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Study of metal–fulvic acid interactions by capillary electrophoresis

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Abstract

Capillary electrophoresis (CE) has been used to study metal–humic substance (HS) interactions. The work was carried out with an aquatic fulvic acid (FA) as a representative HS. Two approaches were attempted for the complexation study. In the first approach, the metal complexation capability of FA by using CE for the determination of free metal cations (Sr^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+} and Al^{3+}), was investigated. The second approach examined the electrophoretic behavior of the FA and its complexes with Hg^{2+} and Fe^{3+}. Based on the obtained results, CE appears to be a promising tool for the characterization of metal–HS interactions.

Keywords: Complex formation; Environmental analysis; Metal ions; Fulvic acids; Humic substances

1. Introduction

Humic substances (HS), relatively stable (one hundred to several thousand years) decomposition products of biological origin [1], play an important role in the fate and transport of toxic chemicals and in nutrient cycling throughout the environment [2]. They are chemically heterogeneous polyelectrolytes with molecular masses ranging from a few hundred to several hundred thousand [3]. Depending on their solubility in water, HS can be divided into three fractions; fulvic acid (FA), humic acid (HA) and humin. Fulvic acid is soluble in water at all pH values, humic acid at pH values over 2 and humin is not soluble in water at all [3]. Humic substances possess a variety of dissociable functional groups, such as carboxylic, phenolic-alcoholic and enolic-OH (polyfunctionality), that are capable of forming many types of potential metal binding sites [1].

The complexation of metal ions to humic substances is of great interest in the understanding of metal ion transport, toxicity and bioavailability of the metal ions [4]. In order to evaluate the importance of metal–HS interactions, various parameters such as the molecular nature of dissolved organic compounds and the characteristics of metal–organic interactions have to be investigated. Numerous studies about metal interactions with HS, applying many different analytical methods, including potentiometry [5], anodic stripping voltammetry (ASV) [6,7], fluorescence techniques [8,9], nuclear magnetic resonance spectroscopy (NMR) [10], electron paramagnetic resonance spectroscopy (EPR) [11], luminescence spectroscopy [12] and ion-selective electrodes (ISE) [13], have been reported. Furthermore, different separation techniques such as ion chromatography, high-performance liquid chromatography (HPLC), size-exclusion chromatography and ultrafiltration, in combination with various detection methods were applied [14–22]. Although the separation techniques employed to characterize humic substances and their metal ion complexes, hitherto, have failed to separate

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these complex mixtures into smaller identifiable fractions, the partial separation that has been achieved, contributes significantly in understanding the nature and properties of HS.

In the last ten years, capillary electrophoresis (CE) has been shown to be a fast, powerful and efficient separation technique for a variety of compounds [23]. Its high separation potential covers a broad field of applications and supplements other separation methods like HPLC [24]. These benefits make CE techniques valuable also for metal speciation investigations with organics like tanning agents and proteins [25–28]. Applications of CE for the characterization of humic substances has also received attention [29–31]. Kopáček et al. [29] examined HS using capillary isotachophoresis (ITP), while Rigol et al. [30] and Garrison et al. [31] investigated the potential of capillary zone electrophoresis (CZE) for the study of HS. However, to our knowledge, no work has been reported where CE has been applied to study the complexation between metal ions and HS.

In this paper, the results from an initial investigation of the usefulness of CE as a new tool to study the interaction between metal ions and HS are presented. An aquatic fulvic acid (FA) was chosen as a representative HS. Two approaches were undertaken, in the first the complexation capability of FA, by using CE for the determination of free metal cations (Sr^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+} and Al^{3+}) was studied. The second approach considered the investigation of migration behavior of FA in its free ionic form and the complexed forms (with Fe^{3+} and Hg^{2+}).

