PRODUCTION OF ANTIBACTERIAL FILTER PAPER FROM WOOD CELLULOSE

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Paper has a visible market-share in hygiene products either in the form of personal hygiene or as food packaging. The designation "hygiene", though it suggests cleanliness, does not imply antibacterial properties; rather it can be stated that hygiene products do not initiate microorganism growth. Antibacterial products could restrict propagation of pathogenic bacteria either by holding bacteria or by trapping and neutralizing them. Most research in this field has been conducted using textile fibers as a substrate, but the present work uses paper instead. The objective was to produce an antibacterial filter paper capable of trapping and neutralizing pathogenic microorganisms using wood fibers. To produce antibacterial paper, chitosan and nanosilver capped with PAA (polyacrylic acid) were deposited on the fiber surface using a layer-by-layer technique. Samples for the tests were prepared from refined bleached softwood (RBSW) kraft pulp. The deposition of antibacterial agents on fiber as well as paper were monitored using a zeta potential analyzer (ZPA), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIRS). The minimum requirement for deposition of the agents was a multilayer comprised of eight alternating layers. The deposition onto fiber or paper had no effect on tensile strength or the pore structure of the substrate.

Keywords: Facemask; Antibacterial; Pathogen; SARS; Toxic

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INTRODUCTION

A need for control of pathogenic microorganisms in contaminated environments has led to the development of antibacterial products. Antibacterial agents are used to kill or inhibit the growth of bacteria, viruses, or fungi. They can be either bactericidal (i.e., killing the pathogenic organisms) or bacteriostatic (inactivating the organisms concerned, thus providing an opportunity to host's immune system to react) (Patel 2009). Antibacterial products not only protect humans from infectious disease, but also could contribute in the healing processes. Therefore, antibacterial products should be a visible portion of consumed items. The market is full of toxic antibacterial materials (inorganic salts, onium salts, antibiotics, formaldehyde derivatives, etc.). Public concern of hazardous materials is a strong driving force for replacing toxic chemicals with materials that are eco-friendly. Chitosan is an ideal alternative, since it is inexpensive, non-toxic,

biodegradable, cationic, and antibacterial (Chen and Chiang 2008; Chirkove 2002; Kim and Kim 2006; Knill et al. 2004; Lee et al. 2003; Wu et al. 2004). Chitosan, the N-deacetylated polysaccharide of chitin, is the second most abundant natural polymer in the world (Wu et al. 2004), after the cellulose/hemicellulose/lignin group. Similar to cellulose it has a β-(1→4) linkage of D-glucosamine units as its backbone. The only difference is replacement of the C_2-hydroxyl group of the cellulose backbone by an acetamide group. Among the natural polymers, chitosan has been used the most in wound dressing (Gupta et al. 2010). Although the actual mechanism of action of chitosan on the microbes is yet unknown, it is reported that the polycationic nature of chitosan plays a significant role deactivating various bacteria or fungi and eventually killing them (Ye et al. 2005).

Silver has been used as early as 1000 B.C. for the treatment of water and as early as 1700 for the treatment of different infections (Rai et al. 2009). It has generally been used and studied the most among the antibacterial agents. However, with the introduction of penicillin in 1940s, its use as an antibacterial agent was reduced. The emergence of antibiotic-resistant bacteria (super bugs) has forced labs to return to silver products. Super bugs have been considered as the worst health treats, even worse than SARS or Bird flu (Patel et al. 2009). Bacteria exposed repeatedly to antibiotics genetically mutate, developing resistance to antibiotics.

The mechanism of silver action involves the inhibition of microbial respiration. Klasen (2000) reported that silver binds to the membranes of bacteria cells, thus rendering the respiration system impaired. Silver is also non-toxic in minute concentrations to humans (Rai et al. 2009).

Nanotechnology is rapidly growing in different fields of science and technology. New nanomaterial technologies have provided fresh opportunities to develop higher performance composite materials. Layer-by-layer (LbL) self-assembly based on electrostatic theory is a simple, practical, and versatile method for deposition of tailored multilayered thin films on substrate surfaces. The process is alternate deposition of oppositely charged polyelectrolytes on substrate surfaces, which results in surface charge reversal after each deposition. The modified substrates demonstrated special functions along with preserving their original structure (Xing et al. 2007).

