Flocculation of Quartz and Kaolin by Alginate-Protamine Complex

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ABSTRACT

A novel bioflocculant, alginate-protamine complex, was developed and was proposed in this study. Alginate and protamine have no flocculating ability solely, however, the mixture solution of alginate and protamine or alginate-protamine complex showed a good flocculating ability. This study reported a flocculating performance of the alginate-protamine complex. The flocculation performance was evaluated by clarification experiment with kaolin and pulverized quartz sand in terms of relative absorbance. The flocculation performance was examined by varying experimental conditions, especially, alginate/protamine mass mixing ratio and pH. The flocculation performance was significantly affected by the mass mixing ratio and pH. The most effective mass mixing ratio was found at 0.4-0.8 in the pH range of 3-7. The flocculation performance was also affected significantly by pH. Good flocculation performance was provided at pH 3-7, however, the performance was getting worse at pH 8-11.

Keywords: flocculation; complex flocculant; solid-liquid separation; sodium alginate; protamine sulfate

1. Introduction

The flocculation of suspensions by chemical flocculants is used to improve solid-liquid separation in mineral processing operations, wastewater treatment, and so on. The currently accepted chemical flocculants are synthesized high-molecular-weight polymers and alum. Both of these flocculants are environmentally undesirable because chemically synthesized polymer flocculants remain in natural environments for long periods without degradation to less harmful forms. Therefore, many studies concerned environmentally friendly biodegradable flocculants have been reported; naturally existing polymers such as chitin or chitosan [1,2], methylated proteins [3-5], extracellular polymers produced by bacteria [6,7], chemically modified natural polymer or polysaccharide [8-14]. Recently, utilization of polysaccharides for flocculants have been especially reported; starch-based flocculant [15], cellulose-based flocculant [16,17], alginate-based flocculant [18,19].

On the other hand, fillet of salmon, cod, and so on have been used for food all over the world. However, for example, fish milt of salmon, cod, sandfish and so on, have been used for food from old times in Japan only, and most salmon milt has been discarded because of an inherent fishy smell, a fast decline of freshness, and difficulty for food processing due to lacking heat coagulation. In the world, a huge amount of fish milt has been discarded. Salmon milts include a lot of protamine as a nucleoprotein, which has a basic group, such as amino or guanidine functional group, and has a positive charge in the neutral pH region. A surface charge of solid particles in the aqueous phase is known as a negative charge mostly. These suggest that protamine can adsorb onto suspended particles surface and can become a flocculant for suspended solid particles. However, judging from the molecular weight of protamine (Mw. ca. 4600), the molecular size of protamine should be relatively small than other proteins used as flocculants. Novel flocculants are needed to have a certain size for making effective flocs in the flocculation process.

As described above, polysaccharides have been employed as bio-flocculants. Major polysaccharides used as bio-flocculant were mainly alginate, chitosan, cellulose, starch, pullulan, xanthan and pectin [20]. Among these, one of the polysaccharides, alginate is a major structural polysaccharide from brown seaweeds. Alginic acid, a linear polysaccharide of β-D-mannuronic acid and α-L-guluronic acid, is a main cell-boundary constituent of brown algae. Alginic acid contains about 4×10^3 mol/g of carboxylic groups [21]. Alginate has been widely used as a viscosifier and gelling agent in food, pharmaceutical and biotechnological industries. Several alginate-based flocculants have been proposed and reported. The major were modified alginates chemically, such as drafted copolymerization; for examples, Tripathy et al. [22] synthesized polyacrylamide grafted alginate. Sand et al. [23] proposed grafting of N-vinyl-2-pyrrolidone onto alginate initiated by potassium peroxymonosulphate/glycolic acid redox initiator. Rani et al. [18] synthesized polymethyl methacrylate grafted alginate (SAG-g-PMMA) by microwave irradiation. Zhang et al. [24] prepared trimethyl quaternary ammonium salt of lignin-alginate polyampholyte with trimethyl quaternary ammonium salt of lignin and sodium alginate using glutaraldehyde as a crosslinker. Tian et al. [25,26] proposed several modification of alginate polymer and applied them not only to be a flocculant but also to be a adsorbent of harmful divalent metal ions. By these ways, cationic properties were added to alginate polymer to bind with solid surfaces charged negatively in aqueous environment.

