Coagulation Mechanism of Salt Solution-Extracted Active Component in *Moringa oleifera* Seeds

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ABSTRACT

This study focuses on the coagulation mechanism by the purified coagulant solution (MOC-SC-PC) with the coagulation active component extracted from *M. oleifera* seeds using salt solution. The addition of MOC-SC-PC into tap water formed insoluble matters. The formation was responsible for kaolin coagulation. On the other hand, insoluble matters were not formed when the MOC-SC-PC was added into distilled water. The formation was affected by Ca^{2+} or other bivalent cations which may connect each molecule of the coagulation active component in MOC-SC-PC and form netlike structure. The coagulation mechanism of MOC-SC-PC seemed to be an enmeshment by the insoluble matters with netlike structure. In case of Ca^{2+} ion (bivalent cations), at least 0.2mM was necessary for coagulation at 0.3 mg-C l^{-1} dose of MOC-SC-PC. Other coagulation mechanisms like compression of double layer, interparticle bridging or charge neutralization were not responsible for the coagulation by MOC-SC-PC.

KEYWORDS

Bivalent cation; coagulation; *Moringa oleifera*; natural coagulant; salt extraction

INTRODUCTION

Moringa oleifera is a tropical plant belonging to the family of *Moringaceae* which is cultivated across the tropical belt. It is known that *M. oleifera* seeds contain a coagulation active compornent, the water extractant is well known as a natural coagulant (*M. oleifera* coagulant : MOC). Many studies (Schulz and Okun, 1983; Olsen, 1987; Jahn, 1988; Muyibi and Okuofu, 1995; Ndabigengesere *et al.*, 1995; Muyibi and Evison, 1996) have been carried out to clarify the performance of MOC extracted with distilled water (MOC-DW) as an alternative coagulant or coagulant aid for water and wastewater treatments. Although MOC-DW has high coagulation activity, it is effective only for high turbid water (Muyibi and Evison, 1995). Another disadvantage of MOC-DW for water treatment is to increase dissolved organic carbon (DOC) in the treated water because DOC is regarded as a source of odor, color and taste, and a precursor of disinfection by-products in drinking water treatment. These disadvantages as a coagulant prevent MOC-DW from application for drinking water treatment (Babcock, 1979; Bull, 1991; Ndabigengesere, 1998; Rook., 1974).

In our previous study (Okuda, 1999), the extraction of coagulation active component from *M. oleifera* seeds using salt solution was found to be more efficient than the conventional method using water. The coagulation capacity of MOC extracted with 1M NaCl solution (MOC-SC) was 7.4 times higher than that of MOC-DW for the removal of suspended kaolin at low turbid sample (50 mg-kaolin l^{-1}). It seemed that the improvement in extraction efficiency by salt was ascribed to the salting-in mechanism, that is, the increase in ionic strength by salt caused the increase in solubility of the active components (White *et al.*,

1968; Voet and Voet, 1990). The coagulation active component have been purified from MOC-SC (Okuda, submitted). The purified coagulant (MOC-SC-PC) from MOC-SC did not increase DOC after coagulation. And MOC-SC-PC was effective even for low turbid water such as 5 mg-kaolin l^{-1} (about 3.5 NTU). These performance of MOC-SC-PC would be suitable for drinking water and other low turbid water treatments. In addition, the active component of MOC-SC was not the same as that of MOC-DW. Molecular weight of the active component of MOC-SC was about 3 kDa, while that of MOC-DW was about 12-14 kDa, and the active component of MOC-SC was not protein such as MOC-DW. And more, the active component of MOC-DW dissolves in deionized water, on the other hand, that of MOC-SC dose not dissolve in deionized water. (Okuda, submitted)

Coagulation mechanism of MOC-DW was reported to be adsorption and neutralization of charges or adsorption and bridging of destabilized particles (Ndabigengesere, 1995). The coagulation mechanism of MOC-SC-PC may be different from that of MOC-DW because its active components is different. The difference may lead to the high coagulation efficiency of MOC-SC-PC for low turbid water. The coagulation mechanism will give us the information about the optimum and practical conditions for coagulation using MOC-SC-PC. The objective of this study is to clarify the coagulation mechanism of the MOC-SC-PC.

