Effects of Incorporating Technique and Silver Colloid Content on Antibacterial Performance for Thermoplastic Films

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ABSTRACT: Antibacterial efficacies of various thermoplastics, such as medium-density polyethylene (MDPE), polystyrene (PS), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) containing nano-silver colloids were studied under a wide range of testing conditions. The effects of nano-silver colloid content and the silver-polymer contact time were of our main interests and quantitatively assessed by shake flask method coupled with a plate-count-agar (PCA) technique using *Escherichia coli* as testing bacteria. Two different methods were used for incorporating the nano-silver colloids into the thermoplastics, these being spray-coating and melt-blending techniques. The experimental results suggested that all neat thermoplastics alone could not generally inhibit the *E. coli* growth, suggesting that all thermoplastics exhibited non-bactericidal behavior. However, neat PVC appeared to show a retarding effect for the *E. coli* growth. In addition, coating silver colloid onto all types of thermoplastic substrates could inhibit the *E. coli* growth up to 99.9% at the optimum silver content of 50 ppm for PS, PET and PVC and of 75 ppm for MDPE. The optimum contact time for all thermoplastics was 150 min. Among the thermoplastics used, PVC exhibited the highest % *E. coli* reduction, and this was confirmed by the higher silver content via Atomic Absorption Spectrometry (AAS) technique. For a given silver content, the spray-coating technique could give better dispersion level of silver throughout the thermoplastic films and this led to more effective antibacterial performance as compared with the dry-blending technique. In PVC sample, the contact angle value appeared to increase with the addition of silver content for both incorporating techniques. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 3456–3465, 2011

Key words: thermoplastics; diffusion; blending; films; coatings

INTRODUCTION

In packaging applications, thermoplastics have been widely used and their microbial contaminations are recently of main concern. The application of antimicrobial agents into the polymer products is one of the methods to prevent food from microbial contaminations.[1] Many antibacterial agents including nisin, nano-silver, triclosan, and sorbic acid anhydride have been used in packaging thermoplastics for inhibition of the bacterial growth in various processing techniques.[2] Methods to incorporate the antibacterial agents into the polymer matrices could be done by either blending into the polymer matrices or coating onto the surface of the polymer products. The antibacterial efficacies are dependent on type, concentration, and diffusability of the incorporated bacterial agent through the polymer matrices, as well as the testing methods used for such evaluations.[3,4]

It has been known that heavy metals like silver, copper, zinc, copper, lead, and thallium have antibacterial properties. Silver exhibits good antibacterial properties and has recently been used in a variety of medical applications and materials industries.[3,5–11] The effectiveness of silver as antibacterial agent was dependent on type and form of silver based-antibacterial agents as well as particle size (aggregation) of the silver particles. Radheshkumar et al.[6] and Dowling et al.[7] prepared three different forms of nano-silver colliods namely nano-silver water dispersion system, nano-silver ethanol dispersed system, and nano-silver/sulfur ethanol dispersion system, which were doped on nonwoven polypropylene/polyethylene (PP/PE). The results suggested that the nano-

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silver particles doped PP/PE with silver/sulfur based ethanol dispersing system showed the smallest silver particles on the nonwoven without aggregation of silver nanoparticles and this gave the best antibacterial performance. Pradeep and Jain[3] investigated the antibacterial performances of silver with different species, which included silver nanoparticles, silver ions and silver chloride colloids. They suggested that the silver nanoparticles could mostly inhibit the nitrification process when testing with autotrophic bacteria. However, for antiheterotrophic bacteria (e.g., *Escherichia Coli*) the silver ions showed higher antibacterial performance than the silver chloride colloids and silver nanoparticles.

Another factor that affects the antibacterial performance of silver based polymeric materials is molecular interaction between the silver and the polymeric substrates which can be influenced by the processes at which the silver particles or ions are incorporated (either by blending, embedding, or coating) into/onto the polymeric substrates.[8–11] Kumar et al.[8] found that the releasing ability of silver ions from silver/polymer composites was associated with ability to uptake water into the polymer matrix. The plasticizing effects could be used to describe mechanism of silver ion release. Their results suggested that polyamide showed a relatively good plasticizing effect as the water uptake in polyamide increased the free volume and the molecular mobility and thus increased the silver ion release. Jeong et al.[9] prepared polymeric material specimens doped with silver atoms by magnetron sputtering technique, and the adhesion of silver atoms on various types of polymeric substrates were studied. The results showed that the polymer which had less rigidity gained better adhesion for silver atom coating. The antibacterial performance was found to be dependent on the thickness of silver coating. Jeong et al.[10] studied antibacterial efficiency of silver nanoparticles coated polyurethane foams prepared by soaking silver nanoparticles solution overnight. Polar-polar interaction between nitrogen atoms of polyurethane molecules and silver nanoparticles was confirmed by FTIR technique and these nanosilver particles bounded PU foam had an ability to eliminate the suspended *E. Coli* in water. Choi et al.[11] demonstrated new synthesis method of silver nanoparticles which were embedded into alkyd resins by free-radical-mediated autooxidation process in vegetable drying oils. The silver embeded resins could kill both *E. coli* and *Staphylococcus aureus* bacteria.

