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Chemical Controls over Natural Organic Matter Aggregation and the Resulting Structures

by

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Introduction

Natural organic matter (NOM), a diverse collection of organic chemical entities derived from the natural decay of biomass in the environment, is ubiquitous in most ecosystems. NOM is known to develop strong interactions with certain heavy metal ions, anions, and small organics (e.g. pharmaceuticals), making an understanding of NOM behavior in solution essential to developing models of how organic and inorganic pollutants move through the environment. However, the structure of NOM in solution and how chemical conditions affect that structure are poorly understood, as are the ways in which chemical conditions affect the interaction of NOM with other NOM molecules, surfaces, and ions. Some metal ions are known to promote the aggregation or flocculation of NOM in solution to produce particles consisting of many molecules. Flocculation is the process by which individual molecules interact strongly with one another to form large particles visible with the naked eye that can settle from solution via gravitational forces. Aggregation is similar, however, a particle aggregate is generally smaller and can remain suspended in solution under the influence of only gravity. This thesis attempts to shed light on the specific types of molecular-scale interactions between NOM molecules, solvent molecules, and other components of the solution; how floccs and aggregates form and grow; and how chemical properties of the NOM, mineral surfaces, and the solution phase affect aggregation/flocculation and the properties of the resulting aggregate or flocc particles.

Before getting into the heart of this thesis, it is necessary to define some critical chemical concepts that govern the behavior of NOM in water and some properties that we typically use to characterize particles formed from NOM. Cations are metal ions that bear a positive charge due to a deficiency of electrons and are found in most natural waters. Since the attractive or repulsive interactions between positive and negative charges often govern the properties of ion-ion, ion-solvent, or ion-solid interactions, the property called the charge density of a cation often has the strongest influence on interactions between cations and other species that may be dissolved or suspended in water. The charge density is the total charge on the ion divided by its volume if we consider the ion to be a perfect sphere with a radius equivalent to the ionic radius. Ionic strength is a property of solutions that estimates the total ionic environment; i.e. how well can the solution compensate for charged surfaces on particles or the driving force for change due to charge. pH is an estimate of the total hydronium ion (H_3O^+) concentration in the solution expressed on a log scale such that one change in pH units is equivalent to a 10-fold change in the concentration of hydronium ion. These parameters (pH, ionic strength, charge density) are often crucial to understanding how ions interact with solids and NOM in aqueous media. In the case of the floccs themselves, we characterize them based on their size, size distributions, and morphology across several length scales. The size of the particle is difficult to determine, but is often reported as either a hydrodynamic radius or a mean particle radius determined by image analysis. The hydrodynamic radius estimates particle size by considering the particle to be a spherical entity consisting of the flocc or aggregate itself and the solvent molecules that surround the particle whose motion is governed by the motion of the particle itself. We also are interested in the particle size distribution, a measure of the variability of the particle size within a specific size fraction of

flocc/aggregate particles. Finally, morphology is the nature of the particle shape, surface texture, etc.

This thesis opens with a review of key literature regarding what is known about NOM structure in solution and what we know about how NOM aggregates or flocculates. Each NOM molecule is different and most NOM samples in solution probably contain both macromolecular structures (large molecules with all atoms part of a single covalent network) and supramolecular structures (several molecules held together through strong physical interactions). Supramolecular structures can form *via* cation bridging between negatively charged sites on two NOM molecules, by hydrogen bonding (a very strong intermolecular force) between oxygenated functional groups *via* water bridges, or by water avoiding regions on NOM molecules clustering together to minimize the system free energy in solution. Supramolecular structures are primarily observed in higher pH ranges where cations with a high charge density are present. Evidence of these structures was observed using techniques such as dynamic light scattering and diffusion ordered nuclear magnetic resonance (NMR) spectroscopy that will be covered in the review. The macromolecular view of NOM with high “molecular” weight was mainly developed based on mass spectrometric methods. However, multivalent cations may increase the observed weight of NOM on a mass spectrometer through coordinative crosslinks; thus, there is a debate over whether the apparent weights are from one macromolecular structure or from smaller entities crosslinked together as a supramolecular aggregate.

To expand on this body of knowledge, original studies performed at Alfred University regarding NOM-NOM interactions in solutions with various cations, ionic strengths, and pH will be discussed. Dynamic light scattering (DLS) was used to quantify the effects of these chemical parameters on the hydrodynamic radii, number of unique hydrodynamic radii, and particle size distribution of NOM aggregates suspended in water. Solutions of Suwanee River NOM were prepared at several pHs and with several types of monovalent and divalent cations. The results suggest that in alkaline earth metal-NOM solutions, the protonation state of NOM seems to dominate the number of unique hydrodynamic radii distributions observed, with more deprotonation leading to fewer unique hydrodynamic radii that are larger on average when Ca^{2+} is present. In contrast, the polarizability of the cation and NOM protonation state seems to dominate the number of unique radii and their relative sizes observed for alkali metal solutions, with polarizable ions leading to fewer size fractions and larger particles as the NOM deprotonates. A model is proposed based on these results in which the number of unique hydrodynamic radii distributions is affected by the percent saturation of available ion bridging sites between functional groups of NOM molecules.

Finally, the work with solution NOM behavior is extended to explore the fundamental chemistry of clay (smectite)-NOM complexation and how it affects the microstructure and morphology of these composite materials. Based on the aggregate work presented earlier, the relative importance of ionic strength vs. the solution pH on composite formation and morphology were examined using helium ion microscopy (HeIM). A series of clay-fulvic acid (FA; fraction of NOM soluble at all pH) samples were prepared at pH 6 such that only the carboxylic acid groups will be deprotonated and then compared to composites

prepared at pH ~2 and pH ~12. The helium ion microscope images give insight into the morphology of the clay coated with NOM and also suggest that Ca²⁺ solutions with low ionic strengths can lead to a previously unobserved “string of pearls” morphology in composites and NOM floccs. There are two proposed mechanisms for smectite-NOM interactions depending on solution pH; hydrophobic interactions between molecular structures that avoid H₂O and ion bridging between charged entities. HeIM results from this suite of data will be compared to work published in the literature.

1. Literature Review - Natural Organic Matter, its Common Interactions in Solution, and Methods for Exploring Such Interactions

a. NOM Fundamentals

i. Nomenclature and Origins

‘Humus’ is a Latin word meaning soil and the term “humic substances” often refers to the organic chemicals left over from natural decay processes in soils and natural waters. Humic substances in the soil make up part of what is known as soil organic matter (SOM), which as defined by Stevenson et al.¹ includes the litter, the light fraction, the microbial biomass, the water-soluble organics and the stabilized organic matter (humus) found in soils. Hayes et al.² used the term soil organic matter to specify the non-living components of the soil, and describe SOM as a heterogeneous mixture of products resulting from the process of humification: the microbial and chemical transformation of organic debris. Humification of organic matter in the environment generally involves oxidation of the organic matter via the breaking of C-H and C-C bonds and making of C-OH and C=O bonds in the chemical structures, leading to smaller and more oxygenated molecules as a function of increasing time. In a broader context, the term natural organic matter (NOM) refers to the organic molecules produced by humification in soils, waters, and sediments and is widely used in studies of the physical and chemical properties of these decay products.

The source of the organic molecules in NOM is largely the remains of vascular plants and algae that are more difficult to degrade via humification. One such material is lignin, a structural material in the cell walls of plants. There are other plant structures that give rise to NOM molecules as well, such as sporopollenins, tannins, black carbon, proteins and aliphatic macromolecules such as algaenans, cutans and suberans³. In the context of this research, lignin is the primary precursor of the NOM we use, which is obtained from the Suwannee River that drains the Okefenokee swamp in southern Georgia.

