

LIST OF FIGURES

| Figure no. | Caption | Page no. |
|---------------------|---|----------|
| INTRODUCTION | | |
| Figure 1 | Molecular structure of monolaurin. | 2 |
| Figure 2 | Molecular structure of 1,2-dilauroyl- <i>sn</i> -glycerol. | 3 |
| Figure 3 | Molecular structure of tristearine. | 3 |
| Figure 4 | Molecular structure of cetyl palmitate. | 4 |
| Figure 5 | Molecular structure of glycerophospholipids. | 4 |
| Figure 6 | Molecular structure of phosphatidylcholine. | 5 |
| Figure 7 | Molecular structure of phosphatidylethanolamine. | 5 |
| Figure 8 | Molecular structure of phosphatidylserine. | 6 |
| Figure 9 | Molecular structure of phosphatidylinositol: | 6 |
| Figure 10 | Molecular structure of phosphatidylglycerol. | 6 |
| Figure 11 | Molecular structure of phosphatidylethanolamine. | 7 |
| Figure 12 | Molecular structure of sphingophospholipids. | 7 |
| Figure 13 | Molecular structure of glycolipids. | 8 |
| Figure 14 | Molecular structure of cholesterol. | 9 |
| Figure 15 | Molecular structure of curcumin. | 10 |
| Figure 16 | Molecular structure of chrysin. | 10 |
| Figure 17 | Molecular structure of oleanolic acid. | 11 |
| Figure 18 | Molecular structure of pyrazinamide. | 11 |
| Figure 19 | A schematic diagram of nanoemulsion. | 14 |
| Figure 20 | A schematic diagram of liposome | 15 |
| Figure 21 | Schematic diagram of the drug loaded SLN (left) and NLC (right) | 16 |
| Figure 22 | Schematic representations and TEM images of different types of NLC formulation (a), imperfect type; (b), amorphous type and (c), multiple type. | 18 |
| Figure 23 | Schematic diagram of different type of drug incorporation models for NLC formulations. A, drug enriched core model ; B, solid | 19 |

| | | |
|------------------|---|-----------|
| | solution model and C, drug enriched shell model. | |
| Figure 24 | Flow diagram for the preparation of NLC using Hot homogenization followed by ultrasonication technique. | 24 |
| Figure 25 | Aggregation method in reducing intensified dispersions and pearl like network in the NLC dispersion with stabilizing effect. | 25 |
| Figure 26 | Representative size distribution of NLC (SLC + TS + PA, 2:2:1, M/M/M) formulation obtained in the DLS measurements. | 27 |
| Figure 27 | Representative TEM image of NLC (CP+TO+PA, 2:2:1, M/M/M) formulation. | 28 |
| Figure 28 | Representative AFM images of the NLC formulations comprising TB + HSPC + BA, 2:2:1, M/M/M (1) and TE + HSPC + OA, 2:2:1, M/M/M (2). | 28 |
| Figure 29 | DSC thermograms of the physical mixture of lipids (solid line) and NLC (SLC + TS + PA, 2:2:1, M/M/M) formulations (dotted line). | 30 |
| Figure 30 | Representative release profiles of incorporated procaine hydrochloride from NLC (Span 65 + SLC + SA, 2:2:1, M/M/M) formulations. | 32 |

CHAPTER 1

| | | |
|-----------------|--|-----------|
| Figure 1 | Surface pressure (π) – area (A) isotherm of SLC (1), TS (2), PA (3) and curcumin (4, panel A) and mixed monolayer of SLC+TS (1:1) and PA (panel B and C). Panel D describes the π -A isotherms of mixed monolayer of (SLC+TS+ PA, component 1) and curcumin (component 2). While pure water was used for A & B, 10 mM aqueous Tween 60 solution was used as subphase in C and D. Mole % of PA (B and C): 5 &10, 0; 6 &11, 10; 7 &12, 20; 9 &13, 80 and 8 &14, 100. Mole% of curcumin (Panel D): 15, 0;16, 80; 17, 40; 18, 50; 19, 60 and 20, 100. Temperature 25 °C. | 45 |
| Figure 2 | Dependence of compression moduli (C_s^{-1}) of (SLC+TS+FA, 2:2:1, M/M/M) monolayer with percentage of area compressed and surface | 47 |

pressure (π) at 25 °C. 10 mM aqueous Tween 60 solution was used as subphase. FAs are mentioned inside the figure.

