

## Color removal by coagulation, flocculation and sedimentation from water containing humic substances with different apparent molecular sizes

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### ABSTRACT

Samples of water with true color of approximately 100 HU, prepared with humic substances of different molecular sizes (filtered through a 0.45  $\mu\text{m}$  membrane with weights in the range of > 100 kDa; 100 to 30 kDa; < 30 kDa) were studied in order to verify the influence of the apparent molecular size on the performance of coagulation, flocculation and sedimentation of flocs formed using aluminum sulfate as coagulant. To achieve the same degree of color removal, the water samples with higher apparent molecular size fractions required lower dosages of aluminum sulfate (approximately 50% of those necessary for the water prepared with the fraction of the smallest molecules). The results of nuclear magnetic resonance (NMR) and Infrared Spectroscopy (FTIR) studies demonstrate that, under the same conditions, the higher the number of oxygen-containing functional groups, the lower the level of color removal becomes.

**Key words** | apparent molecular size of humic substances, coagulation, color, flocculation, sedimentation

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### ABBREVIATIONS

EESCUSP: School of Engineering of São Carlos of  
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### INTRODUCTION

Until some decades ago, the removal of color from water was only performed for esthetic reasons, being recommended that did not exceed  $15 \text{ mg L}^{-1}$  on the platinum cobalt scale. Little was known about the factors that were responsible for color in natural waters and their possible harmful effects. With the development of this research field, it was noted that the compounds that were responsible for coloring superficial waters include different types of natural organic matter (NOM), produced in different environmental systems. NOM can be leached from soil, peat land, dispersal of sediments,

liberated from plankton, or from bacterial activity. (Thurman 1985; Buffle 1988; Hayes *et al.* 1989). A large part of NOM in superficial waters is composed of stable species, known as humic substances (HS), from various origins, which contribute from 50% to 70% of dissolved carbon in such waters (Aiken 1985).

Di Bernardo (1983) claims that for the majority of natural surface waters clay is the prime cause of turbidity and HS responsible for the coloration. The author also explains that when turbidity is high compared to color, the main mechanism of coagulation is the adsorption of the positive aluminum species by the colloidal particles. When water presents low turbidity compared to color, the clay particles become covered by HS and other organic compounds. Initially, the positive species and the precipitate resulting from the coagulant hydrolysis react with the dispersed HS and form particles that may settle. When the

HS are not efficiently removed during the steps of water treatment, they can cause several problems such as: i) serving as substrates for microorganism growth; ii) protecting some species of bacteria, which reduces the efficiency of the disinfection process; iii) complexing with heavy metals such as Fe, Mn, Pb and others, making their removal difficult; iv) provoking corrosion in piping; and v) producing substances with unpleasant taste and odor, some of which are toxic and potentially carcinogenic when pre-oxidation is performed using free chlorine.

Processes involving coagulation with aluminum salts (Dempsey *et al.* 1984), iron and polyelectrolytes, membrane (ultrafiltration, microfiltration, and nanofiltration), oxidation, biofiltration and sorption of HS in ion-exchange resins and activated carbon have been extensively studied as forms of HS removal in natural waters. However, few papers correlate the results obtained with the structural characteristics of HS (Odegaard *et al.* 1999).

The presence of the functional groups and higher or lower concentrations of aromatic chains may influence the efficiency of HS removal. This is also true when the great variation of molecular size and the proportion of fulvic and humic acids are considered (Campos *et al.* 2005; Edzwald & Benschoten 1990). The use of different analytical techniques (ultrafiltration, NMR, FTIR Spectroscopy, for example) is of great importance in the understanding of such properties. This understanding helps to explain the results obtained during water treatment by coagulation, flocculation and sedimentation.

The present work was performed with the objective of comparing the results acquired during coagulation with aluminum sulfate, flocculation and sedimentation tests, carried out on waters that presented the same level of color. The water samples were prepared with HS, extracted from peat, of different apparent molecular size and, consequently, different structural characteristics.

