

List of Figures

Figure No.	Figure Caption	Page No.
Figure 1.1:	Representation of plasma drug concentration versus time profile after administration of conventional drug (red line) and controlled drug release formulation (blue line)	2
Figure 1.2:	Types of nanocarriers currently in use for the delivery of anticancer agents. (a) Liposomes are self-assembling structures composed of phospholipid bilayers mimicking the structure of cell membrane. (b) Dendrimers are highly branched synthetic polymer with nanometer scale dimension. (c) Polymeric nanoparticles are nano sized solid polymeric matrices. (d) Polymeric micelles are composed of amphiphilic block copolymers forming nanosized core/shell structure in aqueous solution. (e) Mesoporous silica nanoparticles and (f) Layered double hydroxide nanoparticles.	8
Figure 1.3:	(a) Schematic illustration of MR-visible hyaluronic acid–ceramide (HACE)-coated nanohybrid liposomes containing DOX. <i>In vitro</i> drug release profiles of the developed formulations; (b) F2 and (c) F3. DOX release was determined at pH 5.5, 6.8, and 7.4. (d) Cellular uptake analysis using MDA-MB-231 cells as visualized by CLSM. DOX solution, F2 and F3 (all 50 µg/ml DOX) were incubated for 2 h. Red and blue colors indicate DOX and DAPI staining, respectively. The length of the scale bar is 10 µm.	9
Figure 1.4:	(a) Formation of empty amphiphilic dendrimers (AmDM) nanomicelles and DOX-encapsulated mDM/DOX nanomicelles. (b) <i>In vitro</i> DOX release behaviour from AmDM/DOX micelles at pH 5.0 and pH 7.4 at 37 °C. (c) The cellular uptake was imaged using confocal microscope following treatment with free DOX and AmDM/DOX in MCF-7R cells. (d) Inhibition of the uptake of AmDM/DOX micelles on MCF-7R cells using specific endocytosis inhibitors. CD: inhibitor of macropinocytosis; genistein: inhibitor of caveolae-mediated endocytosis; CMZ: inhibitor of clathrin-mediated endocytosis. (e) Inhibition of doxorubicin efflux by AmDM/DOX nanomicelles was determined in MCF-7R cells.	15

Figure 1.5:	Schematic diagram showing formation of LDH structures by isomorphic substitution of Mg^{2+} of brucite by Al^{3+} .	18
Figure 1.6:	<i>In vitro</i> drug release profiles for $[Li_xAl_2(OH)_6][drug]_x \cdot yH_2O$ LDHs: (a) release of diclofenac at pH 4 and pH 7 and (b) release of gemfibrozil at pH 4 and pH 7.	21
Figure 2.1:	Reaction scheme showing synthesis of various LDHs with varying interlayer anions and intercalation of drug molecules into the interlayer gallery of LDH.	26
Figure 2.2:	(a) Reaction scheme showing synthesis of various Zn-Fe based LDHs with varying interlayer anions; (b) intercalation of drug molecules into the interlayer gallery of LDH; and (c) dispersion of the drug intercalated LDHs into PCL matrix to form PN-R nanoconjugate.	28
Figure 2.3:	(a) Reaction scheme showing synthesis of various Li-Al based LDHs; and (b) intercalation of DNA molecules into the interlayer gallery of LDH.	30
Figure 3.1:	(a) XRD patterns of LN, LN-R; LC, LC-R; and LP, LP-R. ‘*’ marks indicates the new set of basal reflections originates from RH intercalated LDH phases; (b) a schematic drawing of LDH structure before and after drug intercalation in LN and LP.	45
Figure 3.2:	XRD patterns of free drug. Pure RH shows its crystalline nature with the characteristics peak at 2θ of 13.46, 14.52, 20.98, 22.62 and 24.1°, which agrees well with the literature.	47
Figure 3.3:	Bright field transmission electron micrographs of (a) LN, LN-R and (b) LP, LP-R.	48
Figure 3.4:	FESEM micrographs of (a) LN and LN-R; (b) AFM topographs of LN and LN-R with height profile,	49
Figure 3.5:	FTIR spectra for drug intercalated LDHs, in comparison with pristine LDHs and raloxifene hydrochloride for (a) nitrate based LDH systems, (b)	50
Figure 3.6:	<i>In vitro</i> drug release profiles for LN-R, LC-R and LP-R with (a) 5% drug intercalation, (b) 15% drug intercalation and (c) 30% drug intercalation. Similar patterns of release profile have been observed suggesting that the extent of drug intercalation does not affect much the nature of release profile. The results presented are mean \pm standard deviation (SD) values obtained from three independent experiments. (d) Schematic drawing of drug release behavior from LN-R and LP-R systems.	52

