SECTION 1

Studies on

Oreochromis mossambicus (Peters)

CHAPTER

3

Effects of Surfactants on Biological Membranes

3.1 Introduction

Biological membranes are complex structures comprising of numerous lipids, proteins, ions etc. The interaction of detergents with the cell membranes has been studied in great details with reference to the isolation, purification and solubilisation of various membrane proteins and enzymes. There are many factors that affect the interaction between membrane components and the surfactants. These include surfactant concentration, asymmetrical distribution of lipids, presence of carbohydrate moieties on the cell membrane and electrostatic and hydrophobic interactions between the lipids and proteins (Lichtenberg, 1983; Higgins, 1987).

Surfactant concentration plays an important role in the damage caused to the cell membrane. At low concentrations there might only be subtle changes in the membrane permeability whereas at higher concentrations there might be drastic effects like lysis or fusion (Jones and Chapman, 1995).

In the present study, the effects of three surfactants-sodium dodecyl sulphate (SDS), Triton X-100 and cetyl trimethyl ammonium bromide (CTAB) at a sub lethal concentration of 1 ppm were studied on the hepatic lysosomal membranes and the erythrocyte membranes (*in vitro* and *in vivo*).

3.2 Studies on Hepatic Lysosomal Membrane

3.2.1 Introduction

Lysosomal damage is well-established as a bio-marker of stress in a wide range of vertebrates and invertebrates (Bayne, 1976; Moore, 1990; Tabata *et al.*, 1990). The damage to the lysosomal membrane may be conceptualised as an increase/activation or decrease/inhibition of the lysosomal hydrolases or labilising/stabilising effects due to changes in membrane permeability effected by the contaminants (Hawkins, 1980). Membrane labilisation is unlikely to occur other than as a stress phenomenon, whereas elevation of activity can occur as a consequence of an increased metabolic demand, for example during gametogenesis, rather than as a stress response (Lowe and Fosstovu, 2000).

Earlier works on surfactant toxicity in fishes have revealed that liver is an important site of accumulation or metabolism of the surfactant (Kimerle, 1981; Kikuchi *et al.*, 1980). Hence liver lysosomes serve as perfect biochemical indices in assessing the impacts of surfactants on the cell membrane. Lysosomes are single membrane-bound organelles that enclose a battery of hydrolytic enzymes like acid phosphatase, beta glucuronidase, cathepsin, aryl sulfatase etc. They have an acid environment which is maintained by a membrane-bound Mg^{2+} ATPase dependent proton pump (Ohkuma *et al.*, 1982). These organelles are best with numerous functions like sequestration of foreign compounds, immune response, protein and organelle turnover, embryonic development, apoptosis etc. If the lysosomal membrane is damaged or destabilised then these marker enzymes are released. Hence the assay of these enzymes can be used as an index of lysosomal membrane damage. The release of lysosomal enzymes is related to necrosis or death of the cell or pathological or stressful conditions (Hawkins, 1980).

3.2.2 Materials and Methods

The fish species were collected from Rice Research Institute, Vyttila, Kochi, Kerala. They were fed on a commercial diet *ad libitum* and were acclimated in aquarium tanks for a month before the experiment. The fish of the size range 15 ± 3 gm and 8.5 ± 0.5 cm were used for the experiments. Six fish each were exposed to a sub lethal surfactant concentration of 1 ppm (1/10 of 96 h LC_{50}) of each of the surfactants (anionic sodium dodecyl sulphate, cationic cetyl tri methyl ammonium bromide and non ionic Triton X-100) in aerated fibre glass tanks. The surfactants were dissolved in tap water and diluted to obtain the required concentration of 1 ppm (APHA). A control group of six fishes was also maintained without any surfactant.

The tap water used had dissolved oxygen content of 7-8 ppm, hardness-below detectable limits, pH 7, temperature 25 ± 3°C and salinity 0 ppt. During the experimental period of 30 days the animals were fed on the same diet so as to avoid the effects of starvation on normal physiological processes and anti oxidant status. The water in the experimental tanks was replaced every 48 h with water containing fresh surfactant so as to avoid any possible degradation of the surfactant. Fishes were deprived of food 24 h before assay. They were sacrificed by pithing (by damaging the brain and severing the spinal cord between the head and the trunk region using a sharp needle) and the liver tissue was removed, washed in ice-cold isotonic sucrose (0.33 M), blotted dry and weighed.

