INTERACTION BETWEEN GELATIN AND NANO-SILVER PARTICLE: FOUNDATION FOR NANO-SILVER IN ANTIBACTERIAL LEATHER

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ABSTRACT. In order to explain the interaction between collagen and nano-silver, gelatin solution was blended with nano-silver particles (AgNPs) with particle size 26 mm, and then the mixture was interacted under different time, pH and temperature. The changes in the process were measured by UV-Vis, fluorescence spectroscopy, dynamic light scattering and FT-IR. The results showed that the main type of reaction between gelatin and AgNPs was electrostatic interaction and the interaction was diffusive encounters. The particle size and distribution of nano-silver would not be affected by gelatin, however, there was dynamic fluorescence quenching of gelatin after nano-silver particle induced. The longer time and lower pH were beneficial for the interaction process while the interaction balanced after 60 min and pH 3.0 resulted in the most drastic interaction. Moreover, nano-silver would not impair gelatin structure during the interaction process. In short, the results in this work might be a foundation and reference for applying nano-silver in antibacterial leather producing.

KEY WORDS: gelatin, nano-silver, interaction process

INTERACȚIUNEA DINTRE GELATINĂ ȘI NANOPARTICULE DE ARGINT: FUNDAMENTUL UTILIZĂRII NANOPARTICULELOR DE ARGINT PENTRU A OBȚINE PIELE CU PROPRIETĂȚI ANTIBACTERIENE

REZUMAT. Pentru a explica interacțiunea dintre colagen și nanoparticule de argint, soluția de gelatină a fost amestecată cu nanoparticule de argint (AgNP) cu dimensiunea particulelor de 26 mm, iar apoi amestecul a fost supus unor interacțiuni în condiții diferite de timp, pH și temperatură. Modificările procesului au fost măsurate prin UV-Vis, spectroscopie de fluorescență, împrăștiere dinamică a luminii și FT-IR. Rezultatele au arătat că principalul tip de reacție dintre gelatină și AgNP a fost interacțiunea electrostatică prin difuzie. Dimensiunea particulei și distribuția nanoparticulele de argint nu au fost afectate de gelatină, cu toate acestea, a existat stingerea dinamică a fluorescenței gelatinei după inducerea nanoparticulelor de argint. Timpul mai lung și pH-ul mai scăzut au fost benefice pentru procesul de interacțiune, în timp ce interacțiunea s-a echilibrat după 60 de minute, iar pH-ul de 3.0 a dus la cea mai drastică interacțiune. Mai mult, nanoparticulele de argint nu afectează structura gelatinei în timpul procesului de interacțiune. Pe scurt, rezultatele acestei lucrări ar putea constitui un fundament și o referință pentru aplicarea nanoparticulelor de argint în obținerea pielii cu proprietăți antibacteriene.

CUVINTE CHEIE: gelatină, nanoparticule de argint, proces de interacțiune

L'INTERACTION ENTRE LA GÉLATINE ET LES NANOPARTICULES D'ARGENT : LES FONDAMENTAUX DE L'UTILISATION DE NANOPARTICULES D'ARGENT POUR OBTENIR UNE PEAU AUX PROPRIÉTÉS ANTIBACTÉRIENNES

RÉSUMÉ. Pour expliquer l'interaction entre le collagène et les nanoparticules d'argent, la solution de gélatine a été mélangée avec des nanoparticules d'argent (AgNP) d'une taille de particule de 26 mm, puis le mélange a été soumis à des interactions dans différentes conditions de temps, de pH et de température. Les changements de processus ont été mesurés par UV-Vis, spectroscopie de fluorescence, diffusion dynamique de la lumière et FT-IR. Les résultats ont montré que le principal type de réaction entre la gélatine et les AgNP a été l'interaction électrostatique par diffusion. La taille des particules et la distribution des nanoparticules d'argent n'ont pas été affectées par la gélatine, cependant, il y avait une extinction dynamique de la fluorescence de la gélatine après l'induction de nanoparticules d'argent. Un temps plus long et un pH plus bas ont été bénéfiques pour le processus d'interaction, tandis que l'interaction a été équilibrée après 60 minutes, et un pH de 3,0 a conduit à l'interaction la plus drastique. De plus, les nanoparticules d'argent n'affectent pas la structure de la gélatine pendant le processus d'interaction. En bref, les résultats de ce travail pourraient être une base et une référence pour l'application de nanoparticules d'argent envers d'obtenir une peau aux propriétés antibactériennes.

