

Comparison of the Performance of Chitosan and a Cationic Polyacrylamide as Flocculants of Emulsion Systems

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ABSTRACT: Food industries contribute significantly to the pollution of water streams. Oil in wastewaters must be removed to prevent fouling of process equipment, to reduce interference with subsequent water-treatment units, and to comply with water-discharge requirements. Polyelectrolytes are frequently used to coagulate and flocculate colloidal systems. A model system of a sunflower oil/water emulsion was used to analyze the effect of: (i) emulsion droplet sizes, (ii) surfactant chain length (sodium dodecyl sulfate and sodium tetradecyl sulfate), (iii) ionic strength, and (iv) pH, on the doses of chitosan (natural cationic polyelectrolyte) and a cationic polyacrylamide, necessary to neutralize electrical charge and to get flocculation. Methods used were: turbidimetry, jar test, colloidal titration, and light microscopy observation. Results showed that the increase of NaCl concentration reduces the doses of chitosan and polyacrylamide to reach zero colloidal charge; these doses increase with the surfactant chain length. pH variation did not show any influence on the chitosan dose necessary to flocculate the system. Redispersion of the emulsion was observed in chitosan and polyacrylamide treatments. Paper no. S1188 in *JSD* 4, 57–63 (January 2001).

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Food industries contribute significantly to the pollution of water streams, due to the large amounts of wastewater generated during food processing and to the important biological activity associated with this kind of wastewater. Besides, oil in wastewaters must be removed to prevent fouling of process equipment, to reduce interference with subsequent water-treatment units, and to comply with water-discharge requirements. Wastewaters with high soluble organic loads or fine suspensions (colloid systems) are difficult to deal with. Fats and oils can block filters by continuous accumulation with time.

When discharged into water streams, emulsified wastewaters undergo incomplete microbial degradation. Variability and high concentrations of food wastewaters limit the application of standard biological treatments. Polyelectrolyte treatments have been shown to reduce the strength of processing wastewaters from red peppers (1), canned vegetables (2), potatoes (3), beans (4), and recovery of organic

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compounds from crustaceous wastes (5) and poultry (6). Information about the effectivity of polyelectrolytes on emulsified wastes is scarce (7).

Natural and synthetic polyelectrolytes, such as chitosan and polyacrylamide, are typical polymeric flocculants. Chitosan is a high molecular weight carbohydrate polymer manufactured from chitin. It is a natural cationic polyelectrolyte formed by *N*-acetyl-D-glucosamine units with β (1–4) glycosidic bounds (8).

Chitosan owes its cationic nature to the free amino groups obtained by removing some of the acetyl groups of chitin (9). Chitosan is a linear polyelectrolyte at acidic pH and it has a high charge density, one charge per each glucosamine unit. It is an excellent flocculant due to its high number of $-\text{NH}_3^+$ groups that can interact with negatively charged colloids and it forms complexes with many metal ions (10).

Acrylamide is a crystalline, relatively stable monomer that is soluble in water and in many organic solvents (11). It undergoes polymerization by conventional free-radical methods, but can also be polymerized photochemically. All current industrial production is believed to be free-radical polymerization. The pH of the reaction medium is also important, since hydrolysis of amide groups can take place at high pH, whereas imidization is favored at low pH and high temperature. By far the greatest current interest is in those polyacrylamides having very high molecular weights ($>5 \cdot 10^6$).

The purpose of the present work was to explore the use of natural and synthetic polyelectrolytes (chitosan and polyacrylamide) as conditioning chemicals for emulsion systems analyzing the effect of different factors such as: droplet sizes of the emulsion, surfactant and oil concentration ratios, surfactant chain length, NaCl concentration, and pH on the optimal doses of the polyelectrolytes.

MATERIALS AND METHODS

The model system of the food waste consisted of sunflower oil/water emulsions (1,333 and 5,000 ppm oil) and NaCl. Stabilizing solutions of sodium dodecyl sulfate (SDS; MW 288.36) or sodium tetradecyl sulfate (STS; MW 316.44) were added as surfactants. The emulsions were prepared in a colloidal mill (AD-35 R; Colmil, Buenos Aires, Argentina), at

maximum speed with different stirring times to analyze the effect of the emulsion droplet size.

