Cape Peninsula
University of Technology

THE SUPERCRITICAL SOLVENT IMPREGNATION OF TEXTILES WITH THERAPEUTIC COMPONENTS

by

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Thesis submitted in fulfilment of the requirements for the degree

Master of Engineering: Chemical Engineering

in the Faculty of Engineering and the Built Environment

at the Cape Peninsula University of Technology

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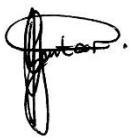
Date submitted: 5 September 2022

DECLARATION

I, Geraldine Joan Lentoor, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



5 September 2022

Date

ACKNOWLEDGEMENTS

I wish to thank:

- My parents, for their unwavering love and support.
- My supervisor Mr T.F.N Madzimbamuto and co-supervisor Prof T.V Ojumu, for their continuous guidance and support.
- Dr Le Roes-Hill, for the antimicrobial analysis performed
- The Chemical Engineering technical staff members, Mrs Hannelene Small and Mr Alwyn Bester for their assistance throughout my experimental runs.
- The High-Pressure Separations group, for their assistance in the lab.
- NRF and Nedbank, for the funding provided during my first and second year.

The financial assistance of the National Research Foundation towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the National Research Foundation.

DEDICATION

Dedicated to my mother and father for their unwavering love, support, and trust.

ABSTRACT

Hospital-acquired infections have become common. One of the mechanisms by which they spread is through adherence to the textiles used as apparel or as bedding. One effective method to reduce the spread of this type of infection is by functionalising the material, i.e. adsorbing antimicrobial agents onto the textile fibre. Current industrial processes used to perform this process are by adsorption of the appropriate antimicrobial agents onto the fibre's surface. This method has several drawbacks – the use of water as a medium for absorption, which requires treatment for re-use. Synthetic compounds are used; these are harmful to aquatic life and accumulate in the food chain. Therefore, investigating an alternative method to functionalise textiles while avoiding these drawbacks is required.

An alternative approach is to absorb the antimicrobial agent into the fibre matrix using a waterless mass-transfer medium such as supercritical carbon dioxide ($scCO_2$). The absorbed antimicrobial agent diffuses to the fibre's surface over time during use. Besides being waterless, this approach could result in higher amounts of antimicrobial agent retention than the conventional methods since the whole fibre matrix, not just the surface, act as the agent's reservoir. $scCO_2$ is particularly attractive in this respect due to its moderate critical parameters: its critical temperature and pressure are 31^{o}C and 7.4 MPa, respectively. Thus, thermal degradation of temperature-sensitive therapeutic components and materials can be avoided—no aqueous waste results directly from this alternative process.

Synthetic compounds generally have a lower lethal concentration value (LC50) than natural compounds (the amount required to be potent to a specific microbe). This means that more of the natural compound is required, by mass, to achieve the same effect as the synthetic. In addition, synthetic compounds also have a higher likelihood of being non-biodegradable. Since the alternative method could show higher retention of the agent inside the fibre matrix, it follows that this method could achieve the same effect as synthetic compounds applied at ambient conditions.

Although some studies have shed light on the feasibility of the sorption of antimicrobial agents into polymeric material using $scCO_2$ as the medium, no comprehensive study could be found that compared the performance of the different commonly used materials used in hospitals when loaded with natural antimicrobial compounds. This work compares the

performance of fabric samples loaded with antimicrobial extracts of the plant, under supercritical and ambient conditions, against specific microbes. The fabric types are lycra, cotton, and polyester. The extracts are the essential oils and resin obtained from buchu (*Agathosma crenulata*), tea tree (*Melaleuca alternifolia*), and hops (*Humulus lupulus*).

The results show that, indeed, all the textile fibres investigated retained a higher amount of the agent within them when the agent was infused under high pressure and subjected to a slow depressurisation. Further, antimicrobial tests showed that the potency was much more persistent than that of control samples. This investigation thus confirms the hypothesis that infusion of bioactive compounds under high pressure using $scCO_2$ results in higher retention of the agents, which can be active over an extended period. In addition, the immersed cotton samples exhibit an activity greater than the polyester and lycra samples. This is despite cotton having the lowest solute retention. It further opens the path to using natural compounds to produce functional textiles.

Contents Page

LIST OF TABLES

LIST OF FIGURES

Glossary

ρ_c	Critical density
AST	Antimicrobial susceptibility testing
CO_2	Carbon dioxide
CP	Critical point
Df	Degree of freedom
GC-MS	Gas chromatography- mass spectrometry
HAI	Hospital-acquired/associated infections
HCW	Health care workers
ICU	Intensive care unit
LC50	Lethal concentration 50%
MDROs	Multi-drug resistant organisms
MIC	Minimum inhibitory concentration
MSA	Measurement system's analysis
ΔP	Pressure drop/ depressurisation
Pc	Critical pressure
ppm	Parts per million
PVT	Pressure-Volume-Temperature
$scCO_2$	Supercritical carbon dioxide
SEM	Scanning electron microscopy
SEM_{stats}	Standard error margin
SSE	Supercritical solvent extraction
SSI	Supercritical solvent impregnation
Tc	Critical temperature
Tp	Triple point
% T	% Transmittance
%wt	(g of solute/g of textile sample) * 100
VLE	Vapour liquid equilibrium

Chapter 1 INTRODUCTION

1.1 BACKGROUND

For millennia, traditional medicines have included flora as the primary active component to treat various ailments. However, microbes and their connection with diseases were only discovered in 1665-83 by Robert Hooke and Antoni van Leeuwenhoek (Gest, 2004). The dawn of the antimicrobial age was instigated by the discovery of the first natural antibiotic by Alexander Fleming in 1928 (Lobanovska & Pilla, 2017). Since these scientific discoveries, ways to reduce the spread of diseases by curbing the spread of pathological microbes have been sought. Various methods to curb the spread have been used, including washing hands, utensils, and safe food processing (Drexler, 2011). The use of semi-synthetic and synthetic chemicals to achieve the same was inspired by the discovery of the first natural antimicrobial. Today, the overuse and misuse of synthetic compounds are blamed for bacteria mutation into multidrug-resistant forms (Mérillon, 2018). The non-biodegradable nature of some synthetic drugs leads to their accumulation in waterways and consequently pose a danger to aquatic life, humans and ecosystems (Uddin, 2014).

Antimicrobial agents act via monotherapy or synergistic pathways. Monotherapy refers to using one antimicrobial agent to treat a pathogen. Many synthetic antimicrobial substances act via monotherapy pathways. Whereas natural antimicrobial substances act via synergistic pathways. This is because natural substances do not owe their activity to one component. Often various components in the substance act in synergy, improving the antimicrobial activity of the natural substance. This synergism makes it more complicated for microbes to develop resistance (Bollenbach, 2015);(Mérillon, 2018). Additionally, natural substances are inherently biodegradable, and the active components are present in such low concentrations that they pose much less environmental risk (Grenni et al., 2018).

Research has shown that textile surfaces such as scrubs, uniforms, bed linen, privacy curtains and lab coats harbour a wide variety of bacteria (Pinon et al., 2013);(Koca et al., 2012);(Fijan & Turk, 2012). Textiles are responsible for the spread of hospital acquired/associated infections, particularly those caused by organisms that are resistant to antibiotics, such as *Klebsiella pneumonia* and *Acinetobacter baumannii*, the latter being notorious for infections in intensive

care units (Alsan & Klompas, 2010). Soft surface infection management can thus provide an opportunity to reduce hospital-acquired infections significantly.

Textile impregnation is a technique that has been used to impart antimicrobial agents onto the textile to produce the required bioactive textiles for surface infection management (Kim et al., 2010). Replacing synthetic with natural antimicrobial substances solves the issues related to the use of synthetic antimicrobial substances. However, natural antimicrobial substances are used in conventional processes such as the pad-dry-cure; the very processes that involve the use of toxic chemicals and large quantities of water (Tawiah & Badoe, 2016),(Innovation In Textiles, 2010). Thus, alternative processes should also be investigated.

1.2 WATERWAY POLLUTION

Antibiotics are antimicrobial agents used against bacteria and fungi present in human or animal hosts (Grenni et al., 2018). They are classified as natural, semi-synthetic and synthetic. Semi-synthetic and synthetic antibiotics are more stable and less susceptible to biodegradation if at all (Grenni, Ancona and Barra Caracciolo, 2018). Thus, the presence of antibiotics results in bioaccumulation in ecosystems.

Human influence is responsible for the contamination of natural ecosystems with antibiotics (Ojemaye & Petrik, 2021). Hotspot environments for antimicrobials include hospitals and textile industries (Kraemer et al., 2019). These environments produce effluent streams that eventually end up in waterways. Hospital effluent is contaminated with high concentrations of a broad range of antimicrobial agents due to the extensive application of antibiotics in the medical sector (Kraemer et al., 2019)(Grenni et al., 2018). In the textile sector, a copious quantity of water is used where processes use up to 150 L of water per kg of textile processed (Innovation In Textiles, 2010). This water forms part of the effluent produced by the textile industry. The textile industry effluent wastewater has a significant chemical waste load since chemicals, dyes and other substances are used (Kraemer et al., 2019). In cases where antimicrobial finishing is performed, the wastewater from this process is contaminated with high concentrations of antimicrobial agents as well as other chemicals. The hospital and textile industry effluent are directed to WWTPs (Kraemer et al., 2019).

Conventional WWTPs do not remove and deactivate all pharmaceutical residues (Ncube et al., 2021). Ncube performed a study on effluent streams produced by WWTP and the leachates from a nearby dumpsite leading to the Klip River situated in Gauteng province. This study confirmed wastewater effluents and dumpsites leachates as hubs for the release of antimicrobials into the surrounding environment (Ncube et al., 2021). Therefore, antimicrobials are present in the effluent streams that are released into the environment. Oyemanye conducted a study around False Bay, situated on the Southside of Cape Peninsula in South Africa (Ojemaye & Petrik, 2021). The study revealed that the effluent streams released from WWTPs into the Bay were treated inadequately, resulting in the contamination of the Bay with pharmaceuticals. In developing countries such as South Africa, wastewater sludge is often used for fertilizer and thus indirectly introduce antimicrobials into the surrounding environment (Ojemaye & Petrik, 2021). Many residents of Africa do not have access to safe drinking water and often use a nearby body of water as their source for cooking, irrigation, and fishing activities (Ojemaye & Petrik, 2021). These very water sources are the dumping grounds for wastewater treatment and pharmaceutical effluent streams.

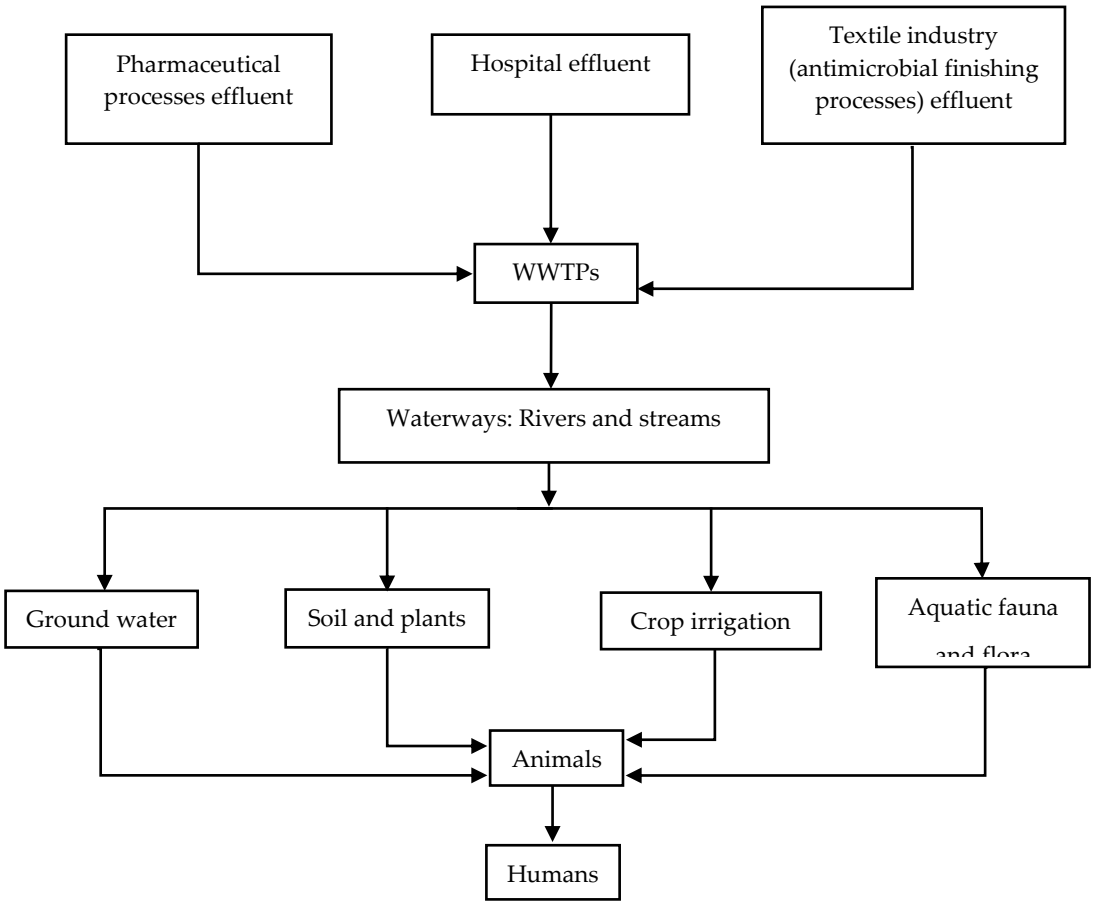


Figure 1: Ecosystem pollution by antimicrobial agents (redrawn from (Hussain et al., 2016))

Waterways contaminated by pharmaceuticals thus pose a threat to biota and human health. Additional sources of antimicrobials in developing countries include dumpsites, septic tank leakages and landfill leachates. Consequently, Africa has a much higher antimicrobial micro contamination when compared to Europe (Ncube et al., 2021). According to Oyemaye, the lack of stringent wastewater discharge limitations in South Africa has allowed for the release of antibiotics and or active metabolites into ecosystems including freshwater and marine (Ojemaye & Petrik, 2021).

The presence of antibiotics in ecosystems has a negative effect on the environment. Antimicrobial agents were developed to stop the growth (bacteriostatic) and or kill (bactericidal) microbes (Grenni et al., 2018). The effect that an antimicrobial has on the microbe is concentration-dependent (Grenni et al., 2018). The minimum inhibitory concentration is the lowest antimicrobial agent concentration needed to visibly inhibit microbial growth (Grenni et al., 2018). If the antibiotic concentration in the ecosystem is higher than the MIC certain microbes essential to ecosystem functions are inhibited or killed, actively diminishing the diversity of the microbial community (Grenni et al., 2018). This is detrimental to the ecosystem as various microbes are involved in crucial ecological functions. Functions include, but are not limited to biomass production, nutrient transformation, organic matter degradation, maintenance of water and soil quality and biodegradation of toxic compounds (Grenni et al., 2018). Disrupting the degradation of contaminants thus aids in the bioaccumulation of contaminants in natural ecosystems.

Conversely, when the concentration is lower than the MIC, the concentration for bacteriostatic or bactericidal action is too low. The low concentration has accelerated the development of drug-resistant microbes (Grenni et al., 2018). The presence of antimicrobials can alter the dynamics and physiology of natural microbial communities (Grenni et al., 2018). Where microbial physiology refers to the structure-function relationships in microorganisms (Cole, 2018). In layman terms, the presence of antimicrobials in the environment affects the way microorganisms respond to environmental stimuli. Antibiotic residues have negative effects on an ecosystem's fauna and flora (biota) (Polianciuc et al., 2020). The effect on biota is dependent on their position in the food chain because of the continued biomagnification of antibiotics as the trophic levels increase (Polianciuc et al., 2020). The transfer of antibiotics

between biota is through consumption of contaminated plants and or water. The presence of antibiotics in plants impact photosynthesis, mitochondria, and potentially delay germination (Polianciuc et al., 2020). Eventually, these antibiotics are consumed by humans and can alter the human microbiome, having adverse health effects (Polianciuc et al., 2020).

Increasing rates of high chemical load influent streams are placing WWTPs under increasing pressure (Bega, 2018). Thus, the presence of a broad range of active pharmaceuticals as contaminants has become more common. This has led to the contamination of more freshwater and marine ecosystems by organic chemical pollutants (Bega, 2018). Many of these contaminants are not easily removed once in the environment (Bega, 2018). The presence of antibiotics often, indirectly leads to bioaccumulation due to the persistent nature of many antibiotics (Ojemaye & Petrik, 2021). Bioaccumulation takes place when the rate of contaminant absorption surpasses the rate of contaminant removal (Ojemaye & Petrik, 2021). The bioaccumulation increases the antibiotic concentration and thus worsens the direct and indirect effects (Grenni et al., 2018).

1.3 CURRENT METHODS FOR TEXTILE IMPREGNATION

Textile impregnation is the saturation of a solid matrix with a functionalizing solute carried by a fluid solvent (Zizovic *et al.*, 2014). Various impregnation methods have been used to impart textiles with antimicrobial properties to develop specialised textiles (Zizovic *et al.*, 2014). Currently, direct application technology and microencapsulation processes are used for textile impregnation.

1.3.1 DIRECT APPLICATION

The direct application technique is one of the oldest means of impregnating textiles. This technique is quite simplistic. An example includes the pad-dry-cure, which simply entails immersing the textile in the aqueous antimicrobial solution (Tawiah & Badoe, 2016). Followed by the padding of the textile through the squeeze rolls and concluded by the drying of the textile. Unfortunately, this technique does not produce a satisfactory fastness due to the weak forces present between the solute and the solid matrix (Tawiah and Badoe, 2016).

1.3.2 MICROENCAPSULATION

Microencapsulation is a physicochemical process used to impregnate textiles. Where microscopic quantities of solid, liquid, or gaseous active agents are covered by a thin polymeric film, forming microcapsules (Yip & Luk, 2016). This technique is applied in the textile industry by immersing textiles in a microencapsulated solution. Upon immersion, the microcapsules form a thin protective layer on the textile (Kolte & Sharma, 2014). In the presence of agitation or mechanical pressure, capsules burst. The burst capsule releases the agent in a slow, controlled manner (Yip & Luk, 2016). This technique has the potential for a relatively high impregnation load. It protects the antimicrobial agents from the elements to which it is susceptible and could cause degradation (Zhu *et al.*, 2018). However, the textile texture is altered by this technique and can lead to the production of harmful components (Tawiah and Badoe, 2016)

Although natural agents can be used against various microbial organisms, it is still essential that the methods in which these agents are used promote fastness, a high impregnation load and deeper penetration of the polymer matrix (Tawiah and Badoe, 2016).

1.3.3 OTHER TECHNIQUES

Other techniques include nanotechnology, the insolubilisation of active substances in fibre, fibre surface modification and the cross-linking technique (Tawiah & Badoe, 2016). Although natural agents can be used against various microbial organisms, it is still essential that the methods in which these agents are used promote fastness, a high impregnation load and deeper penetration of the polymer matrix (Tawiah and Badoe, 2016).

1.4 ADVANTAGES AND DISADVANTAGES OF CURRENT METHODS

Direct application techniques are simple techniques with relatively low material costs (Tawiah & Badoe, 2016). A few drawbacks are associated with using these current techniques (Tawiah & Badoe, 2016). These include the limited depth of solute penetration, use of toxic organic solvents, unwanted reactions (Casas et al., 2018), heterogeneous dispersion, low impregnation load, thermal degradation of thermally labile compounds, effluent waste production, products with solvent residue, and use 150L of water for 1 kg of textile (Innovation In Textiles,

2010). The water-intensive nature of these methods aid in the micro-contamination of waterways with effluent waste streams and deteriorate the environment (Carneiro et al., 2010)

Water has become scarce worldwide. This scarcity is principally attributed to climate change and an increase in the world population that places a greater demand for water in the agricultural sector (Misra, 2014). Current impregnation techniques use significant quantities of water (Series and Science, 2017). It is, therefore, preferable to develop processes that are water-free (Montero *et al.*, 2000).

1.5 PROPOSED ALTERNATIVE PROCESS ROUTE: SUPERCRITICAL SOLVENT IMPREGNATION

Supercritical solvent impregnation has been presented as a sustainable, green alternative for the impregnation process (Casas et al., 2017). Carbon dioxide is often used as the solvent, but other substances are also common, such as propane and butane (Belinsky, 2011). Supercritical solvents have many advantages, without the drawbacks associated with the current techniques. Advantages include operating at a low temperature and thus preventing thermal degradation, anhydrous impregnation, easier penetration and impregnation of the polymer matrix, and homogeneous distribution achieved with no waste production (Casas, Mantell and Ossa, 2017).

Carbon dioxide has a plasticizing effect on polymers. This effect is usually accompanied by a decrease in the glass transition temperature of the polymer (Kikic and Vecchione, 2003). The sorption of carbon dioxide into the polymer matrix causes the swelling up of the fibre and changes the physical properties of the polymer. This swelling effect and the near to zero surface tension, low viscosity and small size of carbon dioxide allows for rapid penetration of the polymer matrix (Weidner, 2018). A relatively rapid sorption process follows this. The density of carbon dioxide can be adjusted to manipulate the solubility of carbon dioxide (Weidner, 2018).

Furthermore, the swelling effect and the decrease in the glass transition temperature also cause the softening of the polymer and result in a deeper, improved molecular diffusion of the impregnate(Weidner, 2018). The adsorption that takes place is either physical or chemical.

Lastly, supercritical fluids such as carbon dioxide can interact with the polymers above their softening point and with polymers in the glassy state (Weidner, 2018). This is important as glassy polymers are generally more difficult to infused into (Jansen, 2020).

1.6 RESEARCH PROPOSAL

1.6.1 PURPOSE OF THE STUDY

The purpose of this study is to compare the performance of fabric samples loaded with antimicrobial plant extracts, under supercritical and ambient pressure conditions; against specific microbes.

1.6.2 PROBLEM STATEMENT

The transmission of infections within hospitals is a serious problem within the healthcare sector (Khan et al., 2015). Various vehicles for the transmission of pathogens have been identified, of which one is the textiles worn and used by the medical personnel (Mitchell et al., 2015). Textiles offer an ideal breeding ground for microorganisms, especially when the textile and humans make contact. The human body release fluids that are a source of nutrients for pathogens provide a moist and warm environment and thus an ideal breeding ground for pathogens (Kim, Kim and Rhee, 2010). One way of reducing the growth of pathogens within textiles is to load the textile fibre with antimicrobial agents via textile impregnation. Both natural and synthetic antimicrobial agents have been used in the development of these specialized textiles. The current textile impregnation processes used have several drawbacks that have become more problematic over the years.

Current processes such as the direct application technique and microencapsulation (see section 1.4) have various drawbacks (Tawiah and Badoe, 2016). In general, these drawbacks include heterogeneous dispersion, a low impregnation load, the use of toxic organic solvents, solvent residue on textiles and inadequate fastness (Casas, Mantell and Ossa-fernández, 2018). The inadequate fastness alludes to the single-use attribute of these textiles; this is because of the weak attractive forces present. The textiles processed by these methods do not retain antimicrobial properties for very long. Therefore, the potency of the textiles against pathogens decreases rapidly. Upon laundering, the agents that leach off the textiles end up in the waterways (Uddin, 2014).

The accumulation of antimicrobial agents in waterways is worse if the agents are synthetic. Synthetic antimicrobial agents are non-biodegradable, have toxic effects and negatively impact ecosystems (Uddin, 2014), and this contributes significantly to the contamination of waterways. All the factors mentioned above make it vital to find an alternative method to produce bioactive textiles. Over the years, microbes have become more resistant to antimicrobial agents (Zizovic, 2018), creating a need for non-synthetic agents that possess antimicrobial activity (Mérillon, 2018). South Africa is host to numerous indigenous flora that possess enormous antimicrobial activity, such as hops, tea tree and buchu essential oil.

1.7 RESEARCH QUESTIONS, AIM, OBJECTIVES AND HYPOTHESIS

1.7.1 HYPOTHESIS

It is hypothesised that the impregnation of textiles with natural therapeutic components will produce multi-use textiles with a better solute loading, a superior fastness and longer-lasting antimicrobial activity compared to current methods.

1.7.2 RESEARCH QUESTIONS

a) How much of the antimicrobial agent can be retained per unit mass of fibre?
b) What is the persistence of the retained solutes?
c) What effect does pressure have on textile impregnation?
d) What is the potency of the impregnated textiles in terms of its antimicrobial activity?

1.7.3 RESEARCH AIM

The aim of this work was to compare the performance of fabric samples loaded with antimicrobial plant extracts, under supercritical and ambient pressure conditions; against specific microbes.

1.7.4 OBJECTIVES

a) To determine the solute retention immediately after textile impregnation.
b) To analyse the persistence of the retained solutes in an isolated, homeostatic environment.
c) To assess the effect of pressure on textile impregnation.
d) To determine the antimicrobial activity of the impregnated textile samples.

1.8 SUMMARY OF RESEARCH APPROACH

This study explores the use of SSI (supercritical solvent impregnation) to infuse textiles as opposed to conventional processing routes. However, the technical feasibility of SSI needs to be assessed first. In this study the technical feasibility is comparison based. Where the effect of high pressure on the impregnation yield, load, textile bioactivity and persistence of the active solutes is compared to that of a simple solution immersion process *Figure 2: Research approach* illustrates the research approach adopted to ultimately assess the solute retention, bioactivity, and persistence of the solutes and compare it to that achieved through ambient solution immersion.

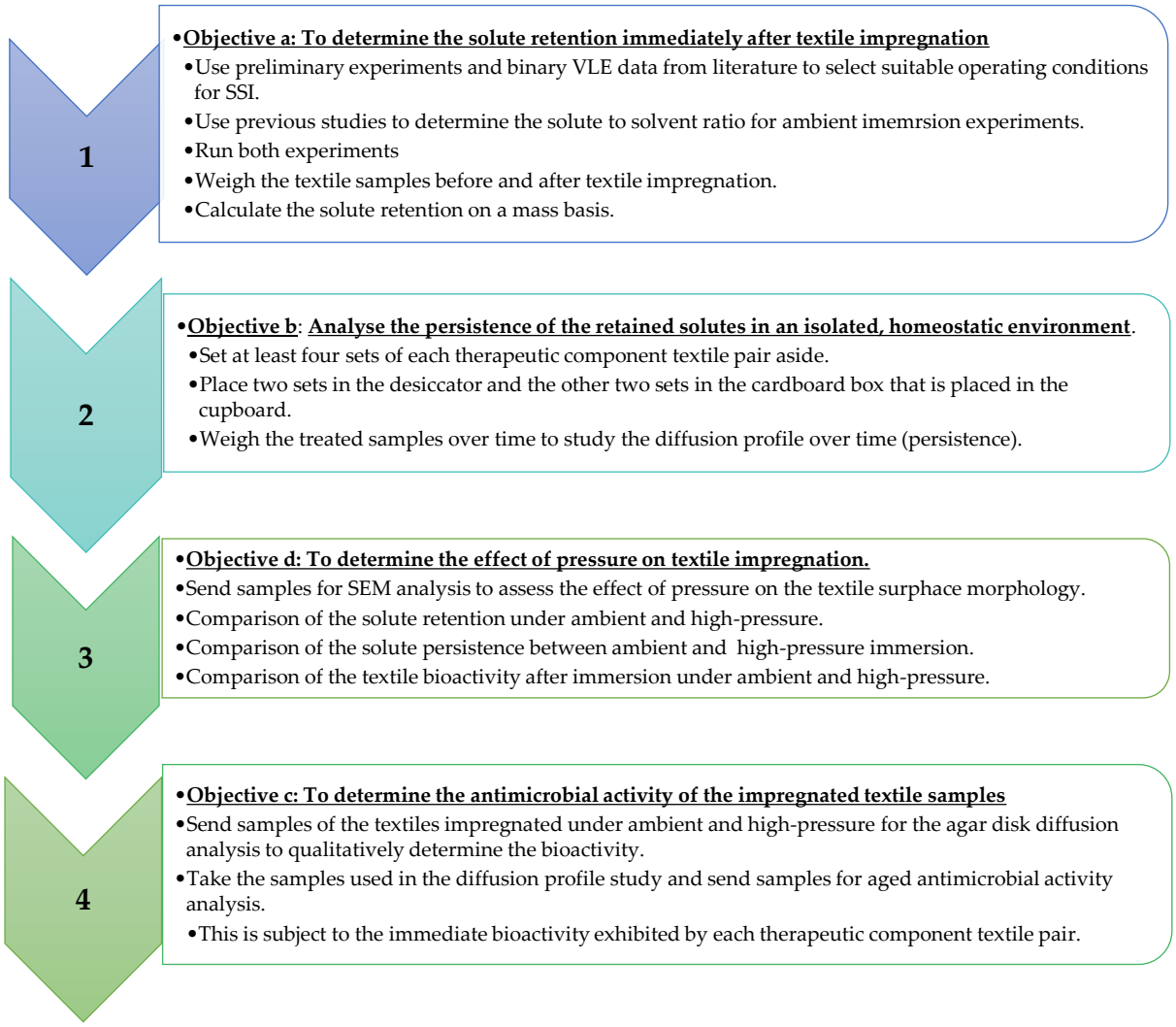


Figure 2: Research approach

1.9 SIGNIFICANCE

A textile that exhibits antimicrobial properties can curb the spread of nosocomial infections (Mariscal et al., 2011), more so a textile that acts as a barrier. When a natural therapeutic component is used, the development of resistant pathogens is slowed down (Mérillon, 2018).

These studies enrich current literature on the $scCO_2$ impregnation of fabric samples loaded with different antimicrobial plant extracts. Investigating this green, innovative process helps fill the existing gap in national processing, research, and development. This allows South Africa to move closer towards being a knowledge-based economy in line with one of South Africa's initiatives.

1.10 LIMITATIONS OF THE RESEARCH PROJECT

- CO_2 is the only supercritical fluid used in this research.
- The textiles are only tested for bioactivity against a select few bacteria.
- The process conditions selected cover a small range of the possibly viable operating conditions.
- A limited range of indigenous therapeutic components are impregnated into the textiles.
- The wash and rub/ abrasion fastness are not assessed.
- The compounds that infused into the textile are not identified.
- The optimum conditions for SSI are not investigated.
- The use of dispersant in SSI is not assessed.

1.11 THESIS DELINEATION

This research focuses on applying South African extracts as therapeutic components. The textiles used are a combination of natural and synthetic textiles and thus may not be easily biodegradable or biodegradable.

1.12 BRIEF CHAPTER OVERVIEW

Chapter 1 is an introduction; used to place the research into context. The chapter covers the motivation behind the impregnation of textiles with antimicrobial properties and the need for an alternative means of processing. The chapter also includes the aim and objectives used to achieve the aim of the research project. Chapter 2 covers the literature review related to

supercritical fluids, the application thereof, specifically, textile impregnation and the principles on which this application is based. Then, Chapter 3 is concentrated on the experimental means by which the aim and objectives are addressed. This is inclusive of the analyses conducted. Chapter 4 covers the discussion and interpretation of the obtained results. Finally, Chapter 5 summarises the outcomes related to the objectives and aim, and other conclusions drawn. This chapter is concluded with recommendations for future studies. This chapter overview is tabulated below in *Table 1: Chapter overview*.

Table 1: Chapter overview

Chapter	Objective	Chapter outline
Chapter 1: Introduction		Describes the study background & motivation, problem statement, research questions, aims and objectives, research questions, significance, and limitations of the study.
Chapter 2: Literature	a,b,c,d	Review of literature on: • Microbes and antimicrobial agents: where literature is provided on the proliferation of pathogens in a hospital setting, the rise of antimicrobial resistant strains. This is to emphasise the impact of HAI and the need to curb its spread. • The shift from synthetic to natural antimicrobial agents to explain the use of natural therapeutic components. • The role hospital textiles play in the frequency of HAI. Subsequent literature is provided on the viability of using biofunctionalized textiles to curb the spread of HAI. • Textile impregnation followed by literature on conventional textile finishing methods. In addition to previous studies on ambient solution textile immersion. • Literature review on supercritical fluids, specifically $scCO_2$ and its application in textile impregnation. This includes a review of previous SSI studies and the factors that affect the technical feasibility of the SSI. This is done to determine a viable range of operating conditions. • The research is focused on the use of natural therapeutic components. Thus, this chapter includes literature on the proven antimicrobial potential of the selected therapeutic components. • The research entails the use of an analytical scale for retention quantification. As such literature is provided on the use of MSA (measurement system's analysis) to assess the capability of the scale.

		• In this research the diffusion, over time, in a homeostatic environment is studied. As such, literature on the hygroscopicity of textiles is included as relative humidity variation affects the mass of the textiles. Additional literature is provided on the use of convective drying to reduce the moisture content of the substrates.
Chapter 3: Experimental: Material and Methods	a,b,c,d	Chapter 3 focuses on the experimental and subsequent analysis methods used to answer the objectives and ultimately the aim of this study. The MSA results are used to validate the quality of the measured data obtained within this chapter.
Chapter 4: Results and discussion	a, b,c &d	As set out in chapter 3, the results from the experiments are collated. A comparative analysis is performed between the high-pressure and ambient immersion processes. This comparison is in terms of the solute retention of the components infused into the substrates, the persistence of the solutes in an isolated, homeostatic environment, the bioactivity and ultimately the effect of pressure on the textile impregnation.
Chapter 5: Conclusion and recommendations	a, b,c &d	Outline of the research findings answering the objectives. In addition, the findings from chapter 4 are used to answer the research questions and, eventually, the research aim. Suggestions for future studies are also included in this chapter.

Chapter 2 LITERATURE REVIEW

2.1 INTRODUCTION

This study is aimed at comparing the performance of fabric samples loaded with antimicrobial plant extracts; under supercritical and ambient pressure conditions; against specific microbes. Chapter 2 aims at establishing what is already known and in the public domain and understanding the gaps that require further research. In addition, providing the theoretical background of the processes, in terms of the thermodynamics, phase behaviour, physical properties and prediction of operation parameters, therapeutic components and polymers functionalised.

2.2 MICROORGANISMS AND ANTI-MICROBIAL AGENTS

Microorganisms play a pivotal role in the environment and are subsequently essential for preserving life (Brown, 2008). It was only between 1665-1683 where certain microorganisms were found to be pathogenic. The golden age of antimicrobial agents was sparked by the discovery of penicillin in the 1940s by Alexander Flemming (Brown, 2008). From the 1940s to the 1980s, the synthesis of semi-synthetic, synthetic, and natural antimicrobials proliferated (Brown, 2008). However, all antimicrobial agents have inherent obsolescence due to the mode of action used by the agents to control the bacteria(Overbye & Barrett, 2005). Recently, the production of antimicrobial agents has since become more and more stagnated. This has been attributed to the rise in antimicrobial-resistant pathogens (Bollenbach, 2015). Over the years, the rate of antimicrobial discovery has declined whilst the rate of antimicrobial-resistant pathogens emergence continues to increase (Jayaraman, 2009). The presence of antimicrobials is said to help the proliferation of resistant bacteria by killing the less resistant bacteria and thereby giving resistant bacteria the edge (Chait et al., 2010).

Furthermore, the continual misuse and overuse of antimicrobial agents and their accumulated presence in the environment (water, soil, and food) have accelerated the rate of resistance against antimicrobials (Overbye & Barrett, 2005). Today, resistance has developed against all types of antimicrobials (Jayaraman, 2009). The indiscriminate resistance suggests that any pathogen can build resistance to any antimicrobial (Jayaraman, 2009). This further supports the inherent obsolescence of antimicrobial agents.

Today, antimicrobial-resistant bacteria have rendered most conventional antimicrobials essentially ineffectual against pathogenic microorganisms (Mittal et al., 2018). To the extent where some agents seem to act as inter-microbial signalling agents and, in some cases, a source of nutrition to microorganisms (Jayaraman, 2009). Researchers and the medical sector face one of the biggest challenges of developing new antimicrobial agents (Mérillon, 2018). The resistance built by microorganisms has given rise to superbugs such as methicillin-resistant *Staphylococcus aureus*, Clostridium difficile, carbapenem-resistant *Klebsiella pneumonia* (Mérillon, 2018). All these multi-drug resistant strains pose a serious threat to healthcare worldwide. Subsequently, new ways to control and eliminate multi-drug resistant pathogens are highly sought. Researchers have shifted focus to using secondary metabolites of compounds extracted from various flora (Mérillon, 2018). Natural therapeutic compounds are gaining traction as an eco-friendly alternative to their synthetic counterpart. Since time immemorial, flora has been fighting against microorganisms by producing secondary metabolites (Emad M. Abdallah, 2007). The use of plant and secondary microbial metabolites aided in the increased human life span in the 20th century. Flora offers the largest biochemical and pharmaceutical source on earth (Emad M. Abdallah, 2007). Medicinal plants provide a rich source of antimicrobial secondary metabolites including saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes, lactones, terpenoids and phorbol esters (Emad M. Abdallah, 2007). Moreover, plant extracts such as essential oils have proven to be effective against drug resistant microorganisms (Mittal et al., 2018).

Today resistant pathogens are unaffected by most of the current synthesized antimicrobial agents. Infectious disease is the 2nd or the 3rd leading cause of death in developed countries (Brown, 2008). In the United States, HAI (hospital-acquired/associated infections) kill more people than AIDS, where superbugs such as *S.aureus* kill approximately 100 000 patients per year (Brown, 2008).

2.3 HOSPITAL-ACQUIRED/ASSOCIATED INFECTIONS

Nosocomial infections, also known as hospital-acquired/associated infections, are infections that were not present on a patient upon admission. Instead, they are contracted during their hospital stay (Sikora A, 2009). Common HAIs include *Streptococcus* spp., *Acinetobacter* spp., *enterococci, Pseudomonas aeruginosa, coagulase-negative Staphylococci, Staphylococcus aureus,*

Bacillus cereus, Proteus mirabilis, Klebsiella pneumonia, Escherichia coli and Serratia marcescens (Khan et al., 2015). These types of infections are recognised contributors to morbidity and mortality rates. Where developed countries like the United States experience approximately 100 000 nosocomial related fatalities per year (Sikora A, 2009). HAIs present an even more significant challenge to developing countries where effective infection control programmes have not been implemented (Allegranzi et al., 2011). Such countries are at a disadvantage, as shown by a prevalence study by WHO (World Health Organisation) that suggested that control systems can deter HAIs by 33% (Samuel et al., 2010).

