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Quantifying Pb and Cd Complexation by Alginates and the Role of Metal Binding on Macromolecular Aggregation

Cristina Lamelas,† Fabrice Avaltroni,‡ Marc Benedetti,§ Kevin J. Wilkinson,‡ and Vera I. Slaveykova*†

Environmental Chemistry and Ecotoxicology (CECOTOX) - ISTE - ENAC, Ecole Polytechnique Fédérale de Lausanne, Station 2, Lausanne, CH-1015, Switzerland, Analytical and Biophysical Environmental Chemistry (CABE), University of Geneva, 30 Quai E. Ansermet, Geneva, CH-1211, Switzerland, and Laboratoire de Géochimie des eaux, Université Denis Diderot, UMR CNRS 7047, 2 place Jussieu 75251 Paris Cedex 05, France

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Introduction

Alginates (AA) are a family of unbranched polysaccharides consisting of 1 → 4 linked, β-D mannuronic acid (M) and α-L guluronic acid (G) residues. They are produced by a number of microorganisms including Pseudomonas aeruginosa, Azotobacter vinelandii, and Azotobacter crococcum and are a principal component of the cell walls of several brown algae.1–3 Depending upon their source,4 they can have different chemical (e.g., complexation) or physicochemical (e.g., biopolymer rigidity) natures due primarily to the relative proportions and sequences of the M and G residues.2 The different macromolecular structures give the polysaccharide different functional roles. For example, in algae, alginates have a structural function, whereas in bacteria, they generally adopt a more acetylated form that serves a protective function.5

Because of their metal binding properties, alginates can be used to remove metals from wastewaters and industrial effluents2,6 and as a matrix to immobilize Pb and Cd complexing particles, including ion-exchange resins6 or microorganisms7 in order to treat contaminated waters. The major difficulties in understanding the metal binding of most natural biopolymers, including alginates, results from their (i) polyelectrolytic properties — at circumneutral pH, the carboxylic sites of alginic acid are ionized, thereby creating an electric field which increases complex stability; (ii) polyfunctionality — a diversity in the chemical nature and the resulting electronic and steric environment of the coordinating sites; and (iii) capacity to change their three-dimensional conformation. The relative importance of each of these effects depends mainly on the physicochemical properties of the external medium including the pH, the ionic strength, and the metal-to-alginate (or metal-to-carboxylic site) ratio. All three effects result in a decreasing metal stability with increasing metal loading of the biopolymer. Nonetheless, few studies have attempted to separate effects due to intrinsic chemical binding from the electrostatic or macromolecular (i.e., conformational) contributions. For the most part, metal binding studies have been performed at high metal concentrations (typically mM, e.g., ref 8), in acidic pH (typically between pH 2.0 and pH 4.0, e.g., refs 8, 9), and at high alginate concentrations (typically between 1 and 5 g L−1, e.g., refs 8, 10, 11). Quantitative information corresponding to low metal-to-alginate ratios, low Pb and Cd concentrations, circumneutral pH values, and lower ionic strengths are missing from the literature, despite their obvious environmental and biological relevance.

The conformation of alginate depends on its hydration, the formation of hydrogen bonds or metallic bridges, and the relative proportion of its principal constituents (i.e., its M/G ratio). For example, guluronic-rich alginates are char-
acterized by a higher affinity for divalent metals because of a "zig-zag" structure that can easily accommodate divalent cations, i.e., the "egg-box model". Biopolymer conformations are typically evaluated by measurements of their dimensions (gyration or hydrodynamic radii, persistence and contour lengths), as determined by intrinsic viscosity, nuclear magnetic resonance, or scattering techniques (e.g., X-ray, neutron, or light). These techniques require the use of biopolymer concentrations (typically \( \geq 1 \text{ g L}^{-1} \)) that are often associated with the formation of molecular aggregates or gels. In more dilute systems, single molecule detection techniques such as transmission electron microscopy, atomic force microscopy, or fluorescence correlation spectroscopy (FCS) are more likely to provide information on the dissociated molecules and the role of metal binding on macromolecular conformations.

The main objectives of this study are therefore the following: (i) to quantify Pb and Cd binding by an alginate and to determine the influence of pH, ionic strength, and metal loading of the binding sites; (ii) to relate aggregation and conformational changes, as determined from measurements of the diffusion coefficients using fluorescence correlation spectroscopy, to the physicochemistry of the medium; (iii) to identify and quantify the intrinsic and electrostatic contributions for Pb and Cd binding by alginate by modeling with nonideal competitive adsorption isotherm (NICA) –Donnan approach. Pb and Cd were studied because of their contrasting complexation properties.

**Materials and Methods**

Alginic acid, AA (Sigma, sodium salt A2033), isolated from *Macrocyctis pyrifera*, was used as purchased without any additional treatment. Stock solutions of an alginate, AA (1 g L\(^{-1}\)), were prepared in Mill-Q water, filtered for partial sterilization (0.22 \(\mu\)m regenerated cellulose, Millipore), and stored at 4 °C for a maximum of 15 days. Experimental solutions, containing between 5 and 100 mg L\(^{-1}\) of AA, were prepared by dilution of the stock solution. Organic carbon was measured using a Schimadzu TOC 500/5000A following acidification with 10 \(\mu\)L of ultrapure HCl (Baker suprapur) per mL of sample.

