Paper A9

The Influences of Carboxyl Groups Content of Collagen on the Chromium (III) Absorption

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Abstract

Gelatin is used as a model of collagen, chloroacetic acid can be a good candidate for a modification agent to introduce additional carboxyl groups and methanol is used as a termination agent to block carboxyl groups, the effect of carboxyl group's content of collagen on the chromium (III) absorption was investigated. Gelatin modified with chloroacetic acid or methanol, the Zeta potential decreases from 4.8 to 2.8 for the former and increased from 4.8 to 6.2 for the latter, and the total organic carbon (TOC) contents increase by 9% and 12%, respectively. Study on chromium(III) absorptions reveals that hide powders modified with the same way show quiet different chromium(III) absorption capacity, for the carboxyl-group-added samples the maximum absorption capacity of chromium (III) (A_{max}) increased from 42.44mg/g to 87.65mg/g (Cr^{3+}/collagen) at 40℃ with pH=4, whilst the carboxyl-group-blocked samples lose its affinity for cationic chromium by the inactivation of carboxylic groups, the A_{max} decreased from 42.44mg/g to 16.34mg/g under the same condition. Further study indicates that the carboxyl-group-blocked or added does only change the chromium absorption capacity but not change the traditional chromium tanning mechanism, the effect of pH value, temperature and time on the chromium absorption process is still as the same the traditional chromium tannage.

1. Introduction

Chromium salts have become the dominant tanning agent for over 100 years due to their mature tanning technology and high performances of final leather, such as high hydrothermal stability, super elasticity, high mechanical strength and high washability.[1] However, in the conventional chrome tanning process, the actual combination ratio of chromium is only 60% -70%,[2] and the Cr (III) in the residual waste caused not only environmental pollution, but serious waste of resources.[3-5] Therefore, how to improve the conventional chrome tanning process, fully utilize the chromium salt resource and decrease the chrome pollution have taken the spotlight for traditional chromium tannage.

According to chrome tanning mechanism, Cr (III) predominantly coordinate with the carboxyl groups of collagen, form fixable crosslinking between collagen fibers, and make the skin into leather, so in the process of chrome tanning, carboxyl group (-COOH) of collagen has played a

major role in improving the hydrothermal stability of collagen.[6] On the basis of this background, Covington et al[3] proved that the crosslinking and coordinating reactions mostly occurred between the chromium (III) and the pendent carboxyl groups of aspartate and glutamate, and aspartate tended to form bi-point bounded chromium complexes, while glutamate showed a tendency to form uni-point chromium complexes. In fact, on the backbone of collagen, the molar ratios of aspartate and glutamate residues to all the amino acids are 42/1000, 73/1000 respectively,[2] Moreover, the coordinating reaction is affected by other factors such as spatial position, degree of ionization as well as coordination field,[7] which means the combining sites between the collagen and the chromium are very limited, so the actual combination ratio of chromium is often lower than 70%. According to the mechanism of chromium tannage, modification of collagen by introducing additional carboxyl groups onto the backbone of collagen is considered to be an efficient approach to enhance chromium exhaustion.[8-10]

In our previous work, an oxazolidine derivative with carboxyl group and oxazolidine ring, which can react with the amino of collagen was synthesized and used as a modification agent, a novel collagen-fiber-based high Cr(III) adsorption material was developed and used for treatment chromium-contained wastewater.[11] In contrast to un-modified collagen fiber, the modified one with extra carboxyl groups showed stronger ability to absorb Cr(III), the maximum adsorption capacity (A_{max}) of Cr^{3+} was increased from 41mg/g to 143mg/g (Cr^{3+}/ collagen) with a pH value of 4.0. The waste water from leather tannary was treated with this modified collagen fiber, the Cr_2O_3 content decreased from 1.76g/L to 0.06g/L,[12-13] this materials show a wide application in treating wastewater from leather-making industry or electro-plating industry.

