Preparation of Antibacterial Cotton Fabric using Chitosan-Silver Nanoparticles

A Thesis Submitted to the Department of Biomedical Engineering and Sciences, School of Mechanical and Manufacturing Engineering (SMME), NUST, Islamabad, in the partial fulfillment of the requirements for the degree of

Masters of Science (MS)

In

Biomedical Sciences

Submitted by

Dooa Arif

NUST201261426MSMME62412F

Supervisor Dr. Muhammad Bilal Khan Niazi

Co- Supervisor Dr. Noaman Ul Haq &

Dr. Nabeel Anwar

School of Mechanical and Manufacturing Engineering (SMME)

National University of Sciences and Technology (NUST)

H-12 Campus Islamabad 2015

Certificate

It is certified that the entire work in the thesis i.e. "**Preparation of Antibacterial Cotton Fabric using Chitosan-silver Nanoparticles**" by Dooa Arif, for award of MS degree in Biomedical Sciences , National University of Sciences and Technology, H-12, Islamabad, Pakistan.; is an original work completed in the presence of my guidance and supervision. Research work is authentic and fulfill the required criteria of MS.

Supervisor: Dr. Muhammad Bilal Khan Niazi

School of Chemical and Material Engineering (SCME), NUST Islamabad.

Co-Supervisor: Dr. Noman Ul Haq (SCME)
Dr. Nabeel Anwar (SMME)

Submitted through

Principal/Dean: _____

School of Mechanical and Manufacturing Engineering NUST Islamabad

Dedication

This thesis is dedicated to my parents who have supported me all the way since the beginning of my studies.

Acknowledgement

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. Special appreciation goes to my supervisor, ***Dr. Muhammad Bilal Khan Niazi*** (Assistant Professor SCME, NUST), for his supervision and constant support.

There are a number of people without whom this thesis might not have been written, and to whom I am greatly obliged.

Dr. Syed Irtiza Ali Shah (Associate professor SMME, NUST) for being an inspiration and source of guidance.

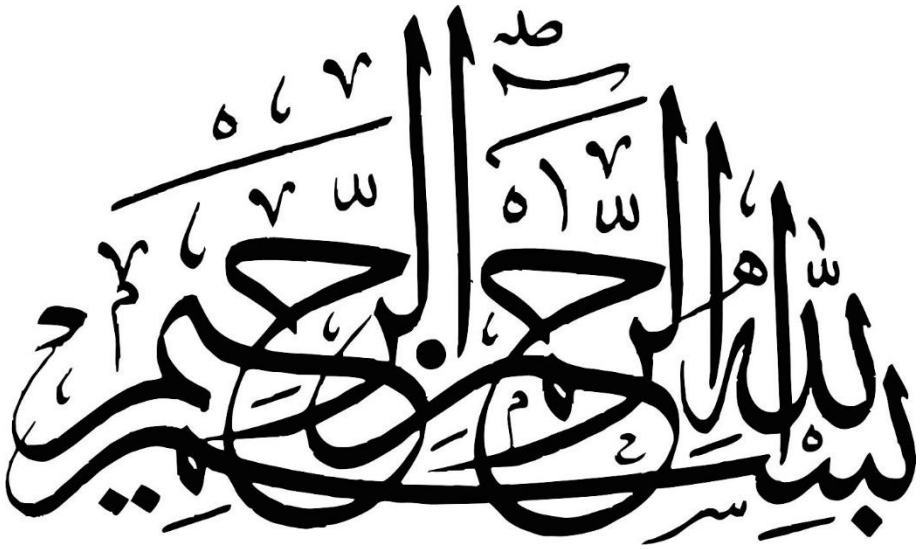
Sidra Rana and ***Mubin Athar*** for their supportive attitude during lab work.

Thanks to all cooperative and supporting staff members of laboratories I visited during my project including SCME, IESE, and SMME (NUST).

Sincere thanks to my friends ***Muhammad Affan Zia*** and ***Danish Umar*** for their kindness and moral support during my study. Thanks for the friendship and memories.

I also thank my Parents and my sisters ***Sundus Arif*** and ***Maryam Arif*** who encouraged me and prayed for me throughout the time of my research.

May the Allah Almighty richly bless you all.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List of Acronyms

AgNPs	Silver Nanoparticles
CS-AgNPs	Chitosan-Silver nanoparticles
XRD	X-ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
UV-Vis	Ultra Violet Visible
SEM	Scanning Electron Microscopy
℃	Degree Celsius

Abstract

The aim of this study was to prepare antimicrobial cotton fabric using chitosan-silver nanoparticles (CS-AgNPs). CS-AgNPs were used as finishing agent for textile made from 100 % pure cotton. AgNPs used in composite were prepared by Turkevich method and CS-AgNPs was synthesized by mixing chitosan solution with silver nanoparticles. Fourier transform infrared (FTIR) spectrometer technique supported the formulation of CS-AgNPs. Cs-AgNPs crystalline peaks obtained by X-ray Diffraction were in perfect agreement with to the JCPDS card no. 89-3722. Two gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and two gram positive bacteria *Bacillus cereus, Staphylococcus aureus* were used to test the bacterial efficacy of synthesized AgNPs and CS-AgNPs. SEM micrograph of cotton fabric revealed the presence of CS-AgNPS on the surface. The presence of small amount of silver nanoparticles in the composite was enough to enhance antibacterial activity significantly as compared with pure chitosan.

Keywords: Chitosan, Antimicrobial, Silver nanoparticles, Cotton, biopolymer.

Chapter 1: Introduction

Textile substrates, especially of natural origin, are splendid source for the suitable growth of microbes as they contribute the conditions like moisture, temperature, oxygen, and nutrients that are necessary for their growth [1].

1.1. Background

Cellulosic fabrics, i.e. linen, cotton and viscose are extensively adopted in the field of clothing. There are numerous reasons for their use such as as renewability, eco-friendly, hydrophilic properties, air permeability and comfortability [2]. However, cellulose-based textiles are carriers of micro-organisms and more vulnerable to microbial attack than synthetic textiles. Due to their properties of porosity, hydrophilic nature along with the ability to maintain water, oxygen, and nutrients, they promote bacterial growth, body odor, and also show loss of their performance properties [3]. Recently, number of antimicrobial-finished textiles have been acknowledged for clinical perspective to obstruct the transmission of pathogens. These products consist of antimicrobial agents such as silver, quaternary ammonium chloride, and chitosan, and show anti-bacterial activity against a wide range of microorganisms [4].

The steady exploration of antimicrobial agents has led to recognition of antimicrobial biomaterials that are devised from the polymer or are the composites of these polymers. Chitosan is one such poly-cationic biopolymer with high antimicrobial activity. It is composed of polymeric 1 → 4-linked 2-amino-2-deoxy-β-D-glucose. It is obtained from the chitin through alkaline deacetylation. Chitin is a natural biopolymer generally found in shells of marine crustaceans and cell wall of fungi [5]. It possess distinctive properties such as biodegradability, nontoxicity, cationic nature, and antimicrobial activity [6].

In agriculture, many of the properties served as a major elicitor of plant defense mechanisms, as a flocculating agent in wastewater treatment, plus an additional additive in food and beverage industry. Currently these agents are utilized in the cosmetic and pharmaceutical industries [7, 8]. The chitosan could be an exemplary chemical for textile industry enduring eco-friendly processes [9].

Similarly silver, in its many oxidation states (AgO, Ag^{+}, Ag^{2+}, and Ag^{3+}), is commonly used as an antimicrobial agent against numerous bacterial strains and micro-organisms [10]. Nano-silver particles are usually smaller than 100 nm consisting of 20-15,000 silver atoms. At nanoscale, silver express notably amazing physical, chemical and biological properties, and antibacterial activity [11]. It is commonly used as antibacterial agent due to low toxicity toward mammalian cells [12, 13].

In the past, a lot of work has been done on the use of silver nanoparticles as antimicrobial textile finish. Zhang and Wu showed antibacterial effects of cotton fabric impregnated with colloidal silver synthesized in one step method using $AgNO_3$ against *S. aureus* and *E. coli* [14]. El-Rafie extracted silver nanoparticles from biomass filtrate of fungus *F. solani,* particle size 3-8 nm with effective antimicrobial activity even if nanoparticles were used in very minute amount of 54 ppm towards the development of hybrid [15].