## MATERIAL AND METHODS

### Isolation of humic substances

The humic substances used to color the studied waters were extracted from peat samples collected in the margins of the Mogi Guaçu River, Brazil. After drying, the peat samples

were coarsely ground in an agate mortar and stored in a closed polyethylene vessel. The method of extraction of humic substances is described as follows: (a) 50 g of peat was added in one liter of KOH at 0.5 M concentration and stir for 4 h at room temperature ( $\sim 25^{\circ}\text{C}$ ) (b) the solution obtained was allowed to settle for 48 h, (c) the supernatant was stored in semi-permeable paper bags and submitted to dialysis in 0.1 N hydrochloric acid solution to eliminate chloride.

### Fractionation of HS

The extracted HS samples were diluted to form a solution of  $1.0\text{ mg ml}^{-1}$ . The solution was filtered firstly through a coarse membrane ( $3\ \mu\text{m}$ ) and afterwards using a membrane of  $0.45\ \mu\text{m}$  (Millipore). The samples were separated into different nominal molecular size fractions by using tangential ultrafiltration (Vivaflow 50, Sartorius group) with *polyethersulfone* membranes. A peristaltic pump provided continuous recirculation of fluid through a spiral thin-channel, producing laminar flow with minimal accumulation of solute on the membrane surface. The ultrafiltration method, applied to the fractionation of humic substances, was the concentration mode (Duarte *et al.* 2001). The flow was  $300\text{ ml min}^{-1}$  with a pressure of 1.5 bar. High flow is recommended to remove humic substances accumulated over membrane (Rocha & Rosa 2003). After 1 L of humic substances solution goes across the membrane it was washed with NaOH solution at concentration 0.5 M during 5 min. After that, the membrane was washed with 2 L of water organic free. The membrane was taken off after 12 L of filtrated material.

The following nominal cut off fractions were obtained:  $>100\text{ kDa}$ ,  $30\text{--}100\text{ kDa}$ ,  $<30\text{ kDa}$ . The transmembrane pressure was maintained at  $\sim 1.5\text{ atm}$  and flow rate of  $300\text{ ml min}^{-1}$  was applied. Samples of all fractions were freeze-dried for NMR and FTIR spectroscopic analyses.

### Fourier-transfer infrared (FTIR) analysis

FTIR spectra of humic substances were obtained for a wave number range of  $4,000\text{ to }1,000\text{ cm}^{-1}$  using a Bomem MB-102 spectrometer. The samples were prepared for analysis

by mixing 100 mg of KBr with about 1.0 mg of the material and then compressing the mixture to form pellets. Spectra resolution was  $4 \text{ cm}^{-1}$ .

### Solid-state nuclear magnetic resonance measurements

The NMR analyses were performed in a VARIAN unit INOVA spectrometer operating at 100 MHz and 400 MHz for  $^{13}\text{C}$  and  $^1\text{H}$ , respectively. A magic-angle spinning at 5 kHz was used in all experiments. Cross-polarization excitation using a radiofrequency ramp (rampCP/MAS) (Cook *et al.* 1996), combined with Total Suppression of Spinning Sidebands (TOSS) acquisition (Dixon *et al.* 1982) was used (Cook 2004). The rampCP/MAS/TOSS  $^{13}\text{C}$  signals were obtained with contact time of 1 ms, acquisition times of 15 ms, and recycle delays of 0.5 s.  $^1\text{H}$  broadband Two-Pulse Phase Modulation (TPPM) (Bennett *et al.* 1995) decoupling of 70 kHz was applied in all experiments.

### Coagulation, flocculation and sedimentation tests

In order to study the influence of each range of apparent molecular size on coagulation, flocculation and sedimentation, three water samples with color of approximately 100 HU were prepared using the three fractions of humic substances and water abstracted from a well on the university campus before chlorination (Water sample I

with HS fraction of apparent molecular size  $> 100 \text{ kDa}$ ; Water sample II with HS fraction of apparent molecular size between  $100 \text{ kDa}$  and  $30 \text{ kDa}$ ; Water sample III with HS fraction of apparent molecular size  $< 30 \text{ kDa}$ ). The main characteristics of the three samples studied are presented in Table 1.

