

LIST OF FIGURES

| Fig. No. | TITLE | Page No. |
|----------|--|----------|
| 1.1. | Strategy for bone metastasis targeted nanocarrier | 5 |
| 2.1 | Bone microenvironment, tumor cell inoculation and colonization | 16 |
| 2.2 | Various nanomaterial-based drug delivery platforms. | 37 |
| 2.3 | Molecular structure of lactide and glycolide based biodegradable polymer | 38 |
| 2.4 | Different method for preparation of PLGA nanoparticles | 40 |
| 2.5 | Nano drug delivery systems in vivo. | 43 |
| 2.6 | Chemical structure of three representative bisphosphonate compounds. | 47 |
| 3.1 | Regressed calibration curve of DTX in ACN: water (70:30) mobile phase at $\lambda_{\max}=230\text{nm}$ | 78 |
| 3.2 | Chromagram of Standard DTX solution in ACN: water (70:30) mobile phase at $\lambda_{\max}=230\text{nm}$. | 79 |
| 3.3 | Chromatogram of Standard DTX in PLGA NP formulation using ACN: water (70:30) mobile phase at $\lambda_{\max}=230\text{nm}$ | 81 |
| 3.4 | Chromatogram of Standard DTX in PBCA NP formulation using ACN: water (70:30) mobile phase at $\lambda_{\max}=230\text{nm}$. | 81 |
| 3.5 | MRM chromatograms of ApppI (a), IPP (b) and ISTD (c) in standard preparation in MCF7 cell line. | 84 |
| 3.6 | MRM chromatograms of ApppI (a), IPP (b) and ISTD (c) in standard preparation in BO2 cell line. | 84 |
| 3.7 | MRM chromatograms of ApppI (a), IPP (b) and ISTD (c) in test preparation in MCF7 cell line. | 85 |
| 3.8 | MRM chromatograms of ApppI (a), IPP (b) and ISTD (c) in test preparation in BO2 cell line. | 86 |
| 3.9 | Calibration curve and regression analysis for ApppI in MCF7 cell line | 88 |
| 3.10 | Calibration curve and regression analysis for IPP in MCF7 cell line | 89 |
| 3.11 | Calibration curve and regression analysis for IPP in BO2 cell line | 90 |
| 3.12 | Calibration curve and regression analysis of coumarin-6 in ACN ($\lambda_{\text{ex}} = 430\text{nm}$; $\lambda_{\text{em}} = 485\text{ nm}$) | 94 |
| 3.13 | Calibration curve and regression analysis of poloxamer P188 in DCM : phosphate buffer (pH 7.4) at 510 nm | 96 |
| 3.14 | Calibration curve and regression analysis of PEG at 500 nm | 99 |
| 3.15 | Regressed calibration curve of ZOL in mobile phase (2.5 L water, 4.7 mL formic acid, pH 3.5) at $\lambda_{\max} = 210\text{ nm}$, Data presented as Mean \pm SD, n = 6. | 102 |
| 4.1 | Preparation scheme of PLGA NP by solvent diffusion - | 109 |

