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Synthesis and Characterization of Silver Nanocomposite as a Food Packaging

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ABSTRACT: Today nanopackaging has been applied in order to increase the quality and safety of foods. In this study the possibility of using silver nanocomposite as food packaging is investigated. Silver nanoparticles were prepared by chemical reduction and to approve the presence of silver nanoparticles, UV-VS spectroscopy was used. The mentioned test showed the formation of silver nanoparticles by exhibiting the typical surface Plasmon absorption at a wavelength of 380 nm as observed. Coating on LDPE was carried out after the formation of nanoparticles. The nanostructure and particle size of silver nanoparticles were confirmed by scanning electron microscopy (SEM). The average of particle size was 48 nm. In addition, the distribution of silver nanoparticles and their crystalline structure are characterised by SAXS and XRD respectively. The X-ray diffraction pattern showed five intense peaks (38.2°, 44.3°, 64.5°, 78.1° and 81.8°) in the whole spectrum of 2θ values ranging from 10° to 90°. The antibacterial activity of the prepared nanocomposite film was evaluated. The results showed favourable antibacterial efficiency against *Escheria Coli* (ATCC 8739) and *staphylococcus aureus* (ATCC 6538).

Keywords: *Antimicrobial Activity, Chemical Reduction, Polyethylene, Silver Nanocomposite, Silver Nanoparticles.*

Introduction

Several new technological applications have appeared on the basis of novel behavior exhibited by several materials at the nanoscale particle size between 1 and 100 nm(Cushen *et al.*, 2014). Reducing the particles size to the nanometer scale increases surface-to-volume ratio and as a result the reactivity of nanoparticles (Ezhilarasi *et al.*, 2013). One of these nanoparticles is nanosilver having exceptional characteristics as biocide that have made them the largest and fastest growing class of manufactured nanomaterials in commercial applications (Martins *et al.*, 2012). Generally, silver nanoparticles are synthesized using various techniques resulting in different shapes and sizes for use in numerous applications. Typical processes include attrition, sol gel processing, soft lithography, mechanical & chemical manipulation tools (Varadan *et al.*,

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2010). Among The mentioned approaches, chemical reduction method was chosen because it has many advantages such as gentle reaction condition, high efficiency, low energy usage, and easy separation method (Liu *et al.*, 2010). This method includes the suspention of silver salt into a solvent and addition of a reductant, with using of stabilizing agents, to prevent aggregation of nanoparticles. Silver nitrate (AgNO3) is the most widely used salt precursor accounting for almost 83% of those reported in studies of general and specific synthesis methods. In this study, silver nitrate was applied as reductant as well. The reason of the high using of AgNO3 is the low price and high stability and chemical stability when compared to other types of silver salts (Lee *et al.*, 2007). Generally, the solvents and reducing agents used in these processes affect the physical and morphological characteristics of manufactured silver nanoparticles (Tan *et al.*, 2007). In some studies the effect of $AgNO_3$ concentration on nanoparticles size was investigated. This is shown by Janardhanan *et al.* who applied chemical reduction method in order to synthesis silver nanoparticles and confirmed that it is feasible to control the size and optical properties of Ag nanoparticles simply by varying the reducing agent concentration (Janardhanan *et al.*, 2009). The main point of using nano packaging is to increase the lifetime of foods by stopping bacterial activities. Several studies have been conducted in this regard. In one of the studies, a method was suggested for the fabrication of antibacterial nanocomposite polymeric films, for use in food packaging. A colloidal solution of silver nanoparticles was prepared by chemical reduction of silver salt using fructose as a reductant. silver nanoparticles were coated on the LDPE surface by plunging of the films in the colloidal silver solutions. Surface morphology of the silver nanocomposite was recognized by FE-SEM and AFM analysis. In the next step antibacterial efficiency of the silver coated LDPE films against S. aureus were evaluated. The bacterial inhibition zone indicates the release of silver ion from the nanocomposite film into the media and preventing the growth of bacteria in the agar medium (Dehnavi *et al.*, 2013). In other studies, Silver nanoparticles were prepared by chemical reduction method and hydrazine hydrate was applied as a reducing agent, then they were monitored by Uv-visible. The average size of AgNPs were identified by transmission electron microscopy (TEM) as well. The results of microbial test showed the effective impact of silver nanoparticles on both gram positive and negative bacteria (Guzmán *et al.*, 2009). Effective antimicrobial nanocomposite packaging materials based on silver nanoparticles (AgNPs) have been presented. This was conducted by Mayorga et al who produced silver nanoparticles (AgNPs) into a mixed microbial cultures based on poly(3-hydroxybutyrate-co-18 mol%- 3-hydroxyvalerate) (PHBV18) was used, which was diluted by melt compounding with a commercial poly(3-hydroxybutyrate-co-3 mol%- 3-hydroxyvalerate) (PHBV3) material.The results showed PHBvs-AgNPs film has a strong antibacterial activity (Castro-Mayorga *et al.*, 2016). In the study that presented by Shrivastava et al silver nanoparticles were prepared in the range of 10–15 nm and the morphology of the nanoparticles was characterized by transmission electron microscopy. The antibacterial activity of nanoparticles were determined as well. They noted that silver nanoparticles in the range of 10–15 nm have a higher antibacterial impact on gram negative than gram-positive bacteria (Shrivastava *et al.*, 2007). Morones et al tested silver nanoparticles in four types of gram-negative bacteria. They reported that silver nanoparticles of 1–10 nm must be compact and connected to each other to have

