Application of nanotechnology for wool and wool blends in medical textiles utilising biopolymers

A thesis submitted in fulfillment of the requirements for the degree of
Doctor of Philosophy

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August, 2012
RMIT UNIVERSITY
DECLARATION

I, Saniyat Islam, certify that:

a. except where due acknowledgement has been made, the work is that of the candidate alone;
b. the work has not been submitted previously, in whole or in part, to qualify for any other academic award;
c. the content of the thesis is the result of work which has been carried out in the School of Fashion and Textiles, RMIT University, between March 2009 and August 2012.
d. any editorial work, paid or unpaid, carried out by a third party is acknowledged.
e. ethics procedures and guidelines have been followed.

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Saniyat Islam

August 2012
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DEDICATION

This work is dedicated to
my parents and family members
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<td>1-butyl, 3-methyl imidazolium chloride</td>
<td>BMIMCl</td>
</tr>
<tr>
<td>American Association of Textile Chemists and Colourists</td>
<td>AATCC</td>
</tr>
<tr>
<td>American Type Culture Collection</td>
<td>ATCC</td>
</tr>
<tr>
<td>Chitosan</td>
<td>CHT</td>
</tr>
<tr>
<td>Colony forming unit</td>
<td>CFU</td>
</tr>
<tr>
<td>Differential scanning calorimetry</td>
<td>DSC</td>
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<tr>
<td>Fourier Transform Infra-Red</td>
<td>FTIR</td>
</tr>
<tr>
<td>Grams per square metre</td>
<td>GSM</td>
</tr>
<tr>
<td>Infrared</td>
<td>IR</td>
</tr>
<tr>
<td>Ionic Liquid</td>
<td>IL</td>
</tr>
<tr>
<td>On weight of material</td>
<td>OWM</td>
</tr>
<tr>
<td>Poly-acrylonitrile</td>
<td>PAN</td>
</tr>
<tr>
<td>Poly-caprolactone</td>
<td>PCL</td>
</tr>
<tr>
<td>Poly-DL-lactic acid</td>
<td>PDLA</td>
</tr>
<tr>
<td>Polyethylene co-vinyl acetate</td>
<td>PEVA</td>
</tr>
<tr>
<td>Polyethylene oxide</td>
<td>PEO</td>
</tr>
<tr>
<td>Poly-lactic acid</td>
<td>PLA</td>
</tr>
<tr>
<td>Poly-L-lactic acid</td>
<td>PLLA</td>
</tr>
<tr>
<td>Poly-methyl methacrylate</td>
<td>PMMA</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>PS</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>PVA</td>
</tr>
<tr>
<td>Room temperature Ionic Liquid</td>
<td>RTIL</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>SNPs</td>
</tr>
<tr>
<td>Tetra-ethyl benzyl-ammonium chloride</td>
<td>TEBAC</td>
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Abstract

The object of the project was to explore ways to utilise wool in medical textiles particularly in wound-dressing materials. Wool was chosen for this study as it possesses the highest moisture regain property (saturation regain is approximately 36%) among all the natural and synthetic polymers. The experimental sets were designed to highlight two major fields for wound-dressings namely the use of nonwovens and films. A biopolymer chitosan (CHT) and silver nanoparticles were chosen for their antibacterial properties to be applied to wool and wool-blend materials to assess their potential use in wound-dressing materials.

Based on the background research, the current research concept was developed to utilise novel techniques and application methods. To develop advanced wound-care products and to characterise the properties and performance of these developed samples, three different routes of processing were chosen for the current research namely are: pad-dry-cure, electrospraying and dissolution and regeneration of polymers to prepare blend films.

For this study, 100% wool nonwoven and wool–viscose (50-50) nonwovens, having weight of 150–400 grams per square metre (GSM) were prepared and post-treated with a range of CHT concentrations varying from 0.1–1.0% on the weight of material.

Firstly, 100% wool nonwoven (400 GSM) was pretreated with 1-butyl, 3-methyl imidazolium chloride (BMIMCl) to dissolve the surface lipid layer and as such increase the wettability of wool fibres for the subsequent application of CHT by the pad-dry-cure method. Then the substrates were treated with CHT concentrations of 0.3–1% and tested for water absorbency and anti-bacterial properties. The BMIMCl treatment was found to have negative impact on the absorbency because an approximately 8% reduction in wool fibre mass was observed. CHT-treatment did improve the water absorbency at lower concentrations of CHT the absorbency was comparable with the untreated control. At higher concentrations of CHT (> 0.5) the stiffness of the treated material increased and absorbency was not further improved. The lowest concentration of CHT-treated nonwoven was tested for antibacterial properties and was found to be excellent. Thus 0.3% CHT-treated samples were found to be optimally absorbent together with excellent anti-bacterial properties.

Secondly, a 100% wool nonwoven (150 GSM) was treated with CHT (0.1–1% OWM) by the pad-dry-cure method without any BMIMCl pretreatment. Blood absorbency and anti-
bacterial efficacy were tested and the results showed that the minimum inhibitory CHT concentration was 0.3% with enhanced absorbency of blood.

Thirdly, a 50-50 wool–viscose nonwoven (150 GSM) was treated with CHT (0.1–1% OWM) by the pad-dry-cure method. Blood absorbency and anti-bacterial efficacy were tested and the results showed again that the minimum inhibitory CHT concentration was 0.3% with increased absorbency of blood. Thus it was concluded that wool nonwoven treated with CHT at lower concentrations (0.3% OWM), proved to be at least comparable with commercially available dressing materials. They therefore were excellent candidates as advanced wound-dressing material where both absorbency and anti-bacterial properties are benchmark features.

The current study also explored the method of electrospaying of chitosan to coat wool nonwoven substrates, a technique that has been introduced for the very first time. Scanning electron micrographs of the coated 100% wool nonwoven (400 GSM) showed excellent coatings with 0.3% CHT (OWM). The coated substrates were also tested for anti-bacterial properties and proved to be outstanding. Thus this novel technique provided excellent coating opportunities for textiles to achieve suitable substrates with enhanced functional properties.

Both CHT and wool are renewable, bio-degradable and bio-compatible, but are complicated to process by directly dissolving in common solvents. A new class of ionic liquid solvent 1-butyl, 3-methyl imidazolium chloride (BMIMCl) has provided an ideal platform to solubilise polymers to achieve the blending operation. The current study blended wool and chitosan utilising BMIMCl as their common solvent and characterised the resultant films. It was shown that wool-CHT (50-50) blend films were homogeneous and exhibited excellent anti-bacterial attributes. Silver nanoparticles (SNPs) were also incorporated into the films by utilising the BMIMCl wool dissolution technique. The 100% films prepared from BMIMCl dissolution and regeneration containing 0.5% (OWM) SNPs proved to possess antibacterial features. Since toxicological behaviour and potential health concern of using SNPs are yet to be comprehensively understood, wool-CHT films are recommended because of non-toxicity, biodegradability and are effective at low concentrations.
Chapter 1: Background Research

1.1: Introduction

Medical textiles have been classified as belonging to a category called 'Medtech', which is one of the main streams of technical textiles [1]. Technical textiles provide significant opportunities for business to achieve sustainable growth to escape from the tough competitive environment faced by traditional textile manufacturers. Technical textile products are mainly used for their performance or functional characteristics rather than for their aesthetics.

Currently, and into the foreseeable future, the role of medical textiles within the healthcare sector is of vital importance. Sustainable and economic growth within that market sector will be driven by the demand for innovative solutions that result from the utilisation of novel materials and technologies. Wound-dressings are a case in point. Within the transitory environment of a wound, numerous interlinked cellular processes facilitate repair of damaged tissue. As a consequence, the functionality of wound-dressings is focusing on the processes of healing and controlling the wound environment, rather than merely covering it. Collectively, these processes can best be described as one component of overall wound-management. This thesis will introduce the textile fibres, fabrics and structures that are utilised in wound-management. In addition, it highlights potential applications of bio-polymers and bio-polymeric materials such as chitin and chitosan. Bio-medical textiles are fibrous structures designed for use in particular biological environments. They are used where their performance depends on bio-compatibility with cells, tissues or body fluids.

Technological advancements have made it possible to use textiles in implantable materials and devices, bio-compatible materials such as textiles used in tissue engineering and intricate neural repairs. Anti-microbial agents traditionally used in other medical textile applications offer protection from cross infections when used in wound-management. The advent of innovative
technologies such as nanotechnology, plasma treatments and special coatings for medical textiles have opened avenues to healing wounds that were considered difficult to manage only a decade ago. To summarise, medical textiles are a strong foundation for successful wound-management aimed at rapid recovery. Recent developments in wound-care products along with the review of medical textiles are reported in this chapter.

1.2: Technical textiles

Technical textiles are defined as “textile materials and products intended for end-uses other than non-protective clothing, household furnishing and floor covering, where the fabric or fibrous component is selected principally but not exclusively for its performance and properties as opposed to its aesthetic or decorative characteristics” [2].

Techtextil [1] gives a definition of technical textiles based on main end-use markets (Table 1.1). These terms classified by Techtextil are market-based but not used universally. However, this classification provides the structure and end-use markets for technical textiles in general.

Table 1.1. End-use based classification of the technical textiles [3]

<table>
<thead>
<tr>
<th>Description</th>
<th>Markets/ applications</th>
</tr>
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<tbody>
<tr>
<td>Agrotech</td>
<td>Agriculture, aquaculture, horticulture and forestry</td>
</tr>
<tr>
<td>Buildtech</td>
<td>Building and construction</td>
</tr>
<tr>
<td>Clothtech</td>
<td>Technical components of footwear and clothing</td>
</tr>
<tr>
<td>Geotech</td>
<td>Geotextiles for landscaping and civil engineering</td>
</tr>
<tr>
<td>Hometech</td>
<td>Technical components of furniture, household textiles and floorcoverings</td>
</tr>
<tr>
<td>Indutech</td>
<td>Filtration, conveying, cleaning and other industrial uses</td>
</tr>
<tr>
<td>Medtech</td>
<td>Hygiene and medical</td>
</tr>
<tr>
<td>Mobiltech</td>
<td>Automobiles, shipping, railways and aerospace</td>
</tr>
<tr>
<td>Oekotech</td>
<td>Environmental protection</td>
</tr>
<tr>
<td>Packtech</td>
<td>Packaging</td>
</tr>
<tr>
<td>Protech</td>
<td>Personal and property protection</td>
</tr>
<tr>
<td>Sporttech</td>
<td>Sports and leisure</td>
</tr>
</tbody>
</table>
It is distinctive for technical textiles that the production is mainly concentrated in highly-developed countries. The production of technical textiles is increasing faster than that of conventional textile products i.e. clothing and household textiles. Technical textiles and household textiles were almost similar in market consumption but were very low compared to apparel usage. PCI Consulting Group [4] reported that, in 2004, the global market for fibres totalled 64 million tons. The main end-uses were apparel (65%), household textiles (18%) and technical textiles (17%). Of the fibres that were consumed worldwide in 2004, synthetic fibres were 40 million and natural fibres 24 million tons respectively. The breakdown was polyester (40%), cotton (36%), polypropylene/other olefins (7%), polyamide (6%), acrylic (4%), regenerated cellulosic fibres (4%) and wool (2%) as shown in Figure 1.1 [4].

![Figure 1.1: Breakdown percentage of usage of total fibre used globally in 2004](image)

Apart from Techtextil, few others have tried to classify the application-based textiles for the end-use markets. Johnson et al. [5] described the multitude of classifications of textile fibres from a modern perspective as shown in Figure 1.2.
The global market of technical textiles was worth US$60,270 million in 2000 and of that market about US$1,077 million (1.6%) was medical textiles alone. The growth of the medical textile market was expected to reach 4.3% by volume by the end of 2010 [3, 6-8]. Therefore new and innovative technological approaches should be addressed for this growing industry to satisfy consumer needs for functionality and hygiene.

In Figure 1.2, medical textiles fall under the classification of 'Medical', which includes stents, human tissue scaffolds, surgical sutures and bandages and other feminine hygiene and incontinence products. This classification shows how the different fibres are prepared for specific end-use applications in particular textile industries. Medical textiles are a key growth area within the technical textiles industry and its applications continue to escalate in diversity with every new innovation or invention. Recent innovations and inventions include application of chitosan–alginate fibres in advanced wound-dressings, use of ultrasonic energy for bleaching cotton-based medical textiles, bio-cidal textiles, utilisation of spider silk as a supportive matrix, novel surgical sutures...
and smart textiles for medical applications. The technologies associated with wound-dressings are pushing the boundaries of design and innovation. Novel wound-management practice requires the use of new materials and methods of drug storage and delivery. In the future, users will be influenced by the availability of dressings that feature properties such as minimisation of healing time, lessening of change over frequency and mitigation of pain on removal. The effectiveness of wound-dressings can be greatly enhanced by incorporating anti-fungal and anti-microbial agents, and by affixing drug storage and delivery systems into their structural matrix.

1.3: Overview of worldwide consumption

Medical textiles have been classified as belonging to a category called “Med-tech”, which is one of the main streams of technical textiles. Technical textiles provide significant opportunities for business to achieve sustainable growth to escape from the tough competitive environment faced by traditional textile manufacturers. Technical textile products are mainly used for their performance or functional characteristics rather than for their aesthetics. End-uses served by technical textiles are numerous and diverse. They include agriculture and horticulture, architecture, building and construction, clothing technology, geotextiles, functional textiles, automotive textiles and medical textiles. Within medical textiles, wound-management has been a niche market that is growing at a rapid pace. Figure 1.3 shows the market segmentation of wound-management in 2008 [9]. Traditional wound-care products are being replaced by innovative advanced wound-care products that are based on advanced technical textiles.
Figure 1.3: Worldwide wound-management product market segment by country in 2008 [9]

Figure 1.4 shows the forecasted growth of traditional and advanced wound-care markets towards 2017 [9]. As discussed earlier, medical textiles are a key growth area within the technical textiles industry. The global wound-management market totalled over US$15 billion in annual sales in 2009; USA and Europe alone account for 62% of the wound-management market. Figure 1.4 shows that the projected use of wound-management products is increasing every year.
As standards of living improve and consumers demand more comfort and quality, the health industry has had to come up with new features utilising technical advancement and innovation to attract and satisfy those consumers.

1.4: What are wounds?

The skin is the largest organ in the body by surface area and weight. The skin consists of two layers: the epidermis and the dermis, as shown in Figure 1.5. Beneath the dermis there is the hypodermis or subcutaneous fatty tissue. The skin facilitates the main functions of providing protection, thermal regulation and sensation. Wounding affects all these skin functions [10-13].

Wound types are diverse, from acute to chronic, traumatic to surgical to disease-driven. Open wounds can be defined as an injury that is exposed due to broken skin. An open wound has a high risk for infection. Cuts, abrasions and puncture wounds are common, and everyone will experience these in their lifetime [14]. Wounds include cuts, grazes, minor burns, abrasions, friction burns, pressure sores, diabetic ulcers and many more [15, 16].

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**Figure 1.5: Anatomy of the skin**

Wound types are diverse, from acute to chronic, traumatic to surgical to disease-driven. Open wounds can be defined as an injury that is exposed due to broken skin. An open wound has a high risk for infection. Cuts, abrasions and puncture wounds are common, and everyone will experience these in their lifetime [14]. Wounds include cuts, grazes, minor burns, abrasions, friction burns, pressure sores, diabetic ulcers and many more [15, 16].
1.4.1 : Phases of wound healing

The healing of an adult skin wound is a complex process requiring the collaborative efforts of many different tissues and cell lineages. The behaviour of each of the contributing cell types during the phases of proliferation, migration, matrix synthesis and contraction, as well as the growth factor and matrix signalism, present at a wound site [17, 18]. The progression of wound healing is not linear and often wounds can evolve both forwards and backwards through these phases depending on intrinsic and extrinsic forces at work within the patient. Generally, wound healing can be divided into three distinct phases. The phases of wound healing are:

- inflammatory phase
- proliferation phase
- maturation phase.

Figure 1.6 shows the different wound-healing phases corresponding to the time it takes to progress for each of the phases.

![Figure 1.6: Different stages of wound healing corresponding to time](image)

The inflammatory phase is the body’s natural response to injury. After initial wounding, the blood vessels in the wound-bed contract and a clot is formed. When haemostasis has been attained, blood vessels then expand to allow essential cells: antibodies, white blood cells, growth factors, enzymes and nutrients, to reach the wounded space. This increases exudate levels, so the surrounding skin needs to be monitored for signs of maceration. It is at this stage that the characteristic signs of inflammation are noticed. The predominant cells at work here are the phagocytic cells such as neutrophils.
and macrophages, mounting a host response for ‘necrotic or sloughy’ tissue [14, 15, 19].

During proliferation, the wound is reformed with fresh granulation tissue which is comprised of collagen and an extracellular matrix and into which a new network of blood vessels develops, a process known as ‘angiogenesis’. Healthy granulation tissue is dependent on the fibroblast receiving sufficient levels of oxygen and nutrients supplied by the blood vessels. Healthy granulation tissue is granular and uneven in texture; it does not bleed easily and is pink or red in colour. The colour and condition of the granulation tissue are often an indicator of how the wound is healing. Dark granulation tissue can be indicative of poor perfusion, ischaemia and/or infection. Epithelial cells finally resurface the wound, a process known as ‘epithelialisation’.

Maturation is the final phase and occurs once the wound has been closed. This phase involves remodelling of collagen from type III to type I. Cellular activity reduces and the number of blood vessels in the wounded area regress and decrease [20].

1.4.2 Problems associated with wounds

Wounds, in general, are open to the external environment and thus can be affected by contaminants and micro-organisms. The presence of bacteria in a wound may result in:

- contamination, where bacteria do not multiply or cause clinical problems
- colonisation, where the bacteria multiply but wound tissues are not damaged; or
- infection, where the bacteria multiply, healing is disrupted and wound tissues are damaged (local infection); bacteria may produce problems nearby (spreading infection) or systemic illness (systemic infection).

Figure 1.7 [21] illustrates the above.
Figure 1.7: Problems associated with presence of bacteria

Infection is a serious problem caused by bacteria that may lead to serious consequences if not addressed. Effective management of wound infection depends on optimising the host (patient) response, reducing the bacterial load and maintaining some general measures such as managing pain and giving psychological support. Figure 1.8 [22] demonstrates the steps of effective wound-management.

Figure 1.8: Effective wound-management

1.4.3 Basic components of a wound-dressing

Wound-dressing serves various functionalities. Depending on the type, severity and location of the wound, the dressing should stem bleeding, absorb exudate, ease pain, debride the wound and promote healing. The ranges of wound-management products and products under development to
address them are even more varied. In general, a multi-layer wound-dressing consists of several layers, each of which performs different functions. Figure 1.9 shows typical multi-layer dressing components.

![Diagram of typical multi-layer dressing components]

Figure 1.9: Components of a typical dressing

The non-adherent layer prevents the dressing from adhering to the wound. The bactericidal layer has an active anti-bacterial finish or agent to kill bacteria present in the wound environment and, in addition, inhibits microorganisms from an external source. The absorbent layer soaks up exudates and gives a cushioning effect for the wound. This typical dressing is often backed by a permeable polymeric layer with adhesive to cover the wound surface.

1.4.4 : Application of textiles in wound-dressings

Medical textiles have numerous and diverse applications within the healthcare sector, such as bandages and dressings, biocompatible implants and tissues, antibacterial wound-dressings and prosthetics. The term ‘bio-material’ can be defined as a “viable material used in fabrication of a medical device and intended to react with biological systems” [23]. The term ‘bio-textiles’ is defined as a “structure composed of textile fibres and designed for use in a specific biological environment (e.g. wound-dressing), where its performance depends on its interactions with cells and biological fluids” [24].
All kinds of textiles such as fibres, mono and multi-filament yarns, woven, knitted, nonwoven, braiding and composite fabrics can be used for medical textiles [3, 25]. Nonwovens are used in large quantities in the wound-management market because of the disposable nature of these products [3]. In addition, there is a rising share of composite materials used in wound-management products. Textiles are combined with films, foam and adhesives by coating and lamination to form structures for composite wound-treatment and healthcare products [26]. Table 1.2 shows different textile fibres and fabric structures and their major applications in wound-management products.

Table 1.2: Application of fibre and fabric structures [27]

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Fabric structure</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton, viscose, lyocell</td>
<td>Nonwoven</td>
<td>Absorbent pad</td>
</tr>
<tr>
<td>Alginate fibre, chitosan, silk, viscone, lyocell, cotton</td>
<td>Woven, nonwoven, knitted</td>
<td>Wound-contact layer</td>
</tr>
<tr>
<td>Viscose, lyocell, plastics film</td>
<td>Woven, nonwoven</td>
<td>Base material</td>
</tr>
<tr>
<td>Cotton, viscose, lyocell, polyamide fibre, elastomeric-fibre yarns</td>
<td>Woven, nonwoven, knitted</td>
<td>Simple non-elastic &amp; elastic bandages</td>
</tr>
<tr>
<td>Cotton, viscose, lyocell, elastomeric-fibre yarns,</td>
<td>Woven, nonwoven, knitted</td>
<td>High-support bandages</td>
</tr>
<tr>
<td>Cotton, viscose, lyocell, elastomeric-fibre yarns,</td>
<td>Woven, knitted</td>
<td>Compression bandages</td>
</tr>
<tr>
<td>Cotton, viscose, lyocell, polyester, polypropylene, polyurethane foam</td>
<td>Woven, nonwoven</td>
<td>Orthopaedic bandages</td>
</tr>
<tr>
<td>Cotton, viscose, plastics film, polyester, glass, polypropylene</td>
<td>Woven, nonwoven, knitted</td>
<td>Plasters</td>
</tr>
<tr>
<td>Cotton, viscose, lyocell, alginate, chitosan</td>
<td>Woven, nonwoven, knitted</td>
<td>Gauze dressing</td>
</tr>
<tr>
<td>Cotton</td>
<td>Woven</td>
<td>Lint</td>
</tr>
<tr>
<td>Viscose, cotton linters, wood pulp,</td>
<td>Nonwoven</td>
<td>Wadding</td>
</tr>
<tr>
<td>Polylactide, polyglycolide, carbon</td>
<td>Spunlaid, needle-punched nonwoven</td>
<td>Scaffold</td>
</tr>
</tbody>
</table>

1.4.5 : Recent developments
Both natural fibres and synthetic polymers are used in medical textiles. Natural carbohydrate polymers such as D-glucans, chitin, chitosan, alginate,
hyaluronan, sulphated polysaccharides and complex hetero-polysaccharides are used. Modified carbohydrate polymers by cellulose solubilisation and derivatisation, cross-linked dextrans, chitosan derivatives and hylans are also being used. Natural and modified proteins such as collagen, gelatin, casein, zein, laminin and elastin are used for medical purposes [28].

