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Complexation Study of Uranyl Ion with Dissolved Organic Matter in Natural Freshwater by Fluorescence Quenching Techniques

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<http://dx.doi.org/10.5772/intechopen.72861>

Abstract

The environmental problem of uranium waste has attracted global attention, and the investigation of its migration behavior in the environment has become an important topic. Uranyl ion is the most distributed form of uranium in water, and its mobility is highly affected by dissolved organic matter (DOM) due to its complexation in aquatic systems. In this study, DOMs in a variety of water samples from the Merrimack Valley of Massachusetts in the USA were studied using fluorescence excitation-emission matrix (EEM) techniques and regional integration analysis (RIA) data treatment method. RIA divided an EEM of DOM into five regions according to its fluorescence features and categorized them as humic-acid-like, fulvic-acid-like and amino-acid-like. Fluorescence quenching techniques were used to study the complexation properties of DOM with uranyl ion in aquatic systems at pH 3.5. Intense peaks in regions III and V were found to be quenched during the titration by uranyl ion. Results obtained showed that the stability constants ($\log K$) were 4.01–4.19 and 3.83–3.97 for regions III and V, respectively. This study provides an easy, nondestructible and effective approach for studying the complexation of uranyl ion with DOM by applying the RIA method with fluorescence quenching.

Keywords: dissolved organic matter, uranyl ion, excitation emission matrix, fluorescence quenching, regional integration analysis

1. Introduction

The mining and refining activities of radionuclides for military and energy application have been dramatically increased since the past century. Uranium is one of the important radionuclides that have been extensively used, and its production capacity will increase to 122,260

tons/year by 2020, which is a 40% increase compared to 2010 [1]. Radioactive waste as a by-product during the production has resulted in environmental contamination hazards to ecosystems and human health from both radioactivity and toxicity [2]. The majority of uranium can be found in the oxidized U (VI) state, while reduced U (IV) is also possible in water, soil and rocks. When dissolved in aquatic systems, uranyl ion (UO_2^{2+}) exists as the free ion in acidic environments, and it may form uranyl hydroxyl and uranyl carbonate ion pairs when in neutral and basic environments [3, 4]. The migration of uranyl in aquatic systems can be affected by a wide variety of factors and dissolved organic matter (DOM) plays an important role in the migration of uranyl ion [5]. Many techniques, including chromatography and mass spectrometry, have been used to study DOM and its interactions with uranium in the environment [5–8]. Fluorescence spectroscopy became an effective technique for studying DOM due to the existence of abundant aromatic groups and conjugated structures in DOM, which provide relatively good fluorescence characteristics [9, 10]. The fluorescence signal from DOM decreases when it forms complexes with certain metal ions, and based on this phenomenon, the fluorescence quenching technique was developed to study metal binding behavior of DOM [11, 12]. The advantages, such as good sensitivity, easy sample preparation and non-destruction to the sample, make the fluorescence quenching technique an effective approach to quantitatively studying the interaction between DOM and metal ions in aquatic samples.

The fluorescence excitation emission matrix (EEM) has been widely used in recent work [13]. It can provide detailed information of analytes in a three-dimensional spectrum by collecting signals from emission scans at different excitation wavelengths within a certain range. In order to interpret the EEM spectra, Chen developed the regional integration analysis (RIA) method [9]. In the RIA method, an EEM is divided into different regions according to fluorescence spectral characteristics and quantitative analysis is conducted by integrating the volume under the peaks in those regions. This RIA method has been used in the study of DOM previously [14, 15].

In this study, a new approach was developed by applying the RIA method to fluorescence quenching techniques to investigate the complexation behavior between uranyl ion and DOM in natural freshwater samples. This procedure was employed to aid in elucidating the binding mechanism of DOM with pollutants such as radioactive metal ions in natural aquatic systems, which will further improve understanding of migration of metal ions in the environment. Therefore, this study was aimed at investigating the fluorescence properties of DOM in natural freshwater samples and the complexation properties between uranyl ion and DOM using the fluorescence quenching technique coupled with the RIA data treatment method.

