Toward Understanding the Efficacy and Mechanism of *Opuntia* spp. as a Natural Coagulant for Potential Application in Water Treatment

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Historically, there is evidence to suggest that communities in the developing world have used plant-based materials as one strategy for purifying drinking water. In this study, the coagulant properties of *Opuntia* spp., a species of cactus, are quantitatively evaluated for the first time. *Opuntia* spp. was evaluated for turbidity removal from synthetic water samples, and steps were made toward elucidating the underlying coagulation mechanism. In model turbid water using kaolin clay particles at pH 10, *Opuntia* spp. reduced turbidity by 98% for a range of initial turbidities. This is similar to the observed coagulation activities previously described for *Moringa oleifera*, a widely studied natural coagulant. Although it has been reported that *Moringa oleifera* predominantly operates through charge neutralization, comparison of zeta potential measurements and transmission electron microscopy images of flocs formed by *Opuntia* spp. suggest that these natural coagulants operate through different mechanisms. It is suggested that *Opuntia* spp. operates predominantly through a bridging coagulation mechanism. Once optimized, application of these readily available plants as a part of point-of-use water treatment technology may offer a practical, inexpensive, and appropriate solution for producing potable water in some developing communities.

Introduction

Waterborne diseases are among the leading causes of morbidity and mortality in developing countries (*1*). Preventable disease burden in these countries can be mitigated with improvements in water quality, sanitation, and hygiene (*2, 3*). In the near term, it is unlikely that the 1.1 billion people without access to an improved water supply will receive clean, piped water from a community treatment plant (*4*). Installation of centralized water treatment can be hindered by high fixed costs and difficulty in operating and maintaining systems, particularly for rural and peri-urban communities (*2*). Point-of-use (POU) technologies that are simple, inexpensive, and can be applied at the household level hold promise for improving water quality and for reducing associated negative health effects, such as diarrheal disease, dehydration, and death (*3*).

Drinking water treatment typically includes coagulation, sedimentation, filtration, and disinfection. Coagulation is a critical step in water treatment processes not only because it removes particles but because it is also removing the microorganisms that are often attached to the particles (*5*). That is, by removing turbidity, coagulants also have the potential to remove pathogens and to significantly improve water quality and, subsequently, human health.

Aluminum sulfate (alum), a common coagulant globally used in water and wastewater treatment, can achieve 90–99% microbial removal under optimal conditions (*6*). However, alum produces large sludge volumes (*7*), reacts with natural alkalinity present in the water, leading to pH reduction (*8*), and demonstrates low coagulation efficiency in cold waters (*6*). In addition, alum has raised a number of concerns including (1) ecotoxicological impacts when introduced into the environment as post-treatment sludge, (2) impacts on human health as a result of consumption in finish water, and (3) the cost of importing these chemicals for developing communities (*9*). Furthermore, optimal implementation of alum requires technical skill and training (*4*). For these reasons, there is a need to design and develop appropriate POU treatment technologies for developing communities. One component of this may be alternative coagulants that are less expensive, inherently benign, renewable, locally available, and readily implementable.

The combination of the concerns with alum and the strong push to meet the drinking water needs of the developing world have led to the recent growing interest in using plant-based natural coagulants in both the developed and developing world (*10–14*). There are reports in the literature (*8–11, 13, 15–18*) and anecdotal accounts from diverse geographic regions of plant-based materials that can effectively remove turbidity from water with little processing. If proven technically robust, these natural coagulants, such as *Opuntia* spp. and *Moringa oleifera* (*M. oleifera*), may offer part of an appropriate approach for water treatment at the household level. Because this technology relies on local materials and local labor, renewable resources, and food grade plant materials and is relatively inexpensive, it can contribute to advancing the goal of sustainable water treatment technologies that are themselves sustainable.

Given the potential of natural coagulants to be part of an appropriate solution to producing potable water, a remarkable number and variety of natural substances have been examined for their coagulation properties. They range from the more widely known seeds of different plant species to bone shell extracts, bark resins, ashes, exoskeleton of shellfish extracts, and natural mineral soils (*19*). The most studied of the natural coagulants is *M. oleifera*, whose efficacy has been reported for turbidity removal (*8, 9, 13, 14, 17, 20–22*) as well as antimicrobial properties (*19, 23*). Analogous reports for *Opuntia* spp. are scarce. *Opuntia* spp. has low water intensity (*24*) and grows in arid, water scarce regions where *M. oleifera* is not found indigenously. The coagulation mechanism and active agent of *M. oleifera* have been discussed in the literature, but the coagulation mechanism and active agent(s) of *Opuntia* spp. have not previously been reported.