(a) Activity of Acid Phosphatase (ACP) (E.C.3.1.3.2) in Various Subcellular Fractions of Liver Tissue

Liver was homogenised in isotonic sucrose at 0° C (10%). The homogenate was centrifuged at 600 g for 10 min in a high speed refrigerated

centrifuge. The sediment of nuclei, unbroken cells and plasma membrane (nuclear fraction) was separated. The supernatant was again centrifuged at 15000 g for 30 min. The 15000 g sediment (lysosomal fraction) and the nuclear fraction were resuspended in citrate buffer containing 0.2% Brij-35. The 15000 g supernatant (soluble fraction) was diluted with an equal volume of double strength buffer. The activity of acid phosphatase was determined in all these fractions (Plummer, 1987).

(b) Rate of Release of Acid Phosphatase from Lysosomal Fraction or Lysosomal Enzyme Release Assay (LERA)

(i) In vitro Studies

Liver from control fishes was homogenised in isotonic sucrose and the lysosomal fraction was obtained as above. The lysosomal pellet was washed, centrifuged at 15000 g for 10 min and again resuspended in sucrose. A definite volume of this suspension was incubated at room temperature and aliquots were withdrawn at various time intervals of 0, 15 and 30 min. The retrieved fractions were stored at 0°C (Control). In order to study the effect of surfactants on the lysosomal membrane, a definite volume of the lysosomal suspension (Test) was incubated in presence of each of the surfactants such that the final surfactant concentration is 1 ppm. Here also aliquots were withdrawn at time intervals of 0, 15, 30 and 45 min. Both the control and test aliquots were centrifuged at 15000 g for 30 min to separate the unbroken lysosomes and the acid phosphatase activity released into the supernatant was determined. Total lysosomal activity was estimated after adding citrate buffer containing Brij-35. For evaluation of the effect of surfactants on enzyme release, the possible effect of surfactant (inhibition or activation) was also taken into account.

(ii) In vivo Studies

Here the hepatic tissue from control animals and from those exposed to surfactant for 30 days were homogenised separately and centrifuged to obtain the lysosomal fraction as described in 3.2.2(a). The rate of release of acid phosphatase was noted at definite time intervals by withdrawing definite aliquots of the suspension as described in *in vitro* methods in 3.2.2(b)(i).

(c) Determination of Enzyme Activity (Anon, 1963)

0.5 ml of para nitro phenyl phosphate (400 mg%) was mixed with an equal volume of 0.1 M citrate buffer of pH 4.8. The enzyme was added and incubated for 30 min at room temperature. At the end of 30 min, reaction was stopped by the addition of 4 ml of 0.1 N NaOH. The absorbance of the solution was measured at 410 nm in a UV-visible spectrophotometer (Hitachi). The amount of p-nitrophenol liberated by the enzyme per hour per mg protein gives the specific activity. Protein was estimated by the method of Lowry *et al.* (1951).

(d) Statistical analysis

Statistical analysis of results was done by ANOVA followed by Least Significant Difference (LSD) (Zar, 1996).

3.2.3 Results

(a) Subcellular activity of acid phosphatase

The subcellular activity of acid phosphatase is given in Table 3.1 and Figure 3.1.

Table 3.1. Subcellular activity of acid phosphatase exposed to 1 ppm surfactants *in vivo*

Groups	Nuclear activity	Lysosomal activity	Soluble activity	Ratio Lysosomal/soluble
Control	5.789 ± 0.92	11.99 ± 0.91	6.1 ± 0.85	1.965
SDS dosed	10.55 ± 0.52	8.89 ± 0.83	7.18 ± 0.9	1.23
Triton dosed	9.09 ± 0.47	2.48 ± 0.82	12.01 ± 0.892	0.207
CTAB dosed	8.87 ± 0.43	6.41 ± 0.88	10.65 ± 0.88	0.591

Values are the mean ± SD of six separate experiments.
Activity expressed as mg p-nitro phenol liberated/h/mg protein in each fraction.

