Properties of nisin incorporated ZrO$_2$/poly (vinyl alcohol)-wheat gluten antimicrobial barrier films

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**Abstract**

The properties of manufactured nisin-ZrO$_2$/poly (vinyl alcohol)-wheat gluten (ZPWG) films were characterized. The formation of hydrogen bonds among the PVA, WG, and ZrO$_2$ units were detected and caused a decrease in the film crystallinity. Also, the degradation rate was increased with WG content. As expected, the addition of WG and ZrO$_2$ influenced the light transmission, tensile properties, gas permeability and water vapor permeability of the films. Nisin-loaded films presented a low initial burst release of the antimicrobial agent, followed by a gradual release. The initial diffusion of nisin ($M_t/M_{\infty}<2/3$) from the ZPWG film was formulated by the Fickian diffusion equation, showing a linear relationship ($R^2>0.998$) between $M_t/M_{\infty}$ and $t^{1/2}$, and the diffusion coefficient $D$ increased with increasing WG content. Antimicrobial activity testing of nisin-ZPWG films with 40 wt% WG showed excellent inhibitory activity against *Staphylococcus aureus*, indicating its potential as a new active food packaging material.

**Keywords**

Antimicrobial barrier films; Drug diffusion; Active food packaging; Food safety.

**1. Introduction**

Currently, more consumers show increasing demand for natural healthy food in the pursuit of high food quality and safety. Researchers have accordingly begun exploring different methods to ensure food quality, safety, and freshness (Erginkaya, Kalkan, & Ünal, 2014; Gómez-Estaca, López-de-Dicastillo, Hernández-Muñoz, Catalá, & Gavara, 2014). One of the most widely accepted methods is the use of active food packaging (Campos, Gerschenson, & Flores, 2011; Silva et al., 2009). Antimicrobial packaging is the most common type of active food packaging (Han & Ahvenainen, 2003; Mari Pau, Gracia, Ramon, Rafael, & Pilar, 2013; Wang, Zou, & Chen, 2009); it can inhibit microbial growth or kill microbes directly using antibacterial ingredients in the packaging films in contact with the surface of the food, thereby prolonging the shelf life (Cha & Chinnan, 2004). In general, commercial nanocomposite barrier packaging is prepared by melt blending methods. These require antibacterial agents to have high thermal stability to ensure that the antibacterial agent does not fail when heated to a molten state of the polymer. Therefore, the majority of antimicrobial agents are inorganic antibacterial agents, such as copper, zinc, and silver, with metals or metal ions loaded onto carrier films (Bruna, Pealoza, Guarda, Rodríguez, & Galotto, 2012). Coating is ideal for producing antimicrobial packaging, overlaying antimicrobial coatings on plastic substrates (Caillet, Millette, Salmiâ©Ri, & Lacroix, 2006; Chetan, Romika, Namita, & Harsh, 2017), addressing the drawbacks of the melt blending process that require high thermal stability of the antimicrobial.

