

# THE FURTHER INVESTIGATION OF TANNING MECHANISMS OF TYPICAL TANNAGES BY ULTRAVIOLET-VISIBLE AND NEAR INFRARED DIFFUSED REFLECTANCE SPECTROPHOTOMETRY

by

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## ABSTRACT

The ultraviolet-visible and near-infrared diffuse reflection (UV-Vis-NIR DR) spectroscopy was employed for further understanding the mechanisms of three kinds of classical tannages, including aldehyde tanning, vegetable tanning and mineral tanning. The NIR DR spectra of glutaraldehyde tanned leather exhibited a decreased peak intensity of amino group at 1475 nm, suggesting that the main reaction groups of collagen in aldehyde tanning are amino groups. Comparing the NIR DR spectra of pickled pelt and vegetable tanned leather, it was proved that the vegetable tanning is based on the mechanism of multiple hydrogen bonding interaction, where the amino groups, carboxyl groups and peptide chains are involved. As for mineral tannages, the tanning mechanisms of chromium sulfate, zirconium sulfate, ferrum sulfate, titanium sulfate and aluminum sulfate were investigated. Based on UV-Vis-NIR DR analysis in the wavelength range of 200 to 2070 nm, it was confirmed that the main reactions involved in mineral tanning are the chelating interactions of metal ions with both the carboxyl groups and the amino groups of collagen, although the reactivity of metal salts toward carboxyl groups is higher than toward amino groups. The reactivity of metal salts toward carboxyl groups of collagen follows the sequence of chromium salt > zirconium salt > ferrum salt > titanium salt > aluminum salt. However, the reactivity of metal salts to amino groups of collagen is in the sequence of zirconium salt > chromium salt > ferrum salt > titanium salt > aluminum salt. Meanwhile, the peptide chain of collagen was also found to play an important role in fixation of metal ions during tanning process.

## RESUMEN

La espectroscopia por reflexión difusa de [radiación] ultravioleta-visible e infrarroja cercana (UV-Vis-NIR DR) fue empleada para la mejor comprensión de los mecanismos de los tres tipos de curtidos clásicos que constan del curtido al aldehído, curtido al vegetal y curtido mineral. Los espectros NIR DR del cuero al glutaraldehído señalaron una intensidad disminuida del grupo amino a 1475 nm, sugiriendo que los grupos principales en la curtición por aldehído son los mismos grupos aminos. Por comparación de los espectros NIR DR de piel piquelada con la de cuero curtido al tanino, se demostró que el mecanismo del curtido al tanino se basa en múltiples interacciones por medio de uniones a través de puentes de hidrógeno, donde los grupos aminos, grupos carboxilos y las mismas cadenas peptídicas están involucradas. En cuanto a curtidos minerales, los mecanismos del curtido con sulfato de cromo, sulfato de circonio, sulfato férrico, sulfato de titanio y sulfato de aluminio fueron investigados. En base al análisis por medio UV-Vis-NIR DR en la región de 200 a 2070 nm se confirmó que las reacciones principales involucradas en el curtido mineral son por interacción de quelación entre iones metálicos con ambos los grupos carboxílicos y los aminos del colágeno, aunque la reactividad hacia los carboxilos es mayor que la ocurrida hacia los grupos aminos. La reactividad de sales metálicas hacia los grupos carboxilos del colágeno siguen la secuencia de sal de cromo > sal de circonio > sal férrica > sal de titánio > sal de aluminio. Sin embargo, la reactividad de las sales metálicas hacia los grupos aminos del colágeno sigue la secuencia sal de circonio > sal de cromo > sal férrica > sal de titánio > sal de aluminio. Adicionalmente, la cadena peptídica del colágeno juega un importante papel en la fijación de iones metálicos durante el proceso de la curtición.