The chemical components making up humic substances are generally classified further based on their solubility in aqueous media. In classical definitions⁴, NOM components that are soluble in basic solutions but precipitate or fall out of solution at pH 1 are known as humic acids (HA). Fulvic Acid (FA) is the operational definition describing the

constituents that remain in solution when the medium is acidified; thus, FA is soluble in all pH ranges. Humins are the fraction of NOM that is insoluble overall. Generally, FA contains more oxygen and is more degraded than HA, which is more oxygenated and degraded than humin.

ii. NOM functional groups

Functional groups are groups of atoms on a molecule that participate actively in chemical reactions. The most critical functional groups on NOM for this thesis work are phenols and carboxylic acids (Figure 1). These groups are important because they can lead to the development of negative charge on the NOM molecule depending on the solution pH. Chemists use the term pKa to denote the pH that leads to 50% of the functional groups in a solution experiencing deprotonation. Often, chemists use pKa as a quantitative estimate of the acid strength. A lower pKa means the substance is more acidic and will more easily give up its proton to become negatively charged. The pKas of phenol and carboxylic acids are ~9-10 and ~4-5, respectively. Each type of functional group tends to undergo the same or similar changes with respect to pH in an aqueous solution and all contain the elements needed to participate in hydrogen bonding with water (in this case O with non-bonding valence [outer] electron pairs). Therefore, these functional groups will experience similar interactions with H₂O and ions almost independent of the overall molecular structure in our NOM.

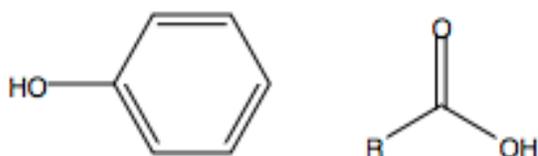


Figure 1. Functional groups of NOM. Phenol (left) and carboxylic acid (right).

iii. Debate about molecular-scale structure: macromolecular or a supramolecular aggregate?

There are two prevailing lines of belief regarding the molecular structure of NOM. In the past, it was uniformly accepted that NOM was macromolecular⁵⁻⁶ meaning NOM is made up of large molecules each having a single covalent network. For instance, Piccolo et al. proposed that NOM consists of coiled macromolecules that have elongated shapes in basic or low ionic strength solutions.⁷ The macromolecular model of NOM was developed predominantly from studies involving a technique called gas chromatography mass spectrometry (GC-MS), which separates mixtures into individual components, ionizes them, and characterizes them based on their mass-to-charge ratio (equivalent to the particle mass if the molecule has a -1 or +1 charge). It wasn't until more recently that NOM became viewed as a self-assembling supramolecular association of molecules held together by strong physical interactions.⁸⁻¹¹ Multivalent cations may increase the weight observed on GC-MS as a result of coordinative crosslinks between OM molecules; thus, these supramolecular structures cannot be ruled out on mass

spectrometric data alone. Currently, it is more widely accepted that NOM consists of both macromolecular structures and supramolecular aggregates, with the relative amounts of each depending on the age of the organic matter and rates of humification reactions.¹² Techniques such as molecular dynamics computer modeling, dynamic light scattering, diffusion ordered NMR spectroscopy, X-ray scattering, and electron microscopy have provided evidence that supramolecular structures exist in NOM. Studies report ion residence times in association with NOM, suggesting linkages may exist between two NOM species in order to form these supramolecular structures.¹³ Since the NOM molecules are varied in structure and origin, NOM is always considered an amorphous material (a material without regular ordering of atoms).

b. Types of NOM-NOM Interactions in Solution

i. Cation Bridging

Organic matter (OM), particularly when critical functional groups are deprotonated (lose a hydrogen ion), is subject to various interactions with cations including alkali metals, alkaline earth metals, zinc, manganese, aluminum and iron; thus, complexation or binding of an organic ligand by a cation controls transport¹⁴, toxicity¹⁵, reactivity and solubility¹⁶ of the metal contaminant. The interactions between two or more NOM functional groups and cations via electrostatic interactions will be referred to as cation bridges in the remainder of this study. Electrostatic interactions occur when atoms associate by attraction between opposing charges (Figure 2) and are directly proportional to the magnitude of the charge and indirectly proportional to the distance between the charges. In the absence of cations, NOM molecules that have lost a hydrogen ion and subsequently develop a negative charge can also repel each other via electrostatic repulsion.

Hong et al.¹⁷ and Ritchie et al.¹⁸ used molecular modeling (approximating the behavior of a chemical system using computers and classical or quantum physics to govern atom behavior and interactions) to show the development of a negative charge on NOM in approximately neutral pH (pH = 7) can be attributed to deprotonated carboxylic groups, which typically have a pKa of ~4.5. Much of the ion interaction with NOM occurs through primarily carboxylate groups (the carboxylic acid functional group with a hydrogen removed) and phenol groups.¹⁹ In additional studies using computation, Tipping et al.²⁰ and Rey-Castro et al.²¹ claim negatively charged carboxylic groups are the prominent binding sites for metal cations^{10-11, 22-24} stating the strongest NOM associating ion is Ca²⁺. Iskrenova-Tchoukova et al.¹³ found in molecular dynamics computer simulations that Ca²⁺ coordinates with NOM carboxylic groups for 0.5ns, an order of magnitude longer than the 0.02-0.05ns residence time of the similarly sized but less charged Na⁺ ions coordinated with NOM. This helps to explain the formation of visible aggregates of NOM in calcium solutions but not in sodium solutions. Numerous sources²⁵⁻²⁷ report a direct relationship between the charge density of the ion and the complexation strength of organic matter in the case of lighter metals. In general, as the charge density increases the complexation strength increases.

Molecular modeling results^{13, 28} also demonstrate four main routes of Ca-NOM

coordination: bidentate and monodentate inner sphere associations in which water molecules surrounding the ion are replaced by one or two oxygen atoms of a carboxylate group and bidentate and monodentate outer sphere associations in which the calcium ion is separated from NOM's carboxylic oxygen atoms by a mono-molecular layer of H₂O. In chemistry, monodentate refers to a coordination by one atom on a chemical entity while bi-dentate refers to coordination by two atoms on the same chemical entity (Figure 3). Kalinichev et al.²⁸ found 35-50 percent of NOM carboxylic groups to be associated with calcium ions in three different simulations performed for the same Temple–Northeastern–Birmingham (TNB) model of NOM in aqueous solutions containing Ca²⁺ ions. The models differed in system size, force fields used to govern the interactions between atoms, and used two different computer models of water. Outer sphere and inner sphere complex formation was also noted by Kalinichev et al.²⁶ and Xu et al.²⁹ following experiments combining NMR spectroscopy and molecular dynamics computer modeling. Specifically, weak outer sphere complexes were formed with Cs⁺ and Na⁺ while strong inner sphere complexes were observed between Ca²⁺ and NOM. Kalinichev et al.²⁶ also observed that molecular size; pH; the number, type, and density of OM functional groups and ionic strength all impact the extent of cation-OM binding. Iskrenova-Tchoukova et al.¹³ found Ca²⁺ can form monodentate complexes with NOM as well at longer Ca²⁺-O_{OM} distances.

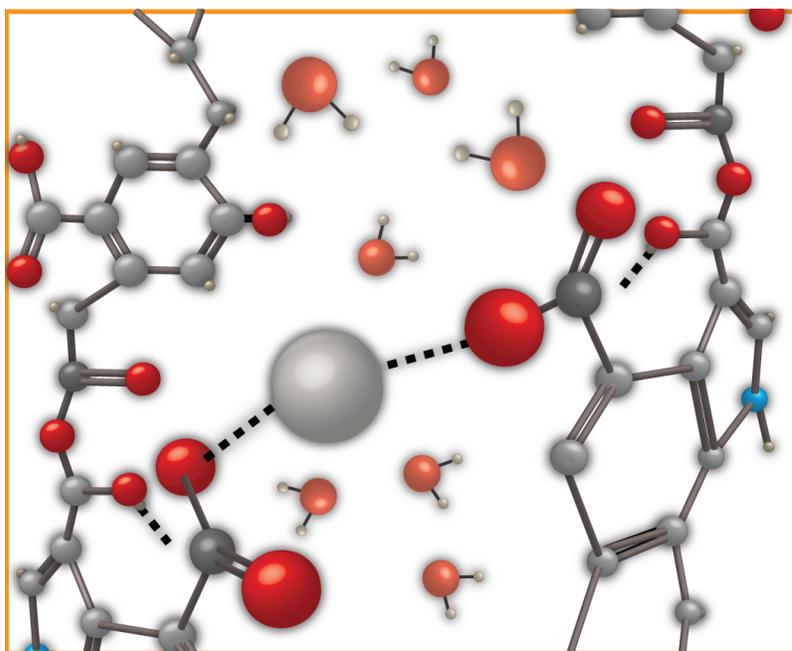


Figure 2. Cation bridge between the negatively charged functional groups of NOM and the positively charged cation. Red atoms are oxygen, grey are carbon, white are hydrogen, and the large grey-brown ball near the center of the image represents a cation. Dashed lines represent an electrostatic interaction.

