Effect of polylactic acid-silver nanoparticle nanocomposite on the kinetics of *Escherichia coli* growth

Samira Molapour Rashedi[1], Mersedeh Fatemeh Yazdan Bakhsh[2], Fahimeh Karimi[3], Mehras Yazdan Bakhsh[4]

1-PhD student in Textile Chemical Engineering, Islamic Azad University, Science and Research Branch, Tehran, Iran

2- Professor at the University of Applied Sciences - Jihad University Karaj - Iran and CEO at the Company of Dara Cellulose Nita Karaj-Iran

3- Doctor of Chemical and Physical Engineering, Islamic Azad University, Tehran Branch and Labor Inspector, Karaj-Iran

4- Master of Mining Engineering, Islamic Azad University of Tehran, South Iran and Director (R&D) at the Company of Dara Cellulose Nita Karaj-Iran

rashedisamira@yahoo.com, yazdanbakhsh1800@gmail.com, fahimehkarimi1388@gmail.com, mehras.yazdanbakhsh@gmail.com

ABSTRACT:

Nowadays the application of biodegradable materials in food packaging has gained increasing attention due to their high potentials. These materials exhibit lower resistance against gases and water permeation and are mechanically weaker. One of the approaches to resolve these problems in their composition with other useful substances. In this study, polylactic acid/silver nanocomposites (0.5 and 2 wt.% nanosilver) were prepared by the solvent casting method. The antimicrobial effect of these films on the growth parameters of Escherichia coli (a Gram-negative bacterium) was investigated by a Solvent casting test using Gompertz and Logistic models which indicated a 4.3% increase in the lag phase duration and a 30.9% decline in the E. coli growth rate as well as 32.6% reduction in the final population of this bacterium. It was also observed that an increment in the silver nanoparticle percentage can enhance the antimicrobial activity of this nanocomposite. A new peak was observed in the FTIR spectrum of the silver nanoparticle-containing nanocomposite at 3620 cm^{-1} for all nanoparticle contents (0.5 and 2 wt.%) which can be assigned to the chemical interactions between silver nanoparticles and polylactic acid. XRD patterns also indicated the peaks related to Ag(111), Ag(200), Ag (220), and Ag (311) representing the presence of crystalline silver nanoparticles.

Keywords: Escherichia coli, polylactic acid, antimicrobial activity, silver nanoparticles, nanocomposite

INTRODUCTION:

Silver nanoparticles (NPs) have gained a significant position in the improvement of antimicrobial properties of textile and paper-based products. Silver NPs are toxic to pathogens. Various mechanisms have been found explaining the mechanisms of action of the silver on microbes [1-2]. Thanks to these diverse mechanisms, microbes failed to develop resistance against Nanosilver. Some of these mechanisms are:

1- Reactive oxygen generation: this mechanism holds for most of the Nanosilver composites placed on semiconductor supports such as TiO_2 and SiO_2. Under such conditions, the particles serve like an electrochemical cell and produce O^{2-} and OH^- ions through oxidizing oxygen atom and water hydrolysis. Both these ions are among the most reactive antimicrobial agents [3-4].

2- Metallic Nanosilver particles can gradually release silver ions which can convert SH- bonds at the microorganism wall to SAg by substitution reactions which will ultimately lead to the destruction of microorganisms. Silver NPs destabilize the membrane plasma which can decline the adenosine triphosphate (ATP) level and end up in the bacterium death [5-6].

3- Silver NPs adhere to the cell membrane and disturb the ordinary functions of the cell such as respiration and substance transport. This hypothesis relies on the high efficiency of nanoparticles as an increase in the nanoparticles level can enhance the adhesion to the cell surface

and hence augment the bactericidal efficiency [7-8].

4- In addition to the cell surface, silver NPs can diffuse into the cell. By bonding with P and S which can be found in the cell components such as DNA, they can kill and destroy the microbe.