This study systematically evaluated the coagulation performance and mechanism of *Opuntia* spp. Through

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examination of variables such as initial turbidity, pH, zeta potential, and floc structure, the coagulant properties of *Opuntia* spp. were compared to that of *M. oleifera*. Possible active coagulant components of *Opuntia* spp. were investigated through experimentation with dissected parts of the plant and with compounds naturally found in the plant.

Background

Candidate Natural Coagulants Studied. *Opuntia* spp., commonly called nopal (Mexico), prickly pear, or cactus leaf (USA) (*25*), grows readily in Mexico, Texas, and other arid and semiarid regions (*26, 27*). Its uses are varied, ranging from paint additive to food source (*26, 27*). There is little information in the literature evaluating its use as a water clarifier, although it has anecdotally been reported.

It is well-known that water quality parameters, such as pH, electrolyte background, natural organic matter concentration, and temperature can significantly affect the optimal performance of various coagulants, both synthetic (*21*) and natural (*9, 21*). As such, it is imperative to consider varying water quality parameters on the potential effectiveness of *Opuntia* spp. as a coagulant to inform optimization of its coagulation behavior. Given that *Opuntia* spp. is commonly eaten (*24*) and is used for medicinal purposes (*28*), there are likely to be few, if any, adverse health effects associated with the residual levels found in finish water, but this would need to be confirmed prior to field testing or implementation.

M. oleifera, a tree that grows throughout the tropics and subtropics (*29*), produces a seed with coagulant properties that has been well studied. Previous studies reported similar turbidity removal efficiencies in comparison with alum, under certain conditions (*9, 12, 13, 16, 17, 20, 30*). Ndabigengesere et al. (*9*) observed that *M. oleifera* performance is mostly unaffected by changes in cation or anion concentration, produces less sludge than alum (*8*), and does not affect the pH of finish waters (*9*). Evidence suggests that *M. oleifera* is nontoxic (*30, 31*) and may even have antimicrobial properties (*19, 23*).

The chemical composition of the active coagulating agent of *M. oleifera* has been debated. Several researchers have described the active component from a water extract as cationic and proteinaceous with an isoelectric point around 10 (*9, 32*). Okuda et al. (*22*) find the active agent from a salt extraction to be a 3 kDa polyelectrolyte that is neither protein nor polysaccharide. However, Ghebremichael et al. (*32*) determine that both the water and salt extract are cationic proteins with molecular weights less than 6.5 kDa. Researchers have suggested the coagulation mechanism for the water-extracted active agent is adsorption and charge neutralization (*9, 33*).

Coagulation Mechanisms. Particles can aggregate and settle out of solution through four basic mechanisms: double layer compression, sweep flocculation, adsorption and charge neutralization, and adsorption and interparticle bridging (*34, 35*). The presence of salts can cause compression of the double layer, resulting in destabilization of particles whereby repulsive electrostatic interactions are overcome by attractive van der Waals forces. Sweep flocculation, or enmeshment in the precipitate, occurs when precipitating coagulant traps suspended particles within a colloidal floc as it forms or settles (*35–37*). Destabilization of particles through charge neutralization can occur when suspended particles in solution sorb to oppositely charged ions. Bridging can occur when a coagulant forms a polymer chain that can attach to multiple particles so that particles are bound to the coagulant and need not contact one another.

Experimental Section

Model Turbid Water. Based on the drinking water composition supplied to a city in Venezuela (*11*), a model drinking

