POLYSACCHARIDE BASED BIODEGRADABLE PARTICLES WITH VARIOUS ARCHITECTURES INTENDED FOR PROGRAMMABLE DRUG DELIVERY

Aiswarya T.T.

Department of Materials Science and Engineering

INDIAN INSTITUTE OF TECHNOLOGY DELHI

MARCH 2025

POLYSACCHARIDE BASED BIODEGRADABLE PARTICLES WITH VARIOUS ARCHITECTURES INTENDED FOR PROGRAMMABLE DRUG DELIVERY

by

Aiswarya T.T.

Department of Materials Science and Engineering

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

MARCH 2025

Dedicated to my family and God

CERTIFICATE

This is to certify that the thesis entitled, “Polysaccharide Based Biodegradable Particles with Various Architectures Intended for Programmable Drug Delivery” being submitted by Ms. Aiswarya T T to Indian Institute of Technology Delhi for the award of degree of Doctor of Philosophy is a record of bonafide research work carried out by her. Ms. Aiswarya T T has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard. The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

Prof. Sampa Saha

Associate Professor

Department of Materials Science & Engineering

Indian Institute of Technology Delhi

Hauz Khas, New Delhi - 110016

ACKNOWLEDGEMENTS

I am incredibly grateful to my supervisor, Prof. (Mrs.) Sampa Saha, for her invaluable guidance, unwavering encouragement, and tremendous support throughout my research journey. Her insightful advice, extensive knowledge, and unfailing support were invaluable in assisting me in deepening my comprehension of polymer chemistry.

I extend my sincere thanks to Prof. J. Jacob, Prof. Leena Nebhani, Prof. Neetu Singh, Prof. B.K. Satapathy, Prof Shib Shankar Banerjee, Prof. A.K. Ghosh, and Prof. Bijay P. Tripathi, as well as all faculty members of the Department of Materials Science and Engineering at IIT Delhi, for their invaluable encouragement and support during my work. I would like to deeply appreciate Dr. Dietmar Appelhans, Institute of Macromolecular Chemistry, for his insightful guidance and valuable input while I worked in his lab at Leibniz Institute of Polymer Research Dresden.

My warmest gratitude is extended to my lab colleagues, whose affection, motivation, and enlightening conversations made my time pursuing research genuinely unforgettable. I would like to acknowledge my seniors, Dr. Shivangi Sharma, Dr. Ifra Mirza, Dr. Agni Kumar Biswal, Dr. Shaifali Dhingra and Dr. Kalpana Pandey for their assistance in the early stages of my research career and up to this point and my juniors, Kunal Verma, Subhashree Subhasmita Pradhan, Manmohan Bharadwaj Mouli Sarkar, Vasanth Kumar, Sumit Kumar, Ashuthosh Kumar, Dharmendra Pandey, Ashank Upadhyay, Rohit Kumar, Ankit Mishra and Sumit Gupta and Dr. Chandrani Sarkar for their continual support. It was a pleasure and an absolute pleasure to work with them. Special thanks go to the technical staff, including Mr. Surender Sharma, Mr. Shivkant, Mr. Ashok Kapoor, Mr. Islam, Mr. Gajraj, Mr. Jitender,Mr. Ashish Sharma, Mrs. Shalini Arora, Mr. Subhash, and Mr. Amit for their immediate assistance whenever needed. I would like to mention the research collaborators, Ms. Manisha Malani and Prof. Jayabalan Nirmal from Birla Institute of Technology and Science-Pilani, Hyderabad, Mr. Nandan Murali, and Prof Soutik Betal from Department of Electrical Engineering, IIT Delhi, Dr. Hemlata Rai and Prof. Shilpi Minocha from Kusuma School of Biological Sciences, IIT Delhi and Prof. Aniruddh Vashisth from University of Washington for their valuable inputs and support.

I am blessed to have my family's unconditional love, support, and motivation which have been the foundation of my Ph.D. journey. I take this opportunity to dedicate my deepest appreciation to my parents Mr. Sadanandan T T and Shylaja T C and my sisters Adv.

Anaswara T T and Anupama T T for their firm trust in me, their patience, and the innumerable sacrifices they made to support me in achieving my goals. I am eternally thankful that the four of you being in my life and for giving me the courage and self-assurance I needed to keep pursuing my dreams. My best friend and better half Dr. Biswajit Mishra have been my anchor throughout this journey. I appreciate you being there for me during the times of uncertainty as well as joyful moments, always there to offer words of assistance and affirmation for that I am forever grateful. I consider myself fortunate to have a family who has never stopped backing me in chasing my ambitions. I would like thank my achamma, ammomma, vellyachans, vellyammas, mamman ,ammai, kuttyatans and chechis for their affection and love. I will always remember my beloved Vellyachan, the Late Adv. Sreedharan T. T., who is undoubtedly proud of my accomplishment from heaven. My friends always have provided me strength and motivation which has contributed to flourishing personally and academically, particularly Meenakshi Verma, Dr. Lukkumanul Hakkim, Nidhi Gupta, and Harshita.

Finally, I would like to convey feelings of appreciation to each person who supported me to complete my thesis, and I truly regret not being able to acknowledge them all individually. I am also extremely grateful to the Department of Science and Technology for providing me financial assistance through DST INSPIRE funds enabling me to move forward with my Ph.D journey without any unhindered Above all, I am thankful to my God for the countless opportunities, insight and inspiration, this journey would not have been possible without your blessings.

Aiswarya

ABSTRACT

Recent research in the development of biomaterials has shifted focus from synthetic biocompatible polymers to natural materials due to their inherent biocompatibility, biodegradability, and compatibility with the metabolic system. Among natural biomolecules, polysaccharides have garnered interest due to their abundance, hydrophilicity, defined structure, ease of functionalization, and selectivity towards specific cell sites. Polysaccharides, as constituents of glycoproteins, glycolipids, and proteoglycans, play crucial roles in cell signalling, immune recognition, proliferation, adhesion, and tumor metastasis. These properties make them ideal for biomedical applications ranging from drug delivery to tissue engineering. Polysaccharide-based biomaterials can mimic biological functions, resist elimination from the body, and perform targeted therapeutic actions, making them promising candidates for advanced therapeutic delivery systems.