Concerning the antibacterial mechanism of silver, the transformation of micron-sized silver particles to nanosilver will enhance the mentioned effects due to an increment in the specific surface and the quantum effects at the nano scale. At higher dimensions, silver is a metal with low toxicity. But when shrinks to the nanometer realm, its bactericidal effect will increase by 99%. Such an increase can be exploited in wound healing and the treatment of infections. So far, silver NPs and their derivatives have been approved to be used as an antibacterial agent in laminated fiber plate [9], fabrics [10], nylon and silk fabrics [11], and polypropylene and polyester synthetic fibers [12]. Silver can be also used in the production of active nanocomposites for packing purposes due to its reinforcing effects on the physicochemical properties of biopolymers. Bio-nanocomposites are a new generation of nanocomposites which contain a natural polymer in combination with inorganic substances in a way that at least the dimension of one component is at the nanoscale, this means that in addition to having nano-scaled components, they possess bioactive compounds which can be decomposed to their constituent subunits at the environment [13-14].In this regard, various studies have been conducted to develop biopolymers from renewable resources. Polylactic acid (PLA) is one of these biodegradable polymers. PLA is derived from lactic acid obtained from the fermentation of raw carbohydrates such as corn, wheat, molasses, and other starch-rich substances. Its monomer is lactic acid from carbohydrate sources. PLA is a relatively hard polymer with Tg of 60-70 ℃ and a melting point of 170-180℃. This polymer is biodegradable and biocompatible. It can be obtained from renewable agricultural sources, hence declining the carbon dioxide emission compared to petroleum-based polymers. This polymer has gained increasing attention due to its higher mechanical and processibility [15-16]. PLA, polycaprolactone, polyglycolic acid, chitosan, alginate, and cellulose are among the biodegradable biopolymers which can replace the synthetic polymers. The building blocks of PLA (lacticacid (2-hydroxy propionic acid)) can be found in D and L states. This polymer can be generated by the fermentation of carbohydrate compounds in the food products and used for the production of thin films for packing purposes. Metallic nanoparticles are among the distinctive groups of nanomaterials which have been employed in various applications such as catalysis, optical sensors, and antibacterial agents [17-18].This study is aimed to investigate the antibacterial properties of silver-PLA nanocomposites prepared through the solvent casting method. The antimicrobial properties of the samples were then tested against *E. coli*. PLA-Ag nanocomposites containing various silver contents were characterized by XRD and FTIR methods. Finally, the absorbance data were modified by Gompertz equations presented by Zwietering et al. (1990) and Logistic methods to estimate and model the microbial growth parameters.

MATERIALS AND METHODS:

Precursors: silver nitrate (AgNO3, 99% purity), sodium boron hydrate (NaBH4), and

Polyvinylpyrrolidone (PVP) with a mean molecular weight of 40000, and PLA (Nature, US), and polyethylene glycol (Merck Germany).To prepare the nanoparticles by chemical reduction method, first, 1,53 g silver nitrate was mixed with 0.933 g Polyvinylpyrrolidone and 250 mL distilled water was added to the mixture, the solution was stirred until complete dissolution. Upon addition of sodium boron hydrate, silver nanoparticles were formed after stirring according to Reaction (1). The colorless solution turned into brown.

$$AgNO_3 + NaBH_4 + 3H_2O \rightarrow Ag + H_3BO_3 + NaNO_3 + 7/2H_2 \quad (1)$$
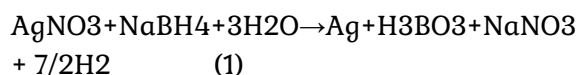
The prepared nanoparticles were separated by centrifugation and washed by distilled water several times followed by drying at 40 °C in an oven for 12 h. in this method sodium boron hydrate and PVP acted as the reductive agent and protective factor, respectively. to prepared the PLA-Ag nanocomposites, the prepared nanoparticles were used as the reinforcement agent and polyethylene glycol was used to prevent agglomeration giving rise to a uniform distribution of silver NPs in the polymer matrix. Then, according to the solvent casting method, PLA solution (5%) was prepared in chloroform. To prepare nanocomposites, a specific amount of silver nanoparticles was added to 10 wt/vol% PLA-PEG solution in chloroform. The AG-PEG solutions were shaken at 1400 rpm. PLA (5%) solution was then added to silver NP and PEG and shaken and sonicated until complete mixture. Finally, the solutions were placed in Petri dishes and dried.(Fig.1)
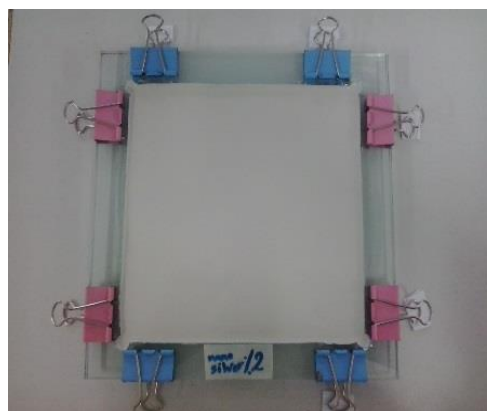

