Supercritical Solvent Impregnation of Textiles with a Therapeutic Component

GJ. Lentoor, TFN. Madzimbamuto and TV. Ojumu

Abstract— Drug resistant bacteria pose a threat to healthcare worldwide. One alternative to mitigate its spread is the use of textiles made from functionalised textile fibre, containing antibacterial agents. Textile impregnation is currently achieved using melt, powder and solution impregnation techniques, each achieving limited persistence of the active components. Impregnation using a supercritical fluid may offer longer persistence due to slower diffusion losses, as the solutes are deposited deeper into the fibre material, faster. This work investigates the hypothesis that supercritical fluid textile impregnation offers longer persistence of natural antimicrobial compounds when compared to current techniques, and is thus a better technique to prepare modified textiles. Samples of textile materials commonly used in healthcare are impregnated at 40-60^{o}C and 100-350 bar, with and without ethanol co-solvent. Natural compounds proven to be effective against pathogens are used. Fastness and antibacterial activities of these samples are compared with those prepared using current techniques.

Keywords—Alpha-acid, Beta-acid, Hops, Polyester, Supercritical Solvent Impregnation, Polycaprolactone, Rayon

I. Introduction

Flora has been used in traditional medicines for the treatment of various ailments. However, microbes and their connection with diseases were only discovered in 1665-83 [1]. The dawn of the antimicrobial age was brought about by the discovery of the first natural antibiotic by Alexander Fleming in 1928 [2]. These discoveries sparked a need to develop means of controlling the spread of pathological microbes. The use of semi-synthetic and synthetic chemicals to control the spread was inspired by the discovery of the first natural antimicrobial [3]. Today, the overuse and misuse of synthetic and semi-synthetic compounds are blamed for the mutation of some bacteria into drug-resistant forms [4][3].

Research has shown that textile surfaces such as hospital scrubs harbour a wide variety of bacteria. Textiles used and worn in hospitals provide a vector for the spread of infection within hospitals including the spread of resistant pathogenic strains [5]. The process of imparting textiles with antimicrobial properties is referred to as 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 [6]. Current techniques used such as powder, melt and solution impregnation [7], microencapsulation and the direct application technique have various downfalls [8]. 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 the inadequate fastness [9]. 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[10]. This 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 makes it consequential for the discontinued use of synthetic agents[11]. 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 the 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 [8]. 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 [6]. 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.

Supercritical solvent impregnation has been presented as a sustainable and green alternative for the impregnation process. This alternative uses carbon dioxide as its solvent. This alternative is gaining attraction due to the numerous advantages eventuated without effectuating the downfalls associated with current techniques. Advantages include anhydrous impregnation, operating at a low temperature thus preventing thermal degradation [9]. Equally, the low viscosity, near to zero surface tension, the small molecular size of the solute result in an easier penetration and impregnation of the polymer matrix and a homogeneous distribution is achieved with no production of waste [12]. This is owing to the plasticizing effect of carbon dioxide, thus swelling the fibres of the polymer which decreases the viscosity of the polymer. Additionally, the plasticization is accompanied by a decrease in the glass transition temperature which softens the polymer. The swelling and softening allow for a deeper, improved molecular diffusion into the polymer matrix [13].

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South Africa is home to various flora that possesses the potential to be harnessed for their established antimicrobial efficacy. Outeniqua hop Region near George, South Africa is home to variations of hops, *Humulus lupulus L., Cannabaceae* [14]. The principle application of hops is in the brewing industry. However, hops are not limited to this application, especially given its documented antimicrobial efficacy. In fact, traditionally, medicines used to cure illnesses contained hops as the principle ingredient [15]. Recent discoveries have further proven that hops extracts act as an inhibitor against pathogens and resistant strains. Pathogens such as *Helicobacter pylori*, *Clostridium difficile* and *Clostridium botulinum*, *Listeria moncytogenes*, *Mycobacterium tuberculosis* and *Staphylococcus aureus* [16]. Hop bitter acids have even been proven to exhibit the potential for anticancer activity [17]. Components of hop bitter acids 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 [18]. The alpha and beta acids contained within hops are unique and inhibit the growth of gram-positive bacteria and Mycobacteria in relatively low concentrations [16]. However, hop bitter 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 the hops due to their higher permeability. Whereas, gram-negative are less susceptible due to their almost impermeable cell membrane due to the structural difference that it has. 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 possess [16]. Thus, hops extract can be utilized as a therapeutic component and impregnated onto textiles.