Liquid aluminum sulfate with 7.28% of  $\text{Al}_2\text{O}_3$  and specific density of  $1.304 \text{ gL}^{-1}$ , with approximately  $652 \text{ gL}^{-1}$  of  $\text{Al}_2(\text{SO}_4)_3 \cdot 14.3 \text{ H}_2\text{O}$  was used as a coagulant. Solutions of NaOH (0.1 M) and HCl (0.1 M) were always used before the alum addition to vary the coagulation pH in the range of 4.0 to 8.5, in order to allow the construction of the coagulation diagram for each water sample (remaining apparent color as function of aluminum sulfate dosage and coagulation pH). Jar test equipment (ETICA) with six square beakers of 2 L that provided velocity gradients in the range of  $10$  to  $1,200 \text{ s}^{-1}$  was used to carry out the tests. Samples of settled water were collected at a point situated at 7 cm beneath the surface of the water in each beaker.

The following parameters were adopted for the tests: rapid mixing (mixing time = 5 s; velocity gradient =  $1,000 \text{ s}^{-1}$ ); flocculation (mixing time = 30 min.; velocity gradient =  $15 \text{ s}^{-1}$ ); sedimentation (settling velocity =  $0.5 \text{ cm min}^{-1}$ ). Color measurements were based on the procedure recommended by the *Standard Methods for the Examination of Water and Wastewater* (1998).

**Table 1** | Main characteristics of the water samples

Characteristic	Water sample I	Water sample II	Water sample III
Apparent molecular size (kDa)	$> 100$	30–100	$< 30$
Total organic carbon ( $\text{mg L}^{-1}$ )	1.716	4.075	4.453
Temperature ( $^{\circ}\text{C}$ )	$20 \pm 1$	$20 \pm 1$	$20 \pm 1$
Turbidity (NTU)	$< 1$	$< 1$	$< 1$
Alkalinity ( $\text{mg L}^{-1} \text{ CaCO}_3$ )	$23 \pm 2$	$24 \pm 1$	$26 \pm 1$
Conductivity ( $\mu\text{s cm}^{-1}$ )	$43.5 \pm 0.5$	$49 \pm 1$	$61 \pm 3$
Apparent color (HU)	$100 \pm 5$	$100 \pm 5$	$100 \pm 5$
pH	6.32 to 6.60	6.39 to 6.59	6.47 to 6.70

## RESULTS AND DISCUSSION

### FTIR spectroscopic analysis

Infrared spectroscopy of all three HS samples of different apparent molecular size ( $>100$  kDa, 30 to 100 kDa,  $<30$  kDa) exhibited similar peaks (Figure 1).

The main absorbance bands were a broad band around  $3,300$ – $3,400$   $\text{cm}^{-1}$  (H bonds, OH groups), one small peak at  $2,920$   $\text{cm}^{-1}$  (C–H asymmetric, C–H stretch of –CH), a well-pronounced peak at  $\sim 1,600$   $\text{cm}^{-1}$  (aromatic C = C and conjugated carbonyl C = O), at  $1,400$   $\text{cm}^{-1}$  (symmetrical stretching of  $\text{COO}^-$ , OH deformation, and C–O stretching of phenolic groups), and  $1,050$   $\text{cm}^{-1}$  (stretching of carbohydrate or alcoholic C–O (Piccolo & Stevenson 1982; Maccarthy & Rice 1985; Piccolo et al. 1992)). These results indicate that the humic fractions contained an abundance of oxygen-containing functional groups. This behavior is confirmed by the  $^{13}\text{C}$ -NMR analyses presented in next section.

