

## Table of Contents

1	Introduction .....	2
1.1	Biopolymers for the design of tumor microenvironment responsive systems .....	3
2	Literature Review .....	6
2.1	Biopolymers with pH-responsive cleavage bonds .....	7
2.2	Biopolymers with pH-responsive protonated chemical groups .....	10
2.3	Biopolymers with Cleavable ROS responsive bond .....	10
2.4	Biopolymers with amphiphilicity transition-ROS responsive bond.....	13
2.5	Biopolymers with enzyme responsive bonds .....	16
2.6	Biopolymers with hypoxia activatable groups .....	18
2.7	Cleavable-bond based targeting .....	23
2.8	pH-dependent Protonation mediated targeting .....	26
2.9	Size dynamic targeting .....	27
2.10	Charge reversal targeting .....	27
2.11	Redox responsive nanomedicine .....	30
2.12	Enzyme responsive nanomedicine.....	33
2.13	Hypoxia responsive nanomedicine .....	35
2.14	Dual stimuli-responsive nanomedicine.....	37
2.15	Stimuli activated surface ligand-mediated targeting.....	38
2.16	Limitations .....	45
2.17	Carriers for localized delivery of TME-Responsive nanomedicine .....	46
3	Objectives and Plan of Work.....	50
3.1	Objectives .....	50

3.2	Plan of Work.....	50
3.2.1	Cetuximab functionalized chitosan/hyaluronic acid-based nanoparticles loaded with cabazitaxel for enhancing anti-tumor efficacy in DMBA-induced breast cancer model in rats through spatial targeting .....	50
3.2.2	Cabazitaxel-loaded redox-responsive nanocarrier based on D-alpha-tocopheryl-chitosan and hyaluronic acid loading for improved anti-tumor efficacy in DMBA-induced breast cancer model.....	51
3.2.3	Hyaluronic acid-oleylamine and chitosan-oleic acid conjugate-based hybrid nanoparticle delivery via. dissolving microneedles for enhanced treatment efficacy in localized breast cancer .....	52
4	Cetuximab functionalized chitosan/hyaluronic acid-based nanoparticles loaded with cabazitaxel to improve the anti-tumor efficacy in DMBA-induced breast cancer models in rats	54
4.1	Introduction .....	54
4.2	Materials and Methods .....	56
4.2.1	Materials .....	56
4.2.2	Synthesis and characterization of succinylated TPGS.....	57
4.2.3	Preparation of chitosan/hyaluronic acid-based nanoparticles.....	58
4.2.4	Physicochemical characterization of nanoparticles .....	60
4.2.5	Analytical method development .....	61
4.2.6	Encapsulation efficiency, drug loading, and degree of conjugation .....	61
4.2.7	Drug release study.....	62
4.2.8	In vitro cell line studies.....	63
4.2.9	In vivo studies in female Sprague Dawley rats.....	66
4.2.10	Statistics .....	67

4.3	Results and Discussion .....	67
4.3.1	Characterization of succinylated TPGS .....	67
4.3.2	Characterization of nanoparticles.....	79
4.3.3	Drug release study .....	86
4.3.4	In vitro assessment of nanoparticle efficacy .....	88
4.3.5	In vivo studies .....	96
5	Cabazitaxel-loaded redox-responsive nanocarrier based on D-alpha-tocopheryl-chitosan and hyaluronic acid for improved anti-tumor efficacy in DMBA-induced breast cancer model .....	106
5.1	Introduction .....	106
5.2	Materials and Methods .....	109
5.2.1	Materials.....	109
5.2.2	Synthesis and Characterization of TPGS-COOH and CSVE .....	110
5.2.3	Formulation of CSVE-based nanoparticles.....	111
5.2.4	Characterization of CSVE-based nanoparticles .....	112
5.2.5	Drug release Study .....	114
5.2.6	Stability of lyophilized nanoparticles.....	115
5.2.7	In vitro cell line studies .....	115
5.2.8	In vivo Studies .....	118
5.2.9	Statistical Analysis .....	121
5.3	Results and Discussion .....	121
5.3.1	Synthesis of TPGS-COOH and CS-VE .....	121
5.3.2	Formulation and characterization of CSVE-based nanoparticles .....	125
5.3.3	Drug release Study .....	133