In this study, we would propose the utilization of a complex of two substances, alginate polymer and protamine as an environmentally preferable flocculant in the aqueous phase, and examine the expression of flocculation ability and the capabilities of the alginate-protamine complex to flocculation of suspended solid particles. There have been few reports about the utilization of complex of alginate and protein as a flocculant. By this method, relative larger size and charged positively polymer as a flocculant could be obtained without some complicated chemical modifications. Bridging flocculation could occur under the situation which flocculant size is larger than the closest possible distance between surfaces of adjacent solid particles. The distance would exist because of electrostatic repulsive force between surfaces which were same charge. It has been pointed out that one of the conditions for having an excellent flocculant is that it is important to have a size exceeding this distance [27]. Kaolin and quartz were chosen model solid particles. The authors investigated especially the expression of flocculation ability of the complex, the influence of the mass ratio of alginate and protamine and pH value of the suspension on the flocculation in the present study.

2. Experimental

2.1. Materials

Protamine sulfate from salmon, Quartz sand and kaolin were purchased from Wako Pure Chemical Industry (Japan). Sodium alginate was purchased from Kanto Chemical (Japan). Hydrochloric acid and sodium hydroxide were used analytical grade reagents. These reagents were used without further purification.

2.2. Preparation of suspension

Kaolin and quartz powder were used for making suspensions to be flocculated. Kaolin was used without further purification. The quartz powder was prepared by the following procedure. The quartz sand was washed with distilled water and was dried at 60°C. Then, it was pulverized for 48 hr using ceramic ball mill. The quartz powder was washed with distilled water repeatedly and was dried at 60°C for 48 hr. The size distributions were measured with a laser-scattering size distribution analyzer (LA-300 HORIBA, Ltd., Japan). The mean particle diameters for kaolin and quartz were 6.5 and 47.3 μm, respectively.

Suspensions of kaolin and quartz powder were prepared as follows. In order to soak the surface of particles thoroughly with water, the desired weight of powder was boiled with 1000 mL of distilled water in an Erlenmeyer flask. A dilute HCl or NaOH solution was added to adjust the final pH in suspension to pH 6.5-7.0. In this study, this suspension was used as a stock suspension for all the experiments. In most experiments, concentrations of both powder suspensions were 3 g/L.

2.3. Clarification experiments

Quartz or kaolin (3 g/L, 100 mL) suspension was poured into a 100 mL glass graduated cylinder. The protamine and alginate solutions prepared at desired concentrations were mixed each 2 mL, respectively. Then, the mixture solution (4 mL) was stirred hardly and 2 mL of the mixture was immediately added to the 100 mL of suspension with a macro pipetter. In the case of adding a single component solution (protamine or sodium alginate), the dosage volume was also employed for 2 mL. The suspension added flocculant was agitated with a magnetic stirrer at 500 rpm for 5 minutes. After this, the suspension was left to stand without agitation for 1 minute, then, a 2 mL sample was taken from the supernatant layer at a position of 2 cm under the liquid surface of the suspension. The absorbance of the sample was measured at 700 nm spectrophotometrically. The flocculation efficiency was evaluated with the relative absorbance, A/A_0, where A and A_0 represent the absorbance of the suspensions in the presence and the absence of flocculant, respectively.

3. Results and discussion

3.1. Flocculation by adding protamine only

Protamine molecule has a positive charge at ca. neutral pH region since protamine is a basic protein. Flocculation ability would be expected in case of adding protamine only as a flocculant. Fig. 1 shows the flocculation effect of protamine. The ordinate corresponds to the relative absorbance, A/A_0. The lower this value, A/A_0, the greater the effect was obtained for clarification. The experiments were conducted at pH 6.9-7.0. As expected, the addition of protamine only expressed flocculation ability, though the clarification effect was not so great for both kaolin and quartz particle. The maximum effect was expressed at ca. 1×10^{-4} g and 3×10^{-4} g of protamine dosage for quartz and kaolin, respectively. Due to positive charge of protamine at weak acid and neutral pH region (pH < 7), particle coagulation was observed in this experiment. However, sufficient flocculation effect was not obtained by addition of protamine only.

