Journal of Chemical Health Risks 3(3): 21-28, 2013
ISSN:2251-6719

Antimicrobial and Barrier Properties of Bovine Gelatin Films Reinforced by Nano TiO_2

R. Nassiri, A. Mohammadi Nafchi*

Biopolymer Research Group, Food Science and Technology Division, Agriculture Department, Damghan Branch, Islamic Azad University, Damghan, Semanan, Iran

(Received: 25 June 2013 Accepted: 30 July 2013)

Abstract: The effects of nano titanium dioxide incorporation were investigated on the water vapor permeability, oxygen permeability, and antimicrobial properties of bovine gelatin films. The nano TiO_2 (TiO_2-N) was homogenized by sonication and incorporated into bovine gelatin solutions at different concentrations (e.g. 1, 2, 3, and 5% w/w of dried gelatin). The permeability of the films to water vapor and oxygen was significantly decreased by incorporating of low concentration TiO2-N to gelatin solutions. TiO_2-N gelatin films showed an excellent antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. These properties suggest that TiO_2-N has the potential as filler in gelatin-based films for using as an active packaging materials in pharmaceutical and food packaging industries.

Keywords: Nanoparticle, Gelatin film, WVP, Oxygen permeability, Antimicrobial Properties

INTRODUCTION

In recent decade, the demand for such environmental friendly polymers is growing and has been focus of many researcher efforts. Sustainable and biodegradable packages are being developed worldwide and make great efforts to produce the packaging material of natural origin (proteins, fats and carbohydrates) to take films or coatings. In comparison, these biopolymers have less adverse effect on environment than synthetic plastic.

Nanoscale science and other related technologies have been the leading technology for the past decade [1]. Incorporation of nanoparticles into composite materials has attracted a great deal of attention due to its ability to enhance polymer properties such as thermal, mechanical, and gas barrier [2].

Inorganic materials due to their ability in harsh process conditions, such as metal or metal oxides have attracted a great deal of attraction recently [3]. ZnO, TiO2, MgO, and CaO among the inorganic materials have particular interesting due to both safe material for animals and human and stability under harsh condition processes [4, 5].

Biopolymers have advantages over the synthetic polymers; biopolymers are biodegradable and renewable materials. Inhibitory to volatile compounds and oils maintain the quality of the food in production and distribution cycle. However, poor mechanical properties and high permeability to water vapor are two main disadvantages of biopolymers that recently nanotechnology helps to solve these problems.

There are some reports about the improvements of

Corresponding Author: R. Nassiri, Biopolymer Research Group, Food Science and Technology Division, Agriculture Department, Damghan Branch, Islamic Azad University, Damghan, Semanan, Iran. Email: nassiri26@yahoo.com

biopolymer by incorporation of nano particles, such as nano zinc oxide [6-8], nano silicon dioxide [9, 10] and nano titanium dioxide [11, 12]. All of mentioned researchers reported that incorporation of nanoparticles improve mechanical properties, as well as barrier and antimicrobial properties of biopolymers.

Bionanocomposites as a new generation of nanocomposites represent the combination of a biopolymer and an inorganic material that shows at least one nanometer scale dimension. These generations of biocomposites have more desirable functional properties like good mechanical strength and low water vapour permeability. Increasing the barrier properties against gases, increasing efficiency in the use of film as an active packaging, increasing heat resistance and transparency and improving appearance of bionanocomposites are other advantages [13, 14].

Titanium dioxide has been widely used as functional filler in UV-absorbers for application in cosmetics, pharmaceutical materials, pigments, and coating materials, [15]. In addition, the use of titanium dioxide nanoparticles is considered to be a viable method for the prevention of infectious diseases through the antimicrobial effects of zinc oxide [12, 16].

MATERIALS AND METHODS

Bovine gelatin (Type B) and nano titanium dioxide were purchased from Sigma Chemical Co (St. Louis, MO, USA). Liquid sorbitol and glycerol were obtained from Liang Traco (Penang, Malaysia). All chemicals were of analytical grade. Environmental scanning electron microscopy (ESEM) (Fig. 1) reveals that TiO_2-N has a dimension in nanometer.

