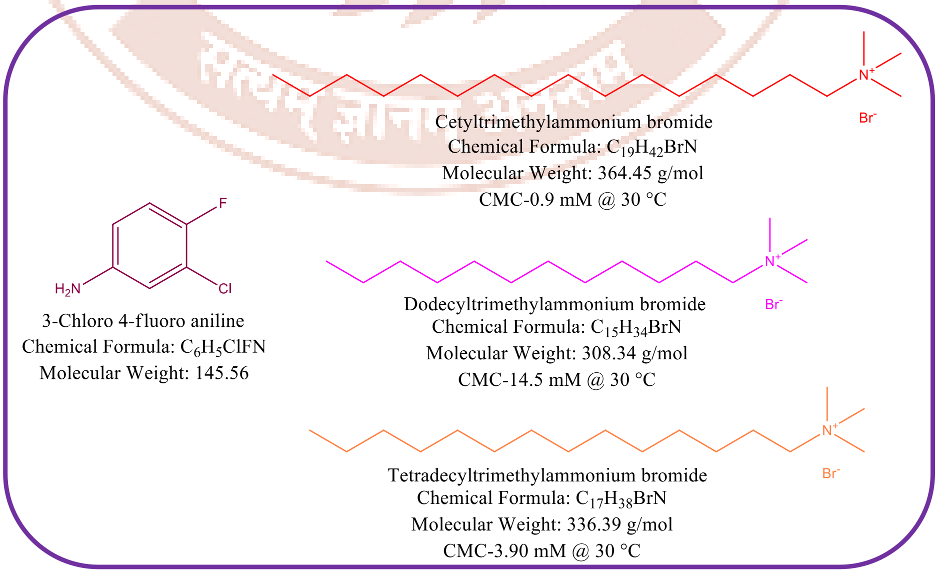


Figure 1. Structural moieties of DTAB, TTAB, CTAB, and 3-Chloro-4-fluoroaniline.

Methods

- **Conductometry**

  The conductance of 3-Chloro-4-fluoroaniline has been evaluated in relation to the concentrations of DTAB, TTAB, and CTAB. Conductance was measured using a digital auto-ranging conductivity meter equipped with an integrated magnetic stirrer. Conductance was assessed in both the presence and absence of a 3-Chloro-4-fluoroaniline solution in the sample. The conductivity of 3-Chloro-4-fluoroaniline at different doses has been assessed with all cationic surfactants.

- **Viscosity**

  The samples' viscosity was assessed utilizing a viscometer. The viscosity of all cationic surfactants, regardless of the presence of 3-Chloro-4-fluoroaniline, was assessed. The viscosity characteristics of the samples were analyzed by altering the concentration of 3-chloro-4-fluoroaniline. A temperature scan was performed to evaluate the effect of temperature on the samples. 3-Chloro-4-fluoroaniline was included until its maximum solubility was attained in the DTAB, TTAB, and CTAB solutions.

- **FTIR**

  The FT-IR spectrophotometer (Model: Perkin Elmer/Spectrum BXw) was utilized to acquire the FT-IR spectra of CTAB both in the presence and absence of 3-Chloro-4-fluoroaniline.

- **NOESY**

  A two-dimensional Nuclear Overhauser Effect Spectroscopy (NOESY) examination was performed on a Fourier Transform Nuclear Magnetic Resonance (FT-NMR) spectrometer (Bruker Avance II, 400 MHz) operating at a proton resonance frequency of 400 MHz. The samples were prepared in appropriate deuterated solvents. The spectra of CTAB with 3-chloro-4-fluoroaniline have been acquired.

- **DLS**

  The size distribution and average hydrodynamic diameter (Dh) of the polymeric micelles were determined via dynamic light scattering (DLS) at a constant scattering angle of 90° using a Zetasizer Nano-Zs (Malvern Instruments, UK). The selected samples were examined using DLS to assess differences in viscosity and solution dynamics.

Results and Discussion:

Micellization Behavior

The critical micelle concentration (CMC) is an essential parameter for comprehending surfactant micellization in aqueous solutions.

