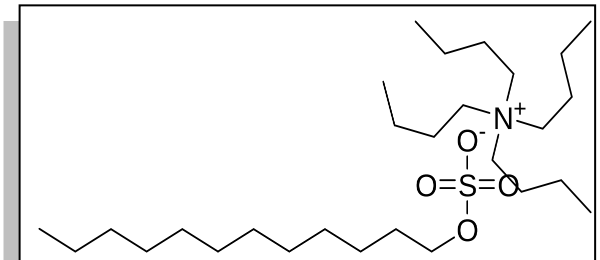
2.1. Materials

SDS (≥ 99 %), TX-100 (ultra) were purchased from Sigma St Louis, USA, SDBS (99%) were obtained from TCI, Japan, MES, a gifted sample (Lion corporation, Tokyo), was recrystallized using dry ethanol before use. TBAB (99%) and TBPB (99%) were purchased from Sigma St Louis, USA. $Ca(NO_3)_2.4H_2O$ (≥ 98%) and $Al(NO_3)_3.9H_2O$ (98%) were purchased from Merck and s. d. fine-chem limited respectively.. All the salts were dried at appropriate temperature before use. Various drugs; AMT (≥ 98 %, Sigma-Aldrich) and curcumin (≥ 97 %, s. d. fine-chem limited) are used as such for the purpose. Drug intermediate AHC is synthesized by standard procedure [141], purified and dried before use.

The water used to prepare the sample solutions was double distilled in an all-glass distillation apparatus. The specific conductivity of the water was in the range $(2\text{-}4) \cdot 10^{-6}$ $S{\cdot}cm^{-1}$. D_2O used in the sample preparation for NMR and SANS is 99.9 atom % D purchased from Sigma-Aldrich St Louis, USA. All other solvents used in study were of high purity available.

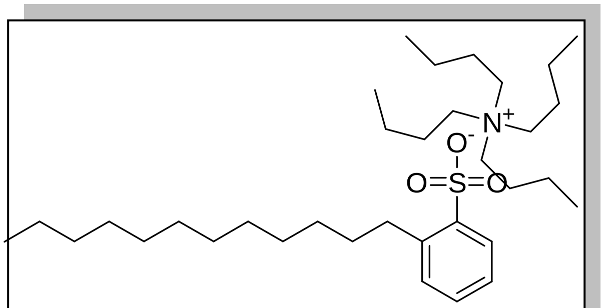
2.2. Synthesis and Characterization of Anionic Quaternary Counterion Surfactants

Two quaternary bromides (TBAB and TBPB) and three conventional anionic surfactants (SDS, SDBS and MES) are used to synthesize various anionic surfactants with quaternary counterions. These surfactants have been prepared by mixing equimolar aqueous solutions of **SDS/SDBS/MES** with **TBAB/TBPB**. The respective clear mixture was stirred at room temperature for 62h. This solution was then extracted with DCM followed by vacuum drying. This results into a viscous mass of a respective quaternary surfactant. The purity of all the synthesized surfactants (**TBADS, TBADBS, TBAMES, TBPDS and TBPDBS)** was confirmed by qualitative (absence of Br^- in aqueous surfactant solution), quantitative elemental (Table 1) analysis (using method Test Eager 200), surface tensiometry (absence of minimum in γ *vs* [surfactant] plot) and ^1H NMR (a few representative NMR spectra are shown in Figures 1-3). Flame photometry has also been used to ensure the absence of Na^+ in the above surfactant samples. The structures of all the above surfactants are shown in **scheme**.