5.3.4	In vitro Study .....	139
5.3.5	In vivo Studies .....	151
6	Hyaluronic acid-oleylamine and chitosan-oleic acid conjugate-based hybrid nanoparticle delivery via dissolving microneedles for enhanced treatment efficacy in localized breast cancer .....	162
6.1	Introduction .....	162
6.2	Materials and methods .....	164
6.2.1	Materials required .....	164
6.2.2	Synthesis of CS-OA, HA-OA, and TPGS-COOH.....	165
6.2.3	Characterization of CS-OA, HA-OA, and TPGS-COOH.....	167
6.2.4	Synthesis of HA-OA/CS-OA based nanoparticles.....	167
6.2.5	Fabrication of Microneedle.....	169
6.2.6	Characterization of HA-OA/CS-OA based nanoparticles.....	171
6.2.7	In vitro drug release of HA-OA/CS-OA based nanoparticles.....	173
6.2.8	Cell culture studies.....	173
6.2.9	Evaluation of MN .....	175
6.2.10	In vivo efficacy: tumor regression, survival analysis, and histological studies 177	
6.2.11	Statistical analysis.....	177
6.3	Results and discussion.....	178
6.3.1	Synthesis and characterization of CS-OA, HA-OA, and TPGS-COOH ....	178
6.3.2	Formulation and characterization of nanoparticles.....	182
6.3.3	In vitro drug release study.....	189
6.3.4	Cell culture studies.....	190

6.3.5	Fabrication and evaluation of dissolving MN.....	196
6.3.6	In vivo studies .....	201
7	Summary and conclusions .....	208
7.1	Future perspective .....	210
	References.....	212
<b>8</b>	<b>Appendices.....</b>	<b>256</b>

## List of Figures

<b>Figure 1.1</b> Graphical illustration of the precise therapeutic delivery mechanism of biopolymer-based nanomedicine tailored to exploit tumor microenvironment stimuli. ____	4
<b>Figure 2.1</b> Mechanism of pH-responsive behavior of Dimethylacrylamide-Trimethyl chitosan; At acidic pH, DMMA-TCM exhibits imine bond cleavage resulting in dissociation of dimethylacrylamide and Trimethyl-chitosan [2]. _____	7
<b>Figure 2.2</b> Synthetic route of N-succinyl-CS and Al-CS [4]. _____	8
<b>Figure 2.3</b> Synthesis of dual pH and redox-sensitive acrylic polymer conjugated thiolated chitosan [5]. _____	9
<b>Figure 2.4</b> Synthesis of pH and redox-sensitive DOX/ $\alpha$ -TOS-HMSN-TK-CMCH-GRP78P [15]. _____	12
<b>Figure 2.5</b> Synthesis of ROS sensitive amphiphilic Poly(propylene sulfide) grafted Chondroitin sulfate (ChS) polymer ChS-g-PPS [16]. _____	14
<b>Figure 2.6</b> Synthesis of ROS-sensitive thioether-bearing polymer and mechanism of ROS mediated increased hydrophilicity [10]. _____	15
<b>Figure 2.7</b> Synthesis of redox-sensitive and CD44 receptor-targeted HASF polymer [21]. _____	16
<b>Figure 2.8</b> Synthesis of hypoxia-sensitive nitroimidazole modified chitosan (Cs-NA) [31]. _____	19
<b>Figure 2.9</b> Synthesis of a 2-nitroimidazole modified carboxymethyl dextran by EDC/NHS mediated carbodiimide chemistry [33]. _____	20
<b>Figure 2.10</b> Synthesis of pH-sensitive adamantane-PEG _____	24
<b>Figure 4.1</b> (A) Conjugation of SA with TPGS. (B) Schematic illustration of preparation of CS-HA-NP and CS-HA-Cmab-NP. _____	58