TABLE 1. Coagulation Activity When Various Forms of *Opuntia* spp. are Added to Turbid Water with pH ∼9.8, Where Greater than 80% Coagulation Activity is "Present" and Less than 30% Coagulation Activity is "Absent"

forms of *Opuntia* spp.	coagulation activity
Fresh Pad	
whole pad (bottom half)	present
whole pad (top half)	present
skin	absent
outer pad without skin	present
outer pad with skin	present
inner pad	present
whole pad: macerated	absent
Dry Pad	
whole pad: dried at 80 °C	present
whole pad: dried at 120 °C	absent

water was formulated by adding 16.4 mg/L KCl, 62.5 mg/L NaHCO₃, 20.0 mg/L MgCl₂·6H₂O, and 37.9 mg/L CaCO₃ to deionized water and mixing thoroughly. Model turbid water was prepared by adding ∼2 g/L kaolin (Sigma Aldrich) to this model drinking water solution and mixing thoroughly. After 24 h of settling, the turbid-water supernatant was decanted and was used as a stable stock solution. This stock solution was diluted with model drinking water to achieve the desired turbidity. Where indicated, the desired pH was attained by adding 1 M HCl or 1 M NaOH.

Waters were defined as low (L) turbidity (0–125 NTU), medium (M) turbidity (125–250 NTU), or high (H) turbidity (250–375 NTU).

Natural Coagulant Preparation. *Opuntia* spp. pads were purchased from local markets in New Haven, Connecticut. Pads were rinsed thoroughly with tap water followed by deionized water. Fresh *Opuntia* spp. was used within two days of purchase and was stored in a refrigerator at 4 °C when not in use. Dissections of fresh *Opuntia* spp. pads were performed by hand: skin was peeled from the pad; the outer pad was considered the outer layer of bright green tissue composed of chlorenchyma (*38*), and the inner pad was considered the inner layer of off-white tissue composed of parenchyma (*38*). Maceration was performed on the entire pad using a Hamilton Beach blender. Results with fresh *Opuntia* spp. are reported only in Table 1; all other results are from dry *Opuntia* spp. Dry *Opuntia* spp. was prepared by cutting fresh *Opuntia* spp. into strips 1 cm in width and followed by drying at ∼60 °C for 24 h. Dry *Opuntia* spp. was then ground in a coffee grinder, resulting in particles ∼300 μm in diameter. Ground *Opuntia* spp. was stored in an airtight container in a refrigerator at 4 °C for up to two weeks. *Opuntia* spp. can maintain more than 80% of its efficacy for up to two weeks stored under these conditions.

M. oleifera seeds of Indian origin were purchased from Seedman, an online seed distributor. Seeds were shipped in dry form, having already been removed from the pod. The exact age of the seeds is unknown, but all seeds were similarly sized, between 0.8 and 1.0 cm. On the basis of previous reports in the literature on efficacy (*9*), seeds were deshelled, and only the kernel was used. Kernels were ground in a coffee grinder to particles ∼300 μm in diameter and stored at 25 °C in an airtight container for up to one month. Previous research has found *M. oleifera* can begin to lose coagulation efficiency after one month stored under these conditions (*39*).

Mucilage Components. Mucilage is a gummy substance produced in cells found both in the chlorenchyma and parenchyma that helps cactus to retain water (*26*). Several groups have reported that the mucilage of *Opuntia* spp. is composed of L-arabinose, D-galactose, L-rhamnose, D-xylose, and galacturonic acid, although reported relative proportions
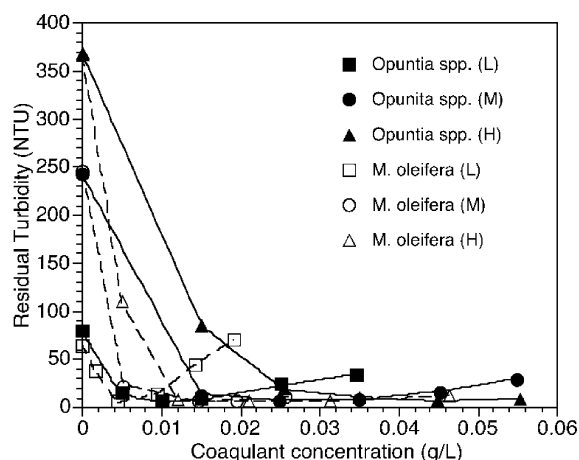
FIGURE 1. Comparison of turbidity removal for coagulants *Opuntia* spp. and *M. oleifera* in waters of low (L), medium (M), and high (H) initial turbidities. For model turbid waters to which *Opuntia* spp. was added, pH was adjusted to ~9.8.

vary (*27, 40, 41*). To quantify the coagulation efficacy of mucilage, individual mucilage components (D,L-arabinose, >99%; D-(+)-galactose, >99%; L-rhamnose, >99%; and D-(+)-galacturonic acid, >97%) were purchased from Sigma Aldrich and were tested independently and in combination.