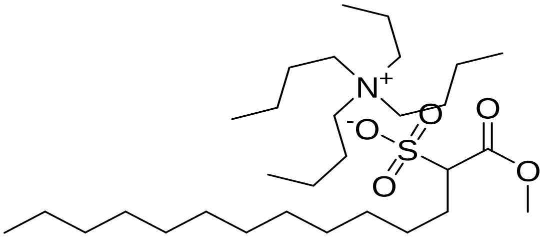
TBADS

tetra-*n*-butylammonium dodecyl sulphate



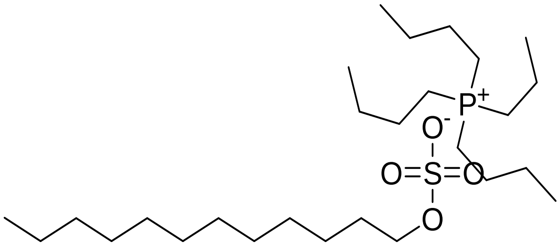
TBADBS

tetra-*n*-butylammonium dodecyl benzene sulphonate



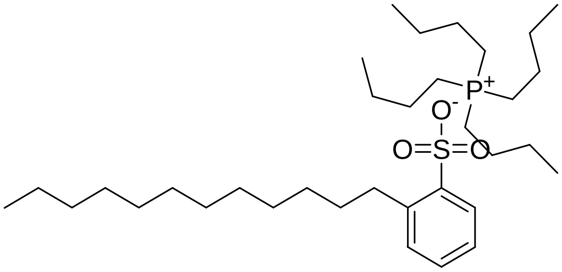
TBAMES

tetra-*n*-butylammonium-*α*-sulfonato myristic acid methyl ester



TBPDS

tetra-*n*-butylphosphonium dodecyl sulphate



TBPDBS

tetra-*n*-butylphosphonium dodecyl benzene sulphonate

Scheme: Structures of Quaternary Counterion Surfactants

Table 1: Elemental analysis showing % composition of each element in different quaternary surfactants

Surfactant	% C	%H	%N	%S	%P
TBADS	65.832	12.034	2.706	6.253	-
TBADBS	70.985	11.543	2.546	-	-
TBAMES	65.367	11.212	2.140	-	-
TBPDS	64.553	11.821	-	5.854	5.942
TBPDBS	69.543	10.850	-	4.963	5.140

