RESEARCH ARTICLE

Novel silicon dioxide -based nanocomposites as an antimicrobial in poly (lactic acid) nanocomposites films

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ABSTRACT

Objective(s): Due to nanocomposites antimicrobial properties, one of the most extensive usages of nano-products is in packing industry. Thus, the production of packages with nanotechnology can effectively prevent against a variety of micro-organisms. In this study, the silicon dioxide nanoparticles the poly (lactic acid) PLA films on antimicrobial and permeability was investigated.

Methods: In order to measure the effect of antibacterial nano-covers, the direct contact of 1%, 3% and 5% silicon dioxide nanoparticles was used. Furthermore, the sample was contaminated with standard strains of gram-negative (Escherichia *coli* –code of 1399 (ATCC 25992)) and bacteria gram-positive (Staphylococcus *aureus*–code of 1431 (ATCC 25923)) provided. Diameters of inhibition zones were measured after 24 h incubation of plates at 37 °C, by using Digital Caliper. Also, the water vapor permeability was investigated according to ASTM E96 and oxygen standards according to ASTM D 3985 standard from film surface.

Results: Comparison the mean diameter of the inhibition zone of Escherichia *coli*, PLA containing 3% silicon dioxide with PLA film containing 5% silica showed no significant difference between the two groups ($P> 0.05$) as well as, the average diameter of Staphylococcus *aureus* ($P> 0.05$). The results showed that the permeability compared to water vapor and oxygen vapor in pure PLA films with PLA containing 1%, 3% and 5% silicon dioxide showed a significant difference ($P <0.05$).

Conclusion: PLA /nanocomposite SiO_2 films have been identified as the most efficient cover in reducing the microbial load and have been useful as active antimicrobial nanopackaging.

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INTRODUCTION

Recently, attention to biopolymers is increased because of increasing environmental awareness, the price of crude oil and the challenges related to global warming. Since the biopolymers are obtained renewable resources and they are biodegradable, therefore using them compared with petroleum-based polymers have the least negative effect on environment. Todays, these compounds are used in different field such as physiotherapy, pharmacy, medicine, coating and packaging material [1]. Among the biodegradable compounds, we can refer to poly lactic acid (PLA). PLA has great potential for substituting with polymers based on crude oil compounds because of its properties including thermo plasticity, biodegradability, biocompatibility, mechanic resistance, high modulus of elasticity and ease of processing [2, 3]. Poly lactic acid is a thermoplastic poly ester with linear chain which is produced by renewable resource. Lactic acid is obtained from fermentation of the plant substrate such as corn as a forming monomer of PLA [4]. Also its use as the material in contact with foodstuff is permitted by FDA [5]. Nanocomposites are known as material of 21 century and it is predicted that its use increase considerably. Industries tend to produce very thin films not only to decrease material and production costs, but also the environmental effects that have on use of packaging. The result-based study

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contribute to develop new active packaging based on nanocomposites material which prevent rancidity, the change or missing color, loss the nutrient material, dehydration, microbial growth, gas production, creating odors and dehydration. Currently, based-biopolymers nanocomposites such as PLA/nanocomposites receive focus of attention in various packaging because of increasing demand from customers and production for stable products environmentally [6,7]. Generally, active packaging has features beyond the inhibition properties and they are obtained by adding active elements and compounds in packaging system. This kind of packaging show good reaction to change of the properties of the package related to internal and external changes of the package environment and therefore it is very important in preserving freshness of food [8]. Active anti-microbial packages made of metal nanocomposites are new generation of nanostructures packaging that are produced from direct combinations of metallic nanoparticles with a background polymer [9]. The antimicrobial activity of a polymer is usually achieved by adding metal particles, metal oxides and organic compounds. Metals and oxidative particles are the most commonly used particles in the development of antimicrobial activity [10, 11]. Silver, copper and zinc oxide nanoparticles are the most widely used metal nanoparticles in the production of antimicrobial films [12]. Silicon dioxide or silica is the most abundant material in the earth's crust. This compound with a SiO_2 chemical formula is structurally similar to diamond, it is a crystalline and white material, it`s melting and boiling point is relatively high and it is found in crystalline and amorphous form in the nature. Nano Silicon Dioxide (SiO_2), which has high heat resistance and a melting point of 1750 °C, can be used to improve the PLA heat resistance. Almost all researchers who have reported the mixing of inorganic silicon dioxide with PLA used it to make a block or film or sheet, rather than a fibrous or filament shape; therefor the PLA / SiO_2 nanocomposite filament should be studied. Meanwhile, nano SiO_2 has environmental protection, safe and non-toxic functions, so the PLA / nano SiO_2 composite filament has an environmentally friendly nature. On the other hand, nano SiO_2 has a very low price and hence can reduce the cost of composite filaments. Also, the nano SiO_2 color is white, so the composite filament is bright and can be colored almost with all colors [13].The aim of this study was to investigate the antimicrobial properties of polylactic acid nanoparticles films with various silicon dioxide on E. *coli* and Staphylococcus *aureus* and determine the permeability property of these films to water and oxygen.

