

Coating PHGH-Modified Starch on Papers to Induce Antimicrobial Properties

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In this work, paper was rendered antimicrobial *via* applying antimicrobial-modified starch as a coating material onto the paper's surface. The antimicrobial starch was prepared by covalently bonding guanidine polymers using a coupling reaction. Two different coating layers were applied onto the paper's surface. The first coating layer contained clay mixed with a latex binder (clay 100 g/latex 20 g). The antimicrobial starches, which possessed different grafting ratios of the antimicrobial agent (30 wt.% and 50 wt.%), were applied as the second coating layer on the paper. The results showed that the coating thickness was approximately constant at 4 μm . In the presence of 0.5 to 1.0 wt.% antimicrobial starch on cellulose fibers, the growth inhibition of bacteria was almost 100%. Additionally, the resulting coated paper exhibited high antimicrobial activities against *E. coli*. Furthermore, the results showed that the coated papers prevented fungal growth.

Keywords: Coated papers; Guanidine polymers; Starch; Antimicrobial properties; AFM; SEM

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INTRODUCTION

Over the past few decades, active packaging has been used for preserving food. Furthermore, packaging materials can be easily contaminated by microorganisms during their manufacture, storage, or use, which further raises the need for antimicrobial agents on the exposed surfaces. Active packaging can be developed by directly incorporating active compounds, such as antimicrobial agents, during papermaking process or coating of papers (Soares *et al.* 2011; Barbiroli 2012). Traditionally, paper has a wide range of applications for food packaging (Soares *et al.* 2011). Different methods can be applied to modify the surface properties of papers to improve their durability or biocompatibility (Buck and Lynn 2010). Because of the hydrophilic and highly porous nature of paper, it can readily undergo microbial attack, which will affect its quality as a packaging material. Coating agents could make the situation worse, as they could be food for microbes (Prasad *et al.* 2010). A coating technology is employed in the paper industry to improve various features of papers, such as printability and smoothness, strength, and water resistance. One approach to overcome this difficulty is to apply a layer of antimicrobial agents onto the surfaces of papers to be used for packaging. As packaging industry is growing, a better understanding of coating properties is necessary. It is known that the conventional coating typically contains pigment particles, latex binders and processing additives (Chinga and Helle 2002; Ghannam 2002; El-Sherbiny and Xiao 2004).

In the coating or surface treatment of paper and board, latex combined with water soluble polymers are the most commonly used binding formulas. Latex has excellent binding properties and provides acceptable structural and strength properties to the paper surface, while the pulpability of the coated paper during recycling is insignificantly affected (Sjoberg *et al.* 2000). Furthermore, pigments, *e.g.*, clay, are a major component of the coating suspension, which greatly impacts the cost, process parameter (such as speed) and end use performance of the coated papers (Wang *et al.* 1996).

Traditionally, starch has been used in paper industry as a strength agent. It has also been used as a sizing agent, which provided better surface properties for papers (Wang *et al.* 1996; Lee *et al.* 2001; Brouwer *et al.* 2002). In this context, cationic starch improves the surface quality of coated papers since it insignificantly penetrates into the paper structure due to its strong electrostatic interaction with the anionic charges on the fiber at the paper's surface. The strong interaction of cationic starch with anionic coating agent is expected to promote the rapid immobilization of the coating agent on the paper surface, which improves coating holdout and optical property (Brouwer *et al.* 2002; Hedman *et al.* 2003).

To improve the coating performance, it is necessary to find other alternatives for the conventional wax-based materials used for coating. The dispersion of water-based coating material develops more environmentally compatible packaging processes and minimizes the recycling cost (Xia *et al.* 2005). In order to increase the application of papers used for packaging, some studies were conducted to improve the antimicrobial performance of papers by using antimicrobial agents or organic compounds (El-Sherbiny and Xiao 2005; Etienne *et al.* 2005; Campos *et al.* 2010). Such antimicrobial agents should be efficient and safe for humans, but persistent against bacteria and fungi (Xia *et al.* 2005).