2. Experimental

2.1. Instrumentation

All experiments were performed on a Beckman P/ACE 5510 instrument (Fullerton, CA, USA) equipped with a diode array detector, an automated sample changer, a liquid thermostated capillary cartridge and a personal computer. The Beckman Gold System (version 8.10) software was used for instrument control and for data collection and processing. Three-dimensional spectral view of elec-

tropherograms was produced using the Beckman ArrayView software. Uncoated fused-silica capillaries of 57 cm in total length (50 cm to the detector)×75 μm I.D.×375 μm O.D. from Polymicro Technologies (Phoenix, AZ, USA) were used. Approximately 0.5 cm of the polyimide coating was burnt off from the capillary to make a transparent window for the detector cell. A positive power supply (anodic injection/cathodic detection) of 20 kV was used and all injections were achieved by applying 0.5 p.s.i. pressure (1 p.s.i.=6894.76 Pa) for 20 s, unless otherwise noted.

A Beckman Φ32 pH meter in conjunction with a Beckman combination electrode was used for all pH measurements. Glass vials (5 ml) and 200 μl polypropylene microvials were used for electrolytes and samples, respectively.

2.2. Reagents

All chemicals were purchased from either Aldrich (Milwaukee, WI, USA) or Fisher Scientific (Ottawa, Canada) in the highest purity available and were used without further purification. Deionized water (>18 MΩ×cm resistance) obtained by treating tap water using reverse osmosis and ion-exchange (Millipore, Model RO 20 and Model SuperQ, Millipore, Mississauga, Canada) was used in preparing all solutions, standards and electrolytes. Individual standard stock solutions (10^{-2} M) of strontium and aluminum were prepared from nitrate salts from Baker (Phillipsburg, NJ, USA); the lead standard was prepared from lead chloride from Fisher Scientific (Ottawa, Canada), and the other cation standard solutions were prepared from National Institute of Standards and Technology standards (NIST, Gaithersburg, MD, USA). All standard solutions were stored in polyethylene bottles. Diluted working standard solutions were prepared daily.

The Rådsla fulvic acid (FA) was isolated from a creek in a coniferous forest in Sweden. The isolation procedure was carried out according to Pettersson et al. [32]. The weight-average molecular weight (M_w) and number-average molecular weight (M_n) of the FA are 2520 and 1580, respectively and the acid capacity is 4.60 mequiv. g^{-1}. The molecular masses were determined by a gel filtration procedure using an HPLC system [33] and the acid capacity was

determined by potentiometric titrations [34]. The FA was dissolved in deionized water to produce a 1000 mg l^{-1} stock solution. This stock solution was stored in a polyethylene bottle at 4°C.

2.3. Procedures

All electrolyte compositions and operating conditions used in this study are presented in Table 1. An electrolyte containing imidazole (E1) as a UV absorbing carrier electrolyte [35] was used for the determination of metal cations with indirect UV detection. An electrolyte (E2) with a strong complexant, ($\pm$)-trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA) [36], that forms UV-absorbing negative metal complexes, was applied for the determination of the studied cations with direct UV detection. For separation and direct UV detection of fulvic acid and its metal complexes an aqueous–isopropanol borate electrolyte (E3) was employed.

Each day before starting analysis or between the use of different electrolytes, the capillary was rinsed with 1.0 M and 0.1 M NaOH, then rinsed with deionized water and the used electrolyte for 15 min each. Between each run, the capillary was flushed with water (2 min), followed by flushing with the running electrolyte (2 min). The electrolytes were prepared fresh daily and before use they were filtered through a 0.45 μm syringe filter and degassed in an ultrasonic bath.

