Turbidity reduction in drinking water by coagulation-flocculation with chitosan polymers

Ampai Soros, James E. Amburgey, Christine E. Stauber, Mark D. Sobsey and Lisa M. Casanova

ABSTRACT

Turbidity reduction by coagulation-flocculation in drinking water reduces microbes and organic matter, increasing effectiveness of downstream treatment. Chitosan is a promising household water coagulant, but needs parameters for use. This study tested the effects of chitosan dose, molecular weight (MW), degree of deacetylation (DD), and functional groups on bentonite and kaolinite turbidity reduction in model household drinking water. Higher MW or DD produced greater reductions. Highest reductions were at doses 1 and 3 mg/L by MW >50,000 or >70% DD (residual turbidity <5 NTU). Higher doses did not necessarily continually increase reduction. For functional groups, 3 mg/L produced the highest reductions by lactate, acetate, and HCl, and lower reductions of kaolinite than bentonite. Doses where the point of zero charge was observed clustered around 3 mg/L. Chitosan reduced clay turbidity in water; effectiveness was influenced by dose, clay type, MW, DD, and functional groups. Reduction did not necessarily increase with MW. Bentonite had a broader effective dose range and higher reduction at the optimal dose than kaolinite. Chitosans with and without functional groups performed similarly. The best of the studied doses was 3 mg/L. Chitosans are promising for turbidity reduction in low-resource settings if combined with sedimentation and/or filtration.

Key words | chitosan, coagulation, turbidity, water

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INTRODUCTION

Effective reduction of turbidity is one of the primary goals in effective drinking water treatment because of potential interference with downstream treatment processes and negative effects on consumer acceptance. Turbidity might interfere with filtration by clogging the filter prematurely. It can interfere with chemical disinfection by creating oxidant demand, UV irradiation by blocking light transmission, and reduce the efficacy of both by providing protection to microbes in aggregates or internal to other particles. Turbidity also has negative impacts on consumer acceptance of water; visible cloudiness in finished water may create the perception for consumers that it is not clean or safe to drink. Turbidity is not necessarily a direct measure of microbial contamination, but microbes are often associated with particles in water. Therefore, removing turbidity serves a two-fold purpose in water treatment: it removes some microbes, while reducing the levels of organic matter and other particles, increasing the effectiveness of downstream treatment processes. For drinking water, the World Health Organization has suggested <1 nephelometric turbidity unit (NTU) for water that will undergo disinfection and <4 NTU for water to be acceptable to the naked eye (World Health Organization 2011). The US Environmental Protection Agency sets the maximum level of turbidity in finished drinking water at

1 NTU and at no time >5 NTU; the vast majority of water treatment plants must be less than 0.3 NTU 95% of the time with a maximum of 1 NTU (United States Environmental Protection Agency 2012).

Turbidity reduction is one part of effective water treatment processes in large-scale centralized treatment plants, small community systems, and at the household level. In areas without water treatment systems or with impaired sources of drinking water, water may need treatment at the household level, or point of use (POU) to render it safe to drink. This household level treatment can include turbidity reduction, which should be followed by POU filtration and ideally disinfection. Turbidity removal removes some microorganisms, but most importantly prepares water for these downstream treatment processes. Coagulation-flocculation, a treatment process where colloids in water are destabilized so they can aggregate and be physically removed, can effectively reduce turbidity when combined with sedimentation and/or filtration. An example of a combined POU system would be one where water is collected in a traditional container (such as a clay jar), coagulant is added, and turbidity can flocculate and settle. The water can then be decanted into a household level filter (e.g., a ceramic pot filter or biosand filter), after which, the filtrate can be disinfected and safely stored and the floc disposed of as waste.

Conventional coagulants used in large-scale water treatment are largely metal salts such as aluminum sulfate, ferric sulfate, and ferric chloride, which depend on the pH of water and precise dosing to produce consistently high coagulation efficiency (Yang *et al.* 2016). When coagulation using metal salts is done, the resulting sludge also contains residual metals that must be properly disposed of so that they do not pollute. These limitations of conventional coagulants make them less suitable for household level water treatment, where people need simple but robust and safe methods to treat their water at home. Organic polymer coagulants are an alternative to metal salts in the household setting.

Chitosan, a biopolymer of D-glucosamine and *N*-acetyl-D-glucosamine produced by deacetylation of chitin, has properties of a promising household-level water coagulant: positively charged when dissolved, non-toxic, and biodegradable. Based on its structure, chitosan could be an effective coagulant for negatively charged particles in water by charge neutralization, electrostatic patch, or inter-particle bridging mechanisms. It has been used for reduction of contaminants in wastewaters (Chi & Cheng 2006; Rizzo *et al.* 2008b; Renault *et al.* 2009). Studies have found that chitosan coagulation can remove turbidity at low doses (1–10 mg/L) (Divakaran & Pillai 2001; Rizzo *et al.* 2008a; Brown & Emelko 2009). For effective treatment of household drinking water, there are parameters for its use that need to be established, including selection of alternative chitosan polymers and optimal dosing. To choose the optimal chitosan polymer, properties that influence coagulation performance, including molecular weight (MW), deacetylation (DD), and the addition of functional groups (Yang *et al.* 2016), need to be understood. Therefore, the purpose of this study was to determine the effect of dose, MW, DD, and the addition of functional groups on the efficacy of chitosans for turbidity reduction of two different clays, kaolinite (a 1:1 clay) and bentonite (a 22:1 clay), in artificial surface water used as a model for household drinking water.

MATERIALS AND METHODS

Selection and preparation of chitosans

A total of 17 chitosans were tested (11 acid-soluble and six water-soluble modified) (Table 1). MW and DD were obtained from the vendor (Table 1). To study the effects of MW on turbidity reduction, five chitosan polymers with different MW and similar DD (~90%) were compared: 5,000, 50,000, 100,000, 600,000, and 1,000,000 Da. To test the effects of DD on turbidity reduction, a set of chitosan polymers with different DD and approximately the same viscosity and MW ($\approx$50,000 Da), were used: 70%, 75%, 80%, 85%, 90%, and 95% DD. Six chitosans modified with functional groups to increase water solubility were tested: chitosan acetate, chitosan lactate, chitosan HCl, carboxy-methyl chitosan, and two commercially available coagulants made of proprietary formulations of chitosan acetate (acetate-SK) and chitosan lactate (lactate-SK).

Stock solutions of chitosan were made for all polymers at 10,000 mg/L (1%). Chitosan powder was dissolved in

Table 1 | Properties of chitosans tested

Chitosans	Molecular weight (Da)	Degree of deacetylation (%)	Viscosity (mPascal-seconds)	Source
MW5,000	5×10^3	90	≤ 5	a
MW 50,000	5×10^4–8×10^4	90	16–30	a
MW 100,000	1×10^5–3×10^5	90	200–500	b
MW 600,000	6×10^5–8×10^5	90	50–200	b
MW 1,000,000	$\geq 10^6$	90	5,501–12,500	a
70% DD	n/a	68–73	≤ 7	a
75% DD	n/a	73–78	≤ 7	a
80% DD	n/a	78–83	≤ 7	a
85% DD	n/a	83–88	≤ 7	a
90% DD	n/a	88–93	≤ 7	a
95% DD	n/a	≥ 93	≤ 7	a
Acetate	n/a	80–95	≥ 5	a
Acetate-SK	n/a	n/a	n/a	c
Carboxymethyl	n/a	80–95	5–300	a
HCl	n/a	80–95	2–200	a
Lactate	n/a	80–95	≥ 5	a
Lactate-SK	n/a	n/a	n/a	c

a, Heppe Medical Chitosan Gmbh, Halle, Germany; b, Acros Organics, Bridgewater, NJ, USA; c, HaloSource, Bothell, WA, USA.

0.5% acetic acid (Roussy *et al.* 2004) and stirred at room temperature until totally dissolved. Stock solutions were stored at room temperature (25 °C). The stock solutions of modified (water-soluble) chitosans were prepared similarly, using deionized water instead of acetic acid. The pH of all stock solutions was 3.5–4.5 with the exception of carboxymethyl chitosan, which was pH 7.5.

