Laboratory Studies on Salinity Induced Flocculation of Dissolved Organic Matter in Estuaries

Midshipman First Class Sofia Daniel and Midshipman 1/C Alexis Lambert, USN, Class of 2026

Advisor(s): Dr. Joseph P. Smith and Mr. Benjamin Hickman

Background and Objectives

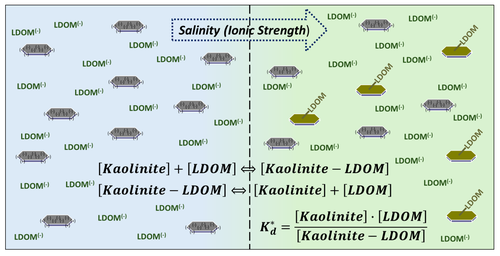

Figure 1. Conceptual diagram for salinity induced flocculation of between kaolinite and laboratory dissolved organic matter (LDOM).

The U.S. Naval Academy is sponsored by the U.S. Defense Threat Reduction Agency (DTRA) to conduct research to improve waterborne hazard mapping and tracking in shallow coastal systems. The aggregation of waterborne suspended particles, known as flocculation, frequently occurs during estuarine mixing of riverine and saltwater. Previous laboratory studies by *Asmala et al. (2014)* observed the flocculation of dissolved organic matter (DOM) within estuaries and determined the process is dependent on salinity. The presence of salt ions increase the ionic strength of riverine water and neutralizes the negative surface charges within DOM molecules. Laboratory studies by *Abolfazli and Strom (2023)* further studied the formation of natural mud-based flocs prior to estuarine mixing and if salinity still could impact flocculation. Flocculation was only present a low salinities, where past 3 to 5 PSU, it had a marginal effect on floc growth. Furthermore, flocs developed due to OM promoting binding among sediments possessing both high surface area and trace inorganic elements, primarily aluminum and silica, that flocculate with riverine colloidal OM (*Sholkovitz, 1976).* In this study, laboratory experiments were conducted to calculate a conditional dissociation constant (K_d^*) for kaolinite clay and laboratory organic matter (LDOM) under increased ionic strength along a simulated low salinity gradient to investigate how flocculation of organic matter and clay particles can potentially affect the partitioning of reactive, dissolved organic waterborne agents in estuarine environments (**Fig. 1**).

Methods and Approach

To calculate a K_d^* for LDOM and kaolinite under increased ionic strength, a stock solution of LDOM was created using HPLC-grade (98%) L-Tryptophan (Sigma-Aldrich) and commercially-available humic acid stock distributed by Root Naturally, LLC, Denver, CO consisting of a minimum of 85% soluble humic acids (SHA) derived from oxidized lignite (humate). A stock solution of kaolinite clay (Sigma Aldrich; ~ 0.1 to 10 µm effective diameter) was also created. Kaolinite is a ubiquitous non-expandible clay comprised of stacked aluminum and silicate layers. These stock solutions were used to perform two 24-hour laboratory incubations. First, a kaolinite titration at a fixed LDOM concentration (3 µmol L-Tryptophan and 40 mg/L SHA) was run in duplicate. The titrations were performed at 5 PSU along a range of kaolinite concentrations from 0-512 mg/L in order to identify the concentration for excess kaolinite surface charge capacity. The determined kaolinite concentration, 20 mg/L, was then utilized to calculate K_d^* for a kaolinite-LDOM complexation using *equation (5)* from *Jarmoskaite et al. (2020)*. Next, a salinity titration was performed from 0-5 PSU (**Fig. 2**) in triplicate using the same LDOM concentration/composition and kaolinite (at a determined concentration of 20 mg/L that provided excess surface charge capacity). Salinity was adjusted for each incubation using a ~70 PSU stock solution created using Instant Ocean Sea Salt. After each incubation, sub-samples were syringe-filtered (0.22 µm) into clean 40 ml amber borosilicate vials and analyzed for both absorbance at a wavelength of 276 nm and excitation emission matrix (EEMs) spectroscopy using a Horiba Aqualog spectrometer (www.horiba.com/int/scientific/ products/detail/ action/show/Product/aqualog-environmental-water-research-analyzer-3497/). Samples were also analyzed for dissolved organic carbon (DOC) using a Shimadzu TOC-L Series Total Organic Carbon Analyzer (www.ssi.shimadzu.com/products/ total-organic-carbon-analysis/toc-analysis/toc-l-series/index.html).

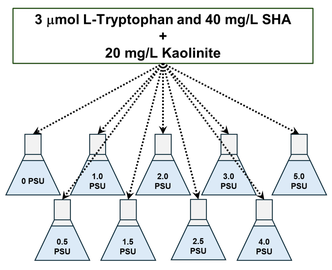

Figure 2. Incubation set-up for salinity titration. A 70 PSU stock solution was used to 'spike' each incubator to the target salinity.

