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Short communication

## Capillary electrophoresis for a “finger-print” characterization of fulvic and humic acids

Susanne Pompe, Karl-Heinz Heise, Heino Nitsche\*

*Forschungszentrum Rossendorf e.V., Institute of Radiochemistry, P.O. Box 510119, 01314 Dresden, Germany*

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### Abstract

Capillary zone electrophoresis is suitable for characterizing fulvic and humic acids of different origins and for developing a “finger-print” catalogue that is based on the functionality of these acids. Several fulvic and humic acids of different origins were investigated by capillary zone electrophoresis. The goal of this investigation was to assign their different functional properties to their origin based on their individual charge-to-size ratios. The electropherograms of the humic and fulvic acids show individual patterns. The substances show differences in their migration behavior and in their UV absorption. These characteristics are due to different charge-to-size ratios and various structural properties of the individual components of humic and fulvic acids caused by their different origin.

*Keywords:* Capillary electrophoresis; Finger-print catalogue; Fulvic acid; Humic acid; Humic substances

### 1. Introduction

Fulvic and humic acids are polyelectrolytic biopolymers. They are the alkaline-soluble part of natural humic substances and play a key role in the migration and retardation of heavy metals in the environment. Depending on their origin and the natural conditions prevailing during their formation, fulvic and humic acids have different structural, physical and chemical properties.

The objective of our work is the characterization of humic and fulvic acids of different origins by means of capillary electrophoresis. Electro-

phoretic techniques are suitable for the separation and characterization of these substances because of their polyelectrolytic properties [1–4]. Capillary zone electrophoresis is characterized by short analysis times, selectivity to the analytes and high resolution. The separation in uncoated capillaries is the result of the combination of an electroosmotic flow that is produced by an applied voltage and the fractionation by different charge-to-size ratios.

The functionality of humic substances depends on their origin and is characterized by their charge and size. This can be observed in the electropherograms and gives the possibility for developing a “finger-print” catalogue for humic substances, given that the electropherograms are clearly distinguishable from each other.

\* Corresponding author.

## 2. Experimental

### 2.1. Apparatus

The investigations were performed using the capillary electrophoresis system P/ACE 2050 (Beckman Instruments, Palo Alto, CA, USA) with a variable separation voltage of 1 to 30 kV and an UV photometer detector. Before sample loading, the capillary (fused-silica, 50 cm × 75 μm I.D.) was conditioned for 2 min with 0.1 M NaOH and then for 2 min with buffer solution. The sample (70 nl) was injected into the capillary by pressure injection. The separation was carried out at 30°C and a voltage of 30 kV. Detection was done on-line at the cathodic site of the capillary at 214 nm.

### 2.2. Reagents

Deionized and filtered water (Millipore, Molsheim, France) with a resistivity of >18.2 MΩ cm was used.

Potassium dihydrogenphosphate–sodiumtetraborate buffer (3 mM KH<sub>2</sub>PO<sub>4</sub>, 6 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>) with pH 8.9 was applied. The buffer chemicals were purity grade Suprapur (Merck, Darmstadt, Germany). All other reagents were “pro analysis” grade (Merck).

### 2.3. Samples

The following samples were investigated: (a) humic acid standard–“Suwannee River” (sample A, International Humic Substances Society, Golden, CO, USA); (b) humic acid standard–“Laurentian” (sample B, Ecolinc, Roxbaro, Canada); (c) commercial humic acid (sample C, Fluka, Neu-Ulm, Germany); (d) commercial humic acid (sample D, Aldrich, Steinheim, Germany); (e) humic acid extracted from loess soil [5] that was isolated in the region near Halle, Germany (sample E); (f) fulvic acid standard–“Suwannee River” (sample F, International Humic Substances Society); (g) fulvic acid standard–“Laurentian” (sample G, Ecolinc).

The solid samples were dissolved in 10<sup>-3</sup> M NaOH with a concentration of 400 mg/l. This

concentration was selected to obtain a good absorption signal. Runs using concentrations of 100 mg/l gave very noisy signals. The clear solutions were used directly for injection; no filtration or any other special sample treatment was necessary.

## 3. Results and discussion

The migration behavior of molecules in capillary zone electrophoresis depends on their charge-to-size ratio. If two humic acids exhibit the same behavior in an electric field, then they are likely to have a comparable charge-to-size ratio. Differences in the intensity of the background, the electrophoretic mobility and absorption behavior are established by structural and chemical differences resulting from the differing origins of the humic acids.

Fig. 1 shows the electropherograms of the individual humic acids (samples A to E). The measurements were reproducible with respect to both the migration time of the peaks and the peak shape. The relative standard deviation of the migration time of the peaks after six separations was maximal 3.3%. All humic acids, except the humic acid from loess soil (sample E), show three superimposed peaks on a broad background. Presently these peaks cannot be assigned to any individual substances because no standards are available for the individual fractions. Further investigations with other analytical methods are planned to identify the peaks, and to possibly derive suitable standards. The humic acid from loess soil shows only two peaks in the electropherogram.

