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Quantitative Study of Be(II) Complexation by Soil Fulvic Acids by Molecular Fluorescence Spectroscopy

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The interaction of the beryllium ion [Be(II)] with one of the major constituent of soil organic matter, fulvic acids (fua), was investigated. The complexation of Be(II) by two samples of fua (amph and gran), at a concentration of 80 mg/L, was monitored by synchronous fluorescence spectroscopy at four pH values (4, 5, 6, and 7). A self-modeling curve resolution technique (evolving factor analysis) was used in the analysis of the sets of spectra collected at increasing Be(II):fua ratio to obtain concentration profiles that ideally correspond to one type of binding site. These profiles were adjusted to a 1:1 complexation model. Quite strong complexation of Be(II) by fua occurs at nearly neutral pH (6–7) (logarithm of the conditional stability constant between 5.6 and 6.5), indicating that fua probably affect markedly the mobility of Be(II) in the environment.

Introduction

Beryllium and its compounds are extremely toxic. Indeed, beryllium is the most toxic of the non-radioactive elements, and there is no effective antidote for its poisoning (1, 2). Nevertheless, due to its unique properties, beryllium is used in the nuclear, aerospace, and electronic industries; its annual production is increasing (1); and some dispersion of the element into the environment is inevitable, either from industrial processing units or waste repositories or as a consequence of accidents [for example, in 1990, in the Soviet Union, several tons of beryllium was released into the atmosphere from a nuclear fuel processing plant (1)]. Also, non-neglectable quantities of beryllium are being released into the atmosphere as a result of the combustion of fuels (3). An indirect indication of the anthropogenic flow rate of beryllium to the environment can be assessed

by the relatively high enrichment factors (atmosphere/soil and freshwater/soil) (4).

Humic substances (hus), which are ubiquitous in soils and natural waters, play an important environmental role both in the inactivation of toxic forms of inorganic ions and in the mobilization/immobilization processes of pollutants (5, 6). However, to the best knowledge of the authors, there are no systematic studies of the interactions of hus and the beryllium ion [Be(II)]. Recently, an experimental procedure was developed for the study of the interaction with Be(II) of the most soluble fractions of hus, fulvic acids (fua), based on synchronous fluorescence (SyF) spectroscopy and self-modeling curve resolution techniques (7). The association of this type of chemometric data analysis with SyF spectroscopy has proved to be a valuable tool for the analysis of the acid–base and complexation [Cu(II) and Al(III)] properties of fluorescent compounds, including fua (8–13). For the study of Be(II) complexation by fua, the use of an experimental technique like molecular fluorescence that measures directly the ligand status rather than metal ion concentrations is a suitable methodology because of the highly complex chemistry of Be(II) in water (1).

This paper presents the results of a study of the interaction of two different fua samples isolated from A_h soil horizons, with Be(II), at four pH values (4, 5, 6, and 7), which are usually found in natural soils. The objective of this work was to obtain a first set of information about the role of fua in the fate of the Be(II) compounds in soils. The fua concentration value (80 mg/L) was chosen to obtain information about the effects of recharge waters containing dissolved organic matter entering surface waters.

Theory

Be(II) forms quite stable and highly fluorescent complexes with some families of compounds, of which salicylic acid and its derivatives are the most well known (7). If Be(II) (or other metal ion) is added to a mixture of fluorescent compounds, which includes a ligand that forms stable and fluorescent complexes with it, an increase of fluorescence above the overall background in a characteristic wavelength range will result as consequence of the complex formation. In principle, if structurally different ligands forming fluorescent complexes with Be(II) (or other metal ion) are present in the mixture, these will be characterized by different fluorescence properties (position and shape of the fluorescent bands).

Information about the complexation can be obtained from a set of spectra collected at successively increasing concentrations of the metal ion, through a data treatment procedure which allows (i) the identification of the number of fluorescent species and (ii) extraction of the spectral variations associated with the corresponding complexes. For the case of fua, which include a complex mixture of ligand structures that will compete for the metal ion complexation, the evolving factor analysis procedure (EFA) previously reported (7, 9), which is a self-modeling curve resolution technique, fulfills these two purposes. Indeed, its first objective is to detect the number of components that provoke linearly independent variations in the spectral data matrix, *i.e.*, the number of fluorescent complex species.