<b>Figure 4.2</b> HPLC Chromatogram of Cabazitaxel (RT- 5.98 min) and Docetaxel (RT- 11.23)	61
<b>Figure 4.3</b> Succinylation of TPGS	68
<b>Figure 4.4 (A)</b> FTIR, <b>(B)</b> DSC, and <b>(C)</b> XRD spectra of TPGS and TPGS-COOH	68
<b>Figure 4.5</b> <sup>1</sup> H NMR spectrum of TPGS Deuterated Methanol.	69
<b>Figure 4.6</b> <sup>1</sup> H NMR spectrum of TPGS-COOH Deuterated Methanol.	69
<b>Figure 4.7</b> <sup>13</sup> C NMR spectrum of TPGS Deuterated Methanol.	72
<b>Figure 4.8</b> <sup>13</sup> C NMR spectrum of TPGS-COOH in Deuterated Methanol.	72
<b>Figure 4.9</b> Mass spectra of TPGS at {m/z (Da) = 300-1200}	74
<b>Figure 4.10</b> Mass spectra of TPGS at {m/z (Da) = 600-1800}	75
<b>Figure 4.11</b> Mass spectra of TPGS-COOH at m/z (Da) in the range of 300-1200	76
<b>Figure 4.12</b> Mass spectra of TPGS-COOH at m/z (Da) in the range of 600-1800	77
<b>Figure 4.13</b> Morphological assessment of CS-HA-NP and CS-HA-Cmab-NP by TEM, SEM and AFM.	80
<b>Figure 4.14 (A)</b> FTIR and <b>(B)</b> DSC spectra of neat CBT, CS, HA, TPGS-COOH, CS-HA-NP, and CS-HA-Cmab-NP.	82
<b>Figure 4.15</b> XRD spectra of neat CBT, CS, HA, TPGS-COOH, CS-HA-NP, and CS-HA-Cmab-NP.	83
<b>Figure 4.16</b> XPS spectra of CS-HA-NP and CS-HA-Cmab-NP.	85
<b>Figure 4.17</b> The drug release profile of CS-HA-NP and CS-HA-Cmab-NP in Phosphate Buffered Saline pH 7.4, Phosphate Buffer pH 6.8, and Acetate Buffer pH 5.5. Comparative graph of release profiles of CS-HA-NP and CS-HA-Cmab-NP at various pH. Data presented as Mean ± SD (vertical bars); n=6.	86

**Figure 4.18** Normalized cell viability of bCS-HA-NP, Neat CBT, CS-HA-NP, and CS-HA-Cmab-NP at various concentrations calculated by MTT assay. Data presented as Mean  $\pm$  SD (vertical bars); n=6. \_\_\_\_\_ 89

**Figure 4.19** Microscopic images of cells imaged using fluorescence microscope after treatment with Free C6, c6CS-HA-NP, and c6CS-HA-Cmab-NP for 6 h. Cells were counter stained with DAPI. Scale bar = 100  $\mu$ m. \_\_\_\_\_ 90

**Figure 4.20** Hoechst33342/PI-stained MDA-MB-231 cells after 24 h treatment with Neat CBT, bCS-HA-NP, CS-HA-NP, CS-HA-Cmab-NP. The yellow and red arrows represent early apoptotic and late apoptotic cells respectively. Cells emitting blue fluorescence are Hoechst positive while those with red fluorescence are PI positive. Scale bar= 100  $\mu$ m. 91

**Figure 4.21** Microscopic images of JC-1-stained MDA-MB-231 cells after treatment with bCS-HA-NP, neat CBT, CS-HA-NP, and CS-HA-Cmab-NP. The control and bCS-HA-NP treated cells showed no signs of depolarization with cationic JC-1 binding to negatively charged polarized mitochondria to spontaneously form J-aggregates (red fluorescence). The CBT and CBT loaded nanoparticles show presence of JC-1 monomer (green fluorescence) due to depleted mitochondrial membrane potential **Scale bar = 100  $\mu$ m.** 92

**Figure 4.22** Images of MDA-MB-231 cells incubated with DCFH-DA after receiving various treatments for 24 h. The green fluorescence is due to the reduction of DCFH-DA to DCF in the presence of ROS (Scale bar = 400  $\mu$ m). \_\_\_\_\_ 94

**Figure 4.23** Cell cycle analysis; graphs showing population distribution of cells in different phases after incubation with various treatment for 24 h. \_\_\_\_\_ 95

**Figure 4.24** Comparative Plasma concentration vs time curve of Neat CBT, CS-HA-NP, and CS-HA-Cmab-NP. Data presented as Mean  $\pm$  SD (vertical bars); n=12. \_\_\_\_\_ 97