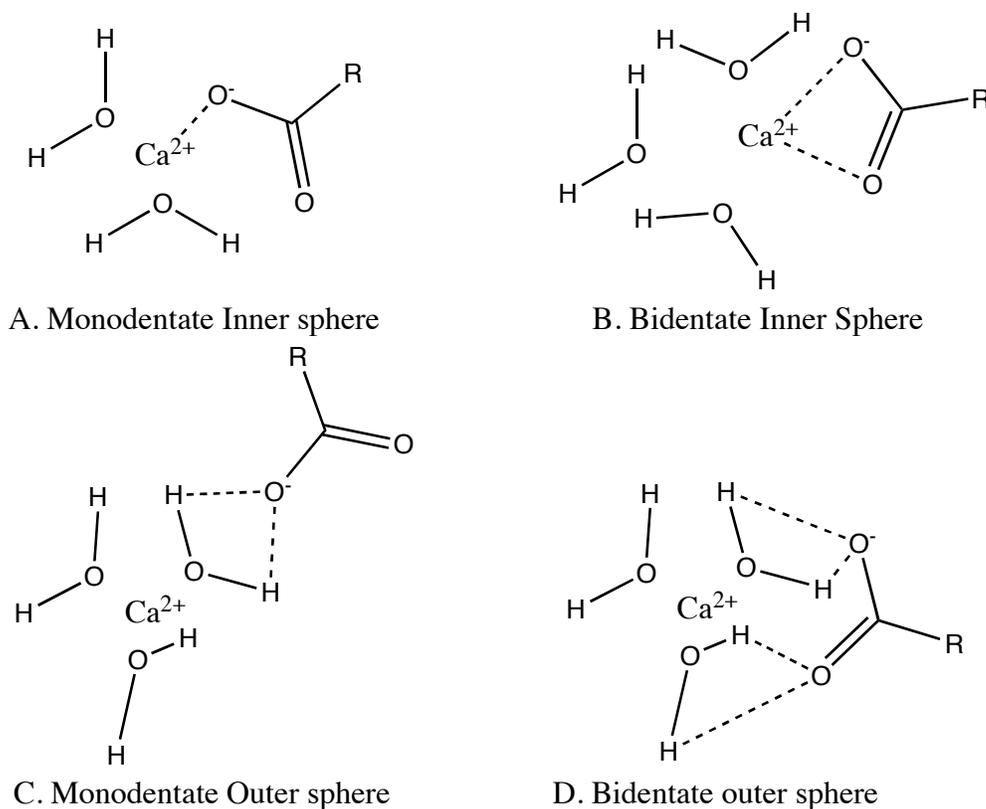


Figure 3. Proposed methods of calcium interaction with carboxylic groups of NOM.

ii. H₂O Bridging via Hydrogen Bonding

NOM functional groups can also form strong interactions within or between molecules via hydrogen bonding. Hydrogen bonding is a special type of intermolecular force where two molecules interact via the sharing of non-bonding valence electrons (those electrons at the greatest distance from the atom's nucleus) between an oxygen or nitrogen of NOM's functional groups with a hydrogen of H₂O or the H of an OH or NH functional group of the NOM with the O of H₂O. In other words, the atoms of the NOM functional group can act as an electron donor or acceptor depending on whether the functional group contains an O-H/N-H bond or not. The lack of regularity in the arrangement of NOM functional groups may prevent the close sequence of functional groups needed for strong intermolecular and intramolecular H-bonding. However, Aquino et al.³⁰ used MD simulations to demonstrate the development of interactions between two NOM molecules via bridging by water molecules. Water molecular bridges (WaMB) are a function of H₂O availability and the distance between NOM functional groups. For instance, Aquino et al.³¹ demonstrated the possibility of six to ten H₂O molecules bridging NOM functional groups 13-14.5 Å apart to stabilize supramolecular structures in the presence of cations. Cations formed indirect and direct complexes with carboxylate groups while the water formed the solvation shell of the cations. In a separate study, Aquino et al.³² used molecular simulations to show this chain-of-H₂O bridging in cation-free HA solutions as well. Stabilizing effects are only present with intermediate water content, for Schaumann et al.³³ indicates that at increased water

content water's plasticizing affects take over and such chain-of-H₂O bridges are short lived. Plasticizers are additives that increase the fluidity of a substance.

iii. Hydrophobic Effects

An alternative NOM-NOM interaction in aqueous solution is the hydrophobic mechanism, where nonpolar regions of the organic molecules organize themselves to avoid contact with the very polar water molecule. Individual organic matter molecules can change conformation (rearrange bond angles to change shape) to facilitate the formation of this hydrophobic interaction⁶, or several molecules can re-arrange their shapes to associate their hydrophobic regions with one another. It is believed that hydrophobic interactions may play a significant role in organic matter aggregation or flocculation in acidic conditions where all OM functional groups are protonated and cation bridging cannot occur.³⁴ It may also be important at basic pH under certain conditions as well. Examples of such rearrangements and inter-molecular associations can be seen in Figure 4 where nonpolar, hydrophobic, aliphatic carbon regions are oriented to the middle, leaving polar regions exposed to water.³⁴

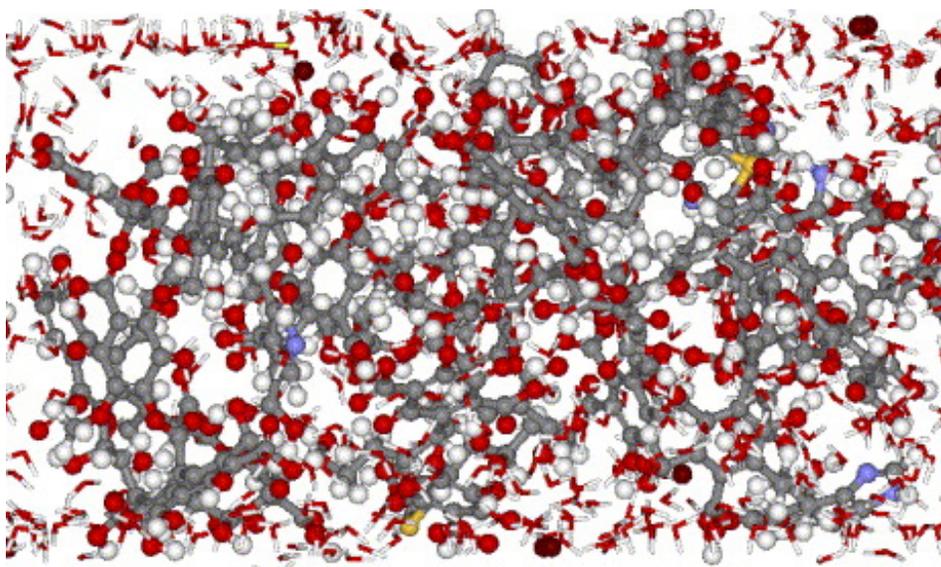


Figure 4. A molecular model of protonated OM in solution. Note the high density of grey and red atoms in the center and OH groups on the outside of the NOM globule. Water molecules are represented using cylinders to reduce visual clutter, and OM molecules are represented in the ball and stick style. Grey denotes C, red denotes O, white denotes H, N and S are blue and yellow, respectively. Image reproduced from Sutton et al. *Environ. Sci. technol.*, 2005, 39 (23), pp 9009-9015.