**Potentiometric Titration of the Alginic Acid.** Automated acid-base titrations\(^{22}\) of the alginate were performed at initial ionic strengths of 0.005, 0.008, 0.02, 0.06, 0.09, and 0.3 M, adjusted by addition of NaNO\(_3\). The pH was monitored with duplicate pH electrodes (Metrohm) and an Ag/AgCl reference electrode (Metrohm), calibrated at pH values 4.0, 7.0, and 9.0. Twenty milliliters of 500 mg L\(^{-1}\) alginate was titrated by 0.1 M HNO\(_3\) and 0.1 M NaOH in a thermostatted cell (20 °C). Titrations were performed under N\(_2\) atmosphere using CO\(_2\)-free NaOH solutions to eliminate buffering due to CO\(_2\) equilibration. Potential measurements were recorded when the rate of linear drift was \(< 0.05 \text{ mV min}^{-1}\). For data analysis, the ionic strength was calculated for each data point by taking into account background electrolyte, free [H\(^{+}\)], and free [OH\(^{-}\)]. Blank titrations of the background electrolyte were performed and subtracted from titration curves for the alginate. Proton binding was quantified by determining the total concentration of carboxylic groups, \([-\text{COOH}]_{\text{tot}}\), and the mean p\(K_a\) of the alginic acid.

**Determination of Carboxylic Group Content and the Apparent p\(K_a\) of the AA.** The concentration of carboxylic acid groups (mol g\(^{-1}\)) was determined from the maximum in the first derivative of the titration curves, corresponding to the equivalent point of the AA titration with 0.1 M NaOH at several ionic strengths (titration curves not shown). Apparent p\(K_a\) values were determined according to

\[
pH = pK_a - x \log\left(\frac{1 - \alpha}{\alpha}\right) \tag{1}
\]

where \(x\) is an empirical parameter (\(> 1\)) and \(\alpha\) is the degree of dissociation calculated from the titration curves obtained at different ionic strengths.

**Metal Complexation by Alginate.** Three sets of experiments were performed to study Pb and Cd complexation by the alginates.

(i) The role of the metal-to-alginate ratio (metal loading) on complexation was studied in two manners: Either the metal concentrations were kept constant at \(10^{-6}\) M while the alginic acid concentration was varied between 5 and 100 mg L\(^{-1}\) or the metal concentration was varied between 5 \(\times\) \(10^{-7}\) and \(10^{-4}\) M using a constant 50 mg L\(^{-1}\) of AA. Solutions were buffered at pH 6.0 using 5 \(\times\) \(10^{-3}\) M MES (2-(N-\(\text{N}\)-morpholino)ethanesulfonic acid, Sigma).

(ii) The effect of pH was studied using a medium containing \(10^{-6}\) M of Pb or Cd as nitrates and 50 mg L\(^{-1}\) of AA. pH was controlled by the addition of appropriate quantities of HNO\(_3\) or NaOH. In the centricron experiments (see below), 5 \(\times\) \(10^{-3}\) M MES (pH values 5.0, 5.5, or 6.0) or 5 \(\times\) \(10^{-3}\) M HEPES (N-(2-hydroxyethyl)piperazine-N'-ethane sulfonic acid, Sigma) (pH 7.0 or 8.0) was employed to maintain constant pH. Metal complexation by the pH buffers was assumed to be negligible, in agreement with the literature.\(^{24}\) Ionic strength was maintained at 5 \(\times\) \(10^{-3}\) M, adjusted when necessary with NaNO\(_3\).

(iii) The influence of the ionic strength, \(I\), was evaluated in the range from \(10^{-4}\) M to \(10^{-1}\) M, adjusted by appropriate quantities of NaNO\(_3\), in a medium containing 50 mg L\(^{-1}\) of AA and 9 \(\times\) \(10^{-7}\) – \(10^{-6}\) M of metal. The pH was maintained at 6.0 by using MES buffer.

**Free Ion Measurements by the Pb\(^{2+}\) and Cd\(^{2+}\) Selective Electrodes.** Cd and Pb complexation of the alginates was investigated using either dynamic titrations or static measurements of free ion activities using Pb\(^{2+}\) (model 9482 BN) or Cd\(^{2+}\) (model 9448 BN) selective electrodes (SE) (ThermoOrion Research). Given the relatively low sensitivity of the Pb\(^{2+}\) SE, a low detection limit Pb\(^{2+}\) selective electrode with an optimized membrane based on the ionophore 4-tert-butylcalix[4]arenetetrasik thioacetic acid dimethylamide was also employed in some of the experiments at low lead concentrations. The detailed membrane composition and electrode preparation procedures are described elsewhere.\(^{25}\) Potentials were measured with a 16-channel electrode monitor (Lawson Labs, Inc.) at room temperature in stirred solutions. The reference electrode was a Metrohm double junction Ag/AgCl reference electrode with 3 M KCl as the reference electrolyte and 1 M KCl as the bridge electrolyte.
The ion selective electrodes (ISEs) were calibrated with a series of Pb$^{2+}$ and Cd$^{2+}$ solutions containing $10^{-6}$ to $10^{-4}$ M free ion. Measurement drift was $<0.3$ mV over 10 min. For each condition, at least 3 replicate measurements were performed. Mean values were used in the modeling and the data interpretation. Relative standard deviations did not exceed 15%.

