LIST OF FIGURES

Figure 1.1: Photograph of normal bone and osteoporotic bone
Figure 1.2: The anabolic action of PTH 1-34 during bone remodeling
Figure 1.3: Structure of recombinant human PTH 1-34
Figure 1.4: Structure of Chitosan
Figure 1.5: Structure of Thiolated Chitosan
Figure 1.6: Structure of PEGylated Chitosan
Figure 1.7: Diagrammatic representation of digestive system of rat and human
Figure 1.8: Diagrammatic representation of (A) intestinal section and the microvilli and (B) different modes of nanoparticle uptake across the epithelial cells lining the microvilli of the intestine
Figure 1.9: Schematic representation of the research strategy
Figure 1.10: A representation of the overall objectives of the thesis

Figure 3.1.1: (A) DLS showing the size distribution of (a) CS NPs and (b) CS-PTH NPs (B) SEM image of CS NPs (C) SEM image of CS-PTH NP
Figure 3.1.2: (A) Atomic Force Microscopic image of CS-PTH NPs showing spherical morphology (B) FT-IR spectra of CS (A), CS -NPs (B) PTH 1-34 (C) and CS-PTH NPs (D).
Figure 3.1.3: (A) SEM of PEG-CS-PTH NPs showing particle size of 200-250 nm(B) TEM image of a 200-250nm PEG-CS-PTH NPs aggregate (C) and (D) TEM image showing that the cluster of PEG-CS-PTH 1-34 NPs are in turn formed of individual particles of approximately 20nm.
Figure 3.1.4: FTIR of the PEG-CS-PTH NPs, PEG 200 and CS-PTH NPs.
Figure 3.1.5: (A) DLS of TCS-PTH 1-34 NPs, (B) AFM images of TCS-PTH 1-34 NPs showing spherical nanoparticles. (C) SEM of TCS-PTH 1-34 NPs showing particle size of 90-100nm

Figure 3.1.6: FTIR of CS,TCS,TCS–PTH NPs, PTH 1-34 and TCS NPs

Figure 3.1.7: Cumulative *in vitro* release of PTH 1-34 from the CS-PTH NPs at pH 3.4, pH 6.8, and pH 7.5 and which is the gastric, intestinal and blood pH of rats (12h fasting)

Figure 3.1.8: Cumulative *in vitro* release of PTH 1-34 from the PEG-CS-PTH NPs at pH 3.4, pH 6.8, and pH 7.5 and which is the gastric, intestinal and blood pH of rats (12h fasting).

Figure 3.1.9: Cumulative *in vitro* release of PTH 1-34 from the TCS-PTH NPs at pH 3.4, pH 6.8, and pH 7.5 and which is the gastric, intestinal and blood pH of rats (12h fasting).

Figure 3.1.10: (A) MTT assay and (B) LDH assay Chitosan nanoparticles concentrations of (A) 0.005 mg/mL (B) 0.0005 mg/mL (C) 0.00005 mg/mL and chitosan nanoparticles concentrations corresponding to the PTH 1-34 loaded concentrations (D) 0.1 mg/mL (E) 0.01 mg/mL (F) 0.001 mg/mL along with negative control (G) Media (H) triton –X 100.

Figure 3.1.11: (A) MTT assay; (B) LDH assay (a) 0.05 mg/mL, (b) 0.005 mg/mL, (c) 0.0005mg/mL PTH 1-34 loaded PEGylated CS NPs and CS nanoparticle concentrations corresponding to the PTH 1-34 loaded concentrations, (d) 0.1 mg/mL, (e) 0.01 mg/mL, (f) 0.001 mg/mL along with (g) media and (h) triton –X 100

Figure 3.1.12: (A) MTT assay (B) LDH assay (a) 0.03 mg/mL, (b) 0.003 mg/mL, (c) 0.0003mg/mL PTH 1-34 loaded TCS NPs and TCS NPs concentrations corresponding to the PTH 1-34 loaded concentrations, (d) 0.5 mg/mL, (e) 0.05 mg/mL, (f) 0.005 mg/mL along with (g) media and (h) triton –X 100.
Figure 3.1.13: (A) Activated partial thromboplastin time APTT, (B) Photograph showing the negligible hemolytic effect of the PTH 1-34 loaded chitosan nanoparticles of concentrations (A) 0.005 mg/mL (B) 0.0005 mg/mL (C) 0.00005 mg/mL and chitosan nanoparticles concentrations corresponding to the PTH 1-34 loaded concentrations (D) 0.1 mg/mL (E) 0.01 mg/mL (F) 0.001 mg/mL along with negative control (G) Saline and positive control (H) triton –X 100 and (C) Prothrombin time test

Figure 3.1.14: Blood compatibility results of the PEG-CS-PTH NPs and CS NPs (A) APTT, (B) PT and (C) Haemolysis assay

Figure 3.1.15: Haemolysis assay (A) 0.03 mg/mL, (B) 0.003 mg/mL, (C) 0.0003 mg/mL PTH 1-34 loaded TCS-PTH 1-34 NPs and TCS nanoparticle concentrations corresponding to the PTH 1-34 loaded concentrations, (D) 0.5 mg/mL, (E) 0.05 mg/mL, (F) 0.005 mg/mL along with negative control, (G) Saline and positive control and (H) triton –X 100 (B) APTT and (C) PT.