- Figure 3** Variation of excess molecular area (A_{ex}) and excess free energy change (ΔG_{ex}^0) with PA and curcumin mole%. For panel A, B: Component 1: SLC+TS (1:1, M/M), Component 2: PA. For panel C, D: Component 1: SLC+TS+PA (2:2:1, M/M/M), Component 2: curcumin. Surface pressure (mNm^{-1}): O, 5; Δ , 10; \square , 15; ∇ , 20; \diamond , 25 and \blacktriangleleft , 30. Temperature: 25 °C. **48**
- Figure 4** Variation in hydrodynamic diameter (d_h) and polydispersity index (PDI) with time for SLNs (SLC+TS+FA, 2:2:1, M/M/M). Samples were stored at 25 °C. FAs are mentioned inside the figure. **50**
- Figure 5** Hydrodynamic size (diameter, d_h) distribution of SLNs comprising different fatty acids. 1 mM SLN formulation of SLC+TS+FA (2:2:1, M/M/M) dispersed in 10 mM aqueous Tween 60 solution were studied. FAs are mentioned inside the figure. Temperature: 25 °C **50**
- Figure 6** FF-TEM images of SLN (SLC+TS+ PA, 2:2:1, M/M/M) in the absence (A) and presence (B) of curcumin. Scale bar: 500 nm. **52**
- Figure 7** DSC thermogram of a 5 mM SLN formulation (SLC+TS+PA, 2:2:1, M/M/M). Scan rate: 2 °C / min. **54**
- Figure 8** DSC cooling thermograms of 5mM SLNs (SLC+TS+FA, 2:2:1, M/M/M, stabilized by 10 mM aqueous Tween 60) with different fatty acids (1, LA; 2, MA; 3, PA and 4, SA). Scan rate: 2 °C /min. **55**
- Figure 9** Absorption (A) and emission (B) spectra of 5 μ M curcumin in different solvents (A1, B1) and SLNs (SLC+TS+FA, 2:2:1, M/M/M) (A2, B2) at 25 °C. Systems: 1&9, n-hexane; 2& 10, aqueous 10 mM Tween 60; 3 & 12, acetonitrile; 4 & 11, ethanol; 5 & 13, SA; 6 & 14, PA; 7 & 15, MA and 8 & 16, LA. λ_{ex} = 419 nm. **57**
- Figure 10** Dependence of the absorption maxima (λ_{max}) of curcumin on dielectric constant (ϵ) of the medium at 25 °C. Red and the blue line correspond to the aprotic and protic solvents respectively. Solvents: **58**

●, hexane; ▲, chloroform; ■, acetonitrile; ▼, DMSO; ○, pentanol, Δ, propanol; □, ethanol and ▽, methanol. 5 μM curcumin was used in recording the spectra.

- Figure 11** Variation in the fluorescence anisotropy (r) with time for curcumin loaded SLNs comprising four different fatty acids. Fatty acids: O, LA; Δ, MA; □, PA and ▽, SA. The concentration of curcumin was 5 μM. Excitation wavelength and emission wavelength were set at 419 and 458 nm respectively. Temperature, 25 °C. **59**
- Figure 12** Dependence of entrapment efficiency (EE) and *in vitro* release profile of curcumin loaded SLN (SLC+TS+FA, 2:2:1, M/M/M) on the fatty acid chain length (C_n) at 25 °C. Systems for right panel: ●, control; O, LA; Δ, MA; □, PA and ▽, SA. **60**
- Figure 13** Inhibitory effect of curcumin loaded SLNs (B) on the growth of *Bacillus amyloliquefaciens*. SLNs without curcumin were used as control (A). Panel C represent the activity of curcumin alone using Tween 60 as control. Fatty acid chain lengths are mentioned inside the figure. **63**