### $^{13}\text{C}$ Solid-State NMR results

The quantification of the absolute quantification of the chemical composition of humic substances cannot be accurately determined in regular  $^{13}\text{C}$  CP/MAS experiments. This is so because the efficiency of the cross-polarization procedure depends on the magnetic dipolar couplings between the  $^1\text{H}$  and  $^{13}\text{C}$  nuclei, which result in different  $^1\text{H}$ – $^{13}\text{C}$  magnetization transfer efficiency for  $^1\text{H}$  rich and poor moieties. This problem is minimized by the use of

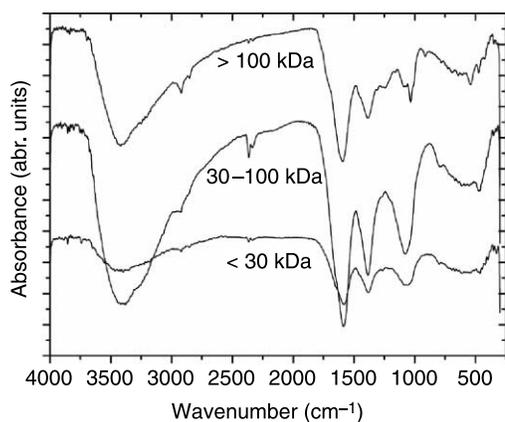
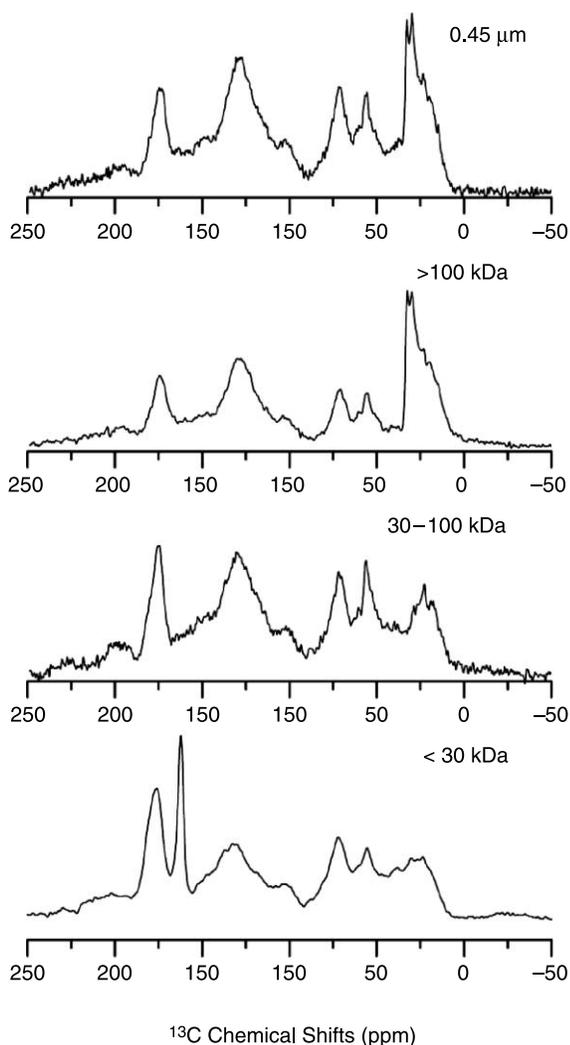


Figure 1 | The FTIR spectra of humic substances of different apparent molecular sizes.

