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Short Communication

Enhanced micellar electrokinetic capillary chromatography separations on anionic polymer-coated capillary with pH-independent electroosmotic flow

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Abstract

Micellar electrokinetic capillary chromatography (MEKC) can be used to separate a variety of anionic, neutral and cationic analytes. Extreme acidic conditions limit the use of MEKC. However, with the use of an anionic polymer-coated capillary with pH-independent flow, the MEKC separations can be achieved over an expanded pH range. An example of this enhancement will be presented using a five-phenol mixture as the sample over a pH range of 3 to 9.

1. Introduction

Capillary electrophoresis (CE) is a rapidly growing analytical technique which separates analytes according to their charge-to-mass ratio. CE distinguishes itself with an extremely high efficiency (approaching or exceeding 1 000 000 theoretical plates), small sample volumes (nl), short analysis time, and simple equipment [1]. The primary limitation of CE is its inability to separate neutral compounds.

In 1985, Terabe *et al.* [2] introduced a new approach to electrophoresis called micellar electrokinetic capillary chromatography (MEKC). A sample is introduced into a fused-silica capillary with a run buffer containing an ionic surfactant, such as sodium dodecyl sulfate (SDS). This method allows small and neutral molecules to be partitioned through the micelle, which acts as a "pseudo" stationary phase. MEKC has the capability of separating anionic, neutral and cationic species within a single run.

One obstacle in the application of bare capillary MEKC is its limited pH range. The ζ potential of the capillary is a function of pH, due to ionization of surface silanol groups. At low pH values, capillary electroosmotic flow (EOF) can approach 0, and increases rapidly in the pH range of 5–10. This leads to reproducibility problems, and often restricts the use of ion suppression or other techniques to enhance selectivity.

A new anionic polymer coating procedure has been developed in our laboratory [3] which produces a capillary with pH-independent EOF. This allows an expanded pH range for the MEKC separations to be carried out. In this paper, a series of phenol samples are used to

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show that reproducible separations can be achieved in the expanded pH range using the new coated capillary.

2. Experimental

2.1. Chemicals

Phenol (registry No. 108-95-2), *m*-cresol (108-39-4), 2-ethylphenol (90-00-6), 3-ethylphenol (620-17-7) and 4-ethylphenol (123-07-9) were all obtained from Aldrich (Milwaukee, WI, USA). SDS (151-21-3) was obtained from Sigma (St. Louis, MO, USA).

2.2. Apparatus

MEKC separations were performed on a laboratory-constructed instrument which included a Plexiglas box, a CZE 1000 PN 30 high-power supply (Spellman, Plainview, NY, USA), a high-power supply local control (Chamonix Industries, Binghamton, NY, USA), and a Spectra 100 UV detector (Thermal Separations, Freemont, CA, USA). The electropherograms were processed on a SP-4400 integrator (Thermal Separations, Freemont, CA, USA). The temperature inside the Plexiglas box was cooled by fan.

2.3. Materials and solutions

Fused-silica capillaries (75 μm I.D. × 375 O.D., PolyMicro Technology, Phoenix, AZ, USA) with opened detection windows were coated with the anionic sodium 2-acrylamido-2-methyl-1-propanesulfate (NaAMPS) polymer using a method developed in our laboratory [3]. The five-phenol mixture sample was prepared by adding 50 μl of each phenol to a 100-ml volumetric flask, and diluting to volume with 10 m*M* phosphate buffer, pH 7.05. 50 m*M* SDS in 10 m*M* phosphate in the range of pH 3 to 9 were used as the run buffers. Samples were introduced hydrodynamically with an elevation of *ca.* 10 cm and an injection time of 3 or 5 s. The applied voltage was 18.0 kV with detection at 210 nm.