Chitin, and its derivative chitosan (CHT), have long been researched for application in wound care. Chitin, poly-1,4-2-acetimido-2deoxy-β-D-glucose, is the second most abundant natural polymer, existing widely in cell walls of fungi and crustacean shell composites. Chitin is commercially produced from the shell waste of crabs, shrimps and other crustaceans through a series of de-proteinisation and de-mineralisation processes. Dry mass of shell waste consists of approximately 15–25% of chitin [29]. Chitin has found application in surgical sutures and it has been demonstrated to expedite healing of wounds [28, 30-38].

Chitosan is achieved by deacetylation of chitin. Chitin and chitosan are polysaccharides that have been reported to have an excellent bio-compatibility and bio-degradability along with biological activities such as anti-microbial activity, low immunogenicity and low toxicity [31, 39-42].

Electrospinning is a textile technique that has increased opportunities to produce chitosan nanofibres that has promising applications in wound-management [43-46]. Nano-scale fibres provide a large surface area to interact with skin, providing the possibility to be utilised as drug storage and delivery mechanisms. This field of electrospinning has great potential in the future for its novelty, cost effectiveness, reproducibility and simplicity.

Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound-dressings have been developed [47]. Storage of drugs such as anti-microbial gentamicine sulphate and metronidazole in di-butarylchitin porous fibres are also possible [48]. Further research is currently in progress using these bio-polymeric materials for new application areas in medical textiles.

1.5: Medical textiles

The requirements for textiles and textile structures used in medical applications are different from those used in clothing and other applications.
The performance of these medical textiles depends on the fibre properties, fabric structures and various finishes used in the manufacturing processes. A variety of manmade and natural fibres are being used in medical textiles and the properties of these fibres are discussed in this section.

Below is a brief account of the major fibres that are used in the manufacturing of medical textiles.

1.5.1 : Manmade fibres

1.5.1.1 : Polyester
Polyester fibre contains recurring ester groups as an integral part of the main polymer chain. The structure shown in Figure 1.10 is polyethylene terephthalate (PET); it consists of ethylene groups and terephthalate groups.

![Ester group in polyester chain](image)

![Terephthalate and ethylene group](image)

**Figure 1.10: Chemical structure of polyester [49]**

The ester groups in the polyester chains are polar in nature, with the carbonyl oxygen atom having a negative charge and the carbonyl carbon atom having a positive charge. The positive and negative charges of different ester groups are attracted to each other. This allows the ester groups of nearby chains to line up with each other in crystalline form and thus forms strong fibres [50].
PET fibres have an excellent resistance to chemicals. They also have very good resistance to acids, but are less resistant to alkali and are not affected by any of the bleaching agents.

Properties, such as high tenacity, high resistance to abrasion and excellent resistance to direct exposure to sunlight make polyester a very popular fibre for medical textiles.

Polyester has an application in preparing surgical gowns, caps, masks and drapes, cloths, surgical hosiery and blankets [26]. Other than these applications, it has also been reported to be used for impervious vascular prosthesis grafting [51, 52] and implants [53-58].

1.5.1.2: Polyamides (nylon 6 and nylon 6, 6)
Polyamides contain recurring amide groups as an integral part of the main polymer chain. Nylon 6 is one of the most common forms of polyamides used as a fibre and, being thermoplastic, can have other end-uses as well. Nylon fibres are also highly crystalline like polyester and this is attributed to the regular and symmetrical polymer chain [50]. Nylon fibres show good chemical resistance towards many chemicals, except for hot mineral acids, and excellent physical properties such as high tenacity and abrasion resistance. The effect of direct exposure to sunlight is moderate for nylon, as strength loss is observed depending on the amount of surface exposed and the size or diameter of exposed filaments. Polyamide fibres are used to manufacture surgical hosiery [26].

1.5.1.3: Polypropylene
Polypropylene is one of the multipurpose polymers that are used as fibre. As a fibre, polypropylene is used to make indoor and outdoor carpeting. Polypropylene is hydrophobic in nature and can be dyed easily during manufacturing. Hence it performs well for outdoor carpet. Structurally, it is a vinyl polymer, except that on every carbon atom in the backbone chain it has a methyl group attached to it. Polypropylene can be made from the monomer propylene by Ziegler-Natta polymerisation (Figure 1.11) and by metallocene catalysis polymerisation [59].
Figure 1.11: Ziegler-Natta polymerisation to make polypropylene [59]

As a fibre, polypropylene has good chemical resistance to acid, alkali, bleaching agents, micro-organisms. It also has high tenacity, elongation and low thermal conductivity. Because of the low thermal conductivity, it is the warmest of all commercial fibres available. It has application in wound-dressing materials [60-65]

1.5.1.4: Viscose (regenerated cellulose)
Lyocell is a fibre mostly known by its brand name Tencel®. It has a soft finish, packs light and is made from cellulose (vegetable matter) or wood pulp. This pulp may be a mix of hardwood trees such as oak and birch, although Tencel is made from eucalyptus trees. This makes it a natural fabric, and it is noted for its durability and strength, in addition to its eco-friendly manufacturing techniques.

Lyocell is made by wood chipping, breaking down the wood fibres with the non-toxic chemical amine oxide and then extruding the material through a spinneret (Figure 1.12). The spinneret produces long fibres, which are then dried and woven into cloth. The company Lenzing Fibres Inc. is the manufacturer of Tencel® fibres – the only company in the United States of America that currently makes these fibres.
Figure 1.12: Process flow chart of Lyocell fibre production

1.5.1.5: Rayon
Rayon was the first manufactured fibre developed from wood or cotton linters and was first known as artificial silk. The manufacture of rayon begins with cellulose, frequently extracted from wood pulp, although any plant material with long molecular chains is suitable. The cellulose is steeped in sodium hydroxide (NaOH), which concentrates some of the cellulose into Na-cellulose, which is then rolled or pressed to remove excess alkali. After pressing, the cellulose is shredded into a phase called ‘white crumb’.

The white crumb is allowed to oxidise, forming shorter molecular chains, and treated with carbon disulfide. The Na-cellulose reacts with this substance, forming yellow crumb due to inorganic compounds that emerge during the chemical process. This yellow crumb is dissolved in an alkali solution, which relaxes the hydrogen bonds in the cellulose, producing a highly viscous substance. This substance gives its name to the manufacturing process, called the ‘viscose process’.
This viscous fluid is allowed to age, breaking down the cellulose structures further to produce an evenly distributed slurry, and then filtered to remove impurities and contaminants. Small air pockets are forced out to ensure a strong, even fibre, and the mixture is forced through a spinneret, which forms many fine, even strands that enter a setting solution to form cellulose filaments: also called rayon. The rayon is stretched to form a strong, evenly drawn out, washed, and then formed into rayon fabric.

This complex process results in a great deal of environmental pollution, inspiring a drive to clean up the industry. The rayon industry has also suffered from the development of cheaper artificial fabrics with a much shorter manufacturing process, such as nylon. Rayon is frequently blended with true synthetic fabrics for various applications, and it is advisable to follow individual care labels on rayon garments, as these blends have specific handling needs.

1.5.1.6: Alginate
Calcium alginate fibre is manufactured from sodium alginate extracted from seaweed. Capable of ion exchange with other metal ions, the fibre gels on contact with solutions of sodium salts and is highly absorbent of water and other fluids. The fibre can be dissolved in weak alkaline solutions. Calcium alginate fibre is available in the form of either continuous filaments or staple fibres. Staple fibres may be presented in the form of textile products such as sliver or fabrics. Fabrics may be of various construction and dimensions. Calcium alginate fibre is used as a material for use in wound-dressings and other wound-management products. In addition, it is also used in diagnostic swabs for microbiological sampling.

1.5.1.7: Hydrogel
Hydrogels are three-dimensional networks formed from hydrophilic homopolymers, copolymers or macromers (preformed macromolecular chains) crosslinked to form insoluble polymer matrices. These polymers, generally used above their glass transition temperature ($T_g$), are typically soft
and elastic due to their thermodynamic compatibility with water and have found use in many biomedical applications [3]. Synthetic monomers used in tissue engineering include, among others, include poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), and polyacrylates such as poly(2-hydroxyethyl methacrylate) (PHEMA). Biological hydrogels have been formed from agarose, alginate, chitosan, hyaluronan, fibrin and collagen, as well as many others [4, 5].

1.5.2 : Natural fibres

1.5.2.1 : Wool
Control of surface chemistry is an important factor in the wool-processing industry. Wool keratin fibres consist of a core of elongated cortical cells within an outer layer of overlapping cuticle cells, each of which is covered by an epicuticle membrane [66-68]. The latter is composed of proteinaceous material, which contains a high level of the amino acid cystine but with a significant proportion (ca. 25%) of the lipid 18-methyleicosanoic acid [69-73]. A schematic diagram of wool-fibre structure according to Leeder [69] is shown in Figure 1.13.
The fibres possess surface free energy, are hydrophobic in nature and are characterised by relatively high inter-fibre friction due to the angularity of the cuticle cell edges. These surface characteristics of wool are unattractive from a textile-processing viewpoint since dyes, softening agents and shrink-proof treatments are usually applied from aqueous solutions and therefore some degree of water wettability is necessary at the fibre surface [74].

Wool is comprised of a central assembly of elongated cortical cells, surrounded by flattened, overlapping cuticle cells. Each cuticle cell consists of an inner region of low-sulphur content (the endocuticle), a central sulphur-rich band (the exocuticle) and a thin 2–7 nm thick outer layer known as the epicuticle [75]. The epicuticle is believed to comprise an outer layer of lipids bonded to an underlying layer of cystine-rich proteins through a thioester linkage [76, 77]. The lipid layer is a methyl-branched 21-carbon fatty acid [70] that has been conclusively identified as 18-methyleicosanoic acid (18-MEA) [78, 79]. It forms a hydrophobic barrier that affects fibre properties such as adhesion and dye uptake. Treatment of the fibre with chemicals such as bases, oxidants or reducing agents removes the lipid layer, resulting in a hydrophilic surface [79, 80].

The wool-fibre surface remains hydrophobic even after repeated solvent extraction. Lindberg [81] reported that the hydrophobic surface could be altered under alcoholic caustic conditions. Kopke and Nilssen [82] suggested that the effect of alkali on wool could be explained if long-chain fatty acids were attached by ester linkages to the wool-fibre surface. Leeder and Rippon [73] treated wool with anhydrous potassium tertiary butoxide in the non-swelling solvent tertiary butanol, which was assumed to confine reaction to the fibre surface. They observed a dramatic reduction in the hydrophobicity of the fibre, which was attributed to the removal of the postulated lipid layer from the fibre surface. Evans et al. [70] showed that the major lipid component removed by alcoholic alkali treatment of wool was a methyl-branched 21-carbon fatty acid and suggested that it was bound to the fibre surface by an ester or thioester bond, as originally proposed by Kopke and Nilssen [82]. The model for the wool-fibre [79] surface proposed consists of a
protein matrix heavily acylated with 18-methyleicosanoic acid, subsequently described as the ‘lipid layer’, which orients away from the fibre and forms a hydrophobic surface. The covalently bound lipid layer has a critical role in determining surface properties of the wool fibre, which in turn are important in many aspects of wool processing. It is often necessary to modify the fibre surface, chemically or physically, to achieve the required end-use. The covalent and non-covalent bonds present in wool structure are shown in Figure 1.14.

Figure 1.14 : Types of covalent and non-covalent bonds in wool [83]

Another aspect of the wool fibre is its intrinsic absorbency property. The crystalline regions of the wool fibres are water impermeable; the amorphous matrix can absorb water up to 45% of its dry mass (for a crystallinity of 25%) without feeling wet [84]. Wool is hygroscopic and able to absorb and desorb large amounts of water as the relative humidity surrounding the fibre changes. The water is believed to be associated with specific chemical groups in the amorphous regions, with polar side groups and peptide groups of the protein chains considered to be the most important [83]. Moisture is drawn into the matrix with dissipation of heat, giving wool fibre a unique comfort-providing property [84].
Recent studies have focused on altering the surface properties of wool with a new class of solvent called Ionic Liquids (discussed later in this chapter in Section 1.9). Yuan [85] studied the effect of IL pretreatment on the dyeing behaviors of wool utilising an enzyme treatment. The results showed that 1-butyl,3-methyl imidazolium chloride (BMIMCl) greatly improves wool dyeability. In later research, Yuan [86] showed that BMIMCl successfully improves the wettability of wool for the hydrophilic modification of wool. BMIMCl is found to be an excellent solvent in the pretreatment step in protease processing. Xie et al. [87] dissolved wool fibre, chitin and chitosan using BMIMCl. Their studies revealed that the solvent is excellent for wool keratin and cellulose fibres and subsequent blending is possible. They also reported preparing membranes and composites using BMIMCl. Another study [88] showed that chitin/IL and chitosan/IL solutions are an efficient, reversible system for fixing carbon dioxide.

The use of BMIMCl has also been investigated within different textile fibre materials including regenerated cellulose [89-91] and bamboo fibres [92]. These attract interest for the development of renewable materials from natural bio-resources for various applications. The dissolution and regeneration of cellulose utilising BMIMCl is expected to become the focus of future developments. The current study utilises this methodology.

The moisture regain property of these fibres and polymers discussed above directly influences the absorbency of the product manufactured. A summary of the moisture regain of the above fibres from various sources are listed in Table 1.3 [93-95].
Table 1.3: Moisture regain property of fibres used in medical textiles

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Moisture regain %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td>0.4%</td>
</tr>
<tr>
<td>Polyamides</td>
<td>4.5%</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>0.05%</td>
</tr>
<tr>
<td>Viscose</td>
<td>11%</td>
</tr>
<tr>
<td>Cotton (mercerised)</td>
<td>8.5%</td>
</tr>
<tr>
<td>Wool (all forms)</td>
<td>13.5%</td>
</tr>
</tbody>
</table>

In addition to the fibres discussed above, other fibres such as cotton, and high-performance fibres such as para-aramid fibres, are also utilised in small quantities in the medical industry for specific end-uses. The above fibres and their properties are utilised in the manufacturing of technical textiles.

1.6: Manufacturing process of technical textiles

The manufacturing and finishing processes of the technical textiles and wound-dressings must be explored in order to determine at what stage of application the current study would fit. Figure 1.15 shows the manufacturing process for technical fabrics that are made of synthetic polymers such as polyesters (PET), polyamides (PA) and polypropylenes (PP). These polymers are processed and made into textiles or textile structures to facilitate the end-use.
In the filament form, the polymer undergoes different manufacturing processes to be finished as technical fabrics, threads or yarns. The medical textiles that are produced by weaving or knitting (both warp and weft) together with the new innovative modern process of 3D knitting undergo various finishing processes before final end-use.

The study so far has dealt with the definitions, scope and manufacturing processes of major fibres used in technical textiles, as well as medical textiles in general.

Recent years have shown vast evidence of research in the area of finishing of textiles to impart functional properties such as anti-odour or anti-microbial textiles for skin-care and so on. There is now an increasing trend for these kinds of finishes because they provide consumers with an added value to the textile products. With steady improvement of technology and application procedures, it has become easier to impart these kinds of value addition to
the consumable textiles. The finishing of textiles is one of the main factors that determine the desired effects for the ultimate consumer product and thus finishing is an essential step in manufacturing. The aesthetic value and the functional properties depend on the performance of the finish applied to the textile substrates.

1.7: Anti-microbial finishing

A variety of species of micro-organisms such as bacteria, fungi, mildew etc. can grow on the textile substrate provided that the substrate contains required nutrients for the micro-organisms to grow. These organisms not only cause undesired smell but also increase the chance of localised and systemic infection. The need to restrain the growth of microbes on the textile substrate and the wound led to the discovery of anti-microbial finishes. Figure 1.16 shows some common micro-organisms which are harmful for humans, as well as for textiles [97].

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive bacteria</td>
<td>Cloth damaging fungi</td>
</tr>
<tr>
<td>Staphylococcus aureus or pyogenes</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Aspergillus fumigatus</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>Trichoderma viride</td>
</tr>
<tr>
<td>diphtheroids</td>
<td>Curvularia lunata</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td>Penicillium species</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Crop damaging fungi</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Fusarium species</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>Rhizoctonia solani</td>
</tr>
<tr>
<td>Pseudomonas pyocyans</td>
<td>Sclerotium rolfsii</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td></td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.16: Some harmful micro-organisms [97]**

Previous studies have shown that these microorganisms can grow on textiles and may cause undesired odour or staining on the textile substrate [98]. To prevent infection, infestation of microbes, malodour and staining or discolouration of textiles, anti-microbial finishing is necessary. There are many anti-bacterial agents that can be incorporated onto textile substrates. These anti-bacterial agents are readily available in the market.
and are becoming more popular with consumers as they become more conscious about health and hygiene of textiles. Some commercially available anti-microbial agents are described in Table 1.4.

**Table 1.4: Commercial anti-microbial agents [99]**

<table>
<thead>
<tr>
<th>Anti-microbial agents</th>
<th>Chemical composition</th>
<th>Company</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanitized-AG</td>
<td>Halogenated phenoxy- and isothiazolinone derivates</td>
<td>Sanitized AG, Switzerland</td>
<td>-</td>
</tr>
<tr>
<td>Actigard</td>
<td>-</td>
<td>Clariant</td>
<td>For floor coverings</td>
</tr>
<tr>
<td>Reputex 20</td>
<td>PHMB</td>
<td>Zeneca Biosides</td>
<td>Durable for cotton</td>
</tr>
<tr>
<td>Sensil 555</td>
<td>-</td>
<td>Senka corporation, Japan</td>
<td>Anti-microbial and deodorant finish for cellulosics</td>
</tr>
<tr>
<td>Q-5700</td>
<td>Quaternary amine incorporated in saline Dow Corning, UK</td>
<td>Thomson Research Associates, Canada</td>
<td>-</td>
</tr>
<tr>
<td>Ultrafresh Range</td>
<td>-</td>
<td>Thomson Research Associates, Canada</td>
<td>Odour protection and anti-staining</td>
</tr>
<tr>
<td>Steri-septic Range</td>
<td>-</td>
<td>Thomson Research Associates, Canada</td>
<td>Cationic, anionic and nonionic are available for cotton and polyvinyl fibres</td>
</tr>
<tr>
<td>Hyfresh Range</td>
<td>-</td>
<td>Daiwa, Japan</td>
<td>-</td>
</tr>
<tr>
<td>Biosil</td>
<td>Organic silicones with tertiary ammonium compounds Toyobo, Japan</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Peach fresh</td>
<td>Tertiary ammonium compounds 3-(trimethoxysilyl propyl dimethyl octadecyl ammonium chloride) Nissihinbo, Japan</td>
<td>For polyester fibres and fabrics</td>
<td></td>
</tr>
<tr>
<td>Aegis microbe shield</td>
<td>Tertiary ammonium compounds PPT, UK</td>
<td>Combat growth candida and yeast that causes thrush</td>
<td></td>
</tr>
<tr>
<td>Sanitan</td>
<td>Tertiary ammonium compounds Kunary, Japan</td>
<td>For polyester fibres and fabrics</td>
<td></td>
</tr>
<tr>
<td>Tinosan Range</td>
<td>Tricosan based on 2,4,4'-trichloro-2'-hydroxylyl-diphenyl ether CIBA specialists chemicals, Switzerland</td>
<td>Durable treatment for cotton, polyester, polyamide, acrylic and their blends with cotton</td>
<td></td>
</tr>
</tbody>
</table>
All these antibacterial agents, when applied to textiles, work in different ways and their behaviour towards the micro-organisms varies from product to product. A live bacteria or fungus generally has a cell wall, which protects it, that is made of mainly polysaccharides. The cell wall or the membrane constrains the body of the micro-organism, which consists of other components such as a variety of enzymes and nucleic acids.Obviously, the cell wall maintains the metabolism of the cell and plays a vital role in maintaining the integrity of the particular microbe. Thus the mortality or growth of the cell depends to a great extent on maintaining the cell wall. Most of the antibacterial agents work under two main principles: inhibition of the growth of the cells (biostatic) and killing of the cell (biocidal). Almost all the commercial anti-microbial agents are biocides. They damage the cell wall or inhibit the metabolism of the cell by stopping nutrients from penetrating the cell; both are a necessity for the survival of the cell.

These anti-microbial agents are either metal or metal salts, quaternary ammonium compounds, polyhexamethylene biguanides (PHMB), triclosan (2,4,4’-trichloro-2’-hydroxydiphenyl ether), regenerable N-halamine and peroxyacid and some synthetic dyes [100]. Other than these a natural biopolymer called ‘chitosan’ has been the focus of recent research in anti-microbial treatment of textiles. In the present study, chitosan was used as an anti-microbial agent to incorporate this property into the developed wool and wool-blend substrates.

1.7.1 : Chitosan as an anti-microbial agent
Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) [100-102]. Chitosan is formed commercially by deacetylation of chitin, which is the compositional element in the exoskeleton of crustaceans such as crabs, shrimps, lobsters etc. Preparation of chitosan from chitin is given in Figure 1.17.
Chitosan has three reactive groups and they are primary (C–6) and secondary (C–3) hydroxyl (–OH) groups and the amino-NH₂ (C–2) group in each repeat of the de-acetylated unit of chitin. Thus it is poly-cationic in nature. The anti-microbial activity of chitosan and its derivatives has been well proven in past studies but the mechanism of the anti-microbial action is yet to be discovered. The most acceptable interpretation is that the anionic cell surface of the microbes interacts with the cationic chitosan, causing extensive cell surface alterations and damage. This leads to inhibition of the metabolism of the cell and results in its death [103]. So far it is considered that chitosan acts as a biocide for some microbes and as a biostatic for others.

