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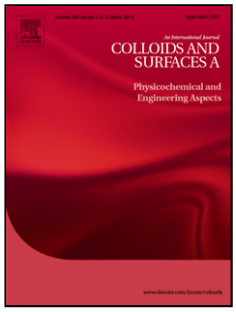

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Cationic Surfactants Based on Ferrocene Containing Thiourea: Synthesis, Self-aggregation, and Antioxidant Properties

Mehwish Naz [a, b], Jahangeer Ali [a], Saira Fatima [a], Saira Tabassum [a], Sameen Nawaz [a], Amin Badshah [a,*], Hongjing Dou [b,*]

[a] *Department of Chemistry, Quaid-i-Azam University Islamabad, Pakistan.*

[b] *The State Key Laboratory of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, P. R. China.*

Correspondence and requests for materials should be addressed to Amin Badshah [a] and Hongjing Dou [b].

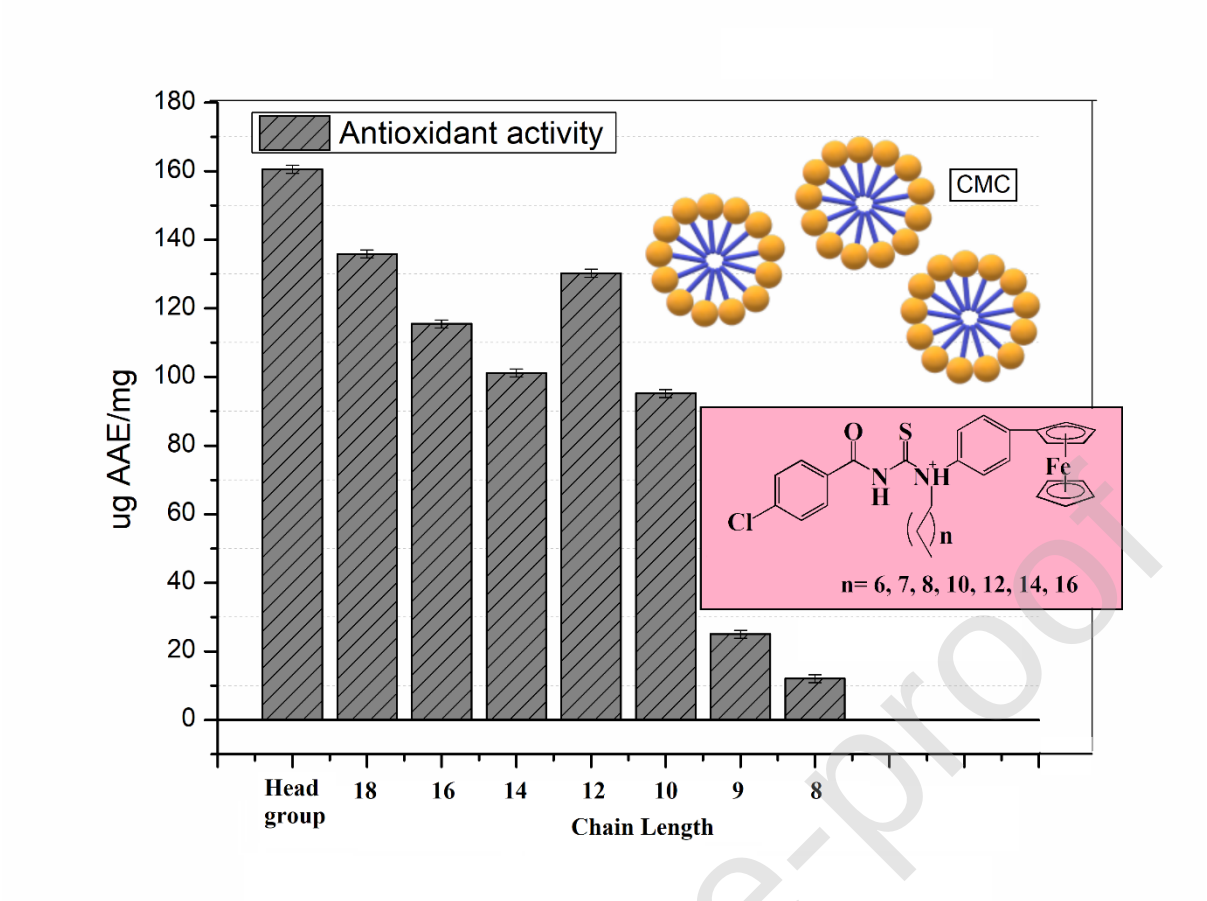
[a] Department of Chemistry, Quaid-i-Azam University Islamabad, 45320, Pakistan.

[b] The State Key Laboratory of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, P. R. China.

Tel: +92-51-9064-2131 [a] , +86-21-34202956 [b]

Email: aminbadshah@qau.edu.pk , hjdou@sjtu.edu.cn

Graphical Abstract



Highlights

- Chain length effects the surfactant aggregation behavior like micellization and critical micelle concentration when studied using same head structure.
- Antioxidant efficacy mainly depend on the head structure in ferrocene based thiourea containing cationic surfactants.
- Antioxidant activity of an already potent head group decrease with decrease in length of added chain due to micellar effect.
- Longer chain surfactants are better candidates for major bioactivities as compared with shorter chain length surfactants in case if chain length is having a role in particular activity.

Abstract

Keeping in view the significance of cationic surfactants in various fields of life since the ancient times, here we are reporting the synthesis of a new ferrocenyl thiourea derivative along with an entirely new series of ferrocene based cationic surfactants in which variable alkyl chain lengths are introduced while keeping the head structure same, thus acting as a key to comparative study of their potential self-aggregation properties and bioactivities. Critical micelle concentration has been determined which is a supporting evidence to the amphiphilic nature of the synthesized compounds. Furthermore, cell cytotoxicity and three types of antioxidant activities also helped depict the therapeutic properties of the synthesized compounds.

Key words: Ferrocene, Thiourea, Cationic surfactants, CMC, surface tension, antioxidant activities.

1 Introduction

Cationic surfactants with their positively charged hydrophilic head and the lipophilic tail duo act as a viaduct between two phases, catalyzing various aquo-organic reactions[1]. Furthermore, the evolution of electrostatic associative assemblance upon interaction with negative small molecules or surfaces makes them a desirable entity for applications in variety of areas. The cationic surfactants acquired prime importance for the first time about 50 years back when their inimitable antibacterial properties came to light [2, 3] and with the passing time their consumption increased and stretched up to millions of metric tons [4]. On industrial level cationic surfactants were regarded as quintessential work pivots because of their supreme implication in oil recovery [5, 6], bactericidal effects [7], and sugar decolorization [8]. They

are constituents of goods ranging from body sprays to sailboat fiberglass, and also they are frequently used in the laundry to keep the clothing softer [9].

