

Multicomponent Antimicrobial Transparent Polymer Coatings

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ABSTRACT: Transparent poly vinyl alcohol (PVA) based coatings with antimicrobial activity against a wide range of microorganisms have been prepared. The films have been prepared by spincoating a mixture of PVA, ammonium dichromate and metal (zinc or silver) precursor from water solutions, and then rendered insoluble by crosslinking under UV illumination. Antimicrobial activity has been tested against different bacteria and fungi, such as *Bacillus atrophaeus*, *Bacillus megaterium*, *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Escherichia*

coli, *Neurospora crassa*, and *Saccharomyces cerevisiae*. It was found that combining the zinc and silver precursors resulted in films which had excellent antimicrobial activity and at the same time exhibited superior environmental stability compared to films containing prepared with single metal precursor. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 122: 1572–1578, 2011

Key words: biological applications of polymers; nanocomposites; thin films

INTRODUCTION

In recent years, there has been increasing interest in antimicrobial coatings for prevention of the spread of infection^{1–3} and water disinfection.³ Many different materials can be used for this purpose, such as silver and silver-containing surfaces, copper and copper alloy surfaces, and various metal oxides exhibiting photocatalytic effects.¹ While detailed mechanisms of antimicrobial activity for some of these materials are still not completely understood and multiple mechanisms have been proposed in the literature, there is general consensus that nanomaterials can either directly interact with cells or produce secondary products, such as reactive oxygen species (ROS) or metal ions, which damage the cells.² Different antimicrobial additives to surface coatings can have different mechanisms of action. In case of silver containing surfaces, antimicrobial activity can occur

due to diffusion of Ag⁺ ions.^{1,2} On the other hand, antimicrobial action of metal oxides mainly occurs due to production of reactive oxygen species, usually under illumination with light with energy higher than the material bandgap,^{1,2} and for some metal oxide materials, such as ZnO, release of Zn²⁺ ions may also contribute to the antimicrobial activity.²

Among different types of coatings exhibiting antimicrobial activity, paints³ and polymer films and fibers^{4–14} are of considerable interest due to simplicity and low cost of the coating preparation. A significant advantage of polymer-based coating containing metals over inorganic metal-containing coatings, is that they can be prepared by solution-based processes, in ambient (as opposed to vacuum deposition commonly used for inorganic materials) and at a low temperature. In these coatings, polymer serves as a binder to ensure uniform and stable surface coverage with active antimicrobial materials included in a polymer matrix. Antimicrobial polymers based on different polymer matrices and antimicrobial material inclusions have been reported.^{4–15} Silver^{4,5,7,8,10,13,14} and metal oxides such as ZnO^{6,11,12} and TiO₂¹⁴ represent common antimicrobial material additives to the polymer. In addition to a variety of antimicrobial materials available there are also a variety of possible polymers that could be used. Among those, poly (vinyl alcohol) (PVA)^{5,7,12–15} is of particular interest, since it is highly soluble in water,

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biologically friendly, easy to process, and optically transparent.⁵ It has been shown that Ag nanoparticle containing PVA films could be prepared by spin-coating PVA + silver nitrate solutions, followed by heating.⁵ However, this procedure resulted in increased absorbance in the visible spectral range with increasing annealing temperature.⁵ While it was shown that Ag nanoparticles could also spontaneously form by refluxing PVA + AgNO₃ solutions at 80°C, depending on refluxing time and/or temperature, increase in visible absorption can also be observed.¹⁵ Other methods to produce antibacterial nanoparticles in a different polymer matrix are also possible, for example photoinduced reduction of metal salts and polymerization of difunctional monomers.⁸