The reaction mechanism of chitosan with wool is of keen interest as the current study explores application of chitosan on wool fibre. It has been reported that application of chitosan on fibres with hydroxyl and/or carboxylic acid groups on surface, such as cellulosic fibres, cellulose acetate, protein fibres (wool keratin), etc., permits the superficial creation of sheath coatings. The coatings tightly adhere to the entire core (fibre), due to the ionic interaction between chitosan and the fibre. They are invisible to the naked eye.
eye, and to optical and scanning electron microscopy (SEM) [104, 105]. However, metachromatism studies revealed they were optically uniform [104, 105]. Chitosan to wool binding is a result of ionic interactions, such as available carboxyl groups in wool forming salts with the free amino groups in chitosan and hydrogen bonding interactions between hydroxyl and amide groups of the wool with the hydroxyl groups of the chitosan. Since chitosan originally is a polyaminocarbohydrate, it is anticipated to have some of the interaction characteristics of both polysaccharides and proteins reason being it contains a polymer chain of substituted glucosidic residues as well as a high proportion of amino residues [106]. These closely supports finding from another study [107] using chitosan for treating woollen fabric.

1.7.2 : Factors affecting anti-microbial activity of chitosan

The anti-microbial activity of chitosan primarily depends on its molecular weight (MW) [108-115] and degree of de-acetylation (DD) [108, 116-118]. Lim [119] extensively summarised the effect of MW on the anti-microbial activity of chitosan as well as the effect of DD. According to Lim’s review, it is complicated to draw a clear interrelationship between the MW and anti-microbial activity of chitosan. Usually the higher the molecular weight of chitosan, the higher its anti-microbial activity. Interestingly enough, over a certain range of MW the anti-microbial activity decreases and in this case it also depends on the DD value of the chitosan used, but in terms of DD the correlation is much more proportional. As the DD increases, which means increase in amino (-NH₂) groups in each individual repeat unit of chitosan, the anti-microbial activity of chitosan increases. This suggests that the protonated amino groups increase with increasing percentage of DD, which evidently increases the chance of interaction between the positively charged chitosan and negatively charged cell wall of the micro-organisms. In addition, the pH [108, 120-122] and strains of bacteria used for testing [116, 123] play a vital role in the anti-microbial activity observed for chitosan.

Other than its anti-microbial activity, chitosan has been extensively studied for other applications such as photography, cosmetics, artificial skin,
dressings and wound healing, food and nutrition, waste-water treatment, dyeing and printing [124]. Chitosan also has been investigated for its capability of forming continuous films [125-128].

1.8: Evaluation of anti-microbial activity

There are several methods available for assessment of the anti-microbial activity of the treated textile substrates. These methods are mainly divided into two groups. The bulk samples are usually tested and evaluated with qualitative procedures to observe the anti-microbial activity, whereas confirmatory or quantitative tests define the anti-microbial activity with percentage reduction giving the efficacy of the anti-microbial agent assessed. The quantitative tests are more time-consuming and give a detailed assessment of the efficiency of the anti-microbial agent and are thus appropriate for a small number of samples. The available standard methods used to evaluate anti-microbial activity are given in Table 1.5.

Table 1.5: Different standard test methods for testing anti-microbial activity

<table>
<thead>
<tr>
<th>Agar diffusion tests</th>
<th>Suspension tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>AATCC TM 147</td>
<td>AATCC TM 100</td>
</tr>
<tr>
<td>JIS L 1902-2002</td>
<td>JIS L 1902-2002</td>
</tr>
<tr>
<td>SN 195920-1992</td>
<td>SN 195924-1992</td>
</tr>
</tbody>
</table>

Two bacterial species *Staphylococcus aureus* (Gram positive) and *Klebsiella pneumoniae* (Gram negative) are recommended in most of the test methods. Strains of these two bacteria, as shown in Figure 1.18, are used to evaluate qualitative and quantitative test methods.
a. *Klebsiella pneumoniae* [129]  

b. *Staphylococcus aureus* [130]

**Figure 1.18 Strains of a. Klebsiella pneumoniae and b. Staphylococcus aureus**

Both of these bacteria are pathogens and precarious for health, thus both require safe handling. Previous studies undertaken evaluating the anti-microbial properties used either the standard procedures or modification of the standard procedures. The modifications involved using different bacterial strains and exposure time and different media to grow the bacterial strains.

**1.9: Ionic Liquids (ILs) – a new class of solvents**

Moving to this new era of innovation, where process and manufacturing demands are emphasising the importance of recyclability, renewability and sustainability, the textile manufacturing industry is actively assessing emerging technologies that embrace the same. Consumers are now more conscious of the origin of garments and garment accessories than ever before. Organic renewable processes are encouraged and they obtain full acceptance when garments are involved least with environmental pollution. The chemical-processing industry in textiles has recently seen research booming into a much needed sector where traditional solvents, predominantly volatile organic compounds (VOCs) responsible for environmental pollution, are being replaced by ‘designer’ solvents called ‘Ionic Liquids’ (ILs). ILs used at room - temperature are often colourless and easy to handle and in addition, can be renewed and reused. The properties of the environmentally benign ILs can be customised according to the need of the process parameters and therefore they are called designer’ solvents. Natural and synthetic polymers are being resynthesised and regenerated.
with the aid of ILs, thus facilitating the exploration of exotic polymer blends and novel application areas. This section highlights recent advancements in textile processing involving ILs. The focus is on how this new class of solvents is pushing the boundaries of knowledge and revamping a broad spectrum of manufacturing industries.

The application of safe and environmentally - benign separation processes has an increasingly important role in the development of clean manufacturing processes and technologies.

Ionic Liquids are comprised entirely of ions. For example, molten potassium chloride is an Ionic Liquid, whereas a solution of potassium chloride in water (a polar solvent) is an ionic solution. Recently, substantial attention has been directed to the use of room temperature Ionic Liquids as solvents for industrial catalytic reactions, which include polymerisations, alkylations and acylations [131-134]. These advances appear to allow the controlled production of desired products from reactants with reduced waste generation through side reactions due to the ability of Ionic Liquids to suppress traditional dissolution phenomena.

1.9.1 : What are ILs?

By definition, ILs are low melting salts with melting points of less than 100°C. Similar to common salt, they consist of 100% cations and anions [135]. However, they are large-volume organic ions, whose low melting points are due to ‘softening’ of their crystal lattices. Due to their interesting dissolving properties for organic and inorganic compounds and polymers, they can replace conventional solvents in many applications. Furthermore, they have excellent stability and are neither volatile nor readily flammable, which gives them a versatile advantage when this class of compounds is used in production processes. The choice of cations and anions contained in an Ionic Liquid is crucial in tailoring its physical and chemical properties in order to meet the requirements of a given production process [90].

In 1934, Graenacher discovered that molten N-ethylpyridinium chloride, in the presence of nitrogen-containing bases, could be used to dissolve cellulose [136]. This was the first instance of cellulose dissolution utilising
ILs. However, this was thought to have little industrial application because the concept of ILs had not been developed at that time. Until recently, the value of dissolution of cellulose and other textile polymers with ionic liquids was being scrutinised based on the understanding of ionic liquids. ILs are non-volatile, non-flammable and thermally stable solvents [137] and as such offer very promising replacement for the traditional volatile organic solvents.

VOCs refer to any compound of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid etc. that participate significantly in photochemical reactions. The Environmental Protection Agency (EPA) defines ILs analytically as organic compounds that have melting points of less than 80°C [138]. Essentially any solvent that can evaporate at room temperature is a VOC candidate. Commonly used industrial solvents ranging from xylene and toluene to carcinogenic benzene are now regulated in the environment. Of particular concern are chlorinated solvents such as tri-chloroethylene and perchloroethylene, which are very useful for solubilising petroleum products and eliminating oil from surfaces, but are persistent environmental contaminants. These solvents have application in textiles as dry-cleaning agents and other treatments. Thus there are growing number of studies investigating alternative solvents that are not environmentally detrimental. ILs are a good candidate in this respect and their rapid emergence as alternative solvents has evolved them in a growing number of applications in the textiles and polymer industry.

An area of potential research in ‘green’ technologies is the application of neoteric solvents [139] including supercritical CO$_2$ [140-142] aqueous biphasic systems [143, 144] and ionic liquids (ILs) [139, 145-148]. ILs offer a highly solvating, yet non-coordinating medium in which a number of organic and inorganic solutes may be dissolved [134, 142]. In an ideal case, many ILs fulfil these requirements and are liquids over a wide temperature range (exceeding 300°C). Melting points as low as –96°C are known [134, 149, 150], thus the usable liquid range may cover that used for conventional synthetic chemistry and low temperature extractions.
1.9.2: How are Ionic Liquids synthesised?

ILs can be derived from a wide variety of complex compounds such as salts of imidazolium, ammonium, pyridinium, isoquinolinium, sulfonium, phosphonium and pyrrolidinium [151]. Ionic liquids based on mono-substituted to penta-substituted imidazolium ions are favourable species for investigation in textiles because of their air and water stability, their wide range of liquidity, the fact that they remain liquid at room temperature, and their relatively favourable viscosity and density characteristics. [152, 153].

This thesis reviews the developments and applications of one significant IL with application for textiles and polymers—specifically, 1-butyl-3methyl imidazolium chloride (BMIMCl) which has the chemical structure shown in Figure 1.19.

![Figure 1.19: Chemical structure of BMIMCl](image)

Figure 1.20 shows the fundamental reaction that takes place for the formation of imidazolium-based room-temperature IL. These reactions are conducted in an anaerobic atmosphere of nitrogen. 1-chlorobutane is added to a vigorously stirred solution of 1-methylimidazole in toluene at 0°C. The solution is heated to reflux at 110°C for 24 hours, after which it is placed in a freezer at -20°C for 12 hours. The toluene is decanted and the remaining viscous oil/semi-solid is re-crystallised from acetonitrile and subsequently recrystallised from ethylacetate to yield a white crystalline solid, which is then dried in vacuo to give BMIMCl. These process parameters might vary from process to process and may change to produce different levels of yield. There are other synthesis methods available [150, 154].
Figure 1.20: Preparation of the imidazolium-halide based ILs [155]

The normalised spectrum obtained from FTIR spectroscopy of BMIMCl is shown in Figure 1.21. The functional groups present and their respective wave numbers (cm\(^{-1}\)) and intensities (%T) confirm the chemical structures given in Figure 1.21. The peak stretching at wave number 3385 (cm\(^{-1}\)) correspond to N–H (2° amines) bonding. The wave numbers from 3138 (cm\(^{-1}\)) to 2872 (cm\(^{-1}\)) refer to O–H stretching. The bending at 1643 (cm\(^{-1}\)) is indicative of C=N and C=C bonding and peak ranging between 696 (cm\(^{-1}\)) to 1567 (cm\(^{-1}\)) correspond to stretching C–C, C–O or C–N bonds.

Figure 1.21 : FTIR spectrum of BMIMCl

1.9.3 : The solvation dynamics

The solvation dynamics [156-159] of BMIMCl and other ILs depend on a number of factors. The solvation model of Abraham is given in Equation 1.
\[ \log k = c + r R_2 + s \pi_2^H + a \alpha_2^H + b \beta_2^H + l \log L^{16} \]  
(Equation 1)

where

- \( R_2 \) is excess molar refraction calculated from the solute’s refractive index.
- \( \pi_2^H \) is solute dipolarity/polarisability.
- \( \alpha_2^H \) is hydrogen bond acidity.
- \( \beta_2^H \) is hydrogen bond basicity.
- \( L^{16} \) is solute gas hexadecane partition coefficient at 298 K.
- \( \log L \) is Ostwald solubility coefficient.
- \( \log K \) is gas liquid partition coefficient.
- \( \log Vg/\log k \) is specific retention volume at a given column temperature or adjusted relative retention time.
- \( r \) is a parameter quantifying the ability of IL to interact with \( \pi \)- and \( n \)-electrons of the solute.
- \( s \) measures the dipolarity/polarisability of the IL.
- \( a \) is a parameter that defines the BMIMCl hydrogen bond basicity.
  (acidic solutes will interact with a basic phase)
- \( b \) is a measure of the hydrogen bond acidity.
- \( l \) describes dispersion forces and indicates how well the IL will separate homologues in any homologous series (e.g., \( n \)-alkanes).

This equation is a linear free-energy relationship that describes the solvation process of a solute as occurring in three stages:

1. A cavity of suitable size is created in the solvent (IL).
2. The solvent molecules reorganise around the cavity; and
3. The solute is introduced into the cavity and the various solute-solvent interactions take place.

Each solute molecule will possess somewhat different solute-solvent interactions due to various acidic, basic, electron-donating, electron-withdrawing and aromatic functional groups. Collectively, the numerical magnitude of each interaction parameter describes the importance of the
individual interactions and thereby characterises the IL. The solvation model uses many solute probe molecules in conjunction with inverse gas chromatography to quantitatively determine the importance of different room-temperature IL interactions as a function of temperature [156]. These results are especially useful in explaining different solvent behaviour between broad classes of room-temperature ILs.

1.9.4 : Recent developments using ILs

A number of studies have been undertaken using Ionic Liquids for textiles and polymer synthesis. These studies are predominantly based on using BMIMCl for dissolution and regeneration of natural protein and cellulosic fibres. Xie et al. [87, 160] first dissolved wool fibre, chitin and chitosan using BMIMCl. Their studies indicate BMIMCl to be an excellent solvent for wool keratin and cellulose fibres, and subsequent blending (in solution form) is possible. They also reported preparing membranes and composites using the abovementioned polymers. The latter study [160] reports dissolution of chitin and chitosan in BMIMCl. In addition, it was demonstrated that, chitin/IL and chitosan/IL solutions are an efficient, reversible system for fixing carbon dioxide.

Hameed et al. [161] reported dissolution and regeneration of wool and cellulose acetate (CA) in BMIMCl. They showed that the CA-rich blend with wool was homogeneous, increased glass transition temperature ($T_g$) and had thermal stability. In another study, Hameed et al. [162] prepared wool/cellulose blend films in BMIMCl coagulated with water. The blending with cellulose contributed significantly to an improvement in thermal stability of the wool and the blends had higher thermal stability as compared to the individual components while retaining the mechanical properties of the plain coagulated wool and cellulose. The enhanced thermal stability and mechanical properties were reportedly due to the intermolecular hydrogen bonding between the components.

BMIMCl was employed as a pretreatment step in protease processing for the hydrophilic modification of wool. The effect of BMIMCl pretreatment
combined with the protease process revealed significant improvement in the efficiency of protease processing. The wettability of wool samples after the combined treatments was improved [163, 164].

Yuan et al. [165] claimed to improve the dyeability of wool using BMIMCl. The physical and chemical properties of the IL-treated wool such as surface morphology, wettability and tensile strength were analysed in addition to the dyeing properties. Critical factors assessed were dyeing rate, dyeing exhaustion at equilibrium, colour depth and colour fastness. The tensile strength of IL-treated wool fibres was slightly decreased when the treatment temperature was less than 100°C. Dyeing kinetics experiments revealed that the IL treatments greatly enhanced initial dyeing rate and shortened half-dyeing time and time to reach dyeing equilibrium. The final exhaustion and colour depth of IL-treated wool were also increased, accompanied by slightly decreased colour fastness.

The development trends in regenerated cellulose fibres are being investigated worldwide. There have been a number of studies reporting dissolution and regeneration of cellulose utilising BMIMCl [90, 135, 166-181]. This method enables the comprehensive use of cellulose by combining two key green chemistry principles: (a) using environmentally benign solvents and (b) to promoting bio-renewable feed-stocks. These properties are useful for the development of renewable materials from natural bio-resources for various applications. The properties such as structural characteristics and application of high-wet modulus in viscose, lyocell, acetate, regenerated cellulose fibres from straws, and regenerated bast fibres have been recently introduced using ILs [182]. It should be noted that the regenerated cellulose fibres with added functional characteristics will become the focus of the future development.

Bamboo fibres have been reportedly dissolved in IL [183]. After dissolution in ionic liquids, bamboo fibres were regenerated with reconstituted solvents such as acetone/water. The regenerated material was more homogenous in microstructure and the fibrous nature of the material disappeared after
dissolution. The crystalline structure of cellulosic bamboo fibre was reported to be decreased after dissolution and regeneration. In addition, it has been suggested that ILs will be the future solvent to synthesise bio-mass such as wood to provide alternative important chemicals, bio-fuel and bio-materials [184]. For a never-ending energy demand in todays world, this alternative solvent system will be investigated further.

Nanofibrillar aerogels have been prepared from cellulose, spruce wood and mixtures of cellulose, lignin and xylan [185]. In this investigation, the lignocellulosic polymers were first dissolved in an ionic liquid and coagulated from solution by adding aqueous ethanol. The resultant formation of the gel was washed with ethanol and liquid carbon dioxide and finally dried by releasing the carbon dioxide from the porous structure at supercritical temperature to obtain the aerogel [185].

It has been possible to electrospin non-derivatised chitosan/cellulose composite fibres from 1-ethyl- 3-methylimidazolium acetate IL [186]. This chitosan–cellulose hybrid film could be useful as an antibacterial reagent for wounds where anti-microbial activity is essential. Wool and wool blends are also being assessed for pre-medicated wound-dressings [187].

ILs provides the possibility of comprehensive utilisation of lingo-cellulosic materials by fractionation in an environmentally-friendly manner. Besides, it enables preparation of various advanced materials, including cellulose derivatives and cellulose composites, which may be a viable alternative to many synthetic polymers that biodegrade slowly. Some of these abovementioned cellulose derivatives and cellulose composites have great potential in industrial applications. For example, the wool/cellulose composite could be used in the textile industry to produce fibrous matrices. This technology also has the potential to revolutionise wool processing by elimination of chlorine-based chemical feed-stocks and reduce dependence on non-renewable resources. Commercialisation of these processes will provide ‘green’ sustainable platforms for other manufacturing industries to embrace this technology in the foreseeable future.
The dissolution of cellulose and other polymers using ionic liquids has provided a new platform for ‘green’ polymer utilisation in the textile industry. A new avenue has been created by ionic liquids for solving the environmental and energy problems that are burning issues around the world. To maintain sustainable development, ILs provide greater flexibility and clean technology in polymer synthesis. In the near future, innovative fibre and fibre blends and composites using ionic liquids will emerge as a result of ongoing research activities. It is quite clear that commercialisation of these processes will benefit the textile and polymer processing industries significantly.

1.10 : Electrospinning

Electrospinning is the process of producing fibrous structures in the nanometer range by subjecting a fluid jet to a high electric field [188]. The origin of electrospinning can be traced back to 1934, when Formhals patented his first invention relating to the process and the apparatus for producing artificial filaments using electric charges [189, 190]. Electrospun nanofibres have very large surface area-to-volume ratio and porous structure as compared to conventional fibres. For this reason, these fibres can be used in adsorption of chemicals and for military and civilian filtration [191]. A wide range of fabric properties such as strength, thickness, weight, porosity and surface functionality can be achieved depending on the specific polymer being used.

1.10.1 : Electrospinning process

Electrospinning is based on the principle of the effect of electrostatic force on liquids i.e. when a suitably electrically charged material is brought near to a droplet of liquid held in a fine capillary, it can form a cone shape (Taylor cone) and small droplets are ejected from the tip of the cone if the charge density is very high.

The basic setup used for electrospinning (Figure1.22) consists of three major components, namely: high voltage power supply, syringe and collector.
1.10.2: High-voltage power supply
Generally, very high DC voltage (usually in the range of 10–30 kV) \[192\] is used as the source of power supply for electrospinning, although there is feasibility of using an AC power source. Free charges are induced in the polymer solution through an immersed electrode. The charged ions of the polymer solution move in response to the external applied electric field towards the collector of opposite polarity.

1.10.3: Syringe
The syringe or pipette is a very fine capillary tube that controls the release of the polymer solution or melt into which a metal electrode is inserted. It is mounted horizontally or vertically on an adjustable electrically insulating stand. A spinneret is connected to the syringe at one end for the production of nanofibres. During the electrospinning process, a syringe pump is used to supply the polymer at a constant and controllable rate.

1.10.4: Collector
The collector or the collecting surface (which may be stationary or moving) is used to collect the electrospun fibres. Surfaces of different geometry and configurations are used to alter the alignment of the nanofibres. The collector is mounted on an insulating stand so that its potential can be controlled.
1.10.5: Operation of the spinneret

The polymer solution held in the syringe is electrified by the application of the very high voltage. When an external electric field is applied to the end of the capillary tube, a charge is induced on the surface of the solution. Now the polymer drop which is held by its surface tension at the tip of the spinneret, experiences an electrostatic repulsion force between the surface charges and the coulombic force exerted by the external electric field. As the electric field intensity is increased, the repulsion force is increased which distorts the hemispherical liquid drop into a conical object commonly known as a Taylor cone.

When the electric field reaches a threshold value, the repulsive electrical force overcomes the surface tension and a charged jet of the solution is ejected from the tip of the Taylor cone. As the jet travels in the chamber away from the spinneret, the solvent is evaporated and the diameter of the re-solidifying polymer is greatly reduced to nanometer size [189]. The charged jet is attracted by the grounded collector plate and is collected randomly on its surface as a nonwoven mat.

1.10.6: Factors affecting electrospinning process

The structure and properties of the electrospun nanofibres is influenced by many factors which are discussed in this section.

