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Investigating Antibacterial Effects of Nano-Ag/titanium dioxide on Polylactic Acid Nanocomposites Produced Using Extrusion Method

Fatemeh Salimi [1], Hamed Ahari [2*], Seyed Amir Ali Anvar [1], Abbasali Motallebi Moghanjoghi [1]

1- Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2- Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Abstract

Background and Objective: Biodegradable polymers generally do not include antibacterial characteristics. This study investigated effects of various concentrations of nano-titanium dioxide containing silver on polylactic acid nanocomposites for their microscopic, chemical, roughness of the coatings and antibacterial characteristics against *Staphylococcus aureus* and *Escherichia coli*.

Material and Methods: Nanometric structure of the coatings was assessed through multiple steps. First, films containing 0.5, 1 and 3% wt% concentrations were produced using extrusion method and then analyzed and compared using scanning electron microscope and energy dispersive spectroscopy. Then, Fourier transform infrared spectroscopy and atomic force microscope were used to investigate functional groups, types of bonds, peak intensity variations and roughness characteristics of the films. Moreover, antimicrobial effects of the coatings was assessed on *Staphylococcus aureus* and *Escherichia coli*.

Results and Conclusion: Results of the scanning electron microscope indicated that the accumulation of nanoparticles in the films increased with increasing concentration of the nanoparticles. This increase affected energy dispersive spectroscopy, resulting in higher levels of titanium and silver nanoparticles. Fourier transform infrared spectroscopy revealed changes in intensity and position of the peaks due to the presence of nanoparticles. Bacterial growth assessments demonstrated that addition of nano-titanium dioxide containing silver to polylactic acid significantly decreased the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria ($p < 0.05$). Based on the results, coatings containing 3 wt% of nano-titanium dioxide with silver showed the greatest decrease in bacterial growth.

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Corresponding author:

Hamed Ahari
Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Tel: +98-9121872334
E-mail: dr.h.ahari@gmail.com

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1. Introduction

One of the most common sources of solid trash in big cities is plastics. Specifically, single-use plastics from food packaging are recognized as a global environmental contamination emergency since they take hundreds of years to decompose. Biopolymers have received significant attentions due to their uses and environmental benefits stemming from their biodegradability and biocompatibility. However, these polymers require property improvements for various uses [1]. From the aliphatic polyesters, polylactic acid (PLA) is a linear, thermoplastic and biodegradable polyester. Therefore, it has recently received the attention of researchers as a safer alternative to conventionally manufactured polymers [1, 2]. The PLA can be shaped into the desired form using standard plastic preparing equipment, producing molded parts, filmsand fibers. Due to its ease of processing, low toxicity and environmental friendly qualities, PLA can be an excellent alternative for packaging. Adding nanoparticles (NP) to the PLA matrix creates novel characteristics and helps overcome their disadvantages [1]. Nowedays, interests have been received by nanotechnology due to the significant functionality of nanomaterials. Because of their microscopic dimensions, engineered NPs can demonstrate unique morphological and processing characteristics, compared to the constituents. Distribution dimensions with the structure of NPs determine their characteristics and uses [3]. Adding NPs changes the molecular mobility within the polymer composite network and creates structures driven by interactions between the NPs and the polymer [4].

Technically, TiO_2 is inexpensive and non-toxic, which includes advantages to most chemicals. It includes a high UV absorption potential and is commonly used as a catalyst for the photodegradation of natural poisons in tissue engineering. Furthermore, it is an antimicrobial agent [5, 6]. This extrusion/thermo-compression technology is widely used in plastic production due to its rapid heating process and ease of operation. Material characteristics of the layers are affected by conditions such as temperature and screw speed during processing. Accurate control of these parameters is essential because excessively high temperatures may adversely affect the film performance. Finding an appropriate balance during film processing is essential to preserve desired characteristics without compromising performance of the films [7]. Herniou et al. produced a biodegradable film of cellulose acetate/corn starch using hot press extrusion with a double-screw extruder in six thermal zones at 90, 100, 105, 110 and 120 °C. The results demonstrated an appropriate biodegradable food packaging [8]. Zibaei et al. prepared a biodegradable PLA film using extrusion method, incorporating various concentrations of polyolefin elastomer (SeNPs; 1.5 % w/w) and triethyl citrate as plasticizer to enhance physical and mechanical characteristics of the film. Fourier transform infrared spectroscopy (FTIR) results of the produced nanocomposites indicated incorporation of SeNPs into the polymer matrix and formation of new covalent bonds. Scanning electron microscope (SEM) results showed that the NPs were well dispersed within the coating [2]. Iacovone et al. assessed effects of incorporating TiO_2 NPs on morphology, physicochemical characteristics and biodegradation of cassava starch-based nanocomposites from the extrusion method expulsion at various screw speeds (80 and 120 rpm). Films prepared at 120 rpm (S120 and S120-TiO_2 NP) showed fully treated starch and homogeneous dispersion of NPs, which resulted in much more flexible nanocomposites than those produced at 80 rpm [9].