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This number is obtained by principal component analysis or by its graphical representation as an EFA plot. This is a chart of the logarithm of the eigenvalues [$\log(EV)$] of a successively increased number of spectra collected as a function of increasing metal ion concentration. Because the spectral variations observed upon the variation of this concentration occur above a constant background spectrum, the first principal component corresponds to this constant spectrum and the following to different fluorescent complex species that provoke "different" variations on the data set. These varying components contain the information about the chemical phenomena under investigation, in the present case, the complexation of Be(II) by fua. The other components correspond to variations due to experimental noise. The characterization of the components corresponding to useful information, *i.e.*, their SyF spectra and SyF profiles, is obtained in the second part of the EFA procedure (secondary EFA), which consists of a least squares adjustment.

Finally, the calculated SyF profiles of the varying components as function of the total Be(II) concentration can be used for the determination of their complexation equilibrium parameters. The complexation reaction between the ligand (L) and Be(II), considering only the formation of 1:1 complexes, can be represented by (charges omitted)



and the corresponding conditional stability constant, K_c , is given by

$$K_c = [\text{BeL}]/([\text{Be}][\text{L}]) \quad (2)$$

where [X] denotes the total concentration of X, for instance [Be] is the concentration of all metal species not bound by the ligand.

Assuming that the enhancement in the SyF signal is proportional to the concentration of the complex, [BeL], the following relation is valid:

$$\frac{I_F - I_0}{I_{\max} - I_0} = \frac{\alpha(I_{\text{FM}} - I_0)}{I_{\max} - I_0} \quad (3a)$$

where the fluorescence intensities are as follows: I_0 , when no Be(II) is present; I_F , throughout the titration; I_{FM} , due to the complex; and I_{\max} , total fluorescence intensity at the end of the titration. α is the fraction of the total ligand (which total molar concentration is represented by C_L) bounded to Be(II) ($=[\text{BeL}]/C_L$). Because the SyF profiles obtained from the EFA analysis of the spectral data are scaled between 0 and 100, *i.e.*, $I_{\max} = 100$ and $I_0 = 0$, eq 3a simplifies to

$$I_F = \alpha I_{\text{FM}} \quad (3b)$$

Considering eqs 2 and 3, and the material balances for the ligand and for Be(II), the following equation is obtained (7), by an approach similar to that described in refs 14 and 15

$$I_F = I_0 + (I_{\text{FM}}/(2K_c C_L))[(K_c C_L + K_c C_{\text{Be}} + 1) - ((K_c C_L + K_c C_{\text{Be}} + 1)^2 - 4K_c^2 C_L C_{\text{Be}})^{1/2}] \quad (4)$$

where C_{Be} is the total molar concentration of Be(II).

TABLE 1

Characteristics of Fua Samples and Soils from Which They Were Extracted

characteristic	fua sample	
	amph	gran
Topological Characteristics		
horizon	umbric Ah	umbric Ah
soil	humic andisol	humic cambisol
bedrock	amphibolite	granite
location	Boqueixón (La Coruña)	Ames (La Coruña)
Elemental Characteristics		
elemental analysis ^a		
N	2.13	1.13
C	44.79	45.40
H	4.04	3.80
Chemical Characteristics		
total acidity (mequiv/g)	7.4	13.6
carboxylic content (mequiv/g)	3.7	5.7
E4/E6	7.67	3.53
molar absorptivity at 280 nm [L (mol of OC) ⁻¹ cm ⁻¹]	304	379
aromaticity (%) ^b	22	26
MW (kD) ^b	1.7	2.0

^a Weight percentages on a dry basis. ^b Obtained from the molar absorptivity at 280 nm (19).

From the nonlinear fitting of this equation to the calculated SyF profiles (I_F as function of C_{Be}), with the fluorescence intensities previously scaled between 0 and 100 ($I_0 = 0$), K_c , C_L , and I_{FM} are calculated (7, 10, 11). The final value for I_{FM} should be around 100. Higher values indicate that either the association of the ligand with Be(II) was not complete at the end of the experimental titration or that deviations from the assumed 1:1 model occur.

Experimental Section

Reagents. Fua were isolated from two different soils of Galicia (NW Spain) by the procedure recommended by the IHSS (16). The characteristics of soils, elemental analysis, total acidity, and carboxylic content obtained respectively by the barium hydroxide and calcium acetate methods (17), the E4/E6 ratio (18), and an estimation of the aromaticity and weight-averaged molecular weight using the molar absorptivity at 280 nm as described in ref 19, are shown in Table 1. The analysis of the characteristics of the two samples shows that they do not differ markedly from other samples described in the literature (5, 19).

