

Solid State NMR Analysis for Hide Powder Tanned by Aluminum, Silicon and Phosphorus Tanning Agents before and after Hydrothermal Denaturation

by

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Abstract

In order to investigate the change of chemical bonds between tanning agents and collagen molecules directly, hide powder tanned by aluminum, silicon and phosphorus tanning agents were prepared. The chemical shifts of Al, Si and P in tanned hide powder were analyzed by solid-state ²⁷Al NMR, ²⁹Si NMR and ³¹P NMR. The results showed that, the chemical shift of Al in aluminum tanned hide powder which interacted with collagen molecules through coordination bond could be regarded as unchanging after hydrothermal denaturation (only slightly moved to high field area). The chemical shift of Si in silicon tanned hide powder which interacted with collagen molecules through hydrogen bond did not change after hydrothermal denaturation. The chemical shift of P in phosphorus tanned hide powder, which interacted with collagen molecules through covalent bond, was obviously shifted to the high field area after hydrothermal denaturation.

Introduction

Tanning is the essential process to transform hides into leather. If tanning is regarded as a kind of chemical reaction, its reactants are raw hides (mainly composed of type I collagen) and various tanning agents (metal tanning agents such as chromium salt and aluminum salt; vegetable tanning agents such as various tannin extracts; organic tanning agents such as glutaraldehyde and oxazolidine; etc.). It is consensus that leather should possess four characteristics after tanning process: (1) an improved hydrothermal stability; (2) resistance of microbial erosion; (3) its hand feeling, color and smell are different from raw hide; (4) it will not be turned back to raw hide after repeated drying and wetting cycles. Because the hydrothermal shrinkage temperature (T_s) of leather is the key index to characterize the tanning effect, and it has a positive correlation with the other three properties,¹ therefore, the study on tanning mechanism and tanning theory usually focuses on the research of the hydrothermal stability of leather.

The key to study tanning mechanism of a certain tanning agent is to find out how the tanning agent improves the hydrothermal stability

of collagen. This requires a clear understanding of the molecular structure of collagen and the composition and structure of the tanning agent. Only after that, the chemical reaction mechanism between them could be studied. Due to the complex and insoluble structure of collagen macromolecules, as well as the complexity and diversity of the composition and structure of various tanning agents, it is very difficult to study the tanning mechanism. As noted by Brown et al, "tanning is more suitably described than defined; tanning chemistry is more like art than science".² So far, according to the research on tanning mechanism of various tanning agents, a lot of theories, such as adsorption, coating, salt bond (ionic bond), hydrophobic bond, hydrogen bond, covalent bond and coordination bond theory, have been put forward.³

In addition to studying the chemical reaction between tanning agents and collagen molecules, another method for the research of tanning mechanism, i.e., the combination mode of tanning agent and collagen, which can be inferred via characterizing the tanned collagen by spectral analysis, could be adopted. At present, the main characterization methods are infrared spectroscopy,⁴ crystal diffraction,⁵ Raman spectroscopy,⁶ molecular simulation,⁷ nuclear magnetic resonance (NMR),^{8,9} etc. Infrared spectroscopy and Raman spectroscopy mainly reflect the hydrogen bond between collagen, which is not enough to fully investigate the interaction between tanning agents and collagen. Crystal diffraction experiment is to measure the distance between collagen molecules by measuring the diffraction angle, which could only reflect the presence of hydrogen bond between collagen molecules. Although molecular simulation can reflect the interaction between collagen and tanning agent, it is not a real material and is still different from the actual situation. Adopting ³¹P NMR, Li Ya et al.⁸ studied the reaction of tetrahydroxymethyl phosphorus chloride (THPC) with polycaprolactam, polyvinyl alcohol and ethylenediamine to simulate the reaction of amide group, hydroxyl group and amino group of collagen with THPC, but it is still not the real reaction of collagen with THPC.

