Surface tension and fluorescence studies of polysaccharide-surfactant solutions: Agar-CTAB

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Received 14 June 2005; accepted 12 October 2005

The interaction of CTAB (cetyl trimethyl ammonium bromide) with the agar has been investigated by surface tension and fluorescence measurements. The plots of the surface tension versus concentration of the surfactants for different polymer concentrations show interesting features of the polymer surfactant binding. The binding capacities of CTAB are found to be dependent on the polysaccharide concentrations. These results are interpreted in terms of a polymer surfactant complex or micelle formed by binding between the surfactant ions and the polymer. The surface tension studies show that the 218 mmol CTAB could be bound to the galactose-anhydrogalactose unit of agar. Fluorescence measurements using pyrene as a photophysical probe have been carried out for agar and CTAB mixtures. The interactions between the neutral agar and cationic CTAB leads to formation of pre-micelles at surfactant concentration lower than CMC (0.9585 mM/L) of CTAB. The aggregation process is caused by the electrostatic attraction. It has been proved, using pyrene fluorescence measurements methods, that CTAB forms induced micelles at concentrations smaller than those needed to form micelles in aqueous solution.

IPC Code: Int Cl.7 C11D; G01N13/00; G01N21/64

The interaction between polymer and surfactants has been a subject of great interest. Initially, the studies related to interaction of proteins associated with natural lipids. Later, it dealt with the interaction involving synthetic surfactants. Adding surfactants to polymer solutions with the formation of polymer surfactant complex can substantially change the physical properties of the starting polymer. This effect can be harnessed to many industrial applications. When a polymer carries hydrophobic groups, the effects are dramatically enhanced. Such polymers are often referred to as associating polymers because of their tendency to self-assemble and in extreme cases they form gels by association of hydrophobes. This interaction is utilized in pharmaceutical applications as well as in colloid science. It is believed that the principal driving force for the binding between an ionic surfactant and a non-ionic polymer is hydrophobic interactions and the process is quite similar to that of self-association of surfactant into micelle. The association of surfactant and polymer chain can be imagined like a necklace, where the surfactants are just like pearls joined to a hydrophobic centre. Actually, the interaction between an ionic surfactant and a non-ionic polymer depends upon the nature of the polymer and surfactants. It has also been suggested that electrostatic interactions between the polymer and polar surfactant groups are important. In all cases, the adsorption of complex polymer/surfactant aggregate structures occurs at the air/solution interface. Therefore, changes in the nature of binding of the surfactant to the polymer, even when it occurs in a single-phase region, are generally evaluated by the surface tension studies.

Agar is chosen as the polymer here. It is a phycocollolid extracted from a red seaweed Gelididiella acerosa (Rhodophyta). Agar is industrially important for its excellent thickening and gelling abilities. The backbone of this phycocollloid is made up of alternating 3-O-linked D-galactopyranose and 4-O-linked 3,6-anhydro-L-galactopyranose. The repeating units in agar are shown in Fig. 1.

Surface tension measurements have earlier been used to study the interaction between surfactants and polymers. Jones reported the interaction between sodium dodecyl sulphate (SDS) and polyethylene oxide (PEO). Svensson et al. have reported the interaction between SDS and amylose and amylpectin from potato. From the surface tension measurements, the saturation concentration (C2), was
determined and total amount of surfactant bound to the polymer was calculated. Miguel and coworkers have reported fluorescence study of the interaction between sodium alginate and surfactants.

Surface tension and fluorescence measurements have been utilized here to characterize the interaction/binding between the nonionic agar and the cationic surfactant CTAB (cetyl trimethyl ammonium bromide). We have already reported the thermal and rheological studies of agar in presence of CTAB and fatty acids in the gel as well as solid state. Therefore, it is appropriate to study the interaction of agar in presence CTAB in solid state by surface tension and fluorescence measurements. The data generated in this study may be useful to explore newer applications of agar, e.g. in pharmaceutical and oil industries.