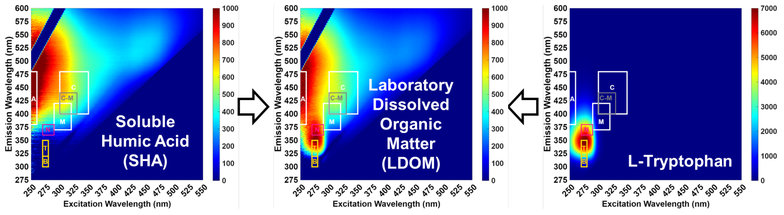

Figure 3. The fluorescent properties of the LDOM mixture (40 mg/L SHA and 3 µmol L-Tryptophan) used in this study represented by excitation-emission matrices (EEMs). The contours represent units of (relative) fluorescent intensity. The highlighted regions of each EEM plot shows chromophoric dissolved organic matter (CDOM) component regions (from *Table 1*, *Boyd et al., 2010*): A & C = humics; M = marine humics; Int. = intermediate C-M; N = unknown; B = tyrosine protein-like; T = tryptophan protein, phenol-like.

The laboratory DOM (LDOM) chosen for this experiment was a mixture of two optically-unique OM end-members (**Fig. 3**) that should be identifiable by their EEMs and interact differently with charged clay surfaces under increasing ionic strength. A data analysis technique known as Parallel Factor Analysis (PARAFAC) organizes EEMs into trilinear arrays in order to identify and model component (fluorophore) intensity variation within a sample population. Chemical interpretation of results is dependent upon the correct number of components being fitted by the PARAFAC model (*Murphy et al., 2013*). In this study, a 3-way, 3-component model was fit to EEMs of samples collected during incubations (**Fig. 4**). The DrEEM toolbox (www. dreem.openfluor.org/) for MATLAB R2025b was used to perform PARAFAC analysis on EEMs.

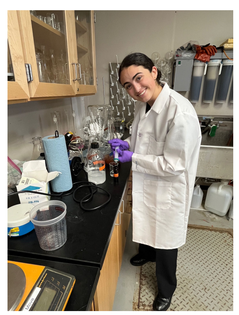

Figure 4. Midshipman 1/C Daniel preparing samples for EEM analysis on the Horiba Aqualog Spectrometer for use in PARAFAC modeling.

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Results and Discussion

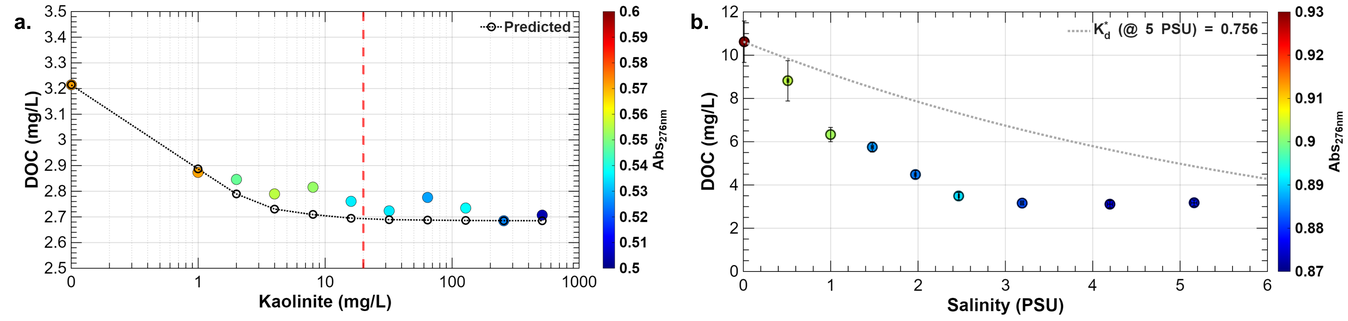

Figure 5. (**a**) DOC concentration (mg/L) contoured by relative absorbance at 276 nm (Abs_{276nm}) vs. kaolinite concentration (mg/L, log scale) for the kaolinite titration and incubation performed at a salinity of 5 PSU. The red dashed line shows the 20 mg/L level of kaolinite determined to provide excess surface charge capacity to not limit flocculation/complexation. The predicated line shows DOC concentrations based on a K_d^* = 0.756 estimated using *equation (5)* from *Jarmoskaite et al. (2020)*. (**b**) DOC concentration (mg/L) contoured by Abs_{276} vs. salinity for the salinity titration/incubation with 20 mg/L kaolinite. The fit line for a K_d^* = 0.756 at 5 PSU is shown for comparison.