Fig.1: Preparation of PLA/Ag using Nanosilver powder

Antibacterial properties of the nanocomposites were assessed by measuring the growth inhibition zone using the dynamic flask method. For this purpose, a Gram-negative bacterium, E. coli (ATCC 2592) was cultured in a nutrient broth medium and incubated for 24 h (Pars Teb Novin 37℃). After 24 h, the bacterium was cultured on nutrient agar solid culture medium and placed in an incubator at 37 ℃ for 24 h. Then, one colony was added to the sterile distilled water and compared with standard 0.5 MC Farland. The colony adding was continued until reaching optical density corresponding to 0.5 MC

Farland.To evaluate the antimicrobial properties of PLA/Ag nanocomposite, 2 2× 1.5 cm² pieces of the film were completely sterilized under UV irradiation. The film pieces were then placed in any flasks containing nutrient broth medium. afterward, 1 cc of the microbial solution (0.5 Mc Farland) was added to all the flasks except the one considered as the control sample. The absorption of the samples was read at 600 nm using a spectrophotometer (WPA, Germany) starting from the lowest concentration. In this way, the sample absorbance was obtained from the lowest to the highest values. After reading the absorbance of the samples, they were incubated at 37 ℃ and 150 rpm for 1 h using a shaker incubator (Meidolth unimax 1010, Germany). The sample absorbance was then read again. The samples were evaluated in one-hour-intervals. Absorbance data were finally modified by Gompertz equations presented by Zwietering et al. (1990) and the microbial growth parameters were modeled [19].

RESULETS AND DISCUSSIONS:
Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was carried out to identify the bonds and describe the Ag NPs interaction with PLA. In this regard, the chemical bonds of PLA/Ag NPs nanocomposite film were assessed by an FTIR spectrophotometer (Thermo Nicolet 6700, US) I the wavenumber range of 400-4000 cm^{-1} at the accuracy of 4 cm^{-1}.
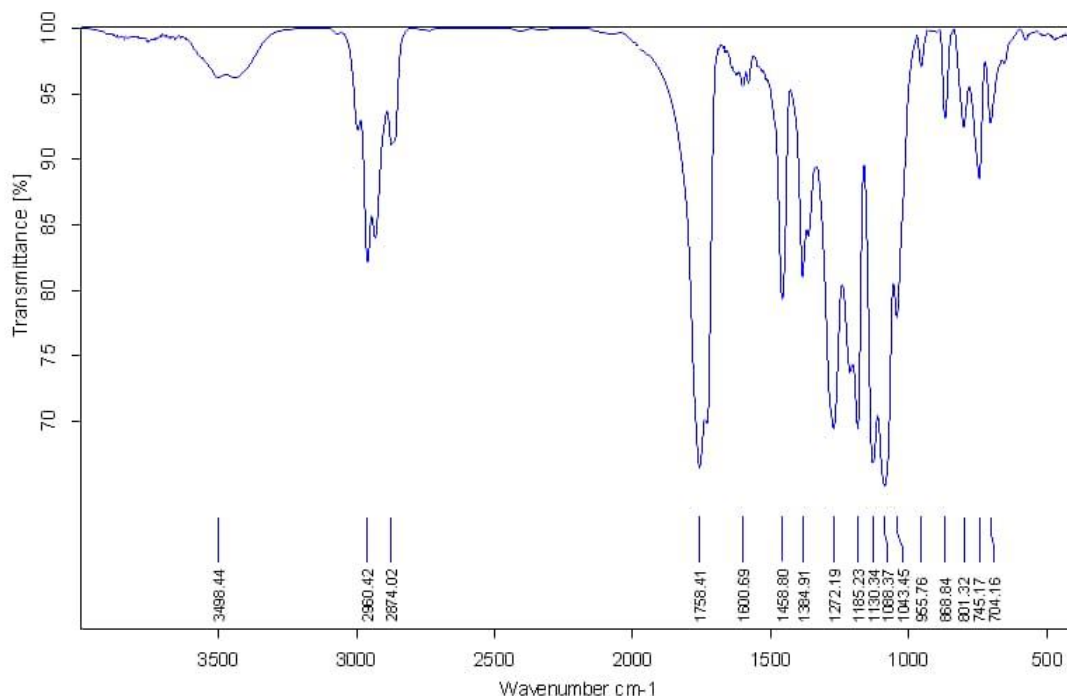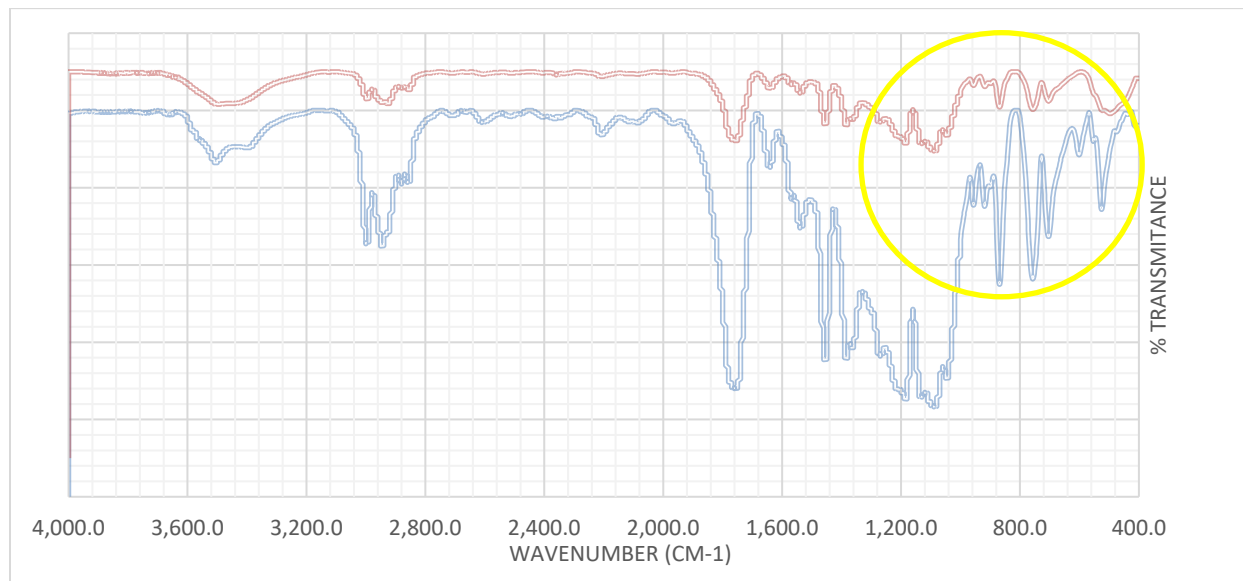

