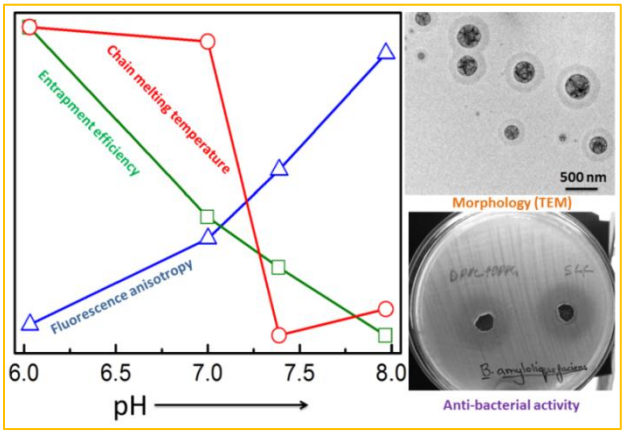
ABSTRACT

The research work auspicious in this thesis entitled "**INTERFACIAL, KINETIC AND MECHANISTIC STUDIES ON DENDRIMER-LIPOSOME INTERACTIONS**" is primarily focused on the two different important aspects; one is to prepare stable liposomes and to study the different kinds of physicochemical properties of these types of liposome and another is to study the interaction with these types of stable liposomes with different generation of PAMAM dendrimer. The first part of the thesis is completely focused on to prepare the stable liposome and investigate the effects of pH, temperature and lipid composition on various physicochemical properties of liposomes. Antibacterial activities were assessed using a gram positive bacteria *Bacillus amyloliquefaciens*. A second part of this thesis is devoted to the interaction of different generations of PAMAM dendrimers with the lipid bilayer interface. The lipid anionic membranes (liposomes) were used to model bio membranes, whereby the compositions of the liposomes were varied together with the chemical nature of the anionic lipids (in combination with DPPC). The type and strength of the interaction was dependent on charge and size of the liposomes as well as the dendrimer generation. The impact of dendrimer concentration and generation on four different kinds of liposomes was investigated using a combination of various physicochemical properties. Finally the dendrimer-liposome complexes, also known as dendriosomes, were explored in terms of its toxicity in healthy human blood cell lymphocyte as well as human red blood cells. Although there are different reports on the interaction studies between dendrimers and liposomes, to the best of the knowledge, no comprehensive and systematic studies have been carried out previously in order to assess the impact of dendrimer generation, concentration as well as the variation of the liposome type.

Chapter I describes about the effects of pH, temperature and lipid composition on various physicochemical properties of liposomes. Four different liposomes with soy phosphatidylcholine (SPC), dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidyl glycerol (DPPG) as well as a 7:3 (M/M) mixture of DPPC+DPPG alongwith 30 mol% cholesterol were studied for this purpose. Antibacterial activities were assessed using a gram positive bacteria *Bacillus amyloliquefaciens*. Although there are scattered reports on the effect of pH on physicochemical studies on liposomes, however, no comprehensive studies have been made to understand the

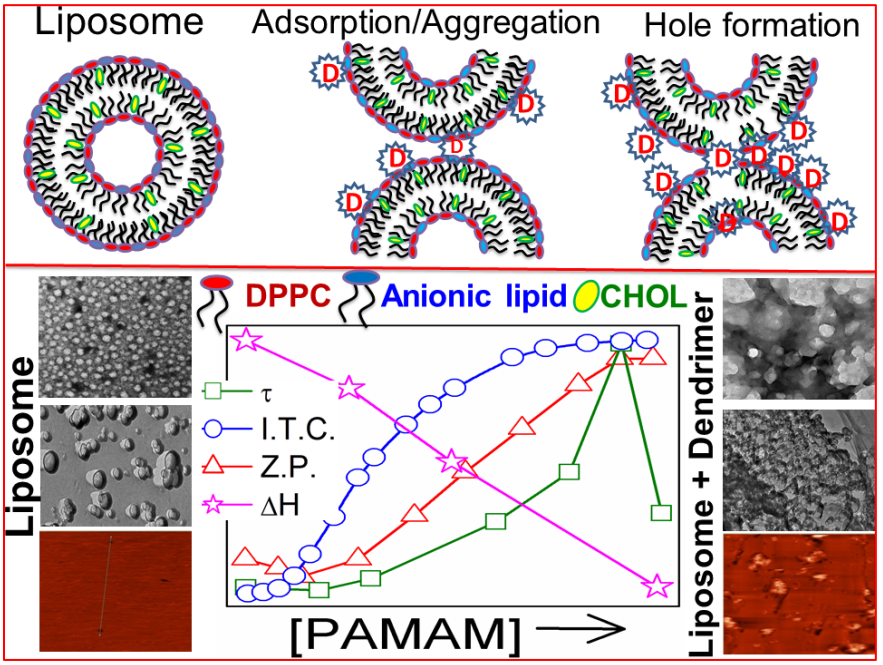
combined effect of pH as well as the charge on liposomes. Besides, exploration on the curcumin loaded liposome as potential antibacterial agent is not so common in the literature.