This thesis explores the fabrication and application of polysaccharide-based materials for drug delivery. Chapter 2 discusses the synthesis of carboxylated nanocellulose fibers for sustained antimicrobial delivery. The nanocellulose was prepared through a two-step process involving citric acid-induced hydrolysis followed by TEMPO-mediated oxidation, resulting in high carboxyl content (~1.12 mmol/g). This high carboxyl content facilitated the capture and release of antibiotics like triclosan and ampicillin sodium, showing high drug loading (>40%) and entrapment efficiency (>80%). Molecular docking studies revealed that the nanocellulose with the highest carboxyl content exhibited strong binding affinity towards antibiotics via hydrogen bonding. Triclosan-loaded fibers displayed sustained antibacterial activity against Escherichia coli and Staphylococcus aureus over a week, and ampicillin-loaded fibers released the drug within 48 hours, demonstrating diffusion-driven release. The nanocellulose-based system offers a green and cost-effective alternative for developing biodegradable nanocarriers with high drug loading and sustained antibacterial effects. Clinical validation was performed by loading the fibers with moxifloxacin hydrochloride to treat bacterial-resistant ocular infections. The moxifloxacin-loaded fibers showed better permeability into Staphylococcus aureus biofilm, with a sustained release of the drug over 40 hours and a reduction in the frequency of dosing.

Chapter 3 details the development of core-shell microspheres with a cellulosic core and an acetalated dextran as shell for stimuli-responsive drug delivery. The acetalated dextran based shell, a pH-sensitive polysaccharide, provided physiological stability, while the porous cellulose core facilitated better drug entrapment and diffusion. A magneto/photo-responsive

microsphere co-loaded with magnetite (Fe_3O_4) nanoparticles, nano zero valent iron (nZVI) particles, and a photoacid generator (PAG) was designed for degradation-assisted release behavior. Upon exposure to an alternating magnetic field (AMF) and UV LED irradiation, these microspheres rapidly degraded, releasing a model drug (curcumin) in less than 50 minutes. The system demonstrated cytotoxicity under external stimulus, validating its potential for targeted cancer therapy. In Chapter 4, the microspheres were modified for radiofrequency (RF) responsiveness by incorporating graphene oxide (GO), enabling on-demand drug release. The GO-loaded microspheres exhibited RF-triggered disintegration and released 98% of the encapsulated drug within 60 minutes, showing promise for precision drug delivery. Chapter 5 introduces bicompartmental (Janus) microparticles with spatioselective conjugation of polymannose (polyMEMA) moieties for targeted drug delivery. The microparticles were fabricated using poly(lactic acid) (PLA) and bromo-functionalized random copolymers through electrohydrodynamic co-jetting, followed by surface-initiated atom transfer radical polymerization (SIATRP) to graft poly(MEMA) brushes. The density of polymannose increased with PLA-Br concentration, enhancing the binding affinity of the particles to mannose receptors expressed on macrophages and dendritic cells. Interaction studies using Concanavalin A and functionalized polymersomes (may act as drug carriers) confirmed their efficient conjugation to polymannose brush modified Janus particles, indicating their potential as targeted drug delivery vehicles. Finally, Chapter 6 concludes the advances in polysaccharide-based biomaterials for targeted drug delivery, highlighting their biocompatibility, biodegradability, and specificity. Systems like carboxylated nanocellulose based fibers, core-shell microspheres, and Janus microparticles demonstrated sustained drug release for applications in antimicrobial therapy, cancer treatment, and immune targeting. The outlook suggests diversifying the polysaccharide types and improving their scalability for *in situ* diagnostics and controlled release, paving an innovative way for sustainable, personalized drug delivery in biomedical arena.

सार

बायोमटेरियल के विकास में हाल के शोध ने अपनी अंतर्निहित बायोकम्पैटिबिलिटी, बायोडिग्रेडेबिलिटी और मेटाबॉलिक सिस्टम के साथ अनुकूलता के कारण सिंथेटिक बायोकम्पैटिबल पॉलिमर से प्राकृतिक सामग्रियों पर ध्यान केंद्रित किया है। प्राकृतिक बायोमोलेक्यूल्स में, पॉलीसेकेराइड ने अपनी प्रचुरता, हाइड्रोफिलिसिटी, परिभाषित संरचना, कार्यात्मकता में आसानी और विशिष्ट सेल साइटों के प्रति चयनात्मकता के कारण रुचि अर्जित की है। ग्लाइकोप्रोटीन, ग्लाइकोलिपिड्स और प्रोटियोग्लाइकन के घटक के रूप में पॉलीसेकेराइड सेल सिग्नलिंग, प्रतिरक्षा पहचान, प्रसार, आसंजन और ट्यूमर मेटास्टेसिस में महत्वपूर्ण भूमिका निभाते हैं। ये गुण उन्हें दवा वितरण से लेकर ऊतक इंजीनियरिंग तक के जैव चिकित्सा अनुप्रयोगों के लिए आदर्श बनाते हैं। पॉलीसेकेराइड-आधारित बायोमटेरियल जैविक कार्यों की नकल कर सकते हैं, शरीर से निष्कासन का विरोध कर सकते हैं अध्याय 2 में निरंतर रोगाणुरोधी वितरण के लिए कार्बोक्सिलेटेड नैनोसेल्यूलोज फाइबर के संश्लेषण पर चर्चा की गई है। नैनोसेल्यूलोज को दो-चरणीय प्रक्रिया के माध्यम से तैयार किया गया था जिसमें साइट्रिक एसिड-प्रेरित हाइड्रोलिसिस शामिल था, उसके बाद TEMPO-मध्यस्थ ऑक्सीकरण हुआ, जिसके परिणामस्वरूप उच्च कार्बोक्सिल सामग्री (~ 1.12 mmol/g) प्राप्त हुई। इस उच्च कार्बोक्सिल सामग्री ने ट्राइक्लोसन और एम्पीसिलीन सोडियम जैसे एंटीबायोटिक्स को पकड़ने और छोड़ने में मदद की, जिससे उच्च दवा लोडिंग (> 40%) और फंसाने की दक्षता (> 80%) दिखाई दी। आणविक डॉकिंग अध्ययनों से पता चला है कि उच्चतम कार्बोक्सिल सामग्री वाले नैनोसेल्यूलोज ने हाइड्रोजन बॉन्डिंग के माध्यम से एंटीबायोटिक्स के प्रति मजबूत बंधन संबंध प्रदर्शित किया। ट्राइक्लोसन-लोडेड फाइबर ने एक सप्ताह में एस्चेरिचिया कोली और स्टैफिलोकोकस ऑरियस के खिलाफ निरंतर जीवाणुरोधी गतिविधि प्रदर्शित की, और एम्पीसिलीन-लोडेड फाइबर ने 48 घंटों के भीतर दवा जारी की, जो प्रसार-संचालित रिलीज का प्रदर्शन करता है। नैनोसेल्यूलोज-आधारित प्रणाली उच्च दवा लोडिंग और निरंतर जीवाणुरोधी प्रभावों के साथ बायोडिग्रेडेबल नैनोकैरियर विकसित करने के लिए एक हरित और लागत प्रभावी विकल्प प्रदान करती है। बैक्टीरिया-प्रतिरोधी नेत्र संक्रमणों के उपचार के लिए फाइबर को मोक्सीफ्लोक्सासिन हाइड्रोक्लोराइड से लोड करके नैदानिक सत्यापन किया गया। मोक्सीफ्लोक्सासिन-लोडेड फाइबर ने स्टैफिलोकोकस ऑरियस बायोफिल्म में बेहतर पारगम्यता दिखाई, जिसमें 40 घंटे से अधिक समय तक दवा की निरंतर रिहाई और खुराक की आवृत्ति में कमी आई। अध्याय 3 में उत्तेजना-उत्तरदायी दवा वितरण के लिए सेल्यूलोज कोर और एसीटैलेटेड डेक्सट्रान शेल के साथ कोर-शेल माइक्रोस्फीयर के विकास का विवरण दिया गया है। एसीटैलेटेड डेक्सट्रान शेल, एक पीएच-संवेदनशील पॉलीसेकेराइड,