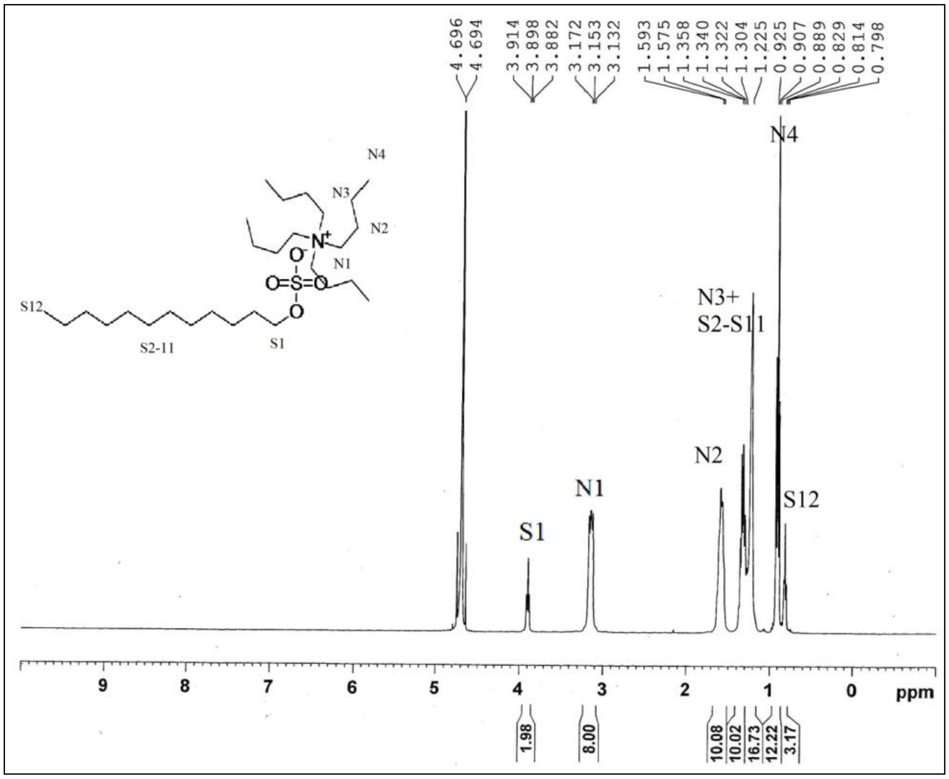


Figure 1: ^{1}H NMR spectra for TBADS

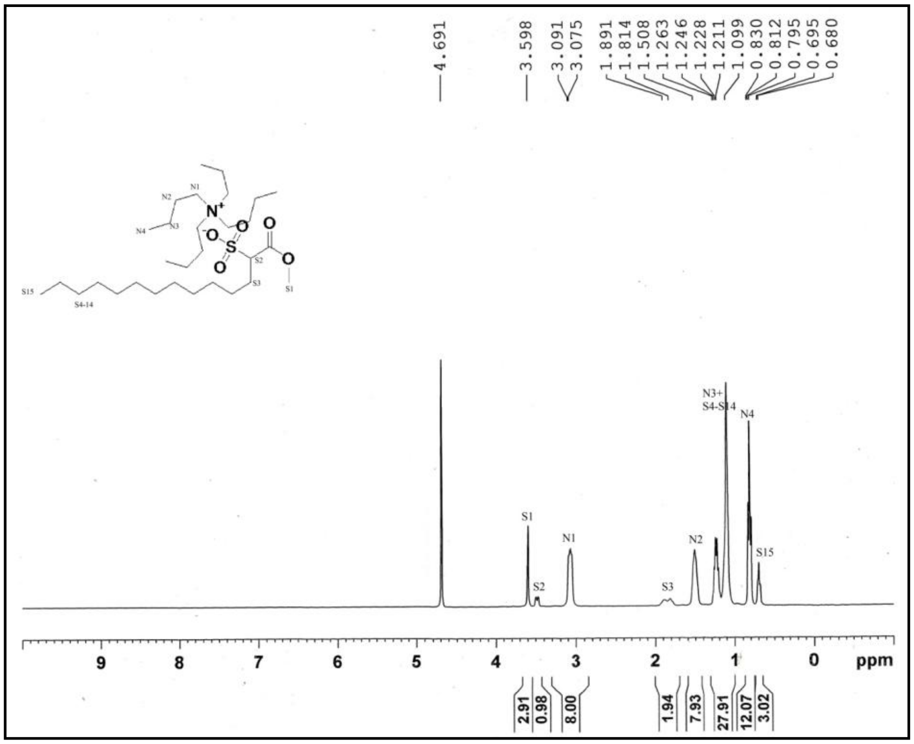


Figure 2: ^{1}H NMR spectra for TBAMES

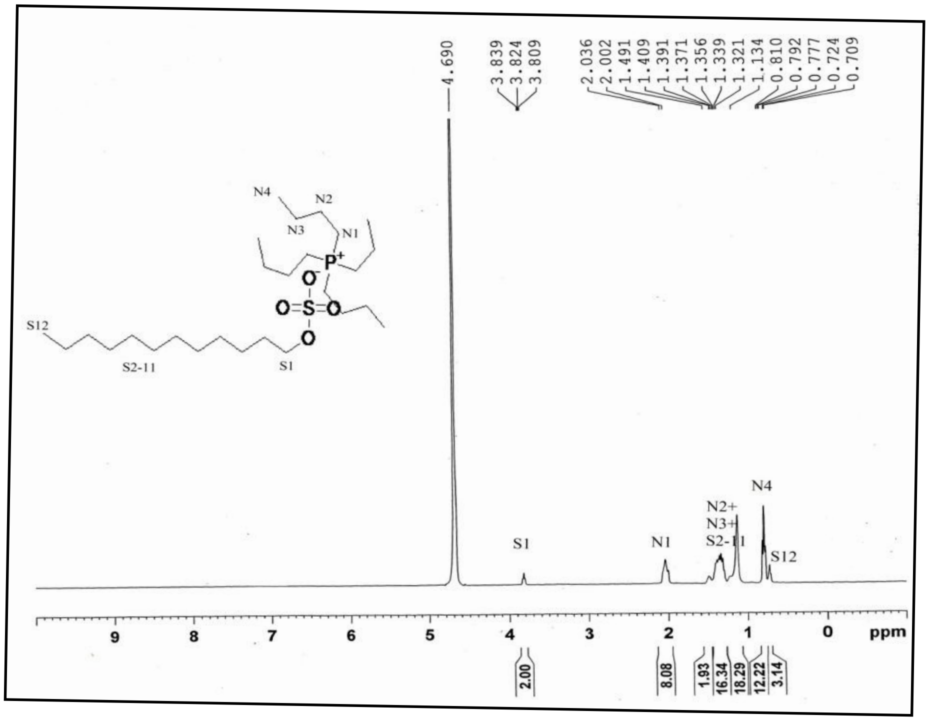


Figure 3: ^{1}H NMR spectra for TBPDS

2.3. Methodology

2.3.1. Sample preparation

Various stock solutions of surfactants have been prepared by weighing the requisite amount of each surfactant in appropriate standard volumetric flask (previously clean and dried). Each stock solution was used to prepare samples with or without inorganic salts. Samples were diluted according to the need of the instrumentation of each methodology.

2.3.2. Conductometric measurements

Conductometric measurements were carried out by using a conductivity meter EUTECH cyberscan CON510 (cell constant 1 cm^{-1}) with an inbuilt temperature sensor. A pre-calibrated conductivity cell was used to obtain specific conductance at an appropriate concentration range. Temperature of the sample solution was precisely controlled by SCHOTT CT 