Results of the kaolinite titration/incubation (**Fig. 5a**) showed a decrease in DOC concentration from 0-16 mg/L and a good fit for a K_d^* = 0.756 (5 PSU). Absorbance at 276 nm decreased as DOC concentration decreased showing that DOC concentration is a good proxy for DOM concentration. Based on the results of the kaolinite titration, a kaolinite concentration of 20 mg/L was chosen for use in the salinity titration to ensure excess surface charge capacity for flocculation/complexation with DOM. Results of the salinity titration/incubation showed a significant decrease in DOC concentration (and Abs_{276nm}) from 0-3 PSU, a result that is consistent with the observed behavior of humic acid with kaolinite during estuarine mixing (*Sholkovitz, 1976*). The loss of DOC to flocculation/complexation with kaolinite does not follow trend predicted for the K_d^* at 5 PSU. Dissociation constants should be estimated at additional salinity values between 0-5 PSU to better predict flocculation/complexation od DOM with kaolinite as ionic strength increases.

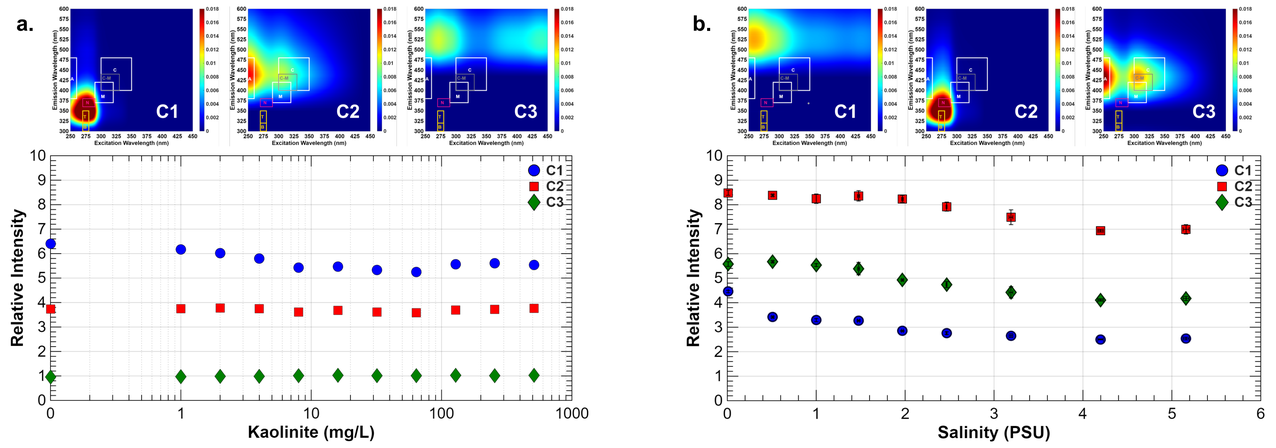

Figure 6. (**a**) Component loadings (relative intensity) vs. kaolinite concentration (mg/L, log scale) for a 3-component PARAFAC model run on EEMs for samples from the kaolinite titration/incubation and (**b**) Component loadings (relative intensity) vs. salinity for a 3-component PARAFAC model run on EEMs for samples from the salinity titration/incubation . The EEMs spectrum for each component is shown above each plot. The highlighted regions of each component EEM shows CDOM component regions (*Table 1, Boyd et al., 2010*).

A 3-component PARAFAC model fit to the initial EEMs from the kaolinite titration/incubation explained 99% of the variance among the EEMs possessing a core consistency of 90% (**Fig. 6a**). Of the three components, C1 showed the highest relative intensity and change with increasing kaolinite concentration followed by C2. These components fall in the CDOM component regions for tryptophan protein, phenol-like DOM (C1) and terrestrial humics (C2), respectively. The 3-component PARAFAC model fit to EEMs from the salinity titration/incubation explains 99% of the variance with an 89% core consistency. Similarly, the C2 component within the tryptophan protein, phenol-like CDOM region possessed the highest relative intensity. Both the C2 component and the C3 component in the terrestrial humic CDOM region (second highest relative intensity) show the largest variation with increasing salinity, specifically between 1-4 PSU. These results are consistent with the laboratory results of previous experiments on salinity-induced flocculation (*Asmala et al., 2014*) which showcased that both proteins and terrestrial humic substances flocculate with kaolinite at lower salinities.

Conclusions and Future Work

- ***The calculated K_d^* for kaolinite and laboratory organic matter under increased ionic strength along a simulated salinity gradient calculated in this study can be applied to field studies of the flocculation of natural organic matter in coastal systems. PARAFAC analysis demonstrated the involvement of both tryptophan-like proteins and terrestrial humics in flocculation with kaolinite at lower salinities.***
- ***Laboratory and field research should continue to investigate how flocculation of organic matter and clay particles can potentially affect the partitioning of reactive, dissolved organic waterborne agents in estuarine environments.***

References: Sholkovitz (1976). *Geochim. Cosmochim. Acta* 40(7); Boyd et al. (2010), *JGR* 115; Murphy et al. (2013). *Anal. Methods* 5; Asmala et al. (2014). *J. Geophys. Res. Biogeosci* 119; Jarmoskaite et al. (2020). *eLife 9.* Abolfazli and Strom, *JGR* 128.