शारीरिक स्थिरता प्रदान करता है, जबकि छिद्रपूर्ण सेल्यूलोज कोर बेहतर दवा फंसाने और प्रसार की सुविधा प्रदान करता है। मैग्नेटाइट (Fe3O4) नैनोकणों, नैनो जीरो वैलेंट आयरन (nZVI) कणों और एक फोटोएसिड जनरेटर (PAG) के साथ सह-लोड किए गए एक मैग्नेटो/फोटो-उत्तरदायी माइक्रोस्फीयर को गिरावट-सहायता प्राप्त रिलीज व्यवहार के लिए डिज़ाइन किया गया था। एक वैकल्पिक चुंबकीय क्षेत्र (AMF) और UV LED विकिरण के संपर्क में आने पर, ये माइक्रोस्फीयर तेजी से विघटित हो गए, जिससे 50 मिनट से भी कम समय में एक मॉडल दवा (कर्क्यूमिन) निकल गई। इस प्रणाली ने बाहरी उत्तेजना के तहत साइटोटॉक्सिसिटी का प्रदर्शन किया, जिससे लक्षित कैंसर थेरेपी के लिए इसकी क्षमता की पुष्टि हुई। अध्याय 4 में, माइक्रोस्फीयर को ग्रेफीन ऑक्साइड (GO) को शामिल करके रेडियोफ्रीक्वेंसी (RF) प्रतिक्रियाशीलता के लिए संशोधित किया गया था, जिससे ऑन-डिमांड ड्रग रिलीज़ संभव हो गई। GO-लोडेड माइक्रोस्फीयर ने RF-ट्रिगर विघटन का प्रदर्शन किया और 60 मिनट के भीतर 98% एनकैप्सुलेटेड ड्रग को रिलीज़ किया, जिससे सटीक ड्रग डिलीवरी की संभावना दिखाई दी। अध्याय 5 लक्षित ड्रग डिलीवरी के लिए पॉलीमैनोज़ मोइटीज़ के स्थानिक चयनात्मक संयुग्मन के साथ द्वि-कम्पार्टमेंटल (जेनस) माइक्रोपार्टिकल्स का परिचय देता है। इलेक्ट्रोहाइड्रोडायनामिक को-जेटिंग के माध्यम से पॉली(लैक्टिक एसिड) (PLA) और ब्रोमीन-फंक्शनलाइज्ड रैंडम कॉपोलिमर का उपयोग करके माइक्रोपार्टिकल्स का निर्माण किया गया, इसके बाद पॉली(MEMA) ब्रश को ग्राफ्ट करने के लिए सरफेस-इनिशिएटेड एटम ट्रांसफर रेडिकल पॉलीमराइजेशन (SIATRP) का उपयोग किया गया। पॉलीमैनोज़ का घनत्व PLA-Br सांद्रता के साथ बढ़ा, जिससे मैक्रोफेज और डेंड्राइटिक कोशिकाओं पर व्यक्त मैनोज़ रिसेप्टर्स के लिए कणों की बंधन आत्मीयता बढ़ गई। कॉनकेनवेलिन ए और कार्यात्मक पॉलीमरसोम का उपयोग करके इंटरेक्शन अध्ययन ने इन माइक्रोपार्टिकल्स के प्रभावी बंधन की पुष्टि की, जो लक्षित वितरण वाहनों के रूप में उनकी क्षमता का संकेत देता है। अंत में, अध्याय 6 लक्षित दवा वितरण के लिए पॉलीसैकेराइड-आधारित बायोमटेरियल में प्रगति का निष्कर्ष निकालता है, उनकी जैव-संगतता, जैव-निम्नीकरणीयता और विशिष्टता पर प्रकाश डालता है। कार्बोक्सिलेटेड नैनोसेल्यूलोज फाइबर, कोर-शेल माइक्रोस्फीयर और जेनस माइक्रोपार्टिकल्स जैसी प्रणालियाँ एंटीमाइक्रोबियल थेरेपी, कैंसर उपचार और प्रतिरक्षा लक्ष्यीकरण में अनुप्रयोगों के लिए निरंतर दवा रिलीज़ को प्रदर्शित करती हैं। भविष्य का दृष्टिकोण पॉलीसैकेराइड प्रकारों में विविधता लाने और इन-सीटू डायग्नोस्टिक्स और नियंत्रित रिलीज के लिए मापनीयता में सुधार करने का सुझाव देता है, जिससे बायोमेडिकल अनुप्रयोगों में टिकाऊ, व्यक्तिगत दवा वितरण का मार्ग प्रशस्त होता है.