One of the most important factors in food packaging is the roughness of the coating surface [10]. Increasing thickness of NPs [11], presence of silver NPs [12] and increasing concentration of TiO_2 NPs [13] cause roughness in the contact surface. Furthermore, roughness of the surface is effective on WVTR [14] and creates further microscopic holes on the film surface, which inhibits movement of water [15]. Studies on PLA coatings containing NPs indicate that pure PLA coatings include no antimicrobial effects. However, when TiO_2 NPs are present, inhibitory effects on bacteria are produced, which depend on the quantity and concentration of the NPs as well as the type of bacteria [1, 16-18]. Barani et al. demonstrated that method of the coating preparation significantly affected antimicrobial characteristics in addition to types of NPs and bacteria [19]. Zandi et al. detected that PE coatings containing TiO_2 included various antimicrobial effects, compared to samples containing ZnO NPs, highlighting effects of nanoparticle type on the antimicrobial efficacy [20]. Furthermore, Dong et al. stated that smaller nanoparticles included greater antimicrobial effects [21]. Jafroudi et al. produced silver nanocomposite packaging using solution-mixing method and investigated antibacterial effects of silver NPs on Gram-negative and Gram-positive bacteria. They concluded that silver NPs with concentrations of 20 and 50 $\mu g.l^{-1}$ included inhibitory effects on *Staphylococcus* (*S.*) *aureus* and *Escherichia* (*E.*) *coli*, while concentrations of 40 to 60 $mg.l^{-1}$ included detrimental effects on *S. aureus* and *E. coli*, respectively [22]. Their results demonstrated good antibacterial effects, but the synergistic effects of adding other nanoparticles to AgNPs were not investigated. Moreover, they did not investigate other biopolymers such as PLA or alternative film production methods. In the current study, four types of PLA coatings with various concentrations of TiO_2 containing silver (Ag@TiO_2) were produced using extrusion method at the Polymer Research Institute, Tehran, Iran. Effects of various concentrations of PLA NPS containing Ag@TiO_2 on morphological structures, roughness of the coatings, condition of the group factors and microbial activity of *S.aureus* and *E. coli* were investigated.

2. Materials and Methods

2.1 Film preparation

Nanocomposites were prepared using extrusion method with an extruder (Bra Bander, South Korea). Operating temperature from the inlet (feeding) to the outlet for PLA was 160–180 °C. The pressure was 12.5 bar (12.5×10^5 pa). Based on Table 1, appropriate quantities of PLA and Ag@TiO_2 were prepared for each sample and added to the input container. Materials were used in several processes, including melting, pressing and mixing and then extruded as a string from the exit part of the extruder. After cooling down, they were processed using granulation machine (Bara Bander, South Korea).

Table 1. Compositions of the prepared films with PLA containing Ag@TiO_2 [14]

Sample	PLA	Ag@TiO_2
Pla	100	0
Pla + 0.5%Ag@TiO_2	99. 5	0.5
Pla + 1% Ag@TiO_2	99	1
Pla + 3% Ag@TiO_2	97	3

Based on Fig. 1, produced granules were heated and mixed before spreading on cooling rollers to form thin film layers.

Figure 1. The film processing. A, extruder machine; b, film layers

2.2 Scanning electron microscopy

An SEM-based approach (Sigma VP, ZEISS, Germany) was used to investigate fracture surface morphology of the samples and report shape, size and distribution of the NPs on the film surfaces. Samples were first immersed in liquid nitrogen for 30 s and then cross-sections were prepared. These were coated with 20-nm layers of gold in preparation for SEM investigations. Images were recorded at magnifications of 1 µm and 20٠ nm [17].

2.3 Energy-dispersive spectroscopy

This study used energy-dispersive spectroscopy (EDX) as a microscopic technique for quantitative analysis of heavier-than-natural chemical elements. Technically, X-rays were used to excite the atoms, producing energy that served as a distinguishing factor for various types of atoms. This energy was represent as a spectrum by EDX. Standard guidelines for quantitative analysis were used in EDX 1 (E1508-98) for this process (sigma VP system, Oxford Instruments, England).

2.4 Fourier-transform infrared spectroscopy

To study structural features, identify functional groups, collect information on the nature of bonds and show possible interaction mechanisms of these nanostructures in films, infrared spectrometry studies with Fourier transform were used on the optimized and control samples. Spectrometer (NEXUS 870, USA) was used for this study. The FITR were achieved in the range of 600–4000 cm^{-1} with a spectral resolution of 4 cm^{-1} [1].

2.5 Atomic force microscopy (AFM)

Samples were cut into dimensions of 10 × 10 mm. Roughness of the samples was assessed using atomic force microscopy (AFM) (Nanotec Electronica, Madrid, Spain) with various scan sizes to provide a detailed analysis of the sample surfaces. Force constant of approximately 40 N/m and fundamental frequency of nearly 300 kHz ±10 were used. Roughness (e.g. Ra and Rq) was analyzed using output of the device [23].

2.6 Antibacterial activity of the films

Discs of each film sterilized by ultraviolet radiation were transferred into tubes containing 10 ml of tryptic soy broth (TSB) with 0.1 ml (10^6 $CFU.ml^{-1}$) of bacterial suspension (*S. aureus* ATCC 25923 and *E. coli* ATCC 25922) and incubated at 25 °C for 24 h. Then, 1 ml of the incubated samples was spread on a plate containing BHIA culture media. After 24 h of incubation at 37 °C, colony forming units (CFU) were investigated [24].

2.7 Statistical analysis

Descriptive statistics for the data were reported as mean ±SD (standard deviation). Mann-Whitney test was used to compare antibacterial activities of the prepared films between the two bacteria. Kruskal-Wallis test was used to compare antibacterial activities of the prepared films against *S. aureus* and *E. coli* within the four groups of the study. Then, Dunn-Bonferroni post-hoc test was carried out to show which pairs of the groups included significant differences in antibacterial activity within the four groups. All statistical analyses were carried out using GraphPad Prism 10 for Windows at a significance level of 0.05.