The increase in the cases of HAIs has led to an increase in antimicrobial resistance, socio-economic disruption, and mortality rate (Khan et al., 2017). HAIs inflate healthcare costs by $ 17 to $ 20 million per year (McFee, 2009). As such, HAIs present a significant challenge globally (Sikora A, 2009). In hospitals, patients and staff are surrounded by various exogenous, pathogenic microorganisms. These microorganisms are brought in by other patients, staff or even visitors and contaminated objects (Collins, 1991), where bacteria are responsible for 90% of the infections (Khan et al., 2015). The rise in the number of drug-resistant microorganisms, along with the presence of new pathogens, has made it more difficult to control the frequency of HAIs (Khan et al., 2015). Multi-drug-resistant microorganisms have become synonymous with a pronounced mortality rate to such an extent. Common examples of drug-resistant bacteria include penicillin-resistant Pneumococci, multi-drug resistant Tuberculosis, methicillin-resistant *S.aureus* (MRSA), vancomycin-resistant *S.aureus* (Khan et al., 2015). Of these pathogens, *S.aureus* and other multi-drug resistant gram-negative bacteria are responsible for a large percentage of HAIs (Khan et al., 2015). The excessive and improper use of broad-spectrum antimicrobials has increased the rate of resistance development and the frequency of HAIs (Khan et al., 2015). The treatment with sub-inhibitory or sub-lethal antimicrobial doses has allowed for microorganism adaptation (Adegoke et al., 2017). This, in turn, has accelerated the rate at which antimicrobial-resistant is built. Pathogenic microorganisms are commonly spread through four vectors: contact (direct and indirect), respiratory droplets, common vehicle, and airborne spread in the air for prolonged periods (Collins, 1991). Studies show that small changes in the pathogen transmissibility through contact drastically affects the spread of HAIs

(Cooper et al., 1999). Moreover, hospital patients are more than likely already immunocompromised and thus more susceptible to rapid microbial proliferation (Collins, 1991). Therefore, in the interest of patient welfare, it is essential to establish control of the transmission of HAIs (Cooper et al., 1999).

2.3.1 HOSPITAL TEXTILES AS A VECTOR FOR HAIS

Healthcare workers (HCW) wear scrubs and lab coats made of textiles used to make everyday clothes. Previous research by (White et al., 2007, Wiener-Well et al., 2011, Borkow & Gabbay, 2019 & Fijan & Turk, 2012) has identified hospital apparel as a vector for acquiring, cross-contamination, and transmitting multidrug-resistant organisms (MDROs). Further research, however, indicates that bioactive textiles are less susceptible to the deposition of microorganisms. In the context of this research bioactive textiles are textiles that have been treated with bioactive compounds and exhibit bioactivity against microbes. Thus, innovative textiles are more suitable for application in healthcare. Particularly since HCW continually encounter bodily fluids of patients. These fluids often nourish and facilitate the transmission of microorganisms, including MRDOs. Particularly MRDOs such as MRSA and *Enterobacteriaceae.*

Microorganisms contaminate other hospital textiles such as bedding, wound dressing, curtains, and other medical textiles, including surgical textiles. In one study by Treakle et al., (2009) 23% and 18% of lab coats tested positive for MSSA and MRSA, respectively. In another study conducted by Wiener-Well et al., (2011), 60% of apparel tested culture-positive for MDRDs. The garment here was taken from over 100 medical practitioners. Another study showed that in the ICU, 86% of a sample of 103 HCW hands were contaminated. The hands were contaminated with *S.aureus* (11%), *Acinetobacter* spp (6%), *enterococci* (2%) and skin flora (70%).

Danzmann et al., (2013) focused on HCWs as the source of infections. In this study, 152 outbreaks were evaluated. These were because of direct contact. These outbreaks were from surgery, neonatology, and gynaecology departments. Of these outbreaks, the most occurring infections were the surgical site infections, hepatitis B and septicaemia. 27 of the outbreaks were caused by hepatitis B, 49 by *S.aureus*, 19 by *Streptococcus pyogenes*. Of these outbreaks 59 (41.5%) involved physicians, and 56 (39.4%) involved nurses.

In a study conducted by Bearman et al., (2012), a 6-log reduction, MRSA was experienced on apparel that was processed to impart non-leaching antimicrobial properties. Despite the study conducted by Bearman, bioactive textiles on their own are not sufficient to curb the spread of microorganisms. This is where the selection of the textiles becomes important. Fluid repellence is a crucial factor that can aid in reducing the spread. When hydrophilic textiles are used, there is a risk that the organic material from bodily fluids could very likely impede the potency of the antimicrobial agent against the microorganisms. Therefore, the use of textiles with hydrophobic properties repels the bodily fluids that may compromise the potency of the bioactive textiles.

2.4 BIO TEXTILES

Today textiles are ubiquitous in healthcare, utilized extensively at all healthcare levels as a protective barrier against various fluids and infections (Schneider et al., 2021). Surprisingly, these textiles act as a vector for the transmission of HAIs. However, under the correct temperature, moisture and nutrient conditions, textiles provide an excellent environment for the accelerated proliferation of microorganisms. Due to their moisture affinity, natural textiles are significantly more likely to have microorganism infestation (Ivanova et al., 2016). In contrast, synthetic textiles are more resistant to infestation due to their hydrophobic nature (Gao & Cranston, 2008). The increase in HAIs is of rising concern. Textiles worn by medical practitioners have been identified as a vector for the spread of infection (Fijan & Turk, 2012). This includes the spread of resistant bacteria. Consequently, a means of reducing the role of textiles in the transmission of infection has been sought after. This has led to the development of bioactive textiles. In the context of this study, bioactive or bio textile refers to any treated textile that exhibits visible antimicrobial activity

2.4.1 BIOACTIVITY OF THE SOLUTE

Antimicrobial agents are generally classified as either bacteriostatic or bactericidal agents. Bacteriostatic agents primarily inhibit the growth of bacteria and keep the bacteria in a stationary phase of growth. Whereas bactericidal agents primarily kill the bacteria. It is important to identify an agent as bacteriostatic or bactericidal agents in vitro. This is simply because data on the agent *in vitro* mechanism of action in conjunction with other pharmacokinetic and dynamic data aid in predicting the potential *in vivo* efficacy of the agents (Rhee & Gardiner, 2004). Since the supercritical solvent impregnation of textiles with therapeutic components aims to produce bioactive, medical textiles, the *in vitro* mechanism of action and efficacy is pertinent to predicting the potential *in vivo* efficacy of the bioactive textiles.

Although this classification of agents exists, bactericidal and bacteriostatic agents do not only kill and inhibit growth, respectively. Bacteriostatic agents can kill bacteria, and bactericidal agents do not always destroy all bacteria. Ergo, the classification is instead based on the extent of eradication and growth inhibition (Rhee & Gardiner, 2004). *In vitro*, microbiological techniques such as minimum bacterial concentration (MBC), time-kill curve and serum bactericidal titter (SBT) are used to as the bactericidal activity of agents. MBC is used to determine the minimum bacterial concentration at which the lowest concentration of an antimicrobial agent that withers completely inhibits growth or results in a ≥99.9 % decrease in the initial bacterial concentration. This technique uses a solid base medium. MIC is another technique that is used. MIC refers to the minimum inhibitory concentration. It is a quantitative susceptibility test that is conducted using a liquid culture medium. The MIC shows the lowest antimicrobial agent concentration required to inhibit visible bacterial growth (Rhee & Gardiner, 2004).

2.4.2 BIOACTIVITY OF BIOFUNCTIONALIZED POLYMERS

Bioactive textiles can inhibit pathogens by contact or diffusion (Risti et al., 2011). However, when diffusion is involved the antimicrobial activity of the textile decreases over time. Bioactive textiles that have bound antimicrobials act by contact, whereas bioactive textiles with leaching antimicrobials act through the stagnated diffusion of the antimicrobial agent (Risti et al., 2011). The antimicrobial agents are chemically bound to the fibre surface to

produce contact bioactive textiles. Examples of bound antimicrobials include the infusion of PHMB (polyhexamethylene biguanide) onto fibres (Schindler & Hauser, 2004). This can be achieved using the pad-dry-cure. The PHMB binds firmly to the fibre's surface and still act as an antimicrobial (Schindler & Hauser, 2004). Another example is where cotton is reacted with methylol-5,5-dimethyldyantoin and then treated with hypochlorite. This treatment forms chloramines on the fibre where the chloramine sites on the fibre act as an antimicrobial (Schindler & Hauser, 2004).

In doing so, chemical bonds are formed between the functional groups. Often, it is the functional groups that are responsible for antimicrobial activity. This chemical attachment could diminish the antimicrobial efficacy of the bio textile (Risti et al., 2011). The contact bio textiles lower the likelihood of resistance and form a barrier against the pathogens. The antimicrobial agents should diffuse out of the textile over time in certain instances. To achieve this, the diffusion method is used to imbed the antimicrobial agents (Risti et al., 2011).The diffusion method is comprised of three steps. First is the solute dispersion in the solvent followed by solute transfer onto the fibre surface. The second is the adsorption onto the fibre's surface (Risti et al., 2011). This forms a boundary layer, and the final step is the diffusion of the solute into the fibre. The diffusion into thermoplastics such as polyester can only occur when the fibre is above its glass transition temperature (Gressier et al., 2019). At or above the glass transition temperature, the polymer chains in the amorphous regions of the fibre become more flexible. Spaces are also created between the macromolecular chains allowing for easier, more rapid diffusion of small molecules (Gressier et al., 2019).

Microbes release biofilms upon contact with substrates such as a polymer (Huang et al., 2016). This biofilm offers an ideal environment for microbial cell growth and accumulation. Now, microbes cannot flourish when the biofilm is defective (Huang et al., 2016). Antimicrobial agents are thus infused into polymers as a technique, to prevent the formation and adhesion of biofilms. Antimicrobial polymers are classified as either active or passive. The biocidal activity is active, and bacteriostatic is passive (Huang et al., 2016). In addition, antimicrobial polymers can be classified as bound or leaching. This is entirely dependent on the type of polymer that was infused.

Passive antimicrobial polymers prevent the adhesion of bacteria by reducing the adhesion. Whilst active antimicrobial polymers kill the bacteria that attach to the polymer (Huang et al., 2016). Thus, passive antimicrobial polymers repel bacteria upon contact, and active antimicrobial polymers kill bacteria upon contact (Huang et al., 2016).

Various antimicrobial susceptibility testing methods are used to quantify the polymer's antimicrobial activity. The method used in this study is detailed in section 3.8.2.

2.4.3 AGAR DISK DIFFUSION METHOD

The discovery of antimicrobial agents was assumed to eliminate infectious diseases. However, that is not the case and microbes have gained resistance against a broad range of antimicrobial agents (Hudzicki, 2012). The need to test the minimum inhibitory concentration thus rose. Over the years a range of disk diffusion and broth dilution methods have been developed to assess the antimicrobial susceptibility of various bacterial strains (Jenkins & Maddocks, 2019). The antimicrobial susceptibility method used in this study is the agar disk diffusion method. The agar disk diffusion method was used to qualitatively assess the antimicrobial activity of treated textile samples against different microbes (Marković et al., 2018). In this method, the antimicrobial disk sample is placed on an inoculated Mueller-Hinton agar plate.

The Mueller-Hinton agar is considered the best medium to use for testing, in part because of the acceptable reproducibility of results attained through its use (Hudzicki, 2012). The working principle of this method is that the moisture is absorbed immediately into the disc from the agar. This is essential to allow the slow antimicrobial agent diffusion out of the disk. The rate of diffusion is dependent on the agent's molecular weight, the solubility of the antimicrobial agent in the agar (Hudzicki, 2012). This is because larger molecules generally diffuse out slower. If the agent inhibits bacterial growth a clear, visible zone, known as the zone of inhibition, is formed around the disk after the incubation period (Hudzicki, 2012).

The zone of inhibition is the clear zone around the disk created because of the activity of the agent against the strain. The depth of the agar affects the zone of inhibition as a shallow layer of agar will generate a wider zone of inhibition than a deeper layer of agar (Hudzicki, 2012). As such it is required that the agar has a minimum depth of 4 mm to avoid unreliable results (Hudzicki, 2012). Inferences are generally made regarding the size of the clear zones

generated. Where the bigger the zone the greater the activity and vice versa. Sometimes, there is bacteria regrowth in the zone of inhibition, this is an indication that the inhibitory effect of the antimicrobial agent has been overcome (Hudzicki, 2012).

2.5 THEORY OF SUPERCRITICAL FLUIDS

2.5.1 SUPERCRITICAL FLUIDS

A supercritical fluid is a fluid that is found at or above its critical point (Belinsky, 2011). The critical point signifies the highest temperature and pressure at which a fluid can exist in equilibrium, as a vapour or a liquid (Belinsky, 2011). Fluid enters the supercritical region where the liquid and gas form a uniform, indistinguishable phase at and above the critical point. As illustrated in *Figure 3*, under constant temperature, the critical pressure defines the upper limit of a two-phase system (Brunner, 1994). Whereas in *Figure 4*, a fluid can exist as a two-phase system whilst at its critical temperature. Moreover, when working with supercritical fluids, the volume is a variable that cannot be neglected (Brunner, 1994). PVT diagrams are utilised to approximate the interdependence of the pressure, volume, and temperature (Brunner, 1994).

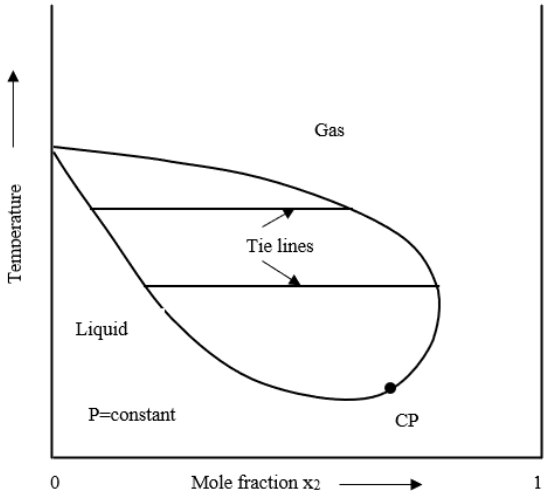


Figure 4: Under constant pressure redrawn from (Brunner, 1994).

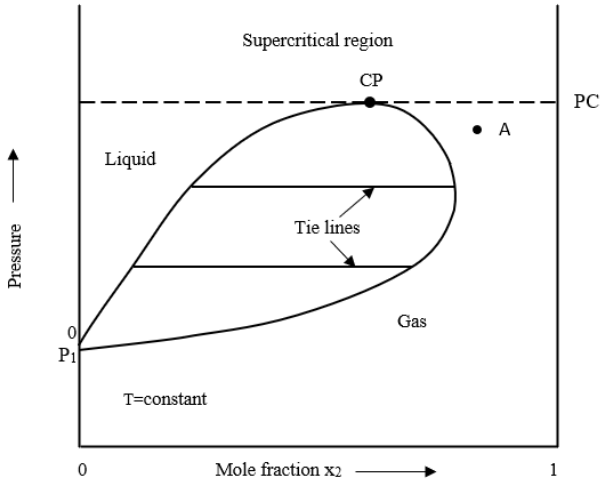


Figure 3: Under constant temperature redrawn from (Brunner, 1994).

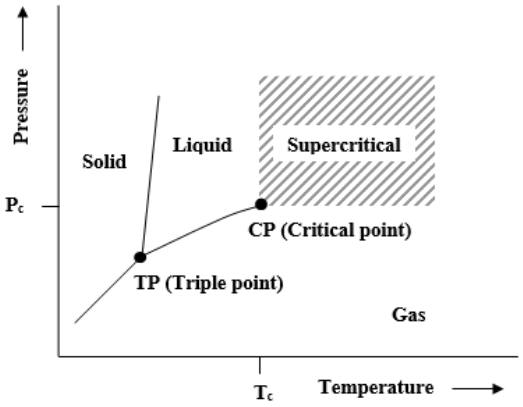


Figure 5: Supercritical state of a pure component (Brunner, 1994)

Supercritical fluids possess tuneable properties comparable to certain properties of a gas and a liquid (Belinsky, 2011). More specifically, it exhibits liquid-like density, gas-like viscosity, and diffusivity greater than a liquid by approximately two orders of magnitude (Belinsky, 2011). A comparison of the physicochemical properties of gases, liquids and supercritical fluids are shown in

Table 1: Physicochemical properties for liquids, gases and supercritical fluids.

Table 1: Physicochemical properties for liquids, gases and supercritical fluids (Belinsky, 2011)

	Liquid T_{atm}, P_{atm}	Supercritical fluid		Gas T_{atm}, P_{atm}
		T_c, P_c	T_c, $4P_c$	
$\rho\ \left(\frac{kg}{m^3}\right)$	600−1600	200−500	400−1000	0.5−2
$\mu(Pa.s)$	$0.2-3\times10^{-3}$	$1-3\times10^{-9}$	$3-9\times10^{-5}$	$1-4\times10^{-5}$
$D\left(\frac{m^2}{s}\right)$	$0.2-2\times10^{-9}$	7×10^{-8}	1×10^{-8}	$1-3\times10^{-5}$

Table 2: Critical properties of common supercritical fluids (Belinsky, 2011)

Solvent	T_c, K	P_c, MPa	$\rho_c, \left(\frac{kg}{m^3}\right)$
ethylene	282	5.04	220
xenon	290	5.84	120
carbon dioxide	304	7.38	470
ethane	305	4.88	200
nitrous oxide	310	7.17	450
propane	370	4.25	220
ammonia	406	11.3	240
n-butane	425	3.80	230
n-pentane	470	3.37	240
isopropanol	508	4.76	270
methanol	513	8.10	270
toluene	592	4.11	290

water	647	22.1	320

Water and carbon dioxide are the two most used supercritical fluids. Supercritical fluid selection depends on the application (Belinsky, 2011). For instance, in the pharmaceutical and food industries, product quality is negatively impacted by high operating temperatures and pressures (Belinsky, 2011). Thus, the fluid selected must operate as a supercritical fluid within the required pressure and temperature range.

From *Table 2: Critical properties of common supercritical fluids*, carbon dioxide, has a critical temperature of 304 K; this is closest to ambient. This, along with its relatively low critical pressure, make it quite popular in industries. Particularly where thermally labile compounds are used. However, supercritical carbon dioxide is not always the most suitable fluid for every process (Belinsky, 2011).

2.5.2 SELECTED SUPERCRITICAL FLUID

Carbon dioxide was selected for the supercritical solvent textile impregnation process. Carbon dioxide has a critical temperature of 304 K and a critical pressure of 7.38 MPa (Belinsky, 2011). Other fluids such as water have a much higher critical temperature and pressure of 647 K and 22.1 MPa, respectively (Belinsky, 2011). The use of fluids with a high critical temperature compromises the thermally labile compounds in the feed, and thus a low-quality product is produced. Moreover, carbon dioxide has a density of 470 $\frac{kg}{m^3}$ at its critical point whereas water has a density of 320 $\frac{kg}{m^3}$ at its critical point (Belinsky, 2011). Thus, carbon dioxide has a greater solvent power at a lower critical point and maintaining carbon dioxide at its operational conditions may prove to be more sustainable.

The carbon dioxide used in this process is extracted from the environment. The use of recycled carbon dioxide does not increase our carbon footprint. Carbon dioxide is non-toxic, found in abundance, inert, non-flammable, cannot be oxidised, and relatively inexpensive (Belinsky, 2011). Furthermore, supercritical carbon dioxide replaces volatile organic compounds so often used as solvents. Some of these organic solvents can potentially contribute to the depletion of the ozone layer (Beckman, 2004).

2.5.3 PHASE BEHAVIOUR

2.5.3.1 DENSITY

Density is dependent on temperature and pressure (Brunner, 1994). The solubility of a solvent is, in turn, dependent on the solvent's density, and the higher the density, the greater its

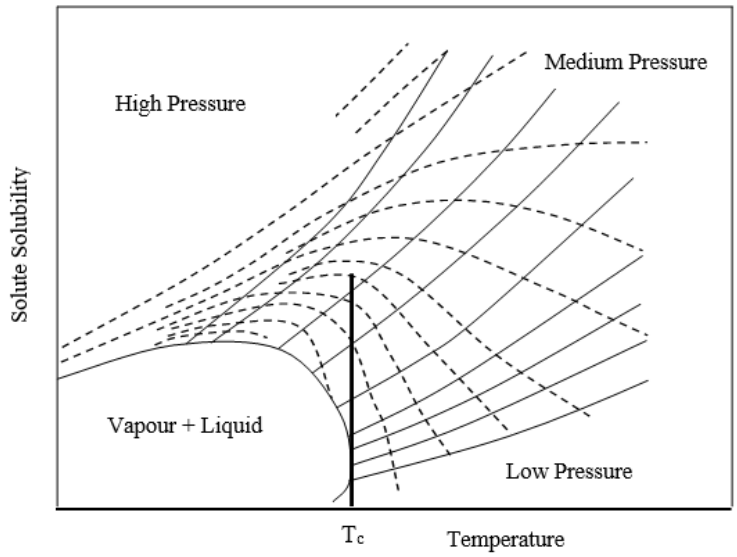


Figure 6:Variations in the solubility of a low-volatility fluid with changes in pressure (perforated lines), temperature and solvent density (solid lines) in the subcritical (T<Tc) and supercritical (T≥Tc) regions. (Redrawn from (Brunner, 2005) which was adapted.

solubility power (Belinsky, 2011).

Figure 6 shows the effect pressure and temperature have on solute solubility. *Figure 6* is split into three regions, low pressure, medium pressure ($\cong 10\ MPa$) and high pressure.

In the low-pressure region, the fluid pressure is lower than the critical pressure and thus subcritical. Here an increase in temperature beyond the critical temperature results in a rapid decrease in the density and solvent power (Brunner, 2005). Similarly, under near-critical pressures, an increase in temperature results in an immediate decrease in density (Brunner, 2005). However, in the high-pressure, supercritical region, a further increase in the temperature results in a very moderate change in the density and solvent power. Here the change in vapour pressure is the controlling factor (Brunner, 2005). The decrease in solvent power is predominantly because of a decreasing density at low pressures. At the same time, fluctuations in the vapour pressure are the dominating variable at high pressures (Brunner, 2005).

In addition, *Figure 6* maps the relationship between density and solubility. From *Figure 6*, the density can be manipulated to optimise the solubility power required by a process. (Brunner, 1994).

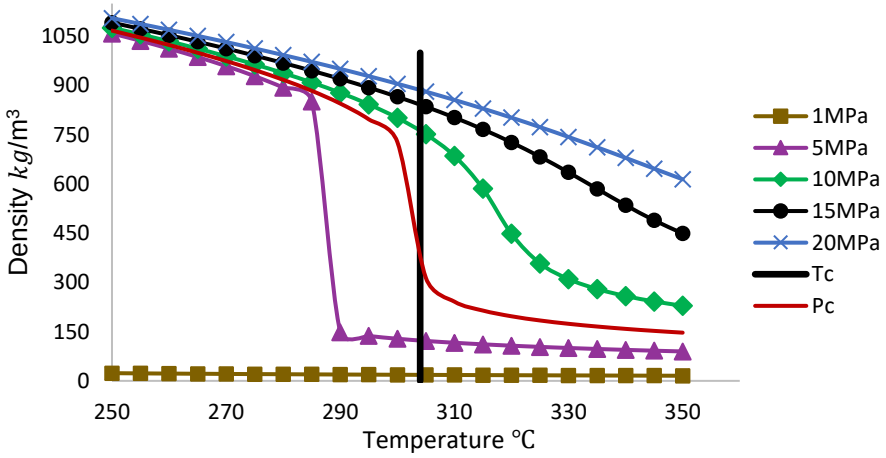


Figure 7: Variation of the density of CO_2 with the temperature at subcritical and supercritical pressures. (Redrawn from (Belinsky, 2011) using data retrieved from (NIST Chemistry WebBook, 2018))

Supercritical fluids are known for the smooth modification of their density (Belinsky, 2011). *Figure 7* shows how the density of a fluid is affected by changes in temperature and pressure. Namely, it shows how the density of carbon dioxide changes rapidly under the subcritical (5MPa) and supercritical pressures as the temperature is increased (Belinsky, 2011). Under subcritical pressures, a sudden change in the density is attributed to the abrupt transition between the gas and liquid phase approaching the two-phase region. As such, the density of the subcritical carbon dioxide is discontinuous when approaching certain temperature conditions (Belinsky, 2011). Whereas, under supercritical pressures, the isobaric transition from a gas-like fluid to a liquid-like fluid is smooth and does not result in density discontinuity (Belinsky, 2011). As a result, in the supercritical region, ranges of physical properties are attainable through manipulation of the temperature and pressure, property ranges that are otherwise unattainable (Belinsky, 2011). This characteristic of supercritical fluids can be used to modify the properties of the supercritical fluid to suit different process steps of the same process without changing solvents.

2.5.4 TRANSPORT PROPERTIES

The hydrodynamics of a supercritical fluid vary drastically from that of fluid in the liquid or vapour phase. This is attributed to the change in property coefficients of a supercritical fluid such as the viscosity, thermal conductivity, surface tension and diffusivity coefficient (Brunner, 1994).

2.5.4.1 VISCOSITY

Parameters such as the pressure drop, mass transfer are viscosity dependent (Brunner, 1994). A fundamental aspect of supercritical carbon dioxide is the effective decrease of the viscosity of a liquid solute saturated in the supercritical solvent (Brunner, 1994). A decrease in viscosity is experienced due to an increase in pressure. An increase in pressure increases the solvent power resulting in a higher solvent saturation point. (Brunner, 1994).

2.5.4.2 DIFFUSIVITY COEFFICIENT

According to (Brunner, 1994), diffusivity coefficients characterise and quantify the molecular transfer in extraction processes. Diffusion is the natural transfer of molecules due to an existing concentration gradient (Brunner, 1994). The diffusion potential is dependent on the concentration gradient, and the diffusion coefficient is used as a proportionality factor. This coefficient relates the diffusion potential to the diffusive flow (Brunner, 1994). The diffusion coefficient is contingent upon the temperature, pressure, density and viscosity of the supercritical solvent, molecular mass and molar volume of the solute and molar volume of the supercritical solvent (Medina, 2012). An increase in pressure decreases the diffusivity, and this is because the number of collisions increases at high pressures because the fluid molecules are closer together. Which effectively reduces the free paths for the solutes and thus inhibits the rapid movement of the fluid molecules (Medina, 2012).

2.5.4.2.1 TEMPERATURE

According to (Medina, 2012), temperature increases increase diffusivity under isobaric conditions. However, the temperature change becomes less significant as the pressure of the system increases. Density is greatly dependent on the temperature and pressure of the system. In the supercritical region, fluid density plays a crucial role. Temperature change increases as the system drop to its critical pressure. Density is simply a measure of the mass occupied per unit volume, and when the pressure of a system is relatively low, the system becomes more

energetic/elastic (Medina, 2012). This is attributed to the induced rapid movement and increase in the kinetic energy of the molecules. An increase in temperature is associated with increased kinetic energy and movement. The inverse is true when the temperature is decreased; thus, the temperature dramatically influences the fluid density (Medina, 2012).

2.5.4.2.2 PRESSURE

The diffusion coefficient is less reliant on the pressure as the temperature decreases, and more reliant on the pressure as the temperature is increased. It is directly affected by viscosity and density. A change in pressure manipulates the density and viscosity. Hence, this is used to understand the effect of pressure on diffusivity. In the supercritical region, pressure affects diffusivity like a normal gas under a high and low temperature. Diffusivity is thus indirectly proportional to variations in pressure. Furthermore, under isothermal conditions, an increase in pressure results in a decrease in diffusivity due to the increase in the fluid density, restricted movement of the molecules and consequent decrease in elasticity and kinetic energy. The pressure is relatively low, the diffusivity coefficient is influenced the most during isothermal pressurisation (Medina, 2012).

2.5.4.3 MASS TRANSFER

Low viscosity and high diffusivity are essential to achieving optimum mass transfer. Mass transfer forms an integral part of supercritical extraction and textile impregnation applications. Optimum mass transfer can only be achieved when it is facilitated by a high diffusivity (Medina, 2012). The successful application of supercritical carbon dioxide as a solvent hinges on an optimum mass transfer, which relies on diffusion. It is of the utmost importance that the mechanism, diffusion upon which mass transfer is based is understood and applied correctly (Brunner, 2005). In textile impregnation, the polymer matrix resists mass transfer. The use of plasticizing agents such as carbon dioxide decreases the resistance offered by the polymer. These agents reduce the resistance by swelling up the fibres of the polymer matrix. It follows that the more supercritical carbon dioxide is used, the lower the resistance to mass transfer becomes. This thus results in an improved mass transfer. Pressure is said to be important to the mass transfer that needs to take place during supercritical extraction and impregnation processes. Specifically, in an extraction process, the interaction with the solid matrix of the feed is taken into account. This interaction cannot be neglected as it affects the

mass transfer process. A study performed on theobromine extraction revealed that in the overall mass transfer, the diffusion coefficient in the solid feed is not effectual. Further, better mass transfer rates are found between the surface of the solid feed and the bulk of the supercritical solvent (Brunner, 2005).

2.6 APPLICATION OF SUPERCRITICAL FLUIDS

Applications of supercritical fluids include the separation of solid or liquid feed. This is referred to as extraction and fractionation, respectively. Supercritical fluids can also be used to attach a solute to a substrate, resulting in substrate impregnation. This impregnation process is applied in various industries.

2.6.1 SUPERCRITICAL SOLVENT EXTRACTION

Implementing supercritical fluids in extraction and separation processes constitutes the central focus of applying such fluids in the industry. As the years have passed, the research on SCF and its application have experienced tremendous growth. It is evolving from pure research to implementation in pharmaceutical, petroleum and food industries, especially concerning its use for extraction. In addition, in the 20th century, as interest in supercritical fluids so did its application. This inspired research into the extraction of various types of feed. Today, research has been conducted on the extraction from multiple types of feed. This research includes the production of hop extracts, extraction from sesame seeds, extraction of essential oils, even the extraction of flavour compounds from milk fat, the extraction of caffeine from coffee beans and black tea leaves. New technologies were even unfolded. An example of this is resins extraction from hops cones (Zizovic et al., 2014).

Supercritical solvent extraction refers to using a supercritical solvent for extraction from a solid feed. This is often to separate the desired component or improve the quality of a product, motivated by commercial gain or regulations. In most cases, this is performed in batch mode. However, it can be operated in a continuous mode, this would be more difficult, but it is achievable (Brunner, 2005). For an extraction process to be feasible, the selectivity should not be 1. A selectivity of 1 would require a difficult, if not impossible, extraction. A value of 1 indicates no significant difference in the solubility of one component over the other in carbon dioxide. One component should be more soluble in supercritical carbon dioxide (Brunner, 2005). The extraction process consists of two sections. The first section is simply the solute

extraction from the solid feed. However, the second section is the total solute separation from the supercritical solvent, carbon dioxide, to achieve a solvent-free solute as the product. In the first section, the supercritical carbon dioxide is pumped through an extractor packed with a fixed bed of solid feed. As the supercritical carbon dioxide is fed in, it is evenly distributed across the bed. After the allotted time for the extraction has been reached, the second stage commences. Separation of the solute, extract from the solvent is required. A series of separators are used to achieve this separation. The solvent density is decreased across the separators by operating the separators at a much lower pressure than the extractor.

As well as increasing the temperature across the separators. This manipulation of the conditions of state reduces the solvent density, lowering the solvent power of carbon dioxide low enough that dissolution of the solute takes place. Through this process, a pure, solvent-free extract is attained (Brunner, 2005).

2.6.2 SUPERCRITICAL SOLVENT TEXTILE IMPREGNATION

Over the years, the use of supercritical solvent impregnation has become more attractive to specific industries such as the textile, pharmaceutical, polymer and plastics industries resulting in an influx of research into the use of this process in different sectors. Studies on the technical feasibility of producing bio-functional textiles have been conducted. Where therapeutic components such as caffeine (Rubio et al., 2010), mango leaf extract (Martinez et al., 2017) and Thymol (Milovanovic et al., 2013) have been explored. Research on textile impregnation of hops has been conducted (Zizovic et al., 2014). However, this study simply focused on solute retention under an array of conditions.

Moreover, the implementation of supercritical fluid technology has been studied to develop controlled drug delivery systems (Bouledjouidja, 2016). These systems would be valuable for the pharmaceutical and medical industries. The development of more advanced medical textiles has also been explored. In 2015, Champeau published an article in which the supercritical solvent impregnation of polymer implants was investigated (Champeau et al., 2015). Furthermore, research has delved into the development of lenses using supercritical technology. This includes the development of therapeutic contact lenses (Costa, Braga, Guerra, et al., 2010) and anti-glaucoma loaded contact lenses (Costa, Braga, Duarte, et al.,

2010). This simply goes to illustrate the current interest in supercritical technology but does not begin to reveal all the research already conducted on this technology

2.6.3 WHAT IS TEXTILE IMPREGNATION

Impregnation refers to the absorption; saturation of a solid matrix with a functionalizing solute that is carried by a liquid or gas phase solvent (Zizovic et al., 2014). Current techniques used such as powder, melt and solution impregnation (Schulte & Lacroix, 2004), microencapsulation and the direct application technique have various downfalls (Tawiah & Badoe, 2016). These downfalls include heterogeneous dispersion, a low impregnation load, the use of toxic organic solvents, the inability to subsequently obtain a solvent-free textile and inadequate fastness (Casas et al., 2018). The inadequate fastness alludes to the single-use attribute of textiles produced. Consequently, non-biodegradable agents often end up in the wastewater and contribute significantly to the contamination of waterways(Uddin, 2014). In conjunction with the high quantity of water used, the synthetic antimicrobial present in wastewater, the current water crisis, and the increasing resistance of microbes make it consequential for the discontinued use of synthetic agents (Zizovic, 2018). Hence, a need has arisen for non-antibiotic agents that possess antimicrobial activity. Subsequently, a shift has been made favouring the use of natural antimicrobial agents, preferably non-antibiotic. Despite this shift away from synthetic agents, certain shortcomings are still experienced. Shortcomings such as the heterogeneous dispersion, a low impregnation load, the use of toxic organic solvents, the inability to obtain a solvent-free textile and the inadequate fastness still come to the fore (Tawiah & Badoe, 2016). In addition, the impregnation techniques used are aqueous and use an enormous quantity in the range of 100-150 L of water per kg of textile processed (Zizovic et al., 2014). Given the current water crisis, there is a definite need to eliminate water from the impregnation process to alleviate some of the strain placed on the limited and depleting water sources. Thus, a green innovative, preferably anhydrous technique is needed that does not require the use of synthetic antimicrobial agents, has a superior fastness, and produces a multi-use textile.

2.6.4 PREVIOUS STUDIES ON SSI OF THERAPEUTIC COMPONENTS

Over the years, the use of supercritical solvent impregnation has become more attractive to specific industries, specifically the textiles, pharmaceutical, polymer and plastics industries.

This has resulted in an influx of research into the use of this process in different industries. Studies on the technical feasibility of producing bio-functional textiles have been conducted. Where therapeutic components such as caffeine (Rubio et al., 2010), mango leaf extract (Martinez et al., 2017) and Thymol (Milovanovic et al., 2013) have been explored. Research on textile impregnation of hops has been conducted (Zizovic et al., 2014). In the infusion of hops polypropylene, polycaprolactone and corn starch xerogel were impregnated with hops extract. All three textiles had a contact time of 5 hours. However, the polypropylene and corn starch process conditions were at 323 K and 29 MPa, whilst that of polycaprolactone was at 308 K and 15 MPa. The highest solute retention of 6.04% was attained when polycaprolactone was used. The lowest solute retention of 2.58% was achieved when corn starch xerogel was used. This study focused on the impregnation yield under an array of conditions and not the bioactivity of the infused polymers. The infusion of mango leaf extract polyphenols into polyester and cotton was studied by (Casas et al., 2017) and (Fernández-Ponce et al., 2018), respectively. Casas obtained the highest polyphenol retention at 500 bar, 55 ℃, contact time of 30 minutes and a fast depressurization rate of 25 bar/min. Whereas, Fernández-Ponce obtained the highest retention under 300 bar, 45 ℃, 6% of co-solvent(ethanol), contact time of 24 hrs and a fast depressurisation of 5 bar/min.

Moreover, the implementation of supercritical fluid technology has been studied to develop controlled drug delivery systems (Bouledjouidja, 2016). Drug delivery systems would be valuable in the pharmaceutical and medical industries. The development of more advanced medical textiles has also been explored. In 2015 Champeau published an article in which the supercritical solvent impregnation of polymer implants was investigated (Champeau et al., 2015). Furthermore, research has delved into the development of lenses using supercritical technology. This includes the development of therapeutic contact lenses (Costa, Braga, Guerra, et al., 2010) and anti-glaucoma loaded contact lenses (Costa, Braga, Duarte, et al., 2010).

2.6.5 PRINCIPLES OF SUPERCRITICAL FLUID IMPREGNATION

This application of supercritical fluids is predominantly dependent on two parameters, density and diffusivity. Fluid density is the property that determines the solvating power of supercritical carbon dioxide. Thus, the higher the density, the higher the solvent power.

Therefore, more solute is required to saturate the supercritical carbon dioxide. Moreover, the diffusivity of supercritical carbon dioxide plays a significant role (Champeau et al., 2015). The ease with which supercritical carbon dioxide diffuses controls the extent of the diffusion of the solute into the polymer. Both these properties are thus essential for successful and efficient textile impregnation. The process of impregnation can be divided into three distinct phases.

