FLOCCULATION BEHAVIORS OF COLLAGEN PROTEIN-AL(III) COMPOSITE FLOCCULANT

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ABSTRACT

Hydrolyzed collagen protein (HCP) was prepared by the hydrolysis of solid skin wastes, and then cross-linked with glycerol triglycidyl ether (GTE) to produce cross-linked collagen protein (CCP). By combination CCP with different amounts of $Al_2(SO_4)_3$, a series of CCP-Al (III) composite flocculants (CCP-Al) were successfully synthesized. A kaolin suspension (5 g/L) was utilized as a model system to investigate the flocculation behaviors of the as-prepared flocculants. When the dosage of the CCP-Al was 50 mg/L, the flocculation extent reached 95% in 20 minutes with the sludge volume ratio lower than 15%. Under the same conditions, the CCP-Al exhibited better flocculation performance than the $Al_2(SO_4)_3$. Scanning electron microscope (SEM) observations revealed that the size of flocs formed by CCP-Al was larger than that of $Al_2(SO_4)_3$, suggesting a better aggregation of flocs.

INTRODUCTION

Collagen protein, which mainly exists in the skin of animals, is one of the most abundant renewable biomass resources in the world, and is commonly used as raw material in leather manufacturing. In the tanning industry, a large amount of skin wastes is generated due to the operation processes of splitting, trimming and shaving, and more than 90% of components contained in these skin wastes are collagen protein.[1] Therefore, effective utilization of these skin wastes is essentially important in viewpoints of environment protection and resources. Skin wastes have been proposed as raw materials to produce cosmetics and feed additive,[2] but these applications are subjected to safety issues. It has been reported that bovine collagen protein intradermal injection could cause keratoacanthoma and benign skin tumor.[3] Besides, there are some limitations on the use of collagen protein as feed additive because of the risk of genopathy.[4] In the European Union, it is strictly restricted for the utilization of animal proteins to feed animals for human consumption. In the US, ruminant protein is prohibited as feed additive to any ruminant.[5] Therefore, to find a proper way to utilize the collagen protein of skin wastes is still a challenge.

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Flocculation precipitation is one of the most efficient methods for water treatment. Flocculant can bring out the aggregation of suspended particles, forming discrete flocs (flocs means the aggregated mass of suspended particles in fluid) in water.[6] Polymeric flocculants, such as polyacrylamide (PAM), polyferric sulfate (PFS) and polyaluminum sulfate (PAS), are the most commonly used high-performance flocculants in water treatment, but the synthesis of these polymeric flocculants often requires hash conditions and/or involves a large amount of toxic organic agents, which are not environmentally friendly. Furthermore, the toxic monomer residues in the flocculants may lead to a secondary source of pollution to water. In Germany, sludge obtained with PAM treatment is not allowed to be discharged into the areas under cultivation by the end of 2013.[7] Currently, some flocculants have been prepared by using natural polymers as the raw materials, such as cationic starch flocculant prepared by etherification with 2, 3-epoxypropyltrimethylammonium chloride (EPTMAC) in the presence of NaOH,[8-10] chitosan-based flocculant prepared by gamma-irradiation-induced grafting,[11] and anionic cellulose flocculant prepared by oxidizing birch cellulose pulp with periodate and subsequently sulfonation.[12] These flocculants are efficient in the treatment of waste water, and most importantly, they are eco-friendly.

Collagen protein contained in skin wastes have abundant functional groups such as $–NH_2$, -COOH and -OH, etc., which can act as the active sites for flocculants to bind with the suspended particles in water. However, the flocculation ability of protein is greatly related with its molecular weight, but the molecular weight of alkali hydrolyzed collagen protein extracted from skin waste is too low. Only these proteins with appropriate molecular weight could exhibit good flocculation performance.[13] Additionally, the surface charge of the collagen protein also needs to increase in order to promote its interaction with the negatively charged particles in wastewater.