1650 thermostat with an accuracy of ± 0.1°C. The cell with an appropriate amount of the solvent (30 ml water) in a vessel was thermostated for at least 30 minutes prior to the measurement. The conductivity runs were carried out by adding concentrated surfactant solution to the 30 ml water. After thorough mixing and equilibration at particular temperature, κ were measured at specific concentration. The cmc and α values for the synthesized anionic surfactants were determined from the intersection point between the two straight lines (in the plot of the [surfactant] *vs.* specific conductance), and the ratio of the slopes of the post-micellar to that of the pre-micellar portions of the straight lines as reported earlier [83,142]. In another set of experiment different fixed concentration of surfactants were taken and the conductometric measurements were performed with varying temperature.

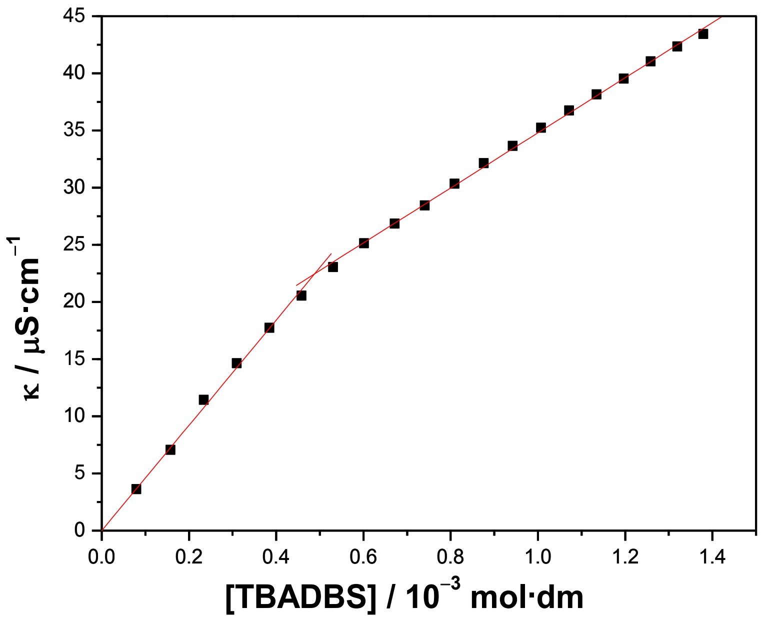


Figure 4: Variation of specific conductance (*κ) vs* concentration plot for TBADBS