In this study, our group has focused on the synthesis of cationic surfactants that are sulfur based quaternary ammonium compounds (QACs) with an adjunct efficacy of ferrocene functionality incorporated into their head group. In spite of potentially applicable pharmacological properties pointed by some researchers, lesser polarity of ferrocene hinders its bioavailability for *in vivo* applications. Hence instead of direct applications ferrocene and its derivatives are usually implicated as a structural entity in designing various drugs. Furthermore, due to the inertness of ferrocene towards atmospheric oxidation, its synthetic derivatives are supposed to be very novel antioxidants, with the respective organic functionalities increasing its bioavailability, and, enhancing the antioxidant efficacy [10].

Ferrocene based QACs are reported to possess variety of applications in biology, specifically as disinfectants and sanitizers [11]. Also since the past few decades, scientists are trying to gain the switchable control over the surface tension of the surfactants by introducing certain redox-active moieties within their structure [12-14] like ferrocene [15] and azobenzene [16]. Such reversible aggregate formation approach can be implicated in controlled drug release [17] and release of various other enclosed species like genes and smaller molecules in response to change in redox properties of external environment [18-20].

Moreover, ferrocenyl surfactants are also of pivot importance and keen interest in most of studies based on the redox behavior of surfactants. These amphiphilic species comprise of a single redox active site with slight modification of head group [21, 22], charge (cationic, anionic, neutral) [12-37] or variation of number [38, 39] and length [29, 39] of hydrophobic chains [40]. Now, in order to synthesize a new series of ferrocenyl surfactants, we have tried to combine the properties of QACs along with ferrocene redox sensitivity, π-conjugation,

electron transfer ability, lipophilicity, lower biotoxicity and more selective cytotoxicity towards tumor cells [12, 13].

Herein, the main aim of this study is to elaborate the effect of chain length on the surfactant properties and behavior like self-assembly and bioactivities, which was achieved by synthesizing a surfactant head structure and attaching different length carbon chains to it. The synthesized surfactants were then used to carry out a comparative study of various surfactant properties like critical micelle concentration (CMC), cell cytotoxicity and different types of antioxidant activities like percentage DPPH inhibition, total reducing power (TRP) and total antioxidant capacity (TAC).

2 Experimental

2.1 Materials

The chemicals used were 1-chlorooctadecane (96 %), 1-chlorohexadecane (95 %), 1-chlorotetradecane (98 %), 4-chlorobenzoyl chloride (95%), ferrocene (98%), 4-nitroaniline (≥99 %), potassium thiocyanate (≥99 %), hexadecyltrimethylammonium bromide (PTC ≥98 %), hydrazine, anhydrous (98 %) and hydrochloric acid (37 %) were obtained from Sigma Aldrich. While the solvents like acetone, ethanol, diethyl ether, petroleum ether, n-hexane and methanol obtained from Merck, Germany were dried applying standard drying methods.

2.2 Synthesis of ferrocene based cationic surfactants

For the synthesis of 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea 0.5 g of potassium thiocyanate was dissolved in 40 mL of dried acetone (0.012 g/mL) and transferred to a 250 mL round bottom flask, and 0.7 mL of 4-chlorobenzoyl chloride (0.049 g/mL) was added under the nitrogen atmosphere. The reaction mixture was kept under stirring for 2 h at a temperature of 50 °C. Then, 1.5 g of ferrocenyl aniline (0.037 g/mL) was added to the reaction mixture and it was kept under stirring for further 4 h at 50 °C [41, 42]. The orange colored precipitates of

1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea were obtained upon cooling in ice which were filtered, washed, dried and recrystallized in acetone to obtain pure product. Subsequently, this ferrocenyl thiourea precursor was used to synthesize ferrocenyl cationic surfactants of different chain length by stirring equimolar ratio of long chain alkyl halides and ferrocenyl thiourea in dry ethanol at 80 °C for 24 h. The progress of the reaction was kept under monitoring by TLC at steady time intervals. After the accomplishment of the reaction, reddish brown precipitates of the product were purified, filtered and dried [13] (Scheme-1).

2.2.1 Characterization

FT-IR spectra of the compounds were recorded using BRUKER (Tensor-37) spectrophotometer (4000-400 cm^{-1}) .While ^{1}H and ^{13}C NMR for all the synthesized compounds were recorded using BRUKER (Advanced NMR) spectrophotometer having a 300 MHz frequency. X-ray measurements were made on a Bruker Kappa APEXII CCD diffractometer equipped with a graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) source. The data collection used ω scans, and a multi-scan absorption correction was applied. The structure was solved by using a SHELXL-97 program and it was refined by full matrix least-squares technique using SHELXL-97.

*1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (**F.R**)*

FT-IR :(cm^{-1}): 3268 (N-H), 3034 (sp^2 -CH), 1713 (C=O), 1263 (C=S), 483 (Fe- C_5H_5); ^{1}H-NMR [300 MHz (DMSO), (δ-ppm)]: 12.53 (1H, (C=S)NH, s), 11.68 (1H, (C=O)NH, s), 7.6-8.01 (8H, Aromatic, m), 4.81 (2H, Cp, s), 4.37 (2H, Cp, s), 4.04 (5H, Cp, s); ^{13}C-NMR: [75 MHz (DMSO), (δ-ppm)]: 178.8, 167.7, 124, 126, 129, 131, 132, 136, 138, 138.5, 69.86, 69.48, 66.8; CRYSTAL DATA: empirical formula: $C_{24}H_{21}ClFeN_2O_2S$ (M =492.79 g/mol): monoclinic, space group $P2_1/c$ (no. 14), a = 15.9706(6) Å, b = 11.4992(3) Å, c = 11.9627(4) Å, β = 96.016(3)°, V = 2184.84(12) $Å^3$, Z = 4, T = 296(2) K, μ(MoKα) = 0.932 mm^{-1}, Dcalc =

1.498 g/cm^3, 17509 reflections measured (4.374° ≤ 2 θ ≤ 54.996°), 5019 unique (R_{int} = 0.0323, R_{sigma} = 0.0362) which were used in all calculations. The final R_1 was 0.0413 (I > 2σ(I)) and wR_2 was 0.1066 (all data).