Preparation of test waters

Two mineral clays, kaolinite, a 1:1 clay, and bentonite, a 2:1 clay, were used to create turbidity in test waters. The kaolinite and bentonite clays were kindly provided by Dr Sterling Weed, Department of Soil Science, North Carolina State University. These clays cause turbidity in natural water and are commonly used to create turbidity in test waters for the evaluation of coagulants (Huang & Chen 1996; Divakaran & Pillai 2001; Roussy *et al.* 2005; Chatterjee *et al.* 2009). Kaolinite and bentonite have different structures that may react differently with chitosan polymers. Bentonite has two silica tetrahedral sheets connected to one aluminum octahedral sheet and expands while kaolinite has one silica tetrahedral sheet connected to one aluminum octahedral sheet and does not expand. Bentonite has higher cationic exchange capacity (CEC) than kaolinite (0.8–1.2 versus 0.03–0.15 meq/g) and bentonite also has larger surface area than kaolinite (40–800 versus 5–40 m^2/g) (Kahr & Madsen 1995; Meier & Kahr 1999).

The test water was artificial surface water prepared based on the recommended parameters from the US Environmental Protection Agency and NSF International for efficacy testing of POU technology (United States Environmental Protection Agency 1987; NSF International 2008). For testing of turbidity reduction, the test water was created by spiking dechlorinated City of Atlanta tap water with: 300 mg/L of total dissolved solids (TDS), 3 mg/L of total organic carbon (TOC), and ≥ 30 NTU of turbidity. Sodium chloride (NaCl) was used as an adjustment material for TDS and tannic acid (University Lake, Chapel Hill, NC) was used as an adjustment material for TOC. Water pH was not adjusted after adding clay, NaCl, and tannic acid and water pH ranged from 7 to 7.5.

Jar test experiments

A jar test method of coagulation testing was used in a conventional paddle blade flocculator apparatus (PB-900, Phipps & Bird, Richmond, VA). The mixing conditions were rapid mixing at 100 revolutions per minute (rpm) ($<1 \times g$) for 1 minute followed by slow mixing at 25 rpm ($<1 \times g$) for 15 minutes and settling for 30 minutes. All experiments were conducted at 25 °C. For turbidity analysis, supernatant was sampled 2 cm from the surface of the water using a pipette and without disturbing the floc. Turbidity was measured by a turbidity meter (Hach 2100AN Turbidimeter, Hach, Loveland, CO). Water pH was also measured before and after the jar test experiment using a pH meter.

Doses of chitosans were 1, 3, 10, and 30 mg/L. These doses were selected because they are in the same ranges as optimum doses of conventional coagulants (2–5 mg/L for aluminum and 4–10 mg/L iron salt coagulants) (WHO 2008) and in the ranges of chitosan effective doses for turbidity reduction in preliminary studies (data not shown). Three replicates, plus one control (no chitosan) for natural settling, were performed for each set of experimental conditions. The parameters tested for effects on coagulation performance were chitosan type and dose (1, 3, 10, and 30 mg/L) and turbidity type (bentonite or kaolinite).

Data analysis

Turbidity reduction was calculated as percent turbidity reduction relative to the natural settling control:

$$\text{Percent reduction} = [1 - \text{sample turbidity}/\text{control turbidity}]*100$$

Statistical comparison of the effects of different chitosans on turbidity reduction was performed using GraphPad Prism (GraphPad, San Diego, CA). Two-way analysis of variance (ANOVA) and Tukey post-test analysis was employed for comparing effects of water-soluble and acid-soluble chitosans and chitosan doses, and one-way ANOVA was used for comparing effects of chitosan doses of each chitosan tested.

Zeta potential measurement

A subset of chitosans that demonstrated high and low reduction of turbidity in jar testing was tested to observe changes in surface charge through the coagulation-flocculation process. Zeta potentials of prepared test water containing bentonite or kaolinite with turbidity of 5, 30, and 300 NTU were also measured. All measurements were done using a Malvern Zetasizer nano ZS (Malvern, Worcestershire, UK). Zeta potential was measured using two different methods. First, water samples were analyzed for their background electrical charges. Second, using a titration method, zeta potential was measured during the titration of water samples with chitosans. For this measurement, water samples containing 5 NTU kaolinite or bentonite at neutral pH were titrated with chitosan stock solution at doses between 0 and 50 mg/L. Water pH was automatically measured during the titration. Titration graphs showing changes in zeta potential while chitosan was being added into the water sample were generated showing chitosan dose and pH at which the point of zero charge (PZC) occurred.

RESULTS

Effects of molecular weight

The effects of chitosan polymer MW on reduction of bentonite turbidity at varying chitosan doses are shown in Figure 1.
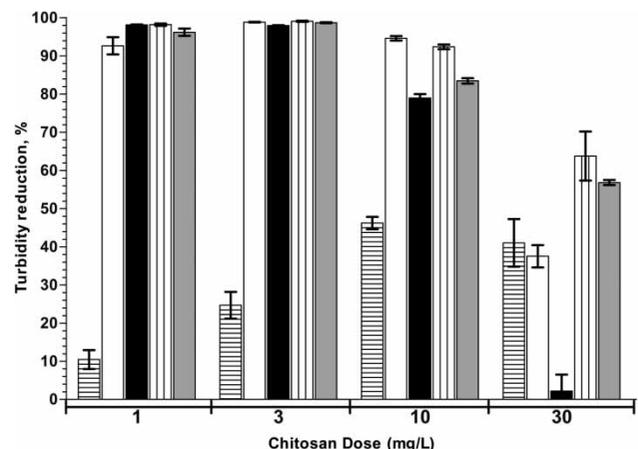

Figure 1 | Effects of chitosan MW (chitosans of 90% DD) and dose on bentonite turbidity reduction (horizontal stripes = 5,000 Da; white = 50,000 Da; black = 100,000 Da; vertical stripes = 600,000 Da; gray = 1,000,000 Da; bars = 95% CI).

Overall, bentonite reduction differed significantly by MW (ANOVA, $p < 0.0001$). In general, higher MW chitosans produced greater turbidity reductions, with poor reductions ($<30\%$) at the lowest MW of 5,000 Da. Even at low MW, there appear to be dose effects. When dose was increased, bentonite reduction by the 5,000 Da chitosan improved, from 10% at 1 mg/L, to 25% at 3 mg/L, to 46% at 10 mg/L.

Higher MW chitosans achieved better turbidity reduction than the 5,000 Da chitosan. The highest reductions in bentonite turbidity were achieved at doses of 1 and 3 mg/L by chitosans of MW $>50,000$. Higher MW chitosans also exhibit dose effects, although it appears that higher chitosan doses did not necessarily result in continually increasing turbidity reduction. Chitosans between 50,000 and 1,000,000 Da gave $>90\%$ bentonite turbidity reduction at 1 and 3 mg/L. At dose 1 mg/L, bentonite reduction differed significantly by MW (one-way ANOVA, $p < 0.0001$); 50,000 Da had significantly lower reduction (92.6%) compared to the larger MW chitosans: 100,000 (98.1%), 600,000 (98.2%), and 1,000,000 Da (96.2%). There were no statistically significant differences in bentonite reduction by 100,000, 600,000, and 1,000,000 Da chitosans ($p > 0.05$) at dose 1 mg/L. The 100,000, 600,000, and 1,000,000 Da chitosans showed similar bentonite turbidity reduction at 1 and 3 mg/L doses. The 50,000 Da improved reduction from 92.62% at 1 mg/L dose to 98.82% at 3 mg/L. At a dose of 3 mg/L, there were no longer statistically significant differences in reduction for 50,000 to 1,000,000 Da chitosans.

Increasing doses may result in diminishing turbidity reduction returns; for higher MW chitosans, bentonite turbidity reductions did not improve but decreased significantly when dose was increased to 10 mg/L or 30 mg/L ($p < 0.05$). Bentonite turbidity reductions at dose 10 mg/L were in the range of 80–90% for MW $>50,000$ Da. For these MW, bentonite reduction by a dose of 30 mg/L decreased by 30–90% when compared to lower doses.