3.2. Expression of flocculation ability by mixture of alginate and protamine

The authors conducted two type clarification experiments to confirm flocculation ability by adding alginate-protamine mixture, that is, separately adding mode and mixed adding mode, respectively. In these experiments, kaolin suspension (3 g/L) was employed as a model solid particle. In first, 2 mL of a protamine solution was added to 100 mL of 3 g/L suspension and the suspension was stirred for 5 minutes. Secondly, 2 mL of an alginate (sodium alginate) solution prepared at the desired concentration was added to the suspension and moreover, the suspension was stirred for 5 minutes. The results were shown in Fig. 2. The open triangle symbol in Fig. 2 corresponds to the results of the separation addition. In this separate addition of alginate and protamine solutions to the suspension, the dosage amount of protamine was fixed at 1.51×10^{-4} g and that of alginate was varied. The variation in the dosage amount of alginate to the system corresponds to the abscissa of Fig. 2. The open circle symbol indicates the clarification experiment result by adding a mixture of alginate and protamine, which dosage amount of protamine is also fixed at 1.51×10^{-4} g. In the case of separate addition, flocculation was not mostly observed in the cylinder, so the relative absorbance, A/A_0, was kept at a high value. On the other hand, in the case of the addition of the mixture, especially the value of A/A_0 became very low at ca. 1.0×10^{-4} g for the dosage amount of alginate. This fact indicated that the mixture of alginate and protamine affected the flocculation of solid particles and clarification of suspension. In the present study for almost runs, the mixture solution was immediately added to the suspension after mixing both alginate and protamine solution. At that time, cloudy (white turbidity) of the mixture solution was not invisible with eyes. However, the two solutions were mixed and the mixture became slightly cloud with time. This suggests that some colloidal matter was formed by mixing protamine and alginate.

Fig. 3 shows the typical results of the change in absorbance (700 nm) of the mixture solution with varying the mixing mass ratio of alginate and protamine. The concentrations of both solutions were 0.15 and 0.3 g/L of alginate solutions and 1.25 and 2.5 g/L protamine solutions in this measurement. The concentration of protamine solution in this case is especially 2-125 folds higher than that in the case of flocculation experiment. The higher concentrations of the protamine solution were used to confirm the formation of colloidal matter. The absorbance of the mixture solution gradually increased with increase in the protamine contents, and abruptly increased when the mass ratio reached at ca. 1.5. The higher concentration of sodium alginate, the higher value of absorbance (open circle in Fig. 3). As the mass ratio increased, the formation of the precipitates was visually observed. Such these cloudy mixture solutions were not used as the flocculant in the flocculation experiments. This result in Fig. 3 suggested that some aggregates or complexes were formed by mixing the both solution and the mixture solution as the flocculant could express flocculation ability even if the precipitation did not occur.

3.3. Effect of mass ratio of alginate and protamine on A/A_0

The clarification experiments were conducted with varying the mass ratio of sodium alginate and protamine in the mixture solution. The results are shown in Fig. 4 for kaolin (Fig. 4a) and quartz (Fig. 4b), respectively. The experiments were conducted at pH 6.9-7.0. Each mixture solution was prepared by varying mass content of alginate in the mixture solution and the mass content of protamine was fixed at the desired value. These values are shown in the legend symbol of Fig. 4. In Fig. 1, data point, Q1, Q2 and Q3 for quartz, and K1, K2 and K3 for kaolin indicate that dosage amount of protamine of these points correspond to 6.0×10^{-5}, 1.5×10^{-4} and 4.0×10^{-4} g for quartz and, 4.0×10^{-5}, 3.0×10^{-4} and 2.0×10^{-3} g for kaolin in ascending order, respectively. The same contents for protamine were employed as the experimental conditions of Fig. 4. As seen in Fig. 1, at data points K1, K3, Q1 and Q3, flocculation was not almost observed. On the other hand, as seen in Fig. 4, due to coexistence with alginate, flocculation ability was mostly enhanced in the same condition of protamine content. For both kaolin and quartz, the degree of clarification was higher in the range of mass ratio ca. 0.3-0.8. Especially, in cases of 4.0×10^{-4} and 6.0×10^{-4} for protamine content in the mixture for quartz and kaolin, the most effective clarification was observed in the experimental range of this study.

In visual observation, large flocs were formed in flocculation process at each optimum condition by adding the mxture solution. This complex should be considered as macromolecular and polyvalent flocculant. These flocculants are known to bridge between particles by chemical binding forces and to form large flocs having high settling velocities.

Ruehrwein and Ward [28] first proposed the basic principle of bridging flocculation in 1952, presenting a model in which a single polymer chain was bridging between two or more particles. Some more vigorous studies about bridging flocculation have been developed [29-33]. The flocculation mechanism in the present study could be bridging flocculation. Protamine molecule consists of about 32-33 amino acids and over 2/3 is alginine. Arginine has carboxylic group (pKa 3.2), amino group (pKa 9.0) and guanidine group (pKa 12.0) [34]. Where, pKa corresponds to logarithmic value of acid dissociation constant of functional group multiplied by minus one (-1). Protamine has positive charge at pH < 7. On the other hand, carboxylic group (pKa 3.2) [35,36] on alginate polymer dissociated with hydrogen ions and alginate molucules were charged negatively (pH > 4). Quartz and kaolin have negative charge at pH > 4. Protamine should adsorb onto the particle surface. However, protamine could not bridge between two particles due to its low molecular weight or small size. Alginate has high molecular weight of ca. 400 kDa, thus, protamine-alginate complex is considered to be a positively charged high molecular weight. The complex could be considered to be adsorbed on the solid surface and to be able to bridge or flocculate the particles.

