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Highly reproducible capillary zone electrophoresis of humic acids in cyclodextrin- or oligosaccharide-modified background electrolytes

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Abstract

Capillary zone electrophoresis has been used for the characterization and separation of humic acids. It was found that addition of saccharides like α-, β-, γ-cyclodextrins, maltose, hydroxyethylcellulose or dextran sulfate in the background electrolyte (50 m*M* $Na_2B_4O_7$, pH 9.6) yields better separation patterns and highly reproducible electropherograms. Electropherograms with higher numbers of peaks and high reproducibility were obtained with α- and β-cyclodextrins or with a mixture of α-+γ-cyclodextrin-modified background electrolytes. Separation was carried out with the cathode at the detector end of the column. Adsorption of humic acids to the capillary wall was diminished using an epoxy-coated capillary tube.

Keywords: Background electrolyte composition; Humic acids; Cyclodextrins; Oligosaccharides

1. Introduction

Humic acids (HAs), natural compounds widely distributed in nature still of unknown structure, are intensively studied. HAs and related fulvic acids (FAs) occur in soils, natural waters, marine and lake sediments, peat, lignin, brown coal and other natural deposits [1–6]. These substances form a group of polyfunctional acids, which have a yellow to brown color and molecular masses ranging between hundreds and hundreds of thousands [11]. It is now accepted that they represent a heterogeneous mixture of compounds that do not have a uniform structural formula [7].

Different methods have been proposed to study the separation, characterization and behavior of HAs [8–11]. Special attention is paid to capillary zone electrophoresis (CZE), mostly with UV–Vis, diode array, or laser-induced fluorescence detection, while as background electrolytes (BGE) usually borate, phosphate [12–14] or a mixture of both [14] are used. An extensive overview of the various background electrolytes can be found in the literature [15–17]. It was the CZE separation technique by which it was found that humic acids are a mixture of many compounds [18]. However, CZE separation of HAs suffers low reproducibility and there is still a need for better methodology.

Compounds containing sugar units are used as chiral selectors in capillary electrophoresis (CE). They also show the ability to modify electroosmotic flow (EOF) and diminish interactions of analytes with the capillary inner wall [19–23]. An important class of such compounds is the cyclodextrin (CDs) [24]. They consist of cyclic oligosaccharides composed of D-glucose units connected to a ring with α-(1–4)-glycosidic bonds. This arrangement of the D-glucopyranose units forms a bucket or ''cone like''

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structure surrounding an inner space, reminiscent of a cage. Many compounds or groups can penetrate the cone and form an ''inclusion'' or ''host–guest'' complex. This is often the basis of chiral separations by CE [25–28]. It is also known that oligosaccharides like maltose, ethylcellulose, hydroxyethylcellulose and dextran sulfate change the viscosity of the solution and improve the separation of the hydroxy and polyhydroxy compounds [29].

As there is a need for a reproducible and more efficient method for separation of HAs and there are no reports in literature concerning BGEs modified with saccharides, the aim of the present study is to investigate the effect of cyclodextrin-modified BGE and the influence of other common oligosaccharides on the effectiveness and efficiency of HA separation by CZE.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade purity. $Na_2B_4O_7$ from Lachema (Brno, Czech Republic). NaOH and H_3PO_4 were from Merck (Darmstadt, Germany). Hydroxyethylcellulose, ethylcellulose, dextran sulfate sodium salt, maltose, saccharose and α-, β-, γ-cyclodextrins were from Sigma–Aldrich (Steinheim, Germany). Mesityl oxide used as an EOF marker for CZE was from Fluka (Buchs, Switzerland). Deionized water used to prepare all solutions was double-distilled from a quartz apparatus of Heraeus Quartzschmelze (Hanau, Germany).

2.2. Humic acids

The HA samples used in this work were: Fluka HA preparative No. 53680 (analysis No. 38537/1 293) (Fluka I), International Humic Substances Society (IHSS) Peat HA Standard (1R103H), coal-derived Czech HA sample from Chemapex (Chomutov, Czech Republic) and coal-derived China HA sample (China).