**Figure 4.25** Change in tumor volume in various treatment groups during 21-day treatment period. Data presented as Mean  $\pm$  SD (vertical bars); n=5 \_\_\_\_\_ 98

<b>Figure 4.26</b> Images of tumors collected from animals after 21-day treatment. In-image cm-scale on left side. _____	99
<b>Figure 4.27</b> Average body weight of various treatment groups during the 21-day treatment period. Data presented as Mean $\pm$ SD; n=5. *** (p-value < 0.001), **** (p-value < 0.0001), ns (p-value > 0.05)_____	99
<b>Figure 4.28</b> Kaplan Meier survival plot of various treatment groups (up to 120 days). _	101
<b>Figure 4.29</b> H&E-stained histological images of normal and tumor tissue after 21-day treatment period (images shown are captured with 4X lens). _____	101
<b>Figure 4.30</b> H&E-stained histological images of organs collected from various treatment groups after 21-day treatment period (images shown are captured with 4X lens). _____	102
<b>Figure 4.31</b> Graphical Summary of Objective 1 _____	104
<b>Figure 5.1</b> Schematic representation of Synthesis of CSVE (A) and TPGS-COOH (B); Preparation of CSVE/HA-based redox responsive nanoparticles (C). _____	110
<b>Figure 5.2</b> FTIR spectra of TPGS, TPGS-COOH, CSO, and CSVE. _____	122
<b>Figure 5.3</b> The <sup>1</sup> H NMR spectra of CSO (black) and CSVE (blue)._____	123
<b>Figure 5.4</b> The <sup>13</sup> C NMR spectra of CSO (black) and CSVE (blue). _____	124
<b>Figure 5.5</b> Morphological assessment (TEM, SEM, and AFM) of CSO/HA NP, CSVE/HA NP, CSVE/HA/DTPA NP, and CSVE/HA/DTPA/Cmab NP. _____	128
<b>Figure 5.6</b> The FTIR spectra of CBT, CSVE, TPGS, HA, CSVE/HA NP, CSVE/HA/DTPA NP and CSVE/HA/DTPA/Cmab NP. _____	129
<b>Figure 5.7</b> The XRD spectra of CBT, CSVE, TPGS, HA, CSVE/HA NP, CSVE/HA/DTPA NP and CSVE/HA/DTPA/Cmab NP. _____	130
<b>Figure 5.8</b> The DSC spectra of CBT, CSVE, TPGS, HA, CSVE/HA NP, CSVE/HA/DTPA NP and CSVE/HA/DTPA/Cmab NP. _____	132

<b>Figure 5.9</b> The XPS spectra of CSO/HA NP, CSVE/HA NP, CSVE/HA/DTPA NP and CSVE/HA/DTPA/Cmab NP.	132
<b>Figure 5.10</b> Drug release profile of CSVE/HA/DTPA NP and CSVE/HA/DTPA/Cmab NP in pH 7.4 phosphate buffer saline, pH 5.5 acetate buffer, and pH 5.5 acetate buffer and Glutathione (10 mM).	134
<b>Figure 5.11</b> Time-dependent change in free thiol concentration in CSVE/HA/DTPA NP suspension after incubation with Acetate buffer (pH 5.5) and GSH	134
<b>Figure 5.12</b> Effect of the storage of Lyophilized nanoparticles on Hydrodynamic size, PDI, Zeta Potential, and Entrapment Efficiency Data presented as Mean $\pm$ SD (vertical bars); n=6. ns (p-value > 0.05)	138
<b>Figure 5.13</b> <i>In vitro</i> cell viability of MDA-MB-231 cells after treatment with Cabazitaxel-loaded formulations for 24 h. Data presented as Mean $\pm$ SD (vertical bars); n=6.	139
<b>Figure 5.14</b> <i>In vitro</i> cell viability of HCT116 cells after treatment with Cabazitaxel-loaded formulations for 24 h. Data presented as Mean $\pm$ SD (vertical bars); n=6.	140
<b>Figure 5.15</b> <i>In vitro</i> cell viability of T47D cells after treatment with Cabazitaxel-loaded formulations for 24 h (n=6).	141
<b>Figure 5.16</b> Fluorescence microscopy images of MDA-MB-231 cells after 6 h incubation with the fluorescent CM6-loaded formulations. The blue fluorescence from DAPI channels shows Hoechst 33342 stained nuclei while the third column shows an overlay image of Hoechst and GFP. The fourth column represents an overlay image of phase contrast, GFP, and Hoechst 33342. Each scale bar represents 100 $\mu$ m. GFP channel showing higher level of green fluorescence in CM6 loaded CSVE/HA/DTPA/Cmab NP treated cells compared to free CM6 and CM6-loaded CSVE/HA/DTPA NP treated cells due to receptor-mediated cellular uptake of CM6 in the cytoplasm. The CM6-loaded CSVE/HA NP also exhibited superior intracellular localization of CM6 compared to CM6-	

