CHAPTER 2

Exploring the Dual Impact of Hydrocarbon Chainlength and the Role of Piroxicam A Conventional NSAID on Soylecithin/Ion Pair Amphiphiles Mediated Hybrid Vesicles for Brain – Tumors Targeted Drug Delivery

Abstract: Development of drug delivery systems, not the drug discovery, has become more cynosures towards the efficacy of drug against the target cells. Modified hybrid cationic vesicles (HCV) were formulated using soylecithin (SLC), ion pair amphiphile (IPA, hexadecyltrimethylammonium-dodecylsulfate, $HTMA^{+}$-DS^{-}), bi-tail cationic surfactant dialkyldimethylammonium bromides (DXDABs, bis-C_{12} to C_{18}) and cholesterol. Piroxicam (Px), a conventional non steroidal anti inflammatory drug, with potent yet unexplored anticancer activity, was encapsulated in the hybrid vesicles. Dual impact of DXDABs and Px on SLC/IPA were scrutinized in the form of monolayer, bilayer and solid supported bilayer. Favourable hydrophobic interaction between SLC/IPA and dihexadecyldimethylammonium bromide (DHDAB) as well as the intercalation of Px molecules between the amphiphiles were noticed through the surface pressure area measurements. Vesicles without and with Px were fairly monodispersed with positive zeta potential (Z. P.) and considerably stable up to two months. Size of the vesicles enhanced with Px incorporation. Vesicles maintain spherical morphology as revealed from the electronic microscopic studies. Differential scanning calaorimetry and FTIR studies confirm the location of Px membrane palisade that enhances the extent of hydration by increasing the proportion of H-bonding. Bilayer thickness and the spacing between two adjacent lamellar phases were investigated by combined small angle neutron scattering and small angle X-ray scattering. Atomic force microscopic studies confirm the Px induced fluidization of membrane bilayer. The entrapment efficiency of

vesicles to host Px depends on the amount of IPA present in the bilayer. Px hosted cationic vesicles showed less than 2% hemolysis. The drug reigned supreme over human Neuroblastoma cell line (SH-SY 5Y) when encapsulated inside the membrane and was non toxic to normal human blood cell lymphocyte (PBMC) as revealed from cytotoxicity assay.

1. Introduction

Vesicles, the aggregates of amphiphiles having bilayer like structure, are widely used as potential drug delivery systems because of its tendency to fuse onto the microbial membrane bilayer. Chemical composition and structure of liposome can be controlled by considering its preparative method that could be useful in various applications. Different properties of liposome's and cell membrane mimic architect offer an useful model system for studying the membrane biophysics, colloidal interaction, photochemistry, cell function, signal transduction and many others.[1-3] Synthetic vesicles find wide range of technical applications, *viz.*, nano sensing, metal encapsulation, and drug delivery, to mention a few.[4,5] It can accumulate both hydrophilic and lipophilic drugs and transfer them to the target cells. However, vesicles suffer from major limitations like the stability and production cost. Susceptibility towards oxidation and microbial attack warrants it modulation by incorporating different substitutes.

Usually naturally occurring biological membranes have negative surface charge and hence well engineered cationic vesicles can be considered as efficient drug delivery systems. The present research group has been pursuing the physicochemical characterization of vesicles with different surrogates, which are stable up to 100 days with superiority than the conventional liposomes.[6-8] The present work endeavours to search for stable cationic vesicles with superior drug delivery capacity.[8] Over the period of time attempts have been made to

modulate cationic vesicles by using bi-tail cationic surfactant with varying chain length (bis-C_{12} to C_{18} or higher).

Ribeiro *et al.*[9] have prepared cationic vesicles in combination with DPPC and dioctadecyldimethylammonium bromide (DODAB) in solutions of different ionic strengths. Dong *et al.*[10] studied the interaction of DNA with cationic vesicle having didodecyldimethylammoniumbromide (DDDAB). Chou *et al.*[11] have made detailed investigation on the catanionic vesicles using dihexadecylphosphate (DHP) and DODAB.

Piroxicam (Px), a non steroidal anti-inflammatory drug (NSAID), can assemble with the enzyme cyclooxygenase-2 (COX-2) in the cell membrane and thereby can reduce the inflammatory function.[12-14] Its potent anticancer activity is worthy to study its efficacy towards the target cell. Its pKa values are 1.86 and 5.42 respectively. Hence in biological condition its half life and activity towards the target cell is substantially reduced. Limited activity in biological conditions, provide a scope to host or entrap Px in suitable delivery systems. Hybrid cationic vesicle (HCV) is considered as a protector as well as a vehicle, for a variety of drugs at physiological environment for better efficiency towards the target cells. However, structural properties and acid-base form of the drug molecule play crucial role and thereby govern the drug-membrane interaction. Membrane mimetic models can avoid the complexity of the biological cells, creating the platform to understand the mechanism by which it interacts with drug molecules. Different attempts have been made to understand the impact of Px or other NSAIDs on different bio-mimetic membranes.[12,14-17] Px induced fluidization of the DMPC and DPPC membranes were already reported. Basak *et al.*[12] had studied the stability of DMPC monolayer in the presence of Px. Whereas Nunes *et al.*[14] had made detail investigation on the impact of all set of oxicams in DPPC membrane. Roy *et al.* have reported the increased orientation order of the lipid chain in presence of NSAID.[17]

However no comprehensive attempts have been made so far where the interactions between hybrid cationic membrane and Px are concerned.

Limited or fragmented knowledge warrants bi-tail cationic surfactants with varying hydrocarbon chain length (bis-C_{12} to C_{18}) in combination with soy lecithin (SLC) and ion pair amphiphile (IPA). IPA, herein prepared by mixing equimolar ratio of two cationic surfactants hexadecyltrimethyalammonium bromide ($HTMA^+Br^-$) and sodium dodecyl sulfate (NaDS) (herein the IPA means $HTMA^+$-DS^-).[7] Bi-tail cationic surfactants were chosen to progressively substitute with previously established three sets of SLC/IPA combinations (1:0, HCV1; 9:1, HCV2 and 7:3, HCV3; M/M)[7] Interaction between hybrid membrane and Px were analyzed in the form of monolayer, bilayer and solid supported bilayer and finally few set of optimised px encapsulated formulations were analysed for biological activity. Mutual miscibility among the components were studied by way of the surface pressure – area measurements. Ideality, nonideality in the mixing processes, Gibbs free energy of mixing was assessed. Physicochemical characterizations of the different hybrid vesicels were assessed by combined dynamic light scattering, zeta potential, electron microscopy, atomic force microscopy, differential scanning calorimetry, FTIR, UV-VIS absorption and emission spectroscopic studies. Entrapment efficiency and the release kinetics of Px from the vesicles were also studied by conventional dialysis bag approach. Finally the toxicity and biocompatiblity of the drug loaded formulations were assessed. It is believed that such a comprehensive set of the studies would shed further light in the development of drug delivery systems in the treatment of brain – tumours targeted drug delivery.

2. Materials and Methods

2.1. Materials

L-α-phosphatidylcholinee (soylecithin, SLC, from soybean) was received from EMD Chemicals, Germany, A. R. grade sodium dodecylsulfate (SDS) [$CH_3(CH_2)_{11}SO_4Na$], hexadecyltrimethylammonium bromide (HTMAB) [$C_{16}H_{33}N(CH_3)Br$], didodecyldimethylammonium bromide (DDDAB) {$[CH_3(CH_2)_{11}]_2N(CH_3)_2Br$}, dimethylditetradecyldiammonium bromide (DTDAB) {$[CH_3(CH_2)_{13}]_2N(CH_3)_2Br$}, dihexadecyldimethylammonium bromide (DHDAB) {$[CH_3(CH_2)_{15}]_2N(CH_3)_2Br$}, dioctadecyldimethylammonium bromide (DODAB) {$[CH_3(CH_2)_{17}]_2N(CH_3)_2Br$}, (3β)-cholest-5-en-3-ol (cholesterol) 1,6-diphenyl-1,3,5-hexatriene (DPH) and piroxicam [4-Hydroxy-2-methylN-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide 1,1 dioxide] were purchased from Sigma-Aldrich Chemicals Pvt. Ltd. (USA). A.R. grade disodium hydrogen phosphate (Na_2HPO_4, $12H_2O$), sodium hydrogen phosphate ($NaH_2PO_4.2H_2O$), sodium chloride (NaCl) and chloroform (HPLC grade) were from Merck Specialties Pvt. Ltd, India. Double distilled water with specific conductance 2-4 μS (at 25^oC) was used for the preparation of solutions. The SH-SY 5Y cell lines were procured from National Centre for Cell Science (NCCS), Pune, India. They were maintained in DMEM F-12 Ham (Hi-Media) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 mg/mL streptomycin, 0.14% sodium bicarbonate and 0.1 mM sodium pyruvate.