TABLE OF CONTENTS

CERTIFICATE i

ACKNOWLEDGEMENTS ii

ABSTRACT iv

LIST OF FIGURES xii

LIST OF TABLES xix

LIST OF ABBREVIATIONS xxi

CHAPTER 1: INTRODUCTION, LITERATURE REVIEW, MOTIVATION, AND OBJECTIVES 1

1.1. Introduction 2

1.2. Mono-and Disaccharide based biomaterials 5

1.3. Carbohydrate conjugated Polymeric nanoparticles 5

1.4. Polysaccharide based nanoparticles 10

1.4.1. Cellulose-based particles 12

1.4.2. Chitosan-based particles 15

1.4.3. Dextran based particles 18

1.5. Effect of shape, size, ligand density, and linker length on cell binding efficiency and cellular uptake 21

1.6. Application of carbohydrate-based nanoparticles 23

1.6.1 Drug delivery 23

1.6.2. Mono/Di-saccharide for drug delivery 23

1.6.3. Polysaccharides for drug delivery 25

1.6.4. Gene therapy 30

1.6.5. Theranostics and Bioimaging 32

1.7. Objective 37

1.8. Thesis organization 37

1.9. Brief overview of each chapter 38

CHAPTER 2A: TEMPO-OXIDIZED NANOFIBRILLATED CELLULOSE AS POTENTIAL CARRIER FOR SUSTAINED ANTIBACTERIAL DELIVERY 41

2A.1. Introduction 42

2A.2. Materials and Methods 46

2A.2. 1. Materials 46

2A.2. 2.Synthesis of citric acid treated NFC 47

2A.2. 3.Synthesis of TEMPO mediated oxidized NFC 47

2A.2. 4.Morphological analysis 48

2A.2. 5. Structural characterizations 48

2A.2.6. Determination of carboxylic acid group content and degree of polymerization (DPv) 48
2A.2. 7. Zeta-potential measurement 49
2A.2. 8. Determination of water dispersibility 49
2A.2. 9. Determination of drug entrapment efficiency and drug loading 49
2A.2. 10. Molecular docking study 51
2A.2. 11.Determination of water uptake (%) 51
2A.2. 12. In vitro release of triclosan and ampicillin sodium from drug loaded samples 51
2A.2. 13. Assessment of antimicrobial activity of triclosan loaded nanofibrillated cellulose .. 52
2A.3. Results and discussion 54
2A.3.1. Carboxyl modification of nano fibrillated cellulose from cellulose pulp 54
2A.3.2. Morphological analysis 56
2A.3.3. Analysis of FTIR, surface charges, water contact angle and water dispersibility 57
2A.3.4. X-Ray diffraction study 60
2A.3.5. N_2 adsorption/desorption isotherm 61
2A.3.6. Drug adsorption and drug entrapment efficiency by NFCs 62
2A.3.7. Water uptake study 67
2A.3.8. In vitro release study 68
2A.3.9. Molecular Docking study 73
2A.3.10. Assessment of antimicrobial activity of drug-loaded system 75
2A.3.11. Analysis of live /dead bacteria 78
2A.4. Conclusion 79
CHAPTER 2B: CARBOXYLATED NANOFIBRILLATED CELLULOSE EMPOWERS MOXIFLOXACIN TO OVERCOME STAPHYLOCOCCUS AUREUS BIOFILM IN BACTERIAL KERATITIS 81
2B.1. Introduction 82
2B.2.Materials and Methods 83
2B.2.1. Synthesis of carboxylated Nanofibrillated cellulose (cNFC) 83
2B.2.2a. Synthesis of cNFC 83
2B.2.2. Morphological and structural analysis 84
2B.2.3. Determination of carboxylic acid group content 84
2B.2.4. Estimation of the sugar content and degree of polymerization (DPv) 84
2B.2.5. Determination of Klason lignin and hemicellulose content 85
2B.2.6. Drug loading 85
2B.2.7. Determination of drug loading and entrapment efficiency 85
2B.2.8. In vitro release study 86
2B.3. Results and Discussion 86

2B.3.1. Synthesis and characterization of cNFC-based fibers 86
2B.4. Conclusions 92
CHAPTER 3: RADIOFREQUENCY-TRIGGERED RELEASE OF THERAPEUTICS FROM GRAPHENE OXIDE-LOADED POLYSACCHARIDE CORE-SHELL MICROSPHERES 93
3.1. Introduction 94
3.2. Materials and methods 96
3.2.1. Materials 96
3.2.3. Preparation of Core Shell Constituents 96
3.3. Characterizations 98
3.4. Results and Discussions 100
3.4.1. Fabrication of Ac-D /CMC based core-shell microspheres 100
3.4.2. Determination of layer compositions 104
3.4.2. Determination of surface functionalities 108
3.4.3. Encapsulation of drug in the microparticles 110
3.4.4. Radiofrequency (RF) triggered drug release 113
3.5. Conclusion 121
CHAPTER 4: MAGNETO-PHOTO RESPONSIVE DRUG DELIVERY CARRIER ORIGINATED FROM ACETALATED DEXTRAN/CARBOXYLATED NANOCELLULOSE BASED CORE-SHELL MICROSPHERES 122
4.1. Introduction 123
4.2. Materials and methods 126
4.2.1. Materials 126
4.2.2. Fabrication of Fe_3O_4 nanoparticles 126
4.2.3. Fabrication and Characterization of core shell Microspheres 126
4.3. Instrumentation 129
4.4. Results and Discussion 130
4.4.1. Synthesis and characterization of the multi-stimuli responsive acetalated dextran/CMC based core shell microspheres 130
4.2.2. Morphological and TGA analysis 132
4.4.2. X-ray diffraction study 135
4.4.3. X-ray photoelectron spectroscopy (XPS) analysis 136
4.4.4. Magnetic properties 138
4.4.5. Synergistic effect of combining ZVI and Fe3O4 in activating PAG 140
4.4.6. Drug loading and release behavior 145
4.4.7. Assessment of cytotoxicity 153
4.5. Conclusion 157

CHAPTER 5: SPATIO-SELECTIVE GRAFTING OF GLYCOPOLYMER BRUSHES ON BI-COMPARTMENTAL JANUS MICROPARTICLES AND THEIR APPLICATION IN ATTACHING POLYMERSOMES 159