Fig 2. FTIR spectrum for PLA

Fig. 3: FTIR spectra of PLA+0.5%Ag ▬▬ and PLA+2%Ag ▬▬

As shown in Fig.2, the absorption band at 704-995 cm^{-1} can be assigned to the fingerprint signals of the PLA structure backbone. The band emerged at 1043-1185 cm^{-1} shows the stretching vibrations of the C-O bond. While the one at 1384-1450 as well as the band at 2874-2960 cm^{-1} could be attributed to C-H vibration indicating the presence of —CH and —CH_3 groups. The absorption band at 1758 cm^{-1} can be assigned to the vibrations of ester carbonyl groups (-C=O).The results of this analysis are in line with the previous reports [20-22]. Ramus et al. [23] also obtained similar results in evaluating PLA/Ag NP nanocomposite. As mentioned earlier, all the observed bonds were similar to previous studies. based on Fig. 3, it can be said that the collective frequency range of 1200-4000 cm^{-1} (marked by yellow color) exhibited similar patterns in both composites indicating the presence of similar functional groups. Fig. 3 also shows some differences in the fingerprint region of the two composites reflecting their different contents. This difference can be ascribed to the various Ag contents.

X-ray diffraction analysis

The structure of the nanoparticles and nanocomposites was characterized by XRD analysis using an X-ray diffractometer operating with CuKα source at the wavelength of 1.54Å (Bruke/D8 ADVANCED) at room temperature and 2θ range of 10-80º at 0.04-degree steps.

The peaks at 2θ= 38, 48, 63, and 77º in Fig. 4 are related to Ag (111), Ag(200), Ag(220), and Ag(311), respectively. these peaks confirm the presence of crystalline silver nanoparticles in the PLA/AG NPs composite (XRD ref no 01-