loaded CSO/HA NP which may be attributed to the amphiphilic nature of the CSVE conjugate. The C-mab pretreated cells exhibited reduced uptake of CM6 which may be due to the competitive binding of C-mab to the EGFR. \_\_\_\_\_ 142

**Figure 5.17** Quantitative assessment of intracellular uptake of green fluorescent CM6 in MDA-MB-231 cells treated with free CM6, CSO/HA NP, CSVE/HA NP, CSVE/HA/DTPA NP, CSVE/HA/DTPA/C-mab NP, C-mab pre-treated CSVE/HA/DTPA/C-mab NP at 0.2  $\mu\text{g ml}^{-1}$  concentrations of CM6 for 6 h using flow cytometry. The blue peak shows the highest cellular uptake of CM6 in CM6-loaded CSVE/HA/DTPA/C-mab NP treated cells compared to free CM6, CSO/HA NP, CSVE/HA NP, CSVE/HA/DTPA NP, and CSVE/HA/DTPA/C-mab NP treated cells. \_\_\_\_\_ 143

**Figure 5.18** Assessment of mitochondrial membrane potential by JC-1 dye in control and treated MDA-MB-231 cells after treatment with Cabazitaxel, CSVE/HA/DTPA NP, CSVE/HA/DTPA/C-mab NP, and C-mab pretreated CSVE/HA/DTPA/C-mab NP. The CSVE/HA/DTPA/C-mab NP treated group showed maximum depolarization (green fluorescence of JC-1 monomer) in cells. In contrast, the control group exhibited an accumulation of JC-1 monomers in negatively charged and energized normal mitochondria to spontaneously form J aggregates (Red fluorescence). \_\_\_\_\_ 144

**Figure 5.19** Quantitative detection of mitochondrial membrane potential in MDA-MB-231 after treatment with the formulations through flow cytometry. The upper right and lower right quadrants represent cells with normal mitochondrial membrane potential and depolarized mitochondria respectively. \_\_\_\_\_ 145

**Figure 5.20** Pattern of mitochondrial distribution in MDA-MB-231 cells using Mitotracker™ Red after treatment with Cabazitaxel, CSVE/HA/DTPA NP, CSVE/HA/DTPA/C-mab NP, and C-mab pretreated CSVE/HA/DTPA/C-mab NP. The uniform distribution of mitochondria was seen in control cells represented by the yellow

arrow while aggregated mitochondria were observed in the treatment group represented by the white arrows. The CSVE/HA/DTPA/Cmab treated cells showed the highest mitochondrial aggregation. \_\_\_\_\_ 147

**Figure 5.21** Combined images of Hoechst33342/PI-stained MDA-MB-231 cells after treatment with the formulations. The yellow, white, and red arrows represent early apoptotic, late apoptotic, and necrotic cells. Cells emitting blue fluorescence are Hoechst positive while those with red fluorescence are PI positive. \_\_\_\_\_ 148

**Figure 5.22** Quantitative assessment of apoptosis by AnnexinV/PI dual staining through Flow cytometry in treated MDA-MB-231 cells. AnnexinV/PI dual staining discriminates the percentage of live (lower left quadrant), early apoptotic (lower right quadrant), late apoptotic (upper right quadrant), and necrotic or dead cells (upper left quadrant). \_\_\_\_ 149

**Figure 5.23** Concentration of Cabazitaxel in plasma, tumor, liver, lung, spleen and kidney after intravenous administration of Cabazitaxel, CSVE/HA/DTPA NP, and CSVE/HA/DTPA/Cmab NP. Data presented as Mean  $\pm$  SD (vertical bars); n=12. \_\_\_\_ 153