3. Results and Discussion

3.1 Microscopic structure

Studying images of SEM (Fig. 2), coatings were seen for the dispersion of NPs as well as their porosity and roughness. It showed distribution of Ag@TiO_2 NPs in the produced coatings, demonstrating that mechanical and thermal processes did not affect the NPs. The Ag@TiO_2 in coatings with a higher percentage of NPs was shown further. Sizes of Ag@TiO_2 NPs ranged 80–130 nm and was nearly spherical. As quantity of Ag@TiO_2 added to the coatings increased, size of the NPs increased due to their accumulation. Evcin et al. reported that size of Ag@TiO_2 particles increased due to accumulation, with sizes ranging 100–400 nm. Size of Ag@TiO_2 increased with the increase of NPs [25]. In another study, Liza et al. compared TiO_2 NPs with Ag@TiO_2. Results indicated that TiO_2 NPs accumulated within the Ag@TiO_2 structure, which could be attributed to the ironic radius of the particles. Therefore, these findings might be similar to those of this study [3]. Several studies have shown that incorporating metal NPs into a polymer matrix can increase surface roughness of the resulting films [26]. Feng et al. prepared composites of PLA and TiO_2 using solution casting method. Their SEM results showed that the surface roughness of the films increased with the addition of TiO_2 NPs. As the quantity of NPs increased, dispersion decreased, leading to a greater accumulation of particles [5]. Sheikh et al. developed PLA/cellulose nanofibers/silver nanocomposite films via in-solution casting. Their SEM analysis revealed that accumulation of particles increased with increases in silver NPs, resulting in further uneven surfaces [27]. Based on Azizi et al. study, polyvinyl alcohol (PVA) and gelatin polymer matrices containing ZnO and TiO_2 NPs showed further uneven surfaces, compared to PVA/gelatin coatings with no NPs.This increased unevenness was attributed to mineral NPs [28]. The SEM results of Jafroudi et al. study indicated that the AgNPs produced by the chemical reduction method were spherical in shape and size of the NPs was 25–40 nm [22]. Based on the study findings, adding NPs to the surface of nanocomposites was effective and increasing their concentration resulted in greater unevenness and particle accumulation.

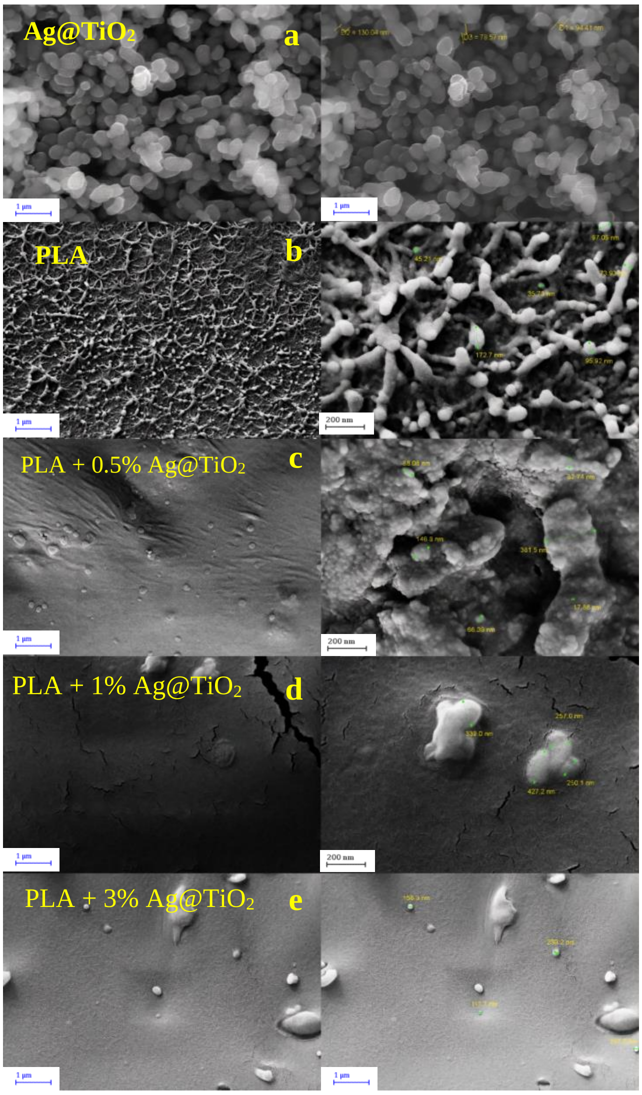

Figure 2. Scanning electron microscopy images of the prepared films at 1 µm and 200 nm. A, Ag@TiO2; b, PLA; c, PLA and 0.5% Ag@TiO2; d, PLA and 1% Ag@TiO2; and e, PLA and 3% Ag@TiO2

3.2. Energy-dispersive spectroscopy

The EDX analysis revealed the constituent elements, their percentage and atomic weight. Based on the results in Fig. 3, PLA coating did not contain NPs [14]. Quantities of Ag and Ti in the coatings were as follows: PLA and 0.5% Ag@TiO_2 (0.8 and 0.6 %, respectively), PLA and 1% Ag@TiO_2 (0.1 and 0.9 %, respectively), and PLA and 3% Ag@TiO_2 (1.2 and 2.5%, respectively). As the quantity of NPs in the coatings increased, quantity of Ti in EDX increased. Absence of the additional peaks in EDX analysis with the high percentages of carbon and oxygen indicated good mixing and correct coatings, suggesting absence of impurities. Study carried out by Liza et al. revealed that as the percentage of NPs increased, quantities of Ti and Ag in EDX increased and no other impurities were observed in EDX [3]. Singha et al. prepared chitosan nanocomposites with Ag/TiO_2 using sol-gel method. Based on the EDX results, carbon and oxygen were the major components and presence of Ag@TiO_2 NPs was verified in the coatings containing Ag and Ti NPs [29].