*4-ferrocenyl-N-((4-chlorobenzoyl)carbamothioyl)-N-octadecylbenzenium chloride (**F.R-18**)*

Quantities: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (0.5 g in 50 mL ethanol), 0.34 mL octadecyl chloride **Yield 75%**, FT-IR (cm^{-1}): 3177 (N-H), 3034 (sp^{2}C-H), 2921 (sp^{3}C-H)1713 (C=O), 1184 (C=S), 720 (-CH_2-), 484 (Fe- C_5H_5); ^{1}H NMR [300 MHz, DMSO, (δ-ppm)]: 12.53 (1H, (C=O)NH, s), 11.68 (1H, (C=S, s)NH), 7.6-8.02 (8H, Ar-H, m), 4.81 (2H, C_5H_5, s), 4.37 (2H, C_5H_5), s), 4.04 (5H, C_5H_5, s), 0.8-1.94 (37H, Aliphatic-H, m); ^{13}C NMR: [75 MHz(DMSO), (δ-ppm)]: 178.8, 167.7, 138, 137.6, 136, 131.6, 131.5, 131.1, 129.2, 129, 126.4, 124, 69.86, 69.47, 66.78, 14.43, 22.57, 26.71, 28.7, 29.18, 29.48, 31.76, 32.5

*4-ferrocenyl-N-((4-chlorobenzoyl)carbamothioyl)-N-hexadecylbenzenium chloride (**F.R-16**)*

Quantities: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (0.5 g in 50 mL ethanol), 0.3 mL hexadecyl chloride **Yield 69%**, FT-IR (cm^{-1}): 3164 (N-H), 3033 (sp^{2}C-H), 2921 (sp^{3}C-H)1732 (C=S), 1183 (C=O), 726 (-CH_2-), 484 (Fe- C_5H_5); ^{1}H NMR [300 MHz, (δ-ppm), DMSO]: 12.53 (1H, (C=O)NH, s), 11.67 (1H, (C=S)NH, s), 7.6-8.02 (8H, Ar-H, m), 4.81 (2H, C_5H_5, s), 4.37 (2H, C_5H_5, s), 4.04 (5H, C_5H_5, s), 0.8-1.97 (33H, Aliphatic-H, m); ^{13}C NMR: [75 MHz, (DMSO), (δ-ppm)]: 178.8, 167.7, 131, 129, 126, 124, 69.86, 69.47, 66.77, 14.43, 22.57, 26.72, 29.18, 29.49, 32.52

*4-ferrocenyl-N-((4-chlorobenzoyl)carbamothioyl)-N-tetradecylbenzenium chloride (**F.R-14**)*

Quantities: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (0.5 g in 50 mL ethanol), 0.28 mL tetradecyl chloride **Yield 76%**, FT-IR (cm^{-1}): 3157 (NH), 3034 (sp^{2}C-H), 2921 (sp^{3}C-H)1735 (C=O), 1183 (C=S), 730 (m, -CH_2-) 484 (Fe- C_5H_5); ^{1}H NMR [300 MHz (DMSO), (δ-ppm)]: 12.55 (1H, (C=O)NH, s) 11.66 (1H, (C=S)NH), s) 7.6-8.02 (8H, Ar-H, m), 4.81 (2H,

C_5H_5, s), 4.37 (2H, C_5H_5, s), 4.04 (5H, C_5H_5, s), 0.8-1.7 (29H, Aliphatic-H, m) ; ^{13}C NMR: [75 MHz (DMSO), (δ-ppm)]: 178.8, 167.7, 138, 137.6, 136, 131.5, 131.1, 129, 127, 126, 124.42, 124.41, 69.86, 69.46, 66.77, 26.72, 28.70, 29.18, 29.36, 29.40, 29.48, 29.51, 31.77, 32.5

*4-ferrocenyl-N-((4-chlorobenzoyl)carbamothioyl)-N-dodecylbenzenium chloride (**F.R-12**)*

Quantities: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (0.5 g in 50 mL ethanol), 0.26 mL dodecyl chloride **Yield 72%**, FT-IR (cm^{-1}): 3153 (N-H), 3032 (sp^2C-H), 2923 (sp^3C-H)1737 (C=O), 1183 (C=S), 722 (-CH_2-), 484 (Fe-C_5H_5); ^{1}H NMR [300 MHz (DMSO), (δ-ppm)]: 12.53 (1H, (C=O)NH, s), 11.66 (1H, (C=S)NH, s), 7.6-8.02 (8H, Ar-H, m), 4.81 (2H, C_5H_5, s), 4.37 (2H, C_5H_5, s), 4.04 (5H, C_5H_5, s), 0.8-1.81 (25H, Aliphatic-H, m); ^{13}C NMR: [75 MHz (DMSO), (δ-ppm)]: 178.8, 167.7, 131, 129, 126, 124, 69.86, 69.47, 66.78, 18.23, 22.53, 26.60, 29.31, 29.42, 29.78, 32.52

*4-ferrocenyl-N-((4-chlorobenzoyl)carbamothioyl)-N-decylbenzenium chloride (**F.R-10**)*

Quantities: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (0.5g in 50ml ethanol), 0.22 mL decyl chloride **Yield 75%**, FT-IR (cm^{-1}): 3154 (NH), 3033 (sp^2C-H), 2925 (sp^3C-H) 1713 (carbonyl), 1182 (thionyl), 729 (-CH_2-), 484 (Fe-C_5H_5); ^{1}H NMR [300 MHz (DMSO), (δ-ppm)]: 12.53 (1H, (C=O)NH, s), 11.69 (1H, (C=S)NH, s), 7.6-8.02 (8H, Ar-H, m), 4.81 (2H, C_5H_5, s), 4.37 (2H, C_5H_5, s), 4.04 (5H, C_5H_5, s) 0.8-1.23 (21H, Aliphatic-H, m); ^{13}C NMR: [75 MHz (DMSO), (δ-ppm)]: 178.8, 167.7, 138.7, 132, 131.5, 129, 126.6, 124, 69.86, 69.47, 66.76, 28.57, 29.1, 29.7, 29.81, 32.50

*4-ferrocenyl-N-((4-chlorobenzoyl)carbamothioyl)-N-nonylbenzenium chloride (**F.R-9**)*

Quantities: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (0.5g in 50 mL ethanol), 0.20 mL nonyl chloride **Yield 67%**, FT-IR (cm^{-1}): 3154 (N-H),3033 (sp^2C-H), 2970 (sp^3C-H,) 1736 (carbonyl), 1229 (thionyl), 728 (-CH_2-), 484 (Fe-C_5H_5); ^{1}H NMR [300 MHz (DMSO), (δ-ppm)]: 12.53 (1H, (C=O)NH, s), 11.66 (1H, (C=S)NH, s), 7.6-8.02 (8H, Ar-H, m), 4.81 (2H,

C_5H_5, s), 4.37 (2H, C_5H_5, s,), 4.04 (5H, C_5H_5, s), 0.8-1.56 (19H, Aliphatic-H, m); ^{13}C NMR: [75 MHz (DMSO), (δ-ppm)]:178.8, 167.7, 131, 129, 126, 124 , 69.86, 69.47, 66.78, 18.23, 22.53, 26.60, 29.31, 29.42, 29.78, 32.52

*4-ferrocenyl-N-((4-chlorobenzoyl)carbamothioyl)-N-octylbenzenium chloride (**F.R-8**)*