Attention to environmental issues has recently increased; millions of tons of food packaging waste is deposited in landfills every year, creating a serious burden on the environment. Consequently, biopolymers have become attractive alternatives...
in active food packaging (Chetan et al., 2017; Khwaldia, Arab-Tehrany, & Desobry, 2010; Suppakul, Militz, Sonneveld, & Bigger, 2003). Structured biopolymers, such as polysaccharides (Durango et al., 2010; Massouda, Green, & Joerger, 2013), proteins (Ramos, Óscar, Fernandes, Silva, Pintado, & Malcata, 2012), and lipids (Debeaufort & Voilley, 2009), have been used to prepare active food packaging (Paula Judith Pérez, Du, Roberto de Jesús, Nilda de Fátima Ferreira, & Mchugh, 2015). Poly (vinyl alcohol) (PVA) is a water-soluble, non-toxic, biocompatible, and biodegradable polymer that shows up excellent film-forming properties, with potential use in food packaging. (LópezedDicastillo, Jordá, Catalá, Gavara, & HernándezMuñoz, 2011). Similarly, wheat gluten (WG) demonstrates a number of characters such as biologically active, biocompatible, biodegradable and non-toxic, and thus it can be applied in active food packaging (El-Wakil, Hassa, n, Abou-Zeid, & Dufresne, 2015). However, PVA and WG are sensitive to acid and thermal changes. Inorganic nanoparticles can be incorporated into polymer matrices to improve the system properties or contribute to the system with new functions. (Li, Suo, & Deng, 2010; Mallakpour & Dinari, 2013). ZrO$_{2}$ is one type of potential inorganic nanoparticle with a high refractive index, high heat resistance, and the possibility of enhancing human immunologic function as a far-infrared material (Haldorai, Zong, & Shim, 2012; Yu, Chiu, Yang, Hsu, & Wu, 2006). In terms of antibacterial properties, nisin is one of a widely used toxicologically safe, natural additive for food antibacterial preservation. (Delves-Broughton, Blackburn, Evans, & Hugenholtz, 1996; Kim, An, Park, Park, & Dong, 2010), can be used to prepare antibacterial materials (Chandrasekar, Coupland, & Anantheswaran, 2016; Meira et al., 2014). In our previous work, we had successfully prepared nisin-loaded poly (vinyl alcohol)/wheat gluten/zirconia (Nisin-ZPWG) nanofibrous and the antimicrobial and release behaviors were investigated. (Wang et al., 2015).

In this experiment, nisin incorporated ZPWG films were fabricated via a coating method. The degradation behavior, light transmission, tensile properties, gas and water vapor permeability of the ZPWG films were studied. In addition, nisin diffusion behavior and the antimicrobial property against representative gram-positive bacterium Staphylococcus aureus (S. aureus) of the nisin-ZPWG films were specific investigational focuses.

2. Experimental

2.1. Materials

PVA (polymerization degree 1750 ± 50, alcoholysis degree 98%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). ZrOCl$_{2}$ 8H$_{2}$O, 99.9% was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Wheat gluten (WG) was purchased from Nanjin Shiye Co., Ltd. (Shanghai, China). Nisin was provided from MP Biomedicals, LLC (Solon, Ohio, USA). S. aureus, ATCC 6538 was obtained from the Industrial Culture Collection Center of China (Beijing, China). All of the other chemical solvents were purchased from Guo yao Chemical Reagent Co., Ltd. (Hefei, China).

2.2. Film preparation

ZrO$_{2}$ sol was made from ZrOCl$_{2}$ 8H$_{2}$O by a sol-gel method (Wang et al., 2014). Assisted by magnetic stirring, PVA was dissolved at 95°C in deionized water for 3 h to prepare a 14.0 (wt%) PVA aqueous solution. WG raw material was dissolved in deionized water at 25°C for 30 min to obtain WG solution. The ZrO$_{2}$ sol was then added to the PVA solution at 25°C by using a NE 1010 syringe pump (New Era Pump Systems Inc., America) at a rate of 1 ml/min and stirring was continued for 1 h to make a mixed solution of 10 wt% ZrO$_{2}$. Subsequently, the prepared WG solution was added to the mixed solution to obtain ZPWG0/ZPWG20/ZPWG60/ZPWG60 film blended solutions with WG contents of 0, 20, 40, and 60 wt%, respectively, based on the weight of PVA. Based on the experiment results, the 40wt%-WG ZPWG mixed solution was selected to prepare nisin-ZPWG films. Nisin-ZPWG blend solutions were obtained by adding nisin to the ZPWG solution with nisin contents of 2, 4, 6, 8, and 10 wt %, based on the percentage of the weight of PVA.

All the film-forming were coated by an automatic film applicator-AFA-III (Hefei Kejing Material Technology Co., Ltd, China) and dried at 35°C for 72 h. Thickness of films depend on viscosity and determined using a digital micrometer (Max-Cal Inc., Tokyo, Japan). The mean value of the thickness of this material is 6 μm when taken at five random locations.