The effects of MW on reduction of kaolinite turbidity at varying chitosan doses are shown in Figure 2. Overall, kaolinite reduction differed significantly by MW ($p < 0.0001$). The highest reductions in kaolinite turbidity were achieved at doses of 1 and 3 mg/L (like bentonite), and higher doses did not produce higher turbidity reduction, except MW 5,000 Da at dose 30 mg/L. Higher MW chitosans
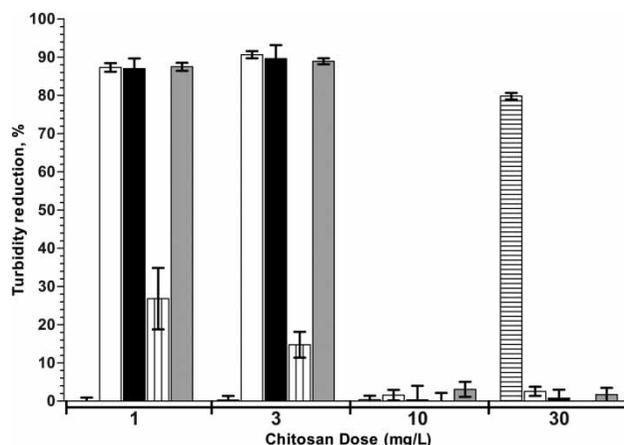

Figure 2 | Effect of chitosan MW (chitosans of 90% DD) and dose on reduction of kaolinite turbidity (horizontal stripes = 5,000 Da; white = 50,000 Da; black = 100,000 Da; vertical stripes = 600,000 Da; gray = 1,000,000 Da; bars = 95% CI).

produced greater turbidity reductions, with poor reductions at the lowest MW.

MW 50,000, 100,000, and 1,000,000 Da chitosans had kaolinite turbidity reduction ranging from 87% to 90% at 1 and 3 mg/L dose. At 1 and 3 mg/L dose, there was no significant difference in kaolinite reduction between 50,000, 100,000, and 1,000,000 Da ($p > 0.05$). MW 600,000 was less effective than the other MWs, with $<25\%$ reduction. The lowest MW, 5,000 Da, performed very poorly ($<1\%$ reduction). All MWs, even those that showed reduction at 1 and 3 mg/L, performed poorly for kaolinite reduction at 10 mg/L and 30 mg/L doses ($<5\%$ reduction for all MWs). The exception was 5,000 Da, which increased from $<1\%$ at lower doses to $\sim80\%$ reduction at 30 mg/L.

For the same MW at the same dose, reduction of bentonite was significantly better than reduction of kaolinite at both 1 and 3 mg/L dose (one-way ANOVA, Tukey's post-test, $p < 0.05$). The exception was 50,000 Da at 1 mg/L, which showed similar reductions of bentonite and kaolinite ($p > 0.05$). At 10 mg/L, reductions of bentonite were significantly better than reductions of kaolinite for the same MW (one-way ANOVA, Tukey's post-test, $p < 0.05$). Reduction of bentonite was significantly higher than reduction of kaolinite for the same MW at 30 mg/L dose (one-way ANOVA, Tukey's post-test, $p < 0.05$) except for 5,000 (kaolinite reduction $\sim80\%$, bentonite reduction $\sim41\%$). There were three MW chitosans that effectively removed both kaolinite and bentonite at 1 and 3 mg/L: 50,000, 100,000, and

Table 2 | Residual kaolinite and bentonite clay turbidity after jar test coagulation with varying molecular weight chitosans (chitosans of 90% DD) at four doses ($n = 9$)

MW (Da)	Clay	Initial turbidity (NTU)	Residual turbidity (NTU) at each chitosan dose (mg/L) (95%CI)				
			0	1	3	10	30
5,000	K	80.8	73.3 (7.12)	74.0 (6.59)	73.1 (8.58)	73.0 (7.06)	14.8 (2.81)
	B	34.4	28.4 (0.39)	25.4 (1.54)	21.4 (3.40)	15.3 (1.11)	16.7 (6.23)
50,000	K	47.5	43.3 (3.41)	5.5 (1.00)	4.1 (0.34)	42.6 (3.40)	42.2 (4.10)
	B	36.7	34.4 (2.74)	2.5 (2.96)	0.4 (0.02)	1.9 (0.86)	21.4 (2.61)
100,000	K	34.2	32.5 (14.52)	4.1 (1.43)	3.2 (2.38)	32.3 (12.58)	32.2 (14.05)
	B	35.6	20.0 (2.57)	0.4 (0.11)	0.4 (0.11)	4.2 (0.23)	19.5 (4.68)
600,000	K	49.1	37.8 (9.56)	30.0 (6.43)	32.3 (10.62)	38.6 (4.68)	41.1 (6.91)
	B	37.9	31.5 (6.01)	0.6 (0.29)	0.3 (0.09)	2.4 (0.25)	11.4 (7.13)
1,000,000	K	55.4	53.4 (5.19)	6.7 (0.52)	5.9 (1.05)	51.7 (3.04)	52.5 (5.81)
	B	37.0	34.1 (1.17)	1.3 (1.24)	0.5 (0.12)	5.6 (0.84)	14.7 (0.26)

B = bentonite; K = kaolinite.

1,000,000 Da. These chitosans could achieve >90% turbidity reduction, and bring residual turbidity from 30 to 70 NTU to <3 NTU for bentonite and <5 NTU for kaolinite. Overall, chitosans >50,000 Da at doses 1 and 3 mg/L brought kaolinite and bentonite turbidity to the <5 NTU standard, and bentonite turbidity to the <1 NTU standard (Table 2).

Effects of degree of deacetylation

Chitosans with six different DDs and comparable MWs (approximately 5 mPa·s viscosity, >50,000 Da) were tested: 70%, 75%, 80%, 85%, 90%, and 95% DD. The effects of polymer DD on reduction of bentonite turbidity at varying doses are shown in Figure 3. Bentonite reduction differed significantly by DD (ANOVA, $p < 0.0001$). All DDs gave bentonite reduction >80% at 1 and 3 mg/L (84.5–99.2%). At 1 mg/L, 70% and 80% DD had significantly higher bentonite reduction than other DDs ($p < 0.05$). All DDs showed highest bentonite reduction at 3 mg/L (~99%). The 85% DD gave statistically significantly higher reduction (99.20%) than other DD at this dose, but the magnitude of difference was very small (98.8 versus 99.2%). All DDs had bentonite reductions of >93% at 10 mg/L. However, chitosans with DD >70% exhibited significantly higher bentonite reduction (~98%) compared to 70% DD chitosan ($p < 0.05$). There were no statistically significant differences among bentonite reductions of chitosans with >70% DD
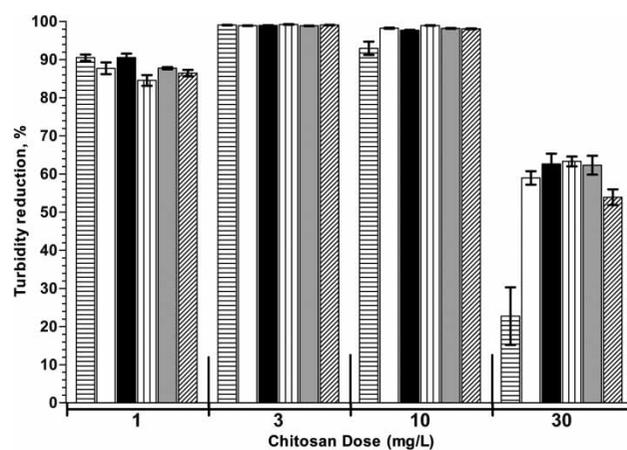
Figure 3 | Effects of polymer degree of deacetylation (5 mPa·s viscosity) on bentonite turbidity reduction (horizontal stripes = 70% DD; white = 75%; black = 80%; vertical stripes = 85%; gray = 90%; diagonal stripes = 95%; bars = 95% CI).

at this dose ($p > 0.05$). When the dose was increased to 30 mg/L, reduction of bentonite dropped significantly. The decrease was greatest for 70% DD (22.8% reduction versus >90% at 1–10 mg/L). While not a huge effect, it appears that the higher DDs tend to be more robust (less likely to overdose) since the lower DD start failing at higher dosages to a greater degree.