1.2. Scope of Study

Currently a lot of effort has been laid upon hybrid of metallic salts and biopolymers on the basis of biopolymer characteristics comprising of metal ion properties. Chitosan occupy high chelating strength for numerous metal ions. This chelation enhance the positive charge density of chitosan and increase adsorption of polycation onto the negatively charged cell surface erupting improved growth inhibition [16]. The metal nanoparticle-chitosan materials are potential candidate in biomedical applications due to advantages of biodegradability, antibacterial and amazing chelating properties. Numerous work has been done using chitosan with different nanoparticles, such as Farouk et al combined the ZnO nanoparticles with different molecular weights of chitosan that proved to be effective antibacterial cotton fabric [17], Elhady et al used different concentrations of ZnO nanoparticles with chitosan at different temperatures that induced antibacterial and UV protection in finished cotton fabrics [18]. As Ag and chitosan both are antibacterial agents, so CS-AgNPs composite material has more antibacterial effect [19]. Wazed et al prepared chitosan loaded silver nanoparticles for polyester fabric in low concentration, the method used was ionic gelation [20]. Youbo et al formulated chitosan silver nanoparticles composite as an antimicrobial finish for tencel/non-woven cotton fabric and checked their activity against *E.coli* and *S. aureus* [21]. Thomas et al have attached chitosan with cotton using coupling

reaction induced covalent attachment and then silver nanoparticles were integrated into chitosan layer by loading Ag(I) ions accompanied by citrate and check their activity with *E.coli* [22].

1.3. Present Study

Against this backdrop a study was undertaken with the aim to prepare an effective antimicrobial textile finish using combination of chitosan and silver nanoparticles. AgNPs prepared by using turkevich method were added to chitosan solution in order to prepare textile finish. The efficiency of the CS-AgNPs was tested on different strains of gram-positive and gram-negative bacteria. Textile that have been coated with this finish developed as resistant to microbial attack. The method applied so far was more convenient and rapid as compared to studies mentioned formerly. The amount of silver nanoparticles was very minute still it showed the significant effect. This study focused on utilizing less use of chemicals to make it eco-friendly and economical.

1.4. Aims and objectives of the study

The present study was aimed to investigate antibacterial activity of Cotton fabric coated with Chitosan-Silver nanoparticles. The main objectives of research were

- Synthesis and Characterization of silver nanoparticles
- Preparation of Chitosan-Silver nanoparticles (CS-AgNPs) antibacterial finish and its characterization
- Coating of CS-AgNPs on Cotton fabric through padding mangle
- Antibacterial activity of Silver nanoparticles, Chitosan, Cs-AgNPs
- Analysis of antibacterial activity of CS-AgNPs coated cotton fabric

Chapter 2: Literature Study

2.1. Introduction

In the evolution of human culture Textile played a major role in technology and artistic development. Most of the development offered by the textile is in protective field. In recent years Hygiene has come at the top of the priority list. Odor is also very important. The bodily fluids produce can lead to the unpleasant odor like sweat. “Consumers are looking for solutions to odor and microbial problem and the unique benefits provided by antimicrobial finish”. Antimicrobial finish is used to remove all the microorganisms from the textile.

Microorganism growth is another factor that has resulted in development of antimicrobial finish. Microbial affliction is a serious threat to both living and non-living things. They cause problems with textile raw materials and processing chemicals, wet processes in the mills, roll or bulk goods in storage, finished goods in storage and transport, and goods as the consumer uses them. Obnoxious smell form the inner garments such as socks, spread of diseases, staining and degradation of textiles are detrimental effects of bad microbes. The consumers are now more concerned of their hygienic life style. This leads to possibility and requirement of extensive antimicrobial textile finished products [23].

2.2. Historical background

2.2.1. Antimicrobial Textile finishes

In the light of fact that consumers are now conscious and very careful about hygienic products R&D institutions are now developing innovative cellulose-based textiles with high added value and durable functional properties as antibacterial, anti-UV, anti-radiation, self-cleaning and easy care to cope with consumers need and to capture major part of market [24–26].

Textiles are used everywhere in the world for numerous purposes. The world per capita fiber consumption was 10.4 kg in 2008 [27]. Antimicrobial treatment used for textiles is not a new experience and have been commenced in the market place for decades. Antimicrobial textile have both indoor and outdoor applications. Tents, tarpaulins, awnings, blinds, parasols, sails and waterproof clothing are some examples of outdoor utilization. There are number of indoor

applications such as shower curtains and mattress ticking [28]. They are also used in some consumer textiles such as sportswear, T-shirts and socks and also in medical settings for instance in bedding [29]. It was found that in 2000, an estimated 100 thousand metric tons of antimicrobial fibers were produced [1].

2.2.2. Nanotechnology

Nanotechnology is briskly expanding by contributing to the development of nanoproducts and nanoparticles (NPs) with unique properties such as size dependent physico-chemical properties which are pretty different from larger matter [30]. The innovative NPs have been exploited in broad dimensions such as medicine, cosmetics, renewable energies, environmental remediation and biomedical devices [31, 32]. Among them, silver nanoparticles (Ag-NPs) have drawn attention due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts [33]. Different types of nanomaterials like copper, zinc, titanium [34], magnesium, gold [35], alginate [36] and silver have come up but AgNPs have proved to be remarkably competent as they hold good antimicrobial potency against a wide variety of bacteria, viruses and other eukaryotic micro-organisms [37].

2.2.3. Silver nanoparticles

Nanoparticulate (colloidal) silver has been known for about 120 years [38]. Silver or silver ions are known as powerful antibacterial agents because they are effective against 650 disease-causing organisms in the body, even at low concentrations [35–37].

Silver, in its many oxidation states (Ago, Ag+, Ag2+, and Ag3+) has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms commonly present in nature [10, 38].

2.2.3.1. Mode of action of Silver nanoparticles against microorganisms

Silver is one of the most effective antimicrobial agents especially in the form of nanoparticles and ions which leads to the destruction of Gram-negative and Gram-positive bacteria and fungi. *Escherichia coli* cell walls were affected by silver nanoparticles by forming pits. Some silver particles accumulated in the walls whereas others pervade into the cells [43]. Silver can affect the structures of the cell that are essential for proper functioning and can disturb the respiratory

enzyme [44]. Studies about silver nanoparticles proposed that they may deliver free radicals, attacking bacterial structures [45].

It was reported that the antibacterial activity of Ag-NPs against Gram-negative bacteria divided into three steps:

(i) Chiefly nanoparticles which lies in the range of 1–10 nm adhere to the surface of the cell membrane and immensely disturb its proper functions, such as permeability and respiration;

(ii) They are capable of penetrating into the bacteria causing more damage to the compounds such as DNA by interacting with Sulfur and phosphorous present in the DNA;

(iii) An additional contribution to the bactericidal effect of Ag-NPs is the release of Silver ions [45].

2.2.3.2. Silver nanoparticles as an antimicrobial textile finishing agent

Silver coatings on textile materials are of great economic interest because of their application in medical sector with the low cost. Textiles modified with silver are used in the treatment of atopic dermatitis and as an antimicrobial wound bandages [46]. Silver coated wound bandages conduct as a barrier against microbial attack and will promote the healing process [43, 44].

Along with the antimicrobial benefits of silver nanoparticles, they are of great interest in electromagnetic fields as a conducting fibre [45 , 46].

2.2.4. Chitosan

Chitosan is a polysaccharide composed of glucosamine and N-acetyl glucosamine linked with a b-1-4- glycosidic linkage [51]. It is one of the most abundant polysaccharides found in nature, derived from marine shells and mollusks. Chitin is converted to chitosan through the process of deacetylation.

Chitosan is polycationic in nature and has capacity to bind with macromolecules at the cell surface of bacteria inhibiting its growth which makes chitosan an antibacterial and antifungal agent [52] . The antimicrobial activity of chitosan depends on various factors comprising the type of chitosan, the degree of deacetylation, molecular weight and other physiochemical properties. The antibacterial activities of chitosan is also sensitive to pH, with higher activity at lower values (pKa 6.5) [53].

