Name of student: **Muzaffar Ul Hassan Mir**

Name of Supervisor: **Dr. Rajan Patel**

Name of center: **Center for Interdisciplinary Research in Basic Sciences**

Title of thesis: **Study the Role of Gemini Surfactants in Protein Stability in Aqueous Medium**.

CHAPTER I. This is the introductory chapter which covers the scope and objective of the proposed work. Various aspects of the work have been explained. An up to date literature survey has been presented to illustrate the work being carried out in this field. Thus, clearly emphasizes the need of the work.

CHAPTER II. Experimental methods and chemicals used in the present investigations along with the calibrations of the apparatus have been detailed out and are presented in the second chapter. Quenching mechanism, binding constant, number of binding site and different thermodynamic parameter have been measured at different temperatures for binary aqueous solvents and ternary solutions.

CHAPTER III. This chapter describes the effect of *N,N'-bis(dodecyloxycarbonylmethyl)-N,N,N',N'-tetramethyl-1,2-ethanediammonium dibromide* (dodecyl betainate gemini or DBG) on the structure and function of Bovine Serum Albumin (BSA). Fluorescence, time resolved fluorescence, circular dichroism and dynamic light scattering techniques were employed. The Stern‑Volmer quenching constants K_{SV} and the corresponding thermodynamic parameters viz ΔH, ΔG and ΔS have been estimated by the fluorescence quenching method. The results indicated that hydrophobic forces were the predominant intermolecular forces between BSA and DBG and the binding is essentially a spontaneous process. Time resolved fluorescence data shows that the quenching follows the static mechanism pathway. It can be seen from far-UV CD spectra that the α-helical network of BSA is disrupted and its content increases from 71% to 79 % at lower concentrations which again decreases to 38 % at higher concentration. DLS measurements have shown that hydrodynamic radius (R_h) decreases in presence of 30 and 40μM of DBG while as it increases when the concentration of DBG was 70 and 100μM. The study of molecular docking indicated that DBG is embedded into subdomain IIA of BSA and binds with the R-914, R-195 and R-217 residues by hydrogen bonding and by hydrophobic interaction.

CHAPTER IV. Herein, spectroscopic approaches viz. fluorescence, time resolved fluorescence, UV-visible, and FT-IR were employed to examine the interaction between ethane-1, 2-diyl bis(N,N-dimethyl-N-hexadecylammoniumacetoxy) dichloride (16-E2-16) and bovine serum albumin (BSA). Fluorescence studies revealed that the 16-E2-16 quenched the fluorescence of BSA through static quenching mechanism which was further confirmed by UV-visible and time resolved fluorescence spectroscopy. In addition binding constant and number of binding sites were also calculated. The thermodynamic parameters at different temperatures (298K, 303K,

308K and 313K) indicated that 16-E2-16 binding to BSA is entropy driven and major driving forces are electrostatic interactions. A decrease of α-helix from 53.90% to 46.20% with an increase in random structure from 22.56% to 30.61% was also observed by FT-IR. Furthermore, the molecular docking results revealed that 16-E2-16 binds predominantly by electrostatic and hydrophobic forces to some residues in sub domains IIA and IIIA of BSA.

CHAPTER V. Fluorescence, UV-visible, Circular dichroism (CD) spectroscopy and surface tension techniques were employed to investigate the interaction between the hemoglobin (Hb) and gemini surfactant, bis (N, N-dimethyl-N-hexadecylammoniumacetoxy) dichloride (16-E2-16). Molecular docking and simulation have also been done to study the changes in the Hb conformation upon interacting with 16-E2-16. The UV-visible study demonstrates the perturbation of the soret/heme band and generates conformational heterogeneity within the heme group in the presence of 16-E2-16. Data shows an increase in the fluorescence intensity of Hb with the addition of 16-E2-16". After the cmc value of the 16-E2-16 the fluorescence intensity increases slowly. Trp fluorescence was largely affected than that of Tyr. The critical micellar concentration of 16-E2-16 in absence and presence of Hb at various temperatures were determined using surface tension plots. The cmc increases with increase in temperature as well as in the presence of Hb. Furthermore, thermodynamic parameters of micellization were also investigated. Docking study reveals that 16-E2-16 binds in the α1 and β2 chain of Hb with predominant forces being hydrophobic and electrostatic. Simulation and CD study confirms the conformational change in the Hb after binding with 16-E2-16.

CHAPTER VI. Refolding of urea denatured cyt c was studied under the influence of ester based gemini surfactants [ethane-1, 2-diyl bis(N, N-dimethyl-N-alkylammoniumacetoxy) dichlorides]16-E2-16, 14-E2-14 and 12-E2-12 (n-E2-n) using ultraviolet and visible absorption, fluorescence and circular dichroism (CD). Upon addition of n-E2-n to the urea denatured cyt c, stabilized molten globule (MG) like state begins to appear which can be an intermediate in protein folding pathway. m values of all the refolded states of cyt c by n-E2-n show marked difference. The higher values of m correspond to the larger hydrophobic chain length, that is refolding ability of the n-E2-n depends on the alkyl chain length.