Fluorescence and Infrared Spectroscopy Study of Humic Acid Complexation with Metal Ions

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Abstract

Humic substances result from the chemical breakdown of animal and plant residues, which include proteins, lipids, lignins, and leaf polyphenols. Humic acids (HA) are high molecular weight (a few hundred to millions of Daltons), hydrophilic, black, organic polymers that are soluble above pH 2. Functional groups such as carboxylic acids and hydroxyl groups attached to aromatic rings make humic acids hydrophilic. Humic acids have been shown previously to have the capacity to bind metal ions, to act as a redox catalyst, to act as photosensitizers, and to act as pH buffers. For these reasons, humic acid combined with metal complexation plays an important role in soils and natural waters by regulating trace metal transport and bioavailability in aquatic systems. As a result of the presence of humic substances in the environment, potentially toxic heavy metals are retained in a bioavailable state for long periods of time. In this study, we used fluorescence and infrared spectroscopy to probe the ability of purchased humic acid to bind to various metal ions, including Cu$^{2+}$, Al$^{3+}$, Mg$^{2+}$, and Mn$^{2+}$, to form organo-metallic complexes. Fluorescence titration analysis and the Stern-Volmer equation were used to calculate the extent of the metal complexation. Initial data indicates quenching of fluorescence intensity for humic acid when Cu$^{2+}$ ions are bound, and enhancement of fluorescence data when Al$^{3+}$ ions are bound to the humic acid. Results from DRIFTS spectroscopy show that the metals bind to the humic acids at carboxylic acid sites.
Introduction

• Simple complexes like humic acids undergo fluorescence.
• Humic acids have high metal binding capacities which can be seen by changes in fluorescence and infrared stretching vibrations of functional groups.
• Simple and complex gaseous, liquid, and solid systems undergo fluorescence.
• Fluorescence is detected from radiation that causes absorption of photons from a sample where excitation has occurred.
• DRIFTS spectroscopy qualitatively identifies functional groups through a beam of radiation striking a powdered sample and being reflected in all directions.
• A wash buffer (EDTA) to clean all glassware is needed because it binds metals and keeps the solutions from being contaminated.
• Humic acids found in natural waters have a concentration of 15 mg/L.
Principle

• The purpose of the experiment is to examine how purchased humic acid binds to metal ions, including Cu$^{2+}$, Al$^{3+}$, Mg$^{2+}$, and Mn$^{2+}$ to form organo-metallic complexes.

• Fluorescence titration analysis and DRIFTS spectroscopy were used to examine the extent to which the metals bind and what functional groups of humic acids that the metals bind to.
Experimental Details

Preparation of Solutions

• Wash solution - An EDTA buffer (pH = 8) was prepared using 3 M NaOH.

• An HA solution was prepared at a concentration of 15 mg/L using 0.1 M NaClO₄ buffer (pH = 4).

• 0.1 M solutions of Cu²⁺, Al³⁺, Mg²⁺, and Mn²⁺ were prepared from their hydrated chloride salts in 0.1 M NaClO₄ buffer (pH = 4).
Experimental Details

**DRIFTS Spectroscopy**
- Instrument: Perkin Elmer Spectrum 1
- Parameters:  
  - KBr pellet 1:10 with sample
  - 32 scans from 4000 – 500 cm\(^{-1}\)
  - Set to %R
- Samples were made from 5.0 mg HA in 4 mL NaClO\(_4\) buffer (pH = 4).
- 1 mL of metal solution was added to each sample.
- Samples were dried in oven and spectrum was then obtained.
Experimental Details