The emulsions were formulated considering the following variables: (i) NaCl concentration from 1 to 100 mM, in order to study ionic strength; (ii) two different surfactants were used: SDS and STS to analyze the chain length effect at a constant concentration (200 ppm) and the ratio between the concentrations of surfactant to sunflower oil; (iii) different agitation times were used during emulsion preparation (15, 7.50, and 3.75 min) to analyze the effect of the emulsion droplet size, and (iv) emulsion pH, was modified adding either NaOH or HCl ranging between 4 and 8.

Chitosan (practical grade obtained from crab shells with a degree of deacetylation 77%; Sigma, St. Louis, MO), and a cationic polyacrylamide of high molecular weight (MW 4.10^6 , Cytec Argentina S.A.) were used as coagulant and flocculant agents. Chitosan solutions (5,000 ppm) were prepared by dissolving chitosan in 1% (vol/vol) acetic acid solution (5) during continuous agitation for several hours, followed by a vacuum filtration to eliminate insolubles. Cationic polyacrylamide (1,000 ppm) was prepared by dissolving the polyacrylamide in distilled water. The flocculation experiments were carried out by adding the desired amount of chitosan or polyacrylamide to the emulsion with continuous agitation using a magnetic stirrer (IKAMAG RCT basic; IKA Laborotechnic, Germany).

The presence of surfactant (SDS or STS) produced negatively charged emulsion. To analyze the flocculation process, different techniques were used: colloid titration, jar test, turbidimetric method, and microscopy observation.

Turbidimetry was measured by a La Motte Turbidimeter, 2008 model (Chestertown, MD) and pH was measured with an electronic pH meter (Model 50100; Hach, EC30 Benchtop, Loveland, CO).

The colloid titration determines the colloidal charge and the isoelectric point of the system (12,13). To determine the concentration of the negative emulsion, a known excess amount of methyl glycol chitosan (MGC; Sigma product number M3150) was added in each test (aqueous solution concentration 0.2094 g/L). The oppositely charged colloids react very nearly stoichiometrically and are neutralized. MGC is a cationic polysaccharide that acts as a positively charged titrant over the entire pH range. The remaining excess of MGC is back-titrated by potassium polyvinyl alcohol sulfate (PVSK; Sigma product number P6000, solution concentration 0.081 g/L), using toluidine blue (TB; Sigma product number T3260) as an indicator. PVSK has a $-\text{SO}_4\text{H}$ functional group that is ionized to $-\text{SO}_4^-$, with the production of a negative colloid (12). MGC and TB were added to emulsion samples during agitation on a magnetic stirrer. Titration was carried out with 10^{-5} eq/L PVSK.

Flocculation assays were performed using the jar test (Decalab, Buenos Aires, Argentina) with six stirrers having a maximum speed of 250 rpm. Different amounts of polyelectrolyte were added to 500-mL aliquots. Samples were stirred at high speed for 3 min and then at 50 rpm for 10 min.

Microscopic observations were done on the emulsions

and on the flocs with a Leitz Ortholux II microscope having a built-in-camera (Leitz, Germany). Micrographs of the emulsions and the formed flocs were obtained using light microscopy. A shutter speed of 1/1,000 s and fast film (ASA 400) were used to avoid microscale movements and distortion effect in the sample. Enough micrographs were digitized to measure droplet sizes with an image analyzer software (Global Lab Image 2.10; Data Translation Inc., MA). Mean, minimum, and maximum radius of droplets were determined on micrographs corresponding to emulsions prepared with different agitation times.

Flocs were also observed with an environmental scanning electron microscope (ESEM 2010; Phillips, The Netherlands) which retains all the advantages of a conventional scanning electron microscope, but removes the high vacuum constraint of the sample environment; samples may be examined in their natural state without modification or preparation and this method is especially designed to observe specimens that must remain hydrated in order to retain their structure.

Statistical analysis. Assays were performed in duplicate and analyses of all samples were performed on the duplicates and averaged. Statistical analyses were carried out using SYSTAT (Version 5.0) software package. Analyses of variance (ANOVA) were performed. Significant differences between means were determined by least significant difference test and were defined at $P < 0.05$.

RESULTS AND DISCUSSION

Influence of droplet size. Emulsions 1 mM NaCl, containing 5,000 ppm oil and 200 ppm SDS were used to analyze the influence of droplet size on the dose of destabilizer necessary to produce flocculation. Emulsions obtained in the colloidal mill showed that as stirring times decreased (15, 7.5, 3.75 min) droplet diameters increased and area/volume ratio (3/R) decreased. Droplet area, minimum, maximum, and mean diameters corresponding to different emulsifying times were obtained from digitized images of the emulsions (Table 1). Initial charge (determined by colloidal titration), and initial turbidity were evaluated for each sample (Table 2). Minimum turbidity was not significantly different for the tested cases; however, the flocs were less compact as initial turbidity decreased.

