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Study on antibacterial wood coatings with soybean protein isolate nano-silver hydrosol

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Abstract

As the new coronavirus pneumonia swept the world in 2020, the demand for antibacterial products significantly increased. In this study, a soy protein isolate nano-silver hydrosol was prepared using an environmentally friendly Ag⁺ in situ reduction process, where the soy protein was ultrasonically blended with polyacrylic resin to obtain a polyacrylate-nano silver antibacterial wood coating. The structure of the soy protein isolate nano-silver hydrosol was assessed, and the structure and antibacterial and mechanical properties of the film were characterized. The results showed that the silver nanoparticles (AgNPs) exhibited good crystallinity and were evenly distributed in the emulsion. The composite film had good antibacterial properties against gram-negative bacteria represented by Escherichia coli and gram-positive bacteria represented by Staphylococcus aureus. With increased nano-silver content, the diameter of the inhibition zone increased from 0 to 30 mm, and from 18 to 50 mm for the two bacteria, respectively. Moreover, the elastic modulus of the film increased from 8.173 to 97.912 MPa, and the elongation at break decreased from 240.601 to 41.038% as the content of AgNPs changed from 0.1 to 1%, respectively. Thus, this study provides a new method for preparing waterborne polyacrylate coatings with excellent antibacterial properties.

Keywords:
Soy protein isolate
Silver nanoparticles (AgNPs)
Polyacrylate
Waterborne wood coatings

1. Introduction

With increasing interest in environmental protection, water-based wood coatings have received widespread attention because of their low toxicity and excellent performance [1–3]. Polyacrylate waterborne coatings are one of the main varieties of wood coatings, with advantages such as strong adhesion, high transparency, and high solid content [4]. The new coronavirus pneumonia in 2020 became a global health crisis, and drastically affected individuals and the world economy. Thus, applying antibacterial coatings on material surfaces may not only effectively protect the base material, but also eliminate disease transmission between people and objects, caused by the spread of microorganisms. This type of mitigation technique is important but is currently less utilized for wood furniture coatings.

Antibacterial coatings can be divided into natural antibacterial coatings, organic antibacterial coatings, inorganic antibacterial coatings, and composite antibacterial coatings, according to the types of antibacterial agents added. Natural antibacterial agents, such as chitosan, have spectral antibacterial properties and good biocompatibility, but begin to carbonize and decompose at 160–180 °C and thus cannot be mass-produced [5–7]. Organic antibacterial agents have strong color permanence and short-term antibacterial effects, with good performance, but utilize small molecules that are toxic [8]. Therefore, researchers are increasingly investigating inorganic antibacterial agents, as they hold potential for development [9]. Among them, AgNPs combine the advantages of inorganic antibacterial materials and nano-antibacterial materials and exhibit low toxicity, high anti-fouling and broad-spectrum antibacterial performance, and strong adsorption capacities [10,11]. Thus, these materials hold promise as super antibacterial materials [12–14]. The biocidal activity of AgNPs is caused by the sustained release of Ag⁺ ions, and their surfaces have photocatalytic properties, which can promote oxidative damage to neighboring cells [15–17].

Current methods for preparing nano-silver can be classified according to physical or chemical preparation. Physical preparation methods include laser ablation, microwave reduction, quenching, and
mechanical grinding [18–20]. Physical methods provide high purity and a simple process but have additional equipment requirements and high production costs [21]. Chemical reduction methods require the addition of a reducing agent, such as sodium borohydride or sodium zirconate, to a silver precursor to reduce the precursor into elemental silver, which further grows into silver particles [22–24]. Silver particles can easily agglomerate, thus stabilizers or dispersants, such as polyethylene glycol, mercaptan derivatives, aniline, long-chain amines, and surfactants, are often added to reduce silver particle agglomeration [25]. Traditional chemical methods have low cost and high yield, but the reagents used therein are harmful to humans and the environment, and some stabilizers and dispersants are even carcinogenic [26]. Therefore, there is a growing demand for non-toxic preparation compounds [27–29].

Soy protein has received extensive attention from researchers because it is non-toxic, inexpensive, biodegradable, and environmentally friendly [30–32]. Soy glycinein (11S globulin) and glycinein (7S globulin) are two important components of soy protein, which are predominant in soy protein [33–35]. In addition, special amino acids such as tyrosine and cysteine from soy protein isolate (SPI) can be used as reducing agents for metal ion precursors. Furthermore, the amino and carboxyl groups on the SPI surface have a strong affinity for silver particles and can be used to stabilize and disperse AgNPs, and stabilize reduced AgNPs in the solution [36].