1.10.6.1: Solution viscosity and polymer concentration

Solution viscosity is one of the most important parameters affecting the diameter and morphology of the electrospun fibres. The amount of the polymer dissolved in the solvent determines the concentration and viscosity of the solution. For successful electrospinning there are upper and lower limits of polymer concentration and viscosity [193]. Below a threshold viscosity value, the jet breaks up and defects such as beads and droplets may be observed [194, 195]. Electrospinning of polyethylene oxide (PEO), polyacrylonitrile (PAN) [196] and poly-DL-lactic acid (PDLA) [197] is difficult below certain concentration levels. Demir et al. [194] used polyurethane in electrospinning and observed that the fibre diameter increases as the third power of solution concentration.
1.10.6.2 Solution surface tension
Surface tension is an influencing factor in the formation of beads. Bognitzki et al. [197] obtained fibres with an average diameter of 1 µm during the electrospinning of polylactic acid (PLA). By using tetraethyl benzylammonium chloride (TEBAC), they found that the diameter was decreased because of the increased surface tension and electrical conductivity that TEBAC exhibits.

1.10.6.3 Solution conductivity
The net charge density of the solution is mainly affected by the applied electrostatic field and the conductivity of the solution. The diameter of the filaments increases with the decrease in net charge density, which makes the charge repulsion force smaller. Baumgarten [198] determined that the jet radius varied inversely to the cube root of the electrical conductivity of the solution. Zong et al. [199] investigated that addition of ionic salts such as KH₂PO₄, NaH₂PO₄ and NaCl to PDLA produces beadless fibre with a relatively smaller diameter.

1.10.6.4 Molecular weight of polymer
The rheological and electrical properties of polymers such as viscosity, surface tension, conductivity and dielectric strength are affected by the molecular weight [199]. The formation of beads when using polymers with too-low a molecular weight and fibres with larger average diameter when using high molecular weight polymers have been reported [200].

Geng et al. [201] investigated that electrospun fibres from lower molecular weight chitosan solution were fragile and usually contained large size beads, while those from higher molecular weight chitosan solutions were rougher, finer and with some bead defects. Gupta et al. [202] used polymethyl methacrylate (PMMA) in electrospinning and observed that as the molecular weight increased, the number of beads were reduced. In addition, uniform fibres were obtained at a lower concentration and with a narrow molecular weight distribution.
1.10.6.5: Volatility of solvent
The nanofibre diameter depends on the solvent evaporation rate or volatility, which in turn is affected by solvent vapour pressure. The electrospinning of poly-L-lactic acid (PLLA) with a highly volatile solvent such as dichloromethane produced nanofibres with pore sizes of 100 nm in width and 250 nm in length along the fibre axis [197].

1.10.6.6: Applied voltage/electric field strength
The external electric field is an important factor governing the morphology and diameter of the electrospun fibres. Shin et al. [203] observed that with the increase in electric field, the polymer jet thins more rapidly and the Taylor cone region becomes shorter and more concave in profile so that the diameter of nanofibres decreases [204]. These observations were borne out also by the work of Deitzel et al. [205].

1.10.6.7: Polymer flow rate
It is observed that, at very high flow rate, more beads are formed, as the jet does not get enough time to fully evaporate the solvent [206]. Also at higher flow rates the deposition pattern of the fibres become more random instead of regular and their diameter increases. This is because the jet is attracted with less charge density towards the collector. At lower flow rates, the jet gets sufficient time for the solvent evaporation and resolidifying of the polymer and its diameter decreases. However, the fibre formation is inconsistent [207]. Megelski et al. [208] observed that the diameter of polystyrene fibre and the pore size increased with an increase in the polymer flow rate.

1.10.6.8: Collector distance and geometry
It has been observed that there is a decrease of the jet diameter by a factor of 5 at a distance of 10 mm from the tip of the cone, indicating a large amount of stretching [209]. Beyond a certain distance, the jet becomes extremely thin and unsteady, leading to the formation of individual and disconnected beads. Lou et al. [210] observed that, when the tip to collector distance is less than 10 cm, the same electrical field cannot properly form the fibres because the solvent is not completely evaporated before the polymer stream reaches the collector.
Several researchers have experimented to align the fibres during electrospinning. Fong et al. [211] electrospun aligned yarns of nylon 6 by rapidly oscillating a grounded frame within the jet. Dersch et al. [212] used a metal frame as a collector to produce well oriented parallel arrays of polyamide nanofibres with an average diameter of 50 nm. Matthews et al. [207] used a rotating mandrel as the collector. Smit et al. [213] used a thin rotating wheel for generating well aligned electrospun nanofibres.

1.10.6.9: Take-up speed
Matthews et al. [207] investigated that a random matrix of collagen fibrils are formed at a rotating mandrel speed of less than 500 rpm whereas when increased to 4500, the fibrils were regularly deposited. Kim et al. [214] investigated that the amount of amorphous region increased with the rotational speed of the mandrel, as higher speeds lead to rapid solidification and deposition of the fibres. They also investigated that there is more regular alignment of fibres at higher speeds.

1.10.6.10: Ambient parameters
The atmospheric conditions such as temperature, relative humidity, vacuum conditions and surrounding gas influence the morphology of electrospun fibres. Higher room temperatures make the electrospinning process quicker and more uniform fibre is produced. Baumgarten [198] observed that at a relative humidity of more than 60%, the electrospun acrylic nanofibres do not dry properly and get entangled on the collector surface. The pore characteristics of polystyrene fibres at varied relative humidity have been studied by Megelski et al. [208] and Casper et al. [215]. It was observed by them that at lower humidity, smooth fibres were produced.

1.10.7: Properties of nanofibres
Electrospun nanofibres possess noticeable differences in their thermal, mechanical and electrical properties as compared to normal fibres. This section highlights the properties of nanofibres.
1.10.7.1: Thermal properties
The thermal properties of nanofibres can be analysed by differential scanning calorimetry (DSC). Zong et al. [199] observed that electrospun nanofibres of PLLA possess lower crystallinity, melting temperature ($T_m$) and glass transition temperature ($T_g$) than semi-crystalline PLLA resins. The low crystallinity can be attributed to the high rate of evaporation and rapid solidification before their collection onto the collector. The decrease in $T_g$ and $T_m$ is due to the large surface area-to-volume ratio of nanofibres. The lower heat of fusion and melting temperature of PEO nanofibres as compared to the PEO powder was attributed to the decreased crystallinity after electrospinning [205].

1.10.7.2: Mechanical properties
The mechanical properties of nanofibres such as tensile strength, elongation and modulus are affected by the surface morphology, pore size and distribution. The tensile strength of polyvinyl alcohol (PVA) fibre aggregate was found to increase with the increasing weight percentage of glyoxal to PVA while the elongation decreased [216]. Dabirian et al. [217] measured the mechanical properties of the yarn and observed that the yarn treated with boiling water under tension showed higher strength and lower strain, which is because of the increase in degree of crystallinity in the treated samples. Fornes et al. [218] observed that the stiffness of nano-composites increased substantially by addition of organo-clays such as montmorillonite.

1.10.7.3: Electrical properties
The electrospun nanofibres containing carbon nanotubes have superior electrical properties (high energy densities and low driving voltages) [219]. The nano-composites of organically modified clay exhibit ionic conductivity that is several orders of magnitude higher than that of the corresponding clay. The intercalating of electro-active polymers into clay minerals can further improve the conductivity [220].

1.10.8: Applications of nanofibres
Nanofibres with high surface area and numerous pores have enormous applications in tissue scaffolds, nano-composites, protective clothing, filtration, electronics and protective clothing.
1.10.8.1: Biomedical applications

Yoshimoto et al. [221] used the biodegradable poly-caprolactone (PCL) in electrospinning and found that the PCL scaffolds provide an environment that supports mineralised tissue formation and may be used for the treatment of bone defects. The degummed silk fibroin nanofibre nonwovens were applied for wound-dressing and found to be favourable for cell attachment, growth and proliferation [221]. Also the biomedical applications of nanofibres for wound-dressing [222] and scaffolds for tissue engineering [223] have been studied.

Matthews et al. [207] produced scaffolds composed of collagen nanofibres and found that the structural properties varied with the tissue of origin, the form and the concentration of the collagen solution. Gibson et al. [224] produced electrospun fibres containing pH-adjusting compounds for use in wound-dressing or for protection from contamination. Electrospun fibre mats were produced from PLA, polyethylene co-vinyl acetate (PEVA) and their blend (50:50) and the potential of the mats were also explored as drug delivery vehicles using tetracycline hydrochloride as a model drug [225]. Huang et al. [226] produced collagen-containing nanofibres and nonwoven fabrics that have potential application in wound healing, tissue engineering and as hemostatic agents.

1.10.8.2: Protective textile materials

For protective clothing applications, nanofibre webs can be directly applied to garment systems [227]. The US Army Natick Soldier Center investigated the potential of nanofibre webs in protective clothing. It was found that nanofibre webs of nylon 6,6, polybenzimidazole, polyacrylonitrile and polyurethane provided good aerosol particle protection, without any change in their moisture vapour transfer properties [228]. Protective clothing for agricultural workers was developed by using electrospun polypropylene webs and laminates produced via melt-electrospinning [229].

Protective garments that reduce soldiers' risk of chemical exposure have been designed from electrospun nanofibres [230]. The layered composite
materials were incorporated with electrospun nanofibres and utilised as protective clothing [231]. Gibson et al. [232] applied nanofibres coatings directly to a polyurethane foam containing activated carbon and suggested that this can be applied as a component for military chemical protective clothing systems.

1.10.9: Limitations and future scopes
The major concern of electrospinning that is yet to be resolved is the commercialisation of the process and enhancement of the productivity. The productivity of electrospinning is very low (10 µl/min–10 ml/min for a single jet [228] and 22.5 ml/min for a nine needle multiple jet) [233] and its production rate needs to be increased multiple times [234]. There is a need to explore the scope of other polymers, their property characterisation and applications. The various factors affecting the morphology and properties of nanofibres also need to be established. The production of various types of novel surfaces (such as porous, hollow, core/sheath and with special features) and their suitability in different applications need to be investigated [235, 236].

An adaptation of electrospinning, named electrospraying, used in automobile paint industry has been of unique interest, as it can be used in an electrospinning setup to achieve a platform for coating textile substrates. Electrospraying is a method of generating a fine mist or aerosol of a specific precursor material using electrostatic charging. When this precursor material (in dissolved form or as a liquid of particular viscosity) passes through a needle, fine droplets are generated by electrically charging the liquid to a very high voltage. Because of the identical electrical charge, these droplets repel each other very strongly. At the tip of the needle, the liquid becomes unstable as it is forced to hold more and more charge. When the liquid can hold no more electrical charge, it disperses into numerous, micron-sized, highly charged droplets at the tip of the needle, as shown in Figure 1.23. In general, these tiny droplets fly about searching for a potential surface on which to land. This surface has an opposite charge to the droplets of the
material. As the droplets fly about, they shrink rapidly as solvent molecules evaporate from their surface.

![Schematic diagram of electrospraying](image)

**Figure 1.23: Schematic diagram of electrospraying [237]**

An electrospraying technique was applied to generate nano-spheres of chitosan for drug-delivery purposes [238]. A study [239] reported that a combination of controlled electrospraying and subsequent freeze drying can produce a fibrous chitosan 3D–network structure from low concentration chitosan solutions. Nanoparticle suspensions of chitosan were first fabricated by electrospraying and then freeze-drying assembled the nanoparticles into fibrous networks. Porous chitosan nanoparticles and cross-linked chitosan capsules for drug delivery were also reported [240, 241]. Solid micro and nanoparticles were prepared from chitosan/acetic acid solution in one step by the electrospraying method [242]. Production of thin films of LiMn$_2$O$_4$ was also reported using electrospraying techniques [243]. To date no study has been conducted to evaluate the coating technique utilising electrospraying techniques onto textile substrates. Utilising this technique for textiles coating has been very recent [244-246] and the current study reports the electrospraying of chitosan to coat nonwoven wool substrates and evaluates the anti-microbial properties of the coated substrates [247].

### 1.11 Silver nanoparticles

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nano-scale level [248]. Nano-materials often show exclusive and
noticeably altered physical, chemical and biological properties compared to their macro-scaled counterparts. One of the most studied nano-materials is nano-silver or silver nanoparticles (SNPs). Since ancient times, among various anti-microbial agents, silver has been most extensively studied and used to fight against infections and prevent spoilage [249]. At present, many studies have been focused on anti-bacterial and multi-functional properties of silver nanoparticles [249-256].

1.11.1: Synthesis of SNPs
SNPs can be synthesised in a wide variety of approaches [250, 253]. A processing chart is shown in Table 1.6.

<table>
<thead>
<tr>
<th>Synthesis of SNP</th>
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<tbody>
<tr>
<td>Physical</td>
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<td>Colloids</td>
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1.11.2: Mechanism of silver action on microbes
The exact mechanism of action of silver on microbes is yet to be established. However, the probable mechanism of action of metallic silver, silver ions and silver nanoparticles has been suggested according to the morphological and structural changes found in the bacterial cells. Silver nanoparticles show efficient anti-microbial properties compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulphur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus-containing compounds such as DNA. When silver nanoparticles enter the bacterial cell, it forms a low molecular weight region in the centre of the bacteria to which the bacteria conglomerates, thus protecting the DNA from the silver ions. The
nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity [257-261].

1.11.3: Effect of size and shape on the anti-microbial activity of nanoparticles

The surface plasmon-resonance plays a major role in the determination of optical absorption spectra of metal nanoparticles, which shifts to a longer wavelength with increase in particle size. The size of the nanoparticle implies that it has a large surface area to come in contact with the bacterial cells and hence, it will have a higher percentage of interaction than bigger particles [261-264]. Nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects, which enhance the reactivity of nanoparticles. Thus, it is corroborated that the bactericidal effect of silver nanoparticles is size dependent [261, 265]. The anti-microbial efficacy of nanoparticles also depends on the shapes of the nanoparticles. This can be confirmed by studying the inhibition of bacterial growth by differentially shaped nanoparticles [261]. According to Pal [264], truncated triangular nanoparticles show bacterial inhibition with a silver content of 1μg, while in the case of spherical nanoparticles a total silver content of 12.5 μg is needed. The rod-shaped particles need a total of 50 to 100 μg of silver content. Thus, silver nanoparticles with different shapes have different effects on bacterial cells.

The discussion above has dealt with currently utilised anti-microbial finishing agents such as chitosan and silver nanoparticles, and ionic liquid and its application in textiles, together with the evaluation of anti-microbial activity, were discussed. Chapter 2 discusses further the research concept and hypothesis made for the present study.
Chapter 2: Research concept and hypothesis

This study aims to investigate the use of wool and wool-blend textile substrates for application in medical textiles, focusing on wound-dressing and wound-care products. It is hypothesized that, since wool fibres provide excellent moisture absorbency properties, and since natural biopolymer chitosan (CHT) or nano materials (silver nanoparticles) provide inherent antimicrobial attributes, when the two are combined, an innovative wound-care product can be produced that should perform better than the current commercial wound care products. Based on the background literature, the current research intends to use novel techniques and application methods to develop the advanced wound-care products and to then characterise their properties and performance.

Three different routes of processing were chosen for the current study:

- pad-dry-cure of developed nonwoven substrates
- electrospraying on the developed nonwoven substrates; and
- dissolution and regeneration of polymers and preparation of blends

2.1: Pad-dry-cure process

In this study, Pad-dry-cure method was used to apply chitosan (CHT) onto the developed nonwoven wool and wool–viscose matrices, which can be used as a contact layer or an absorbent layer in a wound-dressing material. Padding is the most common method for applying chemical formulations onto textile substrates involving continuous processing and it is well known for its economical use of water, energy and time [266]. The padding technique encompasses contacting the substrate with the formulation, generally by immersion and subsequent removal of the excess formulation with squeeze rollers [267, 268]. Generally, a sequence of pad-dry-cure is followed for textile finishing. The proposed dressing material is schematically shown in Figure 2.1.
Figure 2.1: Schematic diagram of the proposed wound-dressing material

The flow of liquids is illustrated in Figure 2.2. As can be seen from the figure, the flow of the liquid, particularly the exudate from the wound, is important as the absorbent core should have maximum absorbency but the liquid, once absorbed, should not flow outwards (to leak through the external covering) or back inwards (carrying contaminants) through the skin-side of the dressing.

Figure 2.2 : Schematic diagram of the proposed wound-dressing material
2.2 : Electrospraying

The current study involves electrospraying of chitosan to coat wool nonwoven substrate for wound-dressing. The coated surface should possess antimicrobial activity and should perform as a wound contact layer. Electrospraying is a method of generating a fine mist or aerosol of intended specific precursor material using electrostatic charging. When this precursor material (in dissolved form or a liquid of particular viscosity), in this study CHT, passes through a needle, fine droplets are generated by electrically charging the liquid to a very high voltage. Because of the identical electrical charge, these droplets of CHT very strongly repel each other. At the tip of the needle, the liquid CHT becomes unstable as it is forced to hold more and more charge. When the CHT solution cannot hold any more electrical charge, it disperses into numerous, micron-sized, highly charged droplets at the tip of the needle, as shown in Figure 2.3.

![Figure 2.3: Schematic diagram of the electrospraying process](image)

A slight modification of the typical setup of the electrospraying unit was done to accommodate the wool sample to be mounted onto the collector plate, as schematically shown in Figure 2.3. In general, these tiny droplets of chitosan fly about searching for a potential surface in which to land and land onto the wool substrate and coat the surface.
2.3: Dissolution and regeneration of polymers and preparation of blends

Chitin is the structural component of the shell of crustaceans and the second most abundant natural polymeric material. Chitosan can be obtained from deacetylation of chitin. Natural wool is another abundant biopolymer, being the fibre that insulates and protects sheep from extreme environmental conditions. It has been proposed that the structure of wool contains about 18 amino acids linked together in ladder-like polypeptide chains \([269, 270]\). The insulating and moisture absorbing properties of wool make it into extremely comfortable textiles. Both CHT and wool are renewable, bio-degradable and bio-compatible, but are complicated to process by directly dissolving in common solvents. This is due to the molecular close chain packing and the various inter and intra-molecular hydrogen bonds \([87]\). It is well established that blending is a convenient technique for developing new polymeric materials with enhanced functional attributes. The extent of intermolecular association plays a vital role in the context of phase behaviour and the properties of polymer blends. The existence of favourable intermolecular interactions between two polymers can promote their miscibility and in addition have a significant effect on the properties of the blends. For functional applications and chemical modifications, it is essential to form a stable homogeneous wool or chitosan solution in order to improve the efficiency of the application. BMIMCl has provided an ideal platform to solubilise polymers to achieve the blending operation. The current study blends wool and chitosan utilising BMIMCl as a common solvent and Figure 2.4 illustrates the schematic diagram of the processing route.
Figure 2.4: Schematic diagram of the proposed wound-dressing material containing wool–CHT blend material

In addition, dissolving wool in BMIMCl provides further opportunities to add additional functional properties. The current study then explores the addition of silver nanoparticles (SNPs) at the dissolved phase of wool and the preparation of regenerated wool films doped with SNPs. Figure 2.5 illustrates the processing route for preparation of these films.
Figure 2.5: Schematic diagram of the proposed wound-dressing containing wool matrix doped with silver nano particles

The developed novel materials are characterised and evaluated for their performance for potential end-use as wound-dressing materials. The methods and experimental setup are discussed in detail in Chapter 3.
Chapter 3: Experimental design and methodology

Chapter 3 discusses the experimental design and methodology followed in the current study in detail. For clarity of the experimental design, all the experiments are broken down into capsules and the methodologies followed in the next section (Section 3.2)

3.1 : Experimental design

3.1.1 : Experimental capsule 1

The flow diagram of experimental capsule 1 is given in Figure 3.1. As discussed in Chapter 2 in the hypothesis, 100% nonwoven wool was developed by a card-crosslapper and subsequent needle punching (400 GSM). The developed nonwoven was treated with BMIMCl at 120°C for 30 minutes. The objective was to get rid of the surface lipid layer and functionalise the wool fibre surface for application of CHT. The sample was then rinsed repeatedly in distilled water to wash off the BMIMCl and dried.

![Figure 3.1: Flow diagram of experimental capsule 1](image-url)
The dried sample was then padded with a series of CHT concentrations (0.3%, 0.5%, 0.75% and 1%) on weight of material (OWM). The developed samples were then dried again and cured. These 100 % wool nonwoven samples were then characterised for morphological and structural changes using Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red (FTIR) spectroscopy and tested for water absorbency and antimicrobial properties.

3.1.2 : Experimental capsule 2

Analysing the results from capsule 1, it was found that BMIMCl pretreatment causes the significant weight reduction of the treated substrates which negatively influences the absorbency property of the developed substrates.

The second capsule involved preparation of nonwoven wool (150 GSM) and treatment with CHT concentration of 0.1%, 0.3%, 0.5% and 1.0% (OWM) by padding, followed by drying and curing, as shown in Figure 3.2.

<table>
<thead>
<tr>
<th>Preparation of wool nonwoven</th>
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<tbody>
<tr>
<td>Card-crosslapper and needle punching</td>
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</table>

<table>
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<tr>
<th>Chitosan treatment</th>
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<tbody>
<tr>
<td>Concentrations of 0.1%, 0.3%, 0.5% and 1.0% (w/w)</td>
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<table>
<thead>
<tr>
<th>Finishing</th>
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<tbody>
<tr>
<td>Drying and curing of CHT-treated substrates (130°C for 5 minutes)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Characterisation of the developed substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM, FTIR, absorbency, anti-microbial properties</td>
</tr>
</tbody>
</table>

**Figure 3.2: Flow diagram of experimental capsule 2**

After curing, the nonwoven samples were then characterised for morphological and structural changes using Scanning Electron Microscopy.
(SEM) and Fourier Transform Infra-Red (FTIR) spectroscopy and tested for blood absorbency and anti-microbial properties.

3.1.3 : Experimental capsule 3

The third capsule involved blends of wool–viscose nonwoven preparation in various ratios. Samples containing 50-50 wool–viscose and 100% viscose (150 GSM) nonwoven materials were prepared by needle punching. The objective was to increase the wicking time as well as facilitate chitosan-cellulose (viscose) interaction. However, 100% viscose was not tested any further as the wet strength viscose was found to be inappropriate for dressing application. Therefore, 50-50 wool–viscose blends were treated with CHT concentrations of 0.1%, 0.3%, 0.5% and 1.0% (OWM) and dried and cured, as shown in Figure 3.3.