Chapter II reported the interaction of different generation poly(amidoamine) (PAMAM) dendrimers and combinations of liposomes. Second, fourth and sixth (2G, 4G, and 6G) generation PAMAM dendrimers were used, which are cationic under normal conditions. Liposomes comprised of soy lecithin + cholesterol (SLC+CHOL) (negative surface charge), DPPC+CHOL (positive surface charge), DPPG+CHOL (negative) and a biologically simulated mixture of DPPC + DPPG (7:3) + CHOL (negative) were used as model bilayers. Silica was used as a negatively charged hard sphere model to make a comparative study. Absorbance (turbidity) at 420 nm, dynamic light scattering, zeta potential measurements on liposome and finally atomic force microscope (AFM) measurements on solid supported bilayers (by vesicle fusion on freshly cleave mica) were performed to study the interactions. Maxima in absorbance and size of liposome was observed upon PAMAM addition. Charge reversal happened with the progressive addition of dendrimer. Interaction between PAMAM with liposome were found to be driven predominantly electrostatic. PAMAM activity was found to be generation dependent as 6G >4G>2G in terms of overall dendrimer concentration. But, interestingly, the order gets reverse when PAMAM activity was considered in terms of total end group concentrations. AFM studies reveal the rupture of bilayer structure upon addition of dendrimer.

In **Chapter III,** interaction of liposomes carrying net negative charges with cationic polyamidoamine dendrimers (PAMAM) of different generations were investigated by combined size, zeta potential, turbidity, electron microscopy, atomic force microscopy, fluorescence

spectroscopy and calorimetric studies. Stability of the liposomes comprising 1, 2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) + dihexadecyl phosphate, DPPC+1, 2-dimyristoyl-*sn*-glycero-3-phosphoglycerol, DPPC+1, 2-dipalmitoyl-*sn*-glycero-3-phosphate and DPPC+1, 2-dipalmitoyl-*sn*-glycero-3-phosphoethanol were checked through their size and zeta potential with the variation of time. Existence of lipid bilayer and subsequent adsorption of dendrimer onto the liposome surfaces were evidenced. Interaction between the dendrimers and liposomes were electrostatic in nature, as evidenced through the charge neutralization of liposomes and its subsequent reversal with increasing dendrimer concentration. Extent of dendrimer-liposome interaction followed the sequence: generation 5 > 4 > 3 in addition to the head group charge, moiety and hydrocarbon chain length of the lipids. Fluorescence anisotropy and differential scanning calorimetry (DSC) studies suggest the fluidization of the bilayer although the surface rigidity was enhanced by the added dendrimers. Thermodynamic parameters of interaction processes were evaluated by isothermal titration and differential scanning colorimetric studies; the binding processes were exothermic in nature. Enthalpy of the transition of the chain melting of lipids decreased systematically with increasing dendrimer concentration and generation. Dendrimer-liposome aggregates were non-toxic to healthy human blood cell lymphocyte as well as in human RBCs suggesting the potential of such aggregates as drug delivery systems against microbial diseases.



In **Chapter IV**, the mutual miscibility and stability of the mixed monolayers of zwitterionic phospholipid, dipalmitoylphosphatidylcholine (DPPC) with negatively charged phospholipids (dihexadecyl phosphate (DHP), 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG), 1,2-dipalmitoyl-sn-glycero-3-phosphate (DPP) and 1,2-dipalmitoyl-sn-glycero-3-phospho ethanol (DPPEth) were investigated at the air-buffer interface. Interaction between the positively charged dendrimer with the monolayers has been studied in detail using surface pressure-area isotherms. Thermodynamic analysis indicates miscibility of the binary mixtures when spread at the air/buffer interface with synergistic interaction between the components. The surface pressure-area isotherms the binary monolayers of DPPC and negatively charged lipids at the air-water interface showed maximum deviation for DPPC : anionic lipid at 7:3 M/M ratio mixed monolayer was more stable than the monolayers individual components. Based on the regular solution theory, the miscibility and stability of the two components in the monolayer were analyzed in terms of compression modulus (C_s^{-1}) and excess Gibbs free energy ($\triangle G^0$) and these physiochemical parameters dependent on phospholipids composition. Stable liposomes were formulated by the binary mixture in 7:3 molar ratio of DPPC with negatively charged phospholipids. Subsequently adsorbed monolayers were generated through vesicles disruption technique. Effects of polyamidoamine (cationic) dendrimers on the adsorption kinetics at the vesicles were followed. Bylayer disintegration and subsequent interfacial adsorption of lipids were followed up through the surface pressure. Time analysis bylayer disintegration kinetics was governed by the lipid head groups, chain length as well as the dendrimer generation an concentration.

The thesis then follows comprehensive summary and conclusion followed by the cited references and off-prints of the published journal articles.