Flocculation of Diatomite by Methylated Egg Albumin

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ABSTRACT

A common and inexpensive protein, egg albumin, was applied to the solid-liquid separation or flocculation of diatomite. Egg albumin was methylated in a 0.1 M HCl methyl alcohol solution at room temperature. About 90% of the carboxylic groups of egg albumin could be methylated within 24 h. The adsorption of egg albumin to diatomite at pH6.8 was remarkably enhanced by methylation. The adsorption constant of methylated egg albumin to diatomite at 30°C was about 100-fold larger than that of native egg albumin however the adsorption constant of methylated egg albumin decreased to about one-hundredth with decreasing temperature from 30 to 6°C. The saturated adsorption amount of egg albumin was also increased by the methylation. The flocculating ability of methylated egg albumin was examined with a diatomite suspension at 6 and 30°C in the pH range from pH2 to pH11. The diatomite suspension was effectively flocculated by the addition of small amount of methylated egg albumin (only 0.5-1 wt-% against diatomite) over a wide pH range from pH 3 to pH10.

Key Words: flocculation; methylated protein, egg albumin, diatomite

INTRODUCTION

The spillage of soil and sand due to the recent deforestation and the waterfront development has become a serious environmental problem. Sedimentation of soil particles in estuary area has caused serious damages on the coastal ecosystem and the coastal fisheries. For example, the extinction of coral reefs has been caused by red soil released from the land. To prevent the inflow of soil particles into the sea an effective separation system for suspended solids in freshwater is necessary.

The flocculation of suspensions by chemical flocculants is used to improve solid-liquid separation in mineral processing operations, water treatment, and wastewater treatment. The currently accepted chemical flocculants are synthesized high molecular weight polymers and alum. Both of these flocculants are environmentally undesirable. Especially, chemically synthesized flocculants remain in natural environments for long periods of time without degradation to less harmful forms. Thus, the use of extracellular polymers produced by bacteria (bioflocculants) for the separation of suspended solids has been a topic of intense research in recent years (1-8). However, for practical application, such types of bioflocculants must be separated or purified from culture media with intricate treatments.

In this study, a common and inexpensive protein, egg albumin, was applied to the solid-liquid separation or flocculation of diatomite in fresh water. Diatomite, used as a model suspended solid in this study, is composed of approximately 90% SiO_2 and it has negative charge above pH 3. Egg albumin, which has an isoelectric point between pH 4 and 5, is also negatively charged at near neutral pH. Therefore, it cannot be expected that a native egg albumin adheres to diatomite surface and it act as a flocculant.

Fraenkel-Conrat and Olcott (9) reported that the carboxylic groups of proteins were readily methylated at room temperature in methyl alcohol containing small amounts of mineral acid (0.02 to 0.1 mol dm^{-3}). The acid-catalyzed reaction of proteins with methanol was a specific one involving only the carboxylic groups; amino, phenolic, thiol, and indole groups and peptide and amide bonds were unaffected. The numbers and the dissociation constants of amino acid residues on an egg albumin molecule are listed in Table 1 (10-13). Since the carboxylic groups account for 78.5 % of the total acidic (negative) groups of egg albumin, the isoelectric point of highly methylated egg albumin shifted to the alkaline side through the blocking of the carboxylic groups (9). Therefore, it is expected that the highly methylated egg albumin will adhere strongly to the negatively charged diatomite surface even at near neutral pH.

In the present study, we prepared an egg albumin having the methylation degree of ca. 90% according to the method reported by Fraenkel-Conrat and Olcott (9). To confirm the effect of methylation on the adsorption behavior of egg albumin to diatomite at near neutral pH, an adsorption experiment was performed at pH 6.8. Since the water temperature of rivers and lakes in the temperate regions varies from about 5°C in the winter to about 30°C in the summer, the effect of temperature on the adsorption behavior of methylated egg albumin to diatomite was also examined at 6 and 30°C. Then we tried to apply the methylated egg albumin to the solid-liquid separation of diatomite in freshwater at 6 and 30°C. Based on the experimental data, the flocculating ability of methylated egg albumin will be discussed.