MOTS CLÉS : gélatine, nanoparticules d'argent, processus d'interaction

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INTRODUCTION

Leather is a useful intermediate product with various applications in downstream industries like furniture, garment and footwear [1]. Footwear made from leather feels comfortable owing to its collagen interwoven structure and characters of breathability and softness [2]. However, leather is known to harbor bacterial growth due to its large surface area and richness in protein (collagen) that enhance bacterial reproduction by providing space and nutrients [3]. Especially, ideal moisture and temperature for bacterial reproduction will be emerged in shoes under the circumstance that sweat from foot skin is absorbed by leather [4]. The large scale of microbial reproduction on leather will not only cause unpleasant odor but also may bring numerous infectious diseases [5-7]. Therefore, antibacterial leather is urgent and necessary for upgrading leather goods quality and generating better experience for customer. Antibiotics can be used to inhibit bacterial growth on leather, but drug-resistant bacteria and compatibility may become a big challenge and obstacle [8, 9].

Silver nanoparticles (AgNPs) are an excellent antibacterial agent with broad-spectrum antimicrobial property and low toxicity [10, 11]. AgNPs have been widely investigated and applied to improve antimicrobial activity of leather by coating or soaking [12, 13]. The AgNPs with particle size of 26 nm was prepared by using sodium borohydride as reductant and benzalkonium bromide as protective agent [14], and this kind of AgNPs was used for producing antibacterial sheepskin [15] and antibacterial leathers [16, 17]. In order to realize the process of making antibacterial fur with AgNPs and help optimize technology parameters, the theoretical research on interaction between keratin and AgNPs was carried out [18]. No matter what kinds of procedures are used to apply nano-silver in antibacterial leather making, the interaction between collagen and AgNPs is essential. Nevertheless, there are few studies on interaction between collagen and AgNPs [19], consequently, antibacterial leather producing with AgNPs was still based on empirical rule rather than scientific foundation.

Collagen could be used for studying the influence of AgNPs on leather structure [20]. However, collagen is thermal sensitive, thus the temperature in the study would be too narrow in order to avoid collagen denaturing [21]. Gelatin is a collagen hydrolysate with almost the same component and structure excluding triple helix structure [22]. Therefore, gelatin could mimic collagen for carrying out reactions under wide temperature and other extreme conditions. Hitherto, the studies on interaction between collagen or gelatin and nano-silver focused on using protein in AgNPs synthesis [23, 24]. Some researchers also paid attention to applying AgNPs for preparing gelatin based antibacterial hydrogel [25, 26]. But the interaction between prepared AgNPs and gelatin in solution to clarify the regulation of the process had not been studied yet. In this work, the gelatin as mimics of collagen was interacted with AgNPs under different time, pH and temperature. The changes in the process were measured by UV-Vis, fluorescence spectroscopy, dynamic light scattering and FT-IR. The results could provide foundation and reference for antibacterial leather with nano-silver.

EXPERIMENTAL

Materials

Silver nitrate, sodium borohydride, hydrochloric acid and sodium hydroxide were research grade chemicals from Chengdu Kelong Chemical Ltd. Benzalkonium bromide solution (5% w/w) was bought from Nanchang Baiyun Pharmaceutical Co., Ltd. Type B gelatin with molecular weight about 100 kDa was purchased from Sigma-Aldrich. Other reagents in the research were research grade.