3. Results and discussion

Using a bare capillary, under normal CE operating conditions, it is impossible to separate the neutral phenol sample consisting of phenol, *m*-cresol, 2-ethylphenol, 3-ethylphenol and 4-ethylphenol. In addition, the neutral phenol sample cannot be separated using the anionic coated capillary with 10 m*M* phosphate buffer, pH 7.05 (Fig. 1). However, with the application of MEKC (*i.e.* addition of 50 m*M* SDS), the neutral phenol sample can be separated on both the bare and anionic coated capillaries (Figs. 2 and 3).

The coated capillary is compared with the bare capillary over a pH range of 3 to 9. The retention times of the five phenols remain stable over the extremes of the expanded pH range on the anionic coated capillary (Fig. 4). However, the bare capillary does not exhibit the same stability (Fig. 5). Under extreme acidic conditions, the retention times in the bare capillary deviate dramatically. At pH 5, the separation takes 30 min, considerable zone broadening occurs, and the separation is not reproducible (Fig. 2B). At pH 4, no separation is achieved

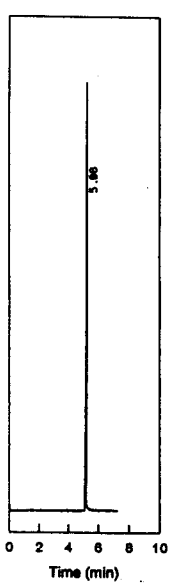

Fig. 1. Attempted separation of five-phenol mixture using anionic coated capillary at pH 7.05 with no SDS.

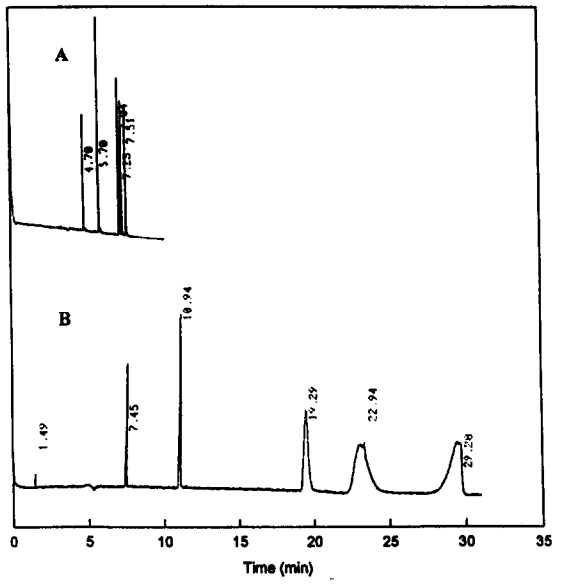

Fig. 2. Examples of the MEKC separations achieved with the bare capillary. Peak order: 1st = phenol; 2nd = *m*-cresol; 3rd = 2-ethylphenol; 4th = 3-ethylphenol; 5th = 4-ethylphenol. (A) Buffer: 50 m*M* SDS in 10 m*M* phosphate, pH 9.18. (B) Buffer: 50 m*M* SDS in 10 m*M* phosphate, pH 5.02.

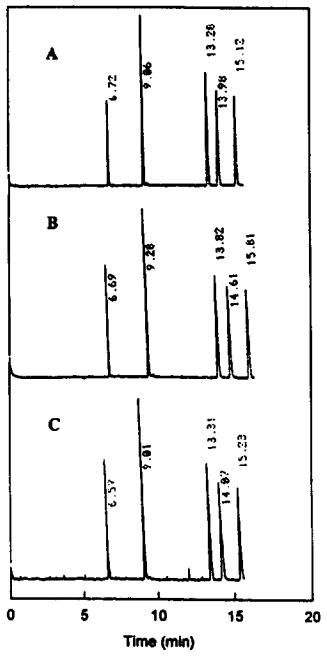

Fig. 3. Examples of the MEKC separations achieved with the anionic coated capillary. Peak order: 1st = phenol; 2nd = *m*-cresol; 3rd = 2-ethylphenol; 4th = 3-ethylphenol; 5th = 4-ethylphenol. (A) Buffer: 50 m*M* SDS in 10 m*M* phosphate, pH 9.18. (B) Buffer: 50 m*M* SDS in 10 m*M* phosphate, pH 5.02. (C) Buffer: 50 m*M* SDS in 10 m*M* phosphate, pH 4.03.

because the migration direction is opposite to that at neutral pH, and detection must be performed at the anodic end [4].