c. Characterizing NOM

i. DLS

Dynamic light scattering (DLS)³⁵ measures the intensity of light scattered by small particles in solution (such as colloids and NOM aggregates) as a function of time. Due to Brownian motion, all molecules in a solution diffuse, which means that the distance between light scattering entities in the path of the beam is constantly changing as a function of time. The rate of diffusion is controlled by temperature, the viscosity of the solvent and the size of the molecules. Thus, constructive and destructive interference of

light scattered by different particles in the light beam causes a fluctuating light intensity measured at the detector. The timescale of these fluctuations is related to the hydrodynamic radius of a molecule through the autocorrelation function, $C(\tau) = A(1 + \beta \int_0^\infty P(\Gamma) \exp(-\Gamma\tau) d\Gamma)$, where $\Gamma = Dq^2$ is known as the diffusion relation and $d_h = \frac{k_B T}{3\pi\eta D}$ is the Stokes-Einstein relation that relates the diffusion rate to the radius of a hypothetical spherical particle diffusing in a fluid with viscosity η . The autocorrelation function is a cross correlation of the signal with itself or the similarity between intensity observations as a function of time. DLS results provide hydrodynamic radii (see introduction for definition) and their radii distributions.

ii. Diffusion Ordered NMR Spectroscopy

DOSY, or diffusion ordered NMR spectroscopy, resolves different compounds based on their differing diffusion coefficients, which relate back to the size and shape of the molecules and the viscosity of the solvent at a particular temperature. The resulting diffusion coefficients can be related back to particle size by applying a model such as the Stokes-Einstein spherical particle model. An experiment by Simpson et al.⁸ highlights how DOSY has contributed to the debate about NOM solution structure. They found that all of the components of IHSS peat standard exhibited a single diffusion coefficient in water. However, the same NOM molecules revealed separate diffusion coefficients when dissolved in ethanoic acid. These coefficients were assigned to lignin, peptide and carbohydrate derived components, respectively. Thus, the acetic acid was capable of dis-aggregating a supramolecular entity, suggesting that this NOM sample is dominated by supramolecular associations rather than macromolecules.

iii. HPSEC

High Pressure Size Exclusion Chromatograph (HPSEC)^{12, 36-37} is often used to determine OM's relative molecular weight distribution. The underlying principle of HPSEC is that molecules in solution are separated by their size or molecular weight due to differing filtration rates. A HPSEC column is a hollow tube packed with small porous polymer beads of varying sizes. As solution travels down a column, particles enter into the pores. A small molecule can penetrate nearly every region of the pore system; thus, the small molecules take longer to travel down the column. Large molecules cannot penetrate the pore system and will elute first, meaning they exit the column first. Thus, passing a sample containing molecules or aggregates of several sizes through an HPSEC instrument will separate those aggregates based on size. A properly calibrated HPSEC column will then give information about the molecular weight of the fractions eluting at each time.

iv. Small-angle X-ray Scattering

Small angle scattering is a measurement technique where elastic scattering of x-rays is recorded as a function of the angle of incidence, which can be used to determine the radius of gyration for the scattering entities in solution. In many ways, the fundamental principle of variations in scattered intensity apply to DLS and small-angle XRS. The amount and angular distribution of this scattered intensity provides size information at low angles 2θ from 0° to 3° when the natural log of the intensity is plotted with respect to the angle-to-wavelength ratio squared. The variations in intensity are a function of

density and differences in particle composition.³⁸ Electron density fluctuations over lengths of 100 Å can produce an appreciable amount of x-ray scattered intensities. Thurman et al.³⁹ analyzed OM size in aquatic solutions adjusted to pH 12. OM sizes reveal that Suwanee River FA and HA molecules have 4.7 to 33 Å radii of gyration corresponding to molecular weights of 1000-1500 Daltons.

V. Electron and Ion Microscopy

Electron microscopy is a technique for taking high-resolution images of materials at very high magnifications by bombarding the surface with high-energy electrons. These electrons stimulate photon emission, secondary electrons (SE), backscatter electrons (BSE) and x-rays for chemical information by energy dispersive spectroscopy (EDS). In scanning electron microscopy, an electron beam is rastered along the surface and the intensity of whatever effect is being detected is used to generate an image (for example, bright regions may correspond to more secondary electron production than dark regions). Helium ion microscopy (HeIM) is a detailed surface imaging and analysis technique with a higher depth of field than is typically obtainable in a scanning electron microscope. This increased depth of field and resolution is the result of a helium ion having a smaller wavelength than the electrons leading to a smaller interaction volume with the sample surface. When the helium ion reaches the sample surface, it can also collide with electrons in atoms of the sample and force them out of orbitals to produce secondary electrons. An SEM typically produces one secondary electron per incident electron, whereas a HeIM can produce up to 3-9 secondary electrons per incident helium ion depending on the topography of the sample.⁴⁰ The high resolution image can then be analyzed in a variety of fashions to give morphology, texture, or even particle sizes and size distributions. HeIM has better sensitivity to light elements for contrast imaging. Unlike SEM, samples analyzed with HeIM do not need to be coated with conductive coatings such as carbon, gold or any other metallic species. Using the electron flood gun, one can neutralize the charging effects and still achieve high resolution imaging.

d. Role of pH, Cation, and Ionic Strength on NOM-NOM Interactions in Solution

Xi et al.¹² used dynamic light scattering (DLS) and high pressure size exclusion chromatograph (HPSEC) to show how solution conditions such as the identity of the cation, ionic strength and pH are critical to the hydrodynamic size of NOM. The effective molecular size distribution of both Biohumic NOM and IHSS (International Humic Substance Society) NOM exhibited no change over the pH range 4–10 and a significant size decrease at low pH values. As the concentration of Na⁺ increased, the effective size of the aggregates was significantly reduced due to coil-to-globule conversion, something akin to Na⁺ leading to increased hydrophobic interactions. HPSEC analysis of the NOM solutions paralleled these findings. Broad molecular weight distributions were seen in cation-free NOM solutions with narrowed distributions seen in the presence of cations. In the presence of Ca²⁺ various NOM types behaved differently. For instance, Singapore NOM size increased with an increase in Ca²⁺ concentration. However in the case of IHSS HA, increasing Ca²⁺ concentration showed competing effects on the effective size. At relatively low Ca²⁺ concentrations and/or solutions with a pH below 2, size reduction occurred similar to that seen in Na⁺ solutions. However,

the authors report that NOM size increased as Ca^{2+} concentration increased as a result of aggregation or chelation between the phenolic groups of the humic acid components of NOM and Ca^{2+} . Baysal et al.⁴¹ proposed similar mechanisms. When sodium is present, it is not clear whether NOM is macromolecular or a supramolecular aggregate; it simply indicates the molecules are very large. When calcium is present, it can be said that supramolecular aggregates exist.

Wu et al.³⁷ investigated the size distribution of the metal-dissolved organic matter (DOM) complexes using HPSEC, UV-Vis and high-resolution inductively coupled plasma mass spectrometry (ICP-MS), a technique that can be used to quantify metal ions by decomposing the NOM complex and ionizing its atoms at very high temperatures. Subsequent results for the Metal-DOM complexes correlated between ICP-MS and HPSEC indicate the following order of decreasing averaged molecular weight: $\text{Cu} > \text{Ni} > (\text{Co}, \text{Zn}, \text{Cr}) > \text{Pb} > \text{Cd}$. Results suggest that the distribution of sizes is closely related to metal binding strength, also supporting the idea of supramolecular aggregation.