Batch Test Procedure. Batch tests were performed on a Model CLM6 Compact Laboratory Mixer made by EC Engineering (Alberta, Canada), according to ASTM D 2035–80 (*42*). For both coagulants, rapid mix, ~335 rpm, was conducted for 1 min, followed by a slow mix, ~35 rpm, for 45 min.

This unit contains six 1 L square jars made from clear sheet acrylic. Each jar was filled with 1 L of turbid water with one of the six jars receiving no treatment, serving as a control against which turbidity removal can be compared for all other jars. Coagulation activity was calculated using eq 1 as defined by ref *22*:

$$\text{Coagulation activity} = \frac{(\text{residual turbidity}_{blank} - \text{residual turbidity}_{sample})}{\text{residual turbidity}_{blank}} \quad (1)$$

Analytical Measurements. Turbidity was measured using a calibrated Hach 2100N Turbidimeter to measure the turbidity of the solution in Nephelometric Turbidity Units (NTU). Zeta potential, a measure of the surface charge of a particle, was quantified by phase analysis light scattering (PALS) using a Brookhaven Instruments Corporation Zeta-PALS. To prepare grids for transmission electron microscope (TEM) analysis, a glow-discharged Formvar- and carbon-coated 100-mesh hexagonal nickel grid was inverted upon a 20 μL sample droplet and allowed to settle for 3 min. Then, unstained samples were blotted on filter paper and air-dried. Stained samples were briefly rinsed on a drop of distilled water and then transferred onto a drop of 1% uranyl acetate in water for one minute; the grid was then blotted on filter paper and air-dried. Grids were examined in a Tecnai 12 Biotwin Morada CCD camera (Olympus Soft Imaging Solutions).

Results and Discussion

Efficacy. The results of batch tests for different initial turbidity and each natural coagulant are presented in Figure 1. *Opuntia* spp. and *M. oleifera* are able to significantly reduce turbidity with removal ranging from 92 to 99% for all tests. Residual turbidity levels for water treated with *Opuntia* spp. are between 5 and 7 NTU, regardless of initial turbidity. Residual turbidity levels for waters treated with *M. oleifera* are ~5
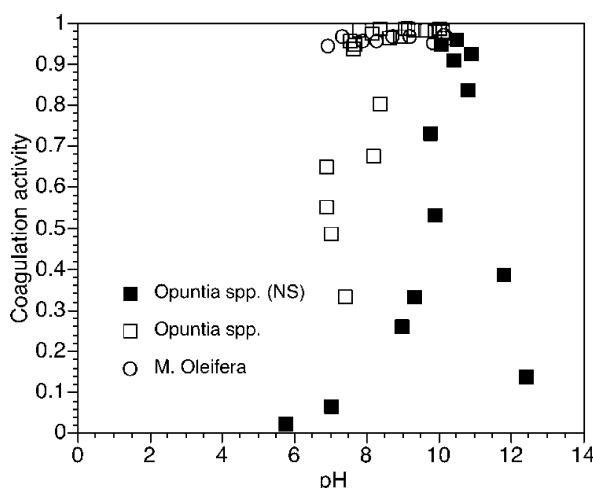

FIGURE 2. Coagulation activity of constant coagulant dose applied to waters of differing pH, with initial turbidities ranging from 162 to 200 NTU. ■: 30 mg/L *Opuntia* spp. and turbid water solution with no salt (NS). □: 20 mg/L *Opuntia* spp. and model turbid water solution. ○: 10 mg/L *M. oleifera* and model turbid water solution.

NTU regardless of initial turbidity, falling within the range reported in the literature (*9, 15, 21*). Each coagulant has an optimal dose that results in the greatest turbidity removal and that varies depending on the water's initial turbidity.

Effectiveness of *M. oleifera* has been examined in natural waters (*8, 15*) and in synthetic clay solutions (*9, 20–22, 32, 33, 39*) absent of natural organic matter (NOM), negatively charged macromolecules (*43*). In the synthetic clay solution used in this study, the optimal dose of *Opuntia* spp. ranges from 5 to 15 mg in low turbidity water, 15–35 mg in medium turbidity water, and 35–55 mg in high turbidity water. Recognizing that coagulant dose can be controlled by levels of NOM, rather than turbidity (*44*), it will be necessary to examine the influence of NOM on turbidity removal by *Opuntia* spp. through coagulation experiments in natural waters where NOM is known to be present or in synthetic turbid waters with NOM added.