Chitosan is a biopolymer with biocompatibility and antimicrobial activity. It can be degraded by enzymes in human body, the degradation products are nontoxic. Chitosan is a polymer which expresses an immense scope of antimicrobial activity by binding to the negatively charged bacterial cell wall followed by attachment to the DNA, inhibiting its replication [54]. Generally for the enhancement of bioactivity on chitosan, it is combined to other bioactive materials, such as drugs.

2.2.4.1. Chemical properties of chitosan

The chemical properties of chitosan are as follows:

- Linear polyamine
- Reactive amino groups
- Reactive hydroxyl groups available

Chelates many transitional metal ions

2.2.4.2. Biological properties of Chitosan

Following are the biological properties of chitosan:

1. Biocompatible
- Natural polymer
- Biodegradable to normal body constituents [55]
- Safe and non-toxic [56]

2. Binds to mammalian and microbial cells aggressively
3. Regenerative effect on connective gum tissue [57]
4. Accelerates the formation of osteoblast responsible for bone formation [58]
5. Hemostatic [59]
6. Fungistatic [60]
7. Spermicidal
8. Antitumor [61]
9. Anticholesteremic [62]
10. Accelerates bone formation [63]
11. Central nervous system depressant [64]
12. Immunoadjuvant

Mode of action of Chitosan

The exact mechanism of the antimicrobial action of chitin, chitosan, and their derivatives is still unknown, but different mechanisms have been proposed.

- Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents [55 , 56].
- Chitosan interacts with the membrane of the cell to alter cell permeability. For example, fermentation with baker's yeast is inhibited [67].
- Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth [68].
- Binding of chitosan with DNA and inhibition of mRNA synthesis occurs through chitosan penetration toward the nuclei of the microorganisms and interference with the synthesis of mRNA and proteins [69].

2.2.4.3. Chitosan as an antimicrobial textile finish

Chitosan and its derivatives have earned much consideration in textile for having an antimicrobial attribute [70]. Chitosan can be affixed chemically to cotton fabric using cross-linking agents glutaricdialdehyde [71] and polycarboxylic acids [72].

Investigations revealed that chitosan can act as a multifunctional textile finishing agent because its antimicrobial activity can be combined with other functions such as dyeing improvement. antistatic and deodorant activity [53].

2.2.4.4. Chitosan and Silver nanoparticle composite material

Chitosan is used as metal nanoparticle-chitosan material in biomedical applications because of its advantages of biodegradability, antibacterial properties, and excellent chelating agent. Both Ag and chitosan are antibacterial agents, so chitosan-Ag nanoparticle composite material has more antibacterial effect. Comparative studies showed that chitosan-Ag nanoparticle composite is much more effective against bacteria than pure chitosan [20].

2.3. Comparative Studies

Following tables showed the previous work related to antimicrobial textile finishes on various fibers, finishing agent used, method that was applied to make the fabric resistant to microorganism and the tested microorganism.

Table 1 Antimicrobial treatment on cotton fabrics

Type of fabric	**Finishing agent used**	**Method used for the application of finish**	**Micro organisms**	**References**
Cotton	Titanium oxide (TiO2) nanoparticles in their two forms, anatase and rutile.	Ultrasonic Irradiation	*Escherichia coli, Staphylococcus aureus*	[73]
Cellulose fibre	Chitosan	UV irradiation	*P.chrysogenum,Escherichia coli*	[74]
Cotton fabrics	Butanetetracarboxylic acid (BTCA) Chitosan	Crosslinking mechanism	*Escherichia coli, Candida albicans, Aspergillusniger*	[75]
Cotton woven fabric	fluoroalkylfunctional water-born siloxane (FAS) , nanosized silver (Ag) (CHT) And a reactive organic–inorganic binder (RB) (CHT).	Sol-gel method	*Staphylococcus aureus, Escherichia coli*	[76]
Cotton canvas	Silver	Photochemical modification	*Staphylococcus Aureus, Escherichia Coli,*	[77]

			Candida albicans, Aspergillus niger	
Cotton fabrics	Nanosilver particles	Biomass of fungus *Fusariumsolani*	*Escherichia coli, Staphylococcus aureus.*	[78]
Woven cotton	Nano-Ag Sub-micro-Ag AgCl	padding-squeezing technique	*Escherichia coli, Bacillus subtillus.*	[79]
Cotton fabrics	Quaternary modified montmorillonite	Clay/enzyme treatment	*Escherichia coli,Staphylococcus aureus, Candida albicans*	[80]
Cotton textiles	Siloxane Sulfopropylbetaine (SSPB)	Covalent bonding	*Escherichia coli, Staphylococcus aureus, Candida albicans*	[81]
Cotton	Chitosan	Fungal chitosan extraction through citric acid production from myceial waste	*Escherichia coli, Candida albicans*	[82]
Cotton	Nano powder of elemental silver Dispersion of AgCl Colloidal silver		*Escherichia coli, S.faecalis, Pseudomonas aeruginosa and*	[83]

			Staphylococcus aureus	
Cotton	Polysulfone microcapsules containing vanillin	Microencapsulation technique	*Staphylococcus aureus*	[84]
woven cotton	Chitosan	surfactant-free emulsion copolymerization		[85]
Cotton	Citric Acid	Dried and cured by microwaves	*Candida albicans* *Escherichia coli,* *Staphylococcus aureus*	[86]
Cotton	Quaternary chitosan.	Reaction with Ionic reactive oligomer IRO	*Staphylococcus aureus,* *Escherichia coli*	[87]
Cotton	zinc oxide sol containing quaternary ammonium salts (DMDAAC-ZnO)	Sol-gel method	*Escherichia coli,*	[88]
Cotton	TiO_2 nanowire	hydrothermal method	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Escherichia coli* *Bacillus cereus* *Candida albicans*	[89]
Cotton	Dimethylol-dimethylhydantion (DMDMH)	Employing a precursor biocidal agent	*Staphylococcus aureus,*	[90]

			Escherichia coli	
Cotton	Chitosan and N-(2-hydroxy) propyl-3-trimethyl ammonium chitosan chloride (HTCC)	Crosslinking using the following agents citric acid (CA), butane tetra carboxylic acid (BTCA), and glutaraldehyde (GA)	*Staphylococcus aureus*	[91]
Cotton	Nanoparticle composed of sodium alginate (SA) and 3-(trimethoxysilyl)propyl-octadecyl dimethyl ammonium chloride (TSA)	Ionic gelation	*Staphylococcus aureus*	[92]
Cotton	Silver nanoparticles	Pad dry cure method	*Pseudomonas aeruginosa.* *Escherichia coli,* *Staphylococcus aureus*	[93]
Cotton	Patchouli oil embedded chitosan–gelatin microcapsules	Coacervation method	*Staphylococcus aureus,* *Escherichia coli*	[94]
Cotton	Nanosized silver colloids	Reduction method using ascorbic acid as reducing agent and polyethylene	*Staphylococcus aureus* *Escherichia coli*	[95]

		glycol s solvent and stabilizer		
Cotton	Silica sols prepared from water glass	Sol gel method	*Escherichia coli*	[96]
Cotton	Didecyldimethylammonium chloride (DDAC), poly-hexamethylenbiguanide, copper and two silver chloride		*Trichophytonrubrum , Trichophytonmentagrophytes and Candida albicans*	[97]
Cotton	N-(hydroxymethyl) acrylamide (NMA), methacrylamide (MA)	Etherification and grafting	*Staphylococcus aureus, Escherichia coli*	[98]
Cotton	Zinc oxide nanoparticles	Wet chemical method		[99]
Cellulose	Acrylic acid (AA) and acrylonitrile (AN)	Graft polymerization	*Bacillus subtilis Escherichia coli and Pseudomonas aeruginosa*	[100]
Cotton woven fabric	Extract of Aervalanata plant and	Microencapsulation	*Staphylococcus aureus Escherichia coli*	[101]
Cotton woven fabric	Organic–inorganic hybrid precursors fluoroalkyl-functional siloxane (FAS) and	Sol gel finishing	*Aspergillusniger Chaetomiumglobosum*	[102]

