

Effect of pH on Al/Zr-Binding Sites Between Collagen Fibers in Tanning Process

by

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Abstract

In this article, near infrared reflection (NIR) spectra were employed to investigate the pH influences on binding behaviors of collagen fiber (CF) towards conventional non-chrome inorganic salts (Al and Zr). Based on the analyses of NIR and corresponding 2D NIR spectra, the binding capacity of Al (or Zr) contributed by each type of functional groups on CF was successfully evaluated at different pH conditions by carried out the aluminum tanning (or zirconium tanning) to native CF deaminated collagen fiber (DACF) and decarboxylated collagen fiber (DCCF), respectively. Our experimental results indicated that; for aluminum tanning, both the carboxyl group and amino group of CF are the active sites responsible for Al-binding and the reactivity of carboxyl group to aluminum is higher than that of amino group. However, the amino binding sites are more influenced with different pH. As for zirconium tanning, amino group and carboxyl group are still the active binding sites while the reactivity of amino group is higher than that of carboxyl group in the pH range of 0.5~1.5 which increases with the increasing tanning pH. Furthermore, based on the analysis of Fourier transform infrared spectra of polycapramides reacted with zirconium at different pH, it was found that the amide group in collagen is responsible for Zr-binding between collagen fibers and its reactivity with zirconium increases with the rise in pH.

Introduction

In leather production, chrome is currently commercialized tanning agent that provides the tanned leather high hydrothermal stability and satisfactory physical properties. However, the use of chrome tanning agents may generate a large

quantity of chrome-containing wastewater and solid wastes, which are difficult to be treated by using common disposal methods.¹ To address this concerned issue, researches have recently focused on the development of environmentally friendly chrome free tannages.²⁻⁵ Among various potential candidates to tanning agents, chrome-free metal salts, such as aluminum tanning and zirconium tanning, show promising perspectives but their tanning mechanisms with collagen are still not fully understood.⁶⁻⁹

Since the tanning pH significantly influences the chemical species of metal complexes and the reactivity of functional groups in collagen, the adjusting of pH during tanning process is essential for achieving high hydrothermal stability and physical properties of non-chrome metal tanned leather, including Al and Zr salts. Until now, the detailed tanning capacity contributed by each type of functional groups hasn't been fully understood at different pH conditions although some previous researches have confirm the reactivity of amino group and carboxyl group in CF towards chrome tanning agent. Therefore, In order to obtain better tanning effectiveness, it is necessary to investigate how the tanning pH influences the reactivity of different functional groups in collagen with aluminum/zirconium.

In our previous study, the mechanisms of metal tannages were successfully investigated by using ultraviolet-visible and near infrared reflectance spectrophotometer (UV-Vis-NIR),¹⁰ since NIR spectroscopy has a high sensitivity to the slight changes in the N-H 1st overtone peak of amino groups (-NH₂) and the O-H 1st overtone peak of carboxyl groups (-COOH) in collagen after tanning.^{11,12} As a result, we has proved that aluminum and zirconium can react with carboxyl group (-COOH), amino group (-NH₂) and amide groups (-CONH-) in collagen,¹⁰ which is consistent with the report of Covington.^{13,14} However, in above-

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mentioned experiments, the leathers were tanned at fixed final pH without variation, which cannot reveal the detailed contribution of each type of functional group to the tanning effectiveness. In the present investigation, a further NIR investigation was carried out to assess the influences of pH on aluminum/zirconium-binding capacity contributed by different functional groups of CF by using collagen fiber (CF), deaminated collagen fiber (DACF) and decarboxylated collagen fiber (DCCF) as the raw materials. Based on the present investigations, the influence behaviors of pH on the reactivity of amino group and carboxyl group are fully revealed, which should be useful for developing high effective aluminum and zirconium tanning agents.

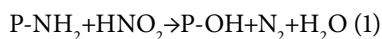
Experimental Procedures

Materials

CF (hide powder) was prepared from bated goat pelts according to the method described in the literature.¹⁵ Different collagen fibers, such as CF, DACF and DCCF, were used for tanning trials. The solutions of aluminum and zirconium (0.1 mol/L) were prepared by dissolving $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ and $\text{Zr}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$ into distilled water at 20°C, respectively. The chemicals used for tanning experiments were of chemical grade, and those used for analysis were of analytical grade.

