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NEOTERIC SUBSTITUTE, ION PAIR AMPHIPHILE, MODULATES PHYSICOCHEMICAL PROPERTIES OF BIOMIMICKING MEMBRANES

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ABSTRACT

Thin bio membranes frequently act as a physical border between the many compartments inside a cell and the continuous surroundings outside the cell.The main aim of the study is Neoteric Substitute, Ion Pair Amphiphile, Modulates Physicochemical Properties of Biomimicking Membranes**.** Under continuous stirring, a 0.1M aqueous solution of SDS was introduced drop-wise to a stoichiometric quantity of aqueous HTMAB solution.Stable vesicle dispersions were prepared using SLC and IPA (HTMA- DS) at varying mole fractions.

Keywords: Neoteric, Substitute, Physicochemical, Amphiphile,

1. INTRODUCTION

Thin bio membranes, which typically act as a physical border to separate the different compartments from the surrounding continuous surroundings, compartmentalise intracellular components. Several physicochemical processes, which in turn rely on the composition of the membrane bilayer, are under the direct control of biological cells. Studies of biological and biomemetic membranes have yielded a wealth of data that may be used to tease apart the membranes' nuanced structure and appreciate their function in natural settings. The functional interface in bio membranes allows for a wide range of applications; these membranes may bind to and regulate enzymes, peptides, and even DNA. Further studies on the membranes themselves reveal that they play an active part in several biochemical procedures including adhesion, signalling, regulating the Na+/K+ balance, fusing cells, etc. What bio membranes are really capable of relies heavily on the materials that make them up (phospholipids, cholesterol, and regulatory protein, etc.).

Phospholipids, a key constituent of cell membranes, may self-assemble into vesicles in the presence of water. Natural phospholipids are often employed in the synthetic formulation of vesicles for the purpose of learning more about their varied physicochemical characteristics; this is because of their amphiphilic nature. During the last several decades, studies of biomemetic membranes have uncovered their potential use in fields including medicine transport, gene therapy, DNA transfection, etc. The drug's caring properties originate from its one-of-a-kind hydrophilic and hydrophobic charisma. Because of their biocompatibility and durability, vesicles have garnered a lot of attention in recent years.

2. LITERATURE REVIEW

Havlíková, Martina &Szabová(2021) Here, we investigate cholesterol's impact on the thermodynamic characteristics of ionic amphiphilic couples in vesicular membranes. Ionic amphiphilic pair system hexadecyltrimethylammonium-dodecyl sulphate, supplemented with 10 mol% dioctadecyldimethylammonium chloride, was selected for a comprehensive investigation of vesicle characteristics. The effects of a wide temperature range (10-80 °C) and a wide range of cholesterol concentrations (0-73 mol%) were investigated. Along with the variation in surface water mobility, we tracked the size distribution, membrane fluidity, and surface layer under these circumstances. We next integrated the obtained values into suitable graphs and looked for correlations between them. It was discovered that the size of vesicular systems is little influenced by temperature in stable systems that satisfy the criteria of unimodal size distribution with a PDI value below 0.3. Nonetheless, when looking at the membrane's hydration and fluidity, researchers detected considerable modifications; these, however, did not have an effect on the vesicular systems' short-term stability. Given these findings, it is feasible to modify the vesicular system's composition with respect to fluidity and membrane hydration while keeping the size distribution and short-term stability constant.

Shobhna, Shobhna&Kumari(2020) In this chapter, we provide a summary of the research on the effects of tiny amphiphilic compounds and new solvents on biomembrane structure and stability. Small amphiphiles and custom new solvent molecules have found widespread use and significance in pharmaceutical industries and biophysics for their ability to alter the physiochemical characteristics of plasma membranes. By altering the structure of biomembranes with molecules of varied size and polarity, such as DMSO, ethanol, acetone, ILs, and DESs, novel routes for drug transport and administration have been made possible. Since the physiochemical nature of the molecules surrounding the biomembrane is just one component among many, including the kind of lipids, the various lipid compositions that make up a bilayer, the bilayer phases, and the temperature, any physical change in the biomembrane is a complex process. So, it is vital to suggest their mode of action, enhance their applicability, and limit their negative effects on the biomembranes by having a full knowledge about all the potential interactions between such compounds or solvents and biomembranes.