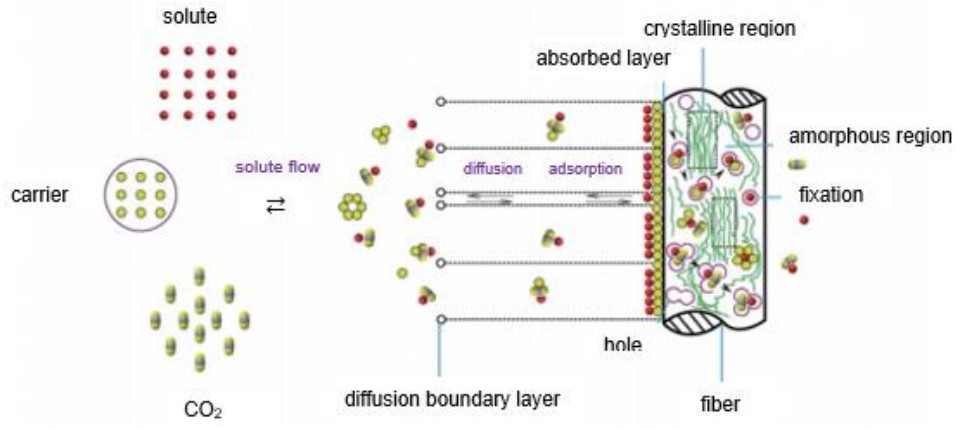


Figure 8: Supercritical solvent impregnation of a fibre (redrawn from (Zheng et al., 2017))

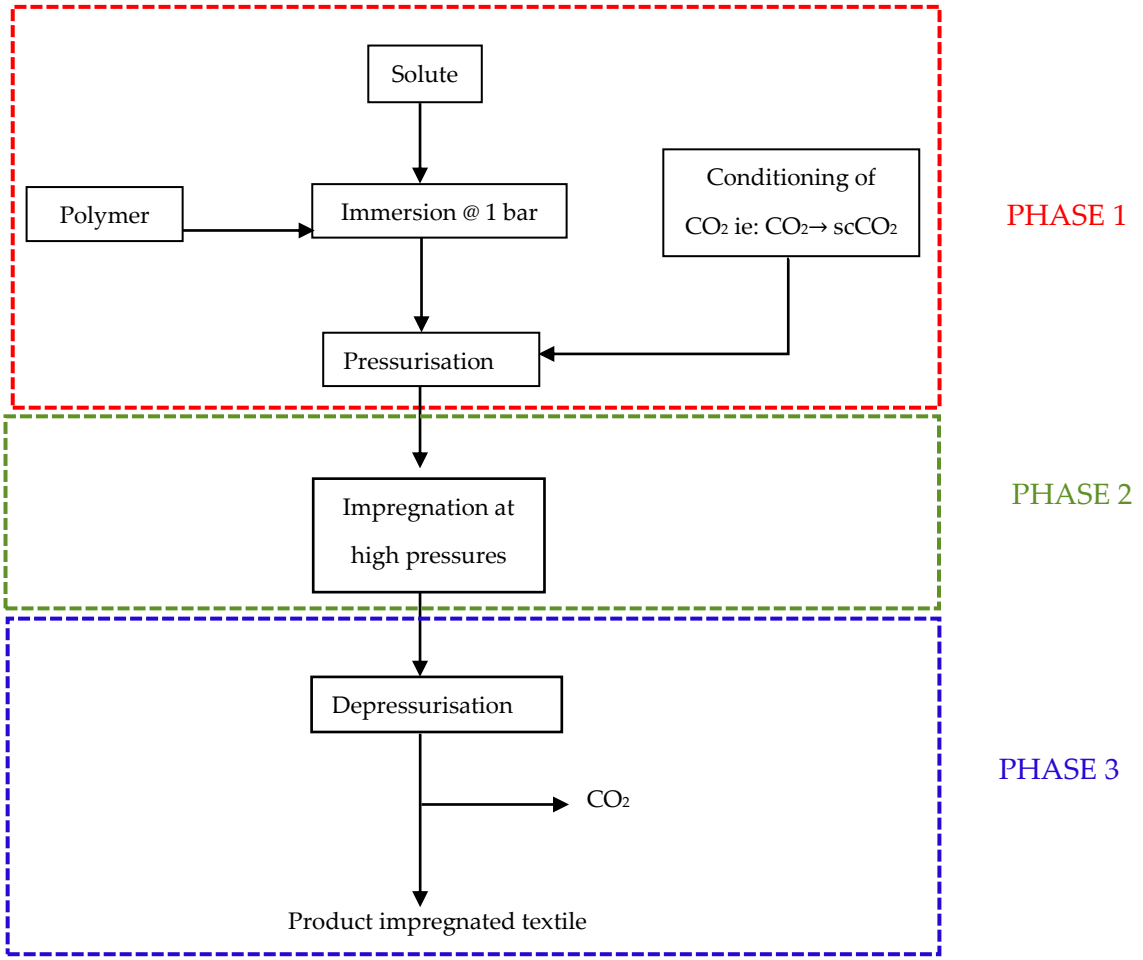


Figure 9: Important phases of an SSI process based on (Weidner, 2018, Champeau et al., 2015 & Hassabo & Osman, 2021)

First, the dissolution of the solute into the supercritical carbon dioxide. The textile is immersed in the solute/impregnate under ambient pressure in this phase. During this immersion, textile impregnation takes place. However, the impregnation is limited by the polymer matrix's low diffusion rate and limited penetration depth. Despite this limitation, immersion is necessary to ensure that the introduced supercritical carbon dioxide remains saturated. Carbon dioxide is then introduced into the system to build up the pressure above that of the critical pressure of carbon dioxide. The supercritical fluid allows for solute dissolution into the solvent because

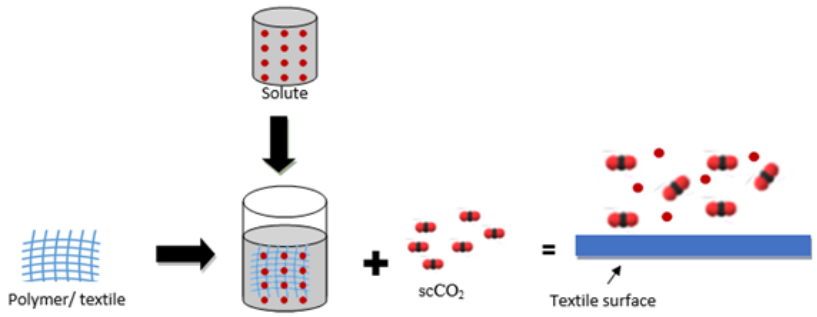


Figure 10: Phase 1 including the dissolution of the solute (based on (Hassabo & Osman, 2021))

of the solvating power that the solvent possesses. This dissolution lowers the mixture's viscosity to lower than that of the solute, allowing for an easier diffusion in phase 2.

The second phase, the contact between the saturated supercritical carbon dioxide and the polymer. During this phase, the carbon dioxide swells the fibres of the polymer matrix. At the same time, the saturated solvent diffuses into the matrix. The saturated solvent is composed of the supercritical carbon dioxide, the dissolved solute, and the modifier if any was used. The second phase is m(Han et al., 2018)ore reliant on the plasticization effect of carbon dioxide on the polymer. Carbon dioxide is known for being a plasticising agent.

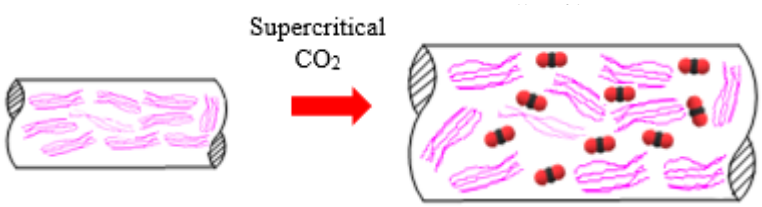


Figure 11: Plasticising effect of supercritical carbon dioxide (redrawn from (Han et al., 2018))

Consequently, the sorption of supercritical carbon dioxide by a polymer swells up the fibres of the polymer matrix. The expansion of the fibre changes the mechanical and physical properties of the polymer. Most significantly, it decreases the transition temperature of the polymer. This decrease in the glass transition temperature is known as plasticization. The reduction of the glass transition temperature softens the polymer. Allowing the saturated carbon dioxide to interact with basic polymer sites induces a more profound, improved molecular diffusion (Kazarian, 2000). Plasticisation is dependent on two factors, the size of the solute and the intermolecular forces between the polymer and scCO2. The intermolecular forces should be comparable to the interactions within the polymer. The size of the solute affects the extent of the glass transition temperature depression. The smaller the solute, the greater the depression. When the glass transition temperature depression is great, the diffusion is thus easier (Alessi et al., 2003).

This effect, in turn, is a function of the solvent density (temperature and pressure). When plasticiser swells up the fibres of the polymer matrix, it provides an easier path for solute diffusion. The attachment that takes place during this phase is physicochemical. Where diffusion is the physical and fastness is the chemical attachment. However, only the physical attachment is essential for a feasible polymer impregnation (Champeau et al., 2015).

The impregnation process is concluded with the depressurization phase. The pressure is consistently reduced during this phase after sufficient contact time between the polymer and saturated supercritical carbon dioxide. The third phase is used to separate the diffused solute from the solvent. The pressure is thus gradually decreased. This reduces the solvent density, as such, the solvent power. As the solvent loses its solvent power, the dissolved solute is deposited in the polymer matrix. The pressure is dropped to below the critical pressure of carbon dioxide. Eventually, the pressure is dropped into the atmosphere. The pressure is reduced in the extractor by venting the vessel. A fraction of the carbon dioxide is vented to the atmosphere. The rest is simply recycled back into the system, where it can be used again. After the impregnation process has concluded, analysis of the impregnated polymer must be performed. This is in terms of solute retention, the surface morphology of the textile, impregnated solute characterisation, and microbial testing depending on the nature of the impregnation. A simple method for determining solute retention is often used (Champeau et al., 2015). This method uses an equation to determine the solute retention

Other factors have been known to affect the impregnation process (Casas et al., 2018). These are:

- Mode of operation

  The supercritical impregnation of polymers can be conducted in a batch mode or in a semi-continuous mode. The textiles are placed in the vessel with the solute in batch mode. Whereas in the semi-continuous mode, the solute is continuously fed with the solvent into the vessel containing the textile. For this experimental work, batch mode was used. The process can then either be static or dynamic. Under static, the vessel is sealed and kept at the predetermined pressure until the contact time has been reached. Whereas with dynamic, there is a continuous flow of carbon dioxide through the vessel.

  In a study performed by Diez Municio (Díez-Municio et al., 2011), a comparison of the solute yield between static and dynamic operations was performed. It was found that dynamic produced a higher yield. This is supported by a study performed by Lauren Comin (Comin et al., 2012). These studies are contradicted by studies performed by (Diankov et al., 2007), (Braga et al., 2008) and (Duarte et al., 2009). Usually, a static

stirrer is used to simulate flow in the vessel during the contact time when static operation is used. A stirrer was used in the studies performed by Diankov, Braga and Duarte but not in the studies performed by Municio and Comin. Based on these studies static operation was selected. However, the pilot plant used for this experimental work is not equipped with a static stirrer as such, the carbon dioxide flow rate is set to 2 kg/hr to stimulate flow through the vessel without dislodging the solute from the polymer. This was proven to work when a low carbon dioxide flow rate is used (Díez-Municio et al., 2011).

- Depressurisation rate

During the depressurisation, the carbon dioxide quickly leaves the polymer leaving the solute behind in the polymer matrix. The rate of depressurization is often used as a means of entrapping more solute into the substrate. The rate of depressurization is dependent on the system at hand, specifically, the affinity of the solute for the substrate and $scCO_2$ (Braga et al., 2011). If the solute has a higher affinity for the substrate, a slow depressurisation rate will entrap more solute within the substrate. However, if the solute has a higher affinity for $scCO_2$ then a slow depressurisation rate would result in the entrapment of less solute as it would still be dissolved in the $scCO_2$ (Braga et al., 2011). In this instance, a fast depressurization rate would entrap more solute within the substrate. However, if the depressurization rate is too fast, the substrate freezes due to the Joule Thompson effect. The freezing could affect the structural integrity of the substrate.

- Molecular size

  The molecular size of the solute greatly affects its solubility in $scCO_2$ and thus affects the solute retention achieved. Generally, the bigger molecules are less soluble in $scCO_2$ (Kim et al., 2019). The lower solubility translates to lower solute retention. It is thus essential to review the solubility of the solute in $scCO_2$ as it would greatly affect the technical feasibility. In addition, the solute concentration is also known to affect the solute retention achieved (Rojas et al., 2020). A higher concentration has been shown to increase solute retention. However, the concentration stops increasing the retention

once the saturation point has been reached. Similarly, low concentrations are known to result in lower solute retention (Rojas et al., 2020).

- Contact time

One of the advantages of supercritical technology is the shorter contact time required. The contact time needed is dependent on the time needed for the polymer solute system to reach equilibrium (Díez-Municio et al., 2011). Batch operation requires a shorter contact time than dynamic operation because equilibrium is reached faster. Studies have shown that although longer contact times of 24 hours are beneficial, only 3 hours are needed for efficient absorption of the solute into the polymer matrix (Casas et al., 2018), (Zalepugin et al., 2020).

- The solubility of $scCO_2$ in the polymer/substrate

The following two mechanisms are crucial for infusing a solute into a substrate. The solubility of the solute in $scCO_2$ and the solubility of $scCO_2$ in the substrate/polymer. According to Kikic & Vecchione (2003), diffusion can be used on its own for textile impregnation. On the condition that the solute is soluble in $scCO_2$ under the selected operating conditions. This is due to the plasticising ability of carbon dioxide and the depressurisation that entraps the solute in the polymer matrix (Kikic & Vecchione, 2003). However, the degree of plasticization is dependent on the morphology and structure of the polymer (Davies et al., 2008). The morphology affects the solubility of $scCO_2$ in the polymer because it is postulated that scCO2 can only diffuse through the amorphous regions of the polymer (Kemmere & Meyer, 2006). A polymer has both crystalline and amorphous regions, as shown in Figure *12*. However, a polymer can be more crystalline than amorphous and vice versa. This is dependent on the degree of crystallinity of the polymer.

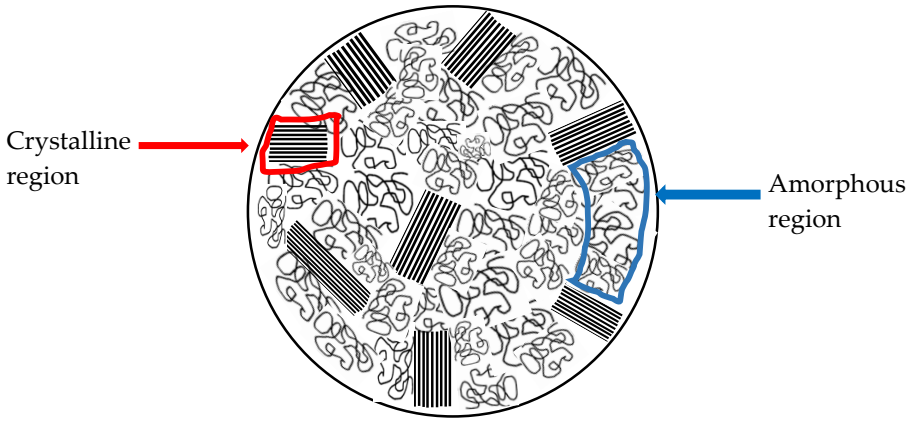


Figure 12: Morphology of polymers (adapted from (Malujda, 2018))

Polymer chains are packed tightly together in the crystalline region, unlike in the amorphous region (James, 2007). The amorphous regions generally have more free volume due to its structural disorder (Bondarenko et al., 2003). Consequently, the crystalline region generally has a higher density and less free volume than the amorphous region (James, 2007). During SSI, the small carbon dioxide molecules diffuse readily through the free volume of the polymer. This takes place in the amorphous regions of the polymers since $scCO_2$ is not soluble in the crystalline regions (Kemmere & Meyer, 2006). Once inside the polymer, the $scCO_2$ increases the free volume found between the polymer chains. This increases the mobility of the polymer chains and decreases the glass transition temperature (Banchero, 2020). The greater the free volume, the greater the diffusion and degree of plasticization. Thus, the degree of plasticization depends on the degree of crystallinity (Kemmere & Meyer, 2006). During depressurisation the carbon dioxide loses its solvent power, and the solute it was carrying is entrapped in the polymer matrix.

However, the solute can only be entrapped if it is soluble in the $scCO_2$ (Kikic & Vecchione, 2003). It is thus essential to review VLE binary data from the literature to determine if the solute is soluble in the $scCO_2$ and under what conditions.

2.7 SELECTED SUBSTRATES

When selecting a textile, the characteristics are of the utmost importance. For instance, this research is focused on textiles for medical purposes. Therefore, certain characteristics are essential. These include hygroscopicity, flexibility, no adherence to a wound, facilitating gas exchange between the wound and the environment, maintaining the optimum temperature

to facilitate blood flow to the wound, and maintaining a moist environment (Zizovic et al., 2014).

Cotton, Lycra, and Polyester were selected as the textile substrates for this research project. Various natural and man-made polymers are used in the medical sector (Qin, 2016). In this study, the impregnation of lycra, polyester and cotton were studied.

Polyester and cotton polymers are used extensively in hospitals in the form of surgical gowns, bedding, protective clothing, medical scrubs, wound dressings, and sometimes as implantable devices (Qin, 2016). At the same time, polyurethanes such as lycra are used as implantable devices and as membranes for wound dressings. It is thus imperative that these polymers are not contaminated, especially the implantable polymers (Brzeska, 2015).

Cotton is a natural cellulosic polymer. This polymer has mostly crystalline regions and a few amorphous regions, with a degree of crystallinity ranging between 70-80% (Clark, 2011). Polyester is a synthetic thermoplastic that has both amorphous and crystalline regions. Crystalline regions are more rigid than amorphous regions. Therefore, diffusion is easier through the amorphous regions. However, benzene rings are present in polyester (Clark, 2011). The benzene rings make the amorphous regions more rigid, making it more difficult to diffuse into (Clark, 2011).

2.7.1 SUBSTRATE MOISTURE SORPTION

Porous substrates (solid materials) such as textiles have bonding/sorption sites that allow moisture sorption between the textile and the surrounding air. Either sorption or desorption can occur until the equilibrium moisture content is reached (Iqbal et al., 2012). The equilibrium moisture content is not fixed. Instead, it is dependent on the relative humidity of the air. Fibres that undergo sorption or desorption in response to the surrounding air relative humidity are known as hygroscopic. Surprisingly, hydrophilic and hydrophobic fibres are hygroscopic (Iqbal et al., 2012). Hydrophilic fibres such as cellulose have more sorption sites and thus are more hygroscopic. However, hydrophilic fibres have poor moisture release and transportation properties, unlike hydrophobic fibres like polyester with fewer sorption sites but good moisture release and transportation properties. Thus, the rate of sorption, moisture release and transportation are dependent on the type of fibre (Iqbal et al., 2012).

Now, moisture is present in the substrate in two forms: bound and free moisture (Richardson et al., 2002). Where bound moisture is the water kept in the capillaries and cavities of a solid material by chemical or physical sorption, this bound water has lower vapour pressure than water under the same pressure (Schaschke, 2014). Bound is considered the equilibrium moisture content of the substrate. Free moisture is the unbound moisture in excess of the equilibrium moisture content (Schaschke, 2014). The free moisture is generally expelled through evaporation.

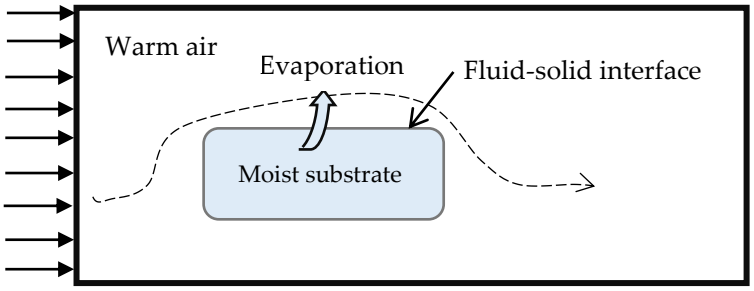


Figure 13: Convective drying of moist substrates (redrawn from (Siagian et al., 2017))

Convective drying is often used to expel the free moisture and some of the bound moisture (Richardson et al., 2002). Drying is a thermal separation process used to separate liquids from materials using evaporation (Tsotsas et al., 2010). During drying processes, water diffuses from regions of high humidity to low humidity regions. Thus, to facilitate the drying of a porous material, the surrounding air must be of lower humidity than the material (Johann et al., 2014). Thermal drying processes consist of heat and mass transfer. Where the transfer of heat takes place from the dry, warm air to the material surface, this heat transfer facilitates the evaporation of surface moisture. The mass transfer takes place in terms of the moisture transfer from inside the material to the surface through capillary flow. Water vapour is transferred into the circulating air (Haghi & Ghanadzadeh, 2005). Convective drying is used as the drying process in this research. A tray dryer is used to circulate warm air over the surface of the textile samples. Forced convection results in transient heat transfer to the material (Johann et al., 2014). This provides the energy needed to evaporate the moisture until the equilibrium moisture content is reached at the respective relative humidity and temperature. During evaporation, the moisture vaporises and diffuses from the material surface to the circulating warm air (Johann et al., 2014). The drying rate is dependent on the rate at which the heat and mass transfer occurs (Theodore & Ricci, 2011).

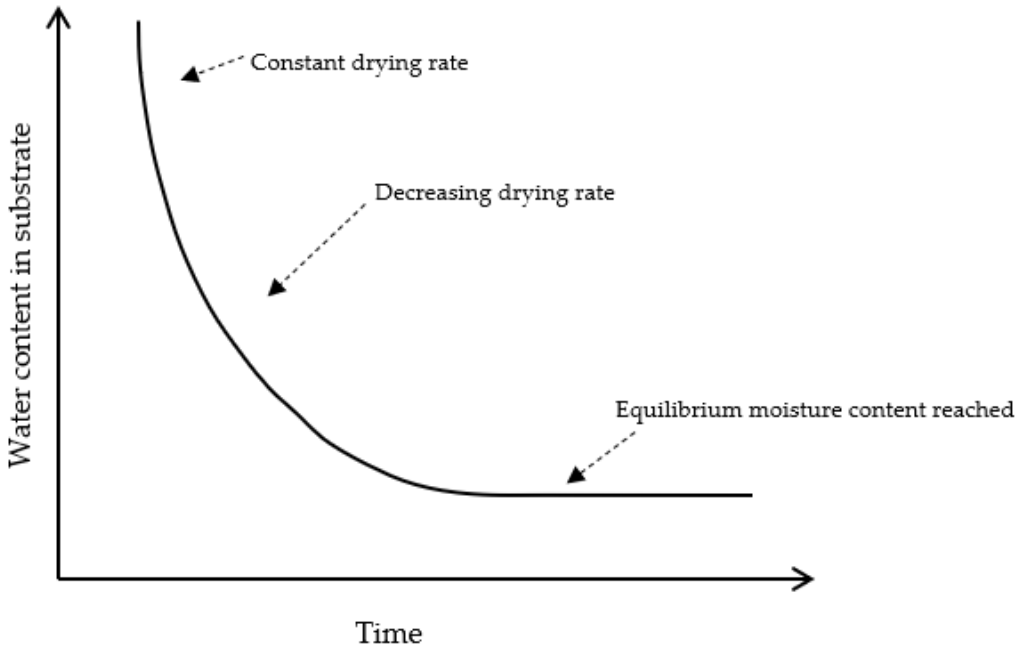


Figure 14: Moisture content during convective drying (redrawn from (Theodore & Ricci, 2011))

In the beginning, there is a thin film of fluid on the surface of the porous material, keeping it wet. During the first drying period, the film evaporates (Theodore & Ricci, 2011). This is the unbound moisture that is evaporated from the material's surface. The moisture diffusion that takes place across the surface of the material is non-Fickian and depends partly on the material's chemistry (Hamdaoui et al., 2014). As the surface moisture evaporates, the substrate moisture seeps up to the surface through capillary action. The diffusion through the bulk of the material is Fickian (Hamdaoui et al., 2014). Eventually, the moisture content is reduced to below the critical moisture content. During the second drying period, a decrease in the moisture content results in a decreased drying rate (Theodore & Ricci, 2011). In the third drying period, the residual moisture is bound to the material by sorption. The drying rate continues to drop as the moisture content lowers to the hygroscopic equilibrium moisture content (Theodore & Ricci, 2011). When the equilibrium moisture content is reached, the pressure exerted by the water on the material is equal to the pressure of water vapour in the air. At this point, the evaporation stops (Theodore & Ricci, 2011); this is illustrated in Figure *14*.

2.8 SELECTED THERAPEUTIC COMPONENTS

2.8.1 HOPS

The hop plant is taxonomically known as *Humulus lupulus L., Cannabaceae*. The *Humulus lupulus* species belongs to the *Cannabinaccae* botanical family (Larson et al., 1996a). The traditional application of hops included using it as an ingredient in medicine synthesis to treat various ailments. Recently, the primary application of hops has been and is in the brewing industry. Hops are used to impart characteristics such as their bitterness, floral, fruity, or citric flavours and aromas (History et al., 1952). However, the hops plant is not limited to the brewing industry. Scientific research has asserted the antimicrobial efficacy of hops bitter acids. Studies have further shown hops bitters to be an inhibitor to pathogens such as Helicobacter pylori, Clostridium difficile, Clostridium botulinum, *Listeria monocytogenes* and Mycobacterium tuberculosis. Thus, the hops species is one of South Africa's natural sources of antimicrobial activity (Examiner & Saucier, 2001).

Furthermore, hop bitter acids induce apoptosis and increase the expression of the cytochrome P450 detoxification enzymes. This action inhibits cell proliferation and angiogenesis. Thus, hop bitter acids exhibit a potential for anticancer activity. As a result, hop bitter acids have arisen as promising molecules for cancer chemoprevention and cancer chemotherapy (Mulvey, 2006).

Hop components such as the alpha-acids (humulones), beta-acids(lupulones), xanthohumol, hop extracts, and isomerized potassium hops extracts have been recognized in the literature as the antimicrobial components (Rój et al., 2015). The alpha and beta acids are unique and inhibit the growth of gram-positive bacteria and Mycobacteria in relatively low concentrations (Examiner & Saucier, 2001). However, these acids are not as potent towards gram-negative bacteria. Literature has attributed this to the difference in the permeability of the cell membrane of the bacteria. Gram-positive bacteria are more susceptible to hops due to their higher permeability.

In contrast, gram-negative are less vulnerable due to their almost impermeable cell membrane. This is due to the structural difference that exists. Although both types of bacteria

have a layer known as the peptidoglycan layer, the gram-negative bacteria have a thick layer of peptidoglycan which gram-positive bacteria do not have (Examiner & Saucier, 2001)

2.8.1.1.1 SYNTHESIS OF TETRA POTASSIUM SALTS

Tetra is the potassium salt found in tetrahydro-iso-acids and is the most antibacterial acid in hops. This salt is quite potent against gram-positive bacteria and some gram-negative bacteria (Caballero et al., 2009). Tetra is quite significant because it is potent against these bacteria at a minimum inhibitory concentration of just 40ppm (Steiner, 2012). Hop bitter acids are mainly comprised of alpha acids (humulone) and beta acids (lupulone). Tetrahydro-iso-acids are not present naturally in hop extracts. In order to produce this acid, the extract is subjected to a series of reactions to produce the tetrahydro-iso-acids. First, the alpha acids undergo isomerisation to form iso-acids. These iso-acids undergo further processing, more specifically hydrogenation, to produce tetrahydro-iso-acids. The synthesis of this acid is illustrated directly below in . Where Rx1 denotes the isomerisation and Rx2 denotes the hydrogenation that takes place.

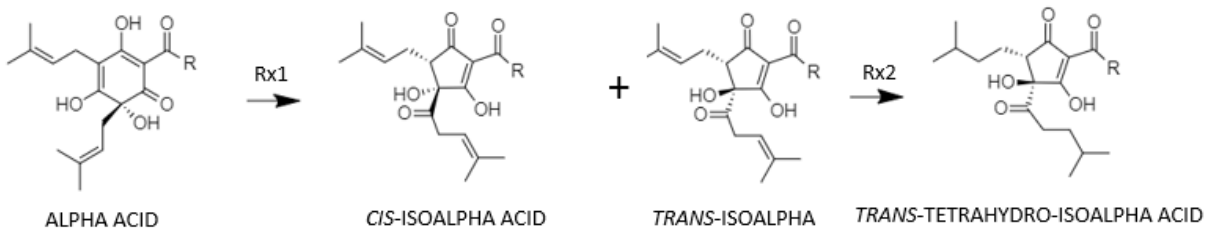


Figure 15: Synthesis of tetrahydro-iso-alpha acid (redrawn from (Bamforth, 2006 & De Keukeleire, 2000))

2.8.2 ESSENTIAL OILS

Numerous essential oils extracted from plants have been shown to inhibit growth and destroy a range of bacteria and fungi. The monoterpenoid components present in the extract damage the cell membrane structures of the bacteria. Thus, these components are considered the principal antimicrobial activity of these extracts is generally attributed to the major monoterpenoid components (Singh et al., 2016).

2.8.2.1 BUCHU OIL

Buchu is taxonomically known as Agathosma Betulina and Agathosma Crenulata. The Agathosma species belongs to the Rutaceae family. Buchu is known for the essential oil that the shrub produces. However, its extract is known to have superior antimicrobial activity. This plant has been a remedy used by the Khoi and San people for a relatively broad spectrum of ailments and is known for its enormous therapeutic potential. Buchu has been used to treat colds and flu, as a diuretic, as a cough remedy, as an antispasmodic, as an antipyretic, an antiseptic and anti-inflammatory agent. Despite this, Buchu's bioactivity was only recently confirmed scientifically. Buchu is made up of a multitudinous range of components. The primary antimicrobial components are Coumarins, Isomenthone (Oils, 1995), Carvacrol, 1,8 Cineole and Terpenen-4-ol (Moolla, 2005).

Studies have shown that both Agathosma Betulina and Crenulata have been proven effective against a range of microbes (Moolla, 2005). This, of course, includes the fungi Candida Albicans. Candida Albicans is a fungus that is classified as a human pathogen, where it is a common inhabitant of the gut, gastrointestinal tract and mouth (Mayer et al., 2013). This pathogen is not an unusual find in healthy adults. Of the human pathogens that exist, Candida Albicans has been known to cause two types of infections. The types are systemic infections proven to be fatal and superficial infections that are not fatal. Even though this pathogen is found in the oral cavity of approximately 75% of the human population, it does not threaten healthy individuals. This is attributed to the benign nature of Candida Albicans in healthy individuals. However, mildly immunocompromised individuals are susceptible to recalcitrant infections of the oral cavity at the hand of Candida Albicans (Mayer et al., 2013).

Furthermore, individuals with HIV are susceptible to these oral cavity infections. Although many individuals are faced with some form of Candidiasis, in cases where the infection is

superficial, there is no threat to that individual's life. However, systematic Candidiasis has a high mortality rate even when the current first-line antifungal therapy is used. This infection can also cause neutropenia and damage to the gastrointestinal mucosa. Candida Albicans can adapt quite rapidly to fluctuations in the conditions of its environment. This is one of the reasons why it is so prevalent. Other individuals susceptible to fatal systemic infections that invade the bloodstream include ICU patients, individuals undergoing chemotherapy and immunosuppressive therapy due to an organ or bone marrow transplant (Mayer et al., 2013). These infections pose a very imminent threat to the quality of life and the survival of individuals. Candida Albicans predominantly cause candidiasis. Thus, the fact that Agathosma Betulina and Crenulata have demonstrated effectiveness against this fungus could mean wonders for the future and create the potential for significant change in the effectiveness of the treatment of infections caused by this morphological, prevalent fungus (Mayer et al., 2013).

2.8.2.2 TEA TREE OIL

Tea tree oil is taxonomically referred to as Melaleuca alternifolia. The potency of this essential oil is mainly attributed to the terpinen-4-ol. This essential oil has a broad spectrum of antimicrobial activity. Although resistant bacteria have become more prominent, what is most concerning is the growing resistance of gram-negative bacteria. Especially since there are no new antibiotics to combat this type of resistant bacteria. Tea tree oil can be used as a disinfectant to prevent the spread of gram-positive and gram-negative epidemic organisms (Cox et al., 2001).

In a study conducted by (Kabir Mumu & Mahboob Hossain, 2018), tea tree oil has proven to be one of the most potent antimicrobial agents. In addition, it is effective against a range of bacteria strains and is an excellent substitute for conventional antibiotics. This includes *Staphylococcus aureus*, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus Vulgaris, Aeromonas hydrophilia, Escherichia coli, Streptococcus pneumonia, Bacillus subtilis, *Klebsiella pneumonia*, Streptococcus agalactiae, S. epidermidis, E. Faecalis, M. avium and H. influenza. Interestingly, in a study conducted by (Singh et al., 2016), the difference in the tea tree oil sensitivity of antibiotic-resistant and non-resistant strains was marginal. This study

included antibiotic-resistant strains such as mupirocin-resistant methicillin-resistant strains and MRSA (Kabir Mumu & Mahboob Hossain, 2018).

2.9 MEASUREMENT SYSTEM ANALYSIS

Statistical analysis forms a large part in analysing and validating experimental data. The aim of this research is in assessing the technical feasibility of SSI. In textile impregnation, the feasibility is determined by calculating the percent solute retention. The measured data is used to determine the solute yield. The difference in mass of the substrate is taken as the solute mass absorbed by the substrate. The difference in mass is minute, and important conclusions are drawn from this data. It is thus imperative that the capability of the measurement system used is assessed prior (as a pre cursor) to experimental work. An analytical Radwag 2012 analytical scale is used as the measurement system throughout this research.

Any measurement system is prone to variation (Theodore & Ricci, 2011), making it essential to discern whether the difference in mass is due to measurement error or if the difference is indicative of the solute mass absorbed. An MSA is used to identify and quantify the sources of variation because all elements of a measurement system can contribute to the total variation (Theodore & Ricci, 2011). Doing so affects the measurement system's capability.

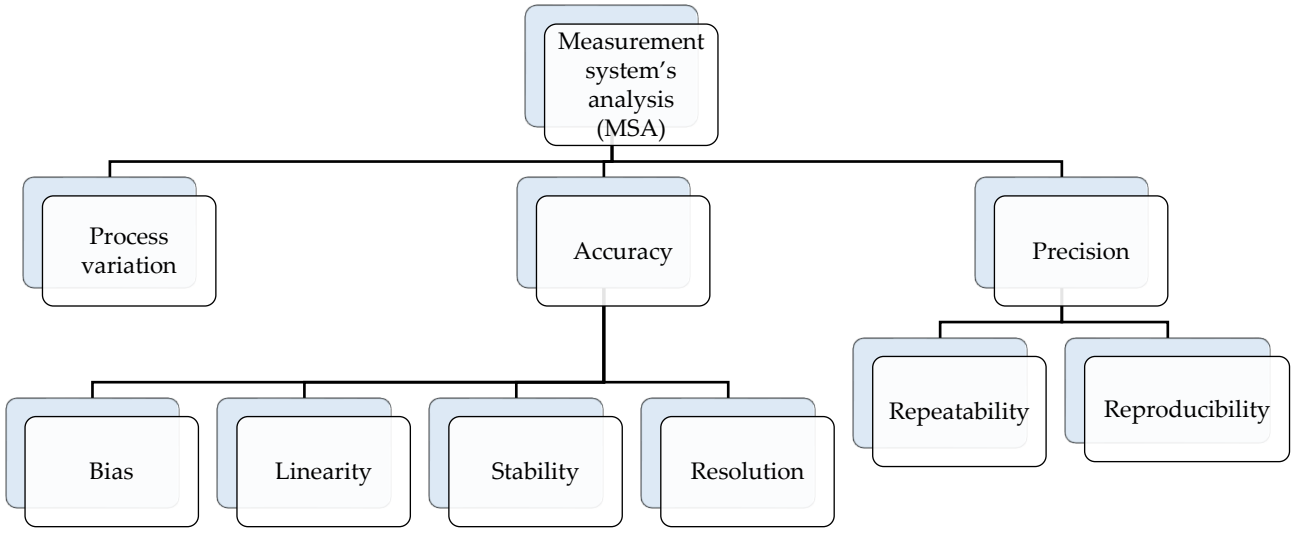


Figure 16: Measurement system's analysis

In an MSA study, an experimental and mathematical method is used to quantify the total variation of the system (Runje et al., 2017). The total variation is the sum of the process and

measurement variation. The measurement variation is quantified by evaluating the accuracy and precision of the measurement system (Runje et al., 2017). The accuracy is influenced by the system's bias, stability, linearity, and resolution. Whilst the precision is dependent on the repeatability and reproducibility of the system (Runje et al., 2017). Repeatability and reproducibility are two crucial metrological characteristics of any measurement system as they identify the sources of variation (Zanobini et al., 2016). Repeatability is a measure of the equipment variation and thus quantifies the contribution of the measurement system to the total variation. However, reproducibility is a measure of the operator variation and its contribution to the total variation (Burdick et al., 2003).

MSA use statistical analysis to assess the measurement system capability. Statistics are used to analyse and characterise population or sample data sets. Studying an entire population is not always possible (Wilkerson, 2008) as such samples statistics are often relied upon to estimate population parameters. These parameter estimations performed and deductions made are only as good as the sample data. Therefore, the inferences and deductions made highly depend on how well the sample data represent the population (Wilkerson, 2008). This type of sample statistical analysis is known as inferential statistical analysis. Inferential statistical analysis bases its deductions on the estimation and hypothesis testing performed. In hypothesis testing, inferences are often based on the samples' mean and standard deviation (Wilkerson, 2008).

The type of hypothesis testing used in this research is the paired student's t-test. A paired t-test is used when two data sets studied are dependent because each value in the one data set is paired with values in the second set. On a paired t-test, the difference of means is the parameter used to make the inferences (Wilkerson, 2008). The student's t-test is the test statistic used in the paired t-test. The student's t is a distribution discovered by W.S. Gosset. The student's t-test is used when the variance is unknown, and the twos sets analysed have a sample size of less than 30 (Wilkerson, 2008). Gosset developed a t-distribution that is used along with the t statistic and degrees of freedom to determine the corresponding p-value (Wilkerson, 2008), where fixed significance level testing is used along with the p-value to either reject or fail to reject the hull hypothesis (Wilkerson, 2008). In fixed significance level testing, the outcome of the statistical testing hinges upon the p-value and how it corresponds

to the set alpha value (Zanobini et al., 2016). In this study, the confidence level was fixed to 95% with a corresponding significance level (α) of 0.05. In the comparison, if the p-value is lower than the α value, the null hypothesis is rejected and the alternative hypothesis is accepted. A p-value lower than 0.05 infers that there's statistically significant differences between the data sets observed (Zanobini et al., 2016). Formulae used to calculate the t-test statistic (Wilkerson, 2008):

$$d = x_1 - x_2$$ Difference

$$\bar{d} = \frac{\sum d}{n}$$ Mean difference

$$S_d = \sqrt{\frac{\sum(d - \bar{d})^2}{n - 1}}$$ Sample standard deviation

$$t = \frac{\bar{d}}{S_d}\sqrt{n}$$ t-test statistic

Steps used in a t-test (Wilkerson, 2008):

State the H_o and H_a

Calculate the test statistic

Determine the p-value

Reject or fail to reject the H_o

State if the difference is statistically significant or not

2.10 PREDICTION OF PROCESS CONDITIONS

Predicting the conditions under which extraction of the bioactive components from the solid matrix and their deposition on and in the matrix of the textile fabric will occur can be laborious and time-consuming if no prior estimation of the phase behaviour of the relevant components is known. The following section describes how the extraction and impregnation conditions could be estimated before the experimentation, saving time and resources.