Quantities: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (0.5 g in 50 mL ethanol), 0.16 mL octyl chloride **Yield 75%**, FT-IR (cm^{-1}): 3027 (sp^2C-H), 3153 (N-H), 2970 (sp^3C-H), 1738 (carbonyl), 1261 (thionyl), 730 (-CH_2-), 484 (Fe-C_5H_5); ^{1}H NMR [300 MHz (DMSO), (δ-ppm)]: 12.53 (1H, (C=O)NH, s), 11.67 (1H, (C=S)NH, s), 7.6-8.02 (Ar-H, m), 4.81 (2H, C_5H_5, s), 4.36 (2H, C_5H_5 , s), 4.04 (5H, C_5H_5, s), 0.8-1.49 (17H, Aliphatic-H, m); ^{13}C NMR: [75 MHz (DMSO), (δ-ppm)]: 178.8, 167.7, 131, 129, 126, 124, 69.86, 69.47, 66.78, 14.39, 18.54, 22.4, 26.7, 28.12, 29.30, 29.43, 29.56, 32.50

2.3 Critical micelles concentration (CMC)

2.3.1 Surface tension studies

A BZY-202 surface tensiometer (automatic) was used for the determination of surface tension with the concentrations of the surfactants ranging between 0.01-0.2 mM in ethanol/water mixture (1:9).

2.3.2 UV-visible spectroscopy

SHIMADZU (UV-1800) UV-visible spectrophotometer was also used for the determination of critical micelle concentration (CMC) for which the absorbance spectra were recorded by varying the concentrations of the surfactants between 0.01-0.02 mM in ethanol/water solvent mixture (1:9).

2.4 Biological evaluation (Bio-activities)

In vitro biological activities for the compounds were performed in purpose to estimate their bioactive potential and antioxidant abilities.

2.4.1 Brine shrimp lethality assay

Cytotoxicity of the tested surfactants was scrutinized in a microtiter (96 well) plate utilizing brine shrimp (Artemia salina) larvae as test insects [43]. Incubation of eggs of *A. salina* (Ocean star, USA) was carried out within the simulated marine water (38 g/L augmented by 6 mg/L dry yeast) at 32 °C for up to 48 h in the presence of light in a specifically designed two-section platter. The maturated nauplii were harvested after incubation using Pasteur pipette which were then shifted to the individual plate wells (10 nauplii per well). In the next step simulated marine water containing 1% surfactant sample dissolved in DMSO (500, 250 and 100 µg/mL) was added to the wells already containing brine shrimps larvae and marine water keeping the ultimate volume of the wells up to 300 µL. Serialized concentrations of the standard drug doxorubicin and 1 % DMSO were contained within the positive as well as negative control wells correspondingly. Subsequently, after an incubation period of 24 h, the percentage of deaths were calculated after counting the shrimps that were alive. LC_{50} (lethal concentration to kill 50 % population) of the compounds was calculated using the table curve V-5.01 software.

2.4.2 Total antioxidant capacity determination

Total antioxidant capacity (TAC) evaluation of the titled compounds was carried out by using the reported method [44] with few alterations. 100 µL of surfactant sample dissolved in DMSO (4mg/mL) was added to 900 µL of the reagent aqueous solution containing 0.6 M H_2SO_4, 4 mM ammonium molybdate ($(NH4)_6Mo_7O_{24}$) and 28 mM Na_2PO_4 (1:1:1). After 90 min incubation of this reaction mixture done at 95 °C it was cooled to ambient temperature. Then the absorbance measurements were made at 695 nm using microplate reader. 100 µL DMSO

was used as a negative control, while ascorbic acid as a positive control for respective calibration curve. Expression of the resultant total antioxidant capacity (TAC) was made as µg equivalent of ascorbic acid as per milligram of dried weight (µg AAE/mg DW).

2.4.3 Total reducing power assay (TRP)

Investigation of the total reducing power (TRP) was made in accordance with the standard protocol [44]. 100 µL of the individual sample (4 mg/mL in DMSO) was mixed with 200 µL of 0.2 M PO_4^{-2} buffer (pH 6.6) and 250 µL of $K_3Fe(CN)_6$ (1 % w/v).The resultant reaction mixture was incubated for 20 min at 50 ^oC followed by acidification of the reaction by adding 200 µL of CCl_3COOH (10 % w/v). The reaction mixture was then centrifuged at 3000 rpm for 10 min.150 µL of the liquid from supernatant floating layer was removed and mixed with 50 µL of $FeCl_3$ (0.1 % w/v)to carry out the optical density measurement at 630 nm. The positive control used was ascorbic acid while the expression of results of total reducing power (TRP) was as equivalents of µg of ascorbic acid per milligrams of dried weight (µg AAE/mg DW).

2.4.4 DPPH free radical scavenging assay

Free radical scavenging assay is a common method to determine the antioxidant capacity of a test compound in which 2,2-diphenyl picrylhydrazyl (DPPH) was used to analyze the scavenging activity of the compounds following the standard protocol [45] with some alterations. 10 µL of the sample solution (4 mg/mL in DMSO) was mixed with 190 µL of DPPH solution in methanol (0.004 % w/v) and incubated for 1 h in dark. Then the microplate reader was used to carry out the optical density measurement at 515 nm wavelength. DMSO was used as negative whereas ascorbic acid was used as a positive control. Initially the screening of the samples was done at 200 µg/mL and those with better inhibition activity *i-e* ≥ 50 % were then screened further for activity at lower concentration (66.6, 22.2 and 7.41 µg/mL)

to carry out calculation of their LC_{50} values. Following formula was used to calculate the percentage inhibition.

% inhibition of the sample under test = Percent scavenging activity = $(1 - {Ab_s}/{Ab_c}) \times 100$

Where,

$Ab_s = \lambda_{max}$ (DPPH solution + sample)

$Ab_c = \lambda_{max}$ negative control (reagent + solvent)

The calculation of LC_{50} was carried out by applying the 2D Version-4 Table curve software.

3 Results and discussion

Synthetic strategy for the preparation of the surfactants is outlined in Scheme-1. Ferrocene functionalized cationic surfactants were synthesized through a three step mechanism. In the first step, ferrocenyl aniline was synthesized by treating 4-nitroaniline with sodium nitrite in the presence of HCl, and then reacting the resultant product with ferrocene in the presence of phase transfer catalyst to obtain nitrophenyl ferrocene. The nitrophenyl ferrocene precursor was then finally reduced to ferrocenyl aniline in the presence of hydrazine and palladium charcoal [12, 40] . In the second step, 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea derivative was synthesized by reacting 4-chlorobenzoyl chloride with potassium thiocyanate which was further treated with ferrocenyl aniline to yield the product. In the third step, the resultant product (**F.R**) was treated with different chain length alkyl chlorides to prepare respective ferrocenyl cationic surfactants comprising different chain length.