	3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (SiQAC)			
Cotton	Silver nanoparticles	Reduction method HBP-NH2 aqueous solution as reducing agent	*Escherichia coli, Staphylococcus aureus*	[103]
Cotton	Fibre-reactive chitosan derivative, O-acrylamidomethyl-N-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (NMAHTCC)	Cold pad-batch method	*Staphylococcus aureus*	[104]
Cotton	Sericin from the cocoons of Bombyxmori silk worms	Extraction with cold ethanol	*Escherichia coli, Staphylococcus aureus*	[105]
Cotton				[106]
Cotton	Chitosan	Radical UV-curing with 2-hydroxy-2-methylphenylpropane-1-one	*Escherichia coli*	[107]
Cotton	nanoparticle composed of sodium alginate (SA) and 3-(trimethoxysilyl)propyl-octadecyldimethylammonium chloride (TSA)	Ionic gelation	*Staphylococcus aureus*	[108]

Cotton	chitosan derivatives	synthesized with 2,3-epoxypropyltrimethylammonium chloride and benzaldehyde as modifiers through formation of Schiff base, reduction, N-methylation and O quaternization.	*Staphylococcus aureus*, *E. coli*	[109]
Cotton woven fabric	Silver chloride (AgCl), reactive organic–inorganic binder (RB)	Pad dry cure method and exhaustion method	*E. coli*, *fungi A. nigerand C. globosum*	[110]
Cotton	Silver nitrate	Ultraviolet Irradiation	*B. subtilis*, *Escherichia coli and Staphylococcus aureus.*	[111]
Cellulose	chitosan-based microcapsules containing grapefruit seed oil extract	Microencapsulation	*Escherichia coli and Staphylococcus epidermidis.*	[112]
Cotton	Silver nanoparticles, 1,2,3,4-butanetetracarboxylic acid (BTCA).	Cross-linking	*Escherichia coli Staphylococcus aureus*	[113]
Cotton	Glyoxal,	Pad dry cure method	*Staphylococcus aureus*	[114]

woven fabric	chitosan		*Klebsiellapneumoniae*	
Cotton	Composite- Chitosan, ZnO nanoparticle	hydrothermal method	*Staphylococcus aureus,* *Escherichia coli*	[115]

Table 2 Antimicrobial treatments on Wool fabrics

Type of fabric	**Finishing agent used**	**Method used for the application of Finish**	**Micro organisms**	**References**
Wool	Biocide polyhexamethylenebiguanide (PHMB)	Pre-treatment with peroxymonosulfate/sulfite	*Escherichia coli*	[116]
Wool	Imidazolium-based ionic liquids	Treatment with the Pre-synthesized anionic agent (AA) (sodium 4-(4,6-dichloro-1,3,5-triazinylamino)-benzenesulfonate)	*Escherichia coli*	[117]
Wool	Chitosan Henna	Immerse the fabric in chitosan solution	*Staphylococcus aureus* *Escherichia coli.*	[118]

Wool	Cetylpyridinium chloride (CPC)	Oxidative Pretreatment to enhance exhaustion of QACs	*Escherichia coli*	[119]
Wool	Acid dye with colloidal silver nanoparticles	Exhaustion method in a one-bath	*Escherichia coli,* *Staphylococcus aureus*	[120]
Wool	Curcumin	Dyeing process (batch/continuous)	*Escherichia coli,* *Staphylococcus aureus*	[121]
Wool	ε-polylysine [homo-poly-amino acid characterized by peptide bond between carboxyl and ε-amino groups of l-lysine]	Electrostatic interactions	*Escherichia coli,* *Micrococcus luteus*	[122]
Wool	Capsaicin	Sol-gel method	*Escherichia coli*	[123]
Wool	Quaternary aminopyridinium salts	Ionic interactions	*Escherichia coli*	[124]
Wool	Chitosan	UV grafting	*Escherichia coli*	[125]

Table 3 Antimicrobial treatments on Linen fabrics

Type of fabric	**Finishing agent used**	**Method used for the application of Finish**	**Micro organisms**	**Reference**
linen fabric	Didecyldimethylammonium nitrate [DDA][NO3]	Padding process	*Aspergillus niger, Chaetomium globosum, Gliocladium virens, Paecilomyces variotii, Penicillium ochrochloron*	[126]
linen fabric	Laccase from the *Ascomycete myceliophthora thermophila*	Pre-treatment of linen with laccase followed by chitosan Application (laccase assisted grafting)	*Staphylococcus aureus, Escherichia coli*	[127]

Table 4 Antimicrobial treatments on Polypropylene fabrics

Type of fabric	**Finishing agent used**	**Method used for the application of Finish**	**Micro organisms**	**References**
Nonwoven Polypropylene	Glycidyl methacrylate (GMA) and then linked to cyclodextrin (CD) or MCT CD (MCT-CD) or quaternary ammonium chitosan derivative (HTCC).	Atmospheric plasma-aided graft copolymerization	*Escherichia coli, Lactobacillus plantarum, Staphylococcus aureus*	[128]
Polypropylene nonwoven fabrics	Poly(2-(N,N-dimethyloamino ethyl) methacrylate)	Melt blown method	*Staphylococcus aureus*	[129]

	(PDAMA) and silver-containing layers			
non-woven polypropylene (PP) textile	Acrylic Acid Gentamicin (aminoglycoside antibiotic) and heparin (anticoagulation agent)	Cold-plasma pre-activated PP Ionic Interactions or Covalent linkages	*Escherichia coli*	[130]
spun bonded nonwoven polypropylene fabric	*Ficusbengalensis, Cleome viscosa,* and *Areca catechu*	Air plasma treatment (PT)	*Staphylococcus aureus Escherichia* *coli*	[131]

Table 5 Antimicrobial treatments on Viscose fabrics

Type of fabric	**Finishing agent used**	**Method used for the application of Finish**	**Micro organisms**	**References**
Viscose	1,2,3,4-butanetetracarboxylic acid (BTCA) Chitosan	Microwave technology	*Escherichia coli and Candida albicans*	
Bamboo viscose	5-chloro-2-(2,4-dichlorophenoxy phenol) and triazine derivative	UV absorbers through Exhaust method	*Staphylococcus aureus Escherichia*	[132]

			coli	

Table 6 Antimicrobial treatments on Nylon fabrics

Type of fabric	**Finishing agent used**	**Method used**	**Micro organisms**	**References**
Nylon Carpet	Colloidal nano silver		*Staphylococcus aureus,* *Escherichia coli*	[133]
Nylon	Anionic carboxylic end groups of polyamides and cationic quaternary ammonium salts	Ionic interactions	*Escherichia coli*	[134]

Chapter 3: Materials and Methods

3.1. Materials

100 % pure Cotton fiber was provided by Kohinoor textile mills, Rawalpindi, Pakistan. Chitosan and Acetic Acid (100 %) was obtained from Sigma-Aldrich (USA). Silver nitrate was obtained from Fisher Scientific (UK), sodium citrate from Etiqueta (CE) and nutrient agar from Merck Specialties (Mumbai).The test strains *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus, Staphylococcus aureus* were taken from clinical isolates. All the chemicals used in this work were of analytical grade and used as received, without further purification.

3.2. Methodology

3.2.1. Synthesis of Silver nanoparticles and Chitosan-Silver nanoparticles (CS-AgNPs)

AgNPSs were prepared by the reduction of silver nitrate solution with sodium citrate using the standard method described by Turkevich [135]. Aqueous solution of silver nitrate (1.0 mmol L^{-1}, 500ml) was heated and stirred gently with a magnetic stirrer up to 90 °C. Then (5.0 mL of 0.3 mol L^{-1}) preheated sodium citrate solution (at 90 °C) was added to the above solution. Appearance of yellow color indicates the presence of AgNPs. Further silver nanoparticles were dried using rotary evaporator and oven drying. After drying fine black particles were obtained. The silver nanoparticles were dispersed in distilled water. The prepared silver nanoparticles solution (1 mL) was added into the chitosan solution (1% w/v in 1% acetic acid) and stirred for 2 hours to obtained CS-AgNPs [136].