MATERIALS

Chemicals

Albumin (from egg), methyl alcohol, sodium chloride, sodium hydroxide, and hydrochloric acid were purchased from Wako Pure Chemical Industries (Japan). Albumin was of practical-grade quality and other chemicals were of reagent-grade quality. They were used with no further purification. Distilled water, boiled for 15 min and cooled under a nitrogen atmosphere, was used in all experiments.

Suspended Solid

Diatomite (Celite, Johns-Manville Co., USA) was used in making the suspensions to be flocculated. According to the data supplied by the company, the diatomite is composed of 92.6% SiO_2. The diatomite was screened by a 80-mesh (0.177-mm) sieve. The undersize fraction was washed repeatedly with distilled water and it was dried at 80°C for 2 days and stored in a desiccator. The average particle size and the specific surface area measured by an air-permeability method were 2.62 μm and 1.02 m^2 g^{-1}, respectively. This particular material was selected because of its strong surface-negative character in water; it may remain negative even when the pH is decreased to as

low as 3 or so. To confirm this point, a clarification or auto-coagulation experiment of the diatomite was conducted in the pH range from pH 1 to pH 8 (Fig.2). The maximum coagulation of the diatomite occurred at about pH 2.5 and the pH well agreed with the isoelectric point of the major constituent of the diatomite, SiO_2 (14, 15).

Methylated Egg Albumin

An aqueous solution of egg albumin (10 g dm^{-3}) was prepared. The egg albumin was precipitated by the addition of HCl solution (0.1 mol dm^{-3}). It was separated from the liquid phase in a centrifuge at 3,000 rpm for 20 min and washed twice with methyl alcohol. Then, it was methylated according to the method reported by Fraenkel-Conrat and Olcott (9). The egg albumin, washed with methyl alcohol, was dispersed in a 100-fold amount of methyl alcohol containing HCl (0.05 mol dm^{-3}) as a catalyst. It was stirred for 24 h at room temperature. The methylated albumin was collected in a centrifuge at 3,000 rpm for 20 min and washed repeatedly with HCl solution (1×10^{-4} mol dm^{-3}). Hereafter, the untreated egg albumin and methylated egg albumin will be abbreviated as OA and MeOA, respectively.

To determine the necessary time to attain an enough methylation degree, a preliminary kinetic experiment was conducted. The methylation reaction

proceeded rapidly and 20 h or so was enough to attain the methylation degree of 90%. From the results, we determined the reaction time for the preparation of MeOA as 24 h.

EXPERIMENTAL METHODS

Potentiometric Titration of Egg Albumin

The degree of methylation was determined from the change in the number of carboxylic groups before and after methylation. The number of carboxylic groups was determined by a potentiometric titration. A solution (0.3 dm^3) containing a certain amount of egg albumin (OA or MeOA) was mechanically stirred at 30°C. The ionic strength of the solution was adjusted to 0.1 mol dm^{-3} by the addition of $NaNO_3$. To eliminate CO_2, the titration was performed under nitrogen atmosphere. After reaching thermal equilibrium, the solution was titrated with a volumetric standard solution of HNO_3 or NaOH (0.1 mol dm^{-3}). The pH of the solution was measured by using a pH meter (Orion Research 520-A). The titration was performed in the pH range of 3 to 12 and the number of protonated acidic groups was determined from the difference between the bulk proton concentrations in the presence of egg albumin and those in the absence of egg albumin.

The net charge of OA and MeOA were calculated as a function of pH according to the method described in our previous study (16). The isoelectric points of OA and MeOA (methylation degree of 90%) were estimated to be about 4.5 and 11, respectively.