3.4. Effect of pH value on clarification efficiency, A/A_0

It is well known that the pH value of the aqueous phase plays an important role in flocculation ability. Influence of pH value on the flocculation ability by adding the mixture of alginate and protamine was investigated. In this experiment, kaolin particle was employed as a model solid particle, and the pH value of the 3 g/L suspension was varied by addition of hydrochloric acid or sodium hydroxide aqueous solutions. The content of protamine in the mixture solution was fixed at 1.5×10^{-4} g and that of alginate was varied. The result is shown in Fig. 5. At pH 3, 5 and 7, A/A_0 value mostly became under ca. 0.1, thus, good clarification by flocculation was observed. On the other hand, at pH 9 and 11, flocculation obviously did not occur in the system. At pH 8, flocculation ability became getting worse with an increase in the mass ratio of alginate and protamine in the mixture solution.

The reason why the flocculation performance became getting worse significantly in the alkali pH region was considered as follows. The reason why the complex flocculant has the flocculation efficiency in pH 7 was described in the section 3.3. On the other hand, in the alkaline pH region (pH 8-11), especially, a hydrogen ion dissociated from the guanidine and the amino groups, which resulting the both functional groups became uncharged state. This should be considered that the complex as the flocculant could dissociate into protamine and alginate and the flocculation performance disappeared.

3.5. Comparison with other polymer base flocculant

There are several reports concerning polymer or biopolymer-based flocculants. Although it was difficult to compare the flocculation efficiency of various flocculants due to several different experimental conditions, an effort was made to compare the present flocculant with other flocculants reported in the literature in terms of kaolin suspension flocculation. The data are summarized in Table 1. From table 1, it is different from the conditions of clarification conditions, especially the stirring method and the settling time. Considering from the viewpoint of the settling time, it is suggested that the flocculant used in this study may have formed flocs with a very fast sedimentation rate since the sedimentation time in this experimental condition is very short (1 min) and the sampling position is 1 cm below the liquid surface. It was concluded that the present flocculant is comparable with other reported flocculants.

We think that the advantages and disadvantages of the present complex flocculant are as follows; Advantage points: (1) In comparison with other reported polymer or biopolymer-based flocculant, some complicated chemical treatments are not required; (2) Since protamine is a component obtained from milt which is discarded sites, it should be an effective use of fisheries waste, and in other words, effective utilization of bioresource; Disadvantage points: (3) The optimum pH conditions are limited to range in weak acidic and neutral pH region. This range is considered to be a somewhat narrow range. (4) Difficulty in storing the two solutions (protamine and alginate) in a mixture state. It could be true that the mixing process for each use is a little complicated, however, the flocculation performance is not extremely inferior, and we think it can be recognized that it is not unsuitable for practical use.

4. Conclusion

The authors investigated the capability of the complex of alginate and protamine as flocculant for clarification of suspension. The flocculation ability was evaluated by clarification experiment with kaolin and pulverized quartz sand as model particles. As a result, the complex of alginate and protamine were confirmed to express the ability as flocculant. Flocculation performance was examined by varying experimental conditions, especially, mass mixture mass ratio of sodium alginate and protamine and pH value. The flocculation performance was greatly affected by the mass mixing ratio of alginate and protamine and pH value. The most effective mass mixture ratio was found at 0.4-0.8 in the case of pH 7 of the suspension and the present experimental concentration (3 g/L). In acidic and neutral pH region (pH 3-7), the flocculation ability is provided better performance, however, in the alkaline region (pH 8-11) the flocculation ability for the mixture was getting worse. The reason might be considered that in these alkaline regions hydrogen ions should be dissociated from amino or guanidine functional groups, then, the positive charge of protamine molecule decreased.

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Captions of figures and tables

Fig. 1. Influence of dosage amount of protamine on the relative absorbance, A/A_0, of quartz (circle) and kaolin (square).