087-0718). In a study on the synthesis and characterization of Ag-chitosan nanocomposite, three peaks at $2\theta=37.9$, 33, and 63.9^o were assigned to Ag(111), Ag (200), and Ag(2200, respectively [24]. Moreover, the peaks emerging at $2\theta=38$, 44, 64, and 77° in a bio nanocomposite containing silver and crystalline micro cellulose were attributed to the silver nanocrystals [25].In a study evaluating the antioxidant activity of silver NPS, the peaks at 38.18, 44.3, 64.55, 77.54 and 81.71 were attributed to Ag(111), Ag(200), Ag(220), Ag(311), and Ag(222), respectively [26-27] the presence of peaks at 38.2, 44.3, 64.5, and 77.4 were assigned to Ag(111), Ag(200), Ag(220), and Ag(311) in the polyaniline/silver nanocomposite [28-29]. The results of the present study are in agreement with the reports of the mentioned works. Fig(4,5).
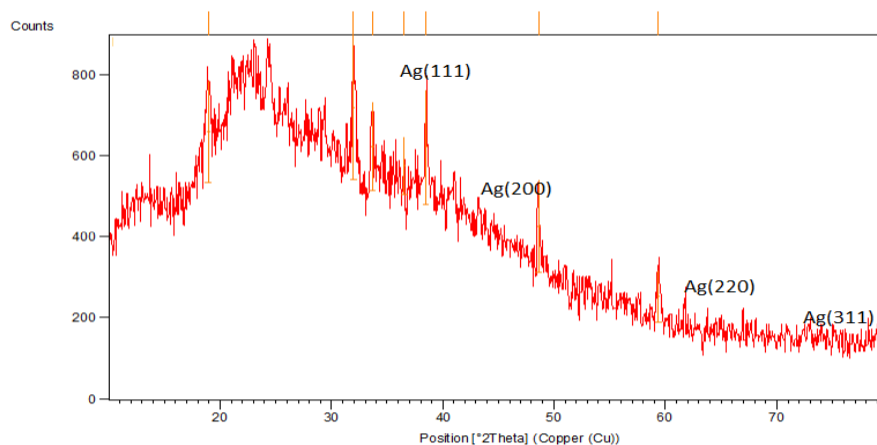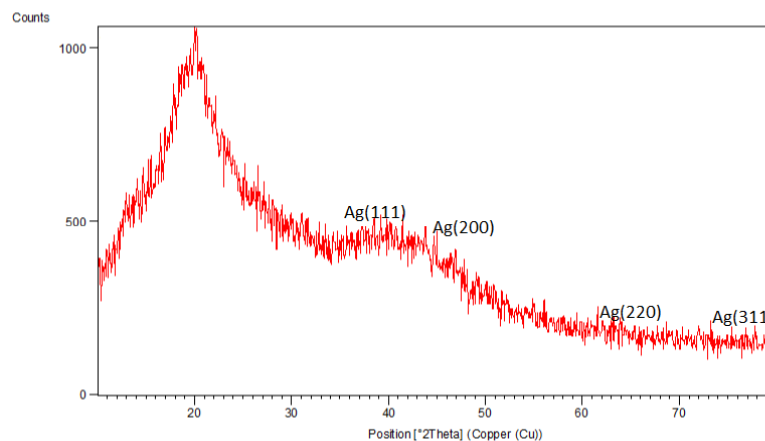

Fig.4.XRD pattern of PLA+0.5%Ag nanocomposite

Fig.5.XRD pattern of PLA+2%Ag nanocomposite

Statistical analysis

The growth parameters of the microorganism were calculated using the solver modeling method by Excel software. The data were then analyzed by one-way variance analysis. The antimicrobial activity of the PLA/Ag NPs nanocomposite, as well as PLA film, was evaluated by determining the growth parameters of the microorganism including A (maximum growth), μ_{max} (microorganism growth in the logarithmic phase), and λ (lag phase time) using Gompertz and logistic models which are two validated mathematical models (Zwietering et al.,1990).

1- Gompertz model

$$Abs_{600nm} = A\,exp\left\{-\exp\left[\frac{\mu_{max}\,\times e}{A}\,(\lambda - t) + 1\right]\right\}$$

2- Logistic model

$$Abs_{600nm} = \frac{A}{\left\{1 + exp\left[\frac{4\mu_{max}}{A}(\lambda - t) + 2\right]\right\}}$$

The Gompertz model results are as follows: Table1.

Table 1- growth parameters of E. coli at contact with PLA/Ag NPs nanocomposite according to Gompertz equation

	Lag phase (h)	Growth rate (h^{-1})	Maximum concentration	RSS	R^2
Control	3.492	0.171	1.601	0.041	0.9887
0.5%	3.491	0.167	1.568	0.039	0.9881
2%	3.644	0.118	1.086	0.017	0.9919

The Logistic model results are as follows: Table2.