MATERIALS AND METHODS

Extraction of active component from *M. oleifera* seed

M. oleifera seeds in dry pods were obtained from Los Baños, Laguna, the Philippines in May 1998. The seeds were removed from the pods and were stored with winged seed covers in our laboratory at room temperature. The winged seed cover was shelled and the kernel was ground to fine powder by using a mortar and pestle just before extraction. Ten grams of seed powder was suspended in 1 liter of 1.0 M NaCl solution and the suspension was stirred using a magnetic stirrer for 10 minutes to extract

coagulation active component. The suspension was then filtered through a filter paper (quantitative ashless; ADVANTEC, 5A) with about 7 μm of pore size. This filtrate is referred to as MOC-SC, and *M. oleifera* coagulant extracted by distilled water instead of 1.0 M NaCl solution in the above procedure is referred to as MOC-DW.

Purification of active component

The coagulation active component in MOC-SC was purified as follows. NaCl in MOC-SC was removed by seamless cellulose dialysis tubing with molecular weight cutoff of 12-14 kDa. As white precipitate was produced in the tube after dialysis, the precipitate was collected by centrifugation at 5,000 rpm for 20 min and was rinsed with deionized water. The rinsed precipitate was suspended in cold acetone and was homogenized using homogenizer (Digital Homogenizer, Iuch) for delipification (White and Smith, 1968). The white precipitate was recovered from cold acetone by centrifugation at 3,500 rpm for 30 min and was rinsed with cold acetone. The precipitate was dissolved into 0.1M ammonium buffer (NH_4Cl-NH_3) at pH 10.5. Insoluble matter in the buffer was removed by centrifugation at 3,500 rpm for 30 min. The supernatant was applied to an anion exchanger column (25 mm I.D. x 30 mm height, packed with 12.2 ml of Amberlite IRA-900) equilibrated with 0.1 M ammonia buffer at pH 10.5. Active component was eluted by increasing ionic strength from 0.15 to 0.25 M with NaCl in the ammonium buffer. The eluate with coagulation active component is referred to as MOC-SC-PC. The details of the purification method were reported in the previous paper (Okuda, submitted).

Preparation of synthetic turbid water

Synthetic turbid water for coagulation tests was prepared by adding kaolin into tap water. Ten grams of kaolin (CP grade, Katayama Chemical) was added into 1 liter of tap water. The suspension was stirred for 1 hour to have uniform dispersion of kaolin particle, then it was stood for 24 hours to allow complete hydration of the particle. Synthetic turbid water with 50 mg l^{-1} kaolin (about 35 NTU) was prepared by

dilution of 1 ml suspension to 200 ml using tap water just before coagulation test.

The effects of inorganic salt on coagulation were evaluated by using inorganic salt solution into distilled water instead of tap water. Inorganic salts studied were KCl, NaCl, NH_4Cl, $MgCl_2$, $CaCl_2$, $BaCl_2$, $MnCl_2$, $ZnSO_4$, $Ca(NO_3)_2$, $Ca(C_9H_{10}NO_3)_2$ and Na_2SO_4. All of these chemicals with chemical grade or special grade were obtained from Katayama Chemical in Japan.

Coagulation test

Jar test was carried out to evaluate coagulation activity of MOC-SC-PC. The synthetic turbid water (200 ml) was filled into 300 ml-beaker placed on each slot in a jar tester (SUGIYAMAGEN : NT-6). MOC-SC-PC was added into each beaker at various doses and was agitated at 150 rpm for 2 minutes. Then mixing speed was reduced to 30 rpm and was kept for 30 minutes. These coagulation parameters were similar to the optimum condition reported in the paper by Ndabigengesere *et al.* (1996). The coagulation pH was kept at 9 by adding ammonia solution in all coagulation tests, which is slightly higher than the optimum pH of 8 in our previous study (Okuda, submitted). After sedimentation for 1 hour, an aliquot of 5 ml was sampled from the mid depth of the beaker and residual turbidity (RTsample) was determined. The same coagulation test was conducted with no coagulant as a control. The residual turbidity in the control was defined as RTblank.. Coagulation activity was calculated based on Lee's equation (Lee *et al.*, 1995) as follows.

