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Antifungal Textiles Formed Using Silver Deposition in Supercritical Carbon Dioxide

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The antifungal properties of two silver-coated natural cotton fiber structures prepared using a supercritical carbon dioxide ($scCO_2$) solvent were examined. Scanning electron microscopy confirmed that the $scCO_2$ process may be used to produce cotton fiber textiles with uniform silver nanoparticle coatings. A version of the Kirby-Bauer disk diffusion test was used to assess the ability of these textiles to inhibit fungal growth. Cotton fabric samples modified with Ag(hepta) and Ag(cod)(hfac) exhibited measurable zones of inhibition. On the other hand, the uncoated fabric had no zone of inhibition. Possible applications of antifungal textiles prepared using $scCO_2$ processing include use in hospital uniforms and wound dressings.

Keywords antifungal materials, disk diffusion test, silver, supercritical carbon dioxide

1. Introduction

Textiles are appealing materials for use in several medical applications, including hospital uniforms and linens; prosthetic valves; and wound dressings (Ref 1-5). One promising innovation is to impart these textiles with antimicrobial properties. Noble metals such as copper, gold, and silver have broad-spectrum antimicrobial activity. For example, silver has several effects on microorganisms, including impeding the electron transport system and preventing DNA replication (Ref 6-8). Nanocrystalline silver provides Ag^0 and Ag^+ ions to the surrounding environment (Ref 9-12). As silver ions are depleted, an equilibrium shift allows additional Ag^0 and Ag^+ ions to be liberated from nanocrystalline silver. In previous studies, silver has demonstrated antimicrobial activity against a broad range of fungi, viruses, and bacteria (Ref 12-15). Processes such as electroless plating (Ref 16), master batch impregnating (Ref 16), IBAD SPI-ARGENT (Ref 3), layer-by-layer deposition (Ref 17), RF-plasma-mediated deposition (Ref 18), dip-pad-dry (Ref 19-21), sol-gel coating (Ref 22, 23), soaking in silver nanosols (Ref 24), and sonochemical coating (Ref 25) have previously been used to fabricate textiles that contain antimicrobial metals.

Supercritical CO_2 ($scCO_2$) is an attractive process for imparting antimicrobial functionality to textile materials. It is a "green" technology, which does not produce any harmful byproducts (Ref 26). In addition, $scCO_2$ processing is relatively inexpensive and is amenable to scale-up for industrial production. Supercritical CO_2 processing is widely used for industrial-scale extraction of chemicals, including coffee decaffeination and fragrance collection (Ref 26). Supercritical CO_2 has also been used for disinfection of medical fabrics (Ref 27). While most commercial applications of $scCO_2$ processing involve the extraction of chemicals, $scCO_2$ processing may also be used to modify the surface (Ref 28) and impregnate the bulk (Ref 29, 30).

At temperatures and pressures above 31.1 °C and 73.8 bar, carbon dioxide exhibits properties of both liquid and gas (Ref 31-34). Supercritical fluids exhibit low viscosity and high diffusivity values similar to gases, but exhibit density values comparable to liquids. Supercritical CO_2 has recently been utilized for producing silver nanoparticle suspensions (Ref 35-37). It is also known that $scCO_2$ can be used as a solvent to dissolve metal-organic precursors to form thin films of metals and metal oxides (Ref 31-34). In previous studies, $scCO_2$ processing has been used to impart polymers (Ref 29) and porous structures (Ref 30) with antimicrobial functionality by impregnating these materials with silver nanoparticles. The use of $scCO_2$ processing to impregnate textiles with antimicrobial materials has not been previously investigated.

Candida albicans is a pathogenic yeast that may infect the skin, mucous membranes, nails, and gastrointestinal tract. The incidence of candidal infection is rising due to the growing number of individuals with suppressed immune function caused by malignancy, HIV infection, antibiotic use, steroid use, or chemotherapy (Ref 38). In addition, common health problems, including diabetes mellitus and obesity, can also predispose an individual to candidal skin infection (Ref 38). *Candida albicans* is major cause of nosocomial infections (infections acquired during medical care); contaminated health care workers and biomaterials are common sources of these infections (Ref 39). For example, *C. albicans* is the most common fungus isolated from surgical wounds; Giandoni et al. demonstrated that asymptomatic candidal infection may delay wound healing (Ref 40, 41). In addition, *C. albicans* is the most commonly isolated fungal species in intensive care unit (ICU) patients; candidal infection is associated with ICU patient mortality (Ref 42). It is interesting to note that *C. albicans* may also cause enzymatic degradation of common textile dyes

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(Ref 43). For example, Vitor et al. demonstrated degradation of Direct Violet 51 azo dye by *C. albicans*, which resulted in removal of color (Ref 43).