As continues work, chloroacetic acid was selected as a modification agent to introduce the additional carboxyl groups use a simple experimental method with a more moderate condition, and methanol as a termination agent to permanent block carboxyl groups in a more desirable pH range. The effect of carboxyl groups changes of collagen on the maximum chromium absorption and chromium absorption mechanism was investigated in detail. The aim of this study is to find out the key issue to affect the chromium uptake in chromium tannage and finally to develop an efficient way for fully utilization of the chromium salt resource as well as decrease the chrome pollution.

2. Experiments

Material and Instruments

The gelatin used as a model of collagen provide by the Chinese Tianjin Bodi Chemical Industry Company. The hide powder was obtained from Nanjing Institute of Forestry Science. The FTIR spectra were measured on a Nicolet 200SXV spectrophotometer from USA. Total Organic Carbon (TOC)-2000 TOC analyzer (Shimadzu, Japan) is used. The HZS-H water bath osciallor/shaker was provided by Donglian electronic technology development Ltd. UV-250PC ultraviolet spectrophotometer was provided by Japan SHIMADZU Instrument Company.

Differential Scanning Calorimetry (DSC) was studied on a PC200-DSC (provided by NETZSCH Instrument Company, German) with a heating rate at 5℃/min under N_2 condition. 100ml chromium sulfate solution with the Cr^{3+} concentration of 0.1g/L and 33% basicity was prepared for experiment.

Modification of gelatin

Introduction of carboxyl group: A 500ml round-bottom, three-necked reaction flask equipped with a mechanical stirrer and thermometer are used as a reactor to prepare the products. The raw materials, gelatin and predetermined amount of deionized water were charged into the flask and soaked overnight. After the gelatin was fully dissolved in water, some sodium chloroacetate was added into the flask. The reaction was carried out at 40°C for 6h with a pH value of 7.5, and then followed by a purifying process in a mixture of water and alcohol with a proportion of 4:1 (v/v). Finally, the modified gelatin was dried by lyophilization. The modification process is described in Figure 1.

$$P\text{-}NH_2 + ClCH_2COONa \xrightarrow{OH^-} P\text{-}NH\text{-}CH_2COONa$$

Figure 1 The scheme of introducing carboxyl group into gelatin

Termination of carboxyl group: Gelatin was dissolved in 4mol/L methanol/HCl solution with a pH value of 4.5, the esterfication was kept at room temperature for 24 hours with stirring. Then an equivalent molar weight of sodium hydroxide was added to neutralize the acid. The gelatin was purified in a mixture of water and alcohol with a proportion of 4:1 (v/v) and washed with cooled water, and then dried by lyophilization. The modification process is described in Figure 2.

$$P\text{-}NH_2 + CH_3OH \xrightarrow{HCl} P\text{-}COOCH_3$$

Figure 2. Blocking the carboxyl group of gelatin by esterfication

Modification of hide powder

The hide powder was modified according to the same methods mentioned above and named as the carboxyl-group-added hide powder and carboxyl-group-blocked hide powder respectively.

The changes of Zeta potential and TOC of gelatin before and after modification

Gelatin and modified gelatin are dissolved into water with a concentration of 0.1g/L. The Zeta Sizer Nano ZS and TOC are used to analyze the changes of Zeta potential and TOC. The Zeta potential and TOC data are averages of three separate measurements.

Coordination between modified gelatin and chromium (III)

Modified gelatin and the trivalent chromium salt were dissolved in water with a certain mole ratio; meanwhile the chromium salt was dissolved in water for the control experiment. pH

values in the solution were controlled to 3.0 and the concentrations of Cr_2O_3 were adjusted to 2.0g/L. After the mixture solutions were stored for 2h, a UV spectrophotometer was used to determine the coordination degree between gelatin and chromium (III).

The A_{max} of modified and unmodified hide powder

0.5g modified/unmodified hide powder and 100ml chromium sulfate with Cr^{3+} concentration 0.1g/L were put into the water bath oscillator. The chromium adsorption reaction was performed as follows: (1) with different pH value at room temperature for 8h, (2) with different temperature at pH=4 for 8h, (3) with different time at 40℃ with pH=4. The Cr^{3+} concentrations in the wastewater were measured by an Inductively Coupled Plasma-Atomic Emission Spectrometry (2100DV ICP-AES, from PE Company, USA). The maximum adsorption capacity (A_{max}) of Cr (III) was calculated by the following equation. The degree of adsorption capacity is expressed as Cr^{3+}mg/g collagen fiber.

