



THE STABILITY OF URANIUM(VI) COMPLEXES OF HUMATES AND FULVATES IN BIPHASIC SYSTEMS

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Received September 25, 2007; in final form March 7,
2008; Accepted April 10, 2008

ABSTRACT

Slight momentary heating of aqueous solutions containing humic substances (HSs) and uranyl ions (UO_2^{2+}) yielded humic flocs that carried the bulk of the ion down with them. These biphasic systems were at near-neutral pH, and after returning to ambient temperatures they remained intact and were analyzed by determining the free uranyl concentration in the aqueous phase. Phosphate enhanced UO_2^{2+} fluorescence was used for this, allowing the determination of the stability constants of HS-uranyl complexes in the floc. The neutral pH of the systems and the relatively unencumbered nature of the analysis provided a realistic picture of the complex formation in natural environments. The log K values of the complexes were found to lie in the 5-7 range and were independent of the total acid content of the humates and fulvates considered. Stability constants obtained for different size fractions of a humic acid were generally smaller than those of the whole material and decreased with molecular size. A comparison of humates and fulvates showed similar trends. The observations suggested that larger HSs polyanions, generally having a greater aliphatic content, provide a cage effect and are able to sequester UO_2^{2+} more effectively than smaller size fractions.

Keywords: humic substances, ultrafiltration, uranyl ion, flocculation, phosphate-enhanced fluorescence

1. INTRODUCTION

Humic substances (HSs), especially humic and fulvic acids (HA, FA), play a major role in the environmental fate of both organic and inorganic materials

[1]. As the degradation products of plant and animal matter in the environment, they are highly functional polyelectrolytes that are ubiquitous in soils, sediments and water. Their specific structural and chemical properties are largely dependent on the parent material and local environment [2,3]. HSs are involved in such processes as pH buffering, transport, soil weathering and nutrient cycling [4].

HA and FA have strong metal complexing abilities [5], largely due to carboxylic acid functionalities [6]. The formation and stability of these complexes have a major effect on the transport of metal ions in environmental matrices [7], but the innate heterogeneity of HSs makes it difficult to gain a detailed understanding of the processes involved [8]. Thermodynamic parameters are of prime importance in assessing the mobility of metals in the subsurface, especially the stability constants of metal-humate complexes, which largely determine the nature of the species being transported. In the case of materials with serious environmental consequences such as U(VI), notably in the form of the uranyl ion, UO_2^{2+} , the stability of humate and fulvate complexes is especially relevant.

Uranium is a naturally occurring trace element found in rocks and soils, but it is also a notorious anthropogenic pollutant that arises from activities such as mining, agriculture, and nuclear fuel processing [9]. Sorption processes on mineral surfaces dictate, to a large degree, the mobility of U(VI) in soils [10-12]. Organic material, especially HA and FA, also play an important role because of the complexation alluded to above, as well as sorption processes [13,14].

A number of experimental approaches have been employed, with mixed results, for the determination of stability constants of U(VI)-HSs complexes. Various models are available for these systems [15-18], which have also been probed with X-ray [19-20], fluorescence [21-22], UV-vis, and IR spectroscopy [23]. Ion-selective electrodes [24], ion exchange [25], and equilibrium dialysis-ligand exchange [26] have also been employed. The majority of these methods yield log K values in the 5 - 6 range, and determinations are generally made at low pH (<5). Agreement among reported values has been reasonable, but each method has inherent limitations. Of these, disturbance of the complexation equilibrium is a common procedural flaw. The low pH customarily employed in these studies has the effect of eliminating competing hydrolysis and carbonate complexation reactions, which narrows the focus to binary U(VI)-HSs systems. Despite these limitations, the methods listed above provide a valuable starting point for the determination of U(VI)-HS stability constants.

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Ternary U(VI) complexes, which must be taken into consideration at environmental pH values, have received relatively little attention. Zeh *et al.* [27] investigated the sorption of UO_2^{2+} on humic colloids under controlled CO_2 partial pressure and between pH 1 and 10. Recently, Sachs *et al.* [28] used time-resolved laser-induced fluorescence spectroscopy (TRLFS) and TRLFS with ultra-fast pulses (fs-TRKLFS) to calculate stability constants at environmental pH values. Both studies implicate the formation of ternary $\text{UO}_2(\text{OH})$ -HA complexes in the reduction of U(VI) mobility by HA.