Preparation of DACF

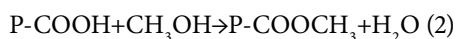
According to the reaction shown in the chemical equation (1), DACF was prepared by reacting CF with NaNO_2 in acid condition, where the removal rate of amino groups in CF is in the range of 40% ~ 50%.¹⁶



In details, 50 g of CF was soaked with 100 mL of distilled water for 36 h, and then 250 mL of 200 g/L NaNO_2 solution was added. The mixture was shaken for 1 h at 25°C, and then 42.6 g of glacial acetic acid was added into the mixture. The deamination was then performed for 24 h at 25°C. The resultant reaction product was vacuum-filtrated, washed five times with 50 mL of distilled water, and three times with acetone aqueous solution (1:1(v/v), 10 min/time), and dried at 40°C for 48 h. The obtained DACF was stored at 25°C with a humidity of 65%.

Preparation of DCCF

According to the reaction shown in the chemical equation (2), DCCF was prepared by esterification, where the removal rate of carboxyl groups in CF is about 90%.¹⁷



30 g of CF was added into the mixed solution of 750 mL methanol solution (2 mol/L) and 5.63 mL concentrated hydrochloric acid,

and reacted for 24 h at 25°C. The mixture was then neutralized to pH 7 with 40% (w/v) NaOH solution and shaken for 30 min. The product was vacuum filtrated, washed five times with 50 ml distilled water, washed three times with acetone aqueous solution (1:1(v/v), 10 min/time), and dried at 40°C for 48 h. Then DCCF was obtained, and the samples were stored at 25°C with a humidity of 65%.

Determination of Precipitation pH of Aluminum and Zirconium

20 mL of 0.1 mol/L aluminum and zirconium solutions were transferred to separate triangular flasks respectively. Standard NaOH solution (0.1 mol/L) was titrated to the solutions at a rate of 1 mL/min under continuous stirring until precipitate was formed. The precipitation pH values of aluminum and zirconium were recorded by pH titrator (ZDJ-5, LEICI, China).

Preparation of Collagen Fiber Samples

0.5 g of CF, DACF and DCCF was tanned for 6 hours by 0.1 mol/L aluminum/zirconium solutions (25°C, 10 mL), which the tanning pH range of aluminum was 0.5~4.0 with an increment of 0.25 and that of zirconium was 0.5~1.5 with an increment of 0.5. Sulfuric acid (1:20(v/v)) was used for pH adjustment in the pickling process. Then, the pH was raised to the precipitation pH of aluminum/zirconium by using sodium carbonate aqueous solution (1:20(w/w)). After basifying, tanning was continued for another 12 h. Subsequently, the tanned collagen fibers were vacuum filtrated, washed five times with distilled water, washed three times with acetone aqueous solution (1:1(v/v), 10 min/time), and dried at 40°C for 48 h. Then the tanned collagen fibers were obtained such as Al-CF, Al-DACF and Al-DCCF. Polycapromamide, which has a similar structure to the amide group in collagen, was used to react with zirconium using the same procedure as above and Zr-polycapromamide was obtained.

Determination of Collagen Fibers by XPS Spectroscopy

The collagen fibers tanned with aluminum (tanning pH=4) and zirconium (tanning pH=1.5) were sampled. Their XPS wide scan spectra were measured by X-ray photoelectron spectroscopy (XSAM800, KRATOS, England) with a binding energy range of 0 to 1100 eV (150mS per 0.65eV), and scanned for 2 times. Meanwhile, photoelectron Al 2p and Zr 3d were observed.

Determination of Collagen Fibers by NIR Spectroscopy and 2D Correlation Analysis

The collagen fibers tanned with aluminum and zirconium was sampled, and their original 1D NIR spectra were measured by using UV-Vis-NIR (UV-3600, Shimadzu, Japan) in the wavelength range of 1000 to 1800 nm. The generalized 2D correlation analysis was performed by using the software of 2D Shige ver 1.3 (developed by Shigeaki Morita). The correlated 2D spectra can be outputted and plotted as the contour maps.

Determination of Denaturation Temperatures of Collagen Fibers by Differential Scanning Calorimetry

The denaturation temperatures of the tanned collagen fibers were measured by using Differential Scanning Calorimetry (DSC, 200/1/H, Netzsch, Germany). The samples were measured in the temperature range of 40°C ~ 200°C at a heating rate of 10°C/min.

Determination of Polycapromides by Fourier Transform Infrared Spectroscopy

The polycapromides reacted with aluminum and zirconium were analyzed by Fourier Transform Infrared spectroscopy (FTIR, Nicolet iS10, Thermo Scientific, USA). Their FTIR spectra were recorded in the wave number range of 4000 ~ 500 cm⁻¹.

Results and Discussion

Precipitation pH of Al³⁺ and Zr⁴⁺

It is well known that pH affects the state of chemical species and molecular dimension of metal complex in water, as well as the reactivity of functional groups in collagen. To obtain adaptable pH range for the penetration and fixation of aluminum and zirconium tanning agents, we first analyzed the precipitation pH of Al³⁺ and Zr⁴⁺. It was found that the precipitation pH values of Al³⁺ and Zr⁴⁺ were 4.11 and 1.9, respectively, when the concentrations of aluminum or zirconium in solutions was 0.1 mol/L. Therefore, in our tanning experiments, aluminum tanning was carried out at an initial pH in the range of 0.5~4.0 and a final pH of 4.1, while zirconium tanning was performed at an initial pH in the range of 0.5~1.5 and a final pH of 1.9.