**Figure 5.24** Body weight and tumor volume of normal SD rats, tumor-bearing SD rats, and formulation treated tumor-bearing SD rats over the 28 days treatment period. Data presented as Mean  $\pm$  SD (vertical bars); n=5. \_\_\_\_\_ 155

**Figure 5.25** Images of the tumors harvested from various treatment groups after end of 28-day treatment period. The line marked on the left side of tumor represents a cm-scale. \_\_\_\_\_ 155

**Figure 5.26** Survival rates of tumor-bearing SD rats after treatment with formulations, day 0 represents the day of administration of first dose after the tumor volume reached  $\sim 500 \text{ mm}^3$ . \_\_\_\_\_ 156

**Figure 5.27 (A)** Histological assessments of tumors isolated from rats after tumor regression study using H&E stain (magnification 4 $\times$ ; scale bar 100  $\mu\text{m}$ ) **(B)** Histological

images of organ tissues after 28 days of treatment regimen. Invasive carcinoma (IC), stromal tissue (ST), proliferated and expanded terminal lobular units (HLU), dilated ducts with inspissated secretions (D), Ductal Carcinoma (DC), Mucin (yellow arrow), Fibroadenoma (F) and breast hyperplasia (H). _____	157
<b>Figure 5.28 Graphical Summary of Objective 2</b> _____	159
<b>Figure 6.1</b> Schematic representation of the a) Succinylation of TPGS, b) synthesis of hyaluronic acid–oleylamine conjugate (HA-OA), and c) synthesis of chitosan–oleic acid conjugate (CS-OA). _____	166
<b>Figure 6.2</b> Schematic representation of the preparation of non-targeted HA-OA/CS-OA based nanoparticles (HA-OA/CS-OA NPT), targeted HA-OA/CS-OA based nanoparticles (HA-OA/CS-OA NPT), free Cabazitaxel drug loaded microneedles (CBT-MN) and targeted HA-OA/CS-OA based nanoparticles loaded microneedles (HA-OA/CS-OA NPT – MN). _____	170
<b>Figure 6.3</b> <sup>1</sup> H NMR spectra of Hyaluronic acid, oleylamine (OA), and HA-OA. _____	179
<b>Figure 6.4</b> <sup>1</sup> H NMR spectra of Chitosan (CSO), Oleic acid (OA), and CS-OA. _____	180
<b>Figure 6.5</b> FTIR spectrum of CS, CS-OA, HA, HA-OA, TPGS, and TPGS-COOH _____	181
<b>Figure 6.6</b> Characterization of HA-OA/CS-OA based hybrid nanoparticles; (A) TEM image of HA-OA/CS-OA NP (B) AFM image of HA-OA/CS-OA NP, (C) TEM image of HA-OA/CS-OA NPT, and (D) AFM image of HA-OA/CS-OA NPT. _____	184
<b>Figure 6.7</b> (A) FTIR and (B) XRD spectra of CBT, HA-OA/CS-OA NP, HA-OA/CS-OA NPT, and HA-OA/CS-OA NPT MN. _____	185
<b>Figure 6.8</b> (A) DSC thermogram of CBT, HA-OA/CS-OA NP, HA-OA/CS-OA NPT, and HA-OA/CS-OA NPT-MN. (B) Surface characterization by XPS analysis of HA-OA/CS-OA NP and HA-OA/CS-OA NPT. (C) <i>In vitro</i> drug release study of HA-OA/CS-OA NP and HA-OA/CS-OA NPT at pH 5.8 and pH 7.4. _____	186

**Figure 6.9** *In vitro* cytotoxicity of HA-OA/CS-OA NP and HA-OA/CS-OA NPT in MDA-MB 231 breast cancer cell line after 24 hours of treatment. Data presented as Mean  $\pm$  SD. \_\_\_\_\_ 189

**Figure 6.10** Fluorescence microscopy images of MDA-MB-231 cells after 6 hours incubation with free C6 and various HA-OA/CS-OA NP, HA-OA/CS-OA NPT, and C-mab-pretreated HA-OA/CS-OA NPT. GFP channel showing a higher level of green fluorescence in HA-OA/CS-OA NP and HA-OA/CS-OA NPT treated cells as compared to free C6. C-mab-pretreated HA-OA/CS-OA NPT treatment showed diminished fluorescence than HA-OA/CS-OA NPT treatment. Images were captured through an inverted fluorescence microscope at 400X magnification and 100  $\mu$ m scale bar. \_\_\_\_\_ 191