Kaolinite reduction differed significantly by chitosan DD and dose ($p < 0.0001$) (Figure 4). All DDs performed best for kaolinite reduction at 3 mg/L (88–93% reduction). At 3 mg/L, 70% and 75% DD had significantly higher kaolinite reductions than 90% and 95% DD ($p < 0.05$), although all of them had reductions >91%. At 1 mg/L, kaolinite
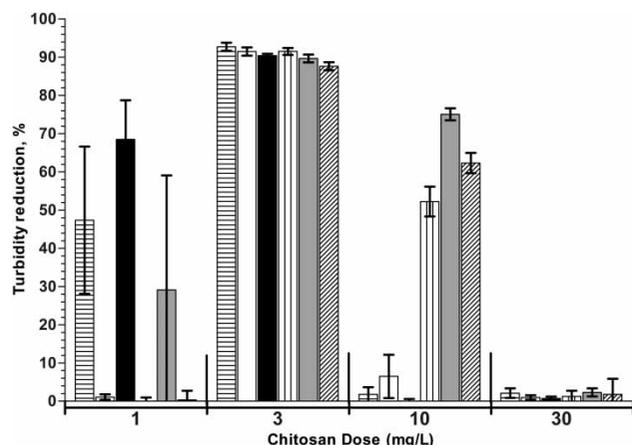
Figure 4 | Effects of polymer degree of deacetylation (5 mPa·s viscosity) on kaolinite turbidity reduction (horizontal stripes = 70% DD; white = 75%; black = 80%; vertical stripes = 85%; gray = 90%; diagonal stripes = 95%; bars = 95% CI).

reductions were poor; only 70%, 80%, and 90% DD reduced kaolinite, with reductions of only 47%, 68%, and 29%, respectively. Higher chitosan doses had decreasing returns; when dose increased from 3 to 10 mg/L, kaolinite reduction decreased. At 10 mg/L, only 85–95% DD showed modest kaolinite reductions (52–75%); 70%, 75%, and 80% DD had very poor reductions (<7%). At 30 mg/L, none of the DDs reduced kaolinite turbidity (reduction <3%).

For the same DD, reduction of bentonite was significantly higher than reduction of kaolinite at 10 and 30 mg/L doses (one-way ANOVA, Tukey's post-test, $p < 0.05$), but still poor compared to 1 and 3 mg/L doses. For the same

DD, reduction of kaolinite was significantly lower than reduction of bentonite at 1 and 3 mg/L (one-way ANOVA, Tukey's post-test, $p < 0.05$). Overall turbidity reduction was lower for kaolinite than bentonite, but the highest reductions of both were at 3 mg/L for all six DDs. For 80% DD at 1 mg/L, reductions of bentonite (90.5% ($\pm$3.9)) and kaolinite (68.5% ($\pm$37.9)) were not statistically different ($p > 0.05$). Based on these results, 3 mg/L was the optimum dose that exhibited the highest reduction of both bentonite and kaolinite turbidity. At this dose, reduction of bentonite by different DD chitosans was similar (99%), and resulted in residual bentonite turbidity <1 NTU (starting turbidity 32–98 NTU). The reduction of kaolinite at 3 mg/L was also similar across DDs (90%) and brought residual kaolinite turbidity to <5 NTU (Table 3).

Effect of modified functional groups

Six chitosans modified with functional groups were tested: carboxymethyl chitosan, chitosan lactate, chitosan acetate, chitosan HCl, and two proprietary commercial coagulants, chitosan lactate-SK and chitosan acetate-SK. Acetate, lactate, acetate SK, lactate SK, HCl, and carboxymethyl were all made as stock solutions in sterile distilled water. The pH of the acetate, lactate, acetate SK, lactate SK, and HCl was between 3.5 and 4.5. The pH of the carboxymethyl stock solution was 7.5. At the doses used in this study, the

Table 3 | Residual kaolinite and bentonite clay turbidity after jar test coagulation at varying chitosan degrees of deacetylation and doses

| DD (%)	Clay	Starting turbidity (NTU)	Residual turbidity (NTU) ($\pm$95% CI, $n = 9$)				
			0 mg/L	1 mg/L	3 mg/L	10 mg/L	30 mg/L
70	K	104.3	97.7 (9.67)	52.2 (76.65)	7.1 (1.18)	95.8 (4.84)	95.5 (5.41)
	B	35.7	32.3 (3.09)	3.1 (0.99)	0.3 (0.03)	2.3 (1.94)	24.9 (7.20)
75	K	50.3	48.6 (4.38)	48.1 (4.58)	4.2 (0.71)	45.3 (6.25)	48.1 (4.86)
	B	34.8	33.6 (2.83)	4.1 (1.67)	0.4 (0.06)	0.6 (0.16)	13.8 (0.87)
80	K	49.0	47.7 (4.10)	15.1 (18.41)	4.6 (0.35)	47.7 (4.93)	47.3 (4.80)
	B	38.1	36.3 (1.44)	3.4 (1.33)	0.4 (0.01)	0.8 (0.10)	13.5 (3.25)
85	K	43.6	41.4 (1.99)	41.6 (3.15)	3.5 (0.78)	19.8 (2.47)	40.8 (3.67)
	B	37.2	35.9 (1.52)	5.6 (2.08)	0.3 (0.13)	0.4 (0.04)	13.2 (2.25)
90	K	33.5	33.7 (2.72)	24.2 (38.76)	3.5 (0.87)	8.4 (2.01)	32.9 (1.84)
	B	37.0	32.8 (3.26)	4.0 (0.47)	0.4 (0.04)	0.6 (0.02)	12.3 (1.58)
95	K	38.4	37.3 (4.14)	37.2 (1.62)	4.6 (0.32)	14.1 (2.76)	36.6 (36.59)
	B	33.8	34.0 (0.80)	4.6 (1.03)	0.3 (0.07)	0.7 (0.15)	15.7 (2.90)
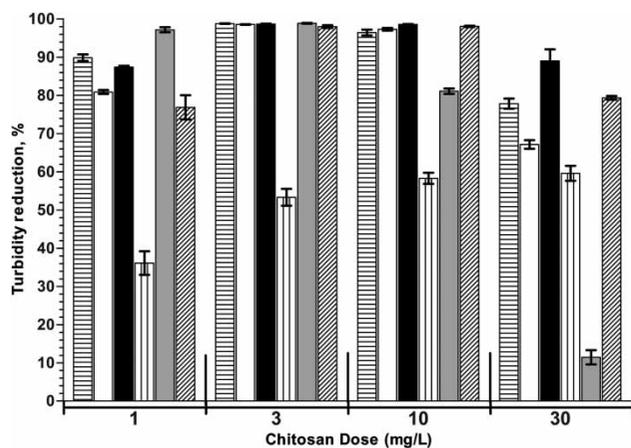
Figure 5 | Effects of polymer modification by functional groups (DD 80–95%, viscosity 2–300 mPa·S) on bentonite turbidity reduction (horizontal stripes = acetate; white = lactate; black = HCl; vertical stripes = carboxymethyl; gray = acetate-SK; diagonal stripes = lactate-SK; bars = 95% CI).

pH of the test water after chitosan dosing ranged from 6.5 to 7.0 (data not shown). Both functional group and dose significantly affected reductions of bentonite turbidity (one-way ANOVA, $p < 0.0001$) (Figure 5). At 1 mg/L, acetate-SK had significantly higher reduction of bentonite (97%) than other functional groups ($p < 0.05$). HCl and acetate had bentonite reduction of 87% and 90% at 1 mg/L, respectively; there was no statistically significant difference between them ($p > 0.05$). Lactate showed significantly better bentonite reduction (81%) than lactate-SK (77%) ($p < 0.05$). Bentonite reductions at 10 mg/L were slightly lower compared to 3 mg/L for HCl, acetate, lactate, and lactate-SK. However,

at this dose, reduction by acetate-SK decreased substantially (99% at 1 and 3 mg/L to 81% at 10 mg/L). Lactate, lactate-SK, and HCl at 10 mg/L also reduced turbidity to <1 NTU. At 30 mg/L, bentonite reduction by modified chitosans was less compared to lower doses; HCl was the best at this dose (89%). The carboxymethyl group was relatively ineffective for bentonite reduction across doses, with only 60% reduction and residual turbidity 12.79 NTU.

The dose of 3 mg/L produced the highest turbidity removals of the dosages examined in this study; at this dose, the lactate, acetate, and HCl functional groups showed high bentonite turbidity reduction (98–99%), similar to unmodified chitosans, and there were no statistically significant differences in reduction between functional groups ($p > 0.05$). There also were no statistically significant differences between lactate and lactate-SK or between acetate and acetate-SK ($p > 0.05$). The exception was the carboxymethyl functional group, which showed lower reductions than other functional groups regardless of dose. Except for carboxymethyl, residual bentonite turbidity was lower than 1 NTU at 3 mg/L (Table 4).