The selection of operating conditions selected is dependent on the solute solubility. Vapour liquid equilibrium data is often used to predict which conditions are more favourable to a high solute solubility. Such data was used to determine the essential oil's operating temperature and pressure for buchu and tea-tree. Both essential oils contain the same target components, namely 1,8 cineole and terpinen-4-ol. Based on the binary VLE data for the target components in Figure *17* -Figure *21*, a temperature of 40℃ and a pressure of 200 bar were estimated as the operating conditions.

Hops contain thermally labile compounds, and it suggests that the operation temperature be kept below 40 ℃ to prevent degradation (Del Valle et al., 2003). In a study performed by Del Valle, it was found that pressure above 200 resulted in higher solute retention. In this study, a pressure of 280 bar achieved the highest bitter acid ratio of 2.7 (Del Valle et al., 2003). As such, an operating pressure of 280 bar was selected.

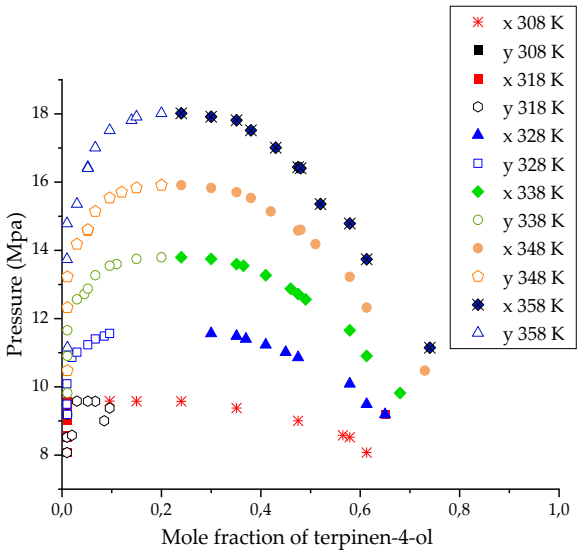


Figure 17: VLE data for carbon dioxide and terpinen-4-ol (Madzimbamuto et al., 2016)

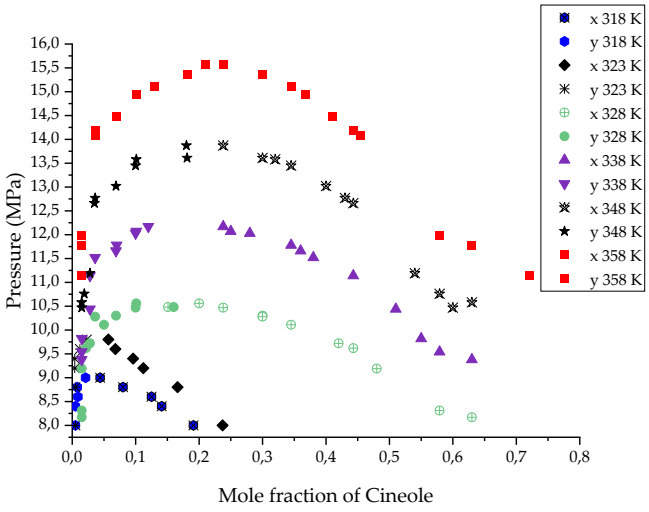


Figure 18: VLE data for carbon dioxide and 1,8 cineole (Madzimbamuto et al., 2016)

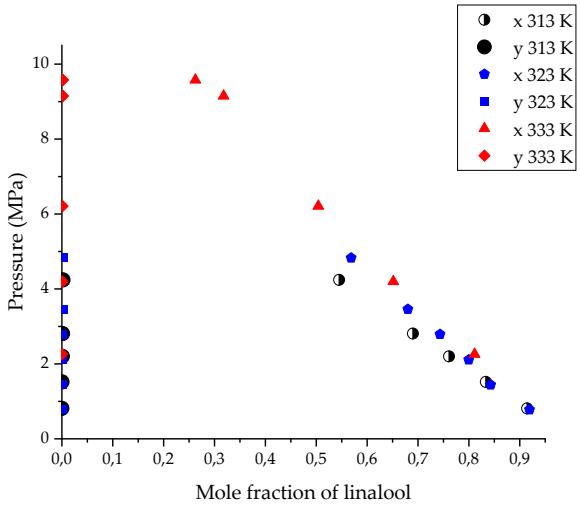


Figure 19: VLE data for carbon dioxide and linalool (Iwai et al., 1994)

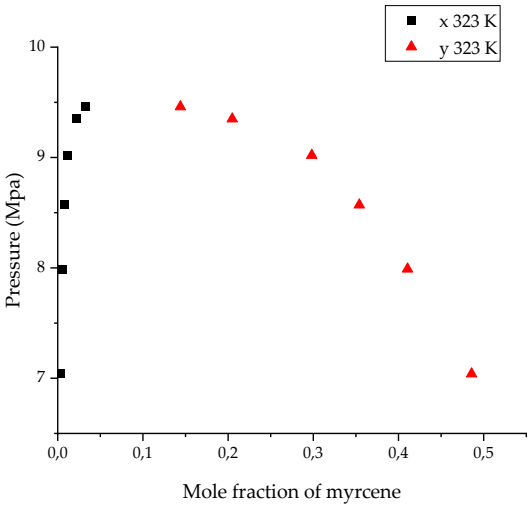


Figure 20: VLE data for carbon dioxide and beta myrcene (Bogel-Łukasik et al., 2009)

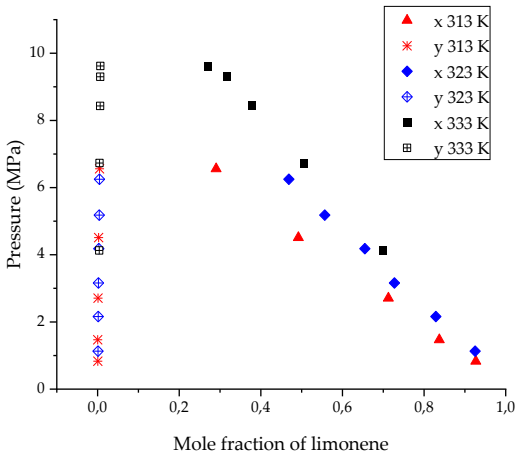


Figure 21: VLE for carbon dioxide and d-limonene (Gironi & Maschietti, 2012)

2.11 CHAPTER OUTCOMES

This chapter started by reviewing literature on disease-causing microbes, their interaction with antimicrobial agents, their presence and subsequent pressure on the hospital setting. This is followed by a review of the literature on the textile impregnation process, in terms of thermodynamics, phase behaviour, physical properties, the prediction of operation parameters and factors that affect solute retention. In addition to the therapeutic components and polymers processed. The key findings of the literature review include:

- To the author's knowledge, research has been conducted on the impregnation of textiles with hops extract. However, the author did not assess the antimicrobial activity of the treated textiles.
- To the author's knowledge, no research has been published on the impregnation of textiles with buchu and tea tree essential oil.
- Based on the revised literature, buchu oil, tea tree oil, tetrahydro-iso-alpha acids and hops extract will be used.
- The microbial strains used in the AST was determined based on the proven bioactivity of the buchu oil, tea tree oil, tetra and hops extracts. As such the immersed textiles are tested against the following strains:

Solute	**Bacterial strain tested against**
Tetra extract and hops extract	*Listeria monocytogenes*, *Staphylococcus aureus*, and *Klebsiella pneumonia* (Bocquet et al., 2018)
Buchu essential oil	*Staphylococcus aureus* and Candida albicans (Moolla, 2005)
Tea tree oil	*Staphylococcus aureus*, *Klebsiella pneumonia* (Singh et al., 2016)

- Polymers have a degree of hygroscopicity. This means that the mass is affected by fluctuations in relative humidity. This is important because the solute retention is measured using the difference between the mass of the polymer before and after immersion. Furthermore, the diffusion profile/solute persistence is studied by monitoring the mass of the textile samples over time. Relative humidity fluctuates thus

it is important to set up a control experiment to account for the effect of moisture on the mass of the textiles over time.

- Binary VLE data was studied for the SSI process. This was done to determine a set of temperature and pressure conditions that favour the solubility of the solute in $scCO_2$. Based on the VLE data it was found that the SSI can take place at 40 ℃ and 250 bar with a contact time of 3hrs to allow for equilibrium to be reached.
- Samples are completely immersed in the solute. This must be done to ensure saturation of the substrate throughout the impregnation process. Lack of saturation would limit the solute retention.
- Due to the dependence of the work on the accuracy and precision of the measurements, and on the large quantity of minute measurements to be made, a measurement system analysis will be carried out to assure the reliability of the measurements, and a determination of the accuracy and reliability of the conclusions. MSA is needed to validate the solute retention measurements taken.

Chapter 3 EXPERIMENTAL: MATERIAL AND METHODS

3.1 PREAMBLE

This Chapter describes the experimental methods used to achieve the objects set in Chapter 1. This chapter details the immersion methods followed in infusing the selected therapeutic components into the substrates, relating to objective a. This chapter details the method used to assess solute diffusion over time. One set in a desiccator and one outside the desiccator in a homeostatic environment. Furthermore, this allows a comparison of the two methods' retentions in response to objective a. This is in response to objective b. The treated samples were also sent for AST and SEM analysis, whose procedures are detailed in this chapter. The AST allowed for the characterisation of the bioactivity in response to objective d. At the same time, the SEM allowed one to determine the effect of pressure on the surface morphology of the substrate in response to objective c.

3.2 MATERIALS

The supercritical solvent impregnation was performed using a range of textiles and natural therapeutic components. The textiles served as the substrate impregnated with the therapeutic components with supercritical carbon dioxide as the solvent. The textiles used include cotton, lycra and polyester. The therapeutic components used were global hops extract and essential oils such as buchu and tea tree oil. *Table 3: Materials* below provides more details on the substrate and therapeutic components used and their sources.

Table 3: Materials

Material	Function	Description	Source
Hops	Solute	Southern star pellets	Anheuser-Busch InBev
Buchu essential oil	Solute		
Tea tree oil	Solute		
Tetra-Iso extract	Solute	Tetrahydro-isoalpha acid extract	Hopsteiner
ICE-4	Standard	International calibration extract	Labor Veritas AG
Carbon dioxide	Solvent	99.9%	Air Liquide
Cotton	Substrate	Woven	Kimix cc
Polyester	Substrate	Knitted	Kimix cc
Lycra	Substrate	Woven	Kimix cc
Ethanol	Solvent	99.9% absolute	Kimix cc
Hexane	Solvent	n-hexane AR	Kimix cc

3.2.1 SUBSTRATE

Lycra, polyester, and cotton sourced from KIMIX were used as the substrates in the immersion experiments. All polymer samples were cut into 9 cm discs and washed using detergent and after that, hexane.

3.2.2 SELECTION OF NATURAL ANTIMICROBIAL AGENTS

Three therapeutic components were embedded into the substrate. The components were hops global extract, buchu and tea tree essential oil. The hops global extract was obtained through the supercritical extraction from Southern Star hops sourced from Inbev plus (Anheuser-Busch InBev).

3.2.2.1 HOPS GLOBAL EXTRACT

The antimicrobial activities of hops have been described in detail in Chapter 2. In an article written by (Karabín et al., 2016), it was found that the hops extract rich in prenylated acylphloroglucinols exhibit an activity superior to that of hops extracts rich in the essential oils. The activity of hops is mainly attributed to the presence and natural antimicrobial properties of acylphloroglucinols. Acylphloroglucinols are the bitter acids of which there are humulone and lupulone. Studies by (Larson et al., 1996b) and (Karabín et al., 2016) describe a

direct relation between the acyl chain length and the activity exhibited. As such, beta acids were more potent than alpha acids.

Moreover, this work suggested that isomerised alpha acids such as trans-iso-humulone possess an even greater potency. An article written by (Simpson & Smith, 1992) found trans-iso-humulone to be 20 times more potent than humulone. However, the activity of lupulone remains the most bioactive bitter acid. This experimental work thus focuses on transferring hops' bioactivity to textiles using $scCO_2$. Lupulone exhibits the highest activity; therefore, Southern Star was selected as it contains the highest percentage of lupulone of the types available. The hops extract was obtained through supercritical solvent extraction performed on the pilot plant at 40 ℃ and 250 bar for 2 hours.

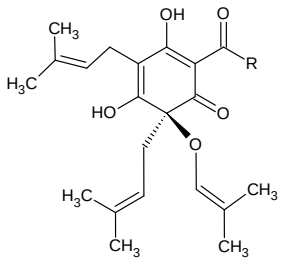


Figure 22: Lupulone redrawn from (Karabín et al., 2016)

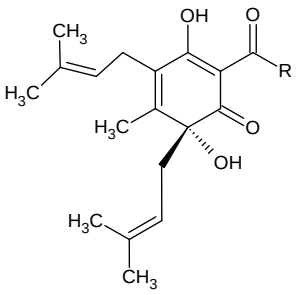


Figure 23: Humulone redrawn from (Karabín et al., 2016)

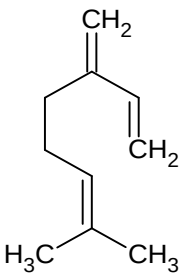


Figure 24: Myrcene redrawn from (Moolla, 2005)

3.2.2.2 BUCHU ESSENTIAL OIL

Buchu's bioactivity was only recently confirmed scientifically. Buchu is made up of a multitudinous range of components. The primary antimicrobial components are Coumarins, Isomenthone (Oils, 1995), Carvacrol, 1,8 Cineole and Terpenen-4-ol (Moolla, 2005). Recent studies have shown that both Agathosma Betulina and Crenulata have been proven effective against a range of microbes such as Candida Albicans, *Staphylococcus aureus* and *Klebsiella pneumonia* (Moolla, 2005).

3.2.2.3 TEA TREE ESSENTIAL OIL

In a study conducted by (Kabir Mumu & Mahboob Hossain, 2018), tea tree oil proved to be one of the most potent antimicrobial agents. In addition, it is effective against a range of bacterial strains and an excellent substitute for conventional antibiotics. This includes *Staphylococcus aureus*, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Aeromonas hydropila, Escherichia coli, Streptococcus pneumonia, Bacillus subtilis, *Klebsiella pneumonia*, Streptococcus agalactiae, S. epidermidis, E. Faecalis, M. avium and H. influenza. Interestingly, in a study conducted by (Singh et al., 2016), the difference in the tea tree oil sensitivity of antibiotic-resistant and non-resistant strains was marginal. This study included antibiotic-resistant strains such as mupirocin-resistant methicillin-resistant strains and MRSA (Kabir Mumu & Mahboob Hossain, 2018).

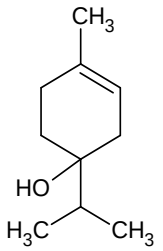


Figure 25: Terpinen-4-ol redrawn from (Moolla, 2005)

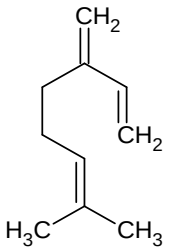


Figure 26: β – Myrcene redrawn from (Moolla, 2005)

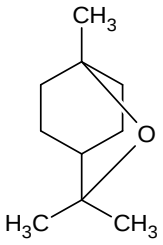


Figure 27: 1,8 Cineole redrawn from (Moolla, 2005)

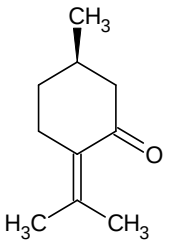


Figure 28: Pulegone redrawn from (Moolla, 2005)

3.3 METHODS & EQUIPMENT

Two main sets of experiments were performed. The first was performed under ambient pressure conditions where ethanol was used as the solvent. The second batch of experiments was used as the solvent performed under high-pressure conditions with a supercritical carbon dioxide fluid. A set of analyses were performed on all the samples to characterise each immersion process accurately. For all experiments performed under high-pressure, the high-pressure pilot plant situated at the Cape Peninsula University of Technology on the Bellville campus was used.

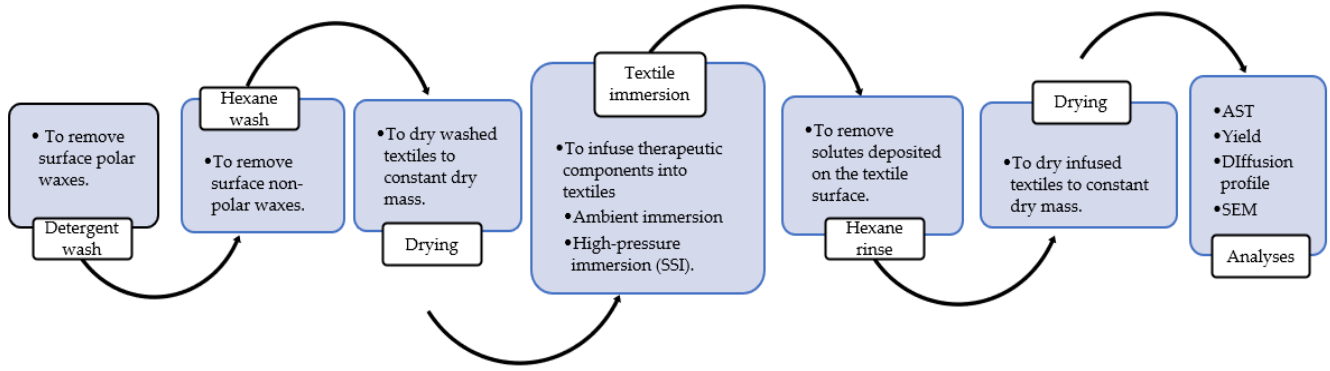


Figure 29: Textile processing overview (pre-treatment and immersion)

3.3.1 PRE-TREATMENT OF SUBSTRATE

Textiles have a layer of polar and non-polar waxes on their surface. The waxes form a hydrophobic protective barrier on the surface of the textile. This barrier of waxes is known to impede any textile treatment process (Menezes & Choudhari, 2011). It is thus imperative that the textiles undergo a dissolution step to remove wax. Non-polar waxes are best dissolved in non-polar solvents. In comparison, polar waxes are best dissolved in polar solvents. A detergent wash followed by a hexane wash was used to scour the textiles.

3.3.1.1 DETERGENT WASH

The water bath was set to 85 ℃ to perform the wash. A detergent to deionised water ratio of 1g:100g (Nad & Muratkar, 2020) was made up and transferred into a round bottom flask with the textile samples. The polyester samples were washed separately because the fabric colour bleeds during the wash process. The round bottom flask was secured in a retort stand and suspended in the water bath. Once the temperature had been reached, the detergent solution

was brought to 80 °C and left for 30 minutes. After 30 minutes, the textiles were thoroughly rinsed in tap water, followed by several washes with deionised water. The textiles were placed in a convective tray dryer until constant dry mass was achieved.

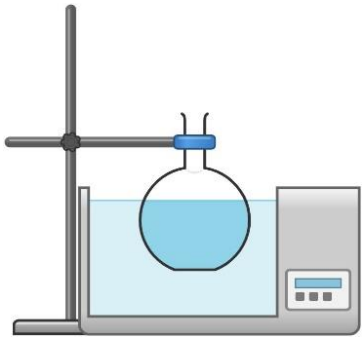


Figure 30: Detergent wash setup drawn using Chemix drawing software

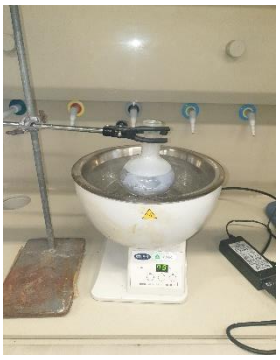


Figure 31: Detergent wash actual setup

3.3.1.2 HEXANE WASH

The detergent washed textiles were placed in a round bottom flask with 400ml of n-hexane. The round bottom flask was secured to a retort stand and suspended in the water bath at 70 °C (the solvent boiling point) for 30 minutes (Agrawal et al., 2007).

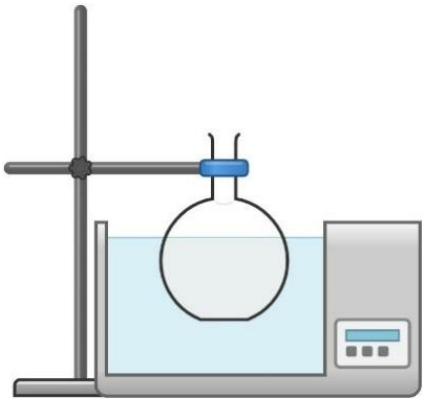


Figure 32: n-hexane wash setup drawn using Chemix drawing software

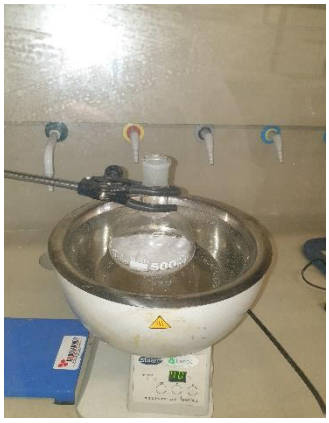


Figure 33: n-hexane wash actual setup

3.3.1.3 CONVECTIVE DRYING

Textile samples were dried in a convective tray dryer to dry the textiles to a constant dry mass. A set of drying experiments were conducted to determine the time needed to dry the textiles soaked with water and hexane to constant dry mass. This was done to simulate the conditions after the first and second wash as well as after the immersion processes. The tray dryer

conditions were set at 40℃ with a fan setting of 7; a higher setting lifted the textiles off the trays. A beaker with the solvent and textile was placed in the dryer to account for bulk drying.

Figure 34: Textile samples in tray dryer

3.4 AMBIENT IMMERSION

For the ambient immersion experiments, 400ml solutions of solute to solvent ratio ranging between 10% and 11%wt were made up. A total of four different solutions were made, each containing one of the four solutes and ethanol as the solvent. 10% wt solutions (Bhatt & Singh, 2018) were made up for buchu and tea tree essential oil solutions. Whereas 11% wt solutions were made up for the hops extract and tetrahydro-iso-alpha acid solutions. All solutions were stirred using a magnetic stirrer. After that, three sets of each textile, a total of 9 samples, were immersed in each solution. After 48 hours (Costa, Braga, Guerra, et al., 2010), the textiles were removed, dried, and sent for SEM and AST (antimicrobial susceptibility) analysis. A set of textiles were also stored to assess the initial solute retention and the retention over time.

3.5 HIGH-PRESSURE PILOT PLANT EQUIPMENT

The high-pressure extraction was conducted in the Separex High-pressure separation pilot plant located on the CPUT Bellville campus. The pilot plant is equipped with:

- High-pressure carbon dioxide piston pump
- Reflux pump
- Co-solvent pump
- Various heat exchangers such as the chiller for conditioning of the solvent and other fluid streams
- Two 5 L high-pressure extraction vessels
- Three high-pressure cyclonic separators
- High-pressure fractionation column

The extractors in the pilot plant are usually used for the extraction of solids. However, for the purpose of this work, the extractors were used for textile impregnation.

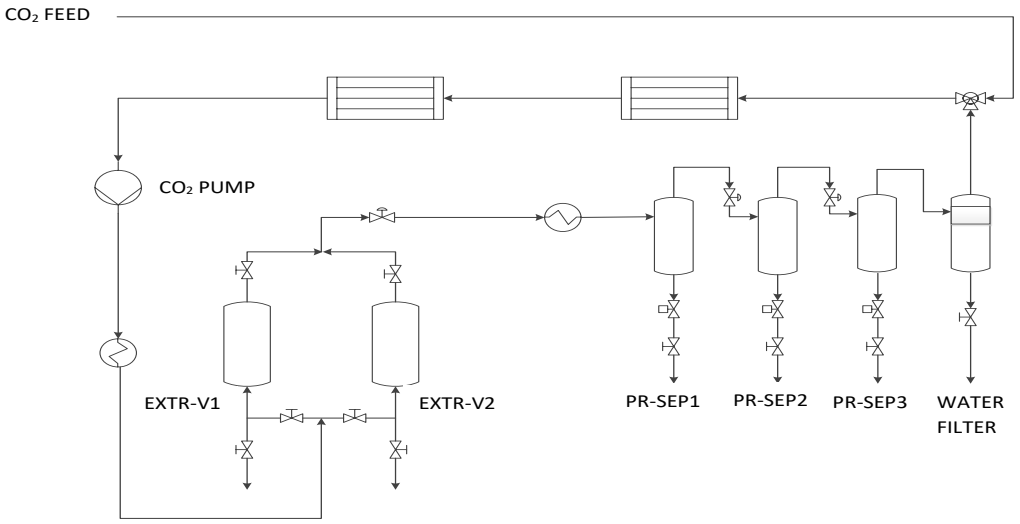


Figure 35: PFD of SFE pilot plant components used for the supercritical solvent impregnation

Figure 35: PFD of SFE pilot plant components used for the supercritical solvent impregnation provides a graphical illustration of the components used for the supercritical solvent impregnation of the textiles. The components used are listed below:

These condensers were used for the liquefaction of gaseous carbon dioxide before it was routed to the carbon dioxide piston pump. The cooling fluid used was a glycol/ water mixture that was supplied by the chiller (C2000). The carbon dioxide routed to the condensers were routed at a consistent pressure of 50 bar.

HP piston pump (P200)

This high-pressure pump was used to pump the carbon dioxide to the extractor vessels at the set pressure. With an adjustable operational flow rate with a range between 1kg/hr and 18 kg/hr. The required minimum inlet pressure of the carbon dioxide was 50 bar, and the maximum allowable outlet pressure of the pump was 700 bar.

Heater (HE3000)

This heat exchanger was used to heat the carbon dioxide that was pumped from the piston pump. This exchanger consisted of an aluminium block through which the carbon dioxide was routed. The design temperature limit of this heater was 350°C with a volume of 72 ml.

HP extraction vessels (A40 & A41)

The extraction vessels were used as the hub for the impregnation process. These vessels had an internal diameter of 131 mm, each a capacity of 5 L with an operating pressure limit of 800 bar. The vessels were also equipped with baskets and electrically heated jackets. These jackets allowed for good temperature control whilst in operation.

Pilot plant experimental work

- Use literature and simulation tools to determine the optimum conditions for textile impregnation of the therapeutic components listed in Table 3: Materials.
- Commence with the impregnation of the various therapeutic components.
- Run analysis for the quantification and characterisation of the solute retention, fastness, antimicrobial activity, and surface morphology.

3.6 SUPERCRITICAL TEXTILE IMPREGNATION

PILOT PLANT START-UP

- Place the sample bottles with the immersed textiles into the extractor basket
- Establish equilibrium
    - Mechanical (pumps)
    - Thermal (set point temperatures)
- Record pressure and temperature conditions at the extractor vessel and separators.
- Analysis
- All collected data is used to determine the antimicrobial efficacy, solute retention and diffusion profile.

3.6.1 THE SUPERCRITICAL TEXTILE IMMERSION METHOD

The supercritical impregnation of textiles with therapeutic components was operated in batch mode. Three sets of each textile were placed in a sample bottle. Then 200ml of the solute was added to the bottle to immerse the textiles. The sample bottle was placed in the extractor basket. The basket was placed in the extractor vessel. Before sealing, the extractor was purged to remove any air. The vessel was closed and charged to 200 bar and 37℃. Once the equilibrium was reached, the vessel was isolated for 3 hours. After 3 hours, the vessel was depressurised at a rate of 3 bar/min. The textiles were removed, washed, and dried in the tray dryer. Finally, the samples were sent for SEM and AST analysis. A set of samples were kept for solute retention analysis—section 2.10 details how the operating conditions were selected.

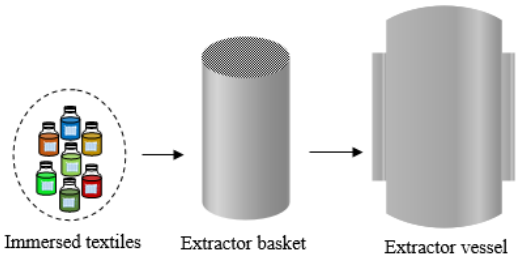


Figure 36: Extractor vessel setup

Figure 37: Sample bottles in the extractor basket

Figure 38: High-pressure pilot plant extractor vessels

Depressurisation rate

During the depressurisation, the carbon dioxide quickly leaves the polymer leaving the solute behind in the polymer matrix. However, rapid depressurisation has been shown to freeze textiles, as shown in *Figure 39*. This was evident by preliminary experimental runs where a rate of 10 bar/min and 5 bar/min froze the textiles. This is not desirable because it damages the textiles. As such, a rate of 2 bar/min was used.

Figure 39: Frozen textile samples after fast depressurisation

Figure 40: CO2 ice formation in extractor vessel due to fast depressurisation

3.7 MEASUREMENT SYSTEM ANALYSIS

In textile impregnation, quantifying the solute transferred into the substrate is integral to determining the technical feasibility. The solute transfer is quantified using the difference in mass of the substrate before and after the final solution immersion phase. Where the difference between the two is attributed to the solute transfer. Each textile sample is weighed using an analytical scale where the difference in mass is often minute. Regardless, it is imperative to quantify the variability and ascertain the capability of the measuring system. Without this analysis, the measured data and conclusions drawn are rendered inconsequential. A statistical experiment was set up to assess the measurement system's capability. Moreover, in this study, the variation between different samples (reproducibility) is not important. What is important is the natural gauge variation (repeatability), the variation in the mass of the same sample due to the gauge capability.

3.7.1 STATISTICAL EXPERIMENTAL SET-UP

- 10 sample bottle lids were numbered 1 to 10.
- Three operators were selected, and one was the person who would be using the measurement system.
- The order of the readings was randomised using the excel randomiser.
- An analytical scale was placed on a concrete bench and weighed all the samples.
- Each reading was triplicated.
- The measurement data obtained from this experiment was used to perform the gauge capability analysis.

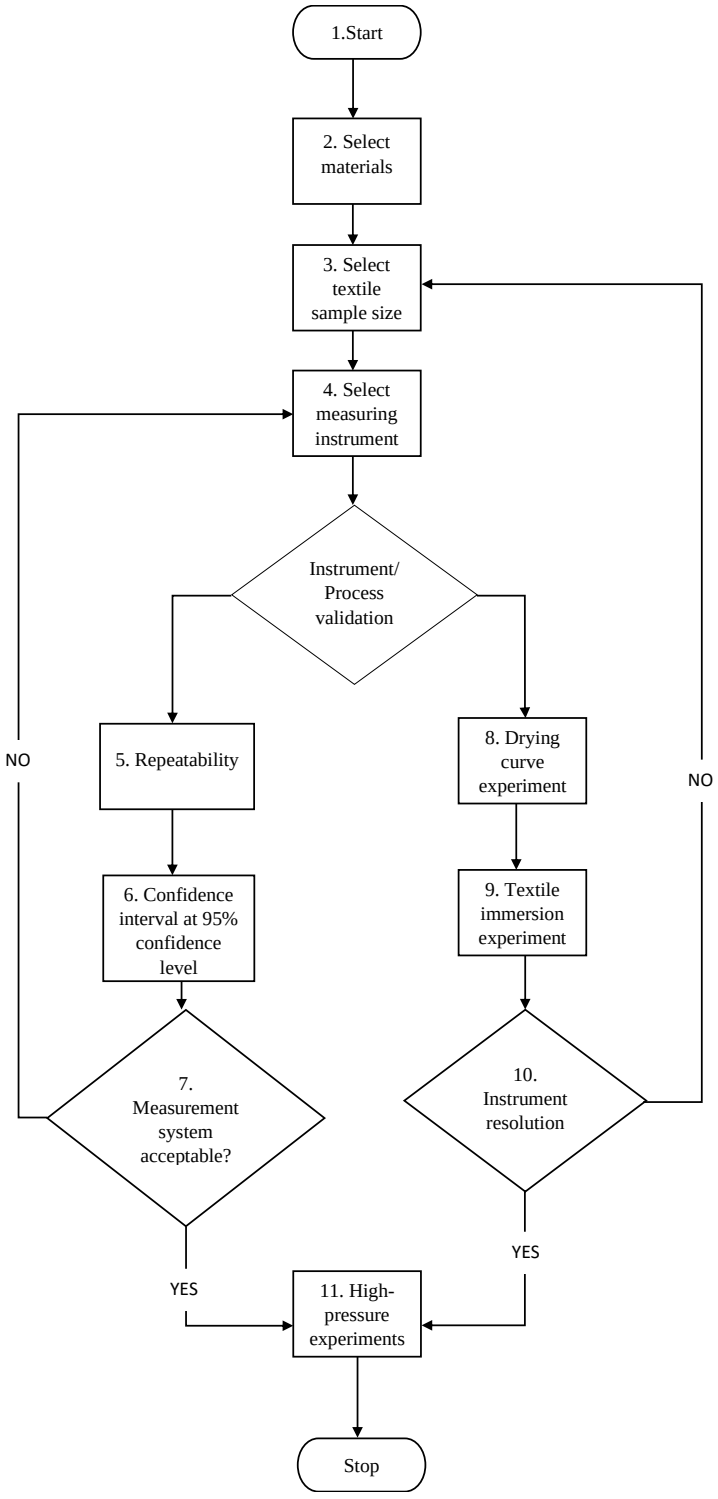


Figure 41: Measurement systems analysis process

If the difference between measures from different investigators was shown to be negligible at a 95 % confidence level, then all 9 readings can be used as one data set. Moreover, this one data set was used to assess the innate variability. A difference of means was used for this. The difference between operators 1 &2, 2&3 and 1&3 was evaluated.

Textile samples of similar size and material to the immersion experiments were used to analyse the drying process. The drying experiment was performed to assess the time needed for the textiles to reach constant mass after being immersed in water, hexane, and ethanol. Additionally, the drying experiment was used to investigate the statistical significance of the measurements taken as the solvent mass on the textile decreased. The measurement system is only deemed acceptable if the innate variability is greater than the confidence interval of the measured data.

3.7.2 THE MEASUREMENT SYSTEM'S ANALYSIS MEASURED DATA

As mentioned in section 2.9 ten samples were measured in triplicate by three different operators. Each sample was weighed using a Radwag 2012 analytical scale. The samples were weighed in random order to remove any bias. The measured sample data is shown in *Table 4: Measured sample data for MSA*

Table 4: Measured sample data for MSA

Sample	Mass of samples								
	Operator 1			Operator 2			Operator 3		
1	15.4280	15.4274	15.4281	15.4284	15.4262	15.4260	15.4274	15.4268	15.4283
2	15.4540	15.4529	15.4532	15.4541	15.4535	15.4521	15.4543	15.4525	15.4537
3	14.8385	14.8376	14.8381	14.8377	14.8388	14.8352	14.8385	14.8370	14.8364
4	15.5970	15.5974	15.5953	15.5978	15.5975	15.5996	15.5960	15.5975	15.5972
5	15.4681	15.4680	15.4675	15.4672	15.4665	15.4675	15.4685	15.4682	15.4669
6	15.4512	15.4509	15.4503	15.4492	15.4495	15.4480	15.4508	15.4497	15.4496
7	15.4077	15.4075	15.4074	15.4082	15.4049	15.4074	15.4075	15.4068	15.4083
8	15.4098	15.4108	15.4095	15.4083	15.4085	15.4058	15.4080	15.4097	15.4090
9	15.7707	15.7729	15.7708	15.7709	15.7759	15.7750	15.7706	15.7723	15.7712
10	15.4866	15.4871	15.4858	15.4858	15.4899	15.4864	15.4860	15.4876	15.4864

Table 5: Results of paired students t-test

Count	30	Operators		
Df	29	1&2	2&3	1&3
Mean		2.76E-03	3.00E-05	2.50E-04
SEM_{stats}		3.60E-04	3.10E-04	1.40E-04
STDEV of differences		1.97E-03	1.70E-03	7.70E-04
t critical	1.70			
t statistic		0.77	0.10	1.74
p-value (one-tail)		0.78	0.54	0.95

Table 6: Descriptive statistical analysis results

Sample	1	2	3	4	5	6	7	8	9	10
Mean	15.4	15.5	14.8	15.6	15.5	15.4	15.4	15.4	15.8	15.5
SEM	3.00E-04	3.00E-04	4.00E-04	4.00E-04	2.00E-04	3.00E-04	3.00E-04	5.00E-04	7.00E-04	4.00E-04
Median	15.4	15.5	14.8	15.6	15.5	15.4	15.4	15.4	15.8	15.5
STDEV	9.00E-04	8.00E-04	1.20E-03	1.20E-03	7.00E-04	1.00E-03	1.00E-03	1.40E-03	2.00E-03	1.30E-03
Sample Variance	8.03E-07	5.68E-07	1.36E-06	1.43E-06	4.32E-07	1.01E-06	1.01E-06	2.04E-06	3.93E-06	1.67E-06
Count	9	9	9	9	9	9	9	9	9	9
Confidence Level (95.0%)	6.89E-04	5.79E-04	8.95E-04	9.18E-04	5.06E-04	7.71E-04	7.73E-04	1.10E-03	1.52E-03	9.92E-04
Upper CI (95%)	15.4	15.5	14.8	15.6	15.5	15.5	15.4	15.4	15.8	15.5
Lower CI (95%)	15.4	15.5	14.8	15.6	15.5	15.4	15.4	15.4	15.8	15.5
CV (%)	1.16E-02	9.70E-03	1.57E-02	1.53E-02	8.50E-03	1.30E-02	1.30E-02	1.85E-02	2.51E-02	1.67E-02

3.7.3 STATISTICAL SIGNIFICANCE

It was important to determine whether or not the data measured in this experiment was of such a nature that the variation shown in the data could be attributed to the factor being investigated, and not to the inherent noise in the data. At the start of the analysis, the null hypothesis assumed that the mean difference was zero and the upper-tailed alternative hypothesis assumed that the mean difference was greater than zero. A paired student t-test was performed using the data in *Table 4: Measured sample data for MSA* to assess the variation of the measurement system and, more importantly, ascertain if the variation is statistically significant.