2. Original Study of NOM Size Distribution in Aqueous Solution

a. Motivation

Many types of industrial processes such as dialysis (a process to purify aqueous fluids) and reverse osmosis filtration (a process often used to desalinate water) involve passing large volumes of water containing dissolved organics and other impurities through a membrane. In these applications, the membrane openings must be very small to be effective, which makes clogging a serious problem. Large colloids or NOM in the aqueous media can lead to membrane fouling, a process that decreases the performance of the filtering membrane by deposition of particles on the membrane surface or within the membrane pores.¹² Thus, it is important to understand the chemical factors that lead to large NOM colloid formation and/or chemical means to destabilize these colloids and protect the filtration membranes. As demonstrated in the literature review, there are a number of important factors that govern ion-mediated aggregation of NOM in solution, including ion bridging, water bridging, and hydrophobic interactions, all of which also depend on the pH and chemical makeup of the NOM molecules. This study explores the fundamental chemical controls over such NOM-NOM interactions in solution with special emphasis on understanding the mean particle sizes and their size distributions as a function of ionic strength, cation charge density, and solution pH.

b. Methods

i. Sample Preparation

Aqueous NOM samples were prepared at pH 2, pH 6, and pH 12 in the presence of cesium, calcium, sodium, strontium, magnesium and potassium. These pH values span the range of pKas that lead to full protonation of NOM (pH 2), deprotonation of only carboxylic acid groups (pH 6), and deprotonation of all carboxylic and phenolic groups (pH 12). Procedures for generating the NOM samples were designed to mimic clay-

NOM composite preparation from earlier studies.⁴² The samples at pH 12 were generated by titrating 50 mg of dry, powdered NOM in 30.0mL of deionized H₂O to pH ~12 using 0.1 M A(OH)_x solution, where A^{x+} corresponds to one of the six cations previously listed. The pH was adjusted and monitored using a pH electrode and autotitration system (Vernier). Once the titration was complete, DI H₂O was added until the total volume of the NOM suspension was 50.0mL and the sample was allowed to equilibrate for at least one hour before DLS analysis. The NOM aggregates at pH 2 were prepared by following the same procedure, however, after 30 minutes of equilibration at basic pH, the samples were brought to pH 2 by addition of 0.1 M HCl. Aggregate suspensions at pH 6 were prepared in one of three ways. The first method entailed taking the NOM solution from its natural pH directly to pH 6 (direct method) using A(OH)_x. The second method involved taking the solution to pH 12 and then down to pH 6 (indirect method) in a similar procedure to the pH2 samples. The third method involved a titration to pH 6 and then ACl_x was added until the ionic strength matched that of the indirect sample. Ionic strength calculations accounted for the free H⁺/OH⁻, Cl⁻, and free/liberated Ca²⁺ as needed based on the specific sample preparation conditions. All final pH equilibrations took place in a dark location in order to avoid potential photochemical reactions. Suspensions were refrigerated and stored in acid washed polyethylene bottles until measurements were taken on the DLS instrument. Most samples were returned to the refrigerator and analyzed several times to gain information about how the sizes and size distributions varied with time.

ii. Sample Analysis

Storage bottles were brought to room temperature and inverted ten times prior to obtaining DLS measurements. A plastic pipette was used to transfer solution to the instrument following a DI water blank. The instrument was operated in absorbing mode with a particle density of 1.05. The solvent refractive index was set to 1.333 (water) and the viscosity range was set from 0.797 at 30°C to 1.002 at 20°C. The particle size range for the study was set from 0.8 nm to 6540 nm determined via a Geom 8 root progression and intensity distribution.

c. Results and Discussion

While we hoped to develop a detailed quantitative understanding of the chemical controls over particle size and particle size distributions, we were only able to draw qualitative conclusions about NOM aggregation from our results. DLS is well suited to size determination in monodisperse samples and all of our NOM solutions contained several different populations of hydrodynamic radii, significantly complicating quantitative interpretation of the results. In addition, the uncertainty as determined by repeat DLS measurements was high enough that statistical size differences between systems were challenging to establish. There did seem to be a correlation between the number of unique hydrodynamic radii distributions, the number of deprotonated oxygen sites on the NOM, and the properties of the cation (Figure 5). However, the charge density of divalent cations was crucial in the alkaline earth metal systems (as predicted by MD modeling) while cation polarizability seemed to be the most important cation property in alkali metal-NOM solutions.

In alkaline earth metal-NOM solutions, the DLS results suggest that the degree of saturation of the possible ion bridging sites dominates the number of unique hydrodynamic radii distributions observed (Figure 5). For a multivalent ion such as Ca^{2+} that has a high enough charge density to easily form ion bridges between NOM molecules^{13, 26}, we hypothesize that increasing numbers of Ca^{2+} ions in solution will lead to more ion bridges formed at fixed number of deprotonated oxygen sites: more ions means a greater likelihood that the ions will locate and remain in a bridging site. Likewise, as the pH increases and more sites are deprotonated, more potential ion bridges between NOM molecules or NOM aggregates can form. Thus, we predict that as the Ca^{2+} concentration and pH go up, smaller particles likely agglomerate to make larger particles via ion bridges between OM^- and OM^- . This is in agreement with the decrease in number of unique NOM particle sizes and a shift to larger particle size in DLS at basic pH when many Ca^{2+} ions are available. We propose that there is a tradeoff between stabilization of NOM molecules and aggregates via DLVO forces (the charge compensation at negatively charged NOM surfaces due to the counterions in solution accumulating near said surface) and growth of aggregates via an increased number of ion bridges to other NOM molecules and aggregates. More multivalent ion NOM solutions need to be assessed to determine the validity of this hypothesis and to determine whether there is a correlation between charge density, pH, and the number of large-sized particles amongst the alkaline earth metals. Other methods of size determination should also be used in an attempt to get more reliable data regarding the actual particle sizes and size distributions.

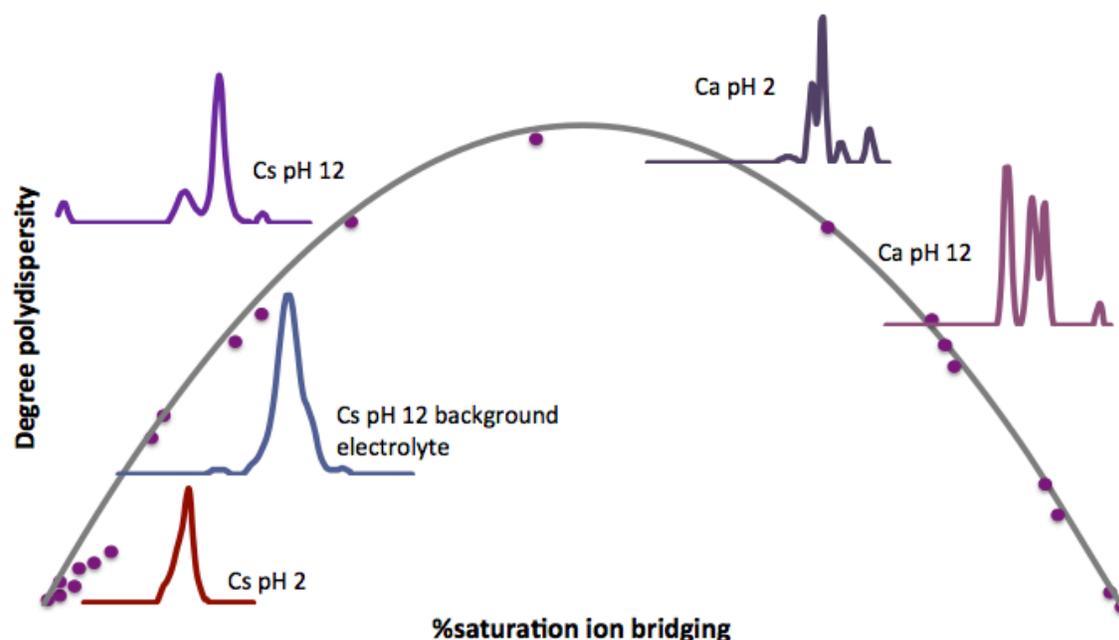


Figure 5. Alkali metal-NOM particles (left side) have fewer unique particle sizes with larger size distributions, as expected for particles with less cation bridging. As the pH increases and ion bridging in alkali systems becomes more likely, we notice more unique particle sizes and narrower distributions. In the case of the divalent calcium systems that readily form ion bridges, acid pHs lead to more unique

particle sizes with narrow distributions and the number of unique sizes decreases with increasing pH. This suggests that basic pH facilitates smaller particles aggregating into larger particles as potential ion bridging sites become saturated.