Based on existing published works, uses of silver as antibacterial agent are still open for wider discussion, especially by taking the physical properties of polymers and the dispersibility of silver particles into consideration to obtain the most antibacterial efficacy from nano-silver incorporated polymeric substrates. Understanding the relationship between the polymer structures with their structural changes during processing and the silver agent would lead to proper material selection for antimicrobial packaging applications. In this present article, antibacterial performance efficacies of various commercial packaging thermoplastics, such as medium-density polyethylene (MDPE), polystyrene (PS), polyethylene terephthalate (PET) and poly(vinyl chloride) (PVC) containing nano-silver in colloid form were studied under a wide range of testing conditions. The effects of nano-silver content and the silver-polymer contact time were of our main interests and quantitatively assessed by shake flask method coupled with a plate-count-agar (PCA) technique using *E. coli* as testing bacteria. Two different methods were used for incorporating the nano-silver colloids into the thermoplastics, spray-coating and dry-blending methods, and the silver dispersibility and the antibacterial performance were then assessed.

EXPERIMENTAL

Materials

Medium-density polyethylene (MDPE, M380RU/RUP, Thai Polyethylene Co., Ltd., BKK, Thailand), polystyrene (PS, Styron 656D267; Siam Polystyrene Co., Ltd, BKK, Thailand), polyethylene terephthalate (PET; Indorama Polymers Public Co., Ltd. BKK, Thailand), and polyvinyl chloride (PVC, Vinyl Thai Co., Ltd., BKK, Thailand), were used as matrices. Nano-silver colloid (designated as SNSE, supplied by Koventure Co., Ltd, BKK, Thailand) was used as the antibacterial agent. The information of thermoplastics, and nano-silver colloid used are given in Table I. *Escherichia coli* (ATCC 25922) was used as testing bacteria.

Preparation of film specimens

The experimental procedures were started by compression molding a thermoplastic for making a film specimen of 0.1 mm thick. Based on the thermal properties in Table I, the processing conditions used for compression-molding all the thermoplastics were designed differently. In this work, the mold pressure, temperature and time were 150 kg cm^{-2}, 160°C and 5 min, respectively, for MDPE, 150 kg cm^{-2}, 170°C and 5 min, respectively, for PS, 150 kg cm^{-2}, 255°C and 1 min, respectively, for PET, and 180 kg cm^{-2}, 180°C and 2 min, respectively, for PVC.

Incorporating method

In this work, the thermoplastics were incorporated with nano-silver colloid using two different techniques, which were spray-coating and direct blending

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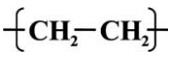
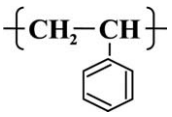
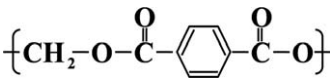
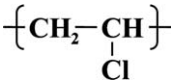
TABLE I
Specifications and Physical and Thermal Properties for Thermoplastics and Silver Colloid

Materials	Supplier/grade	Physical and thermal properties	Chemical structure
Medium density polyethylene (MDPE)	Thai Polyethylene Co., Ltd. (Thailand) / M380RU/RUP	Density 0.940 g·cm^3; hardness = 52; T_m = 126 °C; T_g = −100°C	
Polystyrene (PS)	Siam Polystyrene Co., Ltd. (BKK, Thailand)/ Styron 656D267	Density 1.05 g·cm^3; hardness = 83; T_g = 95°C	
Polyethylene terephthalate (PET)	Indorama Polymers Public Co., Ltd. (BKK, Thailand)/RAMAPET N3	Density 1.42 g·cm^3; hardness = 83; T_g = 75°C; T_m = 250°C	
Polyvinylchloride (PVC)	Vinyl Thai Co., Ltd. (BKK, Thailand)/ SIAMVIC 258RB	Density 1.380 g·cm^3; hardness = 78; T_g = 82°C	
Silver colloid	Koventure Co., Ltd. (BKK, Thailand)/ Nano Silver Ethanol-sol-SNSE 1000	Initial concentration = 1000 ppm; particle size range = 3–5 nm	–

techniques. For the first technique, the thermoplastic substrates (films) were preheated at a temperature of 120°C for 5 min before spray-coated with nano-silver colloids using a constant spraying condition (spraying distance of 10 cm and spraying temperature of 25°C, spraying time of 10 s and volumetric flow rate of silver colloid of 5 mL/min). The spraying area covered by the nano-silver particles was 10 × 10 cm^2 for each required dosage. In the blending technique, the thermoplastic films were prepared by directly blending nano-silver colloid with thermoplastic powder for a determined silver content in a tidy beaker. The mixture was then dried in a vacuum oven at a temperature of 25°C for 1 h, and kept in desiccators for 7 days. The dried mixture was compression-molded to obtain silver blended thermoplastic films with 0.1 mm thick. The prepared films were finally cut into square samples of 2.5 × 5 cm^2 for further antibacterial analysis.