To maintain high transparency of the coating and at the same time obtain good environmental stability, methods different from heating the PVA are of interest. Therefore, here we investigated the addition of a crosslinking agent, ammonium dichromate,¹⁶ to obtain insoluble PVA antibacterial coatings after UV curing. Similar procedure has been used previously for preparing PVA gate dielectric film in thin film transistors.¹⁶ However, the use of crosslinking agents to prepare antibacterial coatings with different additives has not been reported. The use of a crosslinking agent enables us to prepare coatings with excellent optical properties which are insoluble in water without heating the substrates. We have investigated different additives to achieve antibacterial properties of cross-linked PVA films. Antibacterial activity of the films with the addition of AgNO₃, zinc acetate and both precursors together has been investigated for a wide range of microorganisms. Our objective is to develop a coating which is effective against a wide range of organisms, rather than optimize the coating composition to obtain optimal results for individual organisms (since minimum inhibiting concentrations of active ingredients are naturally different for different organisms). Due to differences in mechanism of antimicrobial action for different additives (silver, metal oxides, chitosan, etc.), investigation of multicomponent coatings^{14,17,18} is a promising method to improve their antimicrobial properties. It has been demonstrated that addition of Ag improves antibacterial properties of TiO₂ under UV illumination,¹⁸ while in the absence of UV illumination addition of AgNO₃ resulted in significantly higher antibacterial activity compared to TiO₂.¹⁴ Since ZnO can exhibit antibacterial activity under ambient light, unlike TiO₂ which requires UV illumination,² here we investigated addition of zinc acetate (alone or in combination with AgNO₃). It was found that the two component coatings exhibited improved properties compared to single component coatings. The coatings exhibited good stability and activity

against a wide range of microorganisms at the same time. The coatings are based on two commonly used metal compounds (silver nitrate and zinc acetate), with PVA as a polymer matrix, which has good chemical resistance, and is biodegradable and biocompatible.¹¹ However, PVA is also water soluble, which can be detrimental in terms of environmental stability of the coatings. This problem was solved by adding a crosslinking agent and UV curing, instead of the usual thermal heating method. Since the coatings are prepared by a simple, low-temperature, low-cost method they can be of interest for disposable protective coverings on surfaces to minimize the spread of infectious diseases.

EXPERIMENTAL

Sample preparation

Glass substrates (2.5 × 2.5 cm²) have been cleaned by sonication in acetone, ethanol, and deionized water. The solutions for spincoating were prepared by dissolving 50 mg of PVA (Aldrich, 87–89% hydrolyzed, $M_w = 13,000$ – $23,000$) in 1 mL of deionized water. The solution was sonicated for 15 min. and then 14.3 mg of ammonium dichromate (Aldrich, 99.999%) in 1 mL of deionized water was added and solution was sonicated for another 15 min. Control sample (cross-linked PVA film on glass substrate) is labeled as PVA. For films containing single type metal ions, 387 mg (labeled as a) or 516.1 mg (labeled as b) of precursor for each metal (silver nitrate, Aldrich, 99.8% or zinc acetate, Aldrich, 99.99%) was added followed by another 15 min of sonication. Since for both compositions similar behavior was observed but more significant brown discoloration was seen for higher Ag concentration, 387 mg of each precursor was used for two component films. Then the films were spin-coated at 1000 rpm for 1 min and crosslinked in a UV crosslinker using energy of 360 mJ/cm² (wavelength 254 nm). The thickness of the films was determined to be ~ 100 nm using a Dektak step profiler. Label I denotes films prepared with zinc acetate only, label II films with silver nitrate only, and label III films with both precursors. The morphology and structure of prepared coatings were examined by transmission electron microscopy (TEM) and selected area electron diffraction (SAED) using a Phillips Tecnai G2 20 STEM and Jeol JEM 2010F TEM. Samples for TEM were prepared by spincoating Poly(methyl methacrylate) (Aldrich, $M_w = 120,000$) in chloroform (1000 rpm for 1 min) on a clean glass substrate, followed by drying with nitrogen gas. Then the PVA-based films were spin-coated, dried, and then lifted off from the substrate and transferred to a TEM grid in chloroform. Crosslinking was