Table *5*: Results of paired students t-test shows the results obtained from the student t-test, ultimately used to either reject the null or fail to reject the null hypothesis. The statistical significance of the measurement system variation is determined by evaluating the p-value and comparing it to the set alpha value. The p-value shows the probability of the null hypothesis reigning true. However, this does not mean that the null hypothesis is true. If the alpha value is 0.05, it simply indicates a less than 5% chance that the null hypothesis is false. It follows that the closer this p-value is to the alpha value, the lower the probability of the null hypothesis being true becomes.

Similarly, the further the p-value is from the alpha value, the greater the probability that the null hypothesis is true. Thus, a high p-value indicates that the variation does not significantly affect the measurement system's performance. The calculations were performed at a confidence level of 95 % and a corresponding alpha value of 0.05. The paired t-test conducted showed corresponding p-values for each paired comparison. Table *5*: Results of paired students t-test shows the p values obtained from the analysis of the measured data in *Table 4: Measured sample data for MSA*. From the three paired t-tests, p-values of 0.7756, 0.5381 and 0.9537 were obtained. All these p-values are greater than the alpha value of 0.05. This infers that the variation experienced is not likely statistically significant. Since the variation is not significant, the null hypothesis cannot be rejected. This further implies that the measurement system is acceptable. However, this alone is not sufficient.

Thus, descriptive statistical analysis results establish a confidence interval for the measured readings. The confidence interval is an interval estimate of population parameters. The

confidence interval is a function of the point estimate and margin of error. Confidence intervals are used to express the population parameter's interval. More significant variation in the sample data leads to a greater confidence interval.

In comparison, a narrow confidence interval is indicative of a small variation within the sample data. A confidence interval is obtained from the sample data collected for each sample. The largest confidence interval of ± 0.001524 is found in sample 9, as seen in Table *6*: Descriptive statistical analysis results. This confidence interval is adopted as the interval for further readings taken. This is because adopting the smallest confidence interval of $\pm\ 0.000506$ does not account for the variation experienced in the repeated measures. The confidence interval is an estimate obtained through statistical analysis of sample data. The sample size was increased to 10 parts with triplicated readings to lower the sampling error; however, increasing the sample size does not negate sampling error; it simply decreases it. Thus, the sample data is never a perfect representation of the population. Therefore, the most considerable confidence interval is adopted so as not to underestimate the variation experienced. Further, measured data is thus presented with this confidence interval as the level of uncertainty.

3.7.4 PRACTICAL SIGNIFICANCE

It is essential to consider the statistical and practical significance when interpreting the data to draw a meaningful conclusion. The practical significance is determined by comparing the confidence interval to the actual process deviation experienced. Samples, similar in size and type of material to those to be used in the main immersion experiments, were used to assess the drying process. The reason for this was twofold; firstly, it provides a means of establishing whether the measurements of the quantity of solvent and solute present on the sample are detected by the measurement system readings and is not eclipsed by the confidence interval.

An experiment was performed to establish the drying curve for drying the samples after immersion in water. Plotting the drying data and including the confidence interval provides information regarding the statistical significance of the measurements. Suppose the confidence interval is broader than the natural variation of the measured data. In that case, the experimental set-up is deemed unsuitable, and a re-configuration of the sample size and selection of a new instrument would be necessary. The purpose of the drying experiments

was thus twofold. Firstly, as a means of evaluating the practical significance. Secondly, to determine the time needed to dry textiles to constant mass.

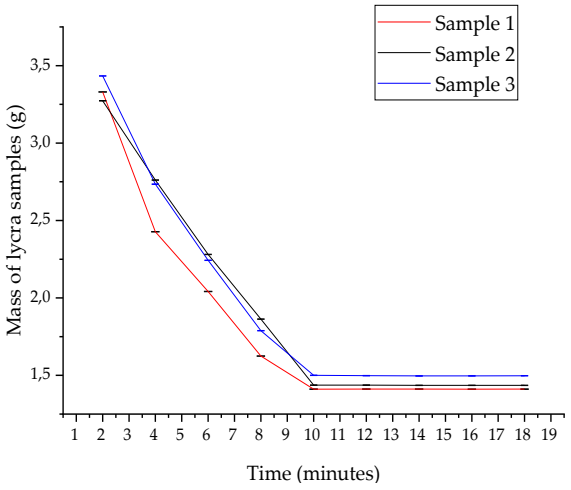


Figure 42: Drying curve for lycra textiles immersed in water (Confidence interval of all readings: ±0.001524 g)

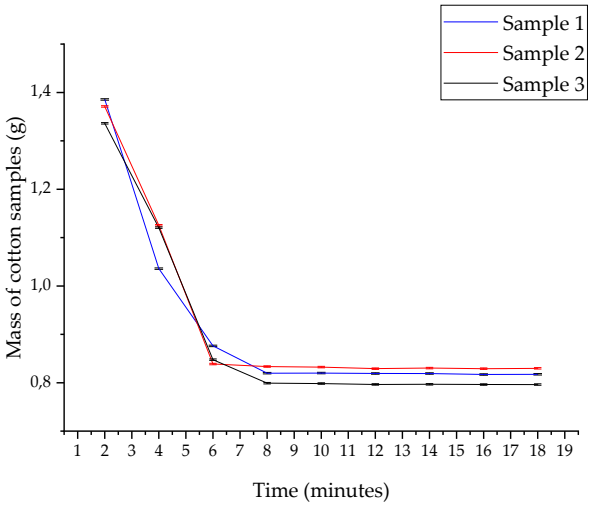


Figure 43: Drying curve for cotton textiles immersed in water (Confidence interval of all readings: ±0.001524 g)

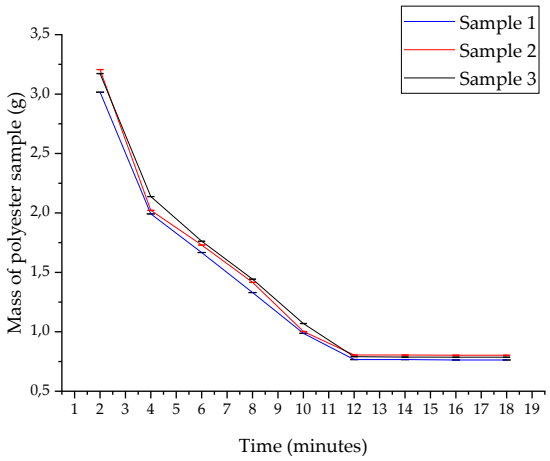


Figure 44: Drying curve for polyester textiles immersed in water (Confidence interval of all readings: ±0.001524 g)

Figure 42, *Figure 43* and *Figure 44* are plots of the drying data obtained when cotton, lycra, and polyester samples were immersed in water. These plots include the confidence interval adopted in section 3.7. From these figures, the confidence interval is hardly discernible in comparison to the loss of water the textiles experience during the drying process. The drying curves illustrated thus indicate that the natural variation of the measured data is insignificant compared to the mass of the sample at each measured point in time until the constant dry mass is reached. The process of measurement, as well as the measurement system, is suitable for the main immersion experiments.

3.8 SAMPLE ANALYSIS

3.8.1 QUANTIFICATION AND ANALYSIS OF THE IMPREGNATION LOAD

To quantify the impregnation load, the impregnated textiles were weighed using an analytical scale to ascertain the solute retention in terms of the mass impregnated. The solute retention was taken from time zero to time infinity. Where time zero was taken from when the textiles were had reached constant dry mass. Additionally, the mass of the textiles before impregnation was at constant dry mass. Before impregnation, this dry mass was used as the point of datum for determining the retention for each textile. Where:

$$Solute\ retention = \frac{mass\ impregnated}{Mass\ of\ the\ textile\ after\ immersion} \quad (2.111)$$

3.8.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antimicrobial efficacy of textiles can be analysed through qualitative or quantitative methods. For this research project, the following factors were used to assess the activity of the extract samples. The Kirby-Bauer disk diffusion method was used to evaluate the bioactivity of the treated samples. The first step consists of preparing the liquid cultures specified in *Table 7: Liquid growth media*.

Table 7: Liquid growth media

Specific liquid growth media (10ml per 50ml glass flask; 5 flasks per strain) were prepared for each test strain:	
Staphylococcus aureus ATCC 29213	Tryptic soy broth
Staphylococcus aureus ATCC 33591	Nutrient broth
Escherichia coli ATCC 25922	Tryptic soy broth
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 700603	Nutrient broth
Listeria monocytogenes ATCC 19111	Brain heart infusion broth
Candida albicans ATCC 90028D-5	Nutrient broth

The growth media were placed in an autoclave for 25 minutes at 120 ℃ and under pressure. The growth media were autoclaved for 25 minutes at 120°C and under pressure. Once cooled, the liquid media were inoculated from an agar plate culture. The liquid cultures were incubated at 37°C overnight, shaking on an orbital shaker at 160rpm. After 24 hours, the cultures were analysed by Gram staining to ensure that the cultures were not contaminated. The optical density of the cultures was determined at 600nm using a Perkin-Elmer Lambda-

25 spectrophotometer and diluted to an OD_{600} = 0.5. These diluted cultures were used in the agar diffusion test described by (Marković et al., 2018). The agar plates were prepared where:

1. For each agar plate, 100µl of the OD600 = 0.5 cultures was spread-plated onto the agar and allowed to diffuse into the agar
2. Each material sample was pre-treated with UV (30 minutes) to kill off any contaminants and placed onto the agar plate containing the test strain
3. For each test strain, commercial antibiotics were used as a positive control. Gentamicin and vancomycin were used for the bacterial test strains, while cycloheximide was used against the yeast test strain (*C. albicans*).
4. Once all samples were plated, the agar plates were incubated at 37°C overnight. The zones of inhibition were measured to determine whether growth inhibition occurred.

3.8.3 DIFFUSION PROFILE

The persistence of the retained solutes was assessed by monitoring the diffusion over time. However, all the polymers used have a degree of hygroscopicity. This meant that the changes in mass observed could be influenced by the varying relative humidity of the surroundings. This variation was explained in Chapter 2, section 2.7.1.

An experiment was set up to observe the effect of moisture on the mass of the textile samples. This experiment placed a set of treated textiles in a desiccator containing silica desiccant. Simultaneously, a set of treated textiles was placed in an isolated environment outside the desiccator. The mass of samples inside and outside the desiccator was taken to establish the diffusion profile of the solutes.

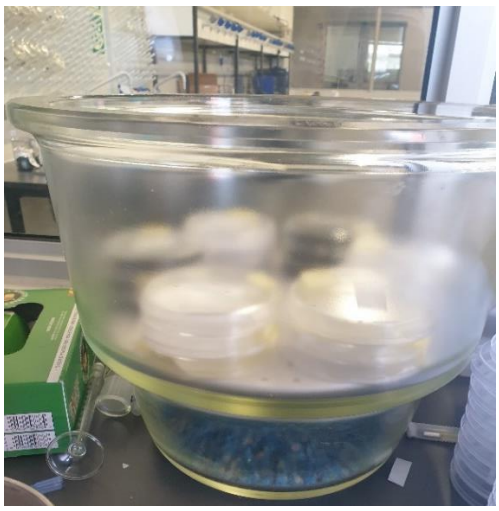


Figure 45: Side view of desiccator, showing silica desiccant

Figure 46: Top view of desiccator

3.8.4 CHARACTERISATION OF THE FABRIC

SEM (Scanning electron microscopy) analysis was used to determine the surface morphology of the fabric before and after immersion at 10 μm, 50μm & 100μm. The morphology was assessed because the $scCO_2$ swells up the polymer fibres. It is necessary to check if the plasticiser had a lasting effect on the surface morphology as this would affect the polymer properties. In addition, FTIR (Fourier Transform Infrared Spectroscopy) was used to validate the identity of textiles used in this research. FTIR produces infrared light to identify the chemical bonds. From this, the spectra produced a distinctive molecular fingerprint profile of the sample. This fingerprint was used to screen and detect components, functional groups and characterize covalent bonding information (Intertek, 2018).

3.9 CHAPTER OUTCOMES

This chapter detailed the materials, methods and equipment used in this research. Specifically, the experimental methods used to achieve the of the study. The following experimental methods and analyses were included in this chapter.

- The pre-treatment of the polymers. This was needed to remove polar and non-polar waxes from the surface of the polymers. Especially since the wax acts as a barrier against the sorption of solutes onto and into the polymer.
- The drying procedure was included and performed since the textile samples need to be dried to constant dry mass after the pre-treatment and immersion steps. This is needed to accurately measure the change in mass.
- The ambient immersion of the polymer samples in the solute solutions. In this procedure, ethanol/solute solutions were prepared where the ethanol acted as the solvent in the immersion process. This was to achieve textile impregnation under ambient pressure and temperature conditions.
- The $scCO_2$ immersion of the polymer samples in the solute. This was to achieve textile impregnation under high-pressure conditions.
- The measurement system's analysis was included. This is due to the dependence of the research on the accuracy and precision of the minute measurements taken. This chapter also includes the results from the MSA. The analysis showed the measurement system to be acceptable for this application. With a confidence interval of ± 0.001524g.

This chapter further includes the sample analysis performed needed to answer the objectives set in Chapter 1. These analyses include:

- The quantification of the solute retained immediately after ambient and $scCO_2$ immersion. The retention was calculated by subtracting the polymer mass before immersion from the polymer mass after.
- The diffusion profile/ solute persistence in an isolated, homeostatic environment. This was monitored by weighing the polymer samples over time and calculating the solute

retention. This was also performed for the samples placed in the desiccator (control experiment).

- SEM analysis on both treated and untreated textile samples under 10, 50 and 100 μm. This analysis was performed to assess the effect of pressure on the surface morphology of the polymer samples. In addition to checking for the presence of solute on the polymer's surface. This was necessary because samples were rinsed after immersion to remove any surface solute as the focus is on the absorbed solute.
- AST using the agar disk diffusion method. This was performed to assess the antimicrobial activity of the treated polymer samples.

Chapter 4 RESULTS AND DISCUSSION

4.1 PREAMBLE

This chapter is aimed at discussing the results obtained from performing the experiments and analyses set out in Chapter 3. This includes the results of the drying experiments and both immersion processes in terms of quantification of the solute retained, solute persistence, SEM analysis and bioactivity of the treated polymer samples. In addition to a detailed comparison of the two processes based on the results obtained.

4.2 DRYING EXPERIMENT RESULTS

Drying experiments were conducted using the tray dryer as shown in Chapter 3. The textile samples are washed in water and hexane. It is thus important to have a good estimate of the drying time needed to get the textile samples to constant dry mass. Textile samples were also immersed in an ethanol solute solution during ambient immersion. The first set of samples immersed using ambient immersion was not rinsed thus the drying time for ethanol needed to be known as well. Three drying experiments were thus conducted, to estimate the drying time needed for textile samples saturated with water, hexane and ethanol. This section contains the results of these experiments.

4.2.1 WATER DRYING CURVE

Textiles have polar waxes on their surface that inhibit the sorption of solutes. A water and detergent solution were used to remove the polar waxes. After the wash, the textiles are rinsed in reverse osmosis water repeatedly. The textiles thus needed to be dried to constant mass. A drying experiment where textiles were immersed in water and dried in a tray dryer was used to estimate the drying time. A total of 9 samples were placed in the dryer. However, in the main experiments, more samples are dried at once. A beaker containing an A4 size textile soaked in water was placed in the dryer to account for this. The mass of each

sample was taken at two minutes intervals.

From *Figure 47: Water drying curve* and *Figure 48: Water moisture content curve*, the first drying

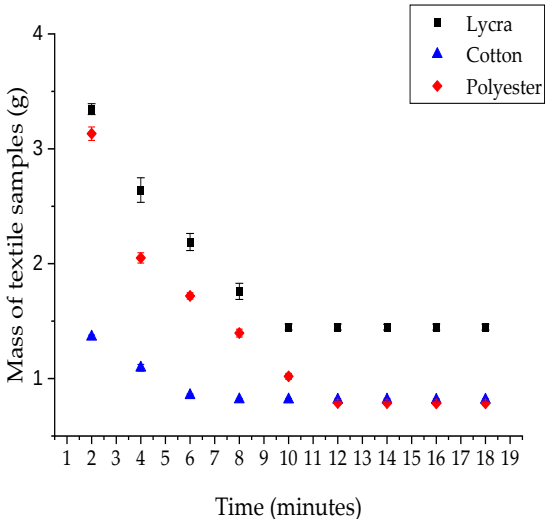


Figure 47: Water drying curve (Confidence interval of all readings: ±0.001524 g)

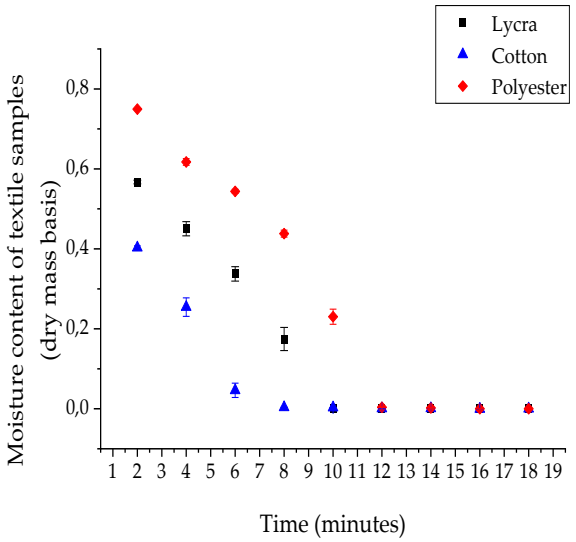


Figure 48: Water moisture content curve (Confidence interval of all readings: ±0.001524 g)

period for cotton and lycra seems to end after 4 minutes. This would indicate that all the unbound moisture evaporated after 8 minutes. The second drying period takes place from 8 minutes to 10 minutes. The drying rate during the first period is faster than the second. This is because, in the second period, evaporation takes place from inside the textile. The drying rate decreases with the moisture content until the constant dry mass of cotton, polyester and lycra is reached by 16 minutes. For polyester, the first drying period seems to end after 4 minutes, after which the second drying period takes place between 10 and 12 minutes.

4.2.2 HEXANE DRYING CURVE

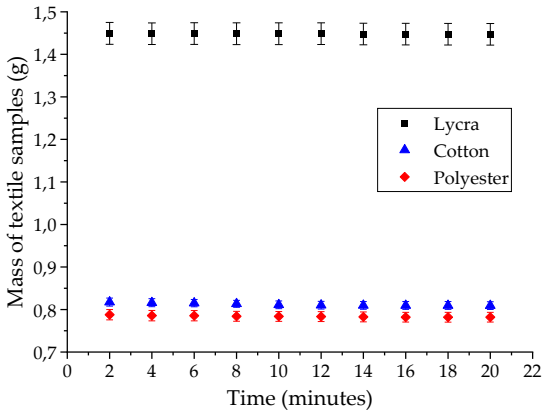


Figure 49: Hexane drying curve (Confidence interval of all readings: ±0.001524 g)

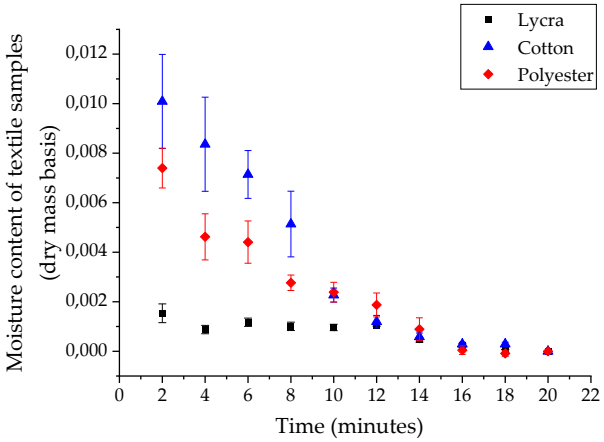


Figure 50: Hexane moisture content curve (Confidence interval of all readings: ±0.001524 g)

From *Figure 49: Hexane drying curve.* It seems that at the 2-minute mark most of the hexane had evaporated. After that, barely any decrease in mass can be seen in *Figure 49: Hexane drying curve* and the constant dry mass seems to be reached within 8 minutes. However, from *Figure 50: Hexane moisture content curve*, the constant dry mass is only achieved after 20 minutes. From the figure, the first drying period for cotton and polyester occurs until the 2-minute mark. After this, the drying rate continues to decrease over time in the second drying period till the 16-minute mark. After this, the evaporation stops, and a constant dry mass is reached after 20 minutes.

Hexane is the most volatile of the three solvents, followed by ethanol and water. The overall drying time for textiles immersed in hexane, ethanol and water is 20, 16 and 20 minutes, respectively. The expectation is that hexane would dry quicker than water; however, this is not the case. This could be explained by the difference in the size of the hexane and water molecule. Hexane is smaller than water; the size of a molecule is a factor that significantly affects the rate of diffusion and thus sorption. Thus, more hexane could have diffused onto and into the textile; therefore, a longer drying time is required to reach a state of constant dry mass.

4.2.3 ETHANOL DRYING CURVE

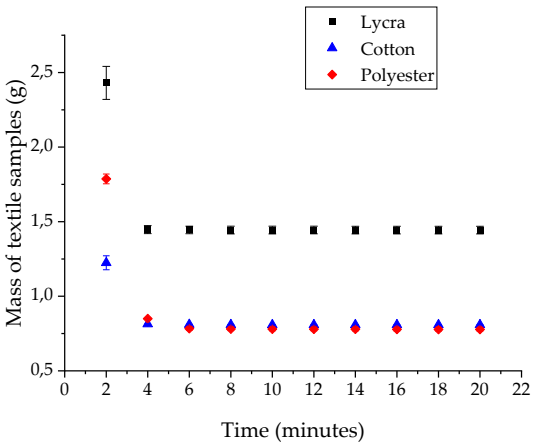


Figure 51: Ethanol drying curve (Confidence interval of all readings: ±0.001524 g)

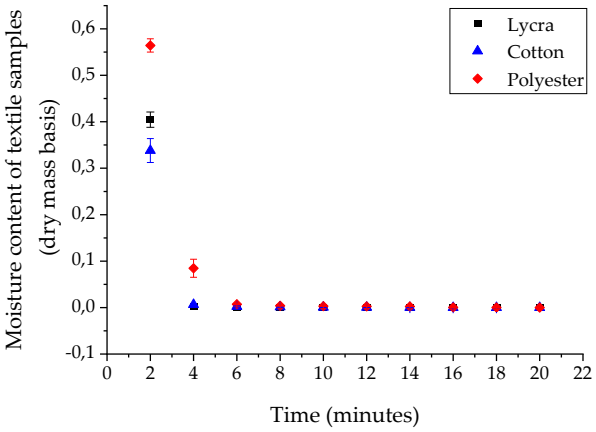


Figure 52: Ethanol moisture content curve (Confidence interval of all readings: ±0.001524 g)

From *Figure 51: Ethanol drying curve* and *Figure 52: Ethanol moisture content curve,* the first drying period for all textiles appear to end after 4 minutes. The second drying period takes place from 4 minutes till the 12-minute mark. After 12 minutes, the drying rate decreases even further until a constant dry mass is reached after 20 minutes.

4.3 QUANTIFICATION OF THE RETAINED SOLUTE

The results for the quantification is reported in terms of the solute retention for the process at ambient conditions, that at high pressure using $scCO_2$ and a detailed comparison of the two.

4.3.1 THE SOLUTE RETENTION OF TEXTILES INFUSED USING AMBIENT IMMERSION

Two sets of results are presented under this section – a set for each of the three runs conducted. In the first run the textile samples were not rinsed after immersion. Whereas in the second run the textiles were rinsed with hexane after immersion to remove surface solutes. Finally run three serves as a repeat of the second run.

4.3.1.1 AMBIENT IMMERSION (FIRST RUN)

Washed textile samples of cotton, lycra and polyester were immersed in a 10% wt solute to ethanol 400 ml solution. These samples were stored for 48 hrs. After that, the textiles were removed, dried to constant mass using the tray dryer at the predetermined conditions and weighed over time.

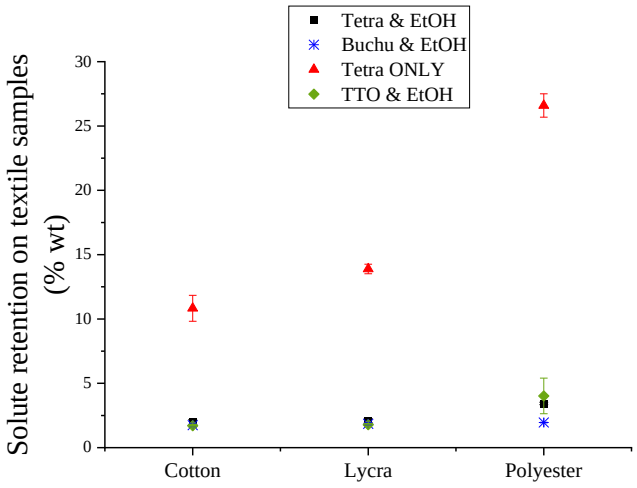


Figure 53: Initial solute retention (% wt) of solute on textile samples

Figure 53: Initial solute retention (% wt) of solute on textile samples shows the data obtained from immersing three different textiles in different therapeutic components. The data is depicted using a scatter plot. From *Figure 53*, it appears that the highest mass of solute was deposited on the textiles when infused with tetra only. The solute retention obtained through infusion with the remaining three therapeutic components appears relatively close. The solute retention seems to be affected by the type of textile used. A question, therefore, arises whether the difference in retention experienced based on the textile type and therapeutic component is statistically significant or not. A statistical tool is needed to assess the significance of the observations at a 95 % confidence level. The student's t-test, as described in sections 2.9 and 3.7, was used to compare the data pairwise. Pairwise comparison between the types of therapeutic component revealed:

For Tetra and EtOH: When the differences are considered pairwise, the solute retention of polyester was statistically greater than that of lycra and cotton samples. However, the retention difference was not statistically different between lycra and cotton samples.

For Tetra only: Pairwise comparison revealed a statistically significant difference between all three textiles. Polyester displayed statistically greater solute retention when compared pairwise to cotton and lycra. Whilst lycra showed statistically greater retention compared to the cotton samples. The pairwise comparison thus suggests a decrease in solute retention in the order polyester> lycra> cotton.

For TTO & EtOH: Pairwise comparison revealed that the solute retention of polyester textiles was significantly greater than the lycra and cotton samples. According to the solute retention data, the cotton samples had retention greater than the lycra samples. However, the pairwise comparison further revealed no statistically significant difference between cotton and lycra samples

For Buchu & EtOH: A statistically significant difference was found between all three textile samples through pairwise comparison. This comparison suggests a considerable decrease in retention in the order polyester> cotton> lycra.

For the therapeutic components: From *Figure 53: Initial solute retention (% wt) of solute on textile* samples, the solute retention varies when different therapeutic components are infused. The pairwise comparison revealed that tetra only textiles yielded significantly greater retention to the other therapeutic components. Further comparison between the therapeutic components and the respective solute retentions suggests that tetra-only textiles yielded significantly more retention than tetra and ethanol textiles. However, there was no statistically significant difference between the TTO and buchu textiles.

4.3.1.2 AMBIENT IMMERSION (SECOND AND REPEAT RUN)

As per the discussion in *section 4.5*, tetrahydro-iso-alpha acids showed no significant bioactivity despite its high solute yield attained on the textiles. Tetrahydro-iso-alpha acids were thus excluded from the SSI and remaining ambient immersion experiments.

The solute retention data in *Figure 54* and Figure *55* was obtained through the ambient immersion of textile samples in a 10% wt solute to solvent solution. Three washed polyester samples, cotton and lycra, were immersed in an isolated environment for 48 hours during the ambient immersion. However, the infused textiles were removed and washed in hexane to remove any surface solutes. Only then were the textiles dried at constant mass using a tray dryer at the predetermined conditions. This experiment was repeated for validation purposes; the data obtained from the repeat is illustrated in *Figure 55.*

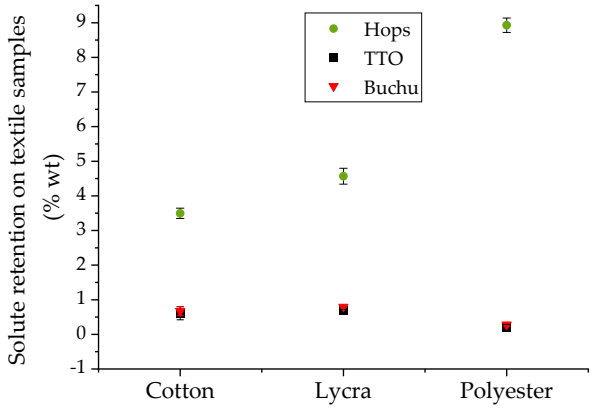


Figure 54: Initial solute retention (% wt) of solute absorbed into textile samples during ambient immersion

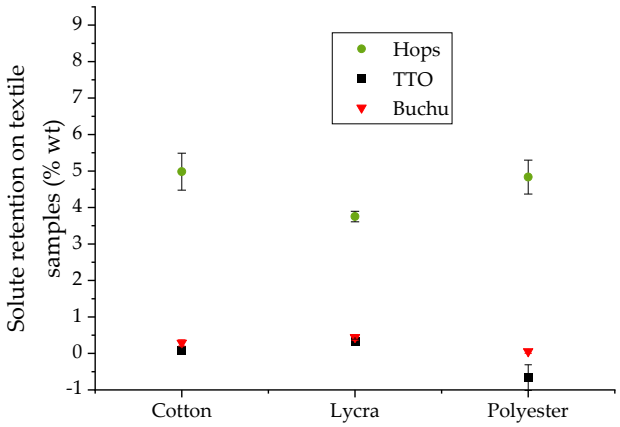


Figure 55: Initial solute retention (%wt) of solute absorbed into textile samples during ambient immersion(repeat)

Figure 54 and *Figure 55* illustrate the solute yield infused into the textiles using scatter plots. Here, the different textiles' solute retention and therapeutic components are emphasised. Differences in the yield are studied across the three types of textiles and when different therapeutic components are infused. Pairwise comparison is used to assess the significance of the differences observed. Through pairwise comparison of the data in *Figure 54*:

- For hops & EtOH: The polyester samples' solute retention was significantly greater than the lycra and cotton samples. In contrast, there was no statistically significant difference between the retention attained on the cotton and lycra samples.
- For TTO & EtOH: No statistically significant difference in solute retention was attained on the lycra and cotton samples nor the cotton and polyester. However, the retention attained on the lycra samples was significantly greater than the polyester samples.
- For buchu oil & EtOH: Through pairwise comparison, lycra and cotton samples had significantly greater solute retention than the polyester samples. Additionally, the pairwise comparison revealed no statistically significant difference between the retention attained on the cotton and lycra samples.
- For the therapeutic components: Pairwise comparison revealed a significant difference between the solute retention attained when different therapeutic components were infused. The retention achieved through hops infusion was significantly greater than that achieved with buchu oil and TTO. The retention of buchu infused textiles was

significantly greater than textiles infused with TTO. This suggested a significant decrease in solute retention in the order: Hops> Buchu oil> TTO.

- For textiles: Pairwise comparison suggested no statistically significant difference between the solute retention achieved across the different textiles.

Furthermore, a noticeable drop in solute retention is observed when comparing the solute retention achieved in *Figure 53*, *Figure 54* & *Figure 55*. This was assessed using pairwise comparisons:

For lycra: Rinsing the immersed textiles seemed to have decreased the solute retention. The pairwise comparison revealed that the retention on lycra samples significantly decreased when textiles were rinsed in hexane after immersion to remove the surface solutes.

For polyester and cotton: Similarly, a significant decrease in retention was found when polyester and cotton textiles were rinsed in hexane after immersion.

Through pairwise comparison, the retention of buchu and TTO on the textiles rinsed in hexane was significantly lower than those not rinsed in hexane after immersion.

The results of the pairwise comparisons suggest that the act of rinsing with hexane significantly decreased the solute retention across all the textile samples. One could postulate that the results in *Figure 53* illustrate the overall solute retention achieved through adsorption and absorption. Whereas *Figure 54* and *Figure 55* show the solute retention achieved through absorption alone. However, an investigation was necessary to test this postulation. An SEM analysis (scanning electron microscopy) was performed on the textile samples rinsed after immersion. In this research, the SEM analysis was used to show if solutes were present on the surface of rinsed textiles and those that were not. The SEM analysis results, and discussion can be found in section 4.4.1.

4.3.2 THE SOLUTE RETENTION OF TEXTILES INFUSED USING SCCO_2

4.3.2.1 SSI (FAST DEPRESSURISATION)

Three sample bottles were filled with 300 ml, each with a different therapeutic component. The washed samples of each textile were immersed in the sample bottles and placed in the extractor vessel. The extractor vessel was shut and charged to 200 bar and 37 ℃ for 3 hours. A

carbon dioxide flow rate of 2 bar/min was maintained to prevent a pressure drop across the vessel. After the contact time, the vessel was depressurised at a relatively rapid, erratic rate due to the malfunction of the ramp function, as shown in *Figure 56*. This resulted in the freezing of the textile samples due to the Joule Thompson effect. This was the reason for the sharp decrease in temperature shown in Figure *57*. According to section 3.6, the operating conditions were set at 250 bar and 40 °C. However, previous experimental runs had shown that operating at 250 bar or above resulted in a significant leak from the extractor lid. This would result in a failed experimental run it if were to occur in the middle of these runs. The operating conditions were adjusted to lower, feasible conditions for the pressurised textile impregnation. The change in pressure and temperature did not drastically decrease the density and, by extension, solvating power of the $scCO_2$. In fact, the adjusted operating conditions lowered the density from 880 kg/m^3 to 856 kg/m^3. The results obtained from this experimental run are illustrated in *Figure 58: Solute retention (% wt) of solute absorbed into textile samples during SSI (fast ΔP).*

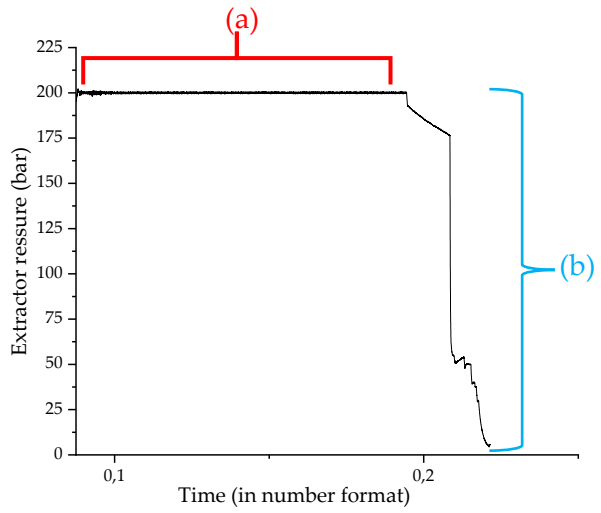


Figure 56: Pressure during contact time (a) and fast depressurisation (b)

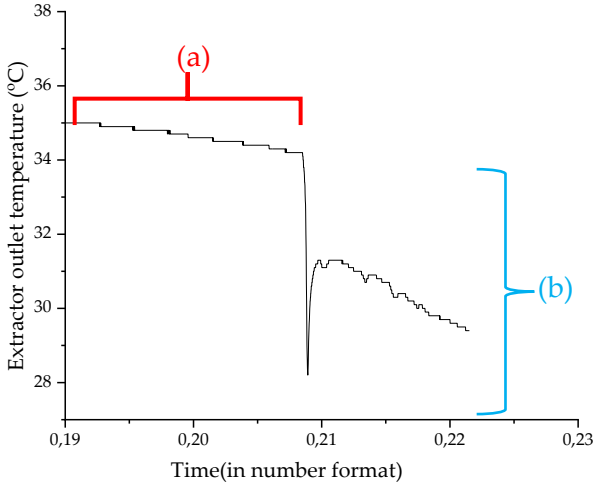


Figure 57: Temperature during contact time (a) and fast depressurisation (b)

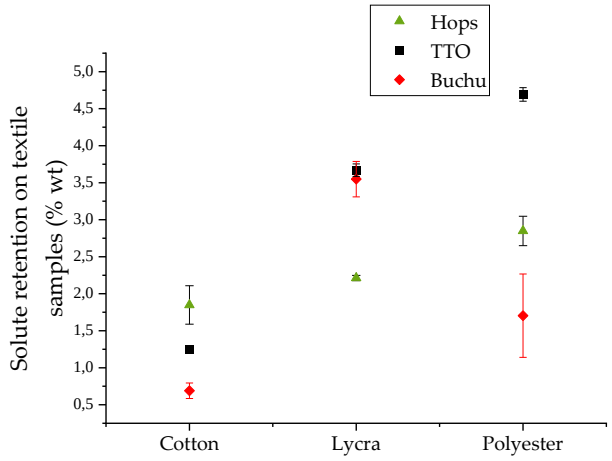


Figure 58: Solute retention (% wt) of solute absorbed into textile samples during SSI (fast ΔP)

Figure 58: Solute retention (% wt) of solute absorbed into textile samples during SSI (fast ΔP) displays the solute retention achieved when samples of polyester, lycra and cotton textiles were immersed at 200 bar and 37℃ for 3 hours using $scCO_2$. In *Figure 58*, the solute retention seems to vary with the type of textile and the type of solute infused. Pairwise comparison was used to study this further:

- No statistically significant difference was shown between the retention obtained when buchu oil and hops were used, nor between hops and TTO. However, the pairwise comparison revealed that the TTO retention was significantly greater than buchu oil.
- For hops extract: No statistically significant difference was found between the retention obtained by polyester and cotton as well as lycra and cotton. Upon comparing polyester and lycra, polyester had significantly greater retention.
- For TTO: A significant difference was found between the retention achieved across all three types of textiles. The solute retention decreased significantly in the order polyester> lycra > cotton.
- There was no significant difference between retention achieved by lycra and polyester and polyester and cotton samples for buchu oil. However, the solute retention on lycra samples was significantly greater than on cotton samples.