Although antibacterial paper products with having different applications has been on the market since 2007 (Adams et al. 2006; Askew 2007; Patel 2009), work on antibacterial filter paper is still scarce (Fujihshima et al. 2000). The present work focuses on developing a filter paper with characteristics of trapping and killing pathogenic microorganisms by evoking the versatile nature of paper.

EXPERIMENTAL

Materials

Bleached kraft softwood pulp was market pulp from Finland. Chitosan was provided by the Seafresh company of Thailand with a deacetylation degree of 89% and molecular weight (Mw) of 8×10^4 Da. Nanosilver in micro-ball form was made by the ISE department of Asian Institute Technology (AIT), then capped with PAA (polyacrylic

acid) to convert its surface charge to negative. Figure 1 shows a microphotograph of micro-ball nanosilver. The antibacterial products were tested using gram negative bacteria (*Escherichia coli*) and gram positive bacteria (*Staphylococcus aureus*). The pulp was soaked in water for four hours, disintegrated according to TAPPI standards, then refined with a PFI mill for 10,000 revs. Chitosan was dissolved in 2% acetic acid at 80 ^{o}C. Nanosilver was prepared as a solution at 0.25 mol/L concentration to the size of micro-balls, as shown in Fig. 1.

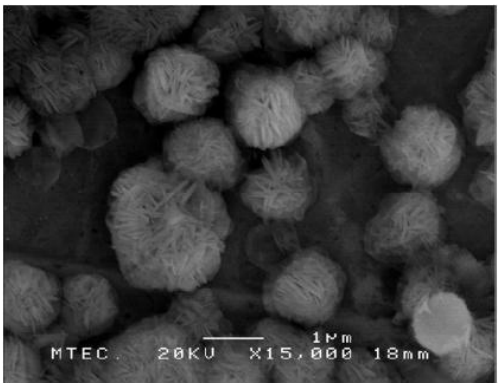

Fig. 1. SEM image of silver nanoparticles as micro-ball

Methods

Fibers surface charge as well as the silver micro-ball particles capped with PAA were negative, but the surface charge of chitosan dissolved in acetic acid was positive. Based on a layer-by-layer technique the chitosan should be deposited on the fiber surface prior to the micro-balls. Chitosan (1% g/g) based on oven-dry pulp was added to a 3000 mL fiber suspension containing 30 g (od) pulp and stirred for 15 min. Then the fibers were washed thoroughly. Subsequently the silver micro-balls were processed in a similar fashion. After each deposition, the surface charge was monitored using a Mutek SZP-06 zeta potential tester. The presence of functional groups was ascertained with a NEXUS 870 FTIR spectroscope.

Figure 2 demonstrates bilayer deposition process. A layer produced by successive deposition of two oppositely charged polymers on a substrate is referred as a 2-bilayer. Re-depositing two more oppositely charged polymers on a 2-bilayer gives rise to a 4-bilayer.

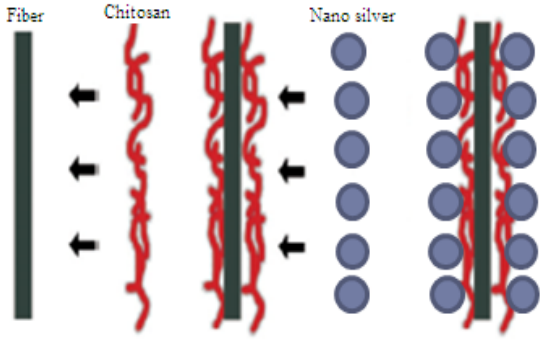

Fig. 2. Schematic image of layer-by-layer process using chitosan (80 KDa) and nanosilver capped with PAA

Assessment of papers for antibacterial properties

The antibacterial properties of the treated papers were assessed using an optical density method (Zhai et al. 2004; Huang et al. 2004; Wang et al. 2005). BHI broth culture was prepared, and 5 mL was added to each test tube. The test tubes containing BHI broth were sterilized in an autoclave for 15 min. The treated paper samples were also sterilized by storing in an oven for 2 h at 150 ^{o}C prior to being added to the test tubes. A dose of 0.01 mL of *S. aureus* suspension was added to the test tubes, then incubated in a shaking bed (160 rpm) for 24 h at 37 ^{o}C. The turbidity of the suspensions was measured at 620 nm using a spectrophotometer (model 722-2000).

