A Collagen-Based Flocculant Prepared From Solid Leather Wastes

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Abstract

Every year a huge amount of solid leather wastes are generated, which brings pollution as well as waste of natural resources. However，the effective utilization of solid leather wastes is still a challenge. Collagen is the main component of solid leather wastes, and its molecular size is large enough. It addition, abundant reactive groups such as amino group and carboxyl group are involved in collagen molecules. These facts suggested that the solid leather wastes could be used as raw material to prepare flocculant. Therefore, a natural flocculant was prepared by incorporating 3-chloro-2-hydroxyproyl three methyl ammonium chloride (CHPTAC) into hydrolyzed collagen protein in this study. Fourier Transform Infrared Spectrometry (FTIR) patterns and other analyses indicated that CHPTAC was incorporated into collagen protein successfully. The flocculation behaviors of as prepared flocculant were conducted with kaolin suspensions as a model system, and it was found that this novel flocculant exhibited significant flocculation ability. When combined with small dosage of Al^{3+}, the flocculation extent of this flocculant could reach 90% within 15 minutes, which is much better than polymeric aluminium and can be compared with polyacrylamide. In addition, the effects of pH, concentration of flocculant and addition dosage of Al^{3+} on flocculation performances were investigated. The experimental results suggested the potential application of collagen-based flocculants can be expected.

Key words: flocculant, collagen, solid leather wastes, flocculation

1 Introduction

In the tanning industry, solid leather wastes generated from the splitting, trimming and shaving are of a huge amount, and the efficient utilization of them is still a challenge. The solid leather wastes can be hydrolyzed to gain collagen, and the utilization of the collagen involved cosmetics, animal feed and nourishment for human (Li, 2002). Despite of the progress that has been achieved, there are some limitations of these utilizations, such as the risk of genopathy. On the other hand, some research focused on preparing flocculant or coagulant with collagen (G.J. Piazza, 2010). Indeed, collagen has abundant reactive groups such as amino group, carboxyl and acylamino group. What’s more, collagen owns a molecular weight of 300 kDa, and its molecular size is large enough (K. Gelse, 2003). Besides, as a

natural and renewable macromolecule, collagen is biodegradable, environment-friendly and relatively inexpensive. (Mirjana G. Antov, 2009) Therefore, the flocculation of collagen can be expected and its flocculating efficiency could be improved by appropriate chemical modification.

Cationization is one of the mostly common modifications of raw materials, including starch, cellulosic derivatives, chitosan and agarose. (Héctor J. Prado, 2010) The cationization can be achieved by the etherification reaction of different reactant with 2, 3-epoxypropyltrymethylammonium chloride (EPTAC). However, the common strategy of this etherification reaction was to prepare the reagent in situ from 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) due to the instability of EPTAC (Héctor J. Prado, 2010).CHPTAC is the reactive intermediate of glycidyl trimethyl ammonium chloride (GTMAC), and CHPTAC could be converted into GTMAC in alkaline environment. The active hydrogen atoms of the amino group from collagen would be replaced by the quaternary ammonium group (CAI Zhao-sheng, 2004; Gamage A, 2007). In this research, the cationic collagen with CHPTAC was prepared and its flocculant ability was measured by kaolin suspension.

2 Material and methods

2.1 Materials

Hydrolyzed collagen was prepared from solid leather wastes by alkaline extraction process. 3-chloro-2-hydroxyproyl three methyl ammonium chloride (CHPTAC) and $Al_2(SO_4)_3 \cdot 18H_2O$ were of analytical reagent. Kaolin (ASP170, from BASF) was a gift from Hangzhou Junyi Chemical Ltd. All of the reagents were used without further purification.

2.2 Preparation of cationic collagen with different cationization degrees

The preparation of cationic collagen was obtained by incorporating 3-chloro-2-hydroxyproyl three methyl ammonium chloride (CHPTAC) into hydrolyzed collagen. 10g hydrolyzed collagen was dispersed in 100mL distilled water, and a certain amount of CHPTAC was added into the solution at 60℃ and stirred for 30min to get it dispersed. Thereafter, NaOH, as a catalyst, was introduced into the system, stirred for 6-10h at 60℃. Then, pH was adjusted fewer than 7 to stop the reaction. The particular parameters were shown in Table.1.

2.3 Preparation of cationic collagen-Al flocculant

The pH of as prepared cationic collagen solution was equilibrated to 4 with HCl. Then, 50%, 100%, 150% and 200 %(w/w) $Al_2(SO_4)_3 \cdot 18H_2O$ were added into cationic collagen solution, respectively. Thereafter the mixture was stirred at a certain rate for 3 h. All samples were dried with freeze-drying. Samples were stored in desiccators.