If the molecular structure of tanning agents can be characterized by NMR, the tanning mechanism can be directly inferred by analyzing

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Manuscript received March 29, 2021, accepted for publication April 25, 2021.

the change of chemical shift of the characterized element in the tanning agent before and after the hydrothermal denaturation of tanned collagen. Covington et al.¹⁰ studied the chemical shift of aluminum (III) before and after hydrothermal denaturation of collagen tanned with aluminum salt by solid-state ²⁷Al NMR, and their results showed that the chemical shift of Al³⁺ did not change. They inferred that there is no cross-linking between aluminum complex and collagen molecules through coordination bonds, and they took this fact as a counter evidence to the cross-linking theory.

In this paper, tanned collagen samples were prepared by aluminum tanning agent, silicon tanning agent and phosphorus tanning agent. The chemical shifts of aluminum, silicon and phosphorus in tanned samples before and after hydrothermal denaturation were analyzed by solid-state NMR. The purpose of this work is to clarify the changes of the interaction between tanning agent and collagen molecules during the process of hydrothermal shrinkage, and to provide experimental basis for the study of tanning theory.

Experimental

Materials and Instruments

Sodium chloride, aluminum sulfate octadecahydrate, citric acid dihydrate, sodium bicarbonate, sulfuric acid, sodium silicate, γ -(2,3-epoxypropoxy) propyl trimethoxysilane (GPTMS), tetrahydroxymethyl phosphorus chloride (THPC), formic acid and other reagents are analytically pure, purchased from Chengdu Kelong Chemical Co., Ltd. White hide powder is provided by Leather Department of Sichuan University. DSC analysis was performed on a differential thermal scanner (Q2000, TA company, USA) and solid-state NMR analysis was performed on 11.7T Bruker Avance III-400.

Aluminum Tanning

Preparation of Tanning Solution¹¹

Take 9.6632 g aluminum sulfate octadecahydrate, fix the volume in 100 mL volumetric flask (No. 1), and draw 33.3 mL from it into another 100 mL volumetric flask (No. 2). Weigh 2.6950g sodium citrate and put the same constant volume into a 100 mL volumetric flask. Take out 54.5 mL and add it into No. 2 conical flask. Add NaOH solution (0.5 mol/L) to adjust pH to 3.5 and place it for more than 24 h.

Tanning Process¹¹

Weigh 0.5 g hide powder into a 25 mL conical flask, add 8 mL NaCl solution (1 mol/L), adjust pH to 2.5 with 1 mol/L formic acid solution, and oscillate at 30°C for 36 h. Then, take 10 mL aluminum tanning solution from No.2 volumetric flask, add it into the conical flask, and vibrate at 30°C for about 30 h. After that, NaHCO₃ solution (10%, w/w) was added in several times, pH was adjusted to 5 within 2 hours, and then oscillated at 30°C for 10 hours. Filter out the hide powder with gauze and keep it in a sealed bag for testing.

Phosphorous Tanning¹²

Put 0.5 g hide powder into a 25 mL conical flask, add 8 mL NaCl solution (1mol/L), and shake overnight at 25°C. Then, add 60% THPC solution in the conical flask, and shake at 40°C for 1 h. After that, add NaHCO₃ solution (10%, w/w) to adjust the pH to 10.0, and continue to oscillate for 1.5 h at 40°C. After standing for 12 h, wash with water, filter out the hide powder with gauze, and store in sealed bag for testing.

Silicon Tanning

Preparation of Silicic Acid Tanning Solution¹³

Sulfuric acid and sodium silicate were used as raw materials to prepare silicic acid solution. Specifically, first, dilute concentrated sulfuric acid to dilute sulfuric acid according to the mass ratio of 1:1, and then cool to room temperature for standby. The sodium silicate weighed according to the mass concentration of 10% is dissolved in water to form a clear and transparent solution. Then, under the condition of stirring, slowly add sodium silicate solution into dilute sulfuric acid solution according to the stoichiometric ratio of sodium silicate to sulfuric acid as 1:1.1, stir evenly, stand still and cool to room temperature before use. The mass concentration of the achieved silica tanning solution is about 3% (equivalent to SiO₂), pH is 2.0-2.5.