5.1 Introduction 160

5.2. Materials and Methods 162

5.2.1. Materials 162

5.2.2. Characterization techniques..... 162

5.2.3. Synthesis of bromo functionalized polylactides (PLA-Br)..... 163

5.2.4. Fabrication of Bicompartmental/Janus Microparticles containing macroinitiator (PLA-Br) in one of the hemispheres..... 164

5.2.6. Selective Conjugation of FITC labelled Concanavalin A to poly MEMA grafted PM50 particles..... 167

5.2.7. Interaction of Psomes with PM50 microparticles 168

5.3. Results and Discussions 168

5.3.2. Interaction of PM 50 microparticles with Con A 178

5.3.3. Investigation of interaction between functionalized polymerosome with microparticles 182

5.3.3.1. Interaction of PM50 with Cy5-Azo-Psome 183

5.3.3.1. Interaction of PM50 with Cy5-β-CD-Psome 184

5.4. Conclusion 185

CHAPTER 6: CONCLUSIONS AND FUTURE SCOPES 187

6.1. Conclusions 188

6.2. Future scopes.....189

BIBILOGRAPHY 191

APPENDIX: I 223

CURRICULUM VITAE: Aiswarya T.T. 225

LIST OF FIGURES

Figure 1.1. Features of carbohydrates----------3

Figure 1.2: Synthesis of PhE-ITA-Glu nanoparticles----------7
Figure 1.3: Schematic representation of the core (PS)-shell (GP) particle formation using RAFT mediated surfactant-free emulsion polymerization (SFEP).----------9
Figure 1.4: Preparation of glycopolymer-grafted polystyrene particles via atom transfer radical polymerization (AGET ATRP)----------10
Figure 1.5: Schematic illustration of the synthesis pathway of CNC-gPEEP via CuAAC "click" reaction and the formation of DOX-loaded nanocrystals----------15

Figure 1.6 : Effect of size, linker length, density and affinity on protein binding----------21
Figure 1.7: Effect of linker length on protein binding----------22

Figure 1.8:Effect of shape on bacterial cell adhesion----------22

Figure 1.9: Efficient co-encapsulation of PTX and SB into Dex-DOCA amphiphilic polymers and schematic illustration of (PTX+SB) NP as a robust nano-platform provided with prolonged circulation, eradicated stromal components and normalized tumor vessels for enhanced drug accumulation and potency in solid tumors----------28

Figure 1.10: Synthesis of polyethylenimine (PEI) derivative. High molecular weight PEI was modified by succinic anhydride. Low molecular weight PEI was conjugated with lactose-bearing galactose groups. These domains were coupled via an amide bond----------32
Figure 1.11 : Schematic illustration of a) the formation of DOX-loaded nanoparticles and b) tumor-targeted drug release behavior of bioreducible nanoparticle----------33

Figure 1.12: (a) Synthetic scheme of hydrophobic 5β-cholanic acid modified GC. (**b)** Schematic illustration of effective self-assembly of DOX-CNPs by hydrophobic interactions between DOX and cholanic acid moieties of GC polymers in mild conditions. **(c)** Synthetic scheme of thiolated glycol chitosan (tGC) by chemical conjugation of Sulfo-LC-SPDP to the amine group of GC polymers. **(d)** Schematic illustration of fthe ormulation process of small and compact Poly-siRNA/tGC complexes. First, weak charge interactions occur between negatively charged Poly-siRNA and positively charged GC polymers. Next, Poly-siRNA/tGC complexes are further stabilized by chemical disulfide crosslinking between tGC and Poly-siRNA. (e) Schematic representation of the combinational delivery process by GC-based nano-platforms. CNP-based delivery platforms exhibit almost similar physico-chemical properties after encapsulation of drugs with extremely different physical features, i.e. DOX and siBcl2. Finally DOX-CNPs and siRNA-CNPs show outstanding tumor accumulation and localization tendencies thereafter similar cellular uptake followed by intracellular trafficking to the site of action, cytosol for the RNAi process and nucleus for DOX action, which will benefit anti-cancer therapeutic effects in a combinatorial way----35

Figure 1.13: Design and characterization of PpIX–GC–NP. (a) The chemical structure of PpIX–GC–NP. (b) Size and shape of PpIX–GC–NP determined by DLS and TEM image. (c) Scheme diagram of self-assembled PpIX–GC–NP and cellular 'on/off' system for synchronous photodynamic imaging and therapy of cancer----------36

Figure 2A.1: Representative FESEM and TEM images of **(a),(e)** NFC**, (b),(f)** TNFC-1, **(c),(g)** TNFC-3 and **(d),(h)** TNFC-5, respectively; (DA represents fiber diameter)---------56

Figure 2A.2: FTIR spectra of NFC and TNFC samples----------57

Figure 2A. 3: Conductometric titration curves for (A) NFC, (B) TNFC-Direct (c) TNFC-1, (D)TNFC-3, and (E)TNFC-5----------58

Figure 2A. 4: Zeta potential values of NFC and TNFC samples----------58

Figure 2A.5: Digital images of NFC, TNFC-1, TNFC-3, TNFC-5 dispersions in water taken at different time intervals----------59

Figure 2A.6:(A) Sedimentation curves of NFC, TNFC-1, TNFC-3, and TNFC-5 over a period of 7 days (B)Hydrodynamic size of TNFC-5 at different time interval determined using dynamic light scattering method----------60

Figure 2A. 7: X-ray diffraction patterns of the original pulp, NFC, and TNFC samples---61

Figure 2A. 8: N_2 adsorption/desorption isotherm of the original pulp, NFC, and TNFC samples----------62

Figure 2A. 9: Triclosan adsorption kinetics (A) Adsorption kinetics, (B) pseudo-first order model (Linear fit), (C) pseudo-second order model (Linear fit), (D) Elovich-chemisorption model (Linear fit) of NFC and TNFC-5----------64

Figure 2A.11: FESEM and mapping image of triclosan loaded TNFC-5 **(a,b),** Energy Dispersive X- ray (EDX) mapping (**c,d,e)** and EDX spectra **(f)**----------65

Figure 2A.12: FESEM and mapping image of ampicillin sodium loaded TNFC-5 **(a,b)**. Energy Dispersive X- ray (EDX) mapping (**c,d,e)** and EDX spectra **(f)**.----------65