4.3.2.2 SSI (SLOW DEPRESSURISATION)

Three sample bottles were filled with 300 ml, each with a different therapeutic component. The washed samples of each textile were immersed in the sample bottles and placed in the extractor vessel. The extractor vessel was shut and charged to 200 bar and 37 °C for 3 hours. A 2 bar/min carbon dioxide flowrate was maintained to prevent a pressure drop across the vessel. After the contact time, the vessel was depressurised manually at an approximate 5 bar/min rate. The depressurisation and accompanying drop in temperature are shown in *Figure 59* and *Figure 60*, respectively. The solute retention results obtained from this experiment is illustrated in *Figure 61.*

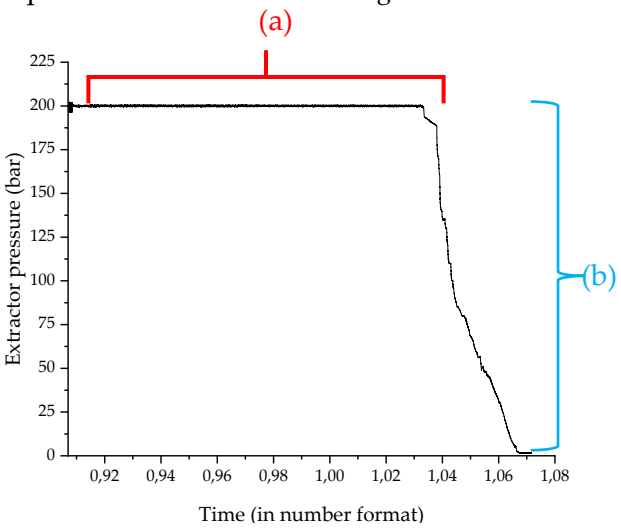


Figure 59: Pressure during contact time (a) and slow depressurisation (b)

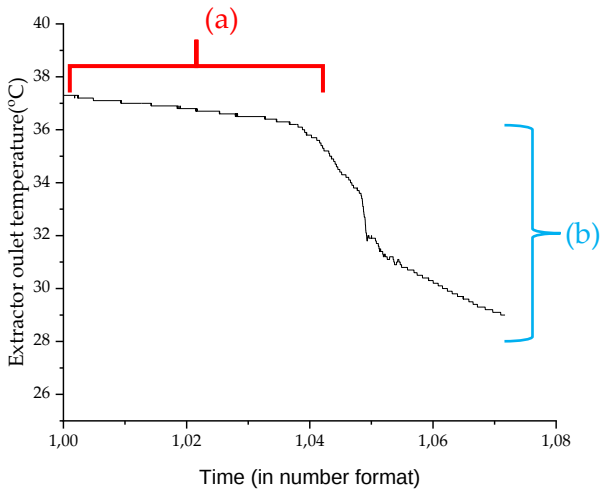


Figure 60: Temperature during contact time (a) and slow depressurisation (b)

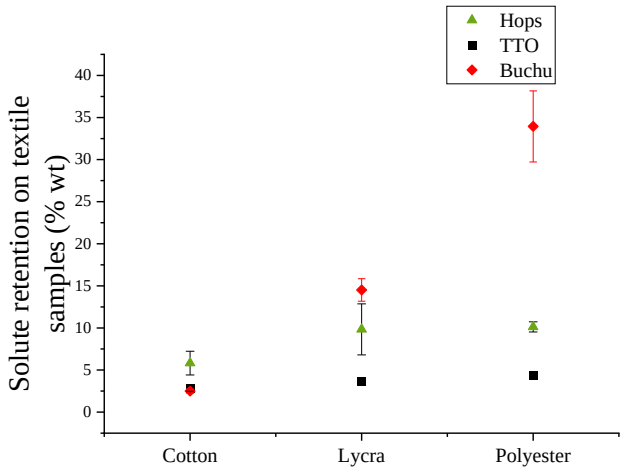


Figure 61: Solute retention (% wt) of solute absorbed into textile samples during SSI (slow ΔP)

Figure 61 shows the data obtained when cotton, lycra and polyester samples were immersed in three different therapeutic components under 200 bar and 37°C. In contrast to the previous

high-pressure immersion, this depressurisation was much slower and controlled at 5 bar/min. From *Figure 61*, the retention seems to vary with the type of textile and the therapeutic component. This appears to be especially true for polyester textiles. Pairwise comparison was used to evaluate this further and determine if the variations are statistically significant. Through pairwise comparison:

Hops were shown to have significantly higher retention when compared to TTO. From *Figure 61*, buchu appears to have a higher retention than TTO and hops. However, this deviation is statistically insignificant.

For Hops & TTO: Pairwise comparison revealed no statistical significance between the retention achieved across the different textiles when TTO and hops were infused.

For Buchu: Unlike the hops and TTO textiles, there is a statistically significant difference in the retention achieved across the textiles when buchu was infused. The pairwise comparison revealed that the solute retention decreases significantly in the order polyester> lycra> cotton.

The pairwise comparison revealed that the slow ΔP polymers *(*Figure 61*)* yielded significantly greater retention to the fast ΔP polymers *(Figure 58).* The same was observed across the different polymers. According to research conducted by (Martinez et al., 2017) and (Belizón et al., 2018), where cotton and polyester were used as the substrate, a fast depressurisation rate improved the impregnation efficiency. However, in this research, the opposite was observed. A significant increase in solute retention across all the slow ΔP textiles was experienced. In the studies conducted by (Martinez et al., 2017) and (Belizón et al., 2018), the textiles were not rinsed to remove any solutes deposited on the fibres' surface. The SEM images at a magnification of 10 μm reported in these articles show the presence of solute molecules on the surface of the fibres. These images are further used in the studies to assert homogenous dispersion when $scCO_2$ is used. The rapid drop in pressure results in an immediate fall in the solvating power of the $scCO_2$. This would mean a relatively rapid loss in the affinity of the solute for the $scCO_2$. The carbon dioxide rapidly drops the solutes. One could postulate that the drop in pressure results in the deposition of the solute mostly on the surface of the fibres. Thus, increasing the total retention and improving the impregnation efficiency instead of increasing the mass of solute absorbed into the fibres. It is also possible that the solute in the

studies conducted by Martinez and Belizón had a greater affinity for $scCO_2$. It is also possible that the solute had a higher affinity for the substrate thus resulting in the higher solute retention when a slow drop in pressure was implemented in this study.

4.3.3 COMPARATIVE ANALYSIS BETWEEN AMBIENT IMMERSION AND SSI

To assess the feasibility of supercritical textile immersion, it is necessary to compare the data obtained from ambient immersion to that of the high-pressure immersion. In this section, a pairwise comparison is used to compare the solute retentions achieved by ambient immersion to those achieved under high-pressure immersion.

The lowest retentions were achieved under the fast depressurisation rate. In fact, for hops, it was found that the solute retention obtained during ambient immersion were higher than the retention attained under a rapid depressurisation. Through pairwise comparison between the ambient immersion and fast depressurisation samples:

- For hops: Pairwise comparison suggested that the solute retentions of the ambient textiles were significantly greater than those obtained SSI with fast depressurisation.
- For TTO: No statistically significant difference was uncovered between the cotton samples. However, the solute retention achieved on the lycra and polyester samples during SSI fast depressurisation was significantly higher.
- For Buchu: No statistically significant difference was shown between the cotton and polyester samples. The lycra samples' lycra SSI fast depressurisation samples displayed significantly greater solute retention than the ambient immersion lycra samples.

Overall comparison between the hops samples revealed that the overall ambient immersion retention was significantly greater than the overall SSI fast depressurisation solute retention attained. Nevertheless, the solute retention obtained by SSI slow depressurisation was substantially greater than its ambient immersion counterpart.

The only statistically significant difference was between the cotton samples regarding the overall retentions on the textiles. Pairwise comparison of the overall retention showed cotton

ambient immersion samples to have significantly greater retention to the cotton SSI fast depressurisation samples.

From section 4.3.2.2, the SSI slow depressurisation retention was greater than the solute retention obtained by SSI fast depressurisation. Therefore, comparing the SSI slow depressurisation to the retention achieved under ambient immersion is necessary. Pairwise comparison was used for the comparison:

- For hops: No significant difference was found between the retention achieved across the types of textiles.
- For TTO and buchu: Pairwise comparison suggests that the SSI slow depressurisation samples yielded significantly greater retention than the ambient immersion samples. This is true for all three types of textiles immersed.
- The overall retention achieved through SSI slow depressurisation was significantly greater than the ambient immersion textiles. However, no significant difference was shown across the overall retention achieved by the cotton, polyester and lycra samples.

From the pairwise comparison results, the following inferences could be drawn. The difference in retention between the SSI slow depressurisation and ambient immersion samples supports the literature that suggests that $scCO_2$ increases the textile impregnation efficiency. However, it is also evident that $scCO_2$ as a plasticising agent alone does not result in higher solute retention. Based on the results obtained in this research, it can be postulated that depressurisation was the factor that greatly influenced the solute retention achieved under SSI. This is evident in the pairwise comparison between the slow and fast depressurisation SSI samples. The effect of the depressurisation rate was so significant that the TTO and buchu cotton samples displayed greater retention under the slow depressurisation. This is significant because cotton has high crystallinity. This type of polymer is relatively rigid and more challenging to infuse than the semi-crystalline and amorphous polymers. In contrast, polymers such as polyester and lycra have more amorphous regions making it easier to infuse solutes. Nevertheless, polyester also has benzene rings, making the amorphous regions more rigid, effectively decreasing the free volume. Based on the degree of crystallinity, one expects the diffusivity to decrease in the order: lycra> polyester> cotton.

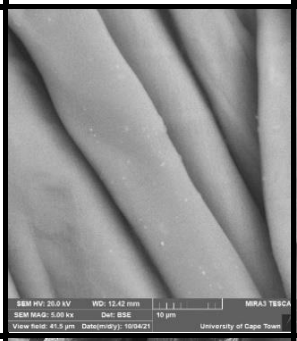
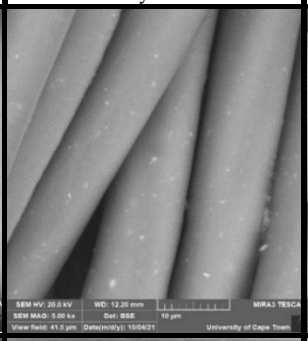
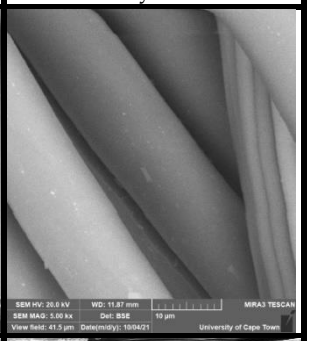
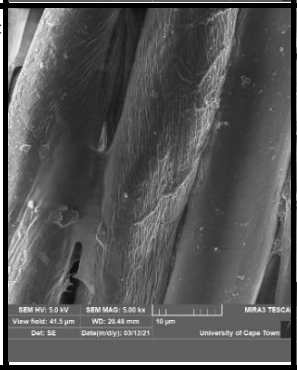
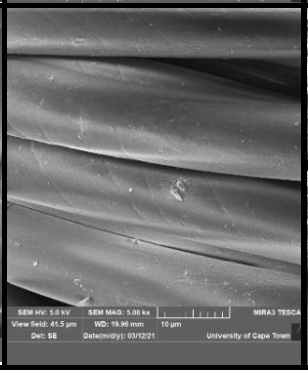
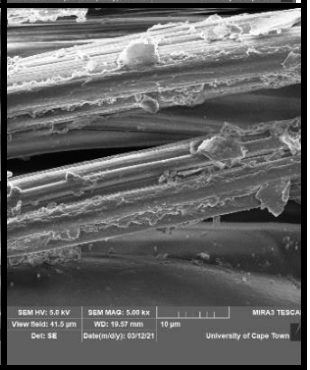
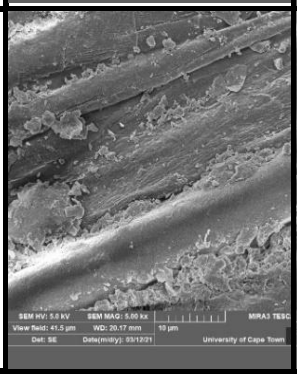
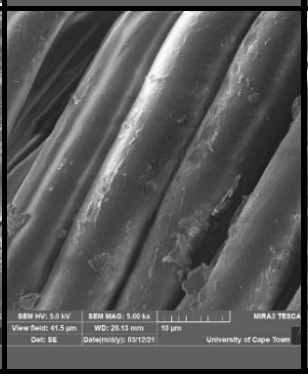
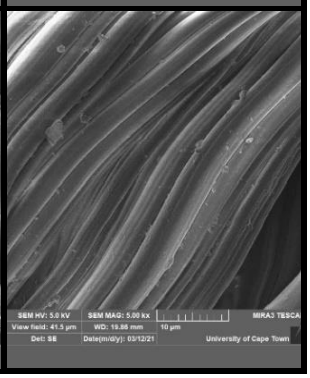
4.4 CHARACTERISATION OF IMPREGNATED TEXTILES

4.4.1 SEM OF TEXTILES INFUSED USING AMBIENT IMMERSION

Table 8: SEM images of ambient immersion textiles (not rinsed) at a magnification of 10μm shows the images taken of the cotton, lycra and polyester samples that underwent ambient immersion but were not rinsed after that. The untreated polyester, cotton, and lycra samples seem to have a relatively smooth surface. However, after it is immersed in buchu & ethanol, the surface of the polyester becomes rough and appears to have many clumps on its surface. This could be attributed to the presence of the buchu and the effect the polar solvent ethanol may have on the surface morphology of the polyester. The surface morphology of the cotton sample seems to have been affected by the immersion in buchu and ethanol. Where striations can be seen on the now rough surface of the cotton sample, the lycra samples seem to be unaffected by the immersion in buchu and ethanol as no visible difference can be seen between the untreated and treated SEM images.

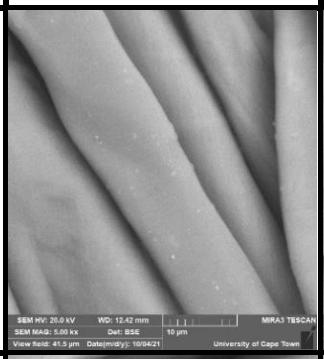
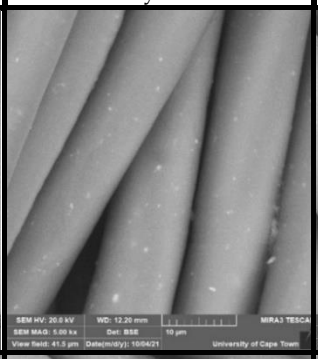
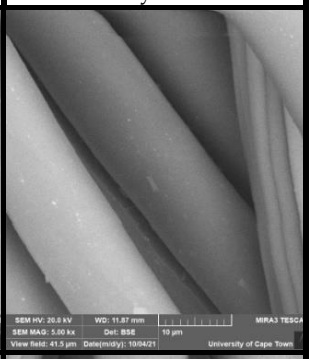
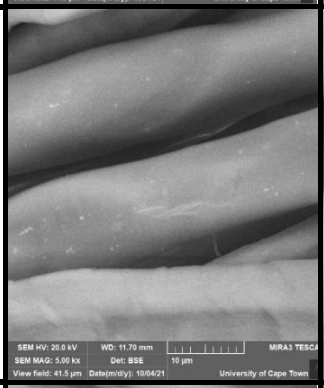
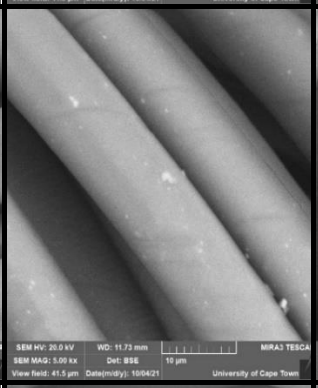
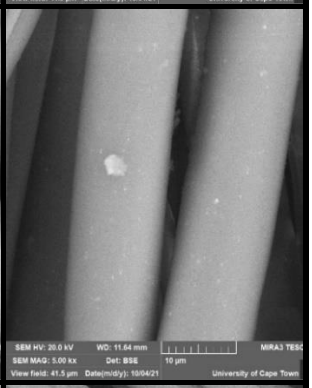
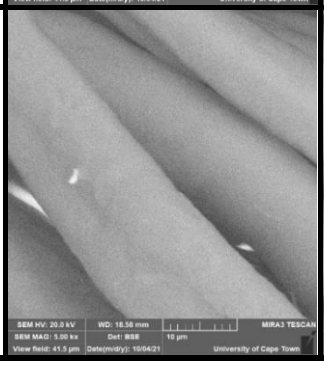
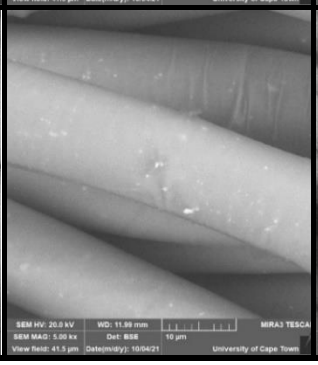
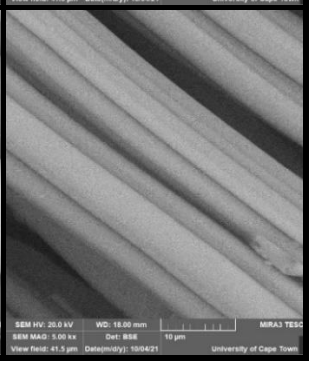
After immersion in TTO and ethanol, the polyester seems unaffected as no visible difference in the morphology can be seen. This contrasts with the buchu and ethanol polyester sample. One could postulate that the difference in morphology of the polyester is due to the buchu in the immersion solution, as the ethanol seems to have no visible effect on the morphology of the TTO and ethanol polyester sample. In addition, a slight difference can be seen on the surface of the lycra sample after immersion in TTO and ethanol. The ambient immersion of cotton in TTO and ethanol seems to have significantly changed the surface of the fibres. What was smooth is now rough. The fibres seem to have been adhered to each other as no gaps are visible between the fibres anymore.

Table 8: SEM images of ambient immersion textiles (not rinsed) at a magnification of 10µm

Ambient immersion (not rinsed)			
	Cotton	Lycra	Polyester
Untreated			
Buchu & EtOH			
TTO & EtOH			

However, the samples in Table 8 were rinsed in hexane after ambient immersion. There is no noticeable difference in the morphology of the cotton, lycra, and polyester samples before and after immersion. This could infer that the rinse with hexane removed all the surface solutes present.

Table 9: SEM images of ambient immersion textile samples at a magnification of 10μm

Ambient immersion (rinsed)			
	Cotton	Lycra	Polyester
Untreated			
Hops			
TTO			

4.4.2 SEM OF TEXTILES INFUSED USING SCCO_2

scCO_2 plasticizes the fibres of a polymer and thus temporarily alters the fibres' morphology. c This is especially true for medical textiles as they were selected based on their properties and using a process to functionalise them alters these properties is thus undesirable. Furthermore, the textiles are rinsed after SSI to remove any surface solutes. Sending samples of the textiles for SEM analysis is often used to confirm the presence of solutes on the surface of the fibres. From the SEM images in *Table 10*, *Table 11* and *Table 12*, no noticeable difference is seen in the morphology of the lycra, polyester and cotton samples under all three magnifications of 10 μm, 50 μm and 100μm. The high-pressure immersion and use of scCO_2

as a plasticising agent did thus not affect the morphology and properties of the cotton, lycra and polyester samples

Table 10: SEM images of untreated and treated samples of cotton under different magnification

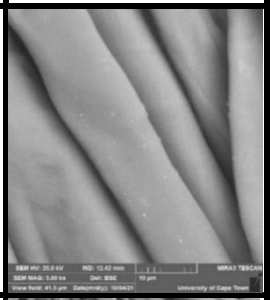
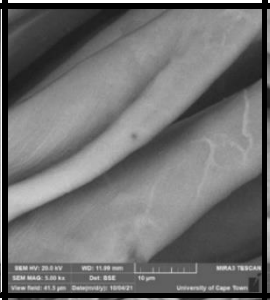
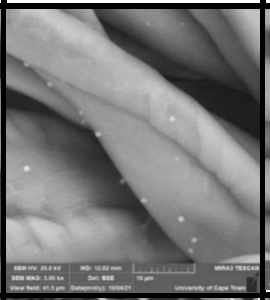
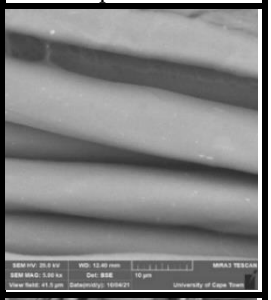
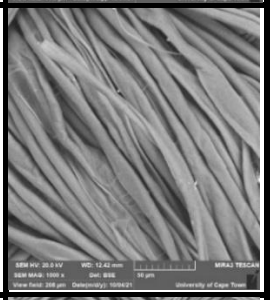
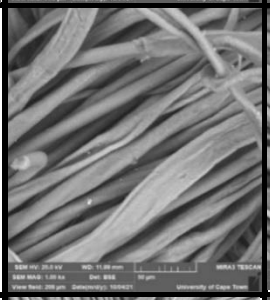
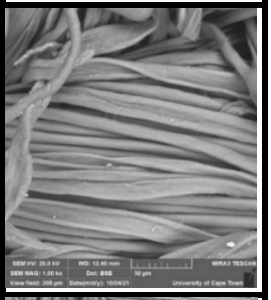
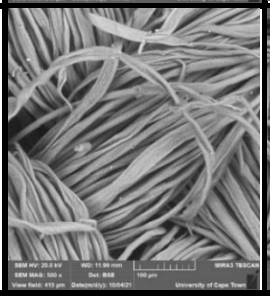
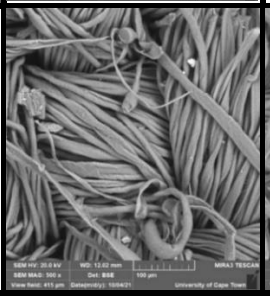
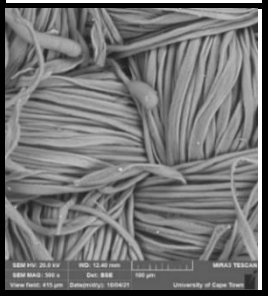
SSI IMMERSION				
Magnification	Untreated	TTO Cotton	Buchu Cotton	Hops Cotton
10 µm				
50 µm				
100 µm				

Table 11: SEM images of untreated and treated samples of polyester under different magnifications

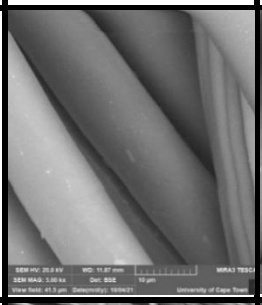
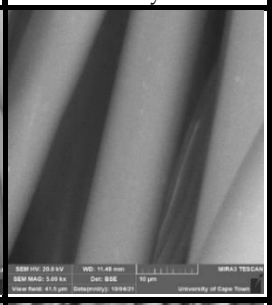
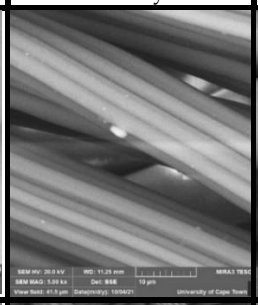
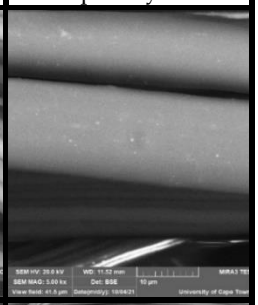
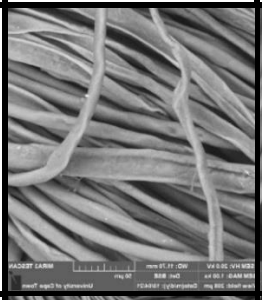
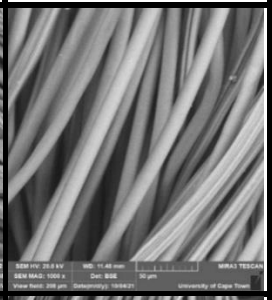
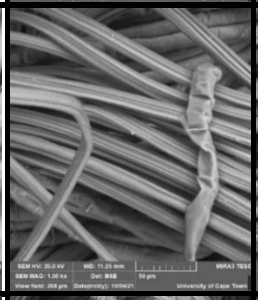
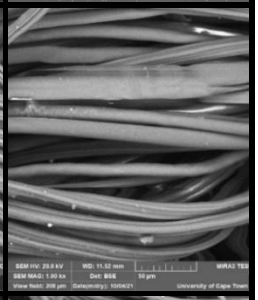
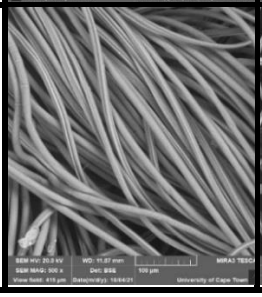
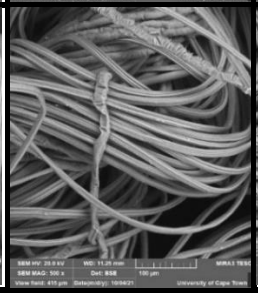
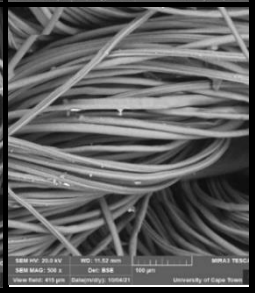
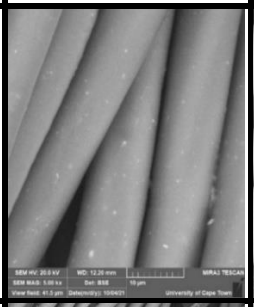
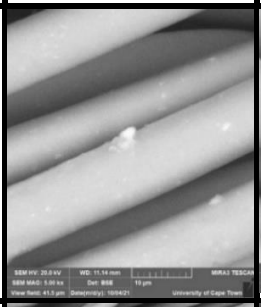
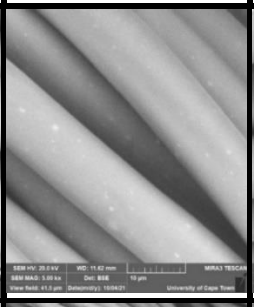
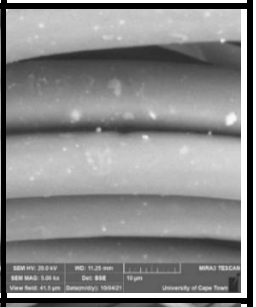
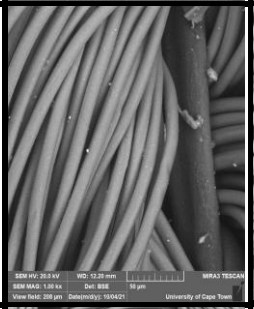
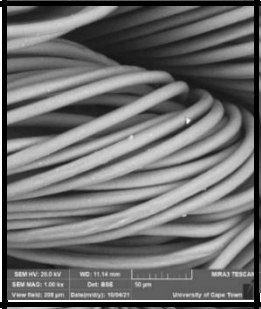
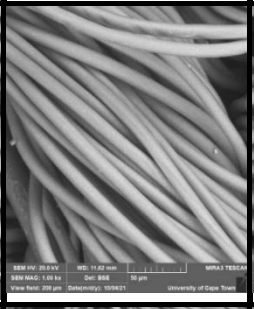
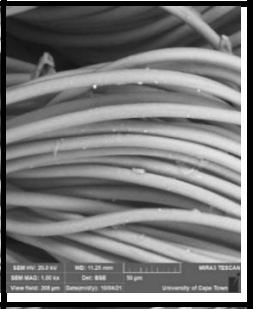
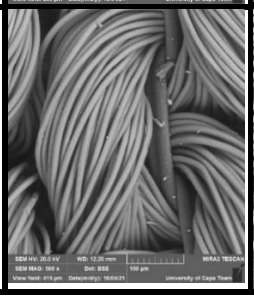
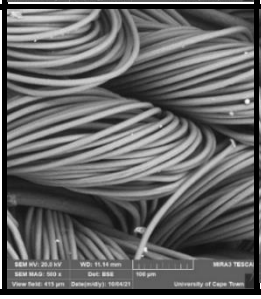
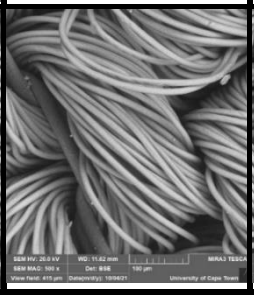
SSI IMMERSION: Polyester				
Magnification	Untreated	TTO Polyester	Buchu Polyester	Hops Polyester
10 µm				
50 µm				
100 µm				

Table 12: SEM images of untreated and treated samples of lycra under different magnifications

SSI IMMERSION: TTO Lycra				
Magnification	Untreated	Treated and washed	Buchu Lycra	Hops Lycra
10 µm				
50 µm				
100 µm				

4.5 BIOACTIVITY OF TEXTILES INFUSED USING AMBIENT IMMERSION (FIRST RUN)

Table 13: Zone of inhibition (mm2) of tetra & EtOH samples shows the data obtained after analysing the tetra & EtOH infused samples using the Kirby Bauer disk diffusion method. This analysis measured the zone of inhibition for three microbial strains; all AST (antimicrobial susceptibility testing) was performed in triplicate. The samples in this table were obtained through the ambient immersion of polyester, cotton and lycra samples in tetra & EtOH according to section 4.3.1.1. In other words, these are the immersed textile samples that were not rinsed in hexane to remove any surface solute deposition.

Table 13: Zone of inhibition (mm^2) of tetra & EtOH samples

Listeria monocytogenes ATCC 19111						
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)
Cotton	11	395.84	10	373.85	10	336.94
Polyester	10	204.99	10	175.93	10	267.82
Lycra	10	235.62	10	235.62	10	235.62
Staphylococcus aureus ATCC 29213						
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)
Cotton	11	395.84	10	452.39	10	452.39
Polyester	10	267.82	10	581.98	10	537.21
Lycra	9	250.54	10	301.59	10	336.94
Staphylococcus aureus ATCC 33591						
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)
Cotton	10	452.39	10	452.39	10	494.02
Polyester	10	494.02	10	452.39	10	412.33
Lycra	10	452.39	10	494.02	10	452.39

In *Table 13*, there is a slight difference in the diameter of samples used in the antimicrobial susceptibility testing. The specific bioactivity of the samples was thus calculated. The specific bioactivity was calculated by dividing the zone of inhibition by the sample area. This specific bioactivity compares all the infused cotton, polyester and lycra samples.

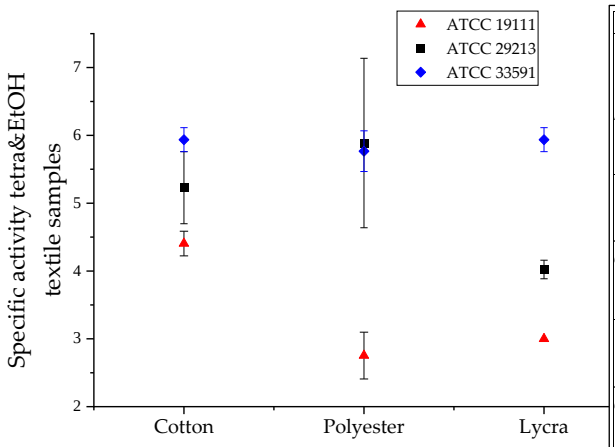


Figure 62: Specific bioactivity of tetra & EtOH infused textile samples

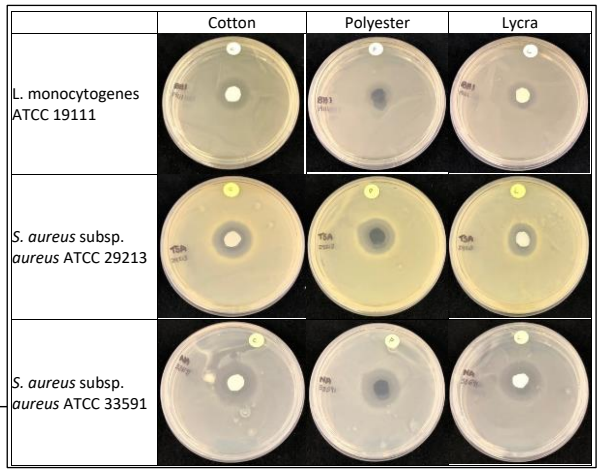


Figure 63: Zone of inhibition around the tetra&EtOH samples

Figure 62 shows the specific bioactivity of textile samples immersed in a tetra & EtOH solution. The specific bioactivity against the three strains appears to be indistinguishable. However, the bioactivity appears to be influenced by the type of textile for all three strains in question. A pairwise comparison was performed to assess the statistical significance of this observation at the 95% confidence level. William Sealy Gosset invented the student's t-test to handle small samples in quality control in brewing (Zabell, 2008). When the t-test was applied to all sets of data, the following features of the data were identified:

- For *S.aureus* (ATCC 29213): The specific bioactivity of the different textiles was statistically similar.
- For *S.aureus* (ATCC 33591): The specific bioactivity of the three different textiles was statistically similar.
- ATCC 29213 and ATCC 33591 are both *S.aureus* strains. However, ATCC 29213 is an MSSA (methicillin-susceptible *Staphylococcus aureus*) strain whilst the ATCC 33591 is an MRSA (methicillin-resistant *Staphylococcus aureus*) strain. Thus ATCC 33591 is a drug-resistant *S.aureus* strain. It would thus be interesting to compare the bioactivity of the two strains of *S.aureus*.
- For *Listeria monocytogenes* (ATCC 19111): The specific bioactivity of the lycra and polyester samples were statistically similar. However, the specific bioactivity of the cotton samples was significantly greater than that of the lycra and polyester samples.

From the pairwise comparison performed, the following inferences and questions are formed. Cotton showed statistically significant superiority over both polyester and lycra in terms of the bioactivity of the solute absorbed into it. Findings in section 4.3.1.1 show that the solute retention of the polyester was statistically greater than that of the cotton and lycra and the solute retention achieved on the cotton and lycra was statistically similar. This infers that the lycra and cotton had similar solute retention. Nevertheless, the cotton samples generated greater inhibition zones, suggesting that the solute diffused better out of the cotton samples.

From *Figure 62* the activity seems to vary depending on the strain tested. It would thus be interesting to compare the specific bioactivity of tetra & EtOH infused samples against different strains. A student's t-test was once again used for this comparison. The following was features of the data was observed:

- For MRSA versus MSSA: The bioactivity of the cotton and polyester samples was found to be statistically similar. However, the bioactivity of lycra infused textiles was significantly greater against MRSA. This finding is significant since MRSA is one of the superbugs that plague healthcare.
- For MRSA versus *Listeria monocytogenes*: Pairwise comparison revealed that cotton, polyester and lycra samples' bioactivity was significantly higher against MRSA.
- MSSA versus *Listeria monocytogenes*: The bioactivity of cotton and polyester infused samples against the two strains was found to be statistically similar. The bioactivity of lycra samples was significantly greater against MSSA.

The overall impression of the comparison between strains is the following. The tetra & EtOH samples seem to decrease bioactivity in the order MRSA> MSSA> *Listeria monocytogenes*. However, these samples were not rinsed in hexane to remove the surface deposition. It is thus possible that ethanol was still on the surface of the textiles during the antimicrobial susceptibility testing. Further experiments are therefore needed to discern if the bioactivity was due to the ethanol and not the solutes. Tetra only infused cotton, lycra and polyester textiles exhibited some activity against *Klebsiella pneumonia*e (ATCC 700603). *Figure 64* provides a visual of tetra-infused polyester's bioactivity against *Klebsiella pneumonia*e. In this figure, there is a visible zone around the circumference of the polymer sample. However,

regrowth has occurred in this zone of inhibition. This suggests that the sample only stops the growth of bacteria but does not kill the bacteria, unlike a bactericidal polymer.

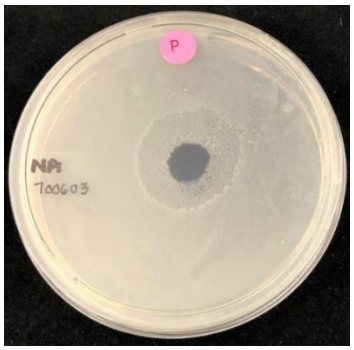


Figure 64: Bioactivity of tetra only infused polyester sample against *Klebsiella pneumonia*e

Whereas tetra only infused cotton, polyester and lycra samples exhibited no bioactivity against the other tested strains. This is despite tetra only textiles having the highest solute loadings indicating that the antimicrobial susceptibility results for the tetra & EtOH are because of the ethanol and not the tetra. The textiles infused with tetra alone made the textile samples more rigid. This could indicate that the tetra crystalised when it was infused and then dried. However, it would help to study the tetra-infused textiles' diffusion profile over time.