XPS Analysis of Al-CF

Figure 1 shows that XPS spectra of Al 2p of the CF and Al-CF(tanning pH=4). It can be seen that the photoelectron peak of the CF does not appear at the binding energy of 75.1 eV, which is attributed as Al 2p, and that of the Al-CF appears clearly (Figure 1a). In Figure 1b, Al 2p peak is divided into two components. The peak at 74.2 eV is attributed to Al-N bonds because this value is in good agreement with previous values,¹⁸ and the component in 75.3 eV indicates the formation of Al-O bonds.¹⁹ As we all know, the main functional groups in CF include amino, carboxyl and amide groups. The polypeptide chain was composed of an amide skeleton in spatial structure. Thus, the main observed functional groups include amino groups and carboxyl groups due to XPS is surface analysis. Therefore, in tanning process, the aluminum ions were combined with the amino groups and carboxyl groups. The Al-containing bonds contents were obtained by calculating the components area, the percentages of Al-N bond and Al-O bond were 29.05% and 70.95% respectively. Thus, binding capacity of Al-carboxyl is higher than that of Al-amino.

Effect of pH on Aluminum-binding Sites Between Collagen Fibers

To investigate the effect of pH on aluminum-binding sites on CF, we observed the changes of main functional groups in CF after aluminum tanning at different initial pH. The N-H 1st overtone peak of amino groups (-NH₂) and the O-H 1st overtone peak of carboxyl groups (-COOH) can be observed by NIR spectrophotometer at 1550 nm and 1700 nm, respectively,²⁰ so the NIR spectra of tanned collagen fibers were measured in the wavelength range of 1000~1800 nm, and compared with those data collected from tanned DACFs and Al-DCCFs.

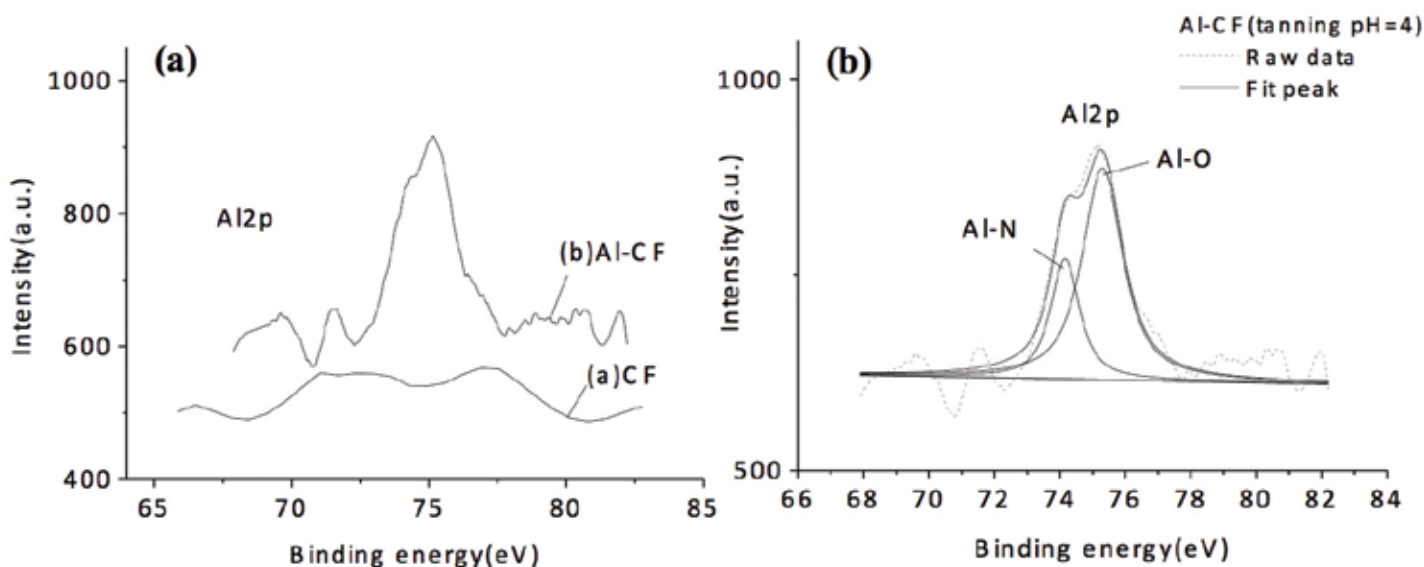


Figure 1. XPS spectra of Al 2p in CF and Al-CF(a) and fit peak of Al 2p(b).

