SUMMARY

1. Physico-chemical nature of milk fat globule membrane (MFGM) proteins isolated from buffalo milk was evaluated. Among the various aspects, electrophoretic characteristics, enzymatic make-up, solubilisation of buffalo MFGM proteins by various membrane solubilising agents as well as separation of proteins by gel permeation chromatography and partial purification and characterisation of 5'-nucleotidase from buffalo MFGM constituted the major parameters.

2. The levels of cholesterol, phospholipid, hexose, homosamine and sialic acid expressed per mg protein in cow and buffalo MFGM were essentially alike.

3. Carbohydrate contents per mg of protein in whole cow and buffalo MFGM and in delipidated proteins were evaluated. It was observed that the extraction of both cow and buffalo MFGM with organic solvents resulted in decrease of hexose to protein ratio, but an increase of sialic acid to protein and homosamine to protein ratios, indicating that considerable amount of hexose is associated with glycolipids in MFGM. The chemical composition of delipidated proteins of cow and buffalo MFGM was essentially similar.

4. Electrophoretic analysis of cow and buffalo MFGM showed the presence of 15 protein components and 6 glycoprotein components in both the membranes. Seven proteins in both cow and buffalo MFGM were observed to be prominent.
The electrophoretic patterns and electrophoretic mobilities of proteins and glycoproteins of buffalo MFOM were essentially similar to the corresponding proteins and glycoproteins of cow MFOM indicating the similarity of the molecular weight profile of the protein components of the above membranes.

6. The enzyme activities associated with buffalo MFOM were evaluated. For a comparative study, the enzyme levels in cow MFOM were also estimated. The specific activities of alkaline phosphomonoesterase, acid phosphomonoesterase, xanthine oxidase, aldolase, ATPase and 5'-nucleotidase in buffalo MFOM were found to be lower than those in cow MFOM. But specific activities of alkaline phosphodiesterase and glucose-6-phosphatase were higher in buffalo MFOM than that in cow MFOM.

6. The effect of washing the milk fat globules with sucrose solution and distilled water prior to the rupture of fat globules on the levels of enzymes associated with both cow and buffalo MFOM was studied. It was noted that MFOM which had been previously washed with sucrose solution had higher specific activities of alkaline phosphomonoesterase, acid phosphomonoesterase, ATPase and aldolase, than MFOM which had been previously washed with distilled water. But the specific activities of xanthine oxidase, alkaline phosphodiesterase and 5'-nucleotidase in MFOM did not depend upon the solution used for washing the milk fat globules.
7. The effect of chilling milk prior to the isolation of milk fat globules on the levels of enzymic activities associated with both cow and buffalo MFGM, showed that the specific activities of enzymes in MFGM isolated from unchilled milk were higher than the corresponding specific activities in MFGM isolated from chilled milk. The above observation indicates that chilling of milk prior to the isolation of MFGM resulted in the loss of membrane materials rich in enzymic activities.

8. In order to find dependence of chemical composition on the method of isolation, the membrane materials from the same milk samples were isolated by churning and freeze-thawing methods. Cholesterol and phospholipid contents of MFGM isolated by freeze-thawing method were comparatively lower than in MFGM isolated by churning method. On the other hand, neutral sugar, hexosamine and sialic acid contents of MFGM isolated by freeze-thawing were higher than those of samples isolated by churning method. The electrophoretic patterns of proteins of MFGM isolated by the above methods also showed some difference. Specific activities of glucose-6-phosphatase and ATPase in MFGM of both species prepared by churning method was less than the corresponding specific activities in MFGM prepared by freeze-thawing. The specific activities of alkaline phosphomonoesterase, acid phosphomonoesterase and alkaline phosphodiesterase associated with MFGM prepared by
freeze-thawing method were higher than the corresponding specific activities associated with MFGM prepared by churning method.

9. NaCl solubilised 40% of buffalo MFGM proteins which were found to be rich in glycoproteins. NaCl also solubilised 70% of alkaline phosphomonoesterase, 45% of xanthine oxidase and 28% of 5'-nucleotidase activities associated with buffalo MFGM. This indicates alkaline phosphomonoesterase is more loosely bound to MFGM than xanthine oxidase and 5'-nucleotidase. Electrophoretic patterns of MFGM proteins solubilised at various concentrations of NaCl were essentially similar showing that no selective solubilisation of MFGM proteins could be achieved in presence of NaCl.