2. Experimental section

2.1. Apparatus

Fluorescence data were acquired by a Perkin Elmer LS55 spectrofluorometer. Instrumental parameters were set as follows: excitation slit—7 nm; emission slits—7 nm; excitation wavelength—200–400 nm (step—5 nm); emission wavelength—250–550 nm (step—0.5 nm); scan speed—600 nm/min; solution temperature—25°C. A WTW pH meter was used to monitor the pH.

2.2. Samples and reagent

Natural water samples were collected from the Merrimack River valley in Massachusetts, USA. The five sampling locations were Haggetts Pond (Andover, MA), Concord River (Lowell, MA), Mascuppic Lake (Tyngsboro, MA), Merrimack River Lowell site (Lowell, MA) and Merrimack River Estuary site (Newburyport, MA). Five liters acid-washed polyethylene bottles were used to store and transfer water samples. Before laboratory analysis, samples were filtered through 0.22 μm Whatman (Maidstone, UK) nylon membranes and stored in Corning polystyrene roller bottles in a refrigerator at 4°C. Uranium atomic absorption standard solution (1000 $\mu\text{g}/\text{mL}$) was purchased from Ricca Chemical (Arlington, TX). The deionized water was obtained from an Elga Purelab water purification system.

2.3. Fluorescence titration experiments

In the titration experiments, a series of accurately measured amounts of uranyl ion standard solution were titrated into a beaker containing a known volume of natural water sample. EEMs of the water sample were collected before the titration and after each addition of uranyl ion solution. The concentration range of uranyl ion was from 0 to 0.68 mmol/L by the end of the titration. Measurements were conducted three times at 25°C ($\pm 1^\circ\text{C}$) and at pH 3.50 (± 0.01) and the same procedure was applied to each of the natural water samples. The experimental pH was maintained at 3.50, because most of the uranyl exists as free uranyl ion at this pH value [16].

3. Theory and analysis

3.1. Theories of RIA

Absorption of light energy may lead to transitions of electronic, vibrational and rotational states of a molecule. As illustrated in **Figure 1**, a simplified Jablonski diagram, an electron may be excited from the ground singlet state (S_0) to different vibrational and rotational energy levels of the first excited singlet state (S_1) by absorption of light at different wavelengths. Excited electrons in S_1 may lose energy via vibrational relaxation and return to the lowest vibrational energy state in S_1 . When electrons return to S_0 from S_1 , they may go back to different vibrational or rotational states by emitting light at different wavelengths within a certain range. Therefore, a fluorophore can be excited by light over a certain range of wavelengths and radiate in another wavelength range. A fluorescence peak follows a near Gaussian distribution [18]. The volume of the peak for RIA in an EEM can be calculated by the integrated area of a fluorophore peak over a range of excitation wavelengths (y axis of EEM) and a range of emission wavelengths (x axis of the EEM) times the fluorescence intensity plotted on the z axis. Based on this theory, quantitative analysis was achieved by the RIA method.

Figure 2 shows a typical EEM of Concord River water sample. Two distinct peaks were observed: one in region III (peak center at excitation—245 nm; emission—440 nm) and one in region V (peak center at excitation—330 nm; emission—440 nm), which were recognized as the fulvic-acid-like region and humic-acid-like region. Amino-acid-like and microbial by-product