**Ultrafiltration by Centricon Devices.** Centricon ultrafiltration devices (Millipore) were used to validate the ISE measurements. Since the 30 kDa membrane cutoff of the centrifuge device was much lower than the average (weight) molar mass of the AA used in the study (e.g., 210 kg mol$^{-1}$), metal-bound AA was assumed to be entirely retained on the membrane, whereas free metal ions and small complexes (e.g., carbonate and hydroxides) passed into the filtrate. As recommended by the manufacturer, the centrifuge devices were washed 3 times with 0.1 M NaOH and rinsed 3 times with MilliQ water to remove organic surfactants from the membranes. Centricon devices were conditioned with an experimental medium by performing at least 5 consecutive runs with 1 mL of the experimental medium. After removing the filtrate, the device was turned over and centrifuged to prevent AA clogging from the membrane. This treatment significantly decreased metal losses to the centrifuge device due to adsorption or unintended removal due to blocking of the pores of the membrane. Filtrates were collected for Pb measurements only after a further 3 consecutive runs using the experimental medium. Metal concentrations in the filtrate were measured by inductively coupled plasma mass spectrometry (ICP-MS, Hewlett-Packard 4500) following acidification with HNO$_3$ (0.2% v/v, Baker instra grade). Typically, 1 mL of experimental medium was centrifuged for 20 min at 4500 rpm (2500 $\times$ g). Controls with solutions containing metal in the absence of AA were run to estimate potential adsorptive losses of metal to the filter and walls of the centrifuge device.

**Determination of the Conditional Stability Constants for Metal–AA Complexes.** Conditional stability constants of the metal–AA complexes, $K_{\text{MAX}} = [-\text{COOM}^+] [\text{M}^{2+}]$ $[-\text{COO}^-]$, were estimated by assuming a 1:1 stoichiometry (with respect to the carboxylic groups). The quantity of metal bound to the alginate, $[-\text{COOM}^+]$, was estimated from the difference between the total metal, $[\text{M}]_{\text{tot}}$, and the free metal ion concentrations, $[\text{M}^{2+}]$. Free $[-\text{COO}^-]$ was determined as the difference between the concentrations of total carboxylic acids and those bound to both metals, $[-\text{COOM}^+]$, and protons, $[-\text{COOH}]$.

**Fluorescence Correlation Spectroscopy.** Fluorescence correlation spectroscopy (FCS) was used to determine the diffusion coefficients, $D$, of the AA. Measurements were carried out with a Confocor Axiosvert 135TV instrument (Carl Zeiss) using an argon ion laser for fluorescence excitation at 514 nm. Calibration of the FCS confocal volume was performed with rhodamine 6G (R6G), which has a known diffusion coefficient of $2.8 \times 10^{-10}$ m$^2$ s$^{-1}$. Twelve 90 s data acquisitions with a 0.5 s delay between measurements were performed in order to optimize the signal-to-noise ratio. Given that AA is not naturally fluorescent, a nonperturbing fluorescent labeling procedure using Rhodamine 123 (R123) was adapted from the literature. After heating a reaction mixture including sodium cyanoborohydride at 70 °C, labeled AA was separated from free R123 by multiple extractions in 70% ethanol in the presence of added NaNO$_3$. A constant amount of labeled AA was added to AA solutions containing Pb or Cd for use in determining average diffusion coefficients by FCS under the same pH, ionic strength, and metal-to-ligand ratios as were employed in the complexation experiments. The labeled AA accounted for $<0.1\%$ of the total AA present in solution, thus minimizing the possible (but never observed) artifacts due to the labeling procedure.

**Determination of Diffusion Coefficients.** In FCS, variations of fluorescence intensity can be attributed to the Brownian diffusion of fluorescent species through a defined confocal volume ($\sim1\ \mu$m$^3$). Intensity variations were analyzed using the autocorrelation function, $G(t)$, for a two-component system (free fluorophore and labeled alginate)$^{20}$

$$G(t) = b + \frac{1}{c} \left[ (1 - y) \left( 1 + \frac{t}{\tau_1} \right)^{-1/2} \right] + \left[ \frac{1 + \frac{t}{\tau_2}}{\left( q \right)^{-1/2}} \right]$$

