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Short Communication

Separation of estrogens and rodenticides using capillary electrophoresis with aqueous–methanolic buffers

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ABSTRACT

Capillary electrophoresis (CE) has proven to be an efficient method for the separation of various charged and neutral analytes. For analytes having limited solubility in water, the CE mode of separation has been micellar electrokinetic capillary chromatography (MECC). However, another approach is the direct addition of an organic solvent to a non-MECC CE separation system. Walbroehl and Jorgenson and also Balchunas and Sepaniak have reported on the use of organics in CE but the focus of their work was using MECC to separate small organic compounds. This work examines the use of aqueous–methanolic buffers in non-MECC CE separations of estrogens and rodenticides.

INTRODUCTION

The fact that most capillary electrophoretic (CE) separations are performed using aqueous separation buffers restricts the range of analytes to those soluble or partially soluble in water. Walbroehl and Jorgenson [1] reported on the use of acetonitrile as a non-aqueous CE medium in the separation of small organic bases (quinoline and isoquinoline), and later used acetonitrile with tetraalkylammonium perchlorate [2]. They concluded that the improved resolution obtained in the separation of small non-polar organic compounds was due to the increased electrophoretic mobilities of the analytes and the decreased electroosmotic flow due to the presence of acetonitrile. Thus, there appeared to be a role for this single-phase non-aqueous CE separation system in resolving non-polar analytes. This conclusion was confirmed by Fujiwara and Honda [3] in the separation of positional isomers of benzoic acid using an acetonitrile-water mixture.

Another approach is the addition of solvents to a micellar electrokinetic capillary chromatography (MECC) system. Here, Balchunas and Sepaniak [4] reported using 2-propanol to extend the sample range and capacity factor of MECC, while Gorse *et al.* [5] and Bushey and Jorgenson [6] demonstrated the use of organics in MECC separations of small organic compounds. But the question still remains as to the role of organic solvents in non-MECC separations of larger molecules of limited water solubility. Thus, this work examines the role of methanol in aqueous non-MECC separation of analytes of biochemical interest, the estrogens and rodenticides.

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EXPERIMENTAL

All capillary electrophoretic separations were performed at 20°C using Isco Model 3850 or Model 3140 variable-wavelength UV CE systems. The columns, 75 μm I.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA), were base treated (0.1 *M* NaOH) and rinsed with water prior to use. Samples were injected on column by vacuum. Acetone, methanol and acetonitrile (EM Science, Gibbstown, NJ, USA) were HPLC grade. Phosphoric acid (Midland Scientific, Omaha, NE, USA), acetic acid, boric acid and sodium hydroxide (Mallinckrodt, Paris, KY, USA), were all ACS-reagent grade. Cyclohexylamino-1-propanesulfonic acid (CAPS) was obtained from Research Organics (Cleveland, OH, USA). Warfarin and coumachlor were obtained from Aldrich (Milwaukee, WI, USA). Estriol, β-estradiol and estrone were obtained from Sigma (St. Louis, MO, USA).

RESULTS AND APPLICATIONS

Since Walbroehl and Jorgenson [1,2] reported a decreasing electroosmotic flow (EOF) with increasing concentration of acetonitrile, while Fujiwara and Honda [3] reported the opposite, we re-examined EOF as a function of percent acetonitrile or methanol. Fig. 1 confirms the effect of acetonitrile and likewise methanol on decreasing the EOF. Shown in Fig. 2 this effect is pH dependent, confirming a more complete

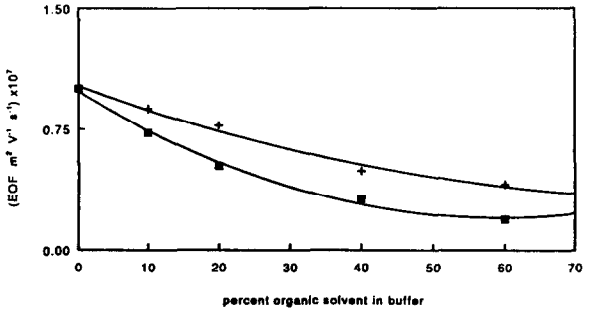

Fig. 1. The effect of methanol (■) and acetonitrile (+) on electroosmotic flow. Conditions: buffer, 50 m*M* sodium borate, pH 10; column, uncoated, 75 μm I.D., 65 cm total, 40 cm effective; 20°C; EOF marker, 0.01% mesityl oxide.