Enzymatic hydrolysis has the advantages of mild hydrolysis reactions, few side reactions, and little damage to amino acids. Therefore, bromelain hydrolysis was used to destroy the peptide bonds in the soybean protein isolate while maintaining the structure and configuration of amino acids. AgNPs were prepared in situ using tyrosine to reduce silver ions and stabilize elemental silver, and were then blended with a polyacrylic resin emulsion under ultrasonication. The corresponding antibacterial coating was then prepared by ultraviolet curing, and its antibacterial performance was evaluated. This green preparation of soybean protein isolate nano-silver hydrosols for antibacterial wood coatings possesses potential and broad development prospects.

2. Experimental

2.1. Materials

Soy protein isolate was obtained from Shansong Biological Products Co., Ltd. (Linyi, China) (protein (%)) ≥ 90). Methylymethacrylate, n-butyl acrylate (BA), ammonium persulfate, sodium dodecyl sulfate (SDS), sodium hydroxide, pentaerythritol triacrylate and bromelain (vitality, ≥300 μg/mg; relative molecular mass, approximately 33,000; light yellow powder) were obtained from Macklin (Shanghai, China). In addition, 2-(methacyryloyloxy)ethyl acetocetate (AEM), OP-10 emulsifier, and silver nitrate were obtained from Aldadin (Shanghai, China).

2.2. Methods

2.2.1. Preparation of soy protein isolate aqueous solution

Ten grams of soy protein isolate and 0.3 g of bromelain were dissolved in 175 mL of distilled water and stirred at 50 °C for 8 h until the solution was clear and uniform. The obtained liquid was then transferred to a dialysis bag, and after dialysis in an 11.5 pH sodium hydroxide solution for two days, the dialysate was exchanged with deionized water for one day, and the dialysate was changed every 2 h. After dialysis, the solution was centrifuged at 9000 rpm for 10 min to remove small amounts of insoluble matter. The concentration of the soy peptide, which was measured by the weighing method, was approximately 1 wt%. The isolate was then stored at 4 °C.

2.2.2. Preparation of soy protein isolate nano silver hydrosol

The soy peptide solution prepared above was diluted up to 0.25 wt%. Then, 20 mmol/L of sodium hydroxide was added to 5 mL of the isolate solution, to adjust the pH to 10. While stirring, 20 mmol/L of silver nitrate solution was added dropwise until different concentrations of silver nitrate formed. The solution was stirred for 10 min until fully mixed, and then the mixed solution was exposed under a 100-W white light. The chemical reaction is shown in Fig. 1.

2.2.3. Waterborne polyacrylate synthesis

A 100-mL alkaline aqueous solution containing 0.4 wt% of NaOH was prepared. MMA, BA, and AEM were washed with the alkaline aqueous solution three times to remove the acrylic monomer inhibitor. The monomer was then washed with 100 mL of distilled water three times to remove the alkaline aqueous solution from the monomer.

An ammonium persulfate initiator (0.042 g) was dissolved in 15 g of distilled water. Then, SDS (0.60 g) and OP-10 (0.30 g) were dissolved in 45 g of deionized water, to form an emulsifier solution for later use.

The MMA (18 g), AEM (5.9 g), and BA (15 g) were then mixed together. The emulsification solution was added to a four-necked flask, and the water-bath temperature adjusted to 80 °C. Then, the flask was filled with nitrogen, and 10 wt% of the monomer and initiator were poured into the four-necked flask and mechanically stirred at 360 rpm for pre-emulsification for 15 min. The monomer and initiator were added dropwise to the four-necked flask at a rate of one drop every 3 s and one drop every 30 s, respectively. The chemical reaction is shown in Fig. 2.

2.2.4. Preparation of antibacterial emulsion

The soy protein isolate nano-silver hydrosol was added to the polyacrylamide solution, and nano-silver content was controlled to 0.1, 0.2, 0.5, and 1 wt% of the polyacrylamide. Then, the 819 L photoinitiator (3 wt % of solid content) and the PETA active diluent monomer (5 wt% of emulsion mass) were mixed with the above antibacterial emulsion, sonicated for 5 min, and then stirred with a magnetic stir bar for 15 min.

2.2.5. Preparation of resin films and wood coatings

To prepare the resin film, the emulsion solution was cast into 45 × 12 × 1 mm polytetrafluoroethylene molds. After removing the water at room temperature for 24 h, the film was irradiated under a 395-nm UV-LED light for 10–15 s. When the films were cooled to room temperature, the corresponding numbers were S2, S3, S4, and S5, and the corresponding nano-silver content was 0.1, 0.2, 0.5, and 1 wt%, respectively.