Glycerol triglycidyl ether (GTE), a kind of epoxy resin, is an effective protein cross-linking agent.[14, 15] Therefore, the molecular weight of HCP can be increased by the cross-linking of GTE. According to the theory of aluminum tannage,[16] Al^{3+} can act as inorganic cross-linking agent to further increase the molecular weight of collagen protein, and the introduction of Al^{3+} also increases the positive charges of collagen protein, so the flocculation performance of GTE cross-linked HCP can be further improved by the combination with Al^{3+}.[17] In the present investigation, HCP was first cross-linked with GTE and then combined with Al^{3+} to prepare composite flocculants, and their flocculation ability was evaluated with the kaolin suspension system.

MATERIALS AND METHODS

Materials and Chemicals

Solid skin waste was obtained from the Ruixing Leather Manufacture (Haining, Zhejiang province) in the trimming and splitting operation of bovine skin, and it was hydrolyzed in the NaOH solution to prepare hydrolyzed collagen protein (HCP). In optimal manner, solid skin waste was suspended in the 3% NaOH solution and hydrolyzed at 70-80°C for 4 h. After drying, powdered HCP was obtained, and the average molecular weight of HCP was about 1.5-4.5 kDa determined by high performance size exclusion chromatography. Glycerol triglycidyl ether (GTE) was purchased from Changshu Jiafa Chemical Ltd. Kaolin (ASP170, BASF, Germany) was graciously provided by Hangzhou Junyi Chemical Ltd. $Al_2(SO_4)_3 \cdot 18H_2O$ was purchased from ChengDu Kelong Chemical Co., Ltd. Other reagents were all analytical grade and used without further purification.

Preparation of CCP-Al

Preparation of Cross-linked Collagen Protein (CCP)

Hydrolyzed collagen protein (10 g) was first dissolved in 90 mL of distilled water, and the pH of the solution was adjusted to 8.0. Then, a defined amount of GTE (10, 15, 20 and 25%, wt%, on the base of collagen protein) was added drop-wise into the hydrolyzed collagen protein solution. The reaction was kept at 55°C for 5 h, and finally, the cross-linked collagen protein (CCP) was obtained.

Combination of CCP with Al^{3+}

The pH of CCP solution obtained above was adjusted to 3.8 with 1 mol/L HCl. Then, a defined amount of $Al_2(SO_4)_3 \cdot 18H_2O$ (mass ratio of $Al_2(SO_4)_3 \cdot 18H_2O$ to CCP was 0.5, 1.0, 1.5 and 2.0, respectively) was mixed with CCP solution, and the reaction was performed at 35°C for 3 h. Finally, a series of cross-linked collagen protein-Al (III) flocculants (CCP-Al) were prepared. CCP-Al composites with different mass ration of $Al_2(SO_4)_3 \cdot 18H_2O$ to CCP, including 0.5, 1.0, 1.5 and 2.0, were referred as CCP-Al-A, CCP-Al-B, CCP-Al-C and CCP-Al-D, respectively.

Molecular Weight Determination

The molecular weight of HCP and CCP was determined by high performance size exclusion chromatography (HPSEC) using the Agilent 1100 Series (Agilent Technologies, Ltd., CA, USA) with a size exclusion chromatography (SEC) column (TSK gel G3000 PWXL, 7.8 ×300 mm i. d. with a particle size of 7 μm, Tosoh, Tokyo, Japan).[18] The calibration curve of molecular weight was established with standard proteins, which were vitamin B_{12} (1355 Da), aprotinin (6500 Da), cytochrome C (12500 Da), bovine trypsin (23300 Da), ovalbumin (45000 Da), bovine serum albumin (67000 Da), and thyroglobulin (670000 Da), purchased from Sigma, USA. The HPSEC operation was performed according to following conditions. Phosphate buffer, with pH 6.8 and containing 0.1

mol/L Na_2SO_4, was used as mobile phase. The sample volume was 20 µL, and the flow rate of mobile phase was 0.8 mL/min, and the detection wavelength was 280 nm.