Table.1 Reaction parameters for preparing cationic collagen

	Hydrolyzed collagen/g	CHPTAC /mmol	NaOH /mmol	Temperature /°C	Time /h
CC1	10	12.5	13.75	60	6
CC2	10	25	27.5	60	8
CC3	10	50	55	60	8
CC4	10	75	82.5	60	10

2.3 Characterization of cationic collagen

2.3.1 Cationization degree

The cationization degree was measured by titration with $AgNO_3$ (Ohbu, K, 1982) 0.30g (W,g) cationic collagen was dissolved in 150mL distilled water, and 5 drops of 10% (w/v) was added to indicated the reaction end. The solution was titrated with 0.1 mol/L $AgNO_3$ (N, mol/L), and the volume of $AgNO_3$ consumed and the blank sample were recorded as V (mL) and V_0 (mL), respectively. The cationization degree (CD) was calculated as follows:

$$CD = \frac{N \times (V - V_0)}{1000 \times W} \times 100$$

2.3.2 Fourier Transform Infrared Spectrometry (FTIR)

Infrared spectra were recorded with an FTIR spectrometer (Nicolet, iS10, USA) using a potassium bromide disc technique.

2.4 Flocculation measurements

Flocculation experiments were conducted in a 100 ml stopped graduated cylinder. In this experiment, 5g/L kaolin suspension was used as a model system, containing a certain amount of as prepared flocculant in a total volume of 100mL, while the pH of the system was adjusted to a required point. The mixture was inverted by 10 times to make the flocculant even and dispersed. Thereafter, the graduated cylinder was settled at 25℃ without disturbing. At the indicated time, a 5mL portion was taken from 10cm below the surface, and the absorption of this supernatant at 550 nm was measured. The absorption was converted to concentration of kaolin with a calibration curve of kaolin, which was established by measuring a range of different concentration of kaolin. The calibration curve revealed that there was a linear relationship between the concentration of kaolin and the absorption of 550 nm. Define flocculant rate (FR) as follow. K_0 and K_t were the initial and final concentration of kaolin, respectively.

$$FR = \frac{K_0 - K_t}{K_0} \times 100\%$$

3. Results and Discussion

3.1 Characterization of cationic collagen-Al (III) flocculant

3.1.1 Cationization degree

Amino group is the main reactive group during the cationization. Under alkaline environment, the active hydrogen atoms of the amino group from collagen would be replaced by the quaternary ammonium group. As it is shown in Table.2, the primary amine content reduced gradually from CC1 to CC4, which indicated that the primary amine was occupied with the increasing dosage of CHPTAC.

3.1.2 FTIR spectrum

The FTIR spectra of hydrolyzed collagen and cationic collagen were shown in Fig1. The band at about 3419 cm^{-1} was assigned to stretching vibration modes of O-H groups and the band at 2937 $cm^{-1}$was assigned to stretching vibration of $–CH_2$- groups (Lihong Fan 2012). The most conspicuous difference between hydrolyzed collagen and cationic collagen was at 1477 cm^{-1}, which indicated that the quaternary ammonium group was introduced into the cationic collagen successfully.

3.2 Flocculation ability evaluation

With the chemical modification of CHPTAC, the cationization degree was increased and the molecular size became larger. The flocculate ability was improved through the combined action with the bridging effect and charge neutralization mechanism. (Wang J P, 2007) The flocculate ability of hydrolyzed collagen (HC) and cationic collagen (CC) was presented in Fig.2. As can be seen from the figures, HC was barely of flocculation ability, which involved with its low molecular weight because of the alkaline extraction process. When flocculate time was up to 10 hours, the flocculate rate of HC was under 20%. In comparison, CC showed superior flocculate ability. Besides, the higher the cationic degree is, the better the flocculate rate. CC4, with the cationic degree of 1.42, presented the great ability of reaching 40% of the flocculate rate within 4 hours. Therefore, we chose CC4 to prepare the cationic collagen-Al flocculate.