2.3. Characterization

2.3.1. Structural analysis

Fourier-transform infrared (FT-IR) spectroscopy analysis of ZPWG films in our previous study had demonstrated that hydrogen bonds are formed among the PVA, WG, and ZrO$_{2}$ units in ZPWG films (Wang et al., 2015). In this study, the X-ray diffraction (XRD) with a D/max-yB rotating diffractometer (Rigaku, Japan) was used to perform the film structural analysis.

2.3.2. Degradation behavior

The degradation of ZPWG film was studied in phosphate-buffered saline (PBS, pH 7.4) at 25°C. Film sample of 20 mm × 20 mm was immersed in glass vials containing 10 ml PBS. Samples were then transferred in a water bath at 25°C. At designated time intervals, samples were removed out of the PBS and rinsed with water, then weighed after dried in a vacuum desiccator. According to the following equation, the weight loss was calculated:

\[
\text{Weight loss(%) = } \frac{W_0 - W_t}{W_0} \times 100\% \quad (1)
\]

where $W_0$ presents the initial weight of the specimen, $W_t$ is the weight of the specimen after a pre-determined immersion time.

2.3.3. Light transmission

Ultraviolet spectrophotometry (UV-754PC, China) was used to measure the light transmission of ZPWG films. For each sample, the absorbance within approximately 400 nm~800 nm was measured and used to calculate the transmittance.

2.3.4. Tensile properties

A hand-held micrometer (BC Ames Co., Waltham, MA, USA) was used to measure the film thickness. The elongation at break ($E_b$) and tensile strength ($T_s$) of specimens was measured on a TA. XT Plus Texture Analyzer (Stable Micro Systems, Co., UK). All films were cropped to rectangular pieces measuring 1 cm in width and 10 cm in length at operating temperature 25°C.
2.3.5. Gas permeability

The oxygen transmission rate of the films was investigated at 25°C and 0% relative humidity (RH) on an N500 gas permeameter (OTR, according to ASTM D1434) (Guangzhou Biaoji Packaging Equipment Co., Ltd China). The gas permeability (OP) was obtained from the OTR (cm^3 m^-2 d^-1 kPa^-1) by Equation (2):

\[ \text{OP} = \text{OTR} \times \text{thickness} \]  

(2)

Similarly, carbon dioxide (CO₂) permeability (CDP) was calculated from the CO₂ transmission rate (CDTR, cm^3 m^-2 d^-1 kPa^-1) by the following equation:

\[ \text{CDP} = \text{CDTR} \times \text{thickness} \]  

(3)

The film thickness and open testing area of samples in three parallel measurements were approximately 100 µm and 50 cm², respectively.

2.3.6. Water vapor permeability (WVP)

WVP of the film was measured following the modified method from Limpan et al. (Limpan, Limpan, Benjakul, & Prasarpnan, 2010). The specimens sealed in beakers containing silica gel at 0% RH, were placed in an incubator containing moderate distilled water. The chamber humidity of the incubator was obtained with a psychrometer to determine the relative humidity when temperature of the incubator was maintained at 25°C. The absorbed moisture was calculated by weighing the beakers at 3-h intervals for 3 days. WVP (g m⁻¹ s⁻¹ Pa⁻¹) was calculated following Equation (4):

\[ \text{WVP} = \frac{\text{wx}}{\text{S} \times \Delta \text{P}} \]  

(4)

where \( x \) is the film thickness (m), \( w \) presents weight gain of the beaker (g), \( S \) presents the area of exposed film (m²), \( t \) presents the time of weight gain (s), and \( \Delta \text{P} \) is the water vapor partial pressure difference (Pa) across two sides from the film, calculated on the basis of the relative humidity.

