CONTENTS
Certificate
Declaration by the candidate & certificate by the supervisor
Copyright transfer certificates
Acknowledgment
Table of contents
List of Figures
List of Tables
List of abbreviations
Preface
Introduction: Cancer & Therapeutics
1.1 Cancer
1.1a Types of cancer
1.1.b Epidemiology
1.2 Breast cancer
1.2.1 Histological subtype
1.2.2 Molecular subtypes
1.3 Common treatments for breast cancer
1.4 Methotrexate
1.5 Challenges in cancer treatment
1.6 Nanoparticles
1.6.1 Polymer-based drug nanocarriers
1.7 Chitosan
1.8 Chitosan nanoparticles synthesis methods
1.8.1 Ionic gelation method
1.8.2 Microemulsion method
1.8.3 Self-assembly
1.8.4 Polymerization
1.8.5 Covalent cross-linking
1.8.6 Precipitation
1.8.7 Spray Drying
1.9 Advantages of ionic gelation over other synthetic methods

Chapter 2: Literature Review
2.1 Overview
2.2 Role of nanotechnology in cancer diagnosis and therapy
2.3 Metallic nanoparticles as drug delivery system
2.4 Limitations of metal and metal oxide nanoparticles in cancer treatment
2.5 Metal Salt nanoparticles
2.5.1 Calcium carbonate nanoparticles ($CaCO_3$-NPs)
2.6 Carbon-based nanoparticles (C-NPs)
2.6.1 Carbon nanotubes (CNT)
2.6.2 Graphene oxide (GO) nanoparticles as drug delivery system
2.7 Limitations of carbon-based nanoparticles in cancer treatment
2.8 Polymer nanoparticles (P-NPs)
2.9 Biopolymer nanoparticles
2.9.1 Chitosan
2.9.2 Chitosan nanoparticles (CS-NPs)
2.10 Advantages of chitosan nanoparticles over metal nanoparticles, metal oxide nanoparticles, and carbon-based nanoparticles in cancer treatment
2.11 Problem Statement & Objective of the Study
Chapter 3: Development and Optimization of Methotrexate Chitosan Nanocarrier for Breast Cancer Treatment
3.1 Introduction
3.2 Experiment Section
3.2.1 Preparation of MTX-encapsulated chitosan nanocarrier
3.2.2 Design of experiments (DOE)
3.2.2a Taguchi orthogonal array design
3.2.2b Statistical optimization and data analysis
3.2.2c Data analysis and validation
3.2.3 Characterization
3.2.3a Physiochemical characterization
3.2.3b Stability studies
3.2.4 *In-vitro* drug release
3.2.5 Animal and treatment protocol
3.2.5.1 *In-vivo* pharmacokinetic studies
3.2.5.1a High-performance liquid chromatography (HPLC)

3.2.6 Toxicity studies and histopathological examinations
3.3 Characterization and Property Evaluation
3.3.1 Validation of the statistical model
3.3.1a Normal probability plots
3.3.1b Main effect plot
3.3.2 Response surface analysis
3.3.2a Influence of independent parameters on particles size 3.3.2b Influence of independent parameters on encapsulation efficiency 3.3.2c Influence of independent parameters on drug loading
3.3.3 Optimization and checkpoint analysis (validation of predicted values)
3.3.4 Physiochemical evaluation
3.3.4a Organoleptic evaluations 3.3.4b UV visible spectroscopy 3.3.4c Fourier transform infrared spectroscopy 3.3.4d X-ray diffraction 3.3.4e Thermogravimetric analysis 3.3.4f High-resolution transmission electron microscopy 3.3.4g Hydrodynamic size and zeta potential 3.3.4h Percent entrapment efficiency and drug loading
3.3.5 Storage stability
3.3.6 *In-vitro* drug release and kinetic model and mechanism
3.4 Animal studies
3.4.1 *In-vivo* pharmacokinetics
3.4.2 Histopathological studies
3.5 Conclusions
Chapter 4: Synthesis of Methotrexate Chitosan Nanoparticles for Breast Cancer Treatment
4.1 Introduction
4.2 Experimental section
4.2.1 Preparation of MTX-loaded chitosan nanoparticles 4.2.2 Physiochemical characterization of nanoparticles 4.2.3 Entrapment efficiency and loading capacity 4.2.4 Stability studies

