

MODIFICATION OF COLLAGEN FOR HIGH Cr (III) ADSORPTION

by

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ABSTRACT

Collagen has an ability to coordinate with chromium (III) salts which is mostly determined by the amount of pendent carboxyl groups on the backbone of collagen. Based on this principle, when an oxazolidine derivative which contains carboxyl group and oxazolidine ring was synthesized and used as a modification agent, a novel collagen fiber-based high Cr (III) adsorption material was developed. In contrast to unmodified collagen fiber, the hydrothermal stability of the modified collagen fiber increased from 63.1°C to 78.8°C and the maximum adsorption capacity (A_{\max}) of Cr³⁺ increased from 41mg/g to 143mg/g (Cr³⁺/collagen) with a pH value of 4.0. In the waste water from a leather tannery treated with the modified collagen fiber, the Cr₂O₃ content decreased from 1.76g/L to 0.06g/L. Investigation of high chromium adsorption mechanism indicates that the increased number of carboxyl groups attached to the backbone of collagen and the intrinsic carboxyl groups of collagen coordinate together with chromium (III) and form uni- or multi-point bound chromium complexes, which seems to contribute to the modified collagen high chromium (III) adsorption.

RESUMEN

El colágeno tiene la habilidad de coordinar con sales de cromo (III) determinada principalmente por la cantidad de grupos carboxilo pendientes en la columna vertebral del colágeno. Basándose en este principio, cuando fue sintetizado un derivado de la oxazolidina, el cual contiene grupos carboxilos y un anillo de oxazolidina, y utilizado como agente de modificación, una novedosa fibra de colágeno basada en alta adsorción de Cr (III) fue desarrollada. En contraste con la fibra de colágeno no modificada, la estabilidad hidrotérmica de la fibra de colágeno modificada aumentó de 63.1°C a 78.8°C y la capacidad máxima de adsorción (A_{\max}) del Cr³⁺ aumentó de 41mg/g a 143mg/g (Cr³⁺/colágeno), a un valor pH de 4.0. En las aguas residuales procedentes de una curtiembre, cuyos cueros fueron tratados con esta modificación de la fibra de colágeno, el contenido de Cr₂O₃ decreció de 1.76g/l a 0.06g/l. La investigación del mecanismo de la alta adsorción del cromo indica que el aumento del número de grupos carboxilo unidas a la columna vertebral de colágeno y los grupos carboxilos intrínsecos del colágeno se coordinan juntos con el cromo (III) y forma enlaces uni o multi-punto con el complejo de cromo, lo que parece contribuir a modificar la alta adsorción de cromo (III) por el colágeno.

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INTRODUCTION

Chromium (III) salts have been shown to be the best inorganic tanning agent for leather-making, because of their excellent tanning characterizations. But in traditional chrome tannages, the utilization ratio of chromium salts is only 65 percent to 70 percent and the abundance of Cr^{3+} in wastewater would cause severe environmental pollution¹. On the other hand, in recent years because of the ultimately recoverable resources of chromium is limited, the price of chromium (III) salts rise intensively and the tendency will likely continue to increase into the future. Moreover the shortage of chromium salt resource would accelerate the rising of its price. Although much effort has been focused on chrome-reduction or high chromium exhaustion tannage so as to eliminate the environmental pollution caused by Cr^{3+} ²⁻⁵, in fact, it is still difficult to achieve over 90 percent chromium exhaustion under normal tanning condition; about 38,000 tons of chromium (III) salts are discarded into water every year in China. So how to fully utilize the chromium salt resource and decrease the chrome pollution is the key issue for leather industry.