2.3.7. Drug diffusion behavior

Drug diffusion behavior was investigated following the methods established in our previous work (Wang et al., 2015). By dissolving 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mg nisin to 9 ml pH 7.4 PBS at 37°C and adding 1 ml Coomassie Brilliant Blue (CBB) solution as chromogenic agent, nisin solutions with concentration of 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 mg/ml were produced. Therefore, the Calibration Curve of the relationship between the concentration and absorbance was obtained. Under the operating temperature of 25°C, using 9 ml PBS and 1 ml CBB as control, the absorbance at 595 nm of every solution above was measured by a spectrophotometer (UV-754 PC, China). According to the experimental data, the linear regression equation between the concentration of nisin and absorbance is determined as the following:

\[ Y = 0.64927X + 0.0418 (R^2 = 0.997) \]  

(5)

where \( Y \) is the absorbance, \( X \) presents the concentration of nisin (mg/mL).

2.3.8. Antibacterial ability investigation

Antibacterial ability of Nisin-ZPWG films was also investigated following the methods in our previous study (Wang et al., 2015). Within limits, there is a proportional relationship between the relative concentration of \textit{S. aureus} and the absorbance of bacterial suspension at 600 nm. After rejuvenating, the concentration of \textit{S. aureus} suspension was set as 1.0 so as to obtain the calibration curve. Then, with the spectrophotometer, every sample to show the absorbance was, respectively, measured at 600 nm with relative concentrations of \textit{S. aureus} at 0.1, 0.2, 0.3, and 0.4. According to the experimental data, the linear regression equation between the concentration of \textit{S. aureus} and absorbance was determined as the following:

\[ Y = 2.4956X + 0.0384 (R^2 = 0.998) \]  

(6)

where \( Y \) is the absorbance and \( X \) is the concentration of \textit{S. aureus}.

2.4. Statistical analysis

Each experiment was repeated three times, and the results were expressed as the means ± standard deviations (SDs). The unpaired Student’s t-test was applied for statistical analysis. A value of \( p<0.05 \) was considered to be statistically significant.

3. Results and discussion

3.1. X-ray diffraction analysis

Figure 1 displays the XRD patterns of PVA, ZrO₂, WG, and ZPWG films with different WG contents. For PVA, typical diffraction peaks 2\( \theta \) = 11.5° and 2\( \theta \) = 19.1° (Figure 1(a)) are ascribed to the orthorhombic structure of PVA. ZrO₂ displays a weak diffraction peak at 2\( \theta \) = 26.9° while a strong peak at 2\( \theta \) = 4.7° (Figure 1(b)), and WG displays diffraction peaks at 2\( \theta \) = 9.8° and 2\( \theta \) = 20.5° (Figure 1(c)). In the ZPWG matrix, weak or no interactions among the PVA, WG, and ZrO₂ units would cause each component to form single-material crystal regions, and the XRD pattern of the film would appear as

![Figure 1. X-ray diffraction pattern of samples (a) Poly (vinyl alcohol) (PVA); (b) ZrO₂; (c) wheat gluten (WG); (d) ZPWG20, ZPWG film blended solutions with WG contents of 20wt%; (e) ZPWG40, ZPWG film blended solutions with WG contents of 40wt%; (f) ZPWG60, ZPWG film blended solutions with WG contents of 60wt%.](image-url)
a simple superposition of the typical diffraction peaks of PVA, WG, and ZrO₂ under the same ratio as mechanical blend (Jia et al., 2007). However, Figure 1(d–f) show that the diffraction peak of WG at 2θ = 9.8° and that of PVA at 2θ = 11.5° have vanished, while the diffraction peak of ZrO₂ at 2θ = 4.7° and that of PVA at 2θ = 19.1° are weakened. The changes imply strong interactions among the PVA, WG, and ZrO₂ units that influence the crystal patterns of the pure materials and cause decreased crystallinity in the films. In comparing Figure 1(d–f), the diffraction peak at 2θ = 19.5° is observed to gradually weaken with increasing WG content, implying that the crystallinity of the films is further decreased with increases in the WG content.

