Short Communication
Capillary zone electrophoresis of humic acids

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Abstract

Capillary zone electrophoresis (CZE) was used for the separation of humic acids. The influence of the buffer solution on the separation of a commercial humic acid was studied. The use of an 8 mmol l^{-1} HCl–59.8 mmol l^{-1} L-alanine buffer (pH 3.17) leads to the separation of humic acids into two fractions. The composition and pH of this buffer and some instrumental parameters such as voltage, injection volume and wavelength of detection were optimized and the quality parameters of the method were determined. Two further commercial humic acids were assayed. The one that showed the highest peak signal was used as a reference for the rough quantification of the others.

1. Introduction

The high structural complexity and the wide range of molecular masses of humic acids have led to the application of different approaches to obtain more information about them. Many techniques and methods have been used for this purpose, most of which are based on the fractionation and characterization of these substances.

Size-exclusion chromatography is the most widely used technique for humic acid studies. There are many papers describing the use of this technique followed by IR [1,2], UV [2,3,4], fluorescence [2] and electrochemical detection [4], pulse polarography [5] and electrophoretic techniques using polyacrylamide gel with den-

sitometry [6], staining [1] or UV detection [7]. Other chromatographic techniques used in these studies are HPLC with fluorescence [8] or UV detection [9], gas chromatography with microwave emission [10] or nitrogen-selective detection [11] and sorption chromatography followed by ^{13}C NMR spectrometry [12].

Because of the ionic nature of the humic acids, it is possible to study them using electrophoretic techniques. Most of these techniques use polyacrylamide gel as a stabilizing medium. Studies have been reported using zone electrophoresis followed by densitometry, IR or pyrolysis–GC–MS [13], paper electrophoresis followed by densitometry [14,15], isoelectric focusing followed by GC [16], staining or densitometry [17] and isotacophoresis followed by densitometry [18]. To our knowledge, only one study of humic acids by a capillary technique, capillary isotachophoresis, has been published [7]. However, there are very few suitable methods for the determi-

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Table 1
Elemental analysis, A_{465}/A_{665} absorbance ratio and absorptivity for three commercial humic aids

Source	C (%)	H (%)	N (%)	A_{465}/A_{665}	Absorptivity
Fluka	45.91	3.73	0.56	4.3080	0.0229
Aldrich	42.17	3.90	0.53	4.7945	0.0196
Janssen	39.17	4.02	0.55	3.6091	0.0193

nation of humic acids. Of the studies of humic acids mentioned above, only one [8] proposes a method for determining them directly from the information obtained in the separation process. In other studies, the carbon percentage is used as a parameter to quantify humic acids.

These substances play an important role in environmental chemistry, mainly forming chelates with metal ions. Moreover, in soils with a high content of organic matter such as peaty soils of Chernobyl, the content of radionuclides was higher than expected [19]. To study the role of organic matter in radionuclide retention it is necessary to develop methods able to determine the different organic matter fractions.

For this purpose, the possibility of establishing a method for the determination of the humic acid content in soils has been studied. CZE was chosen as a technique with high separation efficiency and resolution, which has been applied in biochemistry for the study of substances with high molecular mass (proteins [20,21], nucleic acids [22] and amino acids [23,24]).

2. Experimental

2.1. Apparatus

A Model 270A capillary zone electrophoresis system from Applied Biosystems was used with a 72 cm × 50 μm I.D. fused-silica capillary filled with the respective buffer. High voltages, from 10 to 30 kV, were applied in both polarities (cathodic injection/anodic detection or *vice versa*). This instrument is equipped with a UV detector with a deuterium lamp working from 190 to 700 nm. The electropherograms were recorded using a Hitachi Model D-2500 integrator.

2.2. Reagents

Washing solutions
Solutions of 0.1 mol l^{-1} NaOH or 0.1 mol l^{-1} HCl were prepared using analytical-reagent grade reagents from Carlo Erba and Merk, respectively.