![Figure 3.3: Flow diagram of experimental capsule 2](image)

After curing, the blend nonwoven samples were then characterised for morphological and structural changes using Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red (FTIR) spectroscopy and tested for blood absorbency and antimicrobial properties.
3.1.4 Experimental capsule 4

For the fourth experimental capsule, 100% wool nonwoven was developed by a card-crosslapper and subsequent needle punching, as shown in Figure 3.4 (400 GSM). The needle-punched wool nonwovens were then coated utilising the electrospraying technique by 0.3% CHT and dried and cured. 0.3% solution was selected based on the results obtained from capsule 2 and 3. The objective was to coat the surface in such a way that CHT could be on the surface of the developed nonwoven wound-dressing as contact to skin layer and wool could be used as an absorbent core and therefore, a higher GSM substrate was used in these experiments.

<table>
<thead>
<tr>
<th>Preparation of wool nonwoven</th>
<th>Card-crosslapper and needle punching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan coating by Electrospraying</td>
<td>concentrations of 0.3% (w/w)</td>
</tr>
<tr>
<td>Finishing</td>
<td>Drying and curing of CHT-coated substrates (130 °C for 5 minutes)</td>
</tr>
<tr>
<td>Characterisation of the developed substrates</td>
<td>SEM, FTIR, absorbency, anti-microbial properties</td>
</tr>
</tbody>
</table>

**Figure 3.4: Flow diagram of experimental capsule 3**

The developed wool nonwoven samples coated with CHT were then characterised for morphological and structural changes using Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red (FTIR) spectroscopy and tested for blood absorbency and antimicrobial properties.

3.1.5 Experimental capsule 5

In this experimental setup, wool and CHT were both dissolved using a common solvent BMIMCl. The blending was achieved by mixing both the
polymer solution in *situ*, as shown in Figure 3.5. After mixing, both the polymers were regenerated using an acetone-water medium and subsequent repeated rinsing in water was carried out to get rid of excess BMIMCl. As BMIMCl is miscible with water in any ratio, water was a suitable medium.

<table>
<thead>
<tr>
<th>Preparation of wool and CHT solution</th>
<th>BMIMCl as common dissolution media</th>
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<tbody>
<tr>
<td><strong>Blending</strong></td>
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<tr>
<td>50-50 (w/w%) mixing of wool and CHT in-situ</td>
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<tr>
<td><strong>Regeneration and film preparation</strong></td>
<td></td>
</tr>
<tr>
<td>Acetone-water media and rinse with water</td>
<td></td>
</tr>
<tr>
<td><strong>Characterisation of the developed substrates</strong></td>
<td>SEM, FTIR, Raman, absorbency, anti-microbial properties</td>
</tr>
</tbody>
</table>

**Figure 3.5: Flow diagram of experimental capsule 4**

100% wool, 50-50 wool–CHT and 100% CHT films were prepared and characterised for morphological and structural changes using Scanning Electron Microscopy (SEM), Fourier Transform Infra-Red (FTIR) and Raman spectroscopy and tested for absorbency and antimicrobial properties. In addition, the dependence of the dissolution phenomenon on temperature was also investigated. The degree of N-acetylation value of CHT, which directly influences the anti-bacterial attribute of CHT, was also determined using FTIR spectroscopy.

3.1.6 : Experimental capsule 6

Furthermore, 100% wool was dissolved in BMIMCl and SNPs were doped to the dissolved wool fibres in *situ* with BMIMCl medium. The wool polymer was then regenerated and films were prepared as shown in Figure 3.6.
100% wool films doped by SNPs were prepared and characterised for morphological and structural changes using Scanning Electron Microscopy (SEM), Fourier Transform Infra-Red (FTIR) and Raman spectroscopy and tested for absorbency and antimicrobial properties.

3.2: Methodology

3.2.1: Preparation of nonwoven substrates
Scouring, the technical term for washing, is the first step in wool processing. This involves washing the wool in hot soapy water to remove dirt, grease and dry plant matter from the fleece. Pristine 18–20µ wool was scoured and then fat extracted using an acetone–ethanol solvent system. Scoured wool was used in this study as it is not modified chemically. Chlorinated or oxidised wool was out of the scope of this project as it introduces few variables such as degree of chlorination and resin residue. The wool nonwoven substrates were prepared using an integrated card-crosslapper with subsequent needle-punching. A SCG-600-60 model integrated card-crosslapper from SHIN CHIANG Machinery Co. Ltd. Was used to prepare laps of 100% wool. Parameters were optimized for uniform lap preparation. The prepared lap was subsequently needle punched using a SNP-50M-6, make SHOUU
SHYNG Machinery Co. Ltd, Down-stroke needle punching machine with a working area of 500mm x 296mm. The needles used were 15 x 18 x 36 x 3 1/2" R222G3027 type with 11mm needle penetration and 400 strokes/minute. Feed rate move step was 1.80mm/stroke. A series of needle punched nonwovens were developed having a mass per unit area of ranging from 150 to 400 GSM. 4 commercially available nonwoven wound-dressing products (A, B, C and D) were sourced to compare with the dressing substrates prepared in this study. Samples A, B and C were absorbent dressings and sample D was an anti-bacterial dressing.

3.2.2 Preparation of chitosan solution

Practical grade chitosan from crab shells (Brookfield viscosity 200 cP, 1% in 1% acetic acid, degree of de-acetylation >85% and molecular weight 190,000->375,000, which is based on the viscosity of 200-2000 mPa.s) was sourced from Sigma-Aldrich Pty Ltd Australia and used as received for the experiments. Chitosan was dissolved in 1% (w/w) acetic acid until a clear solution was achieved. Freshly prepared solutions were used for all the experiments.

3.2.3 Treatment with BMIMCl

BMIMCl was sourced from Sigma-Aldrich and used as received. The wool samples were separately treated with BMIMCl in a 100ml beaker at 120°C with continuous stirring for 30 minutes. Temperature was monitored and maintained using a thermometer and hotplate. The samples were washed multiple cycles with water until the IL was removed completely.

3.2.4 Padding of chitosan solution

Nonwoven samples were padded with chitosan solution of concentration 0.1%, 0.3%, 0.5%, 0.75% and 1% respectively. Chitosan was dissolved in 0.1% acetic acid (w/w %) and freshly prepared solutions were used for all the experiments. An 80% pick up ratio was recorded for all samples. In order to standardise and eliminate bias, the control (untreated) was padded with water at 80% expression, dried and cured similar to CHT-treated samples.
3.2.5: Curing treatment
After padding, all chitosan treated wool and wool blend samples were dried at 60°C and cured using a Warner-Mathis stenter at 130°C for 5 minutes.

3.2.6: Surface characterisation
To characterise the effect of BMIMCl pretreatment and deposition of chitosan and to observe the morphological nature of padded and coated wool substrates and regenerated films, an FEI Quanta 200 environmental scanning electron microscope (ESEM) was used.

3.2.7: Fourier Transform Infra-Red (FTIR) and Raman spectroscopy
Infrared (IR) and Raman spectroscopy are commonly employed vibrational techniques to determine phase change or modified surface characterisation. Both these techniques are complementary but differ in selection rules and often produce complementary spectral information about the same material. IR spectra are a result of absorption of radiation, while Raman spectra are generated by inelastic scattering energy. For Raman, when a photon particle is scattered inelastically by a molecule, it may gain or drop and as such scattering can be recorded at higher or lower frequencies. The frequency displacement from the exiting radiation thus corresponds to a characteristic molecular mode of vibration and hence bands that appear weak in the IR spectrum will be strong in the Raman spectrum and vice versa. With the aim of making comprehensive vibrational band assignments, it is valuable to have both IR and Raman data. For example, in the case of wool fibre, the main-chain skeletal bands are highlighted in the IR spectra, while the side-chain features are noticeable in the Raman spectra. So the developed substrates and films were characterised by both FTIR and Raman spectra as required.

3.2.8: Water absorbency test
An indicative test is done to show the quantity of water that each of the wool substrate absorb. Samples of 2cm x 2cm were placed flat on petri dishes respectively. Water droplets were gradually put on top of each sample until the fabric was at a ‘completely wet’ status. The same test was repeated on each sample for five times and the average reading was recorded. The
percentage mass of water that each sample could absorb is measured according to the follow formula:

$$\text{water absorbed} = Y - X$$

where, $Y$ is mass of sample when ‘completely wet’, and $X$ is initial mass of dry sample.

3.2.9 : Blood absorbency test

Similar to the water absorbency test, a method was developed to test the absorbency of the different developed materials; defibrinated (coagulant removed) horse blood was used as it resembles human blood and is used in forensic studies. Defibrinated horse blood was sourced from Australian Ethical Biological Pty Ltd. The pH of the blood was recorded to be 7.2. Identical sample sizes and procedures for calculation were followed in this method. Average of five measurements was reported.

3.2.10 : Electrospraying

The chitosan solution was placed into a 5ml syringe with a metal syringe needle (23 Gauge), which was connected to a syringe pump (KDS 100, KDS scientific) as shown in Figure 3.7. Needle-punched wool fabric was mounted on a metal plate at a distance of 15cm from the needle. The electrospraying was performed under an applied voltage of 15~25 kV with a solution flow rate of 4.0 ml/h.

During electrospraying, the chitosan particles were coated onto the wool fabric mounted on the collector.

Figure 3.7: Spray formation of CHT on wool substrate
3.2.11: Antibacterial Testing
Escherichia coli (E. coli) ATCC 11229, a Gram negative bacteria, was used as the test specimen organism. Bacterial inoculums were prepared to obtain a suspension in an exponential growth of $10^8$ colony forming units (CFU) ml$^{-1}$ in 5mL of nutrient broth which was a modified tryptone soy broth from Oxoids. A tryptone soya agar (from Oxoids) was used as the nutrient agar for agar plates. Antibacterial tests were carried out with both coated and uncoated wool. The uncoated wool fabric was used as a control sample against the coated one. Fabric swatches of 4cm diameters were cut and then sterilised using UV light. Each fabric swatch was placed in a conical flask. AATCC 100-2004 (clause 10.2) test standard was followed to perform the antibacterial test. A series of diluted solutions were prepared as $10^0$, $10^1$, $10^2$ and $10^3$ times with sterile incubation; the $10^2$ dilution was taken to compare the coated and uncoated swatches. The plates corresponding to $10^0$ and $10^1$ times had uncountable colonies and that for $10^3$ times had too few colonies for the control swatch. The percentage reduction of the bacteria by the fabric specimen treatment was calculated with the following formula [271]:

$$\text{Reduction in CFU\%} = \left( \frac{B - A}{B} \right) \times 100\%$$

Where, $B$= average number of bacterial colony in untreated substrate

$A$= average number of bacterial colony in chitosan treated/coated substrate

3.2.12 Silver nanoparticles
Silver nanoparticles suspended in aqueous buffer (size distribution 40 nm) with a density of 0.9900 g/cm$^3$ was sourced from Sigma-Aldrich Pty Ltd and used as received for the experiments. Several concentrations (0.1%-1% OWM) of SNPs were tested for antibacterial assessment based on previous studies [272, 273].

3.2.13 Film preparation of wool and CHT and blends from BMIMCl
The regenerated wool, chitosan and blends were prepared in the following way. To obtain 5 wt% of wool solution in BMIMCl, about 1g of wool was spread into 20mL of BMIMCl in a 100mL flask, and the mixture was heated
at temperature range 100°C to 130°C and stirred using a magnetic stirrer until the wool samples were completely dissolved and showed a clear and viscous wool solution.

To dissolve the chitosan, 1g of CHT was mixed with 20 mL BMIMCl, heated and stirred at 100°C to 130°C obtain a 5 wt% completely homogeneous CHT solution. The dissolved wool and chitosan could be regenerated in water as BMIMCl is completely miscible with water in any ratio. This can be done by pouring the viscous solution into de-ionised water and coagulated several times. The individual regenerated wool and CHT films were obtained by casting the viscous solution between two circular microscopic slides and then soaked in water bath to allow BMIMCl diffusion from the films. During this process, water was changed several times to confirm that the BMIMCl had been removed completely from the sample. After washing with deionised water several times for the complete removal of the BMIMCl, the regenerated wool and CHT films were then dried in a vacuum oven to get flat blend films. The wool–CHT blend films were prepared by the mutual mixing of wool/BMIMCl and CHT/BMIMCl solutions. This was done by mixing same dissolved weight of both the polymers in BMIMCl. For example, 50-50 wool–CHT blend films were prepared by making wool solution (0.5 g in 10 mL BMIMCl), CHT solution (0.5g in 10mL BMIMCl) and mixing them together to get a total of 50-50 blends in 5 wt% solution. The mixture solution was stirred again at 100°C again for 2 hours in order to ensure the complete intermixing. Regenerated wool/CHT blend films were obtained in the same way as that of regenerated wool and were characterised to study the changes in structure and properties with composition.

The results obtained from experimental capsules 1, 2, 3 and 4 are discussed in Chapter 4 and from capsules 5 and 6 are discussed in Chapter 5.
Chapter 4: Results and discussion 1

4.1: Wool nonwoven treated with BMIMCl and padded with chitosan (experimental capsule 1)

4.1.1: Surface morphology

The developed nonwoven wool substrates were characterised for morphological change using SEM. Figure 4.1 shows the random needle-punched fibre orientation of the wool substrate.

Figure 4.1: Nonwoven needle-punched wool substrate
Figure 4.2: (A) SEM pictures of untreated wool substrate (B) SEM pictures of BMIMCl treated wool substrate

Figure 4.2(A) shows the presence of scales on wool fibres without the BMIMCl pretreatment. The presence of scales plays a critical role in limiting liquid absorption for wound-dressing applications, because the epicuticle layer located on the wool surface is hydrophobic in nature. After the BMIMCl treatment, the deterioration of the wool scales is visible, as shown in Figure 4.2(B) and 4.3.

Figure 4.3: Smooth wool fibre surface achieved as a result of BMIMCl treatment
A smooth fibre surface is achieved as the scales are dissolved. This is supported by a decrease in sample weight by 8% after the BMIMCl treatment. The dissolution of scales contributes to the liquid-absorbing ability of the wool fibres, as the hydrophobic barrier is damaged.

After the BMIMCl treatment, the nonwoven samples were further treated with different CHT concentrations and surface morphological structures of the treated substrates are shown in Figure 4.4.

Figure 4.4: BMIMCl treated wool substrate with: (A) 0.3% chitosan; (B) 0.5% chitosan; (C) 0.75% chitosan (D) 1% chitosan
It is clearly visible from Figure 4.4 that individual fibres were coated successfully and the presence of chitosan made the smooth outer surface of the wool more pronounced. It should be noted that the difference in chitosan concentration is not observable in the above SEM images. However, the feel of the treated textiles show that for 0.75% and 1%, the CHT-treated samples were stiff and rigid, whereas the 0.3% and 0.5% CHT-treated samples had comparable handling as for the untreated samples.

The presence of CHT on the treated samples was characterised by FTIR spectroscopy.

4.1.2 : FTIR Analysis

To analyse the effect of BMIMCl treatment and subsequent CHT-treatment, the treated samples were investigated for structural change using FTIR and the spectra obtained are shown in Figure 4.5.

![Figure 4.5: FTIR Comparison of the different treatment stages (a) wool (fat extracted); (b) BMIMCl treated; (c) BMIMCl and CHT-treated (1%)](image)
As can be seen from the Figure 4.5 (c) the spectrum corresponds to the sample that has undergone BMIMCl-treatment and contains 1% CHT. The large stretch at 3269 cm\(^{-1}\) corresponds to the amine (–NH\(_2\)) band and 1627cm\(^{-1}\) refers to the –OH band from the structure of chitosan. No significant change could be observed when the substrate was pre-treated with BMIMCl except a new band was visible at 1238 cm\(^{-1}\) (Figure 4.5)(c), which refers to the –CH\(_3\) deformation of CHT. Hence Figures 4.3 and 4.4 suggest the presence of CHT in the treated substrates as expected.

4.1.3 : Water absorbency test

Figure 4.6 shows the dry and the wet status of the developed wool nonwoven samples.

![Figure 4.6](image)

**Figure 4.6 : (A) initial sample at normal condition, (B) sample completely wet**

Figure 4.6(B) shows the appearance when the wool substrate is completely wet throughout the sample. This is the status where the maximum amount of water is held by the sample. The results are shown in Table 4.1.
Table 4.1: Results for liquid uptake

<table>
<thead>
<tr>
<th></th>
<th>Untreated wool</th>
<th>BMIMCl treated</th>
<th>IL+ 0.3% CHT</th>
<th>IL+ 0.5% CHT</th>
<th>IL+ 0.75% CHT</th>
<th>IL+ 1% CHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHT (%)</td>
<td>0%</td>
<td>0%</td>
<td>0.3%</td>
<td>0.5%</td>
<td>0.75%</td>
<td>1%</td>
</tr>
<tr>
<td>Average initial mass (g)</td>
<td>0.0310</td>
<td>0.0317</td>
<td>0.0346</td>
<td>0.03183</td>
<td>0.031</td>
<td>0.0335</td>
</tr>
<tr>
<td>Average mass at ‘completely wet’ status (g)</td>
<td>0.4125</td>
<td>0.2831</td>
<td>0.3984</td>
<td>0.3545</td>
<td>0.3462</td>
<td>0.3354*</td>
</tr>
<tr>
<td>Average amount of water absorbed (g)</td>
<td>0.3815</td>
<td>0.2514</td>
<td>0.3638</td>
<td>0.32267</td>
<td>0.3152</td>
<td>0.3019</td>
</tr>
</tbody>
</table>

*a complete wet’ status could not be achieved when the sample was treated with 1% chitosan. Water was unable to penetrate into the core of the fibre mass regardless of the amount of water applied. A graph was plotted to compare the liquid uptake and is shown in Figure 4.7.

Figure 4.7: Comparison of water uptake by wool substrates
The BMIMCl treatment was found to contribute to the lower liquid uptake of wool substrates, as can be seen from Figure 4.7. It has been claimed in several studies [86, 165, 274] that BMIMCl treatment was able to improve wettability and dyeing behaviour of wool, although a reduction in liquid uptake was found in this study. This can be best explained by the reduction in weight of the BMIMCl-treated samples. The sample weight dropped by 8% and consequently the liquid-holding capacity was reduced. As a result, a reduction in liquid uptake was recorded for the BMIMCl-treated sample only. The water uptake was calculated on the basis that the water that is held by the pores and interstices of the entangled fibres in a nonwoven matrix as well as by the fibre itself. The BMIMCl treatment not only reduced the fibre weight but also decreased the available pore volume which explains the reduction of water uptake (about 30%) between the untreated and treated samples.

CHT-treatment increases the liquid uptake of wool compared to the samples treated with BMIMCl alone. This can be explained by the increased weight of samples due to CHT deposition. As mass increased, wool was able to hold greater amount of water. However, it was observed that the higher the CHT concentration, the more the wool samples became rigid and stiff. This can be attributed to the film formation of CHT on the substrate. For the CHT-treated samples, the 0.3%, 0.5% and 0.75% samples show similar liquid uptake, whereas 1% shows a lesser amount. This was due to water being unable to penetrate into the inner structure of the wool. As a result, liquid uptake was limited. This is supported by the observation that a completely wet status could not be achieved for the 1% CHT-treated sample even with further addition of water.

4.1.4 : Antibacterial testing

All CHT-treated samples were tested for antibacterial performance. Figure 4.8 shows the anti-bacterial effect of chitosan treated samples against the control of 0.3% concentration as it was the lowest concentration of chitosan tested. All other samples (0.5%, 0.75% and 1%) showed similar findings.
As can be seen from the figure, the 0.3% CHT-treated samples were excellent in terms of anti-bacterial efficacy. No growth was observed for the treated samples tested.

To summarise, BMIMCl treatment, although it increases the wettability of the wool compared to the untreated one, the water absorption capability decreased and hence this pretreatment process was not continued. 0.3% CHT-treated samples were excellent in terms of anti-bacterial properties and showed comparable liquid uptake [275].

Figure 4.8: (A) showing complete growth of bacteria and (B) showing no growth
4.2: Wool nonwoven treated with chitosan (experimental capsule 2)

4.2.1: Surface morphology

Scanning electron micrographs were used to characterise the effect of chitosan treatment on wool substrates. Figure 4.9(A) and (B) shows the untreated and CHT-treated samples of wool substrate.

![SEM pictures of (A) untreated wool substrate; (B) CHT-treated wool substrate](image)

**Figure 4.9**: SEM pictures of (A) untreated wool substrate; (B) CHT-treated wool substrate

Figure 4.9(A) shows the presence of scales of wool fibre on the untreated sample. After the CHT treatment, a relatively smooth fibre surface was achieved, as the scales were covered by the film formed by CHT, as shown in Figure 4.9(B). This is supported by the increased sample volume, as the percentage of CHT increases in developed samples.

4.2.2: FTIR analysis

The FTIR spectra of the untreated and CHT-treated samples were compared, as shown in Figure 4.10. The chemical formulation of CHT consists of the carbohydrate structure with three reactive groups. These are the primary (C-6) and secondary (C-3) hydroxyl (-OH) groups and the amino-NH$_2$ (C-2) group in each repeat of the de-acetylated unit of chitin.
Chitosan with a degree of de-acetylation of >85% displayed a strong vibration at 1629 cm\(^{-1}\), which has previously been assigned to amide I vibration [276, 277]. However, since only 15% or less of the nitrogen atoms occur as amides, the remaining atoms being amines, this assignment is unlikely to be accurate. Amine deformation vibrations usually produce strong to very strong bands in the 1638–1575 cm\(^{-1}\) region [278]. Therefore it is proposed that the band at 1512 cm\(^{-1}\) is N–H bending vibration overlapping amide II vibration and that the 1629 cm\(^{-1}\) band is amide I vibration [279]. In addition, C-N stretching vibrations occur in the 1190–920 cm\(^{-1}\) region and overlap the vibrations from the carbohydrate ring of chitosan. N-H stretching also occurs in the 3315–3215 cm\(^{-1}\) region overlapping the –OH stretch from the carbohydrate ring [279]. Thus the CHT-treated wool samples show the amine groups from CHT, which will contribute to anti-bacterial performance.
The FTIR bands of CHT-treated samples with assignments are shown in Table 4.2.