$A_{max}= (C_1-C_2)\ V\times10^3/W$

Here, (C_1-C_2) is the concentration change of chromium (III) (g/L); V is the volume of solution (L); W is the weight of collagen fiber (g); A_{max} (Cr^{3+}/collagen) is the maximum adsorption capacity of Cr (III).

3. Results and discussion

FTIR spectra of modified/un-modified gelatin

Figure 3 is the FTIR spectra of gelatin and modified gelatins. Table 1 lists the changes of special groups and the corresponding adsorption peaks in FTIR.

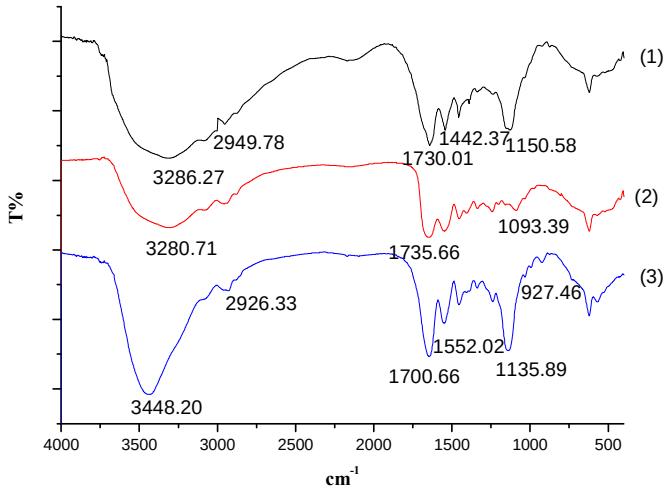

Figure 3. FTIR spectra of the tree kind of gelatin
(1. carboxyl-group-blocked gelatin; 2. unmodified gelatin; 3. carboxyl-group-added gelatin)

Table 1. Special group and their adsorption peaks of FTIR

Adsorption peaks(cm^{-1})	Changes of strength	Groups
3448	w→s	υ(N-H) of -NHR
2926	w→m	γas(C-H) of -CH2-
1700	w→s	υ(C=O) of -COOH
1552	m→s	υa() of -COOH
1442	m→s	υas(C-H) of –CH3
1150	w→s	γ(C-O) of -COOR
1135	w→s	υ(C-N) of -N
927	w→m	υ(O-H) of -COOH

Comparing with the FTIR spectra of (2), it was found that the adsorption peaks of (3) at 1700 cm^{-1} assigned to υ(C=O) of -COOH, 1552 cm^{-1} assigned to υa() of -COOH, 927 cm^{-1} assigned to υ(O-H) of -COOH, and 3448 cm^{-1} assigned to υ(N-H) of -NHR, 1135 cm^{-1} assigned to υ(C-N) of -N obviously become stronger after modification, which indicates that additional carboxyl groups are introduced on the backbone of collagen after modification with chloroacetic acid. For carboxyl-group-blocked gelatin (1), two new adsorption peaks at 1150cm^{-1} assigned to γ(C-O) of -COOR and at 1442 cm^{-1} assigned to υ_{as}(C-H) of $–CH_3$ occur , which reveals that the carboxyl groups of gelatin are partly blocked by the methanol.
From the analysis of FTIR spectra, we can find that it is feasible to add and block the -COOH of collagen via a simple modification method.

The changes of Zeta potential and TOC of gelatin before and after modification

In gelatin solution, the electric properties of particles largely depend on their dissociation degree of side chain carboxyl and amino groups.[14-15] So, the changes of -COOH content can be directly characterized by the analysis of Zeta potential.
TOC analysis is another method to measure the change of carboxyl group content for gelatin.[16] Table 2 shows the difference in average Zeta potential and TOC value.