The fact that the chromium (III) salts have an ability to coordinate with carboxyl groups of collagen and form crosslinking between collagen fibers were first proved by Gustavson et al in 1924. In further works, they found that 90 percent of the total amount of chromium incorporated with collagen in the form of unifunctional complexes, and the remainder of the combined chromium i.e. 10 percent Cr_2O_3 was combined with collagen in the form of bifunctional complexes¹. Working from this background, Covington et al later proved that the crosslinking and coordinating reactions mostly occurred between the chromium (III) and the pendent carboxyl groups of aspartate and glutamate, aspartate tended to form bi-point bound chromium complexes, while glutamate showed a tendency to form uni-point chromium complexes². In fact, on the backbone of collagen, the mole ratio of aspartate and glutamate residue to all amino acids are 42/1000, 73/1000 respectively⁴, which means the combining sites between the collagen and the chromium are very limited, that is why the actual combination ratio of chromium is often lower than 70 percent.

It has been demonstrated that collagen modified via introducing different functional groups on the backbone of it showed selective adsorption to different metal ions⁶⁻⁷, for example, collagen fiber immobilized vegetable tannins showed higher adsorption to Pb(II) , Hg(II) and UO_2^{2+} ⁸. But for the adsorption to Cr^{3+} , the modification of collagen by introducing carboxyl groups was believed to be an efficient approach to enhance the chromium adsorption⁹⁻¹¹.

On this basis, in this work, a new kind of oxazolidine (OXD) compound with carboxyl groups and an ability to react with the amino groups of the collagen was synthesized and applied to the modification of collagen fiber. In contrast to unmodified collagen fiber, the hydrothermal stability of

modified collagen fiber was increased from 63.1°C to 78.8°C and the maximum adsorption capacity of Cr^{3+} was increased from 41mg/g to 143mg/g (Cr^{3+} /collagen). In the waste water from leather a tannery treated with this modified collagen fiber, the Cr_2O_3 content decreased from 1.76g/L to 0.06g/L. Investigation of high chromium adsorption mechanism indicated that the increased number of carboxyl groups attached to the backbone of collagen and the intrinsic carboxyl groups of collagen cooperated together with chromium(III) and formed uni- or multi-point bound chromium complexes, which were considered to contribute to the modified collagen fiber high chromium(III) adsorption.

The aim of this study is, on one hand, to explore high chromium (III) adsorption mechanism; on the other hand, to develop a novel chromium (III) recovered method from wastewater for leather-making industry or electroplating industry.

EXPERIMENTS

Materials and Instruments

The collagen fiber was recovered from the by-product of leather-making (shaving) and it had been pretreated without using chrome; The wastewater which contained chromium salts (Cr_2O_3 content is 1.76g/L) was received from Xinshi leather tannery (in China). The modification agent was an oxazolidine derivative (OXD) and it was prepared as below:

A 500 ml round-bottom, four-necked reaction flask equipped with a mechanical stirrer, nitrogen inlet, thermometer and condenser was used as a reactor to prepare the products. The raw materials: trihydroxymethylaminomethane, glyoxylic acid (HOOC-CHO , a compound with a $-\text{COOH}$ and a $-\text{CHO}$, received from Hoechst Company, German), formaldehyde (mole ratio 1:1:1) and distilled water were charged into the flask. The reaction was carried out at 85°C for 3-4h with pH value 8-9, followed by a ring-closing reaction at 90°C under vacuum condition (250-280 Pa); the end-point of the reaction was monitored by calculating the removed water. Finally, a viscous liquid was obtained. The synthesis is described in figure 1.

The elemental analyses of OXD were performed on a Carlo Earo 1106 instrument (Carlo Earo Company, German), the calcd/found value for C, H and N are listed as follows: C% (44.44/44.40), H% (5.82/5.79), N% (7.41/7.44). The FTIR spectra were measured on a Nicolet 200SXV spectrophotometer from America. Figure 2 shows the FTIR spectra of the OXD and Table 1 lists the special groups and the corresponding adsorption peaks in FTIR.

Both of the elemental analysis and FTIR confirmed the proposed structure of the modification agent (OXD).

