Characterizing the interaction between uranyl ion and fulvic acid using regional integration analysis (RIA) and fluorescence quenching

Bingqi Zhu a, David K. Ryan b, *

a Zhejiang Institute for Food and Drug Control, Hangzhou, Zhejiang 310000, China
b Department of Chemistry, University of Massachusetts Lowell, Lowell, MA 01854, USA

Abstract

The development of chemometric methods has substantially improved the quantitative usefulness of the fluorescence excitation-emission matrix (EEM) in the analysis of dissolved organic matter (DOM). In this study, Regional Integration Analysis (RIA) was used to quantitatively interpret EEMs and assess fluorescence quenching behavior in order to study the binding between uranyl ion and fulvic acid. Three fulvic acids including soil fulvic acid (SFA), Oyster River fulvic acid (ORFA) and Suwannee River fulvic acid (SRFA) were used and investigated by the spectroscopic techniques. The EEM spectra obtained were divided into five regions according to fluorescence structural features and two distinct peaks were observed in region III and region V. Fluorescence quenching analysis was conducted for these two regions with the stability constants, ligand concentrations and residual fluorescence values calculated using the Ryan-Weber model. Results indicated a relatively strong binding ability between uranyl ion and fulvic acid samples at low pH (log K value varies from 4.11 to 4.67 at pH 3.50). Fluorophores in region III showed a higher binding ability with fewer binding sites than in region V. Stability constants followed the order, SFA > ORFA > SRFA, while ligand concentrations followed the reverse order, SRFA > ORFA > SFA. A comparison between RIA and Parallel Factor Analysis (PARAFAC) data treatment methods was also performed and good agreement between these two methods (less than 4% difference in log K values) demonstrates the reliability of the RIA method in this study.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The use of uranium in military and energy applications along with its increased mining and refining activities have caused uranium to become a potential hazard to both environmental and human health (Bleise et al., 2003). Uranium is soluble in water as uranyl ion (UO2²⁺) in low pH environments, and uranyl hydroxyl and uranyl carbonate complexes when the pH is higher (Meinrath, 1997; Vandenhoeye et al., 2010). The migration and sorption of uranyl ion in soil and aquatic systems can be affected by a wide variety of reactions, especially by its interaction with dissolved organic matter (DOM) (Schmeide et al., 2003; Shin et al., 2001). Many approaches have been applied in understanding the structure of DOM (Mopper et al., 2007; Ryan and Zhu, 2013) and the mechanism by which it binds to metals (Mibus et al., 2007; Möser et al., 2012). However, most of these techniques, such as chromatography and mass spectrometry, require specific sample pretreatment procedures that alter the sample or disturb the equilibrium between the metal ion and DOM. Fluorescence spectroscopy, therefore, has become a widely used technique for studying DOM in aquatic samples because it provides a relative rapid analysis requiring little or no sample pretreatment and does not degrade the sample or disturb the equilibrium of interest. The presence of abundant aromatic structures in DOM provides reasonably good fluorescence characteristic and a means to probe various properties of these important molecules (Chen et al., 2003; Hudson et al., 2007).

A fluorescence quenching phenomenon, or a reduction in the fluorescence intensity, has been observed when certain metal ions are bound to DOM and a method was developed to quantitatively monitor the interaction between the DOM and selected metals (Ryan and Weber, 1982a, b; Saar and Weber, 1982). Originally, only excitation and emission wavelengths that produced the maximum signal were used in this analysis, and these wavelengths were obtained by scanning the emission at a fixed excitation wavelength...
and vice versa (Ryan and Weber, 1982a, b). Subsequently, synchronous fluorescence, which scans at a constant offset between excitation and emission wavelengths, was applied revealing more fluorophores in the samples (Esteves da Silva et al., 1998; Hays et al., 2003). In recent years, fluorescence excitation-emission matrix (EEM) fluorescence spectroscopy has been much more widely used in the characterization of aquatic samples (Baker, 2001; Coble, 1996; Henderson et al., 2009). Based on the use of EEMs, parallel factor analysis (PARAFAC) was developed and successfully applied in the fluorescence quenching study of DOM when it is bound with metal (Ohno et al., 2007; Stedmon and Bro, 2008; Wu et al., 2011; Yamashita and Jaffé, 2008; Zhu et al., 2014). A new method called regional integration analysis (RIA) was proposed by Chen (Chen et al., 2003) to help interpret EEM data. They divided an EEM into different regions according to fluorescence spectral features exhibited by DOM and integrated the volume under the peaks in those regions to get quantitative information. The application of the RIA data treatment method to fluorescence EEMs has been used to investigate the composition and transformation of humic and fulvic acid from a land fill and in animal manure (Chai et al., 2012; Yu et al., 2011). Furthermore, this method can be applied in the investigation of complexation between metal ions and DOM with the potential to become a new approach to better understand the mechanism of binding.

