Suppression of solute–wall interactions in humic acid capillary electrophoretic analysis by its diluted solution as background electrolyte

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Abstract

To analyze humic substances by capillary electrophoresis diluted humic acid (HA) solutions were used as a background electrolyte, resulting in the improved reproducibility of the analysis by suppressing the analyte interactions with capillary walls. Two HA solutions were investigated: standard HA solution and the Baltic Sea sediment HA extract. Characteristic shape electropherograms were obtained. Areas of the characteristic regions correlated linearly with the concentration of the injected HA sample.

Keywords: Background electrolyte; Capillary electrophoresis; Humic acid

1. Introduction

Capillary electrophoresis (CE) offers a unique possibility to separate and detect humic substances (HS) in aqueous solution within a wide pH range, close to the environmental conditions, and to obtain information about its structure and properties [1]. The pH of the natural waters is mainly neutral (pH 5–7). One of the most important part of HS is humic acid (HA). The chemical structure of HAs is very complicated and depends on their source. HAs consist of a mixture of a large number of homologous molecules with similar but not identical properties [2]. At pH 7 HS carry a negative charge because of the carboxylic group deprotonation. Presence of a negative charge in the case of uncoated capillaries permits HS separation by CE with positive polarity at the injection side [3]. The mean electrophoretic mobility of the HS corresponds to the distribution of the electrophoretic mobilities of the individual HS molecules and thus, takes polydispersity of the analyte samples into account [4].

In electropherograms HA usually exhibit one very broad non-reproducible peak (“humic hump”) which shape is strongly influenced by the composition of the background electrolyte (BGE) as well by the “chemistry” of the capillary walls [5]. The choice of the buffer for separation plays important role in the formation of the electrophoretic patterns [6–11]. Due to the nature of humic samples (polydisperse, heterogeneous, reactive), possible interferences with the buffer constituents are obvious and can hardly be fully avoided [1]. Typical BGEs usually consist of borate, acetate, carbonate, phosphate or alanine buffers. In HA analysis by CE sorption of HA on the walls of uncoated silica capillaries occurs that causes usually long retention times and a need for relatively high sample concentrations [3,6]. Modification of the capillary walls and addition of certain ingredients to the buffer can, to some extent, reduce the sorption problem [3,12–14]. Thus, Fetsch et al. [14] demonstrated that the adsorption of HA on an uncoated capillary wall can be eliminated by adding magnesium(II) salts. Recently, various modified BGEs were used to improve the reproducibility [12,15,16]. Schmitt et al. [17] and de Moraes and Rezende [18] have used ordinary buffers (acetate, phosphate, carbonate, borate) with the HA additive as a BGE at different pH values to describe the affinity of several ionic pesticides [17] and naphthalene [18] to the HA. Lloyd et al. [19] have used albumin at near-neutral pH values. In this condition albumin has negative charge and has tendency to adsorb to fused-silica capillaries. They found that
this method achieve better resolution for cationic analytes than for the resolution of anions.

In the present work we made an attempt to use the electrolytic nature of HA (negatively charged at neutral pH) and to employ the diluted HA solution as BGE in the CE analysis of HA. The diluted standard HA (S-HA) was used as BGE in the analysis of S-HA and the diluted Baltic Sea sediment HA (B-HA) was used as BGE for the analysis of B-HA. We expected that HA as BGE is “saturating” the capillary walls with HA, avoiding additional sorption of HA from the sample to the walls and so, improving the reproducibility of the analysis.

2. Experimental

2.1. Materials

Analytical grade NaOH was purchased from BDH (UK), HCl from Riedel de Haën (Germany), nitromethane and standard HA (lot no. 53680) from Fluka (Germany). S-HA stock solution (5 mg/ml) was prepared as follows: 0.125 g of S-HA was dissolved in small quantity of 0.1 M NaOH. After dissolution, the pH was adjusted to pH 7 with 0.1 M HCl and diluted to volume 25 ml.