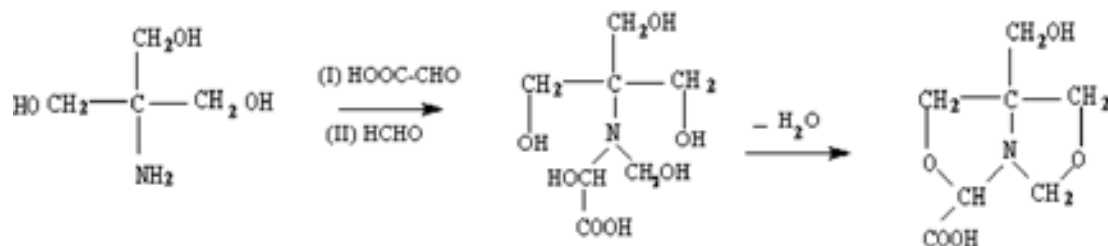


Figure 1. Synthesis of modification agent (OXD)

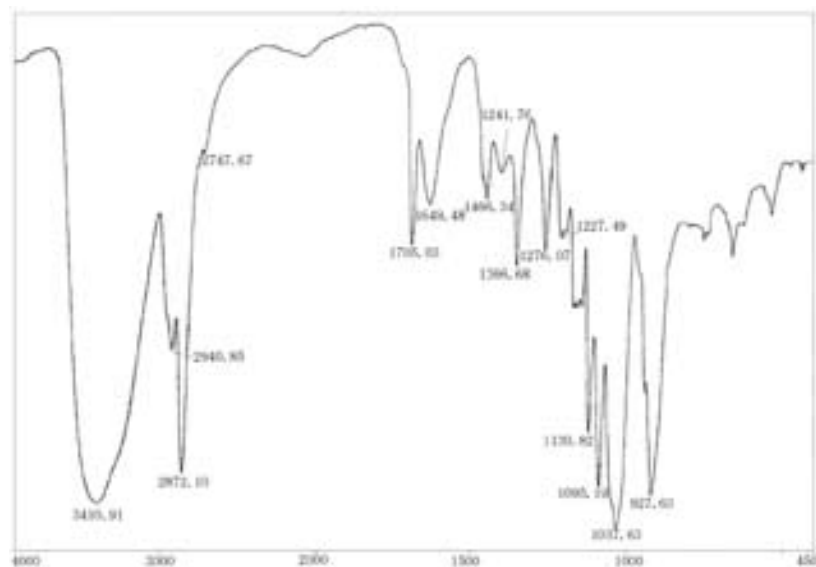


Figure 2. FTIR spectra of the modification agent

TABLE I
Special group and their adsorption peaks of FTIR

Adsorption peaks (cm ⁻¹)	Strength	Groups
3410	s*	v(O-H) of -OH
2940	s	γ _{as} (C-H) of -CH ₂ -
2872	s	v _s (C-H) of -CH ₂ -
2747	w	v(O-H) of -COOH
1705	s	v(C=O) of -COOH
1649	s	v _a (CO ₂) of -COOH
1466	m	δ(C-H) of -CH ₂ -
1421	w	v(C-O) and β(O-H) of -COOH
1276	m	β(O-H) of -CH ₂ -OH
1227	s-m	v(C-O) and β(O-H) of -COOH
1130,1366	s	v(C-N) of -N<
1095	s	v(C-O-C) of -CH ₂ -O-CH ₂ -
1037	s	v(C-O) of -CH ₂ -OH
927	m	γ(O-H) of -COOH

(*s means strong; m means medium; w means weak.)