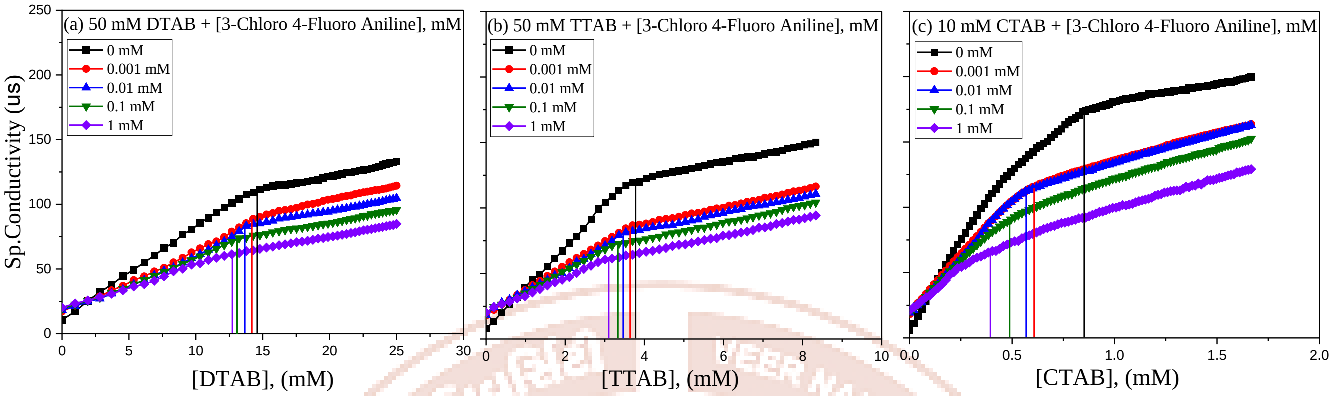


Figure 2. Sp.conductance plots for (a) 50 mM [DTAB], (b) 50 mM [TTAB], and (c) 10 mM [CTAB] in varying concentrations of [3-Chloro-4-fluoroaniline] at 30 ˚C.

Cationic surfactants, specifically Dodecyltrimethylammonium bromide (DTAB), Tetradecyltrimethylammonium bromide (TTAB), and Cetyltrimethylammonium bromide (CTAB), exhibit varying micellization behavior influenced by their hydrophobic chain length and the presence of aromatic substituents. This study explored the physicochemical and bactericidal properties of these surfactants in the presence of 3-Chloro-4-Fluoroaniline using methods such as conductometry, viscosity measurements, spectroscopy, and dynamic light scattering (DLS). The interactions between the cationic surfactants' head groups and the aromatic amine resulted in notable changes in critical micelle concentration (CMC), micellar dimensions, and aggregation behavior, with CMC values decreasing in the order DTAB < TTAB < CTAB, suggesting enhanced hydrophobic interactions with longer alkyl chains.

Solution Behavior

Viscosity is the primary determinant of a solution's phase behavior. It is highly crucial in practical analysis. Subsequently, we could proceed with further data analysis to characterize the data.

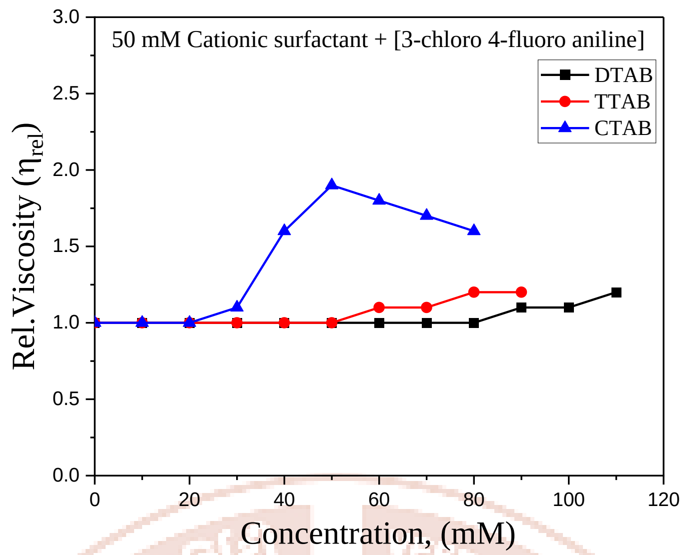


Figure 3. Rel. Viscosity (η_{rel}) vs. Concentration (mM), [DTAB], [TTAB], and [CTAB] with and without 3-Chloro-4-fluoroaniline @ 30 °C Temp.