Aluminum tanning usually needs an initial pH of 2.5~3.0 to promote the penetration rate of aluminum in pelts, while needing a final pH of about 4.0 to help the fixation of aluminum in pelts. Therefore, to obtain more details of the effect of pH on aluminum-binding sites between collagen fibers, Al-DACFs and Al-DCCFs which tanning pH were 2, 3 and 4, respectively, were chosen for analysis. The NIR spectra of aluminum tanned DACF (Al-DACF) are shown in Figure 2(a). It can be seen that aluminum tanning results in some changes in the reflectivity of the NIR spectrum of DACF. For a better detection of these changes, the corresponding second derivative spectra of Al-DACF were analyzed by UV Probe software.²¹ It is obvious that the intensity of second derivative peaks of carboxyl groups (-COOH) in Al-DACF at 1700 nm was much lower than that of DACF (Figure 2(b)). In addition, the peak intensities of carboxyl groups in Al-DACF at pH 2, 3 and 4 were almost the same. By comparing the peak intensities of Al-DACF, it can be found that

the carboxyl groups in DACF can react with aluminum and have similar reactivity with aluminum in the pH range of 2~4. The NIR spectra and corresponding second derivative spectra of aluminum tanned DCCF (Al-DCCF) at different tanning pH are shown in Figure 2(c) and 2(d), respectively. As shown in Figure 2(d), the intensity of second derivative peaks of amino groups (-NH₂) in Al-DCCF at 1549 nm was lower than that of DCCF, and the peak intensity of amino groups greatly decreases when DCCF was tanned at pH 3 and pH 4. These phenomena suggest that the amino groups of DCCF can react with aluminum in all the pH range of 2~4 and have much higher reactivity in the pH range of 3~4. Moreover, the decrease in the intensity of second derivative peaks of carboxyl groups in Al-DACF was larger than that of amino groups in Al-DCCF, suggesting that the carboxyl group in collagen should be the main Al-binding site. Besides, the Al-amino binding sites change more obviously with different pH.