Measurement of antibacterial performance

Shake flask method was suitable for quantitative assessment of bacterial reduction, which follows the test standard of ASTM E-2149 (2001). Thermoplastic film samples of 5 × 5 cm^2 in size were used in this method. Nutrient broth (NB) was used as a growing medium for *E. coli* bacteria and peptone solution (prepared by 1 g/L peptone, pH 6.8–7.2) was chosen as a testing medium. In this work, the bacteria were cultivated in 5 mL of NB at 37°C for 24 h. The film samples and initial suspended bacteria of 10^8 cfu/mL were placed into a 250 mL flask with 50 mL of peptone solution. The flask was shaken on a reciprocal shaker at a speed of 100 rpm at 37°C ± 0.5°C at various contact times (30, 90, 150, and 210 min). Plate count agar (PCA) technique was then used for quantitative assessment of testing specimens. A 10-fold serial dilution technique was also used for ensuring a reliable accounting of the bacteria colonies (usually ranging from 30 to 300 colonies).[4] Hundred microliters of bacterial suspension after the shaking were placed over the agar into sterilized Petri dishes. The inoculated plates were then cultivated at 37°C ± 0.5°C for 24 h before calculating the viable cell count of the testing bacteria and evaluating the antibacterial efficacies using eq. (1).[5,12]

$$\% R = \frac{A - B}{A} \times 100 \quad (1)$$

where R is the reduction of bacteria (%); A is average number of bacterial colonies from thermoplastics without nano-silver colloid (CFU/mL); B is average number of bacterial colonies from thermoplastics incorporated with nano-silver colloid (CFU/mL)

Measurement of contact angle

Contact angle measurement was carried out to quantitatively indicate changes in sensitivity, surface chemistry and roughness, and surface energy of polymers doped with silver colloids. The contact angles of deionized water on polymer surfaces were measured by a drop method using a Contact Angle Goniometer, Model 100-00 from Ramé-hart Instrument Co. (NJ). Wettability property was evaluated comparing between neat polymer with and without Ag colloids. The results were given by microscopic images and the contact angle values were averaged

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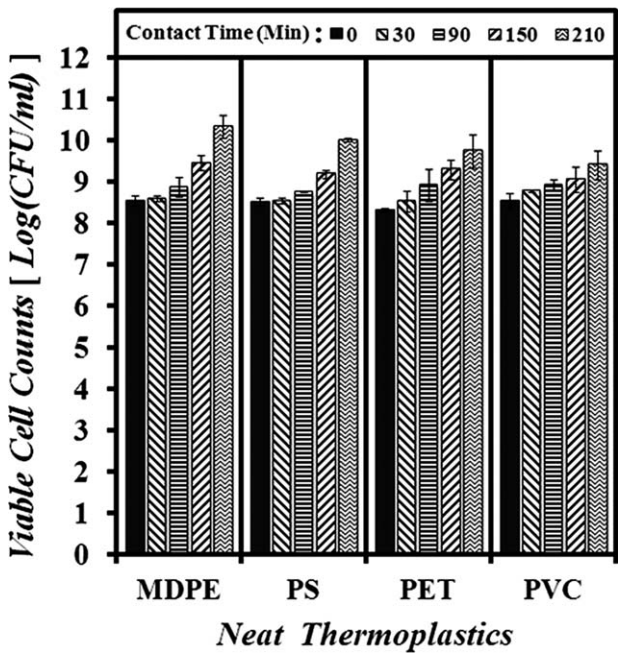

Figure 1 Viable cell count for neat thermoplastic films for various contact times.

for advancing stages of drops for five times/100 μL droplets using three independent samples.

SEM mapping analysis

Silver dispersion level on thermoplastic film surfaces were analyzed using back scattering scanning Electron Microscope using JEOL (JSM-6301F) SEM machine with a gold sputtering device at 15 kV accelerating voltage. The surfaces of thermoplastics films at silver concentration of 100 ppm were examined.

RESULTS AND DISCUSSION

Antibacterial performance and silver morphology in neat thermoplastic films

Figure 1 shows viable colony count for *E. coli* for different thermoplastics under a wide range of contact times without nano-silver colloid. It can be seen that all thermoplastics had similar viable count of *E. coli*, and the *E. coli* growth appeared to increase with increasing contact time. This suggested that all thermoplastics used alone exhibited nonbactericidal