In contrast, cation polarizability and the number of deprotonated oxygen sites on NOM seems to dominate the number of unique hydrodynamic radii observed for alkali metal-NOM solutions. Polarizability is related to the size of the ion electron cloud such that ions with large electron clouds are more polarizable than smaller ions. Thus, Cs^+ has the highest polarizability followed by K^+ and finally Na^+ . Both sodium and potassium seem to yield relatively similar numbers of unique hydrodynamic radii and similar transitions in the number of radii and mean particle size with time (Figure 6). Based on the low number of ion bridges formed by these ions in the MD studies, this is likely a DLVO stabilization effect or evidence of weaker, short-lived water bridges between NOM molecules. However, the Cs-NOM sample increased in the number of unique particle sizes and seems to develop larger particles with increasing time. Since monovalent ions are not as good at forming ion bridges, it may be that the more polarizable species can distort their electron clouds to make ion bridge formation between two NOM OM^- groups more favorable. Thus, as pH increases and more OM^- groups are formed, we might expect larger aggregate sizes for Cs^+ vs. Na^+ or K^+ , in agreement with the DLS results.

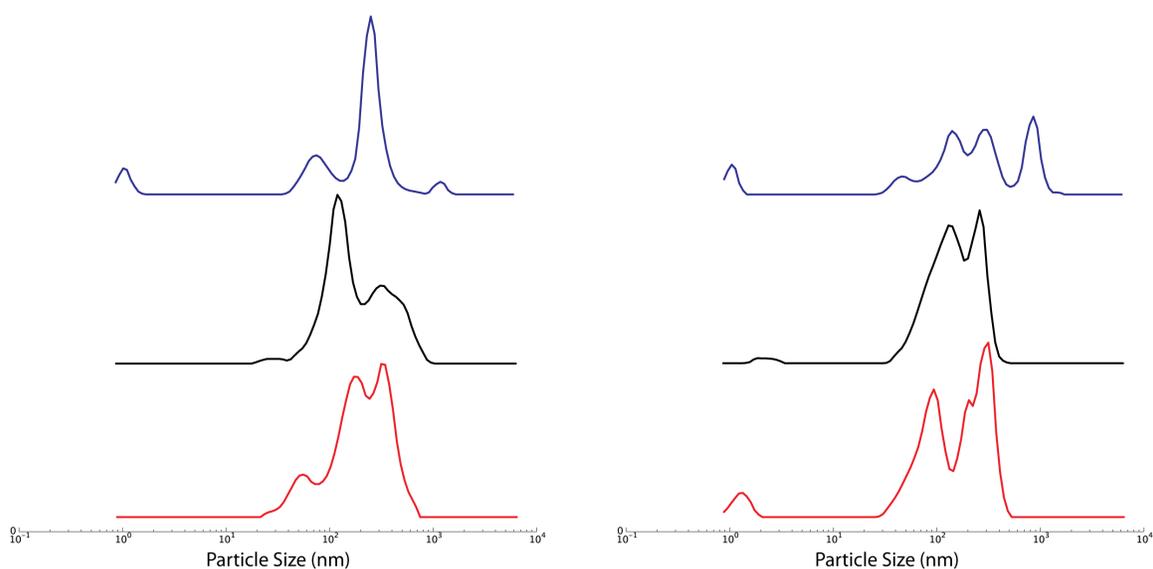


Figure 6. DLS results for alkali metal-NOM aggregates prepared at pH 12. The Cs-NOM results are in blue, the K-NOM in black, and the Na-NOM in red. The data on the left column represent ~1 hour after preparation and the right column is after five days of equilibration.

3. Morphology of Clay-NOM complexes

a. Motivation

While it is well established that NOM interacts with metal ions (particularly heavy metals) and organic pollutants, other commonly suspended components in our natural waters such as clay minerals can also act like nature's sponges, adsorbing metals and

organic molecules. It has been shown that NOM can interact with and coat clay platelets⁴², but how these more complicated organic-inorganic composite materials interact with ions and other pollutants and how solution chemistry affects these interactions, or even composite formation itself, are not well understood. Thus, a complete picture of pollutant transport requires that we explore these more complicated organic-inorganic hybrid materials. Previous studies by our research group into the fundamental chemical controls over clay-NOM composite formation⁴² revealed that the valence of the ion, the protonation state of the NOM, and the ionic strength of the solution all play critical roles in NOM-clay association. However, it was unclear from our data what NOM functional groups are most important in this process. It was also unclear what the relative roles of pH and ionic strength are on the formation and microstructure of clay-NOM composites. In this research project, a series of clay-NOM composite samples prepared at pH 6 via a variety of pH histories and solution ionic strengths are studied with helium ion microscopy and compared to composites previously prepared at pH 2 and pH 12 in an attempt to address both issues.

b. Introduction to Clay

Clays are phyllosilicate minerals frequently found in soils and suspended in water around the globe. The primary structural unit of a phyllosilicate is a silicate tetrahedron, a triangular pyramid, with oxygen atoms at the vertices and a silicon atom at the geometric center. Each silicate tetrahedron shares three of its vertices with other silicate tetrahedra such that the linked tetrahedra form two-dimensional planar sheets with widths and lengths at least one thousand times larger than the sheet height. In the case of clay minerals, two of these silicate sheets sandwich a sheet of octahedrally coordinated cations. These tetrahedral and octahedral sheets are bonded via strong electrostatic interactions (commonly called ionic bonds) to form the basic structural unit called a layer (Figure 7). Clays all contain some degree of isomorphic substitution, where other cations of similar size but different charge insert into the octahedral or tetrahedral positions within the layers. As a result, the total positive charge from the cations in the layers may not balance the negative charges from the anions (negative ions) in the clay structure, giving a permanent negative charge on each layer.⁴³ To build a three-dimensional particle we can hold or see, the layers stack upon one another with charge-balancing cations between layers to combat the electrostatic repulsions between adjacent layers. Water can also reside in this interlayer and the amount of water at different vapor phase water activities depends largely on the charge density of the cation.

The clay used in this study is a low iron smectite or swelling clay that has the ability to expand and contract in the presence of water called hectorite. It is a natural hectorite found in California and develops charge by Li⁺ substitution for Mg²⁺ in the octahedral sheet. This particular hectorite also has a rather high fluoride content. The structural formula for the hectorite in this study is $(\text{Na}^{+0.19} \text{Mg}^{2+0.07} \text{Sr}^{2+0.01} \text{K}^{+0.01})^{+0.36} \bullet [(\text{Mg}_{2.65}\text{Li}_{0.35})^{-0.35} (\text{Si}_{3.99}\text{Al}_{0.01})\text{O}_{10}(\text{F}_{1.1} (\text{OH})_{0.9})]$.

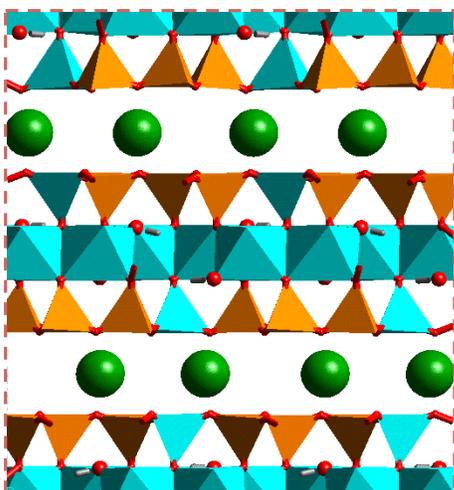


Figure 7. The layer structure of muscovite mica, a phyllosilicate mineral quite similar to the clay used in this study. Octahedrons (Blue) are cations coordinated to oxygens of silicon (yellow) tetrahedrons. The lights blue tetrahedrons show isomorphous substitution of aluminum in place of silicon.

c. Methods

Clay-fulvic acid composites were prepared at pH 6 using a procedure that is extremely similar to that developed by Ferguson et al.⁴² for the pH 2 and pH 12 comparison set. Clay-fulvic acid composites at pH 6 were prepared in one of three methods. The first method entailed taking the clay-NOM suspension from its natural pH (~4.5) directly to pH 6 (direct method) using $A(OH)_x$. The second method involved taking the suspension to pH 12 with the same base solutions and then down to pH 6 with HCl (indirect method). The third method involved a titration to pH 6 with base solution followed by ACl_x addition until the ionic strength matched that of the indirect sample (background electrolyte method). As in the previous study, ionic strength calculations accounted for the free H^+/OH^- , Cl^- , and free/liberated Ca^{2+} as needed based on the specific sample preparation conditions. Once titrated to the final pH, samples were allowed to equilibrate in the dark for ~24 hours. The samples were centrifuged in 80mL centrifuge tubes for 11 minutes at 10,000rpm. The supernatant solution was decanted and two cycles of re-suspension in DI water were completed in order to rinse away excess salts and non-bound NOM molecules. Samples were centrifuged and the supernatant solution was poured off after each rinse. Samples were freeze-dried after the final rinse cycle. The composites were then ground using a mortar and pestle to less than 100 μ m and stored in a dry desiccator until transport to PNNL for analysis.