In previous work by the authors, the modification of starch with a type of guanidine polymer, *i.e.*, poly(hexamethylene guanidine hydrochloride) (PHGH), was performed, and the optimum conditions for achieving the best antimicrobial performance were investigated (Guan *et al.* 2008a,b). PHGH-modified starch showed excellent antimicrobial and antifungal performance when applied to cellulose fibers (Guan *et al.* 2008a,b). However, using antimicrobial starch as a coating component on papers has not been previously reported.

In this paper, PHGH-modified starch was prepared based on previous methods used by the authors (Guan *et al.* 2008a,b) and applied as an antimicrobial agent and the main component of a coating formula for paper coating as stated in the experimental section. The main focus of this paper was on the antimicrobial and antifungal properties of the paper coating. Although some aspects of PHGH-modified starch were studied in the past (Ziaee *et al.* 2010), the main novelties of the present work are the studies on the antimold properties of this polymer and on the impact using this polymer on coating papers.

EXPERIMENTAL

Materials

Two different types of latex (9750 and 8879), clay, and printing papers were obtained from Stora Enso Company Ltd. Potato starches, glycerol diglycidyl ether (GDE), Luria-Bertani (LB) broth, LB agar, and phosphate buffered saline (PBS, pH 7.4)

were all purchased from Sigma-Aldrich. Poly(hexamethylene guanidine hydrochloride) (PHGH) was synthesized according to the procedures described in previous work (Guan *et al.* 2008a).

Preparation of PHGH-modified Starch

To create branched antimicrobial polymers, PHGH was grafted onto starch according to Guan *et al.* (2008a). Initially, 2.4 g of GDE was added dropwise within 20 min to 200 mL of starch solution (10%, aqueous), while the pH was adjusted to 10 using 1 N NaOH. After 30 min of reaction, 8 g of PHGH was added to the solution. The reaction continued prior to neutralizing with HCl (Guan *et al.* 2008a). The grafting percentage of PHGH onto the starch was determined using a thermo-gravimetric analyzer (TGA) SDT Q600 (TA instruments, New Castle, DE, United States).

Preparation of Coated Papers Using PHGH-modified Starch

Coatings (55% solid) were applied onto the paper's surface using a wire rod drawdown coater (K303 Multicoater, RK print coater Instrument Ltd). Two different types of paper were used in this test: regular printing paper and non-sized laboratory handsheets made from bleached sulfite pulps. Two different coating layers were applied on these papers. The first layer of coating was clay mixed with latex (clay 100 g/latex 20 g) using different latex samples. PHGH-modified starches with different grafting ratios were applied as the second layer on top of the first layer. The thickness was approximately 4 μm , and the speed of coating was adjusted at 10 m/min in all cases. All samples were dried at 105 °C after the applying each layer (Ghannam 2002).

Antibacterial Tests

The antibacterial activities were tested against Gram-negative *Escherichia coli* (*E. coli*, ATCC 11229) bacteria based on adopting the American type culture collection (ATCC-100) method (Ziaee *et al.* 2010).

1-The minimum inhibition concentration (MIC) was used to determine the minimum concentration of modified starch required to deactivate bacteria in solutions. The MIC was interpreted as the lowest concentration that could inhibit the visible growth of bacteria compared with that of the control samples.

2-The shaking flask method was used for evaluating the antimicrobial activities of the handsheets made from bleached fibers treated with the antimicrobial polymer. The method is often used for evaluating the antimicrobial activity of textile products with non-releasing reagents. In this method, paper scraps and bacterial culture (10⁶ CFU/mL) were mixed and shaken at 200 rpm at 37 °C for 1 h; afterwards, this culture was seeded on an agar plate. The number of colonies was counted and three replicates were made for each sample. The inhibition of cell growth was quantified based on,

$$\text{Growth Inhibition of Bacterium (\%)} = (A - B)/A \times 100 \quad (1)$$

where *A* and *B* are the number of colonies detected in the control and treated samples, respectively (Guan *et al.* 2007).

