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Characterizing the interaction between uranyl ion and soil fulvic acid using parallel factor analysis and a two-site fluorescence quenching model

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ABSTRACT

In this study, a two-site fluorescence quenching data treatment was developed based on the Ryan–Weber model to investigate natural organic matter complexation with uranyl ion. Parameters for each ligand site including stability constant (log K), ligand site concentration (C_L) and residual fluorescence intensity (I_{res}) were determined using this approach. Parallel factor (PARAFAC) analysis was used to obtain quantitative data for peaks from fluorescence excitation emission matrix (EEM) spectra. This model was tested using a Cu^{2+} titration with a mixed system of tyrosine and tryptophan to simulate two ligands. Relatively good agreements with literature values (within 3.29% for log K values) indicated the validity of the model. The model was further applied in the complexation study between soil fulvic acid (SFA) and UO_2^{2+} since two components were predicted using core consistency diagnoses (CORCONDIA) for our SFA sample. Results for 20 mg/L SFA at pH 3.50 for the peak at $\lambda_{ex} = 330$ nm, $\lambda_{em} = 450$ nm were log $K = 4.49$, $C_L = 5.05$ μmol/L, and $I_{res} = 15.1$ and for the peak at $\lambda_{ex} = 240$ nm, $\lambda_{em} = 450$ nm were log $K = 4.56$, $C_L = 4.56$ μmol/L, and $I_{res} = 1.2$.

1. Introduction

Uranium is very widely distributed around the world and the majority can be found in two oxidation states U(VI) and the reduced form U(IV) in water, soil and rocks. Interest in the study of uranium has been ongoing for decades since it is a bi-product of nuclear fission in power plants and military research giving rise to highly radioactive nuclear waste [1]. The existence of uranyl ion (UO_2^{2+}), a predominant form of dissolved uranium in aquatic systems, is highly dependent on the pH, inorganic ligands, sorption by minerals and interaction with organic matter [2,3].

Fulvic acid is one of the most abundant and reactive components of dissolved organic matter (DOM) in the environment. Due to various and abundant functional groups in its molecular structure, fulvic acid can interact with metal ions to form coordination complexes [4]. Interaction between uranyl ion and fulvic acid is an important research topic in order to develop a method to monitor and predict the transport of the former in aquatic systems.

Many approaches and techniques have been attempted in the study of interactions between uranyl ion and fulvic acid [5–8]. The fluorescence quenching method, developed by Ryan and Weber [9,10], has been shown to provide the advantages of sensitivity, and freedom from disturbing the equilibrium being investigated and gives a rather unique perspective on binding by measuring a property of the ligand, therefore it has been used in many studies [11,12]. One important point to be noted, the Ryan–Weber model was originally developed for a single site interacting with a single metal ion in solution [9], which precludes its use in a multi-site complexation study. Due to the complicated structure of fulvic acid, more than one fluorophore has been detected through fluorescence lifetime measurements [13]. Likewise, multiple binding sites have been postulated [14–17] that can react with metal ions individually or simultaneously in a system. Thus, the mechanism of metal complexation should be represented using a multi-site model. In order to study interactions measured by the fluorescence of different sites with various metal ions, synchronous scan fluorescence and excitation–emission matrix (EEM) fluorescence techniques have been applied in fluorescence quenching studies [12,14,15,17,18]. However, synchronous fluorescence fixes the $\Delta\lambda$ between excitation and emission wavelengths for a scan which may not match the optimum excitation and emission wavelengths in a sample. Therefore, synchronous scans may miss valuable information. Fluorescence EEM spectra, which display all emission signals at every possible excitation wavelength in a three dimensional spectrum, provide the most complete picture of the fluorescence response. EEM data can be analyzed using parallel factor (PARAFAC) analysis, a multi-way decomposition and principal component analysis method [19,20]. PARAFAC treatment of EEM data collected for DOM at various metal

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concentrations allows different fluorophores to be quantitatively identified. A fluorescence quenching curve can be obtained by plotting the fluorescence intensity of a fluorophores against the metal ion concentration in a titration experiment. This curve can be fitted by the Ryan–Weber equation in order to obtain metal ion–DOM interaction parameters. Based on the EEM and PARAFAC techniques, previous studies proved that more than one fluorophore was undergoing quenching when metal ion complexation was taking place [14,16,17]. To better understand the interaction between metal ions, multiple binding sites and quenchable fluorescence signals in DOM, we developed a new methodology borrowing from several past studies. First, EEM spectra were employed in order to get a complete picture of all possible fluorescence signals available. PARAFAC analysis was then used to systematically and thoroughly evaluate the EEM data. Based on the PARAFAC analysis a modified two-site model was developed and tested using Cu^{2+} with a tryptophan and tyrosine model compound mixture. Finally, the newly developed procedure was used to study the interaction between uranyl ion and fulvic acid.