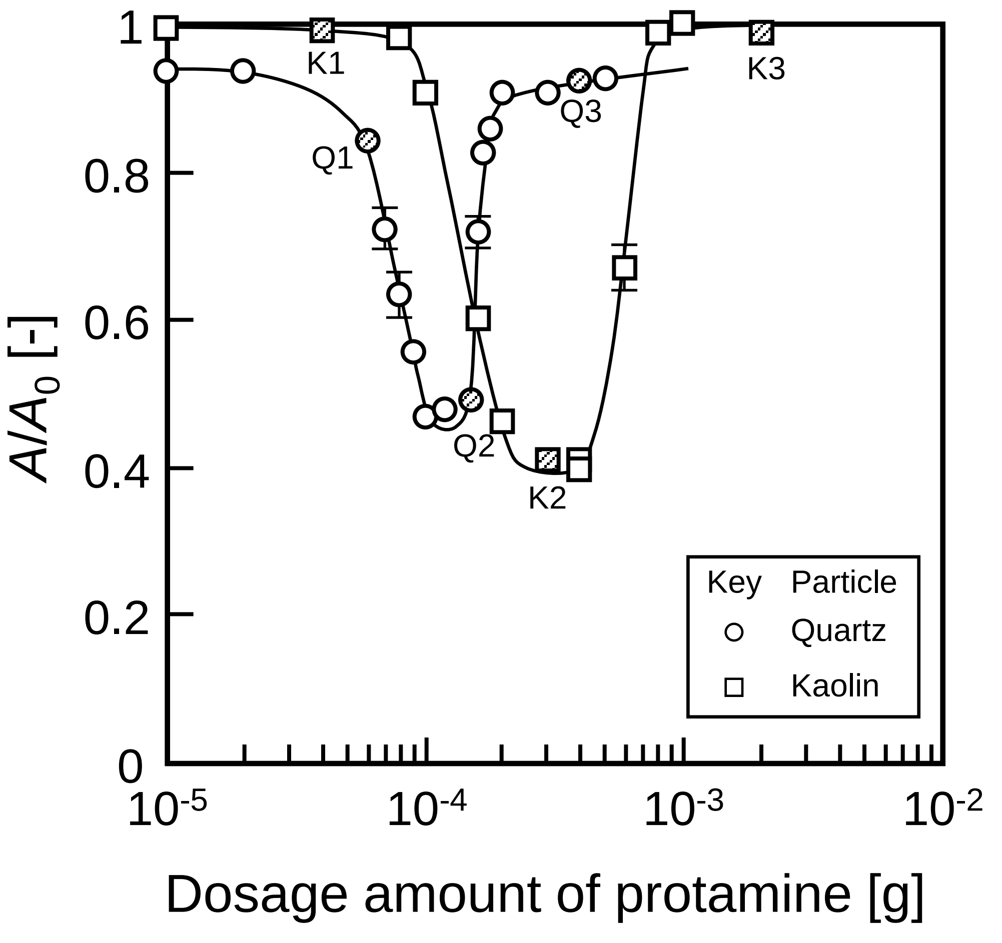
Fig. 2. Influence of addition mode of alginate and protamine on the relative absorbance , A/A_0. The symbols of open triangle and circle correspond to separately adding and mixing before adding mode, respectively.

Fig. 3. Change in turbidity of the mixture solution at 700 nm with varying the mixing mass ratio of Alginate (Alg) and protamine (Pro). Experiments were conducted with addition of protamine solution to 100 mL sodium alginate solution. Open triangle : (0.15 g/L Alg, 1.25 g/L Pro); Solid triangle (0.15 g/L Alg, 2.5 g/L Pro); Open circle (0.3 g/L Alg, 2.5 g/L Pro)

Fig. 4. Influence of mass ratio of sodium alginate (Alg) and protamine (Pro) on the relative absorbance, A/A_0, for (a) kaolin and (b) quartz particles. Each symbol corresponds to protamine content of the mixture.

Fig. 5. Influence of pH value of the kaolin suspension and mass ratio of sodium alginate (Alg) and protamine (Pro) in the mixture on the relative absorbance, A/A_0, of kaolin suspension. The content of protamine is 1.5×10^{-4} g.

Table 1 Comparison of flocculant efficiency and clarification method of kaolin suspension with other polymer based flocculant



