SYNTHESIS AND CHARACTERIZATION OF CHITOSAN NANOPARTICLE AND ITS APPLICATION ON TEXTILES USING LAYER-BY-LAYER SELF-ASSEMBLY APPROACH FOR ANTIMICROBIAL ACTIVITY

by

MD. S. WAZED ALI

Department of Textile Technology

Submitted

In fulfillment of the requirements of the degree of

Doctor of Philosophy

to the

Indian Institute of Technology, Delhi
June 2011

DEDICATED

TO

MY PARENTS & GRANDPARENTS

CERTIFICATE

This is to certify that the thesis entitled **"Synthesis and Characterization of Chitosan Nanoparticle and its Application on Textiles using Layer-by-layer Self-assembly Approach for Antimicrobial Activity"** being submitted by **Mr. Md. S. Wazed Ali,** to the Indian Institute of Technology, Delhi, for the award of the degree of **Doctor of Philosophy** in the department of Textile Technology, is a record of bonafide research work carried out by him. Mr. Ali has worked under our guidance and supervision and fulfilled all the requirements for the submission of the thesis.

The results contained in this thesis have not been submitted, in part or full, to any other university or Institute for the award of any degree or diploma.

Dr. Mangala Joshi
Professor
Department of Textile Technology
Indian Institute of Technology, Delhi
Hauz Khas
New Delhi – 110016
India

Dr. S. Rajendran
Senior Scientist
Institute for Materials Research
& Innovation (IMRI)
The University of Bolton
Deane Road, Bolton BL3 5AB
United Kingdom

ACKNOWLEDGMENTS

I wish to express my hearties gratitude and sincere regards to my respectable supervisors, ***Prof. Mangala Joshi*** *and* ***Dr. S. Rajendran*** *for their constant inspiration, encouragement, invaluable guidance and help that they bestowed on me right from the inception to the successful completion of this endeavor.*

I am very much grateful to my SRC members, Prof. M L Gulrajani, Prof. Kushal Sen and Dr. Prashant Mishra for their constructive criticism and valuable suggestions.

I am thankful to the University of Bolton, UK for awarding 'Bolton Fellowship' to carry out this PhD research programme at IIT Delhi, India. I am grateful to the Commonwealth Scholarship Commission (Association of Commonwealth Universities), London for awarding 'Split-Site Doctoral Fellowship' -2008 to conduct part of the PhD research work at Institute for Materials Research and Innovations (IMRI), University of Bolton, UK.

I am indebted to Prof. V. K. Kothari, Prof. S. M. Ishtiaque, Prof. P. K. Banerjee and Prof. Kushal Sen who, as the Head of the Textile Department at various stages of my research, allowed me to use all the facilities available in the department and also my thanks go to all the faculty members, research scholars of the Textile Department, IIT Delhi.

I gratefully acknowledge the help and suggestions by Prof. B L Deopura, Dr. Abhijit Majumdar, Dr. Amit Rawal, Dr. Sourabh Ghosh, Dr. Samrat Mukherjee & Dr. Dipayan Das as and when required.

I extend my sincere thanks to Dr. & Mrs. Rajendran, Prof. Subhash Anand, Dr. Taheer Shah, Mr. Rafique, Mrs. Jesmin, Mr. Abu Bakkar, all the faculty members, research scholars of the IMRI, University of Bolton, UK for each kind of help I got during my stay at UK.

I gratefully acknowledge the help and cooperation given by Nottingham University and University of Cardiff, UK for XPS (EPSRC) facility. The assistance received from M/S Orbit Research Associates Pvt. Ltd, Delhi, for carrying out testing on goniometer for contact angle measurement is also acknowledged.

I am thankful to the staff members of all the laboratories of Textile Department, IIT Delhi for extending their helping hands whenever needed. In the same breath, I thank the staff members of the laboratories of the Institute for Materials Research and Innovation (IMRI), University

of Bolton, UK. Special thanks to Mrs. Sunita Verma, Mr. Girish, Mr. Vinod, Mr. Rajkumar, Mr. Amarjeet, Mr. Kala, Mr. Khattar, Mr. Omprakash, Mr. Mukundan, Mr. Biswal from IIT Delhi and Mrs. Donna, Mr. Sairam, Mr. Akbar and Mr. Alan from University of Bolton, UK.