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## INTRODUCTION

Diffuse reflection (DR) spectroscopy is recognized to be an important tool for analysis and characterization of a wide variety of organic and inorganic solid compounds, and it has been used in many fields such as catalysis,<sup>1</sup> pharmaceuticals<sup>2</sup> and food industry.<sup>3</sup> In the ultraviolet-visible (UV-Vis) spectral range (200-800 nm), DR spectroscopy is able to characterize the specific electron transition behaviors of solid compounds,<sup>4</sup> especially transition metal compounds.<sup>5</sup> In the near-infrared (NIR) spectral range (780-2526 nm), the most distinguished advantage of DR spectroscopy is its extremely high sensitivity toward the functional groups of -NH<sub>2</sub>, -NH-, -OH and -COOH involved in the formation of hydrogen bonds,<sup>6</sup> where even a slight change of those functional groups can be observed.<sup>7,8</sup> Additionally, DR spectroscopy is extremely efficient, and can perform on-line analysis.<sup>9</sup> Considering all these advantages of DR spectroscopy in analysis of solid compounds, we intend to use ultraviolet-visible and near-infrared diffuse reflection (UV-Vis-NIR DR) spectroscopy to investigate the mechanisms of some typical tanning processes in this study.

As we know, the functional groups of collagen, such as amino group (-NH<sub>2</sub>), carboxyl group (-COOH) and peptide chain (-CONH-), would participate in the reactions with tanning agents (aldehydes, vegetable tannins, metal salts, etc.) in tanning processes.<sup>10</sup> During these reactions, the functional groups of collagen are consumed, which inevitably leads to the destruction of hydrogen bonds in collagen since these functional groups are involved in the formation of hydrogen bonds for the structural stabilization of tropocollagen molecules.<sup>11</sup> Due to the fact that all these functional groups have specific reflecting peaks in NIR spectra range, it is possible to use NIR DR spectroscopy to investigate the reactions involved in tanning process. Additionally, the ultraviolet-visible DR spectra can provide detailed information of the metal complexes when metal salts are used as tanning agents. Therefore it is feasible in principle to investigate the chemical reaction or mechanism of tanning process by using UV-Vis-NIR DR spectroscopy.

In this study, pelts were first tanned by three kinds of typical tannages, including aldehyde tanning, vegetable tanning and mineral tanning. Subsequently, the leather samples were analyzed by UV-Vis-NIR DR, and the mechanisms of these tannages were discussed in detail. In our knowledge, it is the first time to employ UV-Vis-NIR DR spectroscopy for analysis of leather.

## EXPERIMENTAL

### Preparation of Leather Samples

Goatskins were used for preparation of leather samples. The pickled goatskins were processed into leathers according to conventional tanning procedures of aldehyde tanning, vegetable tanning and mineral tanning. Herein, seven kinds of leathers were prepared, including glutaraldehyde tanned leather (noted as GA-CF, GA 3% based on the weight of pickled pelt, final pH=6.5), bayberry tannin tanned leather (noted as BT-CF, BT 30 %, final pH=3.5), chromium sulfate tanned leather (noted as Cr-CF, Cr<sub>2</sub>O<sub>3</sub> 2.1 %, final pH=5.0), aluminum sulfate tanned leather (noted as Al-CF, Al<sub>2</sub>O<sub>3</sub> 3.0 %, final pH=5.0), ferric sulfate tanned leather (noted as Fe-CF, Fe<sub>2</sub>O<sub>3</sub> 4 %, final pH=5.0), zirconium sulfate tanned leather (noted as Zr-CF, ZrO<sub>2</sub> 5.0 %, final pH=3.5), and titanium sulfate tanned leather (noted as Ti-CF, TiO<sub>2</sub> 6.0 %, final pH=3.5). The untanned pickled pelt was used as control (noted as CF). All samples were drying under atmosphere and stored at 25°C with a humidity of 65%.

### UV-Vis-NIR DR Determinations

All UV-Vis-NIR DR spectra were acquired on the UV-3600 UV-Vis-NIR spectrophotometer (Shimadzu, Japan) equipped with an integrating sphere attachment (LISR-3100, Shimadzu, Japan), of which the internal surface is coated with white BaSO<sub>4</sub>. The UV-Vis-NIR spectrophotometer was equipped with a photomultiplier tube for detection of UV-Vis spectroscopy, and the InGaAs and PbS detectors for detection of NIR spectroscopy. After a piece of leather sample was placed in the sample cabinet of UV-3600 UV-Vis-NIR spectrophotometer, two individual light beams passed through the reference (R) hole and the sample (S) hole, and irradiated on the BaSO<sub>4</sub> standard sample and the testing sample, respectively. The light beams reflected from the standard sample and the testing samples were collected by the integrating sphere attachment. The whole analysis process was completed within several minutes. The obtained spectra were analyzed using UV Probe software package, and the corresponding second derivative of each spectrum was calculated by the following equation:<sup>12</sup>

$$A_{\text{second derivative}}(i) = \frac{A_{\text{original}}(i + \Delta\xi) - 2A_{\text{original}}(i) + A_{\text{original}}(i - \Delta\xi)}{\Delta\xi^2}$$

Where  $A_{\text{original}}$  is the reflectivity of original DR spectrum, and  $A_{\text{second derivative}}$  is the second derivative value.  $i$  is the wavelength of differential window, and  $\Delta\xi$  is the differential window width, which is infinitely close to zero.