Characterization of the synthesized compounds was carried out through single crystal XRD, FT-IR, ^{1}H and ^{13}C-NMR spectroscopy.

3.1 Spectroscopic analysis

The proton NMR spectrum of ferrocenyl thiourea (**F.R**) shows two characteristic signals for N-H protons at 12.5 ppm and 11.68 ppm confirming the formation of thiourea. The signals for aromatic protons appear in the region between 7.57-8.02 ppm. The ferrocene signals lie in the region between 4.04-4.81 ppm. (Figure S1a). In case of ferrocenyl cationic surfactants containing aliphatic carbon chain the appearance of additional alkyl chain signals at 0.8-1.9 ppm and 3.5-3.9 ppm confirm the attachment of the long chain at N-H of thiourea adjacent to the aromatic ring. There is also a slight shift in the value of two N-H peaks lying at 11.66 ppm and 12.54 ppm respectively which might be due to the influence of the attached chain (Figure S2a).

In case of ^{13}C NMR spectra of ferrocenyl thiourea, the appearance of two extra signals at 168 ppm and 167 ppm that correspond to the carbonyl and thionyl group carbon atoms respectively, confirmed the thiourea formation. The signals in the region of 124-138 ppm are assigned to aromatic carbon atom. The region between 66.78-84.58 ppm corresponds to the Cp ring carbon atoms of ferrocenyl moiety. (Figure S1b). ^{13}C NMR of ferrocenyl cationic surfactants show a bulk additional signals of long chain carbons in the region of 14-33 ppm that correspond to the aliphatic C-atoms. The ferrocene carbons give four signals in the region of 66-85 ppm. Chemical shift values of all the aromatic ring carbons lie in the region of 124-139 ppm. Signals at 178.7 ppm and 167.6 ppm are specified for carbonyl and thionyl carbon atoms respectively (Figure S2b).

FT-IR studies depict that the N-H band appears in the range of 3150-3270 cm^{-1} and sp^2 (C-H) band emanates at about 3027-3034 cm^{-1}. The region between 2910-2970 cm^{-1} is asssigned to sp^3 (C-H), while the corresponding carbonyl (C=O) and thionyl (C=S) signals lie in the region of 1710-1740 cm^{-1} and 1180-1270 cm^{-1} respectively. The intensity of carbonyl band appears to be low due to the H-bonding between the carbonyl group oxygen atom and the H-atom of N-H group next to the thionyl moiety. While the characteristic band for long-chain methylene (-

CH_2-) for the surfactants lie in the range of 720-730 cm^{-1} and the peaks for Fe-Cp lie at 483 cm^{-1} for thiourea and at 484 cm^{-1} for the ferrocene-based cationic surfactants (Figure S3, Figure S4) [46, 47].

3.2 Single crystal XRD studies

The crystallization of ferrocenyl thiourea monohydrate precursor in acetone yielded orange colored small sized crystals. The crystal data shows that the crystal has monoclinic system with space group $P2_1/c$ (Table-1). The crystal structure diagram with numbering scheme (Figure-1a) shows that the ferrocene Cp rings A(C15-C19) and B(C20-C24) are fully eclipsed. Whereas A(C15-19) and D(C3-C8) are more or less planar to each other while C (C9-C14) is slightly out of plane. Ferrocene moieties lie *trans* to each other according to the crystal packing along the b-axis, Moreover, according to the packing along c-axis they lie perpendicular to each other (Figure-1c,d). Figure 1-b shows the molecular packing with a cube exhibiting minimal repulsion. Supramolecular interactions can also be seen within the crystal structure; and water molecule is also incorporated with thiourea structure through intermolecular H-bonding between the oxygen atom of the water molecule and the H-atom of one of the N-H group. Intramolecular H-bonding between the oxygen atom of the carbonyl group (C=O) and the H-atom of the N-H group (next to the thionyl C=S group) is also present within the structure (Figure-1a).

3.3 Critical micelle concentration (CMC) determination

Surface tension studies were performed in order to determine the CMC points. Plot of surface tension (γ) as a function of concentration of synthesized series of new ferrocene based cationic surfactants are shown in Figure-2. A linear decrease in the surface tension value with each successive addition of surfactant was observed and onset of micellization that trend deviates.

The point of deviation in plots of γ *versus* concentration gave the critical micelles concentration in the ethanol water mixture (1:9). The plot depicts the clear indication of earlier micellization in case of new ferrocene based cationic surfactants having longer alkyl chain length and follow the trend **F.R-8** > **F.R-9** > **F.R-10** > **F.R-12** > **F.R-14** > **F.R-16** > **F.R-18**. (Table S1, Figure 2).

UV-visible spectroscopic studies were also employed to verify the critical micelle concentration values of the synthesized compounds in ethanol-water mixture (1:9). For this purpose, absorbance *versus* concentration profile was recorded, which obeying Beer-Lambert law exhibited sharp rise in the beginning but at a certain point a slowdown in the absorbance increment rate was obtained for the equal increase in concentration. The hinderance in absorbance can be attributed to the formation molecular aggregates that prevent the UV-radiations to penetrate the inner micellar structures. The data thus obtained was used to plot absorbance *versus* concentration curves for the respective surfactants (Figure-3). The points of inflection in the graphs indicated the micelle formation for particular surfactant at that concentration and thus labeled as CMC. The CMC values as calculated by UV absorbance method are in accordance with the values already determined through surface tension method (Table S1). It was concluded from the CMC data of the seven surfactants that decreasing alkyl chain length has a direct impact on micellization. Since, the process of aggregation is more facile when compounds have higher hydrophobicity therefore the surfactants with longer chain formed micelles at comparatively lower concentration and CMC ultimately increased with a decrease in chain length. [48].

3.4 Bio-activities

The cytotoxic activity against the brine shrimp larvae was performed using three different sample concentrations, out of which 500 µg/mL and 250 µg/mL were high enough to kill all the brine shrimp larvae population, so the results could not have been utilized for a comparative

cytotoxicity effect of surfactants. For this purpose another lower sample concentration set was employed which produced desired results pretty enough for comparative study of the cytotoxicity effect, and longer chain compounds like F.R-18 and F.R-16 cytotoxicity was comparable to that of the reference drug doxorubicin.

By considering the impact of alkyl chain hydrophobicity, the molecules comprising longer alkyl chains were expectedly more toxic, which is also reported in the previous studies. But the long chain is not the only factor, cationic head group also has some contribution in this cytotoxic activity mechanism, due to the presence of ferrocene and thionyl functionalities. This can be seen from the cytotoxic activity of ferrocenyl thiourea that is not containing any long chain but still shows remarkable cytotoxicity. Upon addition of long chain to the ferrocenyl thiourea cytotoxic activity is enhanced, which can be attributed to the long chain as well as the positive charge of the head group. All the surfactants showed greater cytotoxic activity than their parent ferrocenyl thiourea but on mutual comparison a descending order decrease in the activity was observed with the subtraction of each ethyl moiety. This respective phenomena can be ultimately accredited to the chain length effect. [49]. (Figure-4, Table S2). The LC_{50} values showed a variable trend with F.R-14, F.R-9 and F.R-8 showing highest LC_{50} values.