RESULTS AND DISCUSSION

It was assumed that the deposition of either chitosan or silver micro-balls on the fiber surface should alter the surface charge of the fibers, thus allowing one to monitor the particle deposition by means of a zeta potential tester. Figure 3 shows that the zeta potential shuttled between positive and negative charge zone, showing an effect of alternate deposition of chitosan and silver micro-balls on the fiber surface.

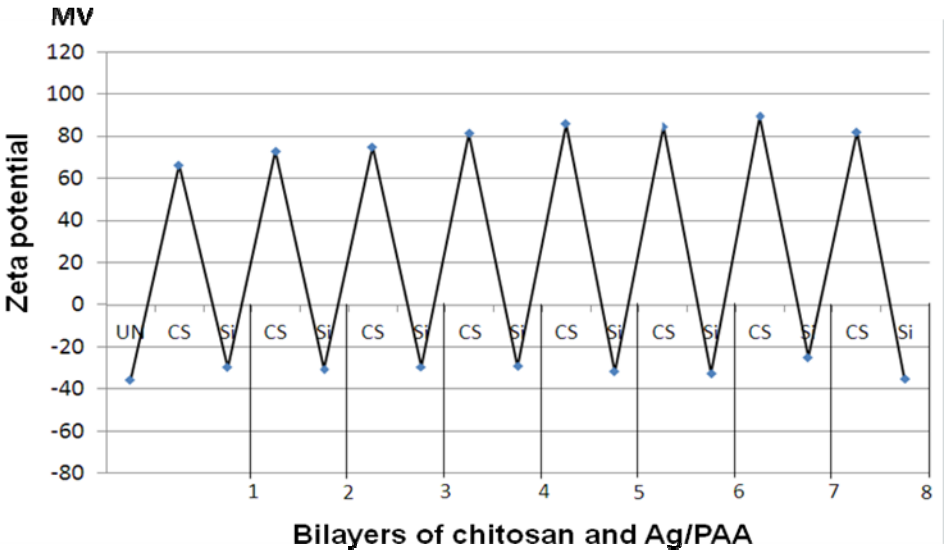

Fig. 3. Zeta potential of fibers surface treated with Chitosan and Ag/PAA

Figure 4 shows SEM microphotographs of untreated and treated fibers, i.e., with optional deposition of a combination of chitosan and silver micro-balls. The image of sample B3, a microphotograph of an 8-bilayer sample, shows signs of numerous micro-balls on fiber surfaces. Such signs are not visible in the B1 microphotograph, or scarcely visible in the B2 microphotograph. Probably, the chitosan deposition on fibers is also higher for an 8-bilayer compared to a deposition of 2- or 4-bilayers.

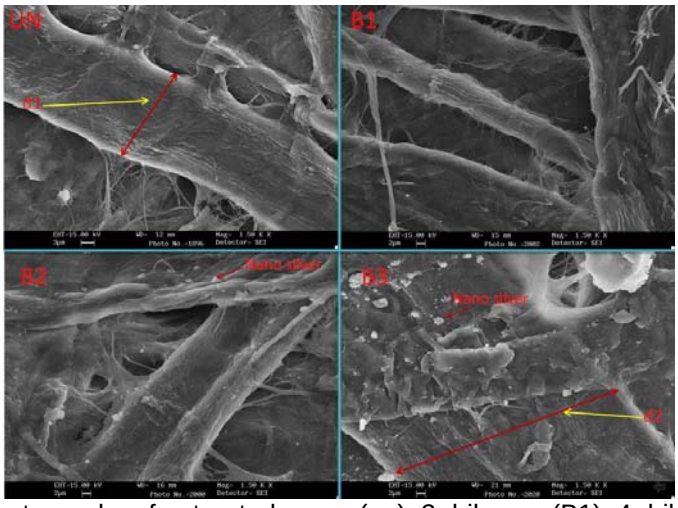

Fig. 4. SEM microphotographs of untreated paper (un), 2- bilayers (B1), 4- bilayers (B2), and 8-bilayers (B3)