4.5.1 DIFFUSION PROFILE OF TETRA ONLY INFUSED TEXTILES

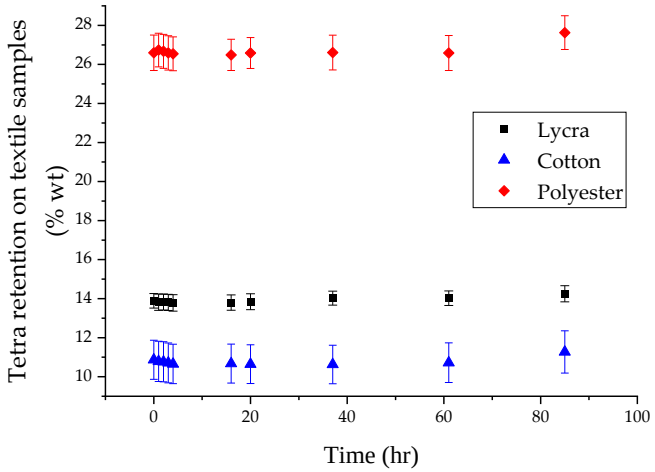
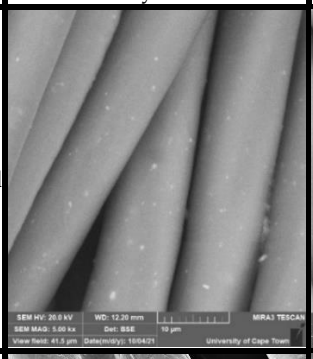
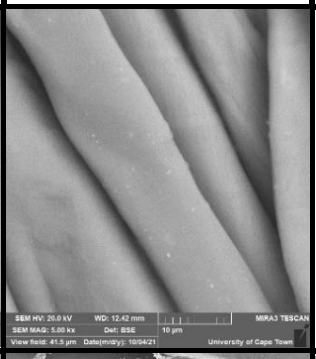
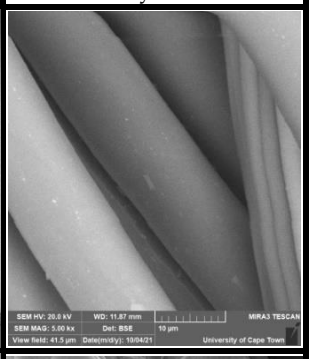
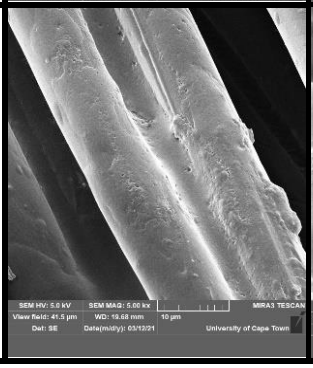
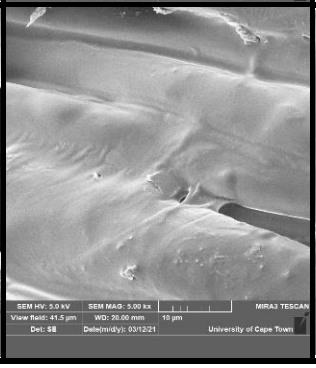
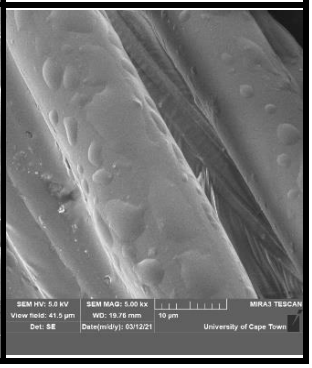


Figure 65: Diffusion profile of tetra only infused textile samples

Figure 65: Diffusion profile of tetra only infused textile samples shows the data obtained by weighing the textile samples over time after infusion with tetra only. The amount of solute absorbed into the fiber here is the sample retention. The rate of solute loss in *Figure 7* suggests

no notable diffusion of tetra from the surface of the solute to the surroundings. This explains the lack of activity as the solute diffuses out of the textile to inhibit or kill the bacteria. This also further supports the postulation that the tetra crystalized after infusion. Samples were sent for SEM analysis to assess the effect of the infusion on the surface morphology of the textiles. The results of the SEM analysis are shown in *Table 14: SEM analysis of tetra only infused textile samples*. From *Table 14*, the infusion with tetra only affected the surface morphology of the cotton, lycra, and polyester samples. More specifically, it seems that the tetra has taken the form of a sludge-like residue on the samples. Furthermore, tetra is a pure aqueous solution of the potassium salts of tetrahydro iso-alpha acids. It thus might just exist as a residue on the surface of the textile samples after drying.

Table 14: SEM analysis of tetra only infused textile samples

	Lycra	Cotton	Polyester
Untreated			
Tetra only			

4.6 BIOACTIVITY OF AMBIENT IMMERSION POLYMERS (SECOND RUN)

Table 15: Zone of inhibition (mm2) of TTO & EtOH textile samples shows the antimicrobial activity exhibited by textiles that were immersed in a TTO and ethanol solution through ambient immersion described in section 4.3.1.1. The Kirby Bauer disk diffusion method was used to assess the AST. The bioactivity is quantified by the zone of inhibition (mm^2) around the textile samples. However, these samples differ slightly in diameter, most by 1 mm. Specific bioactivity shown in *Figure 66* is thus used to compare the activity exhibited against different strains by different textiles. Specific bioactivity is simply the zone of inhibition (mm^2) divided by the area of the textile sample in the petri dish. Although all AST analysis is performed in triplicate, the sample size is still relatively small. The student t-test assesses the observations' significance in Table *15* and *Figure 66*.

Table 15: Zone of inhibition (mm^2) of TTO & EtOH textile samples

Staphylococcus aureus ATCC 29213						
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)
Cotton	10	676.23	10	628.32	10	628.32
Polyester	10	776.76	10	537.21	10	676.23
Lycra	10	581.98	10	581.98	10	676.23
Staphylococcus aureus ATCC 33591						
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)
Cotton	11	760.27	11	760.27	11	760.27
Polyester	10	581.98	10	628.32	10	725.71
Lycra	10	452.39	9	494.02	10	452.39

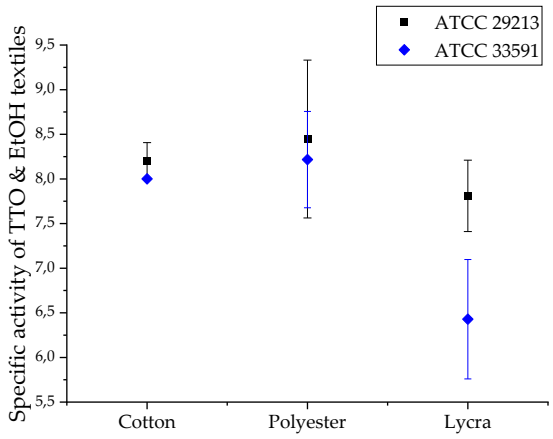


Figure 66: Specific bioactivity of TTO & EtOH infused textile samples

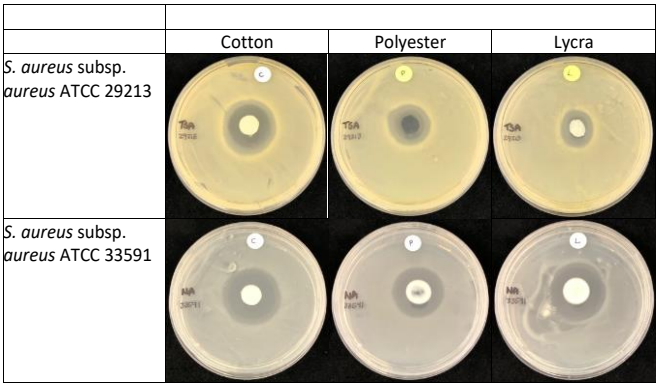


Figure 67: Zone of inhibition around TTO & EtOH samples

Figure 66 shows the specific bioactivity exhibited by TTO & EtOH infused textiles against selected bacterial strains. The specific bioactivity was determined by dividing the zone inhibition area (shown in Table *15*) by the textile sample area. The following features of the data were uncovered through pairwise comparison:

- For *S.aureus* (ATCC 29213): The specific bioactivity of the different samples was found to be statistically similar
- For *S.aureus* (ATCC 33591): The specific bioactivity of the different samples was found to be statistically similar
- For ATCC 29213 versus ATCC 33591: The specific bioactivity of the different textile samples against the two *S.aureus* strains was found to be statistically similar.

From these features, the following inference can be made. Despite initial observations, the TTO & EtOH infused textiles exhibit the same activity against both the methicillin-resistant and susceptible strains of *S.aureus*. However, these textiles were not rinsed in hexane after ambient immersion. Thus, the activity exhibited may be due to the ethanol present. The possibility of ethanol being responsible for bioactivity needs to be ruled out with further experiments. In the other ambient immersion and SSI experiments, samples were rinsed in hexane to remove the surface solute deposition. This would also remove the ethanol from the ambient immersion textiles. As shown in *Figure 64*, AST performed on rinsed TTO and buchu ambient immersion textiles exhibited zero bioactivity against the selected bacterial strains. This would support the postulation that the activity

exhibited by the TTO & EtOH, Tetra & EtOH was due to the ethanol present on the samples. However, most of the sorption in ambient immersion is adsorption onto the surface. Thus, rinsing the textiles in hexane may have significantly decreased the solute retention below the minimum inhibitory content needed for the textiles to show activity against the bacterial strains.

Three cotton, lycra, and polyester samples were immersed in a Southern Star hops extract and ethanol solution. After the ambient immersion, the samples were removed and rinsed in hexane to remove any solute that may be present on the surface of the samples. *Table 16* shows the bioactivity the infused textiles exhibited against selected bacterial strains. However, not all the samples used in the AST analysis are of the same diameter; some differ by 1 mm. The specific bioactivity was calculated to compare the bioactivity of the infused cotton, lycra and polyester samples. The specific bioactivity is simply the zone of inhibition (mm^2) around the sample divided by the area of the sample in the petri dish included in Table *16*.

Table 16: Zone of inhibition of hops extract infused textile samples

	Staphylococcus aureus ATCC 33591					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)
Cotton	10	537.21	10	452.39	10	412.33
Lycra	10	235.62	11	357.36	10	235.62
Polyester	10	336.94	7	188.5	9	316.52
	Listeria monocytogenes ATCC 19111					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)	Disk diameter (mm)	Zone of inhibition(mm^2)
Cotton	10	452.39	10	494.02	10	452.39
Lycra	10	412.33	10	373.85	10	412.33
Polyester	9	351.86	8	402.12	8	329.87

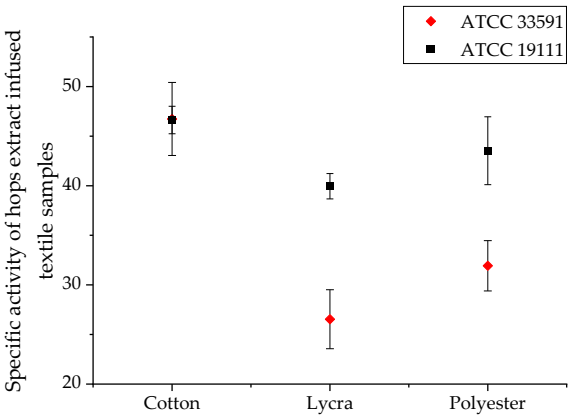


Figure 68: Specific bioactivity of hops extract infused textile samples

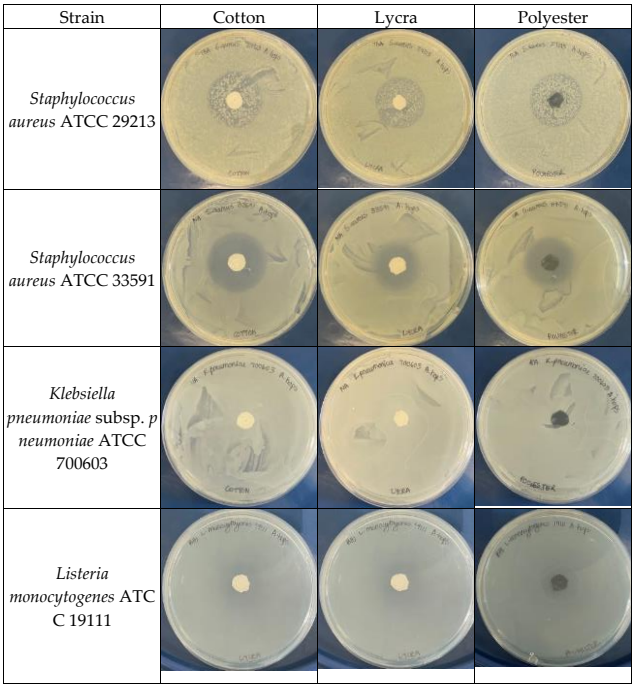


Figure 69: Zone of inhibition around ambient hops immersion (rinsed) samples

Figure 68 shows the specific bioactivity of the hops infused cotton, polyester and lycra samples against a selected range of bacterial strains. The cotton, polyester and lycra samples exhibited no bioactivity against *S.aureus* (ATCC29213), the MSSA (methicillin-susceptible *S.aureus* strain). Nevertheless, all samples showed activity against the MRSA (methicillin-resistant *S.aureus* strain). From *Figure 68*, there appears to be a correlation between the activity and the textile type and the activity and the bacterial strain. These observations were assessed using the student's t-test to test the statistical significance at the 95 % confidence level. From the pairwise comparison, the following was found:

- For *S.aureus* (ATCC 33591): Pairwise comparison showed the activity of the infused cotton samples to be significantly greater than the infused lycra and polyester samples. However, the activity exhibited by the lycra, and polyester samples were statistically similar.
- For *Listeria monocytogenes* (ATCC 19111): The activity exhibited by the infused cotton, lycra and polyester samples were statistically similar.

- For *S.aureus* (MRSA) versus *Listeria monocytogenes*: The cotton and polyester samples' activity was statistically similar. Whilst the activity of the infused lycra samples was significantly greater against the *Listeria monocytogenes* strain.

Some infused polymers exhibit bacteriostatic activity, stopping microbial growth but not killing the microbes. Thus, regrowth can occur as shown in *Figure 70*. The same was observed for the following infused polymer samples

- Tetra only (cotton, polyester, lycra) vs *K. pneumoniae* subsp. *pneumoniae* ATCC 700603*
- Buchu (cotton, polyester, lycra) vs *S.aureus* subsp. *aureus* ATCC 29213*
- Tetra only (cotton, polyester, lycra) vs *S.aureus* subsp. *aureus* ATCC 29213
- Buchu (cotton, polyester, lycra) vs *S.aureus* subsp. *aureus* ATCC 33591*

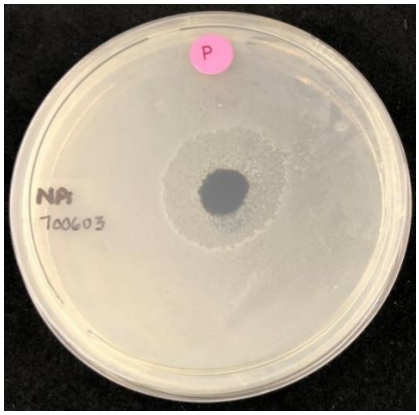


Figure 70: Bacteriostatic (passive) polymer

No activity was observed for the following infused polymers:

- Buchu (cotton, polyester, lycra) vs *C. albicans* ATCC 90028D
- Tea tree (cotton, polyester, lycra) vs *E. coli* ATCC 25922
- Tetra only (cotton, polyester, lycra) vs *L. monocytogenes* ATCC 19111
- Tea tree (cotton, polyester, lycra) vs *K. pneumoniae* subsp. *pneumoniae* ATCC 700603
- Tetra (cotton, polyester, lycra) vs *K. pneumoniae* subsp. *pneumoniae* ATCC 700603
- Tetra only (cotton, polyester, lycra) vs *S.aureus* subsp. *aureus* ATCC 29213
- Tetra only (cotton, polyester, lycra) vs *S.aureus* subsp. *aureus* ATCC 33591

Moreover, it is important to note that the disk diffusion method is dependent on the dissolution of the antimicrobial agent into the solid media. When the solute diffuses out the polymer, it encounters the bacterial strain. The activity of the solute against the strain is shown by the zone of inhibition that forms around the polymer sample. The zone of inhibition created depends on the solute diffusion out of the polymer. If the solute does not diffuse out, no zone of inhibition will form, explaining the lack of activity seen from the Buchu samples.

The question now becomes how this compares to the activity of the textiles hops infused SSI textiles.

4.7 BIOACTIVITY OF POLYMERS INFUSED USING SCCO_2

This section details the bioactivity of 2 sets of SSI samples. Where the one set was subjected to slow depressurisation and the second to fast depressurisation. In this research the slow depressurisation samples were sent for immediate analysis whereas the fast depressurisation samples were sent for aged analysis

4.7.1 HOPS EXTRACT (SLOW DEPRESSURISATION)

Table *17* shows the bioactivity of the cotton, lycra and polyester samples treated using SSI and slowly depressurised according to section 4.3.2.2. The agar disk diffusion method was used to assess the AST. The bioactivity is quantified by the zone of inhibition (mm^2) around the textile samples. However, these samples differ ever so slightly in diameter, most by 1 mm. Specific bioactivity shown in *Figure 71* is thus used to compare the activity exhibited against different strains by different textiles. Specific bioactivity is simply the zone of inhibition (mm^2) divided by the area of the textile sample in the petri dish.

Table 17: Zone of inhibition of SSI hops infused textile samples

	Staphylococcus aureus ATCC 29213					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition (mm^2)	Disk diameter (mm)	Zone of inhibition (mm^2)	Disk diameter (mm)	Zone of inhibition (mm^2)
Cotton	11	867.08	11	1425.5	12	593.76
Lycra	8	911.85	8	753.98	8	753.98
Polyester	10	267.82	10	494.02	10	829.38
	Staphylococcus aureus ATCC 33591					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition (mm^2)	Disk diameter (mm)	Zone of inhibition (mm^2)	Disk diameter (mm)	Zone of inhibition (mm^2)
Cotton	11	565.49	11	611.83	11	565.49
Lycra	8	263.89	9	508.94	8	402.12

Polyester	8	402.12	9	427.26	8	402.12
	Listeria monocytogenes ATCC 19111					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition (mm^2)	Disk diameter (mm)	Zone of inhibition (mm^2)	Disk diameter (mm)	Zone of inhibition (mm^2)
Cotton	11	477.52	11	435.9	11	357.36
Lycra	9	388.77	8	356.21	9	388.77
Polyester	8	263.89	9	250.54	9	250.54

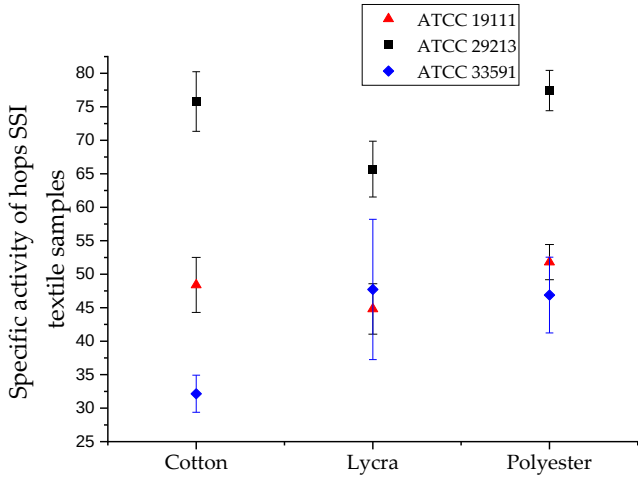


Figure 71: Specific bioactivity of hops extract infused textile samples (immediate AST analysis)

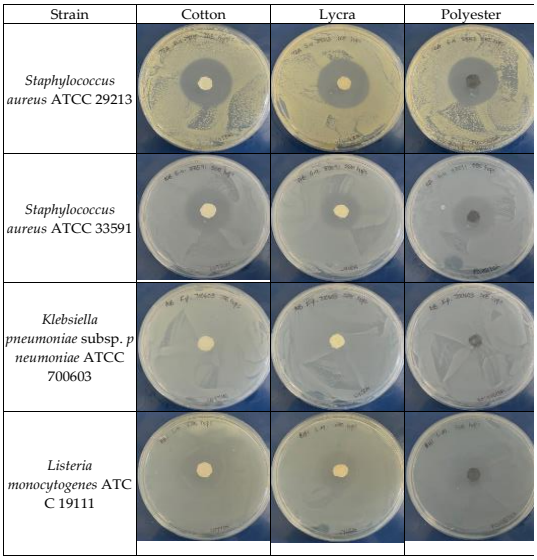


Figure 72: Zone of inhibition of SSE hops samples

Although all AST analysis is performed in triplicate, the sample size is still relatively small. As such, the student t-test is used to assess the statistical significance of the correlations made from *Figure 71* at the 95 % confidence level. From the pairwise comparison, the following features of the data was revealed:

- For *S.aureus* (ATCC 33591): The activities exhibited by the infused cotton, lycra and polyester samples were statistically similar.
- For *S.aureus* (ATCC 29213): The activities exhibited by the infused cotton, lycra and polyester samples were statistically similar.
- For *Listeria monocytogenes* (ATCC 19111): The infused cotton and lycra samples exhibited statistically similar activities. The activity of the infused lycra samples was significantly greater than that of the polyester samples. The activity exhibited by the infused cotton samples was significantly greater than the infused polyester samples.

- For MSSA (ATCC 29213) versus MRSA (ATCC 33591): The activity of the infused cotton and polyester samples against the two strains were statistically similar. However, the activity of the infused lycra samples was significantly greater against the MSSA versus MRSA. Suggesting that the textiles are more effective against the methicillin-susceptible *S.aureus* strain.

- For MSSA (ATCC 29213) versus *Listeria monocytogenes* (ATCC 19111): The infused cotton and polyester samples' activity against the two strains was statistically similar. However, the activity of the infused lycra samples was significantly greater against the MSSA when compared to the activity exhibited against *Listeria monocytogenes*.

- For MRSA (ATCC 33591) versus *Listeria monocytogenes* (ATCC 19111): The activity of the infused lycra samples was found to be statistically similar. Now, the infused cotton and polyester activity was significantly greater against the MRSA. Suggesting that the infused cotton and polyester are more active against the methicillin-resistant *S.aureus*.

However, one cannot forget the activity of the hops textiles infused through ambient immersion. The difference in solute retention achieved by the two methods may not be significant, but that does not imply that the activity of the two is similar. Before conducting the pairwise comparison, the SSI textiles exhibited bactericidal activity against the MSSA while the ambient immersion hops textiles showed none. Through pairwise comparison, the following additional features of the data in *Figure 71* were unveiled:

- For MRSA (ATCC 33591): The activities exhibited by the cotton, lycra and polyester samples infused through SSI and ambient immersion textiles were statistically similar.

- For *Listeria monocytogenes* (ATCC 19111): the ambient immersion and SSI infused cotton and lycra samples were found to be statistically similar. The ambient immersion polyester samples' activity was statistically greater than the SSI slow depressurisation polyester samples.

From the last feature, the following inference could be drawn. The depressurisation rate depends on the solute's affinity for the solvent and how that compares to the affinity of the solute for the substrate. Thus, the retention of the SSI hops fast depressurisation textiles was studied over a 130-hour period where the mass of the samples was taken every few hours.

The mass data was used to calculate the retention over time shown by *Figure 74: Solute retention (wt%) of hops extract on textile samples over time.* This data was collected to study the diffusion of the infused hops extract. This was done for two reasons. Firstly, when the samples are sent for AST analysis, they are not analysed within the hour; therefore, the yield over time is studied to approximate the yield at the analysis time.

Secondly, the textiles' bioactivity depends on the diffusion of the infused solute out of the substrate. Thus, studying the diffusion profile would provide insight into the activity exhibited by the infused cotton, lycra and polyester samples. From *Figure 74: Solute retention (wt%) of hops extract on textile samples* over time, it appears as though there is no significant drop in the yield of the hops infused into the cotton, lycra and polyester samples over time. However, the samples weighed over time were placed in an isolated environment and then weighed over the 130-hour period. Now textiles are porous substrates; both hydrophobic and hydrophilic textiles experience changes in mass due to a change in the moisture content as the relative humidity of the environment changes. The environment in which the samples were stored was not isothermal. This could explain the increase in mass of the samples and yield over time as changes in the relative humidity result in a shift in the equilibrium moisture content of the textiles. As such, samples were placed in a desiccator whilst other samples were placed in the same isolate environment as before, both sets of samples were weighed over time. This data is depicted in *Figure 75* and *Figure 76*. In *Figure 75*, the retention of hops in the textiles appears to only decrease gradually and rather steadily over time. In *Figure 76*, the changes in yield seem to be more erratic and at times increase, such as at 175 hrs. This change could be attributed to an increase in the relative humidity of the surroundings. Nonetheless, the solute retention on the textiles does not vary greatly. Thus, samples of the aged SSI hops infused textiles were sent for AST.

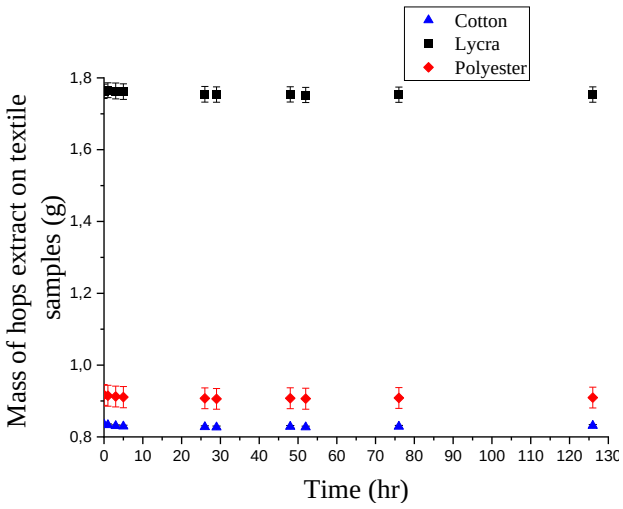


Figure 73: Mass of hops infused textile samples over time (Confidence interval of all readings: ±0.001524 g))

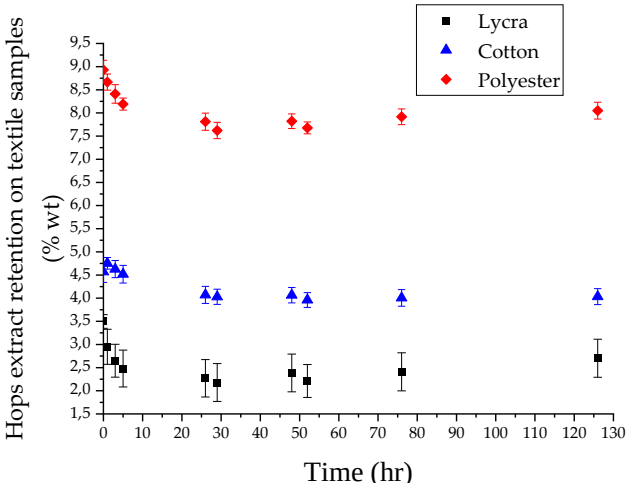


Figure 74: Solute retention (wt%) of hops extract on textile samples over time

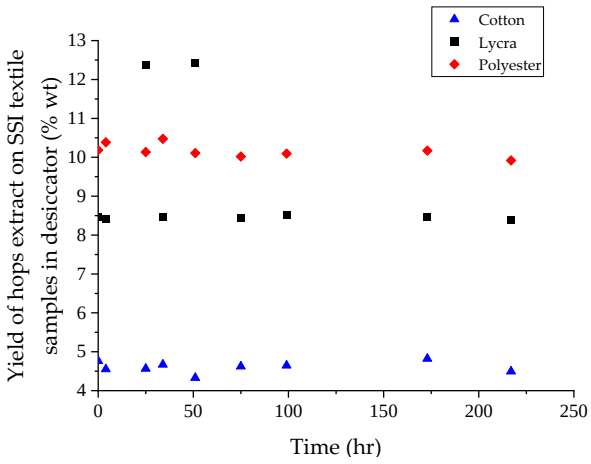


Figure 75: Solute retention (% wt) of hops infused textile samples in the desiccator over time (SSI)

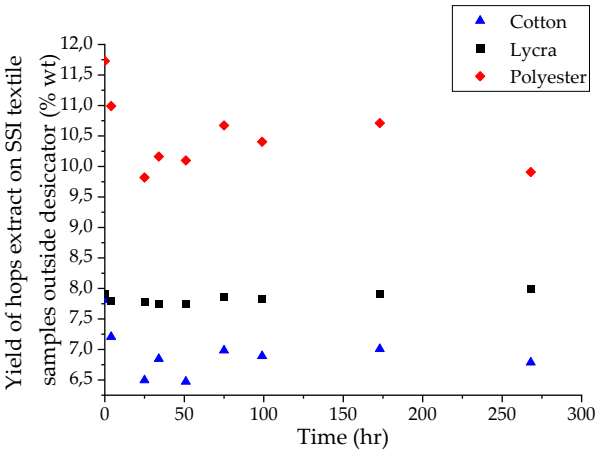


Figure 76: Solute retention (% wt) of hops infused textile samples outside the desiccator over time (SSI)

4.7.2 HOPS EXTRACT (FAST DEPRESSURISATION)

Table 18: Zone of inhibition (mm2) of SSI (fast depressurisation) hops infused samples shows the activity of the aged hops infused textile samples against the same set of bacterial strains as before. Once again, the specific bioactivity was calculated and used in the student's t-test to assess the statistical significance of the observations made at the 95% confidence level. The results of the AST analysis are shown in *Figure 77.*

Table 18: Zone of inhibition (mm²) of SSI (fast depressurisation) hops infused samples

	Staphylococcus aureus ATCC 29213					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition (mm²)	Disk diameter (mm)	Zone of inhibition (mm²)	Disk diameter (mm)	Zone of inhibition (mm²)
Cotton	10	829.38	10	725.71	10	829.38
Lycra	9	725.71	9	596.9	9	691.15
Polyester	10	829.38	10	691.15	10	725.71
	Staphylococcus aureus ATCC 33591					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition (mm²)	Disk diameter (mm)	Zone of inhibition (mm²)	Disk diameter (mm)	Zone of inhibition (mm²)
Cotton	11	395.84	10	581.98	10	581.98
Lycra	10	267.82	10	267.82	10	412.33
Polyester	10	336.94	10	581.98	10	412.33
	Listeria monocytogenes ATCC 19111					
Sample	1		2		3	
	Disk diameter (mm)	Zone of inhibition (mm²)	Disk diameter (mm)	Zone of inhibition (mm²)	Disk diameter (mm)	Zone of inhibition (mm²)
Cotton	11	477.52	11	477.52	11	565.49
Lycra	10	452.39	9	467.31	9	508.94
Polyester	9	508.94	9	351.86	9	427.26

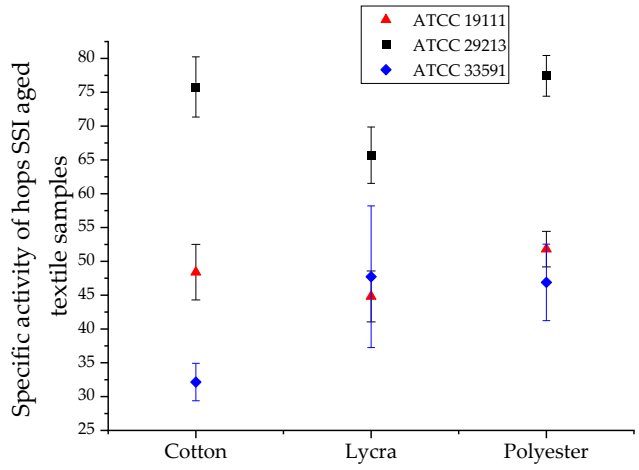


Figure 77: Specific bioactivity of hops extract infused textile samples (aged)

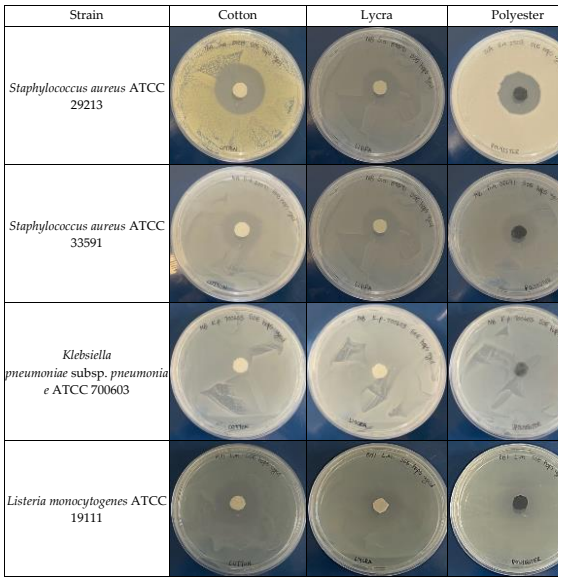


Figure 78: Zone of inhibition around SSI hops aged samples

The following features of the data in *Figure 77* could be drawn:

- For *S.aureus* (ATCC 33591): The activities exhibited by the infused cotton samples were significantly greater than the lycra and polyester samples. Then the activity of the infused lycra samples was significantly greater than the polyester. This suggested decreased activity in the order: cotton> lycra> polyester.

- For *S.aureus* (ATCC 29213): The activities exhibited by the infused cotton was statistically similar to the polyester, and the polyester was statistically similar to the lycra. However, the activity of the infused cotton samples was significantly greater than the lycra samples.
- For *Listeria monocytogenes* (ATCC 19111): The activities exhibited by the infused cotton, polyester and lycra samples were statistically similar
- For MSSA (ATCC 29213) versus MRSA (ATCC 33591): The activity of the infused and polyester samples against the two strains were statistically similar. However, the infused lycra and cotton samples' activity was significantly greater against the MSSA versus MRSA. This suggests that the textiles are more effective against the methicillin-susceptible *S.aureus* strain.

For MSSA (ATCC 29213) versus *Listeria monocytogenes* (ATCC 19111): The infused cotton and polyester samples' activity against the two strains was statistically similar. Whilst the activity of the infused lycra samples was greater against the *Listeria monocytogenes*.

A pairwise comparison was then performed between the immediate and aged AST results. This comparison yielded the following features:

- For *S.aureus* (ATCC 33591): The activities exhibited by the infused cotton and polyester aged and immediate samples were statistically similar. Whilst the activity of the infused lycra immediate samples was significantly greater than the aged lycra samples.
- For *S.aureus* (ATCC 29213), the infused cotton, polyester and lycra aged activities and immediate samples were statistically similar.
- For *Listeria monocytogenes* (ATCC 19111): The activities exhibited by the infused cotton, lycra aged and immediate samples were statistically similar. In contrast, the activity of the infused polyester immediate samples was significantly greater than the aged polyester samples.

4.7.3 TEA TREE OIL (SLOW AND FAST DEPRESSURISATION)

A set of the SSI TTO slow ΔP samples were sent for AST. The results of this analysis are shown in *Figure 79.*

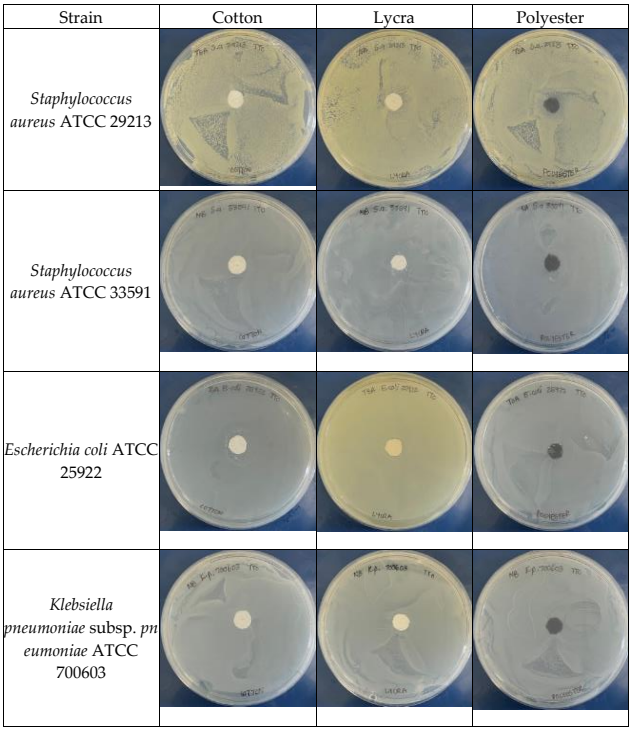


Figure 79: Zone of inhibition around SSI TTO (immediate) samples

A disk diffusion test was performed on the samples in *Figure 79*. It appears these samples did not exhibit any activity against the strains. The activity is measured by the zone of inhibition. As is evident from *Figure 79*, there is no zone of inhibition around the samples.

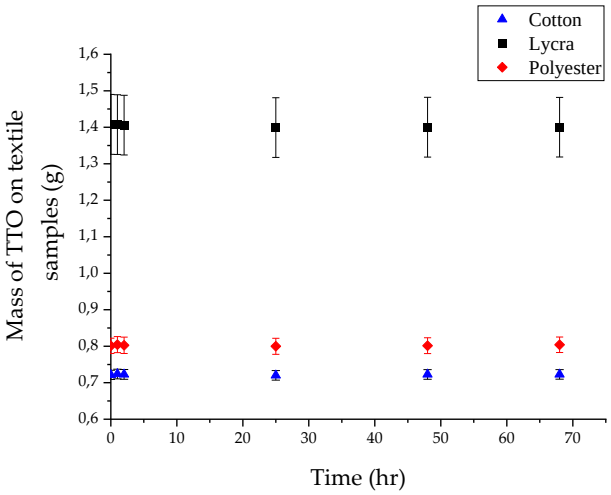


Figure 80: Mass of TTO infused textile samples over time

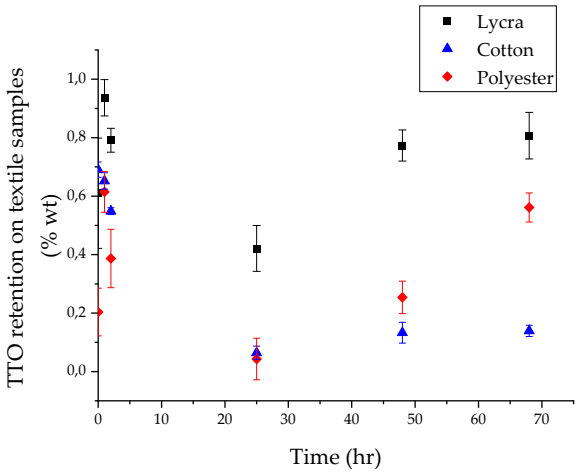


Figure 81: Retention (% wt) of TTO on textile samples over time

When the antimicrobial activity of the aged TTO infused textile samples were assessed, it was found to exhibit bactericidal behaviour against selected strains. This is contrary to the results of the immediate samples shown in *Figure 79*. The specific bioactivity of the aged textile samples is shown in Figure *82*.