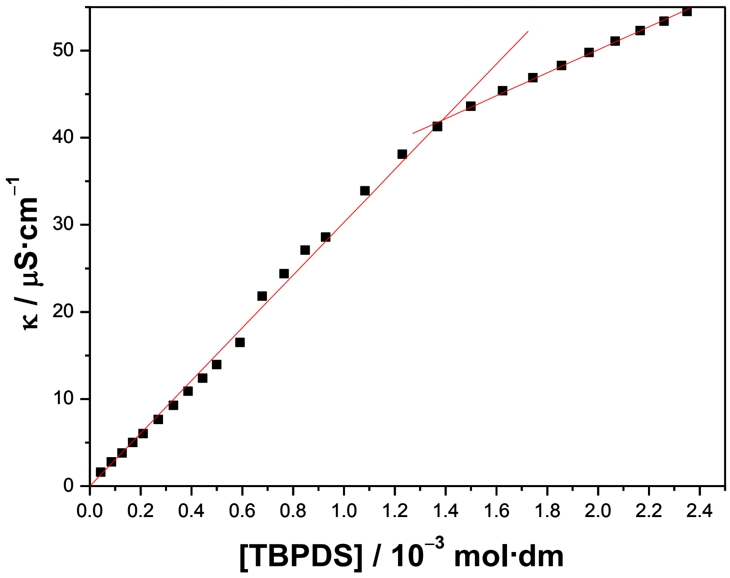


Figure 5: Variation of specific conductance (*κ) vs* concentration plot for TBPDS

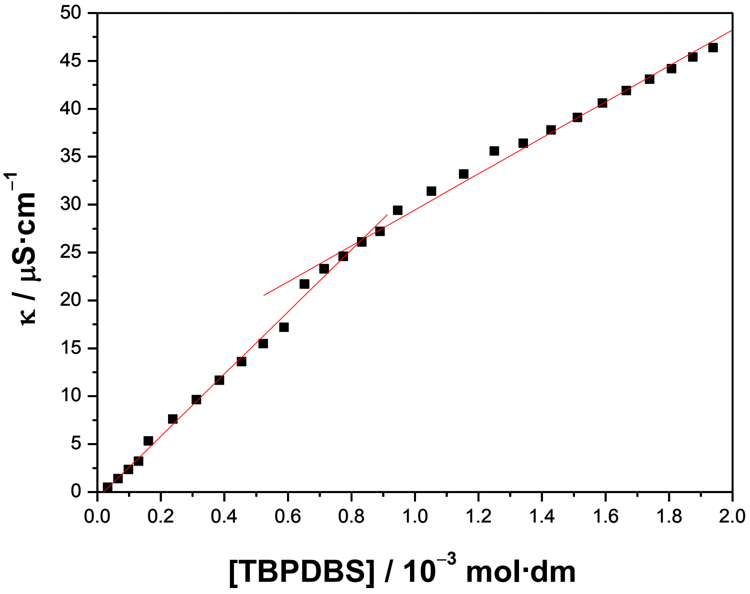


Figure 6: Variation of specific conductance (*κ) vs* concentration plos for TBPDBS

2.3.3 Tensiometric measurements

Tensiometric measurements were carried out using Du-Nouy Tensiometer (Model No-STT-78; manufactured by S. C. Dey and Co, Calcutta INDIA) adopting the ring detachment technique at a controlled temperature, accurate within (0.1°C). Prior to detachment, the platinum ring with gold joints was carefully lifted. Thorough stirring followed by a 10 min interval for equilibration was allowed after each addition. The γ values were directly noted from the instrument accurately within a limit of 0.1 Nm-1. The experiments were conducted at 30°C and were duplicated to check reproducibility. The variation of γ *vs* [surfactant] plot was used to ensure purity as well as for the determination of cmc.