Lower values of initial colloidal charge were obtained for emulsions with higher droplet sizes due to their lower

TABLE 1
Effect of Stirring Times on Mean, Minimum, and Maximum Radius and Total Area of Droplets in Emulsions Containing 5,000 ppm Sunflower Oil, 1 mM NaCl, and 200 ppm Sodium Dodecyl Sulfate (SDS)

Stirring time (min)	Mean radius (mm)	Maximum radius (mm)	Minimum radius (mm)	Total area (mm ²)
15	0.00248	0.00319	0.00158	$1.86 \cdot 10^{-5}$
7.5	0.00331	0.00407	0.00206	$6.77 \cdot 10^{-5}$
3.75	0.00519	0.00602	0.00405	$1.13 \cdot 10^{-4}$

TABLE 2
Effect of Droplet Radius on Colloidal Charge, Minimum, and Initial Turbidity (corresponding to the addition of doses of polyacrylamide and chitosan necessary to reach zero colloidal charge) in Emulsions Containing 5,000 ppm Sunflower Oil, 1 mM NaCl, and 200 ppm SDS^a

Droplet mean radius (mm)	Initial turbidity (NTU)	Initial colloidal charge (mequiv/L)·10 ³	Polyacrylamide		Chitosan	
			Zero colloidal charge dose (ppm)	Minimum turbidity (NTU)	Zero colloidal charge dose (ppm)	Minimum turbidity (NTU)
0.00248	3998	-1.24	120	35	65	66
0.00331	2947	-0.86	100	23	43	24
0.00519	2370	-0.78	85	12	36	26

^aSee Table 1 for abbreviation.

area/volume ratio. Considering that coagulation and flocculation are surface related phenomena, a decrease in area to volume ratio decreases the number of available sites for polyelectrolytes, making the probability for bridge formation lower.

The doses to reverse the sign of the colloidal charge decreased for both polyelectrolytes as the mean droplet diameter increased. In all cases, the reached minimum turbidities were lower than 2% of the initial ones (Table 2). A direct relationship between the interfacial area of the colloidal system and the amount of chitosan and polyacrylamide, required to obtain optimum destabilization, was observed. Differences between destabilizer doses to reverse the sign of the colloidal charge were significant ($P < 0.05$), being the doses of polyacrylamide higher than those of chitosan. These results could be attributed to the higher charge density of chitosan in respect to the used polyacrylamide. Charge density rates of these destabilizers (measured by titration of their electrical charge with PVS_K) was 1.64.

Influence of surfactant and oil concentration. The influence of oil and surfactant concentrations on polyelectrolyte doses was analyzed using a factorial experimental design with two variables (oil and surfactant concentration) at two levels (2 × 2). In all cases NaCl concentration was maintained at 1 mM. Two concentrations (50 and 200 ppm) of the SDS emulsifier were tested; oil concentrations were maintained at two levels: 1,333 and 5,000 ppm (Table 3).

Figures 1 and 2 show colloidal charge and turbidity curves as functions of polyacrylamide and chitosan doses,

respectively. A sharp relationship between colloidal charge and turbidity was observed; while the emulsion colloidal charge was close to zero, turbidity remained at the minimum values. In all the tested cases the application of polyelectrolyte doses higher than those giving the minimum turbidity led to emulsion restabilization and to an abrupt turbidity increase. This emulsion restabilization was accompanied by a reversal of the charge of the colloidal particles. Results agree with several works (4,9,14,15) that reported

TABLE 3
Polyacrylamide and Chitosan Doses Necessary to Reach Zero Colloidal Charge (optimum dose) in Emulsions Containing 1 mM NaCl and Formulated Following a (2 × 2) Factorial Design (factors were oil and SDS concentrations each one at two levels)^a

Oil concentration (ppm)	SDS concentration (ppm)					
	Initial colloidal charge (mequiv/L)·10 ³		Optimum dose of polyacrylamide (ppm)		Optimum dose of chitosan (ppm)	
	50	200	50	200	50	200
1,333	-0.21	-0.65	27	117	3.5	28
5,000	-0.45	-1.24	28	120	7.5	65

^aSee Table 1 for abbreviation.