Antimold Activities

The resistance of handsheets against fungal growth was determined by series of antifungal tests using *Chaetomium globosum* (*C. globosum*) according to TAPPI T 487.

Random test specimens were prepared by cutting 50-mm² sections from the sterilized handsheets. Each specimen was inoculated with *C. globosum* and incubated for 2 weeks at 28 °C. The resistance was determined by visual examination and the results were reported in the form of percentage of deactivated fungus,

$$\text{Growth Inhibition of Fungus (\%)} = (C - D)/C \times 100 \quad (2)$$

where *C* and *D* are the area of the fungus detected from the control and treated samples, respectively (Pinzari *et al.* 2006).

Atomic Force Microscope Analysis

To investigate the effect of PHGH-modified starch on *E. coli* growth, an atomic force microscope (AFM) using a Nanoscope IIIa (Veeco Instruments) was employed. Initially, fresh *E. coli* from the LB broth were separated from the supernatant by centrifuging the bacterial suspension (108 CFU/mL) at 5000 rpm for 1 min. Then, the bacteria were twice washed with the PBS buffer solution and then re-dispersed in distilled water. The bacterium prepared by this procedure was denoted as fresh *E. coli*. Treated *E. coli* was prepared by mixing the fresh *E. coli* with PHGH-modified starch solution at the concentration just above the MIC, as stated above. The mixture was shaken for 30 sec. Fresh and treated *E. coli* solutions were deposited onto a silicon wafer (University Wafer, Boston, MA, United States) and air-dried. AFM images were obtained in tapping mode using a silica probe (NP-S20, Veeco Instruments) with settings of 512 pixels/line and a 1-Hz scan rate (Ziaee *et al.* 2010). The AFM sample preparation procedure most probably led to killing *E. coli* cells as cells were dried after treating with distilled deionized water, implying that the AFM analysis was conducted on dead fresh and treated *E. coli* cells in this work.

Scanning Electron Microscope Analysis

Images of unmodified and modified *C. globosum* with PHGH-modified starch prepared for the antifungal test were taken using a scanning electron microscope, SEM (JEOL, JSM-6400, Japan). Initially, unmodified and modified *C. globosum* were fixed in 6% glutaraldehyde for 2 h prior to washing with sodium cacodylate buffer. Then, the samples were fixed in 1% OsO₄ for 1 h, washed again with the same buffer solution, dehydrated in ethanol, dried, and coated with gold (Ziaee *et al.* 2010).

RESULTS AND DISCUSSION

The results from previous work by the authors showed that the grafting percentages of PHGH for different starches ranged from 12 to 20 wt.% (Ziaee *et al.* 2010). The first layer (*i.e.*, clay/latex mixture) had an average basis weight of 5.9 g/m², whereas the second layer (PHGH-modified starch) varied from 1.9 g/m² to 2.8 g/m². Because different starches had different grafting levels, the amount of PHGH varied from 2 to 20 wt.% on papers, which applied different types of PHGH-modified starches.

Effect of PHGH-modified Starch on the Morphology of *E. coli*

Figure 1 shows AFM images of untreated and treated *E. coli* with PHGH-modified starch at the minimum inhibition concentration (MIC). Figures 1A, 1B, and 1C

show the section images, topography, and height, respectively. For fresh *E. coli*, the surface membrane of *E. coli* was observed, and there were no indentations and grooves on the cell's surface. Also, there were no residues leaked around the *E. coli* structure in the images. In the height and section images, *E. coli* cells appeared elliptical with high middle and low end shape with height differences of around 200 nm. After treating with the PHGH-modified starch, the *E. coli* cell was collapsed, and the cell membrane was completely destroyed, as evidenced in Fig. 1. The significant losses of intracellular components from the bacterial cells caused the membrane to collapse, leading to a reduction in height to 100 nm. This was consistent with the previous findings (Guan *et al.* 2007, 2008a,b; Ziaee *et al.* 2010). The cell membrane of *E. coli* is composed of proteins and phospholipids. The bacterial membrane is stabilized by sodium and potassium ions and phospholipids in the cell. The PHGH-modified starch replaced the metal ions, bound with acidic phospholipids, and induced phospholipids phase separation, thus damaging the cell membranes (Wu *et al.* 1999; Kawabata and Taylor 2007; Guan *et al.* 2008a).