$$\text{Coagulation Activity} = (\text{RTblank} - \text{RTsample}) / \text{RTblank}$$

Analytical methods

All of the analytical methods are following to the Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF, 1989). Turbidity was measured using a turbidimeter (ANA-148,

Tokyo Photoelectric). pH was determined using a pH meter (F-8, Horiba). Total dissolved organic carbon (DOC) was determined using a Total Organic Carbon Analyzer (TOC-500 and TOC-5000, Shimadzu). Zeta potential was measured with a particle electrophoresis apparatus (mode1 Mark II, Rank Brothers).

RESULTS AND DISCUSSION

Formation of insoluble matters

Figure 1 shows solubility of the white precipitate formed after the delipification step during purification of MOC-SC. The white precipitate was added into NaCl solutions to determin the solubility of the precipitate, it was determined by DOC after the removal of insoluble precipitate at each NaCl concentrations. The coagulation activity was also calculated at optimum dosage of each extractants using same method as coagulation test for MOC-SC-PC. Solubility of the white precipitate and coagulation activity of extractants were almost zero without NaCl and increased with the increase in the concentration of NaCl. It is clear that the active component does not dissolve into water without NaCl or other salts.

When MOC-SC-PC was added into tap water with no kaolin, insoluble matters (soft and white precipitate) appeared in it. The insoluble matters must be the active component for coagulation because the active component can not dissolve into water without salts. The salt concentration after addition of MOC-SC-PC into water without salts was too low to dissolve the active component. Stock solution of MOC-SC-PC with 100 mg-C l^{-1} contained approximately 0.3 M NaCl and 0.1 M ammonium. It was diluted to 500 times for optimum dose to give 0.6 mM NaCl and 0.2 mM ammonium buffer which was not enough to dissolve the active component.

Figure 2 shows the formation of insoluble matters as a function of MOC-SC-PC dose and corresponding coagulation activity. The dose was impressed as final carbon concentration of MOC-SC-PC in tap water after addition of MOC-SC-PC, the amount of insoluble matters was defined as turbidity caused by the

insoluble matters after addition of MOC-SC-PC into tap water without kaolin and mixing at 30 rpm for 30 minutes (no sedimentation). The turbidity increased with the increase in MOC-SC-PC dose and resulted in the increase in coagulation activity. Figure 3 shows the relationship between the formation of insoluble matters and coagulation activity as affected by pH. The insoluble matters appeared in the higher pH than 8 with corresponding coagulation activity.

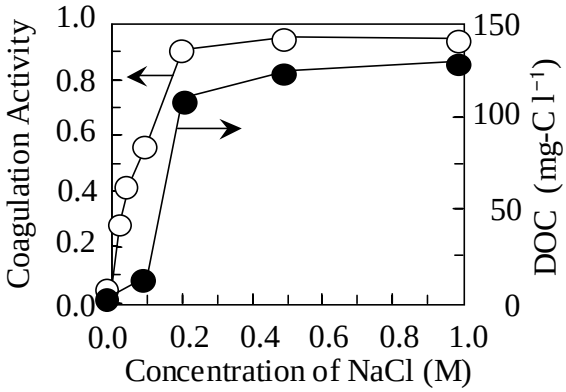

Figure 1. Relationship between concentration of NaCl and solubility (DOC) and coagulation activity of white precipitates after dialysis of MOC-SC.

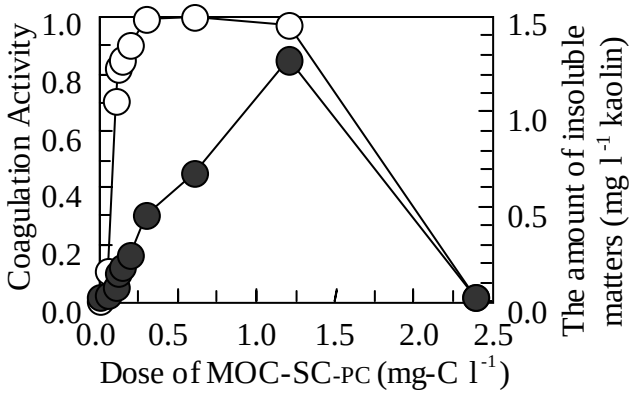

Figure 2. Formation of insoluble matter in tap water and coagulation activity as a function of MOC-SC-PC dose.