Isoelectric Point Determination of Samples

The isoelectric point (pI) of HCP and CCP-Al was measured by zeta-potential approach.[19] Briefly, the sample with volume of 100 mL and concentration of 0.5 g/L was passed through polytetrafluoroethylene filter membrane, and then divided into 10 portions. The pH value of each portion was adjusted to 3.0-8.0 with 0.1 mol/L NaOH and/or 0.1 mol/L HCl. The zeta potential of each portion was detected by Zetasizer Nano ZS90, Malvern, UK. The pI of the sample was the pH point where the corresponding zeta potential was 0 mV.

Flocculation Performance Evaluation

Flocculation Ability

Flocculation experiments were carried out at constant room temperature. The concentration of kaolin was 5.0 g/L and the total volume was 100 mL. The pH of kaolin suspension was first adjusted to 7.0, and then fully mixed with a certain dosage of the as-prepared flocculant. Then, this resultant suspension was transferred to a cylinder (28 mm in top diameter and 27 cm in height). After settling 20 minutes, 5 mL of sample was taken from a height of 10 cm below the surface for measurement of absorbance at 550 nm (UV1800PC, Mapada, China) and zeta potential. The final concentration of kaolin was measured based on the linear relationship between the absorbance at 550 nm and the concentration of suspended kaolin. The SEM observation of flocs was performed using JSM-5900LV scanning electron microscope (JEOL Ltd., Japan). For comparison, the flocculation abilities of HCP and CCP were also tested. The flocculant extent (FE) was calculated as:

$FE= (C_0-C_t)/C_0\times100\%$ (1)

Where, the C_0 and C_t were the initial and final concentration of kaolin, respectively.

Settling Velocity

At the beginning of settling, the height of suspension was recorded as H_0. During settling process, the height of sludge was recorded as H_n after every minute. The sludge volume ratios (SVR) at every minute were calculated as formula (2), and the change of SVR along with time was defined as the settling velocity.

$SVR= H_n/H_0\times100\%$ (2)

RESULTS AND DISCUSSION

Molecular Weight of Collagen Protein Before and After Cross-linked

As shown in Figure 1, the molecular weight of collagen protein was substantially increased after cross-linked with GTE. The molecular weight of hydrolyzed collagen protein (HCP) was lower than 4.5 kDa while it was gradually increased with increasing amount of GTE. When the dosage of GTE was 20%, the collagen proteins with molecular weight distribution between 10-20 kDa and 20-30 kDa were considerably increased. However, the increase in molecular weight of collagen protein was limited when further increasing the dosage of GTE. Therefore, the 20% dosage of GTE was selected as the optimal amount to prepare CCP, and CCP without specific description herein after was prepared with 20% GTE.

The flocculation ability of HCP and CCP (200 mg/L) to kaolin suspension is given in Figure 2. It can be observed that HCP has almost no flocculation ability, and the flocculation ability of CCP was significantly increased but the settling time was still too long. In addition, the pIs of HCP and CCP are 3.64 and 3.82 respectively (Table 1), which is somewhat lower than the needed value for the treatment of negatively charged wastewater.[20] These facts indicated that CCP still needs to combine with Al (III) in order to increase its pIs to promote the charge neutralization ability to wastewater.

As shown in Figure 3, the introduction of Al (III) has greatly increased the flocculation ability of CCP-Al. The flocculation extent of CCP-Al-B was around 70%, which was much higher than those of HCP, CCP and polyacrylamide (PAM). However, the flocculant abilities of CCP-Al-B and $Al_2(SO_4)_3$ were almost the same when the dosage was higher than 50 mg/L, as shown in Figure 4. These facts suggested that the flocculation ability of CCP-Al is attributed to the synergistic effect of CCP and Al^{3+}, and its flocculant extent was also dosage dependent.

The pIs of Samples

The pIs of HCP and CCP were increased due to the combination of Al (III), as presented in Table I. The pIs of HCP and CCP are 3.64 and 3.82 respectively, and these values

TABLE I
Isoelectric point of samples.