There is a relationship between pH and coagulation activity for both coagulants (Figure 2). Data are reported for the ranges over which the synthetic turbid waters were stable with and without background electrolytes at pH 6.9–10.2 and pH 5.8–12.4, respectively. The coagulation activity of *Opuntia* spp. is greatest in basic waters. In the model turbid water containing background electrolytes, *Opuntia* spp. operated with greater than 98% turbidity removal from pH 8–10. This highly efficacious range is narrowed to a pH of right around 10 if the model turbid water does not contain background electrolytes. This is likely due to greater electrostatic repulsion between particles in the absence of background electrolytes, causing coagulation to be more difficult. Similarly, Zhang et al. (*18*) have reported that *Opuntia* spp. is most effective at pH 10 and is least effective at pH 6. *M. oleifera* removes greater than 94% of turbidity across the entire pH range where the synthetic turbid water solution with electrolytes was stable, consistent with the finding that *M. oleifera* components extracted in tap water are effective in waters pH 4–9 (*20*).

Mechanism. For both coagulants, the optimal dose for a given water increases as the initial turbidity increases. This behavior is inconsistent with a sweep flocculation mechanism (*37*) but is consistent with adsorption and charge neutralization as well as adsorption and bridging mechanisms (*35*). Previously, Ndabigengesere et al. (*9*) measured the zeta potential of a kaolin suspension treated with nonshelled water-extracted *M. oleifera* seeds to determine surface
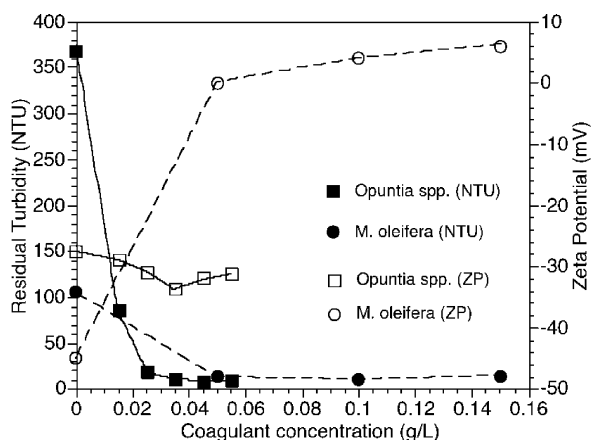
FIGURE 3. A standard batch test was performed on *Opuntia* spp. (NTU_0 = 368, pH adjusted to 9.8). Zeta potential measurements were taken after floc formation, directly after slow mix. Turbidity measurements were taken after settling. *M. oleifera* data adapted from Ndabigengesere et al. (*9*).
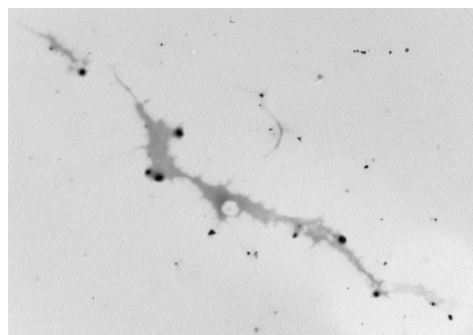

a) Image of *Opuntia* spp. floc, with stain; 52.5μm x 36.75μm.

b) Image of *Opuntia* spp. floc, without stain; 75 μm x 52.5 μm.

c) Image of *M. oleifera* floc, with stain; 50 μm x 35 μm.

FIGURE 4. TEM Images of flocs produced by coagulation experiments in model turbid water, $NTU_0 \sim 250$, pH $\sim$ 8.5. *M. oleifera* concentration = 0.0400 g/L. *Opuntia* spp. concentration = 0.0284 g/L.

potential (Figure 3). These studies suggested that *M. oleifera* predominantly operates through a charge neutralization mechanism because the zeta potential of kaolin particles increases as the *M. oleifera* dose increases, and the optimal *M. oleifera* dose corresponds to zero zeta potential (*9*).