B-HA was extracted from the sediment of the Baltic Sea (Haapsalu Bay, Northwest of Estonia). Experimental procedures are reported in Übner et al. [20]. Solid residue (gel, contains (Haapsalu Bay, Northwest of Estonia). Experimental procedures are reported in Übner et al. [20]. Solid residue (gel, contains only B-HA) was stored at 4 °C. In the present study, the non-lyophilized B-HA was used to avoid changes in their molecular structure. Stock solution of B-HA (10 mg/ml) was prepared by weighing a given amount of B-HA gel and diluting it to a fixed volume using Milli-Q water (Millipore). The value of pH was adjusted with 0.2 M NaOH solution to pH 7. Colloidal stock solution was stored for more than 10 days at 4 °C.

B-HA and S-HA working solutions were prepared daily by diluting the appropriate amount of stock solution in Milli-Q water. The electrolyte was filtered through a Millipore 0.45 μm membrane filter. The concentration of HA fraction in BGE was 0.1 mg/ml.

2.2. Equipment and procedures

All the CE experiments were performed with an ISCO CV4 Capillary Electropherograph model 3850, which was thermostated with forced air by a fan. The bare fused-silica capillary 75 μm i.d. (Polymicro Technologies, Phoenix, AZ, USA) had a total length of 80 cm (48 cm to the detector). The UV absorbance signal of the solution was recorded at 226 nm. The UV detector signal was digitized by “Mini-16” analogue to digital converter (Keithley, Mertabyte, Taunton, MA, USA) and stored on Pentium PC hard disc for later processing by the procedures written in-house in Matlab (MathWorks, Natick, MA). The equipment and procedures used are described in our previous work [21,22].

The plug of HA solution (B-HA or S-HA) was hydrodynamically injected (during 15 s) into the capillary filled with the corresponding HA solution as the electrolyte. After injection, high voltage (20 kV) was applied. During all experiments, the current values were in the range of 1–3 μA.

The velocity of electroosmotic flow (EOF) was estimated by the use of nitromethane as a marker compound. The calculated EOF mobility value for S-HA at the concentration of 0.2–0.8 mg/ml was (1.24 ± 0.01) × 10⁻³ cm²/V s and for B-HA was (1.36 ± 0.05) × 10⁻³ cm²/V s. All experiments were done in triplicate and performed at room temperature.

3. Results and discussion

Differences in the electrophoretic mobility and absorption behaviour of the HA of different origins are caused by their structural diversity [6,23]. Fetsch et al. [9] suggested that electrophoretic patterns could be obtained only after a critical concentration of HA in the capillary is achieved. For that reason highly concentrated (0.2–1 mg/ml) HA samples are usually required. Due to the sorption of HA on the fused-silica capillary wall, the effective HA concentration is about 0.035 mg/ml [9] and the resulted electropherograms have a rather wide migration time range and a strong fronting and tailing effect [3].

According to CE method the HA constituents are separated in accordance with their mass-charge ratio due to EOF within the capillary. Keuth et al. [24] have found that addition of hydroxycarboxylic acids (tartaric acid, malic acid or citric acid) to the borate buffer at pH 8.6, separation of HA into two or three zones may be obtained. The resulted separation was attributed to an interaction between the HA and the added hydroxycarboxylic acids.

When we performed CE using a standard humic acid solution (S-HA) at pH 7 as the BGE without adding any sample, a steady baseline was obtained (as it is in the case of any other BGE). When now S-HA solution was injected to that capillary, characteristic detector signals were obtained (Fig. 1).

All electropherograms of S-HA revealed three areas: a sharp system peak (migration time 2.6 min) that is related to the EOF of the system, a wide positive area with three definite peaks (Region 1), and a broad negative area (Region 2).

![Fig. 1. Electropherograms of S-HA using diluted S-HA as BGE (0.1 mg/ml) at different concentrations of injected S-HA: (A) 0.2 mg/ml; (B) 0.4 mg/ml; (C) 0.6 mg/ml; (D) 0.8 mg/ml. Separation voltage 20 kV; hydrodynamic injection, 15 s; detection at 226 nm; fused-silica capillary 80 cm × 75 μm.](image-url)
To rationalize the obtained signals, we may suggest that the processes in the column filled with HA solution as a BGE (lower concentration) after the injection of the HA sample (higher concentration) proceed as follows: the injected HA (as Na-salt) forms a band of higher concentration at the injection area (anodic end of the capillary). Because of its higher concentration, the injected HA sample has higher concentration of negatively charged molecules. That improves the formation of humic pseudomicelles, which are larger and have lower mobility than EOF (as it has been demonstrated before by de Moraes and Rezende [18] for HA being as ionic micelles in aqueous running buffer).