Karmakar, Gourab&Nahak(2018) High stability of NLCIPA formulations was confirmed by analytical methods such as dynamic light scattering, differential scanning calorimetry, transmission electron microscopy, fridge fractured electron microscopy, and atomic force electron microscopy, with the NLCIPA containing 30 mol% SLC being the most effective. Oleanolic acid (OA) loaded NLC and NLCIPA formulations were subjected to an experimental assessment of their interfacial, solution phase, and thermal properties, with results showing that OA accumulated on the palisade layer and drug acquisition was greater in NLCIPA than in standard NLC. As if that wasn't encouraging enough, NLCIPA formulations were also shown to be much more stable in their release of OA. During cytotoxicity experiments on hepatocellular carcinoma, hepatocyte-derived carcinoma, and colorectal carcinoma cancer cell lines, the OA loaded NLCIPA showed more cytotoxic activity than the NLC. So, NLCIPA formulations might be seen as a potentially useful alternative to standard NLC for enhancing OA's therapeutic effectiveness as an anticancer medication.

Guha, Pritam& Roy, Biplab&Karmakar(2015) A new class of chemicals called on-pair-amphiphiles (IPAs) may one day serve as an innovative replacement for phospholipid. Synthesized by combining equal parts of aqueous solutions of hexadecyltrimethylammonium bromide (HTMAB) and sodium dodecylsulfate (SDS), IPA was tested as a possible replacement for the naturally occurring phospholipid soylecithin (SLC). Cholesterol and SLC/IPA mixtures of varying concentrations were used to create vesicles. The results of surface pressure () - area (A) readings were analysed to see how IPA affected SLC. The thermodynamic parameters were calculated, and it was determined that the ratio of SLC to IPA determines the degree to which the components mix at the interface. During the course of 100 days, the hydrodynamic diameter, zeta potential, and polydispersity index were tracked to gain insight into the solution behaviour of the bilayers in the form of vesicles. Electron microscopic analyses also looked at the vesicles' size and shape. Unusual behaviour was seen in systems with 20 and 40% IPA concentrations. The hydrocarbon chain length and the density of the head group packing were found to have an effect on the thermal behaviour of the vesicles as measured by differential scanning calorimetry. The vesicles' ability to entrap the cationic dye methylene blue (MB) was measured by measuring their entrapment efficiency (E.E.). It was discovered that the vesicles' ability to entrap the dye varied with the quantity of IPA. For certain stable dispersions, the E.E. was shown to be in excess of 80%.

Panda, Amiya&Possmayer(2005) In terms of sensitivity and longevity, surfactant ion selective membranes constructed utilising various sets of catanionic species have been proven to be superior to the membranes developed before. Coacervates of cetyltrimethylammonium (CTA+) ion with dodecylsulfate (DS-), dodecylbenzenesulfonate (DBS-), dioctylsulfosuccinate (AOT), deoxycholate (DC-), and taurochenodeoxycholate (TCDC) ions in 1:1 molar ratio were used as the carrier complexes in the membrane While the membrane electrodes' electrochemical behaviour was not always Nernstian, linearity and abrupt breaks at the crucial micelle concentration were seen in plots of cell emf against log[surfactant] (CMC). Surface pressure ()-

area (A) measurements were used to examine the film behaviour of complex aggregates (coacervates) at the air-water interface. The experimental data was analysed to determine the limiting area per molecule, the maximum feasible surface pressure, the compressibility of the film, and other similar properties. In compressed monolayers doped with a fluorescent probe (NBD-PC), epifluorescence imaging revealed the formation of probe-excluded areas.

3. METHODOLOGY

3.1 Materials

L-α-phosphatidylcholine (soylecithin, SLC, from soybean) was obtained from EMD Chemicals, Germany. A. R. grade sodium dodecylsulfate (SDS) [CH3(CH2)11SO4Na], hexadecyltrimethylammonium bromide (HTMAB) [C16H33N(CH3)Br], 1,6-diphenyl-1,3,5-hexatriene (DPH), 7-hydroxycoumarin (7HC) and (3β)-cholest-5-en-3-ol (cholesterol) were the products of Sigma-Aldrich Chemicals Pvt. Ltd. (USA). Products from Merck Specialties Pvt. Ltd, India included A.R. grade disodium hydrogen phosphate (Na2HPO4, 12H2O), sodium hydrogen phosphate (NaH2PO4.2H2O), sodium chloride (NaCl), methylene blue (MB), and chloroform (HPLC grade). All compounds were utilised as supplied, despite claims of purity levels of 99.9% or more. For making solutions, we utilised double-distilled water with a specific conductance of 2-4 S (at 25 o C).