ramped-CP that permits a more uniform magnetization transfer along the different  $^1\text{H}$  and  $^{13}\text{C}$  nuclei in the sample (Cook et al. 1996; Knicker et al. 2005). However, it is important to emphasize that even ramp-CP/MAS is not truly quantitative, but it is perfectly adequate for the cases where a comparison between the degree of each chemical constituents of samples with similar origins, such as the present report (Cook 2004; Novotny et al. 2006; Novotny et al. 2007). Besides that, for usual spinning frequencies ( $\sim 5$  kHz), spinning sidebands of chemical groups with high chemical shift anisotropy (typical for aromatic and carboxyl groups) can overlap with the lines from other groups, and this can influence in the determination of the relative amount of each constituent in a given substance. Due to that, it is usually necessary to acquire NMR spectra using high spinning frequencies ( $>15$  kHz) or, alternatively, acquire the spectrum using Total Suppression of Spinning Sidebands (TOSS) (Dixon et al. 1982). In order to minimize pulse imperfections, composite  $\pi$  pulses were applied in the TOSS pulse sequence (Novotny et al. 2006; Novotny et al. 2007). Thus, the combination of ramped-CP with TOSS acquisition (ramp-CP/MAS/TOSS) allows obtaining spectra where the amplitude at a given frequency reflects, with reasonable accuracy, the relative amounts of alkyl (0–45 ppm), methoxyl and *O*-alkyl (45–90 ppm), di-*O*-alkyl and aromatic (90–162 ppm), carboxyl and esters (162–190 ppm), and ketones, quinones and aldehydes (190–250 ppm) (Cook 2004). Thus, by integrating the spectra at the frequency regions specified above, and normalizing the results by the integral of the full spectrum, it is possible to estimate the relative amounts of the corresponding chemical groups in the samples. Figure 2 shows the NMR spectra obtained for samples fractionated at different apparent molecular sizes and the relative intensities for each spectral region are shown in Table 2. It is possible to observe from Table 2 that the relative intensities of the spectrum for the regions 0–45 and 90–162 ppm decrease for samples fractionated at smaller apparent molecular sizes. Because these spectral regions are associated to aliphatic and aromatic groups, respectively, this behavior might be attributed to aggregated aromatic rings with long alkyl side chains, as proposed by Schulten & Schnitzer (1993) for the structure of humic substances. The contrary behavior (increase of the intensity as a function of



**Figure 2** |  $^{13}\text{C}$  ramp-CP/MAS/TOSS NMR spectra of HS fractions with distinct molecular sizes.

apparent molecular size) is observed in the 45–90 and 162–190 ppm regions. This shows an increase in the fraction of O-alkyl and carboxyl groups. Together with the observed increase of the relative amount of aromatic groups, this suggests an increase in the humification degree.

### Coagulation, flocculation and sedimentation tests

Based on the apparent color removal observed on the coagulation diagram for each water sample, three efficient regions were selected for analysis in this paper: dosage of coagulant between 16 and 32  $\text{mg L}^{-1}$  of  $\text{Al}_2(\text{SO}_4)_3 \cdot 14.3$

**Table 2** | Percentage NMR intensities (%) observed along the different spectral regions for samples fractionated with different apparent molecular sizes. The percentages were obtained by integrating the line intensities and normalizing them by the total spectrum area

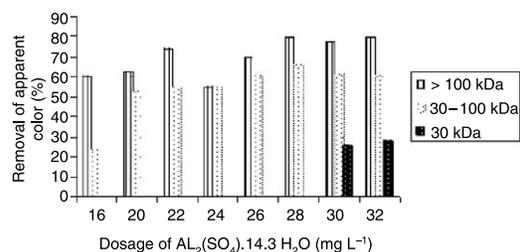
Fractions (kDa)	Percentage NMR intensities (%) relative to different spectral regions				
	0–45 ppm	45–90 ppm	90–162 ppm	162–190 ppm	190–250 ppm
> 100	23.0	12.5	50.0	9.5	5.0
30–100	15.0	23.5	38.0	15.0	8.4
<30	8.5	37.3	34.0	16.6	3.7

The biggest error margin is lower than 10% of the absolute value.

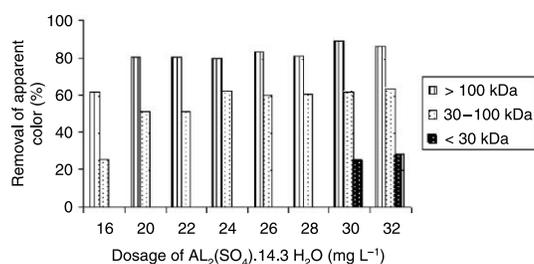
$\text{H}_2\text{O}$  and coagulation pH ranges of 6.20 to 6.50; 6.51 to 6.80 and 6.81 to 7.20.