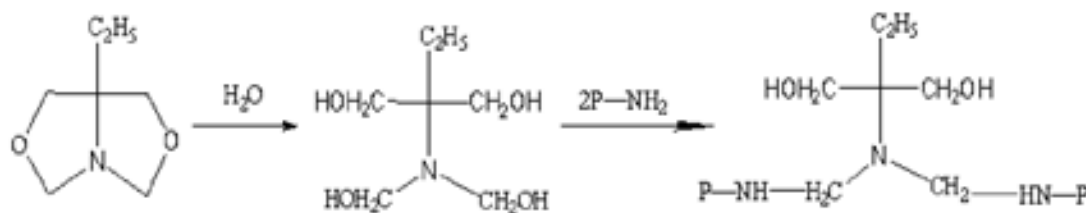


Figure 3. The interaction between the oxazolidine ring and the collagen fiber

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) at a heating rate at 5°C/min under N₂ condition; HZS-H water bath oscillator was provided by Donglian electronic technology development Ltd.

Modification of collagen fiber

The collagen fiber, 200 wt% water and 1-4 wt% OXD (on the weight of the collagen fiber) were charged into the water bath oscillator at a temperature of 35°C-40°C; The reaction was carried out at an initial pH value of 3.0-3.5 for 2h and followed another 2h at a pH value of 4.0-4.5. The hydrothermal stability of specimen, which determines the interaction degree between the modification agent and collagen, was measured by DSC analysis.

Coordination between OXD and chromium (III)

Modification agent (OXD) and the trivalent chromium salt were dissolved in water with a mole ratio of OXD: Cr³⁺ = 4:1; meanwhile the chromium salt was dissolved in water in the control experiment. The solution pH values were controlled to 3.0 and the concentrations of Cr₂O₃ were adjusted to 2.0g/L. After the solutions were stored for 2h and 4h, a UV (ultraviolet) spectrophotometer was used to determine the coordination between OXD and chromium (III).

The maximum chromium (III) adsorption capacity of modified and unmodified collagen fiber

1g modified collagen fiber/unmodified collagen fiber and 100ml chromium sulfate solution (Cr³⁺ concentration was 2.0g/L) with a pH value of 3.0 and 4.0 were separately put into the water bath oscillator, the chromium adsorption reaction was performed at 30°C for 5h. Finally, an Inductively Coupled Plasma-Atomic Emission Spectrometry (2100DV ICP-AES, from PE Company, USA) method was used to measure the Cr³⁺ concentration in the wastewater. The maximum adsorption capacity (A_{max}) of Cr(III) was calculated by the following equation. The degree of adsorption capacity is expressed as mg Cr³⁺/g collagen.

$$A_{\max} = (C_1 - C_2)V \times 10^3 / W$$

Here, (C₁-C₂) 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} is the maximum adsorption capacity of Cr (III).

The adsorption ability of collagen fiber to Cr (III) from wastewater

An adsorption column with 100cm (high) ×5cm (diameter) was filled with modified or unmodified collagen fibers and used as a fixed phase, the mobile phase was the wastewater from leather tannery with a pH value of 3.8-4.0 and a Cr₂O₃ content of 1.76g/L. The flow time was about 30min from top to bottom, and the experiment operation was repeated for 3-4 times. The Cr₂O₃ content in the extracted water was measured by a 2100DV ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry) from PE Company (USA).

RESULTS AND DISCUSSION

Interaction between the modification agent and collagen fiber

It has been reported that the oxazolidine ring of oxazolidine is prone to hydrolyze to produce active N-hydroxymethyl groups which can rapidly react with the pendent amino groups of collagen, and form a crosslinking structure between the collagen fibers at a proper pH value and temperature. The reaction mechanism is outlined in Figure 3¹².

In order to examine the above reaction between the modification agent and collagen fiber, collagen fiber was treated with 4 wt% OXD (based on dry collagen fiber weight) initially and then examined by DSC to analyze the changes of its hydrothermal stability.

Figure 4 shows the DSC patterns (heating curve) of treated and untreated collagen fibers. It can be seen that the denature temperature of modified collagen fiber is 78.8°C and the denature temperature of untreated collagen fiber is 63.1°C. The changes of the denature temperature revealed that the crosslinking reaction mentioned above occurred between the two components.