3.3 Fourier-transform infrared spectroscopy

The FTIR analysis is a technique used to detect and identify NPs. The FTIR can detect changes in the composition of all functional groups through alterations in the vibrational states of molecules. Similarly, the FTIR results indicated that the presence of Ag@TiO_2 NPs affected fabrication of the films. Based on Fig. 4, PLA showed characteristic stretching for C=O at approximately 1749 cm, asymmetric -CH3 at 2995 cm, symmetric -CH3 at nearly 2946 cm and C-O at 1079 cm [1, 14, 27]. Similarly, PLA and 0.5% Ag@TiO_2, PLA and 1% Ag@TiO_2, and PLA and 3% Ag@TiO_2 showed similar peaks, but the presence of Ag@TiO_2 NPs caused changes in intensity and position of some peaks. In PLA and 3% Ag@TiO_2 film, a shoulder appeared at the peak nearly 1749 cm [14]. The peak appearing at 1184 cm in PLA was nearly 1186 cm in PLA and 0.5% Ag@TiO_2, 1193 cm in PLA and 1% Ag@TiO_2, and 1268 cm in PLA and 3% Ag@TiO_2. This change was characterized by greater intensity and width, which was attributed to the bond of Ag@Tio_2, verifying presence of Ag@Tio_2 NPs. Peaks in the range of 600–1000 cm^{-1} were associated with the anatase Ti-O phase [14, 30].

Based on Nayak et al. study, PLA/TiO_2 samples demonstrated higher peak intensities and sub-peak areas, compared to PLA fibers [1]. Sheikh et al. results on the mechanical and thermal characteristics of PLA/CNF/Ag nanocomposites demonstrated effects of nanofillers on the peak intensity [27]. Feng et al. reported that PLA/TiO_2 coatings showed higher absorption peak intensities and sub-peaks, compared to PLA, indicating presence of surface hydroxyl groups in TiO_2 NPs. Furthermore, results showed that basic structure of the films did not disappear [5]. Li et al.