2. Experimental section

2.1. Apparatus

A Perkin Elmer LS55 spectrofluorometer was used to obtain fluorescence data. A 10 mm quartz cuvette was used for all measurements. 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 monitor the pH.

2.2. Reagent

L-Tryptophan and L-tyrosine (Sigma-Aldrich Company, St. Louis, Missouri) were dissolved in de-ionized water and transferred to a volumetric flask after stirring for 2 h. Cu^{2+} solution was prepared by dissolving cupric chloride (Fisher Scientific, Pittsburgh, PA) in de-ionized water and transferred to a volumetric flask. Concentrated sodium chloride (Sigma-Aldrich Company, St. Louis, Missouri) was prepared following the same procedure. A well-characterized soil fulvic acid (SFA) was obtained from Dr. James Weber, Department of Chemistry, University of New Hampshire, Durham, NH, USA. SFA solutions were prepared by dissolving solid SFA in de-ionized water then filtering through a 0.2 μm Whatman (Maidstone, UK) Nylon membrane filter. 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.

2.3. Fluorescence quenching measurements

In order to test the validity of the model, a fluorescence titration experiment of the interaction between Cu^{2+} and a mixed L-tryptophan and L-tyrosine solution was conducted. A series of known amounts of Cu^{2+} were titrated into the L-tryptophan and L-tyrosine mixture and EEMs were recorded after each addition plus the EEM before Cu^{2+} addition. Experimental solutions were transferred manually to the quartz cuvette with a pipette and transferred back to the beaker after the fluorescence measurement. In this experiment, the concentrations of L-tryptophan and L-tyrosine were fixed while the concentration of Cu^{2+} increased incrementally. The change in fluorescence intensity due to the metal added was monitored. The ionic strength was fixed at 0.01 mol/L with NaCl. All measurements were conducted in triplicate at 25 °C (±1 °C) and at a pH of 6.00 (±0.01).

In the titration measurement of the interaction between uranyl ion and fulvic acid, the SFA solution was added to a beaker and followed using the same method described above. Constant stirring was maintained with a magnetic stirrer and a pH meter was used to continuously monitor the pH. Then a series of known amounts of uranyl ion were titrated into the beaker. EEMs were recorded after each addition of titrant as well as the EEM of SFA before uranyl ion addition. Experimental solutions were transferred manually to the quartz cuvette with a pipette and transferred back to the beaker after fluorescence measurements. The concentration of SFA was fixed at 20 mg/L and the concentration of uranyl ion increased from 0 to 0.8 mmol/L during the titrations. The ionic strength was fixed at 0.01 mol/L NaCl. All measurements were conducted in triplicate at 25 °C (±1 °C) and at a pH of 3.50 (±0.01).

The SFA concentration of 20 mg/L, gave a relatively low absorbance (UV–Vis absorbance is less than 0.05) at the emission wavelengths used in this study. For this reason the inner filter effect cannot be considered as a factor of importance and hence there was no need for its correction in this case.

2.4. PARAFAC data treatment

PARAFAC modeling has been described in detail by Bro [19]. 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.

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{ijk}, i = 1, \ldots, I; j = 1, \ldots, J; k = 1, \ldots, K; \quad (1)$$

These three elements can be represented in Eq. (1) above. x_{ijk} is the intensity of fluorescence measured when sample i is excited at wavelength k and emission occurs at wavelength j. Three factors a, b and c in the equation will be the individual outcomes after calculation and represent the concentration, emission spectrum and excitation spectrum of fluorophore f. ε_{ijk} is the residual value that is caused by noise or other unexplained variations. F represents fluorophores, for example, the intensity at a designated excitation and emission wavelength of a sample will be the sum of the intensity of all fluorophores, plus the residual values at that point [20]. The PARAFAC modeling was conducted by MATLAB (version 2010b, MathWorks, Natick, MA) with the N-way toolbox [21].

2.5. Two-site complexation modeling

Using PARAFAC analysis as a tool to decompose a fluorescence EEM spectrum into separate fluorophores, it is then reasonable to treat the different fluorophores with a modified Ryan–Weber model. The sample used in our experiments gave two well-defined peaks from the PARAFAC analysis. Therefore, for each peak location we assumed that one binding site existed. During the titration process, the intensity of each peak decreased differently corresponding to two different stability constants involved in these processes.

In a solution system, binding reactions between metal ion and ligand sites can take place in a 1:1 ratio as follows

$$M + L1 \leftrightarrow ML1$$
$$M + L2 \leftrightarrow ML2$$

M represents metal ion in the solution and *L1* and *L2* represent two metal-free ligand sites corresponding to fluorophore one and two. *ML1* and *ML2* are metal-bound species at these sites. With the reactions above, two stability constants can be described as follows:

$$K_1 = [ML1]/[M][L1] \quad (2)$$

$$K_1 = [ML2]/[M][L2]. \quad (3)$$

K_1 and K_2 are conditional stability constants at fixed pH for site one and site two, respectively, reflected in the EEM spectra. [*ML1*] and