Figure 2A. 13: Progressive change in water uptake (%) of TNFC-5, AMP-TNFC-5 and TCS TNFC-5 samples with respect to time----------67

Figure 2A. 14: In vitro release profiles of triclosan loaded NFC, TNFC-1, TNFC-3, and TNFC-5 (A) cumulative release (CR) profiles (B) first order model (C) Higuchi model and (d) Ritger peppas model fitted curves obtained from panel A----------69

Figure 2A.15: Representative FTIR spectra for TNFC-5 and TCS-TNFC-5 (drug loaded) samples----------70

Figure 2A.16: In vitro release profiles of ampicillin sodium loaded TNFC-5 (A) cumulative release profile(B) first order (C) Higuchi model and (D) Ritger Peppas fitted curves-------72

Figure 2A.17: 2D plot showing interactions between cellulose receiving various treatments and antibiotics (triclosan and ampicillin)----------75

Figure 2A.18: Antimicrobial activity (zone of inhibition (left axis) and cell growth (right axis)) of triclosan loaded sample (TCS-TNFC-5) against (a) E.coli (b) S.aureus and

ampicillin loaded samples (AMP-TFC-5) against (c) E.coli (d) S.aureus. Control represents TNFC-5----------77

Figure 2A.19: Digital images representing zones of inhibition against *S. aureus and E. coli* after treating with triclosan loaded TNFC-5----------77

Figure 2A.20: Digital images representing zones of inhibition against *S. aureus and E. coli* after treating with (A)ampicillin sodium drug (B) ampicillin sodium loaded TNFC-5------78

Figure 2A.21: SEM images of bacteria after treatment with control (TNFC-5) and triclosan loaded TNFC-5, S.aureus (a,b) and *E. coli* (c,d)----------79

Figure 2A.22: Fluorescent images of live and dead bacteria of E.coli and S. aureus cells after treatment with TNFC-5 and triclosan loaded TNFC-5. Green fluorescence represents live bacteria stained with fluorescein diacetate (FDA) whereas red fluorescence represents dead bacteria stained with propidium iodide (PI)----------79

Figure 2B.1: Representative FESEM (a,c) and TEM (b,d) images of NFC and cNFC, respectively. DA represents diameter----------88
Figure 2B.2: FTIR spectra of nano fibrillated cellulose (NFC), carboxylated nano fibrillated cellulose (cNFC), cNFC-Moxifloxacin and Moxifloxacin (Mox)----------89
Figure 2B.3: Field Emission Scanning Electron Microscope (FESEM) and mapping image of Moxifloxacin loaded cNFC (a,b). Energy Dispersive X- ray (EDX) mapping (d, e, f, g) EDX spectra (c)----------90
Figure 2B 4: In vitro release profiles for Moxifloxacin loaded cNFC and NFC- (A) cumulative release profile, (B)zero order fitting (C) first order (D) Higuchi model, and (E) Ritger Peppas fitted curves. CR represents cumulative release----------91
Figure 3.1: Synthesis of acetalated dextran by modifying dextran: ^{1}H-NMR spectra of dextran (a) Acetalated dextran (b)----------101

Figure 3.2: (a) Schematic representation of formation of GO loaded core-shell microsphere systems, (b) optical images taken at different time intervals after the addition of primary emulsion to water containing PVA. (i) 0 hr (ii) 1 hr (iii) 2 hr (iv) 3 hr and (v) 4 hr respectively, (c) schematic representation of plausible mechanism of the formation of core-shell microsphere system----------103

Figure 3.3: (a) FESEM and (b) HRTEM image of GO----------104

Figure 3.4: FESEM images of GO loaded core-shell microsphere systems. (a) and (b) for FESEM and cross-sectional FESEM images of Ac-D-GO/CMC (GO in the shell), (c) and (d) for FESEM and cross-sectional FESEM images of Ac-D/CMC-GO (GO in the core) respectively----------105

Figure 3.5: CLSM image of core-shell microsphere (Ac-D/ CMC) in which blue dye (poly(9,9-di-n-octylfluorenyl-2,7-diyl)) was added to the Ac-D phase----------106

Figure 3.6: Raman spectra for (a) blank Ac-D/CMC particles, and (b) their corresponding optical image taken during Raman mapping----------107

Figure 3.7: Raman spectra of (a) Ac-D /CMC (GO in shell) and (d) Ac-D/CMC-GO (GO in core) and (b-c) show their corresponding optical images taken during the point-by-point mapping----------107

Figure 3.8: PXRD analysis for GO incorporated core-shell microspheres----------108

Figure 3.9: XPS analysis of synthesized microparticles (a) comparative C1s spectra of neat Ac-D, neat GO, Ac-D/CMC-GO and Ac-D-GO/CMC, (b) comparative O1s spectra between neat Ac-D, neat GO, Ac-D/CMC-GO and Ac-D-GO/CMC----------109

Figure 3.10: Surface charges of microspheres measured at pH 7.4----------110

Figure 3.11: FESEM images of different GO/curcumin systems loaded with their cross sections. (a) S1,(b) S2,(c) S3,(d) S4, (e) S5 and (f)S6 (a-i, b-i, c-i, d-i, e-i, and f-i, represent their corresponding cross-sectional images)----------112

Figure 3.12: CLSM and DIC images of microspheres, when curcumin was loaded in (a, b) shell and (c,d) core----------113

Figure 3.13: (a) Applicator used for RF ablation (b)Thermal imaging using IR camera during RF heating (c) RF heating response of samples----------114

Figure 3.14: *In vitro* drug release from the core-shell microspheres S1-S6 without (a) and with (b) RF exposure----------114

Figure 3.15: Representative FESEM micrographs for (a-S1, b-S2, c-S3, d-S4, e-S5, f-S6) after RF treatment during *in vitro* release study----------115

Figure 3.16: NMR spectra of acetalated dextran layer after RF exposure----------116

Figure 3.17: Swelling index of microspheres over time (a) without and (b) with RF exposure.----------116

Figure 3.18: TGA thermograms for microspheres before and after RF exposure (a) Ac-D/CMC-GO, (b) Ac-D-GO /CMC----------118