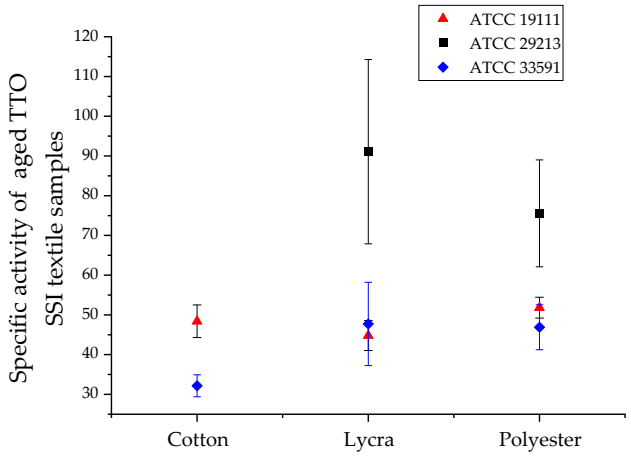


Figure 82: Specific bioactivity of TTO infused textile samples (aged)

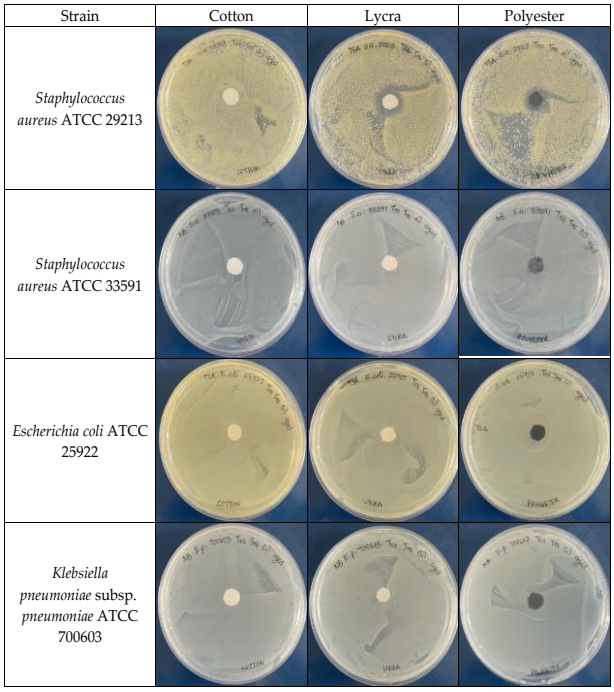


Figure 83: Zone of inhibition around SSI TTO aged samples

Figure 82 shows the data obtained when aged TTO infused textile samples treated using SSI were sent for AST. The following features were discovered by performing a pairwise comparison of the activity shown by *Figure 82*:

- For MSSA (ATCC 29213): The infused cotton samples showed no activity. The infused lycra samples exhibited significantly greater activity than the infused polyester samples.
- For MRSA (ATCC 33591): The infused cotton samples exhibited significantly greater activity than the lycra samples. The cotton and polyester and lycra, and polyester showed statistically similar activities.
- For *Listeria monocytogenes*: The cotton, lycra and polyester samples exhibited statistically similar activities.
- For MSSA versus MRSA: The infused lycra samples' activity was statistically similar. The activity of the infused polyester samples was significantly greater against the MRSA versus MSSA. The infused cotton samples only showed activity against the MRSA strains.
- For MSSA versus *Listeria monocytogenes*: The infused cotton samples only exhibited activity against *Listeria monocytogenes*. The activity of the infused lycra and polyester samples was significantly greater against *Listeria monocytogenes*.
- For MRSA versus *Listeria monocytogenes*: The infused cotton and polyester samples exhibited statistically similar activities against both bacterial strains. The activity of the infused lycra samples was significantly greater against *Listeria monocytogenes*.

From the AST results, the following observations are noted:

- TTO aged versus TTO immediate: The samples sent for immediate AST were taken from the slow ΔP set of samples. Whilst the samples sent for aged analysis were taken from the fast ΔP set of samples. Interestingly the latter set exhibited activity against some of the strains. Whereas the set sent for immediate analysis exhibited no activity. This is surprising given the higher solute retention on the slow ΔP samples. One could postulate that the slow ΔP could have resulted in a higher retention of the components that do not exhibit activity. At the same time lowering the retention of the target antimicrobial components. This assumption would mean that the target components

have a low affinity for the substrate. A compositional analysis of the retained solute after slow ΔP and fast ΔP could be performed to assess this assumption.

- What is not explained is the difference in activity between the buchu and TTO samples, especially since both essential oils contain 1,8-cineole and terpinene-4-ol just in different concentrations. The observation seems to be unaffected by the solute retention because the buchu polymers exhibited no activity even when the buchu retention was significantly greater than the TTO polymers. Nevertheless, TTO polymers exhibited an activity against certain strains.

  However, differences exist between buchu and TTO. Works by Togashi et al.,(2008) has demonstrated that β-myrcene and terpinen-4-ol interact synergistically to increase the activity of tea tree essential oil. Moreover, tea tree oil, partly owes its activity to its ability to disrupt the microbial permeability (Cox et al., 2000). In addition, the content of the similar components is not the same across buchu and TTO. This would require further analysis of the compounds infused into the polymers.

- Despite cotton consistently retaining the lowest % of solute, cotton samples generally exhibited the greatest zone of inhibition. Except for the aged TTO samples, only the lycra and polyester samples showed activity against MSSA. One could postulate that the solutes had a higher affinity for the polyester and lycra polymers. If these solutes had a higher affinity for the polyester and lycra polymers, this would slow down the diffusion rate out of the sample during AST. This would ultimately affect the zone of inhibition generated. This assertion is supported by the trend in solute retention observed during fast depressurisation versus slow. The polyester and lycra samples' diffusion profile also suggests a slower polymer diffusion than cotton.

4.8 CHAPTER OUTCOMES

Objectives a,b,c and d were addressed in this chapter. The main aim of this chapter was to detail and discuss all the results obtained from performing the experiments and analyses detailed in Chapter 3. The results for each of the two processes were presented in 5 main parts; quantification of the solute retained by the polymer, persistence of the retained solutes, antimicrobial activity of the treated polymer samples and the effect of pressure on textile

impregnation. The fifth part, a comparison between and across the two processes, solutes, and polymers in terms of solute retention, solute persistence, and antimicrobial activity of the treated samples. These results are needed to answer the objectives and ultimately the research aim. Key observations in this chapter include:

- The process of rinsing the samples after ambient immersion significantly decreased the solute retention across all the textile samples.
- Solute retention was achieved across all textiles and with all solutes under ambient immersion, even after rinsing once in hexane.
- Solute retention was achieved across all textiles and with all solutes when SSI was performed at 200 bar and 37 ℃ for 3 hours.
- The solute retention increased significantly when the depressurisation rate was slow.
- SEM images showed no solute on the surface of the textiles after rinsing. This was applicable to all textile samples- ambient immersion and SSI.
- SEM images of the untreated and SSI textile samples were very similar, with no visible change in the surface morphology.
- Ambient immersion tetra only samples had the highest solute retention but exhibited no activity against the strains.
- Ambient immersion tetra and ethanol solution samples exhibited activity against the strains.
- Despite showing notable activity as an essential oil, buchu oil-infused textiles showed no activity against the selected strains.
- Cotton samples exhibited the greatest activity against the selected strains.
- None of the ambient immersion samples that were rinsed in hexane after immersion exhibited activity.
- Tea tree oil and ethanol ambient immersion samples exhibited activity against *S.aureus* ATCC 29213 and ATCC 33591 as well as *Listeria monocytogenes* ATCC 19111.
- SSI hops infused textile samples exhibited activity against all selected strains.

- From the SSI results: The optimum depressurisation rate depends on the solute's affinity for the solvent versus its affinity for the polymer/substrate.
- SSI aged hops infused samples exhibited activity against the selected strains.
- None of the SSI TTO and buchu samples sent for immediate analysis exhibited activity against the respective strains.
- SSI aged TTO textile samples exhibited activity against *S.aureus* ATCC 29213 and ATCC 33591 and *Klebsiella pneumonia*e ATCC 700603.

Chapter 5 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The research aim was to compare the performance of fabric samples loaded with antimicrobial plant extracts under supercritical and ambient pressure conditions; against specific microbes. This study compared two methods of impregnation of textile fibre, the immersion method, and the use of scCO2 as a medium at high pressure. The two methods were compared in terms of solute retention, bioactivity, solute persistence, and the effect of pressure on textile impregnation.

In order to achieve the research aim the following objectives were formulated:

a) To determine the solute retention immediately after textile impregnation.
b) To analyse the persistence of the retained solutes in an isolated, homeostatic environment.
c) To assess the effect of pressure on textile impregnation.
d) To determine the antimicrobial activity of the impregnated textile samples.

The results detailed in Chapter 4 show that these objectives and research aim outlined in Chapter 1 were fulfilled, and thus, the associated research questions were answered.

In response to objective a: To determine the solute retention immediately after textile impregnation.

Samples of lycra, cotton and polyester were immersed using ambient immersion and supercritical textile impregnation. These two processes were performed according to the method laid out in Chapter 3. The immersed samples were rinsed in hexane and dried to constant mass. Once constant dry mass was reached the samples were weighed to determine the mass after immersion. The difference between the mass of the samples before and after immersion was the mass of the solute retained by the samples. This was used as the measure of solute retention. The conclusions related to this objective are as follows:

- The immediate solute retention of $scCO_2$ immersion polymers was greater than that of the ambient immersion polymers. Specifically, for those $scCO_2$ immersion polymers that underwent the slower depressurisation. Most notably for the polyester samples.

- The use of $scCO_2$ allowed for greater solute absorption, especially for the polymers depressurised slowly.
- Slow depressurisation favoured the solute retention, suggesting that the solutes had a greater affinity for the polymers.
- Three hours contact time was shown to be sufficient for the solute impregnation of polymers under $scCO_2$
- This research further supports the inference that only physical attachment is needed to achieve textile impregnation using $scCO_2$.
- This research reveals that rinsing the infused textiles in hexane effectively removes the solute deposited by adsorption.

In response to objective b: To analyse the persistence of the retained solutes in an isolated, homeostatic environment.

Sets of the immersed samples from both ambient immersion and SSI were placed in cardboard boxes and stored in a cupboard. However, research shown in section 2.7.1 revealed that all samples were subject to a change in mass as the relative humidity changed. Thus, a control was conducted simultaneously to eliminate moisture as a disturbance variable. The control experiment was conducted as specified in Chapter 3. Where samples of the ambient immersion and SSI were placed in a desiccator containing silica desiccant. All samples were weighed over time and the approach from objective a was used to determine the mass of the solute retained over time. The conclusions related to this objective are as follows:

- The retained solutes diffuse slowly out of the polymers without moisture.

In response to objective c: To assess the effect of pressure on textile impregnation.

A detailed comparison was performed between the results from ambient immersion versus SSI. This comparison was mainly in terms of the effect of pressure on the solute retention, solute persistence, surface morphology of the samples and the bioactivity of the treated samples. In addition, to how pressure affected the ability of the textile samples to retain the solutes. However, the samples sizes are quite small thus a statistical tool, the student's t-test

was used to evaluate the significance of the differences found between ambient immersion and SSI results. The conclusions related to this objective are as follows:

- The $scCO_2$ seemed to have a temporary plasticization effect on the polymers as no morphology was seen on the SEM images taken.
- This research further shows the plasticizing effect of $scCO_2$. Under ambient immersion polyester, a thermoplastic retained a relatively low solute retention. This was significantly increased using $scCO_2$.

In response to objective d: To determine the antimicrobial activity of the impregnated textile samples.

The purpose of infusing the solutes into the textile samples was to impart the bioactivity of the solute to the textile. Thus, it was essential that the antimicrobial activity of the samples be determined. The agar disk diffusion method was used to qualitatively determine the antimicrobial activity of the ambient immersion and SSI textile samples against the selected strains. The conclusions related to this objective are as follows:

- Textiles treated with buchu essential oil do not exhibit visible activity against the selected microbial strains.
- All Southern Star hops extract infused polymers exhibited superior bioactivity, followed by TTO infused polymers.
- This work reveals that Southern Star hop extract's infusion produces textiles that have initial and lasting bioactivity against MSSA, MRSA and *Listeria monocytogenes*.
- Cotton samples generally exhibited greater bioactivity despite having generally attained solute retention lower than the lycra and polyester samples.
- Despite its scientifically proven antimicrobial potential, Buchu essential oil-infused polymers showed little to no activity against the selected strains.
- Ethanol seems to have been responsible for the bioactivity exhibited by the ambient textiles that were not rinsed in hexane after immersion. Especially since the textiles rinsed in hexane showed no activity.

5.2 RECOMMENDATIONS

The retention of an active ingredient within a textile material in a real setting is dependent on a few additional factors, such as washing of material, and also on conditions of service such as rubbing and attrition. The results from this work could be further enhanced by carrying out It would therefore be helpful to study the wash and rub fastness of the infused textiles. This research does not investigate which components were infused into the textiles. Further research could thus be focused on just that. Further research could be conducted to ascertain how temperature and humidity conditions affect the rate of solute diffusion out of the textile.

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Chapter 7 APPENDIX

7.1.1 FTIR ANALYSIS RESULTS

FTIR (Fourier Transform Infrared Spectroscopy) was used to validate the identity of textiles used in this research. FTIR produces infrared light to identify the chemical bonds. From this, the spectra produced a distinctive molecular fingerprint profile of the sample. This fingerprint was used to screen and detect components, functional groups and characterize covalent bonding information (Intertek, 2018).

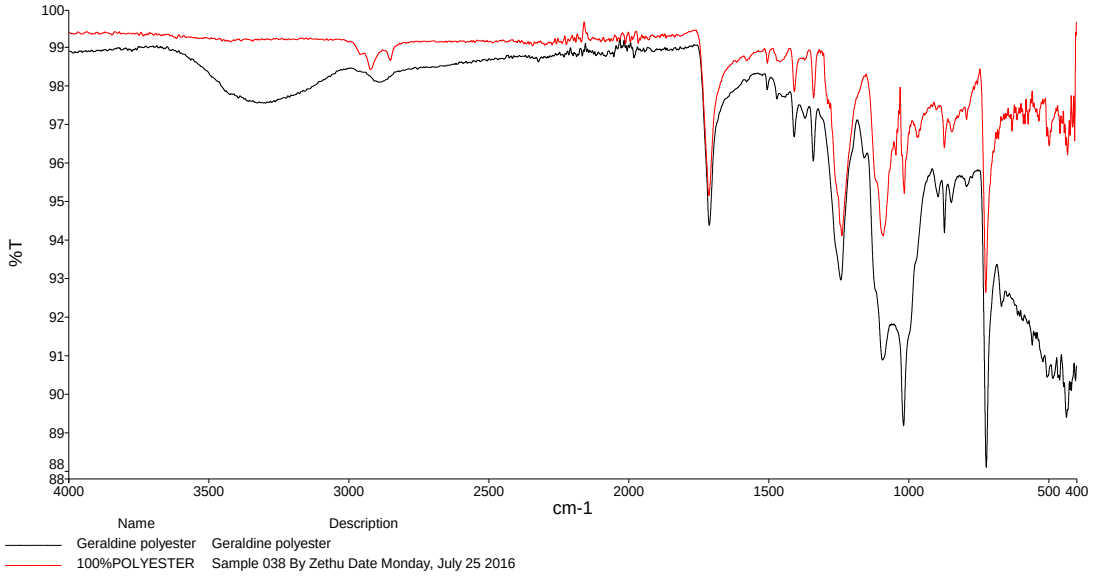


Figure 84: FTIR analysis of untreated polyester sample

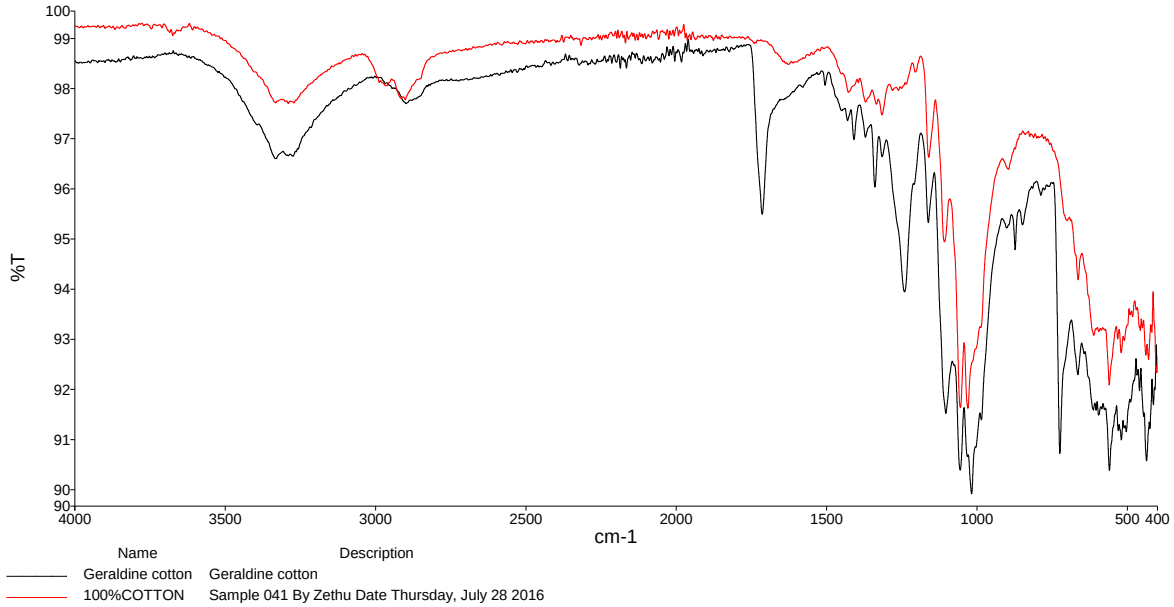


Figure 85: FTIR analysis of untreated cotton sample

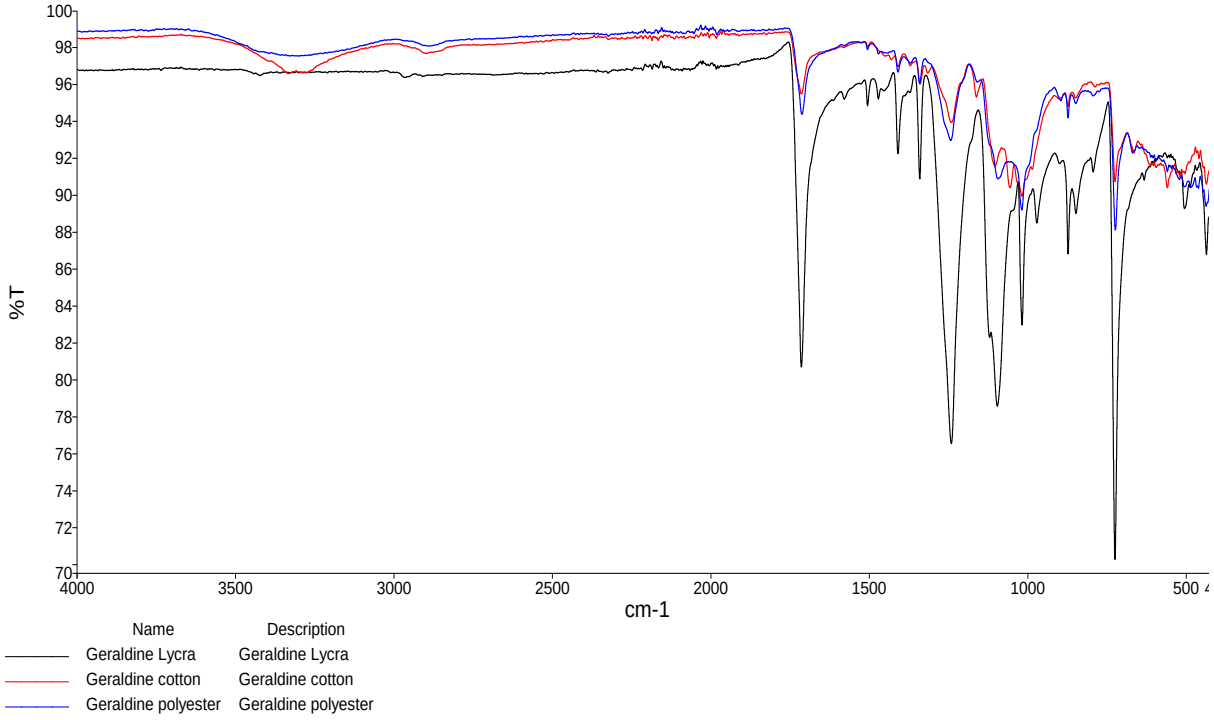


Figure 86: FTIR analysis of untreated cotton, polyester and lycra samples

7.1.2 ETHANOL

Table 19: Mass of textiles after immersion in ethanol

	Lycra Mass (g)			Cotton Mass (g)			Polyester Mass (g)		
Time(min)	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
2	2.2551	2.4009	2.6347	1.3044	1.1420	1.2270	1.8500	1.7400	1.7711
4	1.4088	1.4335	1.4990	0.8103	0.8145	0.8154	0.8599	0.8416	0.8481
6	1.4087	1.4319	1.4933	0.8062	0.8114	0.8128	0.7627	0.8017	0.7854
8	1.4070	1.4318	1.4933	0.8059	0.8090	0.8124	0.7612	0.7992	0.7821
10	1.4070	1.4318	1.4931	0.8055	0.8078	0.8124	0.7596	0.7991	0.7814
12	1.4072	1.4317	1.4930	0.8055	0.8078	0.8123	0.7593	0.7987	0.7814
14	1.4068	1.4318	1.4930	0.8055	0.8070	0.8121	0.7589	0.7986	0.7811
16	1.4067	1.4318	1.4929	0.8054	0.8070	0.8120	0.7574	0.7970	0.7797
18	1.4067	1.4315	1.4928	0.8054	0.8069	0.8120	0.7568	0.7971	0.7799
20	1.4066	1.4315	1.4928	0.8055	0.8069	0.8120	0.7568	0.7969	0.7795

- Confidence interval of all readings: ±0.001524

7.1.3 WATER

Table 20: Mass of textiles over time after immersion in water

	Lycra Mass (g)			Cotton Mass (g)			Polyester Mass (g)		
Time(min)	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
2	3.3299	3.2730	3.4336	1.3856	1.3711	1.3362	3.0164	3.2061	3.1727
4	2.4275	2.7612	2.7347	1.0359	1.1255	1.1205	1.9919	2.0217	2.1377
6	2.0415	2.2811	2.2434	0.8762	0.8387	0.8477	1.6667	1.7299	1.7621
8	1.6248	1.8636	1.7887	0.8197	0.8335	0.7991	1.3299	1.4157	1.4431
10	1.4111	1.4372	1.5003	0.8200	0.8323	0.7983	0.9872	1.0025	1.0697
12	1.4116	1.4367	1.4979	0.8191	0.8292	0.7965	0.7664	0.8046	0.7909
14	1.4116	1.4357	1.4963	0.8190	0.8303	0.7968	0.7660	0.8043	0.7874
16	1.4110	1.4357	1.4968	0.8171	0.8291	0.7963	0.7630	0.8030	0.7864
18	1.4112	1.4354	1.4974	0.8174	0.8298	0.7962	0.7630	0.8029	0.7865

- Confidence interval of all readings: ±0.001524

7.1.4 HEXANE

Table 21: Mass of textiles after immersion in hexane

	Lycra Mass (g)			Cotton Mass (g)			Polyester Mass (g)		
Time(min)	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
2	1.4121	1.4373	1.4988	0.8215	0.8313	0.7994	0.7659	0.8077	0.7896
4	1.4114	1.4369	1.4970	0.8201	0.8299	0.7980	0.7631	0.8050	0.7886
6	1.4117	1.4373	1.4976	0.8205	0.8265	0.7980	0.7631	0.8050	0.7881
8	1.4111	1.4372	1.4976	0.8197	0.8232	0.7972	0.7627	0.8027	0.7869
10	1.4111	1.4372	1.4974	0.8182	0.8219	0.7931	0.7625	0.8021	0.7868
12	1.4113	1.4375	1.4972	0.8174	0.8211	0.7921	0.7619	0.8018	0.7865
14	1.4106	1.4365	1.4964	0.8164	0.8209	0.7918	0.7612	0.8010	0.7857
16	1.4103	1.4362	1.4960	0.8164	0.8208	0.7912	0.7609	0.8004	0.7846
18	1.4099	1.4362	1.4957	0.8164	0.8208	0.7912	0.7608	0.8004	0.7844
20	1.4098	1.4361	1.4956	0.8161	0.8205	0.7911	0.7610	0.8005	0.7843

- Confidence interval of all readings: ± 0.001524

7.2 GC-MS ANALYSIS OF ESSENTIAL OILS

RT	Library/ID	Area Pct
13.2013	.alpha.-Thujene $$ Bicyclo[3.1.0]hex-2-ene,	0.0841
13.5383	.ALPHA.-PINENE $$ DIHYDRO-para-CYMENE	1.2932
15.7366	sabinene	0.0799
16.8224	MYRCENE	2.1372
17.5605	.alpha.-phellandrene	0.1415
18.1542	.alpha.-terpipene $$ .ALPHA.-TERPINENE	0.413
18.9993	LIMONENE	22.9407
19.0634	1,8-CINEOLE $$ EUCALYPTOL	2.3228
19.3362	CIS-OCIMENE	0.0565
19.8764	TRANS-.BETA.-OCIMENE	0.7456
20.406	.gamma.-terpinene	0.6446
21.9624	.alpha.-terpinolene $$ Cyclohexene, 1-meth	0.1641
22.6684	2-.BETA.-PINENE $$ Bicyclo[3.1.1]heptane,	0.3615
23.2407	.alpha.-terpinolene $$ Cyclohexene, 1-meth	0.3892
24.856	2-.BETA.-PINENE $$ Bicyclo[3.1.1]heptane,	0.1734
26.5248	Cyclohexanone, 5-methyl-2-(1-methylethyl)-	10.8225
26.9634	Cyclohexanone, 5-methyl-2-(1-methylethyl)-	18.7279
27.1131	.delta.-terpineol	0.092
27.2789	CIS-ISOPULEGONE	2.8479
27.3217	TRANS-ISOPULEGONE	1.3681
27.4608	TERPINEN-4-OL	0.3983
28.0117	.BETA. FENCHYL ALCOHOL	0.0731
28.0919	TRANS-DIHYDROCARVONE	0.1275
28.7391	(+)-3-CARENE	0.3019
29.1884	Cyclopentanecarboxylic acid, 3-methylene-,	0.0948
29.413	Pulegone $$ Cyclohexanone, 5-methyl-2-(1-	5.1492
29.4665	Campholic acid $$ Cyclopentanecarboxylic a	0.044
29.7875	(1RS,4SR)-8-hydroxy-p-menthan-3-one $$ C	0.5826
30.1833	2-FLUORO-4,7,7-TRIMETHYLTRICYCLO[2.2.1	11.3187
30.2474	Maleic hydrazide	0.085
30.4026	Dodecane (CAS) $$ n-Dodecane $$ Ba 51-09	0.046
30.5951	Cyclohexanamine, N-ethyl- (CAS) $$ N-Ethyl	0.3701
31.0283	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-	12.7666
31.5311	ethyldimethylthiophene	0.2637
31.7504	2-Cyclohexen-1-ol, 2-methyl-5-(1-methyleth	0.0473
31.8788	3-Cyclohexen-1-ol, 4-methyl-1-(1-methyleth	0.0661
32.2853	ethyldimethylthiophene	0.1059
32.4939	P-MENTHA-8-THIOL-3-ONE	0.4842
32.7506	8-MERCAPTO-p-MENTHANE-3-ONE	1.3601
33.0769	2-Cyclopenten-1-one, 2-(2-butenyl)-4-hydro	0.0769
33.1357	METHYL EUGENOL	0.0491
33.7294	(.+-.) 3-exo-Hydroxycineole	0.0736
33.9113	5,7-dimethyl-1-azacyclo[3.2.2]azine $$ Imid	0.1197
35.2644	Ethyltetramethylcyclopentadiene $$ 1,3-Cyc	0.0977
40.4312	Hexadecanoic acid (CAS) $$ Palmitic acid $$	0.0923

Figure 88: GC-MS of buchu essential oil

RT	Library/ID	Area Pct
13.565	.ALPHA.-PINENE, (-)- $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- (CAS) $$	3.0056
16.817	MYRCENE	1.195
17.5711	.alpha.-phellandrene	0.1867
18.2237	.alpha.-terpipene $$ .ALPHA.-TERPINENE	5.9607
18.6141	PARA CYMENE	0.5655
18.7585	Benzene, 1-methyl-4-(1-methylethyl)- (CAS) $$ p-Cymene $$ 1-Methyl-4-is	2.5214
19.2078	1,8-CINEOLE	42.6803
19.8925	TRANS-.BETA.-OCIMENE	0.275
20.561	.GAMMA.-TERPINENE	11.777
22.0212	TERPINOLENE $$ PARA-MENTHA-1,4(8)-DIENE	1.8067
24.626	Bicyclo[2.2.1]heptane, 2-chloro-1,3,3-trimethyl-, endo- (CAS) $$ Norborna	0.1008
26.5462	.GAMMA.-TERPINENE	0.0662
26.7708	2-Isopropylfuran	0.6275
27.1933	.delta.-terpineol	0.0802
27.6373	TERPINEN-4-OL	21.6155
28.0919	.BETA. FENCHYL ALCOHOL	3.3335
28.7498	2-.BETA.-PINENE $$ Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (CA	0.3128
28.9477	CAMPHENE	0.0655
29.1991	Cyclopentanecarboxylic acid, 3-methylene-, 1,7,7-trimethylbicyclo[2.2.1]h	0.1229
30.6807	2-Butanone, 4-(5-methyl-2-furanyl)- (CAS) $$ 1-(5'-METHYL-2'-FURYL)BUT.	0.0696
31.5364	(S)-2-methylene-1-cyclohexanol $$ Cyclohexanol, 2-methylene-, (S)- $$ (S)	0.0969
32.5527	(-)-ISOLEDENE	0.0444
32.6543	.alpha.-Copaene $$ Tricyclo[4.4.0.0(2,7)]dec-3-ene, 1,3-dimethyl-8-(1-met	0.0822
33.2908	.ALPHA.-GURJUNENE	0.0283
33.5368	.BETA.-CARYOPHYLLENE	0.0857
33.8952	(+)-Aromadendrene $$ 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethy	0.2853
34.0236	VALENCENE	0.0544
34.2963	AROMADENDRENE	0.1013
34.5317	.gamma.-Gurjunene $$ Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7	0.0523
34.8098	.delta.-Selinene $$ Naphthalene, 2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(	0.0498
34.9061	Ledene $$ 1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-t	0.4187
34.9542	4-Chloro-2-fluoroaniline $$ Benzenamine, 4-chloro-2-fluoro-	0.5735
35.0024	.delta.-Cadinene $$ Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(	0.2489
35.1414	Aromadendrene	0.1065
35.4516	.delta.-cadinene (armoise-Maroc) $$ .DELTA.-CADINENE	0.3793
35.5479	1S,CIS-CALAMENENE	0.1027
35.7833	CADINA-1,4-DIENE	0.05
36.7353	SPATHULENOL	0.0319
36.8958	(-)-GLOBULOL	0.1447
37.0509	.gamma.-Gurjunene $$ Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7	0.1179
37.2274	(3E)-1-cyclopentylidene-3-methyl-3-penten-2-one $$ 3-Penten-2-one, 1-cy	0.0595
37.4734	3,4,5,6,7,8-Hexahydronaphthalen-1(2H)-one, 7-methyl- $$ 1(2H)-Naphthal	0.0493
37.5215	.beta.-Selinene $$ Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-r	0.2134
37.5804	isospathulenol	0.0882
37.7141	.alpha.-Cubebene $$ 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, 3a,3b,4,	0.0445
37.8157	(3,5-dimethyladmantyl)phosphinic acid dichloride $$ Phosphonic dichloride	0.0368
37.9387	N-(P-TOLYL)-PROPIONIC ACID AMIDE $$ Propanamide, N-(4-methylphenyl	0.0265
40.4312	Hexadecanoic acid (CAS) $$ Palmitic acid $$ Palmitinic acid $$ n-Hexadeco	0.0585

Figure 89: GC-MS of TTO

7.3 HPLC ANALYSIS OF SOUTHERN STAR HOPS EXTRACT

Southern Star hops extract (extracted @ 250bar)		
Cohumulone	mg/g	16.62
N+ adhumulone	mg/g	44.27
Colupulone	mg/g	3.55
n+ adlupulone	mg/g	3.46

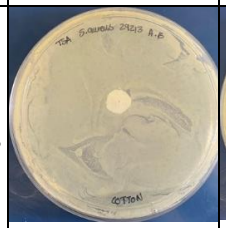
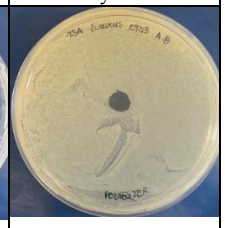
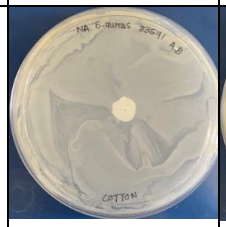
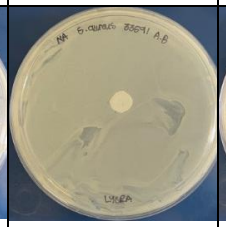
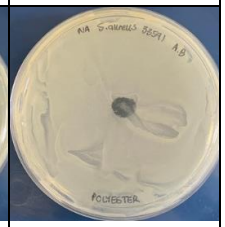
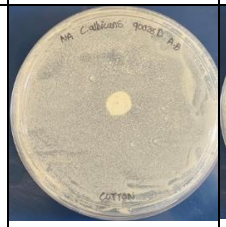
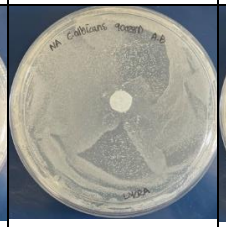
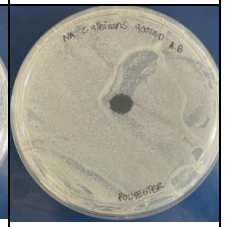
Strain	Cotton	Lycra	Polyester
Staphylococcus aureus ATCC 29213			
Staphylococcus aureus ATCC 33591			
Candida albicans ATCC 90028D-			

Figure 90: AST results of buchu ambient immersion (rinsed) samples

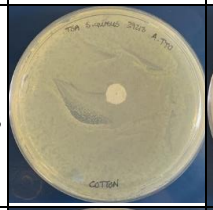
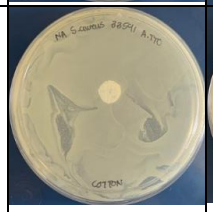
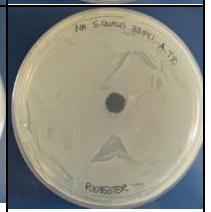
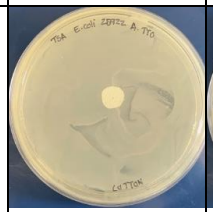
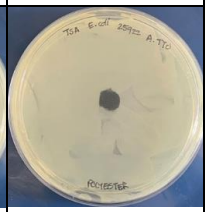
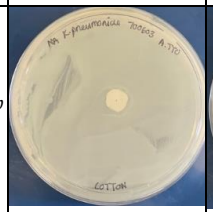
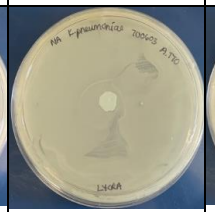
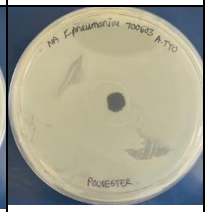
Strain	Cotton	Lycra	Polyester
Staphylococcus aureus ATCC 29213			
Staphylococcus aureus ATCC 33591			
Escherichia coli ATCC 25922			
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 700603			

Figure 91: AST results of TTO ambient immersion (rinsed) samples

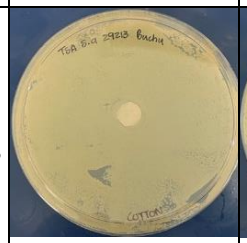
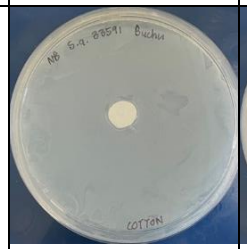
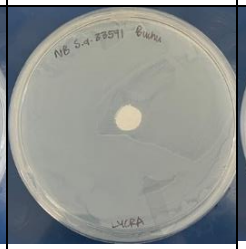
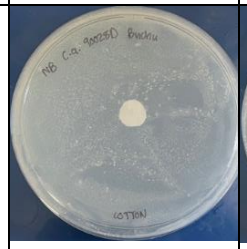
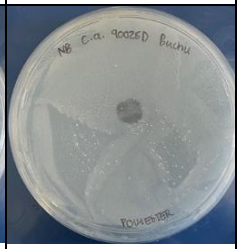
Strain	Cotton	Lycra	Polyester
Staphylococcus aureus ATCC 29213			
Staphylococcus aureus ATCC 33591			
Candida albicans ATCC 90028D-			

Figure 92: AST result of SSI buchu samples sent for immediate analysis

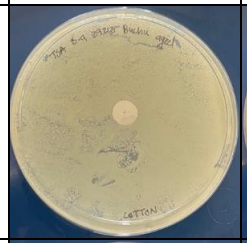
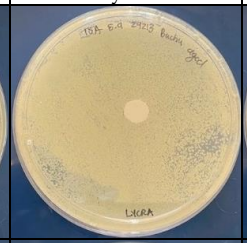
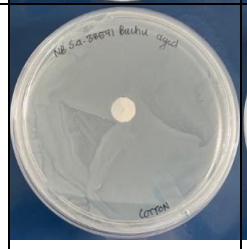
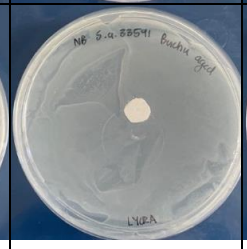
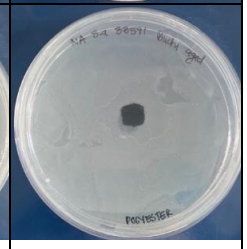
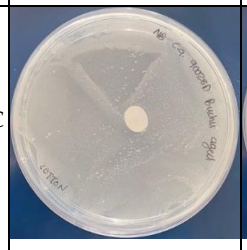
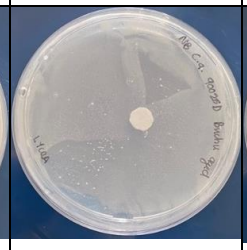
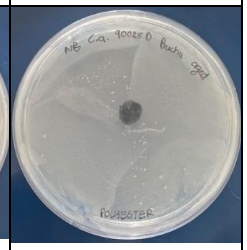
Strain	Cotton	Lycra	Polyester
Staphylococcus aureus ATCC 29213			
Staphylococcus aureus ATCC 33591			
Candida albicans ATCC 90028D-			

Figure 93: AST result of aged SSI buchu samples sent for analysis