where $b$ is the limiting value of $G(t)$ for $t \rightarrow \infty$ (usually $b = 1$), $c$ is the average number of fluorescent particles diffusing into the confocal volume, $y$ is the fraction of the signal due to the second species in solution, and $\tau_1$ and $\tau_2$ are the diffusion times of the two species inside the confocal volume. The parameter $q$ is a structural parameter that is the ratio of the transversal, $\omega_\perp$, to longitudinal, $\omega_\parallel$, radius of the confocal volume, $q = \omega_\parallel / \omega_\perp$. Values of $q$ are obtained from a calibration of the apparatus with rhodamine-6G (R6G). Diffusion coefficients were calculated from measured diffusion times, $\tau$ (eq 3)

$$D = \frac{\omega_\parallel^2}{4\tau}$$

For macromolecules that are rigid spheres or random coils, diffusion coefficients, $D$, can be related to an equivalent hydrodynamic radius, $r_h$, using the Stokes–Einstein relationship$^{28}$

$$r_h = \frac{RT}{6\pi \eta \nu DN_a}$$

where $R$ is the gas constant, $N_a$ is Avogadro’s number, $T$ is the absolute temperature, and $\eta$ is the viscosity of the solvent. Changes in the hydrodynamic radius are used to evaluate conformational changes or aggregation of the alginate.

**Data Modeling by the NICA–Donnan Approach.** The electrostatic and intrinsic components of the ion binding constants to AA were estimated by applying the NICA–Donnan model$^{21,28}$ In that model, alginate is assumed to behave as a Donnan soft particle (often referred to as a Donnan “gel”), a reasonable assumption given the polyelectrolyte properties of AA. The macromolecular charge (related mainly to the presence of carboxylic groups) leads to an electrostatic Donnan potential, $\psi_D$, that is assumed to be homogeneously distributed throughout the Donnan soft
particle. Under such conditions, the concentration of ion, \( i \), in the soft particle, \( c_{i,D} \), is related to its concentration, \( c_i \), in the bulk solution by a Boltzmann factor

\[
c_{i,D} = c_i \exp \left( \frac{z_i F \psi_D}{RT} \right)
\]

where \( z_i \) is the ionic charge of \( i \) and \( F \) is Faraday’s constant (C mol\(^{-1}\)). To simplify the notation, the electrostatic term, i.e., the ratio of \( c_{i,D} \) to \( c_i \), is replaced by \( Z_{iD} \) and used in eq 6 to relate the salt concentration, \( c_s \), and charge, \( Q \)

\[
(Z_{iD})^2 + \left( \frac{Q}{[\ell_D]} \right) Z_{iD} + 1 = 0
\]

where \( Z_{iD} \) is the electrostatic term for monovalent salt cations, \( Q \) is the charge of the AA in equivalents per kilogram, and \( [\ell_D] \) is the specific volume of the AA soft particle in liters per kilogram (Donnan volume).

The charge was obtained from the acid–base titration of the AA at several salt concentrations. The Donnan volume was estimated from the construction of a master curve relating the charge to the pH in the soft particle, \( \mathrm{pH_D} \), following a procedure described previously.\(^{30}\) The master curve obtained in this manner is free from electrostatic contributions. It was analyzed to obtain a distribution of intrinsic proton affinity constants using the NICA\(^{21}\) isotherm that was incorporated into the Fit software.\(^{30}\)

The NICA approach takes into account both molecular heterogeneity and competition between H\(^+\) and Cd\(^{2+}\) or Pb\(^{2+}\)

\[
Q_{i,t} = Q_{\text{max}} \frac{\left( \bar{K}_{\ell_i} c_{i,D} \right)^n \left( \sum \left( \bar{K}_{\ell_i} c_{i,D} \right)^y \right)^p}{\sum \left( \bar{K}_{\ell_i} c_{i,D} \right)^y 1 + \left( \sum \left( \bar{K}_{\ell_i} c_{i,D} \right)^y \right)^p}
\]

where \( Q_{i,t} \) is the total amount of component \( i \) bound to the AA, \( p \) is the intrinsic heterogeneity of the AA, \( n \) is the ion specific heterogeneity, \( \bar{K} \) is the median intrinsic stability constant, and \( Q_{\text{max}} \) is the total site density. Electrostatic interactions are taken into account by the Donnan approach, since they are incorporated into eq 7 through the concentration of ions in the soft particle, \( c_{i,D} \).

**Results and Discussion**

**Effects of the pH.** Because protons are known to compete with metals for the binding sites of natural biopolymers,\(^{31}\) carboxylic groups were quantified and their apparent dissociation constant, \( K_a \), determined. Carboxylic groups were quantitatively deprotonated above pH 6.0 (Figure 1). An apparent p\(K_a\) value of 3.44 was obtained, in good agreement with the literature for alginic acid containing brown algae (p\(K_a = 3.49\))\(^{32}\) and values corresponding to the carboxylic groups of the mannuronic (3.38)\(^{33}\) and guluronic acids (3.65).\(^{33}\)

The concentration of carboxylic acid groups determined here, \( 4.4 \times 10^{-6} \text{ mol kg}^{-1} \), corresponded well to values obtained previously by \(^1\)H NMR (4.5 \times 10^{-6} \text{ equiv kg}^{-1})\(^{34}\) and potentiometric titration (4.39 \times 10^{-6} \text{ mol kg}^{-1}).\(^{8}\) This concentration of carboxylic acid groups also corresponds well to theoretical estimates that can be calculated from the alginate structural unit (4.4 \times 10^{-6} \text{ mol kg}^{-1}), if the water content (19.8%) is taken into account.