Buffer solutions
Twelve buffers were used at different concentrations: 2-(N-morpholino)ethanesulphonic acid (MES)--NaOH, HCl–tris(hydroxymethyl) aminomethane (Tris), citric acid–citrate, HCl–imidazole, HCl–glycylglycine, HCl–glycine, HCl–L-alanine, HCl–L-leucine, HCl–L-serine, HCl–L-lysine, HCl–β-alanine and L-aspartic acid. All were of analytical-reagent grade from Merck, except for MES which was from Sigma. For the preparation of buffer solutions, Culligan Ultrapure GS doubly deionized water of 18.3 MΩ cm resistivity was used.

Buffers were prepared by mixing a measured mass of the compound with a volume of 0.1 mol l^{-1} HCl or NaOH solution in various proportions to obtain the appropiate pH and concentration. The solutions were filtered through a 0.22-μm nylon filter (Scharlau).

2.3. Samples

Commercial humic acids from Fluka, Aldrich and Janssen were used. Some of their characteristics, such as elemental composition, ratio of absorbances at 465 and 665 nm (A_{465}/A_{665}) and absorptivity (expressed in terms of absorbance at 400 nm per mg C l^{-1} in a 1-cm cell [25]) are given in Table 1. Solutions were prepared by dissolving the humic acid in 10^{-3} mol l^{-1} NaOH.

2.4. Capillary electrophoresis procedure

The capillary was washed with 0.1 mol l^{-1} HCl for 2 min, then the buffer solution (59.8 mmol l^{-1} in L-alanine and 8 mmol l^{-1} in HCl at pH 3.17) was applied for 5 min to condition the capillary. In both steps the solution was introduced into the capillary by means of the vacuum. The sample was injected for 12 s (48.6 nl) by the vacuum technique, and separation was achieved using a voltage of 15 kV with anodic injection and cathodic detection. Each sample was injected in triplicate. Detection was carried out at 215 nm by means of an optical window for UV detection 50 cm from the injection site. The column temperature was set at 40°C.

3. Results and discussion

3.1. Buffer selection

The reagents used to study the effect of the buffer composition on the separation were chosen according to the literature [7,26] and taking into account the presence of different functional groups.

Some buffer solutions showed an electropherogram with overlaping bands (MES–NaOH, critic acid–citrate and HCl–imidazole) or with low sensitivity (HCl–Tris). Of the amino acids assayed, only those with a pK between 2.2 and 2.4, containing only one carboxylic acid group and one amino group in the α-position (L-alanine, L-glycine, L-leucine and L-serine) gave good results when the buffer was prepared at pH ≈ 3. The electropherograms obtained with these buffers were similar and included four fractions, referred to as A, B, C and D according to their migration time. HCl–L-alanine buffer was chosen for the determination studies as it gave the narrowest peaks. Fig. 1 shows the electropherogram obtained with a commercial humic acid from Fluka.

Only fractions A and D were attributable to humic acids, because the peaks corresponding to fractions B and C were independent of the humic
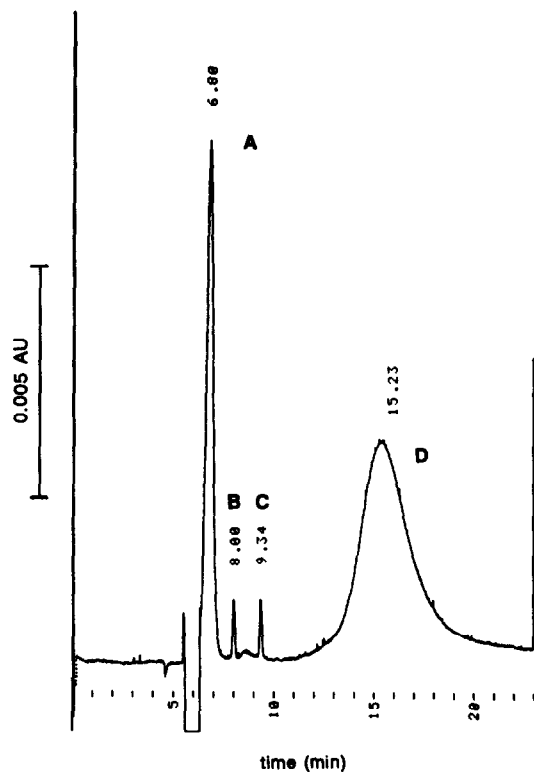

