Chapter 1: Introduction

1.1. Concept of Controlled Release Drug Delivery
   1.1.1. Need for Controlled Delivery Systems
       1-2
   1.1.2. Demerits of Controlled Release Systems
       2-3

1.2. Fundamental components of controlled delivery formulations
   3-20
   1.2.1. Polymers
       4-12
       1.2.1.1. Chitosan
       5-7
       1.2.1.2. Carboxymethyl chitosan
       7-8
       1.2.1.3. Phosphorylated chitosan (PCTS)
       8-9
       1.2.1.4. Soy flour
       9-12
   1.2.2. Active Agents
       12-15
       1.2.2.1. Isoniazid
       12-13
       1.2.2.2. Curcumin
       13-15
   1.2.3. Reinforcing agents
       15-19
       1.2.3.1. Montmorillonite (MMT)
       16-17
       1.2.3.2. Cellulose whiskers (CW)
       17-19
   1.2.4. Crosslinking agents
       19-20

1.3. Role of micro- and nanoparticles in controlled release drug delivery
    20-22

1.4. Fabrication techniques of nanoparticles for controlled delivery formulations
    22-30
   1.4.1. Evaporation or Extraction of Solvent Based Process
       23-25
       1.4.1.1. Coacervation phase separation method
       23-25
       1.4.1.1.1. Simple Coacervation Method
       23-24
       1.4.1.1.2. Complex Coacervation Method
       24-25
   1.4.2. Emulsion Based Process
       25-27
       1.4.2.1. Single emulsion
       25-26
       1.4.2.2. Double emulsion technique
       27
   1.4.3. Ionotropic Gelation Technique
       28
1.4.4. Desolvation method
1.4.5. Spray Drying
1.4.6. Other fabrication techniques

References

Chapter 2: Literature Search

2.1. Isoniazid: An antituberculosis drug
2.2. Curcumin: An anticancer drug
2.3. Natural polymers for controlled drug delivery formulations
2.4. Montmorillonite (MMT) and Cellulose whisker (CW) in Biomedical Applications
2.5. Glutaraldehyde and Genipin as Crosslinkers
2.6. Objectives and plan of work

References

Chapter 3: Experimental

3.1. Material used
3.2. Methods
3.2.1. Section A: Preparation of Nanoparticles/microparticles loaded with hydrophilic drug, Isoniazid
3.2.1.1. Preparation of cellulose whisker (CW)
3.2.1.2. Preparation of Isoniazid loaded Chitosan–CW microparticles by microencapsulation method
3.2.1.3. Preparation of Isoniazid loaded Chitosan Montmorillonite nanoparticles using ionic gelation method
3.2.1.4. Synthesis of carboxymethyl chitosan
3.2.1.5. Preparation of Isoniazid loaded CMC-MMT nanoparticles by ionic gelation method
3.2.1.6. Synthesis of phosphorylated chitosan (PCTS)
3.2.1.7. Preparation of Isoniazid loaded PCTS-MMT nanoparticles by ionic gelation method
3.2.1.8. Preparation of isoniazid-loaded SF nanoparticles by desolvation method

References
3.2.2. Section B Preparation of nanoparticles loaded with hydrophobic drug, Curcumin.

3.2.2.1. Preparation and characterization of curcumin loaded chitosan/MMT nanoparticles for controlled drug delivery applications 73-74

3.2.2.2. Preparation and characterization of curcumin loaded carboxymethyl chitosan/MMT nanoparticles for controlled drug delivery applications 74-75

3.2.2.3. Preparation and characterization of curcumin loaded phosphorylated chitosan/MMT nanoparticles for controlled drug delivery applications 76-77

3.2.3. Preparation of calibration curve

3.2.3.1. Calibration curve of Isoniazid 77

3.2.3.2. Calibration curve of Curcumin 77

3.2.4. Calculation of Process Yield 78

3.2.5. Calculation of Drug loading efficiency and Encapsulation efficiency of the nanoparticles 78

3.2.6. Fourier Transmission Infra-red Spectroscopy (FTIR) study 73

3.2.7. X-ray diffraction (XRD) study 78

3.2.8. Particle size determination 78

3.2.9. Scanning electron microscopy (SEM) study 79

3.2.10. Transmission emission microscopy (TEM) study 79

3.2.11. Water Uptake Studies 79

3.2.12. In vitro drug release studies 79-80

3.2.13. Isolation of Lymphocytes, culture and treatment 80

3.2.14. Cytotoxicity experiments 80

3.2.15. Statistical analysis 81

3.2.16. Cell culture 81

3.2.16.1. Preparation of the cell lysate 81

3.2.16.2. Preparation of samples for LDH activity assay 81

3.2.16.3. LDH assay 81-82

3.2.16.4. Lipid Peroxidation Assay 82

3.2.16.5. Reduced glutathione assay 82

3.2.16.6. Catalase assay 82-83

3.2.16.7. Superoxide dismutase 83
Chapter 4: Results and Discussion (Part 1)

4.1. Section A-Preparation and characterization of isoniazid loaded chitosan/cellulose whisker microspheres for controlled drug delivery applications.