Sample Preparations

Nano-silver Particle (AgNPs) Preparation

0.015 g silver nitrate was dissolved in 100 mL distilled water and warmed by DF-101S water bath heater (Wuhan Ke'er instrument Company) at 30°C for 15min. 0.4 g benzalkonium bromide solution was blended with 100 mL distilled water and then added into silver nitrate solution with intense stirring for 30min to form mixed

solution. Subsequently, 200 mL solution containing 0.0074 sodium borohydride and 0.8 g benzalkonium bromide solution was dropped into the mixed solution with intense stirring for 1.5 h to obtain silver nano-silver particle (AgNPs). Particle size of the AgNPs was 26 mm and silver concentration was 0.024 g/L or 8.25×10^{-11} mol/L.[14]

Interaction between Gelatin and Nano-silver Particles under Different pH

20 mL gelatin solution with concentration 1 g/L was mixed with 20 mL AgNPs solution with particle size of 26 nm; then 0.01 mol/L hydrochloric acid or 0.01 mol/L sodium hydroxide was used to adjust the pH of each mixed solution to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0; finally, the solutions were stirred at 30°C for 120 min.

Interaction between Gelatin and Nano-silver Particles under Different Time

20 mL gelatin solution with concentration 1 g/L was mixed with 20 mL AgNPs solution with particle size of 26 nm; then the solutions were stirred at 30°C for 0.5, 6, 15, 30, 60 and 120 min.

Interaction between Gelatin and Nano-silver Particles under Different Temperature

Gelatin solution was blended with different AgNPs and the gelatin-AgNPs solutions with silver concentration 0, 8×10^{-12}, 1.6×10^{-11}, 2.4×10^{-11}, 3.2×10^{-11} and 4×10^{-11} mol/L were prepared. The gelatin-AgNPs solutions were stirred at 20, 30 and 40°C for 60 min.

Testing Methods

UV-Vis Measurement

Lambda 25 UV-Vis spectrometer (Perkin Elmer, America) was used to measure gelatin-AgNPs solution at 25°C with wavelength from 200 to 800 nm and scanning rate 240 nm/min.

Fluorescence Determination

The fluorescence emission spectra of gelatin-AgNPs solution were tested at 25°C or 20, 30 and 40°C corresponding to the interactive temperature by using F-4010 fluorescence spectrometer (Hitachi, Japan). The excitation and emission wavelengths were 278 nm and 307 nm; wavelength range was from 285 to 400 nm; scan rate was 60 nm/min; excitation and emission slit widths was 5.0 nm.

Zeta Potential Test

Zeta potential of gelatin-AgNPs solution was tested by Nano-ZS laser particle size analyzer (Malvern, UK). The solution was firstly filtered through 0.45 µm microporous membrane and then transferred to a polystyrene cube for the measurement. All measurements were performed in triplicate and the solution was equilibrated for 5 min before the measurement at 25°C.

FT-IR Measurement

The gelatin-AgNPs solution stirred at 30°C for 120 min was lyophilized, and the dried sample was ground with potassium bromide and made into thin sheets. Nicolet 10 FT-IR (Thermo Scientific Corporation, America) was used to scan in wavenumber range from 500 to 4000 cm^{-1} for 32 times, and the data was recorded.

RESULTS AND DISCUSSION

Influence of Time on the Interaction between Gelatin and AgNPs

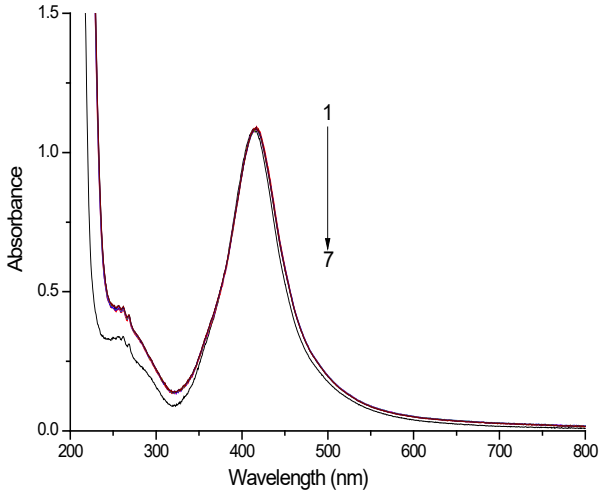

Figure 1. UV-vis spectra of gelatin-AgNPs solution interacted for different time (2 to 7 representing 0.5, 6, 15, 30, 60 and 120 min interaction time respectively and 1 representing AgNPs)

The absorption peak of AgNPs usually locates at about 420 nm in UV-Vis spectrum and there is no peak of gelatin in the same place. Since AgNPs is a metastable system, it will be affected by environmental factor and result in UV-vis spectrum change. The peak intensity trends to weak and the peak width becomes wider when the stability of AgNPs is damaged [27]. As shown in Figure 1, the peak intensity and width of gelatin-AgNPs solutions were almost the same as solo AgNPs with interaction time increasing, indicating AgNPs particle size and distribution were not impacted by gelatin [28].