No differences were observed between free metal concentrations determined in the absence or presence of pH buffers. Furthermore, good agreement was found between measurements of metal activities made using the ion selective electrodes and those performed on the centricon filtrates for pH < 6.0. Above pH 6.0, Pb concentrations determined with the ISE were systematically smaller than those determined with the centrifuge devices because of the presence of carbonate and hydroxide complexes.

Metal complexation by the alginates is influenced by the pH through (i) competition due to the protonation of binding sites, (ii) modification of the overall molecular charge, and (iii) competition by simple inorganic ligands such as carbonates and hydroxides. An increase of pH from 4.0 to 5.5 led to a significant increase in bound Cd (analysis of variance (ANOVA), Tukey test, \( p < 0.05 \)), while a further pH increase from 5.5 to 8.0 had no effect on metal complexation (Figure 2). On the other hand, protons did not appear to significantly affect AA binding of Pb over the entire pH range of 4.0 to 8.0 (ANOVA, Tukey test, \( p < 0.05 \)). In fact, although Pb (and not Cd) effectively outcompeted the proton for \(-\text{COO}^-\) sites (see below), in the pH range 4.0 to 8.0, conditional stability constants increased from ca. 10\(^{3.6}\) to 10\(^{4.3}\) M\(^{-1}\) for

**Figure 1.** Experimentally determined \( Q (\mathrm{pH}) \) and \( Q (\mathrm{pH_D}) \) curves for Donnan model, [AA] = 500 mg L\(^{-1}\), \( I = 0.01 \text{ M (○), 0.02 M (■), 0.1 M (◇), and 0.3 M (■) \). The open symbols and the solid line of the master curve (MC) represent the data points following the Donnan correction to remove the electrostatic contribution.**

**Figure 2.** Metal bound to alginate (mol kg\(^{-1}\)) as a function of pH: [AA] = 50–55 mg L\(^{-1}\), \( I = 5 \times 10^{-3} \text{ M, [M]_{tot} = 9 \times 10^{-7} \text{ to 10^{-4} M for Cd (□) or Pb (■). Dashed lines represent metal bound as predicted by the NICA–Donnan model. Standard deviations are given when larger than the symbol size (N = 3).**
a 1:1 Cd–alginate complex and from $10^{4.9}$ to $10^{5.7} \text{ M}^{-1}$ for the Pb–AA complex. In other work, a fourfold increase of bound Pb was observed when pH was increased from 2.5 to 3.5 ($2 \times 10^{-4} \text{ M Pb}$, $[\text{AA}] = 500 \text{ mg L}^{-1}$ and $I = 0.1 \text{ M}$). Similar tendencies were observed for Cd adsorption by a Ca alginate and an AA-rich fungal biomass. An increase in cadmium uptake by the AA-rich *Fucus spiralis* was observed over the pH range of 1.0 to 4.0 and was followed by a plateau between pH values 4.5 and 6.0 ($[\text{Cd}]_{\text{tot}} \leq 2.5 \times 10^{-3} \text{ M}$). Finally, maximal Cd adsorption to AA-immobilized cells was observed at pH 6.0 ($[\text{AA}] = 2 \text{ g L}^{-1}$; $[\text{Cd}]_{\text{tot}} = 10^{-4} \text{ M}$, pH varied from 3.0 to 8.0). 36

Average diffusion coefficients of the AA were evaluated under identical conditions (pH, $I$) to those used in the metal complexation experiments (Figure 3). Values increased by ca. 10% as the pH was decreased from pH 6.0 to pH 4.0, corresponding to a ca. 10% decrease in the hydrodynamic radii of the AA. No significant variation in $D$ was observed between pH values 8.0 and 6.0. The small observed decrease in molecular size was consistent with a small compression due to the protonation of the carboxylic groups, leading to a reduction of the intramolecular repulsion. This explanation is consistent with data from the acid–base titrations, demonstrating that the degree of dissociation decreased from ca. 100% at pH 6.0 to ca. 62% at pH 4.0 (Figure 1, $I = 5 \times 10^{-3} \text{ M}$). Such a large and significant reduction in negative charge would be expected to allow the molecule more internal flexibility. Indeed, hydrodynamic radii calculated from the Stokes–Einstein equation ranged from 6.0 nm (pH 8.0) to 5.7 nm (pH 4.0) in the presence of Cd and from 6.3 nm (pH 8.0) to 5.6 nm (pH 4.0) in the presence of Pb.

Few other measurements of diffusion coefficients (or molecular sizes) of alginate can be found in the literature. Where available, values of radii were obtained by dynamic light scattering (DLS) and were generally much larger than those observed here. Indeed, DLS has a much larger bias than FCS toward larger molecules and molecular aggregates because of a dependency of the scattered light intensity on the sixth power of the radius. For highly polydisperse systems such as the alginate examined here and under the condition of a single fluorescent label on each AA molecule, the FCS signal should provide a more accurate measurement of the diffusion coefficient.