2.2. Methods

2.2.1. Preparation and Isolation of Ion Pair Amphiphile (IPA)

IPA, also known as hexadecyltrimethylammonium-dodecylsulfate ($HTMA^+$-DS^-) was prepared by the combination of equimolar mixture of two oppositely charged surfactants in water medium.[18,19] An aquous solution of 0.1M hexadecyltrimethylammonium bromide

(HTMA) was progressively added to equimolar amount of sodium dodecyl sulfate (SDS) with constant stirring whereby a white semi solid got precipitated. The precipitate was then extracted by chloroform. After the eveporation of chloroform, the white soild powder (IPA) was dried under vacuum and thus obtaind salt free IPA. IPA was charectarised by means of ^{1}H-NMR, XRD and by FTIR.[7,18-20] Data obtaind were found to be similar with the previously published results and hence are not discussed further.

2.2.2. Preparation of Vesicles

Modified hybrid small unilamellar vesicles (SUVs) were prepared by the conventional thin film generation and rehydration technique.[4,7] Quantitative amount of SLC, IPA, bi-tail cationic surfactant and cholesterol were dissolved in chloroform: methanol (7:3, V/V) in a round bottom flask followed by solvent evaporation in a rotary evaporator. Trace amount of solvent was finally removed by the stream of nitrogen (N_2). The thin film was then rehydrated for 1h in PBS (0.1 M Na_2HPO_4, 0.1M NaH_2PO_4, 100mM NaCl, pH 7.4) at 70 ^{o}C, well above the chain melting temperature of all the lipidic components. After rehydration, freeze-thaw sonication processes were repeated for 4 cycles. Finally, it was extruded using 0.45 micron cellulose nitrate membrane filter (Whatman Gmbh, Germany). Px loaded vesicles were obtained by mixing appropriate amount of drug into the lipid mixture before the generation of the thin film such that the final ratio of lipid and dye was 200:1. Total phospholipids concentration was kept at 2 mM and was diluted depending on the type of experiment.

2.2.3. Surface Pressure (π) – Area (A) Isotherm

Surface pressure-area isotherm was constructed with a Langmuir surface balance (micro trough X, Kibron, Finland). The trough was filled with the PBS solution at pH = 7.4 with 100 mM NaCl with the barrier made of Teflon to avoid contamination.[19] Isotherms were

obtained by carefully spreading the droplets using a Hamilton (USA) micro syringe. Pure as well as mixed lipidic components were dissolved in chloroform (1.0 $mgmL^{-1}$) and were spread over the subphase with an amount of 10 μL. To prevent the entry of dust particles, a Plexiglas box was used which covered the stage and trough. Solvent was allowed to evaporate approximately 20 min. All the π – A isotherms were recorded at a subphase temperature of 25 ± 0.5 ^{o}C with a lateral compression rate of 5.0 mm min^{-1}. To ensure reliable result, each set of experiment as performed twice.

2.2.4. Dynamic Light Scattering (DLS) Studies

To get the knowledge about hydrodynamic diameter (D_h), zeta potential (Z. P.) and PDI, DLS measurement was carried out for different combination of vesicles.[21] Dynamic light scattering spectrometer (Zetasizer Nano ZS90 ZEN 3690, Malvern Instrument Ltd., U.K.) was used for such measurement. A He-Ne laser was used having emission wavelength 632.8 nm and all the data were recorded at a scattering angle 90^{o}.

2.2.5. Electron Microscopic (TEM/FF-TEM) Studies

Morphological information of the vesicles were obtained from the TEM studies, carried out in the form of both normal and freeze fractured (FF) TEM.[22, 23] For normal TEM, one drop of dilute vesicle dispersion was placed on Formver carbon-coated 300 mesh copper grid and the excess liquid was removed from the edge of the grip and the sample was allowed to dry for 10 min before performing the experiment. Hitachi H-600 transmission electron microscope (Japan) was used to view the morphology of the vesicle. For FF-TEM measurement, FR-7000A (Hitachi High Technologies Ltd., Japan) was used at -150 ^{o}C for freeze fracturing process. The sample was placed on the sample holder and frozen in liquid propane. Replication of the sample was done by evaporation using Pt-C. The replica was then

positioned on 300 mesh copper grid, dried and taken to electron microscope (H-7650, Hitachi High Technologies Ltd., Japan) with an acceleration voltage of 120 kV.

2.2.6. Scattering (SANS and SAXS) Studies

2.2.6.1. Small Angle Neutron Scattering (SANS)

Scattering intensities of the different sets of vesicles with and without drugs in D_2O were recorded by SANS instrument at the Dhruva Reactor, Trombay, India.[24] Sample-to-detector distance was set at 1.8 m. Crystalline BeO filtered beam that provides mean wavelength (λ) 0.52 nm was used. A one dimensional position sensitive detector (PSD) was used to record the angular distribution of the scattered neutron. The scattering vector Q ($Q = 4\pi\sin\theta / \lambda$) for this instrument was 0.018 to 0.35 $Å^{-1}$.

2.2.6.2. Small Angle X-ray Scattering (SAXS)

SAXS measurements were performed in NANOSTAR U SYSTEM with a detector VANTEC-2000 (Bruker AXS GmbH, Karlsruhe, Germany) to further clarify the bilayer architect.[25, 26] Samples were placed in a capillary tube and the X-ray wavelength was set to 1.5 Å with energy of the incoming beam 12 keV. Distance of the two sample detectors were set at 63.4 and 95.2 cm respectively leading to the Q range 0.01-0.3 $Å^{-1}$. The obtained raw data were first radially averaged, normalised for the acquisition time and the background correction for the PBS was done to obtain the scattering curve for the samples.

2.2.7. Differential Scanning Calorimetry (DSC) Studies

A Mettler Toledo differential scanning calorimeter (DSC 1, STARe system, Switzerland) was used where two identical pans were loaded with sample and PBS respectively.[27]

Samples were scanned with two different scan rates 5 ^{o}C and 2 ^{o}C with complete heating and cooling circle. The result so obtained was further processed with STAR^{e} software.

2.2.8. FTIR Studies

FTIR spectra of vesicles with and without drug and the drug in PBS alone were recorded with a Perkin Elmer Spectrum two FT-IR spectrophotometer equipped with a zinc selenide and a KBr beam spliter at ambient temperature (Perkin Elmer, Inc. MS, USA).[28,29]

2.2.9. Atomic Force Microscopic (AFM) Studies

The vesicular dispersion with and without drug was sonicated for ~ 7 min at 37 ^{o}C. A mica plate was placed into the cell and covered by 20 μL dispersions. After 45 min of waiting about 1000 μL of PBS was added into the cell and the AFM experiment was started using a Nanoscope III MM-AFM under ambient condition. Surface hardness and topography of the membrane was studied by adopting contact mode.

2.2.10. Determination of Entrapment Efficiency (EE)

Drug loaded vesicles dispersions were filled into the cellulose dialysis bag (MWCO 12 KDa) and dialyzed in PBS with constant stirring (60 rpm) under sink conditions for about 15 min.[30] Thus the un-entrapped drug was removed from the formulations and the absorption spectra of collected samples were recorded through UV-VIS spectrophometer (UVD-2950 Labomed Inc., USA). The EE was calculated by considering the following equation:

$$\text{E.E. (\%)} = \frac{T-C}{T} \times 100 \qquad (1)$$

where, T is the total amount of drug presents both in the sediment and supernatant, C is the amount of drug detected only in the supernatant. T and C values were determined colorimetrically.

2.2.11. In Vitro Drug Release Studies

The release of Px from the vesicular systems was quantified by measuring the Uv-VIS absorption spectra. The experiment was carried out by dialysis method as our group has been reported previously.[8]

2.2.12. Hemolysis Assay

Hemocompatability of the free and drug loaded vesicles were studied to understand the biocompatibility of the vesicles and drug.[31-33] EDTA stabilized freshly prepared blood sample was collected from human subject after obtaining the details. Guidelines provided by the Indian Council of Medical Research (ICMR), New Delhi, India was followed. Blood sample was centrifuged for 5 min at 1600 rpm to renounce the plasma, buffy coat and the top layer of the cell. The remaining RBC was then washed with sterlie isotonic PBS at least 5 times and then diluted with PBS. Dilute suspension of RBC was then mixed with PBS and distilled water as negative and positive control. Besides, the RBC suspension was mixed with different set of hybrid vesicles with or without drug. The mixture was gently vortexes and incubated for 2 h. The incubated mixtures were then allowed to centrifuge at 1600 rpm for 5 min; the supernatant was colorimetrically assessed at 541 nm.