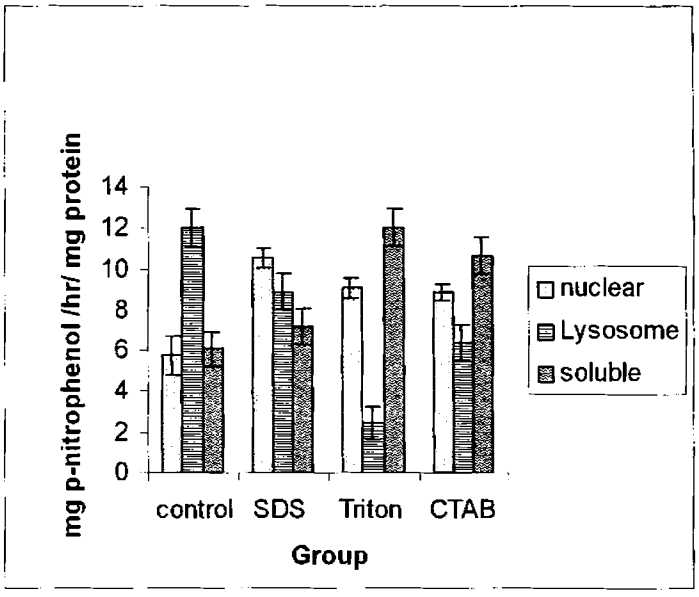

Figure 3.1. Subcellular activity of acid phosphatase in *O. mossambicus*

One way analysis of variance (ANOVA) revealed an overall significant change ($P<0.001$) in the ACP activity in the nuclear ($F = 157.82$) (Table 3.1a), soluble fractions ($F = 156$) (Table 3.1b) and lysosomal ($F = 327.06$) (Table 3.1c) fractions of the experimental animals.

Table 3.1a. ANOVA for nuclear ACP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	72.08802	3	24.02934	157.8178	4.34E-14	3.098393
Within Groups	3.0452	20	0.15226			
Total	75.13322	23				

Table 3.1b. ANOVA for soluble ACP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	145.2006	3	48.4002	156.0006	4.85E-14	3.098393
Within Groups	6.205128	20	0.310256			
Total	151.4057	23				

Table 3.1c. ANOVA for lysosomal ACP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	290.8049	3	96.93495	327.0631	3.71E-17	3.098393
Within Groups	5.9276	20	0.29638			
Total	296.7325	23				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

Subsequent comparisons between different groups were done by Least Significant Difference (LSD) analysis, the results of which are given in Table 3.1d.

Table 3.1d. Results of LSD analysis for subcellular acid phosphatase activity

Groups	P value		
	Nuclear	Lysosomal	Soluble
Control x SDS	P < 0.001	P < 0.001	P < 0.001
Control x Triton	P < 0.001	P < 0.001	P < 0.001
Control x CTAB	P < 0.001	P < 0.001	P < 0.001
SDS x Triton	P < 0.001	P < 0.001	P < 0.001
Triton x CTAB	NS	P < 0.001	P < 0.001
SDS x CTAB	P < 0.001	P < 0.001	P < 0.001

NS – Not significant

There was a significant increase (P<0.001) in nuclear and soluble ACP in all the surfactant treated groups when compared to the control. On the other hand a significant decrease was noted in lysosomal ACP of surfactant exposed groups when compared to the control.

Comparison between surfactants revealed no significant differences in the nuclear acid phosphatase activity in animals exposed to Triton X-100 and CTAB, but there were significant differences (P<0.001) between the surfactants with respect to the lysosomal and soluble enzyme activity.

The lysosomal ACP activity in surfactant dosed fishes was lower than that of control but the soluble fraction activity was highly increased. This indicates damage to the lysosomal membrane on exposure to surfactants. The ratio of ACP activity in lysosomal fraction to that in the soluble fraction or Lysosomal stability index (LSI) was the lowest for Triton X- 100 (0.207). It was followed by CTAB which had an LSI of 0.591 and the anionic SDS had an LSI of 1.23. This indicates that the nonionic surfactants are the most damaging followed by the cationic and then the anionic, the lysosomal stability index for the control group was 1.965.

(b) Lysosomal enzyme release assay (*in vitro and in vivo*)

The time dependent release of ACP from lysosomes *in vitro* and *in vivo* is given in Tables 3.2 and 3.3 and Figures 3.2 and 3.3.

Table 3.2. LERA *in vitro*

Time (min)	Control	SDS	Triton X-100	CTAB
0	1.06 ± 0.21 (8.48)	0.957 ± 0.03 (7.66)	1.77 ± 0.06 (14.16)	1.01 ± 0.05 (8.1)
15	1.135 ± 0.02 (9.1)	1.063 ± 0.01 (8.7)	1.915 ± 0.054 (15.32)	1.17 ± 0.01 (19.36)
30	1.21 ± 0.01 (9.68)	1.276 ± 0.02 (10.21)	2.27 ± 0.02 (18.16)	1.33 ± 0.02 (10.64)

Values are the mean ± SD of six separate experiments expressed as mg p-nitrophenol/h/mg protein.
In brackets is represented ACP release as % of total activity.