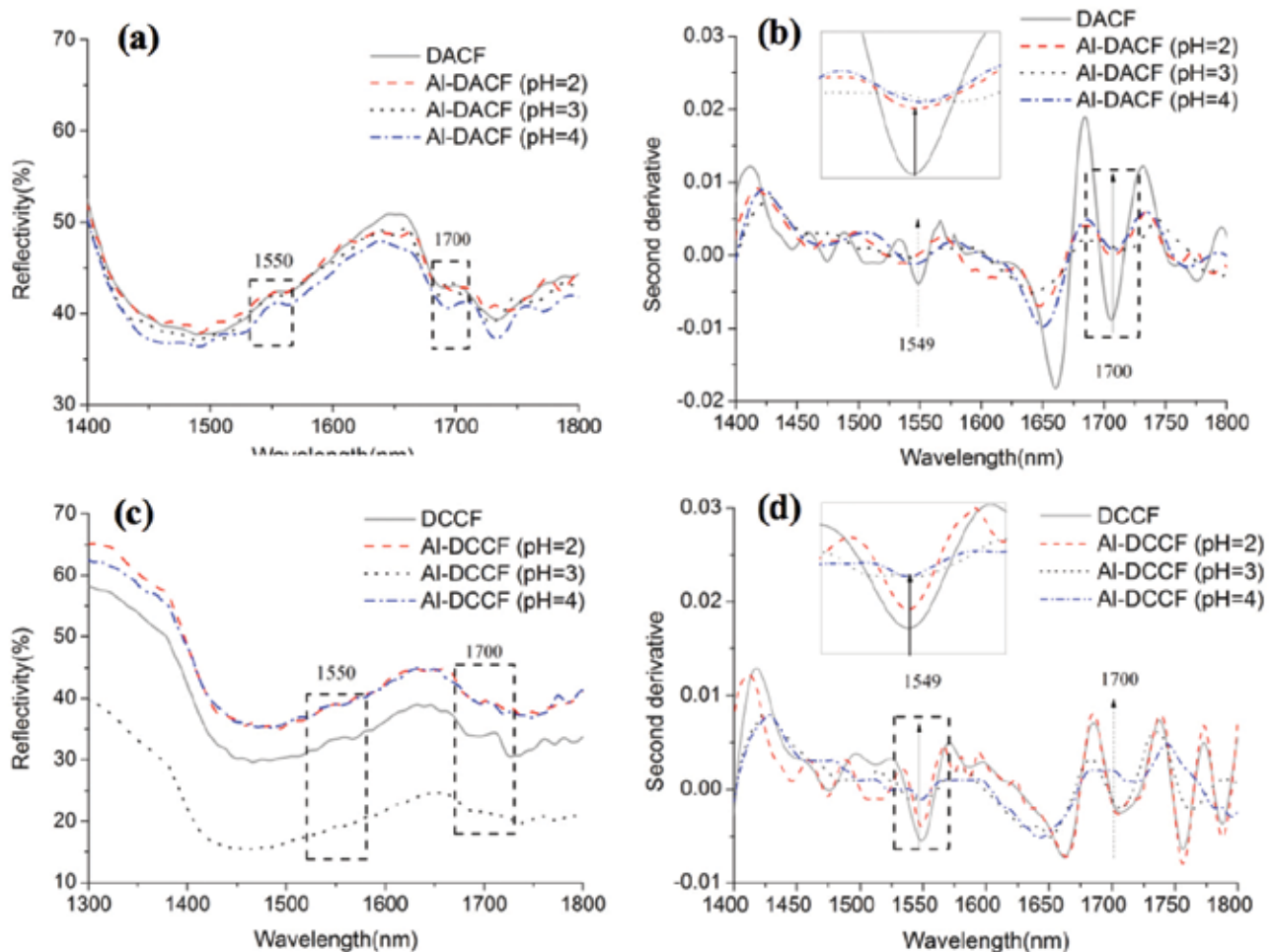


Figure 2. NIR spectra (a) and corresponding second derivative spectra (b) of aluminum tanned DACF (Al-DACF) at different tanning pH; NIR spectra (c) and corresponding second derivative spectra (d) of aluminum tanned DCCF (Al-DCCF) at different tanning pH. (↑: intensity decreases after aluminum tanning.)

To better investigate how the functional groups of collagen play a role in aluminum tanning, we determined the denaturation temperatures of different aluminum tanned collagen fibers. As listed in Table I, it is evident that the denaturation temperatures of Al-CFs are higher than those of Al-DACFs and Al-DCCFs when using tanning pH 2, 3 or 4. This suggests that both the carboxyl groups and amino groups in CF are aluminum-binding sites in collagen fibers. Additionally, the denaturation temperatures of Al-DACFs obtained by using tanning pH 2, 3 and 4 are nearly the same, which reveals that the reactivity of carboxyl groups in DACF is similar to that in the pH range of 2~4. This result may be due to the fact that AlSO_4^+ (cation) is the predominant species of aluminum ions, and its percentage is almost in the same state in the pH range of 2~4 (Figure 3). Furthermore, it is interesting to observe that the increase in

tanning pH exhibits positive effect on the denaturation temperatures of Al-DCCF. This fact means that the amino groups in DCCF react easier with aluminum at pH 3~4 than at pH 2. The reason may be that the percentage of $\text{Al}(\text{SO}_4)_2^-$ (anion) improves with an increase in pH (Figure 3). These results strongly confirm our previous conclusions drawn from NIR spectra (see Figures 1 and 2).

pH	Denaturation temperature (°C)		
	Al-CF	Al-DACF	Al-DCCF
2.0	64	59.1	59.4
3.0	74.8	63.1	65.5
4.0	75.7	62.8	71.5

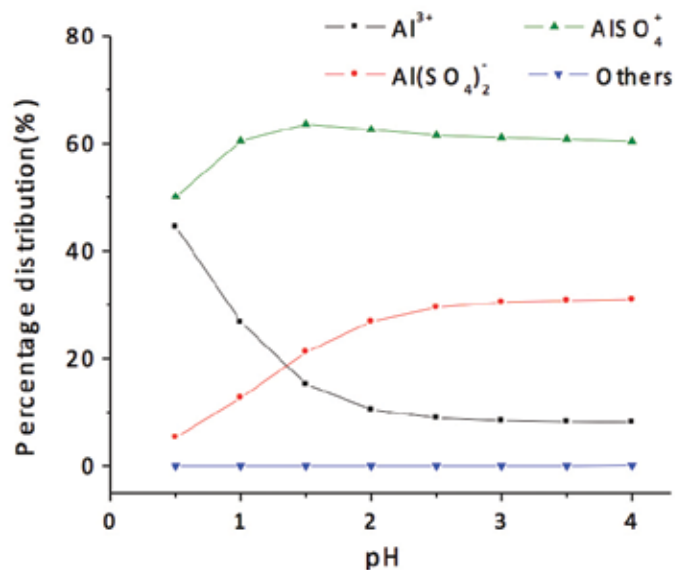


Figure 3. Distribution of Al(III) species in aqueous solution (calculated by using Visual MINEQL 2.40b version, NIST database. Concentration of Al(III) = 0.1 mol/L.)