**Table 4.2: FTIR bands of CHT-treated samples with assignments**

<table>
<thead>
<tr>
<th>Wave number (cm(^{-1}))</th>
<th>Vibrational assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3272</td>
<td>O-H and N-H stretch</td>
</tr>
<tr>
<td>2921</td>
<td>C-H stretch</td>
</tr>
<tr>
<td>1629</td>
<td>amide I</td>
</tr>
<tr>
<td>1512</td>
<td>N-H bending from amide I and amide II</td>
</tr>
<tr>
<td>1389</td>
<td>CH(_3) symmetrical deformation</td>
</tr>
<tr>
<td>1076</td>
<td>asymmetric stretch C-O-C and C-N stretch</td>
</tr>
<tr>
<td>1017</td>
<td>skeletal vibration of C-O stretching</td>
</tr>
</tbody>
</table>

Further SEM analysis shows film formation of chitosan onto wool fibre, as can be seen from Figure 4.11.

**Figure 4.11: CHT-treated wool substrate with 1% CHT**

A Raman spectral comparison was carried out for wool at different stages of processing as shown in Figure 4.12.
Figure 4.12: Raman spectra of (a) pristine wool; (b) fat extracted wool; (c) 0.5% CHT-treated wool substrate; and (d) commercial CHT

Figure 4.12 shows the Raman spectra of wool at different processing stages. These comparisons establish the common phenomenon of wool and CHT interactions. CHT forms a sheath film on the wool fibres as the CHT-treated wool takes on the Raman spectral profile of CHT alone. The distinctive Raman shift of the O–H towards a higher number confirms possible hydrogen bonding between wool and CHT. In addition, the distinct peak at 3050 cm\(^{-1}\) of \(=\text{C–H}\) which was visible for both pristine and fat extracted wool was absent in the 0.5% CHT treated wool sample which further confirms the sheath film formation of CHT.

It is evident from Figures 4.10, 4.11 and 4.12 that the nonwoven substrate was padded successfully with CHT and the presence of CHT can be identified as a film on the fibre surface of wool.

4.2.3 : Blood absorbency test
As described in the experimental section, Figure 4.13 shows the dry and the saturated state of the samples.
Figure 4.13: (A) initial sample at dry condition (dry); (B) sample at saturated status

Figure 4.13 (B) shows the appearance when the wool substrate is saturated with absorbed blood. This is the status at which the maximum amount of blood is held by the tested sample and the value was recorded. A graph was plotted to compare the blood absorbency and this is shown in Figure 4.14.

Figure 4.14: Comparison of blood absorption by wool substrates

The CHT-treatment was found to affect the blood absorption of wool substrates, when compared with the untreated samples as shown in Figure 4.14. The values were normalised to calculate the amount of blood absorbed for 1 cm³ of material with approximately identical mass.
At lower concentrations of chitosan (0.1% and 0.3%), absorption of the liquid (water/blood) is affected by a combination of wool and CHT. Swelling behaviour of CHT is an established fact [128, 280]. This is reflected in Figure 4.13 where a significant rise in absorption is noticed as compared to untreated sample. The higher blood absorbency can be ascribed to the chemical structure of CHT since it possesses hydrophilic hydroxyl (–OH) groups and strongly hydrophilic amine groups (–NH₂) leading to increased hydrogen bonding [281-283]. However, at CHT concentrations of 0.5% and 1%, a decline in absorption is noticed. In this case, the CHT can be considered to be present on the fibre as multiple layers. This can be explained schematically as shown in Figure 4.15. Upon contact with liquid, the outermost CHT layer swells and restricts further liquid influx.

![Figure 4.15: Schematic diagram of CHT inhibiting liquid absorption by swelling](image)

Therefore, the inner CHT layers will come into contact with the liquid only through diffusion from outer to inner CHT-layers. On further addition of liquid, a relaxation (swelling) process of the outer CHT layers will occur and this will
block the transport of the liquid into the void volume of the innermost layer. Hence, diffusion of liquid through the CHT coating plays a significant role in achieving saturation [284, 285]. In addition, this phenomenon could also restrict the amount of liquid available for wool fibre for absorption. The combined effect is a reduction in the total amount of liquid absorbed by the samples in spite of being treated with higher CHT concentration [286].

In addition, it was observed that, for higher CHT concentrations, the wool nonwoven substrates were more rigid and stiff with a reduction of thickness. This can be attributed to the film formation characteristic of CHT application by pad-dry-cure process. Accordingly, at higher concentrations, CHT solidifies when cured, locking the nonwoven matrix at a reduced loft and higher density. The effect of padding process on loft reduction is insignificant as evident from the negligible change in loft recorded for control (untreated) samples that were padded with water alone. The decreased loft of the treated material has a constraining effect on the movement of the fibre mass and thereby can also restrict liquid absorption. Since the nonwoven substrate is considered to be a porous matrix, the pore size distribution may contribute to the observations. The study of the variations introduced by the CHT-treatment and subsequent pad-dry-cure process is suggested as future work.

Thus analysing the absorbency results, 0.1% and 0.3% CHT-treated samples were better in terms of blood absorption of all the CHT-treated samples tested.

4.2.4 : Antibacterial testing
All the CHT-treated samples were tested for antibacterial properties. 0.1% CHT-treated samples showed bacterial growth on agar plates, as shown in Figure 4.16, hence were concluded not to possess antibacterial attributes.
The Minimum Inhibitory Concentration (MIC) is a significant value that indicates the lowest amount that an antimicrobial agent needs in order to inhibit bacterial growth. This MIC depends on molecular weight, degree of deacetylation and several other factors [119, 287, 288]. Figure 4.17 shows the antibacterial effect of 0.3% CHT-treated samples in agar plates against the control (untreated) samples.

**Figure 4.16:** Bacterial growth on agar plate corresponding to 0.1% CHT- treated sample

**Figure 4.17:** (A) control shows complete growth of bacteria; and (B) 0.3% shows no growth
As can be seen from Figure 4.17, the 0.3% CHT-treated wool nonwoven proved excellent in terms of antibacterial efficacy. The 0.5% and 1% CHT-treated samples also showed excellent anti-bacterial attributes; however, in this study, the MIC of CHT was found to be 0.3%. Since 0.1% of CHT-treated samples were not active biologically and 0.5% and 1% CHT-treated samples were rigid and stiff in terms of handling, this experimental set concludes that the 0.3% CHT-treated sample is superior in absorbency together with antibacterial attributes. Thus it can be used as a suitable substrate for the absorbent core component in the construct of a wound-dressing ensemble.
4.3: Wool–viscose blend treated with chitosan (experimental capsule 3)

4.3.1 Surface morphology
Scanning electron micrographs were used to characterise the effect of the chitosan treatment on wool–viscose substrates. Figure 4.18(A) and (B) shows the untreated and CHT-treated sample of wool–viscose substrate.

![SEM pictures of (A) untreated; and (B) CHT-treated wool–viscose substrate](image)

Figure 4.18: SEM pictures of (A) untreated; and (B) CHT-treated wool–viscose substrate

Figure 4.18(A) shows the presence of scales of wool fibre on the untreated sample. After the CHT treatment, a relatively smooth fibre surface was achieved, as the scales were covered by the film formed by CHT. This is supported by the increased sample volume as the percentage of CHT increases in the developed samples.

4.3.2 FTIR spectroscopy
The FTIR spectra of the untreated and CHT-treated samples were compared, as shown in Figure 4.19. The chemical formulation of CHT comprises the carbohydrate structure and three reactive groups, which are the primary (C–6) and secondary (C-3) hydroxyl (–OH) groups and the amino–NH₂ (C–2) group in each repeat of the de-acetylated unit of chitin.
Chitosan with a degree of deacetylation of >80% displayed a strong vibration at 1629 cm\(^{-1}\), which has previously been assigned to amide I vibration [276, 277]. However, since only 15% or less of the nitrogen atoms occur as amides, the remaining atoms being amines, this assignment is unlikely to be accurate. Amine deformation vibrations usually produce strong to very strong bands in the 1638–1575 cm\(^{-1}\) region [278]. Therefore it was proposed that the band at 1512 cm\(^{-1}\) is N–H bending vibration overlapping amide II vibration and that the 1629 cm\(^{-1}\) band is amide I vibration [279]. In addition, C–N stretching vibrations occur in the 1190–920 cm\(^{-1}\) region and overlap the vibrations from the carbohydrate ring. N–H stretching also occurs in the 3315–3215 cm\(^{-1}\) region overlapping the –OH stretch from the carbohydrate ring [279]. The FTIR bands of CHT-treated samples with assignments are shown in Table 4.3.
Table 4.3: FTIR bands of CHT-treated samples with assignments

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<tr>
<td>2921</td>
<td>C-H stretch</td>
</tr>
<tr>
<td>1629</td>
<td>amide I</td>
</tr>
<tr>
<td>1512</td>
<td>N-H bending from amine I and amide II</td>
</tr>
<tr>
<td>1389</td>
<td>CH₃ symmetrical deformation</td>
</tr>
<tr>
<td>1076</td>
<td>asymmetric stretch C-O-C and C-N stretch</td>
</tr>
<tr>
<td>1017</td>
<td>skeletal vibration of C-O stretching</td>
</tr>
</tbody>
</table>

Further SEM analysis shows film formation of chitosan onto both wool and viscose fibres, as can be seen from Figure 4.20.

![SEM image of wool–viscose substrate with 1% CHT](image)

**Figure 4.20**: SEM image of wool–viscose substrate with 1% CHT

It is evident from Figures 4.19 and 4.20 that the substrate was padded successfully with CHT and the presence of CHT can be identified as a film on the fibre surface of wool–viscose.
4.3.3 : Blood absorbency test

As described in the experimental section, Figure 4.20 shows the dry and the saturated state of the samples.

Figure 4.21 : (A) initial sample at dry condition (dry); and (B) sample at saturated status

Figure 4.21(B) shows the appearance when the wool–viscose substrate was saturated with absorbed blood. This is the status at which the maximum amount of blood was held by the tested samples and the value was recorded. A graph was plotted to compare the blood absorbency and is shown in Figure 4.22.
As shown in experimental capsule 3 (Figure 4.14) that CHT-treatment plays a significant role in blood absorption of the treated substrates. A similar trend can be noticed for Figure 4.22. The values were normalised to calculate amount of blood absorbed for 1 cm$^3$ of material with approximately identical mass. The CHT-treatment was found to contribute to the higher blood absorption for lower concentration (0.1% and 0.3%) of CHT-treated wool–viscose substrates when compared to the untreated samples. This was attributed to increased hydrogen bonding provided by the hydrophilic hydroxyl groups and the amine group of CHT [281]. However, this increase in blood absorption did not continue for higher CHT concentrations (0.5% and 1.0%) as expected, which can be explained by the swelling behaviour of CHT by absorbing liquid as discussed in section 4.2.3 [128, 284, 285]. In addition, it was observed that the for higher CHT concentration, the wool–viscose samples were more rigid and stiff as which can also be attributed to the film formation of CHT on the substrate.

Figure 4.22: Comparison of blood absorption by wool–viscose substrates

As shown in experimental capsule 3 (Figure 4.14) that CHT-treatment plays a significant role in blood absorption of the treated substrates. A similar trend can be noticed for Figure 4.22. The values were normalised to calculate amount of blood absorbed for 1 cm$^3$ of material with approximately identical mass. The CHT-treatment was found to contribute to the higher blood absorption for lower concentration (0.1% and 0.3%) of CHT-treated wool–viscose substrates when compared to the untreated samples. This was attributed to increased hydrogen bonding provided by the hydrophilic hydroxyl groups and the amine group of CHT [281]. However, this increase in blood absorption did not continue for higher CHT concentrations (0.5% and 1.0%) as expected, which can be explained by the swelling behaviour of CHT by absorbing liquid as discussed in section 4.2.3 [128, 284, 285]. In addition, it was observed that the for higher CHT concentration, the wool–viscose samples were more rigid and stiff as which can also be attributed to the film formation of CHT on the substrate.
Thus analysing the absorbency results, 0.1% and 0.3% CHT-treated samples were better in terms of blood absorption of all the CHT-treated samples tested in this capsule.

4.3.4 Anti-bacterial testing
All the CHT-treated samples were tested for antibacterial properties. The 0.1% CHT-treated samples showed bacterial growth on agar plates, as shown in Figure 4.23, and hence it was concluded that they did not possess an anti-bacterial attribute.

![Image of bacterial growth](image)

**Figure 4.23: Bacterial growth on agar plate corresponding to 0.1% CHT-treated sample**

The Minimum Inhibitory Concentration (MIC) is a significant value that specifies the lowest amount that an anti-microbial agent needs in order to prevent bacterial growth. This MIC is governed by the molecular weight, degree of deacetylation and several other factors [119, 287, 288]. Figure 4.24 shows the antibacterial effect of 0.3% CHT-treated samples in agar plates against the control (untreated) samples.
As can be seen from Figure 4.24, the 0.3% CHT-treated wool–viscose nonwoven proved excellent in terms of antibacterial efficacy. The 0.5% and 1% CHT-treated samples also showed excellent antibacterial attributes; however, in this study the MIC of CHT was found to be 0.3%. Since 0.1% of CHT-treated samples were biologically inactive and 0.5% and 1% CHT-treated samples were rigid and stiff in terms of handling, this experimental set concludes with a similar finding as for experimental capsule 2, that the 0.3% CHT-treated sample is superior in absorbency together with antibacterial attributes. Thus it can be used as a suitable substrate for the absorbent core component in the construct of a wound-dressing ensemble.
4.4: Electrospraying of CHT on wool substrates (experimental capsule 4)

4.4.1: Surface morphology

Scanning electron micrographs were used to characterise the coating of chitosan on wool substrates after electrospraying. Figure 4.25 shows the untreated sample of wool substrate. The scales of the wool are pronounced.

![Untreated wool substrate](image)

**Figure 4.25: Untreated wool substrate**

Figures 4.26 and 4.27 illustrate the chitosan-coated samples at different magnifications. It is clearly visible from the figures that individual fibres are coated and the presence of chitosan has made the scales on wool more pronounced. Figure 4.26 shows the surface deposition and coating of chitosan on the individual fibres.
In some cases, as shown in Figures 4.28 and 4.29, chitosan formed a bridging film on the neighbouring wool fibres. This was due to the random fibre structure of the needled nonwoven. The applied voltage, needle to collector distance and viscosity of chitosan play important roles for successful coating.
Applied voltage, solution flow rate and nozzle collector distance were the key factors affecting the electrospraying process.
4.4.2 : Antibacterial testing:

The coated and uncoated substrates were tested for their antimicrobial efficacy. Figure 4.30(A) shows the full bacterial growth on the agar plate for the control sample, which is the uncoated wool substrate. Figure 4.30(B) shows the agar plate containing the CHT-coated sample with no bacterial growth.

![Figure 4.30: Agar plates showing (A) growth of bacteria with the control sample; and (B) no growth of bacteria from the CHT-coated sample](image)

All the coated samples using electrospraying showed similar results i.e. the bacterial reduction percentage was found to be 100%. Since the CHT deposition was on the surface only, excellent antimicrobial efficacy against the Gram negative bacteria shows these samples can also be adapted to be used as potential absorbent core and the wound-contact layer in wound dressings [247].

The best performing samples from experimental capsule 1, 2, 3 and 4 were compared against 4 commercially obtained wound-dressing samples for blood absorption and the results are shown in Figure 4.31. The absorption
values were normalised to calculate amount of blood absorbed for 1 cm³ of material.

![Graph showing comparison of blood absorption with commercial samples]

**Figure 4.31: Comparison of blood absorption with commercial samples**

As can be seen from Figure 4.31, the developed samples were comparable in terms of blood absorbency. Commercial samples A, B and C were only absorbent dressings and only sample 4 was a silver-based anti-bacterial dressing (as claimed by the manufacturer). 100% wool samples (400 GSM) treated with 0.3% chitosan for both pad-dry-cure and electrospraying techniques were the best absorbents. However, the 150 GSM, 100% wool and 50-50 wool–viscose nonwovens were comparable with the commercial samples. In addition, the developed samples from the current study were also proved to be possessing outstanding anti-bacterial properties. Thus the samples developed in the current study show promising potential to be adopted commercially.
Chapter 5: Results and discussion

5.1: Regenerated wool–CHT blend from BMIMCI (experimental capsule 5)

The three-dimensional molecular binding structure of wool comprises intermolecular hydrogen, ionic and physical forces of attraction and, significantly, sulphur-containing disulphide bonds. These cause keratin fibres to be insoluble in most solvents and, in addition, to exhibit greater stability towards chemical and physical reactions than other types of proteins [269]. Therefore, in order to dissolve wool, these inter and intra-molecular interactions must be disrupted. It has been reported that the high chloride concentration in BMIMCI is highly efficient in breaking down the highly networked hydrogen bonding and thereby dissolving natural polymers [135]. In the case of wool, the dissolution of wool might be occurring due to the rupture of the disulphide bonds present in the protein structure of wool by BMIMCI. Both wool and chitosan contains many functional groups where both proton accepting and donating groups are present. BMIMCI exists as dissociated BMIM$^+$ and Cl$^-$ ions when subjected to an elevated temperature higher than room temperature. The free Cl$^-$ anions complex is associated with hydroxyl protons of wool and chitosan and the free BMIM$^+$ cations are associated with hydroxyl oxygen. Such a complex association disrupts the hydrogen bonding and leads to their dissolution in BMIMCI solution.

5.1.1: Regenerated wool from BMIMCI

The morphology of the 100% wool regenerated from BMIMCI as described in Chapter 3 was investigated using SEM and is shown in Figure 5.1. The regenerated wool film shows a diffused fibrous structure, as reported by previous studies [87, 161, 162]. Generally, the structure of the film is rough and shows diffused fibre-like assembly.
Figure 5.1: Diffused structure of wool at various locations of film regenerated from a BMIMCl solution

Although it appears to be a clear viscous solution of wool when dissolved by BMIMCl, the morphological study actually shows that very small amounts of fibres are not dissolved completely and remnants of the fibres are still visible, as shown in Figures 5.2 and 5.3.
Figures 5.2 and 5.3 clearly show traces of remnants of undissolved wool fibre in the prepared film from the dissolution process. Although earlier studies [87, 161, 162] showed similar results characterising regenerated wool under SEM, the undissolved wool was not reported. These undissolved
fibres could be the cumulative result of the type of ionic liquid used, temperature and the fineness of the wool fibre, as well as the maximum yield achieved from the BMIMCl. As the dissolution occurs in a system where elevated temperature and ionic liquid medium is involved, it is very difficult to visibly determine the maximum yield point (maximum amount of fibre dissolved in a particular amount of ionic liquid). In addition, as the BMIMCl starts dissolving the wool, the colour of BMIMCl turns from pale yellow to a dark yellow, which makes it even more difficult to detect undissolved fibres. This pale to dark yellow colour is a result of disruption of disulphide linkages (sulphur is yellow). Furthermore, it can be observed from Figure 5.3 that the original scale structure of wool has been diminished by the reaction of BMIMCl during dissolution but fibre-like remnants are still visible. This concept of regeneration differs from the traditional solvent-based spinning systems, as after dissolution, in this case, no extrusion process is involved. Thus, the fibre-like remnants can only be defined as undissolved or semi-dissolved fibres retained after the dissolution and regeneration phenomena. The regenerated wool and natural wool are further characterised by using FTIR spectroscopy, which may show changes during the dissolution and regeneration process.

5.1.2 : FTIR analysis

Figure 5.4 shows the distinction between the natural wool (fat extracted) and the regenerated wool film from the BMIMCl solution.

The peptide bond is the most abundant bond within a keratin protein. It is formed by a condensation/dehydration reaction between the carboxyl groups of the first amino acids with the amino group of the second amino acids. The bond has an unusual property that has a marked effect on the rigidity of a polypeptide chain and consequently on the folding of the polypeptide chain. It has partial double-bond character, which is caused by the resonance of electrons rapidly moving between the oxygen and nitrogen to make the C-N bond a partial double bond C=N. The consequence of this arrangement is that the peptide bond is very rigid because C=N is much less flexible than a C-N bond [289].
Figure 5.4: Comparison FTIR spectra of (a) natural wool; (b) wool film regenerated from BMIMCl solution

For the plain regenerated wool, the broad peak at 3367 cm⁻¹ is due to the intramolecular hydrogen bonding of hydroxyl groups collectively from the amino acids and the stretching vibrations of N–H groups [290, 291]. In the general secondary structure of a protein, the α-helix is in a right or left-handed coiled conformation, in which every backbone N–H group is hydrogen bonded with the backbone C=O group of the amino acid. The two absorption bands at 2875 and 2962 cm⁻¹ refer to the H–C–H asymmetric and symmetric stretch, which can be seen as distinct peaks at 2800–3000 cm⁻¹ region.

A distinct shift of the 3276 cm⁻¹ absorption band to 3367 cm⁻¹ can be seen as a result of increased hydrogen bonding, as a shift towards higher wave number refers to this phenomena [161, 162]. The vibrations of the atoms in the 750–1750 cm⁻¹ region are often referred to as the ‘fingerprint’ region of
the wool fibre, as it contains the major amide bands, C–H deformation and cystine oxides. The three amide structures available in wool fibre are given in Table 5.1.