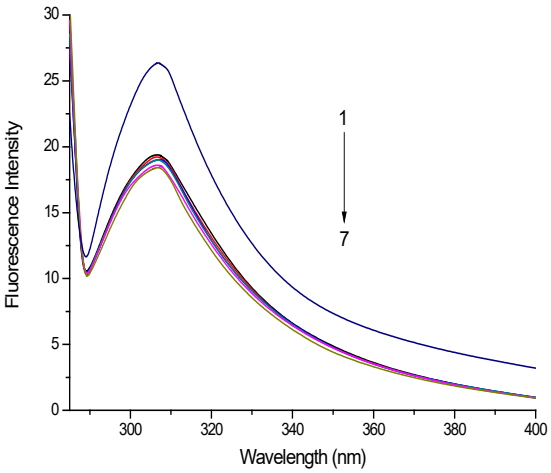

Figure 2. Fluorescence spectra of gelatin-AgNPs solution interacted for different time (2 to 7 representing 0.5, 6, 15, 30, 60 and 120 min interaction time respectively and 1 representing gelatin)

Gelatin contains tyrosine and phenylalanine but tyrosine content is more than 10 times than phenylalanine, consequently, the intrinsic fluorescence of gelatin mainly comes from tyrosine. Protein structure transforming will change gelatin fluorescence intensity [29]. The fluorescence intensity of gelatin interacted with AgNPs for different times was shown in Figure 2. It was clear that AgNPs inducing caused obvious fluorescence quenching and fluorescence intensity of gelatin decreased with interaction time prolonging but nearly balanced after 60 min.

As there was no change in UV-Vis spectra but fluorescence intensity decreasing, in other words, structure of AgNPs remained but gelatin conformation was affected. It could infer that the interaction was that AgNPs was adsorbed on gelatin surface. This adsorption might alter spatial structure of gelation and led to fluorescence quenching. However, the adsorption interaction was too weak to impact AgNPs particle size and distribution.

Influence of pH on the Interaction between Gelatin and AgNPs

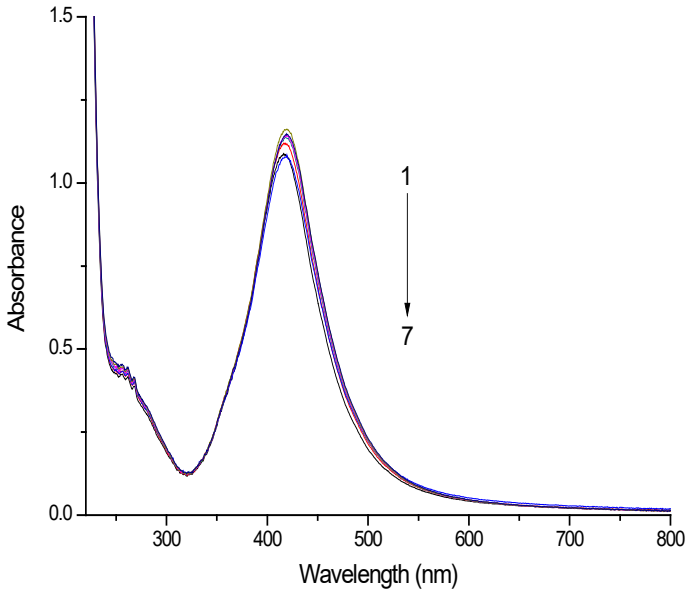

Figure 3. UV-vis spectra of gelatin-AgNPs solution interacted under different pH (1 to 7 representing pH values 9.0, 8.0, 7.0, 6.0, 5.0, 4.0, 3.0)

UV-vis spectra of gelatin-AgNPs solution interacted under different pH (Figure 3) showed lower pH benefited for the interaction between gelatin and AgNPs because smaller absorbance and violet shift was observed during interaction with pH reducing.