HeIM images were collected and compared for these samples and similar samples previously prepared at pH 2 and pH 12 (Table 1) using the Zeiss Orion helium ion microscope housed at Pacific Northwest National Laboratory. For this study, the instrument was operated at an optimal imaging voltage of 30kV using aperture size of 10 μ m. An Everhart-Thornley (E-T) detector was used to obtain the microscope images.

Table 1. pH excursions for composite samples and their respective ionic strengths.

Name	pH History	OM-type	Ionic Strength
MH	nat	N/A	N/A
MHNOM-2	nat→pH12→pH2	SR NOM	0.04±0.02
MHNOM-12	nat→pH12	SR NOM	0.004±0.001
MHFA-6-dir	nat→pH6	Fulvic Acid	8x10 ⁻⁷
MHFA-6-indir	nat→pH12→pH6	Fulvic Acid	0.0006±0.0002
MHFA-6-backgr	nat→pH6	Fulvic Acid	0.0005±0.0002

d. Results and Discussion

Overall, the HeIM results suggest that the appearance and morphology of the pH 6 samples do not vary significantly between the different preparation methods and are in general more similar to the results for the pH 12 clay-NOM composites than for the pH 2 composites. Independent of the counterion, we see HeIM images at pH 6 and pH 12 dominated by smooth, flat structures and edges of stacked platelets that resemble base clay without any NOM present (Figure 8). However, if one increases the magnification, these samples appear to have a rough and hummocky surface unlike base clay exposed to the same grinding and preparation procedures. This suggests a surface coating is present. Since the pH 6 and pH 12 samples are so similar, it is unlikely that this coating is the result of clay dissolution and precipitation of amorphous silica that can occur at basic pH, meaning that this coating must be a thin coating of NOM. The presence of NOM is consistent with SEM/EDS spectroscopy for the pH 12 samples published in a previous study.⁴² In contrast, at pH 2 the alkali metal systems exhibit significant curling of the platelets, a rougher texture, and regions where the signal from the clay is completely obscured by NOM in EDS⁴² (a spectroscopic technique that identifies the composition of a sample in an SEM using the characteristic X-ray energies emitted by atoms under the electron beam) suggesting thicker NOM coatings. The Ca-hec-NOM sample at pH 2 is different from all the others, with thick coatings that have morphological features of the NOM floccs. We hypothesize that this flocc forms from residual solution-phase NOM as the pH drops and ionic strength rises during the acidification process. This NOM flocc agglomerates on the clay platelet surfaces, yielding an NOM-rich and highly heterogeneous composition unlike any other set of conditions.

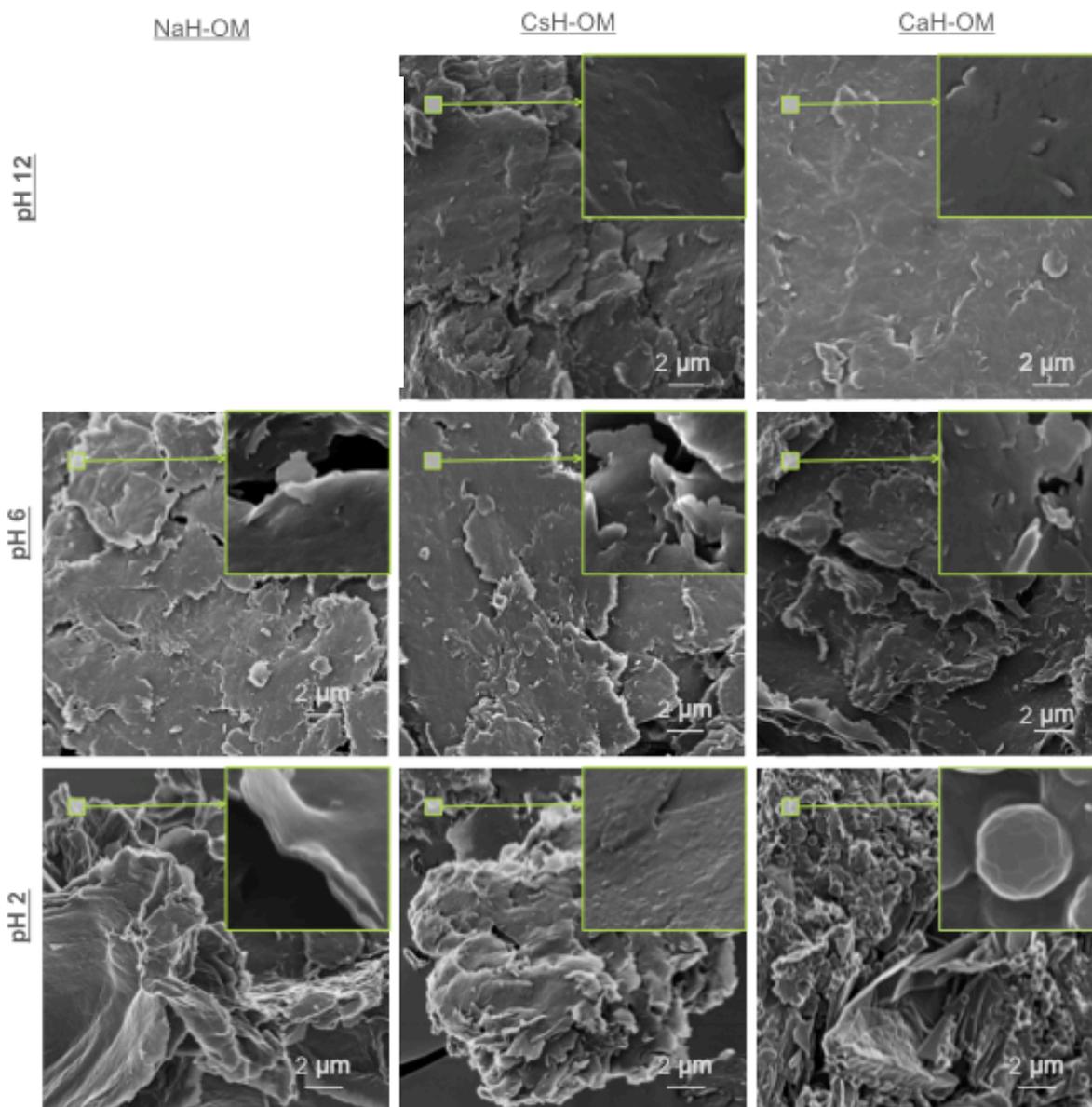


Figure 8. Morphologies of composites at pH 2, 6 and 12. pH 6 sample images are from the direct pH method. pH 2 and 12 inset fields of view are 500 nm unless otherwise stated. Ca pH 2 and pH 6 inset fields are 1.00 μm .

Taken together, the HeIM results suggest that ion bridges between carboxylic acid groups and clay surface-bound cations are the most important drivers in clay-NOM composite formation at pH 6 and higher and that ionic strength is not terribly important to composite formation in this pH range. At pH 6 and higher, both the clay surface and the NOM are negatively charged and should repel one another. The hummocky textured surface coating of NOM can only form if there are positive charges to moderate these repulsive forces between the clay and NOM; strong evidence of ion bridging playing an important role at these pHs. Since the pH 6 and pH 12 results are similar, we can conclude that if ion bridging is important, mono- and bi-dentate complexation of the cations by the carboxylate groups must be the dominant clay-

cation-NOM interaction, since these are the only functional groups deprotonated at both of these pH conditions. While ionic strength may be important to floc formation at other pHs, the lack of variability with ionic strength or pH history in the pH 6 sample set suggests that neither factor has much influence on clay-NOM association at pH 6 or higher.