FTIR spectra also confirmed deposition of chitosan on the fibers (Fig. 5). The broad absorption band at 3487 cm^{-1} in the figure is attributable to the stretching frequency of the hydroxyl group (OH^-) of the untreated sample, which corresponds to the combined band peaks of NH/OH stretching, indicating deposition of chitosan film on fibers. The absorption for untreated fibers in the range of 400 cm^{-1} to 600 cm^{-1} is less than the treated fibers, which also suggests a contribution from the deposited layers.

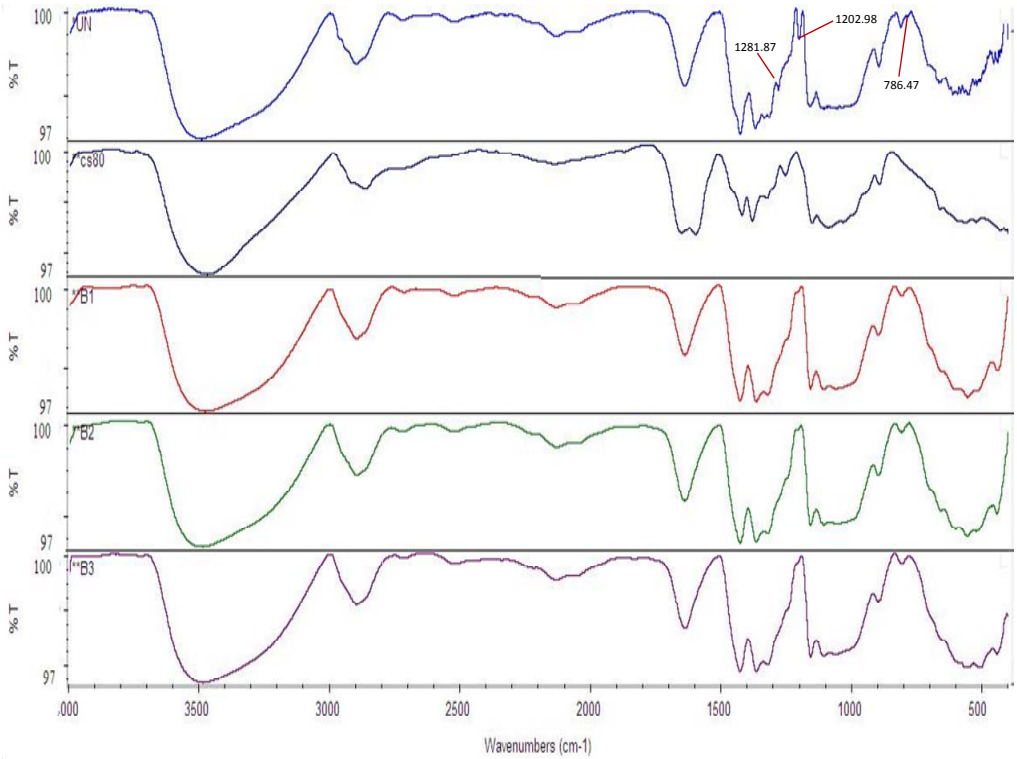

Fig. 5. FTIR spectra of chitosan (cs80), untreated paper (un), and treated papers (B1, B2, & B3)

Figure 6 shows the air permeability of sheets made of the treated vs. untreated samples. The objective in measurement of permeability of the samples was to highlight the fact that the network permeability could be better controlled in paper than woven fabric. Developing a desired pore structure in fabric is very difficult compared to paper. The plots suggest that air permeability decreases with increase in basis weight, which is due to loss in the number of pores with increase of the network fiber population. Air permeability of treated samples increased with the extent of treatment due to loss in fines (Fig. 6). As was highlighted earlier, the pulp was washed after each deposition stage. The washing obviously frees the pulp from fines; thus it contributes to developing a more open fibrous network.