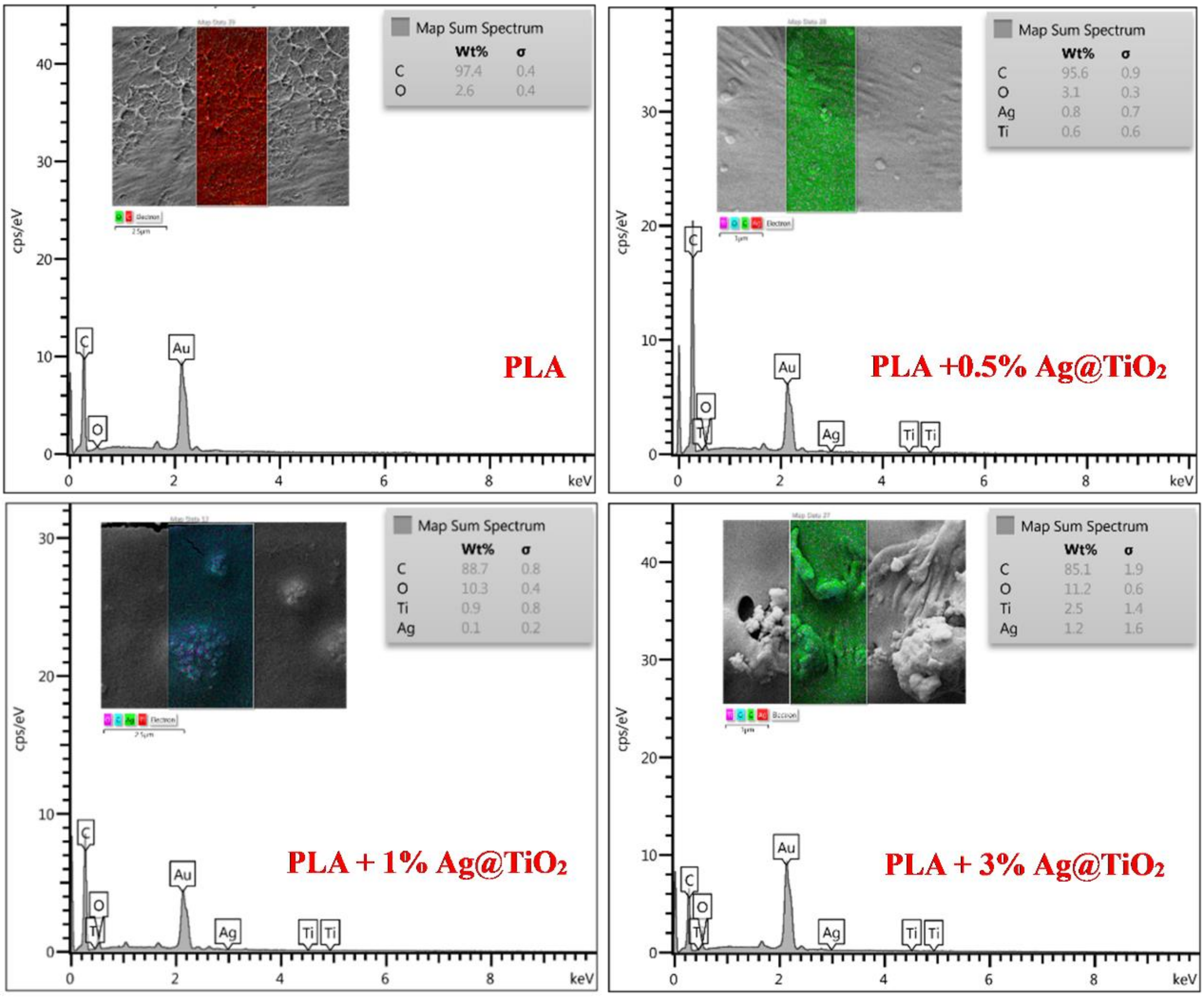

Figure 3. Energy-dispersive spectroscopy of the prepared films

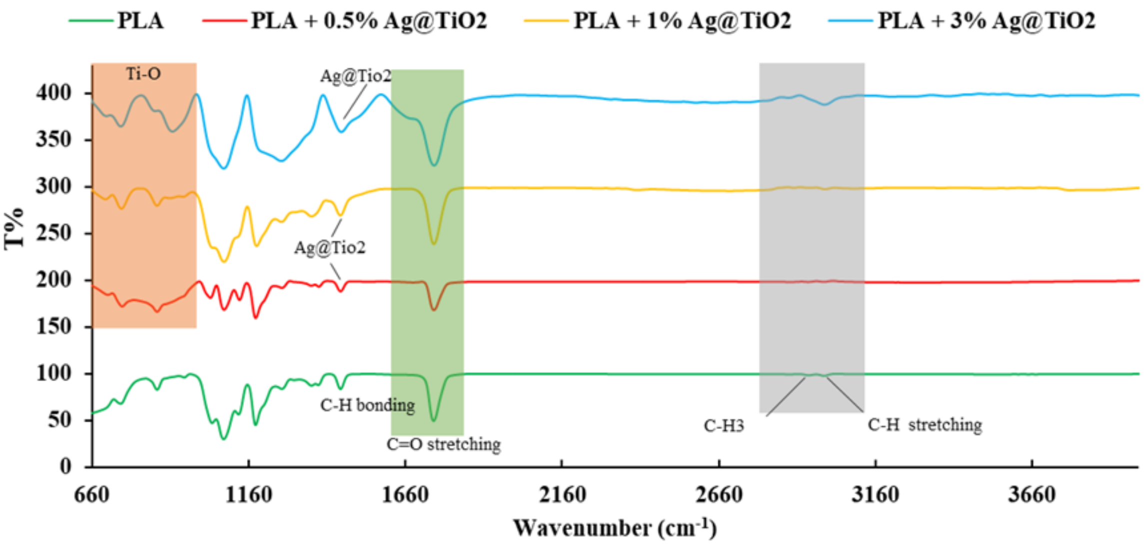

Figure 4. Fourier-transform infrared spectroscopy of the fabricated films. Line colors showing various agents transmittance in Fourier-transform infrared spectroscopy as follows: green, PLA film; red, PLA and 0.5% Ag@TiO_2 film; yellow, PLA and 1% Ag@TiO_2 film; and blue, PLA and 3% Ag@TiO_2 film

Prepared PLA coatings containing Ag and TiO_2 NPs via solvent volatilization method. Their FTIR results indicated that as the concentration of NPs increased, band intensity of the NP composite films increased as well. This was attributed to the faster nucleation and densification of these coatings, compared to pure PLA due to the presence of Ag and TiO_2 [17]. In another study, presence of TiO_2 NPs in a chitosan complex demonstrated increases in interactions between the particles and appearance of a novel peak was due to the TiO2-OH bonds [23]. Results of a study by Gonzalez et al. on PLA coatings containing 100 and 21-nm TiO_2 at various percentages indicated no changes in band intensity as a function of NP content or size under the processing conditions [16]. Findings suggested that while the intensity of the peaks changed due to the presence of Ag and TiO_2 NPs within the PLA, overall structure of the coatings was maintained and interactions between the NPs and PLA were established. Furthermore, not much differences were seen between the various percentages of TiO_2. Small differences could be attributed to the larger NP sizes in coatings with higher percentages, as demonstrated by the SEM results. This increase in size, resulting from NP accumulation, might affect the intensity of peaks in FTIR.

3.4 Atomic force microscopy

Findings from the AFM investigations are present in Fig. 5 and Table 2. Roughness (Ra) for the PLA, PLA and 0.5% Ag@TiO_2, PLA and 1% Ag@TiO_2, and PLA and 3% Ag@TiO_2 coatings were observed at 0.4724, 0.7862, 2.143 and 6.983 nm, respectively. The RMS roughness (Rt) values for PLA coating were 0.6221 nm, for PLA and 0.5% Ag@TiO_2 were 1.176 nm, for PLA and 1% Ag@TiO_2 were 3.77 nm, and for PLA and 3% TiO_2, were 8.456 nm. The PV-Rt values for PLA coating were 6.269, PLA and 0.5% Ag@TiO_2 were 18.62, PLA and 1% Ag@TiO_2 were 43.10 and PLA and 3% Ag@TiO_2 were 49.47. It was concluded that addition of NPs and increases in the concentration of titanium dioxide resulted in increases in surface roughness of the layer. Comparisons of the Rt, Ra and PV_Rt parameters supported this conclusion. The NPs contributed to a larger surface area in the nanocomposite due to their small sizes and specific structures, which could increase surface roughness. Adding NPs to the polymer matrix enhanced contact points between these particles and their environment within the nanocomposite. These contacts could act as starting points to form surface structures with high roughness. Li et al. carried out a study on the identification of thin layers of silver NPs with various thicknesses using AFM. They detected that increases in the thickness of the NPs were correlated with increases in surface roughness [11]. Ahmadi et al. carried out a study on polycaprolactone (PCL)-based films integrated with hairy cellulose nanocrystals (HCN) and silver NPs for tilapia active packaging uses. Results showed that while the silver NPs increased the surface roughness, presence of HCNs decreased it. Decreases in surface roughness were linked to decreases in the contact area and more importantly, decreases in the NPs migration rate. Results demonstrated that at a fixed concentration of silver NPs, addition of HCN to the layers decreased the surface roughness [12].

Surface roughness is one of the important parameters in food packaging. Increased roughness of the surface enhances the contact area, ultimately providing greater inhibitory characteristics against bacteria [10]. Additionally, surface roughness is effective on WVTR, which depends on factors such as materials, production methods and types of NPs and their concentrations [14]. Increasing the surface roughness creates further microscopic holes in the films, which can inhibit water movement through the film surface [15]. Rahma et al. synthesized TiO_2 NPs using spin coating and drop casting methods. The AFM results for two concentrations of 60 and 120 g/l showed that the surface roughness depended on the concentration of the solution. Increases in TiO_2 concentr-ation were associated with corresponding increases in sizes of the NPs [13]. Based on the results from SEM in Fig. 2, distribution of NPs on PLA with various Ag@TiO_2 concentrations varied significantly, which could affect the coating surface. This study revealed that as the quantity of NPs increased, their sizes increased as well, and leading to greater surface roughnesses. Results also showed that the bacterial growth decreased with increases in surface roughness.