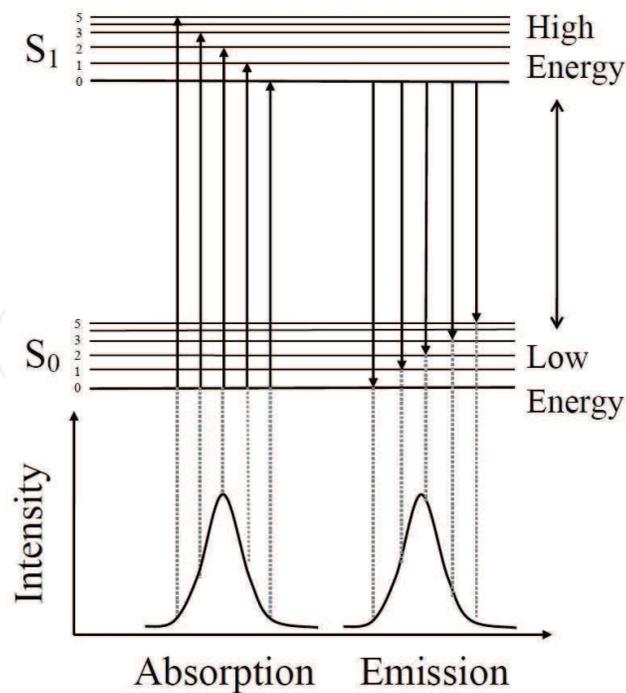


Figure 1. Jablonski diagram for fluorescence [17].

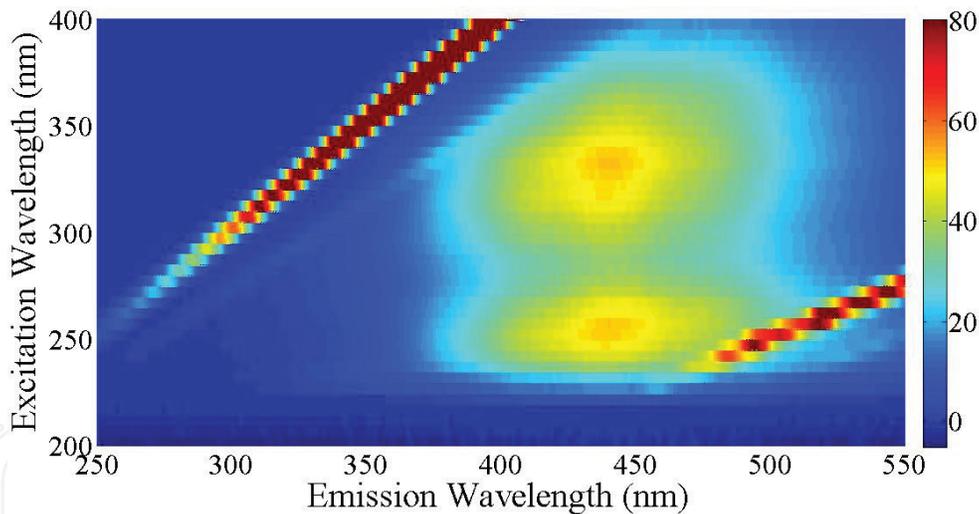


Figure 2. Fluorescence EEM spectrum of Concord River water sample.

related substances are categorized in region IV and were absent. This region includes tryptophan (excitation—280 nm; emission—348 nm), tyrosine (excitation—274 nm; emission—303 nm), and phenylalanine (excitation—257 nm; emission—282 nm).

3.2. RIA data treatment method

According to the RIA method [9], five regions were defined, as demonstrated in **Table 1**. The peak volume was calculated by integration of the area multiplied by the height of the area. A blank EEM spectrum of deionized water was subtracted from sample EEMs to remove any Raman scattering effect. The first-order Rayleigh scattering effect was eliminated by setting

Region	Excitation wavelength (nm)	Emission wavelength (nm)	Description
I	200–250	280–330	Amino-acid-like
II	200–250	330–380	Amino-acid-like
III	200–285	380–550	Fulvic-acid-like
IV	250–400	280–380	Amino-acid-like and soluble microbial by-product-like
V	285–400	380–550	Humic acid-like

Table 1. RIA definition of five regions in an EEM [9, 17].

values in that region to zeros. The second-order Rayleigh scattering effect was eliminated by an interpolation method, which allowed the replacement of the old data by new values in the second-order Rayleigh scattering region until a smooth surface was obtained. MATLAB software (version 2010b, Mathworks, Natick, MA) was used to conduct the RIA.