Figure 3.1.16: Diagram depicting the molecular mechanism of the anabolic effect of PTH 1-34 on human primary osteoblast cell.

Figure 3.1.17: 3 and 7 day bone specific alkaline phosphatase detected by the ALP assay# indicates the p< 0.05 when compared to 3 day CS-PTH NPs treated HOB cells and*indicates p value< 0.05 when compared to 7 day CS-PTH NPs treated HOB cells.

Figure 3.1.18: 3 and 7 day bone specific alkaline phosphatase detected by the ALP assay. # indicates the p< 0.05 when compared to 3 day PEG-CS-PTH NPs treated primary human osteoblast cells and *indicates p value< 0.05 when compared to 7 day PEG-CS-PTH NPs treated primary human osteoblast cells

Figure 3.1.19: 3 and 7 day bone specific alkaline phosphatase detected by the ALP assay. # indicates the p < 0.05 when compared to 3 day TCS-PTH 1-34
NPs treated HOB cells and * indicates p value < 0.05 when compared to 7 day TCS-PTH 1-34 NPs treated HOB cells.

**Figure 3.1.20:** cAMP assay *indicates p value< 0.05 when compared to 7 day CS-PTH NPs treated HOB cells

**Figure 3.1.21:** cAMP assay*indicates p value< 0.05 when compared to 7 day PEG-CS-PTH NPs treated HOB cells.

**Figure 3.1.22:** cAMP assay * indicates p value < 0.05 when compared to 7 day TCS-PTH 1-34 NPs treated HOB cells.

**Figure 3.1.23:** Calcium assay *indicates p value< 0.05 when compared to 7 day CS-PTH NPs treated HOB cells.

**Figure 3.1.24:** Calcium assay*indicates p value< 0.05 when compared to 7 day PEG-CS-PTH NPs treated HOB cells.

**Figure 3.1.25:** Calcium assay * indicates p value < 0.05 when compared to 7 day TCS-PTH 1-34 NPs treated HOB cells

**Figure 3.1.26:** Osteocalcin assay *indicates p value< 0.05 when compared to 7 day CS-PTH NPs treated HOB cells.

**Figure 3.1.27:** Osteocalcin assay *indicates p value< 0.05 when compared to 7 day PEG-CS-PTH NPs treated HOB cells.

**Figure 3.1.28:** Osteocalcin assay * indicates p value < 0.05 when compared to 7 day TCS-PTH 1-34 NPs treated HOB cells

**Figure 3.1.29:** The pharmacokinetic profile of PTH 1-34 after oral administration of the CS-PTH NPs and PTH 1-34 in Female Sprague dawley rats (overnight fast); *(Inset: Hemolysis observed in the initial 30 min of blood draw).*

**Figure 3.1.30:** The pharmacokinetic profile of PTH 1-34 after oral administration of the PEG-CS-PTH NPs, CS-PTH NPs, PTH 1-34 in Female Sprague dawley rats (overnight fast)

**Figure 3.1.31:** The pharmacokinetic profile of PTH 1-34 after oral administration of the TCS-PTH NPs, CS-PTH NPs, PTH 1-34 in Female Sprague dawley rats (overnight fast)
**Figure 3.1.32:** *In vivo* NIR image of rats orally administered with ICG-PEG-CS-PTH NPs is compared to the *in vivo* NIR image of rats orally administered with ICG-CS-PTH NPs. The areas marked A & B are the junction of stomach and duodenum and the small intestine respectively.

**Figure 3.1.33:** *In vivo* NIR image of rats orally administered with ICG-TCS-PTH NPs is compared to the *in vivo* NIR image of rats orally administered with ICG-CS-PTH NPs
LIST OF TABLES

Table 1.1: World Health Organization criteria for the diagnosis of osteoporosis based on bone mass or density category criteria
Table 2.1: List of Cell culture Medium
Table 2.2: List of Cell lines
Table 2.3: List of Biological assay Kits and reagents
Table 2.4: List of Chemicals
Table 2.5: Depicts the different kinetic models of drug release
Table 2.6: Concentrations of NPs tested
Table 2.7: Details of the animals used for the experiments
Table 3.1: Kinetic modeling of CS-PTH NPs
Table 3.2: Kinetic modeling of PEG-CS-PTH NPs
Table 3.3: Kinetic modeling of TCS-PTH NPs
Table 3.4: Shows the cross reactivity of the Rat PTH 1-34 to Human PTH 1-34 ELISA Kit
Table 3.5: In vivo pharmacokinetic parameters of CS-PTH NPs obtained from plasma after oral administration in female Sprague dawley rats
Table 3.6: In vivo pharmacokinetic parameters of PEG-CS-PTH NPs obtained from plasma after oral administration in female Sprague dawley rats
Table 3.7: In vivo pharmacokinetic parameters of TCS-PTH NPs obtained from plasma after oral administration in female Sprague dawley rats