Table.2 The primary amine content and CD of CCs

Samples	Amine content /mmol/100 g	CD /mmol/g
Collagen	62	0.84
CC1	34	1.15
CC2	15	1.23
CC3	4	1.39
CC4	0	1.42

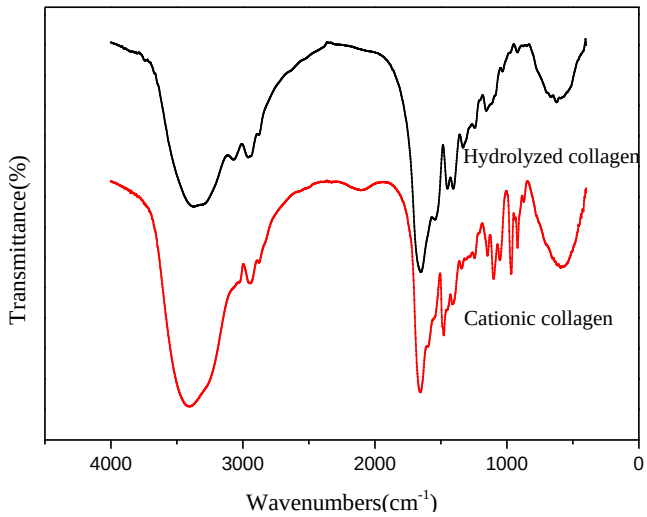

Fig.1 The FTIR spectra of hydrolyzed collagen and cationic collagen

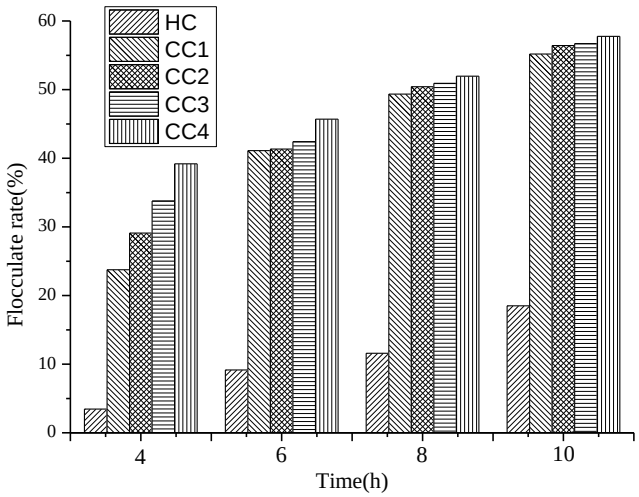

Fig.2 Effect of time on flocculation rate of HC and CC (pH=7; 300mg/L)

3.2.1 Addition dosage of Al^{3+}

Flocculant rate was chosen as the main index to value the flocculation ability of these collagen-Al flocculants. As a tanning agent with long history, $Al_2(SO_4)_3$ is commonly used with Cr^{3+} and vegetable tannin in the tanning process, which was known as combination Al-Cr tanning and vegetable and aluminum combination tanning. Al^{3+} could form coordination compound with collagen. Thus, the crosslink could happen between molecular, as a result the molecular size became bigger. This phenomenon contributed to the excellent flocculate ability of cationic collagen-Al. A series concentration of 20mg/L, 50mg/L, 100mg/L and 200mg/L were chosen to investigate the effect of dosage of Al^{3+}. According to Fig.3, it was obvious that with the increasing dosage of Al^{3+}, the flocculate ability raised at first. However, it went down when the dosage of Al^{3+} was higher than 100%. The reason here was that too much Al^{3+} made the binding sites occupied. Therefore, we chose this cationic

collagen-Al with 100% Al^{3+} to investigate the effect of concentration of flocculate and different pH of system on the flocculate ability.

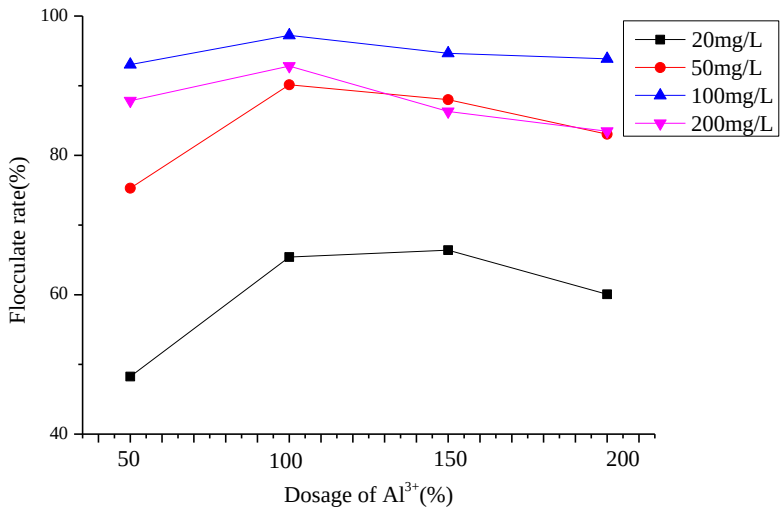

Fig.3 Effect of addition dosage of Al^{3+} on flocculation rate CC (pH=7; 15min)