Tanning Process¹³

Weigh 0.5 g hide powder into a 25 mL conical flask, add 8ml NaCl solution (1mol/L), adjust pH to about 2.0 with 1 mol/L formic acid solution, and oscillate at 30°C for about 36 h. Then, add 15% γ - (2.3-epoxy propoxy) propyl trimethoxysilane (GPTMS) by the weight of hide powder, shake for 1 h, and then add 15% silica tanning solution (equivalent to SiO₂) by the weight of hide powder, shake for 5 h. After that, NaHCO₃ solution (10%,w/w) was added in several times, pH was adjusted to 5 within 2 h, and then oscillated at 30°C for 10 h. Filter out the hide powder with gauze and keep it in a sealed bag for testing.

DSC Analysis

DSC analysis was performed on Q2000, TA company, USA. Take 5 mg of white hide powder (reference sample) and samples tanned with aluminum, silicon, phosphorus tanning agents respectively, seal them in aluminum plate. The scanning temperature was 25~120°C, the heating rate was 5°C /min, the flow rate of nitrogen was 10mL / min, and the onset temperature of DSC curve was taken as the hydrothermal denaturation temperature (T_d). Three times were repeated for each kind of tanned sample.

Solid State NMR Analysis

The samples BEFORE hydrothermal denaturation were those hide powder sealed in bags after tanning, and the samples AFTER hydrothermal denaturation were those hide powder after DSC test. Solid state NMR analysis was performed on 11.7T Bruker Avance III-400.

Table I
 T_d of hide powder and aluminum, silicon and phosphorus tanned collagen

Samples	White hide powder	Aluminum-tanned hide powder	Silicon-tanned hide powder	Phosphorus-tanned hide powder
T_d (°C)	61.5	73.2	84.3	85.6

^{27}Al NMR Analysis

Take 10 mg of hide powder before and after hydrothermal denaturation, seal and label as Al-1 and Al-2 respectively. The test conditions were as below: 4 mm three resonance probe, rotation frequency 10 kHz, resonance frequency 130.4 MHz, single pulse sequence, pulse width 0.4 μs (ca. $\pi / 6$), relaxation time 1 second. The chemical shift of 1 mol/L $\text{Al}(\text{NO}_3)_3$ solution is referred to 0 ppm.

^{29}Si NMR Analysis

Take 10 mg of hide powder before and after hydrothermal denaturation, seal and label as Si-1 and Si-2 respectively. Test conditions were as below: using a 7 mm probe, the resonance frequencies of ^1H and ^{29}Si are 399.33 MHz and 79.33 MHz respectively. ^{29}Si MAS NMR single pulse sampling, $\pi / 2$ pulse is 6.0 μs , sampling delay is 60 s, rotational speed is 5 kHz, kaolin calibration (-91.5 ppm).

^{31}P NMR Analysis

Take 10 mg of hide powder before and after hydrothermal denaturation, seal them and label as P-1 and P-2 respectively. Test conditions were as below: resonance frequency 202.8 MHz; high energy decoupling, repetition time 4 s; pulse width 2 μs (ca. $\pi / 4$); 85% H_3PO_4 as reference sample. Other conditions are the same as ^{27}Al NMR analysis.

Results and Discussion

Results of DSC Analysis

The hydrothermal denaturation temperatures (T_d) of hide powder tanned with aluminum, silicon and phosphorus tanning agents from DSC analysis are listed in Table I.

Analysis of ^{27}Al NMR Result

It is known from previous work¹¹ that, when pH value is less than 2.5, there are few single Al^{3+} complexes in aluminum tanning solution, instead of that, aluminum ions will mainly form linear poly-ion complexes with 2~4 Al^{3+} (as shown in Fig. 1a); when pH value is about 4.0, spherical " Al_{13} " complexes with 13 Al^{3+} will be the main components (as shown in Figure 1b). The chemical shifts of linear polyion complexes with 2~4 Al^{3+} are in the range of 2~5 ppm, while the chemical shifts of spherical " Al_{13} " complexes are about 72 ppm. The chemical shifts of these two kinds of aluminum complexes are positively correlated with the number of Al^{3+} .¹¹