the highest antimicrobial effects (Morones *et al.*, 2005). The antibacterial activity and acting mechanism of silver nanoparticles (SNPs) on Escherichia coli ATCC 8739 were investigated by Li et al. (2010) who analyzed the growth, permeance, and morphology of the bacterial cells following treatment with SNPs. Their result represented that using of 10 µg/ml SNPs had significant fatal effect on E.coli bacteria. A certain study that was carried out by Cho et al, they evaluated the antimicrobial activity of silver nanoparticles which were prepared using different stabilizer, for example sodium dodecyl sulfate (SDS) and poly-(N-vinyl-2-pyrrolidone) (PVP), for gram positive (S. aureus) and gram negative (E.coli) by measuring the Minimum Inhibitory Concentration(MIC). The MIC of Ag-NPs for S. aureus and E. coli were 5 and10 ppm, respectively (Cho *et al.*, 2005). Antibacterial properties of AgNPs can be improved by increasing the percentage of concentration or decreasing the size of silver nanoparticles, however, the migration rate of nanoparticle was increased significantly. Simon et al presented a physicochemical perspective on the potential migration of engineered nanoparticles (ENPs) from packaging to food and they also investigated the effect of nanoparticle size on migration from polymer packaging to food, The results represented that any detectable migration of ENP from packaging to food will take place in the case of very small ENPs with a radius of 1 nm (Simon *et al.*, 2008). In other studies Song et al evaluated the migration of Ag from nanosilver polyethylene composite packaging in to food simulants at different temperatures and times.They revealed Ag migration increased as the temperature gradually increased (Song *et al.*, 2011). The aim of this study is to fabricate silver/polyethylene nanocomposite films for use as antimicrobial food packaging. For this purpose, a colloidal silver nanoparticle solution was prepared by reduction of silver salt using Trisodium Citrate. Silver nanoparticles were coated on the surface of treaded LDPE films. The silver nanocomposite films were examined by SEM, XRD and UV-Visible analysis and the quantity of coated silver on the films as well as silver ion release from the fabricated films was measured. In addition, the antibacterial properties and migration of Ag from nanocomposite films were determined.

Materials and Methods

- Synthesis of silver NPs

First of all, 0.0849 gr AgNO3 was dissolved in 500 ml distilled water. In the next step 5 gr of Trisodium Citrate solution was added to 100 ml boiling AgNO3.The solution was placed at 90°C for 2 hours. Finally the solution's color changed to red.