**Effects of the Ionic Strength.** Ionic strength can influence the metal binding of polyelectrolytes by screening their molecular charges with a resulting reduction in the Coulombic contribution to the stability constant, $K_{\text{coul}} (K = K_{\text{int}}, K_{\text{coul}})$, e.g., refs 8, 10, 41. A 100-fold increase in ionic strength resulted in a 3-fold decrease in bound Cd and a slight decrease in bound Pb (Figure 4).

These data are consistent with the hypothesis that the intrinsic component of the overall binding constant ($K_{\text{int}}$) is larger for Pb than for Cd. In this case, the relative effect of the electrostatic component would be much greater for Cd, in agreement with literature data showing that for different biosorbents, the effect of Na is much more pronounced for the adsorption of weakly bound metals. 41

Diffusion coefficients for the alginate increased by approximately 25% when the ionic strength increased from $10^{-3}$ to $10^{-1} \text{ M}$ for pH 6.0 (Figure 5).

The increase in $D$ corresponded to a decrease in the hydrodynamic radius from 6.6 to 5.3 nm. These variations are consistent with a screening of the negative charges on the AA, leading to a reduction in the intramolecular repulsion and a compression of the diffuse layer. The charge screening effect was also seen in the titration curves at pH 6.0 (Figure 1). Interestingly, a similar, linear trend against $I^{-1/2}$ has been observed for intrinsic viscosity ($\eta$) measurements of alginate. 1,42,43

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**Figure 3.** Effect of pH on average diffusion coefficients of the alginates in the presence of Cd (C) and Pb (O); $[\text{M}]_{\text{tot}} = 9 \times 10^{-7}$ to $10^{-3} \text{ M}$, $[\text{AA}] = 50 \text{ mg L}^{-1}$, $I = 5 \times 10^{-3} \text{ M}$. Standard deviations are given when larger than the symbol size ($N = 3$).

**Figure 4.** Variation in the amount of metal bound to the alginates (mol kg$^{-1}$) as a function of ionic strength of the medium. $[\text{AA}] = 50–55 \text{ mg L}^{-1}$, pH = 6.0, $[\text{M}]_{\text{tot}} = 9 \times 10^{-7}$ to $10^{-6} \text{ M}$ for Cd (C) or Pb (O). Dashed lines represent bound metal as predicted by the NICA–Donnan model. Standard deviations are given when larger than the symbol size ($N = 3$).

**Figure 5.** Influence of the ionic strength on the average diffusion coefficients for the alginates in the presence of Cd (C) or Pb (O); $[\text{M}]_{\text{tot}} = 10^{-6} \text{ M}$, $[\text{AA}] = 50 \text{ mg L}^{-1}$, pH = 6.0. Standard deviations are given when larger than the symbol size ($N = 3$).
Effects of Metal Loading. The quantity of bound metal increased linearly with increasing concentrations of Pb\(^{2+}\) and Cd\(^{2+}\), reaching a nearly constant value above \(10^{-5}\) M (Figure 6). For equivalent concentrations of free metal, Pb complexation by the AA was about 10× greater than for Cd binding over the entire concentration range examined (four orders of magnitude). This observation is in good agreement with the existing literature on alginate-containing biomasses. For 2.2 \(\times 10^{-5}\) M of carboxylic acid groups (i.e., 50 mg L\(^{-1}\)), the proportion of bound Pb increased linearly with [Pb\(^{2+}\)] until leveling off above 1.8 \(\times 10^{-5}\) M. Average stability constants for the Cd–algin complex were not affected by the metal loading (\(K_{\text{CdAA}} = 10^{5.2 \pm 0.2}\) M\(^{-1}\)). Below saturation of the AA Pb binding sites, i.e., [Pb] corresponding to \(<80%\) of filled sites; Figure 6), a value of \(10^{6.6 \pm 0.4}\) was obtained for the value of \(K_{\text{PbAA}}\).

It is difficult to compare the above stability constants with literature results, given that the experiments were not performed using the same alginates, nor in the same intervals of pH, ionic strength, or metal-to-ligand ratios. In particular, the metal binding properties of the AA are very dependent on its guluronic acid content. In this paper, the AA had an M/G ratio of 1.61 and an \(F_{\text{GG}}\) value of 0.24, indicating that the effective GG complexing unit accounted for ca. 24% of the molecule (values determined by \(^1\)H NMR at 90 °C\(^{45,46}\) following thermal acid depolymerization\(^7\)). Despite the above variability, cadmium and lead binding constants were of the same order of magnitude as those obtained previously. For example, values of log \(K_{\text{PbAA}} = 5.12\) M\(^{-1}\) and \([-\text{COOH}]_{\text{tot}} = 3.02 \times 10^{-3}\) M (pH = 2.5–3.5),\(^9\) log \(K_{\text{PbAA}} = 5.79\) M\(^{-1}\) (\(I = 0.1\) M, pH = 3.2, [Pb\(^{2+}\)] = 10\(^{-1}\) M, [AA] = 500 mg L\(^{-1}\)),\(^8\) and log \(K_{\text{GA}} = 3.17\) M\(^{-1}\) ([Cd\(^{2+}\)] = 5 \(\times 10^{-4}\) M, \(I = 0.1\) M, pH = 2.0–5.0)\(^48\) have been determined previously. Finally, for a composite material containing alginic acid, Seki and Suzuki\(^9\) found a log \(K_{\text{PbAA}}\) of 5.13 M\(^{-1}\) (\(I = 0.1\) M, pH = 3.5, [Pb\(^{2+}\)] = 2 \(\times 10^{-4}\) M, [AA] = 200 mg L\(^{-1}\)).