3.2.2. Preparation of Chitosan-Silver nanoparticles (CS-AgNPs) treated Cotton fabric

Fine Cotton fabric was immersed in an aqueous solution. An aqueous solution contains CS-AgNPs along with penetrant Keirlon XCJ (mixture of non-ionic surfactants) and an antifoaming agent MONOELAST NT (Non-ionic functional poly siloxane). Buffer INVATEX AC (Acidic components based) was added to maintain the pH of the solution then passed through padding mangle with 70 % wet pick up. The padded samples were dried and cured at 150 °C for 2 min.

3.3. Characterization of AgNPs and CS-AgNPs

3.3.1. X-ray Diffraction (XRD)

XRD analysis of Silver Nanoparticles, Chitosan and CS-AgNPs was achieved using XRD model theta-theta STOE (Germany).

3.3.2. Ultraviolet Visible spectroscopy (UV-Vis)

UV-vis shows the absorbance spectra of silver nanoparticles. UV-vis spectrum of (CS-AgNPs) was carried out on UV-2800 BMS Scientific Technical Corporation (PVT) LTD.

3.3.3. Fourier Transform Infrared Spectroscopy

FTIR analysis were performed to investigate any structural changes in the CS-AgNPs [136]. The infrared spectra were recorded on Perkin Elmer, spectrum 100 FTIR spectrophotometer.

3.3.4. Scanning Electron Microscopy (SEM)

The visual images of CS-AgNPs were produced by scanning electron microscopy using JEOL JSM-6042 A (Japan). The working distance was of 10mm which was maintained thereby using 5kV acceleration voltage with a variety of various magnifications. The CS-AgNPs fabric was coated by gold using sputtering technique before obtaining measurements.

3.3.5. Antibacterial activity of CS-AgNPs by Disc diffusion method

AATCC Test method 147 (DIN EN ISO 20645-2001) is a qualitative method for the evaluation of bacteriostatic activity of the antimicrobial material [137]. The antimicrobial activities of the fabric were tested against strains of Gram-negative microorganisms. *Escherichia coli, Pseudomonas aeruginosa*, and Gram-positive *Bacillus cereus, Staphylococcus aureus.* During qualitative measurements the samples were placed forming a circular zone hence the antimicrobial activity of AgNPs, chitosan and CS-AgNPs was tested using modified agar diffusion assay (disc test) [138]. Plate inspections were carried out to note down the zone of inhibition at 38°C for 24 hours. Any prominent zone of inhibition around the samples was recorded as an inhibitory effect against microbial species. The coated fiber samples were tested in order to detect the presence or absence of bacterial growth in the contact region. Two different weave of cotton fabrics were tested against microorganisms.

3.3.6. Antibacterial evaluation of CS-AgNPs coated cotton fabric using quantitative method

Antibacterial testing was carried out by using the shake flask method ASTM E2149. Tests were conducted against the Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. One gram of fabric was placed in a conical flask with 0.5 ml of bacterial inoculum in 50 ml of sterile broth. The flask was shaken at 37ºC at 150 rpm for 24 hours at shaking platform. After incubation of 24 hours contact time the solution was serially diluted. The diluted solution was plated on nutrient agar and incubated for 24 hours at 37° C. The reduction of colonies was calculated using the following equation:

$$R (\%) = (B-A) \times 100/B$$

Where A is the number of bacterial colonies survived after contacting with treated sample and B is the number of colonies present in untreated control sample.

3.3.7. Washing fastness test

CS-AgNPs coated cotton fabrics were tested after repetitive washings in AATCC Atlas Launder-O-Meter Standard Instrument. This is used broadly on laboratory scale for assessing laundry result. One wash that is carried out in Launder-O-Meter (ISO-105-CO1: 1989 (E) Standard method) is equivalent to five home laundry washings. The washing cycles time span was 30 min and solution utilized was of SDC standard detergent with the concentration of 5g/L (pH 10.6) previously heated to 40° C to provide a liquid ratio of 50:1. Subsequently samples were wash off in cold distilled water and squeezed and dried at room temperature. The quality of coated samples were assessed after 20 washes.

Chapter 4: Results and Discussion

4.1. Characterization of Silver nanoparticles , Chitosan and CS-AgNPs

4.1.1. X-ray diffraction (XRD) of Silver nanoparticles

The X-ray diffraction patterns of the chitosan, CS-AgNPs and Silver nanoparticles were shown in **Figure 1**. The chitosan showed peak at 10° and 20°correspond to a hydrated crystalline structure and the peak at 20° an amorphous structure of chitosan, respectively [139–141].

In the XRD pattern of CS-AgNPs, the peaks at 10° and 20° showed the characteristics peaks of chitosan and the peaks obtained at 38°, 44°, 64° and 77° in CS-AgNPs are referred to Silver nanoparticles

In the X-ray diffraction patterns of the silver nanoparticles are shown in **Figure 1**. Peaks were obtained at 2 theta values of 38°, 44°, 64° and 77° that correspond to the (111), (200), (220) and (311) reflections of face centered cubic (fcc) silver which are in perfect agreement to the JCPDS card no. 89-3722 [142]. Similarly, no spurious diffraction was observed that indicates the crystallographic impurities in the sample [143]. Coincided peaks confirmed the fcc structure of pure silver metal. The (111) peak was detected in the sample which is the high intense peak of fcc materials. The high degree of crystallinity of the silver nanoparticles was revealed by the intensity of peaks. However, the broad diffraction peaks were expressing the small size of crystals [144].

The average crystallite size of the AgNPs was calculated from the XRD line broadening using the Scherrer equation [145].

$$B = 0.93\lambda / (L \cos \theta)$$

where λ is the wavelength of the incident X-rays (λ = 1.54060A°), L is the full width at half maximum of the (111) diffraction, and θ is the angle of diffraction. The diameter of AgNPs is 27 nm

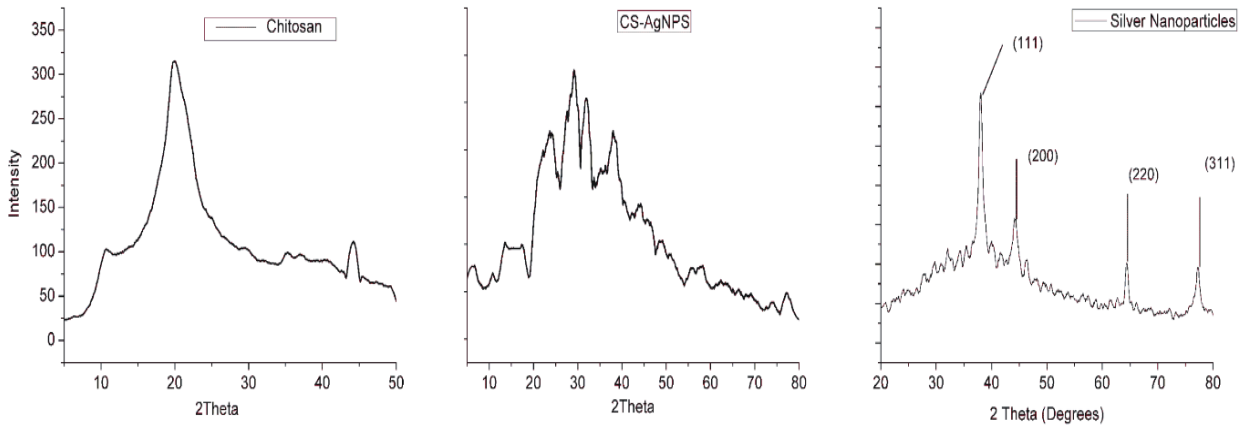

Figure 1 XRD of Chitosan, CS-AgNPs and Silver Nanoparticles

4.1.2. UV-Vis spectroscopy of CS-AgNPs

UV-vis spectroscopy was performed to study the structural characterization of AgNPs present in CS-AgNPs. Surface plasmon resonances (SPRs) dominated the optical spectra of the metal nanoparticles thereby transferring to longer wavelengths with an increment in particle size [146]. UV-vis spectroscopy is a reliable method for detecting the presence of one-dimensional metallic nanostructures [147], [148]. Furthermore, the size and shape characteristics rely on the absorbance pattern of AgNPs [149]. The SPR peaks are inversely proportional to the symmetry of nanoparticles, as the total number of SPR peaks decreases the nanoparticle symmetry rises[150].