Samples	Isoelectric point
Hydrolyzed collagen protein (HCP)	3.64
Cross-linked collagen protein (CCP)	3.82
CCP-Al-A	3.98
CCP-Al-B	4.81
CCP-Al-C	5.07
CCP-Al-D	5.34

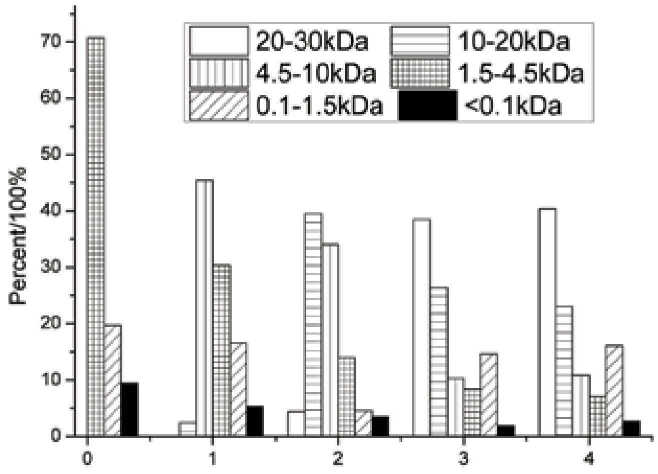

Figure 1. Molecular weight distribution of HCP and CCP 0: HCP; 1, 2, 3 and 4: CCP cross-linked with 10%, 15%, 20% and 25% (w/w) GTE.

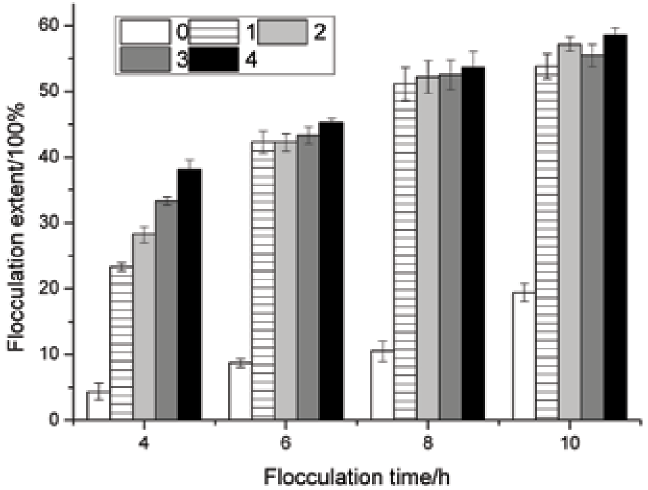

Figure 2. Flocculation ability of HCP and CCP (200 mg/L) 0: HCP; 1, 2, 3 and 4: CCP cross-linked with 10%, 15%, 20% and 25% (w/w) GTE.

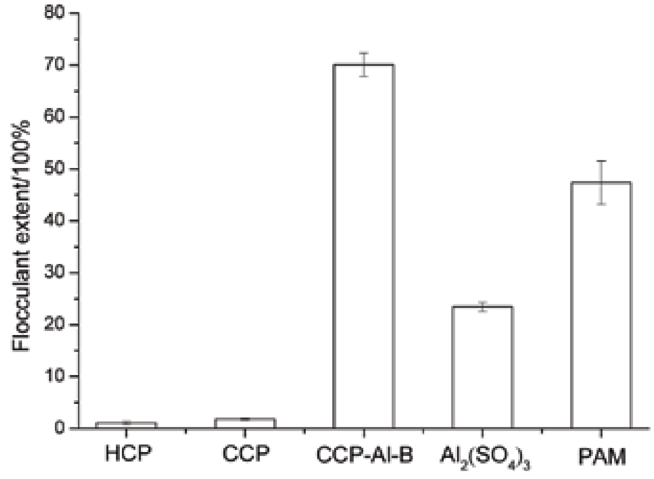

Figure 3. Flocculation ability of HCP, CCP, CCP-Al-B, $Al_2(SO_4)_3$ and PAM (20 mg/L, settled for 20 minutes).