Lukacs and Jorgenson [5] have shown for CZE that a decrease in pH from 8 to 3 is accompanied by a significant decrease in the electroosmotic velocity in fused-silica capillaries. In MEKC, the EOF is more resistant to changes in pH as a result of the absorption of SDS to the inside wall of the capillary [6]. However, the EOF decreases dramatically below pH 5.5 because of the decrease in ζ potential due to dissociation of silanol groups on the capillary wall [4].

EOF is generated by the application of high voltage causing the migration of cations in the direction of the cathode. The SDS micelles are anionic and electrophorese toward the anode. The net velocity of the micelle (ν_{mc}) is thus dependent on the velocity vectors of the EOF (ν_{eo}) and the electrophoretic velocity of the micelle (ν_{ep}) [2] as stated in Eq. 1 below:

$$\nu_{mc} = \nu_{eo} + \nu_{ep} \quad (1)$$

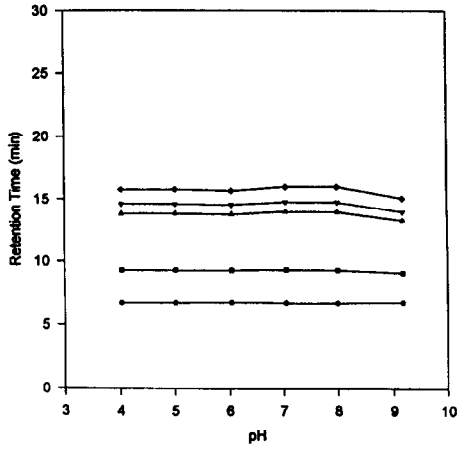

Fig. 4. Independence of anionic coated capillary retention time on pH. ● = Phenol; ■ = *m*-cresol; ▲ = 2-ethylphenol; ▼ = 3-ethylphenol; ◆ = 4-ethylphenol.

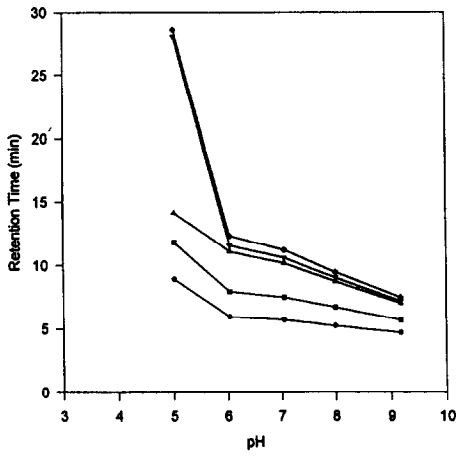

Fig. 5. Dependence of bare capillary retention time on pH. Symbols as in Fig. 4.

(In the case of anionic surfactants at neutral pH, the net velocity is directed towards the cathode.) The electrophoretic velocity of the micelle can be related to the charge and frictional properties of the particle by the simple relationship [7]:

$$\nu_{ep} = qE/f$$

where q is the net charge, E is the electric field strength and f is the translational friction coefficient. The electrophoretic velocity of the micelle remains constant because the charge on the micelle does not change under the conditions defined [6]. As a consequence, the net velocity of the micelle is determined by the EOF. If the EOF is controlled such that the net flow of the micelle is 0, it would be possible to achieve a stationary phase in MEKC. This would be equivalent to the traditional stationary phase of liquid chromatography. Investigation of this phenomenon is currently being pursued by our research group.

MEKC in a bare capillary at low pH will exhibit weak EOF. This means the net velocity of the micelle will be reversed or too slow, and no separation will be possible. In the case of the anionic coated capillary, the EOF remains constant with changes in pH because the surface charge density of the capillary is independent of the pH. This enables separations of analytes with low pK_a values via MEKC in the expanded pH range.

4. Acknowledgements

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