Coordinating reaction between modification agent and chromium (III)

UV spectrum is one of the efficient techniques for investigating the coordinating reaction between modification agent and chromium (III). If the modification agent does coordinate with chromium (III), the UV spectrum of chromium-OXD solution will differ from that of the chromium solution. Figure 5 shows the UV spectrum of chromium-OXD solution and chromium solution. It can be seen that the UV characteristic peak values of chromium solution with different

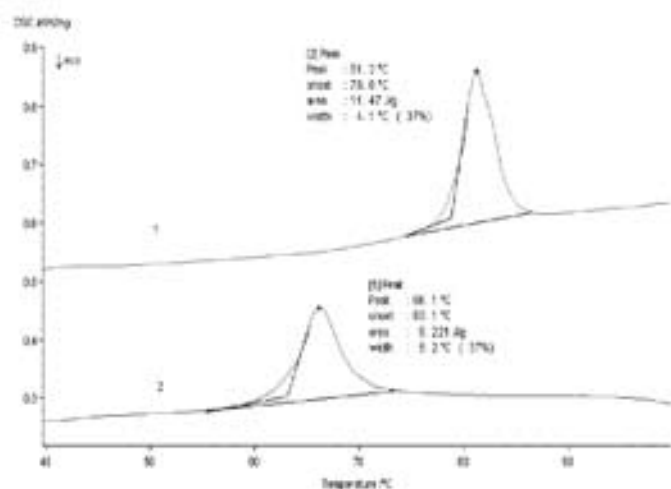


Figure 4. The DSC curves of collagen fibers
(1: collagen fiber treated with OXD; 2: untreated collagen fiber)

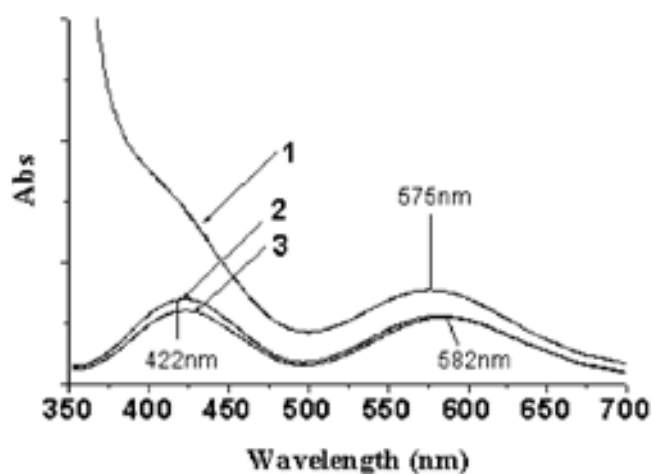


Figure 5. UV spectrum of chromium-OXD solution with pH=3.0
(1. Chromium-OXD solution; 2. chromium solution stored for 2h;
3 chromium solution stored for 4h)

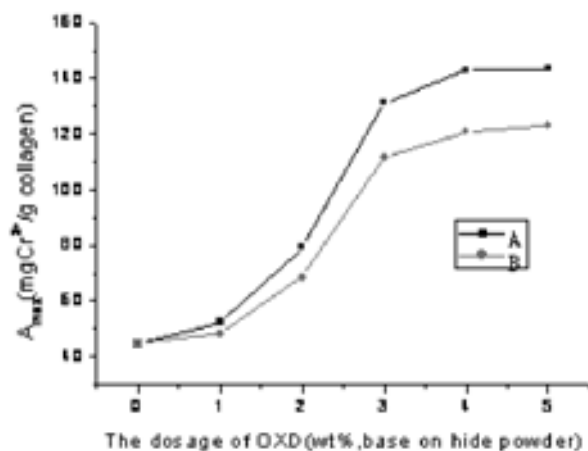


Figure 6. The effect of the dosage of OXD and pH value
on the adsorption of chromium (III)
(A: with pH value of 4.0; B: with pH value of 3.0)

storage time show no changes. Whilst the adsorption peak of chromium-OXD solution at 582nm has shifted to 575nm and another adsorption peak at 422nm shifted to a low wavelength range implying that OXD does coordinate with chromium (III) at pH 3.0.