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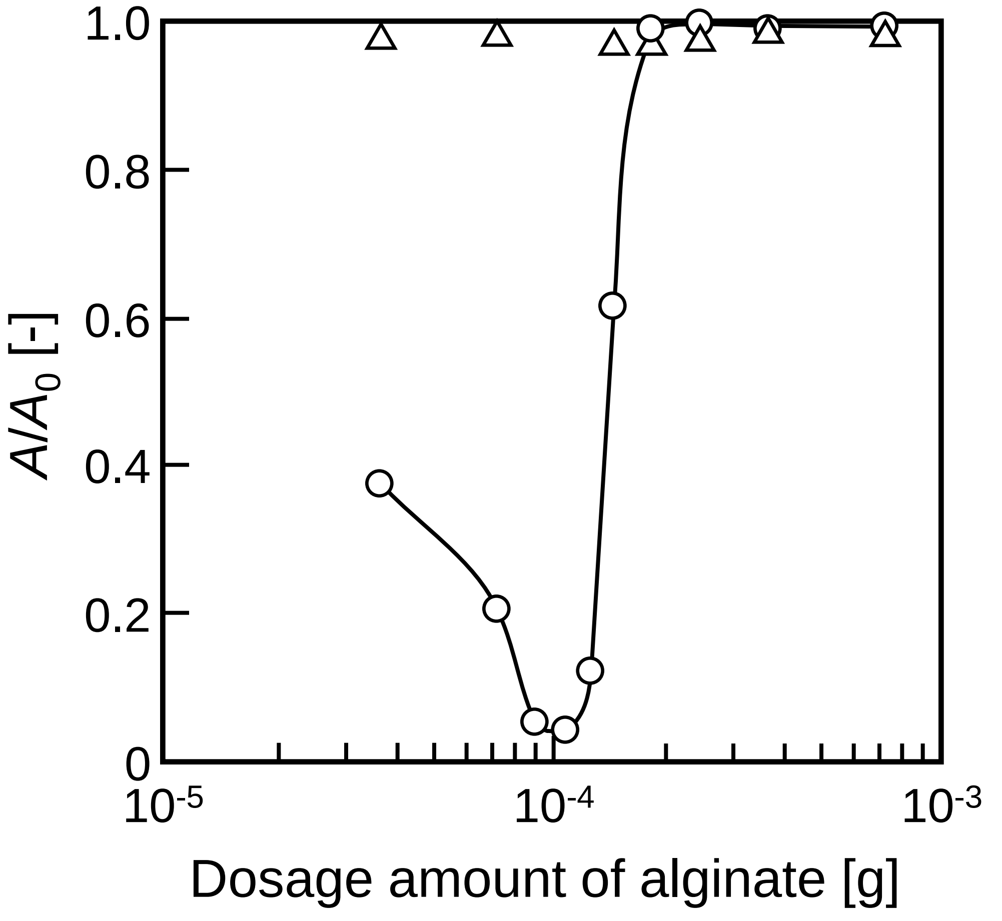

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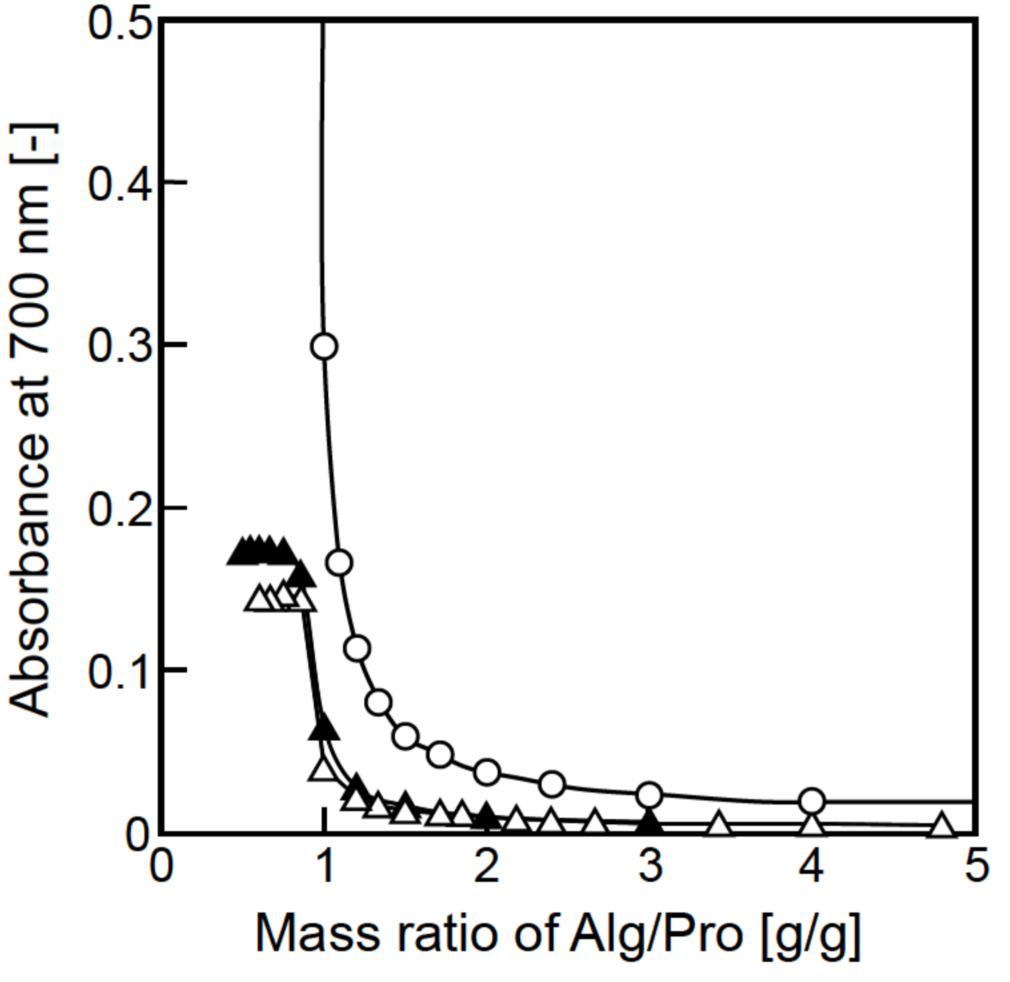

