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Antibacterial Activity of Silver Nanoparticles Colloidal Sol and its Application in Package Film

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Abstract. Using starch as the stabilizing agent, glucose as reducing agent, nano-size silver particles colloidal sols have been synthesized via ultrasonic irritation in aqueous solution. The nanoparticles size distribution in typical colloidal sol is from 15 to 45 nm. The silver nanoparticles-PE blend film with silver content of 86 ppm was prepared by mixing silver nanoparticles colloids sol with the PE master batch and then by film blowing. The silver nanoparticles-PE blend film and the prepared nano-silver sol show effective antibacteial activity to the Staphylococcus aureus and Escherichia coli. The pear fruit package application of the silver nanoparticles-PE blend film has demonstrated that the film has effective fresh preservation ability for the packaged fruits and vegetables.

Introduction

It is well known that silver nanoparticle is a promising antibacterial material and has been used for years in the medical field for antibacterial or antimicrobial applications[1-5]. Silver nanoparticles colloidal solutions have an increased interest due to their large surface area of silver particles, antibacterial effectiveness against a broad spectrum of bacterial strains. The silver nanoparticles may be used in form of colloidal sols or doping agents for a lot of composite materials with polymers or papers matrix or coat fibers[6]. Especially, as an additive for antibacterial packaging materials, it can be used extensively in pharmacy, food, fruits and vegetables packaging elements[7-10].

Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles as stable, colloidal dispersions in water or organic solvents[8]. Commonly used reductants are borohydride, citrate, ascorbate, and elemental hydrogen[12-15]. The reduction of silver ions in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. Initially, the reduction of various complexes with silver ions leads to the formation of silver atoms, which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal silver particles.

In the global efforts to reduce generated hazardous waste, green chemistry and chemical processes are progressively integrating with modern developments in science and industry[11]. The green synthesis of silver nanoparticles involves three main steps, which must be evaluated based on green chemistry perspectives, including: (1) selection of solvent medium, (2) selection of environmentally benign reducing agent, and (3) selection of nontoxic substances for the silver nanoparticles stability. Based on this approach, a clean, non-toxic, environmentally acceptable chemistry synthesis procedure has been developed. H_2O is utilized as the environmentally benign solvent throughout the preparation. Glucose is used as the reducing agent, and starch is selected as the stabilizing agent. The silver nanoparticle-PE antibacterial plastic films are prepared by spraying the nano-size silver particles colloidal sol into the grains surface of the PE master batch and then blowing film. The

Escherichia coli (CPCC 100038) and Staphylococcus aureus (Bac-38) were chosen to be the experimental bacteria species as they are easy to grow and are representatives of gram-negative and gram-positive bacteria. The antibacterial activity of the prepared nano-size silver particles colloidal sol and the silver nanoparticles-PE blend film were evaluated by experiments.

Materials and Methods

Materials and Bacterial Strains. All chemical regents used in this work were of analytical grade and they were used without further purification. Silver nitrate ($AgNO_3$) was purchased from Beijing Chemical Factory (Beijing, China). Glucose and starch were supplied by Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). PE Plastic granules batch were purchased from Sinopec Beijing Yanhua Petrochemical Co., Ltd. (Beijing, China). De-ionized water was used to prepare aqueous solutions.

Staphylococcus aureus (Bac-38) and Escherichia coli (CPCC 100038) were provided by Institute of Medicinal Biotechnology Academy Medical Sciences & Peking Union Medical College (Beijing, China). Nutrient agar medium(PH=7.4) were provided by Beijing Shuangxuan Microbe Cultere Medium Products Factory (Beijing, China), and its ingredient is 10 g peptone, 5 g beef extract, 5 g NaCl, 15 g Agar powder, 1000 ml distilled water. Other materials such as 75% alcohol, 0.85wt% saline PBS, autoclave and ultraviolet sterilization lamps were used in the experimental process.