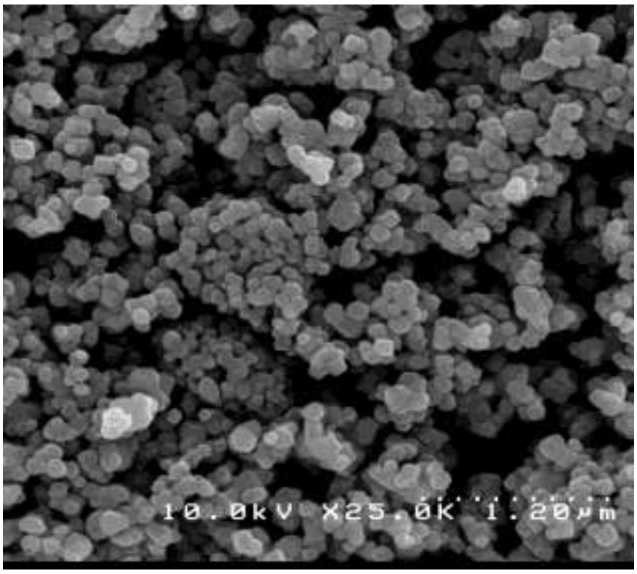

Figure 1: ESEM micrograph of nano TiO_2

Film preparation

TiO_2-N was dispersed in water at different concentrations (1%, 2%, 3%, and 5%; w/w of total solid), stirred for 1 h, and then sonicated in an ultrasonic bath (Marconi model, Unique USC 45 kHz, Piracicaba, Brazil) for 30 min to ensure homogenization was completed. The solution was used to prepare the aqueous gelatin dispersion at 8% (w/w). A mixture of sorbitol and glycerol (3:1) at 40% (w/w) of total solid was added as plasticizer in accordance with Abdorreza and his colleagues (2011) [17]. Gelatin nanocomposites were heated to 58±2 °C to prevent denaturation of proteins and held for 1 h. A portion (45 g) of the dispersion was cast on plates fitted with rims around the edge to yield a 16×16 cm^2 film-forming area. Films were dried under controlled conditions in a humidity chamber (25 °C and 50% RH). Control films were prepared similarly but without addition of nanoparticles. Dried films were peeled and stored at 23 ± 2 ºC and 50 ± 5% relative humidity (RH) until experimentation.

Water vapor permeability

Water vapor permeability (WVP) tests of the films were carried out following the modified method [18] of ASTM standard E96-05 [19].The test cups were filled with water 1.5 cm below the film. A plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapour transmission through the film per unit time (g/h). Six samples per treatment were tested. The slopes yielded regression coefficients of 0.99 or greater. The WVP of film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapour pressure difference across the film.

Oxygen permeability (OP)

Oxygen permeability measurements were performed on films with Mocon Oxtran 2/21 (Minneapolis, USA) equipped with a patented colometric sensor (Coulox®) and WinPermTM permeability software. Measurements were done using the ASTM standard method D3985-05 [20]. The films were placed on an aluminum foil mask with an open area of 5 cm^2 and were mounted in diffusion cells. Tests were carried out at 25ºC temperature, atmospheric pressure, and 50% RH using 21% oxygen as test gas. Transferred oxygen through the films was conducted by the carrier (N_2/H_2) gas to the colometric sensor. Measurements were carried out on "convergent by hour" mode to reach the steady state of oxygen transmission. The permeability coefficients in cc-μm/(m^2 day atm) were calculated on the basis of oxygen transmission rate in steady state taking into account the films thickness.

Antimicrobial assay

Antimicrobial activity test on the films was carried out using the agar diffusion method according to Maizura and his colleagues (2007) [21]. Antimicrobial effects of the films were determined by inhibition zone against *E. coli* and *S. Aureus* on solid media.

Statistical analysis

ANOVA and Duncan's Post Hoc tests were used to compare means of barrier and antimicrobial properties of gelatin based films at the 5% significance level. Statistical analysis was conducted using GraphPad Prism 6 (GraphPad Software Inc., 2236 Avenida de la Playa, La Jolla, CA 92037, USA).

RESULTS AND DISCUSSION

Effects of nano-TiO_2 on water vapour permeability

The results of WVP studies are presented in Table 1. The significant decrease in WVP after the addition of TiO_2-N may be attributed to the greater water resistance of TiO_2-N compared with the bio composite matrix, so that the incorporation of these nanoparticles to the matrix introduces a tortuous pathway for water vapor molecules to pass through [18].

Table 1: Water vapor permeability (WVP) of bovine gelatin nanocomposites

TiO_2-N (%)	**WVP × 10^{11} [g m^{-1} s^{-1} Pa^{-1}]**
0	8.90±0.31a
1	7.10±0.33b
2	3.98±0.45c
3	2.62±0.33d
5	1.61±0.13e

Values are mean (n=5) ± SD. Different letters in WVP column values represent significant difference at 5% level of probability among bovine gelatin films.

Effects of nano-TiO_2 on oxygen permeability

The results of oxygen permeability are presented in Table 2. Oxygen permeability coefficient significantly decreased by addition of TiO_2-N. The inorganic TiO_2-N particles are more water resistance than the biocomposite matrix; therefore incorporation of these nanorods to the matrix could likely introduced a tortuous pathway for oxygen molecules to pass through [18]. Based on the Nielsen's [22] simple model on tortuosity, it is possible to describe the permeability reduction in TiO_2-N incorporated biocomposites. He proposed that each layer of filler particles be positioned perpendicularly oriented to the diffusion pathway. The reduction in permeability coefficients means that oxygen should travel in a longer diffusive path.