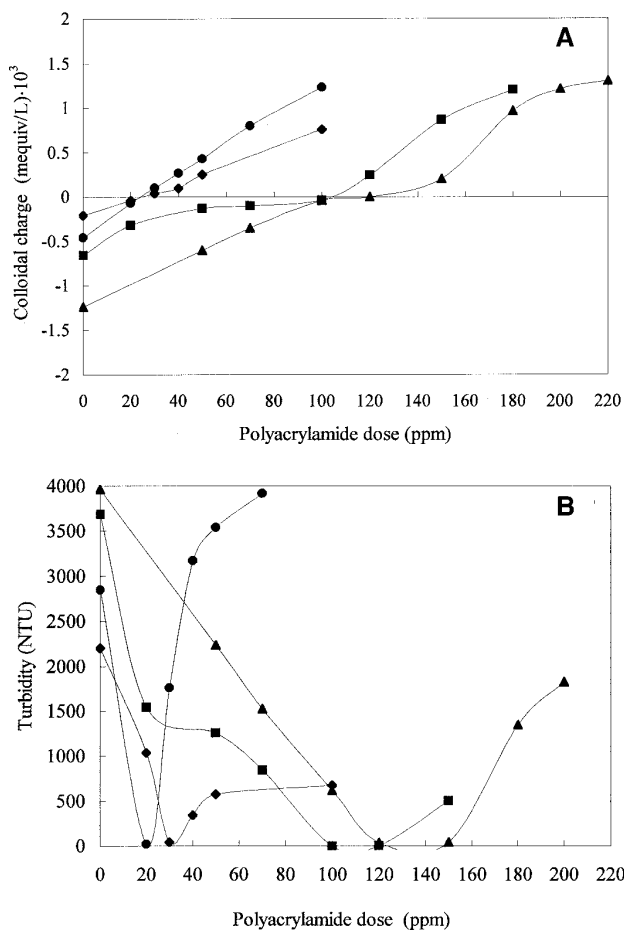


FIG. 1. Colloidal charge (A) and turbidity (B) as a function of polyacrylamide dose, (▲) 5,000 ppm oil and 200 ppm sodium dodecyl sulfate (SDS), (■) 1,333 ppm oil and 200 ppm SDS, (●) 5,000 ppm oil and 50 ppm SDS, (◆) 1,333 ppm oil and 50 ppm SDS.

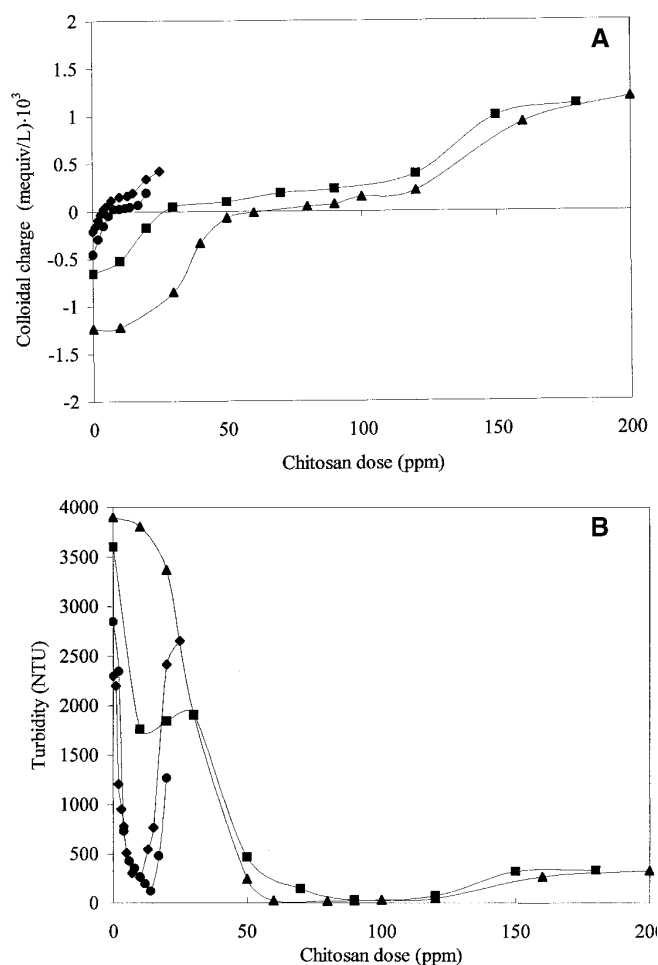


FIG. 2. Colloidal charge (A) and turbidity (B) as a function of chitosan dose, (▲) 5,000 ppm oil and 200 ppm SDS, (■) 1,333 ppm oil and 200 ppm SDS, (●) 5,000 ppm oil and 50 ppm SDS, (◆) 1,333 ppm oil and 50 ppm SDS. See Figure 1 for abbreviation.

restabilization of colloid suspensions due to excessive treatment with polyelectrolytes.