Synthesis of Silver Nanoparticles Colloids and its Characterizations. Firstly, starch was added to the deionized water under heating and magnetic agitation. Then, $AgNO_3$ aqueous solution (0.15mol/L) was added into above solution after starch completely dissolved. Finally, glucose aqueous solution (0.4mol/L) was added. The reaction was processed in a KQ500DB Ultrasonic Cleaner (Kunshan Instruments Co., Ltd. China) at the temperature of 70 Celsius degree, and the power of ultrasonic is 300W. Several minutes after reaction, the solution became a little light yellow, illustrating that silver nanoparticles were already generated gradually.

The UV-Vis absorption specula were obtained in the range between 300nm and 600nm using an ultraviolet-visible spectrophotometer (UV-2501PC, Shimadzu, Japan). Distilled water was used as the blank and all samples were diluted five times before the measurements. Particle size was measured using a Zeta Sizer Nano Series (Malvern, England). The Transmission electrical microscope(TEM) images were obtained using a transmission electron microscope (H-700, Hitachi, Japan) and its sample was prepared by drying a drop of the silver colloidal solution on a TEM copper grid. The X-ray diffraction (XRD) pattern of silver nanoparticles colloidal sol after dried is recorded by an X-ray power diffractometer (D8, Bruker, Germany).

Preparation of Nanosilver-PE Blend Plastic Film. The prepared silver nanoparticles colloidal sol was sprayed onto the surface of the PE granules batch homogenously, then drying the mixture at 110 Celsius degree for 1 hour in an electrothermal constant temperature blast oven (Central Experimental Furnace Co., Ltd. Tianjin, China). The silver content in the mixture of silver nanoparticles sol and PE granules is 86ppm. Finally, the mixture of nanosilver-PE blend granules was used as raw material and was blown to be the silver nanoparticles-PE blend film by Single Screw Plastic Extruder (Laiwu City Ke Cheng Plastic Machinery Co., Ltd. Shandong). The PE granules batch which is commercial obtained and did not add silver nanoparticles sol was blown to be the PE film as the blank reference. The thickness of the silver nanoparticles-PE blend film and PE film was about 40 micrometer, which is determined by a FTS-S3C surface profile meter.

The Evaluation of the Antibacterial Efficiency. To evaluate the antibacterial efficiency, The bacteria suspensions of Escherichia coli (named as Solution 1) and Staphylococcus aureus(named as Solution 2) were prepared by gradient diluting to a concentration in the range from 1 to2×10^6 CFU/mL using normal saline which contained 75wt% salt respectively. The Solution 3 and Solution 4 were prepared by mixing 200μL silver nanoparticles colloidal sol after the high-pressure sterilization and 200μL Solution 1 and Solution 2 respectively. Firstly, injecting 50μL of Solution 1, Solution 2, Solution 3 and Solution 4 respectively onto the surface of the fresh plate medium and smearing homogeneously by glass rod, after keep them on the clean bench about 5minutes, and then, putting them into the 37 Celsius degree bacteriological incubator about 18 hours, finally, obtaining the bacterial colony by visual inspection. Fig. 4 gives the results.

The antibacterial property of the silver nanoparticles-PE blend film was tested according to the Chinese standard of QB/T2591-2003 by using Escherichia coli (CPCC 100038) bacterium[16]. 0.2 mL of the solution with the Escherichia coli concentration of 10^6 CFU /mL was added drop by drop on the surface of the silver nanoparticles PE blend sample film, then covered it with a piece of sterile film, eliminated air bubbles and then, putting it into the 37 Celsius degree bacteriological incubator for 24 hours. Afterwards, the silver nanoparticles PE blend sample film was flushed 20 times by 0.85 wt% normal saline water, then absorbing 0.5mL lotion into a plate to incubate, finally, counting the bacterial colony by visual inspection. Also, the results are given in Fig. 4, marked as (f). According to the same test procedures, using the PE film as sample film to get the bacterial colony as blank reference, marked as (e) in Fig. 4.

Fresh Preservation Evaluation of the Silver Nanoparticles-PE Blend Film by Pear Fruit Package. Commercially obtained pears were packaged with the silver nanoparticles-PE blend film bag and PE film bag respectively. The bags with pears were kept in a constant temperature of 16 Celsius degree. The weight loss, hardness, total soluble solids, pH (total organic acids), and visual appearance of the pears were measured after stored 35 days. Two pears were put into one bag, and ten bag of pears were tested.The average results were given in Table1. The hardness of the pears were tested by a friut hardness tester.