## RESULTS AND DISCUSSION

### Comparison and Analysis of DR Spectra of CF, GA-CF and BT-CF

The NIR DR spectra of CF, GA-CF and BT-CF are shown in Figure 1. In the whole NIR spectral range, the NIR DR spectra of GA-CF and BT-CF exhibit more intensive reflectivity as compared with that of CF, and BT-CF has the highest reflectivity. A detailed description of the strengthened reflectivity of tanned skins is difficult, but a reasonable explanation is that the cross-linking effect of glutaraldehyde and vegetable tannin between collagen molecules increases the reflectivity of leather toward light beam. Obviously, the vegetable tannin has stronger filling effect than that of glutaraldehyde, and therefore the BT-CF results in a higher reflectivity in DR spectra. The specific N-H 1<sup>st</sup> overtone ( $2\nu_{(N-H)}$ ) peaks of acylamide groups ( $-\text{CO}-\text{NH}_2$ ), peptide chains ( $-\text{CONH}-$ ), amino groups ( $-\text{NH}_2$ ), and the O-H 1<sup>st</sup> overtone ( $2\nu_{(O-H)}$ ) in carboxyl groups ( $-\text{COOH}$ ) are clearly observed at 1450 nm, 1475 nm, 1549 nm and 1700 nm, respectively<sup>13-15</sup>. Additionally, the combination peak of N-H and C=O in peptide chains is found in the range of 2000 to 2100 nm.<sup>16</sup>

Subsequently, the second derivative spectra from Figure 1 can be obtained using the calculation of the equation defined in experiment section,<sup>12</sup> which can solve the problems of overlapping peaks and baseline variations. Figure 2 shows the NIR DR spectra (a) and the corresponding second derivative spectra (b) of GA-CF and CF. As shown in Figure 2(b), the second derivative peaks of GA-CF at 1475 nm ( $-\text{CO}-\text{NH}_2$ ) and 1549 nm ( $-\text{NH}_2$ ) are remarkably decreased, while the second derivative peak of carboxyl group at 1700 nm has no considerable change. These observations indicate that glutaraldehyde mainly reacts with the amino groups of collagen rather than the carboxyl groups during tanning process. This conclusion consists with the conventional mechanism of aldehyde tanning.<sup>17-20</sup>

As for the bayberry tannin tanned leather (BT-CF) its NIR DR spectra in the range of 1400 to 2070 nm and the corresponding second derivative spectra are shown in Figure 3. Compared with CF, the second derivative peak of carboxyl group of BT-CF at 1700 nm is obviously decreased (Figure 3b), which should be attributed to the formation of hydrogen bonds between carboxyl groups ( $-\text{COOH}$ ) of collagen and phenolic hydroxyls of bayberry tannin. The second derivative peak of amino groups ( $-\text{NH}_2$ ) at 1549 nm is also decreased, which suggests that the amino groups of collagen have interacted with bayberry tannin *via* hydrogen bonding. Additionally, the combination peak of N-H and C=O in peptide chains shows a blue shift from 2046 to 2034 nm (Figure 3d), indicating that the peptide chains is also involved in the formation of hydrogen bonds with bayberry tannin. Based on these results, it is reasonable to conclude that collagen interacts with tannin

