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43-kDa coagulant active protein from moringa oleifera seed: Isolation, purification, characterization and evaluation their turbidity removal efficiency

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ABSTRACT

Moringa Oleifera seed contain admirable coagulation properties for the water clarification. In presents study coagulation active compound have been isolated and purified, and was characterized by SDS-PAGE method. The isolated active agent from Moringa Oleifera is cationic protein, have 43-kDa molecular weight. The coagulation/flocculation activity of protein was examined on clay suspension (model turbid water). Comparison study was performed on jar test apparatus between the coagulant active protein and alum using model turbid water. The dosage of coagulant active protein was optimized and showed better coagulation efficiency compared to alum for turbidity removal.

KEYWORDS

Moringa Oleifera;
SDS-PAGE;
43-kDa;
Clay suspension;
Coagulant active protein;
Coagulation/Flocculation.

INTRODUCTION

Coagulation/flocculation followed by sedimentation, filtration and disinfection is used in water treatment. Turbidity removal is an important step in water treatment, achieved by coagulants. Many coagulants are widely used in water treatment, based on their chemical characteristics. Coagulants are classified mainly in inorganic, composite of inorganic, synthetic organic polymer and natural coagulants. Primary coagulants are based on aluminium and iron (III) salts. Aluminum salts namely alum and polyaluminum chloride (PACl) are the most common synthetic chemical coagulants used in water and wastewater treatment[1-8].

Before introduced the synthetic chemical coagulants natural coagulants were used. It is obtained from microorganisms, animals, plants, vegetables and derivatives of the mineral origins. Natural coagulants are economically and environmentally more acceptable than chemical coagulants[1]. Along with plant materials, Moringa oleifeira has shows good efficiency as primary coagulants on water clarification belonging to the family of Moringaceae have 14 species[1-4, 6]. The plant is non-toxic and contain natural organic polymer, due to this reason, it is suggested as coagulant on small scale in developing countries[1, 6].

In Moringa Oleifera the seed kernels of Moringa Oleifera contain major quantities of the water-soluble

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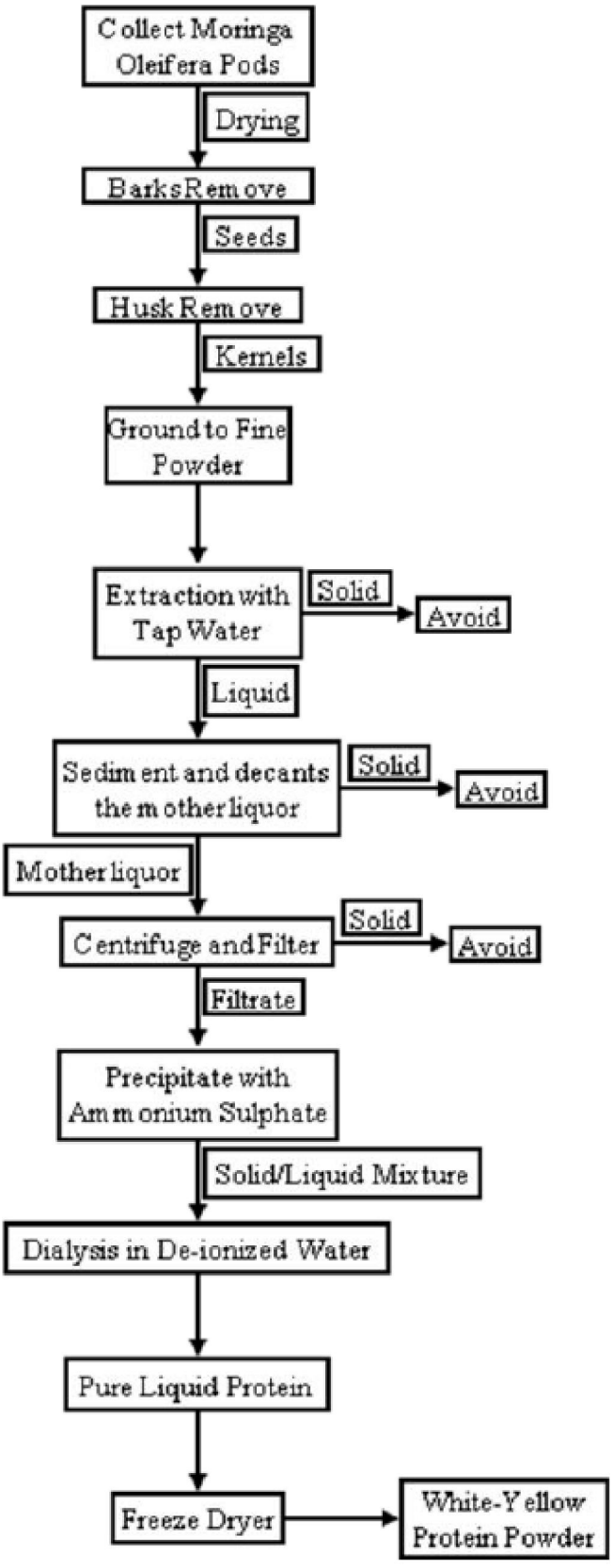
proteins have positive charge and acts as a natural cationic polyelectrolyte during treatment. This protein has been proposed to bind the predominantly negatively charged particles. Mechanisms of coagulation with protein consists adsorption and charge neutralization of the colloidal charges[6, 9]. Active agent was purified and characterized using different techniques namely; dialysis, ultra-filtration, lyophilisation, ion exchange, chemical precipitation and electrophoresis[1, 3-10].

