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Short communication

Interactions of Pb^{2+} with fulvic acid by electrophoretically mediated on-capillary microanalysis

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Abstract

Electrophoretically mediated microanalysis (EMMA) was used to monitor the on-column complexation of Pb^{2+} and fulvic acid (FA). Electropherograms revealed several characteristic regions, the areas of which correlate with the metal concentration. The analysis of the electropherograms suggests that at least two different complexes are formed. Therefore, the EMMA is a prospective technique for structural investigation of humic substances (HS).

Keywords: Capillary electrophoresis; Electrophoretically mediated microanalysis; Fulvic acid; Interaction of Pb^{2+} with humic substances

1. Introduction

Humic substances (HS) constitute an important reservoir of organic carbon in aquatic and terrestrial ecosystems. The structure and character of HS has only been roughly defined. HS have a great potential for reactivity with many natural and anthropogenic chemicals [1]. The mobility and transport of metal ions in the environment are strongly influenced by their complexation with HS [2–4]. The migration behaviour of metallic elements in natural ecosystems is of particular importance because of severe problems of environmental pollution [5].

Depending on their solubility in acidic media, HS can be divided into fractions. It is well known that one of those fractions, fulvic acid (FA), has a greater affinity towards metal ions and is capable of forming stable complexes. Of the variety of metal ions, Pb^{2+} forms quite stable complexes with FA in the neutral pH range [6,7].

During recent years, capillary electrophoresis (CE) has been recognized as a useful method in the area of metal analysis [1,8–17]. Two different approaches are used: on-column complexation, where a soluble ligand is present in the running electrolyte and weak complexes are rapidly formed [9,10,15,16], and pre-column complexation, where a strong ligand is added to the sample to form complexes before CE analyses [18,19].

In recent years, a new general method – electrophoretically mediated microanalysis (EMMA) – where a chemical on-column reaction with a simultaneous electrophoretic separation of the products, has been developed. This method has been used to study many different objects [20–23]. Exhaustive reviews on the EMMA method have recently been published [24,25].

In this work, we make an attempt to apply the EMMA approach in order to explore the complexation of Pb^{2+} with FA and to understand the potential of the method for HS studies.

2. Experimental

2.1. Materials

Analytical grade reagent $Pb(NO_3)_2$ was purchased from Aldrich, NaOH from BDH (UK) and HCl from Riedel de

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Haën, Germany. HS were extracted from the sediment of the Baltic Sea (Haapsalu Bay, Northwest of Estonia). Experimental procedures are described in a previous paper by Übner et al. [26]. FA solution was stored at 4 °C. FA was employed as a background electrolyte (BGE) component. FA solutions were prepared daily by dissolving an appropriate amount of the FA solution (depending on its concentration) in Milli-Q water. The electrolyte was filtered through a Millipore 0.45 μm membrane filter. The concentration of FA in BGE was 0.1 mg/ml, and the pH was adjusted with 0.2 M NaOH solution to pH = 7 (Na-salt form). Standard metal ion solution was diluted from the 0.01 M $Pb(NO_3)_2$ stock solution, using Milli-Q water. Pb^{2+} solutions were prepared in the range 1×10^{-4} to 6×10^{-4} M.

2.2. Equipment and procedures

All the CE experiments were performed with an ISCO CV^4 Capillary Electropherograph model 3850, which was thermostated with forced air by a fan. The bare fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) had a total length of 80 cm (48 cm to the detector). A positive power supply (anodic injection/cathodic detection) of 20 kV was used. The capillary (75 μm i.d.) was rinsed with 1.0 M NaOH and Milli-Q water (Millipore) for 5 min and with a carrier electrolyte for 15 min at the beginning of each day. Between all electrophoretic separations, the capillary was rinsed for 5 min with 0.1 mM NaOH, 0.1 mM HCl, Milli-Q water, and BGE (FA solution). Experiments were performed at 20 ± 1 °C. The UV absorbance signal of the solution was recorded at 226 nm. The UV detector signal was digitized by the "Mini-16" analogue to the digital converter (Keithley, Mertabyte, Taunton, MA, USA) and stored on Pentium PC hard disc for later processing, using the procedures written in-house in Matlab (MathWorks, Natick, MA).