Fig. 1. Electropherogram of a sample of 100 μg ml^{-1} humic acid from Fluka. Conditions: buffer, 8 mmol l^{-1} HCl–59.8 mmol l^{-1} L-alanine (pH 3.17); temperature 40°C; applied voltage, 15 kV; anodic injection and cathodic detection; injection volume, 48.6 nl; detection at 215 nm; attenuation, 2^4 mV.

acid content and they sometimes appeared when a blank was injected.

To optimize the HCl–L-alanine buffer solution, nine solutions were prepared with different pH values (between 2.7 and 3.2) and concentrations (5.4–11.6 mmol l^{-1} for HCl and 14.4–88.8 mmol l^{-1} for L-alanine). Considering the criteria of efficiency, resolution and reproducibility (R.S.D. ≤2%), the buffer chosen was 8 mmol l^{-1} in HCl and 59.8 mmol l^{-1} in L-alanine and had a pH of 3.17.

3.2. Quality parameters

Linearity, limit of quantification and precision were determined using the described procedure. Linear relationships were obtained for peak area

(or peak height) *versus* humic acid concentration (from 5 to 100 μg ml^{-1}) with correlation coefficients better than 0.99. In relation to fraction A, higher sensitivity was obtained when the peak height was used. For fraction D both peak height and peak area gave similar sensitivities. On the other hand, the slopes obtained on different days had good reproducibility (R.S.D. < 4% for peak height and R.S.D. < 8% for peak area). Concentrations below 5 and 20 μg ml^{-1} were not suitable for determining the contents of fractions A and D, respectively.

The precision for the measurements of fractions A and D was calculated by injecting different solutions with 20 and 80 μg ml^{-1} of humic acid for fraction A and 80 μg ml^{-1} for fraction D. The recovery of humic acid was measured in quadruplicate over 20 days. The R.S.D. was better for peak-area measurements (2–4%) than for peak-height measurements (6–7%).

Because of the difficulty in obtaining a certified reference material, the validation of the method was carried out by calculating the recovery for the Fluka humic acid in a simple matrix such as bottled drinking water. The values obtained were satisfactory for fraction A (96.44%) but lower for fraction D (86.02%), perhaps owing to matrix effects. However, humic acids are normally extracted with an alkaline medium and they are precipitated at acidic pH. The humic acid obtained in this way can be dissolved in 10^{-3} mol l^{-1} NaOH, diminishing these matrix effects.

3.3. Capillary electrophoresis of other commercial humic acids

Commercially available humic acids from Aldrich and Janssen were used. The electropherograms obtained for these samples were similar to those from Fluka, as shown in Fig. 1. The recoveries obtained for the Aldrich sample were 92.43% for fraction A and 80.79% for fraction D, whereas those obtained for the Janssen sample were 75.67% and 79.24%, respectively.

The contents of fractions A and D were determined using Fluka humic acid as a reference. The content of the fractions observed for

Aldrich humic acid was higher than that obtained for Janssen humic acid. This may be related to the different characteristics or origins of the humic acid samples. On the other hand, the humic acid from Fluka showed the highest signal and it was selected for use as reference for further determinations of humic acid contents.

4. Conclusions

CZE is a useful technique for studying humic acids, because two fractions can be separated, different commercial humic acids show similar electropherograms and there is a linear relationship between the signal obtained and concentration, which allows a rough determination of these substances.

5. References

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