Reduction of kaolinite turbidity differed significantly by functional group and dose ($p < 0.0001$) (Figure 6). At 1 mg/L kaolinite, reduction was low for all functional groups except acetate-SK (82%). At 10 mg/L, HCl had 85% reduction, but other functional groups had <3%. At 30 mg/L, all functional groups had <3% reduction. As with bentonite, the highest kaolinite reductions were at

Table 4 | Residual kaolinite and bentonite clay turbidity after jar test coagulation at varying doses for chitosans modified with different functional groups

| Chitosan type	Clay	Initial turbidity (NTU)	Residual turbidity (NTU) (mg/L) (95% CI, $n = 9$)			
			0	1	3	10
Carboxymethyl	K	36.7	34.2 (0.76)	34.0 (0.74)	33.9 (1.27)	33.7 (1.14)
	B	34.6	31.7 (1.81)	20.2 (3.31)	14.8 (2.00)	13.2 (0.83)
Lactate	K	48.7	48.8 (42.07)	49.1 (37.72)	5.5 (4.11)	49.1 (40.32)
	B	34.6	32.7 (1.62)	6.2 (0.19)	0.5 (0.07)	0.9 (0.35)
Lactate-SK	K	41.2	41.8 (9.51)	40.8 (12.55)	5.1 (3.06)	41.1 (7.72)
	B	36.3	35.0 (2.20)	8.1 (4.47)	0.7 (0.50)	0.7 (0.24)
Acetate	K	34.0	33.4 (2.10)	32.9 (0.29)	28.0 (13.29)	32.4 (2.00)
	B	33.6	32.3 (5.79)	3.3 (1.47)	0.4 (0.07)	1.1 (0.73)
Acetate-SK	K	64.7	61.9 (5.35)	11.0 (18.61)	24.3 (14.42)	61.3 (3.98)
	B	36.8	35.3 (0.22)	1.0 (0.87)	0.4 (0.06)	6.7 (0.90)
HCl	K	45.6	45.2 (34.65)	44.1 (32.25)	3.8 (2.07)	6.4 (1.86)
	B	32.0	30.0 (2.11)	3.8 (0.52)	0.4 (0.14)	0.4 (0.09)
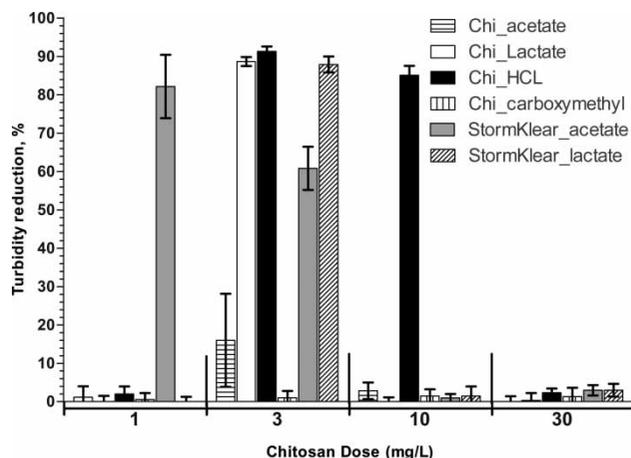
Figure 6 | Effects of polymer modification by functional groups (DD 80–95%, viscosity 2–300 mPa·S) on kaolinite turbidity reduction (horizontal stripes = acetate; white = lactate; black = HCl; vertical stripes = carboxymethyl; gray = acetate-SK; diagonal stripes = lactate-SK; bars = 95% CI).

3 mg/L, but only for some functional groups. At this dose, lower reductions were seen with carboxymethyl (<2%), acetate (16%), and acetate-SK (60%). Lactate, lactate-SK, and HCl reduced kaolinite by 88–91% with residual turbidity 3.80–5.45 NTU (Table 4) and reductions were not significantly different within dose ($p > 0.05$).

As with unmodified chitosans, chitosan polymers with functional groups demonstrated poorer reductions of kaolinite than bentonite turbidity. At 1, 10, and 30 mg/L, reduction of bentonite was significantly higher than reduction of kaolinite (one-way ANOVA, Tukey's post-test, $p < 0.05$). For the same functional groups, reduction of bentonite was significantly higher than reduction of kaolinite at a dose of 3 mg/L (one-way ANOVA, Tukey's post-test, $p < 0.05$). The exception was HCl; reductions of bentonite and kaolinite at 3 mg/L were not statistically significantly different ($p > 0.05$).

Measurement of zeta potential

All water samples exhibited negative zeta potential (Table 5). Water with kaolinite turbidity of 30 and 300 NTU had the lowest zeta potentials (most negative values). Zeta potentials of water with kaolinite were statistically significantly different between turbidity levels ($p < 0.05$). The zeta potential of water with kaolinite turbidity 5 NTU differed significantly from 30 and 300 NTU. Water with

Table 5 | Zeta potential of water of varying turbidities ($n = 9$)

Turbidity source	Turbidity (NTU)	pH	Zeta potential, mV (±95%CI)
Kaolinite	5	4	−18.29 (2.97)
Kaolinite	5	7	−27.83 (1.27)
Kaolinite	30	7	−35.26 (2.67)
Kaolinite	300	7	−35.59 (0.78)
Bentonite	5	7	−24.99 (3.63)
Bentonite	30	7	−26.24 (2.19)
Bentonite	300	7	−25.28 (1.10)

bentonite turbidity, however, had zeta potentials that were not statistically significantly different across turbidity values ($p > 0.05$). Water with kaolinite had more negative zeta potentials than water with bentonite at 300 and 30 NTU. At 5 NTU, zeta potential for water with bentonite and kaolinite was not significantly different ($p > 0.05$).

Zeta potential values and PZC of chitosan coagulation were measured by the titration method (Table 6). Chitosans that provided high and low turbidity reduction (MW 100,000 Da, 70% DD, 95% DD, and modified chitosan HCl) were selected as representatives to: (1) observe zeta potential over the course of the coagulation process and (2) determine the dose at which the water/coagulant mixture reached the PZC (Figure 7). These doses at which the PZC was observed clustered around 3 mg/L, the dose that resulted in the highest turbidity reductions in jar test experiments. The dose at PZC of chitosan MW 100,000 Da for kaolinite (4.61 mg/L) was higher than that

Table 6 | Chitosan dose and pH required to reach points of zero charge (PZCs) during coagulation (test water 5 NTU)

Chitosan	Turbidity source	Zeta potential of chitosan (mV) (95%CI, $n = 9$)	Chitosan dose at PZC (mg/L)	pH at PZC
5,000 Da	Kaolinite	6.61 (5.28)	Not measured[a]	7.44
100,000 Da	Kaolinite	88.93 (10.88)	4.61	7.2
100,000 Da	Bentonite	88.93 (10.88)	2.62	7.5
95% DD	Kaolinite	19.84 (12.00)	1.88	7.3
70% DD	Kaolinite	41.98 (2.73)	2.15	7.2
Carboxymethyl	Kaolinite	−29.64 (2.20)	Not measured[b]	8.08
HCl	Kaolinite	30.37 (10.07)	2.19	7.2

[a]Reached ζ −2.14 mV at 50 mg/L.
[b]Reached ζ −26 mV at 50 mg/L.

of bentonite (2.62), however both PZCs were still close to the jar test effective dose of 3 mg/L.

DISCUSSION

Chitosan polymers effectively reduced kaolinite and bentonite clay particle turbidity in water by coagulation-flocculation-sedimentation at low doses. The effectiveness of reduction was influenced by dose, clay type, polymer MW, polymer DD, and the presence of added functional groups.

Higher MW chitosans were more effective than lower MW, but reduction did not necessarily increase significantly with increasing MW above a certain level. For the same MW at the same dose, reduction of bentonite, a 2:1 clay, was significantly better than reduction of kaolinite, a 1:1 clay, at the optimal dose. Chitosans from 50,000 to 1,000,000 Da had bentonite reductions ranging from 80 to 99% at doses from 1 to 10 mg/L. At the dose that performed best in this study, bentonite reduction was not improved with increasing MW above 100,000 Da. The smallest MW (5,000 Da) performed poorly (<50% reduction at every dose tested). This is consistent with other findings that bentonite reduction increased as MW of chitosan increased (Roussy *et al.* 2005; Chen & Chung 2011). MW 50,000, 100,000, and 1,000,000 Da chitosans had kaolinite turbidity reduction ranging from 87% to 90% at 1 and 3 mg/L dose, and the smallest MW (5,000 Da), performed worse than it did for bentonite (<1% reduction).