In this study, we examined the antifungal properties of two silver-coated cotton fiber structures that were prepared using silver precursors dissolved in $scCO_2$. Two different silver precursor materials, Ag(hepta) and Ag(cod)(hfac), were investigated. A variation of the Kirby-Bauer disk diffusion test was utilized to assess the ability of these textiles to inhibit growth of *C. albicans*. The Kirby-Bauer disk diffusion test is a National Committee on Clinical Laboratory Standards (Wayne, PA) procedure for assessing antimicrobial activity of materials (Ref 44), which has previously been used to assess the antimicrobial performance of textile materials (Ref 45). The prescribed method of the Kirby-Bauer assay was followed with the exception that silver-coated fabric was used instead of disks containing antibiotic agents. The results of this study suggest that $scCO_2$ process may be used to coat textiles with antifungal silver for a variety of medical applications.

2. Experimental Procedure

In this work, modification of cotton fabrics using $scCO_2$ was investigated. The silver precursors dissolved in $scCO_2$ were able to diffuse into the dense fiber network of the woven cotton structures and react with the cotton to deposit thin films and particles of silver. The size and density of the deposited particles may be controlled by altering the deposition conditions. A schematic of the $scCO_2$ system used for deposition of the silver nanoparticles can be seen in Fig. 1. Pressurized CO_2 (99.99% purity) was pumped into a stainless steel cylindrical-shaped reactor, which was heated with heating tape. The volume of the reactor used in this study was ~110 mL. Hydrogen was also introduced into the reactor to reduce the precursor material to metal. The inside of the reactor was observed through a sapphire viewing window. The pressure and temperature of the $scCO_2$ were monitored with a pressure gage and a thermocouple, respectively.

The experimental procedure for the fabrication of silver-coated cotton fabrics is shown in Fig. 2. 2.0 cm × 2.0 cm cotton pieces and either 10 mg of Ag(hepta) or 2.1×10^{-4} mol/L of Ag(cod)(hfac) were placed in the preheated reactor before it was filled with pressurized CO_2. The pressure and temperature of the dissolution process were 21 MPa and 40 °C, respectively. The supercritical fluid color remained clear after dissolution of the precursor. The cotton fabric samples were left in the chamber with the dissolved precursor for between 10 and 15 h, allowing the dissolved precursor to diffuse into the individual cotton fibers. After the diffusion process, the reaction chamber was allowed to vent. The temperature was then raised to 80 °C. Supercritical CO_2 with hydrogen gas (0.7 MPa) was introduced into the reaction chamber for reduction of the impregnated precursor. The decomposition time was 6 h. The decomposition pressure and temperature utilized in this study were 22 MPa and 80 °C, respectively. After the reaction chamber was vented, the $scCO_2$ fluid was replaced with fresh compressed CO_2, which was used for rinsing the materials. Scanning electron microscopy of the silver-coated cotton fabrics was performed using a S3200 system (Hitachi, Tokyo, Japan), which was equipped with a Robinson backscattered electron detector and an energy-dispersive X-ray spectrometer.

The antifungal properties of the Ag(hepta)- and Ag(cod)-(hfac)-coated fabrics were examined using *C. albicans*. The prescribed method of the Kirby-Bauer assay was followed with the exception that silver-coated fabric was used instead of disks containing antibiotic agents (Ref 44). Untreated cotton fabric was used as a negative control in these experiments. All fabrics were cut into ~0.50 cm × ~0.25 cm rectangles. 1% w/v Bacto yeast extract (BD Diagnostics, Sparks, MD), 2% w/v Bacto peptone (BD Diagnostics, Sparks, MD), 2% w/v dextrose (Mallinckrodt Chemical, St. Louis, MO), and 2% w/v granulated agar (BD Diagnostics, Sparks, MD), commonly known as YPD agar, were used as growth media. The microorganism used in this study was *C. albicans* strain DUMC 117.00 (Duke University Medical Center, Durham, NC). A 0.5 McFarland Standard of *C. albicans* in sterile deionized water was prepared. The turbidity was verified using a Stasar II spectrophotometer (Gilford Instruments, Oberlin, OH). Sterile cotton-tipped applicator sticks were used to spread the cell suspension onto the agar plates. A different applicator stick was used for each plate. The fabrics were placed on the plates after inoculation. Triplicates of the same type of fabric were placed on each plate. Different types of fabrics were placed on separate plates. After the fabrics were placed on the plates, the plates were incubated at 37 °C for the duration of the study. The plates were examined at 0, 12, 24, 36, and 48 h after inoculation. Examination consisted of measuring zones of inhibition of cell growth on the agar and obtaining images of the fabrics to detect growth on the fabrics. An EZ4 D dissection stereo-microscope (Leica Microsystems, Bannockburn, IL) was used for imaging of the samples.