Table 2- growth parameters of E. coli at contact with PLA/Ag NPs nanocomposite according to the Logistic equation

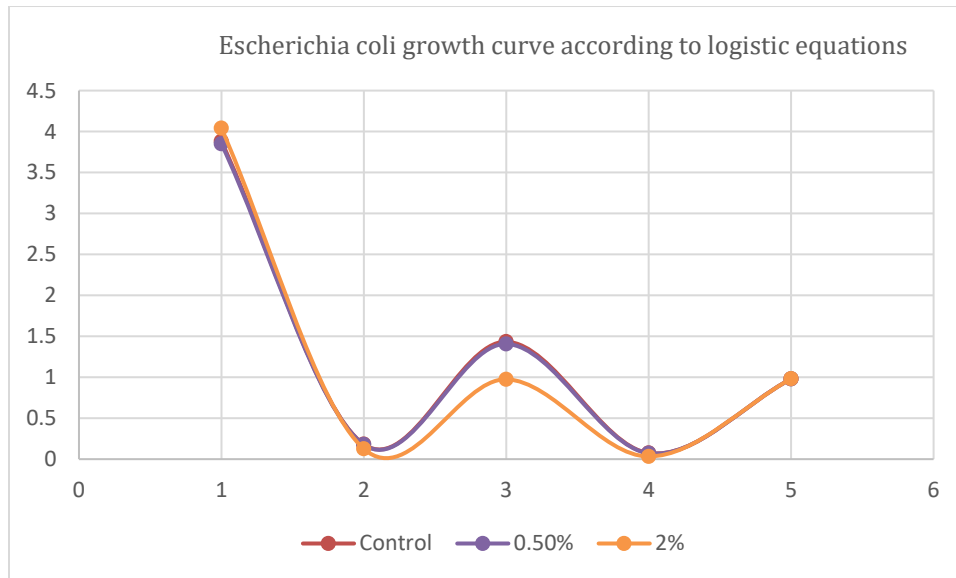|	Lag phase (h)	Growth rate (h^{-1})	Maximum concentration	RSS	R^2
Control	3.88	0.182	1.434	0.075	0.9795
0.5%	3.85	0.178	1.4065	0.072	0.9796
2%	4.04	0.126	0.9744	0.033	0.9818

Fig.6.Growth of E. coli at contact with PLA/Ag NPs nanocomposite according to Logistic equation

Regarding an increase in the lag phase from 3.49 to 3.644 h, it can be said that the use of PLA/Ag NPs nanocomposite enhanced the lag phase duration. The use of the mentioned nanocomposite incremented the lag phase time by 4.3%, compared to the control sample. The longest lag phase time was observed for the film containing 2% nanosilver while the lowest value was detected in the film having 0.5% silver NPs which can be assigned to the experimental error or the quality of the prepared film. (Fig.6)The results also indicated that the application of PLA/Ag nanocomposite led to a significant drop in the growth rate of the microorganism from 0.171 to 0.118 h-1. The lowest growth rate was observed in the microorganism exposed to 2%Ag films while the fastest growth was detected u the control samples (with no nano-silver). These results showed a 30.9% decline in the growth rate of

the microorganism due to the antimicrobial activities of nanosilver on *E. coli*. These results are in line with the reports by Fernandez et al. expressing that the application of nano-silver-containing bags for chicken packaging can decrease the microbial growth by 40% [30]. Among the nanoparticles used in the food packaging, silver NPs have gained considerable attention due to their high antimicrobial strength. The inhibitory effects of Ag NPs against viruses and fungi will lead to increased health and durability of the food products. The amount of the transferred nanoparticles posed no risk on human health and only declined the population of E. coli [31].

ACKNOWLEDGEMENTS

CONCLUSION:

In this study, high-purity and highly crystalline silver nanoparticles were prepared by the chemical reduction method and then characterized by the XRD method. The PLA/AG films were then prepared with various amounts of Ag NPs (0.5 and 2%) which were evaluated by XRD and FTOR tests.The application of PLA/Ag NPS nanocomposites caused a 4.3% increase in the lag phase time from 3.492 to 3.644 h. a 30.9% and 32.16% decline was also observed in the bacterial growth rate (from 0.171 to 0.118 h-1) and the final population of E. coil (from 1.601 to 1.86), respectively. therefore, the use of these nanocomposites for packaging purposes can increase the lag time phase and decrease the growth rate and final population of E. coli.FTIR results showed that the addition of silver NPs to the nanocomposite structure lead to the emergence of a new peak at 3620 cm^{-1} indicating the chemical interaction of Ag NPs with PLA in addition to the normal peaks related to polymeric compounds such as C-O, C-H, stretching O-H, carbonyl C=O, C-O bonds and symmetric and asymmetric bonds of CH$_3$ and –CH-.XRD analysis also revealed several peaks at 2θ= 38, 48, 63, and 77° which could be assigned to Ag (111), Ag (200), Ag (220), and Ag (311), respectively; indicating the presence of nanocrystals of silver.