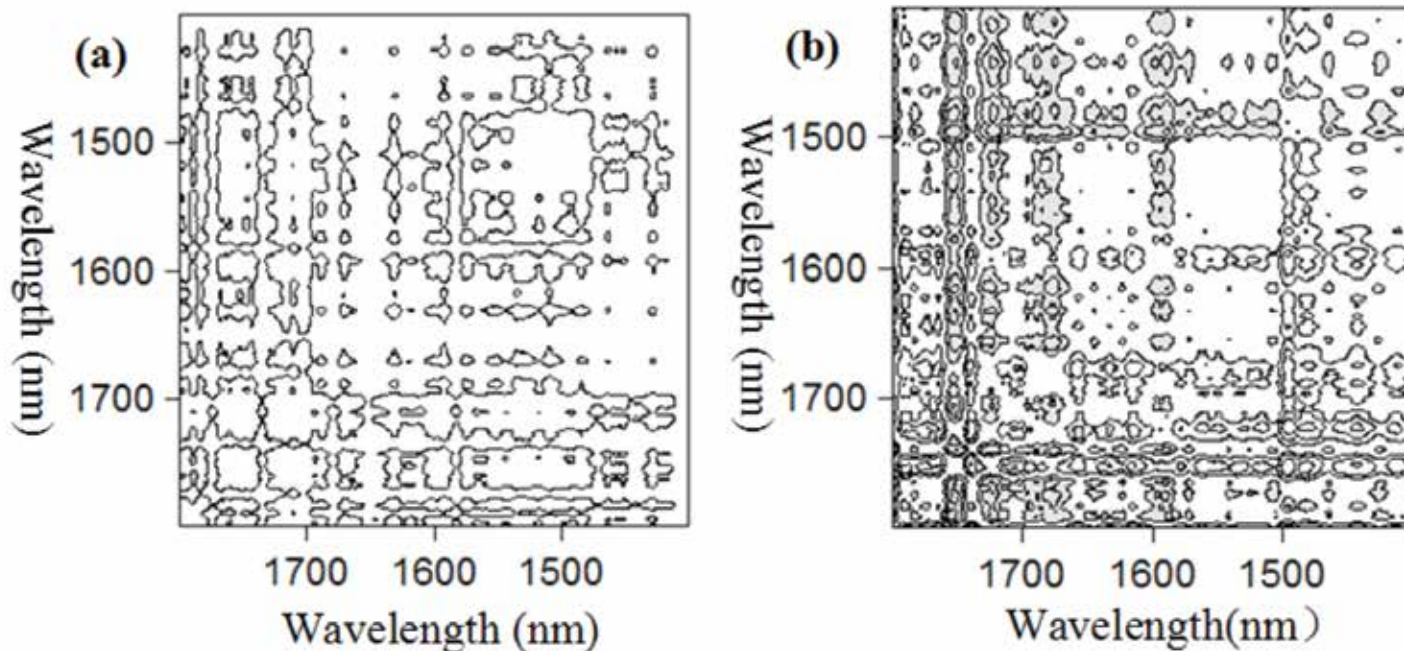


Figure 4. 2D NIR synchronous contour map (a) and 2D NIR asynchronous contour map (b) of aluminum tanned CF whose initial tanning pH varied from 0.5 to 4.0.

From the XPS analysis, the results show that the binding capacity of Al-amino is lower than Al-carboxyl. However, it can be found that the amino takes more change than carboxyl to binding with aluminum at different pH. In response to these phenomena, the rates of change of amino groups and carboxyl groups are explored with pH change by 2D NIR spectra analysis.

In 2D NIR spectra analysis, it can explain the direction and rate of changes between different kinds of functional groups with the intervention condition. In our experiment, the intervention condition is pH. The 2D spectra of aluminum were collected from tanning pH=0.5 to pH=4 in two stages which consist of the first phase from pH=0.5 to pH=2 with an increment of 0.5 and second phase from pH=2 to pH=4 with an increment of 0.25. In 2D contour map, it provides a 2D synchronous map and a 2D asynchronous map. The blank regions are defined as the positive correlation intensity, while the gray-colored ones are regarded as the negative correlation intensity.²² Both of the 2D contour maps are characterized by two vertical axes(λ_1, λ_2), and the correlations of different functional groups are able to be observed by different color peaks.