Results and discussion

The Characterization of Nano-size Silver Particles Colloidal Sol. Panacek et al[17] reported that the antibaceterial activity of silver nanoparticles was found to be size dependent, the nanoparticles of size 25nm possessed highest antibacterial activity. The synthesis technology parameters were controlled to make the average size of silver nanoparticles be about 25 nm. The typical synthesis technology parameters are: The concentration of the starch aqueous is 0.005Wt%, and the $AgNO_3$ aqueous is 0.10mol/L, and the glucose aqueous is 0.4mol/L. The weight ratio of starch to $AgNO_3$ is 2.0:1. The synthesis temperature is 70 Celsius degree. The synthesis reaction time is 180 minutes. The ultrasonic power is 300 W.

Figure.1. shows the UV-Vis absorption spectra of the prepared typical silver nanoparticles colloidal sol, which reveals a single band with maximum absorption at 403 nm. It is well reported that the size and the shape of metal nanoparticles determine the spectral position of the plasmon band as well as its width[18]. As the particles grow bigger, the plasmon band broadened and shift to longer wave length. It is indicated that the size of the silver nanoparticles is less than 50 nm, This result is with agreement with Tali Dadosh[19]. Fig.2 is the TEM images of silver nano-particle sol. The test results of the Zeta Sizer Nano Series indicated that the size of the silver nanoparticles is from 15 to 45nm. The nanoparticles are mostly spherical in shape. Fig. 3 is the XRD pattern of typical sol after dried and ground. The diffraction pattern is in agreement with the values of standard, pure, crystalline silver structure (JCPDS 4-0783). The XRD pattern confirms that the structure of resultant silver nanoparticles is the face-centered cubic (FCC) crystal structure. However, only the diffraction peak at 2θ =38.1°, marked by the indices (111) is presented. There is a broad reflection at about 20°, which is due to the low crystalline soluble starch.

The Antibacterial Activity of Silver Nanoparticles Colloidal Sol and Silver Nanoparticles-PE Blend Film. The antibacterial activity of the prepared silver nanoparticles colloidal sol has been determined by using E. coli and S. aureus bacteria. The typical results are given in Figure 4. (b) and (a) are the photographs of the samples with and without the silver nanoparticles sol after bacterial culture of E. coli. (d) and (c) are those of the S. aureus. The bacterial colony of E. coli and S. aureus on the samples with nano silver nanoparticles sol are far less than those of the samples without nano silver nanoparticles sol. (f) and (e) are the photographs of the samples of silver nanoparticle-PE blend film and PE film after bacterial culture of E. coli. The bacterial colony of E. coli on the silver nanoparticle-PE blend film sample is far less than those of the PE film sample. It is concluded that the silver nanoparticles-PE blend film has effective antibacterial activity.

Fresh Preservation Evaluation of the Silver Nanoparticles-PE Blend Film. The pears were packaged by the silver nanoparticles-PE blend film bag and PE film bag respectively. Table 1 gives average values of the weight loss, fruit hardness decrease, total soluble solids, pH (total Acid) of the pears after stored 35 days. Fig. 5 is the photographs of the pears after stored 35 days. Obviously, the

silver nanoparticles-PE blend film show effective antibacterial and antimicrobial activity compared to the PE film. It has been confirmed[3] that the silver nanoparticles have large surface area, which provides better contact with microorganisms and bacteria. The nanoparticles get attached to the cell membrance and also penetrate inside the bacteria. The bacterial membrance contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity.

It is reported that the silver nanoparticles are effective to a wide spectrum of bacterial strains such as Enterococcus faecalis, staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, staphylococcus epidermidis, staphylococcus aureus, Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter spp., fungi like Aspergillus, Penicillium and Trichoderma et al[20-23] The presented synthesis method allows obtaining of stable silver nanoparticles colloidal sol with very good antibacterial and antifungal activity. The reaction reagents used in the synthesis process are nature and non toxic. The silver nanoparticles colloidal sol can be used extensively in pharmacy, food, fruits and vegetables packaging elements.