I would like to express my heartiest thanks and gratitude to all my friends and colleagues for their direct and indirect help. I am especially thankful to Mr. Saikat Banerjee, Mr. Sohel Rana, Mr. Amitava Bhattacharya, Mr. Syamal Maity, Mrs. Shibani, Mrs. Kasturi Saha, Dr. Raghunath Reddy, Dr. Roli Purwar, Dr. Brojeshwari Das, Dr. Kaushik Bal, Dr. Naveen Padaki, Mr. Jagannath, Mrs. Sreedevi, Mrs. Rosina, Mr. Atanu, Mr. Avik, Mr. Avishek, Mrs. Mr. Jayant, Sagarika, Mr. Subhasish, Mr. Subhodeep, Dr. Animesh Kuley, Dr. Kartick Samanta, Mr. Debajit, Mr. Indrajit , Mr. Aziz & Mr. Sayeed, Mr. & Mrs. Arun, Mrs. Sangita at IIT Delhi and Mr. Zafar, Mr. M. Uzun, Dr. Imran Patel, Dr. Rashid Masood, Mr. Karthick, Mr. Zameer, Mr. Manzoor & Mr. Rocco at the University of Bolton, UK. Thanks also go to my friends Mr. Brijesh & Dr. Sangita for their constant cooperation and help.

I am grateful to my grandparents, brothers, sisters, uncles, aunties, in-laws, and other relatives for their continuous help, support and encouragement.

I gratefully acknowledge the unconditional support and cooperation by my uncle, Nurul Islam; grandmother, A. Khatun; sister, S. Yasmin and brother, Wasef Ali throughout my entire study years. I cannot forget the name of my little niece 'Fiza' & sisters Ria, Dina & Moon who had been a constant source of ecstasy by their love and sweet smile.

My heartfelt thanks to my parents (Late A. Latif and S. Begam) who are the constant source of enthusiasm, patiently stood beside me during my entire study cycle. It would have been impossible to come at the present position without their consistent support and cooperation.

Lastly I am left with lack of words to acknowledge my strength, motivation and stable force, my wife 'P. Sultana'. I am deeply indebted to her for all the pains she bore to make me smile, helped me to take the pressure for the completion of this thesis and more of this, always stood beside me with a smile during the storms of momentary depression.

Finally, thanks to 'ALMIGHTY' for HIS impeccable guidance and support, which helps me to remain focused in entire period of my journey.

Md. S. Wazed Ali

ABSTRACT

Chitosan, a biopolymer derived from a component found in crustacean shells called chitin, is extensively finding use in medicine and medical textiles for drug delivery and other applications because of its biocompatibility, biodegradability and inherent antimicrobial properties due to its polycationic nature. In this study, an attempt has been made to synthesize chitosan in the form of nanoparticles which is expected to have an enhanced antibacterial activity over bulk chitosan. Ionotropic cross-linking using sodium tripolyphosphate (TPP) was the chosen route for synthesis of chitosan nanoparticles (CSN). It has been investigated and found that ionic gelation of cationic chitosan macromolecules offers a flexible and easily controllable process for systematically and predictably manipulating the particle size and surface charge of chitosan nano-particles which is an important property for antimicrobial effect. Variations in chitosan to TPP weight ratio, pH of chitosan and TPP solution, chitosan solution concentration, temperature of chitosan solution and stirring speed during nanoparticle formation were examined systematically for their effects on nanoparticle size, shape and intensity of surface charge, so as to enable faster synthesis of chitosan nanoparticles with desired properties to have optimum antimicrobial property.