This work mainly focused on the interactions between fulvic acid, a major component in DOM, and uranyl ion. The objectives of this study were to (a) use EEMs to explore fluorescence properties of these fulvic acid samples (b) investigate the binding behavior and stability constants for binding between uranyl ion and fulvic acid, and (c) compare the RIA and PARAFAC data treatment methods.

2. Experimental section

2.1. Reagent

Isolated samples of a well-characterized soil fulvic acid (SFA) and Oyster River fulvic acid (ORFA) were obtained from Dr. James Weber, Department of Chemistry, University of New Hampshire, Durham, NH, USA. Commercially available Suwannee River fulvic acid (SRFA) was also used in this study. Fulvic acid solutions were prepared by dissolving fulvic acid in water and stirring for 2 h. These solutions were then filtered through 0.2 μm Whatman (Maidstone, UK) Nylon membrane filters and transferred to volumetric flasks. Uranium atomic absorption standard solution (Ricca Chemical Co., Arlington, TX) was obtained as a 1000 ppm solution. The de-ionized water used throughout these experiments was 18.2 MΩ de-ionized water obtained from an Elga Purelab Option-Q water purification system.

2.2. Apparatus

A Perkin Elmer LS55 spectrofluorometer equipped with a xenon light source was used to obtain fluorescence data. A 10 mm quartz cuvette was used as the sample cell. Solution temperature was maintained at 25 °C using a constant temperature water bath (VWR Scientific, Boston, MA). A WTW InoLab pH Level 2 pH meter was used to measure the pH. A Perkin Elmer Spectrum 100 model FTIR spectrometer was used to conduct infrared analysis and spectra were measured using 2–4 mg of SFA, ORFA and SRFA in KBr pellets. The spectra were collected from 4000 to 400 cm⁻¹ averaging 4 scans with a resolution of 4.0 cm⁻¹. A Perkin Elmer Lambda 35 UV–vis spectrometer was used for absorbance measurements.

2.3. Titration experiments

Titration experiments were used to investigate the interaction between uranyl ion and fulvic acid. The SFA solution was placed in a beaker with constant stirring maintained by a magnetic stirrer and a pH meter was used to monitor the pH. Then a series of known quantities of uranyl ion were titrated into the beaker in microliter amounts. EEMs were recorded after each addition of titrant, as well as the EEM of SFA without uranyl addition. The concentration of SFA was remained essentially constant at 20 mg/L and the concentration of uranyl ion increased from 0 to 0.8 mmol/L during the titrations. The same protocol was carried out for the measurement of ORFA and SRFA. All measurements were conducted three times at 25 °C (±1 °C) and at a pH of 3.50 (±0.01). The 20 mg/L concentration of each fulvic acid gave a relatively low absorbance (lower than 0.04 absorbance units for all three fulvic acids) at the emission wavelengths in the vicinity of 450 nm. For this reason the secondary inner filter effect is not significant. Although the absorbance of fulvic acid samples was more significant at typical excitation wavelengths used, studies have been reported that a low concentration of 20 mg/L of fulvic acid has a negligible primary inner filter effect (Hudson et al., 2007). The fluorescence intensity presented a relative linear relation with the concentration of fulvic acid within the range lower that 20 mg/L, this ensures the soil fulvic acid was not too concentrated. It should also be noted that since the experiments were conducted with an essentially constant concentration of soil fulvic acid throughout, any primary inner filter effect that might be present was constant throughout and had no effect on the results. Experiments were conducted at pH 3.50, because most of the uranyl ion is not hydrolyzed at this pH value (Esteves da Silva et al., 1996; Esteves da Silva et al., 1998; Zhu et al., 2014).

2.4. RIA and PARAFAC data treatment

For each EEM obtained, the background Raman scattering from water was removed by subtracting a blank EEM spectrum. The first order Rayleigh scattering was removed by setting the values to zeros, then the second order Rayleigh scattering was removed using an interpolation method. This interpolation method allowed input of new values to replace the data that are in the second order Rayleigh scattering region. Using this method, a smooth surface for an EEM was obtained.