MATERIALS AND METHODS

In this study, Poly (lactic acid) (PLA) films with 0%, 1%, 3% and 5% silicon dioxide nanocomposite were a commercial grade supplied by Cargill Dow LLC, Minneapolis, USA by extrusion method which were made of (PLA (with a specific gravity of 1.25 g/cm^3 (210 °C, 2.16 kg) and a melting temperature of 170 °C and the SiO_2 from Wacker-Chemie GmbH (Hydrophilic Wacker HDK® T40, with the density of 2.20 g/cm^3, BET surface area of 360–440 m^2/g).

Studied Microorganisms

Escherichia *coli* (gram negative) with code 1399 (ATCC 25992) and Staphylococcus *aureus* (gram positive) with code 1431 (ATCC 25923) from the collection center of industrial Iranian fungi and bacteria are used.

Activation method of used bacteria

In order to activate Escherichia *coli* (ATCC 25922) and Staphylococcus *aureus* (ATCC 25923), the bacterium was cultured in a nutrient broth and incubated for 24 hours at 37 °C (Pars Teb Novin, Iran). After 24 hours, given bacterium was placed into a 4-zone nutrient agar medium and incubator 37 °C for 24 hours. Then, a colony of bacterium was added to sterile distilled water and compared to 0.5 McFarland standards until its turbidity become equal 0.5 McFarland.

Preparation of half McFarland microbial suspension

McFarland standards are prepared by adding a specific volume of 1% sulfuric acid solution and 1.175% barium chloride to obtain a barium sulfate solution with specific optical density. Usually 0.5 McFarland standards that contain 9.95 ml sulfuric acid 1% and 0.05 ml barium chloride 1.175% are most commonly used. The McFarland standard produces an opacity equivalent to a bacterial suspension containing 1.5×10^8 cfu/ml. To prepare a microbial suspension, 24 hours of culture was required from each bacterium. Therefore, 24 hours before the experiment, storage culture was carried out in a sloped culture medium of nutrient agar, and then the culture was washed with a ringer solution and the concentrated microbial suspension was diluted with ringer until the opacity of the solution was equal with opacity of 0.5 McFarland solution (optical absorption of 0.132 and an optical transmission rate of 74.3 at wavelength of 600 nm).

The study of antimicrobial effect of films

In order to investigate the antimicrobial effect of poly lactic acid silicon dioxide nanocomposite film on the studied bacteria, surface culture and spherical slices of the film were used. For this purpose, 0.1 ml of microbial suspension of each bacterium equal to 0.5 McFarland is prepared under sterile conditions and under the hood and while transferring to the Muller Hinton Agar culture medium by L-bar, is distributed at the surface of the culture medium. Then, spherical slices of films with concentrations of 0%, 1%, 3% and 5% silicon dioxide with a diameter of 5 mm were placed on the culture. Culture media (3 replicates for each bacterium) with spherical slice of films (1 slice in each culture medium) were placed in oven at 37 °C and after 24 hours, the diameter of inhibition halo of growth as an indicator of antimicrobial activity of films were measured using digital caliper with a precision of 0.01 millimeters. To ensure the uniform growth of bacteria on a plate surface, a cultured plate without film was considered for each of the tested bacteria. Also, a plate without bacterium was used to ensure non-contamination of the culture media.