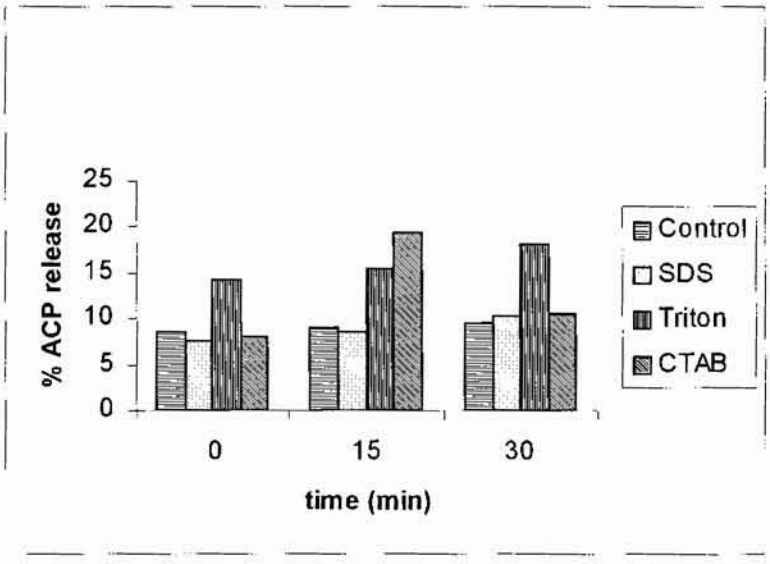

Figure 3.2. Percentage release of ACP from lysosomes *in vitro*

Two way ANOVA (comparing ACP release with time) on *in vitro* studies revealed an overall significant change ($P<0.05$) in the experimental groups (Table 3.2a).

Table 3.2a. Two way ANOVA for LERA *in vitro*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Time	0.006	2	0.003	19.82	0.002	5.15
Between Groups	0.022	3	0.007	51.76	0.0001	4.76
Error	0.001	6	0.0001			
Total	0.0284	11				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

Subsequent LSD analysis (Table 3.2b) reflected significant differences ($P<0.05$) in the release of ACP at time intervals of 0-15, 0-30 and 15-30 min.

Table 3.2b. Results of LSD analysis

ACP release with time	P value
0-15	$P < 0.05$
0-30	$P < 0.05$
15-30	$P < 0.05$

Comparison of surfactant treated groups with control and also among themselves is given in Table 3.2c.

Table 3.2c. Results of LSD analysis for *in vitro* studies on LERA

Groups	P value
Control × SDS	P < 0.001
Control × Triton	P < 0.001
Control × CTAB	P < 0.001
SDS × Triton	P < 0.001
Triton × CTAB	NS
SDS × CTAB	P < 0.001

NS – Not significant

The rate of release of ACP was significantly different ($P<0.05$) in all the surfactant treated groups when compared to the control. Also there were significant differences ($P<0.05$) between the surfactant with respect to ACP release.

The results of *in vivo* studies on enzyme release are given in Tables 3.3 and Figure 3.3.

Table 3.3. LERA *in vivo*

Time (min)	Control	SDS	Triton X-100	CTAB
0	0.702 ± 0.23 (5.6)	0.798 ± 0.02 (6.38)	0.922 ± 0.04 (7.38)	0.8 ± 0.095 (6.38)
15	0.936 ± 0.04 (7.5)	0.979 ± 0.11 (7.83)	1.064 ± 0.01 (8.51)	1.596 ± 0.05 (12.77)
30	1.035 ± 0.02 (8.28)	1.138 ± 0.08 (9.11)	0.993 ± 0.05 (7.94)	1.862 ± 0.04 (14.89)

Values are the mean ± SD of six separate experiments expressed as mg p-nitrophenol/h/mg protein.
In brackets is represented ACP release as % of total activity.