It is noticed that humic acids A and D exhibit comparable electrophoretic behavior. The first peak of humic acid A is more pronounced than the one in D. One possible reason for this behavior is that humic acid A contains a larger amount of this fraction than humic acid D. Due to their similar migration behavior in the electric field, humic acid A and D may possess an approximately similar size as well as a similar number of dissociated groups. Carboxylic groups and some

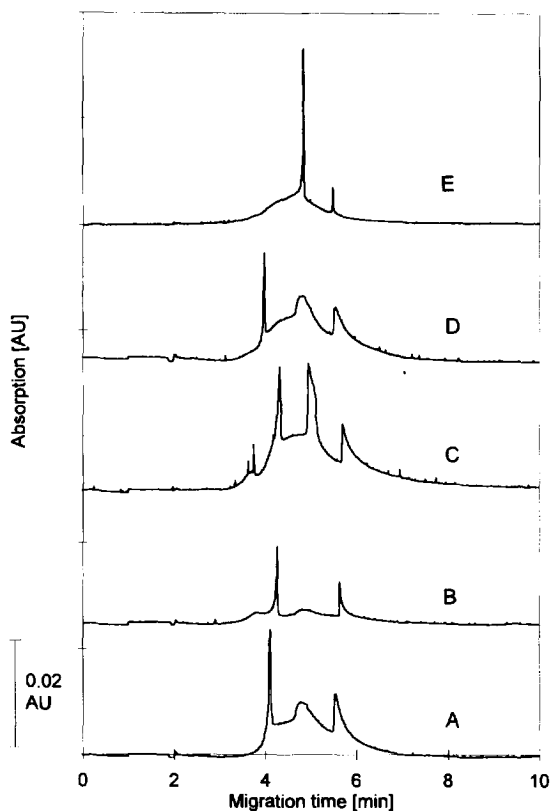


Fig. 1. Electropherograms of the investigated natural humic acids. A = Humic acid Suwannee River; B = humic acid Laurentian; C = humic acid Fluka; D = humic acid Aldrich; E = humic acid extracted from loess soil.

phenolic groups that have other substituents or additional aromatic groups on the benzene ring are the charge carriers of humic acids at the experimental pH of 8.9. At this pH they are deprotonated.

The electropherograms of humic acids B and C are very different from the other electropherograms and show dissimilarities when compared to each other. The first peak of these two humic acids appears at a later migration time than the first peaks of humic acids A and D. The second and the third peaks of humic acid C have longer migration times than the corresponding peaks of all other measured humic acids. The differences in the migration times ( $\geq 5\%$ ) are larger than the relative standard deviations ( $\leq 3.3\%$ ) for the migration time. Therefore, these differences

might be attributed to the different humic acids. This different behavior originates from the different charge-to-size ratios of the individual humic acid fractions.

Although all the investigated humic acids had the same analytical concentration, there are differences in the UV absorption in the electropherograms. This may be caused by: (a) variation in the individual aromatic carbon content; (b) the presence of a different number of UV-active groups; and (c) variation in the concentrations of the individual fractions. The cause of these differences lies in the presence of different precursor substances and/or unequal conditions during humification, which are characteristic for the origin of the humic acids.

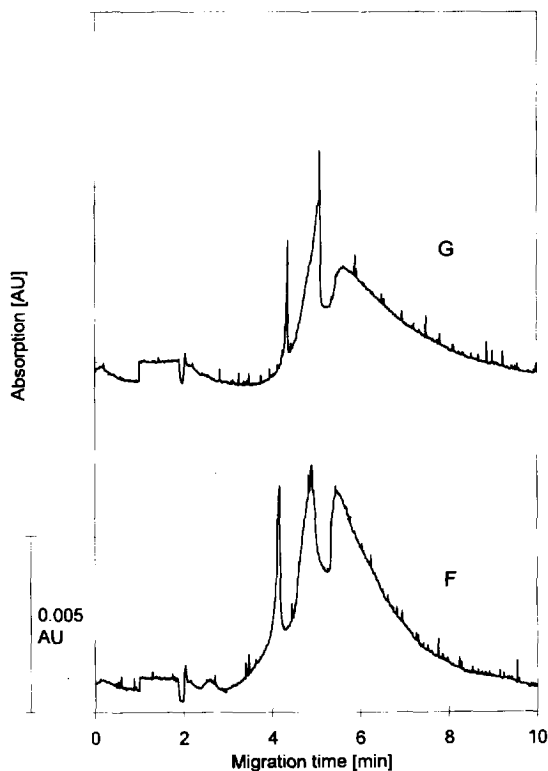


Fig. 2. Electropherograms of the investigated natural fulvic acids. F = Fulvic acid Suwannee River; G = fulvic acid Laurentian.

Electropherograms of the fulvic acids (samples F and G) are shown in Fig. 2. There are marked differences in the relative proportions of the individual fractions and in the absorption intensities of fulvic and humic acids from the same origin. However, there are no marked differences in the migration times. Fulvic acids have a higher content of carboxylic groups and a smaller molecular mass than humic acids from the same origin [6]. Due to the higher charge-to-size-ratio, fulvic acids must have longer migration times than humic acids. In addition to these fractions with higher migration times, however, there are also fractions with low charge-to-size ratios in fulvic acids. The migration times of the investigated humic and fulvic acids are not clearly distinguishable. Nevertheless, the electropherograms for fulvic acid show a larger fraction with a higher charge-to-size ratio than the humic acids. One cannot exclude the possibility that there are more pronounced differences between other natural humic and fulvic acids. The smaller absorption intensity of fulvic acids signifies their smaller aromatic content.

#### 4. Conclusions

This study shows that it is generally possible to characterize fulvic and humic acids of different origins by capillary electrophoresis. The fulvic and humic acids investigated exhibit characteris-

tic, individual electropherograms under the separation conditions applied. Further investigations are underway to specify the individual physico-chemical properties using other analytical methods. We are currently studying if the interaction of these acids with radionuclides and heavy metals can be predicted solely by using their "finger-print" electropherograms.

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