3.2 Methods

3.2.1 Preparation and Isolation of IPA

Disodium hydrogen phosphate (Na2HPO4, 12H2O), sodium hydrogen phosphate (NaH2PO4.2H2O), sodium chloride (NaCl), methylene blue (MB), and chloroform were all available from Merck Specialty Pvt. Ltd., India (HPLC grade). Notwithstanding assurances of purity of 99.9% or above, all chemicals were used in their given forms. Double-distilled water of 2-4 S specific conductance was used in all of our solutions (at 25 o C).

3.2.2 Preparation of Vesicles

Thin film technology was used to create small unilamellar vesicles (SUVs). At a 10:0, 9:1 molar ratio, SLC was added to IPA. With 30 mol% cholesterol, an 8:2:7:3:6:4:5:5 ratio is also possible. Chloroform was used to dissolve a measured quantity of SLC, IPA, and cholesterol in a round-bottom flask, and the solvent was then evaporated using a rotary evaporator. At last, the nitrogen flow eradicated the remaining solvent (N2). After obtaining the thin film, it was rehydrated in PBS (0.1 M Na2HPO4, 0.1 M NaH2PO4, 100 mM NaCl, pH 7.4) at 70 o C for 1 hour, which is above the chain melting point of all the lipidic components. After being flash-frozen at 20 oC, it was allowed to defrost in an ultrasonic water bath at room temperature for 15 minutes.

4. RESULTS

4.1 Surface Pressure (π) – Area (A)

Isotherm As studying pure and mixed lipidic monolayers at the necessary combination is not achievable in the form of a bilayer, a Langmuir-Blodgett trough was used instead. Moreover, a variety of physicochemical properties, including molecular structure and subsequent interaction between components, limiting area, excess thermodynamic potential, film compressibility, etc., were assessed on the basis of these data. While monolayers are just half of a bilayer and have a flat rather than curved shape, the knowledge gained from studying them may be successfully applied to other membrane systems, such as liposomes or cell membranes. 31 In this context, surface pressure-area isotherms were recorded for SLC +IPA combinations at the air-PBS buffer (pH 7.4) interface in the presence of 30 mol% cholesterol. Figure 1 displays the -A isotherms for various mixtures of SLC+IPA containing 30 mol% cholesterol. For SLC, the limiting area was shown to be 1.07 nm2 molecule-1, whereas for IPA it was 1.08 nm2 molecule-1.

It was determined that the results were consistent with those reported in the published papers. Unsaturated lipid monolayers appear as a uniformly expanding liquid phase. At atmospheric pressure, IPA has a limitation area of 1.08 nm2 molecule-1; at the interface, the limiting area per alkyl chain is 0.54 nm2 molecule-1; this value is bigger than the theoretically stated value of 0.20 nm2 molecule-1 per single chain. It is possible that the IPA's greater head group bulk is due to the tighter packing of molecules at the interface, as shown by this bigger limiting value. Moreover, these distinctions may be attributed to the hydrocarbon chains' non-parallel orientations. The cholesterol with the smallest lift-off area (0.3 nm2) tends to interdigitate itself into the parallel-oriented bilayers.

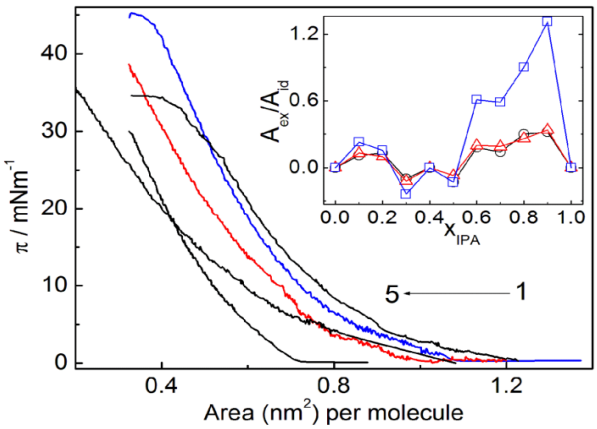

Figure 4.1 Surface pressure (π) – area (A) An air-buffer interface isotherm for SLC+IPA monomolecular films with 30 mol% cholesterol.