4.1.1. Effect of variation of CW and GA concentration on the different properties of isoniazid loaded chitosan CW microparticles

4.1.2. Fourier Transmission Infra-red Spectroscopy (FTIR) study

4.1.3. X-ray diffraction (XRD) study

4.1.4. Scanning electron microscopy (SEM) study

4.1.5. Transmission electron microscopy (TEM) study

4.1.6. Swelling Study

4.1.7. In vitro Release Studies

4.1.8. Cell Viability Study

4.2. Section B-Preparation and characterization of isoniazid loaded chitosan/montmorillonite nanoparticles for controlled drug delivery applications

4.2.1. Effect of variation of surfactant concentration on the different properties of chitosan nanoparticles

4.2.2. Fourier Transmission Infra-red Spectroscopy (FTIR) study

4.2.3. X-ray diffraction (XRD) study

4.2.4. Scanning electron microscopy (SEM) study

4.2.5. Transmission electron microscopy (TEM) study

4.2.6. Swelling Study
4.2.7 *In vitro* Release Studies
4.2.8 Cell Viability Study
4.2.9 *In vitro* wash-off test for evaluation of mucoadhesive property
4.2.10 *Ex vivo* mucoadhesive test

4.3 *Section C*-Preparation and characterization of isoniazid loaded carboxymethyl chitosan/montmorillonite nanoparticles for controlled drug delivery applications

4.3.1 Nuclear Magnetic Resonance (NMR) study
4.3.2 Effect of variation of MMT and GA concentration on the different properties of isoniazid loaded CMC-MMT nanoparticles
4.3.3 Fourier Transmission Infra-red Spectroscopy (FTIR) study
4.3.4 X-ray diffraction (XRD) study
4.3.5 Scanning electron microscopy (SEM) study
4.3.6 Transmission electron microscopy (TEM) study
4.3.7 Swelling Study
4.3.8 *In vitro* Release Studies
4.3.9 Cell Viability Study

4.4 *Section D*-Preparation and characterization of isoniazid loaded phosphorylated chitosan/montmorillonite nanoparticles for controlled drug delivery applications

4.4.1 Nuclear Magnetic Resonance (NMR) study
4.4.2 Effect of variation of MMT and GA concentration on the different properties of isoniazid loaded CMC-MMT nanoparticles
4.4.3 Fourier Transmission Infra-red Spectroscopy (FTIR) study
4.4.4 X-ray diffraction (XRD) study
4.4.5 Scanning electron microscopy (SEM) study
4.4.6 Transmission electron microscopy (TEM) study
4.4.7. Swelling Study
4.4.8. In vitro Release Studies
4.4.9. Cell Viability Study

4.5. Section E-Preparation and characterization of isoniazid loaded soy flour/montmorillonite nanoparticles for controlled drug delivery applications.

4.5.1. Effect of variation of MMT and GA concentration on the different properties of SF nanoparticles
4.5.2. Fourier Transmission Infra-red Spectroscopy (FTIR) study
4.5.3. X-ray diffraction (XRD) study
4.5.4. Scanning electron microscopy (SEM) study
4.5.5. Transmission electron microscopy (TEM) study
4.5.6. Swelling Study
4.5.7. In vitro Release Studies
4.5.8. Cell Viability Study

References

Chapter 5: Results and Discussion (Part 2)

5.1. Section A-Preparation and characterization of curcumin loaded chitosan/MMT nanoparticles for controlled drug delivery applications

5.1.1. Effect of variation of MMT and genipin concentration on the different properties of curcumin loaded chitosan-MMT nanoparticles
5.1.2. Fourier Transform Infra-red Spectroscopy (FTIR) study
5.1.3. X-Ray Diffraction (XRD) Study

xix
5.1.4. Scanning electron microscopy (SEM) study 141
5.1.5. Transmission electron microscopy (TEM) study 141
5.1.6. Swelling Study 142-143
5.1.7. In vitro Release Studies 143-144
5.1.8. Cell Viability Study 144-145
5.1.9. Study of toxicity related parameter (lipid peroxidation and lactate dehydrogenase (LDH) activity) and Antioxidant status 146
5.1.10 In vitro wash-off test for evaluation of mucoadhesive property 147
5.1.11. Ex vivo mucoadhesive test 148

5.2. Section B-Preparation and characterization of curcumin loaded carboxymethyl chitosan/MMT nanoparticles for controlled drug delivery applications 148-157

5.2.1. Effect of variation of MMT and genipin concentration on the different properties of curcumin loaded CMC-MMT nanoparticles 148-150
5.2.2. Fourier Transform Infra-red Spectroscopy (FTIR) study 150
5.2.3. X-Ray Diffraction (XRD) Study 151
5.2.4. Scanning electron microscopy (SEM) study 151-152
5.2.5. Transmission electron microscopy (TEM) study 152
5.2.6. Swelling Study 152-153
5.2.7. In vitro Release Studies 153-154
5.2.8. Cell Viability Study 154-156
5.2.9. In vitro wash-off test for evaluation of mucoadhesive property 156-157
5.2.10. Ex vivo mucoadhesive test 157

5.3. Section C-Preparation and characterization of curcumin loaded phosphorylated chitosan/MMT nanoparticles for controlled drug delivery applications 157-166
5.3.1 Effect of variation of MMT and genipin concentration on the different properties of curcumin loaded PCTS-MMT nanoparticles

5.3.2 Fourier Transform Infra-red Spectroscopy (FTIR) study

5.3.3 X-Ray Diffraction (XRD) Study

5.3.4 Scanning electron microscopy (SEM) study

5.3.5 Transmission electron microscopy (TEM) study

5.3.6 Swelling Study

5.3.7 *In vitro* Release Studies

5.3.8 Cytotoxicity Study

5.3.9 *In vitro* wash-off test for evaluation of mucoadhesive property

5.3.10 *Ex vivo* mucoadhesive test

References

Chapter 6: Conclusion and Future Scope

Appendices

xxi