2.4. Preparation and analysis of sample mixtures

In the investigation of the complexing capability of FA at different pH values, the series of sample solutions were prepared by mixing $5 \cdot 10^{-5}$ M single metal ion and 10 mg l^{-1} FA, which gives a [FA]/[Me] ratio of 0.92 (based on the acid capacity of the FA). Polyethylene bottles were used to avoid adsorption of metal cations onto container walls. The samples were then adjusted to different pH values with NaOH and shaken for approximately 24 h in ambient temperature. Next, the pH was checked prior to the CE determination using indirect (electrolyte E1) and direct (electrolyte E2) UV detection under conditions shown in Table 1. The analysis of metal ions by the two different detection modes were performed the same day. Samples with pH>6 were not analyzed using the E1 electrolyte due to poor resolution of the investigated metal ions and sodium (from NaOH used for the pH adjustment). Mercury was only analyzed using the E2 electrolyte as there were problems with detection and separation when using electrolyte E1 and indirect UV detection. The sample solutions were also analyzed after several months aging (stored at 4°C). Quantitation of the individual metal ions was based on calibration runs. Three aqueous standards were employed to fit a calibration curve, using the linear regression analysis of the corrected peak area. Correlation coefficients of obtained curves were better than 0.994. The concentration of metal ion complexed to FA was calculated from the difference between the total and

Table 1
Experimental conditions for CE analysis

Conditions	Electrolyte E1	Electrolyte E2	Electrolyte E3
Electrolyte composition	20 mM H$_3$BO$_3$, 5 mM imidazole, pH 4.5 (adjusted with acetic acid)	20 mM Na$_2$B$_4$O$_7$, 5 mM CDTA[a], pH 8.6	10 mM Na$_2$B$_4$O$_7$, 10% isopropanol, pH 9.0
Detection	Indirect, 200 nm	Direct, 200 nm	Direct, scanned 200–400 nm, a scan rate of 4 Hz (step with 4 nm)
Polarity[b]	(+)	(+)	(-)
Voltage	20 kV	20 kV	20 kV
Current	10 μA	50 μA	25 μA
Injection mode	pressure, 30 s	pressure, 20 s	pressure, 20 s
Temperature	25°C	25°C	30°C

[a] ($\pm$)-trans-1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid.
[b] (+) indicates anodic injection/cathodic detection.

the determined free metal ion concentration. All results reported are means of two CE replicates, which were reproducible within 5%.

For the study of migration behavior of fulvic acid and its complexes with Fe^{3+} and Hg^{2+}, FA (100 mg l^{-1}) in 0.1 M NaOH, in absence and presence of the individual ions (10^{-5} M), were prepared. After approximately 24 h shaking in an ambient temperature, and pH measurement (pH 12), CE experiments were performed using electrolyte E3 (Table 1).

3. Results and discussion

3.1. Complexation capability study

The initial investigations of the usefulness of CE for the characterization of metal–HS interactions were focused toward the study of the complexing capability of fulvic acid. The following cations; Sr^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+} and Al^{3+}, were used. The formation of metal–FA complexes (M–FA) was examined considering a complexation scheme between FA and metal ions (M) represented as (charges have been omitted for simplicity):

$$M + FA \rightleftharpoons M - FA \qquad (1)$$

Capillary electrophoresis using two modes of detection was applied for the analysis of free metal ions in the presence of FA. The determination was based on the assumption that the used CE methods analyzed the uncomplexed metal cations and as a result the amount of metal ion bound with FA in each solution could be calculated. Two representative electropherograms are shown in Fig. 1.

The influence of pH on the complexation of metal ions with 10 mg l^{-1} FA is shown in Fig. 2. The results obtained from CE measurements with direct detection (Fig. 2b) yield a lower complexation compared to that achieved with indirect detection (Fig. 2a). This discrepancy may be explained by the possibility of the additional (to the reaction M + CDTA$\rightleftharpoons$M–CDTA), competitive reaction (2) occurring during the CE run:

$$M - FA + CDTA \rightleftharpoons M - CDTA + FA \qquad (2)$$
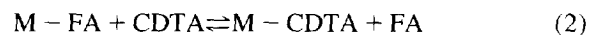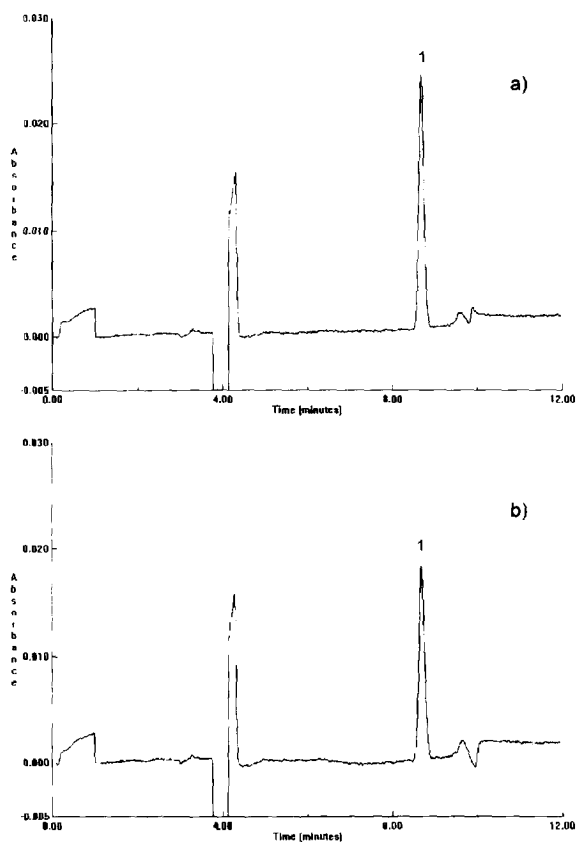
However, as can be seen from Fig. 2, the same order

Fig. 1. Electropherograms of $1 = Pb^{2+}$ solution, $5 \cdot 10^{-5}$ M (a) without and (b) with 10 mg l^{-1} FA at pH 3.2, obtained with electrolyte E2 and under conditions shown in Table 1. See Section 2 for more details.

of complexation for the investigated metals was obtained by both CE methods ($Al^{3+} > Hg^{2+} > Cu^{2+} > Pb^{2+} > Sr^{2+}$). These results are in agreement with what have been generally stated for M–HS interactions; that at any given pH and ionic strength, trivalent cations are stronger complexed than divalent cations. Additionally, that divalent cations which form strong coordination complexes, e.g., Cu^{2+}, complex to a greater extent than weakly coordinated ones, e.g., Ca^{2+} and Mg^{2+} [1]. Although the complexation sequence is in accordance with reported studies, the obtained results for copper probably gives erroneously low estimates of complexation capacity. This underestimation suggests that the lability of Cu^{2+}–FA complexes is an important contribution to this discrepancy.
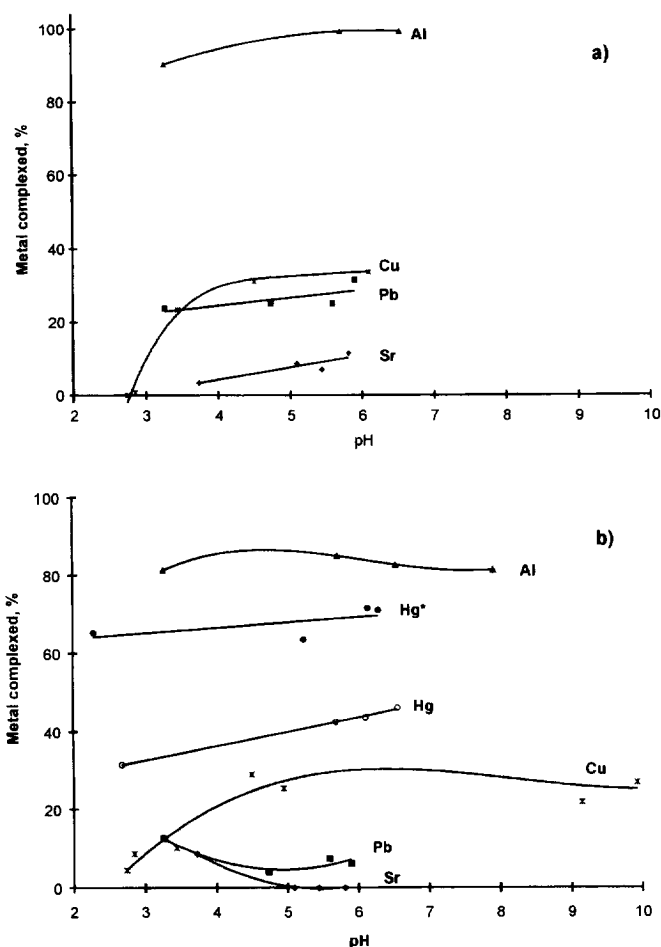
Fig. 2. Effect of pH on Sr^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+} and Al^{3+} complexation by FA (a) obtained by CE with indirect UV detection (electrolyte E1), (b) CE with direct UV detection (electrolyte E2). Total metal concentration: $5 \cdot 10^{-5}$ M, FA concentration: 10 mg l^{-1}. Electrolyte compositions and CE operating conditions are shown in Table 1. * Results obtained after an aging time of 5 months.