Charge reversal cannot be explained by the Gouy-Chapman model for the double layer because purely coulombic attraction will not permit an attraction of counter-ions in excess of the original surface charge of the colloid. It is

therefore necessary to consider additional energy terms arising from specific chemical interactions in order to explain this phenomenon (16).

Nonsignificant differences in the doses of polyacrylamide ($P > 0.05$) to obtain zero charge points were observed for emulsions that had the same surfactant concentration but different oil content (Table 3). In the case of chitosan, the differences in the doses were significant ($P < 0.05$). A linear relationship between the optimum dose of chitosan and the initial emulsion charge was obtained, indicating that the dominant mechanism was charge neutralization rather than bridging; however, in the case of polyacrylamide, this linear relationship was not observed.

Axberg *et al.* (7) found that the main part of the added polyelectrolyte reacts with the surfactant dissolved in the aqueous phase and only a small amount of the surfactant is associated with the emulsion droplets.

For cases in which the polymer and the adsorption site are of opposite sign, it has been postulated (17) that the polymer assumes a flat configuration on the colloidal surface, rather than a configuration with many segments extending into the solution. Electron paramagnetic resonance has been applied to polymer colloid systems to determine polyelectrolyte configuration on colloidal surfaces (17); this technique has shown that polyelectrolyte segments often adsorb primarily in trains, rather than in the loop configuration. However, according to the bridging mechanisms, without a significant number of loops extending away from the polymer, it is impossible for particles to flocculate. These evidences would confirm that charge neutralization is one of the main mechanisms in polyelectrolyte treatment.

Influence of the surfactant chain length and NaCl concentration. To study the influence of surfactant chain length, the behavior of emulsions 1, 10, and 100 mM NaCl formulated with 200 ppm SDS or STS and 5,000 ppm sunflower oil was analyzed (Table 4). Emulsions 1 mM NaCl prepared with 200 ppm SDS needed 120 ppm polyacrylamide to reach zero colloidal charge; for a similar emulsion containing 200 ppm STS, a dose of polyacrylamide of 165 ppm was necessary to reach zero colloidal charge. Redispersion was observed with doses above 150 and 200 ppm polyacrylamide for emulsions formulated with SDS and STS, respectively.

TABLE 4
Initial Colloidal Charge of Emulsions Containing Different Concentrations of NaCl (1, 10, and 100 mM) and Doses of Chitosan and Polyacrylamide to Reach Zero Colloidal Charge in Systems Formulated with 5,000 ppm Oil and 200 ppm SDS or Sodium Tetradecyl Sulfate (STS)

NaCl concentration (mM)	Surfactant	Initial colloidal charge (mequiv/L) · 10 ³	Zero colloidal charge dose (ppm)	
			Polyacrylamide	Chitosan
1	SDS	-1.24	120	65
	STS	-1.71	165	94
10	SDS	-1.08	105	48
	STS	-1.31	148	60
100	SDS	-0.76	83	33
	STS	-0.83	125	36

Table 4 shows that the polyacrylamide dose necessary to reverse the sign of the colloidal charge was lower for emulsions with SDS than for those with STS in the range of 1 to 100 mM NaCl.

Doses of chitosan were proportional to the initial emulsion colloidal charge; however, when polyacrylamide was applied this linear relationship was not observed. In the case of chitosan treatment, emulsions containing STS also showed a higher affinity for the natural polyelectrolyte, evidenced by the higher chitosan dose necessary to get zero colloidal charge compared to SDS emulsions.