Table 2. The average Zeta potential and TOC

gelatin	Unmodified gelatin	Carboxyl-group-added gelatin	Carboxyl-group-blocked gelatin
Zeta potential	4.8	2.8	6.2
TOC(mg/L)	41.48	45.61	46.46

It can be seen that the Zeta potential of unmodified gelatin is 4.8, while this value decrease to 2.8 for the carboxyl-group-added gelatin and rises to 6.2 for the carboxyl-group-blocked gelatin. The reason may be that after termination of -COOH, the positive charge of gelatin relatively increases with the decrease of -COOH content, so the isoelectric point of carboxyl-blocked

gelatin is increased. On the contrary, introduction of -COOH increases the negative charge of gelatin, therefore the isoelectric point of carboxyl-blocked gelatin is declined.

The modification reaction can be further confirmed by TOC analysis. In comparison with the unmodified gelatin, the TOC values for two modified samples increase by 9% and 12%, respectively, implying that the -COOH added or blocked method is valid and successful.

Denature temperature of hide powders

DSC is employed to analyze the denature temperature of hide powders before and after modification, and the results are illustrated in figure 4.

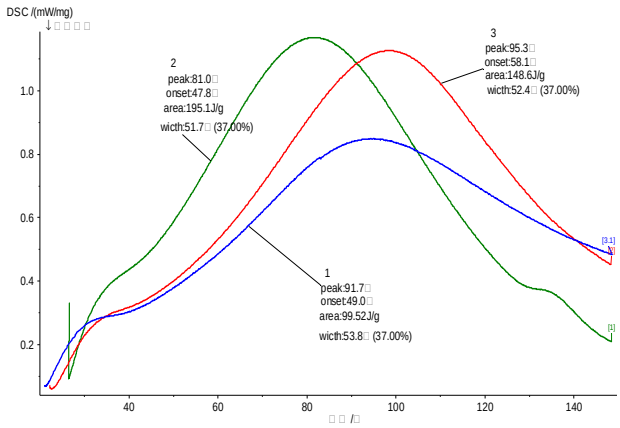

Figure 4. The DSC curves of hide powders
(1. unmodified hide powder; 2. carboxyl-group-blocked hide powder;
3. carboxyl-group-added hide powder)

It can be seen that the DSC peak shape has no essential change before and after modification, indicating that introducing or blocking -COOH does not change the inherent structure of collagen,[12] slight changes of the thermal denature temperature (slightly increase for the carboxyl-group-added hide powder and slightly decline for carboxyl-group-blocked hide powder) should be attributed to the changes of ionic forces, i.e. the introduction of extra -COOH causes the ionic force of collagen chains increase; inversely, carboxyl-group-blocked reaction decreases the ionic force.

Coordination reaction between modified gelatin and chromium

UV spectrum can be used to investigate the coordinating reaction between collagen and chromium (III). Once the carboxyl groups of collagen do coordinate with chromium (III), the UV spectrum of mixed solution would differ from the chromium solution and shift to a low wavelength.[12] Figure 5 shows the UV spectrum of chromium-unmodified gelatin solution, chromium-carboxyl-group-added gelatin solution, chromium-carboxyl-group-blocked gelatin solution and chromium solution, and Table 3 lists the changes of the absorption peak.

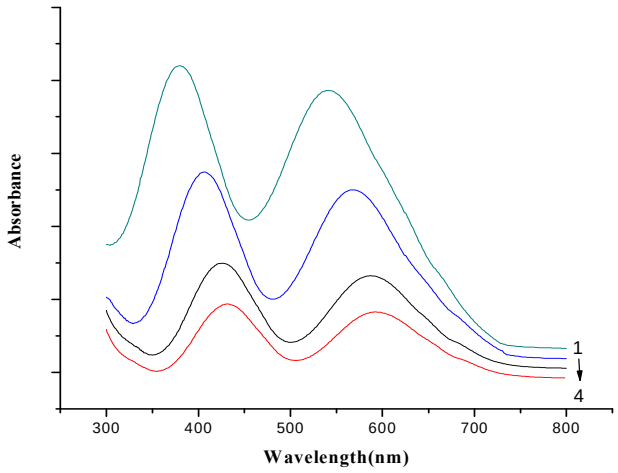

Figure 5. UV spectrum of chromium-gelatin solution with pH=3.0
(1. Chromium-carboxyl-group-added gelatin solution; 2. Chromium-gelatin solution; 3. Chromium solution; 4. Chromium-carboxyl-group-blocked gelatin solution)