By comparing the isotherms of pure SLC and pure IPA, there was a clear deviation in the case of mixed monolayers ((SLC+IPA). For systems with = 0.2, we observed elongated isotherms, with the limiting area being 1.21 nm2 molecule-1. At a starting concentration of 0.3, the mixed monolayer entered a condensed condition that persisted up to a concentration of 0.5.

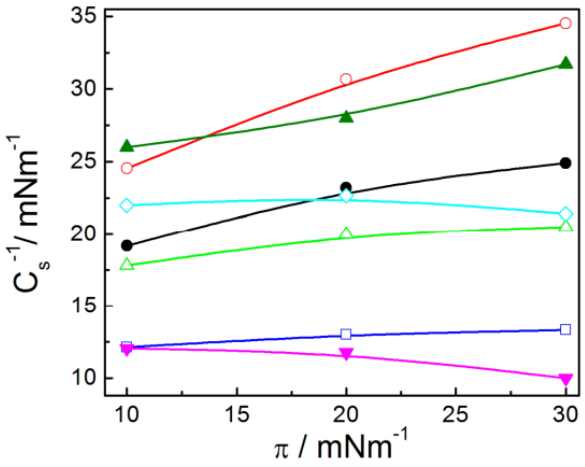

Figure 4.2 Variation in the compressibility modulii (C_S-1) Using the surface tension of SLC+IPA pseudo binary monomolecular films. Cholesterol at a concentration of 30 mol.

All the mixed monolayers in the current study were in the LE phase, with compression moduli ranging from 12.5% to 50% mNm-1. C_S^{-1} Surface pressure was found to raise values for = 0.1 and decrease them for = 0.5. Lack of dependency on surface pressure C_S^{-1} was found for hybrid monolayers with 20 and 40 mol% IPA, correlating well with prior work. Lowest C_S^{-1} values were obtained for both the IPA-only system and the system including 30% IPA. These monolayers were more compressible than their saturated counterparts because of their unsaturation. IPA had lower compressibility than SLC because the acyl chain was saturated. This is a frequent enough observation to make.

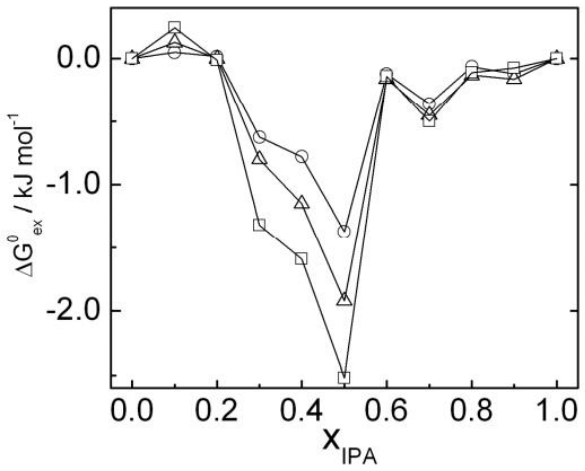

Figure 4.3 Variation of excess free energy (ΔG^0_{ex}) for the SLC+IPA mixed monolayer systems (in presence of 30 mol% cholesterol) with the mole fraction of IPA (x_{IPA}) at different surface pressure (mNm-1) : O, 10 ; Δ, 20 and □, 30. Temp. 25 o C.