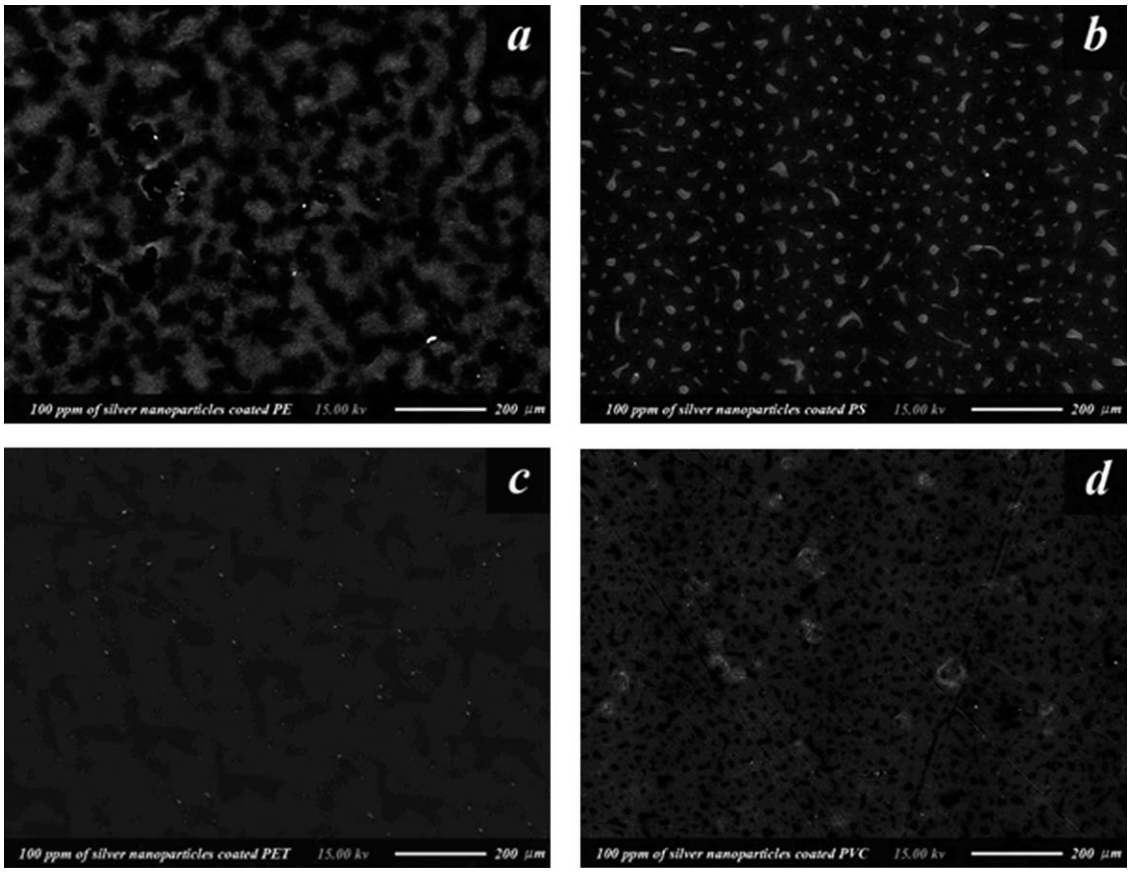

Figure 2 SEM micrographs showing silver dispersion level by silver spray coating technique (a) MDPE, (b) PS, (c) PET, and (d) PVC.

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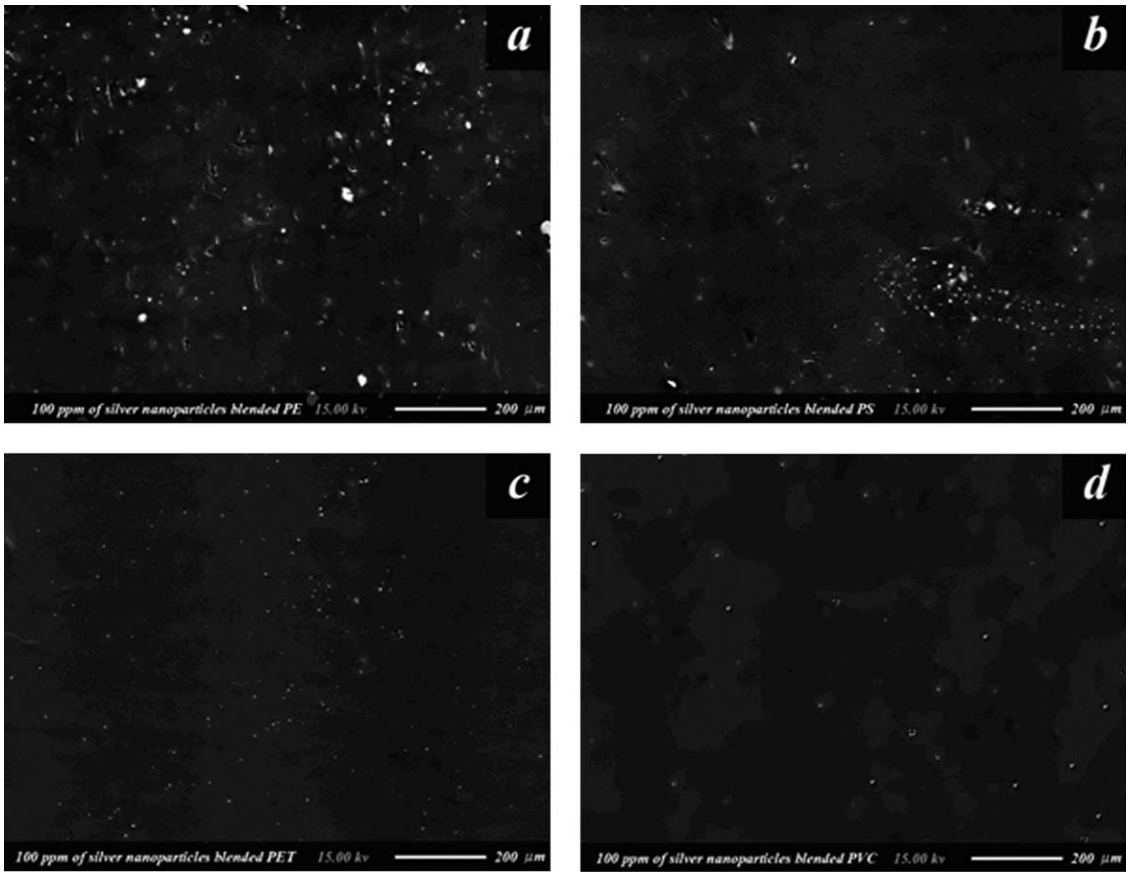