The specialised textiles produced are intended for medical use. As such, certain traits of the textile are essential. A medical textile needs to allow for gas exchange, maintain optimal tissue temperature to facilitate blood flow to the wound, maintain a sufficiently moist environment, and not adhere to the wound as not to hinder the healing process [19].

During the preliminary experiment, the first extractor was used for an integrated extraction-textile impregnation process. The extractor was operated at a temperature of 50 ℃ and a pressure of 300 bar with a constant CO_2 flow rate of 3 kg/hr. The integrated process took place over 2 hours. Although a preliminary study was conducted, insufficient data was recorded. Thus an accurate yield could not be definitively established. What was evident was that if impregnation took place the loss of solute after impregnation was very low over 24 hours. The main aim of this research is to determine the textile impregnation yield of hops extracts into polymer woven textile using supercritical CO_2 and to determine the antimicrobial activity thereof. Whereas, the main objectives of this study focus on determining the technical feasibility, optimum conditions for total extraction, the impregnation yield of a sample of textile, antimicrobial efficacy, the effect of textile packing on impregnation load and fastness and the effect of carbon dioxide on the surface morphology of the textile. Where textiles such as PCL(polycaprolactone), polyester and rayon will be used.

The development of a process model makes up an indispensable section of this study. The process model is developed to validate the experimental data, assess the technical feasibility and impregnation performance. The accuracy, reliability of the model is dependent on the mathematical equations used [20]. In process simulation, thermodynamic models use these equations and interaction parameters to simulate a process. Models include activity coefficient models, equations of state, octanol-water partition coefficients, electrolyte mixtures, adsorption equilibria and group contribution methods for the estimation of pure component properties [20]. It is imperative that the correct model is selected. But the model cannot perform reliable simulations without experimental data to illustrate the real phase behaviour of the system. Vapour-liquid equilibrium data is used to describe the phase behaviour [21]. In other words, VLE data is used to describe the distribution of components present in the vapour and liquid phase once equilibrium has been reached and is therefore needed to predict the feasibility. It is thus imperative that VLE data for the main system components at different temperature and pressure conditions are acquired. The accuracy of the model prediction can be improved by fitting the thermodynamic model to the VLE data. This is achieved through the regression of the thermodynamic model [22].

II. Materials and Methods

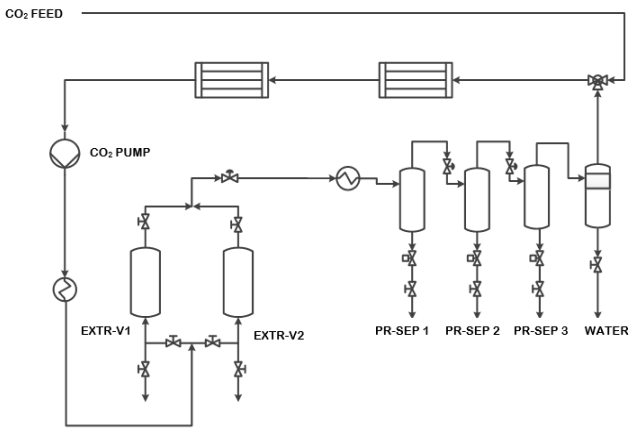

Fig. 1: PDF of Separex Pilot plant

A. Materials

In this study, South African hop pellets from Inbev were used as the raw therapeutic material. These pelleted hops were ground to produce a uniform feed; this feed was placed inside the extractor basket. In terms of the textile, polyester, PCL and rayon were used. The carbon dioxide cylinders used are 99.997% pure and were sourced from Air Liquide.

B. High-Pressure Extraction Procedure

The high-pressure extraction was conducted in the Separex High-pressure separation pilot plant located on the CPUT Bellville campus. The pilot plant consists of two 5L extraction vessels among other equipment. Before the extraction took place preparation of the feed was first to be conducted. This preparation is in terms of hops and textiles. 600g of hops were ground and weighed before being placed in the extractor.