An alternative approach. It has been observed in our laboratory that the addition of UO_2^{2+} to a dilute HS solution, followed by slight warming (by $\sim 10^\circ\text{C}$), causes the HS to form a floc that settles out of solution in a matter of minutes. This gentle phase separation produces a system that closely mimics the status of HSs in many natural waters of moderate ionic strength, where the bulk of the material is present as a solid phase and a minimal amount remains in solution. The complexed UO_2^{2+} is largely carried down with the floc, while the free uranyl ion remains in the liquid phase. Free exchange occurs between the two phases, without the interposition of a barrier or a separation medium. The assumption in using this system for the determination of stability constants is that floc formation occurs independently from uranyl-HS complexation and does not substantially alter the equilibrium of the latter. The free uranyl species in the supernatant can be determined by luminescence spectroscopy (aided by phosphoric acid), and the stability constant of the complex carried down in the flocculation determined by Eq. (1) [29]:

$$K = \frac{\frac{M_T}{L_c} - 1}{M} \quad (1)$$

Here, M_T is the total U(VI) concentration added, M is the free U(VI) in solution measured by luminescence spectroscopy, and L_c is the ligand concentration.

The results presented below pertain to stability constants of uranyl - HS complexes in biphasic systems at low ionic strength, near-neutral pH, and ambient atmospheric conditions. The HSs include humate and fulvate standards, isolated humates, and different size fractions of the same humate. The luminescence quantification of UO_2^{2+} was validated by means of ICP-AES measurements.

2. MATERIALS AND METHODS

2.1. Materials

Humic and Fulvic Acids. Nordic Aquatic humic acid (NAHA), Nordic Aquatic fulvic acid (NAFA), Suwannee River humic acid (SRHA), Suwannee River fulvic acid (SRFA), Leonardite humic acid (LHA), Soil humic acid (SHA), and Peat humic acid (PHA), were all purchased from the International Humic Substances Society (IHSS) and used as received. A humic acid isolated from the living alga *Pilayella littoralis* (PLVHA) was purchased from the Humic Acid Research Group at Northeastern University (Boston, MA) and used as received. A Leonardite humic acid isolated from a crude material purchased from Horizon Ag Products (Kennewick, WA) was also used. The extraction was carried out according to the procedure published by the IHSS [30].

Other Materials. $\text{UO}_2(\text{NO}_3)_2$ purchased from Spectrum Chemical (Gardena, CA), H_3PO_4 from J. T. Baker (Phillipsburg, PA), and NaOH, HCl, HF, AgNO_3 from Fisher Scientific (Fair Lawn, NJ) were all used as received.

2.2. Methods

Aqueous HSs solutions (*ca.* 100 mg L^{-1}) were prepared, by adding a minimal amount of 0.01 M NaOH to the solid HSs and shaking to assist dissolution. $\text{UO}_2(\text{NO}_3)_2$ was then added to give a UO_2^{2+} concentration of $6.175 \times 10^{-4} \text{ M}$. This solution was left to equilibrate for 60 min, heated to $30\text{-}35^\circ\text{C}$ and set aside for the humate to flocculate. Following adequate time for reaction and settling, the suspension was centrifuged, the supernatant removed, and the UO_2^{2+} determined fluorimetrically by a standard addition procedure. For the measurement procedure, the fluorescence signal of the uranyl in the supernatant was enhanced by the addition of phosphoric acid to a solution pH around 1. This also ensured the presence of the UO_2^{2+} ion free of any hydroxo or carbonate complexes that may affect quantification, and it removed any remaining traces of dissolved HS. All aqueous solutions were prepared with doubly deionized water treated with a 0.22 mm Millipore filter system to a resistivity of at least 10 MW cm.

Luminescence Spectroscopy. All luminescence intensity measurements were made with a SLM-AMINCO Model 8100 fluorescence spectrophotometer. The excitation and emission bandpass settings were 8 nm, and the cell temperature was 20°C . The excitation

wavelength for U(VI) was 292 nm, and the emission was observed at 495 nm.

Acidity. The total acid content of the humates was determined by a conductance slope method, which has been described previously [31]. Briefly, the procedure involved dissolution of HA in a small excess of NaOH, followed by conductometric titration with standard HCl. A plot of solution conductance vs. added HCl yielded a curve with three distinct regions, arising from: (i) the titration of excess OH⁻, which gave a steep decrease in conductance as OH⁻ was replaced by Cl⁻; (ii) the protonation of humate, which resulted in a gradual increase in solution conductance as the humate anion was replaced by Cl⁻; (iii) the addition of unreacted HCl, which sharply increased the conductance. Extrapolation of each region revealed the exact beginning and end of the humate titration, allowing the total acid content to be determined.