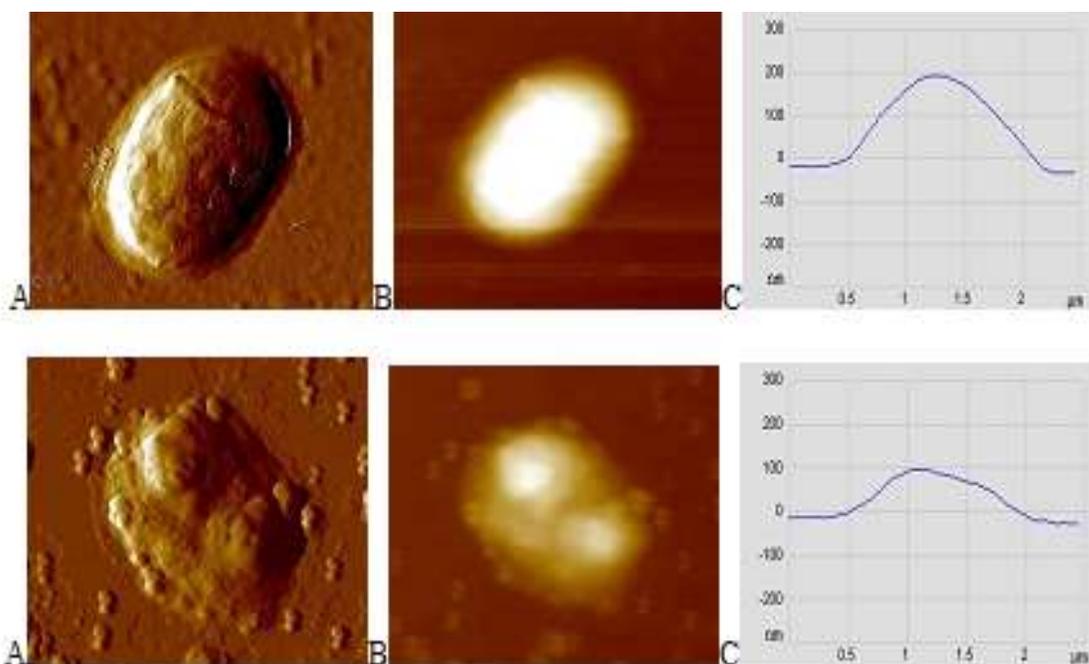


Fig. 1. Morphology of *E. coli* untreated (upper image) and treated (lower image) with cationic starch (Ziaee *et al.* 2010)

Antimicrobial Performance of Coated Papers

The previous study showed that the MICs of the unmodified starch were more than 2000 ppm. In contrast, the MIC should be as low as 32 ppm for the PHGH-modified starch (Ziaee *et al.* 2010). Such results suggested that the PHGH modification of starch significantly increased its antimicrobial performance (Ziaee *et al.* 2010).

Figure 2 shows the growth inhibition of bacteria (%), determined by the shaking flask method, *versus* the amount of PHGH for the coated papers using different latex types. As can be seen, applying about 1 g/m² PHGH resulted in complete growth inhibition (*i.e.*, 100%). At all dosages, the growth inhibition of the papers coated with latex 9750 and modified starch appeared to be higher than that of latex 8879 and modified starch. It is inferred from these results that the overall performance of latex

9750 and modified starch was better than that of latex 8879 and modified starch. This may show that the overall charge density of latex 9750/modified starch was more cationic than that of latex 8879/modified starch. Therefore, it may be concluded that the charge density of latex 9750 was more neutral than that of latex 8879 (*i.e.* latex 8879 had a more anionic charge density).