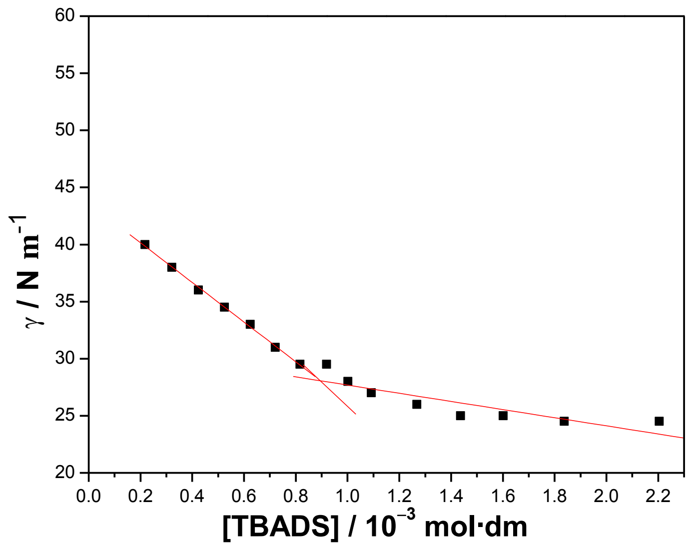


Figure 7: Variation of surface tension (*γ) vs* concentration plots for TBADS

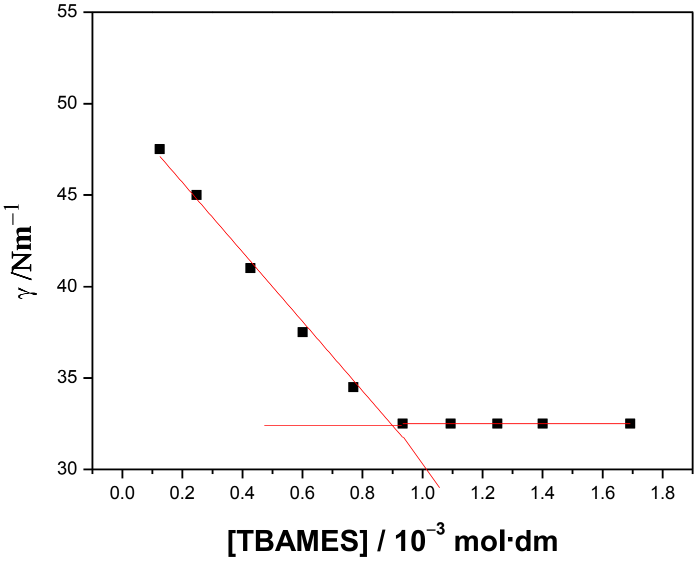


Figure 8: Plot of [TBAMES] vs S.T to get cmc

2.3.4. Cloud Point (CP) measurements

The CP values were obtained by placing sample tubes, containing surfactant solutions with a fixed concentration of surfactant in presence or absence of inorganic salt, into a temperature-controlling water bath. The sample was heated and temperature of onset of turbidity (visual observation) was noted. The system was allowed to cool and temperature at which the disappearance of turbidity took place was also noted. The average of two readings was taken as the cloud point (CP). This procedure was repeated for the same sample and nearly two concurrent values (within ± 0.1°C) were considered as the final CP. Similar CP measurements were also made by diluting the samples using either double distilled water or surfactant stock solution (in presence of additive) to collect CP data in order to observe [surfactant] - [additive] interplay.

2.3.5. DLS measurements

DLS measurements were performed for the characterization of the particle size distributions of micellar solutions, using a Brukhaven 90 plus particle size analyser at different temperatures. The measured quantity in DLS experiments is the time dependent fluctuation in the scattered intensity from the solution undergoing random, Brownian motion. Analysis of the intensity fluctuation allows the determination of the diffusion coefficients, which in turn yield the particle size through the Stokes-Einstein equation. Nonnegatively constrained least squares (NNLS) method has been used to obtain diameter probability distribution function.The solid laser operated at 660 nm with a maximum power output of 15 mW with the scattering angle 90° was used. The sample was filtered (0.22 µm) to avoid interference from dust particles. The correlation functions were analyzed by the method of Contin and Cumulant to have an idea of mean diameter and polydispersity index. The instrument was calibrated before measurements with both air and a standard solution (12.88 mS at 25 °C).