Since the clarification was due to sedimentation in the experimental study, sweep coagulation was the main mechanism of coagulation with aluminum sulfate. Figures 3–5 present the best removal results of apparent color as function of coagulant dosage for each water sample and range of pH.

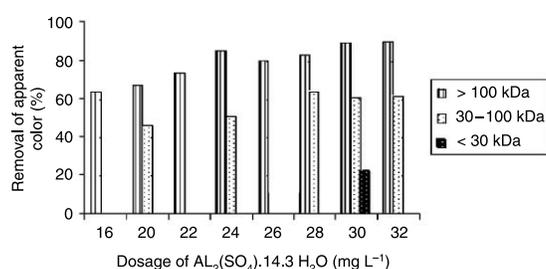
It can be seen in Figures 3–5 that for all aluminum sulfate dosages and coagulation ranges pH, the apparent molecular size had a significant influence on the color removal. This is because the removal percentage decreased with the decrease of the apparent molecular size according to: (> 100 kDa) > (30 to 100 kDa) > (<30 kDa). Since sweep coagulation was the main coagulation mechanism in the three conditions of pH studied, it can be seen in these figures the little influence of the coagulation pH on the efficiency of apparent color removal. It can also be observed in Figures 3 to 5 that water samples I and II presented a



**Figure 3** | Removal of apparent color for each sample of water at different dosages of aluminum sulfate (pH range: 6.2 to 6.5; settling velocity = 0.5  $\text{cm min}^{-1}$ ).



**Figure 4** | Removal of apparent color for each sample of water at different dosages of aluminum sulfate (pH range: 6.51 to 6.80; settling velocity = 0.5 cm min<sup>-1</sup>).



**Figure 5** | Removal of apparent color for each sample of water at different dosages of aluminum sulfate (pH range: 6.81 to 7.10; settling velocity = 0.5 cm min<sup>-1</sup>).

considerable color removal for all aluminum sulfate dosages. However, this result was not observed for water sample III. In fact, only for aluminum sulfate dosages higher than 28 mg L<sup>-1</sup> was observed a satisfactory removal. This behavior may be explained considering the FTIR and NMR results. FTIR and NMR spectra of the three fractions of humic substances (> 100 kDa, 30 to 100 kDa e < 30 kDa) are qualitatively very similar (similar absorption bands in the FTIR or lines in the NMR spectra were observed for all samples). However, the percentage intensity analysis of the NMR spectra showed that the three fractions of HS have similar chemical groups, but with different concentrations. NMR also showed that the smaller the HS apparent molecular size, the higher the concentration of the groups containing oxygen, with the smallest apparent molecular size fraction presenting the highest concentration of these groups. These smaller molecules are formed mainly by small stable micelles, which remain dispersed due to the repulsion of their negative charge that originates from the dissociation of the large amount of groups with oxygen linked to its structure (Collins *et al.* 1986; Owen 1995; Piccolo *et al.* 1996a,b). Therefore, it is necessary to increase the amount of aluminum precipitate in order to achieve destabilization of these structures.

## CONCLUSIONS

Based on the research work carried out, it was concluded that:

- Humic substances with different apparent molecular sizes behave differently during coagulation, flocculation and sedimentation;
- The higher the apparent molecular size of the humic substances used to prepare the water samples with the same true color of approximately 100 HU, the more efficient the removal of color;
- The NMR and FTIR results showed that for the fractions of humic substances with apparent molecular sizes within the intervals of 30 – 100 kDa and < 30 kDa, which presented higher proportions of bond oxygen groups, resulted in the lowest removal of apparent color. These groups have great amounts of negative charges, which favor the repulsion between their electrons and the colloidal particles, and inhibit the process of coagulation.

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