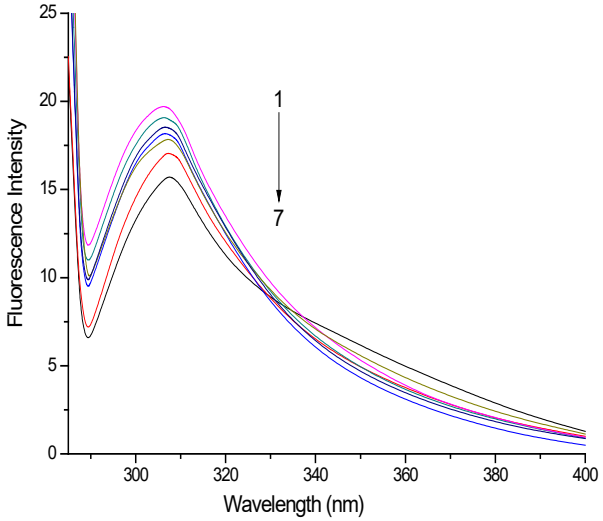

Figure 4. Fluorescence spectra of gelatin-AgNPs solution interacted under different pH (1 to 7 representing pH values 9.0, 8.0, 7.0, 6.0, 5.0, 4.0, 3.0)

Fluorescence intensity of gelatin interacted with AgNPs under different pH was shown in Figure 4, and the results illustrated higher fluorescence intensity of gelatin was obtained under higher interaction pH. The isoelectric point of gelatin used in the study was 9.6 based on experimental measurement, thus protein chain trended to unfolding under high pH. Consequently, more tyrosine residues were exposed to generate more intense fluorescence. Gelatin chains became folding with pH reducing and resulted in fluorescence intensity decreasing on one hand. On the other hand, gelatin folding with pH reducing might promote the absorption interaction and resulted in more tyrosine residues being hidden.

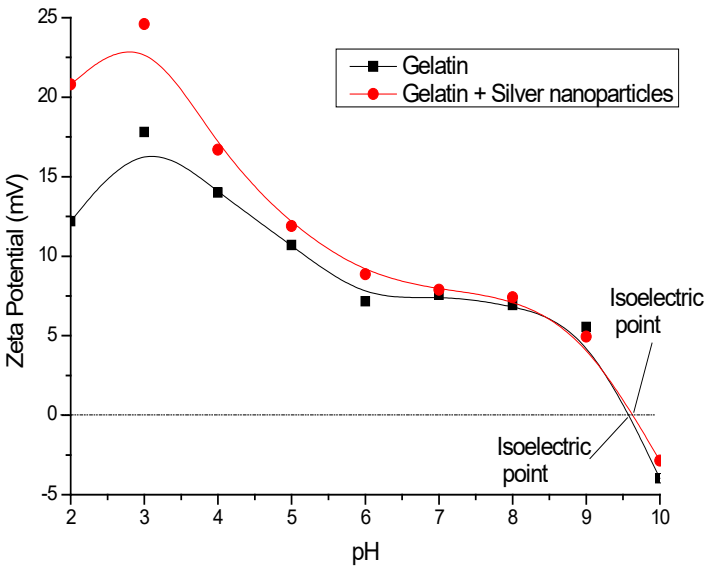

Figure 5. Zeta potential of gelatin-AgNPs solution interacted under different pH

Figure 5 showed Zeta potential of gelatin-AgNPs solution interacted under different pH. In Figure 5, there were two stages, the first one illustrated Zeta potential enlarged from pH range 2 to 3, the second one demonstrated Zeta potential diminished with pH ascending from pH range 3 to 10. Since Zeta potential of AgNPs was positive as cationic surfactant benzalkonium bromide was applied in the preparation procedure, the Zeta potential gelatin-AgNPs was larger than pure gelatin below isoelectric point as AgNPs was absorbed by gelatin. In addition, the differences of Zeta potential between gelatin-AgNPs solution and pure gelatin narrowed down with pH increasing, indicating lower pH facilitating the interaction process. However, much lower pH representing too hydrogen ion would impair colloidal electrical double layers and might have negative effect on system stability, thus the Zeta potential was maximum under pH 3.0.