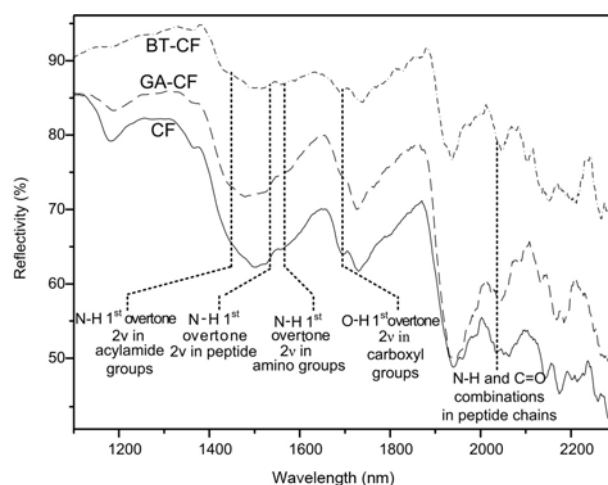


Figure 1. NIR DR Spectra of CF, GA-CF and BT-CF

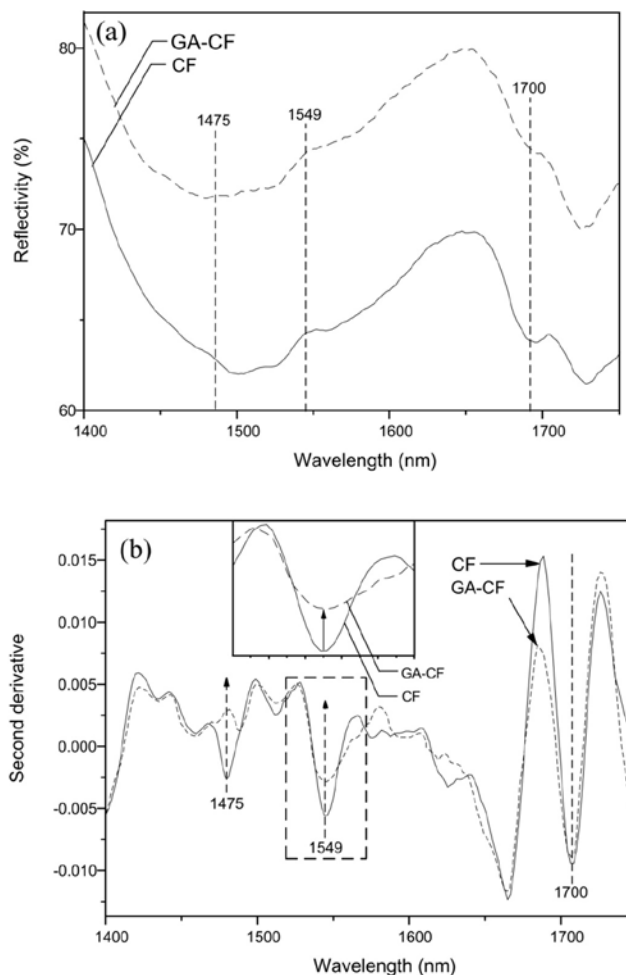


Figure 2. NIR DR Spectra (a) of GA-CF (dash) and CF (Solid) from 1400 to 1750 nm and corresponding derivative spectra (b); (↓: intensity decrease after glutaraldehyde tanning)

via multiple hydrogen bonding during vegetable tanning process. The amino groups ( $-\text{NH}_2$ ), carboxyl groups ( $-\text{COOH}$ ), and the peptide chains ( $-\text{CONH}-$ ) of collagen all participate in the formation of hydrogen bonds. Consequently, the NIR DR spectra provide new persuasive evidences for the suggestion of multiple hydrogen bond mechanism of vegetable tanning.<sup>21-25</sup>

### Comparison and Analysis of DR Spectra of Metal Salts Tanned Leathers

Figure 4a shows the UV-Vis DR spectra of leathers tanned by five commonly used metal salts. Both the chromium sulfate tanned leather (Cr-CF) and ferric sulfate tanned leather (Fe-CF) exhibit double peaks in the wavelength range of 200 to 1400 nm. These double peaks should be derived from the  $d \rightarrow d^*$  electronic transition of  $\text{Cr}^{3+}$  or  $\text{Fe}^{3+}$  complexes.<sup>26</sup> Such an electronic transition behavior cannot be observed in the UV-Vis DR spectra of aluminum sulfate tanned leather (Al-CF), zirconium sulfate tanned leather (Zr-CF) and titanium sulfate tanned leather (Ti-CF) since the  $d$ -orbitals of central atoms of these metal complexes are empty, and thus no  $d \rightarrow d^*$  electronic transition happens<sup>26</sup>. Additionally, the NIR reflectivity of Cr-CF is the highest and that of Fe-CF is the next, then followed by Zr-CF, Ti-CF and Al-CF, which is quite consistent with their tanning effect,<sup>27,28</sup> as shown in Figure 4(b). These facts suggest that chrome has the strongest cross-linking effect among the metal salts investigated, and also well explain the highest shrink temperature of chrome tanning leather.