Figure 3 SEM micrographs showing silver dispersion level by silver blending technique (a) MDPE, (b) PS, (c) PET, and (d) PVC.

behavior, which could not inhibit the *E. coli* growth. However, it was observed that the increasing magnitude of the *E. coli* growth with increasing contact time (i.e., differences of changes in viable colony counts from 0 to 210 min) for all thermoplastics used were not the same. The increasing rate of *E. coli* for PVC was smaller than that for the other thermoplastics, suggesting that the PVC had an ability to retard the growth of *E. coli* cells. A possible reason for this was associated with chlorine atoms residing on the PVC film surface, which had occurred during the film preparation in the hydraulic hot press, which may then release during the antibacterial test. This claim was supported by Julia et al.[13] who observed that the *E. coli* cells in broccoli florets significantly reduced when dipping in the chlorine solution.

Figures 2 and 3 show SEM mapping images for silver particles dispersed on the four thermoplastic films via spray-coating and blending techniques, respectively, using a silver concentration of 100 ppm. In general, it was found that the quantity of silver particles from the coating technique was more apparent than that from the blending technique as one would expect. This was because the majority of the silver particles in the blending technique in Figure 3 were trapped within the thermoplastics during the blending. Considering the characteristics of silver coated surfaces in Figure 2, it was noticeable that the silver particles on nonpolar thermoplastics (MDPE and PS) formed a number of particle clusters or islands. This was probably due to incompatibility between polar silver colloids in ethanol solution and nonpolar thermoplastics during the spray coating. This incompatibility had then led to poor wettabilities and formed the particle clusters. Work by Silapasorn et al.[14] suggested that polarities of thermoplastics and the antibacterial agents used were one of the main paramaters to affect the antibacterial performances evaluated. On the other hand, the silver coating on the PET and PVC thermoplastics appeared to show relatively good compatibilities of polar silver colloids in ethanol solution on the polar thermoplastics, leading to better dispersions of silver particles on the thermoplastic surfaces as compared with those obtained in the MDPE and PS matrices.

Effect of incorporating technique for silver deposition onto thermoplastic films

Figure 4 shows the effect of incorporating technique on antibacterial performance for silver colloid of 100

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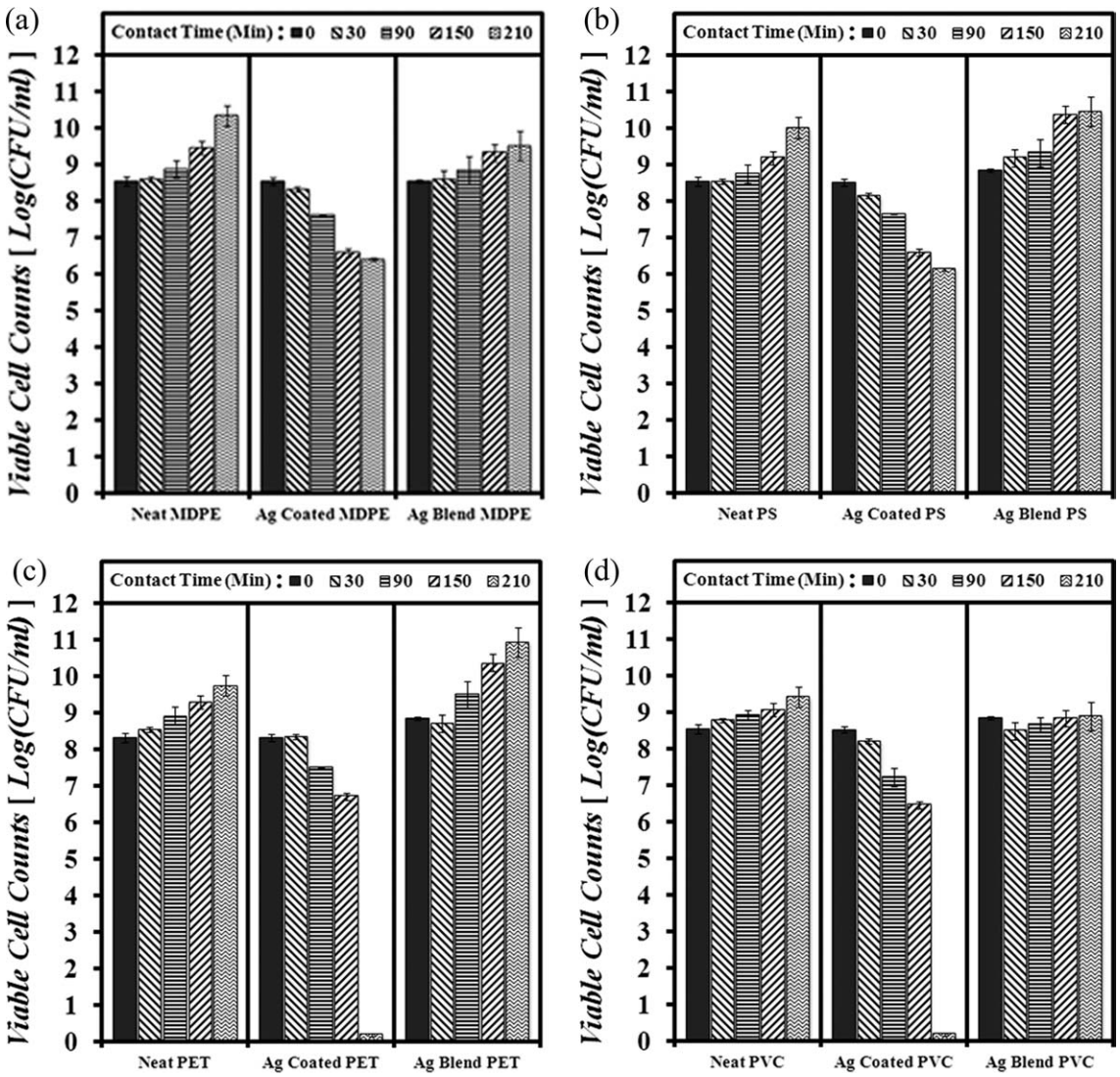