DD, the number of amino groups ($-NH_2$) along the chitosan chain, helps create cationic sites along the chitosan polymer due to their deprotonation when dissolved in water near neutral pH. Positively charged sites on the polymer can attach to negatively charged colloids, resulting in coagulation. Because higher DD results in higher positive charge on the polymer, higher reduction of bentonite and kaolinite would be expected from higher DD chitosans. This was observed only to a limited extent in this study; when dose was held constant, polymer DD had minimal impact on turbidity reduction. At 3 mg/L, reduction of bentonite and kaolinite were not significantly influenced by DD; higher DD chitosans had 99% bentonite reduction with residual turbidity <5 NTU, and 90% kaolinite reduction with residual turbidity ≤7 NTU. Effects of DD on kaolinite
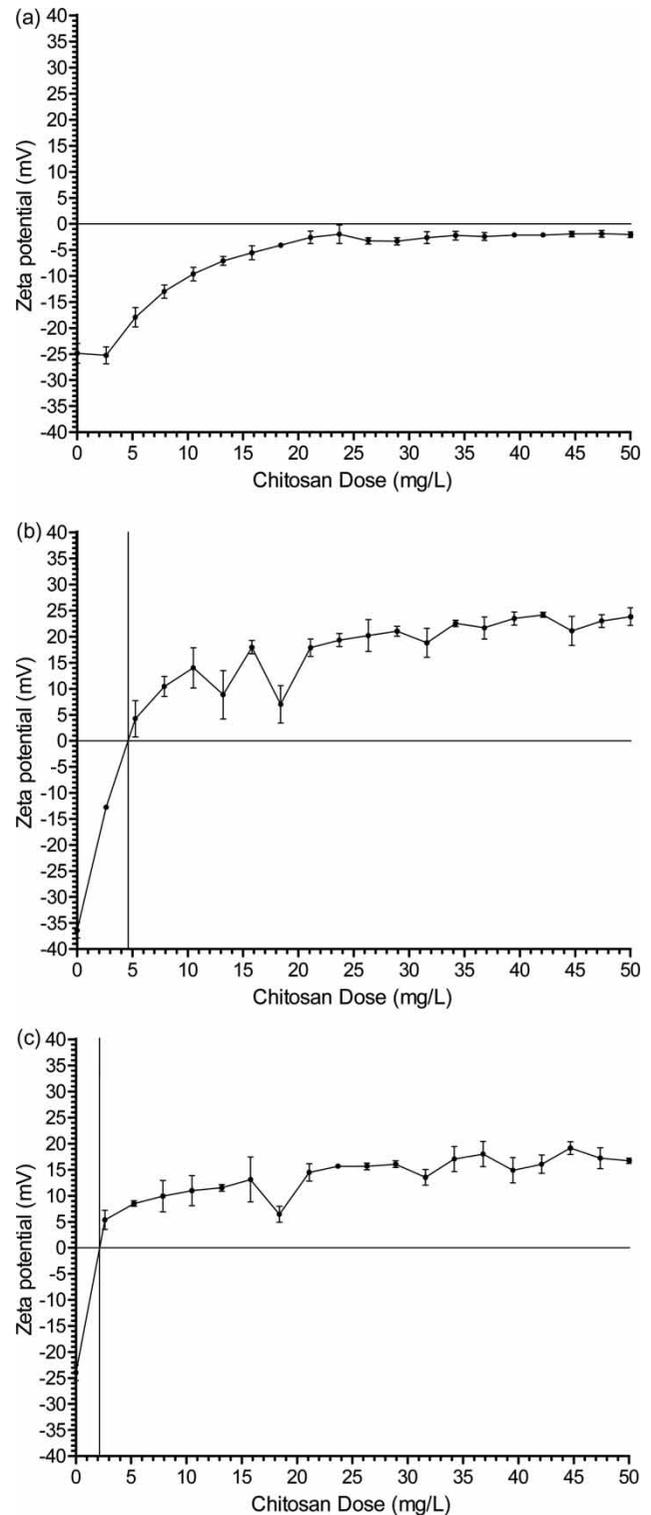

Figure 7 | Zeta potentials and points of zero charge (PZCs) of kaolinite turbidity coagulation by (a) chitosan MW 5,000 Da, (b) MW 100,000 Da, and (c) 70% DD (black circles mean zeta potential; connecting line = weighted mean zeta potential; vertical line = PZC; bars = 95% CI).

reduction at doses other than the optimum were variable. There was better kaolinite reduction by lower DD at a dose of 1 mg/L, however at the higher dose of 10 mg/L, higher DD were more effective than lower DD.

Above about 80% DD, further increases in the DD may not greatly affect coagulation (Yang et al. 2016). Chen & Chung (2011) also observed that MW influenced bentonite reduction more than DD. Using a pyrene-fluorescein probe method to study polarization of the microenvironment of chitosan polymers at varying DD, they found that DD had little effect on polarization and therefore likely limited effects on bentonite flocculation. In this study, measured zeta potential of chitosans did not differ significantly by DD. At similar MW, chitosan polymers may possess similar 'effective' charge even though the actual numbers of $-NH_2$ groups are different; as a result, different DD chitosans produced similar turbidity reductions at the optimum dose. Possible reasons for this outcome are that all positively charged sites might not be available (or could be redundant) depending on the size and charge density of the particles, or that coagulation needs interaction between negative charges of colloids and positive charges of chitosan but does not require complete charge neutralization.

Water-soluble chitosans (modified with functional groups) performed similarly to acid-soluble (unmodified) chitosans. The functional groups on modified chitosans reduce the intramolecular hydrogen bonding of the chitosan molecule; it can then interact with water similarly to chitosan protonated by acetic acid, resulting in similar coagulation properties. Maximum reductions of bentonite turbidity were at doses 1–10 mg/L and kaolinite at 3 mg/L. This is similar to the optimal dose range for bentonite observed in previous studies, although they found the optimum dose range of water-soluble chitosans was broader than that of acid-soluble chitosans (Chen & Chung 2011). As observed for acid-soluble chitosan, water-soluble modified chitosans demonstrated better reduction of bentonite compared to kaolinite turbidity. However, the turbidity reduction of each functional group varied; HCl, acetate, and lactate were more effective than carboxymethyl chitosans. Chitosan HCl produced high bentonite reductions between 1 and 30 mg/L and high kaolinite reduction at 3 mg/L. Acetate-SK was best at the lowest dose and worst at the highest; acetate also had lower removal at the highest

dose. Lactate, a slightly stronger acid, was also less effective at the highest dose. The carboxymethyl group had the poorest turbidity reduction among water-soluble chitosans. When test water was titrated with carboxymethyl chitosan to determine the PZC, it was not reached even at doses up to 50 mg/L; it was the only chitosan where this was observed. Carboxymethyl was worst at the lowest dose and kept improving at higher dosages. Also, simply increasing the solubility of the polymer in water does not necessarily increase turbidity reduction efficacy.

The best of the studied chitosan dosages for reduction of both bentonite and kaolinite turbidity was 3 mg/L, although there was a broader effective dose range for bentonite than kaolinite. A dose range of 1–10 mg/L was effective for bentonite reduction (80–99% reduction with residual turbidity ≤5 NTU). When dose was increased to 30 mg/L, bentonite reduction dropped sharply. Kaolinite reduction was more sensitive to dose than bentonite. Generally, reduction of kaolinite only occurred at doses of 1 and 3 mg/L (≈82–93%, residual turbidity 3–7 NTU). Other studies also reported similar optimal doses in the 0–10 mg/L range (Pan et al. 1999; Huang et al. 2000; Chatterjee et al. 2009; Pontius 2016) and differential bentonite and kaolinite reduction by the optimal dose (Huang et al. 2000). Chen & Chung (2011) observed better bentonite reduction with higher MW chitosan, with the highest bentonite reduction (92%) by a 300,000 Da polymer at doses similar to those used in this study (2.5 mg/L). Although MW and dose can be selected to maximize turbidity reduction as much as possible, the combinations of dose and MW that achieve reductions >90% may, in practice, be able to produce finished waters of similar quality, particularly when combined with downstream treatment processes.