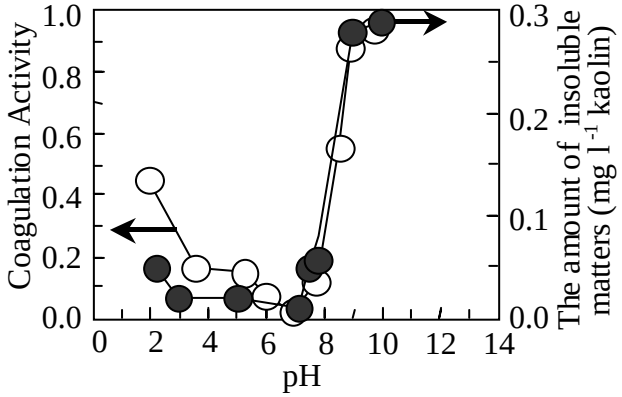

Figure 3. Formation of insoluble matter and coagulation activity of MOC-SC-PC as a function of pH. (Dose = 0.2 mg-C l^{-1})

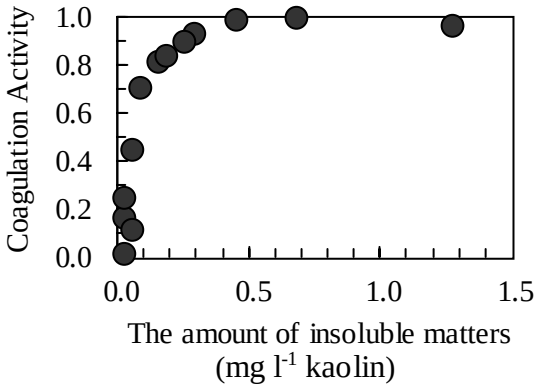

Figure 4. Correlation between coagulation activity and the formation of insoluble matters.

The correlation between coagulation activity and the formation of insoluble matters is summarized in Figure 4 from the data used in Figure 2 and 3. Increase in the amount of insoluble matters resulted in the increase in coagulation activity, which suggests that the formation of insoluble matter is responsible for the coagulation of kaolin.

Effect of salt on the formation of insoluble matter

The insoluble matters were not formed in distilled water instead of tap water without kaolin. The formation may result from an association between the active component in MOC-SC-PC and some ions in tap water. Figure 5 shows the coagulation activity at 0.3 mg-C l^{-1} dose of MOC-SC-PC in various 0.4 mM salt solutions with distilled water. MOC-SC-PC showed high coagulation activity in $MgCl_2$, $CaCl_2$ and $BaCl_2$ solutions and low activity in KCl, NaCl and NH_4Cl solutions. The coagulation activity was remarkable only in solutions with bivalent cations like Mg^{2+} Ca^{2+} and Ba^{2+}. The activity did not depend on anions like Cl^-. The result was confirmed by experiments using other salt solutions like $MnCl_2$, $ZnSO_4$, $Ca(NO_3)_2$, $Ca(C_9H_{10}NO_3)_2$ and Na_2SO_4 (Data is not shown).

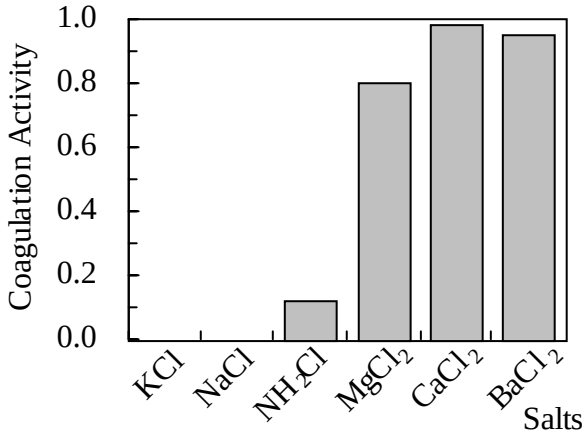

Figure 5. Coagulation activity of MOC-SC-PC with various salts. (Dose = 0.3 mg-C l^{-1}, salt concentration = 0.4 mM)