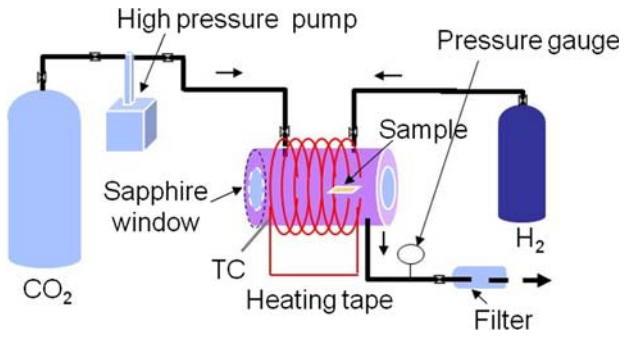

Fig. 1 Schematic of the $scCO_2$ system

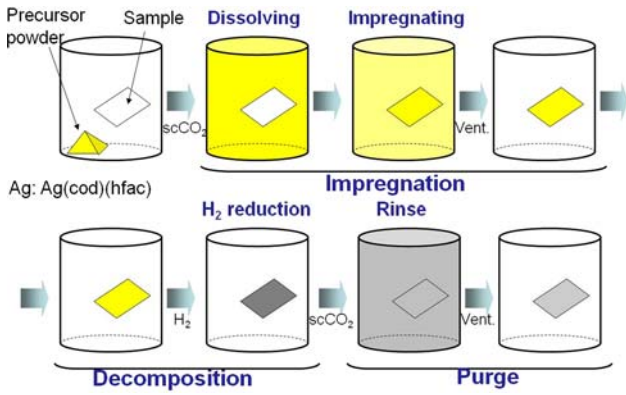

Fig. 2 Procedure for fabrication of silver-coated cotton fabrics

3. Results and Discussion

Scanning electron microscopy images of the three fabrics can be seen in Fig. 3. The silver-coated fabrics demonstrated both (a) a thin layer of silver over the entire fabric surface and (b) a scattering of silver aggregates on regions of the fabric surface. The appearance of streaks on the fibers was caused by electron beam-initiated swelling of the cotton fibers. A increase in volume of the cotton fiber caused fissures to form in the silver film; these features appear as streaks in the electron micrographs. Additional scanning electron microscopy images of the silver-coated fabrics showing both silver aggregates and electron beam-induced fissures are provided in Fig. 4. In this figure, the Ag(hepta)-modified fabric exhibited a greater number of silver crystals on its surface than the Ag(cod)-(hfac)-modified fabric. The energy dispersive X-ray spectra for Ag(hepta)-modified fabric, Ag(cod)(hfac)-modified fabric, and uncoated fabric can be seen in Fig. 5. All three fabrics exhibited trace amounts of copper and aluminum. The energy dispersive X-ray spectra of the uncoated cotton demonstrated that the material contained no silver. Energy dispersive X-ray spectra also indicated that the Ag(hepta)-modified fabric and Ag(cod)(hfac)-modified fabric contain similar amounts of silver. The cotton is primarily made up of cellulosic polymer, which contains a significant amount of hydroxyl groups on the surface. In the presence of hydrogen, it is likely that the silver precursors reacted at the surface to reduce the metal to Ag^0 and oxidize the functional ligands. This mechanism is often found in other deposition techniques, including chemical vapor deposition and atomic layer deposition. The oxidized ligands were subsequently dissolved in the $scCO_2$ and removed from the