3.3. Curve fitting modeling

The curve fitting was carried out based on the nonlinear regression analysis of the experimental titration curves and the complexation model [12]. The modeling has been described in our previous study [17]. Briefly, based on the assumption of 1:1 complexation ratio between metal and ligand, a reaction can be described as follows:



where M is the free metal ion; L is the metal-free ligand site of a fluorescent species; and ML is the metal-bound species.

A stability constant for the reaction is represented in Eq. (2), and mass balances for metal and ligand can be represented in Eqs. (3) and (4), respectively. Fluorescence intensity is proportional to the concentration of fluorophores during the titration, and this phenomenon can be represented by the Ryan-Weber model [11, 12], Eq. (5). By combining and rearranging the above equations, fluorescence intensity I and C_M are represented in Eq. (6):

$$K = [ML]/[M][L] \quad (2)$$

$$C_M = [M] + [ML] \quad (3)$$

$$C_L = [L] + [ML] \quad (4)$$

$$[ML]/C_L = (I - I_{res}) / (I_L - I_{res}) \quad (5)$$

$$I = [(I_{res} - 100) / 2K C_L] \{ (K C_L + K C_M + 1) - [(K C_L + K C_M + 1)^2 - 4K^2 C_L C_M]^{1/2} \} + 100 \quad (6)$$

where K is the conditional stability constant; $[ML]$ is the concentration of metal-bound species; $[M]$ is the concentration of free metal ion; $[L]$ is the concentration of metal-free ligand; C_M is the total concentration of metal in the solution; C_L is the total concentration of ligand in the solution; I_L is the fluorescence intensity of ligand without any addition of metal; I is the fluorescence intensity of unbound ligand during the titration process; and I_{res} is the residual fluorescence intensity at the end of the titration.

With the fluorescence quenching data obtained in the experiment, nonlinear regression of the titration curve was conducted using Eq. (6) and K , C_L and I_{res} values were obtained.

4. Results and discussion

4.1. Fluorescence quenching analysis

The fluorescence quenching phenomenon was observed when aliquots of uranyl ion standard solution were titrated into natural water samples. As shown in **Figure 3**, the two distinct peaks in region III and region V were found to be quenched.

As the incremental concentration of uranyl ion in solution increased, the fluorescence signal of each region was decreased to varying degrees. The volumes of the solution were calculated after each addition of uranyl ion, and based on the change of solution volumes and the amount of uranyl ion added, quenching curves were plotted. Uranyl ion binding capacities for natural water samples were studied via fluorescence quenching phenomena for these regions. **Figure 4** shows the fluorescence quenching curves of the Concord River water sample titrated with uranyl ion and good reproducibility of the quenching curves were obtained in regions III and V. Fluorescence intensities during the titration were adjusted to a percentage relative to the initial intensity of the sample before the titration started. The fluorophores in region V show a higher residual fluorescence value than in region III, which might be caused by several factors: (1) the binding site associated with fluorophores in region V has a lower binding affinity for uranyl ion, which makes it less quenchable. (2) The fluorophores in region V may have a high fluorescence efficiency in their bound form, which enables the complex to absorb and emit significantly even when it is bound with metal. (3) There could be nonbinding fluorescent DOM in this spectral region producing a high residual fluorescence. Compared with the Concord River water, similar trends were observed for other natural water samples. This related trend represented a relatively similar DOM composition between these different samples.

Nonlinear regression analysis using the Ryan-Weber equation [11, 12] was conducted, and the results are presented in **Table 2**. In another experiment, the interaction between Cu^{2+} and tryptophan was studied, and a very good agreement was obtained between our results and literature reports by using the RIA method coupled with the fluorescence quenching technique. This proved the reliability of the application of the RIA method to fluorescence quenching analysis.