To get insight to the tanning mechanisms of these metal salts, the corresponding second derivative spectra are obtained using the calculation of the equation defined in experiment section, as shown in Figure 5.<sup>12</sup> As for the characteristic peak of amino groups (1549 nm) of collagen in Figure 5a, the peak intensity decreases in the sequence of Zr-CF > Cr-CF  $\approx$  Fe-CF > Ti-CF > Al-CF. Accordingly, zirconium ion has the highest reactivity toward the amino group of collagen, and that of aluminum ion is the lowest. However, the reactivity of metal ions with carboxyl groups of collagen is in the sequence of chromium salt > zirconium salt > ferric salt > titanium salt > aluminum salt, as shown in Figure 5b. These facts strongly suggest that chromium ion, ferric ion and zirconium ion react with both amino group and carboxyl group of collagen during tanning, even though chrome and ferric are conventionally regarded as mainly chelating with carboxyl group. Aluminum ion exhibits very limited reactivity with amino and carboxyl group, which is consistent with the weak tanning effect of aluminum salt.<sup>29</sup> It is well-known that  $\text{Cr}^{3+}$  exhibits the highest chelating ability with carboxyl groups of collagen, and  $\text{Al}^{3+}$  has the lowest chelating ability with the carboxyl groups. These are due to the fact that  $\text{Cr}^{3+}$  and  $\text{Al}^{3+}$  form inner-orbital and outer-orbital complex with the carboxyl group, respectively. The inner-orbital complex is more stable than outer-orbital complex.<sup>26</sup>