Water vapor permeability test

The water vapor permeability was measured using the ASTM E96 standard. For this experiment, glass cups with an inner diameter of 3 cm and a height of 3.5 cm were used. 10cc deionized water was spilled in each cup and it can create a 100% humidity in the interior of the cup, so that the inside humidity of the cup increases beyond outside humidity. At first, pieces of film were cut off from the healthy parts of the film in size of the external mouth of the cup. Three replicates were prepared from each sample. 10cc deionized water were poured into the each cup and the films were placed on the outer mouth of the cup and sealing was done using paraffin and the film was fixed on the cup. There is a gap between the films and the water level inside the cup. Then the cups were placed inside the desiccator containing active silica gel .Prior placing the cups into the desiccator, weighing was done and recorded as the initial weight. Then the cups were placed in the desiccator and weighing was done every 2 hours. And therefore 7 weights was recorded for each cup. After finishing the work, the thickness of each film was accurately measured at 5 random points and used to perform the calculations. The amount of weight loss of the cup is equal to the amount of water passed through the film. To calculate the permeability to water vapor, at first curve of the water passing through the film was plotted in a time unit and the curve slope was calculated in curve linear part ($\Delta m/\Delta t$(. Then the obtained value is divided by the area of the film. This value is water vapor transmission rate (WVTR) (equation2). Equation1 show the slope of the curve in linear part. The linear slope is water vapor transmission rate.

$$\text{Slope of curve} = \Delta m/\Delta t \qquad (1)$$

Then the value of WVTR is calculated by equation 2.

$$\text{WVTR} = (\Delta m/\Delta t)/\,A = \textit{(slope of curve) / (area of film)} \qquad (2)$$

In order to obtain WVP according to Equation 3, the WVTR value is multiplied by the mean thickness of the film (Ɣ) and the resulting value is divided by the pressure difference in water vapor on the two sides of the film.

$$\text{WVP} = ((WVTR \times Ɣ))/\Delta p \qquad (3)$$

In the other words, WVP value can be obtained by equation 4.

$$\text{WVP} = ((\Delta m/\Delta t)/A \times Ɣ)/\Delta p \qquad (4)$$

Oxygen Permeability test

The rate of permeability to oxygen was measured according to ASTM D 3985 standard and it was calculated by multiplying the amount of oxygen transfer rate (OTR) in uniform flow mode, in the average film thickness and divided it into pressure difference between two levels. Oxygen transfer rate is the amount of oxygen gas that passes through the film thickness at a given time. Before the test, the films were immersed in a desiccator containing a saturated solution of magnesium nitrate at 25 °C (relative humidity 55%). The test sample is installed in a way that separates the sides of the test chamber. One of the levels of the film is in contact with the nitrogen atmosphere and its other surface is in contact with oxygen. A barometer sensor, mounted on the nitrogen atmosphere, measures the amount of oxygen passing through the atmosphere in the nitrogen atmosphere. When the oxygen concentration at the nitrogen atmosphere reaches a constant extent, the test is complete.

Statistical analysis

The software used to analyze the data was SPSS software package and Excel spreadsheet under Windows. Significant difference ($P<0.05$) was evaluated by ANOVA. Tukey test was used to confirm the existence of a significant difference between the means.