- Figure 3.7:** Linear fitting of the drug release data to various kinetic models, (a) zero order model, (b) first order model, (c) Elovich model, (d) modified Freundlich model and (e) **53**
- Figure 3.8:** (a) Al 2p and (b) Mg 2p XPS spectra for pristine LDHs and drug intercalated LDHs. The vertical lines indicate the peak position/binding energy; (c) **56**
- Figure 3.9:** DSC thermograms for (a) free drugs and drug intercalated LDHs, (b) pristine LN, LC and LP systems; in pure LC degradation of carbonate species **58**
- Figure 3.10:** (a) Relative cell viability of HeLa cells after incubation of pure nitrate, carbonate and phosphate LDHs (LN, LC and LP respectively) having different concentration, (b) *In vitro* cytotoxicity of free drug and drug intercalated LDHs against HeLa cells with different time intervals; The results presented are mean \pm standard deviation (SD) values obtained from three independent experiments, and (c) fluorescent images of AO/EB staining of control, free drug, and drug intercalated LDHs. **61**
- Figure 3.11:** *In vivo* tumor suppression performances and systemic toxicity of pure RH and drug intercalated LDHs in comparison to control. (a) Photographs of the mice of different experimental groups at 0 day and at 21 days, (b) excised solid tumors at the 22nd day, (c) relative changes in tumor volume of pure drug and drug intercalated LDHs with time, inset figure shows relative changes in tumor volume of pristine LN and control (PBS) treated groups, and (d) changes in body weight of the animals of the different treatment groups with time, where, *P < 0.05, **P < 0.01, ***P < 0.001. **65**
- Figure 3.12:** Histopathological analysis of (a) liver (b) kidney and (c) spleen of tumor bearing Balb/c mice treated with control (saline), pure RH, LN-R and LP-R (all tissues: 200 \times). The investigation reveals that the free drug administered mice resulting in bile ductular proliferation and congested portal vein in portal triad (shown by red arrows). Mice administered LP-R shows dilated venous radical with mild congestion (red arrows). Mice treated with pure drug exhibits cloudy degeneration of the tubular epithelial cells in the kidney (shown by blue arrows). A slight damage of tubular cells is also noticed for mice treated with LP-R (blue arrows). However, other organs of mice administered saline and drug intercalated LDH nanoparticles shows no obvious toxicity. **63**

Figure 4.1:	Energy dispersive X-ray spectrum of ZN, ZC and ZP LDH nanoparticles.	73
Figure 4.2:	(a) High-resolution transmission electron microscopic image of platelet like shape of ZN, ZC and ZP LDH nanoparticles. (b) SAED patterns of ZN, ZC and ZP	74
Figure 4.3:	SEM images of pristine ZN, ZC and ZP LDH nanoparticles.	74
Figure 4.4:	(a) Powder X-ray diffraction patterns of pristine PCL, pristine ZN, ZN-R and PN-R, ‘★’ marks indicate the new set of basal reflections which originates from RH intercalated LDH phases; (b) TEM images of ZN-R and PN-R samples, inset shows the SAED patterns of ZN-R and PN-R; (c) Zeta potentials of the developed samples; and (d) AFM topographs of ZN-R, PCL and PN-R with corresponding height profiles exhibiting relative surface roughness.	76
Figure 4.5:	DLS particle size distribution of ZN, ZN-R, PCL-RH and PN-R which were found to be 100 ± 4 , 110 ± 3 , 315 ± 4 and 332 ± 3 nm with polydispersity index (PDI) of 0.19, 0.22, 0.17 and 0.20 respectively.	77
Figure 4.6:	(a) Cumulative drug release profile for raloxifene intercalated LDH systems (ZN-R, ZC-R and ZP-R), raloxifene embedded in PCL (PCL-RH) and PCL coated ZN-R (PN-R) in PBS at pH ~ 7.4 at 37 °C. The results are plotted as mean \pm SD values obtained from three different set of experiments; (b) Linear fitting of the drug release data to (i) Zero-order, (ii) first-order, (iii) Higuchi, (iv) modified-Freundlich modified, and (v) Korsmeyer–Peppas model; and (c) Schematic representation of possible drug release mechanisms; (i) in drug intercalated LDHs, (ii) in PN-R.	79
Figure 4.7:	(a) Fe 2p and (b) Zn 2p XPS spectra for pristine LDHs and drug intercalated LDHs. The vertical lines indicate the peak position/binding energy.	83
Figure 4.8:	Comparison of solid-state UV–vis spectra of pristine LDHs (ZN), pure drug (RH), drug intercalated LDHs (ZN-R) and polymer nanohybrid (PN-R).	83
Figure 4.9:	DSC thermograms of free drugs and drug intercalated LDHs, (a) nitrate LDH systems, (b) carbonate LDH systems, (c) phosphate LDH systems, and (d) pure polymer and polymer nanoconjugate.	85
Figure 4.10:	TGA thermograms of ZN, ZN-R, pristine PCL, pure drug and for PN-R systems.	85
Figure 4.11:	FTIR spectra of pristine LDH, pure drug and drug intercalated LDHs for (a) ZN systems, (b) ZC systems and (c) ZP systems.	87