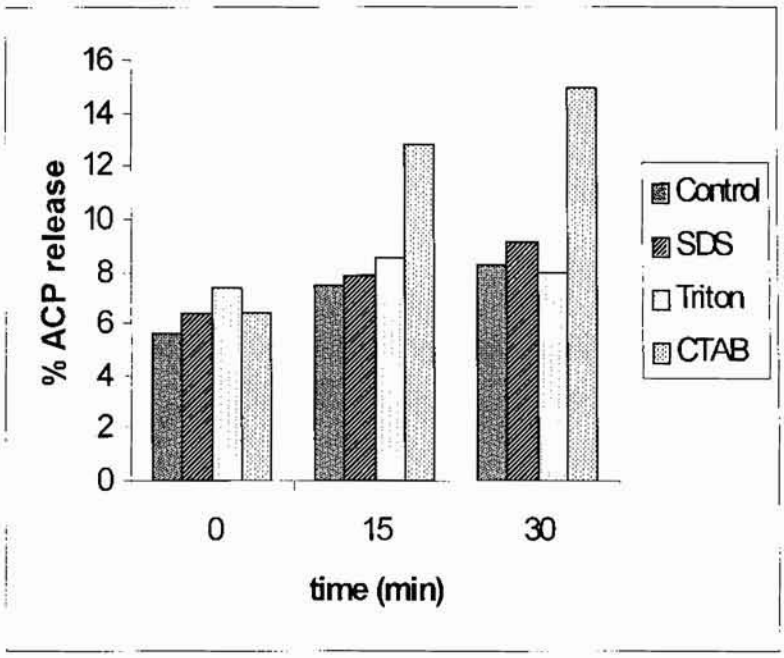

Figure 3.3. LERA *in vivo*

Two way ANOVA on *in vivo* studies showed that there was no significant difference in the enzyme release with time. But the surfactant exposure was found to induce an overall significant change (Table 3.3a).

Table 3.3a. Two way ANOVA for LERA *in vivo*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Time	0.013856	2	0.006928	3.7	0.08967	5.15
Between Groups	0.036192	3	0.012064	6.5	0.026316	4.76
Error	0.011226	6	0.001871			
Total	0.061274	11				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

Comparison by LSD analysis (Table 3.3b) revealed that only CTAB exposure caused significant release ($P<0.05$) of ACP when compared to control. Also ACP release in CTAB dosed fish were significantly different ($P<0.05$) from that in animals exposed to Triton X-100 and SDS.

Table 3.3b. Results of LSD analysis for *in vivo* studies

Groups	P value
Control × SDS	NS
Control × Triton	NS
Control × CTAB	$P < 0.05$
SDS × Triton	NS
Triton × CTAB	$P < 0.05$
SDS × CTAB	$P < 0.05$

NS – Not significant

3.2.4 Discussion

The interaction of surfactants with the lysosomal membrane involves two aspects-first there are hydrophobic as well as hydrophilic interactions with membrane lipids and proteins, and second is the interaction with the membrane lipids causing peroxidation.

The concentration of the surfactant plays a major role in the interaction with the cell membrane. The membrane is rendered leaky at a much lower concentration than the one required for complete solubilisation. Also this concentration increases with an increase in the critical micellar concentration (CMC) of the surfactant. Thus a surfactant of low CMC causes release of the cell/ cytoplasmic protein by intercalating into the membrane bilayer without much

solubilisation. Thus the decreasing order of toxicity of surfactants is TX-100 > CTAB > SDS.

Triton X-100, the non-ionic surfactant and CTAB, the cationic surfactant have comparatively much lower CMC than SDS, the anionic surfactant. This might have caused increased release of the acid phosphatase enzyme into the soluble fraction. This is evident from an LSI of 0.207 for Triton, 0.593 for CTAB and 1.23 for SDS.

Results of LERA *in vitro* revealed significant increase in ACP release with time. This demonstrates direct interaction of surfactants with the lysosomal membrane. The effects of Triton were significantly different from that of SDS and CTAB, which were similar in labilising effects. But *in vivo* studies on LERA presented a different result. Here only the cationic surfactant CTAB induced significant membrane labilisation when compared to the control. It could be inferred that the metabolism of the surfactant *in vivo* leads to a change in toxicity pattern.

It has been proved that the toxic effects of surfactants on fishes are manifested through a decrease in the surface tension and that the toxicity of the surfactant depends on the length of the alkyl chain and the chemical structure (Swedmark *et al.*, 1971). The non ionics like Triton and the cationic CTAB have long alkyl chains which results in a rapid reduction of surface tension than the anionic SDS.

In addition, damage via peroxidation of lipids in the cell membrane cannot be overruled. Though lysosomal membrane has only 25% lipids, it is still susceptible to the deleterious effects of peroxidation. The works by Desai *et al.* (1964) indicate that lysosomes treated with methyl linoleate exhibited rapid peroxidation which resulted in 50% release of the aryl sulfatase enzyme in one

hour. Cyto chemical observations on the size and conglomeration of the ACP containing particles in the cortical neurones and the purkinge cells of X-ray irradiated rats also support the sensitivity of the lysosomes to free radicals.

Thus it may be concluded that surfactant interactions with the cell membrane lipids and proteins as well as lipid peroxidation are key factors in the damage caused to the lysosomal membrane *in vitro* whereas metabolism of the surfactant is a key factor in deciding *in vivo* toxicity.

3.3 Studies on Erythrocyte Membrane

3.3.1 Introduction

Haematological profile has played a significant role in assessing the impacts of chemicals or toxicants. The commonly studied parameters include WBC count, haemoglobin content, hematocrit, packed cell volume, RBC membrane stability etc. Of these the stability of the erythrocyte/RBC membrane is a simple and precise method in evaluating the toxicities of pollutants because the release of haemoglobin can serve as a criterion for haemolytic effects in presence of pollutants.