Agar, that was extracted in our laboratory from Gelidiella acerosa, and characterized by IR, NMR studies, was used during the studies. This decision was based on the surface tension of double distilled water was measured to be 69.2 mN/m at 27.1°C, while that of 0.01% (w/v) Oxoid agar (Oxoid Agar No.1 (Oxoid, UK; Lot/Ch-B: 810501-2) and Difco agar (Difco Laboratories, 0140-01, Detroit Michigan, USA) was 54.8 mN/m and 51.2 mN/m, respectively. The agar that was used in this investigation (AS042901) had surface tension value (in 0.01%, w/v) 65.1 mN/m, indicating thereby the latter was associated with minimum surface-active contamination.

Materials and Methods
The agar that was used in this investigation was extracted from a seaweed Gelidiella acerosa (Forsskal) J. Feldmann & G. Hamel (Rhodophyta, Gelidiales) by following a method by Rolda et al. with modification. CTAB (Analytical Grade, S.D fine Chemicals, India), was used as received. The weight average molecular weight of the agar determined by intrinsic viscosity measurements using an Ostwald viscometer and found to be $2.74 \times 10^5$ D.

For surface tension measurements, the agar sols were prepared by heating agar powder in water with constant stirring. Then, the surfactant solutions were added slowly to the agar sol under agitation. For all the preparations, double distilled water was used. The experiments were performed at 27.1°C for selected agar concentrations 0.01% to 0.16% (w/v) having 0.00392 μM to 15.31 mM of the surfactants.

Surface tension measurements
The surface tension in the air-water at 27.1°C (±0.5°C) (Kraft point of CTAB), was measured by the static Wilhelmy plate using dynamic contact angle tensiometer (DCAT 21, Dataphysics, Germany). To ensure removal of surface-active contaminants, all glassware in contact with the sample were cleaned in chromic acid and rinsed with double distilled water. The platinum plate was washed in double distilled water, heated on a Bunsen flame and left to cool at room temperature. The solutions were prepared with the double distilled water. Experiments were carried out in triplicate.

Fluorescence measurements
Steady-state fluorescence measurements were performed on air-equilibrated solutions using a Perkin-Elmer Luminescence spectrometer LS-50B. Pyrene was excited at 334 nm and the detection wavelengths were $I_1 = I_{373}$, $I_2 = 384$ nm, $I_3 = 475$ nm. Excitation and emission slits were 3.0/3.0 nm, respectively.

The solutions containing the probe were prepared by transferring a sufficient amount of a methanol stock solution of pyrene to a flask under a stream of nitrogen. After this, agar solution was added and the total volume completed to 3.0 mL with water. After
wards, the surfactant (CTAB) was added in small amounts. The final pyrene concentration used for the measurements was \( \equiv 1.60 \times 10^{-6} \) mol/L, corresponding to an absorbance 0.025 at 334 nm. The concentration and the optical density are low enough not to interfere with the system or affect it otherwise.

**Results and Discussion**

Surface tension is a measure of the amount of surfactant in solution. With increasing concentration of the surfactant (CTAB), the surface tension will decrease till the critical micelle concentration (CMC) is reached. At CMC, the surfactant starts to aggregate, generating a break in the curve. Beyond CMC, the surface tension value is not changed with the increase in concentration. The CMC was calculated to be 0.9585 mM at 27.1°C (Fig. 2), which tallies well with the value reported in the literature.

Surface tension as a function of total concentration of CTAB at three different agar concentrations 0.01%, 0.08% and 0.16%, has been studied. As the total CTAB concentration increased in the polysaccharide sols, the surface tension did not decrease as much as it did in water. The surface tension at the saturation concentration was the same as of the CMC in water.

| Table 1 — Saturation concentration and binding capacity of agar-CTAB at 27.1°C |
|-----------------|------------------|------------------|
| Agar concentration (%) | Saturation concentration, mM | Binding capacity, mmol CTAB/galactose-anhydrogalactose |
| 0.01 | 1.0232 | 209 |
| 0.08 | 1.5848 | 252 |
| 0.16 | 2.0417 | 218 |

In order to reach the saturation concentration, more amount of CTAB was required in case of higher polysaccharide concentration as reported by Lundqvist et al. in the case of amylose and amylpectin. The saturation concentration was calculated from the plots as the total concentration of CTAB at the breakpoint in the surface tension plots. The data is presented in Table 1. The saturation concentration values were plotted against the polysaccharide concentrations that are shown in Fig. 3. It shows that with the polymer concentration, the saturation concentration increases linearly. From the slope, it is possible to calculate the amount of CTAB molecules bound to agar. The maximum binding in this case is 218 mmol CTAB per mole of 1,3-β-D-galactose-α-L-3,6 anhydrogalactose units of the agar polymer at 0.16% concentration.