2.2.13. In Vitro Cytotoxicity Studies on Human Blood Cell Lymphocyte (PBMC) and Human Neuroblastoma Cell Line (SH-SY 5Y)

Cells were seeded in 96-well plates at a density of 5 x 10^3 cells per well in 200 µL culture medium. After 24 h, the cells were treated with PBS, vesicles and drug encapsulated vesicles with increasing concentration of drug. After 24 h, media was replaced with MTT solution (10 µL of 5 mg/mL/well) prepared in PBS and incubated further for 3 h at 37 ^{o}C in a humidified incubator with 5% CO_2. Then 50 µL isopropanol was added to each well and plates were gently shaken for 1 min and absorbance was recorded at 595 nm (for MCF 7) and

620 nm (for SH-SY 5Y) by micro titre plate reader (Thermo). The experiments were repeated three times independently. Results were presented as mean of triplicates from three independent experiments.

3. Results and Discussion

3.1. Surface Pressure (π) – Area (A) Isotherm.

With the quest to understand the mixing of hydrocarbon chain, surface pressure (π) - area (A) isotherms of the individual as well as mixed components were obtained (Figure 1, panel A and B). A bilayer can be considered as superimposition of two monolayers. Nature of the molecular interaction between the components, lift-off area, excess free energy, film compressibility, *etc.*,[7,20] could define the nature of bilayer. The behaviour of mixed monolayer in the presence of Px would certainly be interesting over here. Px induced fluidization or repulsive interaction in lipid monolayer has already reported.[12,14,17] Interfacial behaviour of the individual components (SLC, IPA, DXDABs and cholesterol) as well as in the form of mixed monolayer was investigated by using Langmuir Balance. Monolayer isotherms for individual components were represented in Figure 1, panel A. SLC with unsaturation in one of its fatty acyl chains was in liquid-expanded state and the lift-off area was at 1.09 $nm^2 molecule^{-1}$. Being unsaturated it does not show any platue in the isotherm while undergoing transition from the gaseous to liquid phase. Lift-off area for the IPA was 1.02 $nm^2 molecule^{-1}$ because of the mismatch in its hydrocarbon chains.[7] The limiting area of IPA per molecule was 0.56 nm^2. Apparently, half of the limiting area per IPA should have been 0.28 nm^2, comparable to the limiting area per alkyl chain. The ideal limiting area per alkyl chain is 0.20 nm[34], quite lower than the value herein calculated. This implies that the bulky head groups of IPA play crucial roles in molecular packing.

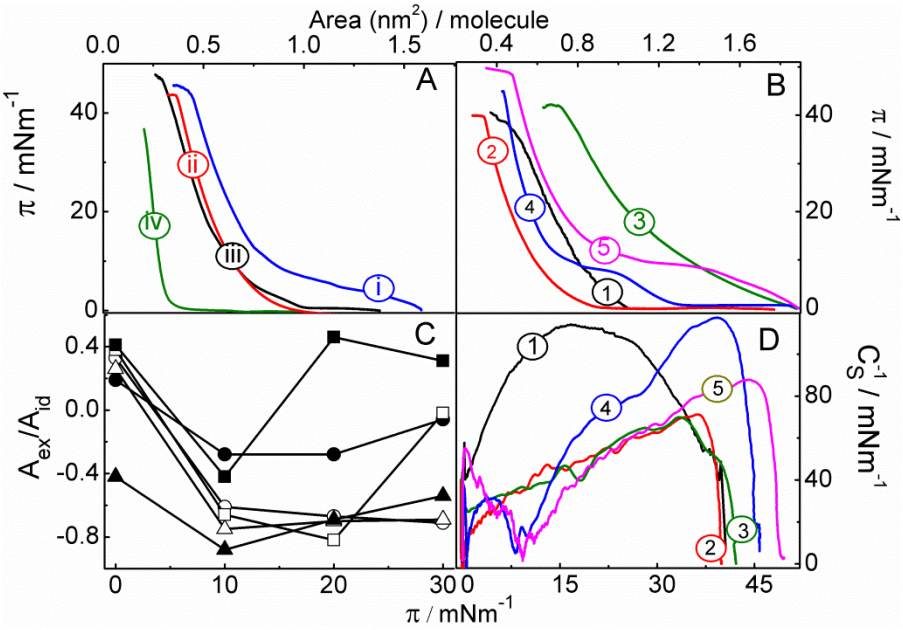

Figure 1. (A) π – A isotherms for the mono momolecular films of (i) DHDAB, (ii) SLC, (iii) IPA and (iv) cholesterol **(B)** mixed spread monolayers at the air-buffer interface: (1) HCV2, (2) 1+DDDAB, (3) 1+DTDAB, (4) 1+DHDAB and (5) 1+DODAB. Bi-tail surfactant amount was 5 mol%. **(C)** Dependence of A_{ex}/A_{id} on π for different sets of SLC/IPA with DHDAB. (○ and ●) HCV1; (Δ and ▲) HCV2 and (□ and ■) HCV3. Amount of DHDAB (○, Δ and □ 5 mole % and ●, ▲ and ■ 10 mole %). **(D)** Variation in the compressibility moduli (C_s^{-1}) with π. Surfactant amount 5 mol%: (1) HCV2; (2) 1 + DDDAB; (3) 1 + DTDAB; (4) 1 + DHDAB; (5) 1 + DODAB. Temperature 25 ºC.

DHDAB also produces SLC like expanded isotherm with lift-off at 1.59 $nm^2molecule^{-1}$. It could achieve surface pressure up to 45.4 mNm^{-1} with limiting area of 60 $nm^2molecule^{-1}$. The expanded isotherm was probably the outcome of electrostatic charge repulsion between the similar head groups.[7] Isotherms of HCV2 in combination with 5 mol% bi-tail surfactants (bis-C_{12} to C_{18}) are shown in Figure 1, panel B. Area condensation occurred with the addition of (DDDAB) (isotherm 2) and DHDAB (isotherm 4) and the vice-versa for ditetradecyldimethylammoniumbromide (DTDAB) (isotherm 3) and dioctadecyldimethylammoniumbromide (DODAB) (isotherm 5). For the isotherms 2 and 4, the limiting area was 0.34 and 0.44 $nm^2molecule^{-1}$ respectively indicating favourable area

condensation/contraction for these two mixed systems than the individual pure components. Favourable hydrophobic interaction resulted area condensation.

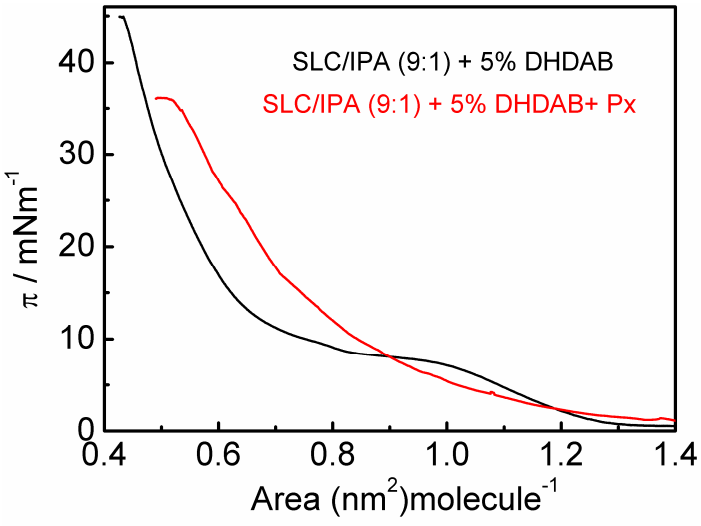

Figure 2. π – A isotherm of mixed monolayer with (Red) and without (Black) Px at 25 ºC

It is known that for acyl chains the constant segmental order parameter exists between C1 to C9. From C9 to C10 or higher, there occurs considerable disorder.[14] DDDAB and DHDAB have the major number of carbon atoms in the ordered state, which facilitate hydrophobic interaction leading to area condensation. Bi-tail cationic surfactants facilitate the dissociation of IPA[7] as the loss of $HTMA^+$ from the monolayer into the bulk. [35] This eventually leads to the area condensation. The expanded monolayer was the outcome for the systems with DODAB. Presence of C9 - C18 carbon atoms in the acyl chain brings relatively more disordered state in the monolayer, and hence produces expanded monolayer. Px significantly alters the π – A isotherms as revealed from Figure 2. Px being negatively charged (in PBS pH 7.4) occupies the bilayer region or better to say Px intercalated itself between the amphiphiles causing the expansion of the isotherm.

Variation in the compressibility modulus (C_s^{-1}), that indicates monolayer elasticity, with surface pressure for the same systems described in panel B are shown in panel D, Figure 1.

Maximum C_s^{-1} values were 113 and 117 mNm^{-1} for HCV2 and HCV2 + 5 mol% DHDAB respectively. Lower C_s^{-1} values for the other three bi-tailed surfactants were noticed when 10 % IPA was used. Fluidization[14,36] of the mixed monolayer (lower C_s^{-1} value) indicates relatively less miscibility or unfavourable packing among the components. As both the hydrocarbon chains of SLC and one chain of the IPA have sixteen carbon atoms, together they exhibit favourable hydrophobic interaction with DHDAB. However lower C_s^{-1} values for all the DXDABs, except DHDAB, were due to the disordered state of the mixed lipidic chains that promote the fluidization.