Both RIA and PARAFAC were the methods that were used to provide quantitative peak information. The RIA data treatment was proposed by Chen (Chen et al., 2003). The volume beneath the peak was calculated by integration of the area multiplied by the height (i.e. fluorescence intensity). EEMs were divided into five regions and the volume of each region was calculated separately. Total volume was the sum of the volume for all regions. The volume percentage was calculated by taking the volume of a certain region divided by the total volume. The RIA calculation was conducted by MATLAB (Version 2010b, MathWorks, Natick, MA).

PARAFAC modeling has been described by Bro (Bro, 1997). Briefly, a series of EEM spectra can be considered as a three-dimensional data array, which is composed of excitation wavelength, emission wavelength and sample number. It is modeled as a three-way array I × J × K, and xijk represents the fluorescence intensity of sample i, measured at emission wavelength j and excitation wavelength k. PARAFAC analysis decomposes multi-way data and turns it into tri-linear components. Excitation, emission spectra and relative intensities of different fluorophores are calculated as outputs (Stedmon and Bro, 2008). The PARAFAC modeling was conducted by MATLAB (Version 2010b, MathWorks, Natick, MA) with the N-way toolbox (Andersson and Bro, 2000).
2.5. Curve fitting modeling

A complexation model proposed by Ryan and Weber (Ryan and Weber, 1982a, b) was applied in the analysis used here. Based on the 1:1 complexation assumption between metal and ligand, a reaction can be represented by the following equation,

\[ M + L \leftrightarrow ML \]

\( M \) represents free or uncomplexed metal ion in the solution and \( L \) represents a metal-free ligand site which is the fluorescent species. \( ML \) represents the metal-bound species at the site. Based on this reaction, a stability constant can be calculated as follows:

\[ K = \frac{[ML]}{[M][L]} \]  

(1)

\( K \) is the conditional stability constant at a fixed pH value. \([ML]\) represents the concentration of metal-bound species, while \([M]\) and \([L]\) represent the concentration of free metal ion and the concentration of metal-free ligand respectively.

Metal and ligand sites follow a mass balance in solution such that,

\[ C_M = [M] + [ML] \]

(2)

\[ C_L = [L] + [ML] \]

(3)

\( C_M \) and \( C_L \) represent total concentrations of metal and ligand in the solution system, respectively.

Since the fluorescence intensity is proportional to the concentration of fluorophores, an equation relating fluorescence and the solution equilibrium can be derived as follows:

\[ \frac{[ML]}{C_L} = \frac{I - I_{res}}{I_L - I_{res}} \]

(4)

where \( I_L \) represents the fluorescence intensity of ligand without any addition of metal. \( I \) is the fluorescence intensity during the titration process. \( I_{res} \) is the residual fluorescence intensity, which may come from the metal-ligand complex fluorescence or some other unquenched material in the fulvic acid sample. It is the unquenchable fluorescence intensity at the end of the metal titration process.

Rearranging equations (1)–(4), a relationship between \( I \) and \( C_M \) is obtained,

\[ I = \frac{(I_{res} - 100)}{2KC_L} \]

\[ \times \left\{ (KC_L + KC_M + 1 - \left[ (KC_L + KC_M + 1)^2 - 4K^2C_LC_M \right]^{1/2}) + 100 \right\} \]

(5)

In our experiment, nonlinear regression of the titration curve was conducted using equation (5) applied to data in the form of \( I \) versus \( C_M \) and \( K, C_L \) and \( I_{res} \) values were obtained.

3. Results and discussion

3.1. Peak volume distribution theories

As illustrated in Fig. 1, a Jablonski energy level diagram, when a molecule absorbs light energy, alternations in the electronic, vibrational and rotational states of the molecule take place. An electron may be excited from the ground singlet state (S0) into different vibrational and rotational energy levels of the first excited singlet state (S1), second singlet excited state (S2) or higher state with different absorption wavelengths. Internal conversion and vibrational relaxation may take place and the excited electron may lose energy and fall back into the lowest vibrational energy state of the first singlet excited state. When electrons return back to the ground electronic state, they may go back to different vibrational or rotational states resulting in the emitted light being of different wavelengths within a certain range. Therefore, a fluorophore can be excited by a certain range of wavelengths and emit wavelength in another wavelength range. A fluorescence peak should have a near Gaussian distribution (Dubnick et al., 2010). The maximum absorption and emission wavelengths follow the Franck–Condon principle determined by the structure of the molecule (Atkins and Friedman, 1997). In quantitative analysis of EEMs, it is meaningful to integrate the area over a range of excitation wavelengths (y axis of EEM) and a range of emission wavelengths (x axis of the EEM) rather than just collecting the maximum peak intensity only. The x-y area of a fluorophore peak in an EEM times the fluorescence intensity plotted on the z axis define the volume of the peak for RIA. Based on this theory, quantitative peak information is obtained using the peak volume in the RIA method.