Solutions of 80 mg/L fua were prepared in 0.1 M potassium nitrate and used within 2 days. For adjusting the titrated solutions to a constant pH value, a 0.05 M decarbonated potassium hydroxide solution was used. These experimental conditions were used in work involving other ions (7–13) and were maintained to get comparable results and to detect trends in these. With lower values of ionic strength it would be impossible to keep it constant along the experiments due to the unknown contribution of fua to ionic strength and to the addition of potassium hydroxide for pH adjustment. A concentrated solution of Be(II) was prepared by dissolving 0.04360 g of beryllium hydroxide in 4.00 mL of concentrated sulfuric acid and diluting with water to 100.0 mL. The Be(II) solutions used in the experiments were obtained by rigorous 1:10 dilution of the concentrated solution with water.

Instruments. pH was kept constant with a PC-controlled system assembled with a Crison MicropH 2002 pHmeter, a Crison MicroBU 2030 microburette, a Philips GAT 130 glass electrode, and a Radiometer K711 (double junction) reference electrode. The experiments were made under nitrogen at 25.0 ± 0.2 °C. The cell was calibrated with two buffer solutions with ionic strength adjusted to 0.1 M ($\text{pH}_1 = 6.784$ and $\text{pH}_2 = 3.883$) (20).

Fluorescence measurements were made with a Perkin-Elmer LS-50 luminescence spectrometer with a flow cell. A Gilson Minipuls-2 peristaltic pump forced the displacement of the titrated solution into the flow cell after each addition of titrant, which was left to stabilize for about 5 min (no fluorescence variations were detected after this stabilization period). During pH and fluorescence intensity measurements, the pump was turned off. SyF spectra were recorded with the following instrument settings: between 250 and 550 nm excitation wavelength; 0.5 nm resolution between points; 7.5 nm excitation and emission slit width; wavelength difference of 35 nm; and scan rate of 200 nm/min. After subtraction of the reference spectrum (pure solution of 0.1 M potassium nitrate) and correction for dilution of the titrated solution (due to the addition of the Be(II) and potassium hydroxide solutions), data spectra were stored on a disk and converted to ASCII format with LAB CALC software (Galactic Industries Co., USA).

Programs and Data Treatment. All software used for data simulations and calculations were developed in this laboratory and previously described (8, 9).

Results and Discussion

Analysis of Spectra. The effect of the presence of Be(II) on the SyF spectra of gran and amph fua at $\text{pH} = 7$ is shown in Figure 1. At all four pH values, the shapes of the spectra are similar, *i.e.*, when Be(II) is present, a major SyF band is observed above the intrinsic background fua fluorescence in the 335 nm wavelength range. As shown by Figure 1, the two fua samples present similar SyF spectra (two main bands located at about 342 and 385 nm), but slight differences are also detected, namely, the band at 342 nm of the amph sample has a relatively higher intensity than for the gran sample.

Figure 2, which refers to the gran sample at $\text{pH} = 7$, shows a set of typical results of the application of the EFA procedure to the experimental data sets. The analysis of the EFA plots for the two fua samples at the four pH values (Figure 2A) shows that only two components are detected for $\text{pH} = 4$ and 5 but three components are detected for $\text{pH} = 7$. One of the detected components corresponds to the SyF spectra of the fua fraction that is not affected by the presence of Be(II), *i.e.*, remains constant, and the others (one or two, depending on the pH) correspond to the spectra resulting from the formation of complexes with Be(II) by different binding sites. The increase of the number of detected components with the pH observed for both samples reveals an increase of available ligand structures for Be(II) complexation. This fact results probably from an increase of the extent of deprotonation of the weaker acid-base structures, making these binding sites more available for complexation.