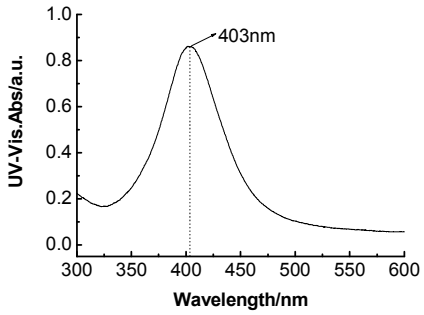

Fig.1 UV-Vis absorption spectrum of the typical silver

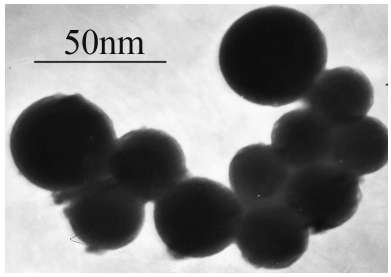

Fig.2 TEM images of the typical silver nanoparticles colloidal sol

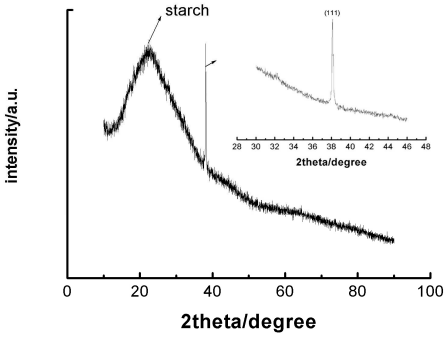

Fig.3 XRD of the typical silver nanoparticles colloidal sol after dried.

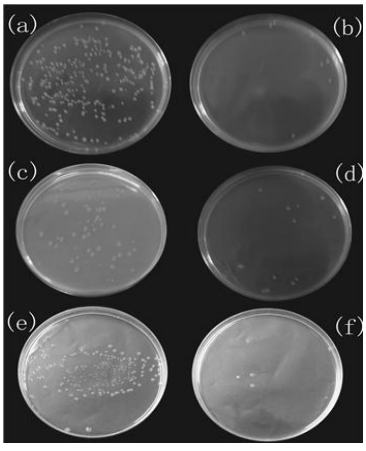

Fig.4 The photographs of samples the samples after bacterial culture. (a) E. coli,without silver nanoparticles sol as blank reference; (b) E. coli,with silver nanoparticles sol; (c) S. aureus, without silver nanoparticles sol as blank reference, (d) S. aureus, with silver nanoparticles sol; (e) E. coli, PE film as blank reference; (f) E. coli, silver nanoparticles-PE blend film.

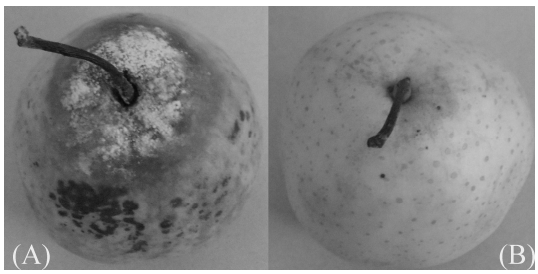

Fig. 5 The photographs of the pears after stored 35 days. (A) packaged by PE film bag, (B) packaged by the silver nanoparticles-PE blend film bag

Tab.1 The weight loss, hardness, total soluble solids, pH of the pears after stored 35 days

	Weight loss/%	Fruit hardness decrease/%	Total soluble solids/%	pH
Packaged with the silver nanoparticles-PE blend film	0.8	10	11	4.6
Packaged with the PE film	1.7	25	10	5.0

Conclusion

Silver particles colloidal sol with the size distribution of 15 to 45 nm was systhesized. The prepared silver nanoparticles sol and the silver nanoparticles-PE blend film with silver content of 86 ppm have effective antibacterial activity to bacteria and effective fresh preservation for the packaged fruits and vegetables.

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