- UV-Visible absorption studies

The synthesis of the AgNPs in aqueous solution was evaluated by recording the absorption spectra at a wavelength range of 350-550 nm (Jyoti, 2016).

-Preparation of antimicrobial nanocomposite film

Nanocomposite film (LDPE) was made by melt-mixing method. Along the screw were various elements in order to induce polymer melting and to achieve suitable dispersion of the nanoparticles in the polymer melt (Bikiaris *et al.*, 2006). Fine dispersion of the minor component in the polymer matrix was observed for all the studied blends with scanning electron microscopy (Takidis *et al.*, 2003).

- Characterization of the AgNPS

Electron microscopy imaging was applied in order to determine the characteristics of nanoparticles. Suspensions were made in solvent within flasks to make samples of electron microscopy. 3 ml of the solution was placed to evaporate the solvent. Samples were covered by gold and

transferred to the Spotter coater device. After 10 minutes the samples were transmitted to the electron microscope case and the images were recorded (Martins *et al.*, 2012).

- *Determination of AgNP loading in the coatings*

The total level of Ag found in each of the coatings (n=3) was determined by total acid digestion of the LDPE film. Nanocomposite film was cut into 2.5 cm×2.5 cm squares and placed in a PTFE vessel containing 10 ml HNO3 (69% HNO3, VWR International, Ireland). The sample was incubated at 120 °C for 5 hours in an oven. After digestion, the acid solutions containing the digested film in the absence of any residual LDPE sampled and 100 µl of the digestate was diluted with 9.9 ml Milli-Q water and analysed immediately using ICPAES (Hannon *et al.*, 2018).

- *The X-ray diffraction (XRD)*

The X-ray diffraction (XRD) measurement was performed on X-ray diffractometer (Panalytical Xpert-PRO 3050/60) operated at 30 kV and 100 mA and spectrum was recorded by CuKa radiation with wavelength of 1.5406 Å in the 2q range of 10-90 (Jyoti *et al.*, 2016).

- *Antibacterial test*

The bacterial strains used in this study were Staphylococcus aureus (ATCC 6538), Escherichia coli (ATCC 8739) that were provided by the Iranian Research Organization for Science and Technology. Each film sample of $10\times10cm^2$ in size was placed on individual sterile flasks. The strains of the bacterium mentioned were incubated in brain heart infusion (BHI) broth at 37ºC for 16 h. 50 mL of each prepared inoculum with the 1/10 diluted broth was added to the flasks containing the test films to obtain inocula of approximately $2\text{-}3\times10^4$ colony-forming units (CFU)/ mL.A flask without a test film was used as a control. The flasks were incubated using a shaking incubator and rotated at 30 rpm and 30ºC. Fraction of 0.1 mL of cell suspension was taken from the flasks and plated on PCA media. The plates were incubated aerobically for 48h at 37ºC. The results were reported as the average values in CFU/Ml (Hong & Rhim, 2012).

- *Migration test*

Butter (10 gr) was coated by nanosilver film and placed at 4°C. Migration test was carried out after one month.

Determination of Ag was carried out with an Agilent 7500cx ICP-MS. A scott spray chamber was used with a micromist nebuliser in order to recognize the sample. Argon was employed as carrier gas. Indium (1 µg ml^{-1}) was used as an internal standard solution to optimize the instrument and was pumped with a peristaltic pump in line with the sample solution. ICP-MS method conditions were: RF power, 1500 W; plasma gas flow rate, 15.00 l min^{-1}; auxiliary gas flow rate, 1.00 l min^{-1} ; carrier gas flow rate, 1.00 l min^{-1}(Song *et al.*, 2011).