Generally, the observed pH dependence of the metal–FA binding shows an increase in the stability of the complexes with increasing pH, which agrees with previous reports [37]. The present study shows that aluminum forms strong complexes with FA in a pH interval from 3 to 8 (Fig. 2). According to Browne et al. [38], FA can combine with and/or facilitate the formation of soluble polynuclear hydroxy-Al ions from pH 5.5. Copper shows a pH dependence between pH 3 and 6, and after that no increase in complexation with increasing pH is observed (Fig. 2b). A similar behavior has been noticed by Ryan and Weber [39]. The complexation of Cu^{2+} with a soil FA showed no significant

increase in complexation strength with an increase in pH from 5 to 7.

The influence of aging, prolonged contact time, on the metal–FA interactions was also investigated. An increase of more than 25%, in the amount of Hg^{2+} bound to FA had occurred after five months (Fig. 2b). The increase in complexation strength over the studied period might be connected to the possible time dependence of the reduction process [1,40], and/or could be related to the macromolecule nature of FA. It has been suggested, that metal ions brought into contact with HS first occupy binding sites in the outersphere of HS macromolecules and that less accessible binding sites in the innersphere of the

macromolecules are reached later [21]. For the Hg–FA mixture studied, a longer contact time than 24 h might be needed. The other metals were also analyzed after 5 months (apart from Cu that was analyzed after 3 months), but did not show the same obvious increase in complexation with time.

3.2. Separation of FA and its mercury and iron complexes

The migration behavior of the FA in the free ionic form and its complexed forms, with Fe^{3+} and Hg^{2+}, was investigated using an aqueous–isopropanol borate electrolyte (E3) and the electrophoretic conditions shown in Table 1. The FA studied was regarded as anionic in a basic solution (pH 12) owing to the dissociation of carboxylic and other functional groups. The high pH of the analyzed samples used in this approach is also justified, because it enhances the UV absorption of HS [41]. Although the FA does absorb UV light at much lower pH values, it is likely that the combination of a short light-path in the capillary and a low pH gives no detectable absorption. Under the applied CE conditions, the migration of the anionic FA and metal–FA mixtures, from the anode to the cathode, is a net result of electroosmotic and electrophoretic movement. This implies that the electrophoretic mobility of the negatively charged FA species was smaller than that of the electroosmotic flow of the solution.