Table 3. The change of the absorption peak

Sample	Chromium solution	Chromium-unmodifie gelatin solution	Chromium-carboxyl-adde gelatin solution	Chromium-carboxyl-blocke gelatin solution
$\lambda_{max,1}$	425	404	385	427
$\lambda_{max,2}$	588	567	544	589

In comparison with UV spectrum of chromium solution, the UV characteristic peaks values of chromium-carboxyl-group-blocked gelatin solution keep almost unchanged which means the carboxyl-group-blocked gelatin has lose its affinity for cationic chromium (III). Whilst the absorption peak of the chromium-carboxyl-group-added gelatin solution and chromium-unmodified gelatin solution have shifted from 425 nm to 385 nm and 404 nm, another adsorption peak shifted from 588 nm to 544nm and 567 nm, respectively. All peaks shifting to a low wavelength imply that the -COOH of collagen does coordinate with chromium (III), the former contains much more carboxyl groups, so the coordination reaction is stronger.

Effects of carboxyl content on the absorption of chromium (III)

Figure 6, 7 and 8 show the maximum absorption (A_{max}) of hide powder to chromium (III) as a function of pH value, temperature and time, respectively.

As can be seen from Figure 6, the A_{max} is increased with the increasing of pH. At any pH value, the A_{max} shows the same order of carboxyl-group-added hide powder > unmodified hide powder > carboxyl-group-blocked hide powder. This is probability because a higher pH value is beneficial for the ionization of the carboxyl groups, and also in favor of the hydrolysis of basic chromium salts, as a result, more ionized carboxyl groups and large size chromium complexes are easy to coordinate together. For the case of at the same pH value, the largest A_{max} for the

carboxyl-group-added hide powder should be attributed to the more carboxyl groups, and the lowest A_{max} for the carboxyl-group-blocked hide powder should be attributed to the decrease of carboxyl groups.

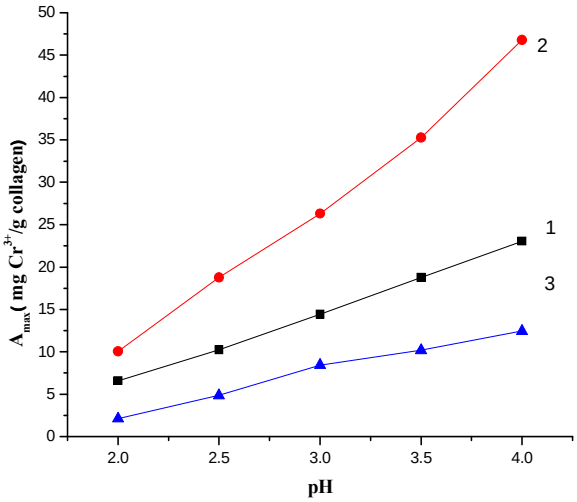

Figure 6. The effect of pH value on the adsorption of hide powders to chromium(III) at 30ºC (1. hide powder; 2. carboxyl-group-added hide powder; 3. carboxyl-group-blocked hide powder)

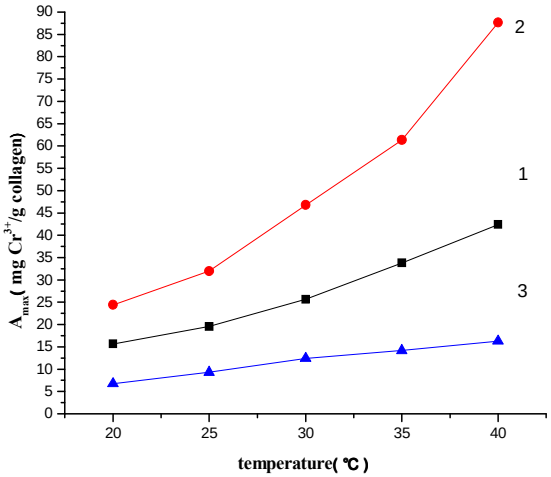

Figure 7. The effect of temperature on the adsorption of chromium (III) at pH=4 (1. Hide powder; 2. carboxyl-group-added hide powder; 3. carboxyl-group-blocked hide powder)