3.5 Antibacterial analysis

Colony method was used to assess the growth of *S. aureus* and *E. coli* and assess antimicrobial characteristics of the prepared films. Results showed that developments of the coatings containing PLA and PLA with 0.5, 1 and 3% Ag@TiO_2 for *S. aureus* after 24 h included 2.56×10^8, 1190, 90, and 70 CFU, respectively.

Table 2. The atomic force microscopy analyses

Group	Roughness	RMS Roughness	Peak-to-Valley Roughness
PLA	0.4724	0.6221	6.269
PLA + 0.5% Ag@TiO_2	0.7862	1.176	18.62
PLA + 1% Ag@TiO_2	2.143	3.77	43.10
PLA + 3% Ag@TiO_2	6.983	8.456	49.47

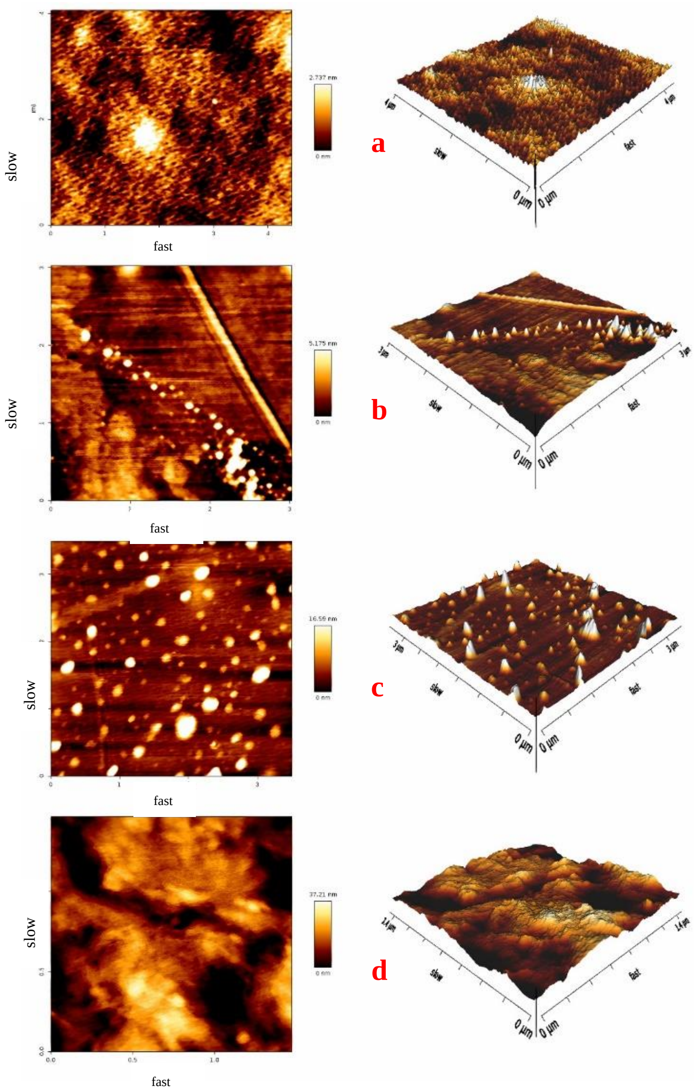

Figure 5. The atomic force microscopy analyses for all groups. A, PLA; b, PLA and 0.5% Ag@TiO_2;, c, PLA and 1% Ag@TiO_2; and d, PLA and 3% Ag@TiO_2

Results from the Kruskal-Wallis test revealed significant differences between the four groups regarding antibacterial activity of the prepared films against *S. aureus* ($p < 0.05$). Dunn-Bonferroni post hoc test indicated significant differences in the means of antibacterial activity between the PLA group and the groups containing 1 and 3% Ag@TiO_2 ($p < 0.05$). Additionally, statistically significant differences were detected between the means of antibacterial activity of the PLA coatings containing 0.5% Ag@TiO_2 and those containing 3% Ag@TiO_2 ($p < 0.05$). However, no significant difference was observed between the other groups ($p > 0.05$). For *E. coli*, a statistically significant difference was detected in the means of antibacterial activity of prepared films within the four groups based on the Kruskal-Wallis test results ($p < 0.05$). Dunn-Bonferroni post hoc test was then used to identify which pair of the groups caused the significant difference between the four groups. Results showed statistically significant differences in the means of antibacterial activity between the PLA group and the groups containing 1 and 3% Ag@TiO_2 ($p < 0.05$). However, no significant difference was detected between the other groups ($p > 0.05$) (Tables 3 and 4). Results from the Mann-Whitney test carried out in the PLA group demonstrated a statistically significant difference in the mean antibacterial activities of the prepared films between the two bacteria ($p < 0.05$). In other groups, no statistically significant difference was detected between the mean antibacterial activities of the prepared films between the two bacteria ($p > 0.05$) (Table 3).