**Figure 6.11** Combined microscopic images of Hoechst33342 / PI-stained MDA-MB-231 cells after 24 hours of treatment with free CBT, HA-OA/CS-OA NP, HA-OA/CS-OA NPT, and C-mab-pretreated HA-OA/CS-OA NPT. The white, yellow, and red arrows represent early apoptotic, late apoptotic, and necrotic cells. Cells emitting blue fluorescence (DAPI channel) are Hoechst positive while those with red fluorescence (PI channel) are PI positive. \_\_\_\_\_ 192

**Figure 6.12** The impact of free-CBT, HA-OA/CS-OA NP, and HA-OA/CS-OA NPT treatment on the mitochondrial membrane potential of MDA-MB-231 cells was assessed using JC-1 staining after 24 hours of treatment. The increase in green fluorescence emission indicates a decrease in mitochondrial membrane potential upon treatment, with the highest decline seen in HA-OA/CS-OA NPT-treated cells. Red fluorescence indicates normal potential. Cells were examined using an inverted fluorescence microscope at 400X magnification. \_\_\_\_\_ 194

**Figure 6.13** Plot denotes the flow cytometric data of free-CBT, HA-OA/CS-OA NP, and HA-OA/CS-OA NPT treated MDA-MB-231 cells. PI staining was done to determine the percentage of cells in different phases of the cell cycle. HA-OA/CS-OA NPT treated cells

showed a significant ( $***p < 0.001$ ) increase in cell population in the G2-M phase in comparison to the control. A significant difference between various treatment groups was also observed. Free-CBT and HA-OA/CS-OA NP ( $***p < 0.001$ ), free-CBT and HA-OA/CS-OA NPT ( $***p < 0.001$ ), and HA-OA/CS-OA NP and HA-OA/CS-OA NPT ( $**p < 0.01$ ) was observed. \_\_\_\_\_195

**Figure 6.14** Photograph and Scanning Electron Microscopy (SEM) images of HA-OA/CS-OA NPT MN \_\_\_\_\_196

**Figure 6.15** Microscopic images of HA-OA/CS-OA NPT MN after exposure to PBS pH 7.4 and dissolution of microneedles in PBS pH 7.4 (calculated in terms of percentage therapeutic payload release w.r.t. time). \_\_\_\_\_197

**Figure 6.16** Images of parafilm layers after insertion of HA-OA/CS-OA NPT MN. The histogram represents the percentage of holes created in each layer of parafilm by microneedles after manual insertion \_\_\_\_\_199

**Figure 6.17 (A)** Microscopic image of rat skin after HA-OA/CS-OA NPT MN insertion. **(B)** Graph representing ex vivo drug permeation across rat skin determined using a Franz diffusion cell. HA-OA/CS-OA NPT-MN showed significant improvement in drug permeation in comparison to CBT-MN and HA-OA/CS-OA NPT loaded PVA/HA blend ( $****p$  value  $< 0.001$ ) **(C)** Representation of percentage drug retention in skin after 48 hours of drug permeation study. \_\_\_\_\_200

**Figure 6.18 (A)** Tumor regression analysis in Tumor-bearing female SD rats; Tumor volume of control, CBT (i.v.), CBT-MN, and HA-OA/CS-OA NPT-MN treated groups during a 14-day treatment period. **(B)** Image of tumors collected from treatment groups after 14 days of treatment. \_\_\_\_\_203

**Figure 6.19 (A)** Body weight of healthy female SD rats and tumor-bearing female SD rats receiving CBT (i.v.), CBT-MN, and HA-OA/CS-OA NPT-MN treatment for a 14-day

treatment regimen. **(B)** The Kaplan-Meier survival probability plot (120 days) of Healthy and tumor-bearing female SD rats treated with CBT (i.v.), CBT-MN, and HA-OA/CS-OA NPT-MN treated groups. \_\_\_\_\_ 203

**Figure 6.20** Histological images of H&E stained healthy/tumor tissues before and after 14-day treatment with CBT (i.v.), CBT-MN and HA-OA/CS-OA NPT-MN. \_\_\_\_\_ 205