Presents studies is focused on isolation and purification of the coagulant active protein from dry Moringa Oleifera seed kernel and characterized by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique. The efficiency assay of coagulant active compound was tested on clay suspension (model turbid water) and compared with alum.

MATERIALS AND METHODS

Materials

Moringa Oleifera was collected from surrounding area of Bhavnagar, district of Gujarat, India for extract the coagulant protein. The protein was extracted by tap water obtained from institute. Alum was used in the present study as chemical coagulant for comparisons studies only (AR grade, S. D. Fine Chemicals, Mumbai, India). Barium chloride (AR grade) purchased from Ranbaxy, India. Ammonium persulphate, N, N methylenebisacrylamide, Sodium dodecyl sulfate, Glycerol and Bromophenol Blue (Molecular Biology grade, S. D. Fine Chemicals, Mumbai, India). Coomassie Brilliant Blue R-250 (Himedia Laboratory, India). β-Mercaptoethenol (Accros), Tris base (AR grade, SRL, India), Acrylamide and TEMED (Molecular grade, Spectrchem, India), Tris glycine and Methenol (AR grade, Fisher Scientific) for SDS-PAGE to determine the molecular weight of the active agent. Dialysis tube from Sigma Aldrich, USA for purifies the coagulant active protein. Clay (Bentonite) was collected from Barmer, district of Rajasthan, India for prepared clay suspension. To prepare the solutions only de-ionized (Milli-Q) water was used obtained from Milli-Q Gradient A-10 water purification system.



Schematic 1 : Block diagram of protein extraction and purification.

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Isolation and purification of coagulant active compound

The pods of Moringa Oleifera were washed to remove impurities which stick on it by tap water and drying in open space at atmosphere temperature for 3-4 days. The seeds were extracted from pods and dried at atmosphere temperature till it appeared brow. The kernels were extracted from husk and ground to fine powder with domestic blender (Maharaja Mixture Grinder, India). To obtain uniform size of powder it was passed from 250-micron mesh. The kernel powder was used for extracted the active agent (Schematic 1). 2.0% w/v of kernel powder was added to tap water and allowed the crude for stirring under 400 rpm on magnetic stirrer (Schott, Germany) for 60 min at room temperature. Solution was sediment for 30 min and decanted the supernatant solution contained coagulant active compound. Centrifuge the mother liquor under 9000 rpm for 120 min at 8 ºC (Kubota 6500, Japan). Filter the supernatant solution thorough whatman filter paper No. 3. The filtrate contained coagulant active protein, was stored at 4 ºC in refrigerator before purified (Schematic 1). To precipitate the protein, ammonium sulphate was added in filtrate solution till saturation reached 100%. Solution was transferred to dialysis tube (7014 M.W., Size: 23 mm x 15 mm) and washed till 100% sulphate removed. The active protein was remaining inside the tube as solution. The solution was Freeze-dried in freeze dryer (Heto FD3, Denmark). Final product was water soluble white yellow fine powder stored at 4 ºC in refrigerator, used for characterization and coagulation studies.

Analytical methods

Molecular weight and purity of the protein were analyzed by standard SDS-PAGE method (12% polyacrylamide gel) (Bangalore Genei, Bangalore, India) and quantitatively estimation of protein was carried out by using Bradford assay (Bio-Rad). Turbidity and pH of the water was measured on TN-100 Eutech turbidity meter, Singapore and pH meter from Toshniwal Instruments Ajmer, India.