Furthermore, the Zeta potential of pure gelatin was 0 when pH was 9.6 but it was 9.7 for gelatin-AgNPs solution. That was to say, isoelectric point of gelatin was 9.6 and isoelectric point of gelatin-AgNPs was 9.7. The increasing of protein isoelectric point indicated more basic amino acids remaining in gelatin-AgNPs. It was evidence that AgNPs were mainly absorbed by gelatin acid amino acids.

Influence of Temperature on the Interaction between Gelatin and AgNPs

Fluorescence Quenching Type Classification

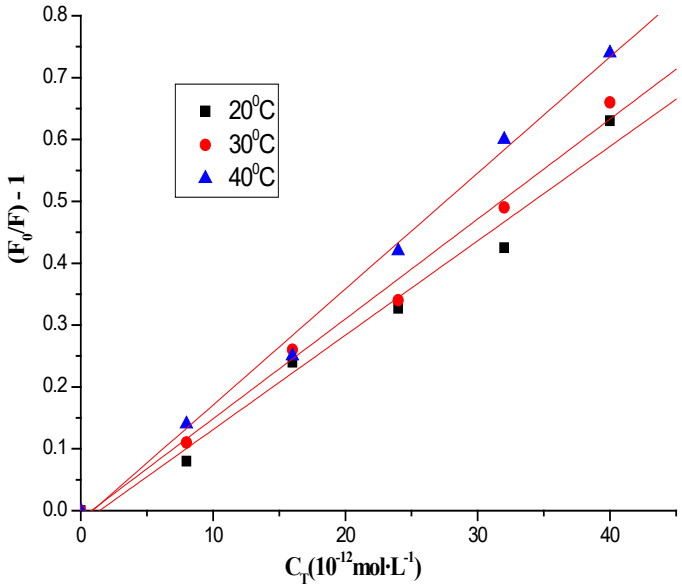

Figure 6. Stern-Volmer relationship between the concentration of AgNPs and fluorescence intensity of gelatin at different temperature

Stern-Volmer relationship between the concentration of AgNPs and fluorescence intensity of gelatin at different temperature was shown in Figure 6, where F_0 and F were the fluorescence intensities of gelatin in absence and presence of AgNPs separately, and C_t was the concentration of the AgNPs in the mixed solution. As shown in Figure 6, more significant fluorescence quenching generated under higher temperature and the slope of each line increased with temperature ascending.

Fluorescence quenching could be divided in to dynamic quenching and static quenching. For both quenching processes, the fluorescence intensity is proportional to the fluorescence quencher concentration. The linear Stern-Volmer equation was [30]:

$$F_0/F = 1 + K_q \tau_0 C_t = 1 + K_{sv} C_t \quad (1)$$

where K_{sv} was the quenching constant and equaled to $K_q \tau_0$, in which K_q was the biomolecular quenching rate constant and τ_0 was the average lifetime of the fluorescent molecules without addition of a quencher. Since dynamic fluorescence depended on diffusion rate, and diffusion rate increased with temperature rising, meaning constant of dynamic quenching (K_{sv}) enlarging with temperature increasing. Therefore, it could conclude the fluorescence quenching of gelatin interacted with AgNPs was dynamic quenching.

Binding Constant and Number of Binding Sites Analysis

The binding constant and the number of binding sites between gelatin and AgNPs could be calculated based on fluorescence intensity changing under different temperature. If there were equal and independent binding sites (n) between gelatin (G) and the fluorescence quenchers AgNPs (S), the quenching reaction between gelatin and AgNPs was as follows:

$$nG + S = G_nS \quad (2)$$

The binding constant K_A was:

$$K_A = C_{GnS} / C_S^n \cdot C_G \quad (3)$$

where C_S= concentration of fluorescence quencher (AgNPs);

C_G= concentration of gelatin;

C_{GnS}= concentration of G_nS.