The calculations of the secondary EFA were similar to those previously reported (7, 9), and typical results are shown in Figure 2B (spectra of the detected components) and Figure 2C (concentration profiles for the second and third components). The calculated spectrum that corre-

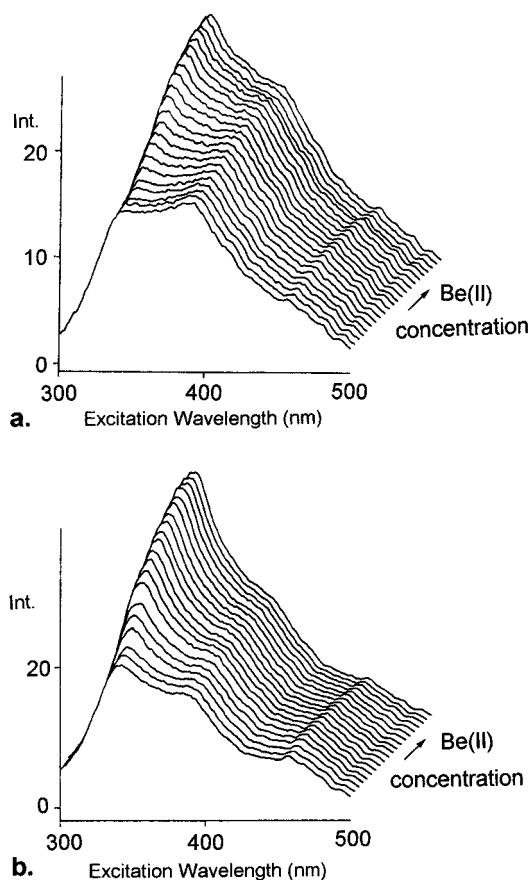


FIGURE 1. Typical synchronous fluorescence spectra as function of Be(II) concentration (between 7×10^{-7} and 5×10^{-5} M): (a) gran ($\text{pH} = 7$); and (b) amph ($\text{pH} = 7$).

sponds to the constant component (Figure 2B.1) is similar to the spectrum of the fua in the absence of Be(II).

For all the experiments, the calculated spectra for the second component are similar to that shown in Figure 2B.2 and characterized by only a well-defined band at 335 nm. The information contained in this component is related to the formation of a relatively high fluorescent complex between fua and Be(II). Since the calculated spectra of the second component for the two samples at the four pH values are quite similar, the same type of ligand structures are assumed to bind Be(II) in all the situations. The shape of the calculated spectra for the third component, when detected, is similar to that shown in Figure 2B.3 and characterized by a well-defined positive band (meaning that it corresponds to enhancement of fluorescence) around 325 nm and by a broad negative band (meaning quenching of fluorescence) around 450 nm. The experimental variations associated with this third component are smaller than for the second component. Therefore, only the information contained in the second component was used for calculations of equilibrium parameters.

From the secondary EFA, the concentration profiles were obtained, as shown in Figure 2C, where the variation of the concentration of the second and third components as function of the Be(II) concentration is represented. This information was used in the calculations of the equilibrium parameters.

Equilibrium Parameters. Table 2 shows the equilibrium parameters obtained by nonlinear adjustment of the data for the coordination of Be(II) by the fua binding sites corresponding to the SyF profiles of the second component,