RESULTS AND DISCUSSION

The effect of Poly (Lactic Acid)/ Silicon Dioxide nanocomposite Films on Escherichia coli

The amount of inhibitory activity of films was measured based on the diameter of the halo formed around the spherical slice of films. The greater the antimicrobial property of the film, the diameter of the halo formed around the film is more. In this test, the diameter of the control film inhibition was zero, which indicates that pure poly lactic acid films do not have antimicrobial activity ($p>0.05$). The results of the study of the effects of films containing 1.3 and 5% silicon dioxide on E. *coli* are shown in (Table 1). The experiments were also carried out in 3 replications and the mean values are as follows. The mean diameter of the inhibition halo of the growth in the poly lactic acid film containing silicon dioxide 1% with a value of 8.3 mm has the lowest and the poly lactic acid film containing 5% silicon dioxide with a value of 10 mm has the highest value. All three percent has inhibitory effect and increasing the percentage of silicon dioxide results in a significant increase in its inhibitory effect on E. *coli* growth ($p <0.05$).

The effect of Poly (Lactic Acid)/ Silicon Dioxide nanocomposite Films on Staphylococcus aureus

The results of the study of the effects of films containing 1, 3 and 5% silicon dioxide on Staphylococcus *aureus* are shown in (Table 1). The experiments were also carried out in 3 replications. As shown in the table below, the mean diameter of the inhibition halo of growth in the poly lactic acid film containing silicon dioxide 1% with value of 13.6 mm has the lowest and the poly lactic film containing 5% silicon dioxide with value of 15 mm has the highest value. All three percent has inhibitory effect and increasing the percentage of silicon dioxide results in a significant increase in its inhibitory effect on Staphylococcus *aureus* growth ($p <0.05$).

In this study, the highest inhibition halo of growth was observed around Staphylococcus *aureus* and the lowest inhibition halo of growth was observed around E. *coli* bacteria. According to these results, the antimicrobial effect of poly lactic acid containing silicon dioxide-containing film on gram-positive bacteria relative to bacteria Gram-negative was more due to the presence of outer membranes in bacteria gram-negative containing hydrophilic lipopolysaccharides.

The results of research by Hosseini and et al showed that gram negative bacteria are more resistant to zinc oxide than gram positive bacteria due to the structural difference of the cell wall of these two groups of bacteria. The main composition of cell wall of the germ positive bacteria is peptidoglycan and small amount of protein, but the cell wall of bacteria gram-negative in spite of less thickness have more complexity and in addition to peptidoglycan contain various polysaccharides, protein and lipids [14]. Also, the cell wall of the bacteria gram-negative has an outer membrane (which covers the outer surface of the wall) and a periplasmic space that is not seen in gram-positive [15]. The hydrophilic surface of the gram-negative membrane, rich in lipopolysaccharide molecules, creates a barrier against the release of antibiotic molecules (natural or synthetic), and enzymes in their periplasmic space can break down the molecules entered into this space from the outside [16] which causes their resistance to antibacterial agents. While

Table 1. Diameter of inhibition zone (mm) in Poly-Lactic Acid Films Silicon Dioxide with various nanocomposite on E. *coli* and Staphylococcus *aureus*

Sample	Diameter of inhibition zone(mm)	
	E. coli	Staphylococcus *aureus*
PLA	-	-
1%PLA/SiO_2	0.3 ±8.3	13.6±0.6
3%PLA/SiO_2	0.3 ± 9.6	14±0.5
5%PLA/SiO_2	0.5 ± 10	15±1.5

gram-positive bacteria lack such a membrane and wall and therefore antibacterial agents (such as phenols) easily destroy the cell wall to damage membrane proteins, interferes with membrane enzyme, secrete cellular compositions, coagulate the cytoplasm and damage the bacteria [17]. All these factors increase the resistance of gram-negative bacteria to gram-positive bacteria.

Water Vapor permeability

Water vapor permeability (WVP) of the film has significant effect on storage life of foodstuff. It is a scale for measuring humidity transport through the material. Ability to control missing water molecule from the product for the film is an important property that is effective on quality of end product [18]. Results obtained of WVP experiments is presented in Fig 1. The experiments were done in 5 replicate and the means values is as follow. As it can see in Fig. 1, the mean of water vapor permeability for the poly lactic acid film containing silicon dioxide 5% with value of 2.21 has the lowest and for the poly lactic film without silicon dioxide with value of 5.12 has the highest value. It shows that increasing the percent of silicon dioxide lead to decrease water vapor permeability.