However, as increasing amounts of *Opuntia* spp. are added to a solution of kaolin particles, zeta potential remains negative and relatively constant (Figure 3). These data suggest that *Opuntia* spp. likely causes coagulation by a mechanism other than charge neutralization. Kaolin, with an isoelectric point of 2.8 (*20*), is known to have a net negative surface charge at pH 10. Zeta potential tests performed on ground *Opuntia* spp. indicate that the isoelectric point of *Opuntia* spp. is ~2 (see Supporting Information), so *Opuntia* spp. also has a net negative surface charge at pH 10. Given that *Opuntia* spp. is able to achieve up to 98% coagulation activity (Figure 2) at this pH, it can be concluded that the electrostatic repulsion between these negatively charged particles is not enough to prevent successful coagulation by *Opuntia* spp., and the mechanism is likely to not be charge neutralization.
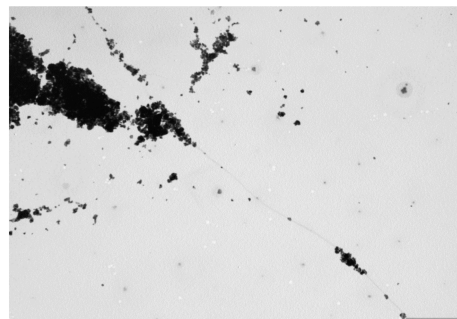
Sweep flocculation is unlikely to be the mechanism of coagulation because of the positive correlation observed between optimal dose and initial turbidity (*37*). Further evidence includes the sensitivity of *Opuntia* spp. to overdosing (see Supporting Information). The observed reduction in coagulation activity when the *Opuntia* spp. dose is too low or too high is consistent with a bridging mechanism (*45*), as a stoichiometric relationship between particle concentration and coagulant dose is expected (*46*).

If the mechanism is not sweep floc or charge neutralization, there are two other possible mechanisms to consider: double layer compression and bridging. Although *Opuntia* spp. is an effective coagulant in solutions of kaolin and deionized water (Figure 2), it is possible that naturally existing ions present in the cactus itself may provide the ionic strength necessary for compression of the double layer and therefore coagulation. However, theoretical analysis of *Opuntia* spp. inorganic cation content (*47, 48*) and experimental analysis from conductivity measurements of water treated with *Opuntia* spp. (see Supporting Information) indicate that the ionic strength provided by *Opuntia* spp. itself is not high enough to cause coagulation as a result of double layer compression.
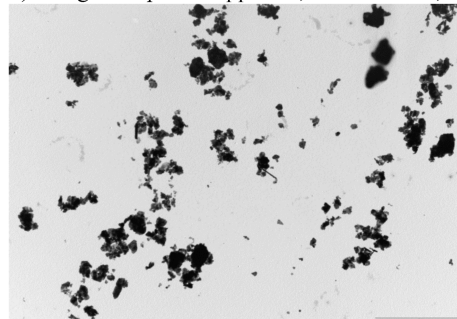
Our results support the hypothesis that the predominant coagulation mechanism for *Opuntia* spp. is adsorption and bridging, whereby clay particles do not directly contact one another but are bound to a polymer-like material from *Opuntia* spp. Adsorption may occur through hydrogen bonding or dipole interactions (*35*). It is likely that natural electrolytes from within the *Opuntia* spp. pad, particularly the divalent cations, which are known to be important for coagulation with anionic polymers (*46*), facilitate adsorption.

Unlike the flocs formed by alum and *M. oleifera* that appear relatively spherical, the flocs formed from *Opuntia* spp. in the presence of kaolin clay are long, thin, and threadlike. Within the one-minute batch test rapid mix (~335 RPM) of *Opuntia* spp., these threadlike flocs are clearly visible. As slow mixing proceeds, the threadlike flocs grow in length and circumference. TEM images of *Opuntia* spp. flocs (Figure 4) show kaolin particles oriented along the axis of these "threads," possibly adsorbed to the polymer-like matrix.