Jafroudi et al. investigated antimicrobial effects of silver nanoparticles and nanocomposites using MIC and MBC as well as the inhibition zone. The MBC results of silver NPs against *S. aureus* and *E. coli* were 45 and 70 $\mu g.ml^{-1}$, respectively, while the MIC results were 25 and 50 $\mu g.ml^{-1}$. Results of the inhibition zone for the nanocomposite containing 24 $\mu g.l^{-1}$ of silver showed 4-mm zones for *E. coli* and 3 to 5-mm zones for *S. aureus*. Their findings highlighted the antibacterial effects of the produced coatings on Gram-positive and Gram-negative bacteria, particularly against Gram-positive bacteria [22]. Results of the inhibitory zone by Nayak et al showed that PLA coatings included no inhibitory zones, while TiO_2 nanocomposites demonstrated two additional inhibitory zones and addition of TiO_2 to PLA increased the inhibitory zones. It was indicated that TiO_2 was specific to some bacteria and included effects on a narrow range of bacteria [1]. Gonzalez et al. prepared PLA polymer composites filled with TiO_2 using two steps of solvent casting followed by hot pressing for antimicrobial packaging and their antimicrobial effects were investigated using *E. coli*. Their results showed that the growth of bacteria was limited [16]. Li et al. prepared PLA films containing TiO_2 and silver using solvent evaporation method and assessed antimicrobial effects on *E. coli* and *Listeria monocytogenes*. Their results showed that the pure PLA film did not affect the growth of either bacterium. However, films containing a certain percentage of nanoparticles demonstrated significant effects ($p < 0.05$). In addition, antimicrobial capacity of the films increased by increasing the quantity of NPs in PLA. Presence of 0.5% wt% nano-Ag in PLA/TiO_2 resulted in decreases in the number of microorganisms [17]. Buddhakala et al. combined PLA with corncob fiber coatings at various percentages of corncob fiber coatings and prepared hot-molded samples.

Table 3. Comparisons between the means of antibacterial activities of the prepared films against *Staphylococcus aureus* and *Escherichia coli*#

Group	*Staphylococcus aureus*	*Escherichia coli*	P-value
Pla	$2.56\times10^8 \pm 21283796.653$	$1.96\times10^8 \pm 25534290.669$	0.04*
Pla+0.5%Ag@TiO_2	$1.19\times10^3 \pm 58.617$	$1.22\times10^3 \pm 44.395$	0.27
Pla+1% Ag@TiO_2	$9\times10^1 \pm 13.453$	$8\times10^1 \pm 12.529$	0.37
Pla+ 3% Ag@TiO_2	$7\times10^1 \pm 6.928$	$7\times10^1 \pm 13.747$	0.82
P-value	0.02*	0.01*	-

Values are reported as Mean±SD; * Significant at the level of p= 0.05.

Table 4. Pairwise comparisons of the antibacterial activities of the prepared films between the four groups of the two bacteria

Group		*Staphylococcus aureus*	*Escherichia coli*
		P-value	P-value
Pla	Pla+0.5%Ag@TiO_2	0.30	0.30
	Pla+1% Ag@TiO_2	0.04*	0.01*
	Pla+ 3% Ag@TiO_2	0.002*	0.007*
Pla+0.5%Ag@TiO_2	Pla+1% Ag@TiO_2	0.30	0.17
	Pla+ 3% Ag@TiO_2	0.04*	0.08
Pla+1% Ag@TiO_2	Pla+ 3% Ag@TiO_2	0.30	0.73

Significant at the level of 0.05.

They investigated selected coatings based on their mechanical and physical characteristics with various quantities of TiO_2 for their antimicrobial effects, specifically against *S. aureus* and *E. coli*. Coatings containing 0.5% TiO_2 showed no inhibitory effects on *E. coli* but coatings containing 2% TiO_2 resulted in inhibition zones of approximately 20 mm for *E. coli* and 30 mm for *S. aureus*. Their results demonstrated that the antibacterial activity depended on the quantity of TiO_2 and coatings lacking TiO_2 demonstrated no antimicrobial effects overall [18]. Barani et al. prepared polyethylene/TiO_2/Ag coatings using melt mixing and sol-gel methods and investigated the inhibition zones of *S. aureus* and *E. coli*. Results showed that the coatings produced by the melt mixing method showed greater efficacies. The largest diameter of the inhibition zone was observed in coatings containing 5% Ag and 2.5% TiO_2, with an inhibition zones of approximately 18.45 mm for *S. aureus* and 16.76 mm for *E. coli* [19]. Using extrusion method, Zandi et al. findings showed that polyethylene coatings containing TiO_2 included stronger antimicrobial effects on *S. aureus* and *E. coli*, compared to those with ZnO. Their study with other studies suggested that the generation of hydrogen peroxide led to cellular deaths. Furthermore, researchers detected that hydrogen peroxide effects on *S. aureus* were more reported than those on *E. coli* due to the protective layers of lipopolysaccharides in the cell walls of Gram-negative bacteria [20]. Cresnar et al. prepared PLA films containing Ag/ZnO and TiO_2 at 0.5, 1 and 2.5 wt% using extrusion method. Their results showed that PLA/Ag coatings included the best growth inhibition effect. Sizes of the Ag NPs within the PLA composite ranged 80–120 nm. They revealed that although uneven surfaces with larger surface areas and further hydrophilic characteristics could inhibit bacteria more effectively, no significant increase in the roughness of the PLA/Ag coatings was observed [10]. Dong et al. stated that the smaller the sizes of NPs, the lower the quantities of MIC and MBC, showing greater antibacterial activities [21]. Based on the results and comparisons by other researchers, it could be observed that addition of Ag@TiO_2 to PLA resulted in significant changes ($p<0.05$). Based on the results, 0.5% Ag@TiO_2 coatings showed the minimum antimicrobial effect. Compared to other research, it was still able to decrease the microbial burden. This could be attributed to the synergistic effect of adding TiO_2 to Ag, as well as the production using extrusion method. Concen-trations of 1 and 3% Ag@TiO_2 demonstrated significant antimicrobial effects on *S. aureus* and *E. coli* ($p<0.05$). However, these two percentages did not show significant differences from each other ($p>0.05$). Similar to the study of other researchers, production of hydrogen peroxide with Ag and TiO_2 NPs caused damages to the cell membrane and ultimately led to death of the bacteria. The antimicrobial effect of coatings in *S. aureus* was similar to that of *E. coli* and presence of peptidoglycan layers in Gram-negative bacteria did not affect it. Additionally, results of SEM and AFM indicated that as the quantity of NPs increased, the particles agglomerated and their roughness increased. Since PLA nanocomposites with 1 and 3% Ag@TiO_2 included the highest roughness, it could be concluded that their inhibitory effects were due to further uneven surfaces, creating greater inhibitory effects. Results of SEM showed that heat during the extrusion production process did not affect the NPs within the PLA composites. Similar to findings from other studies, the smaller sizes of the NPs contributed to the effective antimicrobial characteristics of the produced nanocomposites.