The fluorescent substance concentration (C_{G0}) was:

$$C_{G0} = C_{SnG} + C_G$$

Then,

$$K_A = C_{G0} - C_G / C_S^n \cdot C_G \quad (4)$$

During the static fluorescence quenching process, the fluorescence intensity is proportional to the concentration of the free fluorescent substance, and the equation is as follows:

$$C_G / C_{G0} = F/F_0 \quad (5)$$

According to (4) and (5),

$$\lg (F0-F)/F = \lg K_A + n \lg C_S \quad (6)$$

The binding constant (K_A) and the number of reaction sites (n) in equation (6) could be calculated from the intercept ($\lg K_A$) and the slope in Figure 7, and the results were shown in Table 1.

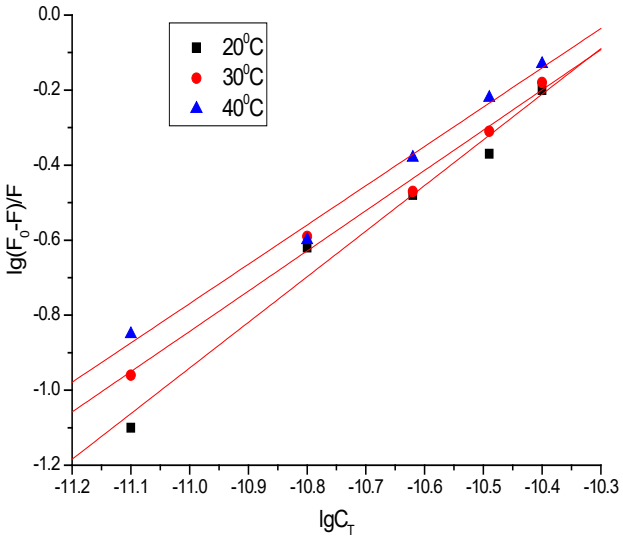

Figure 7. Relationship between the fluorescence intensity of gelatin and the different concentration of AgNPs

Table 1: Number of interaction sites (n) and interaction constants (K_A) at different temperatures

T (°C)	K_A	n
20	2.75×10^{11}	1.22
30	9.15×10^{10}	1.07
40	5.7×10^{10}	1.05

In Table 1, binding ratio of gelatin and AgNPs at different temperatures was about 1:1 and decreased with temperature. The relationship between reaction constant and temperature was negative. K_A decreased from 2.75×10^{11} at 20°C to 5.7×10^{10} at 40°C, indicating the interaction between gelatin and AgNPs was diffusive encounters.

Thermodynamic Functions Calculation

The $\Delta H_m^\ominus$, $\Delta G_m^\ominus$ and $\Delta S_m^\ominus$ could demonstrate the main type of the reaction between gelatin and AgNPs based on Van't Hoff equations.

$$\Delta G_m^\ominus = \Delta H_m^\ominus - T\Delta S_m^\ominus \quad (7)$$

$$\Delta G_m^\ominus = -RT\ln K \quad (8)$$

$$\ln[K_{T2}/K_{T1}] = \Delta H_m^\ominus (T_2-T_1)/RT_1T_2 \quad (9)$$

where T_1, T_2 and T were the temperatures of the reaction, $\Delta H_m^\ominus$ was considered as a constant when the temperature slightly changes. Combining the reaction constants (K_A) from Table 1, $\Delta H_m^\ominus$, $\Delta G_m^\ominus$ and $\Delta S_m^\ominus$ can be calculated by equations (7), (8) and (9), separately. The results were shown in Table 2.

Table 2: Thermodynamic functions of gelatin with AgNPs at different temperatures

T (K)	$\Delta H_m^\ominus$ (kJ/mol)	$\Delta G_m^\ominus$ (kJ/mol)	$\Delta S_m^\ominus$ (J/mol·K)
293	-37.3	-69.7	100.6
303	-37.3	-63.5	86.5
313	-37.3	-64.4	86.6

In Table 2, ΔG_m^{Θ} at each temperature was below zero, showing that the reaction between gelatin and AgNPs was spontaneous. According to the thermodynamic regulation of reaction types [31], the main type of reaction between gelatin and AgNPs was electrostatic interaction.