Figure 3.19: Schematic representation effect of RF on microsphere----------118

Figure 3.20: Cell viability of S1system----------120

Figure 4.1: Schematic representation of the synthetic procedure of formation of core-shell microspheres, (A) AcD-FZPA/CMC (B) AcD/CMC-FZPA----------130

Figure 4.2: TEM images of (A) nZVI nanoparticles and (C) Fe_3O_4 nanoparticles; and FESEM images of (B) nZVI nanoparticles and (D) Fe_3O_4 nanoparticles----------131

Figure 4.3: CLSM image of microsphere having blue polymeric dye incorporated in AcD layer of the AcD/CMC microspheres (without incorporating any active agents)----------132

Figure 4.4: Representative FESEM images of (A) AcD-FZPA/CMC (PAG, ZVI and Fe_3O_4 in shell) and in (B) AcD/CMC-FZPA (PAG, ZVI and Fe_3O_4 in core) (*inset showing their*

corresponding cross-sectional images). Arrows indicate the existence of additives in designated areas----------132

Figure 4.5: FESEM and EDX mapping spectra of core shell microspheres having active agents (A) AcD /CMC-FZPA (PAG, ZVI and Fe_3O_4 in core) and (B) AcD-FZPA /CMC (PAG, ZVI and Fe_3O_4 in shell)----------133

Figure 4.6: TGA of various microspheres----------134

Figure 4.7: Representative XRD spectra of core shell microspheres having magnetic nanoparticles incorporated in shell (AcD-FZPA/CMC) and core (AcD/CMC-FZPA), along with neat AcD, Fe_3O_4 and ZVI nanoparticles----------135

Figure 4.8: XPS analysis of AcD-FZPA/CMC and AcD/CMC-FZPA microspheres (a) Representative XPS survey spectra and their corresponding (b) Deconvoluted C1s spectra, (c) Deconvoluted Fe2p spectra, and (d) Deconvoluted O1s spectra for both type of microspheres----------137

Figure 4.9: MFM phase images of core-shell microspheres having magnetic nanoparticles incorporated in core (A) AcD/CMC-FZPA and in shell (B) AcD-FZPA/CMC and VSM of (C) neat Fe_3O_4 and neat ZVI nanoparticles and (D) Ac-D-FZPA/CMC----------139

Figure 4.10: pH (a) and evolution (b) of released chloride ion from different systems upon UV and AMF exposure for 10 min----------141

Figure 4.11: Representative (A) EPR spectra and (B) NMR spectra of various systems (i-iv) after UV/AMF exposure for 10 min. (C) Photolysis of R–Cl monitored by UV-vis spectroscopy recorded after UV/AMF exposure for 10 min----------143

Figure 4.12: Plausible mechanism of acid generation to deprotect acetalated dextran during UV and AMF exposure----------144

Figure 4.13: CLSM and DIC images of AcD/CMC microspheres when curcumin was incorporated in (A, B) shell and (C, D) core (*Green stain indicates curcumin*)----------146

Figure 4.14: Schematic representation of the set-up where microparticles were treated with cancer cells under UV/AMF exposure----------147

Figure 4.15: *In vitro* release study of different systems (S1-S6) under UV exposure (a) and combined exposure of UV and AMF (b)----------144

Figure 4.16: (a)Schematic representation of disintegration of the microspheres and drug release under UV/AMF exposure. (b) Representative optical (b-i) and FESEM(b-ii) images of AcD-FZPA /CMC and optical (c-i) and FESEM(c-ii) images of AcD /CMC-FZPA under UV/AMF exposure for various time periods.(*circles highlighted the disintegration of AcD layer))*. Both the systems were loaded with curcumin drug----------149

Figure 4.17: Evolution of change of temperature (a) and swelling index (b) of various systems (S1-S6) under UV/AMF exposure.----------150

Figure 4.18: GPC traces of dextran as starting material used for AcD synthesis (a) and post triggered AcD by UV/AMF (b)----------151

Figure 4.19: *In vitro* release of DOX from DOX-loaded AcD-FZPA/CMC (a), CLSM image of Dox loaded AcD-FZPA/CMC microspheres(b),Cell viability of DOX-loaded AcD-FZPA/CMC microspheres treated with HepG2 cells, evaluated using MTT assay. Samples were exposed to UV/AMF for 60 min(c)----------153

Figure 4.20: Cell metabolic activity (MTT assay) for various systems (i-v) against HEK293 (a) and HepG2 cells (b) evaluated using MTT assay----------154

Figure 4.21: Live dead staining images (fluorescence) of HepG2 cells incubated with (a,b) AcD-FZPA/CMC microspheres (no drug) and (c,d) DOX-loaded AcD-FZPA/CMC microspheres. *(b and d showed the overlay images of a and c, respectively)*----------.155

Figure 5.1: ^{1}H-NMR spectrum of BMGL----------166

Figure 5.2: ^{1}H-NMR spectrum of Poly(LA-ran- BMGL)(a) and PLA-OH(b)----------167

Figure 5.3: ^{1}H-NMR spectrum of Poly(LA-ran- BMGL)(a) and PLA-OH(b)----------167

Figure 5.4: ^{1}H-NMR spectrum of MEMA----------169

Figure 5.5: Bright field and Field emission scanning electron microscopic (FESEM) images of Poly (MEMA) brush-modified Janus microparticles, P50 at different monomer concentrations (a, e) 0.017mmol, (b, f)0.051mmol, (c, g) 0.085mmol and (d, h) 0.171 mmol)----------171

Figure 5.6: Bright field images of Poly (MEMA) brush-modified Janus microparticles, P50 varied polymerization time(a) 0.5 h, (b) 1 h, (c) 3 h, (d) 5 h , (e) 7 respectively----------172

Figure 5.7: FTIR-ATR spectra of PLA, P50 and PM50 respectively----------173

Figure 5.8: Bright field images of janus microparticles, P10 (a), P25 (b) and P50 (c) and Poly (MEMA) brush-modified Janus microparticles, PM10 (d),), PM25 (e) and PM50 (f).----------174

Figure 5.9: Field emission scanning electron microscopic (FESEM) of janus microparticles, P10 (a), P25 (b) and P50 (c) and Poly (MEMA) brush-modified Janus microparticles, PM10 (d), PM25 (e) and PM50 (f)----------174