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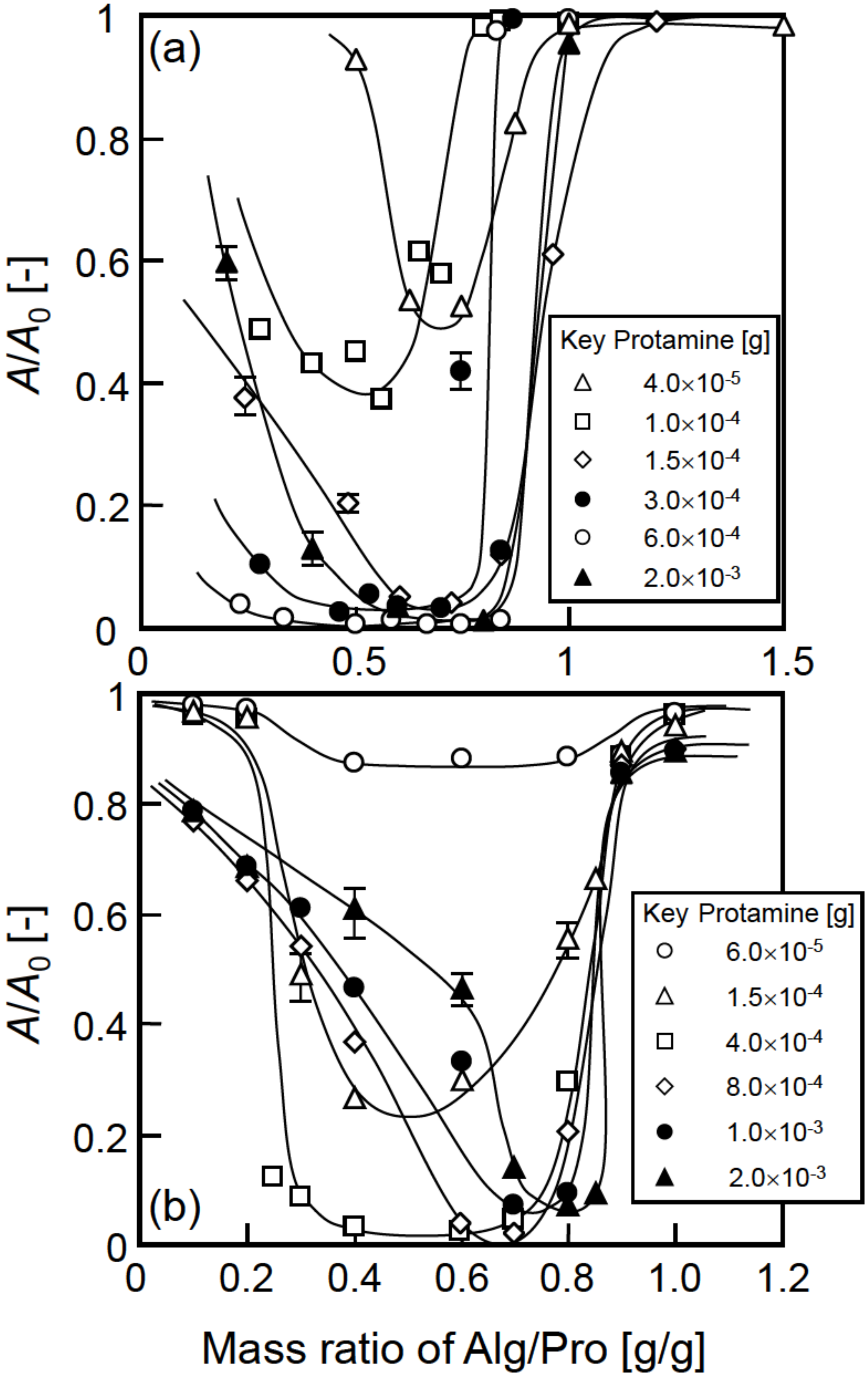