Adsorption Experiments of Egg Albumin on Diatomite

Suspensions of diatomite were prepared as follows. In order to soak the surface of the solid particles thoroughly with water, 0.2 g of diatomite powder was boiled with 10 ml of water in a 50 ml flask. An amount of dilute HCl or NaOH solution was added so as to adjust the final pH in the suspension to pH 6.5 – 7.0. An appropriate amount of water was added so as to form finally a 50 ml suspension when combined with a prearranged amount of egg albumin solution to be added later. After reaching thermal equilibrium at 6 or 30°C, a prearranged amount of MeOA or OA solution was added to the suspension. The suspension was stirred for the necessary time to attain the adsorption equilibrium, and then diatomite was separated from the liquid phase in a centrifuge at 4,000 rpm for 20 min. The pH and egg albumin concentration of the liquid phase were measured. The concentration of egg albumin was determined by a general UV spectrophotometric method at 210, 225, or 280 nm using Hitachi U-1500 spectrophotometer. The amount of egg albumin adsorbed to diatomite was determined from the difference between the egg albumin concentrations in the initial and the equilibrium states. A control experiment was conducted in the absence of MeOA and OA, and it was confirmed that the diatomite particles were completely removed from the liquid phase by the centrifugation and made no effect on the UV

spectrophotometric measurement. Preliminary kinetic experiments for the egg albumin-diatomite system were performed for 40 min. The adsorption reaction proceeded rapidly and 10 min or so was enough to attain the equilibrium. The adsorption curve showed a plateau from 15 to 40 min. From the results, we determined the contact time for the adsorption experiments as 30 min.

Clarification Experiments of Diatomite

A suspension (20 ml) containing 0.2 g of diatomite was boiled in a 100 ml flask. An amount of dilute HCl or NaOH solution was added so as to adjust the final pH in the suspension to an expected value. An appropriate amount of water was added so as to form finally a 50 ml suspension when combined with a prearranged amount of egg albumin solution to be added later. After reaching thermal equilibrium at 6 or 30°C, a prearranged amount of OA or MeOA solution was added to the suspension and it was stirred for 15 min. The suspension was poured into a 50 ml glass cylinder and was left to stand for 1 min. A 1 ml sample was taken from the supernatant layer at the position 1 cm under the surface. The absorbance of the sample was measured by a Hitachi U-1500 spectrophotometer at 700 nm. At this wavelength, the absorbance of the sample, A, was almost proportional to the concentration of solid, T. For the judgment of flocculating power, the relative turbidity, T/T_0, or A/A_0 was

employed, where T_0 and A_0 denote T and A in the absence of the flocculant, respectively. It must be noted that all the adsorption experiments and the clarification experiments were conducted without any addition of salt to the diatomite suspension, since we purposed to use MeOA for the separation of suspended solid in freshwater at near neutral pH.

RESULTS AND DISCUSSION

Adsorption of MeOA and OA to Diatomite

To examine the effect of methylation on the adsorption behavior of egg albumin to a negatively charged surface at near neutral pH, the adsorption experiments of MeOA and OA were conducted with diatomite. Figure 1a shows the adsorption isotherms of MeOA to diatomite at pH 6.8 and at two different temperatures (6 and 30°C). The enlarged adsorption isotherms in the lower concentration region of Fig. 1a were shown in Fig. 1b. The methylation degree of MeOA used in this experiment was 91%. The adsorption isotherm of OA at pH6.8 and at 30°C is also shown in the figures. The ordinate of the figure, X, represents the amount of MeOA or OA adsorbed to 1 dry-g of diatomite, and the abscissa of the figure, C_e, represents the concentration of MeOA or OA in liquid phase at equilibrium. As expected above, a remarkable change in the adsorption behavior was brought about by the methylation. The adsorption isotherm of MeOA at 30°C rose steeply at very low equilibrium concentrations and demonstrated the high adsorption affinity of MeOA to the negatively charged solid surface. A flocculant, which has high adsorption affinity to suspended solids, has the advantage of lowering the residual