3.2. Characterization of fulvic acid samples

Three fulvic acids (SFA, ORFA and SRFA) were used in this study. An FTIR measurement was conducted in order to understand the structure and compare the differences among these fulvic acids. The spectra given in Fig. 2 show that these fulvic acids share similar spectral features implying structures that are very close to one another. A very broad peak appears around 3400 cm\(^{-1}\) usually attributed to the O–H stretching from alcohols, phenols and carboxylic related groups and N–H stretching from amide and amine functional groups. A peak around 2900 cm\(^{-1}\) is due to C–H asymmetric and symmetric stretching. Peaks around 1620 cm\(^{-1}\) and 1720 cm\(^{-1}\) can be attributed to the C=O stretching vibrations which might be from aromatic rings and carboxylic, ketone or ester groups, respectively. Peaks from 1000 cm\(^{-1}\) to
1400 cm$^{-1}$ are possibly due to C–O stretching, $-\text{CH}_3$ and $-\text{NH}_2$ deformation vibrations (He et al., 2011b; Huo et al., 2008). The complex heterogeneous nature of fulvic acid samples gives rise to the rather broad peaks observed.

Fluorescence EEMs exhibited and two distinct peaks for all three samples as shown in Fig. 3. Excitation wavelengths for all samples were observed around 220 nm and 330 nm with the emission at 450 nm. For these two peaks, the fluorescence intensity increased in the order SFA < ORFA < SRFA. However, an opposite sequence was observed in UV–vis absorption measurements. The highest absorption was observed for SFA, and the lowest absorption was observed for SRFA throughout the region from 220 nm to 600 nm. The quantum yield follows SRFA > ORFA > SFA. Stewart and Wetzel reported the same phenomenon and noted that higher molecular weight components of DOM gave weak fluorescence but absorbed strongly while low molecular weight components exhibited the opposite effect (Stewart and Wetzel, 1980, 1981).

Based on the EEMs of the fulvic acid samples, an analysis was performed following Chen’s method (Chen et al., 2003). As shown in Fig. 4, five regions were delineated and the volume of each region was calculated following the definition in Table 1. The separation line between regions V and III was changed from 250 nm to 285 nm in order to better differentiate the two peaks (Chen et al., 2003; Coble, 1996). Aromatic amino acids refer to tryptophan, tyrosine and phenylalanine, which are the three amino acids that have aromatic rings and emit fluorescence. Phenylalanine exhibits a low quantum yield compared to tryptophan and tyrosine (Chen, 1967). Therefore, tryptophan ($\lambda_{\text{ex}}$: 280 nm/245 nm, $\lambda_{\text{em}}$: 348 nm) and tyrosine ($\lambda_{\text{ex}}$: 274 nm/230 nm, $\lambda_{\text{em}}$: 303 nm) were primarily considered in the analysis. As a result, aromatic amino acid like structures were monitored at region I, II and IV.

Fig. 2. FTIR spectra of SFA, ORFA and SRFA.

Fig. 3. Fluorescence EEMs of SFA (A), ORFA (B) and SRFA (C). Concentrations were fixed at 20 mg/L and measured at 25°C.

Fig. 4. Delineation of five regions in the EEM of fulvic acids with their excitation and emission wavelength boundaries as described by Chen et al., 2003.