To prepare the wood coatings, the emulsion was coated on a wooden board by brush coating. Subsequently, the wooden board was placed in an 80 °C oven for about 30 s. After removing the water, the wood coating was irradiated with a 395 nm UV-LED light for 10–15 s. The above steps were then repeated two times, and then we tested the performance of the paint film after 10 days, after it was completely dry.

2.2.6. Testing and characterization

The soy protein isolate solutions containing different concentrations of AgNO3 were reacted under a 100 W incandescent lamp for 24 h. Then, the solution was diluted 10 times, and the fluorescence was measured using a fluorescence spectrophotometer (Hitachi, Japan) with an excitation wavelength of 280 nm.

The soy protein isolate nano-silver hydrosol was analyzed by a Hitachi u-3010 UV spectrophotometer. Wavelength detection was 300–600 nm, the scanning speed was 300 nm/min, and the step length was 1 nm. Deionized water was used as the blank control.

The morphologies of the AgNPs were observed using a JEM-2100 transmission electron microscope (TEM, JEOL, Japan) at an operating voltage of 200 kV. The hydrosol was diluted to about 0.5 wt% with deionized water and coated on carbon thin-film-modified copper grids. A selected area electron diffraction (SAED) was used for crystal structure analysis, and energy dispersive X-Ray spectroscopy (EDX) was used for elemental analysis.

The surface morphologies of the polycarbonate and composite films were assessed by a scanning electron microscope (SEM) (Se3400N,
Hitachi Ltd., Tokyo, Japan), at a voltage of 20 kV, after coating with gold.

*S. aureus* and *E. coli* were selected as the representative gram-positive bacteria and gram-negative bacteria, respectively, to test the antibacterial properties of the polyacrylate nano-silver composite film.

Tensile tests were conducted using an electronic universal testing machine (UTM 2203, Sansi Zongheng Technology Co., Ltd.), where 45 × 10 mm (length × width) rectangular films were tested at a rate of 10 mm/min.

The surface static contact angle (CA) was determined by using a full automatic video microcontact angle measuring instrument (OCA20, German, DATA, PHYSICS) with distilled water and organic solvent n-hexadecane as the test liquid at room temperature. The CA of each sample at 10 s was measured five times in different positions. The mean was calculated, and the surface energy calculated using the Owens two-component method.

The performance of the wood coating was assessed according to its abrasion resistance, hardness, adhesion, and glossiness, in accordance with the GBT4893.8-2013, GB/T 6739-2006, GBT4893.4-2013, and GBT4893.6-2013 standards, respectively.

3. Results and discussion

3.1. Fluorescence spectrum analysis of SPI-AgNPs hydrosol

Fig. 3 shows the fluorescence spectrum analysis results of the SPI-AgNPs hydrosols with different AgNO$_3$ concentrations. The fluorescence intensity of the sol reflected the tyrosine content, and the phenolic hydroxyl group on the tyrosine residue had a strong electron-donating ability, reducing silver ions in situ to obtain AgNPs. When the AgNO$_3$ concentration in the mixed solution increased from 0.10 to 2.00 mmol/L, fluorescence intensity gradually decreased. In addition, the fluorescence intensity was unchanged after reaching 1.60 mmol/L, indicating that the reducing ability of tyrosine reached its limit at this time. Therefore, when the AgNO$_3$ concentration was 1.40 mmol/L, the Ag$^+\,$ in the system could be completely reduced to Ag$^0$. In the follow-up study, we uniformly used 1.40 mmol/L of AgNO$_3$ to prepare the corresponding polyacrylate nano-silver composite material.

3.2. Ultraviolet visible spectrum analysis of the SPI-AgNPs hydrosol

Fig. 4 shows the UV-Vis spectra of the SPI-AgNPs hydrosol as a function of illumination time. The soy protein isolate solution with a concentration of 0.25 wt% was colorless and transparent. After adjusting the pH to 10, and adding 1.40 mmol/L of the silver nitrate solution, the solution became milky white. After the solution was irradiated under a 100 W incandescent lamp for 1 h, the solution changed from milky white to light yellow. The solution became bright yellow after 3 h, dark gray after 4 h, and dark brown after 6 h. After further illumination, the color...
of the hydrosol did not change significantly. The observed solution color changes were caused by the surface-plasmon resonance (SPR) of the AgNPs in the visible-light range \[37, 38\]. This was due to the deepening of the solution color, as the number of AgNPs increased with increased light time, and the absorption peak of the AgNPs tended to increase with exposure time. Studies have also shown that the shape and size of AgNPs will affect the position of the characteristic peaks \[39\]. Thus, characteristic peaks between 320 and 450 nm are generally associated with spherical nano-silver, which was verified in the nano-silver transmission diagram in this study. In addition, in the spectrum, no peaks near 335 and 560 nm were observed, indicating no aggregation of nanoparticles \[40\].