Results and Discussion

- *Result of Ultraviolet–Visible Spectroscopy (UV–Vis)*

In the UV-Vis spectrum; broad Surface Plasmon Resonance (SPR) peak was observed at 370 nm that confirmed the synthesis of AgNPs (Figure 1)**.**

- *Result of X-ray diffraction (XRD)*

The crystalline nature of nanoparticles was confirmed by X-ray diffraction. The XRD pattern of the synthesized AgNPs is presented in Figure 2. The pattern showed five intense peaks (38.2°, 44.3°, 64.5°, 78.1° and 81.8°) in the whole spectrum of 2θ values ranging from 10° to 90°

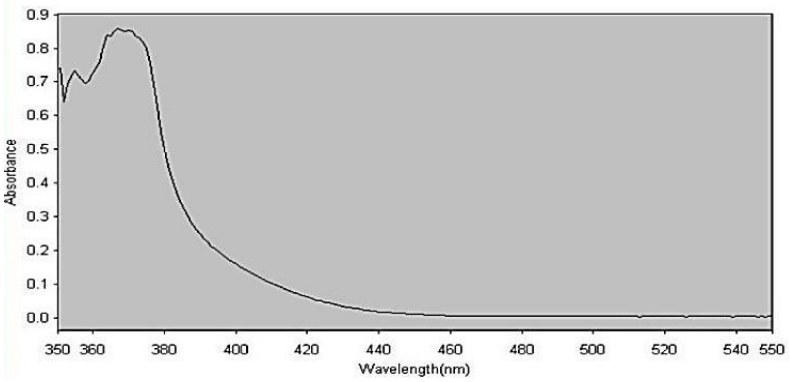

Fig. 1. UV-vis spectra of AgNPs.

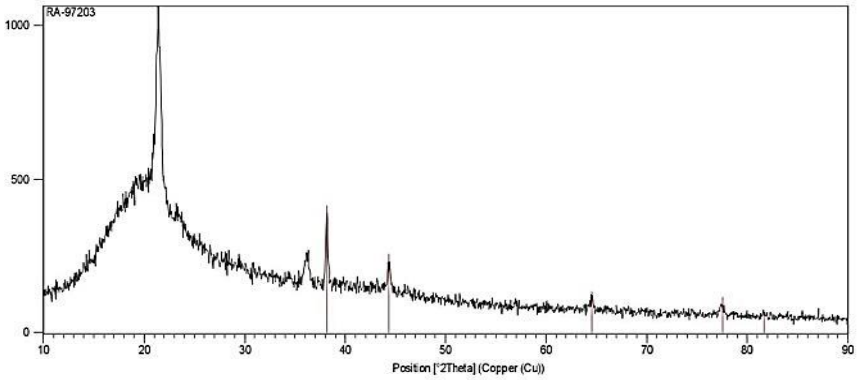

Fig. 2. The X-ray diffraction (XRD) of AgNPs.

- Result of SEM analysis

In order to measure nanoparticles and particle dispersion, SEM analysis was employed to determine the type of chemical bond. The distribution of silver nanoparticle with 20 KV, 30 and 60 KX is measured. As shown in Figures 3 and 4 the average size of particle and the film thickness were 48 nm and 33.67µm respectively. Stability of the Ag coated film was evident by SEM electron microscope. The points that were inspected with higher magnification, the presence of aggregation was evident that was due to the lack of a perfect solution within the sample coatings using the acetonitrile as solvent.

- Result of antibacterial activity of silver/ nanocomposite

coating of Silver nanoparticle on the LDPE film exposed an interesting antibacterial activity in the test. The antimicrobial activity of silver nanoparticles synthesized by chemical reduction was investigated against pathogenic organisms such as S. aureus, E. coli. The results indicated that the released silver from the coated film is enough to create antimicrobial activity in the incubated solution (Figure 5).

-Result of migration test

The migration of silver from nanosilver–polyethylene composite film into butter was evaluated using ICP-MS to determine the silver concentrations. The test result indicated that silver nanoparticles did not release after one month.