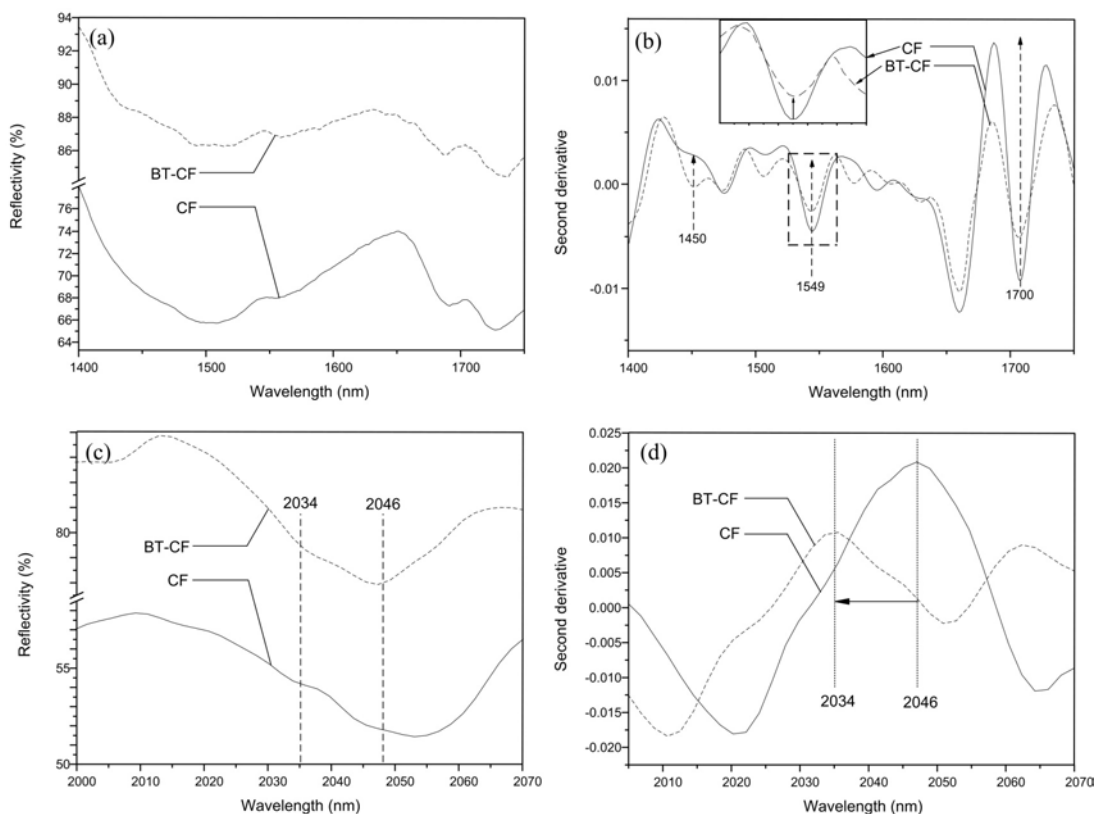


Figure 3. NIR DR Spectra (a) of BT-CF (Dash) and CF (Solid) from 1400 to 1750 nm and corresponding second derivative spectra (b); NIR DR Spectra (c) of BT-CF (Dash) and CF (Solid) from 2000 to 2070 nm and corresponding second derivative spectra (d) (↓: intensity decrease after vegetable tanning)

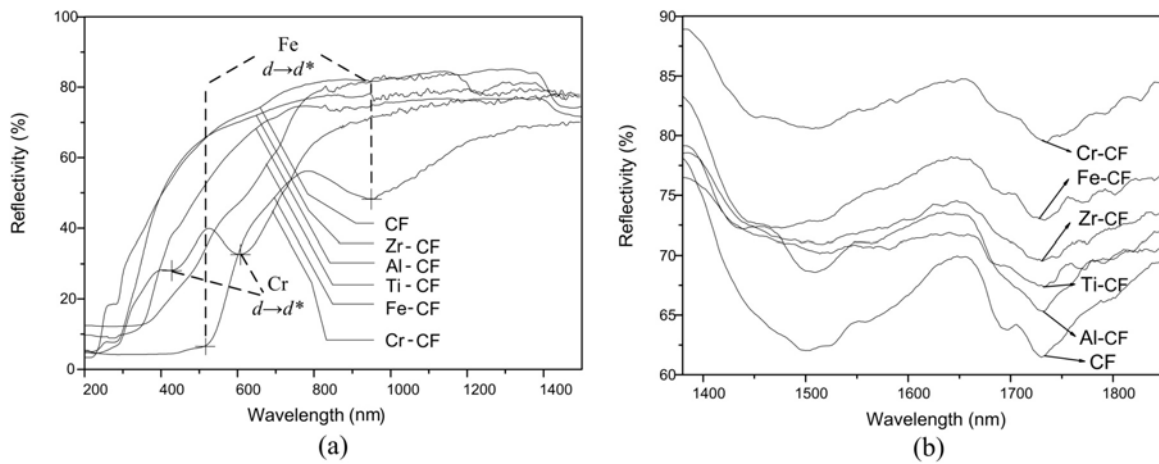


Figure 4. UV-Vis (a) and Near infrared (b) DR Spectra of metal salts tanned leathers (Cr-CF, Fe-CF, Zr-CF, Ti-CF and Al-CF)