3.2. Degradation behavior study

Figure 2 illustrates the weight losses of ZPWG films with different WG contents in PBS (pH 7.4, 25°C). Time for the degradation experiment was 24 days until the weight measurement became difficult and entailed greater error since the films totally degraded and dispersed in PBS for longer time periods. PVA is one of the few water-soluble and biodegradable ethylene polymers, while WG is a biodegradable natural polymer material. As shown in Figure 2, with increasing WG content, the weight loss of the ZPWG films is significantly increased, indicating an increased degradation rate. In general, semi-crystalline polymers contain crystalline and amorphous regions and usually the degradation of semi-crystalline polymers happens to begin at the amorphous regions since the segments of the macromolecules packed more loosely and are more easily decomposed by water molecules (Wang et al., 2015). When the crystallinity of the polymer is reduced, the sizes of the crystalline regions are also decreased, and the majority of the polymer is amorphous, which leads to faster degradation. As mentioned in the XRD analysis (Figure 1), the crystal structure of PVA was destroyed by the incorporation of WG and ZrO₂ units; the crystallinity of the films was further reduced with increasing WG contents. Meanwhile, WG has very good degradation performance and is easily degraded into peptides even amino acids, so adding WG to PVA promotes the degradation of the films and the degradation rate is relative to the WG content.

3.3. Light transmission

The light transmission rates of pure PVA and ZPWG films with different WG content are presented in Figure 3. The ZrO₂ content in all ZPWG films is 10 wt%, and the content of WG is 0 wt%, 20 wt%, 40 wt%, and 60 wt%, respectively. In Figure 3(b–e), Figure 3 shows that the pure PVA film has the highest light transmittance. After adding a small amount of ZrO₂ sol, the light transmittance of the PVA/ZrO₂ film (Figure 3(b)) is decreased because the ZrO₂ is a good opacifier with a high refractive index, which reduces the light transmittance of the film. From Figure 3(b–e), the light transmittance of the films is reduced as the WG content is increased, which may be related to the reduced crystallinity of the films and the lighter color of WG itself.

3.4. Tensile properties

Figure 4 shows the mechanical properties of pure PVA and ZPWG films with different WG contents. Figure 4(a) depicts the tensile strength (Tₛ) and Figure 4(b) shows the elongation at break (Eₓ) for films with uniform 10 wt% ZrO₂ contents. From Figure 4(a), the pure PVA film has the lowest Tₛ, and the Tₛ is improved with the addition of ZrO₂ sol, mainly because of the formation of strong hydrogen bonds and Zr–O–C bonds between the PVA and ZrO₂ which strengthen
the intermolecular forces. Moreover, pure PVA has a single-chain structure that slides easily; this phenomenon is improved after forming network structures with ZrO₂, thereby increasing the $T_s$ of the PVA/ZrO₂ film. When the WG content is increased from 0 to 40 wt%, the $T_s$ of the composite film increases continually; when the WG content reaches 60 wt%, the $T_s$ of ZPWG film tends to decrease. This amount of new hydrogen bonds formed among the PVA, WG, and ZrO₂ sol; higher hydrogen bonding densities correlate to higher material strength. More complex crosslinked network structures are formed among the PVA, WG, and ZrO₂ sol with increasing WG levels; the increased degree of crosslinking degree can also increase $T_s$. However, excessive crosslinking is caused by the decreased crystallinity, impeding alignment and thereby decreasing $T_s$. When the WG content reaches a certain value, the $T_s$ of the composite film therefore decreases.

From Figure 4(b), the pure PVA exhibits the maximum $E_{br}$ and the $E_b$ of the composite film is decreased with the addition of ZrO₂ sol and increases in WG content. This is because of the strong hydrogen bonds and complex network structure forming among the components, which impairs segment movement and thus decreases the deformation of the composite material with increased strength. This effect intensifies with increases in WG content; finally, the composite material shows brittle fracture.