Oxygen permeability

In addition to WVP, oxygen permeability is one of the most important properties of polymer of food packaging, because the presence of oxygen is an important factor in creating reactions such as oxidation and rancidity of lipids, help to growth of the micro-organisms, etc. This indicator is determined by the devices designed for this purpose. As it can see in the (Fig. 2), the mean of oxygen permeability for the poly lactic acid film containing silicon dioxide 5% with value of 96.26 has the lowest and for the poly lactic film without silicon dioxide with value of 215.14 has the highest value. It shows that increasing the percent of silicon dioxide lead to decrease oxygen permeability.

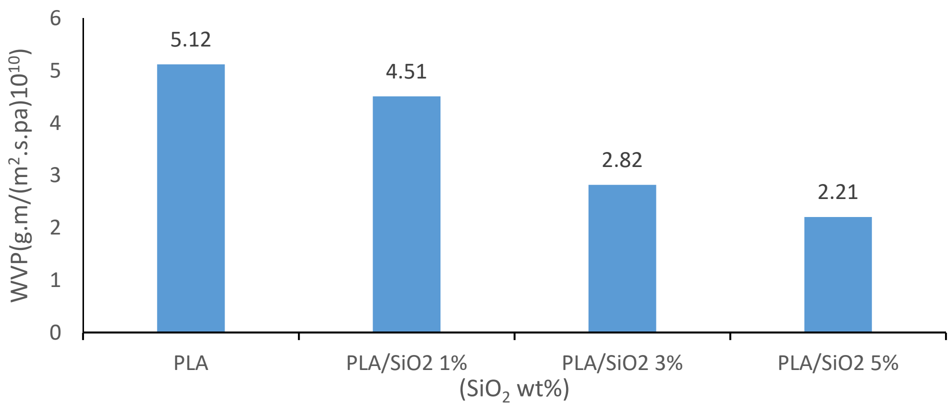

Fig1. The mean of water vapor permeability in in poly lactic acid silicon dioxide nanocomposite

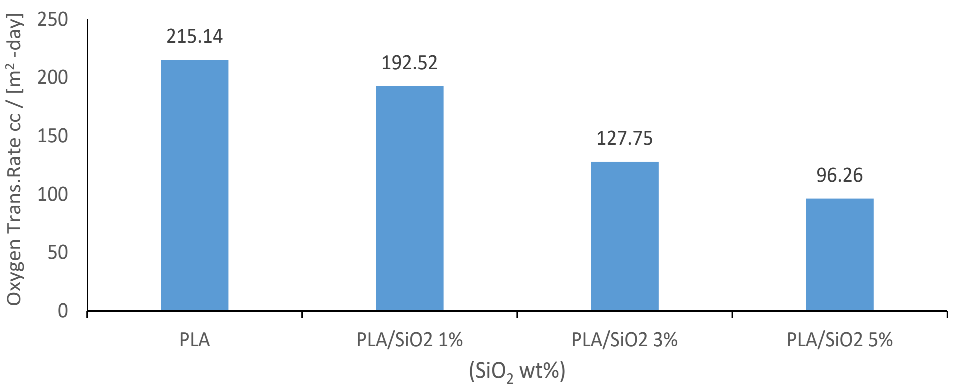

Fig. 2. The mean of oxygen permeability in in poly lactic acid silicon dioxide nanocomposite

CONCLUSION

Comparison of the mean diameter of inhibition halo of growth of Escherichia *coli*, the poly lactic acid 3% silicon dioxide nanocomposite film with the poly lactic acid film 5% silicon dioxide nanocomposite show that that the mean of these two groups is not significant (P> 0.05), While the other groups compared with each other, indicate that the mean diameter of the inhibition halo of growth of Escherichia *coli* bacteria is significant between these groups (P <0.05).