CHAPTER 2

- Figure 1** Surface pressure (π)- area (A) isotherms of pure lipids using water (panel A) and 2mM aqueous Tween 60 solution (panel B) as the subphase at 25°C. Systems: 1 & 4, TS; 2 & 5, OA; 3 & 6, CP. **73**
- Figure 2** Surface pressure (π) – area (A) isotherm of CHR 10 (blue) and CHR 16 (red) using water (dotted line) and 2 mM aqueous Tween 60 solution (solid line) as subphase (panel A). Panel B represented the $\pi - A$ isotherms of mixed lipidic system (CP+TP+OA, 2:2:1, M/M/M) in absence (black) and presence of 50 mole% CHR 10 (blue) & CHR 16 (red) using 2mM aqueous Tween 60 as subphase. Temperature 25 °C. **74**
- Figure 3** Variation of elasticity moduli (C_s^{-1}) with % compressed area upon addition of 50 mole% different LCDs of chrysin on the mixed lipidic **75**

system (CP+TP+OA, 2:2:1 M/M/M). 2mM aqueous Tween 60 was used as subphase. Different systems have been mentioned inside the figure.

- Figure 4** Variation of excess molecular area (A_{ex}) and changes in excess free energy (ΔG_{ex}^0) of the mixed lipidic system with the mole% OA (panel A, B) and chrysin, LCDs of chrysin (panel C, D). For panel A, B: Component 1: CP+TP (1:1, M/M), Component 2: OA. For panel C, D: Component 1: CP+TP+OA (2:2:1, M/M/M), Component 2: chrysin and LCDs of chrysin. Surface pressure for panel A & B (mNm^{-1}): O, 5; Δ , 10; \square , 15; ∇ , 20; \diamond , 25 and \triangleleft , 30. Systems for panel C & D: O, CHR; Δ , CHR 8; \square , CHR 10; ∇ , CHR 16 and \diamond , CHR 18. Temperature: 25 °C. **76**
- Figure 5** Variation of hydrodynamic diameter, PDI and zeta potential with time for 1 mM NLC formulation (CP+TP+OA, 2:2:1, M/M/M) stabilized by 2 mM aqueous Tween 60 solution in the absence and presence of chrysin and LCDs of chrysin at 25 °C. Individual systems are mentioned inside the figure. [Chrysin] & [LCDs of chrysin]: 10 μ M. **79**
- Figure 6** TEM (panel A, B) and FF-TEM (panel C, D) images for NLC (CP+TP+OA, 2:2:1, M/M/M) formulations in the absence (panel A, C) and presence (panel B, D) of chrysin. The scale bars are mentioned inside the figure. **82**
- Figure 7** AFM image of NLC (CP+TP+OA, 2:2:1, M/M/M) formulation where panel A and B represented the two and three dimensional view respectively. Panel C represented the roughness profile for the NLC formulation. Height scale is given inside the figure. Scan area: (1X1) μ m. **83**
- Figure 8** DSC thermogram of a 5 mM NLC formulation (CP+TP+OA, 2:2:1, M/M/M, stabilized with 2 mM aqueous Tween 60 solution). Inset represented the DSC thermogram of the corresponding physical mixture. **85**