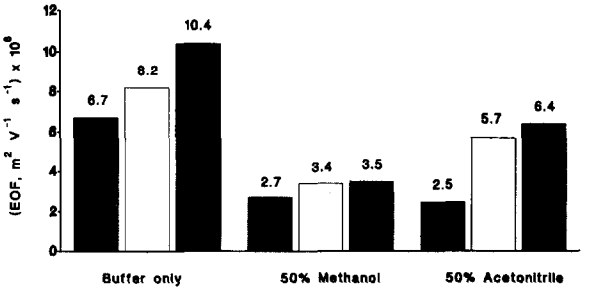

Fig. 2. Addition of methanol and acetonitrile to common capillary electrophoresis buffers: effect on electroosmotic flow. Conditions: final buffer concentration, 10 m*M*, not adjusted for ionic strength differences; column, uncoated, 75 μm I.D., 70 cm total, 45 cm effective; EOF marker, 0.01% mesityl oxide. Hatched bars = sodium acetate, pH 5; open bars = sodium phosphate, pH 7; solid bars = sodium borate, pH 9.

study by Kenndler and co-workers [7,8]. Thus, the organic solvent component of the separation buffer serves a dual purpose by solvating the analyte and by lowering the EOF, which can aid the resolving power of the system.

The first application of this work is the CE separation of the estrogens. These analytes have very limited solubility in water and are generally prepared in acetone. Fig. 3 demonstrates the effect of increasing methanol in the separation

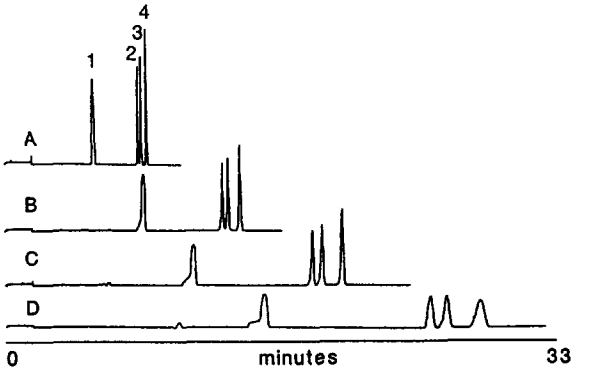

Fig. 3. Effect of increasing methanol concentration in buffer on the separation of steroids. (A) 0% methanol in buffer; (B) 10% methanol; (C) 20% methanol; (D) 30% methanol. Buffer: 100 m*M* CAPS, pH 11.5; 21 nl injection (4 ng). Peaks: 1 = acetone; 2 = estriol, 0.2 mg/ml; 3 = β-estradiol, 0.2 mg/ml; 4 = estrone, 0.2 mg/ml.

buffer on the resolution of the estrogens. Although 20% methanol is the practical limit of the organic solvent component because of the compromise between the loss of peak efficiency versus the limit of detection, this example does show promise should the sample contain interfering compounds.

The second application is the rodenticide, warfarin and its related compound coumachlor. Since compounds very similar to these are used clinically as anticoagulants, this separation is of biochemical interest. Fig. 4 shows the separation of these compounds as a function of increasing percent methanol in the separation buffer. As in the example of the steroids, there is a loss of peak efficiency at a gain of peak resolution, demonstrating a practical limit to the percent methanol.

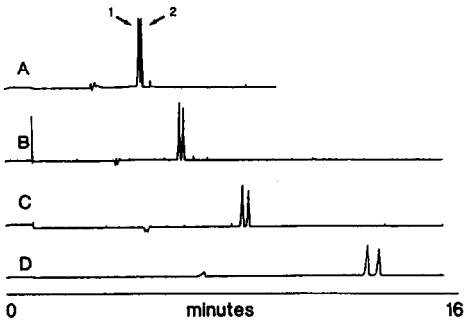

Fig. 4. Effect of increasing methanol concentration in buffer on the separation of rodenticides. (A) 0% methanol in buffer; (B) 10% methanol; (C) 20% methanol; (D) 40% methanol. Conditions: buffer, 20 m*M* sodium phosphate, pH 6.5; column, uncoated, 75 μm I.D., 70 cm total, 45 cm effective; 357 v/cm: 237 nm. Peaks: 1 = coumachlor, 100 μM; 2 = warfarin, 100 μM.

CONCLUSION

This work demonstrates that for the estrogens and rodenticides the addition of methanol to a non-MECC separation buffer improves resolution but at a loss of peak efficiency, and increased separation times. Thus, this approach may be limited to only specific applications of biomolecules.

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