performed after the transfer onto the TEM grid. Thermogravimetric analysis (TGA) was performed in nitrogen atmosphere using a TA instruments Q5000 thermogravimetric analyzer with a heating rate of 10°C/min. Samples for Fourier transform infrared (FTIR) experiment were prepared by mixing the polymer with infrared grade KBr (99+%, International Laboratory, USA) and pressing into pellets. The spectra were collected by a Shimadzu FTIR-8300 Fourier transform infrared spectrophotometer. X-ray diffraction (XRD) measurements were performed at a glancing angle using a Bruker AXS SMART CCD diffractometer. Transmission of the polymer coating was studied using a Cary 50 Bio UV-Vis Spectrophotometer. Environmental stability of the coatings was tested by immersing the samples into distilled water for 24 h and then testing the concentration of Zn and Ag using inductively coupled plasma optical emission spectrometer (ICP-OES, Spectro Ciros). To test the stability under different pH, ammonia (to achieve pH = 8) or acetic acid (to achieve pH = 5.6) were added to distilled water before immersing the samples.

Tests of antibacterial activity

For antibacterial testing, the following organisms were used: gram-negative bacteria (*Escherichia coli* XL1-Blue, *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* ATCC 14028, *Pseudomonas aureginosa* ATCC 13388), gram-positive bacteria (*Bacillus atrophaeus* ATCC 9372, *Bacillus megaterium* ATCC 13402, *Staphylococcus aureus* subsp. *aureus* ATCC 11632, *Enterococcus faecium* ATCC 19434), and fungi (*Neurospora crassa*, *Saccharomyces cerevisiae*). Culture broth (Luria-Bertani broth for *E. coli*, *Salmonella*, *Pseudomonas*, *Bacillus*, *Staphylococcus*; Brain Heart Infusion broth for *Enterococcus*; or Yeast malt broth for *Saccharomyces*, water for *Neurospora crassa*) was used as culturing nutrient source for culturing bacteria, while culture agar (Luria-Bertani agar, Brain Heart Infusion agar, Yeast malt agar or *Neurospora* culture agar) was used for testing the antibacterial activity of the films. The conditions for culturing microorganisms were: *E. coli*, *Salmonella*, *Pseudomonas*, *Staphylococcus* 24 h, 37°C; *B. atrophaeus*, *B. megaterium* 24 h, 30°C; *Saccharomyces cerevisiae* 48 h, 30°C; *Neurospora* 24 h, 24°C. Repeated tests of antibacterial activity were performed. About 200 μL or 800 μL of bacteria (5×10^6 CFU/mL or 1.25×10^6 CFU/mL) was placed onto the coated plates and incubated for 4 h. Culture agar was then added on the top of the bacteria, or the bacteria were extracted and serial dilution was performed and 20 μL of the diluted bacteria were evenly spread onto a culture agar in triplicate to ensure reproducibility of the results. The samples were then kept at 37°C (for *E. coli*, *Salmonella*, *Pseu-*

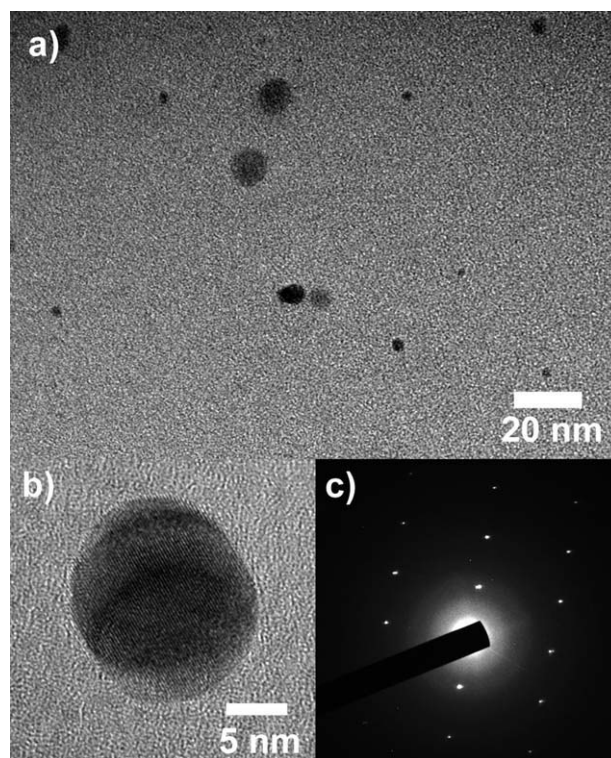


Figure 1 (a,b) TEM and high resolution TEM images of coating III; (c) representative SAED pattern.