A_{ex}/A_{id} *vs.* surface pressure (π) plots (panel C, Figure 1) indicate the extent of miscibility among the amphiphiles. Ideal area of the mixed system can be calculated as:

$$A_{id} = x_1A_1 + x_2A_2 \quad (2)$$

where, A_{id} is the theoretically calculated average area per molecule. A and x represent the area per molecule and mole fraction of the components respectively. Suffices 1 and 2 represent component 1 (SLC/IPA, M/M + 30 mol% cholesterol) and component 2 (bi-tailed cationic surfactant + 30 mol% cholesterol) respectively. Deviation from the ideality (A_{id}) was calculated from the excess area (A_{ex}) as:

$$A_{ex} = A_{12} - A_{id} \quad (3)$$

where, A_{12} is the experimentally obtained area per molecule for the mixed monolayer. Positive deviation indicates repulsive interaction and vice versa. HCV1 and HCV3 with 10 mol% DHDAB execute positive A_{ex}/A_{id}. Electrostatic repulsion between the head groups result in the fluidization of the monolayer. However, HCV2 with 5 and 10 mol% DHDAB exhibited attractive interaction.

To prepare stable vesicle dispersion, one need to further consider the mixed monolayer studies and subsequent evaluation of different thermodynamic parameters of the interfacial mixing processes, viz., excess Gibbs free energy (ΔG_{ex}^{0}) that measures the extent of interaction among the components in mixed system with reference to the interaction of the components before mixing. The values of ΔG_{ex}^{0} can be determined by using the following calculation:

$$\Delta G_{ex}^{0} = \int_{0}^{\pi} (A_{12} - A_{id})\, d\pi \qquad (4)$$

Negative ΔG_{ex}^{0} indicates spontaniety of the mixing processes and vice versa. The hydrocarbon chain mixing between DxDABs with HCV2 are shown in Figure 3. In the present study, the chain mixing between component 1 (SLC/IPA/cholesterol) and component 2 (bi-tail cationic surfactants) was considered. Spontaneity of chain mixing in the absence (panel A) and presence of piroxicam (panel B) was also recorded. Magnitude of the negative free energy change was more profound for DDDAB and DHDAB, indicating better mixing of hydrocarbon chains in presence and absence of Px. However, DTDAB and DODAB exhibit less mixing between chains. Hence from monolayer study it was possible to assess the optimum stable vesicular system for carrying out the further studies. Minimum chain miscibility for DTDAB was noticed, like the other experiments, which indicates that DTDAB is not a suitable agent for the preparation of vesicles. Negligible ΔG_{ex}^{0} values for DDDAB and DHDAB in absence and presence of Px were recorded. An appreciable change in ΔG_{ex}^{0} was observed in the mixed monolayer having the drug and DODAB. The hydrophobic interaction between component 1 and DODAB in presence of Px become the predominant factor, that leads to the negative free energy change. In all the cases the maximum spontaneity was observed in 30 mNm^{-1}, which is also the pressure of the biological cell membrane. Being in the palisade layer, Px can induce expansion of the polar head groups.

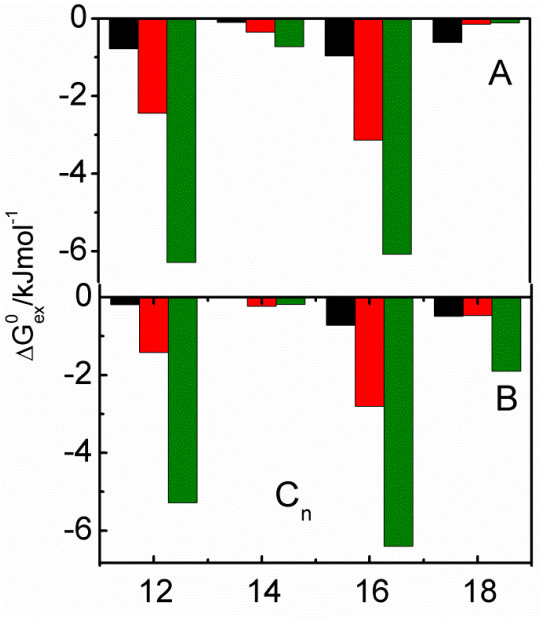

Figure 3. Gibbs excess free energy (ΔG^0_{ex}) for the interfacial mixing of lipids. Systems (HCV2) with the chain length of bi-tail cationic surfactant (5 mol%) at different surface pressures. Surface pressure (mN/m): 10 (black), 20 (red) and 30 (green). Panel A: mixed monolayer without Px. Panel B: mixed monolayer with Px.

3.2. Dynamic Light Scattering (DLS) Studies

Figure 4 describes the dependence of hydrodynamic diameter (d_h), zeta potential (Z. P.) and polydispersity index (PDI) of the vesicles on the composition and hydrocarbon chain length of the double tailed cationic surfactant. Vesicle size did not vary appreciably with the bi-tail cationic surfactant chain length for the system HCV1 and HCV2. Impacts of bi-tail cationic surfactants were insignificant upto 10 mol% IPA. However, size abruptly increased for HCV3. Excepting a few, sizes of the vesicles were in the range of 250 to 300 nm. 30% IPA in combination with SLC brings more disorder in bilayer as compared to 10% IPA. Hence added bi-tail cationic surfactant along with 10% IPA produced smaller vesicles than the 30% IPA.

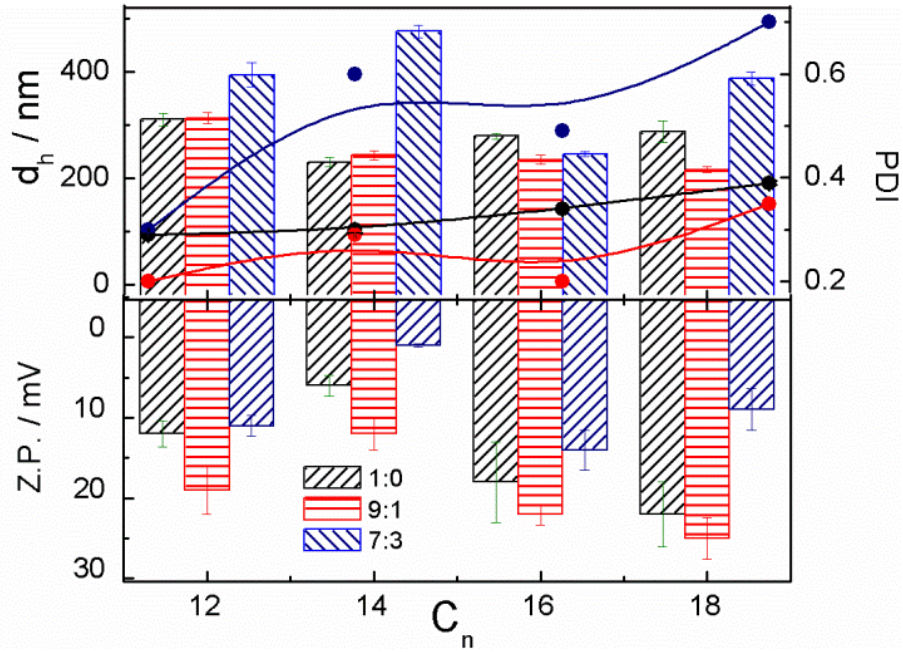

Figure 4. Variation of hydrodynamic size (d_h), zeta potential (Z. P.) and PDI of SLC/IPA hybrid vesicles with the bi tail surfactant chain length at 25 ºC. d_h and PDI were indicated by bars and solid lines respectively. SLC/IPA mole ratios are indicated in the figure and the bi-tail cationic surfactants amount was fixed to 5 mol%.

Z. P. holds the identity of the vesicles that prevents coagulation. Z. P. values were in the range of 10 to 28 mV for obvious reason. Z. P. increased with increasing hydrocarbon chain length. It is expected that with increasing chain length, inductive effect (+I effect) of the surfactant increases, which favours the charge separation between the counter ions around the polar surface leading to increased Z. P. Except 30% IPA, other vesicles were fairly monodispersed (PDI values less than 0.4). Px, being small and rigid, preferentially occupies the bilayer which causes enhanced d_h value. Although DLS studies provide the information concerning state of the vesicle with and without Px, but not adequate to come across the insight of bilayer which warrants further studies like electron microscopy.

3.3. Electron Microscopic Studies (TEM/ Freeze Fractured (FF)-TEM*)*

To figure out the size, morphology and the existence of the bilayer,[7,8,37] electron microscopic studies were performed in the form of normal TEM and freeze fractured-TEM measurements. Impact of Px could also be revealed from such studies. Representative TEM and FF-TEM microphotographs of HCV2 with 5 mol% DHDAB are shown in Figure 5, panel A_1 and B_1 respectively. Spherical morphology of the vesicle was confirmed from both the normal and FF-TEM studies. Additionally the formation of bilayer could also be established. Size of the vesicle was found to be comparable with DLS measurement.