Effects of amount of carboxyl groups and pH value on the adsorption of chromium (III)

The amount of carboxyl groups on the backbone of modified collagen fiber was determined by the dosage of OXD. Figure 6 shows the effects of carboxyl groups' content of collagen and the pH value on the maximum adsorption of chromium (A_{max}).

Comparing to the A_{max} of the unmodified collagen fiber and modified collagen fiber, the latter shows a rapid increasing tendency as an increase as the dosage of OXD, especially under higher pH condition. The reasons can be explained that the modification agent reacts with the residue amino groups of collagen, on one hand, releasing some carboxyl groups, on the other hand, introducing additional carboxyl groups onto the backbone of collagen, as a result, provides more combining sites for the chrome ions, so the A_{max} of modified collagen fiber is superior to the unmodified one. When the dosage of modification agent exceeds 4 percent, the A_{max} values for both higher and lower pH values appear to be the highest and reach 143mg and 123mgCr³⁺/g collagen, respectively. This implies that the chromium adsorption is also affected by space effect and repulsion of chrome ions, when the adsorption-desorption equilibrium established, the collagen fiber is enclosed fully by chrome ions, the space effect and repulsion of Coulomb force retard the further adsorption of other chrome ions.

We also find that the chromium adsorption is also related to the pH value, and a higher pH value results in higher chromium adsorption (as shown in Fig. 6). This reason may be explained in that a higher pH value is beneficial for the ionization of the carboxyl groups, also beneficial for the further hydrolysis of basic chromium salts, as a result, more ionized carboxyl groups and large size chromium complexes are easy to coordinate together, that is why the chromium adsorption is greater for the higher pH value than for the lower pH value.

Another experiment was performed to determine the adsorption ability of modified collagen to chromium (III) from wastewater. The changes of Cr₂O₃ content of wastewater treated with modified or unmodified collagen fiber is described in Table 2.

It can be seen that the adsorption ability of modified collagen fiber to chromium (III) from wastewater is related to the number of carboxyl groups of the collagen, when the collagen fiber is treated with 4 wt% OXD, the Cr₂O₃ content of wastewater decreases from 1.76g/L to 0.06g/L.

TABLE II
The changes of Cr₂O₃ content of wastewater treated with modified/unmodified collagen fiber and regenerated collagen fiber

Cr ₂ O ₃ content (g/L)	Unmodified collagen	Collagen modified with OXD			
		1%	2%	3%	4%
In original wastewater	1.76	1.76	1.76	1.76	1.76
Wastewater treated with modified/unmodified collagen fiber	1.32	1.21	0.88	0.23	0.06
Wastewater treated with regenerated modified collagen fiber		1.31	0.92	0.32	0.11

This saturated chromium (III)-adsorbed collagen fiber was immersed in acidic solution with pH 2.0-2.5, the adsorbed chromium (III) would be desorbed and recovered for reuse¹³⁻¹⁴; in this case, acid has some effect on the ionization degree of carboxyl groups but has little effect on the structure of the desorbed chromium (III) collagen fiber because of the modification of OXD, so it can be regenerated after a simple neutralization. Table 2 also gives the adsorption ability of regenerated modified collagen fiber to chromium (III), similar adsorption tendency and ability can be observed compared with the original modified collagen fiber.

Possible high chromium adsorption mechanism of modified collagen fiber

In the theory of chromium complexes, the basic chromium (III) salts are coordinated with collagen in the form of uni- and bi-point bound chromium complexes rather than in the form of full chemical fixation, and part of it is also held by physical adsorption. This coordinating reaction is believed to be reversible and affected by many factors, such as temperature, pH value, space position, and amount and ionization degree of carboxyl groups as well as coordination fields together with the materials used during the coordination reaction^[1]. Once the coordination equilibrium is established, the chromium physically adsorbed to the collagen or coordinated with collagen would be "desorbed" and returned back to the wastewater under suitable condition. Thus, it is difficult to achieve high chromium-adsorption ratio for unmodified collagen.