High metal concentrations or high metal-to-ligand ratios could also be expected to affect macromolecular conformations because of the formation of metal bridges and the subsequent aggregation of the molecules, in line with the “egg–box model”\(^49\). For low metal additions, i.e., \([\text{M}]_{\text{tot}}/\text{[COOH]}_{\text{tot}} < 0.1\), no modification of the biopolymer structure was observed by FCS. On the other hand, for \([\text{M}]_{\text{tot}}/\text{[COOH]}_{\text{tot}} > 0.1\), a large (\(>2\times\)) decrease in \(D\) was observed in the presence of both Cd\(^{2+}\) and Pb\(^{2+}\) (Figure 7), corresponding to an increase in the hydrodynamic radius from ca. 6.2 nm at low M/L ratios to ca. 8 nm at \([\text{M}]_{\text{tot}}/\text{[COOH]}_{\text{tot}} = 0.38\) (eq 44). For \([\text{M}]_{\text{tot}}/\text{[COOH]}_{\text{tot}} > 1\) (data not shown), irreproducible \(D\) values were found because of a large polydispersity that was likely caused by the formation of small numbers of very large aggregates.

Application of the NICA–Donnan Approach to Model \(H^+\), Cd\(^{2+}\), and Pb\(^{2+}\) Binding by AA. To take into account pH and ionic strength effects and to estimate the electrostatic contribution to the proton and metal binding, a relatively simple but numerically efficient Donnan approach was employed. This Donnan approach has been previously applied to interpret proton binding by alginates,\(^50–52\) algal biomass,\(^41,51,53\) bacterial cell walls,\(^54\) and humic substances.\(^55,56\) Charge curves obtained as a function of pH for different ionic strengths merged nicely into a single master curve (MC), \(Q = f(\text{pH})\) (solid line and open points in Figure 1). In the modeling, it is assumed that the Donnan volume, \(V_{\text{D}}\), is mainly dependent on the ionic strength and independent of the pH. Indeed, Donnan volumes, determined empirically from the master curve\(^29\) (\(V_{\text{D}} = a I^{b}\), \(a = 0.747\), and \(b = 0.841\); Figure 8), were well-correlated with \(I\) in the range 0.001–0.1 M. This result indicated that the swelling and shrinking of the AA depended primarily on the ionic strength.
and was consistent with FCS observations of a decreasing hydrodynamic radius with increasing ionic strength (Figure 5) at circumneutral pHs. Moreover, the charge curves (Figure 1) and the relative changes of the hydrodynamic radii (Figures 3 and 5) support the observation that the Donnan potentials depended to a larger extent on ionic strength rather than pH under the studied conditions.

To confirm that the values of Donnan volume that were used to obtain the master curve were physically realistic, equivalent spherical Donnan radii, \( r_D \), were calculated according to 29

\[
r_D = \frac{3 M_w}{4 \rho AA N_a^2 \eta} \left(1 + \rho_{AA} \eta_D \right)
\]

where \( \rho_{AA} \) is the bulk density of AA (2 kg dm\(^{-3}\))\(^1\), \( M_w \) is its average (weight) molar mass (210 kg mol\(^{-1}\)),\(^3\) and \( N_a \) is Avogadro’s number. Radii calculated from the Donnan volumes (eq 8) were much larger than hydrodynamic radii, \( r_h \), measured by FCS (Figure 9, solid symbols; \( R^2 = 0.94 \), slope = 15.8 ± 2.2). Donnan radii were linearly related to, but nearly twice as large as values, obtained when the thickness of the double layer, \( k^{-1} \), was added to the hydrodynamic radii (Figure 9, open symbols; \( R^2 = 0.99 \), slope = 1.9 ± 0.1). It is currently unclear to what factor, other than a systematic experimental error, the difference of 1.9× can be attributed.

 Nonetheless, changes in the Donnan volume, \( \Delta V_D \), with ionic strength were proportional to the changes of the intrinsic viscosity of AA, \( \Delta [\eta] \), according to 29

\[
\Delta [\eta] = \zeta \Delta \nu_D
\]

In this case, the value of structural parameter \( \zeta \) was found to be 0.26 (\( R^2 = 0.93 \)), near the theoretical value of 0.25 that is expected for a sphere-like particle.\(^1\) The above considerations support the use of the Donnan approach and confirm that the Donnan volume approximation was physically realistic for the AA under the given conditions of pH and I. The attractiveness of the Donnan soft particle approximation is that it is fairly consistent with the protonation data, the FCS measurements, and the literature viscosity data.