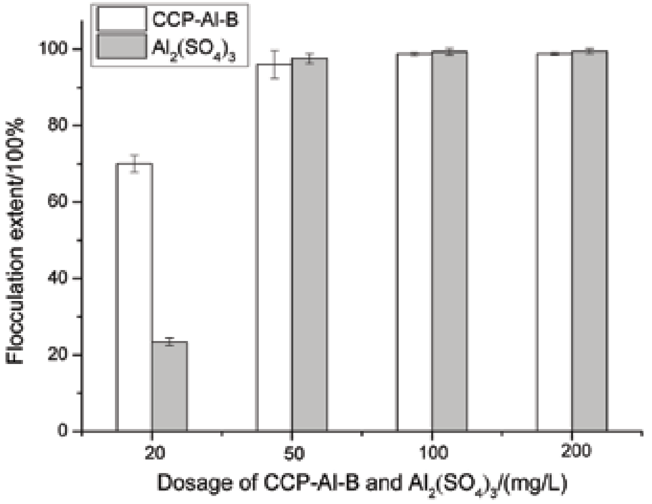

Figure 4. Comparison between CCP-Al and $Al_2(SO_4)_3$ (settled for 20 minutes).

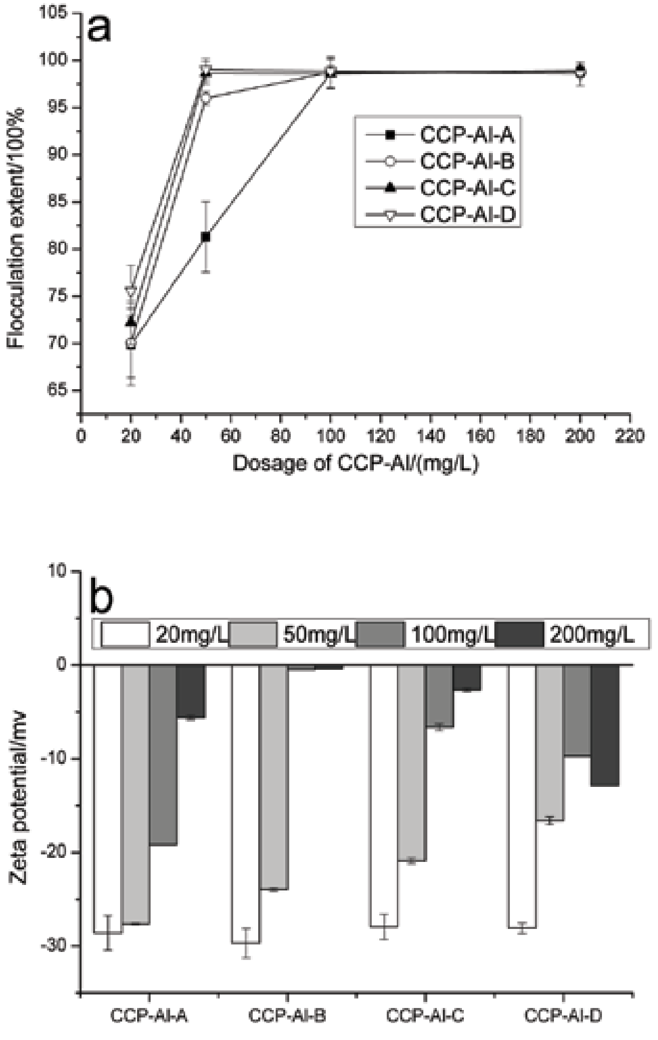

Figure 5. Effect of dosage of CCP-Al on flocculant rate (a) and zeta potential (b) (settled for 20 minutes).

were gradually increased with the increasing the content of Al (III). As shown in Figure 3, CCP-Al with high pI is more effective in flocculation than HCP and CCP due to the stronger charge neutralization ability. Moreover, the pI of CCP-Al-B was considerably increased from 3.98 to 4.81, resulting in a remarkable increase of flocculation ability.