Antioxidant activities of the compounds were tested through DPPH inhibition assay, TRP assay and TAC assay. The results obtained through all the three experiments showed approximately similar antioxidant activity trend as observed through cytotoxicity assay, with the longer chain surfactants showing comparatively greater cytotoxicity and an overall decreasing activity trend was observed with the decrease in chain length. Herein the ferrocenyl thiourea (F.R) parent group activity in the three graphs (Figure-5, S5 Table S3) depicts that the main contributor towards the antioxidant activity is probably the surfactant head group comprising ferrocene antioxidant potential which is overall enhanced by the additional head group alkylation by enhancing its bioavailability. Upon addition of the hydrophobic chain the antioxidant activity

of the long chain surfactants remains the same but as the chain length decreases the antioxidant activity decreases rapidly. This phenomena can be attributed to the micellar effect since the concentration of the surfactant samples for antioxidant activity was very much higher than their individual CMC values. At such higher concentration the long rate of micelle degradation was higher as compared with micelle formation in case of longer chain surfactants, leading to higher monomer to micelle ratio. while in case of shorter chain surfactants there were enough micelles to overall masking the bioavailability of the head group.[48-51]

5 Conclusions

Successful synthesis of ferrocene containing thiourea based cationic surfactants homologous series has been carried out, with complete characterization of the compounds using different techniques. The studies showed that the alkyl chain length effects the surfactant overall properties like self-aggregation and biological properties. Furthermore it is also concluded that longer chain length surfactants are more bio-applicable as compared with the shorter chain length surfactants. Antioxidant potential of the surfactants was solely a property of head structure containing ferrocene, which ultimately showed a decline with the reduction of the added chain length. These experimental studies can be extended to carry out further analytical comparison of more surfactant properties. Head structure, experimental conditions and solvent compositions can also be varied to carry out more detailed studies.

Supplementary data

The crystallographic structural data for ferrocene containing thiourea compound being reported in this paper is submitted in the Cambridge Crystallographic data center with supplementary publication number CCDC-1909730. For which the crystallographic data copies will be accessible at deposit@ccdc.cam.ac.uk.

Credit Author Statement:

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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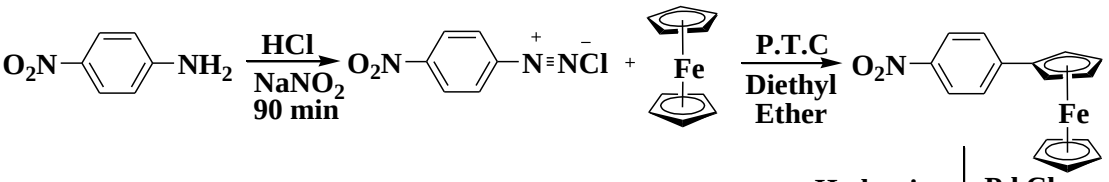
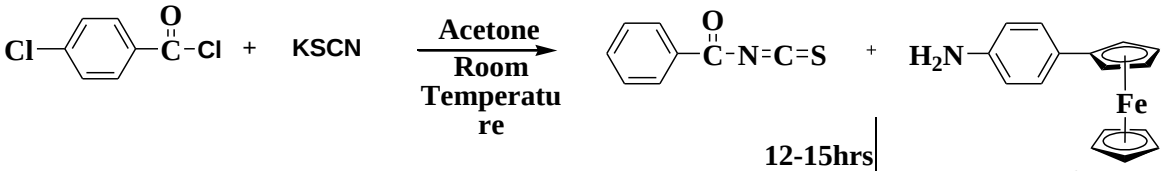
Scheme 1: Synthetic scheme for ferrocenyl thiourea cationic surfactants.