3.3. TEM analysis of the synthesized AgNPs

As shown in Fig. 5(a), the AgNPs were approximately 10 nm in size, and the morphology was spherical, with uniform dispersion and no agglomeration. This indicated that the AgNPs exhibited good stability. The lattice parameters, observed by HRTEM, showed that the interlayer spacing was 0.23 nm, which corresponded to the lattice constant of the (101) plane of nano-silver.

The SAED pattern was clear, indicating that the nanoparticles were highly crystalline. The rings were also attributed to the diffraction of the (111), (200), (220), and (311) planes of face-centered cubic (FCC) silver \[41\]. The EDX spectrum showed obvious characteristic absorption peaks for silver, which further confirmed that the product was nano-silver.

3.4. Film analysis using SEM

The micro-morphologies of the film surfaces were further observed by SEM, and the results are shown in Fig. 6. The surface of the polyacrylate film was smooth, with almost no phase separation or cracking. The results showed that the film exhibited a high crosslinking density and good phase mixing between the soft and hard segments when cured by ultraviolet light \[42\]. Furthermore, the AgNPs did not affect the smoothness of the film.

Fig. 7 shows that the nano-silver was uniformly dispersed on the surface of the polyacrylate nano-silver composite film without large-scale agglomeration.

3.5. Antibacterial activity tests

The contact antibacterial method was used to test the antibacterial performance of the polyacrylic resin-nano silver composite film. Pure polyacrylic resin was used as a reference material to test the
antibacterial ability of the composite film for at least 24 h. As shown in Fig. 8, the prepared polyacrylic resin-nano silver composite film had excellent resistance to both *E. coli* (gram-negative bacteria) and *S. aureus* (gram-positive bacteria). This was the result of bacterial structural damage and death, caused by the interactions of the nano-silver with the cell membrane, which destroyed the respiratory chain binding enzymes, inhibited the ribosomal subunit proteins, and ATP production processes related to enzyme expression, which consequently affected DNA replication. In addition, with increased nano-silver content, the antibacterial effect of the composite film on *E. coli* and *S. aureus* improved.

In this study, a higher inhibition zone was observed for gram-positive *S. aureus* compared to gram-negative *E. coli*. This observation was in excellent agreement with earlier studies [43–45]. This was attributed to the cell wall structure of gram-negative bacteria, which consists of an outer membrane composed of lipids, proteins, and lipopolysaccharides (LPS), and acts as a barrier, providing effective protection against antibacterial agents. By contrast, the walls of gram-positive bacteria do not contain an outer membrane [46,47].

### 3.6. Evaluation of mechanical properties

The mechanical properties of the films were evaluated and the results are shown in Fig. 9 and Table 1. With increased AgNPs content, the elastic modulus and tensile strength increased from 8.173 and 5.311 MPa to 97.912 and 6.092 MPa, respectively, while the elongation at break decreased from 240.601 to 41.038%. These results were mainly attributed to the small average particle size and large specific surface area of the nanoparticles. In addition, the dispersed nano-silver particles acted as reinforcement sites for the film, effectively improving the strength and modulus of the film. Thus, the introduction of AgNPs...
significantly enhanced the mechanical properties of the polyacrylate films.

3.7. Contact angle and surface energy analysis test of coating films

The influence of AgNPs content on the contact angle and surface energy of the coating film is shown in Fig. 3. With increased AgNPs content, the water contact angle of the film decreased gradually, the change of n-hexadecane contact angle did not change significantly and the surface energy increased. These results are mainly caused by the fact that AgNPs is a hydrophilic metal, and the increase of its content leads to the increase of hydrophilicity (Table 3).

The surface energy is calculated by the Owens two-liquid method [48]:

\[ \gamma_s = \gamma_s^D \gamma_s^P \]
\[ \gamma_L = \gamma_L^D \gamma_L^P \]

where \( \gamma_s \) is the surface energy of the solid, \( \gamma_L^D \) is the dispersion force of the solid, \( \gamma_L^P \) is the polar force of the solid; \( \gamma_s \) is the surface energy of the liquid, \( \gamma_L^D \) is the dispersion force of the liquid, \( \gamma_L^P \) is the polar force of the liquid.