The red blood cell is bounded by a single membrane and it has a low protein to lipid ratio whereas the ratio of cholesterol to lipid is high. Lysis of RBC membrane by surfactants has been studied by Kirkpatrick *et al.* (1974). It was observed that surfactants like Triton X-100 and sodium dodecyl sulfate exerted protective effects against osmotic shock on the membrane at concentrations of 1×10^{-5} to 6×10^{-5} M but at higher concentrations lysis was the rule (Helenius and Simons, 1975).

The present study focuses on the impacts of three surfactants –anionic sodium dodecyl sulfate, cationic cetyl tri methyl ammonium bromide and the non ionic Triton on the red cell membranes of the teleost *Oreochromis mossambicus in vitro* and *in vivo* (30 days exposure).

3.3.2 Materials and Methods

Oreochromis mossambicus of size 15 ± 3 gm and 8 ± 0.5 cm were used for the experiments.

Collection of blood: Blood was collected from the cardinal vein in plastic syringes containing citrate as the anti coagulant (Michael et al., 1994). Fresh saline solutions – isotonic (0.85%) and hypotonic (0.5%) were prepared.

Stock suspension of RBC was prepared after washing the cells thrice with isotonic saline. Then different volumes of the suspension was mixed with distilled water to hemolyse the cells and centrifuged at 1000 g for 5 min. The absorbance of the supernatant was read at 540 nm against distilled water blank. The dilution giving a suitable absorbance for 100% hemolysis was selected. Also a suitable volume of blood giving a suitable absorbance for 100% hemolysis was noted.

The experiment was done with each of the three surfactants as described below.

(1) To 0.1 ml of the stock RBC suspension in a centrifuge tube, 5 ml of isotonic saline was added and incubated for 30 min at room temperature. Then it was centrifuged at 1000 g for 5 min. The absorbance of the supernatant was read at 540 nm. This gives the absorbance of the "blank"(B).

(2) To 0.1 ml of the stock RBC suspension in a centrifuge tube, 4.5 ml of distilled water was added and incubated for 30 min at room temperature. To this 0.5 ml of the surfactant was added (such that the final surfactant concentration was 1 ppm). Then it was centrifuged at 1000 g for 5 min and the absorbance of the supernatant was read at 540 nm. This gives the absorbance corresponding to 100% hemolysis (H).

(3) To 0.1 ml of the stock RBC suspension in a centrifuge tube, 4 ml of hypotonic saline and 0.5 ml of distilled water was added and incubated for 30 min at room temperature. Then 0.5 ml of the surfactant was added (such that the final surfactant concentration was 1 ppm). Then it was centrifuged at 1000 g for 5 min and the absorbance of the supernatant was read at 540 nm. This gives the absorbance of the "control" (C).

(4) To 0.1 ml of the stock RBC suspension in a centrifuge tube, 4 ml of hypotonic saline and 0.5 ml of surfactant was added (such that the final surfactant concentration was 1 ppm) and incubated for 30 min at room temperature. This was followed by the addition of 0.5 ml of distilled water and was centrifuged at 1000 g for 5 min. The absorbance of the supernatant was read at 540 nm and this gives the absorbance corresponding to "test"(T).

Calculations

% Hemolysis in the control(X) = C-B/H-B x 100
% Hemolysis in the test (Y) = T-B/H-B x 100
% labilisation by test = Y-X /X x 100

3.3.3 Results

The results are given in Table 3.4 and Figure 3.4.

Table 3.4. Percentage hemolysis in *O. mossambicus* on exposure to 1 ppm surfactants *in vitro* and *in vivo*

Group	% Hemolysis *in vitro*	% Hemolysis *in vivo*
Control	0	0
SDS	14.99 ± 0.5	10.1 ± 2.5
TRITON	61.45 ± 1.3	64.2 ± 3.5
CTAB	24.40 ± 1.5	32.5 ± 5.0

Values are the mean ± SD of six separate experiments

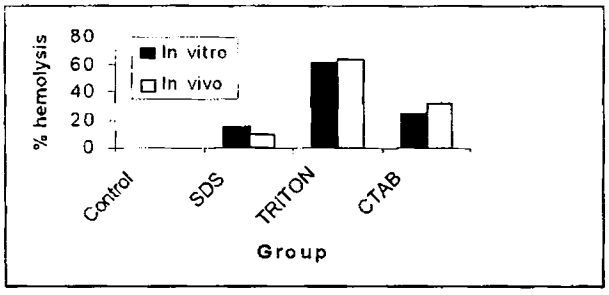

Figure 3.4. **Percentage hemolysis in *O. mossambicus* on exposure to 1 ppm surfactants *in vitro* and *in vivo***

In vitro studies indicated that the exposure to 1 ppm of surfactants had a labilising effect on the RBC membrane (Table 3.4 and Figure 3.4). SDS, the anionic surfactant induced 14.99% labilisation, for the cationic CTAB it was 24.398% and the non ionic Triton produced a labilising effect of 61.45%.

