Summary
Surfactants are gaining relevance as major aquatic pollutants as they are now the most abundant group of anthropogenic pollutants. The present study investigated the biochemical impacts of three surfactants viz. anionic sodium dodecyl sulfate (SDS), cationic cetyl trimethyl ammonium bromide (CTAB) and non ionic Triton X-100 on a freshwater adapted euryhaline teleost *Oreochromis mossambicus* and the marine cyanobacterium *Synechocystis salina* Wislouch.

8.1 Studies on *Oreochromis mossambicus*

Exposure to surfactants was found to be highly stressful to the animals. Studies on lysosomal and erythrocyte membranes revealed that interaction of surfactant with the cellular membranes depends on the structure of the surfactant, critical micellar concentration and hydrophile-lipophile balance. It was observed that lysosomal stability index (LSI), decreased significantly on surfactant exposure both *in vitro* and *in vivo*. The control animals exhibited an LSI of 1.965. Triton exposure caused maximum release of acid phosphatase from lysosomes as reflected in an LSI of 0.23. Cationic surfactant CTAB was next to Triton in labilising effects with an LSI of 0.591. SDS, the anionic surfactant, caused minimum labilisation as evident from an LSI of 1.23. *In vitro* studies on the release of ACP at definite time intervals from the lysosomes also recorded significant increases in the release of the enzyme from surfactant-incubated lysosomes than from control whereas in *in vivo* studies only CTAB dosed animals exhibited significant increase in ACP compared to the control.
Studies on erythrocytes *in vitro* also confirmed the membrane damaging effects of the surfactants. Triton induced maximum hemolysis of 61.59%. CTAB caused 24.99% and SDS induced 14.99% hemolysis.

Studies on peroxidation potential of surfactants revealed that severe oxidative stress was experienced by fish exposed to these compounds. The antioxidant enzymes like catalase, superoxide dismutase and glutathione reductase were significantly increased particularly in hepatic tissues of all the surfactant-treated groups. The levels of reduced glutathione significantly increased in SDS-treated animals whereas those exposed to Triton had comparatively less amounts of this non-enzymatic anti oxidant. The levels of conjugated dienes and malondialdehyde were significantly increased in hepatic tissues of Triton and CTAB dosed groups.

Thus interaction of surfactants with the biological membranes has severe consequences as obtained from studies on lysosomes, erythrocytes and peroxidation studies. The lysosomal and erythrocyte studies revealed that the non ionic surfactant, Triton, had maximum membrane damaging potential. Here the long unbranched, non ionic and hydrophobic structure of the surfactant together with its low critical micellar concentration (CMC) is responsible for the maximum labilising action. CTAB, the cationic one, has the lowest CMC but is branched and ionic when compared to Triton. Hence its interaction with the membrane is limited due to the steric factors and the positive charge, which limits interaction with only the negative charges. SDS, the anionic surfactant, is a short chain alkyl sulfate and has the highest CMC compared to Triton and CTAB. Moreover its interaction is limited to cationic sites on the membranes. Thus it causes least damage compared to the other two surfactants.
Thus surfactant interaction with the cellular membranes depends on structure of the surfactant, charge, critical micellar concentration and hydrophile-lipophile balance. Results from membrane stability studies on lysosomes and erythrocytes *in vitro* indicate that the order of toxicity is non ionic > cationic > anionic for membrane labilising effects. But on examining the results of lipid peroxidation studies it is found that cationic surfactant imparted the maximum oxidative stress. Here it must be taken into account that *in vivo* metabolism of the surfactant also plays a key role in peroxidation potential. Cationic surfactant is not at all metabolised by fish and hence is the most toxic. Triton is mainly excreted as glutathione conjugates. This depletes glutathione levels which makes the animals exposed to Triton more prone to peroxidation. SDS, the anionic surfactant, is largely metabolised in hepatic tissues by β and ω oxidation and thus causes decreased peroxidation. This is reflected in very low malondialdehyde levels comparable to controls in SDS-treated fish. Thus it is evident that surfactant metabolism is a major determinant of the extent of toxicity.

Influence of surfactants on the branchial membrane-bound enzymes like Na\(^+\)-K\(^+\) ATPase and Mg\(^{2+}\) ATPase revealed significant differences in the susceptibility of these enzymes to the surfactants. Na\(^+\)-K\(^+\) ATPase was insignificantly activated by the anionic SDS whereas Triton and CTAB caused insignificant inhibitions. Mg\(^{2+}\) ATPase was significantly inhibited by all the three surfactants in a similar manner. It is thought that the surfactants interfered with the ATPase activities by affecting the membrane integrity. It is evident that membrane lipids play a critical role in the activity of membrane-bound enzymes. The ability of surfactants to cause peroxidation can result in lipid damage and subsequent inactivation of these enzymes.
Metabolic activities of the animals subjected to surfactants exhibited significant changes when compared to the control group. A typical stress-adaptation pattern was reflected in all the key biochemical processes. Hepatic enzymes like alanine transaminase and aspartate transaminase were significantly increased on surfactant exposure. Alkaline phosphatase also showed significant increase compared to control. Reserve energy stores viz. glycogen, lipid and protein were significantly mobilised in surfactant exposed groups. This clearly points out the fact that surfactant exposure is stressful. Stress calls for an increased ATP demand which is evident from reserve food mobilisation. Also transaminases play an important role in stress because they can convert amino acids to keto acids which can be chanelled into Krebs' citric acid cycle.