Figure 2 is the ^{27}Al NMR spectrum of collagen samples tanned with aluminum salt before and after hydrothermal denaturation. It can be obtained that, the chemical shifts of Al in the collagen samples tanned with aluminum salt before and after hydrothermal denaturation are 4.1 ppm and 2.9 ppm, respectively. Although

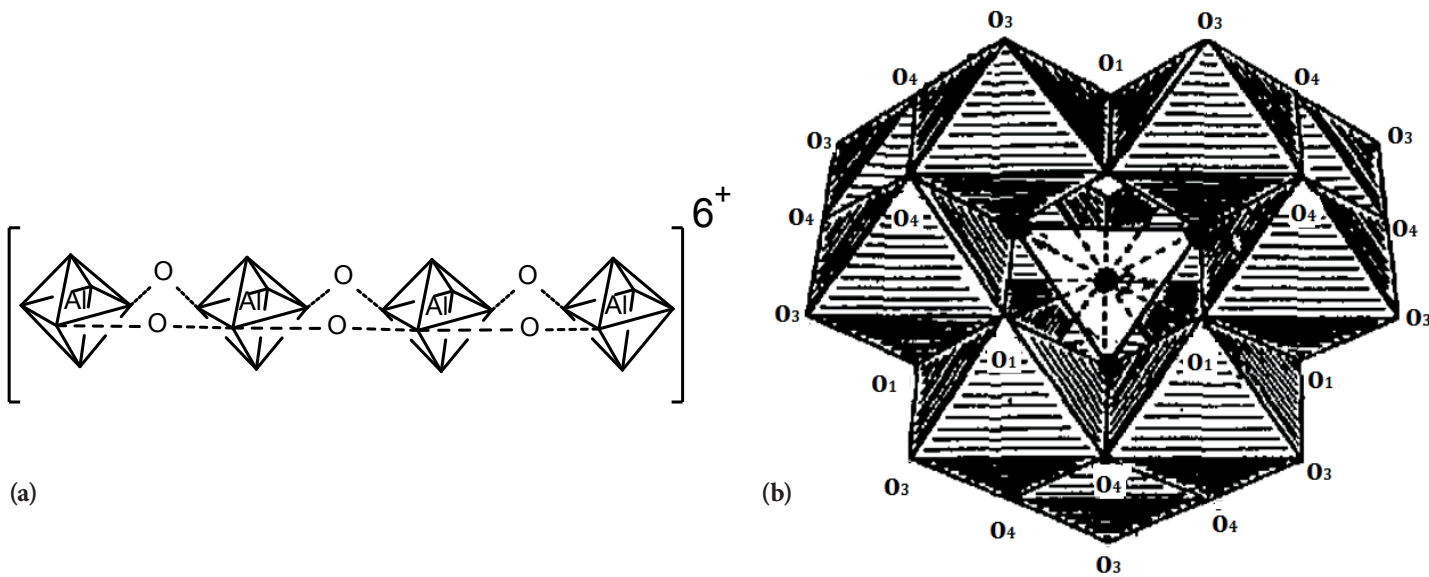


Figure 1. Structure of two kinds of aluminum complexes in tanning solution ((a) linear; (b) " Al_{13} ")

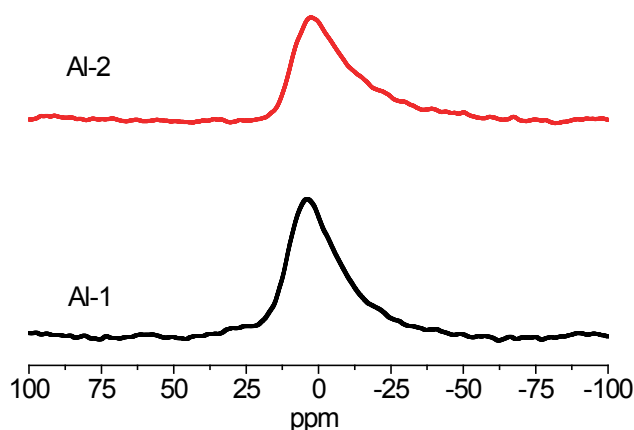


Figure 2. ^{27}Al NMR of aluminum-tanned collagen before and after denaturation (Al-1 —before denaturation, Al-2 —after denaturation)