If the surface energy of the liquid and its dispersion force and polar force are known, and the contact angle of the liquid on the solid surface is measured, there are two unknowns in the formula. Therefore, the two liquids used in this experiment are water and n-hexadecane, and the relevant parameters are shown in Table 2.

3.8. Performance of the wood coatings

The performance results of the wood coatings with different AgNPs content, such as abrasion resistance, adhesion, glossiness, and pencil hardness, are shown in Table 2. There were no significant changes in abrasion resistance and pencil hardness of the wood coatings. However, the glossiness of the coatings slightly improved with increased AgNPs content. In addition, the adhesion properties of the polyacrylate nanosilver coatings with different AgNPs content reached level 1 or higher. This was attributed to the hydrophilic groups in the water-based acrylic
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silver precursor was reduced to elemental silver in situ. It was then ul-

4. Conclusion

Table 1
Mechanical properties of the mixtures.

| Group          | Elastic modulus (MPa) | Elongation at break (%) | Tensile strength (MPa) |
|----------------|-----------------------|-------------------------|------------------------|
| 0.1 wt% AgNPs  | 8.173                 | 240.601                 | 5.311                  |
| 0.2 wt% AgNPs  | 36.467                | 162.331                 | 6.518                  |
| 0.5 wt% AgNPs  | 62.946                | 175.789                 | 7.463                  |
| 1.0 wt% AgNPs  | 97.912                | 41.038                  | 6.092                  |

Table 2
Dispersion power and polar power of n-hexadecane and distilled water.

| Liquid            | Property    | γp | γD |
|-------------------|-------------|----|----|
| n-Hexadecane      | Non-polarity| 0  | 27.6|
| Distilled water   | Polarit y   | 51 | 21.8|

Table 3
The contact angle and surface energy of different AgNPs content coatings.

| Group          | Static contact angle (°) | Surface energy (mJ/m²) |
|----------------|--------------------------|------------------------|
|                 | Distilled water | n-Hexadecane          |
| 0.1 wt% AgNPs  | 78.65                    | 11.06                  | 34.38                  |
| 0.2 wt% AgNPs  | 77.03                    | 10.23                  | 35.03                  |
| 0.5 wt% AgNPs  | 74.19                    | 11.18                  | 36.60                  |
| 1.0 wt% AgNPs  | 69.55                    | 13.17                  | 39.17                  |

resin in the coating, which chemically reacted with the hydroxyl groups of the wood surface, providing good adhesion strength (Table 4) [49].

The optimal feeding concentration of the silver ions was 1.4 mmol/L, according to the fluorescence intensity of the solution. The UV–Vis spectrum indicated that as the illumination time increased, the absorption peak gradually increased, and more nano-silver was generated. In addition, the 320–450 nm absorption peak corresponded to spherical nano-silver, which was in good agreement with the subsequent TEM images. The SEM images also showed that the nano-silver was uniformly distributed in the polyacrylate film, and its surface morphology was not affected. The polyacrylic resin nano-silver composite film thus showed excellent resistance to both E. coli and S. aureus bacteria. With increased AgNPs content, the tensile strength and elastic modulus gradually increased, while the elongation at break gradually decreased. When AgNPs content was 0.5 wt%, the film exhibited ideal properties, with an elastic modulus, tensile strength, and elongation at break of 62.946 MPa, 7.463 MPa, and 175.789%, respectively. In addition, the wear loss, adhesion, glossiness, and pencil hardness values after 100 revolutions were 0.003 g, 0, 26.9, and 5H, respectively.

Not only do antimicrobial coatings protect the surfaces, but they also help stop the spread of pathogens that can cause disease. The nano-silver polyacrylate wood antibacterial coatings explored in this paper have wide application value in the fields of wooden floors, wooden furniture, cabinets, wooden doors, and so on.

CRediT authorship contribution statement

Bin Feng: Conceptualization, Data curation, Writing-Original draft preparation. Sibo Zhang: Methodology, Software. Di Wang: Formal analysis, Validation. Yalong Li: Investigation, Methodology. Pai Zheng: Formal analysis, software. Long Gao: Investigation, software. Da Huo: Investigation. Lei Cheng: Resources, software. Shuangying Wei: Supervision, Writing - Reviewing and Editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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