Characterization of coagulant active protein

Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) (12%) electrophoresis (Vertical Dual Mini Gel System, Bangalore Genei, Bangalore, India) under reducing condition was done as described by sambrook et al.[11]. Relative molecular weight was determined by performing the SDS-PAGE of protein with molecular weight standards under reducing condition. Molecular weight of isolated active agents was determined using protein molecular weight marker (6-16% Gradient PMWB, Bangalore Genei, Bangalore, India). The proteins were detected by staining the gel with 0.1% Coomassie brilliant blue R-250[7].

Model turbid water

Coagulation experiments were conducted using model turbid water prepared by reported method[12]. The typical analysis of model turbid water was 165 NTU (initial turbidity) and pH 6.83 (initial pH).

Coagulant activity assay

Jar test apparatus (Pooja Scientific Instruments, New Delhi, India) is widely used in coagulation/flocculation process. This study consists of batch experiments involving rapid mixing, slow mixing and sedimentation. All the coagulation experiments were conducted in 1000 ml glass graduated beaker using a square jar test apparatus. For each experiments, known amount of coagulant dosages were added to beaker containing clay suspension. Water sample was first stirred rapidly at 140 rpm for 1 min and then at a slow speed at 40 rpm for 8 min followed by sedimentation for 30 min after dosing and supernatant water used to measure final turbidity and pH.

RESULTS AND DISCUSSIONS

Molecular weight determination by SDS-PAGE

To isolate the active agent from Moringa Oleifera, ammonium sulphate was used to precipitate the protein[1, 13]. Active agents from Moringa Oleifera are responsible of water treatment. Maikokera et al.[10, 14] studied of interaction of the coagulant protein with an anionic surfactant SDS reported that the active agents are water-soluble proteins of molecular weights between 6000 and 16,000.

Coagulant active agent was isolated from Moring Oligofera seed kernel by molecular sieving method (SDS-PAGE) under reducing condition. The picture of

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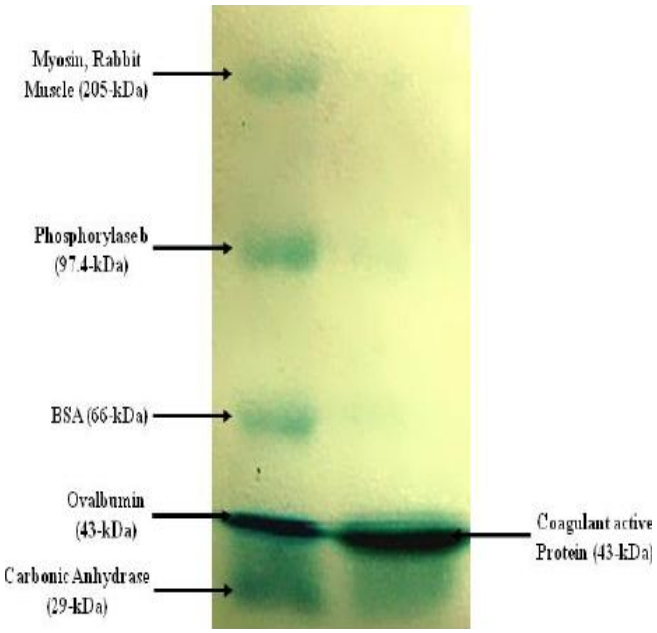
the gel was showed the migration of a sample with molecular weight standards ranging from 29 to 205-kDa namely Carbonic Anhydrase 29-kDa, Ovalbumin 43-kDa, Bovin Serum Albumin 66-kDa, Phosphorylase b 97.4-kDa and Myosin, Rabbi Muscle 205-kDa (Schematic 2). These results shows that isolated protein was monomeric of 43-kDa and monomers retain their coagulant properties.



Schematic 2 : SDS-PAGE.

Performance of polymer on turbidity removal with pH

Turbidity removal is main aim of this study through coagulation/flocculation by coagulant active compound (protein) and efficiency of the protein compared with alum. For these experiments, model turbid water was used has 165 NTU turbidity and pH 6.83. Mechanism of the turbidity removal is strongly related with pH value[15]. Final pH of the treated solution was acidic and maximum turbidity removed at the neutral or acidic pH region[16]. Changes in residual turbidity and pH of water samples after dosing were investigated in details. Dosage of the protein was varied in step to see the efficiency for turbidity removal with pH. The pH and turbidity removal study was investigated in detail showed in Figure 1.