3.2.2 Effect of concentration of as prepared flocculants

The concentration of flocculants has a significant effect on flocculate rate. Generally speaking, flocculate rate would rise with the increasing concentration of flocculants. When the concentration goes up to a certain level, flocculate rate would go down because of the function of protective colloid. Charge neutralization mechanism plays an important part in kaolin flocculate as kaolin suspension is a system of negative charge. Excessive flocculates would wrap up the kaolin particles rapidly and the charge of particles would turn into positive. Then, a new charge balance was built up and the flocculation process stopped. Fig.4 was illustrated the trend described above. From the figure, when the concentration went up, the flocculate rate raised until the concentration was up to 100mg/L. From that point, the flocculate rate went down as the concentration of flocculant increased. Hence, the appropriate concentration of flocculant was 100mg/L.

3.2.3 Effect of different pH of system

Cationic collagen-Al performed a better flocculate ability with a lower pH. As it was shown in Fig.5, lower pH was better than higher pH for the cationic collagen-Al. With the increasing pH, the flocculant rate reduced slightly. The reason was that with a higher electric charge density of cationic collagen-Al, the charge neutralization would be more useful. At the mean time, the molecular of the flocculate would be more stretched, which was good for the bridging effect.

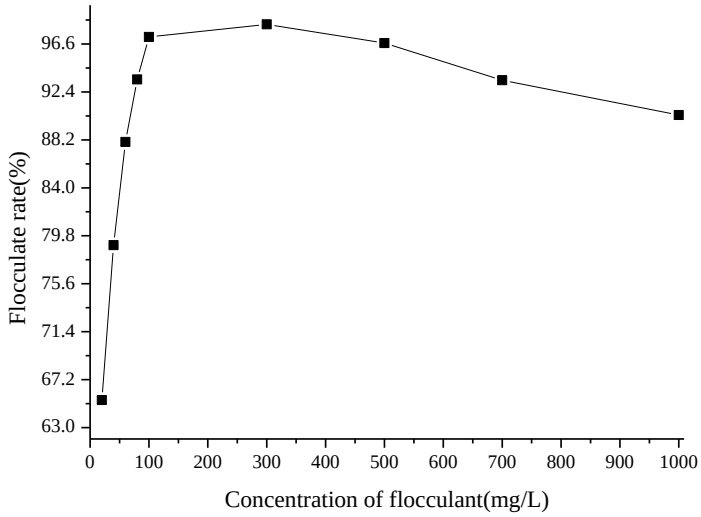

Fig.4 Effect of concentration of cationic collagen-Al (pH=7; 15min)

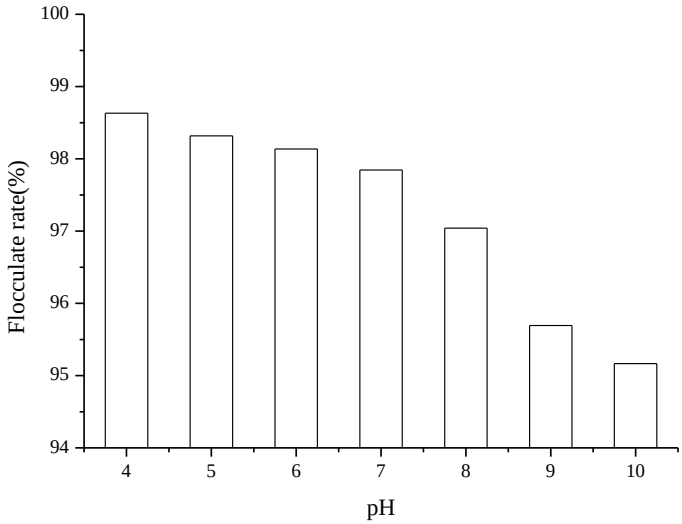

Fig.5 Effect of different pH of system on flocculate rate (100mg/L; 15min)

4. Conclusion

A novel flocculant was prepared from solid leather wastes, which was biodegradable and inexpensive. Hydrolyzed collagen was modified by incorporating 3-chloro-2-hydroxyproyl three methyl ammonium chloride (CHPTAC) into it. The flocculate ability of as prepared flocculant was investigated with 5g/L kaolin suspension. The addition dosage of Al^{3+}, concentration of flocculant and effects of pH were investigated. Results indicated that the best addition dosage of Al^{3+} was 100% and the most appropriate concentration of flocculant was 100mg/L. Besides, the lower pH of kaolin suspension was，the higher the flocculate rate was. The experimental results suggested this novel flocculant showed significant flocculation ability.

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