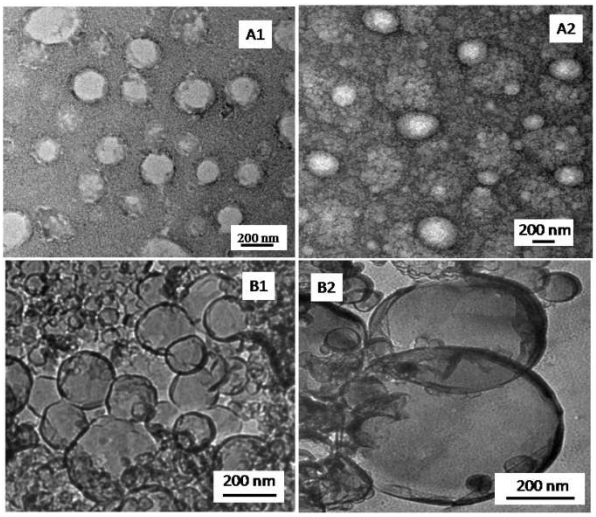

Figure 5. Conventional (A_1 and A_2) and freeze fractured (B_1 and B_2) transmission electron micrographs of HCV2/DHDAB in the absence (A1, B1) and presence (A2, B2) of piroxicam.

Influence of Px on the size as well as the morphology were revealed from the TEM and FF-TEM images shown in Figure 5 (panel A_2 and B_2). Heterogeneity in the vesicle sizes were due to the accumulation of Px in the bilayer. Thus spherically shaped vesicles offer Px an safe location in the bilayer zone.

3.4. Scattering (SANS and SAXS) Studies

3.4.1. Small Angle Neutron Scattering (SANS)

Bilayer thickness is an important parameter of the vesicles as it modulates the cellular activities by randomly altering its composition. The typical bilayer thickness under appropriate physiological condition is reported to lie in the range of 4 – 6 nm.[38-40] However, additives, like cholesterol and other amphiphile can change its thickness that eventually alter its biological function. Neutron (SANS) scattering has become one of the powerful prevalent techniques to measure the bilayer thickness.[39] Figure 6 shows some representative SANS plot, where the scattering intensity I(Q) of vesicles are plotted against scattering vector Q = 4 π Sinθ/λ; where 2θ is the scattering angle and λ is the wavelength of the neutrons. I(Q) values decreased with increasing Q that indicates lowering of scattering with the increase of scattering angle.

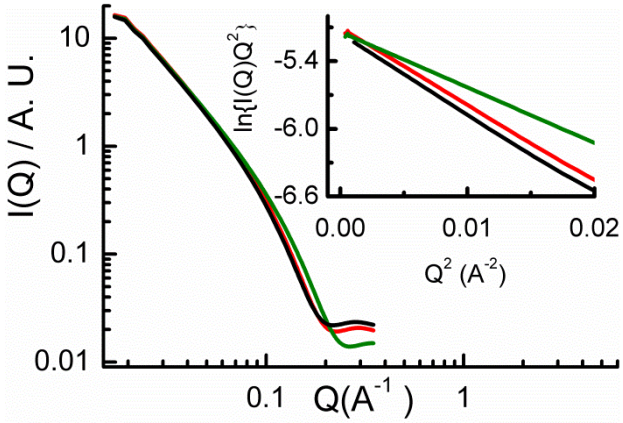

Figure 6. Variation of SANS intensity with scattering vector Q for HCV1 (black), HCV2/DHDAB (red) and HCV2/DHDAB/Px (green) at 25 ºC. Inset: Kratky plot for the same set of systems. Q value set between the region of 0.0013 Å^{-2} ≤ Q^2≤ 0.02 Å^{-2}.

3.4.2. Kratky Plot Analysis

Kratky plot is one of the important and simplest ways to get the bilayer thickness (Figure 6, inset).[41] Scattering intensity of vesicles within a small range of scattering vector can be written as:

$$I(Q) = I(0)\, Q^{-2} \exp(-Q^2 R_g^2) \quad (5)$$

where, I (0) is a constant and R_g is the radius of gyration. R_g can be calculated from the Kratky plot represented in Figure 5 B (inset). From the R_g value, one can easily evaluate the bilayer thickness parameter d_g as:

$$d_g^2 = 12\, R_g^2 \quad (6)$$

A careful assessment leads to select the region of 0.0013 $Å^{-2} \leq Q^2 \leq 0.02$ $Å^{-2}$ for fitting the experimental graphs. The driven Kratky plot from the experimental SANS data maintained the linear function and fitted well up to $Q^2 = 0.02$. At larger values of Q, deviation from linearity was observed (data not shown). The calculated values of R_g and d_g are summarized in Table 1 derived from equation 5.

Table 1. SANS data for the different cationic vesicles in the absence and presence of Px at 25 ºC. DXDAB's concentration was set to 5 mol%.

Vesicles	**R_g / Å**	**d_g / Å**
HCV1	139.25	40.87
HCV2/DDDAB	148.72	42.24
HCV2/DHDAB	156.49	43.33
HCV2/DODAB	163.72	44.32
HCV2/DHDAB/Px	158.27	63.58

3.4.3. Small Angle X-ray Scattering (SAXS)

Amphiphile molecules get self assembled spontaneously to form micro structure of different shapes. Identification of such structures like bilayer lamellar phase could be identified by SAXS studies.[40,42] SAXS data of cationic and Px loaded vesicles are graphically shown in Figure 7, where scattering intensities are plotted against the scattering vector Q.

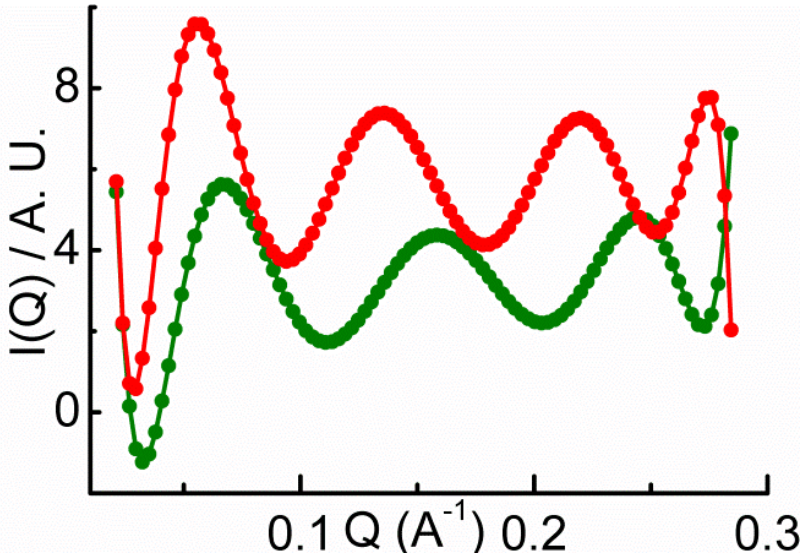

Figure 7. SAXS profiles of the cationic vesicles with and without Px. HCV2/DHDAB (red) and HCV2/DHDAB/Px (green) at 25 °C.

The periodic bilayer distance (d) can be evaluated form the equation, $d = 2\pi/Q$. The first order diffraction peak for HCV2/DHDAB and the same with Px appears at Q = 0.06 and 0.05 respectively, which correspond to d spacing of 125.7 and 104.7 Å respectively. Appearance of more than one Braggs diffraction peaks with equal spacing ($\Delta Q = 0.08$ Å^{-1}) indicate existence of vesicles.[25,42] Relative broadening of the diffraction maxima for Px loaded system (HCV2/DHDAB/Px) correspond to low electron density around the head group region due to the intercalation of the drug. Accumulation of Px in the palisade layer

causes the amphiphilic head groups to move apart from each other as already discussed in monolayer section.

3.5. Differential Scanning Calorimetry (DSC) Studies

Functionality and/or stability of vesicles are governed by the state of the lamellar phase. Exogenously added compounds can alter the activity and the state of the lamellar phase[43] which can be detected through DSC measurements. Comprehensive DSC studies on the thermotropic features of the vesicles bilayer with and without Px are expected to enable in understanding the impact of the drug on the membrane bilayer. Figure 8 describes the thermotropic features of SLC bilayer, hybrid bilayer, cationic and drug loaded cationic bilayers. Phase transition temperature (T_m) of SLC and hybrid vesicles appeared at -20 ºC,[44] that was completely abolished upon the addition of DXDABs'and Px. The disappearance of specific T_m indicates the heterogeneity of the bilayer region due to the insertion of surfactant and Px. Broadening of $\Delta T_{1/2}$ with less heat change was observed when DDDAB, DTDAB and DODAB were introduced into SLC/IPA, compared to DHDAB. The mixing of SLC/IPA with DHDAB produces relatively less compressible or more compact lamellar phase than the other three bi-tail surfactants and thus associated with lesser heat change.