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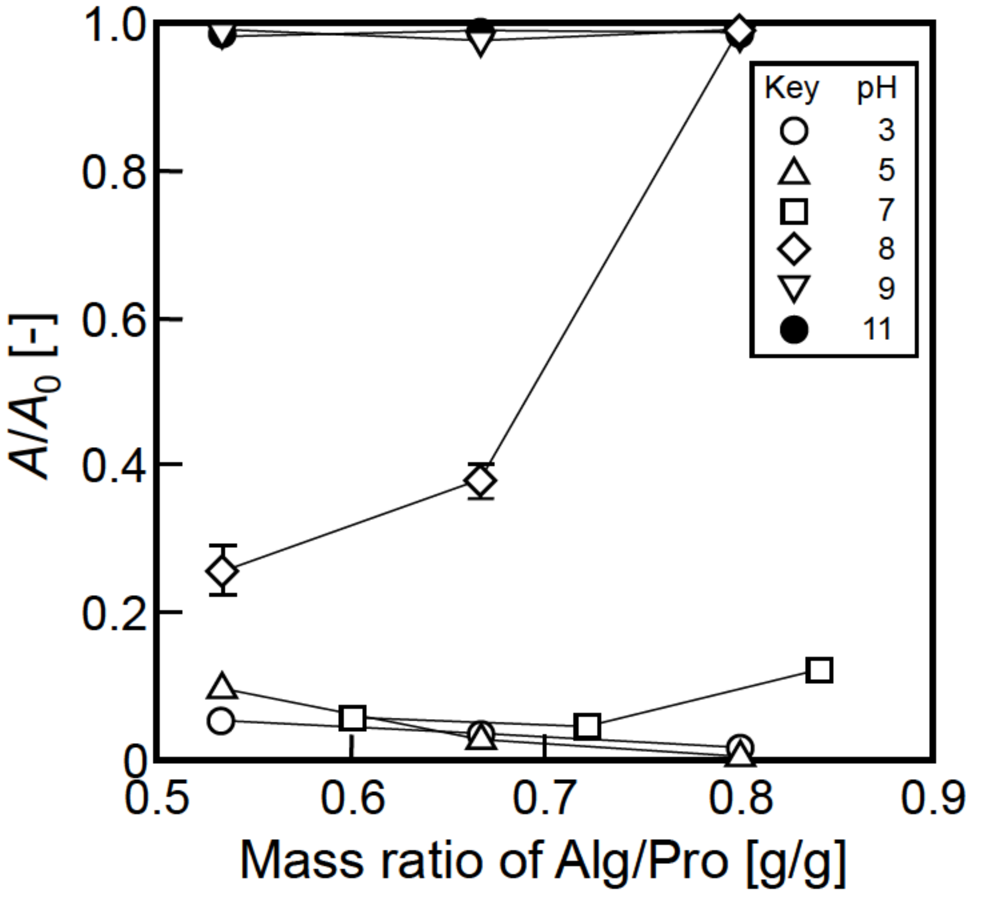

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Table 1
Comparison of flocculant efficiency and clarification method of kaolin suspension with other polymer based flocculants

base polymer	modification	initial concentration	clarification method	flocculation efficiency	Ref.
polyarcylamide	$Al(OH)_3$ hydrid	2.5 g/L	mixing by 10 times of inversion, settling for 5 min	90 %	[37]
starch (potato, wheat, maize)	2-hydroxypropyl trimethylchloride	1 g/L	stirring for 15 min, settling for 20 min	99 %	[38]
chitosan	grafted polyacrylamide	5 g/L	fast stirring (300 rpm, 15 min), medium stirring (120 rpm, 1min), slow stirring (40 rpm, 5min)	90 %	[39]
chitosan	grafted polyacrylamide	5 g/L	slow stirring (40 rpm, 5 min), fast stirring (120 rpm, 2 min)	98 %	[40]
celullose	dicarboxy celullose from bamboo pulp	5 g/L	adding $CaCl_2$ (200 rpm, 3min), adding flocculant (40 rmp, 7min), settling for 30 min	95 %	[41]
alginate	grafted polyacrylamide	2.5 g/L	strring (50 rpm, 5 min), settling for 15 min	80 %	[42]
alginate	complexing with protamine	3 g/L	strring (500 rpm, 5 min), settling for 1 min	95 %	this study