### 3.5. Gas permeability

Figure 5 illustrates the OP (A) and CDP (B) of the pure PVA and ZPWG films with different WG contents. As shown in Figure 6, the pure PVA has the highest OP and CDP; with the addition of ZrO₂ sol, both OP and CDP are decreased. As the content of WG increases, the OP and CDP show continuous decreases. During the test process, gas pressure exists between the two sides of the film. The gas molecules pass through the film from the high-pressure side to the low-pressure side via a single-
molecule diffusion process. With the addition of WG and ZrO$_2$ sol into the PVA matrix, hydrogen bonds and a complex network structure are formed among the PVA, WG, and ZrO$_2$ species, creating a more compact structure in the composite film and a relative decline in the diffusion channels available to gas molecules. Therefore, the OP and CDP of the film are decreased with increasing WG content. When the content of WG reaches 60 wt%, the decreasing tendency in the gas permeability is weakened. This may be from the reduced crystallinity and the increased degree of disorder in the structure.

### 3.6. Water vapor permeability

The WVPs of pure the PVA and ZPWG films with different WG contents are displayed in Figure 6, respectively. PVA is a hydrophilic polymer material containing many –OH groups that could form hydrogen bonds with water molecules. Therefore, PVA shows strong water absorption and high WVP. The WVP is reduced from 1.465 $\times$ 10$^{-10}$ to 1.32 $\times$ 10$^{-10}$ g m$^{-1}$ s$^{-1}$ Pa$^{-1}$ with the addition of ZrO$_2$ sol, probably since the strong hydrogen bonds formed of the PVA and ZrO$_2$ sol. With the addition of WG to the film, denser hydrogen bonds and more complex network-like structures are formed. As the WG content increases, the compactness of the film structure increases and the WVP decreases accordingly. However, as the WG content reaches 60 wt%, the WVP does not change significantly.

### 3.7. Nisin diffusion behavior

As showed in Figure 7(a), the accumulative release level of nisin from the ZPWG films with different WG contents were calculated according to Equation (7) and are plotted versus time. Each curve shows a similar phenomenon that there is an initial burst release before reaching a plateau at diffusion equilibrium after the accumulative release percentage increases. This suggests nisin-ZPWG films hold a good controlled release behavior, and the slight initial burst release phenomenon is ascribed to the dissolution of nisin near the film surfaces; the entrapped nisin the inner core would require more time to diffuse.

An initial model for describing nisin diffusion from nisin-ZPWG films can be deduced from Fick’s second law:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

(7)

where $C$ (kg/m$^3$) presents the nisin concentration in the film, $x$ (m) is the coordinate that measured in the direction of transport, $D$ (m$^2$/s) is the diffusivity or diffusion coefficient and $t$ (s) is the time (Williams, 1977). There were assumptions to follow: (1) The shape of film was a planar sheet. (2) Concentration of nisin in the aqueous medium was zero and the dispersion behavior of nisin in the film is uniform. (3) The diffusion of nisin occurred one-dimensionally under non-steady-state and non-concentration-dependent conditions. Following the above assumptions, the classical solution is obtained as Equation (8):

$$C = \frac{4C_0}{\pi} \sum_{n=0}^\infty \frac{1}{2n+1} \exp\left\{ -\frac{D(2n+1)^2t}{h^2} \right\} \sin\left( \frac{(2n+1)\pi x}{h} \right)$$

(8)

where $C_0$ (kg/m$^3$) is the initial nisin concentration and $h$ (m) presents the thickness of the film. After integrated over the film thickness, Equation (9) expressed in linear form as (Choi et al., 2005; Moditsi, Lazaridou, Moschakis, & Biliaderis, 2014; Yoshida, Bastos, & Franco, 2010):

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^\infty \frac{8}{(2n+1)^2} \exp\left\{ -\frac{D(2n+1)^2t}{h^2} \right\}$$

(9)

where $M_t$ ($\mu$g) and $M_\infty$ ($\mu$g) is the nisin diffused amount at time $t$ and equilibrium, respectively. For $M_t/M_\infty < 2/3$, $M_t/M_\infty$ can be presented as follows:

$$\frac{M_t}{M_\infty} = 4\left( \frac{Dt}{h^2} \right)^{1/2} = k\sqrt{t}$$

(10)

where the slope of the linear regression of $M_t/M_\infty$ versus $t^{1/2}$ is $k$ (1/s$^{1/2}$) (Malley, Bardon, Rollet, Taverdet, & Vergnaud, 1987; Peppas, 1985; Williams, 1977). Consequently, the diffusivity is calculated as follows:

$$D = \left( \frac{kh^2}{4} \right) \pi$$

(11)