| | | |
|------------------|---|-----------|
| Figure 9 | Change in the thermodynamic parameters for NLC formulations in the presence of different mole % of OA (panel A). Panel B represents the change in the thermodynamic parameters for the NLC (CP+TP+OA, 2:2:1, M/M/M) formulation with the incorporation of chrysin and LCDs of chrysin. The systems are mentioned in the figure. | 86 |
| Figure 10 | Crystallinity index (CI%) of the NLC formulations (CP+TP+OA, 2:2:1, M/M/M) in the absence (panel A) and the presence (panel B) of chrysin and LCDs of chrysin. The individual systems are mentioned in the figure. | 87 |
| Figure 11 | Entrapment efficiency (EE%), drug loading (DL%) capacity (panel A) and release profile (panel B) of chrysin and different LCDs of chrysin form NLC formulation at 25 °C . systems (panel B): O, CHR; Δ , CHR 8; \square , CHR 10; ∇ , CHR 16 and \diamond , CHR 18. | 89 |
| Figure 12 | Simple diffusion of chrysin and LCDs of chrysin in 2 mM aqueous Tween 60 solution through dialysis membrane (12kD) at 25 °C. Systems, \circ , CHR, Δ , CHR8; \square , CHR10; ∇ , CHR16 and \diamond , CHR 18.. 10 μ M chrysin and the LCDs were used for the experiment. | 90 |
| Figure 13 | Percentage cytotoxicity at three different concentration of chrysin and LCDs of chrysin loaded in the NLC formulation (CP+TP+OA, 2:2:1, M/M/M) against human neuro blastoma cell lines (SHSY5Y). Blank NLC formulation is taken as control. The individual systems are mentioned inside the figure | 93 |

CHAPTER 3

| | | |
|-----------------|---|------------|
| Figure 1 | Surface pressure (π) – area (A) isotherm of pure lipidic components and OLA using water as subphase at 25 °C. The different systems were mentioned in the figure. | 102 |
| Figure 2 | Surface pressure (π) – area (A) isotherm of mixed lipidic system (SLC/IPA+TS+PA, 2:2:1, M/M/M) in the absence (red) and presence (black) of IPA using water as subphase. Corresponding C_s^{-1} | 102 |

vs. % compressed area has been presented in the inset of the Figure.
Temperature 25 °C

- Figure 3** Variation in A_{ex} (panel A & C) and ΔG_{ex}^0 (panel B & D) of the mixed lipidic system with mole % of IPA (panel A, B) and OLA (panel C, D). For panel A, B: Component 1: SLC & Component 2: IPA. For panel C, D: Component 1: SLC/IPA (30:70)+TS+PA (2:2:1, M/M/M) & Component 2: OLA. Surface pressures (mNm^{-1}): O, 5; Δ , 10; \square , 15; ∇ , 20; \diamond , 25 and \triangleleft , 30. Temperature: 25 °C. **104**
- Figure 4** Variation in size (d_h), PDI and zeta potential (Z.P.) with time for 1 mM NLC_{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) stabilized by 2 mM aqueous Tween 60 solution in the absence (panel A) and presence (panel B) of OLA at 25 °C. SLC/IPA ratios for different systems have been mentioned inside the figure. [OA]: 10 μ M. **107**
- Figure 5** TEM (panel A, B & C) and FF-TEM (panel A1, B1 & C1) images for (A) conventional NLC (SLC+TS+PA, 2:2:1, M/M/M); (B) NLC_{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) and (C) NLC_{IPA} in presence of OLA. SLC/IPA ratio is 30 : 70 (M/M) in NLC_{IPA} . The scale bars are mentioned inside the figure. **109**
- Figure 6** AFM images of (A) conventional NLC (SLC+TS+PA, 2:2:1, M/M/M); (B) NLC_{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) and (C) NLC_{IPA} in presence of OLA. SLC/IPA ratio was 30 : 70 (M/M) in NLC_{IPA} . Corresponding three dimensional view and roughness profiles were also presented in the Figure. **110**
- Figure 7** DSC cooling thermograms for NLC_{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) formulations having different SLC/IPA ratio in absence (panel A) and presence (panel B) of OLA. SLC/IPA (M/M) ratios (panel A & B): 1, 100 : 0; 2, 40 : 60; 3, 30 : 70 and 4, 20 : 80. [NLC_{IPA}] and [OLA] : 1 mM and 10 μ M respectively. Scan rate, 2.5 °C min^{-1} . **111**
- Figure 8** Representative heating cooling DSC thermogram for NLC_{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) formulations having SLC/IPA **112**