The higher dose necessary to reach zero colloidal charge in STS emulsions could be attributed to the higher bonding energy between STS and the polyelectrolyte in comparison to that of SDS. The reaction between the surfactant and both polyelectrolytes (chitosan and polyacrylamide) is not purely ionic. In that situation, a constant ratio independent of hydrocarbon chain length would be observed; however, this ratio increases with chain length. To analyze the interaction between the surfactant and chitosan in the absence of oil, aqueous solutions containing 200 and 50 ppm SDS (without oil) and 1 mM NaCl were prepared. Increasing chitosan doses were tested; solutions became turbid at 30 and 10 ppm chitosan, respectively. These results were attributed to the bonding of the free surfactant to the polyelectrolyte, thus, polyelectrolyte neutralizes the charges of both the solubilized surfactant and the surfactant that is adsorbed on the surface of the droplets surface.

The effect of the ionic strength was analyzed in systems with different NaCl concentrations and the necessary doses of chitosan and polyacrylamide to reach zero colloidal charge are also shown in Table 4. Chitosan and polyacrylamide doses necessary to produce emulsion destabilization decreased as NaCl increased for the tested concentrations.

Of particular importance in colloid science is the fact that the thickness of the double layer depends markedly on the ionic concentration. As the salt concentration increases, the thickness of the double layer decreases rapidly, entering in the zone of intermolecular range where van der Waals forces are predominant and agglomeration of the particles is produced. Thus, in an aqueous solution of a 1:1 electrolyte at 25°C the values of $1/\kappa$ are: at 0.1 mM, 31.4 nm; at 1 mM, 9.6 nm; at 10 mM, 3.0 nm; and at 100 mM, 0.96 nm, $1/\kappa$ being the thickness of the double layer, identical to the parameter of the Debye-Hückel theory of electrolytes in which $1/\kappa$ is identified to the radius of the ionic atmosphere (18).

Influence of pH. An emulsion with 200 ppm SDS, 5,000 ppm sunflower oil, and 1mM NaCl was used to analyze pH effect by adding HCl or NaOH. The pH of the original emulsion (control) was 5.8.

An emulsion of pH 3.5 and the control emulsion of pH 5.8 were treated with increasing polyacrylamide concentrations (50–200 ppm); minimum turbidity (1.6 NTU) was attained with 150 ppm of polyacrylamide. The emulsion was still clear with 180 ppm polyacrylamide addition; however, higher doses of the polymer produced restabilization of the system and turbidity increased.

In another experiment the control emulsion was brought to pH 8 with NaOH and doses of polyacrylamide ranging between 70 and 300 ppm were added. An important decrease in turbidity values was obtained with 180 ppm and this situation was maintained up to 250 ppm; pH was not a significant factor for the minimum turbidity values reached in polyacrylamide treatments. In all cases, the systems were clarified and then higher doses restabilized the emulsions; however, concentration ranges of polyacrylamide that produced flocculation varied with emulsion pH. For the control emulsion (pH 5.8) the range of polyacrylamide concentration that lead to minimum turbidity was 120–150 ppm, and for acid and alkaline pH the ranges were 150–180 ppm and 180–250 ppm, respectively. The range to reach minimum turbidity was shifted to higher polyacrylamide concentrations for both acid and alkaline emulsions, being wider the range for alkaline emulsions.

The analysis of pH effect on emulsions treated with chitosan showed that chitosan doses necessary to reverse the colloidal charge were 67, 65, 68, and 64 ppm for pH 4, 5.8, 7, and 8, respectively. These results indicated that pH has no significant effect ($P < 0.05$) on the optimum chitosan doses in the tested range.

Qualitative analysis of flocs. Flocs obtained by chitosan treatment were open, nonadhesive, and had good quality that allowed the filtration of the clarified liquid. However, flocs obtained by polyacrylamide treatment had a mucous aspect, being oily to the touch, they adhered to the vessel walls and were difficult to filtrate. Figure 3 shows the typical flocs observed by ESEM.

Time to reach minimum turbidity. Mechanisms of action. In the case of chitosan addition, turbidity reached low final values immediately once agitation was stopped, and no significant modifications in the turbidity values were observed with time. When polyacrylamide was added to the emulsions, turbidity decreased rapidly, but not instantaneously. The whole operation time for chitosan treatment was shorter than that for polyacrylamide.

Charge neutralization, can be considered, as the mechanism of flocculation by oppositely charged polymers; however, in some cases polymer bridging plays at least a minor role (19,20).

The results obtained in the present work show that charge neutralization in chitosan treatment is probably the most relevant mechanism; polyacrylamide is acting mainly by charge neutralization, although a bridging mechanism would be present as well.