Flocculation Ability Evaluation

Effect of Dosage of CCP-Al

CCP-Al exhibited good flocculation ability with low dosage, as presented in Figure 5a. For all CCP-Al, the flocculation extent was increased from 70% to 95% when the dosage was increased from 20 mg/L to 100 mg/L. These facts indicated that CCP-Al was an effective flocculant featured with low dosage. On the other hand, the flocculation ability of CCP-Al was in the order of CCP-Al-A < CCP-Al-B < CCP-Al-C≈CCP-Al-D, which is consistent with their molecular weight.

Moreover, the zeta potential of the flocculation system after CCP-Al introduced was also determined.[21] As shown in Figure 5b, the zeta potential was gradually increased with the increasing dosage of flocculant. Especially for CCP-Al-B, the zeta potential almost reaches zero when the dosage was 100 mg/L. In the case of CCP-Al-D, however, the zeta potential was reversely decreased when the dosage was increased to 200 mg/L, which suggests an over charge neutralization in suspension solution that leads to some loss of flocculation ability at higher dosage of flocculant.

These facts suggested that the flocculation efficiency of a flocculant is greatly related to the molecular size and charges, and the molecular size is the most essential factor.[22] Therefore, the effect of bridging action and charge neutralization should be the flocculation mechanism of the as-prepared flocculant.

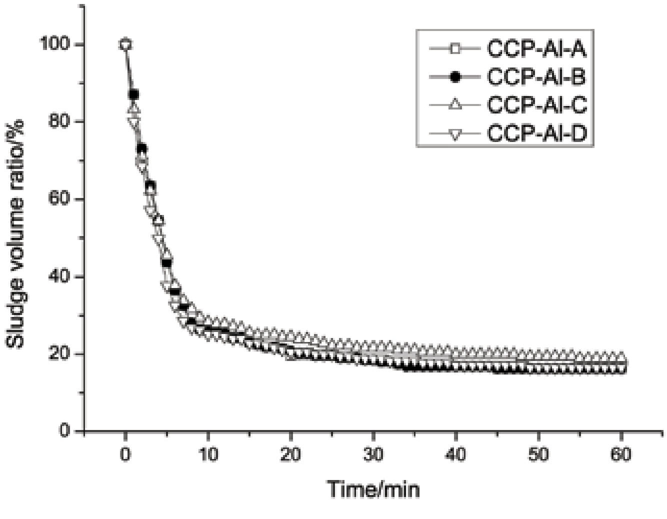

Figure 6. Settling velocity curves of CCP-Al (50 mg/L).