The plug of metal solution was hydrodynamically injected (during 15 s) into the capillary filled with the FA electrolyte, followed by the application of high voltage (20 kV). During all experiments, the current values were in the range of 1–3 μA. The velocity of the electroosmotic flow (EOF) was estimated by the use of nitromethane as a marker compound. The calculated EOF mobility was equal to $(8.53 \pm 0.28) \times 10^{-4}$ cm^2 V^{-1} s^{-1}. All experiments were performed in triplicate.

3. Results and discussion

The introduction of $Pb(NO_3)_2$ to the FA column resulted in similar shape, reproducible electropherograms at different metal ion concentrations (Fig. 1). The blank FA solution (without Pb^{2+}) as a BGE revealed a stable steady baseline. Peaks appeared only when Pb^{2+} solution was injected.

Based on characteristic features, an electropherogram can be divided into three main regions: region 1—consisting of a wide symmetrical peak; region 2—a negative peak (vacancy); and region 3—a triangular-shape peak.

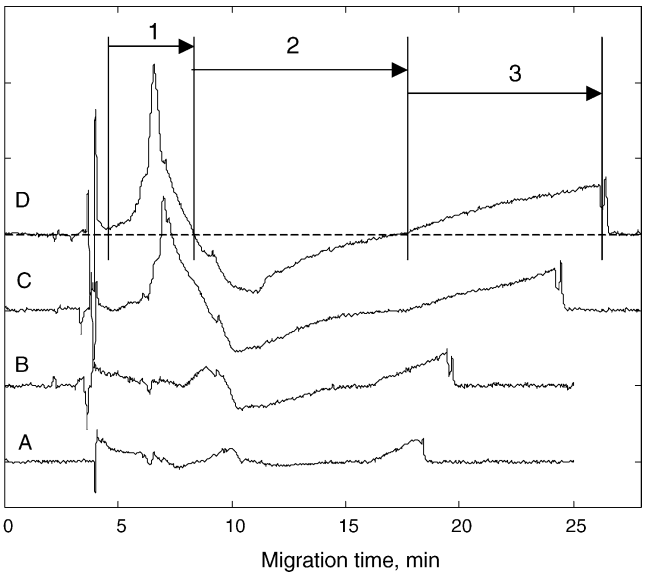

Fig. 1. Electropherograms with Na-fulvate as BGE at different concentrations of Pb^{2+}: (A) 1×10^{-4} M; (B) 2×10^{-4} M; (C) 4×10^{-4} M; (D) 6×10^{-4} M. Separation voltage, 20 kV; hydrodynamic injection, 15 s; detection at 226 nm; fused-silica capillary 80 cm × 75 μm.

The hypothetical model of the processes occurring in the column filled with the FA solution after the hydrodynamic injection of the zone of Pb^{2+} solution may be described as follows. As in a traditional EMMA process, the capillary is filled with the BGE solution – Na-fulvate – and the reactant (Pb^{2+}) is introduced into the capillary inlet as a separate band at the anodic end of the capillary (Fig. 2A).

Upon application of an electric field, the two bands merge due to the differences in their electrophoretic mobilities. The positively charged Pb^{2+} ions having a positive electrophoretic mobility move rapidly toward the cathode (−) through the FA solution. The migration velocity of Pb^{2+}-ions and FA-anions is opposite under the selected conditions. The two zones mix, and the complexation reaction occurs. Simultaneously, resultant products migrate away from the reaction zone and separate because the electrophoretic mobilities of the products

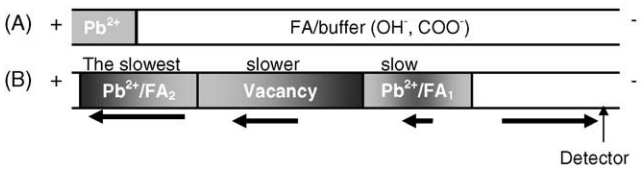

Fig. 2. On-column reaction of Pb^{2+} and FA. (A) The capillary is filled with Na-fulvate (pH = 7) solution and metal solution is injected; (B) electrophoretic separation of the ions and migration of the products towards the detector after mixing of the analyte zone, the FA solution zone. The shading of Pb^{2+}/FA zones approximately expresses the concentration of complexes along the capillary. Arrows represent the value and direction of migration velocities of different reaction zones.

differ from the mobility of FA anions (Fig. 2B). Zones of complexes moving at different velocities, result in a complex electropherogram signal. Thus, a detector can individually determine the relative amounts of different products [20].