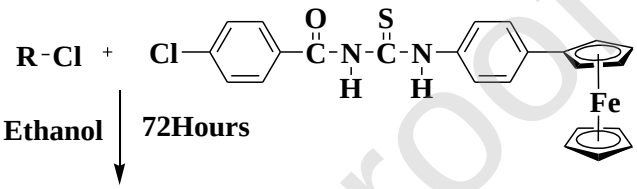
Step 1



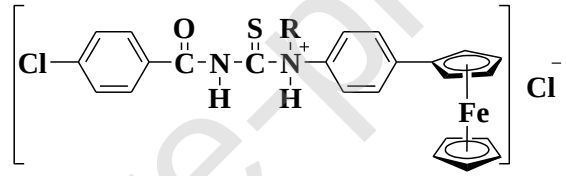
Step 2

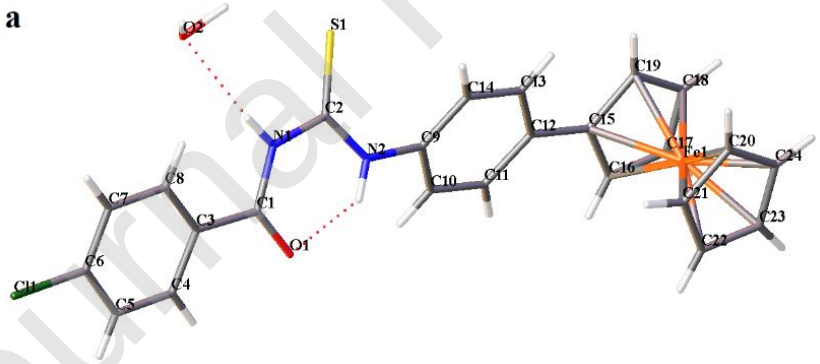


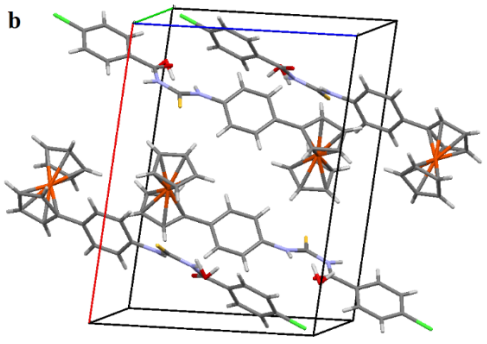
Step 3



R= $C_{18}H_{37}$, $C_{16}H_{33}$, $C_{14}H_{29}$, $C_{12}H_{25}$, $C_{10}H_{21}$, C_9H_{19}, C_8H_{17}







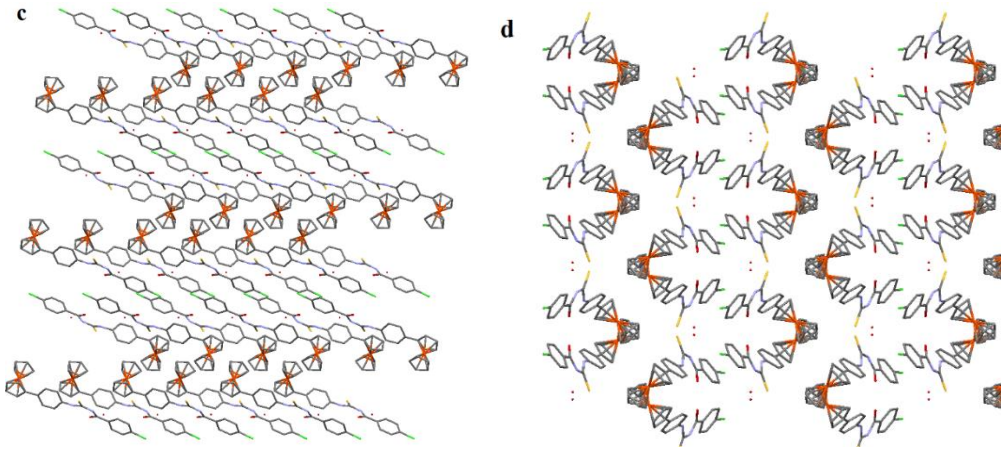


Figure 1: 1-(4-ferrocenylphenyl)-3-(4-chlorobenzoyl)thiourea (**F.R**) (a) molecular structure, (b) molecular packing within the unit cell, (c) molecular packing along b-axis, (d) molecular packing along c-axis.

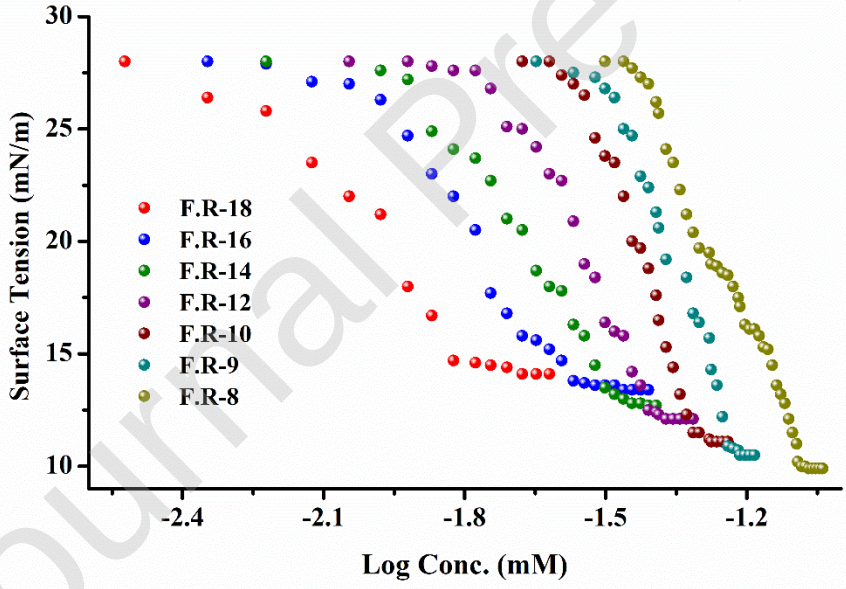


Figure 2: Surface tension *versus* concentration graph showing chain length dependency of CMC.

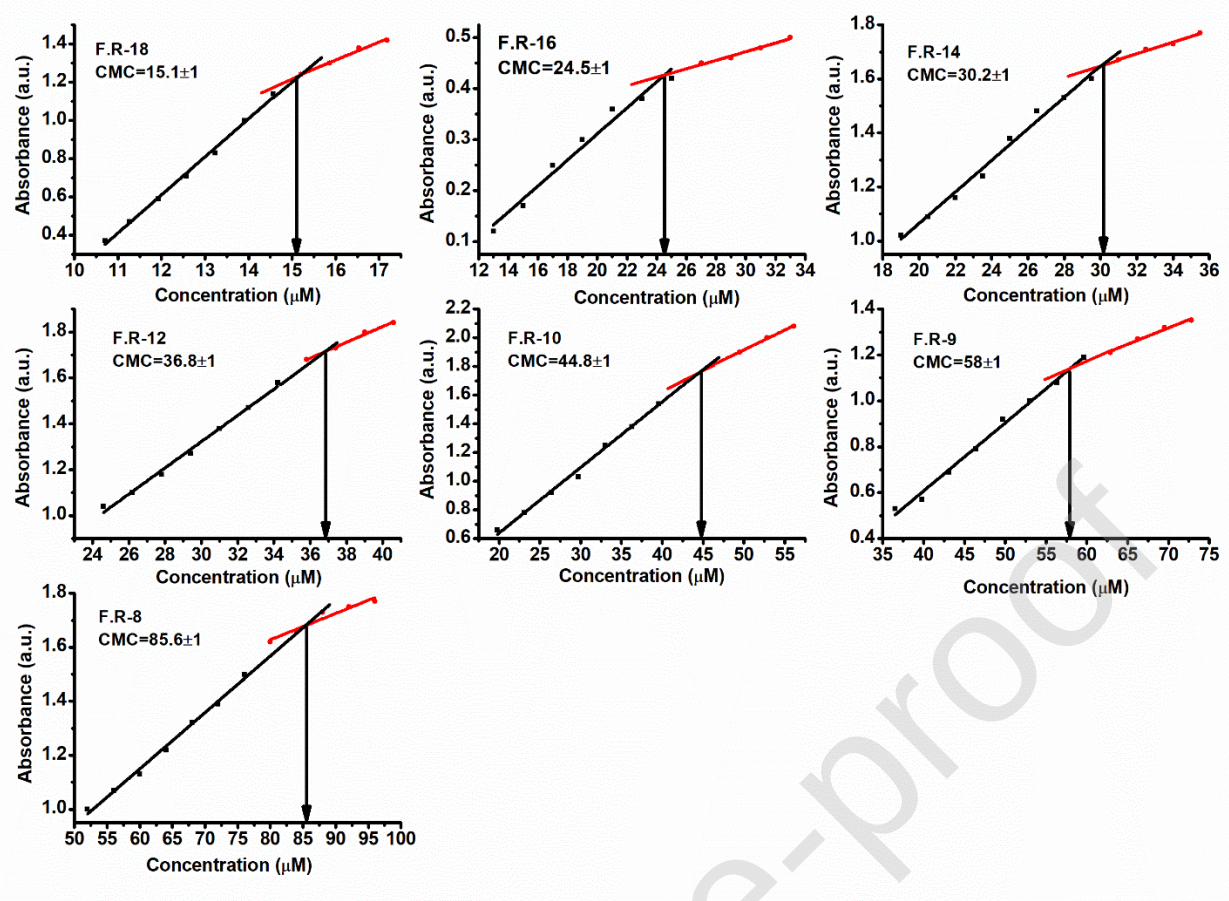


Figure 3: Absorbance *versus* concentration plots for the surfactants CMC determination.