| | | |
|------|---|-----|
| | nanoprecipitation method | |
| 4.2 | Particle size and zeta potential measurement reports of PLGA NP, PLGA-PEG20 NP and PLGA-PEG-ZOL NP | 121 |
| 4.3 | FTIR spectra of a) PLGA b) PEG c) PLGA-PEG and d) PLGA-PEG-ZOL | 123 |
| 4.4 | ^1H NMR spectrum of PLGA-PEG-ZOL | 124 |
| 4.5 | In-vitro release of DTX in pH 7.4 PBS containing 0.5 % tween 80 and 10% ethanol | 125 |
| 4.6 | DSC study of (a) DTX, (b) PLGA-PEG-ZOL, (c) Mixture of DTX and PLGA-PEG-ZOL and (d) DTX loaded PLGA-PEG-ZOL NP | 126 |
| 4.7 | Cryo TEM images of PLGA NPs (a) unconjugated (b) ZOL-conjugated | 127 |
| 4.8 | Colloidal stability studies using salt induced aggregation using (a): Na_2SO_4 and (b): CaCl_2 . (c): Serum stability study of nanoparticulate formulations in PBS (pH 7.4) containing 1% FBS. | 128 |
| 4.9 | In vitro bone binding affinity of ZOL solution and PLGA-PEG-ZOL NP | 129 |
| 4.10 | PEGylated PBCA NP formation, drug entrapment mechanism (a to d) and characterization by cryoTEM (e) and particle size analysis (f) | 143 |
| 4.11 | A) Alkyl cyanoacrylate molecule B) anionic and nucleophilic initiation of polymerization process of alkyl cyanoacrylates. | 144 |
| 4.12 | Effect of temperature change on particle size of PBCA | 145 |
| 4.13 | Effect of stirring speed on particle size of PBCA NP | 146 |
| 4.14 | Effect of surfactant concentration on particle size and entrapment efficiency of PBCA NPs | 147 |
| 4.15 | Turbidimetric measurement for particle formation with time | 148 |
| 4.16 | Effect of pH on particle size and % drug entrapment in PBCA NP | 150 |
| 4.17 | Effect of % DTX in monomer on % drug entrapment and % drug loading in PBCA NP | 151 |
| 4.18 | Particle size and zeta potential measurement reports of PBCA NP, PBCA-PEG20 NP and PBCA-PEG-ZOL NP | 153 |
| 4.19 | In-vitro drug release of DTX and DTX loaded PBCA NP | 155 |
| 4.20 | FTIR spectra of a) PBCA b) PEG c) PBCA-PEG and d) PBCA-PEG-ZOL | 156 |
| 4.21 | ^1H NMR spectra of PBCA-PEG-ZOL conjugate | 157 |
| 4.22 | Gel Permeations chromatogram of PBCA and PBCA-PEG-ZOL polymer | 158 |
| 4.23 | TEM image of a) PBCA NPs, b) PBCA-PEG-ZOL NP | 159 |
| 4.24 | DSC study of (a) DTX, (b) PBCA-PEG-ZOL, and (c) DTX | 160 |

| | | |
|------|---|-----|
| | loaded PBCA-PEG-ZOL NP | |
| 4.25 | Colloidal stability study using salt induced aggregation using (a): Na ₂ SO ₄ and (b): CaCl ₂ . (c): Serum stability study of NP formulations in phosphate buffer saline (pH 7.4) containing 1% FBS. | 162 |
| 4.26 | In vitro bone binding affinity assay of ZOL solution and PBCA-PEG-ZOL NP | 164 |
| 5.1 | Phagocytic uptake of 6-coumarin loaded PEGylated PLGA NP formulations by mouse macrophage cell line RAW264 after incubation for 60, 120 and 240 min using FACS as estimation technique. | 179 |
| 5.2 | Phagocytic uptake of 6-coumarin loaded PEGylated PBCA NP formulations by mouse macrophage cell line RAW264 after incubation for 60, 120 and 240 min using FACS as estimation technique. | 181 |
| 5.3 | Phagocytic uptake histograms of 6-coumarin loaded NP formulations by mouse macrophage cell line RAW 264 after incubation for 60, 120 and 240 min using FACS as estimation technique. | 182 |
| 5.4 | Microscopic evaluation of phagocytic up takes of PBCA NP and PBCA-PEG20 NP using confocal microscope. (a to c) show images for PBCA NP and (d to f) shows images for PBCA-PEG20 NP. (a) & (d) coumarin6 loaded NP uptake (b) & (e) nucleus stained using Hoechst 33342 and (c) & (f) overlapping images | 182 |
| 5.5 | Endocytic uptake of 6-coumarin loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP in human breast cancer cell line (a) MCF7 after incubation for 30, 60 and 120 min using FACS as estimation technique. | 185 |
| 5.6 | Endocytic uptake of 6-coumarin loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP in human breast cancer cell line BO2 after incubation for 30, 60 and 120 min using FACS as estimation technique. | 186 |
| 5.7 | Endocytic uptake histogram of control cells, 6-coumarin loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP in human breast cancer cell line BO2 after incubation for 120 min using FACS as estimation technique. | 186 |
| 5.8 | Endocytic uptake of 6-coumarin loaded PBCA-PEG20 NP and PBCA-PEG-ZOL NP in human breast cancer cell line (a) MCF7 after incubation for 30, 60 and 120 min using FACS as estimation technique. | 188 |
| 5.9 | Endocytic uptake of 6-coumarin loaded PBCA-PEG20 NP and PBCA-PEG-ZOL NP in human breast cancer cell line BO2 | 188 |