REFERENCES:

[1] Abbas, M., Naeem, N., Iftikhar, H., & Latif, U. (2018). Synthesis, characterization and antimicrobial properties of silver nanocomposites. *IntechOpen*.

[2] Deshmukh, S. P., Patil, S. M., Mullani, S. B., & Delekar, S. D. (2019). Silver nanoparticles as an effective disinfectant: A review. *Materials Science and Engineering: C, 97*, 954-965.

[3] Sanpui, P., Murugadoss, A., Prasad, P. D., Ghosh, S. S., & Chattopadhyay, A. (2008). The antibacterial properties of a novel chitosan–Ag-nanoparticle composite. *International journal of food microbiology, 124*(2), 142-146.

[4] Shahmoradi, S., Golzar, H., Hashemi, M., Mansouri, V., Omidi, M., Yazdian, F., … & Tayebi, L. (2018). Optimizing the nanostructure of graphene oxide/silver/arginine for effective wound healing. *Nanotechnology, 29*(47), 475101.

[5] Kratochvíl, J., Kuzminova, A., & Kylián, O. (2018). State-of-the-Art, and perspectives of, silver/plasma polymer antibacterial nanocomposites. *Antibiotics, 7*(3), 78.

[6] Uskoković, V., Tang, S., Nikolić, M. G., Marković, S., & Wu, V. M. (2019). Calcium phosphate nanoparticles as intrinsic inorganic antimicrobials: In search of the key particle property. *Biointerphases, 14*(3), 031001.

[7] Gorrasi, G., Sorrentino, A., & Pantani, R. (2015). Modulation of biodegradation rate of poly (lactic acid) by silver nanoparticles. *Journal of Polymers and the Environment, 23*(3), 316-320.

[8] Salas-Papayanopolos, H., Morales-Cepeda, A. B., Sanchez, S., Lafleur, P. G., & Gomez, I. (2017). Synergistic effect of silver nanoparticle content on the optical and thermo-mechanical properties of poly (l-lactic acid)/glycerol triacetate blends. *Polymer Bulletin, 74*(12), 4799-4814.

[9] Kim, S., & Kim, H. J. (2006). Anti-bacterial performance of colloidal silver-treated laminate wood flooring. *International Biodeterioration & Biodegradation, 57*(3), 155-162.

[10] Chen, C. Y., & Chiang, C. L. (2008). Preparation of cotton fibers with

antibacterial silver nanoparticles. *Materials Letters*, *62*(21-22), 3607-3609.

[11] Lee, H. J., Yeo, S. Y., & Jeong, S. H. (2003). Antibacterial effect of nanosized silver colloidal solution on textile fabrics. *Journal of Materials Science*, *38*(10), 2199-2204.

[12] Dubas, S. T., Kumlangdudsana, P., & Potiyaraj, P. (2006). Layer-by-layer deposition of antimicrobial silver nanoparticles on textile fibers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *289*(1-3), 105-109.

[13] 13-Munteanu, B. S., Aytac, Z., Pricope, G. M., Uyar, T., & Vasile, C. (2014). Polylactic acid (PLA)/Silver-NP/VitaminE bionanocomposite electrospun nanofibers with antibacterial and antioxidant activity. *Journal of nanoparticle research*, *16*(10), 2643.

[14] 14-Fortunati, E., Rinaldi, S., Peltzer, M., Bloise, N., Visai, L., Armentano, I., … & Kenny, J. M. (2014). Nano-biocomposite films with modified cellulose nanocrystals and synthesized silver nanoparticles. *Carbohydrate polymers*, *101*, 1122-1133.

[15] 15-Fortunati, E., Armentano, I., Zhou, Q., Iannoni, A., Saino, E., Visai, L., … & Kenny, J. M. (2012). Multifunctional bionanocomposite films of poly (lactic acid), cellulose nanocrystals and silver nanoparticles. *Carbohydrate polymers*, *87*(2), 1596-1605.

[16] 16-Cabedo, L., Luis Feijoo, J., Pilar Villanueva, M., Lagarón, J. M., & Giménez, E. (2006, February). Optimization of biodegradable nanocomposites based on aPLA/PCL blends for food packaging applications. In *Macromolecular Symposia* (Vol. 233, No. 1, pp. 191-197). Weinheim: Wiley-VCH Verlag.