The data on particle size and zeta potential was obtained by dynamic light scattering (DLS) and electrophoretic mobility measurement of the chitosan nanoparticles respectively. UV-VIS absorption of bacterial suspension was measured at 610 nm to evaluate the bacterial reduction. Shake flask method (AATCC-100) was also used to quantify the antibacterial efficacy. It was interesting to observe that pH of the chitosan and TPP solution has a significant role in controlling the nanoparticle shape and size and thus can be fine tuned to achieve optimum antimicrobial activity.

Solution pH of both chitosan and TPP was demonstrated to be the most critical factor in controlling particle size, surface charge and even shape as revealed by the SEM and TEM micrograph. At very strong acidic condition of chitosan solution, cross-linking was less resulting in lower conversion of chitosan to chitosan nanoparticles which was reaffirmed by colloidal titration of the surface groups of the chitosan nanoparticles with a negatively charged polyelectrolytes (poly (vinyl sulfate kalium salt). The study demonstrated that best antimicrobial activity against bacteria *Staphylococcus aureus* was achieved when the chitosan nanoparticles were synthesized at chitosan solution pH fixed at 6.0 and the TPP solution pH at 8.9. It was found that variation in size and surface charge of chitosan nanoparticles could be achieved by changing the process parameters during ionotropic gelation synthesis route which resulted in significant variation in their antimicrobial activity.

Silver loaded chitosan nanoparticles (CSN-Ag) were obtained by adding a solution of silver nitrate ($AgNO_3$) to the chitosan nanoparticle suspension during chitosan nanoparticle synthesis. Silver loaded chitosan nanoparticles have comparatively higher average size (165 nm) with higher polydispersity index (PDI). Different concentrations of $AgNO_3$ were used for preparation of CSN-Ag to optimize the loading of silver on chitosan nanoparticle. The conversion of some of the silver ion to silver nanoparticles was confirmed by the presence of broad Plasmon resonance peak in the UV-VIS spectra.

The antimicrobial activity of chitosan gets much enhanced in nanoparticle form, as indicated by reduction in minimum inhibitory concentration (MIC) value. MIC value was determined by using shake flask method (AATCC-100) against

staphylococcus aureus bacteria. MIC value of CSN is 10 times lower than bulk chitosan in suspension form and 50 times lower in powder form. Silver loaded chitosan nanoparticle shows 50 times less MIC when it is in suspension form and 500 times less when it is in powder form. These particles additionally show a release mechanism as evident from a clear zone of inhibition. The antibacterial mechanism of CSN on *Escherichia coli* was confirmed by AFM images of nanoparticle treated bacteria at different interval of treatment time.

The physical, chemical and thermal characterization of synthesized chitosan nanoparticles (CSN) and silver loaded chitosan nanoparticles (Ag-CSN) vis-à-vis bulk chitosan has been done. The structure of CSN and Ag-CSN was studied by FTIR, XRD, DSC, and TGA analysis. FTIR analysis confirms the cross-linking of chitosan with TPP. X-ray diffraction pattern reveals the amorphous characteristics of chitosan and silver loaded chitosan nanoparticle. The synthesized nanoparticles were found thermally more stable than bulk chitosan. The introduction of silver ion in chitosan backbone has enhanced the thermal stability of the chitosan nanoparticle which was confirmed by the TGA studies. The other morphological characteristics of the chitosan–TPP nanoparticles were examined using the TEM technique.

Layer-by-later (L-b-L) technique on textiles is an emerging route to impart newer functionalities and properties on textile surfaces without compromising on fabric properties such as breathability, texture and handle. The attachment of the first layer in L-b-L technique depends solely on the interaction of the polymers with a charged textile surface. Thus, for strong gradual build-up of multilayer of nanoparticles, initial few bi-layers were made up of linear polyelectrolytes. To