| | | |
|------|---|-----|
| | after incubation for 30, 60 and 120 min using FACS as estimation technique. | |
| 5.10 | Endocytic uptake histogram of 6-coumarin loaded PBCA-PEG20 NP and PBCA-PEG-ZOL NP in human breast cancer cell line BO2 after incubation for 120 min using FACS as estimation technique. | 189 |
| 5.11 | Characterization of route for NP uptake using various endocytosis inhibitors on BO2 cell line and 6-coumarin loaded PLGA-PEG-20 NP and PLGA-PEG-ZOL NP. | 194 |
| 5.12 | Characterization of route for NP uptake using various endocytosis inhibitors on BO2 cell line and 6-coumarin loaded PBCA-PEG-20 NP and PBCA-PEG-ZOL NP. | 195 |
| 5.13 | Confocal microscopic evaluation of NPs up take of PLGA-PEG20 NP and PLGA-PEG-ZOL NP using LysoTracker Red [®] and Hoechst 33342. Figure (a & b) shown overlapping images for PLGA-PEG20 NP and (c & d) shown overlapping images for PLGA-PEG-ZOL NP. Yellow color indicated NP in lysosomal compartment. | 196 |
| 5.14 | Confocal microscopic evaluation of NPs up take of PLGA-PEG20 NP and PLGA-PEG-ZOL NP using LysoTracker Red [®] and Hoechst 33342. Figure (a) shown overlapping images for PBCA-PEG20 NP and (b) shown overlapping images for PBCA-PEG-ZOL NP. Yellow color indicated NP in lysosomal compartment. | 196 |
| 5.15 | Intracellular association of NPs with endosome-lysosome compartments and possible route of trafficking. (a) Early endosomal release - route without involvement of lysosome (b) Immediate endosomal exocytosis (c) Late endosome-lysosome association and release by lysosome rapture. | 199 |
| 5.16 | PLGA-PEG20 NP and PLGA-PEG-ZOL NP retention time in intracellular compartment after uptake in BO2 cell line after incubation for control, 60, 120 and 240 min using FACS as estimation technique. | 200 |
| 5.17 | PBCA-PEG20 NP and PBCA-PEG-ZOL NP retention time in intracellular compartment after uptake in BO2 cell line after incubation for control, 60, 120 and 240 min using FACS as estimation technique. | 201 |
| 5.18 | Cytotoxicity study PBCA NP without DTX in MCF7 after 48 h and 72 h using MTT assay. | 204 |
| 5.19 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP in MCF7 cell line after 48 h using MTT assay. | 207 |
| 5.20 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX | 208 |