concentration of flocculant in liquid phase. However the adsorption amount of MeOA at 6°C was considerably lower than that at 30°C. Phillips *et al*. (17) have studied the interfacial behavior of two proteins, yeast alcohol dehydrogenase without and with the coenzyme nicotinamide adenine dinucleotide, at a Pt surface. They reported that both proteins were strongly absorbed to the Pt surface via chemisorption and the adsorption amount decreased markedly with decreasing temperature. The similar temperature dependences were observed in the adsorption of lysozyme to porous hydrogel membranes (18), the adsorption of insulin variants to C_8 bonded silica (19), the adsorption of lysozyme and human serum albumin to porous silica (20), and the adsorption of bovine serum albumin to polyethylene and poly(ethylene oxide)-grafted polyethylene surfaces (21).

According to the literature (17, 18, 20), the experimental results in Fig. 1a were described by the Langmuir adsorption isotherm:

$$K = X / (X_S - X) C_e \qquad [1]$$

or

$$X = \{P - (P^2 - 4C_d C_i X_S)^{0.5} \} / 2C_d$$

$$P \equiv C_d X_S + C_i + 1/K \qquad [2]$$

where K, X_S, C_i, and C_d are the adsorption constant, the saturated adsorption amount, the initial concentration of MeOA or OA, and the concentration of diatomite, respectively. A nonlinear least-squares method was applied to find the adsorption constant, K, and the saturated adsorption amount, X_S. The values of K and X_S that gave the best fit with the experimental data in Fig. 1a are listed in Table 2. The solid lines in Figs. 1a and 1b represent the theoretical curves calculated from Eq. [2] with the parameters in Table 2. The correlation coefficients between the experimental and the predicted value were 0.986 (MeOA, 30°C), 0.996 (MeOA, 6°C), and 0.998 (OA, 30°C). The adsorption constant of MeOA at 30°C was about 100-fold larger than that of OA at 30°C. However, the adsorption constant of MeOA at 6°C was only about one-hundredth of that at 30°C.

The saturated adsorption amount of MeOA-diatomite system at 30°C was about 4-fold larger than that of OA-diatomite system. Kamytshny *et al*. reported that the glucose oxidase chemically modified by palmitic acid ester of N-hydroxysuccinimide displayed a much higher adsorption affinity to hydrophilic silica forming more compact surface layers compared to the native glucose oxidase (22). On the other hand, Matsumoto and Inoue (23) reported that a native OA molecule in an aqueous system is almost spherical with

diameter ca. 5 nm based on small-angle X-ray scattering measurement. Based on the molecular size, the diatomite must have the specific surface area of more than 100 m^2 g^{-1} and it is much larger than the specific surface area measured by an air-permeability method (1.02 m^2 g^{-1}). The result suggests that egg albumin molecule was denatured through the methylation and MeOA molecules are adsorbed to diatomite surface in its aggregate form.

Flocculating Ability of MeOA

To determine the necessary dosage of MeOA to flocculate the diatomite at near neutral pH, a preliminary clarification experiment was conducted at pH 6.8 and at 30°C. The addition of small amount of MeOA, only 0.5-1 wt-% against diatomite, was enough to flocculate the diatomite suspension. The remarkable formation of large floc that settled quickly was observed at the dosage of 1-3 wt-%, while an excess addition of MeOA brought about the redispersion of diatomite suspension. The floc formed at the dosage of 1-3wt-% has enough strength and it could be separated by filtration with a 32-mesh (0.5 mm) sieve.