The UV-vis spectrum of CS-AgNPs was demonstrated in **Figure 2**. A single peak aroused at 434nm in the spectrum of CS-AgNPs due to an excitation in the surface plasmon vibrations of silver atoms [136]**.**

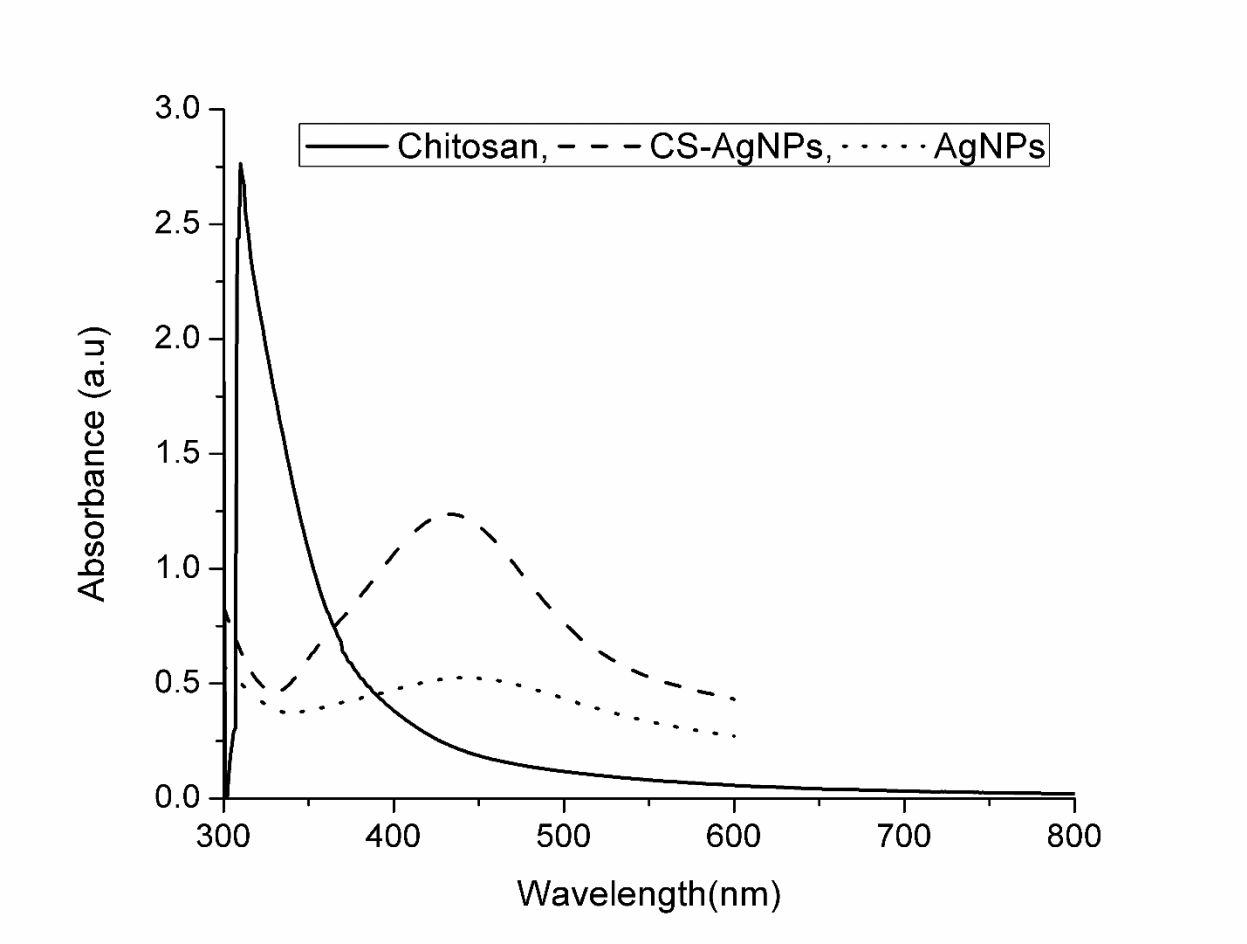

Figure 2 UV-Vis Spectrum of Chitosan, CS-AgNPs and Silver Nanoparticles

4.1.3. FTIR of Silver, Chitosan and Chitosan-Silver nanoparticles

The characteristic peaks of chitosan were observed at 3398 cm^{-1} (OH stretch overlapped with NH stretch), at 2877 cm (shows the presence of sp^3 C-H stretch), at 1601 cm^{-1} (shows the presence of C=O vibration of amide group) , at 1384 cm^{-1} (shows CH stretching), and at 1091 cm^{-1}(shows C-O-C stretch) and 667cm^{-1}(shows stretching vibration of CH bend out of plane)[22], [151].

The FTIR analysis of silver nanoparticles shows peaks at 3436 cm^{-1} (O-H Stretching vibrations), 2922 cm^{-1} (C-H stretching vibrations), 1590 cm^{-1} (N-H bending of primary amines) [142] 1082 cm^{-1} (C-N stretching vibrations of all amines), 909 cm^{-1} and 850 cm^{-1} (C-H bending vibrations out of plane).

The characteristic peaks of CS-AgNPs were present at 3212 cm^{-1} (O-H stretching vibration), 1638 cm^{-1} (C=O stretch, N-H bending for primary amides), 1037cm^{-1} (C-N stretching band for all

amines), 722.56cm^{-1} (C-H bending out of plane).All these peaks were available in the composite. (**Figure 3**)

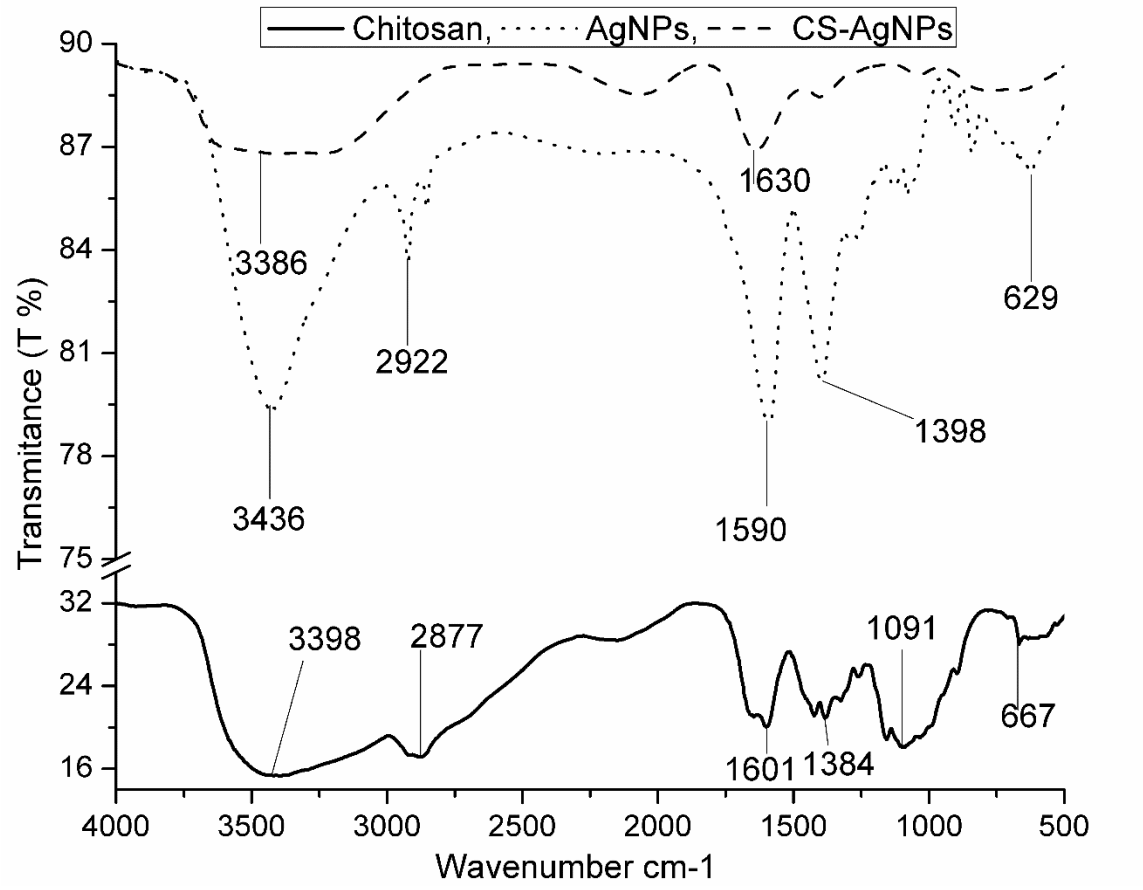

Figure 3 FTIR analysis of Chitosan, Silver nanoparticles and Chitosan-Silver nanoparticles