Moreover, the textiles were washed in hexane, dried to constant mass and weighed before being placed in the extractor prior to start-up. Once the set temperature conditions were established the carbon dioxide solvent was introduced into the pilot plant. The pressure was built up using the solvent piston pump. When the set pressure was reached the continuous extraction process commenced and the static ended. The operating conditions for the extractor were be 50℃ and 300 bar. In this study, the extraction and textile impregnation took place in the same vessel. This is one of the reasons for only using 600g of hops. Before start-up, in the extraction basket, approximately 3 layers of glass marbles were placed on top of the hops. This was to provide a physical barrier between the hops and the textiles. The textiles were then placed on top of the marbles. A layer of cotton was placed as a top layer, above the textiles to prevent excessive surface textile impregnation when the system was pressured down atmosphere. During the continuous extraction process, carbon dioxide was be fed in at 3 kg/hr. This extraction process was also conducted with ethanol as the co-solvent. During this operation, 0.154 kg of ethanol was injected into the extractor prior to pressuring up. Once continuous extraction had commenced ethanol was fed in at a constant rate of 0.6 kg/hr.

C. High-Pressure Textile Impregnation

The textile impregnation process was conducted in the same extraction vessel as the extraction process. Thus the extraction process took place simultaneously with the textile impregnation process with an overall contact time of 3 hrs, under the same temperature and pressure conditions. Also, this integrated extraction-textile impregnation process was operated in batch mode. After the contact time had been reached the piston pump was switched off and the depressurisation commenced at a rate of 5 bar/min. The depressurisation was achieved by using the ramp function of the automatic relief valve that is connected to the extractor vessel. Once the entire plant had been depressurised the textile sample and hops were removed. The textiles were then washed with hexane to remove the hop extract that was just deposited on the surface of the textile. Thereafter, the textile was dried to constant mass, weighed and sent for analysis. It is of the utmost importance that the textiles be kept in an environment that is not very dependent on the external weather conditions.

D. Quantification and Analysis of the Impregnation Load

To quantify the impregnation load, the impregnated textile was simply weighed to determine the textile impregnation yield. This weighing took place over a few hours to assess how the textiles were able to hold onto the solutes. HPLC (high-performance liquid chromatography) technique is used to quantify the impregnation load. After the textile was been impregnated it undergoes extraction using a predetermined quantity of ethanol. This extraction took place in an ultrasound bath for approximately 30 minutes to recover all the impregnated agents. More cycles can be run, replacing the ethanol each time to ensure that all the agents are recovered. The extract was then dried in a rotary drier, after which the extract was once again dissolved in a predetermined quantity of ethanol before being frozen at -4℃. This all took place in the absence of light. The extract was analysed and the impregnated components were thus identified[9]. The textile also underwent an H-NMR and FTIR analysis. Since HPLC cannot identify the impregnated solutes H-NMR (hydrogen nuclear magnetic resonance) a qualitative analysis, was used to determine the structure of the impregnated molecules. Then FTIR (Fourier transform infrared spectroscopy) was used to identify the functional groups of the impregnated molecules. Thus all three types of analyses were used to characterise the impregnated molecules.

E. Antimicrobial Susceptibility

The Kirby Bauer disc diffusion technique was used. This technique is used quite often due to its simplicity and efficiency. In this technique, the suspension of the isolate was prepared to a particular McFarland standard. An appropriate agar such as the Müller Hinton agar was placed in a Petri dish. The standard was then spread evenly onto the agar. The impregnated textiles were placed onto the surface of the agar. The Petri dish was incubated for 16 to 24 hours at a temperature of 35℃. After the incubation period, the diameter of the zones of growth inhibition around the textiles was measured to the nearest millimetre. The clear zone around the textile indicated the susceptibility of a pathogen to the impregnated textile [23].

F. Characterisation of The Fabric

SEM (Scanning electron microscopy) analysis was used to determine the surface morphology of the fabric. This was due to the plasticizing effect of carbon dioxide. The plasticizing effect swells up the fibres of the polymer and the impregnated molecules could affect the morphology of the textile as well. Morphology of the structure changes the characteristics of the fibre. It is important to know the characteristics of the impregnated fibre. This analysis also shows whether the textile has truly been impregnated. [9]. Also, FTIR (Fourier Transform Infrared Spectroscopy) was used for qualitative and quantitative analysis of samples. FTIR produced an 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 [24].

G. Fastness

The fastness was determined in terms of wash fastness. Wash fastness was determined by analysing the desorption of the extract from the impregnated textile. To do this, procedures such as the ISO 105 - C01 procedure was used.

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