In 2D synchronous map the autopeaks, which appear along the diagonal, represent the autocorrelation of functional group vibrations with the changes of condition. In our researches, the changing condition was pH. The cross-peaks (marked as $\Phi(\lambda_1, \lambda_2)$) represent two kinds of functional groups which appear at the wavelength of λ_1 and λ_2 ($\lambda_1 < \lambda_2$). The positive cross-peaks in a synchronous map indicate that the functional groups at λ_1 and λ_2 increased or decreased simultaneously with pH change while negative cross-peaks demonstrate the functional groups change conversely with pH change. In asynchronous spectra, only off-diagonal cross-peaks appear and were marked as $\Psi(\lambda_1, \lambda_2)$, they

represent sequential-order change of the functional groups at λ_1 and λ_2 . When $\Phi(\lambda_1, \lambda_2) > 0$, $\Psi(\lambda_1, \lambda_2) > 0$ or $\Phi(\lambda_1, \lambda_2) < 0$, $\Psi(\lambda_1, \lambda_2) < 0$, functional group λ_1 vary prior to group λ_2 . When $\Phi(\lambda_1, \lambda_2) > 0$, $\Psi(\lambda_1, \lambda_2) < 0$ or $\Phi(\lambda_1, \lambda_2) < 0$, $\Psi(\lambda_1, \lambda_2) > 0$, functional group λ_1 vary after λ_2 with pH change.

Figure 4(a) and 4(b) show the 2D NIR correlation contour maps of CF tanned with aluminum at the initial pH in the range of 0.5~4.0 and a final pH of 4.1. In the 2D synchronous contour map (Figure 4(a)), the autopeaks $\Phi(1550, 1550)$ and $\Phi(1710, 1710)$ appearing along the diagonal are apparent. This indicates that the N-H in amino group and the O-H in carboxyl group both vary with pH. Moreover, the cross-peak (off-diagonal peak) $\Phi(1550, 1710)$ is positive, suggesting that the amino group and the carboxyl group at 1550 nm and 1710 nm, respectively, decreases simultaneously with the change in pH.²³ These results mean that the amino groups and carboxyl groups in CF should both be the aluminum-binding sites between collagen fibers. In the 2D asynchronous contour map (Figure 4(b)), the cross-peak $\Psi(1550, 1710)$ was positive, which indicates that the change in amino groups with pH is prior to that in carboxyl groups.²³ This fact implies that the carboxyl groups in CF should react with aluminum later than the amino groups. Therefore, though the binding capacity of Al-amino is lower than Al-carboxyl, Al-amino has the more obvious influence to the thermal stability due to the more rapid rate of change from pH=0.5 to pH=4.

Our result that the carboxyl group and amino group in collagen fibers can all combine with aluminum, is in agreement with previous studies.^{14,24} Our current findings about the effect of pH on aluminum-binding sites between collagen fibers expand prior research,^{24,25} which should be useful to give tanners some suggestions on choosing proper pH for aluminum tanning.

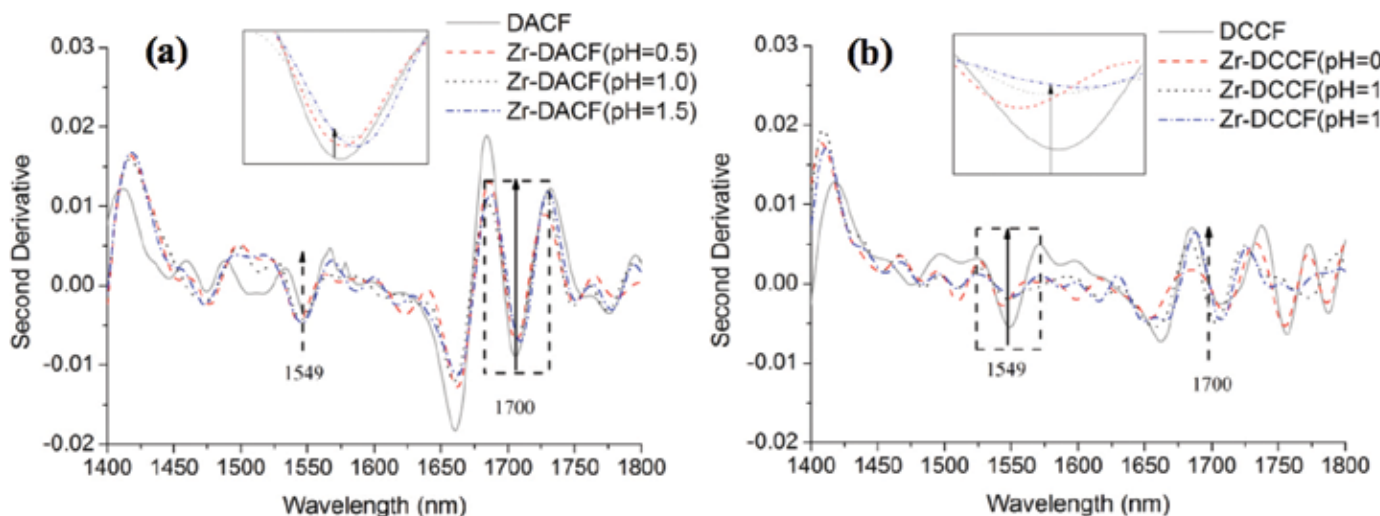


Figure 5. Corresponding second derivative spectra of zirconium tanned DACF (Zr-DACF) (a) and zirconium tanned DCCF (Zr-DCCF) (b) at different tanning pH. (↑: intensity decreases after zirconium tanning.)