Thus it may be concluded that surfactants even at sub lethal concentrations are stressful.

8.1.1 Conclusion

The action of surfactants on the cell membranes largely depends on their structure, critical micellar concentration, charge and hydrophile-lipophile balance. In addition ability for oxidative damage via peroxidation also is a critical factor affecting membrane interaction. The peroxidation potential in turn is largely dependent on surfactant metabolism. On the other hand it may be understood that
inhibition of branchial enzymes and readjustments of metabolic machinery to defend the stressful situation is largely controlled by the piscine stress hormones.

Thus the cationic surfactant CTAB is hydrophobic with a low critical micellar concentration and is not at all metabolised and thus causes maximum stress to the organism. Triton, the non ionic surfactant, is the second toxic as damage via oxidative stress is aggravated by excretion of this compound as glutathione conjugates and this in turn depletes glutathione. The low critical micellar concentration of Triton and its hydrophobicity add to its toxicity. SDS, the anionic surfactant, is more hydrophilic, has higher critical micellar concentration and is metabolised by β and ω oxidation which makes it less toxic compared to the cationic and the non ionic surfactants.

8.2 Studies on *Synechocystis salina* Wislouch

Exposure to surfactants was found to be stressful to the cyanobacterium though the responses were not strictly concentration-dependent. Studies on growth inhibition revealed that the cationic surfactant was the most inhibitory where a concentration-dependent decrease in growth was observed. In case of both the anionic and non ionic surfactants the growth inhibition was insignificant. The chlorophyll content was found to decrease significantly especially at higher concentrations of all the surfactant-treated cultures. The maximum decrease in chlorophyll content was noted at higher concentrations tested viz. 0.8 ppm and 1 ppm. Here too the cationic surfactant was found to cause maximum decrease in chlorophyll content at higher concentrations. Studies on protein estimation revealed that there was significant decrease in the protein content of all the cultures exposed to the surfactants. The decrease in protein content was found to be dose-dependent and was significant at higher concentrations of all the
surfactants tested. The carbohydrate content showed fluctuating trend irrespective of the concentration in surfactant-treated cultures. Cultures exposed to CTAB showed a significant increase in carbohydrate. Lipid content of the cultures also showed irregular changes which were independent of the dose of the surfactant. SDS and CTAB treated cultures showed an overall decrease in lipid content whereas Triton treatment resulted in higher lipid levels at higher concentrations.

8.2.1 Conclusion

It may be deduced that among the surfactants CTAB inhibited the growth of the cyanobacterium in a significant manner. The chlorophyll content was found to be decreased only at higher surfactant concentrations which could be due to the fact that solubilisation of chlorophyll-lipid-protein complexes required higher surfactant concentration. The protein content was decreased significantly in surfactant treated groups which implied membrane damage in the protein synthesizing machinery. The carbohydrate content showed wide fluctuations in surfactant- treated cultures. In CTAB treated cultures, there was an increase in carbohydrate at all concentrations tested. The increase in carbohydrate might be an adaptive mechanism to meet the stress imposed by the surfactant. Lipid content decreased in SDS and CTAB dosed cultures whereas Triton treated ones showed a significant increase. The increase in Triton dosed cultures suggest that probably the surfactant is used as a carbon source by the cyanobacterium and utilized for lipid synthesis.

Thus studies on this cyanobacterium reveal that biochemical processes like protein and chlorophyll synthesis are more or less affected in a dose dependent manner i.e., at higher concentrations there is maximum inhibition. Whereas carbohydrate and lipid content showed fluctuations irrespective of the
concentrations. Regarding the relative toxicity, it may be concluded that the cationic surfactant is the most damaging. This could be due to selective solubilisation and increased penetration of the hydrophobic moiety in the lipopolysaccharide cell wall of this gram-negative species.

Thus the major findings are

♦ Surfactants at sub lethal concentrations are stressful.

♦ Order of toxicity for *O. mossambicus* is Cationic (CTAB) > non ionic (Triton) > anionic (SDS), and for *S. salina* cationic is the most toxic.

♦ Main target of surfactant action is the cellular membranes. Damage to membranes is largely caused by peroxidation of lipids thus inducing oxidative stress.

♦ Stress-oriented metabolic readjustments are made in the hepatic enzyme profile and other hepatic biochemical parameters.

### 8.3 Future Perspectives in Research

♦ Oxidative stress potential of cationic and non ionic surfactants is clearly established from the present study.

♦ Oxidative stress can lead to carcinogenesis and mutagenesis.

♦ A study on carcinogenic and mutagenic effects of surfactants is relevant in this regard.

♦ And piscine cell lines can then substitute mammalian cell lines in *in vitro* studies on carcinogenicity.