the pH value of tanning solution is increased to above 4.5 in the later stage of aluminum tanning process, and at this pH value, the aluminum complexes in tanning solution should be spherical “ Al_{13} ” complexes theoretically.¹¹ However, it can be seen from Figure 2 that, both the aluminum complexes in hide powder before and after denaturation exist as linear polyion complexes with 2~4 Al^{3+} . In other words, there are no “ Al_{13} ” complexes in aluminum tanned hide powder. The possible inference for this fact is that, either “ Al_{13} ” complexes are too large to penetrate into collagen, or “ Al_{13} ” complexes are transformed into linear complexes with 2~4 Al^{3+} after hide powder leaving tanning solution. In a word, linear complexes composed of 2~4 Al^{3+} play the dominating role in tanning process.

It can also be seen from Figure 2 that the chemical shift of Al in aluminum tanned collagen hardly changes after denaturation (in ^{27}Al NMR spectrum, the small difference between 2.9 ppm and 4.1 ppm might be caused by the shielding effect of the conformational change on the central Al^{3+} ion caused by the hydrothermal denaturation of collagen). This result is consistent with that of Covington et al.¹⁰ The explanation given by Covington et al. is that there is no cross-linking between the aluminum complexes and the collagen side groups.

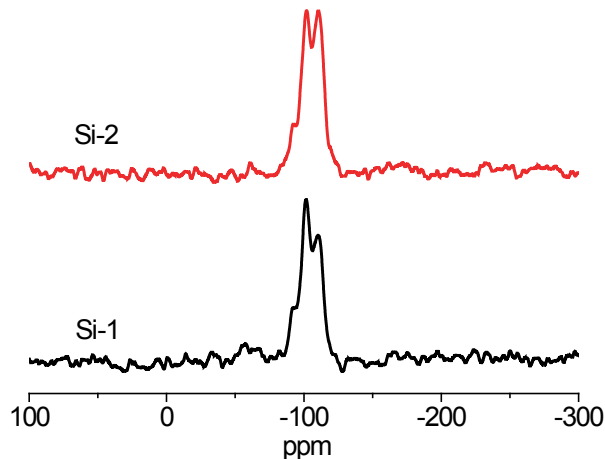


Figure 3. ^{29}Si NMR of silicon tanned hide powder before and after denaturation (Si-1 —before denaturation, Si-2 —after denaturation)

However, we speculated that, there might be another possibility, that is, after the hydrothermal denaturation of the collagen, the coordination bonds between the aluminum complexes and the collagen side groups are not broken.

Analysis of ^{29}Si NMR Results

Zhang et al.¹³ considered that silicic acid tanning agent mainly produced tanning effect on collagen through hydrogen bonds. It can be seen from Figure 3 that, both the chemical shift of Si in the collagen before and after hydrothermal denaturation is about -100 ppm. This result indicates that Si-O bonds and Si-Si bonds of silicon tanning agent in tanned collagen are not broken after hydrothermal denaturation. From this result, we could speculate that, the hydrothermal denaturation might be caused by the destruction of hydrogen bond among the tanned collagen molecules.

Analysis of ^{31}P NMR Results

Relevant studies suggest that the tanning mechanism of THPC is shown in Figure 4: each molecule of THPC contains four hydroxymethyl groups, which can form multi-point cross-linking with the amino groups of collagen molecules via covalent bonds.^{12,14}

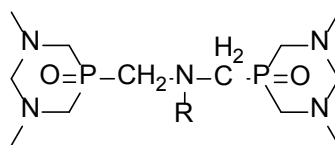
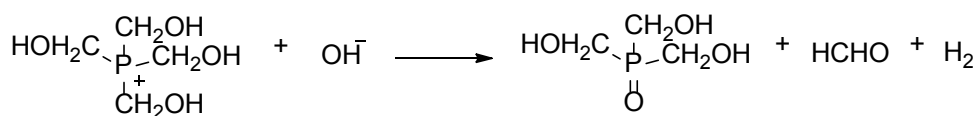