Both the vesicles without and with Px exhibited exothermicity (Figure 8). Exothermic nature for HCV2/DHDAB was due to the hydration of the amphiphilic head groups around the vesicle surface. Px caused enhanced exothermicity reflecting higher extent of hydration. Results suggest the location of Px near the polar head group region (palisade layer) of the bilayer where it influences the extent of hydration by parting the polar head groups.

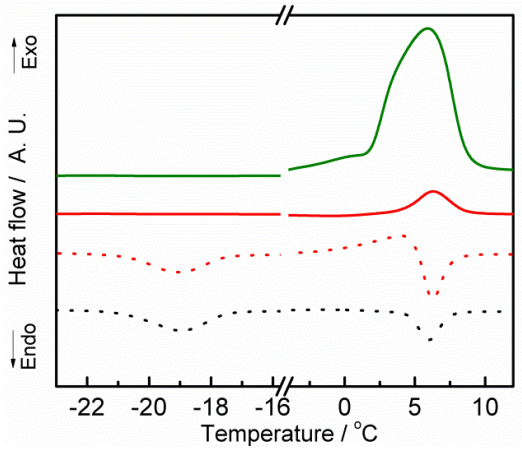

Figure 8. DSC thermograms of HCV1 (black dotted line), HCV2 (red dotted line), HCV2/DHDAB (red solid line) and HCV2/DHDAB/Px (green solid line). Scan rate: 2 ºC/min.

Other thermodynamic parameters such as changes in enthalpy (ΔH), heat capacity (ΔC_p) and half peak width ($\Delta T_{1/2}$) of SLC/IPA bilayer on addition of bi-tail cationic surfactants are shown in Figure 9. Acyl chains of DHDAB in SLC/IPA bilayer are in more order state relative to other bi-tailed cationic surfactants, the chain mismatch of DDDAB, DTDAB and DODAB with SLC/IPA causes the fluidization of the bilayer.[17]

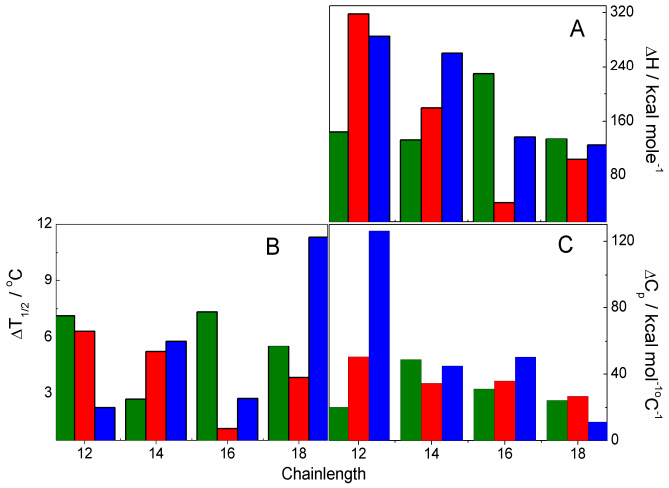

Figure 9: Variation of enthalpy (ΔH; panel A); half peak width ($\Delta T_{1/2}$, Panel B) and heat capacity (ΔC_P, Panel C) with bi-tail surfactant chainlength (bis-C_{12} to bis-C_{18}). Vesicle composition: SLC/IPA (1:0, Green); SLC/IPA (9:1, Red) and SLC/IPA (7:3, blue).

3.6. FTIR Studies

Phospholipids having different functional groups with different stretching frequencies act as the fingerprint in IR-zone.[45] Such groups are $-CH_2$, $-CH$, PO_4^{2-}, etc. FTIR spectra of SLC vesicles in combination with 5 mol% DHDAB and 5 mol% DHDAB + Px are shown in Figure 10. The antisymmetric and symmetric stretching frequencies indicate the hydrocarbon chains to move from the ordered to disordered states with the corresponding frequencies of 2918.5 and 2851.3 cm^{-1} respectively for the SLC vesicles.

The trans/gauche isomerisation needs to be considered as per as the $-CH_2$ stretching frequency is concerned. Increasing stretching frequency implies gauche conformation with more disordered state in the acyl chain.[46] Addition of DHDAB into the bilayer upshifted the antisymmetric and symmetric stretching frequency to 2925.3 and 2854.3 cm^{-1}, hence gauche conformation regain supremacy over the trans conformer. Similarly, increased stretching frequency (2927.7 cm^{-1}, antisymmetric) with relatively broad spectra was observed in case of added Px. Upshift in the $-CH_2$ stretching frequency with added DHDAB and Px make major population of the hydrocarbon chain in gauche form as depicted in the inset of Figure 10. Results suggest that the preferential position of Px was in the palisade layer of the bilayer region with disordered state.

The state of the membrane polarity could be known form the stretching frequency of phosphate group which is vital in the formation of aggregates and H-bonding. Lower is the frequency of the phosphate group higher is the extent of H-bonding with surface water molecule/hydration.[46] Zwitterionic head group of SLC is relatively less hydrated and for which a weak band appears at 1227 cm^{-1}. DHDAB downshifts the peak (1224 cm^{-1}) supporting the process of hydration as also reflected from DSC studies. Px downshifted the peak to lowest frequency (1220 cm^{-1}), reflecting greater hydration.

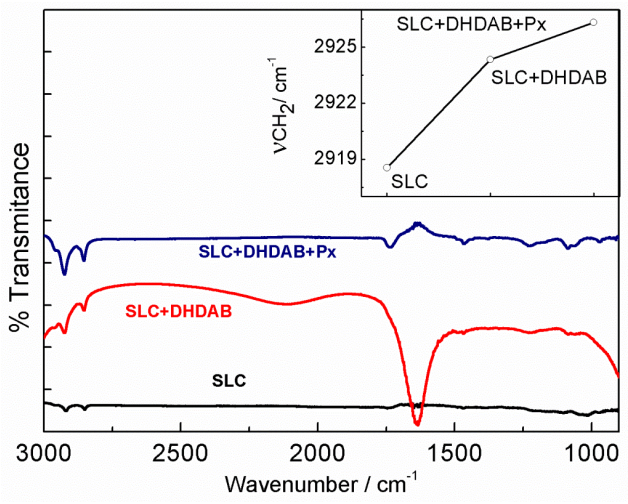

Figure 10. FTIR spectra of HCV1 (black), HCV2/ DHDAB (red) and HCV2/ DHDAB/Px (blue). Inset: Increment of $-CH_2$ stretching frequency with addition of DHDAB and Px on SLC bilayer.

Px is capable of modifying the orientation and the number of H-bonded water molecules in the palisade layer[14] and confirmed its position around the palisade layer. Px causes the formation of hole which facilitates the process of roughening the surface and causes higher extent of hydration as noticed in DSC. Thus the state of the acyl chain as well as the polar head group could be assessed when bi-tail surfactants and Px were added.

3.7. Atomic Force Microscopic (AFM) Studies

Atomic force microscopy is a direct imaging tool to visualise lipid bilayer with high resolution. It is well known that vesicles spontaneously transform into a solid supported bilayer (SSB),[47] when they are placed on freshly cleaved mica. The nanoscale properties of membrane and its ability to form solid supported bilayer (SSB) onto the mica substrate can be accessed from such studies. With an aim to understand the interaction of drug with model membrane, AFM studies were carried out as represented in Figure 11. Panel A and A1 elucidate the surface morphology and corresponding height analysis. Px occupying the palisade layer, reduces the compact packing of the lipid head groups.

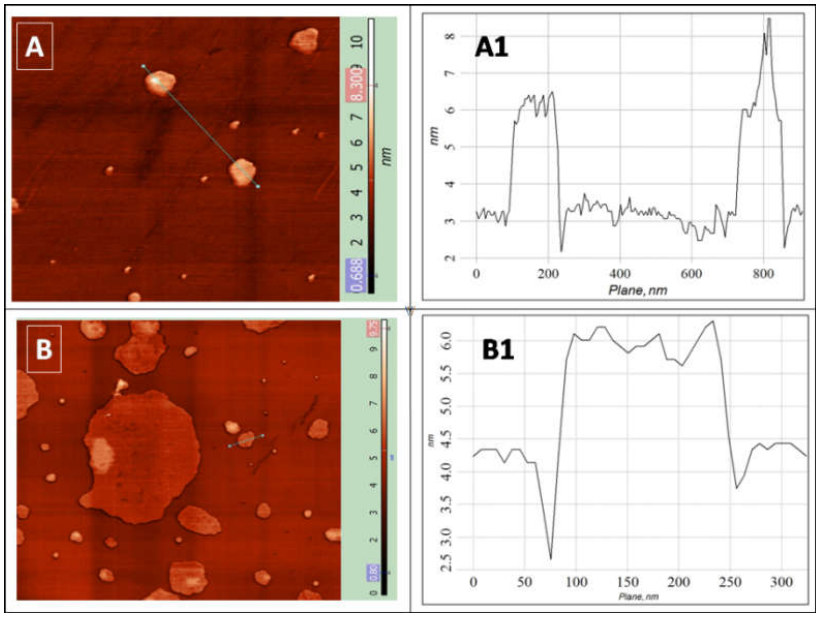

Figure 11. AFM images of solid supported bilayers. Images were recorded in liquid cell by tapping mode. Systems (A) HCV2/ DHDAB/Px (B) HCV2/DHDAB. Scan area: 2x2 μm^2. Panel A1 and B2: Height analyses of the bilayer surface of system A and B.