domonas, *Staphylococcus*, and *Enterococcus*) or 30°C (for *B. atrophaeus*, *B. megaterium*, and *Saccharomyces*) or 24°C (for *Neurospora*) overnight, and then the formation of colonies was determined by observation under an optical microscope. No UV illumination of the samples was performed.

RESULTS AND DISCUSSION

For all film compositions, transparent films with transmittance exceeding 75% across entire visible range were obtained. Since it was proposed that PVA can serve as a reducing agent^{5,15} and a stabilizer for formation of Ag nanoparticles,¹⁵ the structure of the films was studied using TEM and SAED. Representative TEM images at different magnification levels and the SAED pattern of an individual nanoparticle for the PVA film with Zn and Ag precursors are shown in Figure 1. Nanoparticles in the film can be clearly observed. From the TEM images, it can be determined that the nanoparticle size is in the range 10–20 nm. From SAED and high resolution TEM, the nanoparticles can be identified as Ag metal. The d spacing values identified from SAED images are 2.39 and 1.46 Å, which are close to the expected values for (111) and (220) planes in Ag. No zinc or zinc oxide particles have been observed, indicating that Zn is likely distributed throughout

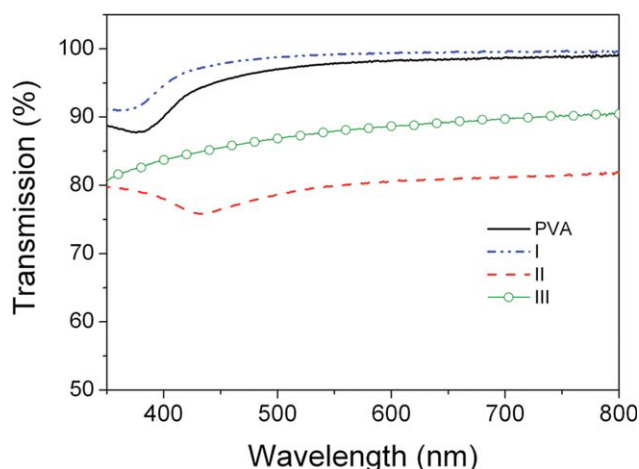


Figure 2 Transmittance spectra of different coatings. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the coating, while silver precursor was reduced and Ag nanoparticles were formed, in agreement with other works on PVA + AgNO₃ materials.^{5,15}

To study further the properties of the obtained coatings, transmittance measurements, FTIR, XRD, and TGA have been performed and the obtained results are shown in Figures 2–4. From the transmittance spectra shown in Figure 2, we can observe that the addition of zinc acetate does not result in any reduction of the transmission. The lowest transmission is obtained for coatings II which contain silver only. The dip in the transmission in the violet–blue spectral region for coating II likely corresponds to the absorption of Ag nanoparticles.^{5,13} It should be noted that in coating III no such dip is observed in spite of the presence of the nanoparticles in the TEM.

XRD patterns of different coatings are shown in Figure 3(a). It can be observed that no characteristic peaks of Ag can be resolved in the XRD spectra, which is likely due to the low thickness of the coatings, combined with small size and low density of the nanoparticles observed in the TEM. The lack of observation of Ag peaks in PVA/Ag samples is in agreement with other literature reports,¹³ although in some cases observation of weak peaks corresponding to metallic Ag has been reported.¹⁹ In coating II, small peaks close to 30° can be observed, which likely correspond to silver nitrate precursor (JCPDS file 74-0947). However, in coatings I and III we can observe a peak at low angles. The peak position is different for the coatings I and III, and the peak is more broad for coating I. No peaks were observed for the pure PVA indicating amorphous structure, even though PVA can crystallize,^{19,20} resulting in several sharp peaks below 40°, the lowest being at ~ 11° for orthorhombic cell.²⁰ It should