Table 2: Oxygen permeability (OP) of bovine gelatin nanocomposites

TiO_2-N (%)	**O.P [cm^3 $\mu m/(m^2$ -day)]**
0	214.41±5.12a
1	201.11±4.01b
2	157.16±4.77c
3	128.72±2.45d
5	95.72±4.16e

Values are mean (n=5) ± SD. Different letters in OP column values represent significant difference at 5% level of probability among bovine gelatin films.

Effects of nano-TiO_2 on antimicrobial activity

Effects of sample films on the growth of *E. coli* and *S. Aureus* were investigated. Figure 2 shows the effects of TiO_2-N contents on antimicrobial activity of different films against *E. coli* and *S. Aureus*. The inhibition zone of control and nano-incorporated films significantly was increased by increasing the TiO_2-N contents. Excellent antimicrobial activity of TiO_2 nanoparticles and the mechanism of the action against the microorganisms have also been demonstrated by other researchers [6, 8, 23-25]. Mechanisms of the antibacterial behavior of TiO_2 have categorized as chemical and/or physical interaction between TiO_2 particles and the cell envelope of microorganism. The Ti^{4+} could penetrate through the cell wall of microorganism and react with interior components that finally affects on viability of the cells [11, 12]. Another possible mode of action is generating of H_2O_2 due to presence of TiO_2 nano particles [26].

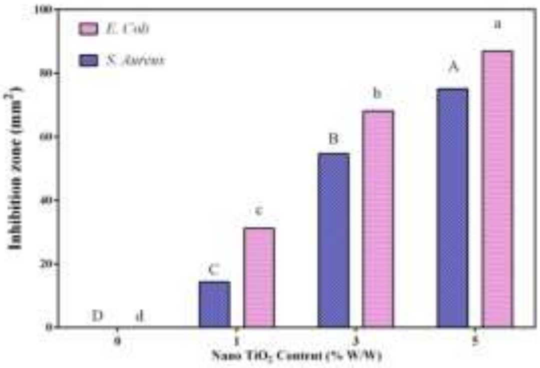

Figure 2: Effects of TiO2-N contents on antimicrobial activity bovine gelatin nanocomposite films against *S. Aureus*. and *E. Coli*. Inhibition zone = total inhibition area – total film area. The bars show mean (n=5) ± SD. Different letters on the bars represent the significant difference at 5% level of probability.

CONCLUSION

In this study, we introduced nano-TiO_2 to the bovine gelatin matrix to fabricate bionanocomposites. By incorporating low level of the filler (~2%), a huge differences between properties was observed especially in water vapour permeability and oxygen permeability of the gelatin films. Nano-TiO_2 incorporated films shows excellent antimicrobial activity against *S. aureus* and *E. coli*. The results showed that under strict regulation, bionanocomposites based on nano-TiO_2 may have potential applications in the medical, pharmaceutical, and food packaging industries.

REFERENCES

1. Narayanamurti V., Frontiers in Nanoscience and Technology in the 21st Century and New Models for Research and Education at the Intersection of Basic Research and Technology. *ASME Conference Proceedings*, *2006*. 1203-1204.

2. Kurian M., Dasgupta A., Galvin M. E., Ziegler C. R., Beyer F. L., A Novel Route to Inducing Disorder in Model Polymer-Layered Silicate Nanocomposites. *Macromolecules*. 2006, *39*, 1864-1871.

3. Fu L., Liu Z., Liu Y., Han B., Hu P., Cao L., Zhu D., Beaded Cobalt Oxide Nanoparticles along Carbon Nanotubes: Towards More Highly Integrated Electronic Devices. *Advanced Materials* , 2005. *17*, 217-221.

4. Stoimenov P. K., Klinger R. L., Marchin G. L., Klabunde, K. J., Metal Oxide Nanoparticles as Bactericidal Agents. *Langmuir,* 2002. *18*, 6679-6686.

5. Lin W., Xu Y., Huang C. C., Ma Y., Shannon K., Chen D. R., Huang Y. W., Toxicity of nano- and micro-sized ZnO particles in human lung epithelial cells. *Journal of Nanoparticle Research*, 2009. *11*, 25-39.

6. Nafchi A. M., Nassiri R., Sheibani S., Ariffin F., Karim A. A., Preparation and characterization of bionanocomposite films filled with nanorod-rich zinc oxide. *Carbohydrate Polymers,* 2013. *96*, 233-239.