10. Distilled water at pH 9.8 was observed to solubilise 40% of buffalo MFGM proteins which had higher carbohydrate content per mg protein than the whole MFGM. The electrophoretic analysis of water solubilised buffalo MFGM proteins showed that lower molecular weight proteins of the membrane were selectively solubilised by distilled water. Isoelectric pH of water-solubilised buffalo MFGM proteins was 3.9 – 4.1. Removal of sialic acid from the water-solubilised proteins resulted in shifting of isoelectric pH to 4.5 – 4.7. Nearly 80% of protein and 70% of phospholipid were eluted along with the void volume when the water-solubilised membrane material was subjected to Sephadex G-200 gel filtration.
11. EDTA could solubilise substantial amount of buffalo MFGM proteins. Electrophoretic pattern of proteins of EDTA solubilised membrane material showed that all the protein components were not equally solubilised by EDTA. Sephadex G-200 gel filtration of the membrane material solubilised by EDTA showed that the solubilised proteins were in a highly aggregated state.

12. 80% of buffalo MFGM protein was solubilised by n-butanol. Extraction of MFGM with n-butanol did not result in change of the electrophoretic pattern of MFGM proteins. Sephadex G-200 gel filtration of the MFGM proteins solubilised in presence of butanol resulted in the elution of proteins in a single peak along with the void volume.

13. Pyridine could solubilise 23% of buffalo MFGM protein which had high carbohydrate content. The pyridine-solubilised proteins could be resolved into five zones by Sephadex G-200 gel filtration showing that such proteins were not in an aggregated state.

14. Triton X-100 and Tween-20 solubilised significant amounts of alkaline phosphomonoesterase, xanthine oxidase and 5' -nucleotidase activities from buffalo MFGM. The above enzymic activities were enhanced in presence of the non-ionic detergents.

15. Sodium deoxycholate solubilised more than 80% of
alkaline phosphomonoesterase, xanthine oxidase and 5'-nucleotidase activities from buffalo MFOM. At lower concentrations of deoxycholate, alkaline phosphomonoesterase was solubilised to a greater extent than xanthine oxidase and 5'-nucleotidase. The activities of the above enzymes were also enhanced in presence of deoxycholate. But ATPase activity of MFOM was lost in presence of deoxycholate. Sodium deoxycholate treatment of MFOM resulted in the cleavage of the membrane lipoproteins into lipids and proteins as observed by Sephadex G-200 gel chromatography. The nature of solubilisation of buffalo MFOM by deoxycholate was observed to be similar to the reported nature of solubilisation of some other biomembranes indicating the similarity of lipid-protein interactions in MFOM to those in other biomembranes.

16. Sodium lauryl sulphate at lower concentrations enhanced the activities of alkaline phosphomonoesterase, xanthine oxidase and 5'-nucleotidase of buffalo MFOM. But at 2 mM concentration, it inhibited the above enzymic activities. The MFOM material solubilised in SDS was resolved into four protein fractions by Sephadex G-200 gel chromatography. The phosphorus containing peak was separated from the major protein peak by the gel chromatography.

17. 5'-nucleotidase from buffalo MFOM was partially purified. Two fractions of the enzymic activity were
separated by Sepharose 4B gel permeation chromatography and they were designated as fraction V and fraction VI. CMP and GMP were found to be the most active substrates for fraction V and fraction VI, respectively. K_m values of fraction V for AMP, CMP and GMP were lower than the corresponding values of fraction VI.

18. The degrees of inhibition of enzymic activities of fraction V and of fraction VI by Ni^{++}, Cu^{++}, Hg^{++} and Zn were distinctly different.

19. Heat stability and energy of activation of fraction VI were found to be higher than those of fraction V.

20. Both fractions did not require Mg^{++} for enzymic activity, but were inhibited by EDTA. Inhibition by EDTA could be relieved in presence of Na^{++}, Mg^{++} or Co^{++}.

21. AMP and ATP were competitive inhibitors of enzymic activity of fraction V and non-competitive inhibitors of enzymic activity of fraction VI.

22. A biphasic modification of 5'-nucleotidase activity of buffalo MFGM (isolated by freeze-thawing procedure) was observed in presence of Concanavalin A. But enzymic activities of fraction V and of fraction VI were inhibited at lower concentrations of Concanavalin A. Concanavalin A at lower concentrations inhibited the enzymic activity of fraction V to a greater extent than that of fraction VI.
In general, the properties of 5'-nucleotidase from buffalo MFGM were observed to be similar to the reported properties of 5'-nucleotidase from cow MFGM. This indicates that in addition to the major proteins and glycoproteins, the minor proteins of cow and buffalo MFGM appear to be very similar.