Introduction

- Fluorescence Spectroscopy – bulk characterization technique for Natural Organic Matter (NOM)
- Used for qualitative and quantitative characterization
- Highly sensitive to the sample conditions
- Thiosulfate (TS) and ascorbic acid (AA) are reductants commonly used to quench oxidation experiments with NOM

Objective

- Analyze fluorescence spectra of NOM with reductants added at a range of concentrations
- Determine absorbance threshold where interference occurs, as well as concentration of preservative for which problems arise
- Make recommendations for preservative use during fluorescence spectra analysis
- Investigate effect of reductants on NOM redox properties with respect to observed fluorescence

NOM Background

- Heterogeneous mixture of organic matter from natural waters, sediments, and soils
- Refractory
- NOM: made of soluble and particulate components (Figure 2), affecting:
  - Biogeochemical processes
  - Water quality, contaminant binding, fertility of soil, and carbon cycle
- Composition and function dependent on origin, temperature, ionic strength and composition of cations, pH, microbial activity, and photochemistry
- Concentration expressed as Total Organic Carbon (TOC)

Fluorescence Background

- Absorption of photon -> excitation of electron to higher energy level (Figure 3)
- Relaxation of electron -> emission of fluorescence (fluorophores)
- Composition specific information on redox state, source, and reactivity information for NOM
- EEMs plots: Intensity of energy emitted at particular emission (λEm) and excitation (λEx) wavelengths (Figure 4)
- FI assess composition differences
- Fluorescence photo-physical phenomenon
- Peak Picking
- Humic substance peaks (A&C)
- Protein/polyphenolic/nitrogen (B&T)

Experimental Methods

- Samples preparation:
  - 3 mg/L TOC - Suwannee River NOM (SRNOM) 2R101N
  - ~7.5 pH, 10 mM phosphate buffer
  - Reductant concentrations range between 5 and 160 mgAA/L and 5 to 160 mgTS/L in NOM solution (< 2% dilution)
  - Overnight storage at 5 °C
- Fluorescence Spectroscopy runs:
  - Jobin Yvon HORIBA FluoroMax – 4
  - λEx 240 – 450 nm; λEm 300 – 550 nm
  - 20°C, duplicates
- Ultraviolet/visible spectra (UV/Vis)
  - CARY 100 Bio, VARIAN – 200-800 nm
  - HACH DR 500 – 200-600 nm
  - TS&NOM, AA&NOM; TS& Distilled Water (DI); AADl
- EEM corrections and analysis in MATLAB®:
  - Blank subtraction, Raman normalized, instrument correction, light screening correction; Matlab code
  - Differential EEMs AA&NOM versus AA&DI plots; peaks A, C analysis; FI analysis

General NOM Results

- Control EEM spectra - 0 mg preservative per liter NOM solution (Figure 5)
- Used in order to determine differential fluorescence interference after preservative addition

NOM + TS Results

- The interference observed only affects low λEx (Figure 8)
- The interference seems to be uniform and independent of λEm
- FI determined to be statistically similar (p_value=0.74)
- Peak A dependent on TS concentration (Figure 9)
- High absorbance observed (Figure 10) in the UV/Vis Spectra below 270 nm, affecting correction factor:
  - 0.10 mgTS/L in DI: statistically similar Peak A values (Average=1.3, p_value=0.20)
  - Correction factor accounts for light screening
  - 0 to 11 mg/L TS in DI: statistically similar Peak A values
  - 10% screening at <78 mgTS/L

NOM + AA Results

- AA absorbs light between 240 nm and 300 nm
- AA does not fluoresce
- AA presence systematically enhances fluorescence intensity in the Peak C region, and quenches fluorescence intensity of Peak A region (Figure 12)
- FI statistically similar for up to 40 mgAA/L (p<0.15) (Figure 13)
- Deducing that AA affects the redox properties of NOM which affect fluorescence

Conclusions

- TS and AA are important for oxidant quenching
- During analysis of samples prepared with these preservatives, there is need to consider constraints for fluorescence characterization
- For TS concentrations above 78 mg/L, it is expected to notice 10% screening of light, and along with it strong effects in the EEM plot below 250 nm λEx.
- It is hypothesized that AA can lead to changes in the redox properties of NOM, changing the photo-physics of the sample

Future Work

- Analyze NOM from other sources (different composition)
- Verify all results through the reproduction of the full experiment
- Perform size exclusion chromatography (SEC) for the AA-NOM samples to determine potential size effects due to redox reactions
- Perform Nuclear Magnetic Resonance experiments, to assess transformations in NOM structure after AA addition
- Publish findings, providing concise description of the constraints in oxidant quenching chemicals
- Analyze the fluorescence spectra for additional preservatives

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References

1) H.R. Schulten, M. Schnitzer, Naturwissenschaften, 1993, 80, 27;
2) www.mdpi.com;
4) J.A. Korak et. al., Water Research, 2014, 49, 328