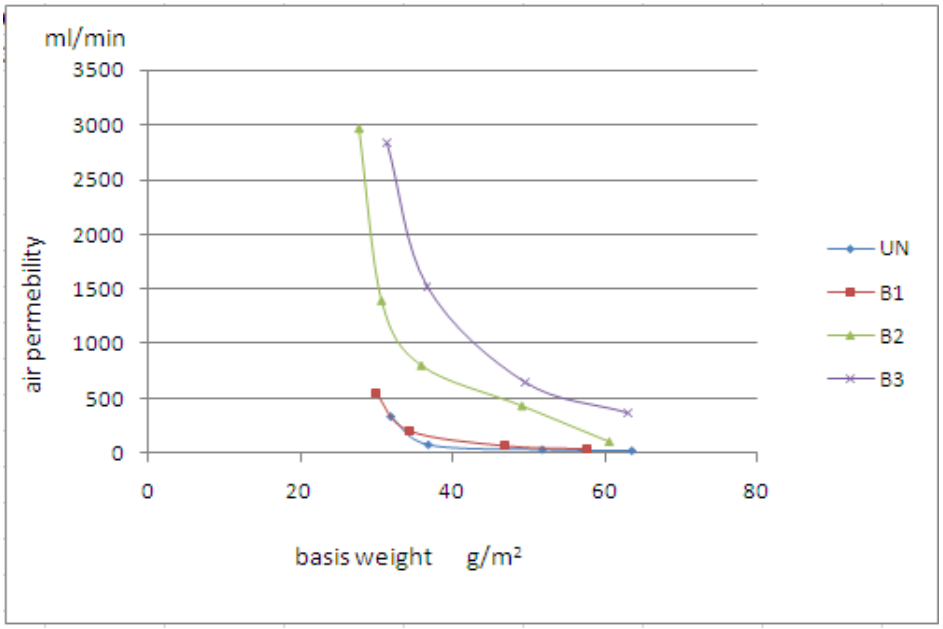

Fig. 6. Influence of antibacterial treatment process on air permeability of paper

Antibacterial paper should resist attrition due to repeated end use as a filter media. This means that high strength is a major requirement for filter paper in general. The antibacterial agents may also mask the bonding agents of fiber surfaces. Figure 7 shows a marginal reduction in tensile strength with the progress of fiber treatment. This is consistent with the previously mentioned loss of fines. Therefore, the strength properties did not suffer from the presence of the antibacterial agents; rather, the observed loss was due to the loss of fines during processing.

The untreated and treated samples were tested for antibacterial properties using two bacteria, namely *staphylococcus- Aureus* (gram positive) and *Escherichia coli* (gram negative). As Figs. 8 and 9 show, the bacterial population decreases with processing, as measured by the optical density (O.D.) method. The higher the number of deposition layer, the stronger is the antibacterial property of the sample. The 8-bilayer treated sample was the most effective one in confronting either of the bacteria, compared to either 2- or 4-bilayer treated samples (Figs. 8 and 9). These observations suggest possibility of developing an antibacterial paper capable of catching and killing the bacteria with better control of its pore structure.