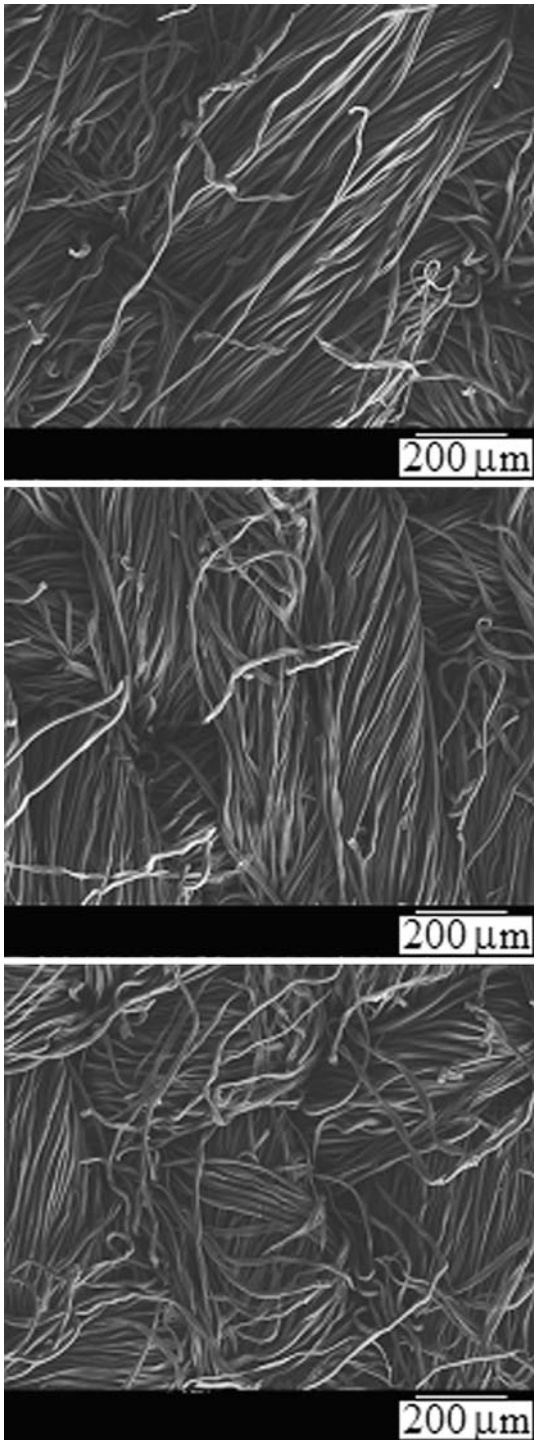

Fig. 3 Scanning electron micrographs of Ag(hepta)-modified fabric (top), Ag(cod)(hfac)-modified fabric (middle), and uncoated fabric (bottom)

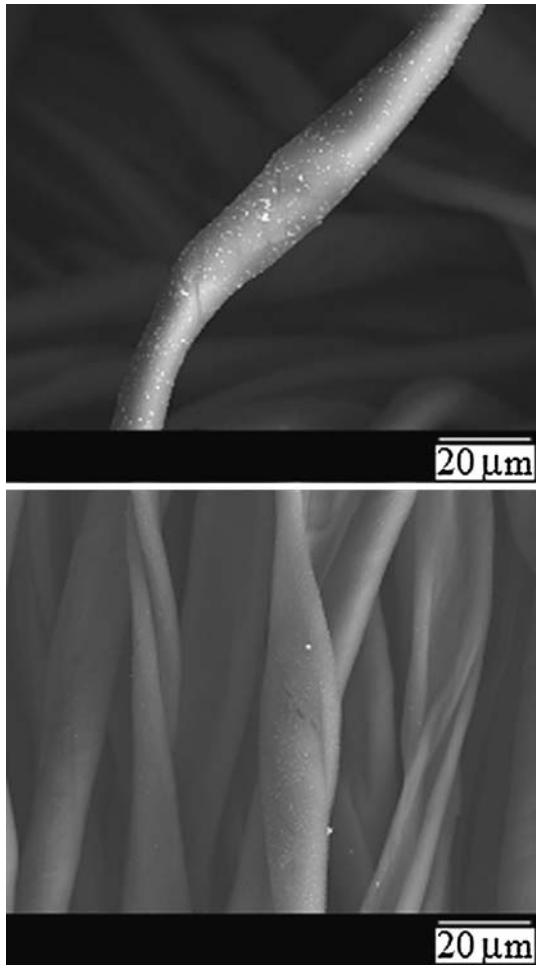

Fig. 4 Scanning electron micrographs of Ag(hepta)-modified fabric (top) and Ag(cod)(hfac)-modified fabric (bottom). The lighter-colored regions on the silver-modified fibers represent silver aggregates