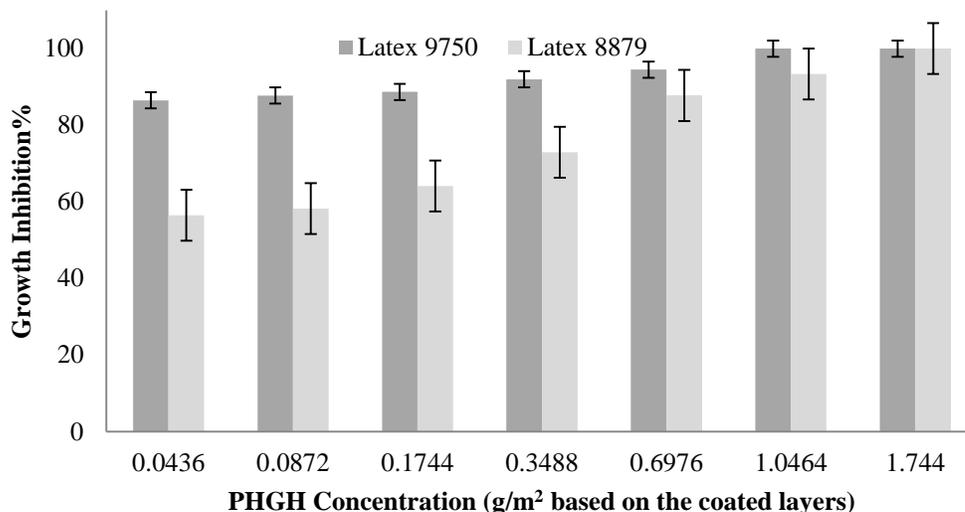


Fig. 2. Growth inhibition of *E. coli* vs. the dosage of PHGH for the different latex samples

Figure 3 shows the effect of latex/clay on the antimicrobial activity. The shaking flask method was performed using different samples containing latex/clay coating layers and the single antimicrobial layer. The growth inhibition was lower for coated papers with the latex/clay layer in comparison with papers with a single antimicrobial layer. The small reduction of growth inhibition (5 to 10%) can be explained by the covering effect of the starches by latex coating. The interaction between the starch and the mixture of clay and latex reduced the amount of starch exposed to the surface, which decreased the growth inhibition. However, the benefit of using the latex/clay layer lies in the cationic nature of the modified starch. At a higher dosage or higher amount of PHGH grafting, the antimicrobial starch tended to agglomerate *via* electrostatic association due to its cationic nature (Guan *et al.* 2008a,b). Such a problem could be eliminated by using cationic latex (*e.g.*, cationic polybutylacrylate latex).

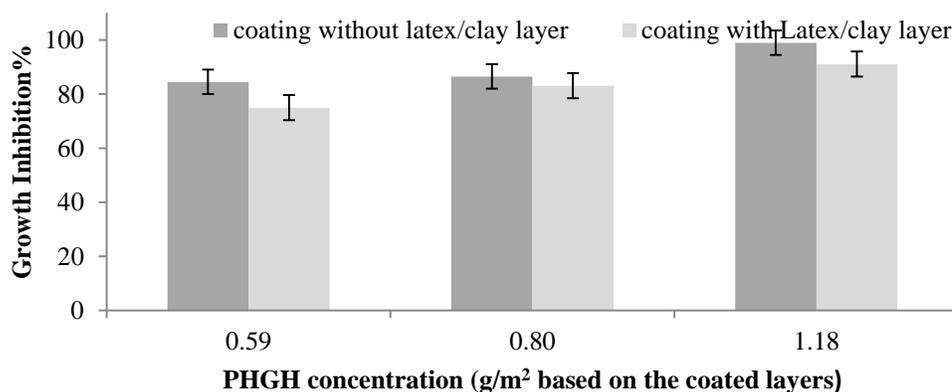


Fig. 3. Growth inhibition of *E. coli* vs. the dosage of PHGH for different coating layers