Solvent used was ethanol/water mixture (1:9)

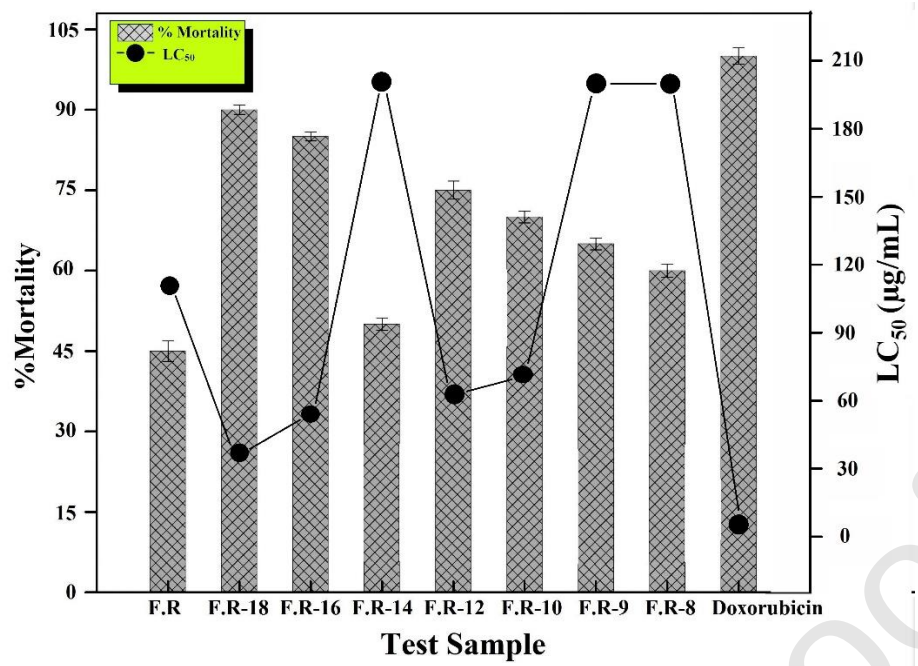


Figure 4: Cytotoxicity profiles of Brine shrimp larvae incubated with synthesized surfactants and the reference drug Doxorubicin (100µg/mL).

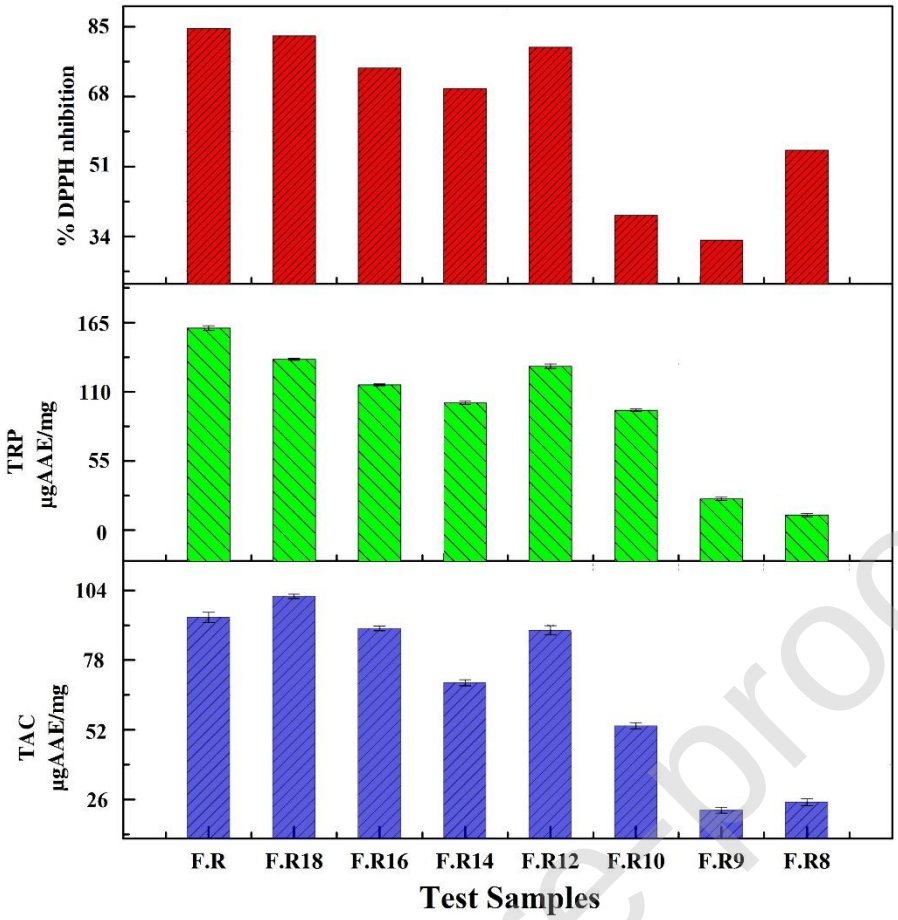


Figure 5: Antioxidant activity of ferrocene based cationic surfactants versus their head group molecule (a) DPPH free radical scavenging assay, (b) Total reducing power assay (TRP), (c) Total antioxidant capacity assay (TAC).

Table 1: Crystallographic and refinement details of **F.R**.

Identification code	F.R
Empirical formula	$C_{24}H_{21}ClFeN_2O_2S$
Formula weight	492.79
Temperature/K	296(2)
Crystal system	Monoclinic
Space group	$P2_1/c$
a/Å	15.9706(6)

b/Å	11.4992(3)
c/Å	11.9627(4)
α/°	90
β/°	96.016(3)
γ/°	90
Volume/Å^3	2184.84(12)
Z	4
ρ_{calc}g/cm^3	1.498
μ/mm^{-1}	0.932
F(000)	1016.0
Crystal size/mm^3	0.51 × 0.17 × 0.14
Radiation	MoKα (λ = 0.71073)
2θ range for data collection (°)	4.374 to 54.996
Index ranges	-20 ≤ h ≤ 14, -14 ≤ k ≤ 11, -15 ≤ l ≤ 15
Reflections collected	17509
Independent reflections	5019 [R_{int} = 0.0323, R_{sigma} = 0.0362]
Data/restraints/parameters	5019/3/286
Goodness-of-fit on F^2	1.026
Final R indexes [I>=2σ (I)]	R_1 = 0.0413, wR_2 = 0.0945
Final R indexes [all data]	R_1 = 0.0637, wR_2 = 0.1066
Largest diff. peak/hole / e Å^{-3}	0.34/-0.33