Sample preparation for CZE analysis was as follows: 5 mg of HA was dissolved in 180 μl of 1 *M* NaOH and the solution was completed with distilled water to 5 ml. For CZE analysis the stock solution was diluted 10 times. All HA work solutions were analyzed after 10 days of preparation, because it was found that after this time the electropherograms were reproducible.

2.3. Apparatus

A Beckmann CZE (Model P/ACE) System 5500 (Palo Alto, CA, USA) equipped with a diode array detection (DAD) system, an automatic injector, a fluid-cooled column cartridge and a System Gold Data station was utilized for all CZE experiments. Epoxy-coated fused-silica capillary tubing of 47 cm (40.3 cm length to the detector)×75 μm I.D. was used. The normal polarity mode of the CZE system (cathodic pole at the side of detection) was applied.

The pH values were measured using a glass G202C electrode, standard calomel electrode K401 of Radiometer (Copenhagen, Denmark) and Precision Digital pH meter OP-208/1 of Radelkis (Budapest, Hungary) while standard buffer solutions of Radiometer and/or Radelkis 5 were used for the calibration.

2.4. Procedure

A sapphire-coated capillary tube was prepared as described earlier [30] and the same conditioning procedure was used. The capillary was washed daily with deionized water for 10 min, and 5 min with buffer solution. In each analysis the capillary was first washed for 3 min with deionized water and for 3 min with BGE. Separation of HAs was done at an elevated temperature as separation patterns were found more reproducible [17]. The temperature 40°C was found as optimal. Hydrodynamic injection of samples was used. The absorbance was monitored at 210 nm. The EOF was determined using 0.1% mesityl oxide under the same conditions of the HA separation. At the end of the working day, the capillary was washed for 1 min with 0.1 *M* NaOH, for 5 min with deionized water and for 3 min with BGE. All HA solutions were filtered using a 0.2-μm filter from Sigma (USA).

3. Results and discussion

In an extensive review [15] the use of many BGEs in this laboratory have been studied and applied. In this work, we have again examined several BGEs like boric acid, phosphate, citrate and acetate. In the search for optimal conditions we found the borate buffer to be the most suitable.

It was found recently, that humic acid components adsorb strongly on the quartz surface of the capillary wall [10]. This adsorption was eliminated with addition of Mg^{2+} salts. However, Mg(II) can react with HAs and therefore another solution was searched for.

Therefore, in this work we have applied a recently new coating developed by Fetsch et al. [10]. This new kind of coating shows low or no adsorption for proteins and we have found no significant adsorption for HAs. Procedure for coating of the capillary is described elsewhere [30].

Examples of the separation for several different samples of humic acids in borate buffer are given in Fig. 1. This figure shows broad shaped peaks which are commonly called humic ''humps'' [31]. It is suggested that the ''hump'' corresponds to the average electrophoretic mobility of a HA mixture. In this figure, no excellent separation is observed and therefore in the next part of this work we have examined the use of various modifiers of the background electrolyte.

3.1. Effect of cyclodextrins

Effect of the addition of individual α-, β- and γ-cyclodextrins and different mixtures of them was studied. In relation to dependence on cavity size and concentration of individual cyclodextrins different results were obtained. Cyclodextrin concentrations were optimized, as optimal we found 30 m*M* α-cyclodextrin, 15 m*M* β-cyclodextrin and 50 m*M* γ-cyclodextrin additions to the BGE. The γ-cyclodextrin shows low or no significant effect on the separation patterns, perhaps because the size of the cavity is not adequate for the formation of inclusion complexes with most of HA components. On the other hand, the electropherograms obtained for the BGE modified with α- and β-cyclodextrins (Figs. 2 and 3, respectively) show separation patterns with more peaks for the same humic acid samples. On the shoulders of the ''hump'' we can observe many separate peaks which are not observed on electropherograms with un-modified BGE. We suggest that this effect can be explained by the formation of inclusion complexes with the HA components.