Fig. 5 shows the percentage distribution of the EEM fluorescence in different spectral regions for the fulvic acids used in this study. High percentage values were obtained for region III (44%–45%) and region V (51%–53%) while very low concentrations of aromatic amino acids were found for region I (close to 0%), region II (0%–2%) and region IV (1%–3%). In our study, higher percentages were found for region III and region V than were obtained in other studies (Chai et al., 2012; Chen et al., 2003; He et al., 2011a; Yu et al., 2011). This might be due to the fact that the samples used in our experiments were isolated fulvic acids that may not contain the full suite of DOM compounds that might be present in an unfractionated sample. Concentrations of aromatic amino-acid are obtained that are lower than other studies (Chai et al., 2012; He et al., 2011a; Yu et al., 2011), which is probably because the samples used in other studies were
from landfills and animal manure and would be expected to be rich in aromatic amino acid compared to the river and soil fulvic acids. Consistent results were obtained from our three samples, showing that they do not only share similar FTIR properties, but also show very similar fluorescence properties. This indicates a related composition and structures among these three fulvic acids.

### 3.3. Fluorescence quenching analysis of fulvic acids

Based on the above results, regions III and V were selected to conduct fluorescence quenching analyses due to the high signals in these regions for all three samples. Region I, II and IV were not used due to their low intensity which would not be able to provide good accuracy. A series of EEMs were collected during the titration of each sample with uranyl ion and the RIA signal volumes for regions III and V were calculated. These volumes were converted to percentages where 100% equaled the volume of the region without any addition of uranyl ion.

The fluorescence intensity was quenched when all of the fulvic acid samples were titrated with uranyl ion. This comes about because a binding interaction takes place between sites on fulvic acid molecules and uranyl ion. This interaction changes the structural conformation of the fulvic acid molecules in the vicinity of the fluorophore and changes the electronic structure as binding occurs resulting in a reduction in efficiency of the quantum yield of fluorophores. Therefore, the fluorescence intensity of fulvic acid decreased during the titration. The fastest quenching rate was observed at the beginning of the titration and it became slower as more uranyl ion was added into the solution system. At the end of the titration, a flat curve was obtained with low, but non-zero values of fluorescence. This residual fluorescence might be due to non-binding fluorophores that continue to fluoresce even after binding has ceased or it could be caused by some fluorophores that cannot be totally quenched when bound with uranyl ion. In this case there is a reduction in the efficiency or quantum yield of the fluorescence for the complex relative to the free ligand. Quenching curves are shown in Fig. 6, the squares and triangles represent regions III and V, respectively.

Based on the quenching curves in Fig. 6, non-linear regression analysis was performed and three parameters (conditional stability constant, $K$, ligand concentration, $C_L$, and residual fluorescence, $I_{res}$) were calculated. Results are shown in Table 2. The values obtained in our study show a good match with other values reported in the literature (Esteves da Silva et al., 1996; Lubal et al., 2000; Shin et al., 2001).

The results obtained for the three fulvic acid samples are relatively close showing only an 8% difference range for stability constants, which matches the similarity of the spectroscopic data in this study. The log $K$ values decrease slightly in the order, SFA, ORFA, SRFA. $C_L$ values increase going in the same order. This indicates more binding ligand sites exist in the SRFA than SFA, but these ligand sites have lower binding strength than the binding sites in SFA. The variation of stability constant among fulvic acids is due to the different structures and chemical compositions since fulvic acids are very complex heterogeneous assemblages. Different stereo-chemical structures, different substituent groups connected to the binding site and different binding groups (-OH, -COOH and $-\text{NH}_2$) are all possible factors that change binding capacities and strength. For example, three different compounds: 3,4-dihydroxyhydrocinnamic acid, 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxybenzoic acid share the same $-\text{COOH}$ and $-\text{OH}$ groups, however, they have different stability constants when they bind with metal (Borges et al., 2005). When $-\text{COOH}$ is connected to different structures, different stability constants will result from the interaction between these carboxylic anions and metal ions (Bunting and Thong, 1970). Therefore, different results were obtained from our fulvic acids. It is important to mention that application of the Ryan-Weber model is based on the assumption that each peak is associated with a ligand site whose average properties are represented by log $K$ and $C_L$ values and the binding ratio between the metal ion and the ligand site is 1:1.

<table>
<thead>
<tr>
<th>EEM region</th>
<th>Excitation (nm)</th>
<th>Emission (nm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>200–250</td>
<td>280–330</td>
<td>Amino acid-like</td>
</tr>
<tr>
<td>II</td>
<td>200–250</td>
<td>330–380</td>
<td>Amino acid-like</td>
</tr>
<tr>
<td>III</td>
<td>200–285</td>
<td>380–550</td>
<td>Fulvic acid-like</td>
</tr>
<tr>
<td>IV</td>
<td>250–400</td>
<td>280–380</td>
<td>Amino acid-like &amp; Solublemicrobial by product-like</td>
</tr>
<tr>
<td>V</td>
<td>285–400</td>
<td>380–550</td>
<td>Humic acid-like</td>
</tr>
</tbody>
</table>

**Table 1** RIA definition of five regions according to Chen et al., 2003.

![Fig. 5](image) **Fig. 5.** Percentage distribution of the EEM fluorescence in the five spectral regions for SFA, ORFA and SRFA.