The height analysis of A1 shows few interesting facets. It helps to understand the rough bilayer surface due to the presence of number of amphiphiles along with unsaturated SLC. Additionally, increase in vertical height to the extent of few nanometers was also noticed. The height increment was due to the intercalation of Px between the lipidic components that pushes the polar head groups to stay away from each other and thereby resulting rough surfaces. Px could also result in the thickening of the bilayer, similarly observed in the SANS studies. Intercalation of Px between the lipid aggregates disrupts the lipid-lipid interaction and thus creates an opportunity to generate rough surface with hole formation on the surface of the bilayer domain.

AFM image of the SSB without Px has been shown in panel B Figure 11. Height analysis of the same shows the surface morphology was rather smooth than the SSB interdigited with

Px. Presence of bi-tail surfactant with similar head groups along with IPA and SLC associated with one unsaturation cause the surface slightly heterogeneous with the bilayer thickness in the range of 4 to 5 nm. The fluid domain of SSB was noticed as the consequence of the supremacy of gauche conformation of the acyl chain marking from FTIR measurement.

3.8. Entrapment Efficiency (EE)

Entrapment efficiency of different set of vesicles (HCV1, HCV2 and HCV3) to encapsulate the drug Px was tabulated in Table 2. The EE was found to be directly linked with amount of IPA and the chain length of bi-tail surfactants.

Table 2. % of EE of cationic vesicles with ascending chainlength and drug concentrations. Where HCV1, SLC/IPA (1:0) ; HCV2, SLC/IPA (9:1) and HCV3, SLC/IPA (7:3)

System	% of Entrapment Efficiency of Px / µM				
	10	**20**	**30**	**40**	**50**
HCV1 + DDDAB	36 ± 1.3	42 ± 0.9	45 ± 1.1	51 ± 1.6	52 ± 1.2
HCV1 + DTDAB	6 ± 2.2	9 ± 1.5	14 ± 1.6	17 ± 2.0	18 ± 1.9
HCV1 + DHDAB	41 ± 0.8	51 ± 1.1	59 ± 0.7	68 ± 1.4	70 ± 1.2
HCV1 + DODAB	42 ± 1.0	50 ± 1.2	58 ± 1.6	69 ± 0.9	70 ± 1.8
HCV2 + DDDAB	52 ± 0.9	59 ± 1.6	67 ± 1.2	75 ± 1.2	77 ± 1.9
HCV2 + DTDAB	7 ± 1.8	13 ± 2.3	16 ± 1.6	21 ± 1.8	23 ± 1.5
HCV2 + DHDAB	59 ± 0.5	68 ± 1.6	81 ± 1.5	92 ± 1.1	93 ± 0.6
HCV2 + DODAB	65 ± 1.2	76 ± 0.9	83 ± 0.8	96 ± 1.4	97 ± 1.8
HCV3 + DDDAB	42 ± 1.5	51 ± 1.9	60 ± 0.5	67 ± 1.6	68 ± 0.7
HCV3 + DTDAB	4 ± 1.6	10 ± 2.6	14 ± 2.6	19 ± 1.4	20 ± 1.8
HCV3 + DHDAB	52 ± 1.1	60 ± 1.4	71 ± 1.6	82 ± 1.4	82 ± 0.8
HCV3 + DODAB	60 ± 1.6	63 ± 1.1	75 ± 0.8	83 ± 0.4	85 ± 1.6

As shown in the table the EE values for the cationic vesicles comprised of DTDAB did not show any significant loading of Px. Throughout vesicle characterization, it was found that DTDAB comprising vesicles did not show any productive information as it was mostly unstable. However DDDAB, DHDAB and DODAB comprising vesicles show reasonable EE. Interestingly it was found that set of vesicles mixed with DHDAB and DODAB resulted

maximum and almost equal EE. Free energy calculation for the chain mixing derived from monolayer study suggests that systems with DHDAB and DODAB in combination with Px produced spontaneous mixing. Both DHDAB and DODAB associated with longer chainlength expected to be in more disordered state creating enough space in the bilayer region to accumulate more drug as compared to DDDAB.

To achieve the maximum E. E., we varied the drug concentration and it was found that 40 μM was the optimum loading concentration. The systems were found to be saturated beyond this concentration as reflected from Table 2. Among all, HCV2 type vesicles results maximum EE other then HCV1 and HCV3. HCV2 accompanied with 10 mol% IPA have a significant role for providing hydrophobic interaction as reflected from monolayer studies. Relatively less EE for HCV1 and HCV3 could be analyzed as the absence of IPA in HCV1 produces less hydrophobic interaction to accumulate enough drugs; whereas HCV3 was being carried with 30 mole % IPA produces rigid bilayer.

3.9. In Vitro Drug Release Studies

An excellent drug delivery must have the ability to undergo sustained release. With an aim to understand the nature of drug release from the hybrid cationic vesicles, HCV2 with varying hydrocarbon chainlength was chosen to quantify the release of Px. HCV2 was chosen on the basis of its highest EE. Figure 12 represents the cumulative release of Px for 48 hr. The results indicated that the release of Px from the vesicles was composition dependent. As one can see from the Figure 12, apart from the native Px (Px in PBS), vesicles comprised of DTDAB involved almost complete drug release with in 5 hr. Whereas other three sets of vesicles mixed with HCV2 + DDDAB, HCV2 + DHDAB and HCV2 + DODAB put on a view of 82, 92 and 60% total drug release respectively in 48 hr. It took almost 3 hr for the

native Px to completely release from PBS, indicating the superiority of hybrid cationic vesicles for the sustained release.

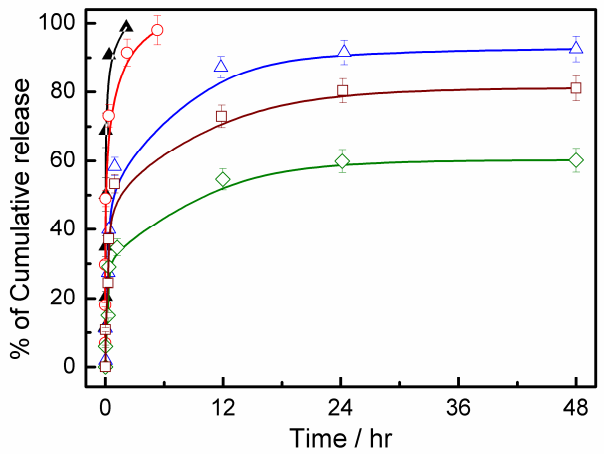

Figure 12. In Vitro release profile of Px from PBS (▲) and from SLC/IPA (9:1, M/M, HCV2) with varying hydrocarbon chainlength. DDDAB (□), DTDAB (○), DHDAB (Δ) and DODAB (◊). All the experiment was performed in PBS and repeated three times. Temperature: 25 ºC

The pattern of drug release could be classified into two steps. The first one was the burst release that happened within 1 hr followed by a sustained release. Vesicles having DTDAB produces maximum burst release (60%) as compared to other three sets of vesicle. The extent of burst release for HCV2 + DDDAB, HCV2 + DHDAB and HCV2 + DODAB were 31, 33 and 24% respectively, then followed a sustain release. The rapid release of Px from HCV2 + DTDAB was due to the instability of the vesicles as well as poor EE. From the results of EE it was found that HCV2 + DHDAB and HCV2 + DODAB have similar EE. However HCV2 + DODAB involve relatively slow release compared to the vesicles having DHDAB and DDDAB. The slow release of Px was due to the favourable hydrophobic interaction that enhanced upon the encapsulation of Px. Which further confirm free energy

calculation. But Px resides in the membrane bilayer of HCV2 + DHDAB and HCV2 + DDDAB creating enough disorder to swipe out the drug.