The increase of surfactant chain length (STS respect to SDS) increased the doses necessary to produce destabilization and to reach zero charge for both polyelectrolytes. This implies that the hydrophobic part of the surfactant plays an important role in the interaction between surfactant and flocculant.

The application of polyelectrolyte (chitosan and polyacrylamide) in doses higher than those giving the minimum turbidity, led to emulsion restabilization and to an abrupt turbidity increase. A close relationship between colloidal

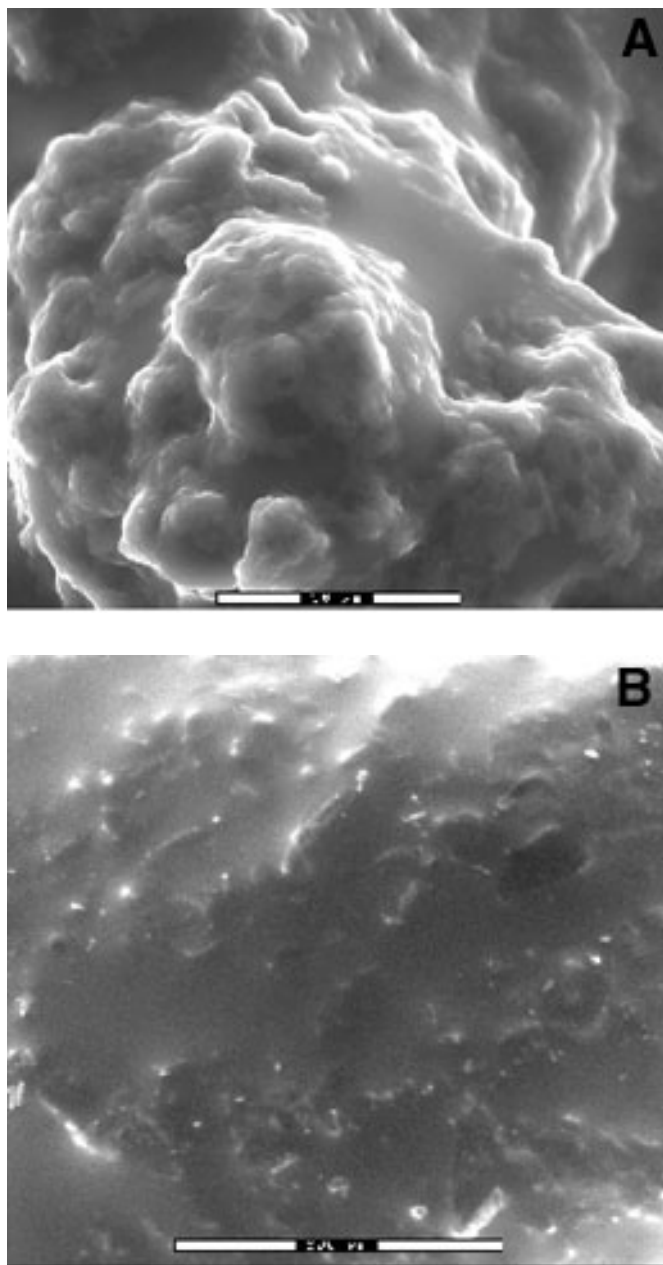


FIG. 3. Micrographs of flocs obtained by environmental scanning electron microscope: (A) chitosan (bar: 50 μm) and (B) polyacrylamide (bar: 200 μm).

charge and turbidity was observed; while the emulsion colloidal charge was near zero, turbidity remained at the minimum values. In all the tested cases, the emulsion restabilization was accompanied by a reversal of the colloidal charge.

The increase of NaCl concentration reduced both the doses of chitosan and polyacrylamide necessary to produce destabilization and flocculation. The system pH (range 4–8) showed no influence on the chitosan dose necessary to flocculate the emulsion. In the case of polyacrylamide, the range of doses to reach the minimum turbidity for alkaline emulsions was wider. For both treatments, the lower the ini-

tial oil and surfactant concentrations, the lower the floc cohesiveness.

Reducing agitation time and consequently dropping surface/volume ratio led to lower initial charges and lower doses of chitosan and polyacrylamide to reach flocculation. Emulsion treated with the optimum chitosan doses were clarified immediately, once the flocculant was added. Polyacrylamide produced a rapid turbidity decrease (but not instantaneous), when the flocculant was added. With regard to the mechanisms of action, charge neutralization is probably the most relevant for chitosan treatment; in the case of polyacrylamide, bridging mechanism, should be also considered.

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