2.3.6. NMR measurements

NMR is a versatile non-destructive technique, useful for the study of surfactant solutions. From the chemical shift changes, broadening and splitting of peaks in present case provides a deeper insight to the micelle arrangement, dehydration of micelles at higher temperature and the attractive interactions leading clouding at the molecular level. NMR spectra were obtained with Bruker Avance 400 Spectrometer at different temperatures (20-70°C). All NMR data were processed using the software package Bruker Topspin 2.1. ^{1}H NMR spectrum was acquired using the standard Bruker pulse program zg30. All the surfactant solutions were prepared in D_2O. About 0.6 ml of solution was transferred to a 5 mm NMR tube and chemical shifts were recorded on the δ (ppm) scale. All the spectra are calibrated with the HOD signal at 4.69 ppm (δ)

2.3.7. SANS measurements

SANS provides information in the length scale (~1-100 nm) corresponding to most of the micellar systems. It is the direct technique used to investigate the structure of and interaction between the particles dispersed in a homogeneous medium. The peculiar neutron's properties like wave-particle duality, compatible wavelength with the inter-atomic distances for the thermal neutrons, compatible energy with the atomic excitation energy, its deep penetration due to charge neutrality, scattering contrasts between neighboring elements in the periodic table, isotope specific scattering and absorption, magnetic moment due to which it can react with magnetic materials, simple interaction with nuclei makes the technique more promising

SANS measurements were carried out using a SANS spectrometer at the Dhruva Reactor, Bhabha Atomic Research Centre, Mumbai, India [143a]. It is a diffraction technique which involves the elastic scattering of neutrons by the sample and measuring the scattered neutron intensity as a function of the scattering angle, 2θ. The spectrometer utilizes BeO filter as the monochromator providing incident neutron beam of mean wavelength (λ) 5.2 Å and wavelength-spread (Δλ/λ) about 15%. The scattered neutrons from the sample are detected using a 1D, 100 cm long, He^3 position sensitive detector. The samples were prepared by dissolving a known amount of surfactant in presence or absence of salt in D_2O and were placed in a quartz sample holder of thickness 2mm. Use of D_2O as solvent instead of H_2O provides better contrast for hydrogenous systems in SANS experiments. Measurements were performed at varying temperatures; below, at and above CP ranging from 25-70°C. Throughout the data analysis, corrections were made for instrumental smearing. The measured SANS data had been normalized to absolute scale using standard procedure [100,143b]

In SANS measurements, the $d\Sigma/d\Omega$ is measured as a function of scattering vector Q [$=4\pi\sin\theta/\lambda$, covering a range of 0.018–0.32 $Å^{-1}$), where 2θ is the scattering angle and λ is wavelength of incident neutrons] For monodisperse micelle solution it can be expressed as [144]

$$d\Sigma/d\Omega = n_m V_m^2(\rho m - \rho s)^2\{\langle F^2(Q)\rangle + \langle F(Q)^2[S(Q) - 1]\} + B \qquad (1)$$

Where n_m and V_m (=Nagg.*v)* are the number density and volume, of micelles respectively, Nagg is the aggregation number and *v* is the volume of surfactant monomer. ρ is scattering length density of micelle (ρm), calculated considering the central core of the micelle consisting of hydrophobic alkyl chains and of solvent (ρs). F(Q) is the *single particle form factor*. S(Q) is the interparticle *structure factor.* B denotes incoherent scattering contributed arising mainly from the hydrogen in the micelle. In the case when the particles asymmetry is not too large and they can be treated by the orientational averaging ($\left|\left\langle F(Q)\right\rangle\right|^2 = \left\langle\left|F(Q)\right|^2\right\rangle$), equation (1) is simplified to

$$\frac{d\Sigma}{d\Omega}(Q) = nV^2\left(\rho_p - \rho_s\right)^2 P(Q)S(Q) + B \qquad (2)$$

where $P(Q)$ is intraparticle structure factor decided by shape and size of the particle and is the square of single particle form factor $F(Q)$

$$P(Q) = \left\langle\left|F(Q)\right|^2\right\rangle$$

$P(Q)$ depends upon *shape and size* of particles and was calculated for the prolate ellipsoidal shape (using ellipsoidal core shell model) of the micelles in case of all anionic surfactants and for oblate ellipsoidal in case of non-ionic surfactants. The hydrophilic headgroup comprising of the sulphate and sulphonate group make up the outer shell.