Figure 2 shows the effect of pH on the flocculating ability of MeOA at 6 and 30°C. The methylation degree of MeOA, the concentration of diatomite, and the concentration of MeOA were 87%, 4 g dm^{-3}, and 0.04 g dm^{-3},

respectively. For the comparison, the results of the clarification experiments without MeOA and with OA were also shown in the figure. In the absence of MeOA, the maximum coagulation of the diatomite occurred at about pH 2.5 and the pH well agreed with the isoelectric point of the major (93%) constituent of the diatomite, SiO_2 (14, 15). In the OA-diatomite system, the diatomite particles were slightly flocculated around the isoelectric point of OA (pH4.5). On the other hand, MeOA effectively flocculated the diatomite particles over a wide pH range from 3 to 10. The T/T_0 value increased steeply at below pH 2.5 (the isoelectric point of SiO_2) and above pH 11 (the calculated isoelectric point of MeOA). It can be considered that both the diatomite and MeOA have the same charge at below pH 2.5 and above pH 11 and thus MeOA lose its flocculating ability. After the clarification experiments, diatomite was separated from the liquid phase in a centrifuge at 4,000 rpm for 20 min, and then the concentration of MeOA remaining in the liquid phase was determined. In the experiments conducted at the pH range of 3-10, the concentrations of MeOA remaining in liquid phase were negligibly small.

CONCLUSION

A common and inexpensive protein, egg albumin, was applied to the solid-liquid separation of diatomite. Egg albumin was methylated according to the method reported by Fraenkel-Conrat and Olcott. About 90% of the carboxylic groups of egg albumin could be methylated within 24 h. The effect of methylation on the adsorption behavior of egg albumin to diatomite at neutral pH was examined. The adsorption of egg albumin was remarkably enhanced by methylation. The saturated adsorption amount of methylated egg albumin to diatomite at pH 6.8 and at 30°C was about 4-fold larger than that of native egg albumin. The adsorption constant of methylated egg albumin to diatomite at pH 6.8 and at 30°C was about 100-fold larger than that of native egg albumin. The adsorption constant of methylated egg albumin decreased to about one-hundredth with decreasing temperature from 30 to 6°C. The flocculating ability of methylated egg albumin was examined with a diatomite suspension. The diatomite suspension was effectively flocculated by the addition of small amount of methylated egg albumin (only 0.5-1 wt-% against diatomite) over a wide pH range from pH 3 to 10.

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TABLE 1

Numbers and Dissociation Constants of Amino Acids on Egg Albumin

Type	Number	p*K*
Negative groups		
β, γ - Carboxyl	51	3.98
Phenolic	10	9.95
Thiol	4	8.50
Positive groups		
Imidasole	5	5.91
ε - Amino	22	10.51
Guanidine	14	12.00

Refs. (10 - 13)