4.1.4. SEM analysis of CS-AgNPs coated Cotton fabric

SEM analysis were executed to explore changes in the topography of fabric after treatment process compared to untreated blank sample. SEM micrograph reveals the presence of CS-AgNPs on the cotton fabric as it is evident from the **Figure 4** (a, b) that untreated sample shows plain smooth surface whereas treated sample has particles dispersed on its surface. As it was mentioned in the literature that untreated cotton fabric showed plain, smooth and uniform surfaces as compared to the treated cotton [152].

The elemental composition of CS-AgNPs coated fabric was studied by energy dispersive analysis of X-rays (EDAX). **Figure 5** depicts the EDAX analysis from a selected area. The EDAX analysis confirmed that the CS-AgNPs contained about 0.10 wt % Ag, 40.03 wt % carbon, and 59.87 wt % oxygen **Table 7**.

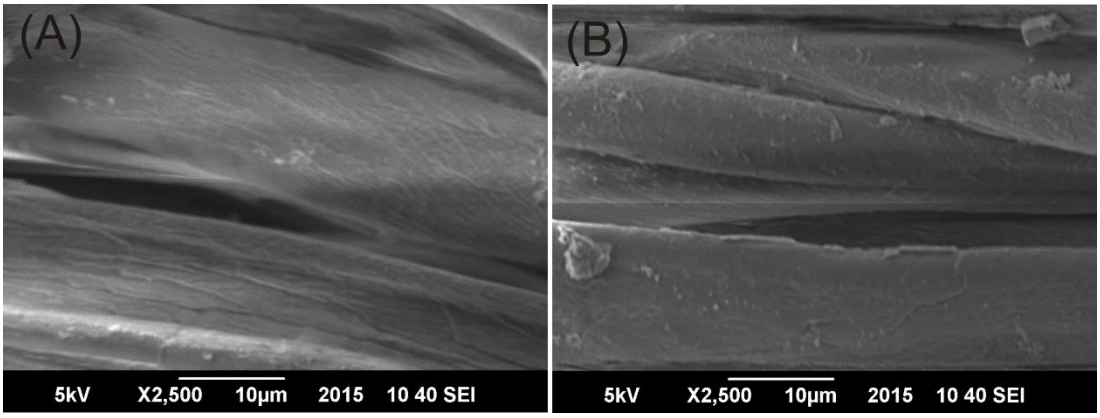

Figure 4 SEM micrographs of Cotton fabric (A) Untreated Cotton fabric (B) Treated Cotton fabric

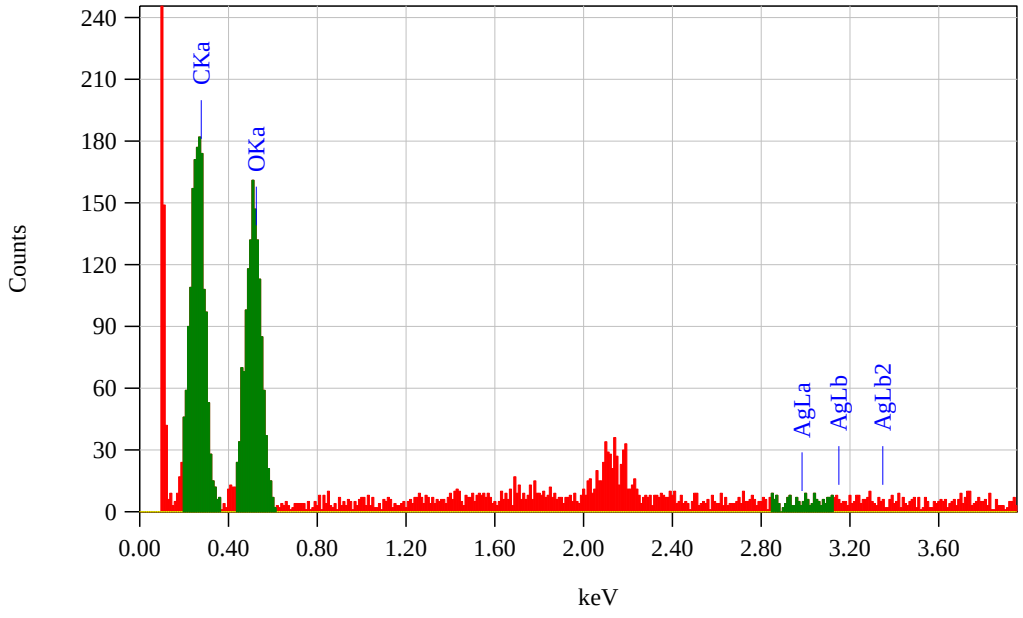

Figure 5 EDAX profile of Chitosan-Silver Nanoparticles (CS-AgNPs)

Table 7 Elemental composition of chitosan–silver nanoparticles

Element	Wt %
CK	40.03
OK	59.87
Ag L	0.10
Total	100.0

4.1.5. Antimicrobial Activity

As the purpose of this Antimicrobial textile finish was to resist the growth of bacteria on to the fabric. First of all the antimicrobial efficacy of AgNps, chitosan and CS-AgNPs were examined independently using discs. **Figure 6** (a, b) showed zone of inhibition of AgNPs, Chitosan and CS-AgNPs against *E.coli* and *B. cereus* prior to the coating on to the cotton fabrics in order to detect their antibacterial potency. **Table 8** presented the diameter measurements of the zone of inhibition. **Figure 6** (c, d, e, and f) explained the antibacterial performance of coated cotton fabrics of two different weaves against gram positive bacteria *Bacillus cereus, Staphylococcus aureus* and gram negative *Escherichia coli, Pseudomonas aeruginosa* bacteria. **Table 9** presented the diameter measurements of the zone of inhibition.

The quantitative method showed bacterial reduction in percentage. The antibacterial activities of cotton fabric containing CS-AgNPs showed high activity with 99.01% bacterial reduction against *S. aureus* and 98.78% against *E.coli* (**Table 10**). The antibacterial efficiency was found to be 98.95% against *S. aureus* and 85.82% against *E.coli* after 20 washes (**Table 11**). The antibacterial activity shown by CS-AgNPs coated cotton fabrics were in line with the literature [20–22].

Previous work has shown that presence of nanoparticles in combination with chitosan has increased the antimicrobial activity of the textile finish [22].

Antimicrobial activity was observed to be increased by adding small amount of AgNPs with chitosan [136]. Antimicrobial activity was increased due to the smaller size of nanoparticles as well. Antimicrobial effect is size dependent. Smaller the size of particles, better the antimicrobial activity [153].

The result indicated that the antimicrobial finish was efficient for resisting the growth of bacteria on cotton fabric. This is due to the synergistic effect of both, AgNPs and chitosan in the textile finish. The outcome suggested that the antimicrobial activity of chitosan can be intensified with inclusion of silver nanoparticles into chitosan.