In this study, the possibility of using silver nanocomposite as a food packaging was investigated. The results indicated that nanocomposite packaging has the potential to increase the shelf life of products due to the reducing number of food pathogens. Antibacterial properties of AgNPs might be improved by increasing the percentage of

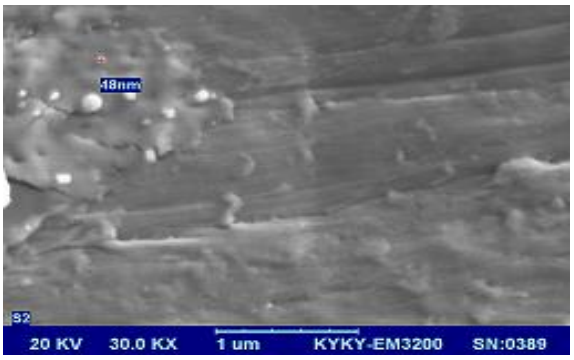

Fig. 3. The SEM image from the surface of the Ag /nanocomposite

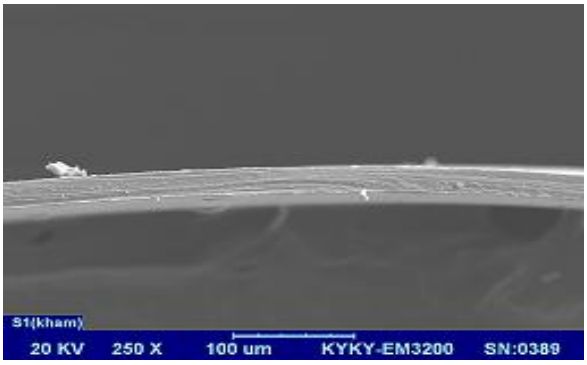

Fig. 4. The SEM image of Silver nanocomposite tickness

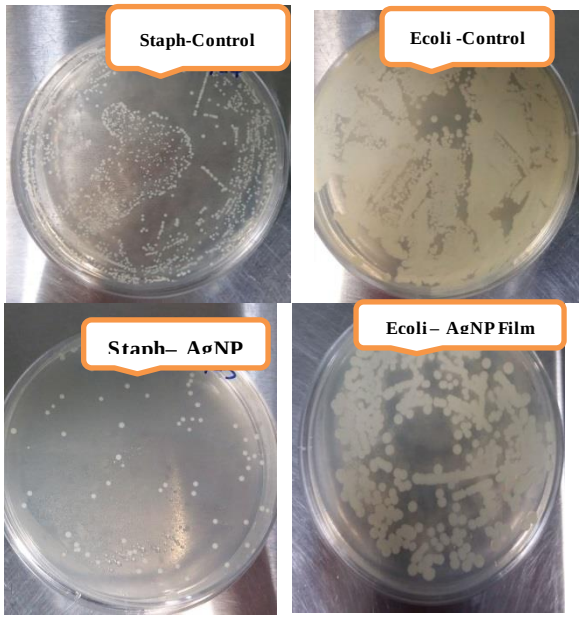

Fig. 5. The result of antibacterial activity of silver nanocomposite.

concentration or decreasing the size of silver nanoparticles, therefore, it is important to detect the size and distribution of the nanoparticles in the packaging material, however, the migration rate of nanoparticles was increased significantly and it might have a toxic effect on human health. It is notable that using of diverse reducing agent can produce different size of nanoparticle that have different antibacterial action. In this study, Trisodium Citrate was used as a reductant. The result of scanning electron microscopy implied the size of 48 nm as the average value of silver nanoparticles. Sileikaite *et al.* (2006) reported that the synthesized silver nano particles from AgNO3 salt were 100 nm when sodium citrate was used as reducing agent. In line with the results obtained Ismail and Jabra (2017) stated the size of 40 nm as the mean value by using glucose as a reductant. In other studies that reported by Jokar *et al.* (2012) silver nanoparticle were prepared by chemical reduction using polyethylene glycol. The average size of AgNPs of nanocomposite film was 5.5 nm. Janardhanan *et al.* (2009) confirmed that by rising the concentration of the ethylene

amine as reducing agent from 77 to 115 mM, the size of nanoparticles decreased. The images obtained from the electron microscope showed that the average particle size of nanoparticle decreased from 75 to 45 nm.