The prolate ellipsoidal shape ($a \neq b = c$) of the micelles is widely used in the analysis of small-angle scattering data because it also represents the other different possible shapes of the micelles such as spherical ($a = b$) and rod-like ($a >> b$). The expression for $P(Q)$ of prolate ellipsoidal particle with semi-major axis a and semi-minor axis $b = c$ is given by

$$P(Q) = \int_0^1 F\left(Q,\mu\right)^2 d\mu$$

Where, $F(Q,\, x) = \frac{3(sin\, x - x\cos x)}{x^3}$ in this $x = Q\left[a^2\mu^2 + b^2\left(1-\mu^2\right)\right]^{\frac{1}{2}}$ and μ is the cosine of the angle between the direction of major axis, *a* and wave vector transfer, *Q*. For oblate ellipsoid (*b*=*c*>*a*), *a* and *b* can be interchanged in above equations.

S(*Q*) depends on the correlation of the particles and hence interaction between the particles. In general, *S*(*Q*) shows several maxima and minima of decreasing amplitude. The first peak in *S*(*Q*) occurs at $Q_{\max} \sim 2\pi/d$, where *d* is the average distance between the particles. For an isotropic system, *S*(*Q*) can be written as

$$S(Q) = 1 + 4\pi n \int [g(r) - 1] \frac{\sin Qr}{Qr} r^2 dr$$

For non-interacting micelles *S*(*Q*) ~1, means in diluted solutions it totally depends on *F*(*Q*). Where, *g*(*r*) is the radial distribution function. It is the probability of finding another particle at a distance *r* from a reference particle centered at the origin.

For charged micelles, S(Q) is calculated by Hayter and Penfold analysis under Mean Spherical Approximation, whereas for non-ionic micelles it is usually calculated for hard sphere potential. If the system is experiencing short range attraction, *S*(*Q*) may be obtained for Baxter's sticky hard sphere potential. The expressions for these *S*(*Q*) may be seen elsewhere [145].

The detailed analysis and relevant expressions are similar as reported earlier [98, 100]. The micellar dimensions (semimajor axis (a), semiminor axis (b=c); the value decided by the length of the surfactant molecule)), *Nagg*($=4\pi ab^2/3v$, where *v* is the volume of the surfactant tail, calculated from Tanford's formula) and the fractional charge α(=Z/Nagg, Z=micellar charge) of micelles are the fitted parameters determined from the analysis. The polydispersity of the micelles is expected due to growth of micelles in many cases.

2.3.8. POM measurements

To observe the micellar morphology, POM experiments were performed using a leica DFC 295 optical microscope fitted with Leica (Germany) Lens (4X, 10X, 20X and 50X magnification). A small drop of the sample solution (fixed concentration) was sealed between a glass slide and a cover slip and images were recorded at different temperatures, using inbuilt leica camera at 20X. The temperature was maintained, using

Linkam heating stage (LST 420) controlled by Leica application suite computer software (LAS-V41).

2.3.9. UV-Visible Spectroscopy

UV-visible spectrophotometer (Lamda-35 Perkin-Elmer) equipped with a quartz cell of path length 1cm was used for absorption measurements of different samples against suitable blanks in the wavelength range of 200-600 nm. This spectrophotometer has a wavelength accuracy of ±0.2 nm and a bandwidth of 2 nm. Data were acquired for the pre-concentrated curcumin samples and the solubilised concentration of curcumin in two phases was found.

2.3.10. AAS measurements

AAS spectrometer (A Analyst 200 Perkin-Elmer) having Ion selective Hollow Cathode lamp as a source was used for the AAS measurements. The instrument uses acetylene and air as fuel. 25 ml of analyte solution was injected and the absorbance readings were noted.