As for the BGE modified with mixtures of cyclodextrins, the electropherograms with the highest number of peaks were obtained for the mixture of

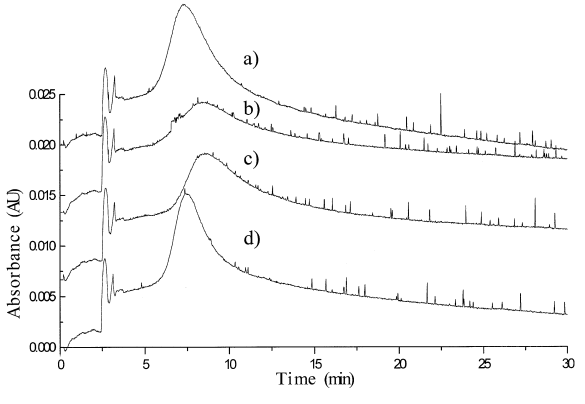

Fig. 1. Electropherograms of different HAs obtained with un-modified BGE (50 m*M* $Na_2B_4O_7$). CZE conditions: pH 9.6, separation voltage 15 kV, hydrodynamic injection 22 s, detection at 210 nm, capillary 47 cm (effective length 40.3 cm)×75 μm I.D. (a) Peat, (b) Fluka I, (c) Chemapex, (d) China HAs.

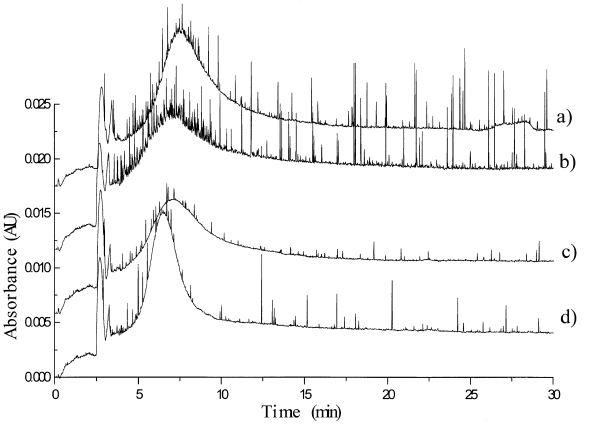

Fig. 2. Electropherograms for different HAs using 50 m*M* $Na_2B_4O_7$ and 15 m*M* β-cyclodextrin. Conditions as in Fig. 1. (a) Peat, (b) Fluka I, (c) Chemapex, (d) China HAs.

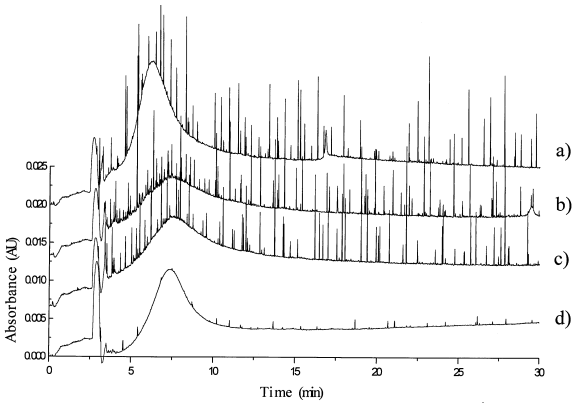

Fig. 3. Electropherograms for HAs with addition of 50 m*M* $Na_2B_4O_7$ and 30 m*M* α-cyclodextrin. Conditions as in Fig. 1. (a) Peat, (b) Fluka I, (c) Chemapex, (d) China HAs.

α-+γ-cyclodextrins (Fig. 4). Electropherograms similar to those obtained when using either α- or β-cyclodextrin-modified BGEs were observed. For the mixture of the three different CDs no further improvement was observed.

3.2. Effect of methanol

The influence of organic modifiers on the BGE were also tested, especially methanol. Addition of different concentrations of methanol (10–30%, v/v) was tested but no significant improvement of the separation patterns was observed.