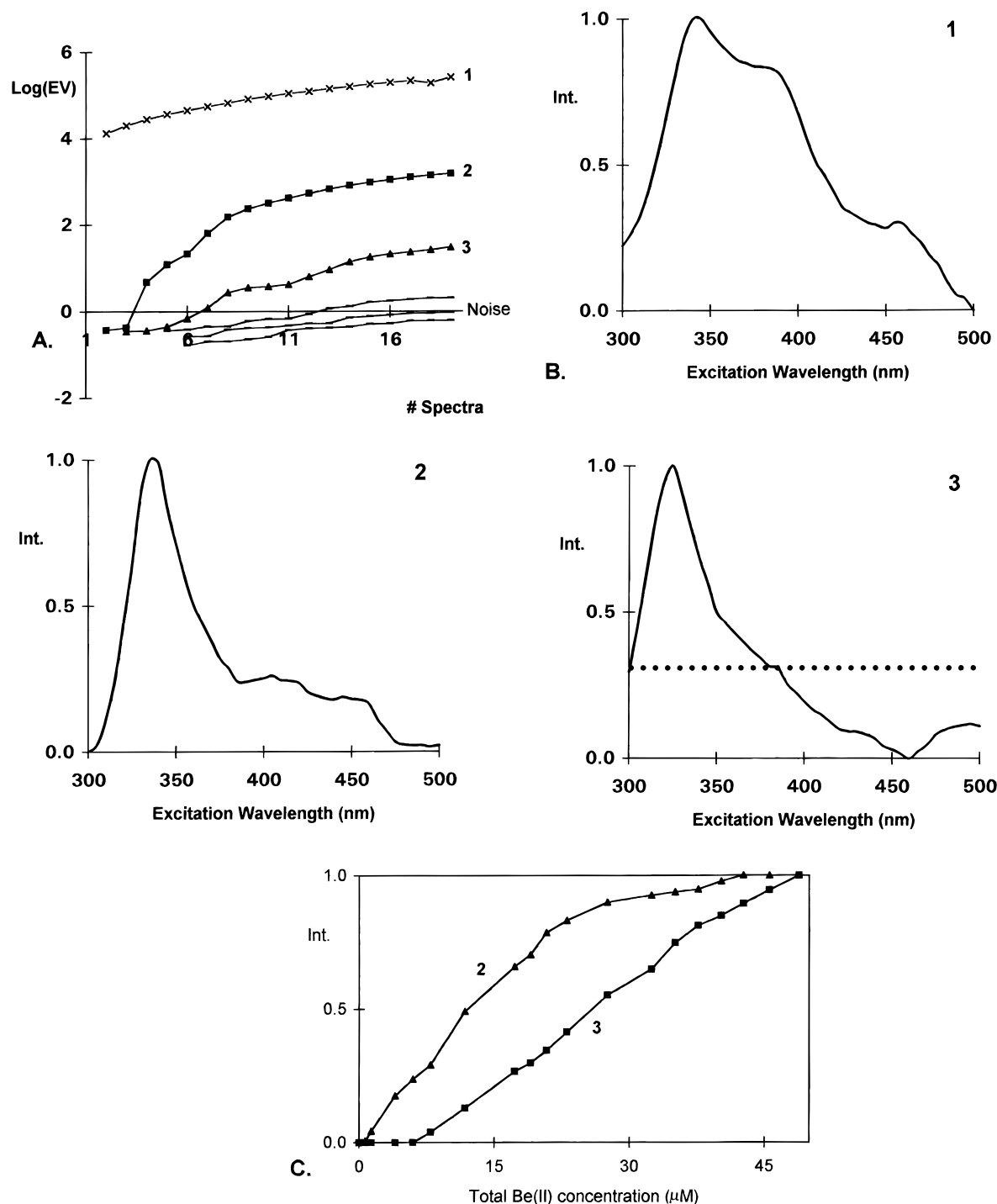


FIGURE 2. Typical results of the EFA procedure (amph fua sample at pH = 7) (each component is represented by the corresponding arabic number): (A) EFA plot; (B) calculated scaled (between 0 and 1) spectra of the components (spectrum B.3 is half-positive and half-negative as shown by horizontal dots); and (C) calculated fluorescence intensity profiles for the second and third components.

as discussed above. The parameters of quality of the adjustment, sum of squares of the residual (SSR), and average deviation (ΔE) show good adjustment for all the cases. Indeed, SSR is always less or about 10, and ΔE is less than 1.

The adequacy of the 1:1 model to describe the complexation of Be(II) by fua can be assessed by the analysis of the I_{FM} parameter. As referred above, an I_{FM} value close to 100 means that the model describes well the complexation reaction. Values in Table 2 show that I_{FM} is around 100 for the experiments at pH = 6 and 7 but higher for lower pH values, particularly for pH = 4 (around 150). This

result, together with the lower $\log K_c$ for lower pH values, suggests that under the experimental conditions used, at the lower pH values, the binding sites are not completely complexed and/or the complexation scheme is more complicated than assumed. At these pH, competition between the proton and the metal ion for the ligand binding sites is likely, which provokes weaker complexation and prevents full saturation of the binding sites at the end of the titration.