But it is not the case for modified collagen. A possible model for combination between chromium and modified collagen can be proposed as seen in Figure 7. The high chromium adsorption mechanism is proposed as follows: in water bath, the modification agent (OXD) hydrolyzes to produce active *N*-hydroxymethyl groups, which react with the amino groups of collagen and create a stable covalent bond between

N-hydroxymethyl group and amino group of collagen; as a result, the hydrothermal stability of collagen increases and the additional functional groups such carboxyl, hydroxyl groups, which supply additional combining sites for chromium (III), are introduced onto the backbone of collagen. In the chromium adsorption process, the carboxyl groups introduced by OXD together with intrinsic carboxyl groups of collagen fiber coordinate with the basic chromium salts, forming cross-linking bonds, giving uni- or bi-functional complexes or chelate complexes (Figure 7), which contribute to the modification collagen fiber's excellent chromium adsorption characteristics.

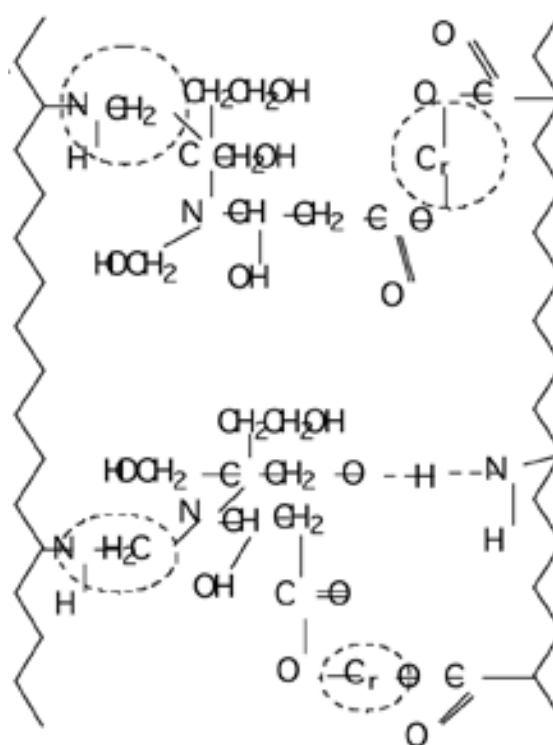


Figure 7. Model of chromium -OXD-collagen combination

Although this work is aimed at the full utilization of chromium salt resources and elimination of the environmental pollution caused by Cr^{3+} in leather-making industry, it is believed that this novel collagen-based adsorption material with higher denature temperatures can also be used for dealing with other metal ions which are discharged in other industrial fields, such as electroplating, ore milling, metal luring and so on.

CONCLUSIONS

(1) It has been demonstrated that the modification agent (OXD) can react with the pendent groups of collagen and create a stable covalent bond. Thus, the hydrothermal stability of collagen increases and the additional carboxyl groups are introduced onto the backbone of collagen.

(2) The chromium adsorption ability of modified collagen is related to the pH value and the amount of carboxyl group. Higher pH values and higher amounts of carboxyl group content are beneficial for chromium adsorption. After modification, the maximum chromium(III) adsorption of collagen fiber increased from 44mg/g to 143mg/g (Cr^{3+} /collagen); the wastewater, which was from a leather tannery, was treated with this collagen-based material, the Cr_2O_3 content in the wastewater decreased from 1.76g/L to 0.06g/L.

(3) Investigation of high chromium adsorption mechanism indicated that the additional carboxyl groups introduced by modification agent together with intrinsic carboxyl groups of collagen fiber coordinated with the chromium, formed cross-linking bonds, uni- or bi-functional complexes, which contributed to the collagen-based adsorption material's high chromium (III) adsorption characteristics.

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