Active Agent. The use of natural coagulants may increase the organic load in waters (*32*), resulting in the possibility for undesired and increased microbial activity. Thus, isolating the active component is critical not only to understand the coagulation mechanism, but also to develop pretreatment practices for potential field implementation. These pretreatment practices would aim to minimize the addition of unnecessary organic material as well as maximize the value by utilizing as much of the plant as possible. For example, it has been established that the active agents of *M. oleifera* are located in the seed kernel, not seed bark (*9, 21*), and the
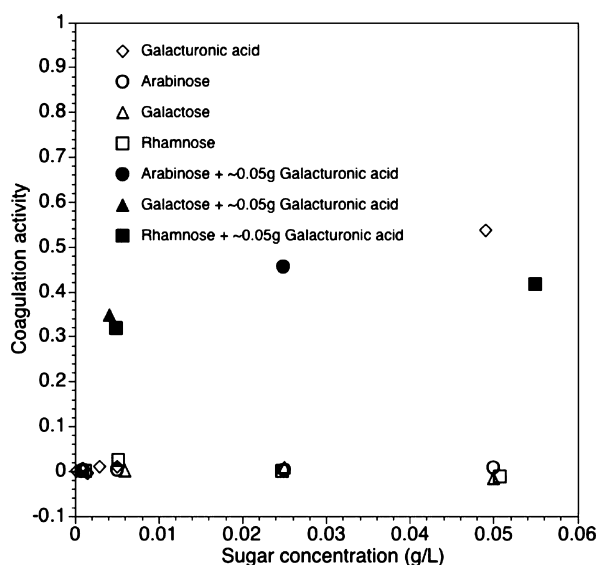
FIGURE 5. Coagulation activity of *Opuntia* spp. mucilage sugar components, individually and in combination with galacturonic acid. Sugars added to a stable solution of kaolin and deionized water, 225–375 NTU, pH adjusted to ~9.8.

valuable oil from the *M. oleifera* seed can be extracted without negatively impacting coagulant properties (*21*).

Parts and forms of the *Opuntia* spp. pad were qualitatively investigated for the presence or absence of coagulation activity (Table 1). As previously defined in an assessment of *M. oleifera* plant components by Ndabigengesere et al. (*9*), greater than 80% coagulation activity is reported as "present" and less than 30% coagulation activity is reported as "absent".

The coagulation agent loses efficacy after maceration or heating above ~100 °C (Table 1). Maceration, a physical process presumably resulting in the breaking of cells, may release a component that hinders coagulation.

Given the working hypothesis that coagulation occurs through a polymer bridge, we considered polymers naturally present in *Opuntia* spp.

Mucilage, found only in some plants, such as *Opuntia* spp., aloe, and okra, is a highly branched (*49*) carbohydrate polymer thought to hold water tightly (*27, 50*). Mucilage cells that produce and store mucilage are present in the outer pad and inner pad (*24, 49*). From the results in Table 1, mucilage may be contributing to the observed coagulation behavior of *Opuntia* spp. In fact, it has been reported that "farmers in some countries use cactus mucilage to clarify drinking water" (*24*), but no further data or analysis are provided to support this statement.

Trachtenberg and Mayer (*51*) report that the viscosity of mucilage from *Opuntia* spp. increases with increasing pH. Molecular weights of 2.3×10^4 (*41*), 3×10^6 (*52*), and 4.3×10^6 (*40*) have been reported for this polymer. Individual components of *Opuntia* spp. mucilage, as reported in the literature, were added to turbid water in isolation and in combination. The results in Figure 5 suggest that mucilage, specifically the galacturonic acid component, may explain some of the turbidity reduction by *Opuntia* spp. Independently, arabinose, galactose, and rhamnose displayed no coagulation activity; however, added in combination with galacturonic acid, these sugars were able to reduce turbidity between 30% and 50%. Galacturonic acid added independently was able to reduce turbidity by more than 50%.

The individual mucilage components in isolation and combination could only account for 50% of the turbidity removal observed when the whole cactus pad was introduced to the turbid water solution. As such, it is likely that there are additional components of the *Opuntia* spp., beyond those found in the mucilage, contributing to the observed coagulation activity. Further studies are needed to determine the other components of the *Opuntia* spp. plant contributing to coagulation.

Note Added after ASAP Publication
This paper published ASAP April 16, 2008 with incorrect address and affiliation designations for some of the authors; the corrected version published ASAP June 12, 2008.

Supporting Information Available
Data used to determine the isoelectric point of *Opuntia* spp., theoretical calculations and experimental estimation of the ionic strength contributed by *Opuntia* spp., and data demonstrating the sensitivity of *Opuntia* spp. to overdosing. This material is available free of charge via the Internet at http://pubs.acs.org.

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