TABLE 2

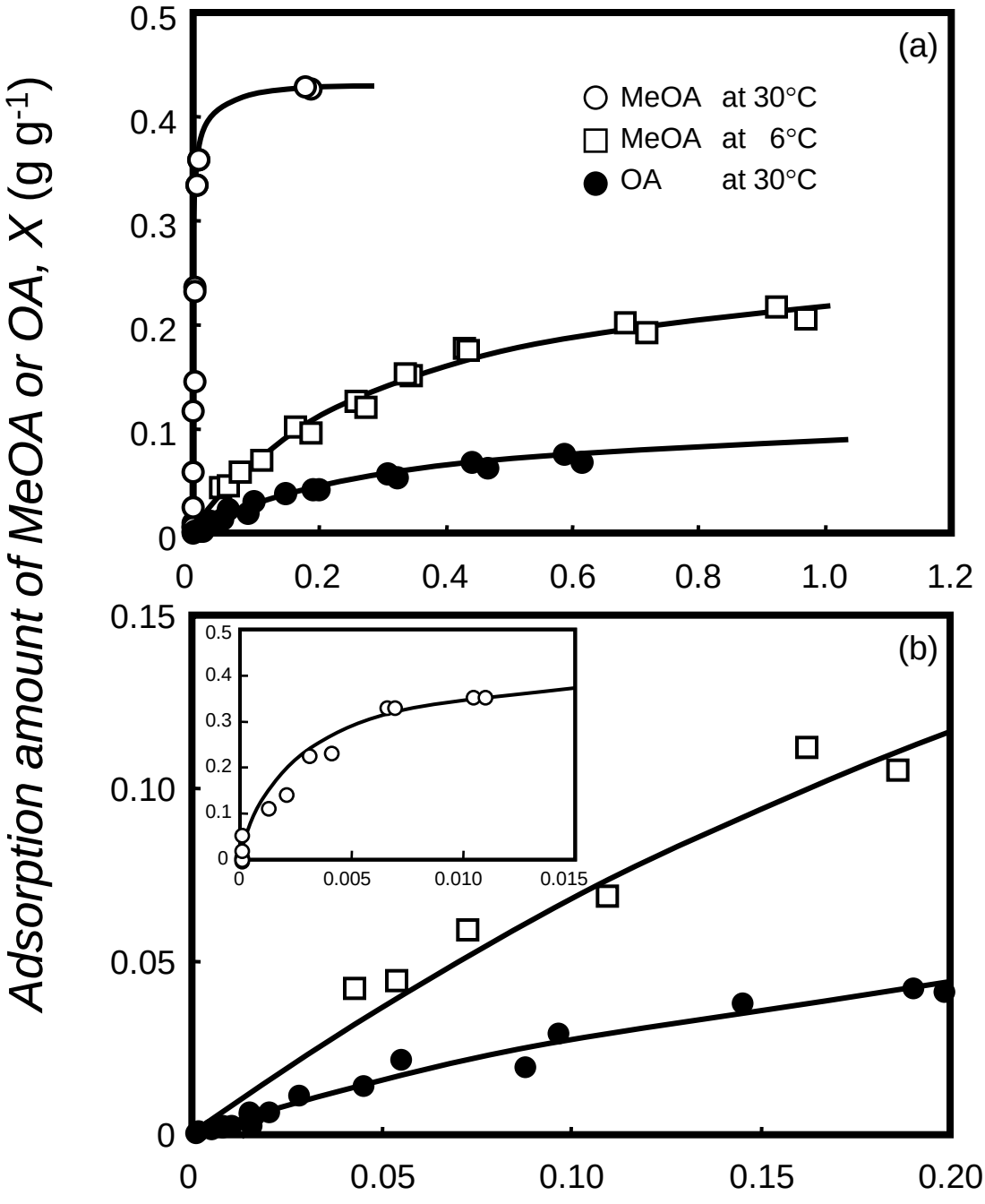
Equilibrium Parameters for MeOA and OA Adsorption of Diatomite

	K (dm^3 g^{-1})	X_S (g g^{-1})
MeOA (30°C)	402 (s.d. = 32)	0.45 (s.d. = 0.018)
MeOA (6°C)	3.14 (s.d. = 0.31)	0.29 (s.d. = 0.022)
OA (30°C)	3.02 (s.d. = 0.14)	0.12 (s.d. = 0.021)