be noted that these peaks were observed in zinc acetate containing samples, and zinc acetate (tetragonal) could also result in a peak at ~ 11° (JCPDS file 21-1467), although for zinc acetate peak positions (without identifying structure or unit cell) have also been reported at 5.4 and 8.9° (JCPDS file 01-0089). Since the peaks observed in our samples do not correspond to any of the individual components reported in the literature, we can conclude that likely interaction between PVA and metal precursor results in a different molecular arrangement (for example, poly(vinyl alcohol) iodine exhibits the first diffraction peak at 8.8°, lower than PVA according to JCPDS file 46-1866).

FTIR spectra of different coatings are shown in Figure 3(b). In all the spectra, we can observe various O–H, C–O and CH₂ modes characteristic for PVA.^{21,22} With the addition of different additives, changes in intensity and shifts of the PVA modes can be observed. In addition, in samples containing

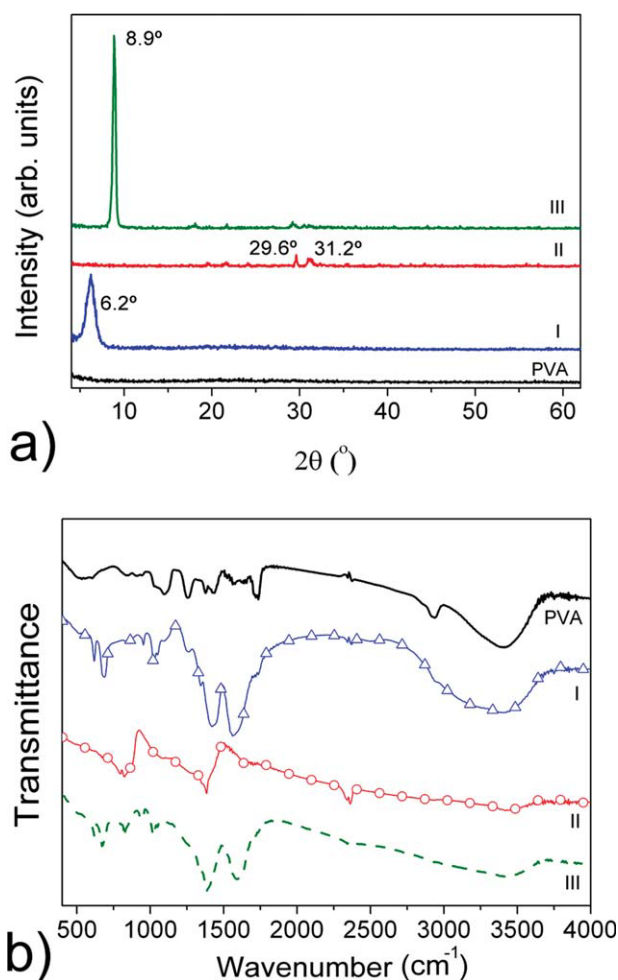


Figure 3 (a) XRD and (b) FTIR spectra of different coatings. The spectra have been shifted to improve clarity. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

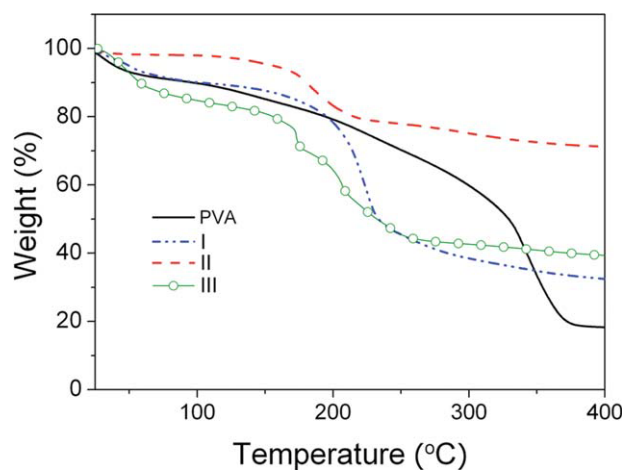


Figure 4 Thermogravimetric analysis thermograms for different coatings. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