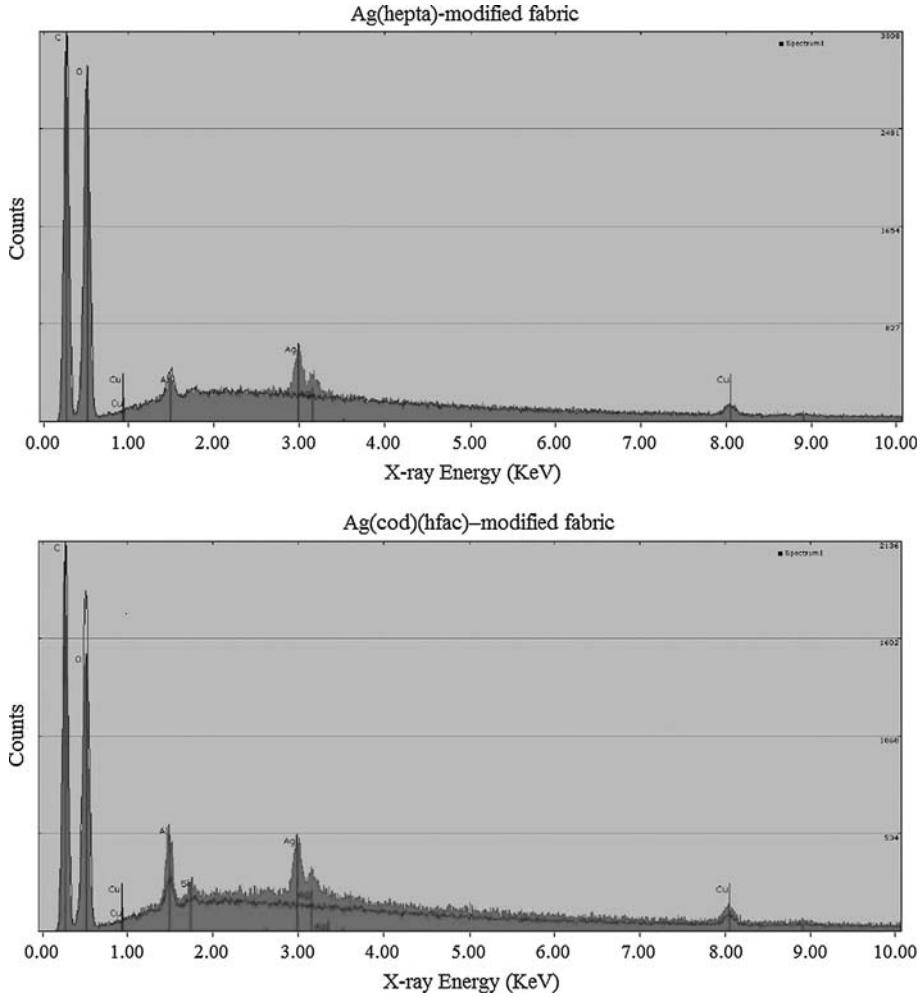

Fig. 5 Energy dispersive X-ray spectra overlay of Ag(hepta)-modified fabric (top) and Ag(cod)(hfac)-modified fabric (bottom). The spectra of the coated fabrics are in gray, and spectrum of the uncoated fabric is in black