Figure 4. Tanning mechanism of THPC

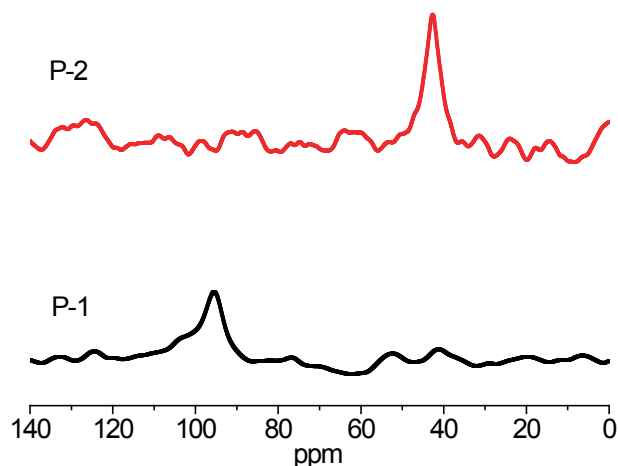


Figure 5. ^{31}P NMR of THPC tanned hide powder before and after denaturation (P-1—before denaturation, P-2—after denaturation)

It can be seen from Figure 5 that, the chemical shift of P in THPC tanned collagen is 95.5 ppm before hydrothermal denaturation, and it moves to 42.7 ppm after denaturation. Since the chemical shift of P in unreacted THPC is 26.5–27.2 ppm, Shao et al. speculated that the P-C bond of THPC was broken during the reaction with collagen, releasing formaldehyde, which was oxidized (as shown in Fig. 4), resulting in the chemical shift of P moving to 95.5 ppm in THPC tanned collagen.¹² Therefore, the results of ^{31}P NMR as shown in Figure 5 can be explained as follows: during the hydrothermal denaturation of collagen, a large number of amino, hydroxyl and carboxyl groups in collagen changed from ordered state to disordered state, which may form hydrogen bonds with oxygen atoms in P = O double bonds; or during the denaturation of collagen, P = O double bonds was reduced to P-O single bonds, resulting in the increase of electron cloud density around phosphorus atoms. Thus, the chemical shift moved to the high field. On the one hand, this result indicated the formation of covalent bonds between THPC and collagen molecules during the tanning process. On the other hand, the chemical bonds between THPC and collagen molecules were changed after hydrothermal denaturation, but they did not completely return to the state before tanning reaction. This conclusion could be deduced from the fact that, the chemical shift of P in denatured THPC tanned collagen was 42.7 ppm, while that in unreacted THPC is 26.5–27.2 ppm.

Conclusion

The chemical shifts of Al, Si and P in hide powder tanned with aluminum, silicon and phosphorus tanning agents before and after hydrothermal denaturation were analyzed by ^{27}Al NMR, ^{29}Si NMR and ^{31}P NMR. From the changes of their chemical shifts, following conclusions could be obtained:

- (1) Linear complexes composed of 2~4 Al^{3+} play the dominated role in aluminum tanning process. The chemical shift of

Al in the complexes reacted with collagen molecules by coordination bonds have no change (slightly moving to high field) after hydrothermal denaturation of collagen. Therefore, it can be speculated that either there are no coordination cross-linking between aluminum complexes and the side groups of collagen molecules, or the coordination cross-linking bonds between aluminum complexes and the side groups of collagen molecules are not broken after hydrothermal denaturation.

- (2) It can be inferred that Si-O and Si-Si bonds are not broken after hydrothermal denaturation of collagen, and the hydrothermal denaturation might be caused by the destruction of hydrogen bonds among the tanned collagen molecules.
- (3) The results show that the chemical shift of P moves to high field obviously after hydrothermal denaturation. It can be inferred that the covalent bonds between THPC and collagen molecules occur during tanning process. After hydrothermal denaturation, the chemical bonds between THPC and collagen molecules are partly broken, i.e., they do not completely return to the state before tanning reaction.

Acknowledgement

This work was financially supported by grants from the National Natural Sciences Foundation (No. 21776231) of China. Thanks to Professor Shen Wen-jie of Dalian Institute of Chemical Physics, Chinese Academy of Sciences for his support in solid state NMR testing.

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