4. Conclusion

Based on the results from EDX, coatings produced via extrusion method were prepared correctly and heat generated during the process did not affect the NPs. As quantity of the NPs increased in the SEM analysis, their sizes increased as well. Furthermore, adding NPs to the PLA matrix enhanced contacts between the NPs and the environment, resulting in increased roughness of the coatings. Increasing roughness of the coatings by creating microscopic holes prevented expansion of water and hence enhanced packaging of the food products. Greater roughness has been shown to inhibit the bacterial growth. Moreover, 1 and 3% Ag@TiO_2 coatings demonstrated better antimicrobial effects against *S. aureus* and *E. coli* due to the increased contact surface areas in the coatings. Results revealed that the combination of Ag and TiO_2 NPs resulted in a better antimicrobial activity due to their synergistic effects. Moreover, comparisons of the current results with results of other studies demonstrated that PLA coatings with Ag@TiO_2 performed better than that those without NPs or Ag or TiO_2 NPs alone did. This improvement could be attributed to the synergy effects of the two NPs within the coating. Addition of Ag@TiO_2 NPs presented an appropriate alternative for food packaging because it effectively caused bacterial cell death without demonstrating toxic effects at low concentrations. Nowadays, consumers increasingly prefer biodegradable packaging to traditional options that contribute to the environmental pollution. Additionally, costs of producing Ag@TiO_2 NPs are lower than costs of producing Ag or TiO_2 NPs alone. This offers a promising method for using biodegradable packaging and decreasing pollution caused by plastics. However, further assessments are necessary.

5. Acknowledgements

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6. Conflict of Interest

The authors declare no conflict of interest.

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بررسی اثرات ضد باکتریایی نانو نقره/ دی‌اکسید تیتانیوم بر نانوکامپوزیت پلی لاکتیک اسید تولید شده به روش اکستروژن

فاطمه سلیمی [1]، حامد اهری [2*]، سید امیرعلی انوار [1]، عباسعلی مطلبی مغانجوقی [1]

۱- گروه بهداشت مواد غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.
۲- گروه علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.

چکیده

سابقه و هدف: پلیمرهای زیست‌تخریب‌پذیر عموماً فاقد خواص ضدباکتریایی هستند. این تحقیق به بررسی تاثیر غلظت‌های مختلف نانودی‌اکسیدتیتانیوم حاوی نقره بر روی نانوکامپوزیت پلی‌لاکتیک‌اسید بر خواص میکروسکوپی، شیمیایی، زبری پوشش‌ها و خواص ضدباکتریایی فیلم‌ها بر روی باکتری‌های گرم مثبت و گرم منفی می‌پردازد.

مواد و روش‌ها: ساختار نانومتری پوشش‌ها طی مراحل متعدد مورد بررسی قرار گرفت. ابتدا فیلم‌های حاوی غلظت‌های ۰/۵، ۱ و ۳ درصد وزنی با استفاده از روش اکستروژن تولید و با استفاده از میکروسکوپ الکترونی روبشی و طیف‌سنجی پراکنده انرژی آنالیز و مقایسه شدند. سپس از طیف‌سنجی فروسرخ تبدیل فوریه و میکروسکوپ نیروی اتمی برای بررسی گروه‌های عملکردی، نوع پیوندها، تغییرات شدت پیک و ویژگی‌های زبری فیلم استفاده شد. در نهایت اثر ضد میکروبی پوشش ها بر روی باکتری‌های *استافیلوکوکوس اورئوس* و *اشریشیا کلی* انجام شد.

یافته‌ها و نتیجه‌گیری: نتایج میکروسکوپ الکترونی روبشی نشان داد که با افزایش غلظت نانوذرات، تجمع نانوذرات در فیلم افزایش می‌یابد. این افزایش همچنین بر طیف‌سنجی پراکنده انرژی تأثیر می‌گذارد و باعث افزایش نانوذرات تیتانیوم و نقره می‌شود. طیف‌سنجی فروسرخ تبدیل فوریه تغییراتی را در شدت و موقعیت قله‌ها در حضور نانوذرات نشان داد. ارزیابی رشد باکتری نشان داد که افزودن نانو دی‌اکسیدتیتانیوم حاوی نقره به پلی‌لاکتیک‌اسید به طور قابل توجهی رشد باکتری های *استافیلوکوکوس اورئوس* و *اشریشیا کلی* را کاهش داد ($p<0/05$). با توجه به نتایج به‌دست‌آمده، پوشش‌های ٪۳ نانودی‌اکسیدتیتانیوم حاوی نقره بیشترین اثر کاهشی را بر رشد باکتری‌ها داشتند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

دریافت ۳ آگوست ۲۰۲۴
داوری ۲۹ آگوست ۲۰۲۴
پذیرش ۳ سپتامبر ۲۰۲۴

واژگان کلیدی

- میکروسکوپ نیروی اتمی
- تبدیل فوریه مادون قرمز
- طیف سنجی
- نانو ذرات
- بسته بندی
- پلیمرها

نویسنده مسئول

حامد اهری
گروه علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.
تلفن: ۹۸+-۹۱۲۱۸۷۲۳۳۴
پست الکترونیک:
dr.h.ahari@gmail.com