Figure 5.10: High-resolution transmission electron microscopy (HRTEM) of janus microparticles, P10 (a), P25 (b) and P50 (c) and Poly (MEMA) brush-modified Janus microparticles, PM10 (d), PM25 (e) and PM50 (f)----------175

Figure 5.11: Differential interference contrast (DIC)of janus microparticles, P10 (a), P25 (b) and P50 (c) and Poly (MEMA) brush-modified Janus microparticles, PM10 (d), PM 25 (e) and PM 50 (f)----------176

Figure 5.12: Calorimetric titration of Con A with (A) PM10 (B) PM25 and (C)PM50 performed at 25 °C----------177

Figure 5.13: CLSM images of FITC-BA Psomes conjugated PM50 microparticles at different pH (a) 5 (b)7.4) and (c) 9 PBS Buffer. *(Green indicate FITC-BA Psomes, Blue indicate mannose decorated compartment of PM50)*----------180

Figure 5.14: CLSM images of Cy5-Azo Psomes conjugated PM50 microparticles at different pH (a) 5 (b)7.4) and (c) 9 PBS Buffer.*(Red indicate Cy5-Azo Psomes, Blue indicate mannose decorated compartment of PM50)*----------182

Figure 5.15: CLSM images of Cy5-β-CD-Psomes conjugated PM50 microparticles at different pH (a) 5 (b)7.4) and (c) 9 PBS Buffer.*(Red indicate Cy5-β-CD-Psomes, Blue indicate mannose decorated compartment of PM50)*----------183

LIST OF TABLES

Table 1.1. Properties and applications of various carbohydrates----------3

Table 2A.1: Comparison of various types of oxidized nanocellulose based drug carrier, their DL and EE----------46

Table 2A.2: Carboxyl content, drug entrapment efficiency and drug loadings of various NFCs prepared under varying concentrations of NaClO----------56

Table 2A.3: Surface area (S_{BET}), Pore volume (V_{total}) and radius of NFC samples obtained from N_2 adsorption/desorption isotherm----------62

Table 2A.4: Parameters and regression coefficients of fitted models to Triclosan adsorption----------66

Table 2A.5: Kinetic parameters and correlation coefficients (R^2), for zero-order, first-order, Higuchi and Ritger-peppas model for triclosan loaded samples----------69

Table 2A.6: Kinetic parameters, correlation coefficient (R^2), for zero-order, first-order, Higuchi and Ritger-peppas model for ampicillin loaded TNFC-5 samples----------72

Table 2B.1: Degree of polymerization, total sugar, hemicellulose and lignin content of pulp, NFC and Cnfc----------88

Table 2B.2: Kinetic parameters, correlation coefficient, for zero-order, first-order, Higuchi model Ritger-peppas model for Moxifloxacin loaded cNFC----------92

Table 3.1: Interfacial Tensions (γ) among different phases and Spreading Coefficients (S)----------101

Table 3.2: Composition, Particle Size, and location of GO----------103

Table 3.3: Compositions, particle size and effect on molecular weight after exposure to RF of different GO/curcumin loaded core-shell microspheres----------111

Table 4.1: Different microsphere systems used as controls and their compositions-------141

Table 4.2: Location of active ingredients including drug in various microspheres--------145

Table 5.1: Concentration of Janus particle (JP), RhB-BA-Fc-Psomes and Cy5-Azo-Psomes----------178

Table 5.2: Mannose units, Stoichiometry of binding (n), Enthalpy of binding (ΔH), and Gibbs free energy(ΔG) for the Interaction of Con A with PM microparticles----------179

LIST OF ABBREVIATIONS

1,4-bis-2-ethylhexylsulfosuccinate (AOT)

2,2,6,6-Tetramethylpiperidinyloxy (TEMPO)

2-chloropropionyloxyethyl methacrylate (CPEM)

2-ethoxy-2-oxo-1,3,2-dioxaphospholane (EOP)

Acetalated-Dextran (Ac-D)

Albumin–fluorescein isothiocyanate conjugate (FITC-BSA)

Alkyl ketene dimer (AKD)

atom transfer radical polymerization (AGET ATRP).

Atomic force microscopy (AFM)

Brunauer–Emmett–Teller (BET)

Carboxymethyl cellulose (CMC)

Cetyltrimethylammonium bromide (CTAB)

Confocal Laser Scanning Microscope (CLSM)

Concanavalin A (Con A)

Deacetylated chitosan (DCH)

Deionized (DI)

Dimethyl sulfoxide (DMSO),

Dimethylacetamide (DMAC)

Dimethylformamide (DMF) and

Doxorubicin (Dox)

Drug loading (DL)

Differential Scanning Calorimetry (DSC)

Dynamic light scattering (DLS)

Escherichia Coli (E. coli)

Entrapment efficiency (EE)

Food And Drug Administration (FDA)

Field Emission Scanning Electron Microscopy (FESEM)

Fluorescence resonance energy transfer (FRET)

Fourier-transform infrared spectroscopy (FTIR)

Gel Permeation Chromatography (GPC)

Graphene oxide (GO)

High resolution Transmission Electron Microscopy (HRTEM)

Isothermal titration calorimetry (ITC)

Itaconic anhydride (ITA)

Mannosyloxyethyl methacrylate (MEMA)

Methotrexate (MTX).

Minimum Inhibitory Concentration (MIC)

Nanofibrillated cellulose (NFC)

Nuclear magnetic resonance (NMR)

Photo acid generator (PAG)

Poly(Lactic-Co-Glycolic Acid (PLGA)

Poly(ethyl ethylene phosphate) (PEEP)

Polyelectrolyte complexation (PEC)

Poly(lactic acid) (PLA)

Polystyrene-block-poly(pentafluoro styrene) (PS-b-PFS)

Powder X-ray diffraction (PXRD)

PS-b-PAA (polystyrene-b-polyacrylic acid)

Radiofrequency (RF)

Reversible addition-fragmentation chain transfer (RAFT)

Reversible deactivation radical polymerization (RDRP)

Staphylococcus Aureus (S. aureus)

Sodium bromide (NaBr)

Sodium hypochlorite (NaClO)

Sodium tripolyphosphate (TPP)

Surface-initiated atom transfer radical polymerization (SI-ATRP)

Ultraviolet-visible (UV)

X-ray photoelectron spectroscopy (XPS)

Zero-valent iron (ZVI)