The study examines the viscosity profiles of surfactants DTAB, TTAB, and CTAB in combination with 3-chloro-4-fluoroaniline, revealing that the additive notably affects micellar microstructure rather than the surfactant aggregation number. At low additive concentrations, viscosity remains stable, indicating that micelles retain their spherical properties and integration of additives is limited. Increasing concentrations of 3-chloro-4-fluoroaniline significantly elevate the viscosity in the CTAB system, suggesting effective solubilization in micelles' hydrophobic core, leading to micellar expansion and rod-like formations. In contrast, TTAB shows minimal viscosity changes, indicating limited micellar development, while DTAB displays slight viscosity variation, likely due to its shorter alkyl chain restricting additive integration. A reduction in viscosity at high additive concentrations in CTAB may stem from micellar reorganization, such as branching or core saturation. Overall, viscosity changes align with the sequence CTAB > TTAB > DTAB, highlighting that additive-induced micellar growth and surfactant chain length primarily govern rheological behavior at constant surfactant concentrations.

Spectral Profile

FTIR

Fourier Transform Infrared Spectroscopy (FTIR) is a technique utilized to identify chemical compounds and characterize molecular structures by assessing the absorption of infrared (IR) light by a sample.

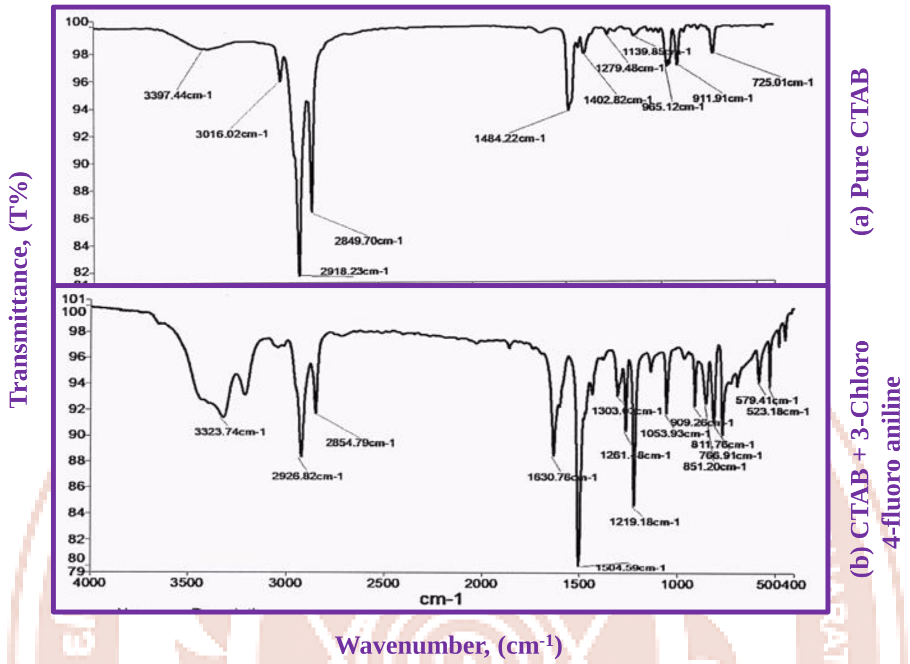


Figure 4. FT-IR spectra of CTAB (a) without and (b) with [3-Chloro-4-fluoroaniline]

(a) The FTIR spectrum of pure Cetyltrimethylammonium bromide (CTAB) reveals significant absorption bands indicative of its structural integrity as a long-chain cationic surfactant. Methylene group stretching is evidenced by peaks at 2918 cm^{-1} and 2849 cm^{-1}, while terminal methyl groups exhibit C–H stretching at 3016 cm^{-1}. Additionally, vibrations of the trimethylammonium head group are represented by bands at 1484 cm^{-1} and 1403 cm^{-1}, and C–N bond vibrations appear at 965 cm^{-1} and 912 cm^{-1}. The pronounced absorption at 725 cm^{-1} corresponds to methane rocking vibrations, confirming a well-organized alkyl chain structure. The clarity of these bands underscores CTAB's purity and aids in the study of molecular interactions and structural changes in systems that use this surfactant.