Figure 4 Viable cell counts for silver containing thermoplastic films for various contact times (a) MDPE, (b) PS, (c) PET, and (d) PVC.

ppm on four different thermoplastics using various contact times, the results being compared with the control samples (those without silver colloid). Generally, it was found that all film samples from the coating technique showed antibacterial behavior, the viable cell count decreasing with increasing contact time, whereas those from the blending technique showed the opposite effect, except for the PVC film in Figure 4(d). The effective antibacterial performance for the coating technique was due to greater silver particles on the thermoplastic film surfaces by the coating technique as compared with those by the blending technique (as also shown by SEM mapping images in Figs. 2 and 3). In the case of PVC, both coating and blending techniques exhibited bactericidal behavior for the contact times from 0 to 210 min, the behavior being much more pronounced with the coating technique. The reason for the silver blended PVC films that gave the *E. coli* inhibition character was related chlorine atom residing on the PVC film surface as already discussed earlier. It was interesting to observe from Figure 4(d) (PVC) that the increases in viable cell count with increasing contact time for neat and silver blended PVC films were different. A smaller increase in viable cell count for the silver blended PVC film was observed. This indication was due to a synergetic effect from the chlorine atom in PVC structure, and silver particles deposited on the PVC film.

Considering the results in Figure 4, the differences in decreasing magnitudes of viable cell count with increasing contact time for different thermoplastics from the coating technique were associated with the dispersions and characteristics of silver particles on the thermoplastic film surfaces as already shown in Figure 2. The silver particles on the MDPE and PS films appeared to form particle clusters, giving rise to larger agglomerations of

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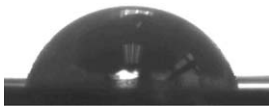
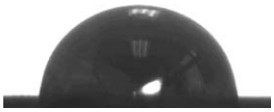
TABLE II
Water Contact Angle Results for PVC with and without Nano-Ag Colloid for Coating and Blending Incorporating Techniques

Incorporating technique	Nano-Ag colloid concentration (ppm)	Contact angle value (°)	Contact angle image
–	0	56.0 ± 4.5°	
Coating	50	80.5 ± 5.7°	
	100	87.3 ± 2.5°	
Blending	50	96.5 ± 1.8°	
	100	83.1 ± 3.2°	

silver particles and these agglomerations were believed to worsen the antibacterial performance. This claim was in line with the work by Dowling et al.[7] who suggested that silver with larger particle size showed lower antibacterial performance. In the case of PET and PVC films, a significant reduction of viable cell count was observed, especially at the contact time of 210 min. The reason was also related to the dispersion level of silver particles onto these two thermoplastic film surfaces as already given in Figure 2.