The curve fit indicated that SLC and IPA do not mix perfectly; in fact, a positive divergence from the optimum behaviour was seen for the system containing 20 mol% IPA. It is possible that the release of HTMA+ from IPA during mixing is related to the repulsive interaction between the polar head groups and the Columbic force. Once again, non-spontaneous mixing was seen when the cis orientation of one of the fatty acyl chains in SLC resisted the IPA's attempts to condense. Deviating from their negative values, ΔG^0_{ex} for = 0.3, 0.4 and 0.5 indicates that stable monolayer formation at their preferred arrangement is based on the addition of IPA to SLC. An associative behaviour develops between SLC and IPA due to the saturation of IPA and the strong van der Waals force of contact and hydrogen bonding between the two. When IPA concentration increased, a stable mixed monolayer formed. The monolayer with 40% IPA deviated from the pattern seen in the systems with 30% and 50% IPA, resulting in a much smaller negative value of ΔG^0_{ex}contrasted with, as opposed to, the other two. An unfavourable interaction between SLC and IPA may be to blame, since HTMA+ tends to dissolve (when is greater) in the presence of quaternary ammonium ion of the choline group and get solubilized into the subphase. Thus, the marginally negativeΔG^0_{ex}likely result from the associative electrostatic interaction between the ammonium ion and in a solution containing 40% IPA.

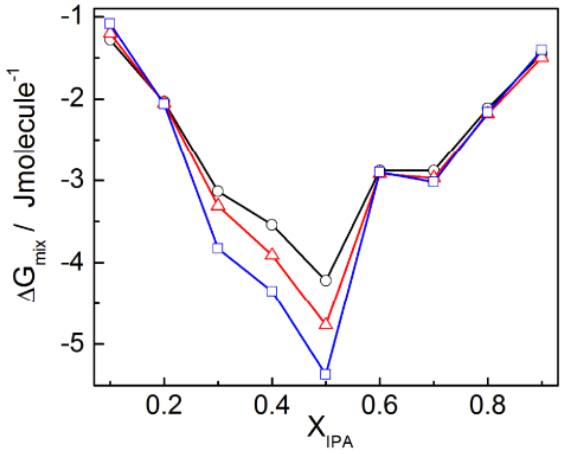

Figure 4.4 Variation of ΔGmix as a function of composition for mixed monolayers of SLC+IPA at 25 o C. Surface pressures (π / mNm-1) are: O, 10; Δ, 20 and □, 30.

The free energy of mixing (Gmix) between SLC and IPA was also determined to characterise the degree of hydrocarbon chain mixing. The free energy of mixing for the monomolecular film of SLC and IPA is shown as a function of the mole percent of IPA in Figure 4.4. The regular solution theory was used to investigate the monolayer interaction of SLC and IPA in addition to the standard monolayer thermodynamic parameters.

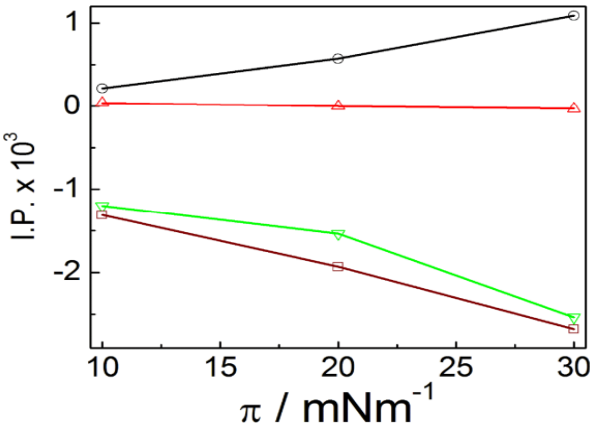

Figure 4.5 Relationship between interaction energy (I. P.) and surface pressure (π) at different IPA mole fraction.

Monolayer and bilayer lipid assemblies have distinct characteristics. while the latter exhibit curvature, the former have a straight orientation. While SLC-IPA vesicles are structurally unique, they may provide clues from mixed monolayer research into the mechanisms behind their robust variety and remarkable durability. While IPA displayed repulsive contact at 10 mole%, it was determined from the - A research that raising the amount of IPA made the system more stiff. The 20 and 40 mol% IPA cases showed some degree of deviation. Such abnormal behaviours defy

current scientific understanding. While the processes of these interactions have yet to be clarified, they may be described using molecular dynamics modelling, which is seen as a promising future direction.