(b) In the mixed system of CTAB and 3-chloro-4-fluoroaniline, the FTIR spectrum exhibits characteristic bands that indicate molecular interactions between the cationic surfactant and the aromatic amine. A broad absorption band around ~3324 cm^{-1} is attributed to N–H stretching vibrations of the aniline group, potentially overlapping with hydrogen-bonded O–H stretching from bound water, suggesting involvement of the amine group in intermolecular interactions. The strong bands at ~2926 and ~2855 cm^{-1} correspond to C–H stretching vibrations from CTAB's long alkyl chain, confirming the surfactant's hydrocarbon tail presence. The persistence of these bands, albeit with slight intensity variations, indicates structural modifications in the alkyl chain due to the aromatic additive's incorporation into the micellar environment. A significant band near ~1631 cm^{-1} is linked to N–H bending and C=C stretching of the aromatic ring, with another band at ~1500–1460 cm^{-1} resulting from aromatic ring vibrations and CH_2 scissoring modes. Observed shifts in these bands compared to pure 3-chloro-4-fluoroaniline suggest strong interactions between the amine group and CTAB's positively charged quaternary ammonium head group, likely through ion–dipole or hydrogen-bonding interactions. Bands in the ~1300–1200 cm^{-1} region associated with C–N stretching further affirm the amine's role in the interaction process. The fingerprint region below 1100 cm^{-1} contains multiple bands, including peaks near ~1054, ~990, ~912, ~852, and ~770 cm^{-1}, attributed to aromatic C–H bending and C–Cl and C–F modes within the substituted aniline ring. The stability of these bands, despite minor shifts, confirms the aromatic compound's structural integrity and its integration into the CTAB assembly. In summary, the spectral features and their modifications indicate that 3-chloro-4-fluoroaniline interacts effectively with CTAB via head-group associations and hydrophobic interactions, leading to a reorganized micellar environment that is crucial for understanding solubilization and binding behavior in surfactant–aromatic systems.

Scattering Profile DLS

Dynamic Light Scattering (DLS) quantifies particle sizes and distributions in solutions by measuring light scattering from their Brownian motion. In surfactant systems, DLS is essential for determining micelle hydrodynamic diameters, offering insights into their size, shape, and aggregation behavior. It aids in detecting micellar growth patterns, including shape transitions and structural changes due to external factors. DLS also reveals polydispersity, aggregation, and interactions between micelles. For instance, in CTAB with 3-chloro-4-fluoroaniline, DLS confirms micellar expansion and structural changes induced by additives, influencing viscosity and CMC.

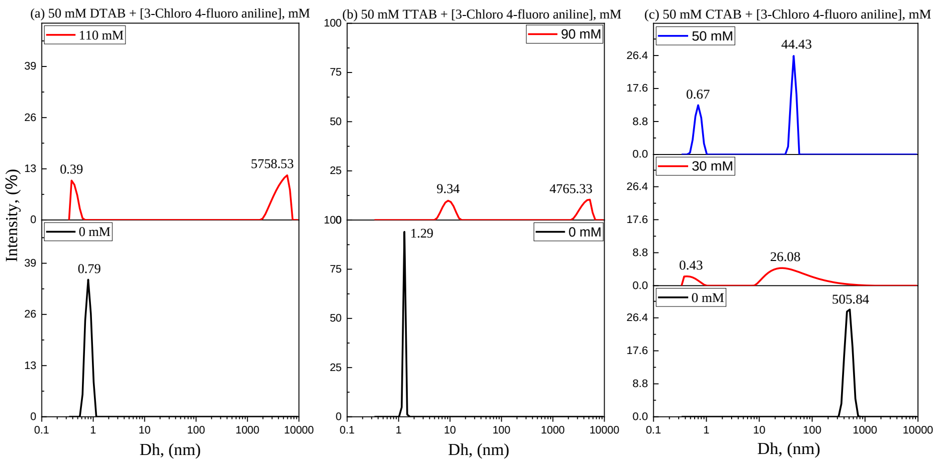


Figure 6. Scattering profile of (a) DTAB, (b) TTAB, and (c) CTAB with and without 3-Chloro-4-fluoroaniline.