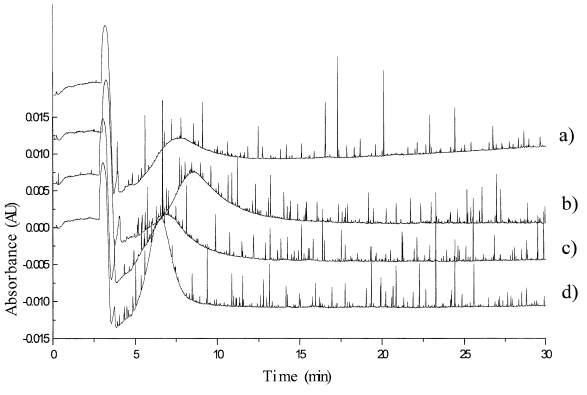

Fig. 4. Comparison of electropherograms for different HAs using a mixture of α-+γ-cyclodextrins in 50 m*M* $Na_2B_4O_7$. Conditions as in Fig. 1. (a) Peat, (b) Fluka I, (c) Chemapex, (d) China HAs.

3.3. Effect of oligosaccharides

Electropherograms for analysis with maltose- and saccharose-modified BGEs did not show any significant differences in comparison with the un-modified BGE.

The solubility of ethylcellulose is limited and therefore for the low concentration achieved (1 m*M*) no improvement was observed. The results obtained using more soluble hydroxyethylcellulose (HEC) are given in Fig. 5. As an optimal we found 7.5 m*M* HEC in BGE. For higher concentrations of HEC in buffer analysis failed because of high current. Using the optimal concentration of HEC for all HA samples studied we obtained electropherograms similar to those obtained for BGE with the additions of the mixture of α- and β-cyclodextrins.

For dextran sulfate sodium salt a concentration equal to 5 m*M* was found to be optimal (Fig. 6). Electropherograms for the dextran sulfate-modified BGE show as in the case of oligosaccharide separation, patterns with many peaks as well but with lower numbers of peaks in comparison with the hydroxyethylcellulose-modified BGE.

In all cases studied above, we suggest that the peaks correspond to the defined compounds present in all HAs which are liberated from the supramolecular structure of the humic acids by the action of cyclodextrins forming inclusion complexes, for example. A higher number of peaks was observed than

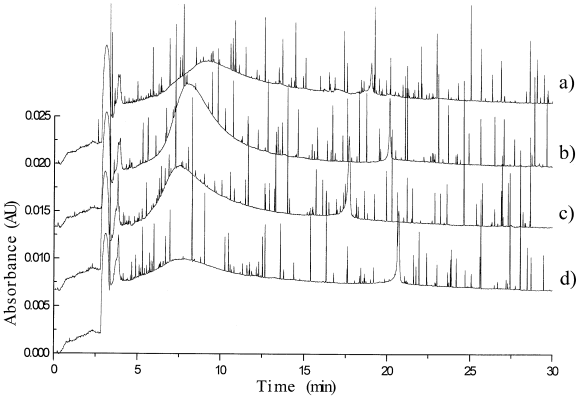

Fig. 5. Electropherograms for different HAs using 7.5 m*M* hydroxyethylcellulose in 50 m*M* $Na_2B_4O_7$. Conditions as in Fig. 1. (a) Peat, (b) Fluka I, (c) Chemapex, (d) China HAs.

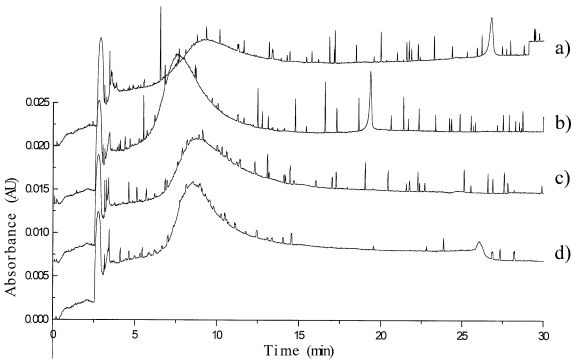

Fig. 6. Electropherograms for different HAs using 5 m*M* dextran sulfate in 50 m*M* $Na_2B_4O_7$. Conditions as in Fig. 1. (a) Peat, (b) Fluka I, (c) Chemapex, (d) China HAs.

those observed by Fetsch et al. [10] where just unmodified BGE was used.