**Table 5.1 : Three amide structures in the wool and their FTIR vibrational assignments [289]**

<table>
<thead>
<tr>
<th>Structures</th>
<th>FTIR vibrations cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amide I</strong></td>
<td></td>
</tr>
</tbody>
</table>
| ![Amide I diagram](image1) | v( C=O) β-pleated sheet 1670 cm⁻¹  
|                  | v( C=O) random coil 1665 cm⁻¹  
|                  | v( C=O) α-helix 1655 cm⁻¹  
|                  | v( C=O) β-pleated sheet 1624 cm⁻¹  |
| **Amide II**     |                                               |
| ![Amide II diagram](image2) | δ( N-H) v(C-N) α-helix 1550 cm⁻¹  
|                  | δ( N-H) v(C-N) β-pleated sheet 1531 cm⁻¹  |
| **Amide III**    |                                               |
| ![Amide III diagram](image3) | v (C-N) δ(N-H) random coil 1249 cm⁻¹  |
|                  |                                               |
| **Cystine oxides** |                                               |
| ![Cystine oxides diagram](image4) | δ(CH₂)(CH₃) 1454 cm⁻¹  
|                  | vs(S-O) cysteic acid 1170 cm⁻¹  
|                  | vs(S-O) cystine dioxide 1125 cm⁻¹  
|                  | vs(S-O) cystine monoxide 1075 cm⁻¹  
|                  | vs(S-O) cysteic acid 1040 cm⁻¹  
|                  | vs(S-O) cystine-s-sulphonate 1024 cm⁻¹  |
As can be seen from Table 5.1, amide I primarily represents the C=O stretching vibration coupled to the in-plane bending of the N-H and stretching of the C-N bonds. It is a complex band and this can be due to either the coupling between two or more similar carbonyl stretching modes or the heterogeneity among the backbone carbonyl groups. Such heterogeneity can arise either from intrinsic basic differences among carbonyl and/or from conformational differences in the strength of the hydrogen bonds associated with the carbonyls. Amide II, due to the coupled N–H in-plane bending and C–N stretching vibrations, is strongly overlapped by bands originating from amino acid side-chain vibrations and is a strong feature in the IR bands. The amide III mode is the plane combination of C–N stretching and NH in-plane bending with the contributions of C–C stretch and CO in-plane bending [289]. The summary of the peaks and the vibrational assignments for both wool and regenerated wool are tabulated in Tables 5.2 and 5.3.

### Table 5.2: FTIR bands of wool fibre (fat extracted) with assignments

<table>
<thead>
<tr>
<th>Wave number (cm⁻¹)</th>
<th>Vibrational assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3276</td>
<td>O-H and N-H stretch</td>
</tr>
<tr>
<td>1629</td>
<td>C-C≡C symmetric stretch and N-H bend from amide linkage</td>
</tr>
<tr>
<td>1522</td>
<td>N-H stretching from amine I and amide II</td>
</tr>
<tr>
<td>1232</td>
<td>asymmetrical C-H bending of the CH₂ group</td>
</tr>
<tr>
<td>1017</td>
<td>sulphur-oxygen stretching region νₜS (S-O cysteine-s-sulphonate) [290, 292]</td>
</tr>
</tbody>
</table>
### Table 5.3: FTIR bands of regenerated wool with assignments

<table>
<thead>
<tr>
<th>Wave number (cm(^{-1}))</th>
<th>Vibrational assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3367</td>
<td>O-H and N-H stretch</td>
</tr>
<tr>
<td>2962 and 2875</td>
<td>C-H stretch</td>
</tr>
<tr>
<td>1625</td>
<td>C=C=C symmetric stretch and C=O stretch from amide linkage</td>
</tr>
<tr>
<td>1598, 1565 and 1534</td>
<td>N-H bending from amide I and amide II</td>
</tr>
<tr>
<td>1384 and 1337</td>
<td>CH stretch and CH(_2) and CH(_3) bending modes</td>
</tr>
<tr>
<td>1167</td>
<td>(\nu)(S-O) cysteic acid</td>
</tr>
</tbody>
</table>

It is noticeable that the peak at 1017 cm\(^{-1}\) is absent in the regenerated wool. The peak in this region as shown in Table 5.2 belongs particularly to the sulphur-oxygen stretching region \(\nu\)(S-O cystine-sulphonate) [290, 292]. As postulated, the disulphide bond needs to break for dissolution and that can be seen in the case of regenerated wool, which postulates those bonds were not restored in the regeneration process.

### 5.1.3: Raman analysis

As a complementary technique, Raman spectral analysis was carried out for the same samples, as it provides more information on the lower end of the spectrum. Figures 5.5 and 5.6 show the Raman spectra obtained for wool (fat extracted) and regenerated wool from the BMIMCl solution.
Figure 5.5: Raman spectrum of natural wool (fat extracted)

The relative Raman shift and the vibrational assignments are listed in Table 5.4.

Table 5.4 : Raman shift and the vibrational assignments of wool (fat extracted)

<table>
<thead>
<tr>
<th>Raman shift (cm(^{-1}))</th>
<th>Vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>430–550 (432 and 552)</td>
<td>(\nu(S-S)) disulphide bond</td>
</tr>
<tr>
<td>1000–1250 (1004)</td>
<td>(\nu(C=S)) phenylaline amino acid</td>
</tr>
<tr>
<td>2100–2250 (2080, 2100, 2166)</td>
<td>(\nu(C \equiv C))</td>
</tr>
<tr>
<td>2800–3000 (2824, 2852, 2881, 2927, 2992, 2999)</td>
<td>(\nu(C-H))</td>
</tr>
<tr>
<td>3000–3100 (3052,3059)</td>
<td>(\nu(=C-H))</td>
</tr>
<tr>
<td>3300–3500 (3338, 3373, 3441, 3467, 3503)</td>
<td>(\nu(N-H)) amines and amides</td>
</tr>
<tr>
<td>3100–3650</td>
<td>(\nu(O-H))</td>
</tr>
</tbody>
</table>
Figure 5.6: Raman spectrum of wool film regenerated from BMIMCl solution

Relative Raman shift values and the corresponding vibrational assignments are listed in Table 5.5.

Table 5.5: Raman shift and the vibrational assignments of wool regenerated from BMIMCl solution

<table>
<thead>
<tr>
<th>Raman shift (cm⁻¹)</th>
<th>Vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>250–400 (305, 318)</td>
<td>δ (C–C) aliphatic chains</td>
</tr>
<tr>
<td>430–550 (415 and 430)</td>
<td>υ(S–S) disulphide bond</td>
</tr>
<tr>
<td>1000–1250 (1185, 1209)</td>
<td>υ(C–S) cysteic oxide and monoxides</td>
</tr>
<tr>
<td>1610–1680 (1632)</td>
<td>υ(C=Н)</td>
</tr>
<tr>
<td>2800–3000 (2881, 2932)</td>
<td>υ(C–Н)</td>
</tr>
<tr>
<td>3300–3500 (3321, 3334, 3363, 3445, 3502)</td>
<td>υ(N–H) amines and amides I and II</td>
</tr>
<tr>
<td>3100–3650</td>
<td>υ(O–H)</td>
</tr>
</tbody>
</table>
As can be seen from Table 5.5, the disulphide bonds are still present, which supports the argument of undissolved or semi-dissolved wool fibre remnants in the film, as in Section 5.1.1. The dissimilarity between the two spectra is due to the chemical changes from dissolution and regeneration which can be further studied using HNMR and NMR technique.

5.1.4 : Regenerated CHT from BMIMCl

CHT was regenerated from BMIMCl as described in Chapter 3 and microscopic studies were carried out for CHT utilising comparable techniques. Regenerated CHT film was characterised for its morphological changes as dissolution and regeneration occur. Regenerated CHT polymeric film shows a different structure as compared to regenerated wool. The appearance can be distinguished as defragmented scaly structure. It is noticeable that although the film visibly seems uniform, the scaly structure is apparent throughout the film region inspected. Figure 5.7 shows the morphological structure of a typical CHT film prepared from BMIMCl at different locations within the film.
Figure 5.7: Appearance of a scaly structure at various locations of the CHT film regenerated from BMIMCl solution

A closer examination with higher magnification within the film shows somewhat even distribution of the surface, as can be seen from Figure 5.8. It is worth mentioning that when BMIMCl is used as a dissolution media, it does not behave as a volatile solvent medium. One of the key advantage of using BMIMCl is its miscibility with water. It is miscible in water at any ratio and after regeneration, the polymers are washed repeatedly and left in a water bath for the excess IL to diffuse in water.
FTIR studies of CHT from crab shells (sourced from Sigma-Aldrich) and CHT of the same regenerated from BMIMCl solution were characterised for any chemical and structural derivatisation of CHT in both forms. FTIR spectra of CHT from crab shells and CHT regenerated from BMIMCl are shown in Figure 5.9.
Figure 5.9: FTIR spectra of (a) CHT from crab shell and; (b) CHT regenerated from BMIMCl.

Both spectra show similar patterns; however, the regenerated CHT produced sharper peaks at 3359 cm\(^{-1}\) and in the 1030–1155 cm\(^{-1}\) regions. The absorption peak in this area indicates stretching of the O–H and N–H bonds at 3359 cm\(^{-1}\) and C–O bonds at 1030–1155 cm\(^{-1}\) respectively. In addition, absorption peaks in the 2880 cm\(^{-1}\) region, around 1550–1590 cm\(^{-1}\) and 1400 cm\(^{-1}\) correspond to C–H stretching, amine groups – (NH\(_2\)) and carboxyl groups (–COO\(^{-}\)) respectively [293]. A band at 3359 cm\(^{-1}\) corresponds to the combined peaks of the NH\(_2\) and O–H group stretching vibration in CHT. The band at 1641 cm\(^{-1}\) is attributed to the CO–NH\(_2\) group. The 1598 cm\(^{-1}\) absorption peak of the – (NH\(_2\)) bending vibration is sharper than the peak at 1641 cm\(^{-1}\), which shows the high degree of deacetylation of the CHT. A shift from 3293 to 3359 cm\(^{-1}\) is shown and the peak is sharper in the regenerated CHT, which indicates that the hydrogen bonding is enhanced [294].
The intensities of the (CO–NH$_2$) band at 1641 cm$^{-1}$ and the (NH$_2$) band at 1598 cm$^{-1}$, which can be observed clearly in pure CHT, increase dramatically, and two new sorption bands at 1422 cm$^{-1}$ and 1322 cm$^{-1}$ appear, which show asymmetrical C–H bending of the CH$_2$ group. Thus it is postulated that the halide (Cl$^-$) of BMIMCl interacts with the ammonium groups of CHT, which serves to enhance both the inter and intra-molecular interaction in regenerated CHT [295]. To summarise, 3429 cm$^{-1}$ (O–H stretching overlapping the N–H stretching), 2921 and 2878 cm$^{-1}$ (C–H stretching), 1641 cm$^{-1}$ (amide II band, C–O stretching of the acetyl group), 1598 cm$^{-1}$ (amide II band, N–H stretching) 1485–1380 cm$^{-1}$ (asymmetrical C–H bending of the CH$_2$ group) and 1029 cm$^{-1}$ (O–bridge stretching) of the glucosamine residue [296] of CHT can be characterised using FTIR spectroscopy. This also indicates that no derivatisation occurred during the dissolution and regeneration stages. The assignments of the bands are listed in Table 5.6.

**Table 5.6 : FTIR bands of CHT and regenerated CHT with assignments**

<table>
<thead>
<tr>
<th>Wave number (cm$^{-1}$)</th>
<th>Vibrational assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3359</td>
<td>O–H and N–H stretch</td>
</tr>
<tr>
<td>2921 and 2878</td>
<td>C–H stretch</td>
</tr>
<tr>
<td>1641</td>
<td>Amide II and C–O stretch of acetyl group</td>
</tr>
<tr>
<td>1598</td>
<td>N–H stretching from amide I and amide II</td>
</tr>
<tr>
<td>1485–1380</td>
<td>Asymmetrical C–H bending of the CH$_2$ group</td>
</tr>
<tr>
<td>1067</td>
<td>Asymmetric stretch C–O–C and C–N stretch</td>
</tr>
<tr>
<td>1029</td>
<td>O–bridge stretching of the glucosamine residue</td>
</tr>
</tbody>
</table>

The CHT sourced from Sigma-Aldrich and regenerated CHT of the same from BMIMCl were further characterised by Raman spectroscopy and the Raman spectra of both forms of the CHT (sourced and regenerated from BMIMCl solution) are given in Figures 5.10 and 5.11.
**Figure 5.10**: Raman spectra of CHT (sourced from Sigma-Aldrich)

**Figure 5.11**: Raman spectra of CHT (regenerated from BMIMCl solution)
The relative Raman shift and vibrational assignments of both CHT (Sigma-Aldrich) and CHT regenerated from the same using BMIMCl are listed in Tables 5.7 and 5.8.

**Table 5.7 : Vibrational assignment of CHT (sourced from Sigma-Aldrich)**

<table>
<thead>
<tr>
<th>Raman shift cm⁻¹</th>
<th>Vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>250–400 (320, 354, 404)</td>
<td>δ (C–C) aliphatic chains</td>
</tr>
<tr>
<td>800–970 (895)</td>
<td>υ(C–O–C)</td>
</tr>
<tr>
<td>1380 (1379)</td>
<td>δ (CH₃)</td>
</tr>
<tr>
<td>1610–1680 (1659)</td>
<td>υ(C=O)</td>
</tr>
<tr>
<td>1500–1900 (1736, 1773, 1799)</td>
<td>υ(C=C)</td>
</tr>
<tr>
<td>1680–1820</td>
<td>υ(C=O)</td>
</tr>
<tr>
<td>2800–3000 (2835, 2884, 2924, 2937, 2994)</td>
<td>υ(C–H)</td>
</tr>
<tr>
<td>3000–3100 (3007, 3018, 3040, 3052)</td>
<td>υ(=C–H)</td>
</tr>
<tr>
<td>3100–3650 (3111, 3120, 3131, 3153, 3172, 3216, 3276, 3283)</td>
<td>υ(O–H)</td>
</tr>
<tr>
<td>3300–3500 (3316, 3373, 3443, 3451, 3465, 3503)</td>
<td>υ(N–H) amines and amides I, II</td>
</tr>
</tbody>
</table>
Table 5.8: Vibrational assignments of Raman spectra CHT (regenerated)

<table>
<thead>
<tr>
<th>Raman shift cm$^{-1}$</th>
<th>Vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>250-400 (259, 278, 324, 351)</td>
<td>δ (C–C) Aliphatic Chains</td>
</tr>
<tr>
<td>550-800 (552, 602, 624, 657, 699, 736, 755)</td>
<td>ν(C–Cl) halogen residue from IL</td>
</tr>
<tr>
<td>800–970 (811, 827, 887, 947, 977)</td>
<td>ν (C–O–C)</td>
</tr>
<tr>
<td>1060–1150 (1057,1116)</td>
<td>ν (C–O–C) Asymmetric</td>
</tr>
<tr>
<td>1380 (1388)</td>
<td>δ (CH$_3$)</td>
</tr>
<tr>
<td>1400–1470 (1419, 1449, 1459)</td>
<td>δ (CH$_2$) δ (CH$_3$) asymmetric</td>
</tr>
<tr>
<td>1610–1680(1659)</td>
<td>ν (C=N)</td>
</tr>
<tr>
<td>1680–1820 (1788)</td>
<td>ν (C=O)</td>
</tr>
<tr>
<td>2800–3000 (2833, 2880, 2915, 2940, 2966)</td>
<td>ν (C–H)</td>
</tr>
<tr>
<td>3000–3100 (3040, 3052)</td>
<td>ν (=C–H))</td>
</tr>
<tr>
<td>3100–3650 (3111, 3157, 3168, 3176, 3200,3224, 3283,)</td>
<td>ν (O–H)</td>
</tr>
<tr>
<td>3300–3500 (3318, 3327,3339, 3375, 3444, 3451, 3464, 3503)</td>
<td>ν (N–H) amines and amides I and II</td>
</tr>
</tbody>
</table>

5.1.5: Calculation of degree of deacetylation from FTIR spectra

The degree of deacetylation (DD) is one of the most important chemical parameters capable of influencing the performance of chitosan in many of its applications [119, 287, 288, 296], one of them being antimicrobial efficacy. Several analytical techniques were developed for DD determination using IR spectroscopy. Quite a few absorption band ratios, such as A1560/A2875, A1655/A2875, A1655/A3450, A1320/A3450, A1655/A1070, A1655/A1030, A1560/A1160, A1560/A897 and A1320/A1420, have been suggested by
several researchers [297] to determine DD by FTIR spectroscopy. Since the CHT used for the current study has DD of > 85% (supplier’s information), two calculation methods were used to determine DD of the CHT and regenerated CHT [297].

\[
\frac{A_{1560}}{A_{2875}} = 0.0125 \times DD + 0.2 \quad (R^2 = 0.99) \quad [298] \quad \text{......... (Equation 1)}
\]

\[
DD = 118.883 - (40.1647 \times \frac{A_{1655}}{A_{3450}}) \quad [299] \quad \text{......... (Equation 2)}
\]

Equation 1 produces a calculated DD value of 70% and Equation 2 suggests a calculated DD of 68% and both the values are lower than the specification provided by the manufacturer. However, for the regenerated CHT samples, the calculated values of DD are 89% and 84%. This increase can be attributed to higher hydrogen bonding caused by dissolution and regeneration phenomenon.

5.1.6 : Temperature-dependent dissolution phenomena

The dissolution phenomena of wool and chitosan were investigated and the total amount dissolved in a particular amount of BMIMCl was calculated as a function of time elapsed to dissolve the sample. A graph was plotted (as shown in Figure 5.12) to show the comparison of wool and chitosan.
Recent studies [300, 301] compared dissolution polymers in a variety of ionic liquids. However, the time it takes to reach the maximum yield was reported differently in several studies [87, 160, 162]. As can be seen from Figure 5.12, the dissolution temperature for CHT started from 90°C and the maximum yield was reached at 130°C. In comparison, the dissolution of wool started from a higher temperature (100°C) and maximum yield was attained at 140°C. This could be because of wool possessing a very strong inter and intra-molecular structure compared to CHT. Also to be noted is that in the case of wool, the maximum amount of dissolution was observed from 120–140°C, which is higher than that previously reported. In addition, the time of dissolution was observed to be much less (4 hours) than in previous studies. This anomaly can be explained by the elevated temperature as well as the fineness and form of wool samples used (powder or normal fibre). For both polymers, the maximum yield was observed to be approximately 5% on the weight of BMIMCl used, which is in good agreement with previous studies [87, 160]. Two equations for the dissolution of wool and CHT in BMIMCl were

\[
y = 0.0056e^{0.0648x} \\
R^2 = 0.991
\]

\[
y = 0.2889e^{0.0373x} \\
R^2 = 0.9829
\]
derived by plotting an exponential curve fit. The generalised equation for exponential scale is \( y = e^{mx} \).

For CHT dissolution, \( y = 0.2889e^{0.0373x} \) \( (R^2 = 0.9829) \) ……… (Equation 3)

For wool dissolution, \( y = 0.0056e^{0.0648x} \) \( (R^2 = 0.991) \) ……… (Equation 4)

Where, in both cases, \( m \) is the dissolution rate of the polymer.

5.1.7: Wool–CHT-blend film

The morphology of the wool–CHT-blend film (50-50 on the weight dissolved in BMIMCl) was investigated using SEM. The morphology of regenerated wool–CHT is given in Figure 5.13. As can be seen, the morphology of the regenerated wool and CHT are significantly changed when blended. Regenerated wool showed a relatively diffused texture by undissolved remnant fibres fused into a conglomerate texture. Regenerated CHT showed flaky structural morphology rather than a homogeneous film, as shown previously in Figures 5.3 and 5.7. Figure 5.13 represents the SEM micrographs of the free surfaces of regenerated wool–CHT-blend films.
A homogeneous morphology was observed in 50-50 wool–CHT blends. It is noticeable that the prepared blend films are not displaying the characteristics of a phase separated structure. With increasing CHT content, the morphology of the blend films changed dramatically from the diffused wool structure to a homogeneous structure. However, the FTIR spectroscopy proves that there are hydrogen bonding interactions between the hydroxyl groups of wool and CHT, which caused partial miscibility between the components. Figure 5.14 and 5.15 show the FTIR spectrum obtained from
50-50 wool–CHT-blend film compared to 100% regenerated wool and 100% regenerated CHT respectively.

Figure 5.14: FTIR spectra of (a) wool–CHT-blend film; and (b) wool regenerated from BMIMCl
As can be seen from both the comparison spectra, the blend spectrum shows similar features in terms of common peaks that were found when the individual components were investigated. Characteristic common peaks from the comparison are listed in Table 5.9.

### Table 5.9: Absorption peaks and vibrational assignments of wool–CHT (50-50) blend film

<table>
<thead>
<tr>
<th>Wave number (cm⁻¹)</th>
<th>Vibrational assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3289</td>
<td>O–H and N–H stretch</td>
</tr>
<tr>
<td>2921</td>
<td>C–H stretch</td>
</tr>
<tr>
<td>1642</td>
<td>Amide II and C–O stretch of acetyl group from CHT</td>
</tr>
<tr>
<td>1587</td>
<td>N–H bending from amine I and amide II of wool</td>
</tr>
<tr>
<td>1374</td>
<td>Asymmetrical C–H bending of the CH₂ group</td>
</tr>
<tr>
<td>1167</td>
<td>ν(S–O) cysteic acid</td>
</tr>
<tr>
<td>1025</td>
<td>O–bridge stretching of the glucosamine residue of CHT</td>
</tr>
</tbody>
</table>
The developed 50-50 wool–CHT film was further characterised by Raman spectroscopy and the Raman spectrum is shown in Figure 5.16.

![Figure 5.16: Raman spectrum of 50-50 wool–CHT blend film](image)

The characteristic bands from the spectrum with their vibrational assignments are listed in Table 5.10.