In our study SEM images with more magnification showed the presence of accumulation due to the lack of solution. In line with the result obtained by Jo *et al.* (2018) who investigated the morphology of the nanocomposite films and the distribution of the AgNP by FE-SEM images and observed the white spots that represent an aggregation of AgNP as confirmed by the results of EDS.

As expected, the effects of silver nanocomposite on both gram positive and negative bacteria is quite confirmed. By increasing the percentages of nanosilver, bacterial load is decreased. The antibacterial activity of these nanoparticles may be related to several procedures. Sawai & Yoshikawa (2004) concluded that induction of oxidative stress due to generation of reactive oxygen species (ROS) may cause the degradation of the membrane structure of the cell. According to these results, silver nanocomposite could affect on pathogenic bacteria (Guzman *et al.*, 2009). In other studies represented by Azlin-Hasim *et al.* (2015) nanocomposite films containing AgNPs could potentially be used as antimicrobial packaging for food applications . In recent study Tavakoli *et al.* (2017) demonstrated that using of different concentration of nano silver could enhance the shelf life of the nuts by decreasing the microbial load. In contrast, Jokar *et al.* (2012) revealed that the antibacterial impact of silver nanocomposite on gram-positive bacteria was more impressive than gram-negative. In another study the activity of silver nanoparticles against E. coli was studied by Sondi and Salopek-Sondi (2004). The bacteriological tests were carried out in Luria–Bertani (LB) medium on solid agar plates and in liquid systems supplemented with different concentrations of nanosized silver particles.The results proved that the treated E. coli cells were damaged. Representing the shape of "pits" in the cell wall of the bacteria, while the silver nanoparticles were detected to accumulate in the bacterial membrane. Mirzajani *et al.* (2011) clarified the mechanism of the effect of silver nanoparticles on *S. aureus*. These authors noted that by adding silver nanoparticles at 20 nm to the *S. aureus* media, the amount of muramic acid released in the environment is increased. According to some works carried out by Jia *et al.* (2013), by increasing $AgNO_3$ concentration, the particles tend to be large and poly-dispersed. The Ag nanoparticles displayed excellent antibacterial property.

As noted earlier by decreasing the silver nano particle size, the migration rate of nanoparticle was increased significantly. According to the European Commission Regulation (EC), migration of a substance to the food should be lower than 60 mg/Kg or 10 mg/L. In the present study, no migration was detected from silver nanoparticles to the food . In study conducted by Bott *et al.* (2011) ICP-MS test was carried out in order to determine the amount of migration from two types of food (aqueous and fatty food) that was coated by LDPE films. All three test films obtained from fatty food simulants, isooctane and 95 % ethanol did not detect silver thus indicating that no silver was released from the polymer matrix even after 10 days at 60°C in 95 % ethanol and 24 hours at 40°C for isooctane. According to Cushen *et al.* (2014) the migration rate of a system increases when nanoparticle size and polymer dynamic viscosity is decreased. Song *et al.* (2011) confirmed that Ag migration is increased as the temperature gradually increases and the maximum migration ratios were 1.70%, 3.00% and 5.60% at 20, 40 and 70ºC, respectively. However, for 95% (v/v) aqueous ethanol, the

migration values of Ag were independent of temperature; the maximum migration ratios were 0.24%, 0.23% and 0.22% at 20, 40 and 70ºC, respectively.

Conclusion

It might be indicated that silver nanoparticle is conveniently synthesized by chemical reduction method. This kind of synthesis is highly recommended because of low energy consumption, and simple separating procedure. The nanoscale produced particles were smaller in size as compared to the nanoparticles produced by companies with better antibacterial properties. The presence of silver nanoparticles was approved in the UV-visible absorption spectra. The formation of silver nanoparticles by exhibiting the typical surface plasmon absorption at the wavelength of 380 nm was observed. In addition, the distribution of silver nanoparticles and their crystalline structure are characterised by SAXS and XRD respectively. The X-ray diffraction pattern showed five intense peaks (38.2°, 44.3°, 64.5°, 78.1° and 81.8°) in the whole spectrum of 2θ values ranging from 10° to 90°.

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