The master curve, free of electrostatic contributions, was fitted by a Langmuir–Freundlich isotherm in order to obtain the intrinsic proton binding characteristics of the AA (Figure 1). The parameters used to describe the charge data are given in Table 1.

Three parameters were sufficient to describe the master curve based upon a single structure containing a single type of functional group (carboxylic) that could interact with protons. The value of the heterogeneity parameter, \( m = n_\nu p \), reflecting the combined effect of ligand-specific (\( p \)) and proton-specific heterogeneity (\( n_\nu \)), was equal to 1, indicating that the alginate had negligible intrinsic chemical heterogeneity with respect to the proton binding sites.

The median intrinsic stability constant value for the interaction of protons, \( \log K_H = 2.72 \), was in good agreement with literature values (\( \log K_H = 2.95 \))\(^,50\) while the total concentration of 4.4 equiv kg\(^{-1}\) of binding sites was slightly larger than the 3.6–3.7 equiv kg\(^{-1}\) found previously for an alginate from a different supplier. Nonetheless, a good fit with the experimental results, as well as practically the same values for the median intrinsic stability constant, were obtained for the Donnan model using a rigid particle assumption (\( \log K_H = 2.58 \)) and the Donnan model when assuming a particle swelling with charge variations (\( \log K_H = 2.60 \))\(^,51\) Both previously obtained values were in good agreement with the value determined here (\( \log K_H = 2.72 \)).

The parameters obtained from the pH titrations (binding site concentration, proton binding constant, and Donnan volume) were used to predict the effect of pH and ionic strength on Cd and Pb binding by the AA. The effects of pH, I, and the metal-to-ligand ratio on Pb or Cd binding by the AA were predicted reasonably well (dashed lines on Figures 2, 4, and 6) by the NICA–Donnan model. For both Pb and Cd, intrinsic stability constants were several orders of magnitude lower than the apparent stability constants (Table 1). The log \( K_{PAA} \) was about twofold larger than that found for Cd, in good agreement with the higher affinity of Pb for carboxylic groups and similar to a value (\( K_{PAA} = 10^{3.80} \) M\(^{-1}\)) found previously.\(^3\) In addition, an improved fit was obtained when Na was considered not only to affect electrostatic interactions but also to compete (weakly) with Pb and Cd for carboxylic binding sites. The ligand-specific heterogeneity parameter (\( p \)) and the ion-specific heterogeneity parameters (\( n_{Cd} \) and \( n_{Pb} \)) were estimated to be 1, again consistent with a chemical homogeneity of the metal binding sites, as observed above for the protons. This result was

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**Table 1. Intrinsic Parameters Obtained by the NICA–Donnan Model Describing H\(^+\), Cd\(^{2+}\), and Pb\(^{2+}\) Interactions with Alginates**

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Q_{\max} ) (equiv kg(^{-1}))</td>
<td>4.34 ± 0.015</td>
</tr>
<tr>
<td>( \log K_H ) (M(^{-1}))</td>
<td>2.7 ± 0.008</td>
</tr>
<tr>
<td>( m = n_\nu )</td>
<td>1</td>
</tr>
<tr>
<td>( Q_p ) (equiv kg(^{-1}))</td>
<td>1.5</td>
</tr>
<tr>
<td>( R^F )</td>
<td>0.982</td>
</tr>
<tr>
<td>( \log K_{PAA} ) (M(^{-1}))</td>
<td>3.5</td>
</tr>
<tr>
<td>( n_{Pb} )</td>
<td>1</td>
</tr>
<tr>
<td>( \log K_{CdAA} ) (M(^{-1}))</td>
<td>1.7</td>
</tr>
<tr>
<td>( n_{Cd} )</td>
<td>1</td>
</tr>
<tr>
<td>( \log K_{NaAA} ) (M(^{-1}))</td>
<td>0.35</td>
</tr>
</tbody>
</table>
reinforced in Figure 6 where, at low concentrations, the slope of bound versus free metal was unity.

In conclusion, the NICA–Donnan model can reasonably account for electrostatic and competition effects describing Pb and Cd complexation by alginate under a broad range of solution conditions ($I = 1 \times 10^{-3}$ to 0.5 M; pH = 4.0 to 8.0; metal-to-ligand ratios of 0.0002 to 0.2). The Coulombic contribution to the metal binding was important, attaining ca. 50% of the binding for Cd$^{2+}$ and ca. 15% for Pb$^{2+}$ (Figure 10).

Discrimination of the intrinsic and electrostatic components of the total binding constants was nonetheless only possible for a large set of experimental data, including molecular diameters, and a large range of physicochemical conditions. Nonetheless, this approach allows for the complete characterization of the Pb and Cd species in different metal fractions: dissolved, bound within the Donnan soft particle, and specifically bound metal.

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References and Notes