At pH 2, NOM-NOM interactions are likely dominated by hydrophobic association mechanisms. Since all of these samples experienced a short equilibration at basic pH, it is reasonable to assume that some sort of thin organic surface coating forms on the clay surface prior to the acidification process. This hydrophobic surface is likely to attract other hydrophobic NOM components during the rapid acidification. Likewise, the formation of thick NOM globules in the Ca^{2+} composite at pH 2 suggest that the organic matter molecules are orienting with their hydrophobic groups in close proximity and their hydrophilic groups facing the aqueous solvent. This leads to formation of globules in solution that can also more easily adhere to the clay surface that already has a thin NOM coating accumulated at basic pH.

We cannot discount the idea that hydrophobic association of the NOM hydrophobic groups with the clay surface occurs at pH 6 and 12. Although the clay surface is hydrophilic, it is less hydrophilic than H_2O , and the system free energy can be reduced by the hydrophobic regions of NOM pushing water away from the clay surface and associating directly with the clay. Recent molecular dynamics simulations by our group that are in the peer review process suggest that the hydrophobic mechanism of NOM-clay surface association can be important even in Ca^{2+} -bearing systems under some conditions.

Finally, we were able to observe a string-of-pearls (SOP) morphology in the Ca-NOM-pH12 sample and the CaHFA-pH6 direct sample that confirm earlier reports that NOM exists in solution as 10-20 nm globules (Figure 9). To our knowledge, this morphology has not been observed previously, but similarly sized NOM globules were reported based on small-angle X-ray scattering and atomic force microscopy studies of NOM solutions.^{39, 44} Since these are the only two samples that exhibit this morphology, we must conclude that SOP formation is favored at very low ionic strength when the carboxylic acid groups are deprotonated and Ca^{2+} is present. The Ca-NOM-pH12 floc was the NOM floc with the lowest ionic strength of any studied previously ($\sim 10^{-3}$).⁴⁵ Likewise, the CaHFA-pH6 direct sample was prepared by adding a very small quantity of $\text{Ca}(\text{OH})_2$ to the system; thus, the ionic strength of this sample is the lowest of all the composites prepared to date ($\sim 10^{-6}$). We conclude that NOM globules in the 10-20 nm range are important solution structures when multivalent ions are present and the solution ionic strength is low. Future studies need to be conducted using other multivalent ions such as aluminum to verify this hypothesis.

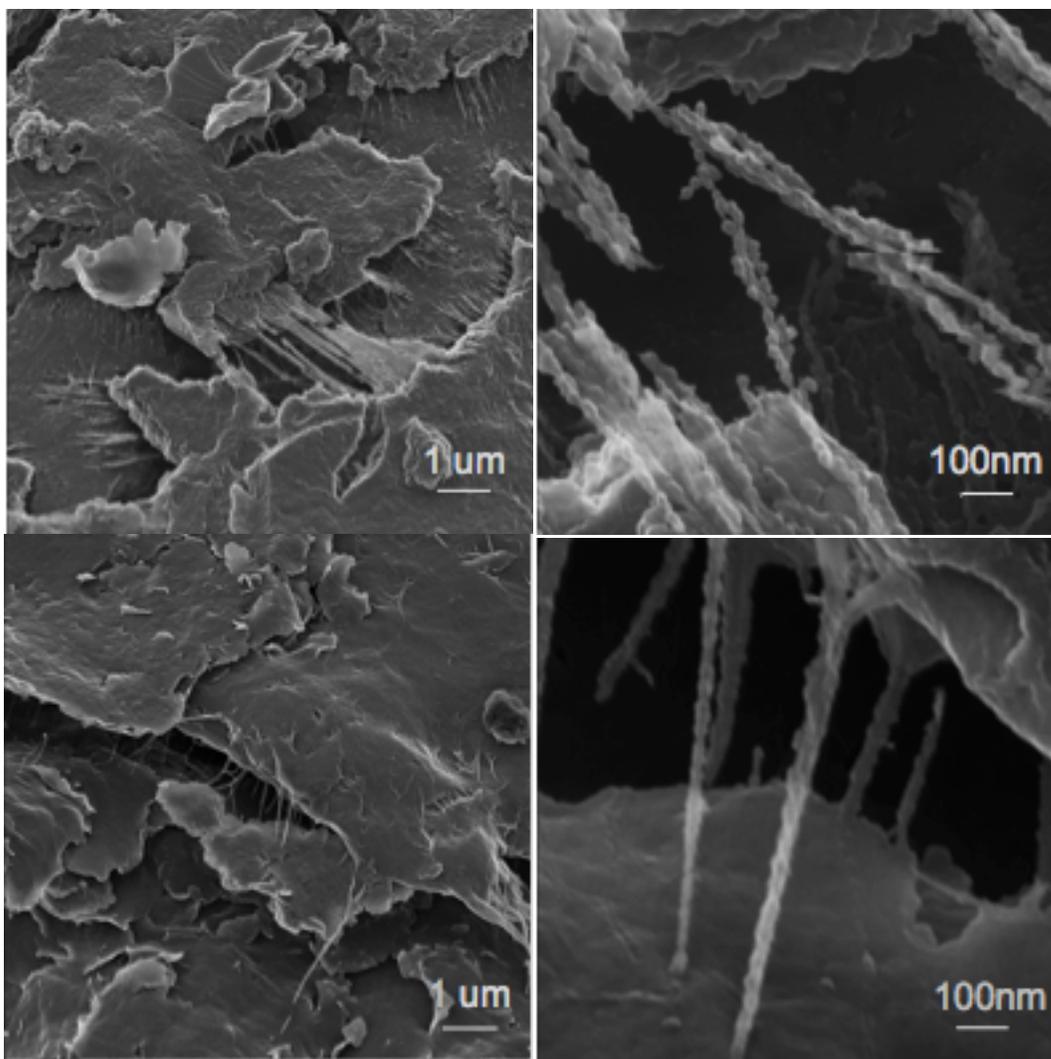


Figure 9. “String of Pearls” morphology observed in the Ca-NOM-12 flocc (top row) and the CaHFA-6-dir sample (bottom row). Images in top row reproduced from Bowers et al. *J. Phys. Chem. C*, 2015, 119 (31), pp 17773–17783.

Take-Homes

Historically, it has been uncertain whether NOM exists as a macromolecular or supramolecular structure; however, it is now widely accepted that NOM exists as both structures. In studying the size distributions of OM in solution, we find that cation polarizability and the NOM protonation state seems to dominate the number of unique hydrodynamic radii observed for alkali metal-NOM solutions. In contrast, the ratio of the number of cations present to the deprotonated NOM sites seems to dominate the number of unique hydrodynamic radii distributions observed in alkaline earth metal-NOM solutions, with more deprotonation leading to fewer unique hydrodynamic radii as smaller entities connect via ion bridges. In studies of clay-OM complexation, we observed that the carboxylic acid groups are the most important functional groups in clay-NOM composite formation and that neither ionic strength nor pH history are important players in complex formation at pH 6 and above. It is likely that ion bridging

is the dominant method of NOM-clay association at these pHs. However, at low pH, the hydrophobic mechanism dominates, as OM molecules are orienting with their hydrophobic groups to avoid as many water molecules as possible. Finally, we obtained evidence in agreement with existing literature about the nature of NOM in solution via the “string of pearl” morphology found at low solution ionic strength in the presence of multivalent cations. This work led to many hypotheses about the fundamental chemistry important in these materials that can be explored by other students in the future.

In a broader context, this work helps to reveal the role of chemistry in NOM-NOM and NOM-clay associations. It shows that multivalent ions can lead to strong NOM-NOM and NOM-surface associations; thus, ion exchange of monovalent for divalent ions may be an important step to protect ultrafiltration and reverse osmosis membranes from fouling by NOM.

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