4.2 Dynamic Light Scattering (DLS) Studies

Vesicle compositions' stability and biodistribution are greatly influenced by their hydrodynamic diameter (dh). The polydispersity index (PDI) represents the size distribution of dispersions and may vary from 0 for a monodispersed system to 1 for a fully polydispersed system. Using size measurement over a period of more than 100 days beginning on the day samples were prepared, we looked at the stability of vesicles with varying compositions of SLC and IPA in conjunction with 30 mol% cholesterol. Figure 6 shows sample size vs time (day) curves.

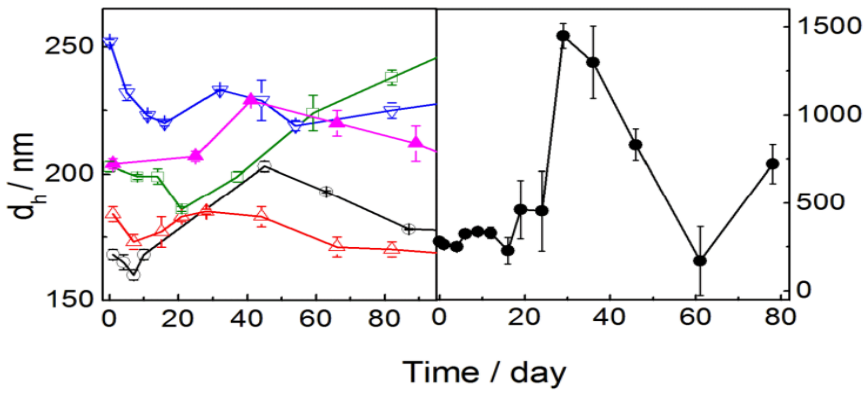

Figure 4.6 Variation in the hydrodynamic diameter (dh) for SLC +IPA (in presence of 30 mol% cholesterol) vesicles with time at 25 o C. Mole fraction of IPA (x_{IPA}):

Figure 4.7 is a graphical representation of the evolution of the PDI values over time. It displays sample results based on day 45 DLS data. The systems with the following SLC/IPA (M/M) ratios successfully generated liposomal dispersions: 5:5, 7:3, and 10:0 wins. Figure 4.6 shows the various systems' dh-time profiles visually. Increased Use of IPA (except x_{IPA}= 0.4) resulting in a decline in polydispersity that has been almost steady throughout time.

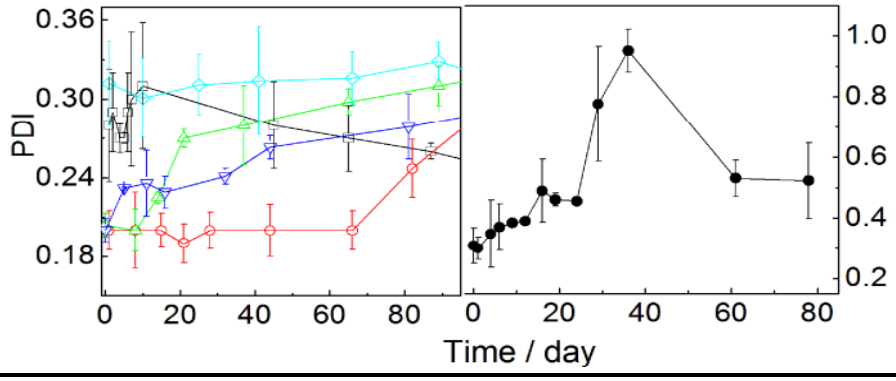

Figure 4.7 Variation of polydispersity index for the SLC+IPA (in presence of 30 mol% cholesterol) vesicles with time.

5. CONCLUSION

Stable vesicle dispersions were prepared using SLC and IPA (HTMA- DS) at varying mole fractions. IPA was shown to have a significant effect on SLC monolayer after a thorough examination into its effects on vesicles was conducted using monolayer tests. Although associative interactions were discovered for certain mixed monolayers, systems with x_{IPA}~ 0.5 not react by scattering vesicles in a steady manner. The systems containing 20 and 40 mol% IPA exhibited several anomalies. This peculiar mutation was caused by the separation of HTMA+ from IPA. The hydrodynamic size of the hybrid vesicles was also measured, and the results showed that the vesicular dispersions were less stable for x_{IPA}= 0.2 and 0.4.

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