The formation of micelles depends on the number and nature of the hydrophobic association sites in an aqueous HA solution and on the concentration of ionized HA at the defined pH. The aggregation of HA particles to larger micelles might be promoted even further by the dielectrophoretic interaction of HA aggregates, as suggested by Kang and Li [25].

As we observed after the EOF peak in the electropherograms a wide hump appears (Region 1). That region forms from negatively charged analyte particles that initially moves against EOF, but carried by EOF towards cathode. It is generally accepted that if there are no interactions with the buffer, the homogenic macromolecules (as well as HA molecules) migrate as a single symmetric broad band [26]. We observed that Region 1 consists of several overlapped peaks (Fig. 1). That indicates the presence of several similar groups of compounds in S-HA, which migrate at very similar velocity. The calculated area value of that hump showed a linear correlation \( y = 35.795x + 15.091; R^2 = 0.9892 \) from the concentration of inserted S-HA. It means that the area definitely reflects the amount of injected S-HA in the sample.

Region 2 expresses the migration of the vacancy of charged particles in the HA background electrolyte (diminished concentration of the HA-anions, which are consumed in the formation of Region 1: injected HA is forming additional complexes (micelles) with the HA from BGE and move together). This assumption is supported by a fact that again, a linear correlation \( y = -9.43x + 1.448; R^2 = 0.9915 \) of the peak area from the S-HA concentration was observed.

The same method was used to analyze the sediment HA from Haapsalu Bay (B-HA), the Baltic Sea. We observed the formation of electropherograms similar to that from S-HA. All signals had considerably lower intensity and the shape of electropherograms was less characteristic.

To test the reproducibility HA electropherograms, several injections of the same sample (S-HA and B-HA, 0.4 mg/ml) were made (Fig. 2A and B). A standard deviation for Region 1 area is 7.39% for S-HA and 7.53% for B-HA. All signals had considerably lower intensity and the shape of electropherograms was less characteristic.

To test the reproducibility HA electropherograms, several injections of the same sample (S-HA and B-HA, 0.4 mg/ml) were made (Fig. 2A and B). A standard deviation for Region 1 area is 7.39% for S-HA and 7.53% for B-HA. Also, all the features of the humic hump are well reproduced on the electropherograms, which confirms the main suggestion about suppressing the capillary wall interference of HA sample by using the same diluted HA as a BGE.

Also, we observed that the peaks of Region 1 of B-HA were not split. The system peak appeared at 2.3 min, which is related to the EOF. The EOF of B-HA moved 10% faster than that of S-HA. It may be related to the lower concentration of HA-anions in the B-HA solution and to a more aliphatic nature of B-HA [22]. In these electropherograms an additional triangular flat peak appears after Region 2. The area of that additional peak did not correlate linearly \( y = 4.721x + 0.23; R^2 = 0.6733 \) with the injected HA concentration. Therefore, the nature of that peak requires additional investigations and was, therefore, neglected by us at this study.

Comparison of the electropherograms of S-HA and B-HA showed the same regularities: the concentration of injected HA solution is in a linear correlation with the area values of selected regions (Region 1—S-HA: \( y = 35.795x + 15.091; R^2 = 0.9892 \); B-HA: \( y = 7.345x - 0.0225; R^2 = 0.9998 \); Region 2—S-HA: \( y = -9.43x + 1.448; R^2 = 0.9915 \); B-HA: \( y = 1.64x - 2.07; R^2 = 0.9898 \)).

4. Conclusions

The results obtained indicate that the diluted analyte solutions can be used as BGE electrolytes for investing complex high polydisperse structures. These BGE enable to reduce the interaction of BGE components to the analyzed compounds as
well as with the capillary walls and their sorption to the walls of uncoated silica capillaries. We observed that by using such approach HA samples afford two characteristic regions in the electropherograms, the areas of which correlate with the concentration of inserted HA. Both, S-HA and B-HA have similar behaviour, indicating that the proposed approach may be of broader use in the analysis of complex mixtures of compounds which themselves may act as electrolytes.

References