zinc precursor (coatings I and III), there are two additional peaks between 600 and 700 cm^{-1} , and the peak at $\sim 610\text{ cm}^{-1}$ indicates Zn—O bending.²¹ The reduction in the intensity of some of the modes with the addition of Ag (coating II and to a smaller degree coating III) likely indicates the interaction between Ag nanoparticles and PVA matrix.²³ In terms of thermal stability of the coatings I and III exhibit similar behavior, while coating II exhibits significantly different behavior and improved stability compared to pure PVA, as shown in Figure 4.

Antimicrobial activity has been tested on both gram-positive and gram-negative bacteria, since they can have different response to antibacterial agents due to differences in their cell wall structure.¹² Obtained results are shown in Table I for the average number of colonies for different organisms (gram-positive bacteria, gram-negative bacteria, and fungi). Obtained results for *N. crassa* (NC), which does not form countable colonies, are shown in Figure 5. All the coatings except pure PVA result in no growth of NC. The labels in Table I are: BA denotes *B. atropheus*, BM denotes *B. megaterium*, SA denotes *Staphylococcus*, E denotes *Enterococcus*, PA denotes *Pseudomonas*, S denotes *Salmonella*, EC denotes *E.coli*, and Y denotes *Saccharomyces*.

Antimicrobial activity against at least some of the organisms tested was observed for Ag and Zn con-

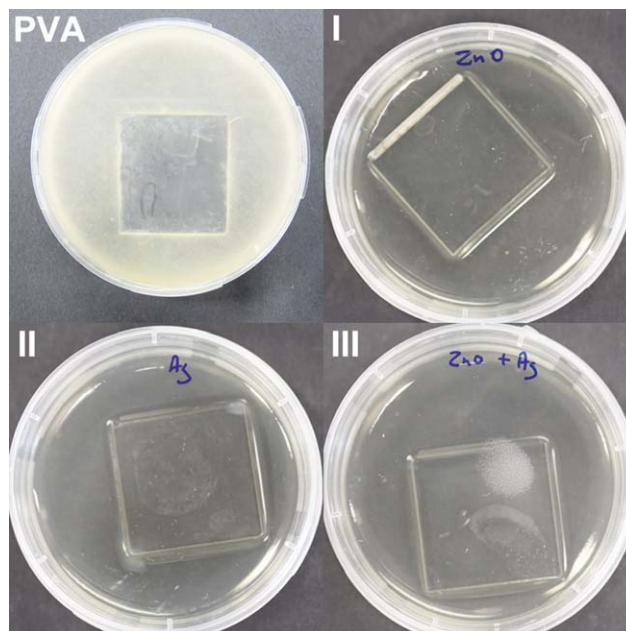


Figure 5 Representative photographs of antifungal activity of different coatings against *N. crassa*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

taining films, in agreement with literature reports,^{4–7} while no antimicrobial activity was observed for PVA film prepared without any metal-containing additives, also in agreement with the literature reports.^{5,12,13} It can be clearly observed that Ag containing films exhibited better antimicrobial activity compared to Zn containing films. The coatings containing both metal precursors exhibited similar activity to coatings containing Ag only. However, coatings containing silver only often exhibited brown discoloration in the Petri dish. Brown discoloration around Ag containing samples in antibacterial testing has been observed in other studies as well.⁴ The discoloration is obvious after 6 h placement of LB broth onto the substrate, as shown in Figure 6, while no obvious discoloration can be observed in samples prepared from solutions containing both zinc acetate and silver nitrate. To further examine environmental stability of the coatings, they were immersed in distilled water and concentration of Ag and Zn ions was determined, and the obtained results are also summarized in Table II. It can be observed that the