| | | |
|------------------|---|------------|
| | (M/M) ratio 30 : 70. [NLC _{IPA}] : 1 mM. Scan rate, 2.5 °C min ⁻¹ . | |
| Figure 9 | Variation in different thermodynamic parameters with the change in SLC/IPA ratio of NLC _{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) in the absence (black) and presence (red) of OLA. The individual systems were mentioned inside the figure. | 113 |
| Figure 10 | Variation in entrapment efficiency (EE%), drug loading (DL%) capacity (panel A) and release profile (panel B) of OLA with the variation in the SLC/IPA ration for NLC _{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) formulation at 25 °C . SLC/IPA (M/M) ratio (panel B): ●, control ○, 100 : 0; □, 20 : 80; Δ, 30 : 70 and ▽, 40 : 60. | 115 |
| Figure 11 | <i>In vitro</i> cytotoxicity of the conventional NLC (SLC/IPA+TS+PA, 2:2:1, M/M/M) and NLC _{IPA} (SLC/IPA+TS+PA, 2:2:1, M/M/M) in absence and presence of OLA on three different cell lines at three different incubation times. The different systems, cell lines and incubation times were mentioned in the figure. NLC _{IPA} having SLC/IPA ratio 30 : 70 was used for the cytotoxicity studies. | 119 |
| Figure 12 | <i>In vitro</i> cytotoxicity of OLA alone on three different cell lines at three different concentrations of OLA. Different cell lines and incubation times were mentioned in the figure. | 120 |

CHAPTER 4

| | | |
|-----------------|--|------------|
| Figure 1 | Variation in d_h and PDI with respect to time for the NLC (HSPC : TS : OA, 2 : 2 : 1, M/M/M) and NLC _{PEG} (HSPC : TS : OA, 2 : 2 : 1, M/M/M) systems in the absence (panel A) and presence (panel B) of PYZ at 25 °C. The different concentration of PEG 2000 was mentioned inside the figure. | 123 |
| Figure 2 | Z.P. vs. time profile for NLC (HSPC : TS : OA, 2 : 2 : 1, M/M/M) and NLC _{PEG} (HSPC : TS : OA, 2 : 2 : 1, M/M/M) systems in the absence (panel A) and presence (panel B) of PYZ at 25 °C. The different concentration of PEG 2000 was mentioned inside the figure. | 131 |

| | | |
|-----------------|--|------------|
| Figure 3 | TEM images of NLC _{PEG} (HSPC : TS : OA, 2 : 2 : 1, M/M/M) having 0.01 (W/V)% of PEG 2000 in the absence (A) and presence (B) of PYZ. Scale bars are given inside the figures. | 132 |
| Figure 4 | AFM image (A) NLC _{PEG} (HSPC : TS : OA, 2 : 2 : 1, M/M/M) having 0.01 (W/V)% of PEG 2000. Panel B and C represented the three dimensional surface morphology and corresponding roughness analysis profile respectively. | 133 |
| Figure 5 | DSC cooling thermograms of NLC (red) and NLC _{PEG} (black) (HSPC : TS : OA, 2 : 2 : 1, M/M/M) having 0.01 (W/V)% of PEG 2000 in absence (panel A) and presence (panel B) of PYZ. The scan rate was fixed at 2 °C min ⁻¹ . | 134 |
| Figure 6 | Variation in EE [^] and DL% of NLC _{PEG} (HSPC : TS : OA, 2 : 2 : 1, M/M/M) formulations with the concentration of PEG 2000. | 137 |
| Figure 7 | Release profiles of the free PYZ and PYZ from NLC (HSPC : TS : OA, 2 : 2 : 1, M/M/M) and NLC _{PEG} (HSPC : TS : OA, 2 : 2 : 1, M/M/M) having 0.01 (W/V)% of PEG 2000 at 25 °C. | 138 |