Effect of pH on Zirconium-binding Sites in Collagen Fibers

Due to the low precipitation pH of zirconium (1.9), little data from tanning pH 0.5~1.5 is available for drawing 2D NIR correlation contour maps of Zr-CF. Therefore, we only analyze the NIR spectra of zirconium tanned collagen fibers. The corresponding second derivative spectra of zirconium tanned DACF (Zr-DACF) and zirconium tanned DCCF (Zr-DCCF) are shown in Figure 5(a) and 5(b), respectively. As shown in Figure 2(a), zirconium tanning only results in a slight decrease in the intensity of second derivative peaks of carboxyl group (-COOH) in Zr-DACF at 1700 nm, while the denaturation temperatures of Zr-DACF even decreases with the rise in tanning pH (Table II). These facts indicate that the carboxyl group should not be the main Zr-binding site between collagen fibers. As shown in Figure 2(b), a decrease in the peak intensity of amino groups in Zr-DCCF at 1549 nm occurs after zirconium tanning. Additionally, the denaturation temperatures of Zr-DACF tanned at pH 1.0 and 1.5 were higher than that of Zr-DACF tanned at pH 0.5 (see Table II). These facts suggest that the amino group in DCCF can react with zirconium in the pH range of 0.5~1.5 and its reactivity enhances when the tanning pH is above 1.0.

Table II
Denaturation temperature of different collagen fibers tanned with zirconium at different pH.

pH	Denaturation temperature (°C)		
	Zr-CF	Zr-DACF	Zr-DCCF
0.5	63.7	63.3	56.5
1.0	64.2	64.1	56.5
1.5	82.7	63.3	61.3

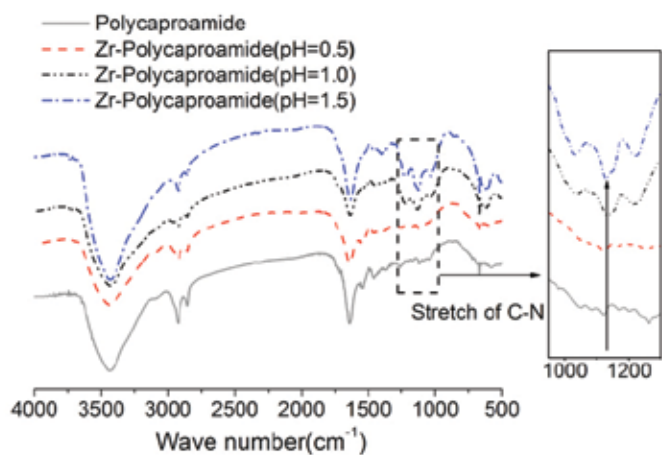


Figure 6. FTIR spectra of polycaproyamide, polycaproyamide reacted with zirconium (Zr-polycaproyamide) at different tanning pH.

It is worth noting that, as we can see from Table II, the denaturation temperatures of Zr-CFs are much higher than those of Zr-DACFs and Zr-DCCFs, especially when using tanning pH 1.0 and 1.5. Hence, it is reasonable to speculate that there should be another functional group in collagen with ability to combine with zirconium. As discussed in previous studies,^{10,14} the amide group of collagen is responsible for zirconium binding between collagen fibers. So we subsequently use polycaproyamide to simulate the amide group of collagen for zirconium tanning. The FTIR spectra of polycaproyamide and polycaproyamide reacted with zirconium (Zr-polycaproyamide) at different tanning pH are given in Figure 6. It can be seen that the rise in pH leads to an increase in the intensity of the broad band between 1000 and 1200 cm^{-1} in the spectra of Zr-polycaproyamide, where this band is attributed to C-N stretching vibration in the polycaproyamide.²⁶ This result proves that the amide group in collagen is one of the main zirconium-binding sites between collagen fibers, and its reactivity with zirconium complexes increases with the rise in pH.

Conclusion

NIR spectroscopy is effective in studying the effect of pH on metal-binding sites between collagen fibers in tanning process, where the inferences drawn from NIR spectra are consistent with those from DSC determination. Increasing pH is beneficial to improve the fixation of aluminum on collagen, because the reactivity of amino groups in collagen can be enhanced with an increase in pH. As for zirconium tanning, increasing pH is also useful to the fixation of zirconium on collagen, since the increase in tanning pH exhibits a positive effect on the reactivity of amino groups and amide groups in collagen.

Acknowledgement

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