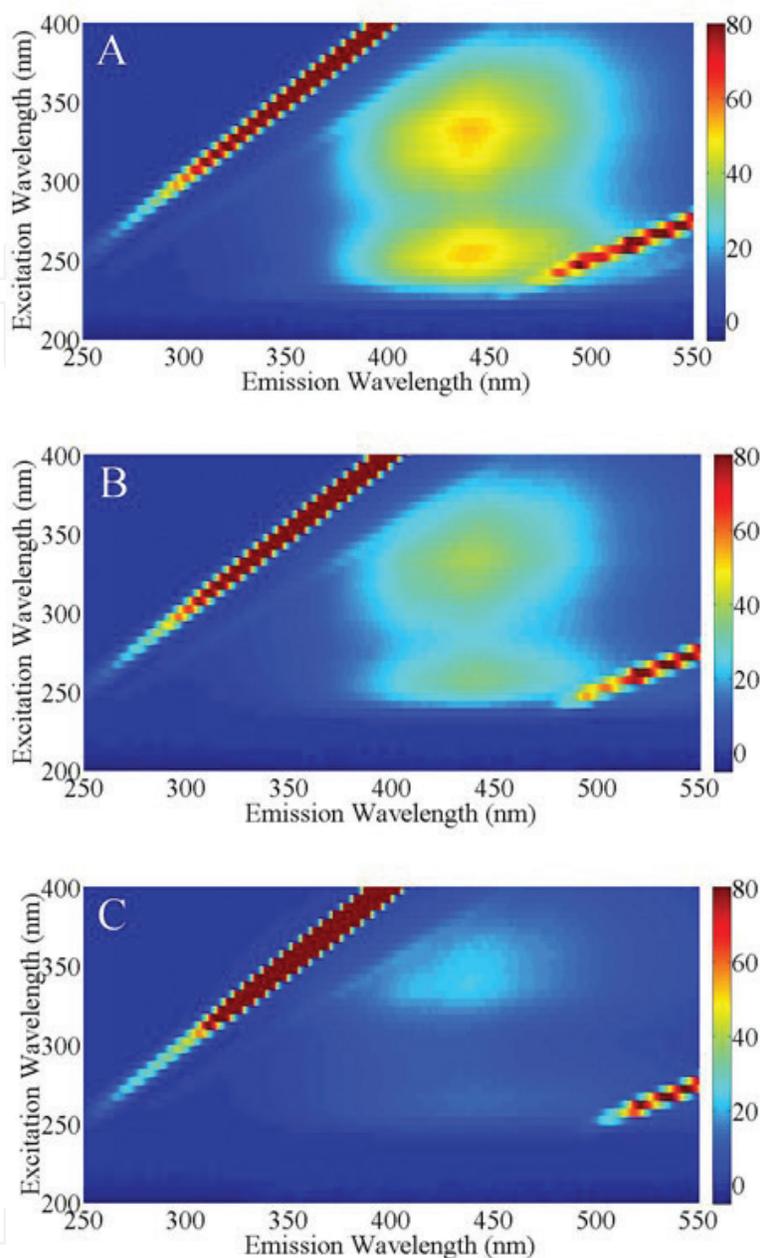


Figure 3. Selected examples of fluorescence EEMs of the Concord River sample during the titration with uranyl ion at concentrations of: (A) 4.2 $\mu\text{mol/L}$; (B) 41.5 $\mu\text{mol/L}$; and (C) 375 $\mu\text{mol/L}$.

4.2. Effect of pH

Uranyl chemistry is highly affected by the pH environment. As shown in **Figure 5**, uranyl mainly exists as free uranyl ion in solutions that have a pH lower than 3.5. In a neutral environment, the majority of uranyl ion is in the form of a uranyl hydroxyl complex and it transforms into a uranyl carbonate complex in basic conditions [16]. Several factors should be considered in studying the complexation of uranyl ion and DOM. First, in an acidic system that has pH values lower than 3.5, the high concentration of hydrogen ion will compete with uranyl ion when