Two types of coated papers were prepared to study the effect of sizing agents on the antimicrobial activity of the modified starch. The regular printing paper was chosen because of its sizing agent content. It can be seen from Fig. 4 that the antimicrobial activities of both papers were close and that the sizing agents did not have any effect on the antimicrobial behavior. Overall, the excellent antimicrobial performance of the coated paper was achieved by the addition of PHGH-modified starch at a very low dosage (2 g of starch containing 12 wt.% of PHGH per m²). PHGH-modified starch is thus a very promising polymer for inducing antimicrobial properties on paper.

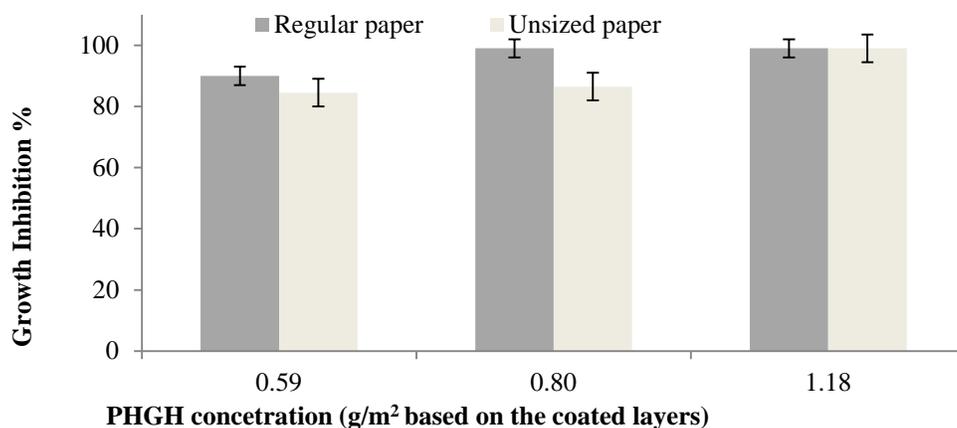


Fig. 4. Growth inhibition of *E. coli* vs. the dosage of PHGH for different sizing agents

Effect of PHGH-modified Starch on the Morphology of *C. globosum*

Figure 5 shows the morphologies of untreated and treated fungi with the PHGH-modified starch observed under SEM. Clearly, the entire body of *C. globosum* can be observed without any further distortion for the untreated sample, while only the spores of *C. globosum* can be found on the surface of the treated sample. Therefore, it is inferred that the PHGH-modified starch was effective at inhibiting the growth of *C. globosum* (Pinzari *et al.* 2006; Ziaee *et al.* 2010).