Figure 4.12:	Percentage cell viability measured through MTT assay of HeLa cells after incubation of pristine LDHs (ZN, ZC and ZP) and pristine PCL with (a) different concentration range, and (b) different time frame.	89
Figure 4.13:	Comparative cell viability study of pure raloxifene, ZN-R, ZP-R, PCL-RH and PN-R against HeLa cells using MTT assay. (b) Fluorescent microscopic images of AO/EtBr staining of the samples treated cells. (c) Relative cell adhesion values of the developed materials after 12 h incubation; (d) Phase contrast images of the HeLa cells grown on top of the indicated substrates after 12 h incubation.	89
Figure 4.14:	(a) Cellular uptake kinetics the developed materials into HeLa cells under different incubation times. (b) Fluorescence microscopic images showing the cellular uptake of rhodamine-B labeled samples into HeLa cells. Cells are exposed to $100 \mu\text{g ml}^{-1}$ or equivalent amount of RdB labeled particles showing various intensity of fluorescence depending on cellular uptake; and (c) Schematic illustration of cellular uptake mechanism considering same HeLa cell with indicated substrate with varying surface potential and roughness.	93
Figure 4.15:	(a) Plasma drug concentration versus time profiles for RH, ZN-R, ZP-R and PN-R evaluated in Charles Foster albino female rats ($n = 4$) after intravenous administration at $10 \text{ mg drug / Kg body weight}$ and equivalent amount in various delivery vehicles.	95
Figure 4.16:	Changes of biochemical parameters as a function time: (a) alanine aminotransferase (ALT); (b) aspartate aminotransferase (AST); (v) urea and (d) creatinine activity at the 7 th day in Charles Foster albino female rats after intravenous administration at $10 \text{ mg drug/Kg body weight}$ and equivalent amount of drug intercalated materials. Results are expressed as $\text{mean} \pm \text{SD}$, $n = 4$.	97
Figure 4.17:	Histopathological examination using hematoxylin and eosin (H & E) staining to evaluate the toxicity of liver treated with control (PBS), pure RH, ZN-R, ZP-R and PN-R at different indicated time intervals.	98
Figure 5.1:	(a) Bright field TEM images of pristine LDH and DNA intercalated LDH (LDH-DNA); (b) HRSEM micrographs of LDH and LDH-DNA, and, (c) AFM topographs of LDH and LDH-DNA with height profile.	103

- Figure 5.2:** (a) Powder X-ray diffraction patterns in the 2θ range of $2-70^\circ$ of LDH and LDH-DNA nanohybrid; (b) Solid state UV-Vis spectra of pristine LDH, naked DNA and LDH-DNA nanohybrid; (c) FTIR spectra of the indicated samples, and, (d) Zeta potential analysis of the samples. **104**
- Figure 5.3:** (a) DNase I protection assay; LDH-pDNA complex and naked pDNA were treated with DNase I for different time intervals. The complexed pDNA was released from the LDH-pDNA nanohybrid after incubation at pH 2; (b) The amount of DNA protected (%) after DNase I treatment was calculated as the relative integrated densitometry values (IDV), quantified, and normalized by that of pDNA values (without DNase I treatment) using Gel Documentation system (Syngene, U.K.), and, (c) Protection against thermal damage; DSC thermogram exhibiting that LDH interlayer gallery space can be used to protect the naked DNA from any thermal damage. **107**
- Figure 5.4:** *In vitro* controlled release experiment and its mechanism; (a) Cumulative percentage DNA release profile from LDH-DNA nanohybrid in PBS at pH ~ 7.4 at 37°C . The data points are plotted as mean \pm SD values obtained from three different set of experiments; (b) Linear fitting of the DNA release data to zero order, first-order, Korsmeyer-Peppas and modified Freundlich model; (c) Schematic representation of possible DNA release mechanisms from LDH-DNA nanohybrid. **109**
- Figure 5.5:** *In vitro* biocompatibility analysis; (a) Relative cell adhesion of LDH compared to control after 12 h incubation; (b) Phase contrast images of the NIH-3T3 cells grown on top of the LDH substrates after 12 h incubation; (c) Comparative cell viability study of LDH against NIH-3T3 cells using MTT assay as a function of concentration and (d) incubation time; Untreated cells are considered as control. The results are presented with mean \pm standard deviation (SD) values obtained from three independent experiments; (e) Fluorescent microscopic images of NIH-3T3 cells after staining with AO/ EtBr dye. **111**

- Figure 5.6:** Cellular uptake kinetics of LDH nanoparticles into HeLa cells. **(a)** The observed rhodamine-B (RdB) fluorescence extracted from the cells in the presence of $100 \mu\text{g ml}^{-1}$ LDH- RdB. The results are presented in mean \pm standard deviation (SD) values obtained from three independent experiments; **(b)** Fluorescence microscopic images showing the cellular uptake of rhodamine-B labeled LDH nanoparticles into HeLa cells. **112**
- Figure 5.7:** Fluorescence microscopy images of GFP expressing in HeLa cells. The images were captured at 48 h after the addition of samples. **113**
- Figure 5.8:** HeLa cells were stained with acridine orange/ethidium bromide after incubation with naked GFP-P53 and LDH-(GFP-P53) complex. Viable cells show green fluorescence. Apoptotic cells show orange and yellow fluorescence. Cells were observed under fluorescence microscope (x400). **114**