When CTAB was added to aqueous sol of agar, the interaction is detected up to a specific concentration denoted as saturation concentration of CTAB. The value of saturation concentration has been found to fall in the range 1.0232 to 2.0417 mM of CTAB, which appears to be sensitive to the agar concentration.

**Fluorescence study**

The dependence of the \( I_0/I_s \) ratio of the pyrene emission as a function of agar concentration at neutral pH is shown in Fig. 4. It can be seen that this ratio, which measures the hydrophobicity of the medium,
Dependence of the $I/I_3$ of pyrene in agar solution (16 mg/L) containing CTAB [$\lambda_{ex} = 334$ nm; detection wavelengths $I_1 = I_3 = 373, I_2 = 384, I_4 = 475$ nm].

decreases with increase of agar concentration. At 0.01 g/L its value reaches approx 0.011. This value is much smaller than that found for pyrene in aqueous solution (1.54-1.69). This behaviour suggests that over the concentration range studied, the pyrene molecules are partitioned between the hydrophobic microdomains of the polymer chain and the aqueous solution. When the concentration of agar is increased, a larger number of polymeric hydrophobic microdomains will be present, to which the highly hydrophobic pyrene molecules will migrate. At even higher concentrations ($\geq 0.016$ g/L) probably, most of the probe molecules will accommodate in the hydrophobic microdomains. The high viscosity of more concentrated agar solutions precludes the experimental determination of this parameter at concentrations above 0.016 g/L.

The behaviour of aqueous solutions of agar when the surfactant CTAB is added can be deduced from the results shown in Fig. 5. The $I/I_3$ ratio shows an initial rapid decay from values near to 0.035 mM to a value around 0.0243, attaining a first plateau at surfactant concentration of ca. 0.87 mM. This slightly more hydrophobic domain is ascribed to the formation of pre-micelles induced by the interactions (probably electrostatic) between CTAB and the charged moieties in agar (mainly sulphate moiety). This decrease is due to the solubilization of the probe in the micelle-like aggregates, which gradually starts to form along the agar chain. The onset of the process is defined as the critical aggregation concentration (CAC)\(^\text{26, 27}\). A second decrease of $I/I_3$ observed between 0.5 and 1 mM is due to the formation of free micelles in the bulk of the solution, as probably the capacity of placing induced micelles on the macromolecular chain is saturated at these concentrations. The concentrations, where this process occurs, characterize the CMC and are quite similar to the value found for CTAB in pure aqueous solution (0.9580 mM). The $I/I_3$ ratio at higher concentrations remains constant at 1.37, typical of pyrene in CTAB micelles.

**Conclusions**

CTAB binds with agar and the extent of binding can be determined by surface tension studies. It has been estimated that at CMC level concentration of CTAB in agar sol (at 27.1°C), 218 mmol CTAB is bound to each repeating unit (1.4-linked galactopyranose 3,6-anhydrogalactopyranose moiety) of agar polymer.

The behaviour of agar in the presence of CTAB resembles that observed for synthetic polyelectrolytes like PSS and its copolymers with hydrophobic monomers\(^\text{26, 29}\). In the presence CTAB, there is an initial electrostatic interaction which leads to the formation of pre-micelles attached to the agar backbone. This interaction is apparent at surfactant concentrations about an order of magnitude below the CMC of CTAB in pure aqueous solution. Only a limited number of such aggregates can be bound to the agar chain. Eventually, above a certain surfactant concentration, a saturation point is reached when all the probe molecules will be solubilized in these microdomains.

It has been proved, using pyrene fluorescence measurements that CTAB forms induced micelles at concentrations smaller than those needed to form micelles in aqueous solution.
Acknowledgement
The authors gratefully acknowledge Dr P K Ghosh, Director, CSMCRI for his kind help and interest in this work. Sincere thanks are due to Mr Ramavtar Meena for his help in the interpretation of some of the fluorescence data.

References