4.2.5 Drug release studies
4.2.6 *In-vitro* studies
4.2.6a Cytotoxic studies 4.2.6b Detection of apoptosis; dual staining method employing acridine orange/ethidium bromide (AO/EBr) 4.2.6c Hemocompatibility studies
4.2.7 *In-vivo* Studies 4.2.7.1 Animal
4.2.7.1a Mammary tumor generation and treatment protocol
4.2.7.2 High-performance liquid chromatography
4.2.7.2a Pharmacokinetic studies
4.2.7.2b Biodistribution studies
4.2.7.3 Confocal laser scanning microscopy (Cellular Uptake)
4.2.8 Statistical analysis
4.3 Results and Discussion 4.3a Physicochemical characterization
4.3b Loading capacity and entrapment efficacy
4.3c Storage Stability
4.3.4 In-vitro drug release studies and kinetic models
4.3.5 Cytotoxic study of nanoparticle 4.3.5a MTT-Assay
4.3.5b Detection of apoptosis by acridine orange/ethidium bromide dual staining
4.3.6 Hemocompatibility studies
4.3.7 *In-vivo* pharmacokinetic and biodistribution
4.3.8 Anti-cancer efficacy
4.3.9 Confocal microscopy
4.4 Conclusions
Chapter 5: Histopathological & Biochemical Assessment using Developed Nano-formulation in Breast Cancer Rat Model
5.1. Introduction
5.2 Experimental section
5.2.1Animal grouping and treatment protocol
5.2.2 Assessment of cytokines as a biomarker (ELISA)

5.2.3 Assessment of renal and hepatic enzymatic function biomarkers
5.2.4 Histopathological study
5.2.5 Statistical analysis
5.3 Results and Discussion
5.3.1 Histopathology of mammary tumors (MNU-induced)
5.3.2 Role of pro-inflammatory cytokines in tumor generation
5.3.3 Role of MTX-loaded nanoparticles in tumor suppression
5.3.4 Role of Meth-Cs-NPs on hepatic & renal biomarker regulation
5.3.5 Tumor histologypathology (after treatments)
5.3.6 Effect of Meth-Cs-NPs on the kidney (histopathology)
5.3.7 Effect of Meth-Cs-NPs on the liver (histopathology)
5.4 Conclusion
Chapter 6: Chitosan Functionalized Fluorescent Calcium Carbonate Nanocarrier Loaded with MTX as Unique Theragnostic Tool for Triple Negative Breast Cancer
6.1 Introduction
6.2 Experimental sections
6.2.1a Plausible mechanisms for (@Cal-Nps synthesis
6.2.1b Surface modification of @Cal-Nps with chitosan and loading of methotrexate
6.2.2 Instrumentation & characterization
6.2.3 Loading capacity and entrapment efficiency
6.2.4 Stability study
6.2.5 Hemolysis assay
6.2.6 *In-vitro* drug release
6.2.7 *In-vitro* cell-line study
6.2.7a Cytotoxic study of nanoparticle
6.2.7b Apoptosis study through AO/EtBr dual staining
6.2.8 *In-vivo* anticancer efficacy
6.2.8.1 Animal grouping and treatment protocol
6.2.8.2 Pharmacokinetics study
6.2.8.3a Tissue biodistribution (Quantitative tissue biodistribution)
6.2.8.3b *In-vivo* fluorescent imaging (Qualitative tissue bio-distribution)

6.2.8.4 Enzyme-linked immunosorbent assays (ELISA)
6.2.8.5 Blood biomarker analysis
6.2.8.6 Tissue histology and immunohistochemistry (IHC) test
6.2.9 Statistical analysis
6.3. Results and Discussion
6.3.1 Synthesis of @Cal-Nps and loading capacity
6.3.2 Optical properties
6.3.3 Hydrodynamic particle size and zeta potential
6.3.4 Fourier transform infrared spectrophotometer analyses
6.3.5 High-resolution transmission electron microscopy & Selected area electron diffraction
6.3.6 Powder X-ray diffraction
6.3.7 Stability studies
6.3.7.1 Photostability
6.3.7.2 pH stability
6.3.7.3 Colloidal dispersion stability
6.3.7.4 Thermal stability
6.3.8 *In-vitro* drug release studies
6.3.9a Cytotoxic assay
6.3.9b Apoptosis study through AO/EtBr dual staining
6.3.10a Pharmacokinetics (PK) and tissue biodistribution
6.3.10b *In-vivo* optical imaging (IVIS)
6.3.11 Hemolysis assay
6.3.12 Anticancer efficacy
3.13 Enzyme-linked immunosorbent assays (ELISA)
6.3.14 Blood biomarker analysis
6.3.15 Immunohistochemistry (IHC) and histopathology
6.4 Conclusions
Chapter 7: Summary & Future Scope
References
Appendix
List of Publications