3.4. Comparison between RIA and PARAFAC methods

PARAFAC has been successfully and widely applied in studies of DOM (Ohno et al., 2007; Wu et al., 2011, 2012; Yamashita and Jaffé, 2008). Therefore, the PARAFAC treatment was conducted to analyze the EEMs obtained during the titration in order to compare with RIA. Extremely close curves were obtained using the PARAFAC data treatment compared with the RIA data treatment. For each titration point a comparison between the RIA method and the PARAFAC method showed values agreed with less than 10% differences for region V and less than 5% differences for region III. This indicated a relatively good agreement between these two methods.

Based on these curves, another set of data was calculated using the Ryan-Weber fluorescence quenching analysis. Results in Table 3 show a relatively close agreement for the log $K$ values, which amounts to less than 4% relative difference. Although relatively large percent differences were found for some values of the $C_L$ and $I_{res}$, the majority of the results for these two parameters show a close correlation.

The different results between these two data treatment methods may be caused by several factors. The most important factor for an accurate result using the RIA method is region selection. A bad boundary selection may result in an inaccurate prediction for the quantitative value of fluorescence since information...
from different fluorophores may overlap each other. If this situation happens, the volume of a particular region may inaccurately represent the mixture information. A good understanding of the fluorescent species such as excitation wavelength and emission wavelength may be required to determine the peak region before using the RIA method. The fluorescence spectral maximum shift in the case of Al-fulvic acid complexation (Ryan et al., 1996) is also another factor that needs to be considered during the application of the RIA method. As a result, further studies need to be conducted in order to uncover the fluorescent properties of fulvic acid samples and binding behavior.

With the results obtained from this study, the agreement between these two methods proved that the RIA data treatment can be applied in metal binding fluorescence quenching analysis as well as quantitative analysis of fluorescence EEM data. If improved procedures can be developed to better avoid the factors mentioned above, then RIA will be a powerful technique in analyzing fluorophores and complex samples such as fulvic acids.

### 3.5. pH effect

pH plays an important role in complexation study of uranyl ion with fulvic acid samples. Uranyl is the most common form of uranium in water. The majority of uranyl exists as free uranyl ion in solutions that have a pH lower than 3.5. When the pH is higher, it exists as a series of uranyl hydroxy and uranyl carbonate complexes (Zhu et al., 2014). When the pH is lower than 3.5, the concentration of the hydrogen ion is much higher than uranyl ion in these experiment. This in turn will increase the protonation of acidic sites on fulvic acid and may reduce the availability of sites for binding with metal ion. Our study showed fulvic acid sample was less quenched when pH gets lower. When pH is higher than 3.5, uranyl hydroxy and uranyl carbonate complexes will dominate the system and hydroxide ion will compete with fulvic acid in binding the free uranyl ion. In these cases, it is difficult to measure the stability constants between free uranyl ion and SFA. Moreover, uranyl itself fluoresces and exhibits a peak at 500 nm–550 nm when it is excited by wavelengths between 250 nm and 450 nm (Vandenhove et al., 2010). Strong fluorescence peaks were observed when the pH was above 3.5 and it reached the highest intensity at pH 5.5. Negligible fluorescence signals and UV–Vis absorbance were obtained for free uranyl ion when the pH is 3.5. Therefore, a pH of 3.50 was maintained throughout the experimental to avoid possible interferences.
4. Conclusions

From this study, relatively strong binding ability was obtained between uranyl ion and three fulvic acid samples. The results described here also demonstrate that RIA is a viable new technique for quantitative analysis of different fluorescence species in metal binding studies. It is notable that the peak region selection is critical for accurate quantitative monitoring of different peaks. Further application of the RIA method can be extended to quantitatively analyze more complicated mixtures.

Acknowledgments

We want to thank Dr. Yunfeng Ling for his support with the Matlab program. We thank the Department of Chemistry at the University of Massachusetts Lowell for supporting the project.

References