Influence of protein as coagulant and compare with alum

Dosage of protein was varied to check its efficiency for turbidity removal. If turbidity was removed near about 80 to 90% after dosing and sedimentation; in this case, coagulation activity was present. Whereas, result was absent if the turbidity has unable to removed more than 30%[1]. Efficiency of coagulant was increased when dosage of the coagulant increased[15]. Figure 1 shows the effect of protein on turbidity removal with varying dosage at fixed turbidity. At 5 and 15 mg/L dosage of the protein, flocks was unable to settled because of its amount was fewer and compact. It might be due to the increased of pH at earlier dosage. However, increased the dosage of protein turbidity was reduced simultaneously. Because of pH of the treated water was reduced[16]. From the results, at 25-95 mg/L of the protein turbidity was reduced from 39-98% at the range of

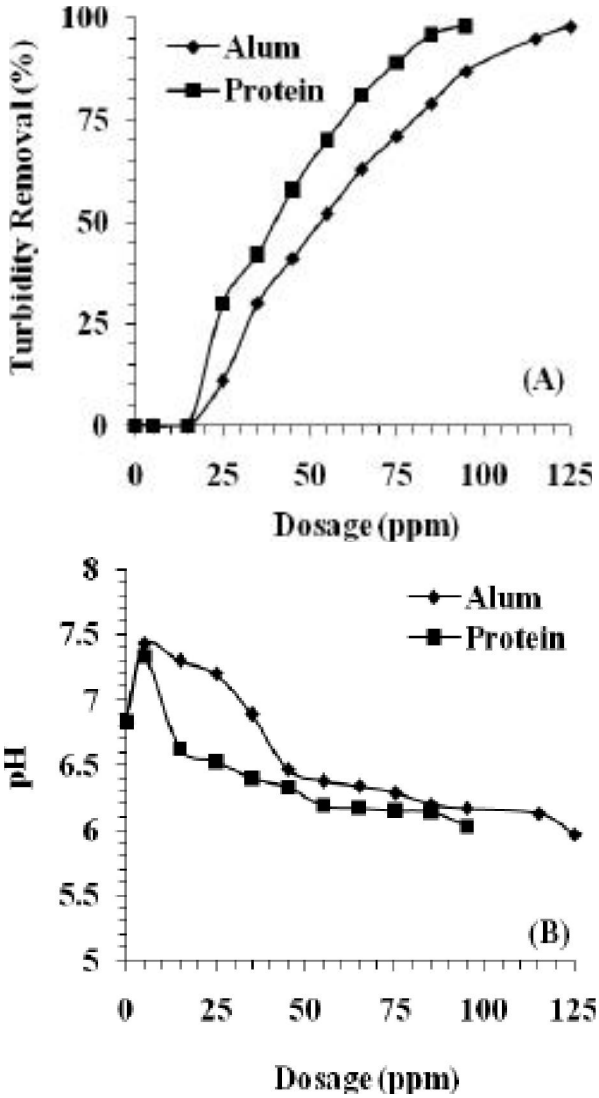


Figure 1 : Turbidity removal efficiency of alum and coagulant active protein.

pH 6.63-6.03 respectively (Figure 1). Results showed that the protein was effective in the range of the pH 7.33-6.05. Results showed that turbidity reduced in acidic pH and sufficient dosage was 95 mg/L for turbidity removal (165 NTU) (Figure 1).

Efficiency of alum was examined with similar model turbid water (165 NTU) described earlier. Dosage of alum was varied to check its efficiency on fixed turbid water. Whereas alum applied on model turbid water in range of 5-125 mg/L. Similar trend was observed with alum applied to turbid water up to 15 mg/L, the residual turbidity remains as such. Turbidity was decreased when dosage of alum increased simultaneously reducing curve followed the trend like protein with range of pH 7.2-5.97 (Figure 1).

While comparing the results it was showed to remove turbidity alum required ~ 25 ppm more dosage than protein (Figure 1).

CONCLUSION

Coagulant active agent was successfully isolated and purified from Moringa Oleifera dry seed kernel, and was characterized by SDS-PAGE has 43-kDa protein. Protein is more effective coagulants than alum and removed up to 99% of turbidity from model turbid water and required ~ 25% less amount of protein for turbidity removal as compared to alum.

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