Figure 7(b) demonstrates the curves of $M_t/M_\infty$ versus time based on Figure 7(a). In Figure 7(b), $M_t/M_\infty$ depends on the composition of the film at a certain time $t$ and increases with increased WG content. Besides, each curve is similar, increasing sharply then followed by a plateau. But in all curves, the linearity related to $t^{1/2}$ as predicted by Equation (10) for the initial percentage of the curve ($M_t/M_\infty < 2/3$) is strong, presenting that the change is approximately described by Fickian diffusion equation. The linear regressions of $M_t/M_\infty$ versus $t^{1/2}$ for the ZPWG films with different WG contents are shown in the inset of Figure 7(b); correspondingly, calculated by Equation (11), the diffusion coefficients $D$ are shown in Table 1. The coefficient

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**Figure 6.** Water vapor permeability of PVA and ZPWG films. PVA, Poly (vinyl alcohol); ZPWG0, ZPWG film blended solutions with WG contents of 0wt%; ZPWG20, ZPWG film blended solutions with WG contents of 20wt%; ZPWG40, ZPWG film blended solutions with WG contents of 40wt%; ZPWG60, ZPWG film blended solutions with WG contents of 60wt%.

**Figure 7(a).** Accumulative release level of nisin from the ZPWG films with different WG contents were calculated according to Equation (7) and are plotted versus time.

**Figure 7(b).** Water vapor permeability of PVA and ZPWG films.
R² > 0.998 indicates that diffusion is predominantly Fickian. And also, Table 1 shows increased D values with increasing WG content. This probably occurs because the film crystallinity is reduced, and the degradation rate is increased with increasing WG contents, which promote nisin diffusion from the ZPWG films.

3.8. Antimicrobial property evaluation

The relative concentrations of S. aureus are presented in Figure 8 when nisin-ZPWG films with different nisin contents were cultured in bacterial solution after incubation at 37°C for 24 h. As shown, the concentration of S. aureus decreases obviously along with the nisin content from 0 to 8 wt%, at which point the rate of decrease slows. The antimicrobial property is inversely proportional to the relative concentration of S. aureus in the bacterial solution after treatment. The proportion of nisin released into the bacterial suspension is increased along with the increase of the nisin contents, which effectively inhibits the reproduction of S. aureus and causes a sharp decrease in the relative concentration of S. aureus for nisin contents below 8 wt%. The performance of the antimicrobial activity tends to be stable when nisin contents are above 8 wt% and this bacteriostatic trend is similar to our previous study (Wang et al., 2015).

4. Conclusions

Properties and antimicrobial activities of Coated Nisin-ZPWG films were studied. The formation of the hydrogen bonds among the PVA, WG, and ZrO₂ units bring a decrease to the film crystallinity. The integrate of WG could promote film degradation; the degradation rate was increased with increasing WG content. The experimental results also showed that the additions of WG and ZrO₂ obviously influenced the light transmission, tensile properties, gas permeability, and water vapor permeability of the films. The light transmission, elongation at break, gas permeability, and water vapor permeability were decreased via the increasing WG content, as the tensile strength was increased with the addition of ZrO₂ sol and increasing WG content. However, for films with WG contents reaching 60 wt%, the tensile strength was decreased. The nisin-loaded films presented low initial burst releases followed by gradual release. The initial
diffusion of nisin (M_f/M_w<2/3) from the ZPWG films was described by Fickian diffusion equation. The diffusion coefficient D increased with increasing WG contents. The experiments indicated that the nisin-ZPWG films possessed good properties of controlled nisin release. A representative ZPWG solution with 40 wt% WG was determined to prepare nisin-ZPWG films for anti-microbial activity testing; these showed excellent antimicrobial property against S. aureus, indicating potential in active food packaging industrialization.

Disclosure statement

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