achieve the strong base layer formation, an attempt has been made to evaluate the effect of different process parameters on the amount of polyelectrolyte adsorbed on a cationized cotton textile substrate via sequential adsorption of negatively charged poly (styrene sulphonate) (PSS) and positively charged poly (allyl amine hydrochloride) (PAH) using layer-by-layer (L-b-L) self-assembly coating process. A considerably different polymer adsorption behavior was observed from thick adsorbed layers to thin adsorbed layer with different degree of layer penetration and ionic pair formation over the pH range (2.5 – 9.0) studied. The amount of polyelectrolyte adsorption on cotton fabric was evaluated by measuring the colour value (K/S) of methylene blue (a cationic dye) absorbed cotton surface. Contact angle measurement revealed that the extent of binding of the oppositely charged polyions on the fabric depends on the pH of the polyelectrolyte solution; 'zipped-up' structure with more ion pair formation was observed at the pH range 4.5 – 6.5. At higher temperature amount of polyelectrolyte adsorbed within the multilayers was higher. An increased deposition of PSS and PAH was observed with increase in electrolyte (NaCl) concentration. The amount of PSS and PAH adsorption increases with increase in concentration up to 0.03 (M) of PSS and 0.01 (M) of PAH concentrations, respectively. A dipping time of 5 min was sufficient to have a maximum deposition of the polyelectrolyte multilayers. These optimized conditions were then used for base layer formation on a cationized cotton surface followed by deposition of body layers via L-b-L technique. The body layer is formed of positively charged chitosan nanoparticles (CSN) /silver loaded chitosan nanoparticle (CSN-Ag) and negatively charged PSS polyelectrolytes which impart the antimicrobial property.

PSS and CSN have been used as anionic and cationic agents, respectively for L-b-L multilayer formation (body layer) on cationized cotton surface already coated with base layer of PSS and PAH. The presence of 'N' on the cationized cotton was confirmed by XPS analysis. Concentration of CSN and number of bi-layers on cotton substrate was varied and tested for effect on bacterial reduction. Bi-layer formation of PSS and CSN was confirmed by XPS measurement of elemental sulphur (PSS) and nitrogen (CSN) of each individual layer of the multilayer coating and also by dyeing method by using negatively charged acid dye. The presence of coating material and the thickness of coating on individual fibre surface was observed under SEM by imaging the fibre cross-section. The results by both parallel streak method (AATCC-147) and colony counting method (AATCC-100) show enhanced antibacterial activity of chitosan nanoparticle coated fabrics when compared with the bulk chitosan (CS) coated fabrics at same concentration. Dyeing with negatively charged acid dyes was carried out to evaluate the difference between the available cationically charged surface active group of cotton surface coated with both the bulk chitosan and its nanoparticles.

The cotton fabric was subsequently coated by L-b-L technique with alternative layers of PSS and CSN-Ag. Different concentration of CSN-Ag with different number of bi-layer has been studied on cotton substrate. XPS measurement technique was used to assess the presence of silver on L-b-L coated cotton surface. Sustained release behaviour of Ag^+ in 'solution A' from CSN-Ag coated fabric was evaluated by atomic absorption spectroscopy. The release of Ag^+ is further substantiated by antibacterial testing which shows a clear zone of inhibition against both Gram-positive and Gram-negative bacteria. Scanning electron microscopy (SEM) shows the deposition of

CSN/CSN-Ag on L-b-L coated fabric surface. SEM micrographs also show the bactericidal action of CSN and CSN-Ag on multilayered cotton surface against both Gram-positive and Gram-negative bacteria.

CS and CSN was also applied on cotton fabric using conventional pad-dry-cure process to compare the difference in bioactivity as compared to the samples produced via L-b-L/coating technique at same concentration. CSN coated samples using L-b-L technique registered almost 50% higher reduction of Gram-positive bacteria vis-à-vis samples produced by pad-dry-cure method at the same concentration. Performance properties of both the L-b-L coated and conventional pad-dry-cure finished fabric was compared. Both of these types of samples were tested for various fabric properties such as air permeability, tensile strength, bending length and whiteness index value along with the antibacterial activity after machine wash for a comparative evaluation of the two technique of application pad-dry-cure vs. layer-by-layer (L-b-L) coating.

TABLE OF CONTENTS