3.10. *In Vitro* Cytotoxicity Studies

3.10.1. On Human blood cell lymphocyte (PBMC) and Human Neuroblastoma cell line (SH-SY 5Y)

Biocompatibility of the hybrid vesicles without or with Px is one of the primary criteria once the drug delivery issue of the vesicles is concerned. RBC is one of the popular membranous systems to check the biocompatibility of drug delivery systems. However, before undertaking the above two cytotoxicity experiments, hemolysis studies were performed. Interaction between RBC and vesicles without or with the drug (positive and negative control) is depicted in Figure 13 as measured through UV-VIS absorption spectra. Hemolysis for both the systems was found to be ~ 2%. Systems having <5% hemolysis are regarded as hemocompatable and hence the studied combinations can be considered as non-toxic to human physiology[32]

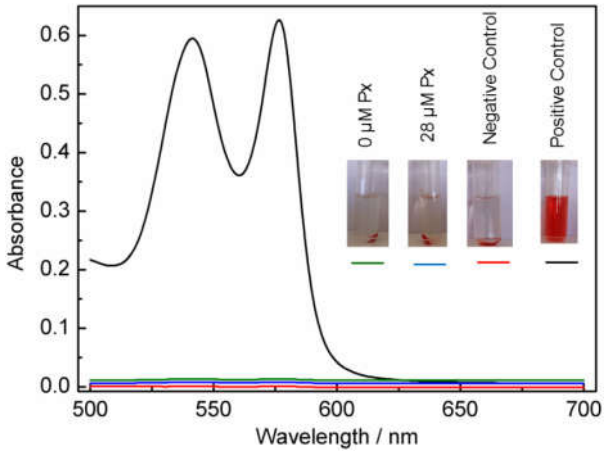

Figure 13. UV-VIS spectra of vesicles with and without Px with respect to the controls. Absorbance measured at 541 nm.

In-vitro cytotoxicity of HCV2 vesicles loaded with Px was assessed on normal human blood cell lymphocytes shown in Figure 14. Compared to the control (PBS), both the blank

and Px loaded vesicles were non-toxic towards normal human blood cell lymphocytes. Same set of formulations were taken to study the impact of Px on human Neuroblastoma cell line (SH-SY 5Y), via MTT analysis[48].

Formulations were non-toxic to normal human blood cell lymphocytes; however when the same set of samples were administered in the SH-SY 5Y cell line (cancerous cell), Px encapsulated vesicles showed substantial cytotoxicity (Figure 15). Px within the experimental concentration range, in PBS, did not show significant cytotoxicity compared to its encapsulated state.

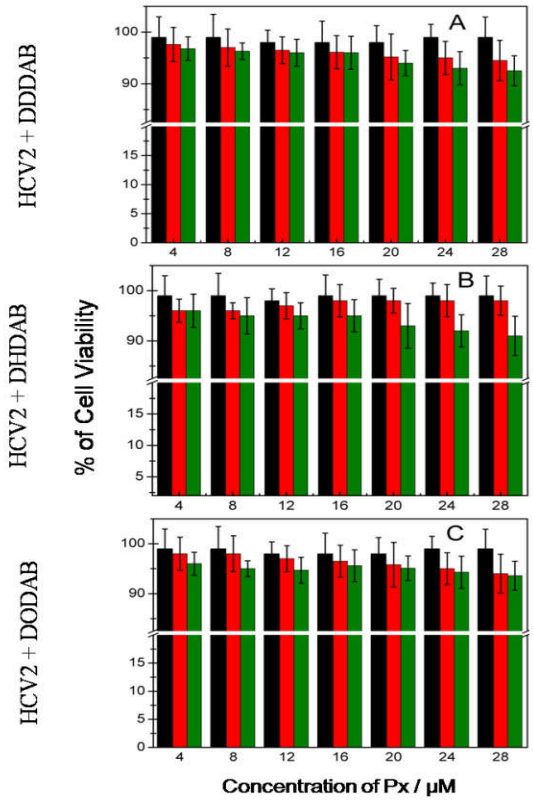

Figure 14. *In-vitro* cytotoxicity studies of vesicles with and without Piroxicam on normal human blood cell lymphocytes. PBS, control (black); HCV2/DxDAB (red) and V2/DxDAB/Px, (green). The mean standard deviations are indicated in the bar.

The activity was studied for 48 h and Px encapsulated HCV2 with varying chainlength show much smaller IC_{50} values (15.65, 21.74 and 25.32 for HCV2/DDDAB/Px, HCV2/DHDAB/Px and HCV2/DODAB) than the values of drug in PBS alone (IC_{50} = 534 μM). Vesicles without Px were substantially nontoxic as reflected from Figure 15. It reveals that the cytotoxicity nature of the drug (with respect to the cancer cell line) gets enhanced when entrapped in the vesicles.

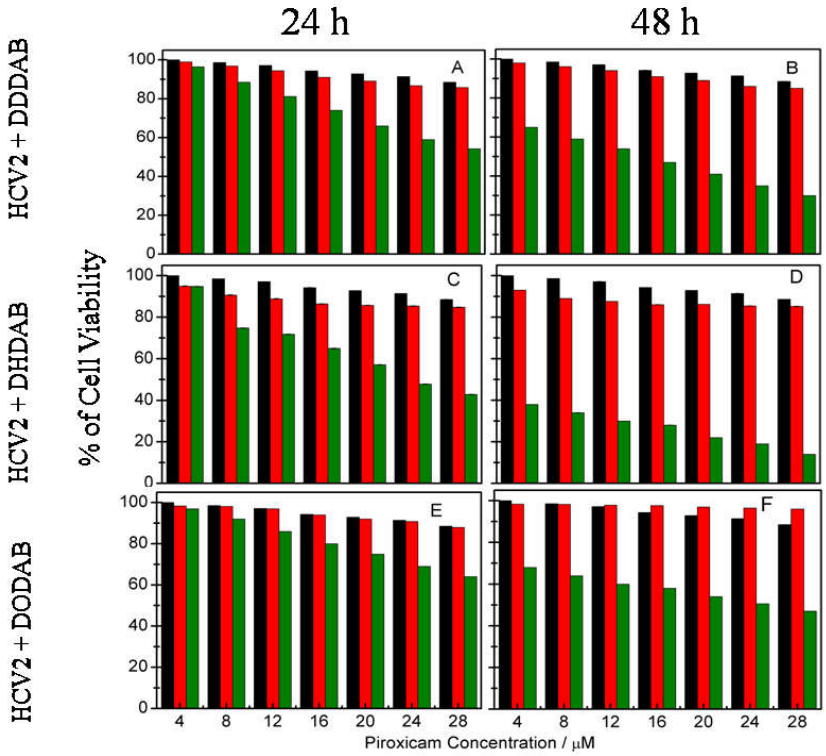

Figure 15. *In-vitro* cytotoxicity studies of vesicles with and without Piroxicam at 24 and 48 h on human Neuroblastoma cell line (SH-SY 5Y). PBS, control (black); HCV2 + 5 mol% DxDAB (red) and HCV2 + 5 mol% DxDAB + Px, (green). The mean standard deviations are indicated in the bar.

The cell line was treated with varied concentration of drug (5 to 28 μM) and HCV2/DHDAB/Px showed highest cytotoxicity. Within 24 h it achieved IC_{50} value 21.74 μM. However it took 48 h to get IC_{50} values 15.65 and 25.32 μM for the systems

HCV2/DDDAB/Px and HCV2/DODAB/Px. The time lag to achieve such values was due to the slow release of Px form the vesicles as compared to HCV2/DHDAB/Px. Such low concentration activity for Px was due to the controlled and sustained release of the entrapped drug from the vesicle.

4. Conclusion

Monolayer studies for the mixed systems (SLC/IPA/bi-tail cationic surfactant + 30 mol% cholesterol) put on a view of attractive interaction between SLC with dihexadecyldimethylammonium bromide (DHDAB) and didodecyldimethylammonium bromide (DDDAB) when 10 mol% IPA was present in the bilayer. DHDAB produce maximum spontaneity in chain mixing as calculated from free energy changes. Morphology of the vesicles with and without Px was revealed from the DLS and electron microscopic studies. Accumulation of the Px in the bilayer was noticed from FF-TEM measurement. Vesicles with positive zeta potential (Z. P.) were stable for substantial time period and maintained monodispersity, except a few combinations. Existence of unilamellar vesicles were confirmed form electron microscopy, SANS and SAXS studies. d-spacing between two adjacent lamellar phases were determined from SAXS. Both DSC and IR studies support the occupation of the palisade layer by small rigid Px molecules. Increased proportion of the gauch conformer of the acyl chains were noticed from FTIR studies. Px fluidizes the bilayer as studied by the AFM measurements. Bilayer thickness of the membrane was found to be around 5 nm as revealed from SANS studies. While the drug hosted vesicles were non-toxic to normal human blood cell lymphocyte (with less than 2% hemolysis), however, it was toxic over Human Neuroblastoma cell line (SH-SY 5Y). Because of the blood brain barrier, the drug avilibity in curing the Neuroblastoma cell line was insignificant. Vesicles of these kinds could be used as drug delivery, gene therapy or other therapeutic agents. Also the efficacy of

drug loaded vesicles towards the Neuroblastoma cell line and its impact on blood brain barrier could be considered as future perspective.

References

References are given in BIBLIOGRAPHY under Chapter II (pp. 154-157).