Evidence for incorporating silver colloids on polymer surfaces can be evaluated through contact angle measurement. In this section, only PVC thermoplastic was selected for evaluation of the contact angle measurement since it exhibited the most effective antibacterial performance among the thermoplastics used. The contact angle values for PVC samples without and with two different silver concentrations (50–100 ppm) from two different incorporating techniques (blending and coating) are given in Table II. It can be seen that neat PVC performs its native hydrophilic property, having a contact angle value of 56°. Once the silver particles were charged the contact angle values significantly increased to around 80.5 to 96.5°, physical changes in deionized water shapes being clearly observed. This suggested that the hydrophilic level of the PVC samples decreased with the presence of silver colloids. The increases in contact angle values, resulting in the decreases in hydrophilicities of the PVC and other polymers due to silver particles were also observed by Balazs et al.[15] and Kaali et al.[16] Balazs et al.[15] also stated that the increases in contact angle due to the silver were also caused by increases in surface roughness due to silver cluster distribution. For the effects of incorporating technique, it was found that the contact angle for the silver coating technique increased with increasing silver contents from 50 to 100 ppb whereas that for the blending technique decreased with increasing silver contents from 50 to 100 ppb. The differences in the contact angle results between these two techniques were associated with level of silver distributions *within* the PVC matrix for the blending technique and *onto* the PVC sample surface for the coating technique.

Effect of silver colloid content

The results in Figure 4 clearly indicated that the coating technique was more effective for incorporating the silver particles onto the thermoplastic films. Therefore, this section focuses on the evaluation of silver content effect on the antibacterial performance

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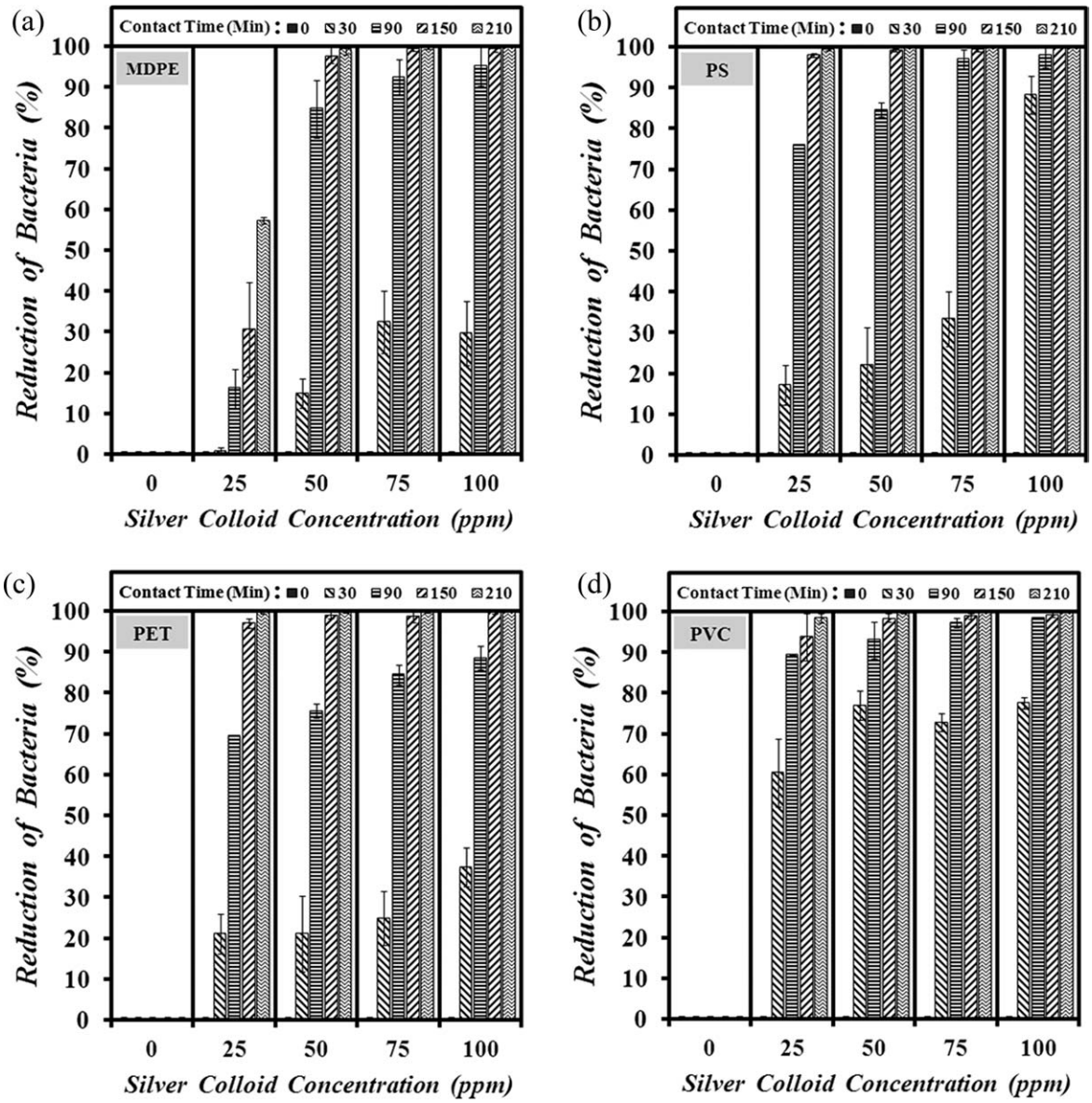

Figure 5 Percentage reduction of *E. coli* for silver containing thermoplastic films for various contact times (a) MDPE, (b) PS, (c) PET, and (d) PVC.

of thermoplastic films produced by the coating technique only. Figure 5 shows the percentage reduction of *E. coli* for different thermoplastic films using contact times from 0 to 210 min. It was observed that as the silver content was increased, the percentage reduction of *E. coli* increased for all thermoplastic films as one would expect. Table III shows the optimum silver content and contact time for all thermoplastic films to achieve 99.9% reduction of *E. coli*. It was found that all thermoplastics had the same optimum contact time of 150 min. The optimum silver content for PS, PET, and PVC was 50 ppm whereas that for MDPE was 75 ppm.