Figure 7 shows the effect of temperature on the adsorption of chromium (III) at pH=4. No matter in any case, all the A_{max} show an increasing tendency as the temperature increasing. This is because the chromium adsorption is a chemical adsorption and an endothermic reaction,[7] therefore, higher temperature is beneficial for the movement and hydrolysis of chromium complex compound, which leads to higher combination ratio.[10] However, at the same

temperature, the A_{max} shows the order of carboxyl-group-added sample>unmodified sample>carboxyl-group-blocked sample, which means that the chromium absorption is dominantly determined by the carboxyl group content of collagen.

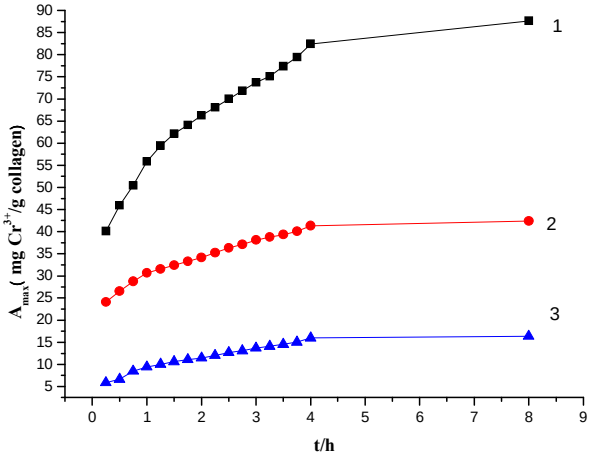

Figure 8. The effect of time on the adsorption of chromium (III) at 40°C and pH=4

(1. carboxyl-group-added hide powder; 2. unmodified hide powder;
3. carboxyl-group-blocked hide powder)

Figure 8 shows the A_{max} as a function of time. Obviously, the chromium absorption process for three samples show similar behavior and can be divided into two stages: a rapid stage and a slow stage. In the first stage, the equilibrium-absorption time is approx 4h, and A_{max} are 82.45mg/g for carboxyl-group-added hider powder, 41.33mg/g for unmodified hider powder and 15.97mg/g for carboxyl-group-blocked hide powder. In the second stage, the chromium absorption tends to decrease, 8h later the improvements of A_{max} show little changes (respectively increase by 6.31%, 2.68% and 2.50%). The rapid absorption rate in the first stage is believed to be large concentration of chromium and large amount of combination points for chromium ions. In the latter stage, the collagen fiber is enclosed fully by chromium ions. So that the space effect and repellence of Coulomb force of Cr(III) retard the further absorption of other chromium ions. Therefore, in the second stage, the absorption rate and A_{max} all tend to decrease.

From the above discussion, it can be conducted that the carboxyl group content is the key issue for chromium absorption, and this behavior can be enhanced or weaken by means of introducing or blocking the carboxyl groups of collagen. And the effect of temperature and time on the chromium absorption is the same as traditional chrome tannage.

4. Conclusions

(1) It has been demonstrated from FTIR, Zeta potential and TOC analysis that the carboxyl groups of collagen can be added or blocked via a simple chemical modification method. UV spectrophotometer study reveals that the carboxyl-group-added gelatin shows an enhanced tendency to coordinate with chromium (III) whilst the carboxyl-group-blocked one shows a weaken tendency.

(2) Hide powders modified with the same way show different chromium absorbing ability, the A_{max} is increased from 42.44mg/g to 87.65mg/g for the carboxyl-group-added sample whilst decreased from 42.44mg/g to 16.34mg/g for the carboxyl-group-blocked sample under the same condition, showing a carboxyl groups content dependency.

(3) The introduction of extra carboxyl groups can efficiently enhance the chromium absorption but not change the traditional chromium tanning mechanism, the effect of time, pH value and temperature on the chromium absorption process is still the same as the traditional chromium tannage.

5. References

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6. Acknowledgments

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