Three characteristic electropherograms are presented in Fig. 3A–C. Because of the extreme complexity of FA, only a partial separation between lower and higher molecular mass fractions was obtained. Under the applied conditions, the signal for hydroxide (from NaOH) appears at a migration time (t_m) of 4.5 min. The analysis of 100 mg l^{-1} FA yielded four peaks, of which one is poorly separated ($t_m = 7.2$ min) and one ($t_m = 7.8$ min) has a much higher intensity than the other peaks (Fig. 3A). As can be seen from Fig. 4a, the peak intensity is gradually decreasing with increasing wavelength from 200 to 400 nm. This UV profile, with little structure, is typical for HS [41]. Because of the large difference in absorption intensity between the peak

with the highest intensity and the other peaks only this one peak is discernible. From the appearance of the electropherogram (Fig. 3A), it seems that there are four sharper peaks at the beginning of a broad peak. This broad peak could be attributed to the adsorption of FA to the capillary walls. Additional experiments with coated capillaries and/or using different organic solvents and concentrations of these solvents, may decrease the possible wall adsorption and increase the separation efficiency.

There is a clear difference in absorption and migration behavior between FA and its mixtures with the metal ions (Fig. 3 and Fig. 4). Fig. 3B shows that the four peaks, seen for the FA, as well can be recognized when analyzing the Hg^{2+}–FA mixture. In addition, the last peak ($t_m = 9$ min) has a much higher intensity than the others. However, these peaks are generally broader and their migration times are longer (higher electrophoretic mobility) than observed for the FA.

The analysis of the Fe^{3+}–FA mixture gives yet another absorption pattern with three distinct peaks (Fig. 3C). The intensity of these peaks are much higher and the peaks migrate closer to each other with shorter migration times compared to the FA. This could indicate that the Fe–FA complexes formed are less negative than the FA and absorb strongly in the UV region. It is well known that Fe^{3+} forms intensively absorbing complexes with polyhydroxy compounds in the UV and Vis region [27]. The difference in absorption behavior between FA and the Fe^{3+}–FA mixture can also be seen in Fig. 4a and b where three-dimensional spectral views of the CE separations are shown. The same UV profile of decreasing absorption with increasing wavelength that is seen for FA, is observed for all three peaks with the Fe^{3+}–FA mixture.