A detailed study of dynamic light scattering (DLS) data for the surfactants DTAB, TTAB, and CTAB in the presence of 3-chloro-4-fluoroaniline highlights the influence of hydrophobic chain length on micellar size and on changes in aggregation. At 0 mM additive, all surfactants exhibit low nanometer hydrodynamic diameters, typical of small spherical micelles. As the additive concentration increases, DTAB shows a moderate shift to larger sizes, suggesting a transition from spherical to elongated micelles, with weak hydrophobic interactions due to its shorter chain. TTAB, on the other hand, exhibits a more substantial increase in hydrodynamic diameter and a broader size distribution, indicative of flexible worm-like micelles formed by enhanced interactions as electrostatic repulsion decreases. Notably, the CTAB system reveals significant changes, with very large sizes observed at higher additive concentrations, indicative of vesicle-like structures or large multimicellar aggregates due to strong hydrophobic and π–π interactions. Overall, the DLS results illustrate a transition from rod-like aggregates in DTAB to worm-like micelles in TTAB and to vesicular structures in CTAB, driven by surfactant chain length and additive interactions.

Biological point of view

Antimicrobial activity is defined as the ability of a substance to inhibit microbial growth or cause cell death, affecting bacteria, fungi, and sometimes viruses. This activity arises from the disruption of key microbial functions, such as cell membrane integrity, wall synthesis, protein activity, and genetic material management, leading to growth inhibition or cellular demise (S. P. Singh, Qureshi, & Hassan, 2021). Specifically, cationic surfactants such as DTAB, TTAB, and CTAB exhibit antimicrobial effects due to their positively charged headgroups, which strongly bind to the negatively charged surfaces of microbial cells. This electrostatic attraction facilitates the infiltration of hydrophobic alkyl chains into the cell membrane, causing membrane damage and the release of intracellular substances, ultimately resulting in cell death. The effectiveness of the antimicrobial action is generally proportional to the length of the hydrophobic alkyl chain; longer chains more effectively penetrate and destabilize the membrane. Thus, the level of antimicrobial activity is a crucial indicator of a compound's capacity to impede microbial survival and reproduction (Fox et al., 2022; Rzycki, Gładysiewicz-Kudrawiec, & Kraszewski, 2024; Sayed, Ezzat, Yassin, & Abdelbacki, 2023).