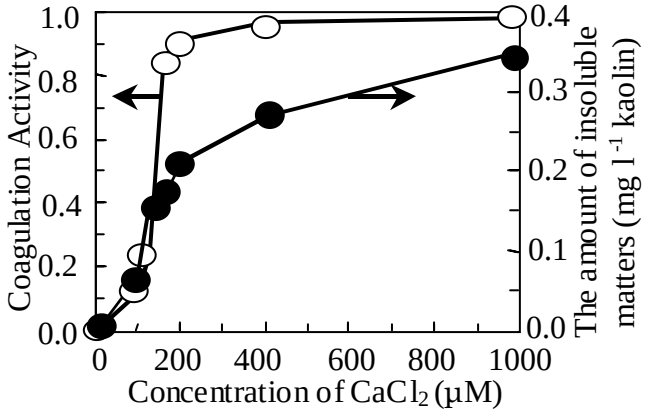

Figure 6. The formation of insoluble matter and coagulation activity of MOC-SC-PC as a function of Ca^{2+} concentration. (Dose = 0.3 mg-C l^{-1})

Figure 6 shows the coagulation activity and the formation of insoluble matters as affected by the concentration of $CaCl_2$ with distilled water at 0.3 mg-C l^{-1} dose of MOC-SC-PC. The formation of insoluble matters increased with the increase in $CaCl_2$ concentration and resulted in the increase of coagulation activity, indicating that the amount of insoluble matter was affected by Ca^{2+} concentration. At least 0.2 mM of Ca^{2+} (bivalent cations) was necessary for coagulation at 0.3 mg-C l^{-1} dose of MOC-SC-PC.

Coagulation mechanism on MOC-SC-PC

Coagulation active component in MOC-SC-PC is supposed to form netlike structure in tap water as illustrated in Figure 7 (b). It removes suspended solids in water by sweep coagulation mechanism, that is, enmeshment of suspended solids like kaolin by the netlike structure (AWWA,1990; Packham, 1965). The bivalent cations might be electrically adsorbed to the active component with negative charge at coagulation pH. The active component might be connected with other component by bivalent cation, which may form the insoluble matter with netlike structure (Figure 7 (a) (b)). On the other hand, monovalent cation does not let two active components connect because it has just one valence. The shortage of bivalent cations to active components may inhibit the formation of netlike structure as shown in Figure 7 (c) resulting in the disappearance of insoluble matters at the high dose of MOC-SC-PC (2.4 mg-C l^{-1}) in Figure 2. In fact, the addition of Ca^{2+} into tap water with MOC-SC-PC at 2.4 mg-C l^{-1} dose resulted in the high coagulation activity.