3.4. Reproducibility

The reproducibility of the electropherograms was studied for different modifiers. Fig. 7 shows 11 measurements of Fluka I HA with β-cyclodextrin-modified BGE during the first 10 min of analysis, Fig. 8 for 10–20 min and Fig. 9 for 20–30 min. In these figures a very good reproducibility is observed with only small deviations or shifted peaks. Such extraordinary good reproducibility confirms that the peaks are not dust particles, bubbles or impurities

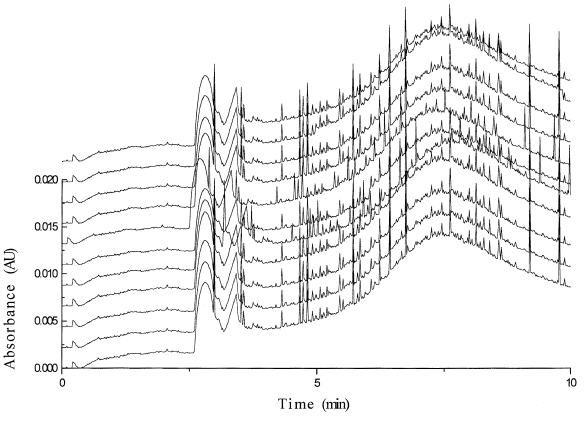

Fig. 7. Reproducibility of electropherograms for Fluka I HA using 15 m*M* β-cyclodextrin in 50 m*M* $Na_2B_4O_7$. Time of analysis: 0–10 min.

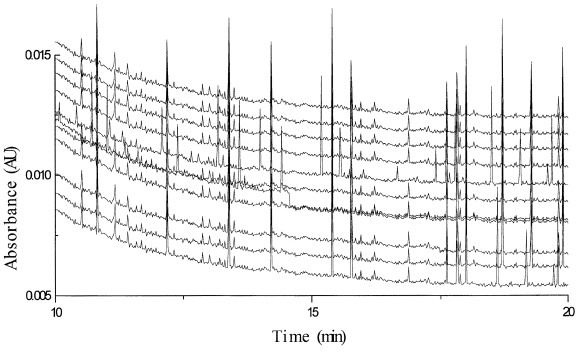

Fig. 8. Reproducibility of electropherograms for Fluka I HA using 15 m*M* β-cyclodextrin in 50 m*M* $Na_2B_4O_7$. Time of analysis: 10–20 min.

and it supports the conclusion that there are many minor compounds in humic acids.

4. Conclusions

The use of a sapphire epoxy-coated capillary and modification of borate buffer with additions of α- or β-cyclodextrins or their mixtures yields stable and reproducible electropherograms that confirm the presence of more than 30 peaks (fractions) in most of the humic acids under study, supporting the fact that humic acids are a complex mixture of many compounds.

The interaction of humic acids with the silica wall can be successfully diminished with coating and

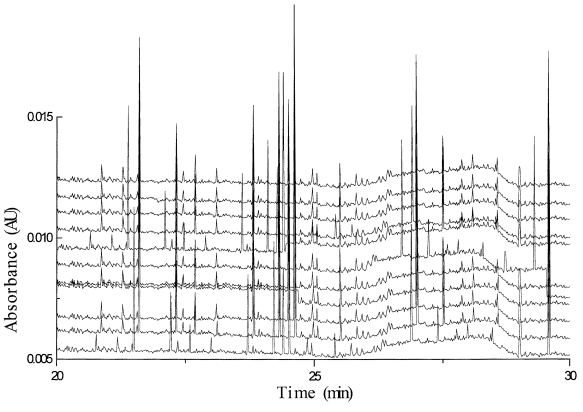

Fig. 9. Reproducibility of electropherograms for Fluka I HA using 15 m*M* β-cyclodextrin in 50 m*M* $Na_2B_4O_7$. Time of analysis: 20–30 min.

addition of modifiers (saccharides) to the BGE improves the separation while highly reproducible electropherograms can be obtained.

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