Size Fractionation. LHA was separated into six size fractions by ultrafiltration [32]. An alkaline solution of LHA was filtered through a 0.2 mm membrane (Gelman Sciences), and the retentate was rinsed with deionized water until colorless washings were obtained. The filtrate was then fractionated with a stirred-cell ultrafiltration apparatus (Amicon) using membranes with successively smaller molecular cut-off values. All separations were carried out in the same manner under a N₂ pressure of 20 psi. The size fractions obtained were: >0.2 mm, 0.2 mm – 100 kDa, 100 – 10 kDa, 10 – 3 kDa, 3 – 1 kDa, and 1 – 0.5 kDa. [The units reported here correspond to the membrane characteristics provided by the manufacturer.]

3. RESULTS AND DISCUSSION

As discussed above, a variety of methodologies have been applied to the determination of the stability constants of U(VI)-HS complexes. Despite the

different approaches used, calculated values are generally similar. Table 1 lists a brief survey of some of the methods used and stability constants obtained, along with pH and ionic strength values where given.

At the low pH values used, it is likely that the HS aggregates were sufficiently collapsed [33] (the "drained sphere" scenario) to reduce the number of exposed reactive sites compared to those available at environmental pH values. The influence of hydroxo- and carbonate-U(VI) complexes on U(VI)-HS interactions would also be eliminated at such low pH.

It was the objective of the present work to generate stability constants under conditions relevant to natural environments. As explained under Materials and Methods, a temporary 10-degree rise in temperature was applied to produce a uranyl-humate floc, and thereby a biphasic system. This effectively constrained the HS to the colloidal phase, where it was in equilibrium with a supernatant containing the remaining free UO₂²⁺. After returning to ambient temperatures, the system retained its biphasic structure and was analyzed fluorimetrically (Fig. 1). The fluorescence emission was enhanced through the addition of H₃PO₄ to the sample solutions after the phases were separated.

Table 2 shows the stability constants of the HS-uranyl complexes, juxtaposed to the total acidities of the humic and fulvic acids used in this work.

As is customary, the carboxyl groups were considered to constitute the metal complexing centers of HSs, and the total acid content was used in place of the ligand concentration, *L_c*, in Eq (1). It may be expected that humates with high total acidity would also have high stability constants, but this was found not to be the case. Table 2 shows no correlation between the measured log K values and total acidity of the corresponding humic substances. It does show notable differences between the stability constants obtained for FA and HA from the same HS source, demonstrating the significance of the nature of the HS considered. In each case the FA had a substantially lower log K values than its HA counterpart.

Table 1 U(VI)-HS stability constants and their accompanying experimental parameters

log K	pH	I (M)	Method	Ref.
4.06 – 6.13	3 – 5	not reported	Potentiometry-ion selective electrode	24
6 – 12	5 – 10	0.02 – 0.2	Equilibrium dialysis-ligand exchange	26
4.3 – 5.3	not reported	not reported	Ion exchange	25
4.70 – 5.07	4	0.1	3D and synchronous fluorescence spectroscopy	22
6.10 – 6.56	4	0.1	Time-resolved laser-induced fluorescence spectroscopy	21

Considering the main structural difference between FA and HA, namely the higher functional group density in the former, these results may be counterintuitive. They may be rationalized by invoking the greater size of the HA polyanion, which allows sequestration of the UO_2^{2+} in the humic matrix. The association therefore had both electrostatic bonding and physical segregation characteristics.

As mentioned above, sorption processes largely dictate UO_2^{2+} mobility in the subsurface. It is therefore instructive to investigate the sorptive capacity of the humic flocs that settle out of solution. To this end the above procedure was repeated with LHA, and the solution and the humic flocs were subsequently kept in contact for a week to allow for sorption to occur after the initial complexation. It was found that the amount of free UO_2^{2+} in solution remained virtually unchanged, indicating that colloidal HA had little tendency to adsorb UO_2^{2+} upon prolonged contact, and that complexation was the primary interaction.

Size Fractions. In order to assess the influence of molecular size on the stability constants of HS-uranyl complexes, LHA was separated into size fractions by ultrafiltration. The total acidity of each fraction was again determined by the conductance slope method and used in lieu of the ligand concentration, L_c . The size fractions were then subjected to the same regimen as described above for whole HA and FA. Table 3 shows the stability constants of the UO_2^{2+} complexes of the fractions.