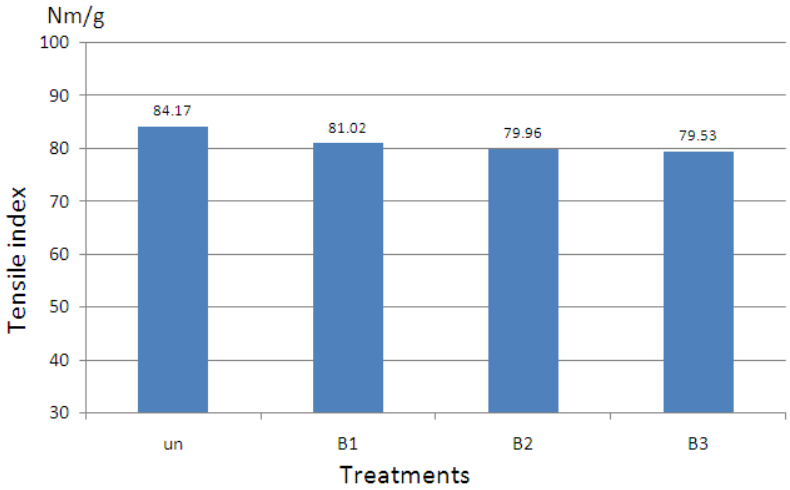

Figure 7. Influence of antibacterial treatment on tensile strength

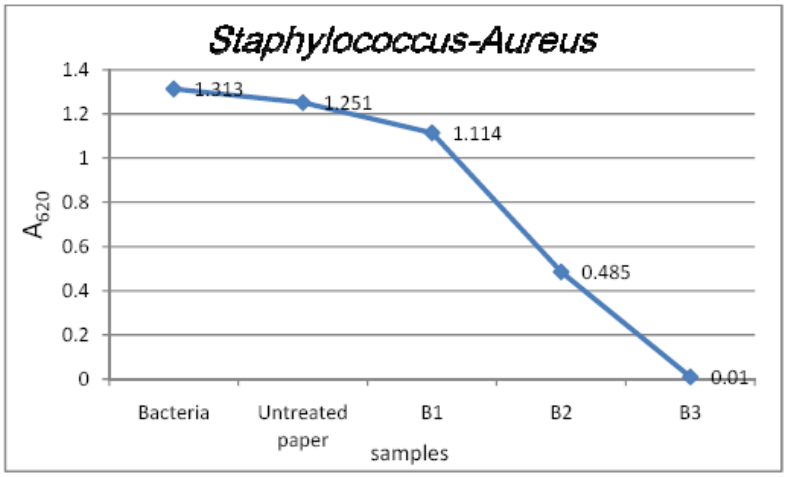

Fig. 8. Antibacterial activities of samples against *Staphylococcus aureus*

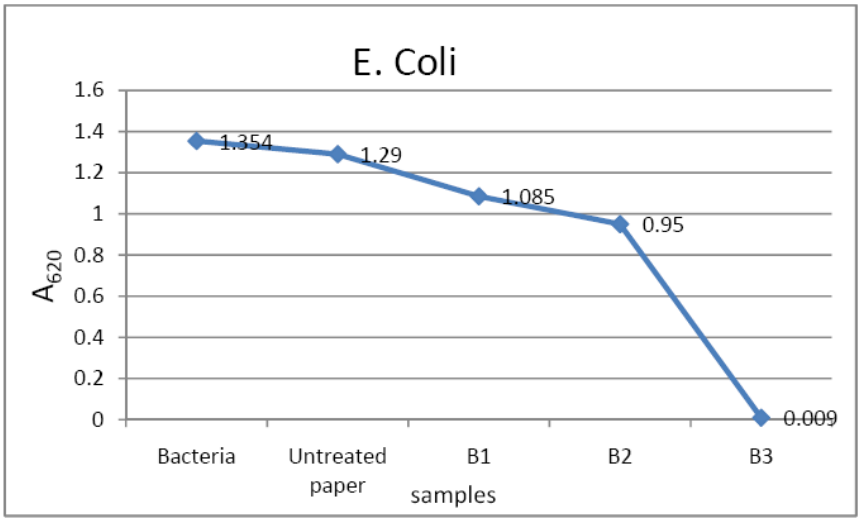

Fig. 9. Antibacterial activities of samples against *Escherichia coli*

CONCLUSIONS

1. One of the important requirements for filter media is a controlled porous structure. Paper has potential to be made in a desired structural form. The present work suggests that it is possible to make an antimicrobial filter paper capable of catching and killing pathogenic microorganisms.
2. Scanning electron microscopy (SEM), zeta potential analysis, and FTIR spectroscopy suggested that the antibacterial agents could be deposited on fiber or on a paper surface without interfering with fiber to fiber bonding. The antibacterial quality of the treated samples was also tested for two different bacteria (i.e., gram positive and gram negative). The results showed that the treated samples have antibacterial quality, in particular the 8-bilayer treated samples.

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