TABLE I
Summary of Antimicrobial Testing (Average Number of Colonies in Three Replicates) for Different Coatings

	BA	BM	SA	E	PA	S	EC	Y
PVA (PVA + ammonium dichromate)	186	113	100	140	147	118	293	290
I (PVA + ammonium dichromate + zinc acetate)	0	0	141	136	97	77	20	7
II (PVA + ammonium dichromate + silver nitrate)	0	0	0	0	0	59	0	0
III (PVA + ammonium dichromate + zinc acetate + silver nitrate)	0	0	12	0	0	0	0	0

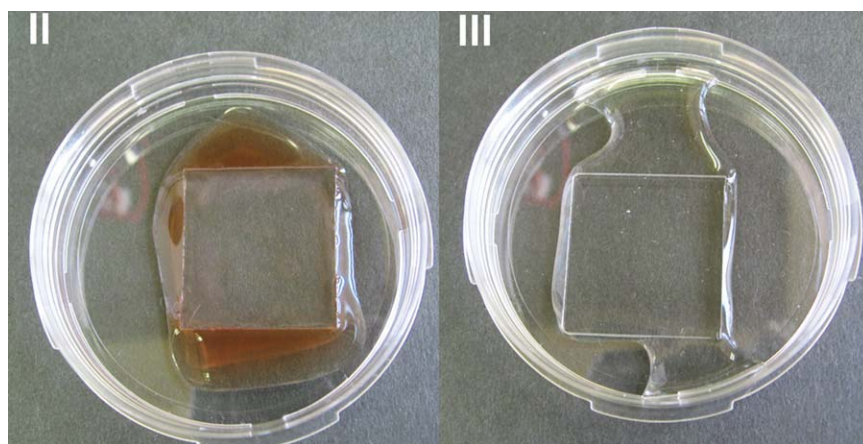


Figure 6 Comparison of stability of coatings II and III after placement of LB broth onto the samples for 6 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

release of Zn and Ag is significantly lower for the two component films compared to the single component films, which would make multicomponent coatings promising for coatings with improved stability and longer lasting antimicrobial activity. Compared to single component coatings, two component coating is more stable not only in neutral pH, but also in acidic and alkaline environments. However, practical application would likely require further optimization of the coating composition to find a compromise between the rate of metal ions release and antibacterial activity.

CONCLUSION

Multicomponent antimicrobial transparent coatings have been prepared using a simple procedure (spin-coating followed by crosslinking under UV illumination). Antimicrobial activity has been examined for nine different microorganisms, namely *B. atrophaeus*, *B. megaterium*, *S. aureus*, *E. faecium*, *P. aeruginosa*, *S. enterica*, *E. coli*, *N. crassa*, and *S. cerevisiae*. In terms of antibacterial activity, the coatings containing zinc only did not exhibit activity against as wide range of

microorganisms as Ag containing coatings. The coatings containing Ag only exhibited excellent antibacterial activity against all organisms investigated (although for *S. enterica* the reduction in the number of colonies rather than complete absence was observed), but they exhibited lower transmission and significant leeching out of Ag. The coatings containing both Zn and Ag precursors exhibited higher transmission compared to Ag-only coatings, and still retained excellent antibacterial activity against wide range of organisms. In addition, the coatings containing two components (Ag and Zn) exhibited improved stability and lower release of the metal ions.

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TABLE II

Summary of Metal Release Results (Metal Concentration in $\mu\text{g/L}$ After 24 h Immersion) for Different Coatings

	PVA	I	II	III
Ag, pH = 5.6	<50	<50	4230	450
Ag, pH = 7	<50	<50	4510	630
Ag, pH = 8	<50	<50	3640	870
Zn, pH = 5.6	<50	950	<50	340
Zn, pH = 7	<50	2330	<50	780
Zn, pH = 8	<50	370	<50	160

The detection limit of the instrument was 50 $\mu\text{g/L}$.

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