7. Alebooyeh R., Mohammadi Nafchi A., Jokar M., The Effects of ZnO nanorods on the Characteristics of Sago Starch Biodegradable Films. *The Journal of Chemical Health Risks* , 2012. *2*, 13-16.

8. Nafchi A. M., Alias A. K., Mahmud S., Robal M., Antimicrobial, rheological, and physicochemical properties of sago starch films filled with nanorod-rich zinc oxide. *Journal of Food Engineering,* 2012. *113*, 511–519.

9. Voon H., Bhat R., Easa A., Liong M. T., Karim A. A., Effect of Addition of Halloysite Nanoclay and SiO2 Nanoparticles on Barrier and Mechanical Properties of Bovine Gelatin Films. *Food Bioprocess Technol,* 2012. *5*, 1766-1774.

10. Torabi Z., Mohammadi Nafchi A., The Effects of SiO2 Nanoparticles on Mechanical and Physicochemical Properties of Potato Starch Films. *The Journal of Chemical Health Risks* , 2013. *3*, 33-42.

11. Chawengkijwanich C., Hayata Y., Development of TiO2 powder-coated food packaging film and its ability to inactivate Escherichia coli in vitro and in actual tests. *International Journal of Food Microbiology,* 2008. *123*, 288-292.

12. Yao K. S., Wang D. Y., Ho W. Y., Yan J. J., Tzeng K. C., Photocatalytic bactericidal effect of TiO2 thin film on plant pathogens. *Surface and Coatings Technology,* 2007. *201*, 6886-6888.

13. Darder M., Aranda P., Ruiz-Hitzky E., Bionanocomposites: A New Concept of Ecological, Bioinspired, and Functional Hybrid Materials. *Advanced Materials,* 2007. *19,* 1309-1319.

14. Ozin G. A., Arsenault A. C., Cademartiri L., *Nanochemistry : a chemical approach to nanomaterials*. Royal Society of Chemistry: Cambridge, 2009.

15. Kumar A. P., Singh R. P., Biocomposites of cellulose reinforced starch: Improvement of properties by photo-induced crosslinking. *Bioresource Technology,* 2008. *99*, 8803-8809.

16. Liu H. L., Yang T. C. K., Photocatalytic inactivation of Escherichia coli and Lactobacillus helveticus by ZnO and TiO2 activated with ultraviolet light. *Process Biochemistry,* 2003. *39*, 475-481.

17. Abdorreza M. N., Cheng L. H., Karim A. A., Effects of plasticizers on thermal properties and heat sealability of sago starch films. *Food Hydrocolloids,* 2011. *25*, 56-60.

18. Yu J., Yang J., Liu B., Ma X., Preparation and characterization of glycerol plasticized-pea starch/ZnO-carboxymethylcellulose sodium nanocomposites. *Bioresource Technology,* 2009. *100*, 2832-2841.

19. ASTM, Standard Test Methods for Water Vapor Transmission of Materials E96/E96M-05. In *Annual Book of ASTM Standards*, Philadelphia, PA, 2005.

20. ASTM, Standard Test Methods for Oxygen Gas Transmission Rate Through Plastic Film and Sheeting Using a Coulometric Sensor D 3985 -05. In *Annual Book of ASTM Standards*, Philadelphia, PA, 2005.

21. Maizura M., Fazilah A., Norziah M. H., Karim A. A., Antibacterial Activity and Mechanical Properties of Partially Hydrolyzed Sago Starch–Alginate Edible Film Containing Lemongrass Oil. *Journal of Food Science,* 2007. *72,* C324-C330.

22. Nielsen L. E., Models for the Permeability of Filled Polymer Systems. *Journal of Macromolecular Science: Part A – Chemistry,* 1967. *1*, 929 - 942.

23. Li J. H., Hong R. Y., Li M. Y., Li H. Z., Zheng Y., Ding J., Effects of ZnO nanoparticles on the mechanical and antibacterial properties of polyurethane coatings. *Progress in Organic Coatings,* 2009. *64*, 504-509.

24. Li X. H., Xing Y. G., Li W. L., Jiang Y. H., Ding Y. L., Antibacterial and Physical Properties of Poly(vinyl chloride)-based Film Coated with ZnO Nanoparticles. *Food Science and Technology International,* 2010. *16*, 225-232.

25. Zhang L., Ding Y., Povey M., York D., ZnO nanofluids - A potential antibacterial agent. *Progress in Natural Science,* 2008. *18*, 939-944.
26. Zhang L., Jiang Y., Ding Y., Daskalakis N., Jeuken L., Povey M., O'Neill A., York D., Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against <i>E. coli</i>. *Journal of Nanoparticle Research* 2010, *12*, 1625-1636.