Table 5.10: Raman vibrational assignments of wool–CHT-blend film

<table>
<thead>
<tr>
<th>Raman shift cm⁻¹</th>
<th>Vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>250–400 (220, 257, 271, 284, 303, 331, 343, 401)</td>
<td>δ (C–C) aliphatic chains</td>
</tr>
<tr>
<td>430–550 (431 and 447)</td>
<td>υ(S–S) disulphide bond</td>
</tr>
<tr>
<td>550–800 (680, 699)</td>
<td>υ(C–Cl) halogen residue from IL</td>
</tr>
<tr>
<td>1000–1250 (1180, 1191, 1211)</td>
<td>υ(C=S) cysteic dioxides and monoxides</td>
</tr>
<tr>
<td>1610–1680 (1623, 1633)</td>
<td>υ(C=N)</td>
</tr>
<tr>
<td>2800–3000 (2881, 2891, 2915, 2931)</td>
<td>υ(C–H)</td>
</tr>
<tr>
<td>3000–3100 (3051)</td>
<td>υ(=C-H))</td>
</tr>
<tr>
<td>3100–3650 (3100, 3111, 3139, 3200, 3268, 3276, 3284)</td>
<td>υ(O–H)</td>
</tr>
<tr>
<td>3300–3500 (3317, 3338, 3363, 3423, 3432, 3441, 3460, 3500)</td>
<td>υ(N-H) amines and amides I and II</td>
</tr>
</tbody>
</table>

As can be seen, the blend material also shows some common peaks that were found when the materials were tested individually and as such has proven to be a proper blended material. The blend was tested for antibacterial properties and Figure 5.17(B) shows the agar plate corresponding to the blend material.
Figure 5.17: (A) showing complete growth of bacteria; and (B) showing no growth

As can be seen from the test results, the wool–CHT-blend film possesses excellent anti-bacterial attributes. Thus with good homogeneity and blend characteristics together with anti-bacterial properties this wool–CHT-blend can be used as a wound-contact layer.
5.2: Regenerated wool fibre doped with silver nanoparticles (experimental capsule 6)

As described in the methods section, the wool fibre was doped with a specific amount of SNPs (0.1–1% OWM) in the wool solution in BMIMCl. The regenerated wool film containing SNPs was characterised by SEM for the morphological structure and presence of SNPs in the film and shown in Figures 5.18, 5.19 and 5.20.

![Surface morphology of wool film containing SNPs](image)

**Figure 5.18**: Surface morphology of wool film containing SNPs

*Saniyat Islam, PhD thesis*
Figure 5.19. Surface morphology of wool film containing SNPs 0.5% (OWM) (shown schematically with arrows)
Figure 5.20 shows the distribution of the silver nanoparticles at different locations of the film prepared. In contrast to the 100% wool, the doped film shows a different morphological appearance. The SNPs show a random distribution on the film. The doping of the SNPs is a critical controlling point in the process of formation of the wool film. Agitation of the wool solution is needed as SNPs are added on the dissolved wool solution. Agitation assures proper dispersion of the particles throughout the solution. However, if not agitated, the doping efficiency is poor and the dispersion of the SNPs is not
achieved. In the current study, a magnetic stirrer was used to provide the agitation action while doping the wool solution with SNPs. The regenerated wool film containing SNPs was further characterised using FTIR spectroscopy. The FTIR spectrum of the regenerated wool film doped with SNPs is given in Figure 5.21.

![FTIR spectrum of regenerated wool containing SNPs](image)

**Figure 5.21: FTIR spectrum of regenerated wool containing SNPs**

The FTIR spectrum of wool doped with SNPs shows similar structures as the regenerated wool. The comparison spectra of the two are shown in Figure 5.22.
Figure 5.22: FTIR spectra of (a) regenerated wool and (b) regenerated wool containing SNPs

The wave numbers and the vibrational assignments are given in Table 5.11.

Table 5.11: Vibrational assignments of the absorption peaks of the wool film doped with SNPs

<table>
<thead>
<tr>
<th>Wave Number (cm(^{-1}))</th>
<th>Vibrational assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3275</td>
<td>O–H and N–H stretch</td>
</tr>
<tr>
<td>2962 and 2875</td>
<td>C–H stretch</td>
</tr>
<tr>
<td>1624</td>
<td>C–C=C Symmetric stretch and C=O stretch from amide linkage</td>
</tr>
<tr>
<td>1598, 1565 and 1532</td>
<td>N–H bending from amine I and amide II</td>
</tr>
<tr>
<td>1384 and 1337</td>
<td>CH stretch and CH(_2) and CH(_3) bending modes</td>
</tr>
<tr>
<td>1238</td>
<td>ν(C–N) δ(N–H) random coil Amide III</td>
</tr>
<tr>
<td>1167</td>
<td>vs(S–O) cysteic acid</td>
</tr>
</tbody>
</table>
The doped films were tested for their anti-bacterial properties. A concentration below 0.5% (OWM) of doped SNPS into wool film did not show antibacterial effect as can be seen in Figure 5.23.

![Figure 5.23: Showing complete growth of bacteria (A) for 0.1%; and (B) 0.3% SNPs (OWM)](image)

The agar plates corresponding to the control and the wool film doped with 0.5% SNPs are shown in Figure 5.24.

![Figure 5.24: (A) showing complete growth of bacteria for control; and (B) showing no growth for the wool film doped with SNPs 0.5% (OWM)](image)
As can be seen from the test results the wool film doped with 0.5% (OWF) SNPs possesses excellent anti-bacterial attributes. Thus, with a good dispersion of the SNPs in the wool matrix together with excellent anti-bacterial properties, this wool film can be used as a wound-contact layer.
Chapter 6: Conclusion

The object of the project was to explore ways to utilise wool in medical textiles, particularly in wound-dressing materials. Wool was chosen for this study as it possess the highest moisture regain property (saturation regain is approximately 36%) among all the natural and synthetic polymers. The experimental sets were designed to cover two major fields of wound-dressing, namely, the use of nonwovens and films. A biopolymer chitosan (CHT) and silver nanoparticles were chosen for their antibacterial properties to be applied to wool and wool-blend materials to assess their potential use in wound-dressing materials. The concluding remarks for all the experimental capsules are discussed in this chapter.

6.1: Experimental capsule 1

For this study, a 100% wool nonwoven substrate (400 GSM) was pretreated with BMIMCl to dissolve the surface lipid layer thereby increasing the wettability of wool fibres for the subsequent application of CHT. The characteristic CHT absorption bands were noted, such as –OH and –NH₂, when the treated substrates were investigated using FTIR spectroscopy that confirmed the presence of CHT on the treated substrate.

The water absorbency/liquid uptake test revealed the absorbency property of the different samples in this capsule. The results show a decrease in the liquid uptake for wool pretreated with BMIMCl and treated subsequently with CHT. This decrease of liquid uptake can be attributed to the decline in mass of the BMIMCl-treated substrates and about 8% decrease was recorded. This negatively influenced the positive water absorption benefits provided by wool fibres. However, the CHT treatment somewhat compensated for the decreased weight, as the absorbency was observed to be improved with the increased amount of CHT deposited onto the CHT-treated nonwoven samples.
The higher water absorbency of chitosan is ascribed to its chemical features as it possesses hydrophilic hydroxyl (–OH) groups and strong hydrophilic amine groups (–NH₂) on the chitosan molecular structure. The amount of water that is bound to the hydrophilic sites in the polymer through hydrogen-bonding and other interactions, indicates the hydrophilicity of the polymer and the strength of intermolecular hydrogen bonding within the network [282, 283]. It was also observed that the handling of the treated samples became stiffer for higher CHT concentrations (0.75% and 1% OWM). The several concentrations of CHT were tested for anti-bacterial performance and the results for those >0.1% showed excellent efficacy. Thus it was concluded that 0.3% CHT-treated samples had good moisture absorbency (comparable with the untreated control) together with excellent anti-bacterial properties. These therefore offer the potential to be used as an absorbent core in a wound-dressing ensemble. The BMIMCl treatment was not continued in subsequent testing (except for the film formation), as it had been shown to cause significant weight reduction for the 100% wool nonwoven samples.

6.2: Experimental capsule 2

Based on the results obtained from experimental capsule 1, the BMIMCl was not pursued for capsule 2 developmental works. Since the wool nonwoven used in experimental capsule 1 was heavy (400 GSM), a second batch of 100% wool nonwoven was developed (150 GSM) to provide a less compact and lighter weight structure, thought to provide a better cushioning effect for the absorbent core component. In addition, a lower concentration of CHT (0.1%) was introduced to test whether it still exhibited anti-bacterial efficacy for the treated substrate. CHT was applied to the developed nonwovens by the pad-dry-cure method with a variation in concentration of 0.1%, 0.3%, 0.5% and 1% (OWM).

The scanning electron micrographs showed a smoother surface of the wool fibres as compared to the untreated fibres. This was due to the film formation of CHT on wool fibre and as such the scales of the wool fibres examined were less pronounced.
The FTIR analysis showed some characteristic IR- vibrations associated with CHT such as hydroxyl (–OH), amine N–H and O–bridge stretching from the glucosamine residue that characterise the poly-cationic nature as well as the structure of CHT.

The absorbency of the treated samples was tested using defibrinated horse blood to simulate blood or exudate absorption from a wound bed. The CHT-treatment was found to affect the blood absorption of wool substrates when compared with the untreated samples as shown in Figure 4.13. At lower concentrations of chitosan (0.1% and 0.3%), absorption of the liquid (water/blood) was affected by a combination of wool and CHT. Swelling behaviour of CHT is an established fact [128, 280]. This is reflected in Figure 4.13 where a significant rise in absorption was noticed as compared to the untreated sample at the two lower CHT concentrations. The increase in blood absorbency can be attributed to the chemical structure of CHT since it possesses hydrophilic hydroxyl (–OH) groups and strongly hydrophilic amine groups (–NH₂) leading to increased hydrogen bonding [281-283]. However, at CHT concentrations of 0.5% and 1%, a decline in absorption is noticed. A plausible explanation based on swelling behaviour of CHT was proposed in Figure 4.14.

Handling properties similar to those already noted in experimental capsule 1 were also observed for the wool nonwoven samples treated with CHT. Higher concentrations such as 0.5% and 1% of CHT- application also resulted in a stiffer and more rigid material for experimental capsule 2. This can be attributed to the film formation characteristic of CHT application by pad-dry-cure process. At higher concentrations, CHT solidifies when cured, perhaps locking the nonwoven matrix into a reduced loft and higher matrix density before it has time to fully recover the loft lost during padding. The decreased loft of the treated material has a constraining effect on liquid absorption. Since the nonwoven substrate is considered to be a porous matrix, a reduced pore size may contribute to the observations. The study of the variations introduced by the CHT-treatment and subsequent pad-dry-cure process is suggested as future work.
Thus analysing the absorbency results 0.1% and 0.3% CHT-treated samples were better in terms of blood absorption of all the CHT-treated samples tested.

All the samples, both untreated (control) and treated, were tested for antibacterial properties and it was found that the MIC of CHT was found to be 0.3%. The 0.1% CHT-treated samples were biologically inactive. Therefore the 0.3% CHT-treated samples were found to be the optimum possessing good absorbency together with excellent anti-bacterial attributes.

6.3 : Experimental capsule 3
A nonwoven sample consisting of wool–viscose (50/50) was developed (150 GSM) to provide a less compact and lighter weight structure, together with a projected extra absorbency. It was also thought that inclusion of viscose would provide a better cushioning effect. In addition, a lower concentration of CHT (0.1%) was introduced to test whether it still exhibited anti-bacterial efficacy for the treated substrate.

The scanning electron micrographs (Figures 4.17 and 4.19) showed the surface of CHT-treated wool and viscose fibres as compared to the untreated fibres. Chitosan treated samples showed a smoother surface since CHT formed a film on both wool and viscose fibres and as such the scales of the wool fibres examined were less pronounced.

The FTIR analysis confirmed some of the characteristic IR vibrations associated with CHT such as hydroxyl (–OH) and amine (–N-H) functional groups which characterise the poly-cationic nature of CHT.

The absorbency of the treated samples was again tested using defibrinated horse blood, to simulate blood or exudate absorption from the wound bed. The CHT-treatment was found to influence the blood absorption of wool substrates when compared with the untreated samples as shown in Figure 4.21. At lower concentrations of chitosan (0.1% and 0.3%), absorption of the
liquid (water/blood) is effected by a combination of wool and CHT. The swelling behaviour of CHT is recognised [128, 280]. This is reflected in Figure 4.21 where a significant increase in absorption is noticed as compared to untreated sample and this can be attributed to the chemical structure of CHT providing increased hydrogen bonding [281-283]. However, at CHT concentrations of 0.5% and 1%, a decline in absorption is noticed. A plausible explanation based on swelling behaviour of CHT was proposed in Figure 4.14.

The similar handling properties experienced in experimental capsule 1 and 2 were also observed for the wool–viscose nonwoven samples treated with CHT. Higher concentrations such as 0.5% and 1% of CHT-application also resulted in a stiffer material for experimental capsule 2. This can be ascribed to the film formation characteristic of CHT application by pad-dry-cure process. Consequently, at higher concentrations, CHT solidifies when cured, locking the nonwoven matrix at a reduced loft and higher density. The decreased loft of the treated material has a constraining effect on the movement of the fibre mass and thereby can also restrict liquid absorption. Thus analysing the absorbency results 0.1% and 0.3% CHT-treated samples were better in terms of blood absorption of all the CHT-treated wool–viscose samples tested.

All the samples, both untreated (control) and treated samples were tested for anti-bacterial properties and it was found that the 0.1% CHT-treated samples were biologically inactive. Hence, the MIC of CHT sample used in this study was found to be 0.3%.

Therefore 0.3% CHT-treated wool–viscose sample was superior in absorbency together with their anti-bacterial attributes and can be recommended as a suitable substrate for the absorbent core component in the construct of a wound-dressing ensemble.
6.4 : Experimental capsule 4

This experimental capsule was aimed at introducing and adapting a coating technique from the automotive paint industry to textiles named ‘electrospraying’. A modification of traditional electrospraying technique was done to facilitate direct coating of the polymer onto the substrate by placing the nonwoven substrate between the spray source and the charged conducting collector. The objective was to get a thin coating of CHT on the top surface of wool nonwoven and to provide a matrix with a wound contact layer (which is the a CHT-sprayed surface) backed by the wool. Based on the results of experimental capsules 1, 2 and 3, the CHT concentration of 0.3% was selected for application on the 100% wool substrate (400 GSM).

After surface coating, the developed samples were characterised for coating performance and were investigated using SEM. Scanning electron micrographs showed that the CHT application through electrospraying resulted in successful fibre coating and deposition of CHT on the surface of the wool nonwoven substrate. The objective of the exercise was to engineer the resultant substrate such that the CHT deposited surface could be used next to the skin and the wool matrix could provide the structure absorbent core-component of the wound-dressing. It was also shown that SEM revealed that the CHT-film bridged between adjacent fibres.

The anti-bacterial property was assessed and results indicated that surface deposition 0.3% CHT by electrospraying on developed nonwoven proved to be excellent.

This technique should be explored in more detail in future research; however, to the best of the authors’ knowledge this was the first time where electrospraying was introduced to coat a textile substrate.

6.5 : Experimental capsule 5

In experimental capsule 1(only), BMIMCl was utilised as a pretreatment step to remove the scales and thereby increase wettability. Experimental capsules
5 and 6 investigated BMIMCl as a dissolution medium used to dissolve wool for producing blended films of wool and CHT (experimental capsule 5) as well as wool films doped with SNPs (experimental capsule 6).

100% wool fibre (fat extracted) was dissolved in BMIMCl and was regenerated from the IL using an acetone-water medium. The three-dimensional molecular binding structure of wool comprises intermolecular hydrogen, ionic and physical forces of attraction and significantly, the sulphur-containing disulphide bonds. To enable the successful dissolution process, these disulphide bonds must be broken. Therefore it was essential that the developed films were investigated for their morphological behaviour. The scanning electron micrographs revealed a diffused fibrous structure. It was shown that small wool fibre remnants remained in the film structure but the scale structure of these remnants was not visible. Such undissolved or semi-dissolved fibre remnants have not been previously reported in the literature. After the dissolution process had started, the colour of the BMIMCl changed from clear to pale yellow, to an amber yellow. This change of colour was attributed to the breakage of disulphide bonds of the keratin structure. This was characterised using FTIR and Raman spectral analysis of the original fat-extracted wool and wool film regenerated from the BMIMCl solution. Within the Raman spectrum, it was observed that the disulphide bands, which were located at 415 cm\(^{-1}\) were lower than original 432 cm\(^{-1}\). This suggested a reduced presence of bonds that supported the observation of disulphide bond breakage. However, the presence of the disulphide band might have risen from the undissolved or semi-dissolved wool fibre remnants. This has been the first instance in which the regenerated wool was characterised utilising Raman spectra.

FTIR analysis showed characteristic wool vibrational bands in the regenerated wool film and these were compared with natural wool (fat extracted) in Figure 5.4 and the distinctions were listed in Table 5.5.

CHT was dissolved using the same method as for wool, and a film was formed. This was characterised using SEM, FTIR and Raman spectroscopy
to identify any morphological and chemical structural changes during dissolution and subsequent regeneration from the BMIMCl solution.

The regenerated CHT was also assessed for the degree of deacetylation (DD%) using FTIR. Calculated DD values of 68% and 70% were recorded for the commercial CHT samples and both these values were lower than the specification provided by the manufacturer. However, for the regenerated CHT samples, the calculated values of DD were 84% and 89%. These values were in close agreement with the specification provided by the supplier.

The regenerated CHT showed a similar FTIR spectral profile to that found in commercial CHT. However, higher hydrogen bonding was noticed in the regenerated CHT in both FTIR and Raman spectra. In addition, the FTIR and Raman spectra also indicate that no derivatisation of CHT occurred during the dissolution and regeneration stages.

Wool and CHT were also blended together at 50-50 ratio (w/w) in a BMIMCl solution and then regenerated as a film. This film was aimed at providing the skin-contact layer of a wound-dressing. This wool–CHT-blend film was also characterised for its potential end-use as a wound-dressing component.

The scanning electron micrographs of the blend film revealed a homogeneous morphological structure, in contrast to the 100% wool (Figure 5.1) or 100% CHT (Figure 5.7) films when investigated using SEM. It is noticeable that the prepared blend films were not displaying characteristics of a phase separated structure. With increasing CHT content (50%), the morphology of the blend films changed dramatically from the diffused wool structure to a homogeneous structure. In addition, the FTIR spectroscopy proved that there were hydrogen bonding interactions between the hydroxyl groups of wool and CHT which could account for this miscibility between the components [162].
FTIR and Raman spectral analysis showed that the blend material shared the spectral profile of both 100% wool and 100% CHT films. Furthermore, an increase in hydrogen bonding interactions was observed in the blend film which was attributed to the homogeneous nature of the film.

The anti-bacterial efficacy of the developed film containing 50-50 wool–CHT was examined and was found to be excellent.

Therefore, it was concluded that this novel film of wool–CHT (50-50) blend showed a homogeneous structure and excellent anti-bacterial properties and as such was suitable to be used as a wound contact layer in a wound-dressing component.

6.6: Experimental capsule 6
The dissolution of wool fibre in a BMIMCl solution opened up opportunities to incorporate other active components into the regenerated wool matrix. This experimental setup aimed to investigate the inclusion of silver nano-particles (SNPs) into the wool whilst dissolved in a BMIMCl solution. The purpose was to dope the dissolved 100% wool with SNPs and regenerate the wool film as described in experimental capsule 5. These novel film structures would contain 100% wool doped with SNPs that could be used as an active wound contact layer for a wound-dressing.

The SEM micrographs showed a random distribution of SNPs on the wool matrix as expected (Figures 5.19 and 5.20). This random distribution should facilitate the anti-bacterial property of the developed film.

FTIR spectroscopy of the developed film against the 100% wool film prepared in experimental capsule 5 showed a similar spectral profile and as such did not show silver interactions with wool fibre. There was no visible conglomeration of silver nanoparticles with dissolved wool during the doping process.
This activated 100% wool film was investigated for its anti-bacterial property and the results indicated that developed film possessed excellent anti-bacterial attributes at 0.5% concentration (OWM). Therefore, it was concluded that regenerated wool film doped with SNPs would provide another avenue to be used as a wound contact layer in a wound-dressing.

6.7: Summary

This research highlights the use of several nonwoven pure wool matrices as the core components in a wound-dressing ensemble, exploiting its outstanding moisture regain properties. The absorbency was comparable with matrices of 50-50 wool–viscose blends. Inclusion of CHT in the matrices, by either a traditional pad-dry-cure or a novel electro-spraying process, imparted excellent anti-microbial attributes with a minimum inhibitory concentration of 0.3% (OWM). In addition, CHT increased absorbency when applied at low concentrations (<0.5% OWM). CHT applied using the electro-spraying technique coated the 100% wool nonwoven sufficiently well to provide outstanding anti-bacterial properties. These combinations have proved to be an innovative development in wound management products.

The use of an environmentally benign IL solvent BMIMCl was investigated to produce a unique wool–CHT blend film. Dissolution and regeneration of wool and CHT from BMIMCl provided homogeneous blend films of 50-50 wool-CHT. The resultant film proved to be bioactive and hence may have the potential to be used as a contact layer for a wound-dressing material. Silver nanoparticles could also be incorporated into 100% wool dissolved in BMIMCl and the resulting doped wool film also exhibited antimicrobial features at relatively higher concentrations (0.5% OWM). This introduces another candidate for consideration as an active wound contact layer. However, CHT being biodegradable, non-toxic and can be used in low concentrations is recommended as toxicological behaviour and potential health concern by using SNPs are yet to be comprehensively understood.
6.8 : Future work

- Since the nonwoven substrate is considered to be a porous matrix, the pore size distribution may contribute to the observations. The study of the variations introduced by the CHT-treatment and subsequent pad-dry-cure process is suggested as future work.
- Application of CHT by electrospraying can be studied further on the natural and manmade woven and knitted structure.
- Shelf-life study of the developed material should be conducted.
- Toxicological testing of SNPs should be conducted before using it.
- Clinical trials can be conducted based on the current finding for future commercialisation of wool-based wound-dressing products.
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