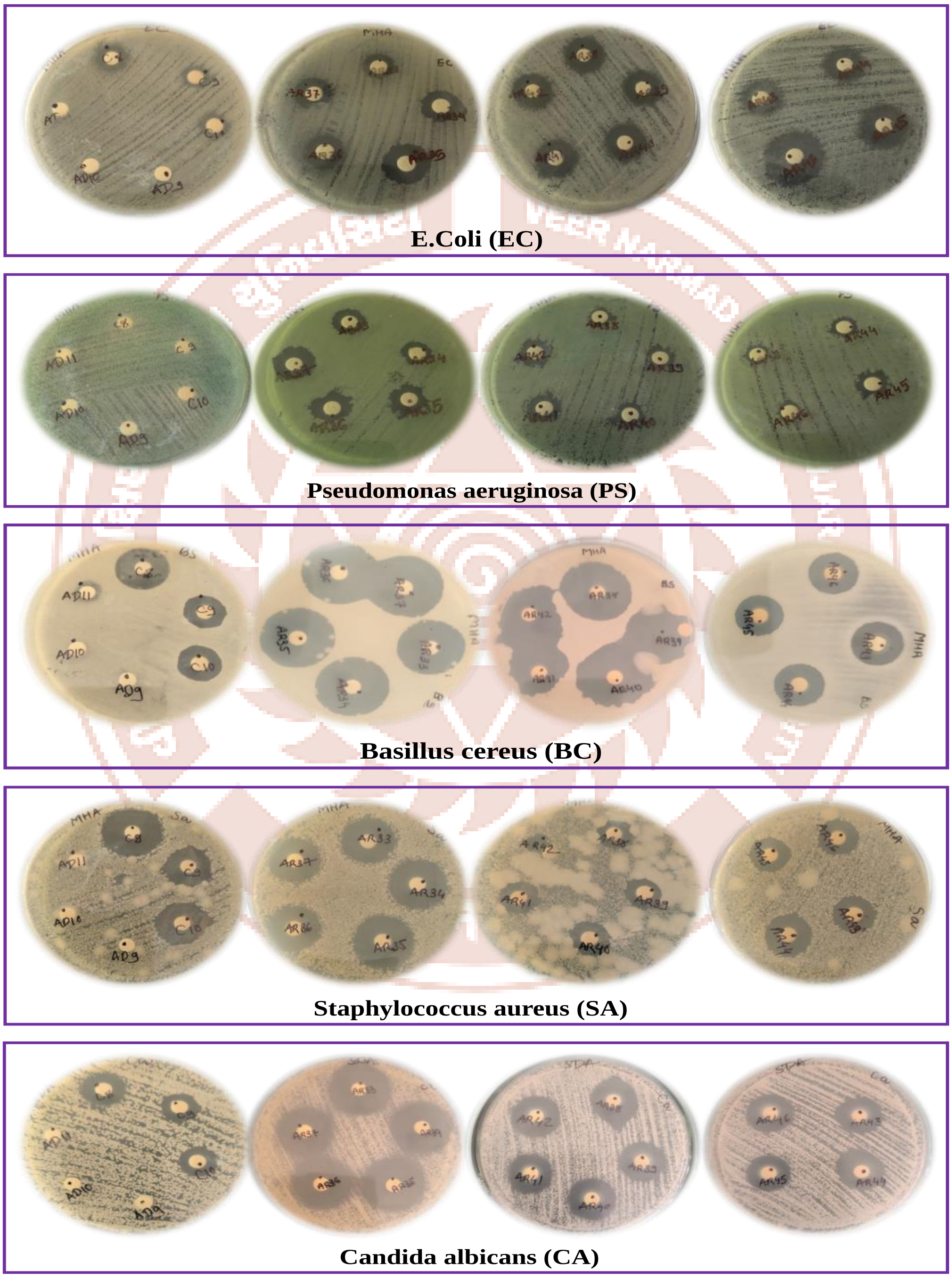


Figure 7. Antimicrobial activity of [50 mM DTAB], [50 mM DTAB + 3-Chloro-4-fluoroaniline], [50 mM TTAB], [50 mM DTAB + 3-Chloro-4-fluoroaniline], and [50 mM CTAB], [50 mM CTAB + 3-Chloro-4-fluoroaniline] on two gram-negative bacteria, *E.coli* and

Pseudomonas aeruginosa, two gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, and a fungus, *Candida albicans.*

Table 1. Sample code and Sample list

Sample code	Sample
C8	50 mM DTAB
C9	50 mM TTAB
C10	50 mM CTAB
AD9	110 mM 3-Chloro 4-fluoro aniline
AD10	90 mM 3-Chloro 4-fluoro aniline
AD11	80 mM 3-Chloro 4-fluoro aniline
AR33	50 mM DTAB + 20 mM 3-Chloro 4-fluoro aniline
AR34	50 mM DTAB + 40 mM 3-Chloro 4-fluoro aniline
AR35	50 mM DTAB + 60 mM 3-Chloro 4-fluoro aniline
AR36	50 mM DTAB + 80 mM 3-Chloro 4-fluoro aniline
AR37	50 mM DTAB + 110 mM 3-Chloro 4-fluoro aniline
AR38	50 mM TTAB + 20 mM 3-Chloro 4-fluoro aniline
AR39	50 mM TTAB + 40 mM 3-Chloro 4-fluoro aniline
AR40	50 mM TTAB + 60 mM 3-Chloro 4-fluoro aniline
AR41	50 mM TTAB + 80 mM 3-Chloro 4-fluoro aniline
AR42	50 mM TTAB + 110 mM 3-Chloro 4-fluoro aniline
AR43	50 mM CTAB + 20 mM 3-Chloro 4-fluoro aniline
AR44	50 mM CTAB + 40 mM 3-Chloro 4-fluoro aniline
AR45	50 mM CTAB + 60 mM 3-Chloro 4-fluoro aniline
AR46	50 mM CTAB + 80 mM 3-Chloro 4-fluoro aniline

Table 2. Antimicrobial Activity profile.