In vivo studies also presented a similar toxicity pattern. Here Triton X-100 was the most damaging producing 64.2% hemolysis. CTAB caused 32.5% and SDS induced 10% hemolysis.

One way analysis of variance revealed that there was an overall significant difference between the surfactants with respect to their haemolytic activity in vitro ($F = 5653.21$, $P<0.001$) (Table 3.4a), as well as in vivo ($F=1343.47$ $p< 0.001$ (Table 3.4b).

Table 3.4a. ANOVA for RBC studies *in vitro*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7239.94	2	3619.97	5653.21	2.61E-22	3.69
Within Groups	9.61	15	0.64			
Total	7249.54	17				

Table 3.4b. ANOVA for RBC studies *in vivo*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8866.92	2	4433.46	1343.473	1.21E-17	3.68
Within Groups	49.5	15	3.3			
Total	8916.42	17				

Subsequent comparisons by multiple comparison (LSD) revealed that haemolytic effects of all the three surfactants were significantly different ($P<0.001$) from control and also from one another (Table 3.4c) in both *in vitro* and *in vivo* studies.

Table 3.4c. Results of LSD analysis for *in vitro* and *in vivo* studies

Groups	P value
Control × SDS	P<0.001
Control × Triton	P<0.001
Control × CTAB	P<0.001
SDS × Triton	P<0.001
Triton × CTAB	P<0.001
SDS × CTAB	P<0.001

3.3.4 Discussion

The results indicated that the non ionic surfactant Triton X-100 was the most damaging to the cell membrane followed by the cationic CTAB and then the anionic SDS both *in vitro* and *in vivo*. The cell membrane composition of RBC and the selective property of the detergents are important in the interactions.

RBC membrane is rich in cholesterol, phospholipids, glycolipids etc. So the surfactant having a higher affinity for these lipid moieties would have an upper hand in the solubilising power. SDS is thought to bind with positive hydrophilic groups or to specific hydrophobic receptors and is suggested that it extracted individual proteins and lipids separately and has only little lipid sensitivity. Triton is supposed to solubilise most proteins and lipids in parallel (Partearroyo *et al.*, 1991). CTAB being cationic would have rendered the membrane leaky by interaction with negatively charged lipids and proteins. Thus it may be inferred that initially all the surfactants solubilised protein and later lipids were selectively extracted. More over the peroxidative effects on the cell membrane (rich in lipids) would have had an added effect on the solubilising capacity of the surfactants. Or it may be suggested that the membrane modifications leading to hemolysis may be a sequel to the generation of activated oxygen species.

3.4 Effects of common industrial surfactants on erythrocyte membrane stability of *Oreochromis mossambicus*

A laboratory study was conducted to assess the impacts of commercial surfactants on RBC membrane. The surfactants tested were

1. Surf: a popular high- priced household detergent powder, anionic with linear alkyl benzene sulfonate (LAS) of 12-15%.
2. Wheel: a low-priced anionic (LAS) household detergent powder
3. Teepol: liquid cleaner, anionic containing 39% w/v C9-C13 sodium alkyl sulfates
4. Extran: liquid cleaner, mixture of various non ionics and alkali.
5. DiTallow Di Methyl Ammonium Chloride (DTDMAC): cationic surfactant (C36) used in fabric softeners, shampoos etc.

3.4.1 Materials and methods

Same as in section 3.3.2. Each of the commercial surfactants were tested at 0.1 %.

3.4.2 Results

Results are given in Table 3.5. It was observed that all surfactants were labilising to the RBC membrane. The non ionic extran induced maximum hemolysis of 40%. Cationic DTDMAC induced 25 % hemolysis. The laundry detergents surf and wheel caused 20% and 16.0 % hemolysis respectively. The anionic teepol had 12.1 % hemolytic activity.