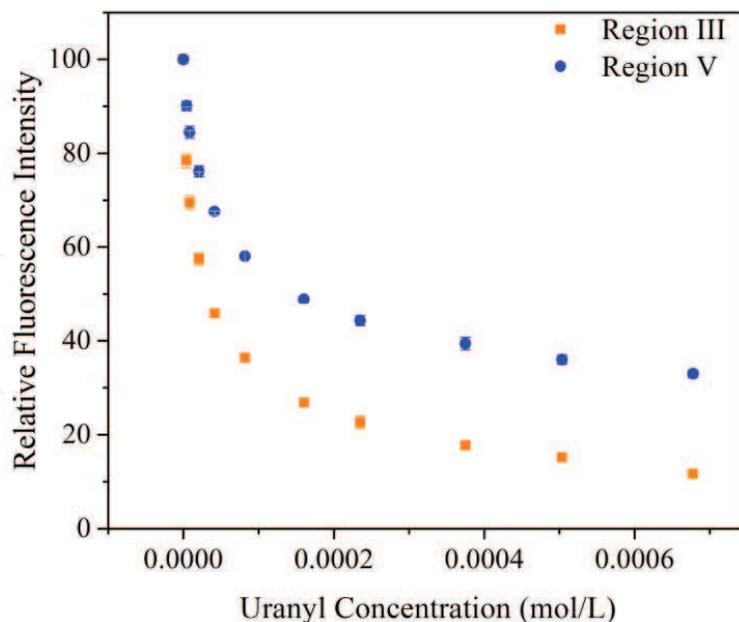


Figure 4. Fluorescence quenching curves for the Concord River water sample titrated with uranyl ion at pH 3.50.

binding with DOM. Study results showed that DOM was less quenched when the pH was lower and more when pH increased. Second, when the pH is higher than 3.5, uranyl mainly exists as uranyl hydroxyl and uranyl carbonate complexes and complexation behavior with DOM may be changed in these situations. Moreover, uranyl was found to exhibit strong fluorescence when the pH was at 5.5; this may cause difficulties in applying the fluorescence quenching techniques [3, 17]. When the pH was maintained at 3.5, uranyl existed as free uranyl ion and had a negligible fluorescence signal; therefore, this condition was selected.

Sample	Region	$\log K (\pm \text{Std. Dev.})$	$C_L (\mu\text{mol/L}) (\pm \text{Std. Dev.})$	$I_{res} (\pm \text{Std. Dev.})$
Haggetts Pond	Region III	4.38 (± 0.03)	*	10.1 (± 0.4)
	Region V	3.83 (± 0.08)	*	35.5 (± 4.9)
Mascuppic Lake	Region III	4.01 (± 0.03)	*	3.4 (± 3.34)
	Region V	3.93 (± 0.07)	4.32 (± 0.93)	34.2 (± 1.3)
Concord River	Region III	4.26 (± 0.02)	*	6.7 (± 0.4)
	Region V	3.93 (± 0.04)	*	23.6 (± 1.7)
Merrimack River Lowell	Region III	4.49 (± 0.05)	*	8.1 (± 0.2)
	Region V	3.97 (± 0.03)	*	28.0 (± 1.7)
Merrimack River Newburyport	Region III	4.08 (± 0.03)	*	3.4 (± 2.3)
	Region V	3.91 (± 0.04)	4.83 (± 3.26)	29.3 (± 0.5)

*Are the values not provided due to extremely small values obtained.

Table 2. Conditional stability constant ($\log K$), residual fluorescence intensity (I_{res}) and ligand concentration (C_L) determined by the fluorescence quenching techniques and the RIA method.