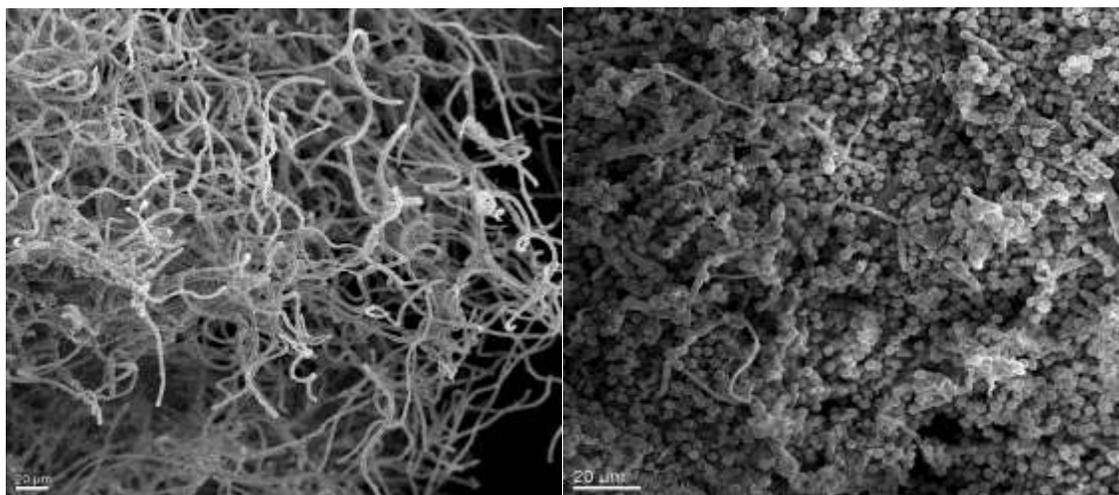


Fig. 5. Morphology of *C. globosum*, untreated (left) and treated (right) with PHGH-modified starch (25 mg/g fiber) (Ziaee *et al.* 2010)

Antimold Performance of the Coated Papers

Figure 6 shows the percentage of inhibition of fungal growth *versus* the PHGH concentration (g/m^2) based on the coated layers. Clearly, increasing the PHGH concentration led to an increase in antifungal efficiency. Using 1 g/m^2 PHGH (based on the weight of coated layers), the antifungal performance was almost complete (around 90%). The inhibition of fungal growth was less for the coated papers using latex 8879 than latex 9750, which corresponded with the antimicrobial performance discussed earlier with *E. coli*.

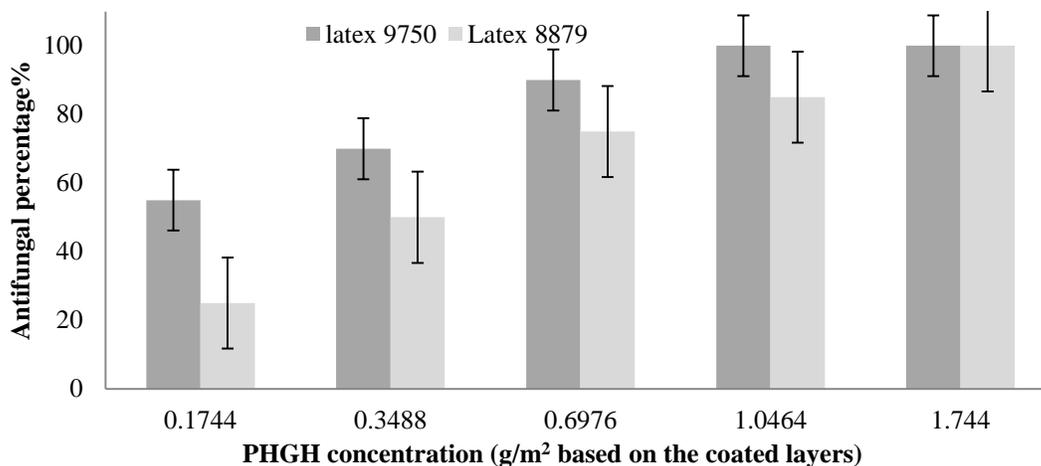


Fig. 6. Antifungal properties of coated papers vs. the dosage of PHGH for different latex samples

CONCLUSIONS

1. Applying modified starch on top of a conventional coated layer is effective at rendering the surface of papers with antimicrobial and antifungal activity.
2. By applying about 1 g/m^2 of PHGH, the growth of *E. coli* and *C. globosum* were nearly inhibited (*i.e.*, 100%).
3. The AFM images demonstrated that the cell membrane of *E. coli* bacterium collapsed upon exposure to the PHGH-modified starch at a concentration higher than the MIC (15.6 ppm). The SEM images also showed that the polymer inhibited the growth of *C. globosum*. Therefore, the PHGH-modified starch rendered the coated papers with antimicrobial and antifungal activity under the specific laboratory conditions.

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