| | | |
|------|--|-----|
| | loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP in MCF7 cell line after 72 h using MTT assay. | |
| 5.21 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP in BO2 cell line after 48 h using MTT assay. | 208 |
| 5.22 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP in BO2 cell line after 72 h using MTT assay. | 209 |
| 5.23 | Cytotoxicity study PBCA NP without DTX in MCF7 after 48 h and 72 h using MTT assay. | 211 |
| 5.24 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX loaded PBCA-PEG20 NP and PBCA-PEG-ZOL NP in MCF7 after 48 h using MTT assay. | 214 |
| 5.25 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX loaded PBCA-PEG20 NP and PBCA-PEG-ZOL NP in MCF7 after 72 h using MTT assay. | 214 |
| 5.26 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX loaded PBCA-PEG20 NP and PBCA-PEG-ZOL NP in BO2 after 48 h using MTT assay. | 215 |
| 5.27 | Cytotoxicity study of DTX solution, ZOL-DTX solution, DTX loaded PBCA-PEG20 NP and PBCA-PEG-ZOL NP in BO2 after 72 h using MTT assay. | 215 |
| 5.28 | Schematic presentation of principle for cell cycle analysis using DNA intercalating fluorescence probe in flow cytometry. | 216 |
| 5.29 | Cell cycle analysis in BO2 cell line after treatment of (a) Control (PBS), (b) DTX solution, DTX loaded (c) PLGA-PEG20 NP and (d) PLGA-PEG-ZOL NP by PI staining using FACS technique. | 219 |
| 5.30 | Cell cycle analysis in BO2 cell line after treatment of (a) Control (PBS), (b) DTX solution, DTX loaded (c) PBCA-PEG20 NP and (d) PBCA-PEG-ZOL NP by PI staining using FACS technique. | 221 |
| 5.31 | Apoptosis estimation in MCF7 cell lines after treatment of Control (PBS), DTX solution, DTX loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP by Annexin V-FITC & PI staining using FACS technique. | 224 |
| 5.32 | Apoptosis estimation in BO2 cell lines after treatment of Control (PBS), DTX solution, DTX loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP by Annexin V-FITC & PI staining using FACS technique. | 225 |
| 5.33 | Apoptosis estimation in MCF7 cell lines after treatment of Control (PBS), DTX solution, DTX loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP by Annexin V-FITC & PI staining | 227 |

| | | |
|------|---|-----|
| | using FACS technique. | |
| 5.34 | Apoptosis estimation in BO2 cell lines after treatment of Control (PBS), DTX solution, DTX loaded PLGA-PEG20 NP and PLGA-PEG-ZOL NP by Annexin V-FITC & PI staining using FACS technique. | 228 |
| 5.35 | Schematic presentation of the mevalonate pathway (MVP) and molecule structures of IPP and ApppI | 229 |
| 5.36 | IPP accumulation after treatment with ZOL solution and PLGA-PEG-ZOL NP along with control (PBS) in MCF7 cell line. | 235 |
| 5.37 | ApppI accumulation after treatment with ZOL solution and PLGA-PEG-ZOL NP along with control (PBS) in MCF7 cell line. | 235 |
| 5.38 | IPP accumulation after treatment with ZOL solution and PLGA-PEG-ZOL NP along with control (PBS) in BO2 cell line. | 236 |
| 5.39 | IPP accumulation after treatment with ZOL solution and PBCA-PEG-ZOL NP along with control (PBS) in MCF7 cell line. | 236 |
| 5.40 | ApppI accumulation after treatment with ZOL solution and PBCA-PEG-ZOL NP along with control (PBS) in MCF7 cell line. | 237 |
| 5.41 | IPP accumulation after treatment with ZOL solution and PBCA-PEG-ZOL NP along with control (PBS) in BO2 cell line. | 237 |
| 6.1 | Mice were administered with ^{99m}Tc labeled DTX and PLGA NPs and the radioactivity was measured after (a) 1 h, (b) 4 h and (c) 24 h post injection. | 251 |
| 6.2 | Mice were administered with ^{99m}Tc labeled DTX and PBCA NPs and the radioactivity was measured after (a) 1 h, (b) 4 h and (c) 24 h post injection. | 254 |
| 7.1 | Strategy for bone metastasis targeted nanocarrier | 261 |