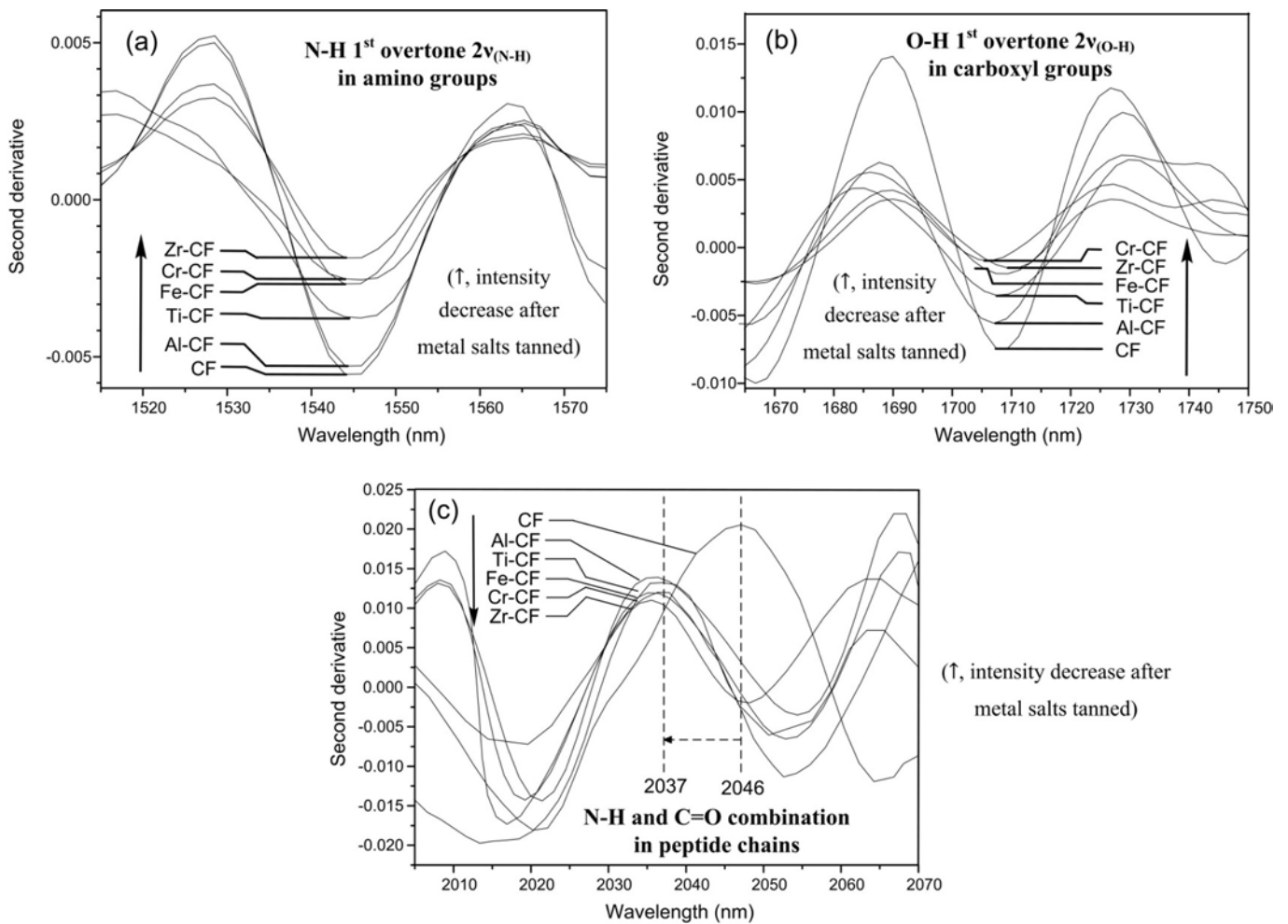


Figure 5. Second derivative spectra from NIR DR of metal tanned leathers

Figure 5a and 5b illustrate that, after tanning by metal salts, the intensity of the second derivative peak of carboxyl groups is decreased more remarkably compared with that of amino groups, which implies that the chelating reaction of metal ions and carboxyl groups should be the predominant reaction in the tanning. Figure 5(c) shows the second derivative peaks from 2000 to 2070 nm assigned to N-H and C=O combination in peptide chains. The peaks are blue shifted from 2046 to 2037 nm after tanning, while their intensity are decreased, which indicates that the vibration frequency of amide in collagen peptide chains is reduced. In general, the interaction between the positively charged metal ion and peptide chain will elongate C=O bond and shorten C-N bond<sup>31</sup>, and as a result, the vibration of N-H and C=O combination is decreased. Therefore, these results provide strong evidence that peptide chains are also involved in tanning reaction, and they may play an important role in the fixation of metal ions.<sup>32,33</sup>

### CONCLUSIONS

In this work, UV-Vis-NIR DR spectrophotometer was successfully used for the investigation of tanning mechanisms of aldehyde tanning, vegetable tanning and mineral tannings. Based on the UV-Vis-NIR DR spectra and the corresponding second derivative spectra, the change of state of the functional groups in collagen can be clearly observed after tanning, and therefore, the groups involved in the tanning processes can be well recognized. So UV-Vis-NIR DR technique is an effective tool to directly obtain the information about tanning mechanisms. In general, the tanning mechanisms presented in this study are consistent with those being proved by other methods in previous works. But some details observed in our work might be novel. For example, the difference of reactivity of typical metal tanning agents toward carboxyl group and amino group of collagen are well summarized in this study. At the same time, the participation of collagen peptide chains in metal tanning has been confirmed. These findings would be valuable in better understanding of tanning mechanisms and for the guidance of effective uses of these tanning agents.

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