**Figure 6.21** Graphical Abstract of Objective 3 \_\_\_\_\_ 206

## List of Tables

<b>Table 2.1</b> Biopolymer for tumor microenvironment responsiveness for site-specificity	21
<b>Table 2.2</b> Targeted nanomedicine responsive to the tumor microenvironment and their drug release mechanisms	39
<b>Table 4.1</b> Composition of CS-HA-NP and CS-HA-Cmab-NP nanoparticles	59
<b>Table 4.2</b> Interpretation of <sup>1</sup> H NMR Spectra of TPGS and TPGS-COOH	69
<b>Table 4.3</b> Interpretation of <sup>13</sup> C NMR Spectra of TPGS and TPGS-COOH	71
<b>Table 4.4</b> Interpretation of the mass (TOF) spectra of TPGS and TPGS-COOH	78
<b>Table 4.5</b> Characterization of CS-HA-NP and CS-HA-Cmab-NP nanoparticles	81
<b>Table 4.6</b> XPS surface atomic percentage of N1s, O1s, C1s, S2p, and P2p in CS-HA-NP and CS-HA-Cmab-NP	85
<b>Table 4.7</b> Correlation coefficients of Cabazitaxel release profile from CS-HA-NP and CS-HA-Cmab-NP at various pH in various fitted release kinetic models	87
<b>Table 4.8</b> Pharmacokinetic parameters of Neat CBT, CS-HA-NP, and CS-HA-Cmab-NP after I.V. administration (6.5 mg/kg IV, n = 12) in female Sprague Dawley rats.	96
<b>Table 5.1</b> Composition, particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency of various formulations.	127
<b>Table 5.2</b> R-square values for mathematical models to determine the goodness of fit.	137
<b>Table 5.3</b> Pharmacokinetic parameters of various formulations determined from plasma concentration vs time plot	151
<b>Table 6.1</b> Composition and properties of various HA-OA/CS-OA NPs prepared during optimization	168
<b>Table 6.2</b> Composition of the various formulations used to prepare MNs	169
<b>Table 6.3</b> Particle size, PDI, zeta potential, EE, and DL of prepared nanoparticles.	183
<b>Table 6.4</b> Fitted release kinetic model for in-vitro drug release of nanoparticles.	188

## LIST OF ABBREVIATIONS

- EPR** : Enhanced permeation and retention effect
- i.v.** : Intravenous
- VES** : Vitamin-E succinate
- CSO** : Chitosan oligosaccharide
- CS** : Chitosan
- HA** : Hyaluronic acid
- NaTPP**: Sodium tripolyphosphate
- EDAC / EDC** : 1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide Hydrochloride
- NHS** : N-hydroxysuccinimide
- Sulfo-NHS**: N-hydroxysulfosuccinimide
- DTPA** : 3,3'-dithiodipropionic acid
- CBT** : Cabazitaxel
- PDI** : Polydispersity Index
- DLS** : Dynamic Light Scattering
- FTIR** : Fourier Transform Infrared Spectroscopy
- SEM** : Scanning Electron Microscope
- SPM** : Scanning Probe Microscope
- AFM** : Atomic Force Microscope
- HR-TEM**: High-Resolution Transmission Electron Microscope
- XRD** : Powder X-ray diffraction
- DSC** : Differential Scanning Calorimetry
- XPS** : X-ray Photoelectron Spectroscopy
- TPGS** : Tocopherol polyethylene glycol succinate
- PBS** : Phosphate Buffer Saline

**FBS** : Fetal Bovine Serum

**MTT** : (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)

**DCFDA**: 2'-7'- dichlorodihydrofluorescein diacetate

**JC-1** : 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide

**PI** : Propidium Iodide

**DAPI** : 4',6-diamidino-2-phenylindole

**DMBA**: 7,12-Dimethylbenz[a]anthracene

**H&E** : Hematoxylin and eosin staining

**AUC** : Area Under Curve

**MRT** : Mean Residence Time

**GSH** : Glutathione

**ROS** : Reactive Oxygen Species

**IC<sub>50</sub>** : Half maximal inhibitory concentration

**C6** : Coumarin-6

**Cmab** : Cetuximab