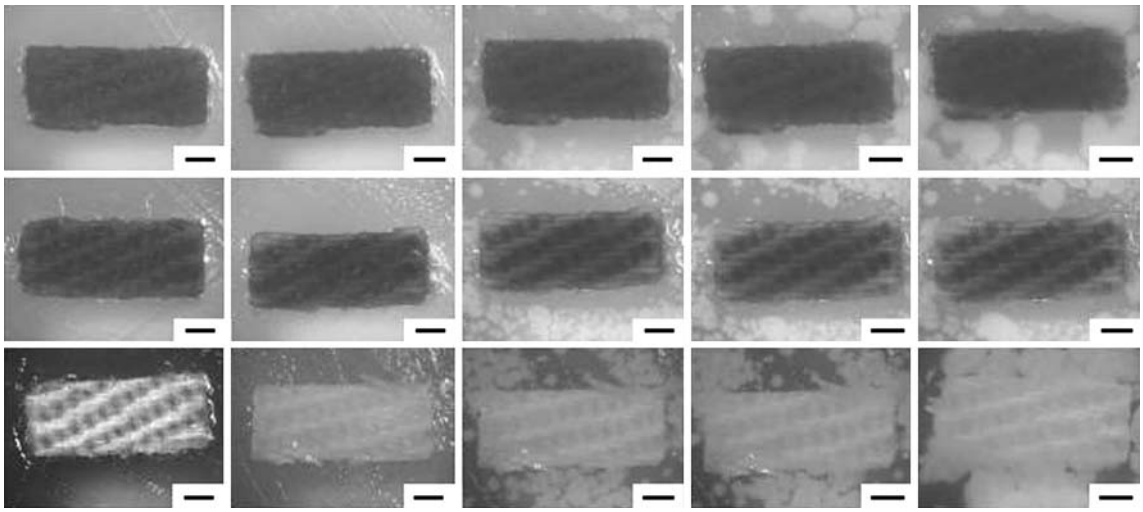

Fig. 6 Ag(hepta)-modified fabric (top), Ag(cod)(hfac)-modified fabric (center), and uncoated fabric (bottom). Images obtained at 0, 12, 24, 36, and 48 h after initiation of the experiment are shown from left to right. Scale bar = 1 mm

system, resulting in silver coatings on the fibers (Ref 24-27). The formation of particles could result from either (a) surface diffusion of silver atoms or small clusters or (b) preferred oxidation/reduction of the precursors at deposited silver sites, which promoted formation of clusters and nanoparticles.

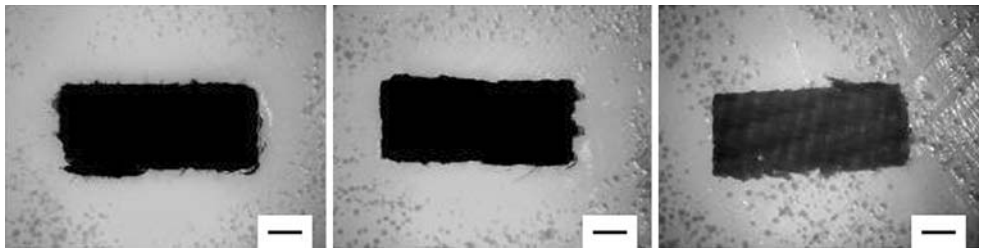

Fig. 7 Optical micrographs showing zones of inhibition for Ag(hepta)-modified fabric (left) and Ag(cod)(hfac)-modified fabric (center) at 12 h. An optical micrograph of the uncoated fabric at 12 h (right) is provided for comparison purposes. Scale bar = 1 mm

Images were obtained at 0, 12, 24, 36, and 48 h after initiation of the antimicrobial susceptibility assay. Images of the silver-coated and uncoated fabrics at these times are shown in Fig. 6. At 12 h, a confluent layer of small colonies formed on the agar. Zones of inhibition of fungal growth were observed in Ag(hepta)-modified and Ag(cod)(hfac)-modified fabrics. Enlarged images of the zones of inhibition of the three fabrics at 12 h are shown in Fig. 7. Zones of inhibition were measured from the edge of the fabric to the first sign of growth. The average radius of inhibited growth was 1.2 mm for both Ag(hepta)-modified and Ag(cod)(hfac)-modified fabrics. The uncoated cotton had no observed zone of inhibition. At 24 h, some fungal cells began to appear within the zones of inhibition for Ag(hepta)-modified and Ag(cod)(hfac)-modified fabric samples. The Ag(hepta)-modified surface exhibited a smaller number of colonies than the Ag(cod)(hfac)-modified surface; this result may be attributed to the arrangement of silver crystals on the fabric surface.

4. Summary

In this study, $scCO_2$ processing was shown to be a viable technique for producing antifungal textiles that may be used in medical applications. While the samples examined in this study were small in size, $scCO_2$ processing can easily be scaled up for commercial production of silver-coated textiles. After exposure to *C. albicans*, fabrics modified with Ag(hepta) and Ag(cod)(hfac) demonstrated measurable zones of inhibition. On the other hand, the uncoated fabric exhibited no zone of inhibition. Use of $scCO_2$ for imparting antimicrobial functionality to materials has several advantages, including relatively low processing temperatures, nonflammable processing materials, and nontoxic reactants. Supercritical CO_2 processing may be used to impart antifungal functionality to textiles used in wound dressings. Hospital uniforms containing antifungal textiles may be used to prevent the spread of fungal infections in hospitals, nursing homes, and other healthcare settings.

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