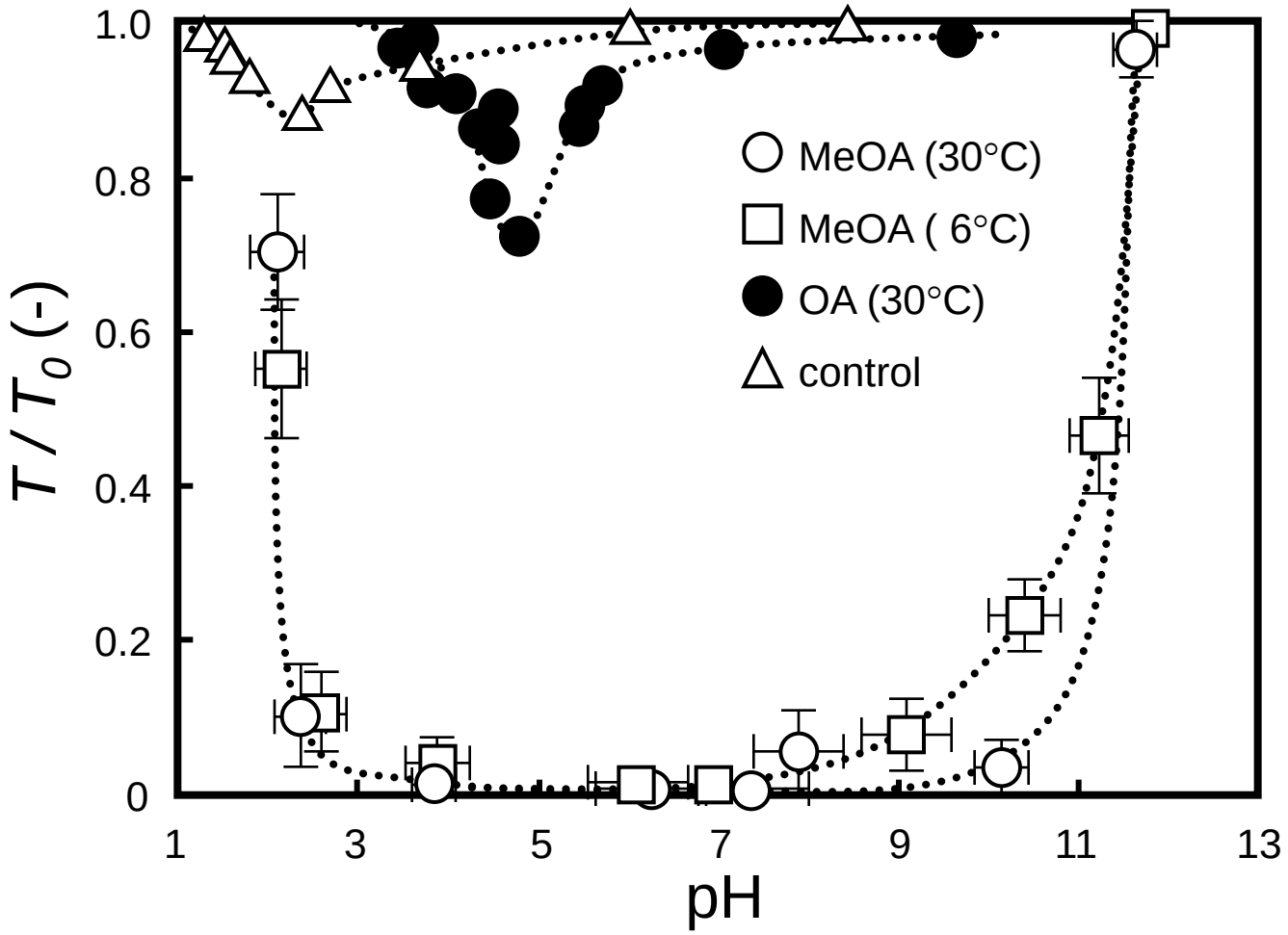
Figure Captions

FIG. 1. (a) Adsorption isotherms of MeOA at 30°C (open circles), MeOA at 6°C (open squares), and OA at 30°C (solid circles) to diatomite at pH6.8. The methylation degree of MeOA and the concentration of diatomite were 91% and 4.0 g dm^{-3}, respectively. The concentration of MeOA and OA was 0.04 g dm^{-3}. The solid lines represent the theoretical curves calculated from Eq. [2] with the parameters listed in Table 2. (b) Enlarged adsorption isotherms in the lower concentration region of Fig. 1a.

FIG. 2. Clarifying efficiencies of MeOA at 30°C (open circles), MeOA at 6°C (open squares), OA at 30°C (solid circles), and without flocculant (open triangles) as a function of pH. The methylation degree of MeOA, the concentration of diatomite, and the concentration of flocculant were 87 %, 4.0 g dm^{-3} and 0.04 g dm^{-3}, respectively.



Seki and Suzuki FIG.1



Seki and Suzuki FIG.2