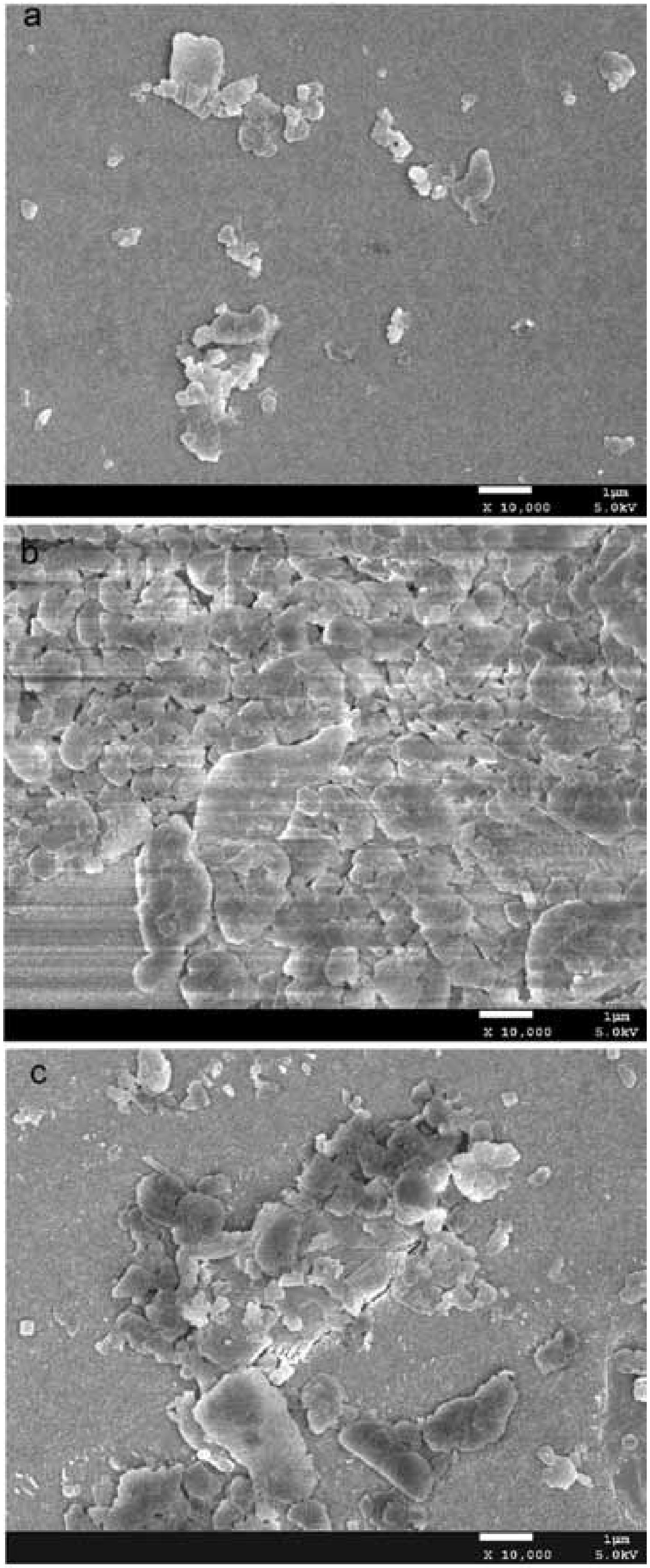

Figure 7. SEM observation of flocs (20 mg/L, settled for 20 minutes) (a) was kaolin particles without flocculation; (b) was flocs formed using CCP-Al-B; (c) was flocs formed using $Al_2(SO_4)_3$

Settling Velocity

Figure 6 illustrates the settling velocity of CCP-Al at the dosage of 100 mg/L. It can be observed that the sludge volume ratios (SVR) were rapidly reduced to ~20% in 10 minutes, and then decreased very slowly. Finally, the SVR reached 15% in 60 minutes. In general, the sludge volume ratios declined along with the increase of the settling time. The settling velocity of as prepared flocculants (CCP-Al) can meet the requirement of wastewater treatment.[23]

SEM Observation of Flocs

Figure 7a is the SEM observation of the kaolin particles without adding any flocculant. Obviously, there were almost no flocs formed if no flocculant was added. Figure 7b and 7c show the formed flocs by CCP-Al-B and $Al_2(SO_4)_3$, respectively. In Figure 7b, the size of flocs formed by CCP-Al-B is apparently bigger, and the flocs closely packed together. It should be noted that the size of flocs formed by $Al_2(SO_4)_3$ was also significantly increased, which confirmed the flocculation ability of $Al_2(SO_4)_3$ as a common flocculant. But its flocculation ability is limited compared with CCP-Al. These facts further proved that the combination of CCP and $Al_2(SO_4)_3$ would significantly improve the flocculation ability of CCP.

CONCLUSION

In the present investigation, biodegradable composite flocculants were prepared through the incorporation of GTE cross-linking collagen protein (CCP) and $Al_2(SO_4)_3$, where these novel flocculants exhibited excellent flocculation ability. It was found that the molecular weight of hydrolyzed collagen protein (HCP) was substantially increased after cross-linking using GTE, and its flocculation ability was therefore improved to some extent. In addition, the combination of CCP with $Al_2(SO_4)_3$ significantly improved the flocculation ability due to the increased molecular size and pIs. The flocculation mechanism of the as-prepared flocculants involves the effect of bridging action and charge neutralization.

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