Other studies (Huang et al. 2000) have also observed differential bentonite and kaolinite reduction by chitosans. The observed differences in reduction of bentonite and kaolinite turbidity may be related to the cationic exchange capacity (CEC) properties of these clays and suggest that charge neutralization is one of the mechanisms underlying coagulation by chitosans, but probably not the exclusive or dominant mechanism. CEC is the ability of a soil particle to retain and exchange positively charged ions; the higher the CEC, the greater the capacity of clay particles to attract positively charged molecules. Bentonite has a CEC ranging

between 0.8 and 1.2 meq/g, which is much higher than that of kaolinite (CEC 0.03–0.15 meq/g) (Kahr & Madsen 1995; Meier & Kahr 1999). Higher CEC may lead bentonite to react more rapidly with a cationic polymer like chitosan while kaolinite reacts slowly, as observed in this study. Higher CEC may also improve coagulation by causing bentonite to attach more effectively to the positively charged chitosan polymer. Bentonite, as an expandable 2:1 clay, also has a higher specific surface area (40–800 m^2/g) than kaolinite (5–40 m^2/g), a 1:1 non-expanding clay (Kahr & Madsen 1995; Meier & Kahr 1999), resulting in greater surface area available for interactions with chitosan polymers. Both the larger surface area and larger surface charge of bentonite compared to kaolinite (Zhang *et al.* 2002) could lead to greater interaction with positively charged chitosan polymers. Visual observation of coagulation flocculation in this study indicated that the reaction of bentonite with chitosan was rapid; flocs were large and they formed and settled quickly (Roussy *et al.* 2005). Kaolinite reacted more slowly with chitosan and produced smaller flocs at the end of the slow mixing period. The more rapid reaction and larger flocs would favor increased removal by sedimentation.

In the coagulation process, doses of a coagulant influence colloid destabilization, a process in which the energy barrier of colloids is reduced so colloids become less stable as the repulsion forces between them decrease, and they are therefore easier to aggregate and physically remove from water. The optimum dose is the lowest dose at which maximum destabilization of colloids occurs, resulting in colloid aggregation and settling. At doses exceeding the optimum, excess coagulant polymers will surround the colloids, causing reversal of electrical charge around them, preventing destabilization and possibly inducing re-stabilization as particles repel each other again (Faust & Aly 1998). From the zeta potential titration graphs, negative zeta potentials of turbidity suspensions decreased in magnitude as positively charged chitosans were added, and charges approached zero as the solution came closer to the PZC. Different chitosans had their PZC at slightly different doses, but all were close to the dose of 3 mg/L observed in jar test coagulation experiments. Above the 3 mg/L dose and above the PZC, turbidity reduction decreased. This may be due to positive charges from surplus chitosan saturating

the clay surfaces, causing reversal of charges and re-stabilization (Huang & Chen 1996; Hu *et al.* 2013).

Coagulation-flocculation can work via multiple mechanisms; depending on the situation, they may occur together, or one mechanism may dominate over others (Bratby 2006). Mechanisms include charge neutralization (state at which the net electrical charge of a colloidal particle is neutralized by the polymer, resulting from association with an equal number of opposite charges), electrostatic patch (state where the particle surface charges are not completely neutralized having both positive and negative regions), and interparticle bridging (where polymers attach to multiple particles and form 'bridges' between particles by extending distances longer than the range of the electrical repulsive forces). The predominant mechanism can be influenced by the properties of the colloid, the surrounding solution, and the polymer (Guibal *et al.* 2006). In this study, nearly all chitosan stock solutions before addition to test water had pH 3.5–4.5, where amine groups would be positively charged. It has been previously hypothesized that for chitosan, charge neutralization predominates at acidic pH, and interparticle bridging at neutral or mild basic pH (Roussy *et al.* 2004). However, it is unlikely that charge neutralization or bridging alone accounts entirely for observed coagulant behavior.

MW 5,000 Da was ineffective at low doses, but reduction improved at 30 mg/L, possibly because the higher dose provided sufficient positive charges to reduce the energy barrier of the colloids to achieve destabilization. Bentonite and kaolinite turbidity reductions at maximum were only 46.2% and 79.8%, respectively, even though the net zeta potential of the coagulation system showed negative values at all doses tested. However, the fact that the zeta potential of the solutions measured was zero at the optimal dose suggests that charge neutralization is taking place (Yang *et al.* 2016). Other investigators have examined the CEC of bentonite and found that effective coagulation occurred even though the positive charges from chitosan were much less than the negative charges of bentonite, and therefore not enough to produce complete charge neutralization (Roussy *et al.* 2005; Chatterjee *et al.* 2009). Charge neutralization is also stoichiometric and susceptible to overdose as coagulant amount increases. Therefore, it can be inferred that charge neutralization is a contributing but

not primary mechanism for coagulation by chitosans in water at near neutral pH.

In a system where a charged coagulant is applied to dispersed particles of opposite charge, the bridging model may explain part but not all of coagulant behavior (Bratby 2006). In this study, MW influenced turbidity reduction more than DD, consistent with previous studies (Huang *et al.* 2000; Chen & Chung 2011). Interparticle bridging needs high MW polymers (Faust & Aly 1998; Yang *et al.* 2016); in this study and others, larger MW chitosans gave higher turbidity reduction than lower MW chitosans (Roussy *et al.* 2004; Chen & Chung 2011). Chen & Chung (2011) observed the highest bentonite reduction (92%) by 300,000 Da at doses similar to this study (2.5 mg/L), and poor reduction by lower MW (27,000 Da) polymers. However, the lack of a definite trend of increased removal with increased MW suggests that interparticle bridging is a contributing but not primary mechanism for bentonite and kaolinite turbidity reduction at near-neutral pH.

The charge neutralization and interparticle bridging mechanisms do not account entirely for some aspects of observed coagulant behavior in this study, such as the lack of chitosan effectiveness at low MW, and the fact that increasingly higher MW chitosans were not consistently and significantly better (which might be observed if bridging were the predominant mechanism). The electrostatic patch model may better explain some of the actions of chitosans observed in this study, since this model requires neither bridging nor complete charge neutralization (Gregory 2005). In the patch mechanism, multiple sections of the polymer adsorb onto multiple sites on the colloid. The result is a particle with a 'charge mosaic' of positively and negatively charged patches. When these positive and negative charge patches on particles align and attract each other, destabilization occurs with particle attachment (Bratby 2006). Furthermore, the variations in charge on the different surfaces of both kaolinite and bentonite particles are more consistent with the patch mechanisms, as the different surfaces of the clay particles themselves have charge mosaics. The patch mechanism may help explain the lack of effectiveness at low MW chitosan (where polymers are too small to create mosaic patches large enough to be effective), why higher DD chitosans did not perform better despite having more positive charge per gram (since the additional positive

charges only created the same sized positively charged patches), and why higher MW chitosans did not outperform the lower MW products between 50,000 and 1,000,000 as bridging theory would suggest. Higher MW products might form larger patches on the particle surface, but not form a greater number of patches; the number and position of patches is what determines how particles attach to each other. While this project was not designed to specifically test and compare coagulation mechanisms, it seems that a patch mechanism model may better explain the results from this study.

Chitosan polymers appear to be promising candidates for turbidity reduction from water at low doses if their coagulation-flocculation ability is combined with sedimentation and/or filtration. Comparison with metal salt coagulants suggests that chitosan can produce turbidity reductions similar to those observed for metal salt coagulants under optimized conditions. Alum flocculation of kaolinite could reduce kaolinite turbidity by 80–90% at neutral pH (Black & Hannah 1961). One study showed that chitosan achieved lower turbidity reductions than metal salt coagulants at neutral pH (Rizzo *et al.* 2008a), but turbidity removal by chitosans was lower overall (40%) than observed in this study. Chitosans could also produce residual turbidity similar to metal salts (Budd *et al.* 2004). Potentially more relevant to POU applications is the work of Preston *et al.* (2010) which evaluated POU coagulation of drinking water in Kenya using alum that could be purchased readily at local markets as a solid and ground into powder. This application of alum showed lower turbidity removal than chitosan in our study. Chitosan coagulation-flocculation has the potential to improve the appearance of water and microbial quality by increasing the effectiveness downstream treatment processes such as filtration (Eikebrokk & Saltnes 2002; Brown & Emelko 2009; Abebe *et al.* 2016). These molecules may be practical alternatives to conventional metal salt coagulants for use in low-resource and point-of-use treatment settings.