As shown in Figure 3, the conditional stability constants calculated for the two fua samples show a similar trend with pH, *i.e.*, they increase from pH = 4 up to pH = 6 but

TABLE 2

Equilibrium Parameters for the Complexation of Be(II) with Fua^a

pH	N	log K _c	C _L	I _{FM}	N _p	ΔE	SSR	ΔBe
Gran								
4	2	4.72 (2)	0.016 (1)	143 (8)	10	0.43	2.93	0.004–0.03
5	3	5.53 (5)	0.047 (7)	110 (10)	12	0.65	8.26	0.01–0.05
6	3	5.97 (9)	0.030 (1)	103 (1)	12	0.41	3.63	0.004–0.05
7	4	5.59 (14)	0.026 (1)	105 (2)	13	0.43	3.39	0.003–0.05
Amph								
4	3	4.70 (10)	0.016 (3)	168 (14)	11	0.30	1.67	0.008–0.04
5	4	5.13 (3)	0.037 (4)	123 (4)	15	0.42	4.27	0.008–0.05
6	3	6.48 (6)	0.037 (3)	96 (1)	12	0.60	4.08	0.004–0.04
7	3	5.92 (2)	0.022 (2)	105 (2)	12	0.78	11.8	0.001–0.05

^a pH, average pH with 0.03 standard deviation; N, number of experiments; log K_c, logarithm of the conditional stability constant; C_L, total concentration of the ligand (mM); I_{FM}, fluorescence intensity value at the end of the titration; N_p, number of points used in calculations; ΔE, average deviation of the estimates; SSR, sum of squares of residuals; ΔBe, Be(II) concentration interval used in calculations (mM). Average, with average deviations in parentheses, of N independent experiments for log K_c, C_L, and I_{FM}, and typical values for the other parameters.

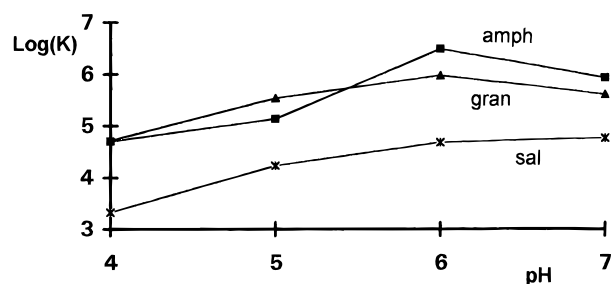


FIGURE 3. Experimental (amph and gran samples) and calculated (salicylic acid, sal) log K_c vs pH.

TABLE 3

Major Chemical Species and Corresponding Logarithm of Stoichiometric Stability Constants, log K (Average Values from Literature), in Mixtures of μM Concentrations of Salicylic Acid and Be(II) in the Range of pH from 4 to 7

species	log K	ref
[Be(OH)] ⁺	−5.40	1, 21
[Be ₂ (OH)] ³⁺	−3.235	1, 21
[Be ₃ (OH) ₃] ³⁺	−8.72	1, 21
[Be(salicylate)]	12.48	1, 21
[salicylateH] [−]	13.1	22
salicylateH ₂	16.02	22

decrease slightly for pH = 7. Figure 3 includes the conditional log K_c for salicylic acid over the pH range of 4–7 calculated from the equilibrium constants in Table 3. The calculated log K_c values were 3.33, 4.23, 4.68, and 4.76 for pH = 4–7, respectively. Salicylic acid was selected for comparison purposes because it has two properties similar to those observed experimentally for the binding sites of fua: (i) it forms fluorescent complexes with Be(II) (7); (ii) the complexes are stable in the acid pH range (4–7). Although the log K_c values for salicylic acid are around 1.5 units lower than for fua, the trend is similar. The common trend is probably a result both of the acid–base properties of the ligand structures and of the Be(II) hydrolysis. The calculated conditional stability constants of the complex of fua with Be(II) are larger than for the fua/Cu(II) system (10, 11).

The calculated C_L for both fua samples are smaller at pH = 4 than at higher pH values. This result is expected because for lower pH some of the binding sites are protonated and unavailable for participating in the complexation reactions.

For higher pH values, smaller variations were found in C_L. The mean value for the two fua samples is around 0.033 mM and corresponds to a concentration of 0.4 mmol/g of fua. This constancy of the C_L parameter for a pH range where the fua binding sites are almost completely deprotonated, particularly noticed for the amph fua sample, is plausible. Indeed, as a similar set of Be(II) hydrolysis products occurs in the pH window between 5 and 7, the hydrolysis probably will not affect markedly the C_L value (15).

These results show that Be(II) associates strongly with soil fua forming soluble complexes in the absence of other potential complexing agents [particularly fluoride (23)] or adsorbents (for example, humic acids). The relative significance of this association when compared to adsorption by inorganic or organic solid phases in soils, however, cannot be estimated because no information on adsorption is available yet. Further research about the overall effect of these agents on the Be(II) speciation is needed to obtain more rigorous estimations about its environmental fate.

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