Sample code	Gram-negative bacteria		Gram-positive bacteria		Fungi
	E.coli	***Pseudomonas aeruginosa***	***Bacillus cereus***	***Staphylococcus aureus***	***Candida albicans***
C8	12	-	20	20	18
C9	11	-	15	14	14
C10	10	-	15	14	14

AD9	-	-	-	-	-
AD10	-	-	-	-	-
AD11	-	-	10	-	-
AR33	36	36	26	51	26
AR34	13	11	27	19	27
AR35	16	13	26	17	26
AR36	14	12	27	16	24
AR37	13	15	27	18	22
AR38	51	30	72	42	21
AR39	15	11	24	15	20
AR40	16	11	23	14	20
AR41	16	12	20	13	20
AR42	16	12	26	13	21
AR43	42	30	19	42	19
AR44	15	10	19	16	19
AR45	17	11	18	15	17
AR46	20	10	18	14	19

Note: *E.coli* and *Pseudomonas aeruginosa* (gram-negative bacteria), *Bacillus cereus* and *Staphylococcus aureus* (gram-positive bacteria), *Candida albicans* (fungi)

Table 2. Proven data on general Antibiotics and Antifungal drugs.

	Gram-negative bacteria		**Gram-positive bacteria**		**Fungi**
	E.coli	***Pseudomonas aeruginosa***	***Bacillus cereus***	***Staphylococcus aureus***	***Candida albicans***
Solvent Control	-	-	-	-	-
Negative Control	-	-	-	-	-
Antibiotics					
Ampicillin	-	-	-	30	NA
Ciprofloxacin	10	20	27	30	NA
Tetracycline	25	14	-	29	NA
Gentamicin	26	14	24	22	NA

Antifungal					
Nystatin	NA	NA	NA	NA	26
Amphotericin-B	NA	NA	NA	NA	19
Clotrimazole	NA	NA	NA	NA	16

The study investigated the antimicrobial activity of various surfactants and their combinations with the additive 3-chloro-4-fluoroaniline against specific microorganisms. Negative controls showed no zones of inhibition, confirming that any observed antimicrobial effects were due to the test samples. Established antibacterial agents like ciprofloxacin and gentamicin demonstrated broad-spectrum efficacy, while ampicillin and tetracycline displayed selective activity. For fungal strains, Nystatin was most effective against Candida albicans, followed by amphotericin-B and Clotrimazole. Among the surfactants, DTAB, TTAB, and CTAB exhibited moderate activity against Gram-positive bacteria and Candida albicans but little to no effect on Pseudomonas aeruginosa, attributed to its protective outer membrane. The standalone 3-chloro-4-fluoroaniline showed minimal activity, but when combined with surfactants, significant synergistic effects were observed, resulting in larger inhibition zones that surpassed those of some antibiotics. TTAB-based combinations yielded the greatest enhancement at lower concentrations, followed by CTAB and DTAB. At higher additive concentrations, antimicrobial activity decreased slightly, likely due to micellar saturation. Overall, surfactant-additive systems proved more effective for antimicrobial action than individual components, suggesting potential for new formulations.

Conclusion

This chapter systematically explores the physicochemical behavior and antimicrobial relevance of cationic surfactants DTAB, TTAB, and CTAB in the presence of 3-chloro-4-fluoroaniline. By comparing these surfactants with varying alkyl chain lengths, the study elucidates how hydrophobicity and additive structures impact micellar organization and performance in aqueous environments. The presence of 3-chloro-4-fluoroaniline significantly alters the micellization and interfacial properties of the surfactants, enhancing micellar stability through hydrophobic interactions, hydrogen bonding, and ion–dipole forces. This leads to a decrease in critical micelle concentration and promotes micellar growth, with the effects increasing from DTAB to CTAB.

From an antimicrobial perspective, the cationic surfactants facilitate strong electrostatic interactions with negatively charged microbial membranes. The hydrophobic alkyl chains enhance membrane penetration and disruption, suggesting that the inclusion of 3-chloro-4-

fluoroaniline improves its solubilization at microbial interfaces, thereby increasing its antimicrobial effectiveness. Notably, CTAB-based systems exhibit superior efficacy due to stronger hydrophobic interactions.

Overall, the research establishes a clear relationship between micellar physicochemical properties and antimicrobial activity for DTAB/TTAB/CTAB–3-chloro-4-fluoroaniline systems, highlighting the importance of surfactant chain length and aromatic additives in modulating micellar stability and antimicrobial performance. These findings present a solid basis for the advancement of surfactant-assisted antimicrobial formulations and enhance our understanding of structure–function correlations in cationic surfactant systems.

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