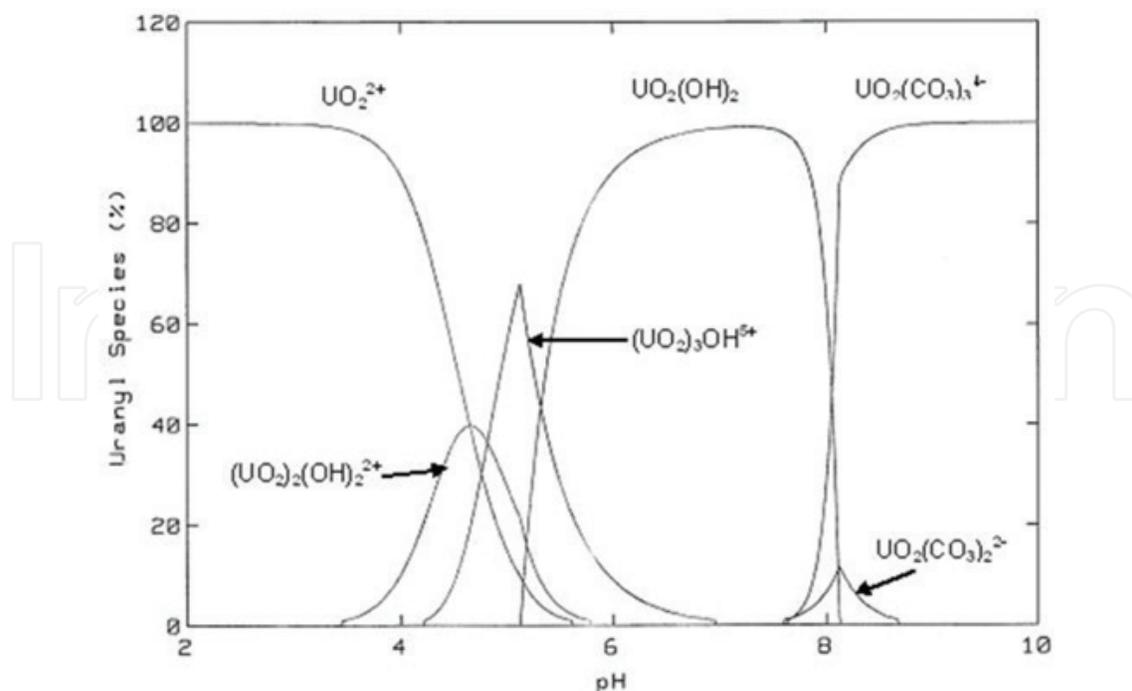


Figure 5. Percentage of chemical species for 0.8 mmol/L uranyl ion in a 0.01 mol/L ionic strength solution and 25°C in equilibrium with the atmosphere. Distributions were calculated with MINEQL + ionic equilibrium program [16].

4.3. Further study

While the fluorescence quenching technique provided an approach to understand the complexation between uranyl ion and DOM, a more complicated situation should be considered in the study of the transport or deposition of uranium with DOM in natural aquatic systems. In this study, the modeling is based on a simplified assumption of 1:1 complexation between uranyl ion and fluorescent DOM ligand. Since DOM has a complicated structure, a fluorophore may be associated with more than one site that is able to bind uranyl ion. On the other hand, other ligands that do not fluoresce may bind with uranyl and compete with fluorescent ligands. The metal ions in natural aquatic systems may compete with uranyl ion in complexation with DOM, which reduces the availability of the DOM that binds with uranyl ion. Other factors that affect the complexation of the DOM and uranyl ion in natural aquatic systems, such as pH, hardness and alkalinity, are highly dependent on the local geology, weather condition and human activity. To better understand these factors and the transport behavior of uranyl ion, further studies should be carried out.

5. Conclusion

The migration of uranium was studied by investigating the binding properties of uranyl ion and DOM. The fluorescence quenching technique coupled with the RIA data treatment was applied in this study. Different water types were studied, including lake, pond, river and estuary. Although challenges existed due to the heterogeneous and complex properties of

DOM and low C_L values obtained, this method provided reliable results in measuring stability constants. From the results, two distinct peaks were observed in region III and region V and the fluorescence quenching of these two peaks was studied. The Concord River water showed the highest stability constants followed by Mascuppic Lake, Haggetts Pond, Merrimack River Lowell and Merrimack River Newburyport. Region V was less quenchable and lower in binding ability than region III. The fluorescence quenching method is easy and nondestructive to the sample and can be used to monitor natural water quality and conduct binding studies. Further study of a more complicated modeling such as multisite binding modeling is needed to better understand the complexation of uranium with DOM in natural aquatic systems.

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