Table 8 Antimicrobial activity of chitosan, silver and Chitosan-silver nanoparticles against gram negative bacteria, *E.coli* and gram positive bacteria, *B. cereus*

Microorganism	**Antimicrobial**	**Zone of Inhibition**
Escherichia coli ***(gram negative)***	Silver nanoparticles	15 mm
Escherichia coli	Chitosan	14 mm
Escherichia coli	Silver and chitosan composite material	21 mm
Bacillus cereus ***(gram positive)***	Silver nanoparticles	17 mm
Bacillus cereus	Chitosan	11 mm
Bacillus cereus	Silver and chitosan composite material	20 mm

Table 9 Zone inhibition diameters (mm) of antimicrobial cotton fabric against gram negative and gram positive bacteria

Microorganisms	**Coated fabric (Duck weave)**	**Coated fabric (percale)**
Bacillus cereus	25 mm	20 mm
Staphylococcus aureus	29 mm	27 mm
Pseudomonas aeruginosa	30 mm	30 mm
Escherichia coli,	24 mm	26 mm

Table 10 Antibacterial efficiency of the control and CS-AgNPs coated cotton fabric

Fabric sample	Test organism	Survival cells (CFU/ml)		Antibacterial Reduction (%)
		Control fabric	Treated fabric	
Cotton fabric finished with CS-AgNPs	*S. aureus*	$458x10^{-2}$	$45x10^{-3}$	99.01 %
	E.coli	$247x10^{-2}$	$30x10^{-2}$	98.78 %

Table 11 Antibacterial efficiency of the control and CS-AgNPs coated cotton fabric after 20 washes

Fabric sample	Test organism	Survival cells (CFU/ml)		Antibacterial Reduction (%)
		Control fabric	Treated fabric	
Cotton fabric finished with CS-AgNPs	*S. aureus*	$458x10^{-2}$	$48x10^{-3}$	98.95 %
	E.coli	$247x10^{-2}$	$35x10^{-2}$	85.82 %

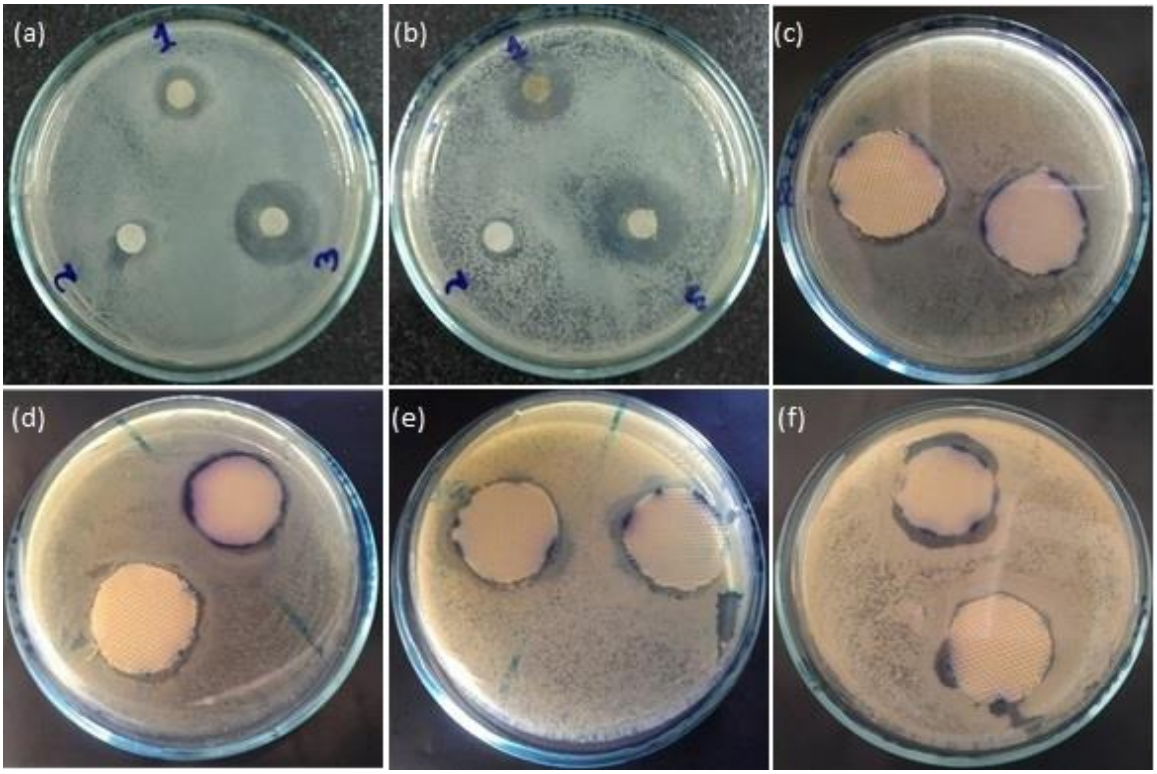

Figure 6 Zone of inhibition of antimicrobial agents against gram negative and gram positive bacteria a) *Escherichia coli* (1) silver nanoparticles (2) Chitosan (3) Chitosan-silver nanoparticles,(b)*Bacillus cereus*(1) silver nanoparticles (2) Chitosan (3) Chitosan-silver nanoparticles, (c) *Bacillus cereus* , (d) *Escherichia coli,* (e) *Pseudomonas aeruginosa,* (f) *Staphylococcus aureus*

4.1.6. Physical properties of CS-AgNPs coated Cotton fabric

The results indicate that tensile strength of the uncoated and the coated fabric was found to be 72. 8 lbs (warp) and weft 57.9 lbs and 75.4 (warp) and 47.5 lbs (weft) respectively. The CS-AgNPs coating is found to increase tensile strength of the fabric in warp direction while there is decrease in weft direction was noticed but there is no effect of finish on weft. There is no effect on the whiteness of finished fabric. It is found that absorbency also remains the same (**Table 12**).

Table 12 Physical properties of Uncoated and CS-AgNPs Coated Cotton fabric

Physical Properties	**Uncoated fabric**	**Fabric coated with CS-AgNPs**	**Test method**
Type of weave	Duck weave	Duck weave	
Fiber content	100 % cotton	100 % cotton	AATCC 20/20A
Tensile strength	Warp: 72.8 lbs Weft: 57.9 lbs	Warp: 75.4 lbs Weft: 47.5 lbs	ASTM D 5034
Tear strength	Warp: 62 N Weft: 23.6 N	Warp: 62 N Weft: 23.6 N	ASTM D1424
Resistance to abrasion	B/W 15000 REV – Not broken	B/W 15000 REV – Not broken	ISO 12947
Resistance to pilling	B/W 125 REV: 4.5 B/W 500 REV: 4.0 B/W 1000 REV:3.5 B/W 2000 REV:3.0	B/W 125 REV: 4.5 B/W 500 REV: 4.0 B/W 1000 REV:3.5 B/W 2000 REV:3.0	ASTM D 4970
Absorbency	3-4 sec	3-4 sec	Drop Method
Whiteness	73.47	73.54	AATCC 110

Crease recovery angle	Warp: 66° Weft: 67°	Warp: 62° Weft: 67°	AATCC 66
Color fastness to rubbing	Dry: 4.5 Wet: 4.5	Dry: 4.5 Wet: 4.5	AATCC 08
Dimensional Stability Top loading machine	Warp: -2.1 % Weft: -1.5 %	Warp: -1.5 % Weft: -1.0 %	BE EN ISO 6330: 2012
Color fastness to washing(Launder-o-meter)	Shade change: 4.5 Staining: 4.5	Shade change: 4.5 Staining: 4.5	ISO 105 CO1

Chapter 5: Conclusion

Chitosan and silver nanoparticle were used to prepare antimicrobial textile finish. The synthesized AgNPs were in spherical shape with particle size of 27 nm. XRD showed high crystalline peaks of silver nanoparticles. FTIR analysis showed the formation of CS-AgNPs by showing its characteristic peaks and UV-Vis analysis showed a single peak at 434 nm in the spectrum of CS-AgNPs verifying the presence of AgNPs. SEM analysis showed the presence of CS-AgNPs on the surface of treated fabric. Investigation on the antibacterial effect of silver chitosan textile finish against *Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus cereus, Staphylococcus aureus* microbes proved that CS-AgNPs is a strong antibacterial agent.

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