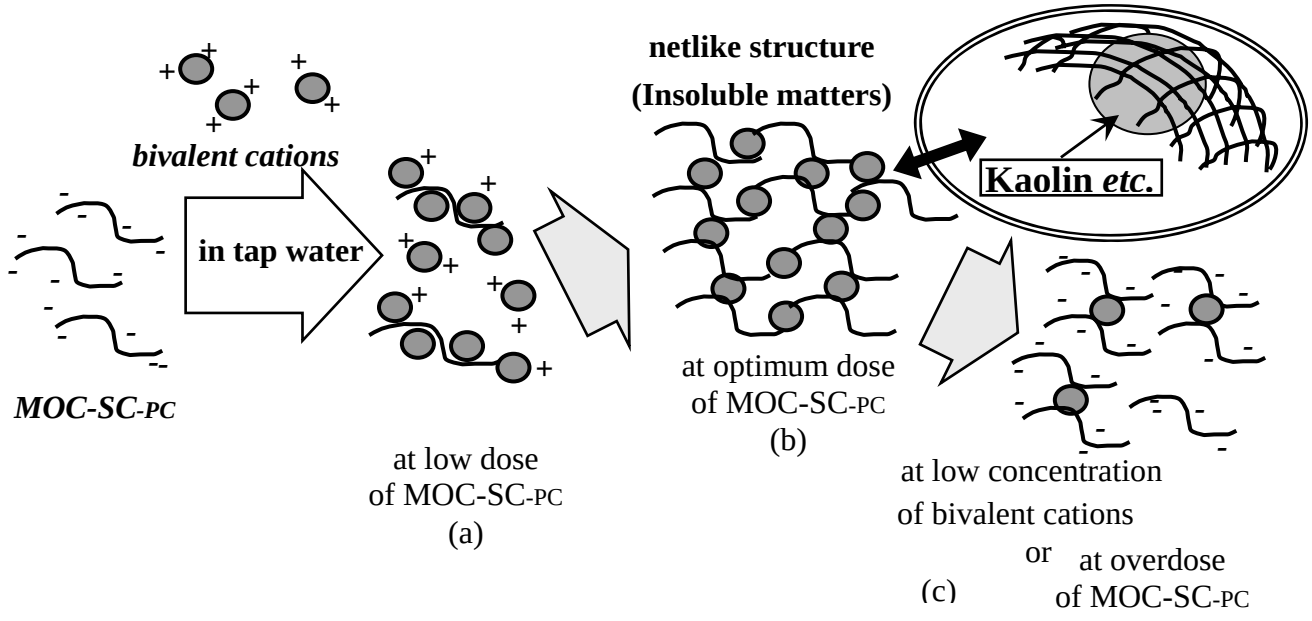

Figure 7. The model for the structures of coagulation active component in MOC-SC-PC.

Figure 8 shows coagulation activity and zeta potential of kaolin particles at pH 9 as a function of MOC-SC-PC dose. There was a slight increase in zeta potential with the increase in MOC-SC-PC dose.

However, zeta potential remained negative at around -25 mV even at the optimum dosage. This value is still too low to make coagulation by the charge neutralization.(Ndabigengesere etc., 1995) Predominant mechanism for kaolin coagulation by MOC-SC-PC, therefore, is not charge neutralization (AWWA,1990; Ndabigengesere etc., 1995). Other coagulation mechanism is the compression of the double layer in kaolin particles with negative charge or the adsorption between kaolin particles and the coagulation active component in MOC-SC-PC to permit interparticle bridging (AWWA, 1990). The former mechanism is known to be effective by the increases in cations. The coagulation active component, however, has negative charge at coagulation pH (9.0). Therefore, it is not likely to occur. The latter mechanism normally functions by the material with high molecular weight like 1,000 kDa (AWWA,1990; Gregory, 1978). It is not likely for the active component in MOC-SC-PC with low molecule weight (3 kDa) to induce interparticle bridging.

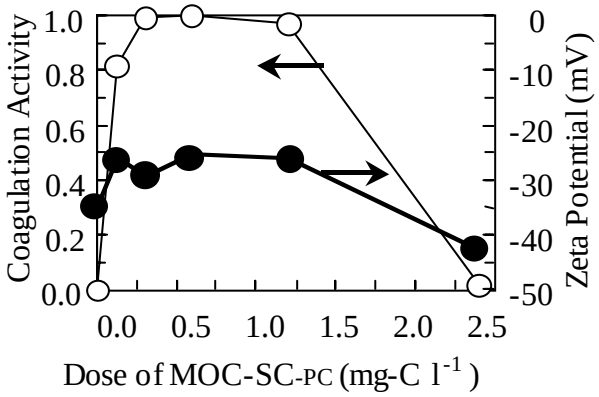

Figure 8. Zeta potential of kaolin particle and coagulation activity as a function of MOC-SC-PC dose.

CONCLUSIONS

The purpose of this study was to clarify the coagulation mechanism of MOC-SC-PC. The specific conclusions derived from this study are as follows:

1) The coagulation mechanism of MOC-SC-PC seemed to be an enmeshment by the insoluble matters formed by the coagulation active component in MOC-SC-PC. Other coagulation mechanisms

like compression of double layer, interparticle bridging or charge neutralization were not responsible for the coagulation by MOC-SC-PC.

2) The formation of insoluble matters was affected by Ca^{2+} or other bivalent cations which may connect each molecule of active component in MOC-SC-PC and form netlike structure.

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