Influence of AgNPs on Gelatin Structure

FT-IR Results

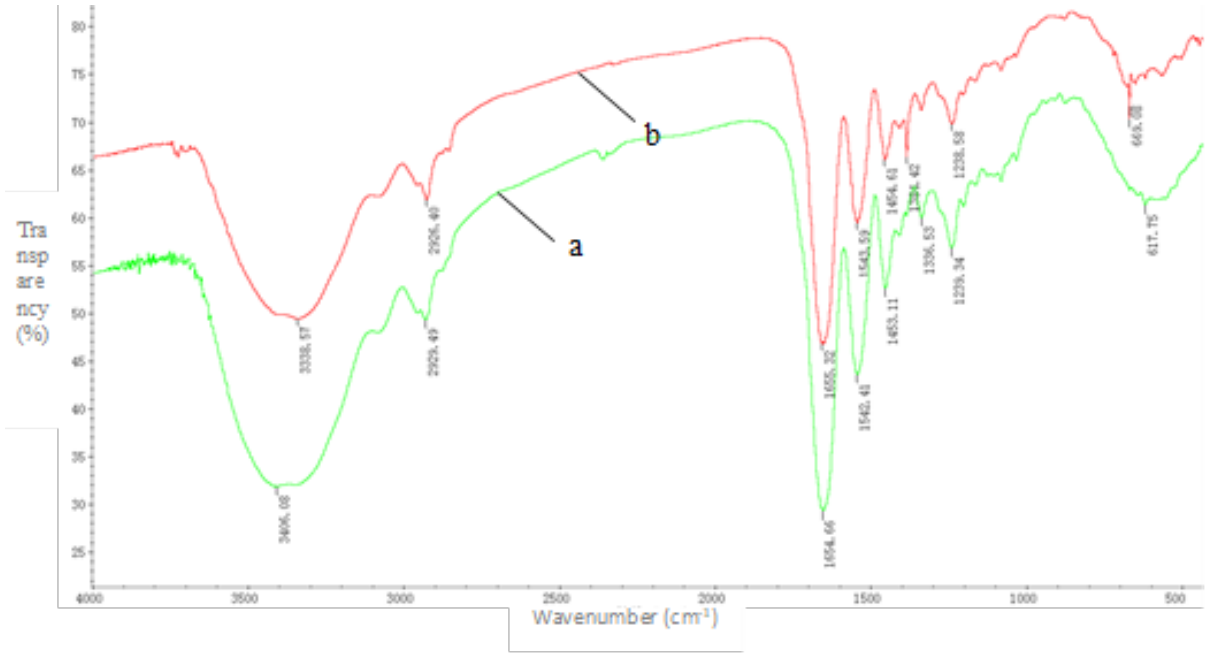

Figure 8. FT-IR image of gelatin interacted with AgNPs (a: pure gelatin, b: after)

The FT-IR image of gelatin interacted with AgNPs was shown in Figure 8. The wavenumber of gelatin-AgNPs at around 1650 cm^{-1} was almost the same as pure gelatin, indicating AgNPs did not impact protein structure during interaction, and the conformation of gelatin was still α helix. However, new peaks emerged at wavenumber at around 1380 cm^{-1} and 669 cm^{-1} after gelatin interacted with AgNPs might be the results that AgNPs reacted with oxygen atom in gelatin through electrostatic interaction.

CONCLUSIONS

When gelatin was blended with nano-silver particle, an interaction was generated but the interaction was weak and no chemical bond was formed. The main type of reaction between gelatin and AgNPs was mainly electrostatic interaction and mainly generated by gelatin acid amino acid. Gelatin could not affect the particle size and distribution of nano-silver, however there was dynamic fluorescence quenching of gelatin under nano-silver particle induced. The longer time and lower pH were beneficial for the interaction between gelatin and nano-silver particle while the interaction balanced after 60 min and pH 3.0 resulted in the most drastic interaction. Moreover, nano-silver would not impair gelatin structure during the interaction process. The results in this work might be a foundation and reference for applying nano-silver in antibacterial leather producing.

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