It is clear from Table 3 that the stability constants of the complexes decreased with decreasing humate size, resulting in lower free UO_2^{2+} concentrations in

solutions containing larger LHA size fractions. That is, the larger LHA size fractions were significantly more effective in complexing UO_2^{2+} than their smaller counterparts.

It should be noted that the primary difference between large and small LHA size fractions, as determined by CP/MAS ^{13}C -NMR (spectra not shown), was a progressive reduction in aliphatic content with decreasing size.

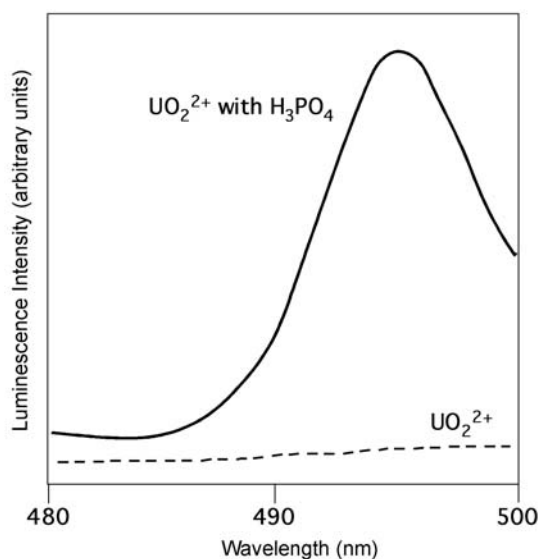


Figure 1 Uranyl luminescence in the presence and absence of H_3PO_4 ; $[\text{UO}_2^{2+}] = 6.175 \times 10^{-4} \text{ M}$; $\text{pH} = 1$

Table 2 Stability constants of HS-uranyl complexes and HS characteristics

Humic Substance	log K	Total acidity of HS (meq g ⁻¹)	%C	%H	%O	%N	%ash
LHA (IHSS)	5.84	5.0	63.8	3.7	31.3	1.2	2.6
LHA (from LHACB)	6.22	4.1	60.1	3.4	35.0	1.4	2.0
SHA	5.96	6.0	50.1	4.3	42.6	3.8	1.0
PLVHA	5.07	8.0	46.6	5.7	42.1	5.7	1.1
PHA	5.89	5.0	56.8	3.6	36.6	3.7	1.7
SRHA	5.69	3.4	52.9	4.1	43.4	1.2	3.5
NAHA	6.30	3.0	54.0	4.8	37.9	5.1	1.4
SRFA	5.22	2.8	53.0	4.4	43.9	0.8	1.0
NAFA	5.59	4.0	52.3	4.0	45.1	0.7	0.5

The number of oxygen-bearing functionalities remain approximately the same among the fractions [34]. The smaller HA size fractions bore a notable resemblance to FA in regard to their carbon distribution. This, together with the effects of total acidity noted above, suggests that size and aliphatic content are major determinants in UO_2^{2+} complexation by humics.

The stability constants reported above represent average values of all uranyl species present in solution under the prevailing conditions. Notably, this includes ternary hydroxo- UO_2^{2+} and carbonate- UO_2^{2+} complexes with the humates and fulvates. The fact that larger log K values were found for larger LHA size fractions and for HA in general compared to FA suggests that sequestration of the uranyl species within the humic structure may play an important role in the stability of the complexes. This may be the case for both doubly charged uranyl cations and singly charged ternary complex ions such as $\text{UO}_2(\text{OH})^+$. Mono- or bidentate attachment of these ions through carboxylate groups on the humate can be enhanced by a cage effect of the surrounding polyanions, especially if these partake in pseudomicellar aggregation [35]. In this manner the aliphatic content can exert its influence by providing the molecular size and amphiphilicity necessary for pseudomicellization. Although the present work provides no direct evidence of this, it is likely that the sequestration concept can be extended to neutral uranyl species such as $\text{UO}_2(\text{OH})_2$.

Table 3 Values of log K for different size fractions of LHA as obtained by UF

LHA size fraction	log K
LHA (whole)	6.22
> 0.2 mm	5.67
0.2 mm – 100 kDa	5.06
100 – 10 kDa	4.61
10 – 3 kDa	4.49
3 – 1 kDa	4.52
1 – 0.5 kDa	4.15

4. ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of the Inland Northwest Research Alliance.

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AES7925

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