It was important to note from the results in Figures 5(a–d) that the percentage reductions of *E. coli* for the four thermoplastic films were not significantly different at the silver contents of 50 to 100 ppm. But, when considering the percentage reduction of *E. coli* at 25 ppm of silver colloid, obviously

TABLE III
Optimum Silver Content and Contact Time to Achieve 99.9% Bacterial Reduction and Antibacterial Behavior of Silver Colloid Coated Thermoplastic Films

	Optimum conditions for 99.9% of bacteria reduction	
Type of spray coated polymers	Silver colloid concentration (ppm)	Contact time (min)
MDPE	75	150
PS	50	150
PET	50	150
PVC	50	150

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TABLE IV
Silver Releasing Content for MDPE and PVC Films with Addition of Silver Colloid for Various Contact Times

Thermoplastics	Ag releasing content [ppb]				
	Contact times (min)				
	0 min	30 min	90 min	150 min	210 min
MDPE with 100 ppm silver colloid	42 ± 3.9	76 ± 2.6	99 ± 3.9	106 ± 4.6	112 ± 5.4
PVC with 100 ppm silver colloid	54 ± 5.5	296 ± 3.1	332 ± 2.2	348 ± 4.4	341 ± 3.9

high %reduction of *E. coli* for PVC was observed. This suggested that the effect of thermoplastic structure on the antibacterial performance was pronounced only at low silver concentration and this effect became suppressed by the silver agent when the silver concentration exceeded 25 ppm. The explanation for this was probably related to formation of silver chloride (AgCl) that occurred during the spray-coating process. The claim can also be substantiated by the work of Impellitteri et al.[17] who suggested that sliver chloride (AgCl) could be formed by the chemical reaction between silver nano-powder and chlorine atoms, that may have occurred from a dehydrochlorination reaction[18] of PVC during the spray-coating process onto the thermally softened PVC film (see Experimental section), and by the work of Min et al.[19] who synthesized AgCl nanoparticles by ion exchange reaction between silver nitrate and HCl vapor. If this was the case, the AgCl would have taken part in killing the *E. coli*. As a result, the percentage *E. coli* reduction for the PVC film would be relatively high as compared with that for the other thermoplastic films.

The differences in antibacterial performance for the thermoplastics shown in Figure 5 could be explained and quantitatively substantiated by assessing the silver content released from the thermoplastic films. The greater the silver released from the films the greater the bacteria reduction rate. Two extreme thermoplastics, which were MDPE and PVC with 25 ppm silver colloid content, were selected and the Atomic Absorption Spectrometry (AAS) was used to investigate the silver content released from the MDPE and PVC films immersed in the peptone solution for a period of time (contact or shaking times). The experimental procedures for the silver content releasing test by AAS technique could be obtained elsewhere.[20] The results are given in Table IV. It can be seen that as the contact time was increased from 0 to 210 min, the silver release increased from 42 ± 3.9 to 112 ± 5.4 ppb for MDPE and 54 ± 5.5 to 341 ± 3.9 ppb for PVC). For any given contact times, the silver release content for the PVC was greater than that for the MDPE, suggesting that the PVC film released more silver to kill the bacteria and thus higher percentage *E. coli* reduction. The low silver releasing rate in the MDPE was probably due to its crystalline structure that made the silver to release from the film[4,6,14] while the higher silver releasing rate in the PVC may be associated with the dehydrochlorination reaction and the formation of the AgCl as mentioned earlier.

CONCLUSIONS

In all thermoplastics used, it was observed that the viable cell count decreased with increasing the content of nano-silver colloid. The contact time at 150 min was sufficient for 99.9% reduction of *E. coli*. Seventy-five ppm silver content was required for MDPE whereas 50 ppm silver content were required for PS, PET, and PVC for achieving the maximum percentage reduction of *E. coli*. For a given nano-silver content, the spray-coating technique was much more effective than that dry-blending technique. The antibacterial performance for silver incorporated thermoplastic films was dependent on the chemical structure and polarity of thermoplastics only at low silver content of 25 ppm. Beyond this content, the performance was influenced by the silver itself. The dispersion and agglomeration of silver particles had significant effect on the antibacterial performance of the thermoplastics used. Among the thermoplastics used, PVC gave the highest percentage reduction of *E. coli* and the results were confirmed by monitoring the silver content released from the thermoplastic films. The results also suggested that loading the silver particles in PVC matrix decreased the hydrophilicity of the PVC evidenced by increases in contact angle values.

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