**Fluoremeter**
- Instrument: Shimadzu RF-5301 PC Spectrofluorophotometer
- Parameters: - Excitation – 320 nm (Cu²⁺), 300 nm (Al³⁺), 310 nm (Mn²⁺, Mg²⁺)
  - Emission- 400 – 500 nm
  - Sampling interval – 0.2
  - Slit width – ex 5 nm  em 5 nm
  - Sensitivity - high
- 4 mL of HA were pipetted into a quartz cuvette and 9 µL of metal were added to each sample
- Excitation was changed from 250 – 360 nm and fluorescence measurements were taken to find $\lambda_{\text{max}}$.
- Increasing increments of metal solutions were added to HA samples and fluorescence spectra were obtained using the $\lambda_{\text{max}}$.
- The Stern-Volmer equation was used to calculate the extent to which metals quenched.
Stern-Volmer Equation

- Used to calculate quenching constant for metals

\[
\frac{\phi_f^0}{\phi_f} = 1 + K_q [Q]
\]

- \( K_q = \frac{m}{b} \)
- \( K_q = \text{quenching constant} \)
- \( m \) is the slope of the graph of \( 1/F \) vs. [ ] of quenching metal
- \( b \) is the y-intercept of the graph
IR- HA with Aluminum(III)
Fluorescence quenching by Al(III)

Stern-Volmer

y = 425.9x + 0.9997
R² = 0.9886
IR- HA with Cu(II)
Fluorescence quenching by Cu(II)

Stern-Volmer

Intensity vs Wavelength (nm)

Spectra

y = 1915.6x + 1.006
$R^2 = 0.9965$
IR- HA with Mn(II)

![Graph showing IR spectra of HA with Mn(II) and HA Control. The x-axis represents Wavenumber (cm⁻¹) ranging from 3450 to 450 cm⁻¹. The y-axis represents % R. The graph compares the two samples, with HA with Mn(II) in pink and HA Control in blue.](image)
Fluorescence quenching by Mn(II)

Spectra

Stern-Volmer

$$y = 238.05x + 1.0505$$

$$R^2 = 0.9585$$
IR - HA with Mg$^{2+}$
Results and Discussion

**DRIFTS Analysis**

- There is sharpening of IR bands at 1624 cm\(^{-1}\) for Cu\(^{2+}\).
- There is sharpening of IR bands at 1629 cm\(^{-1}\) and broadening at 3384 cm\(^{-1}\) for Al\(^{3+}\).
- There is sharpening of IR bands at 1626 cm\(^{-1}\) and broadening at 3401 cm\(^{-1}\) for Mn\(^{2+}\).
- There is sharpening of IR bands at 1629 and 3417 cm\(^{-1}\) for Mg\(^{2+}\).
- Sharpening and broadening of bands at the wavenumbers shown show that the metals bind to the humic acid at carboxylic acid sites and on –OH groups.
- The binding of metal ions produced the sharpening of the peaks at 1624-1629 cm\(^{-1}\) because it decreased the routes for vibrational relaxation to a lower energy level.
- The broadening of the OH peaks at 3384, 3401, and 3417 cm\(^{-1}\) is caused by the binding of the metal ions or by increased hydrogen bonding.
Results and Discussion

Fluorescence Titration Analysis

- $\text{Al}^{3+}$
  $K_q = 426 \text{ L/mol}$
- $\text{Cu}^{2+}$
  $K_q = 1904 \text{ L/mol}$
- $\text{Mn}^{2+}$
  $K_q = 227 \text{ L/mol}$
- $\text{Cu}^{2+}$, $\text{Al}^{3+}$, and $\text{Mn}^{2+}$ showed quenching when fluorescence titration analysis was performed.
- There was no quenching observed when fluorescence titration analysis was performed with $\text{Mg}^{2+}$.
- $\text{Cu}^{2+}$ was found to have a higher quenching constant, therefore, it is shown to bind more readily to humic acid than $\text{Al}^{3+}$ and $\text{Mn}^{2+}$.
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References

- Yi, C.; Conklin, M.; Betterton, E. Competitive Complexation of Trace Metals with Dissolved Humic Acid Environmental Health Perspectives Supplement, 10780475, 1995, 103.