Also in comparison of mean diameter inhibition halo of growth of Staphylococcus *aureus*, the poly lactic acid 1% silicon dioxide nanocomposite film with the poly lactic acid 3 and 5% silicon dioxide nanocomposite film show that the mean of these two groups is not significant. Also the comparison of two group poly lactic acid films 3% silicon dioxide nanocomposite film with the poly lactic acid 5% silicon dioxide nanoparticle show that the mean of these two groups is not significant (p<0.05). While the other groups compared with each other, indicate that the mean diameter of the inhibition halo of growth of Staphylococcus *aureus* is significant between these groups (P <0.05). Comparison the water vapor and oxygen permeability of pure poly lactic acid with poly lactic acid with silicon dioxide nanoparticle 1%,3% and 5% show that the means of the films is not significant (p>0.05), while the comparison of means of other percent with each other is significant (p<0.05).

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CONFLICTS OF INTEREST

The author declare that there is no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Lunt J. Large-scale production, properties and commercial applications of polylactic acid polymers. Polymer Degradation and Stability. 1998;59(1-3):145-52.
2. Drumright RE, Gruber PR, Henton DE. Polylactic Acid Technology. Advanced Materials. 2000;12(23):1841-6.
3. Garlotta, D., *A literature review of poly (lactic acid).* Journal of Polymers and the Environment, 2001. **9**(2): p. 63-84.
4. Jamshidian M, Tehrany EA, Imran M, Jacquot M, Desobry S. Poly-Lactic Acid: Production, Applications, Nanocomposites, and Release Studies. Comprehensive Reviews in Food Science and Food Safety. 2010;9(5):552-71.
5. Ahmed J, Varshney SK. Polylactides—Chemistry, Properties and Green Packaging Technology: A Review. International Journal of Food Properties. 2011;14(1):37-58.
6. Neethirajan S, Jayas DS. Nanotechnology for the Food and Bioprocessing Industries. Food and Bioprocess Technology. 2010;4(1):39-47.
7. Weiss J, Takhistov P, McClements DJ. Functional Materials in Food Nanotechnology. Journal of Food Science. 2006;71(9):R107-R16.
8. Tabari M. Characterization of a new biodegradable edible film based on Sago Starch loaded with Carboxymethyl Cellulose nanoparticles. Nanomedicine Research Journal, 2018. 3(1): 25-30, Winter 2018.
9. Chaudhry Q, Scotter M, Blackburn J, Ross B, Boxall A, Castle L, et al. Applications and implications of nanotechnologies for the food sector. Food Additives & Contaminants: Part A. 2008;25(3):241-58.
10. Silvestre C, Cimmino S, Pezzuto M, Marra A, Ambrogi V, Dexpert-Ghys J, et al. Preparation and characterization of isotactic polypropylene/zinc oxide microcomposites with antibacterial activity. Polymer Journal. 2013;45(9):938-45.
11. Marra, A., Biodegradable PLA composites with different fillers for food packaging application. 2015.
12. Azeredo HMCd. Nanocomposites for food packaging applications. Food Research International. 2009;42(9):1240-53.
13. Wu G, Liu S, Jia H, Dai J. Preparation and properties of heat resistant polylactic acid (PLA)/Nano-SiO2 composite filament. Journal of Wuhan University of Technology-Mater Sci Ed. 2016;31(1):164-71.
14. Hosseini MH, Razavi SH, Mousavi MA. Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. Journal of Food Processing and Preservation. 2009;33(6):727-43.
15. Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology. 2003;80(3):223-30.
16. Shan B, Cai Y-Z, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology. 2007;117(1):112-9.
17. Kalemba D, Kunicka A. Antibacterial and Antifungal Properties of Essential Oils. Current Medicinal Chemistry. 2003;10(10):813-29.
18. Li XH, Xing YG, Li WL, Jiang YH, Ding YL. Antibacterial and Physical Properties of Poly(vinyl chloride)-based Film Coated with ZnO Nanoparticles. Food Science and Technology International. 2010;16(3):225-32.