Table 3.5. Effects of common industrial surfactants on RBC membrane of *O.mossambicus in vitro*

Surfactant	% hemolysis
Control	0
Surf	20.0 ± 5.0
Wheel	16.0 ± 4.5
Teepol	12.1 ± 3.5
Extran	40.0 ± 5.5
DTDMAC	25.0 ± 2.5

One way analysis of variance revealed an overall significant differences between the surfactants with respect to their hemolytic activity ($F = 97.36$, $P < 0.001$, Table 3.5 a).

Table 3.5a Anova for RBC stability in vitro

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2801.421	4	700.3553	97.36172	7.26E-15	2.75
Within Groups	179.8333	25	7.193333			
Total	2981.255	29				

Subsequent LSD analysis indicated that the hemolytic effects of all surfactants differed significantly from control and from one another ($P<0.001$). Comparison of hemolytic property of industrial surfactants with that of SDS, triton and CTAB has been represented in fig. 3.5 a

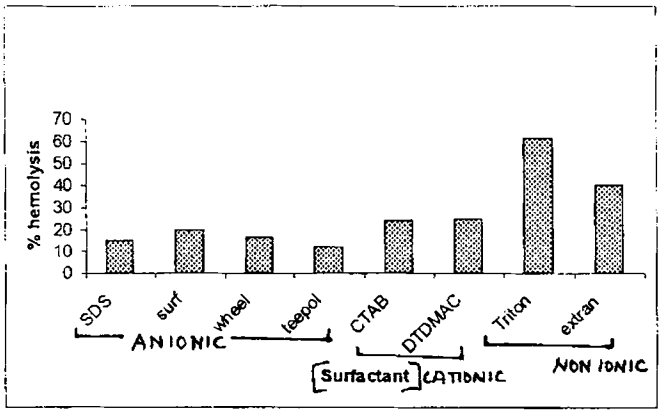

Figure 3.5a. Comparison of hemolytic property of surfactants based on charge

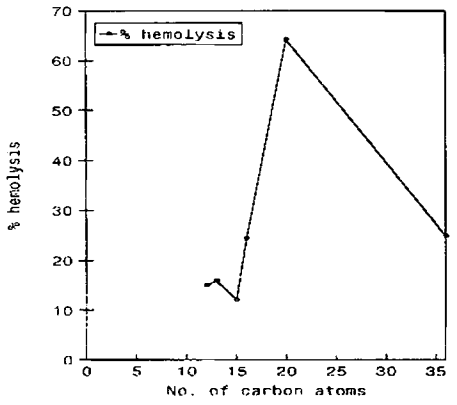

Figure 3.5b. Hemolytic property of surfactants vs carbon chain length

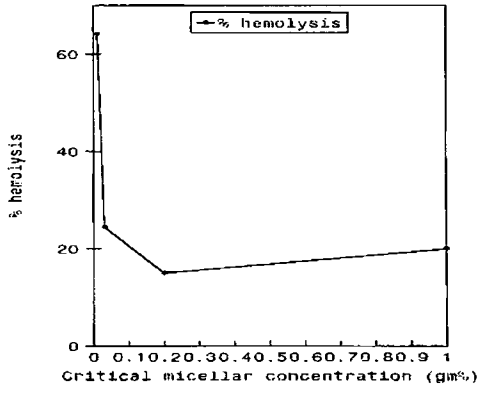

Figure 3.5c. Hemolytic property of surfactants vs critical micellar concentration (CMC)

It was observed that non ionics were the most labilising when comparison is made on the basis of the surfactant charge (fig.3.5 a). Thus, extran > DTDMAC > LAS (Surf, wheel) > SDS> Teepol. It is observed from fig. 3.5 b that as carbon chain length increases, there is an increase in hemolytic potential probably due to a concomitant decrease in critical micellar concentration (CMC) of the surfactants. Hence the decreasing order of toxicity is C20 > C15 > C12 > C36. But beyond C20 it is indicated that an increase in carbon chain has no significant impacts on hemolytic effects So DTDMAC with 36 carbon atoms is the least toxic. Fig 3.5 c indicates relationship between CMC and % hemolysis.

DISCUSSION

It may be inferred from the present study that non ionics are the most labilising to the RBC membrane. The non ionics are capable of both hydrophilic (via ethylene oxide side chain) and hydrophobic (via alkyl chain) interactions with the membrane. On the other hand the interaction of cationics are restricted to the anionic sites and vice-versa. Moreover non ionics show more lipid sensitivity than anionics and cationics. It is to be recalled that RBC membrane has a high lipid : protein ratio. Thus, the damage caused by lipid peroxidation would be the maximum for non ionics compared to cationic and anionic surfactants. Thus it may be concluded that charge of the surfactant, CMC and peroxidative potential are the deciding factors in causing RBC membrane labilisation.