Two humps (regions 1 and 3) and a vacancy (region 2) indicate that with the Pb^{2+} ions, FA can form two different types of complexes, which have an overall negative charge. Due to the complexation of the molecules of free FA, their concentration drops in the reaction zone (region 2). This zone also moves along the capillary towards the anode as a vacancy hump on the detector signal. The bulk EOF flow of the buffer is stronger than the movement of the complexes and it carries complexes and vacancy zones towards the cathode. The detector is placed near the cathodic end of the capillary and the recorded electropherogram appears in time as a mirror image of the real process.

The hump at region 1 is probably due to the fastest (i.e. the slowest in the coordinate system, which moves with EOF) migrating complex. The total migration time of the hump in region 1 decreases when the Pb^{2+} concentration increases, suggesting that the complex had been able to bind more metal ions and had become more positive. Thus, its overall velocity towards the cathode increases. The symmetrical form of the complex peak suggests that the complex formation process is reversible.

Fig. 3 represents the areas of regions 1 and 2 as a function of injected metal concentration. Peak areas have linear correlation with the Pb^{2+} concentrations ($R^2 = 0.9319$ and 0.9585, respectively). Region 2 expresses the range of negative optical density compared to the FA-buffer solution at the rest of the capillary and its amplitude is related to that of region 1, which supports the suggestion presented above that removing of FA anions from the reaction zone forms the vacancy. Thus, the vacancy zone should migrate at the speed of the free FA anion.

Region 3 characterizes the migration of the slowest (i.e. the fastest in the coordinate system, which moves with EOF) complex. In fact, it should be the complex with a higher charge to mass ratio than the free FA, because its movement consists of two components: one migrates to the anode and the other one moves to the opposite direction due to the bulk of EOF.

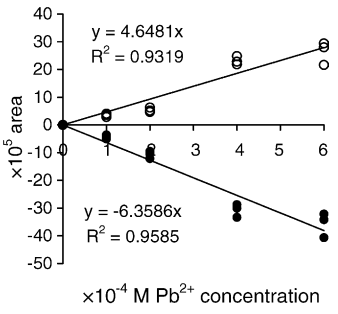

Fig. 3. Dependence of the area of regions 1 (open rings) and 2 (filled rings) on the concentration of inserted Pb^{2+} ions.

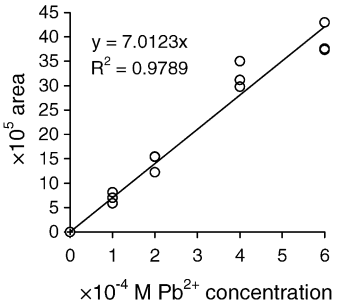

Fig. 4. Dependence of the area of region 3 on the concentration of inserted Pb^{2+}-ions.

The area shows almost a linear correlation ($R^2 = 0.9789$) with metal concentration (Fig. 4). The triangular form of the hump suggests that the complexation in this case is a first order irreversible process. Metal ion concentration moving through the FA solution decreases because ions are consumed in the reaction and thus they decrease the amount of the formed complexes.

It is known that the presence of metal cations increases intramolecular bonding and folding of macromolecules [1]. According to Sekaly et al. [27], two different complexing sites exist in HS: the strong and the weak binding sites. Weak binding sites include carboxylate and phenolate functional groups, and strong binding sites (N- and S-containing groups) form strong complexes with metals. As the metal concentration is increased, the strong binding sites become saturated and the excess metal binds to the weaker sites, forming weak complexes, which are labile. Other authors also suggest the formation of two kind of complexes [28,29]. The reactions of HS with metal ions were found to occur in two pathways: in the main pathway, both carboxyl and phenolic hydroxyl groups participate simultaneously and in the minor one, only carboxyl groups are involved [30]. The results that were obtained indicate that the complexation with weak and strong binding sites that migrate with different speed occurs simultaneously.

4. Conclusions

EMMA on-column complexation offers a chance to follow the chemical reactions of oppositely charged Pb^{2+} and FA ions inside the capillary. The method enables characterization of the HS fractions. From those electropherograms, we can conclude that Pb^{2+} cation is related to the formation of at least two different FA complexes, each with a different mobility. The concentration of Pb^{2+} is related to the electropherograms areas of regions 1–3. An almost linear dependence of the peak area of region 3 on the Pb^{2+} concentration was obtained. We can assume that electropherograms that were obtained reflect the complexing nature of FA components. The approach may be applied to study the interaction of HS with different compounds and metal ions and to analyze the structure of HA.

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