[17] 17-Yang, W., Fortunati, E., Dominici, F., Giovanale, G., Mazzaglia, A., Balestra, G. M., … & Puglia, D. (2016). Synergic effect of cellulose and lignin nanostructures in PLA based systems for food antibacterial packaging. *European Polymer Journal, 79*, 1-12.

[18] 18-Segura González, E. A., Olmos, D., Lorente, M. Á., Vélaz, I., & González-Benito, J. (2018). Preparation and characterization of polymer composite materials based on PLA/TiO2 for antibacterial packaging. *Polymers*, *10*(12), 1365

[19] 19- Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Van't Riet, K. J. A. E. M. (1990). Modeling of the bacterial growth curve. *Applied and environmental microbiology*, *56*(6), 1875-1881.

[20] 20- Rhim, J. W., Park, H. M., & Ha, C. S. (2013). Bio-nanocomposites for food packaging applications. *Progress in polymer science*, *38*(10-11), 1629-1652.

[21] 21-Zhang, Z., Zhao, B., & Hu, L. (1996). PVP protective mechanism of ultrafine silver powder synthesized by chemical reduction processes. *Journal of Solid State Chemistry*, *121*(1), 105-110.

[22] 22-Yang, H., Chen, T., Wang, H., Bai, S., & Guo, X. (2018). One-pot rapid synthesis of high aspect ratio silver nanowires for transparent conductive electrodes. *Materials Research Bulletin*, *102*, 79-85.

[23] 23 -Ramos, M., Fortunati, E., Peltzer, M., Dominici, F., Jiménez, A., del Carmen Garrigós, M., & Kenny, J. M. (2014). Influence of thymol and silver nanoparticles on the degradation of poly (lactic acid) based nanocomposites: thermal and morphological properties. *Polymer degradation and stability*, *108*, 158-165.

[24]   24- Wankhede Y.B., Kondawar S.B., Thakare S.R., More P.S. 2011. Synthesis and characterization of silver nanoparticles embedded in polyaniline nanocomposite. Advanced Materials Letters 4(1), 89-93.

[25]   25-Khan, Z., Al-Thabaiti, S. A., Obaid, A. Y., & Al-Youbi, A. O. (2011). Preparation and characterization of silver nanoparticles by chemical reduction method. *Colloids and Surfaces B: Biointerfaces*, *82*(2), 513

[26]   26-Shameli, K., Bin Ahmad, M., Jazayeri, S. D., Sedaghat, S., Shabanzadeh, P., Jahangirian, H., … & Abdollahi, Y. (2012). Synthesis and characterization of polyethylene glycol mediated silver nanoparticles by the green method. *International journal of molecular sciences*, *13*(6), 6639-6650.

[27]   27- Daphedar, A., Ritti, M., Patil, N., Patil, S., Kakkalameli, S., & Chandrashekharappa Taranath, T. (2020). Synthesis and characterization of Silver nanoparticles from fruit extract of Michelia Champaca L.: Their antioxidant and antibacterial activity. *International Journal of Nano Dimension*, *11*(3), 267-276.

[28]   28- Abouelsayed, A., Anis, B., & Eisa, W. H. (2020). Terahertz, Infrared, and UV−Vis Spectroscopy Study on Silver@ Polyaniline Core@ Shell Nanocomposites: Optical and Electronic Properties. *The Journal of Physical Chemistry C, 124*(33), 18243-18256.

[29]   29- Benzaoui, K., Ales, A., Zaoui, A., Benyoubi, F., Mekki, A., & Bakli, H. (2020, June). Numerical and Experimental Study of the Shielding Effectiveness of Polyaniline-Silver (PANI-Ag) Nanocomposite using Coaxial Open-Ended Waveguide. In *2020 IEEE International Conference on Environment and Electrical Engineering and 2020 IEEE Industrial and Commercial Power Systems Europe (EEEIC/I&CPS Europe)* (pp. 1-5). IEEE.

[30]   30- Fernández, A., Soriano, E., López-Carballo, G., Picouet, P., Lloret, E., Gavara, R., & Hernández-Muñoz, P. (2009). Preservation of aseptic conditions in absorbent pads by using silver nanotechnology. *Food Research International*, *42*(8), 1105-1112.

[31]   31. Sharma, R., Jafari, S. M., & Sharma, S. (2020). Antimicrobial bio-nanocomposites and their potential applications in food packaging. *Food Control, 112*, 107086.