The results described above suggest that the presence of metal ions changes the net charge of the FA and consequently its electrophoretic properties. Similar indications of metal complexation have been noticed with proteins [28]. In addition, the total peak area of the M–FA mixtures was considerably larger than for the FA. This suggests that the molar absorptivity coefficient of the M–FA mixtures was larger than that of the FA due to the formation of the M–FA complexes.
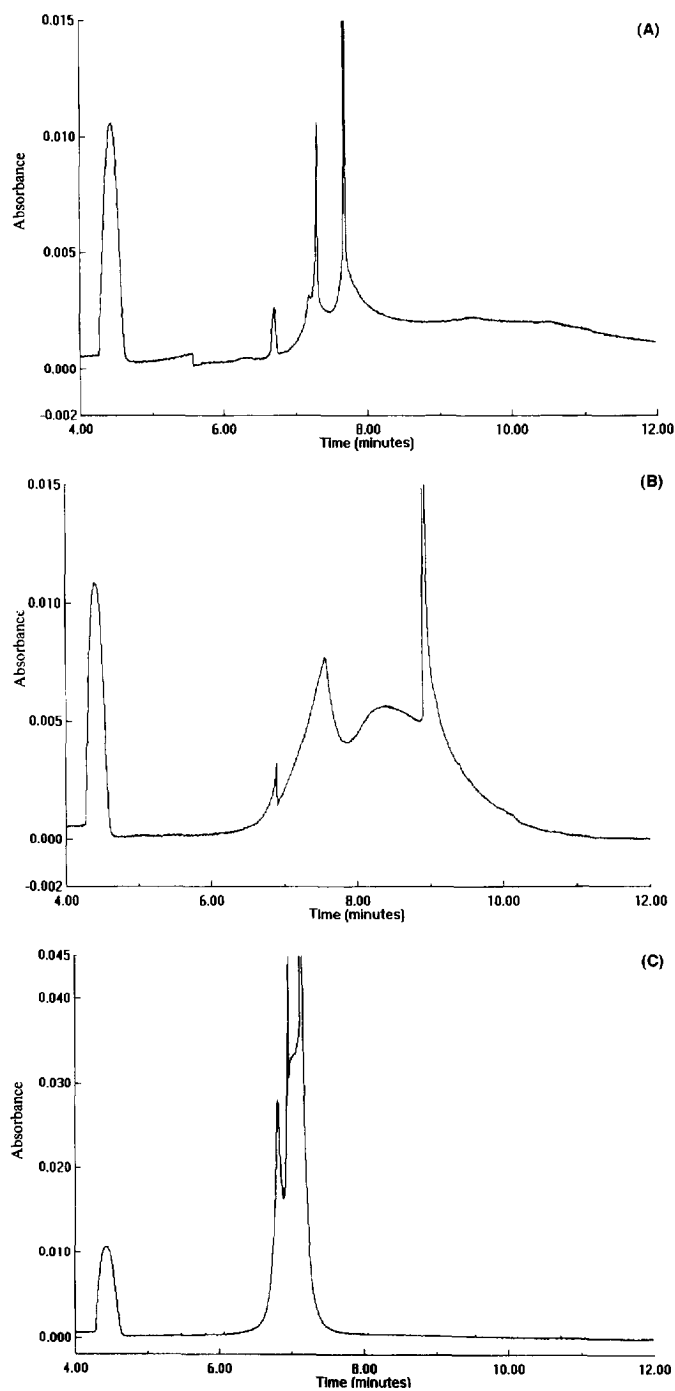
Fig. 3. Electropherograms of (A) FA, (B) Hg^{2+}–FA and (C) Fe^{3+}–FA mixtures using CE with electrolyte E3 and under conditions shown in Table 1. Total metal concentration: 10^{-5} M, FA concentration: 100 mg 1^{-1}, pH 12.
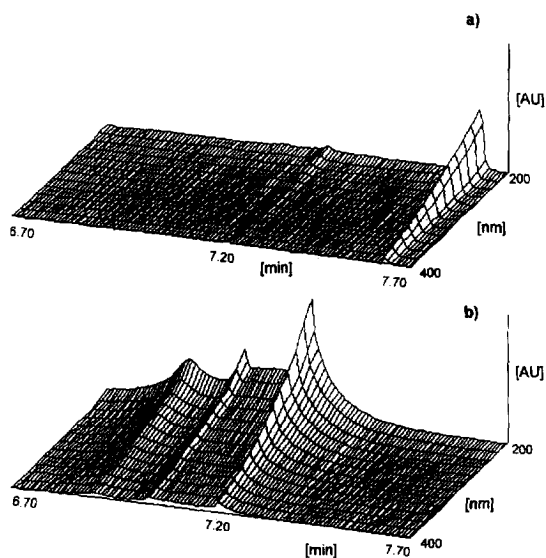
Fig. 4. Three-dimensional spectral view of CE separation of (a) FA and (b) Fe^{3+}–FA mixtures. Separation conditions and other parameters are the same as in Fig. 3.

4. Conclusions

From the results reported in this work CE appears to be a promising technique for studying the interactions between metal ions and humic substances. The obtained order of M–FA complexation; $Al^{3+} > Hg^{2+} > Cu^{2+} > Pb^{2+} > Sr^{2+}$, agrees with that reported using other methods [1,37]. The obtained low FA complexation capacity for copper suggests that the lability of the Cu–FA complexes makes an important contribution to this discrepancy. These preliminary results also suggest that CE can provide data for calculation of conditional stability constants for M–HS complexes, that could be used in speciation modeling.

CE offers the possibility to distinguish between higher and lower molecular mass compounds in HS. More research is necessary to obtain better separation of the FA fractions and explain the difference in absorption behavior between the FA and M–FA complexes. Application of ultrafiltration prior to the CE separation and/or the coupling of CE with mass spectrometry might provide better characterization of these complex metal–HS compounds. In addition, future studies could include FA and HA of different

origin, to investigate the feasibility of using CE for "fingerprinting" of HS.

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