CONCLUSIONS

- Chitosan polymer MW affected bentonite and kaolinite turbidity reduction, with higher MW more effective than lower MW.

- DD of chitosans had less impact on bentonite and kaolinite turbidity removal than did MW.
- Low doses of chitosan (1–10 mg/L) were effective for removing up to 93% of kaolinite and 99% of bentonite turbidity.
- Of the doses tested, 3 mg/L gave the highest removal of both bentonite and kaolinite turbidity.
- Acid-soluble chitosans were as effective as water-soluble chitosans for bentonite and kaolinite turbidity removal.
- The optimum dose range for effective bentonite and kaolinite turbidity removal was similar for acid- and water-soluble chitosans.
- Interparticle bridging and charge neutralization played a role in bentonite and kaolinite turbidity coagulation, but the electrostatic patch model may explain observed coagulation behavior.
- Measured points of zero charge of chitosans during bentonite and kaolinite turbidity coagulation were close to the optimum chitosan doses obtained from jar test experiments.
- Chitosans have the potential to serve as effective alternative coagulants for the removal of turbidity from water.
- Bentonite, a 2:1 clay, and kaolinite, a 1:1 clay, responded somewhat differently to chitosan coagulation-flocculation and sedimentation for turbidity reduction, perhaps due to their differences in structure, surface charge distribution, and reactivity with water and dissolved ions in water.

ACKNOWLEDGEMENTS

REFERENCES

Abebe, L. S., Chen, X. & Sobsey, M. D. 2016 Chitosan coagulation to improve microbial and turbidity removal by ceramic water filtration for household drinking water treatment. *International Journal of Environmental Research and Public Health* **13** (3), 269.

Black, A. & Hannah, S. A. 1961 Electrophoretic studies of turbidity removal by coagulation with aluminum sulfate. *Journal of the American Water Works Association* **53** (4), 438–452.

Bratby, J. 2006 *Coagulation and Flocculation in Water and Wastewater Treatment*. IWA Publishing, London.

Brown, T. J. & Emelko, M. B. 2009 Chitosan and metal salt coagulant impacts on Cryptosporidium and microsphere removal by filtration. *Water Research* **43** (2), 331–338.

Budd, G. C., Hess, A. F., Shorney-Darby, H., Neemann, J. J., Spencer, C. M., Bellamy, J. D. & Hargette, P. H. 2004 Coagulation applications for new treatment goals. *Journal of the American Water Works Association* **96** (2), 102–113.

Chatterjee, T., Chatterjee, S. & Woo, S. H. 2009 Enhanced coagulation of bentonite particles in water by a modified chitosan biopolymer. *Chemical Engineering Journal* **148** (2), 414–419.

Chen, C.-Y. & Chung, Y.-C. 2011 Comparison of acid-soluble and water-soluble chitosan as coagulants in removing bentonite suspensions. *Water, Air, & Soil Pollution* **217** (1–4), 603–610.

Chi, F. H. & Cheng, W. P. 2006 Use of chitosan as coagulant to treat wastewater from milk processing plant. *Journal of Polymers and the Environment* **14** (4), 411–417.

Divakaran, R. & Pillai, V. S. 2001 Flocculation of kaolinite suspensions in water by chitosan. *Water Research* **35** (16), 3904–3908.

Eikebrokk, B. & Saltnes, T. 2002 NOM removal from drinking water by chitosan coagulation and filtration through lightweight expanded clay aggregate filters. *Journal of Water Supply: Research and Technology-Aqua* **51** (6), 323–332.

Faust, S. D. & Aly, O. M. 1998 *Chemistry of Water Treatment*. CRC Press, Boca Raton, FL.

Gregory, J. 2005 *Particles in Water: Properties and Processes*. CRC Press, Boca Raton, FL.

Guibal, E., Van Vooren, M., Dempsey, B. A. & Roussy, J. 2006 A review of the use of chitosan for the removal of particulate and dissolved contaminants. *Separation Science and Technology* **41** (11), 2487–2514.

Hu, C.-Y., Lo, S.-L., Chang, C.-L., Chen, F.-L., Wu, Y.-D. & Ma, J.-l. 2013 Treatment of highly turbid water using chitosan and aluminum salts. *Separation and Purification Technology* **104**, 322–326.

Huang, C. & Chen, Y. 1996 Coagulation of colloidal particles in water by chitosan. *Journal of Chemical Technology and Biotechnology* **66** (3), 227–232.

Huang, C., Chen, S. & Pan, J. R. 2000 Optimal condition for modification of chitosan: a biopolymer for coagulation of colloidal particles. *Water Research* **34** (3), 1057–1062.

Kahr, G. & Madsen, F. 1995 Determination of the cation exchange capacity and the surface area of bentonite, illite and kaolinite by methylene blue adsorption. *Applied Clay Science* **9** (5), 327–336.

Meier, L. & Kahr, G. 1999 Determination of the cation exchange capacity (CEC) of clay minerals using the complexes of copper (II) ion with triethylenetetramine and tetraethylenepentamine. *Clays and Clay Minerals* **47**, 386–388.

NSF International 2008 *NSF Protocol P248 Emergency Military Operations*. Microbiological Water Purifiers, Ann Arbor, MI.

Pan, J. R., Huang, C., Chen, S. & Chung, Y.-C. 1999 Evaluation of a modified chitosan biopolymer for coagulation of colloidal particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **147** (3), 359–364.

Pontius, F. W. 2016 Chitosan as a drinking water treatment coagulant. *American Journal of Civil Engineering* **4** (5), 205–215.

Preston, K., Lantagne, D., Kotlarz, N. & Jellison, K. 2010 Turbidity and chlorine demand reduction using alum and moringa flocculation before household chlorination in developing countries. *Journal of Water and Health* **8** (1), 60–70.

Renault, F., Sancey, B., Badot, P.-M. & Crini, G. 2009 Chitosan for coagulation/flocculation processes – an eco-friendly approach. *European Polymer Journal* **45** (5), 1337–1348.

Rizzo, L., Di Gennaro, A., Gallo, M. & Belgiorno, V. 2008a Coagulation/chlorination of surface water: a comparison between chitosan and metal salts. *Separation and Purification Technology* **62** (1), 79–85.

Rizzo, L., Lofrano, G., Grassi, M. & Belgiorno, V. 2008b Pre-treatment of olive mill wastewater by chitosan coagulation and advanced oxidation processes. *Separation and Purification Technology* **63** (3), 648–653.

Roussy, J., Van Vooren, M. & Guibal, E. 2004 Chitosan for the coagulation and flocculation of mineral colloids. *Journal of Dispersion Science and Technology* **25** (5), 663–677.

Roussy, J., Van Vooren, M., Dempsey, B. A. & Guibal, E. 2005 Influence of chitosan characteristics on the coagulation and the flocculation of bentonite suspensions. *Water Research* **39** (14), 3247–3258.

United States Environmental Protection Agency 1987 *Guide Standard and Protocol for Testing Microbiological Water Purifiers*. Registration Division, O.o.P.P., Criteria and Standards Division, Office of Drinking Water, USEPA, Washington, DC.

United States Environmental Protection Agency 2012 *Title 40 – Protection of Environment, Section 141.73 – Filtration*. United States Government, USEPA, Washington, DC.

WHO 2008 *Guidelines for Drinking-Water Quality*. World Health Organization, Geneva.

World Health Organization 2011 *Guidelines for Drinking-Water Quality*. World Health Organization, Geneva.

Yang, R., Li, H., Huang, M., Yang, H. & Li, A. 2016 A review on chitosan-based flocculants and their applications in water treatment. *Water Research* **95**, 59–89.

Zhang, Y., Nakamura, F., Sakamoto, Y. & Nishida, K. 2002 Coagulation behaviors and coagulant dosage control of kaolin and bentonite suspensions. *Environmental Engineering Research* **39**, 1–7.