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Capillary electrophoresis methods development and sensitivity enhancement strategies for the separation of industrial and environmental chemicals

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Abstract

This paper focuses on capillary electrophoresis (CE) methods development and sensitivity enhancement strategies for the separation of charged and neutral species of interest to industrial and environmental laboratories. Areas addressed include: (a) free solution and micellar electrokinetic capillary chromatography (MECC) methods development strategies; (b) the use of coated capillaries with MECC and reversed polarity to improve separation speed; (c) size separation using a sieving polymer; (d) the use of specific and selective detection techniques to detect environmentally important analytes and metabolites; (e) automated sample concentration techniques to enhance sensitivity by >1000×. Numerous analyte classes are used to illustrate solutions for industrial and environmental problems. These classes include chlorinated phenols, phenoxy acid herbicides, aromatic acids, isomerized α- and β-hop acids, nitroaromatic and nitramine compounds, acidic dyes, linear benzene alkyl sulfonates (LAS), inorganic anions, and charged polymers. Procedures to improve resolution and selectivity are discussed along with methods to enhance sensitivity of detection. Finally, unified methods development strategies to separate and detect analytes in the industrial and environmental setting are described.

Keywords: Method development; Sensitivity enhancement; Phenols; Nitro compounds; Pesticides; Alkylbenzene sulfonates, linear; Organic acids; Dyes

1. Introduction

Increasingly, reports are being published on the use of CE for industrial and environmental applications. These reports include the use the free solution electrophoresis (FSE) mode for the separation of chlorophenols [1] and phenoxy acid herbicides [2]. Micellar electrokinetic capillary chromatography (MECC) was applied to the separation of phenols [3], agrochemicals [4], phenolic carboxylic acid and flavonoids [5], hop bitter acids [6], and disperse basic dyes [7]. FSE and MECC were used to determine numerous organics (phenols, benzidines, aliphatic amines, polynuclear aromatics, herbicides, dyes, and phenoxyacid herbicides) [8]; phenols, nitoaromatic and nitramines [9,10]; phenols, anilines, and polynuclear aromatics [11]; numerous industrial analytes (oligomers, anionic surfactants, cationic monomers, complexing agents, alkylsulfates, sulfonates, formaldehyde/naphthalene sulfonic acid condensation products, amines, and metal-bipyridine complexes) have been analyzed by various CE techniques [12]; acidic and basic dyes [13]; dyes in synthetic foods [14]; anionic/nonionic surfactants [15], and linear alkyl benzene sulfonates (LAS) [16]. MECC with coated capillaries is also finding increas-

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ing applications in the industrial area with normal polarity for LAS separations using Triton-X treated capillaries [17] and polynuclear aromatics with reversed polarity [18].

Initially introduced as a technique for the separation of biological macromolecules, CE has attracted much interest in the environmental and industrial areas. The mechanisms responsible for the separation differ from those in chromatography. Therefore, CE represents an orthogonal and complementary technique to chromatography [19–21]. A number of authors have written well received CE reviews that are related to the goals of this paper. Review topics include: environmental samples [22], organic pollutants [8], industrial analysis [23], selectivity optimization with MECC [24–26], metal ion analysis [27], and low-molecular-mass ions [28]. More detailed coverage of topics relevant to the main goals of this paper can be found in books written or edited by some of the following: Li [29], Jandik and Bonn [30], Weinberger [31], Camilleri [32], and Landers [33].

In free solution electrophoresis (FSE), electroosmotic flow (EOF) can be used to great advantage at any pH higher than weakly acidic (e.g., pH>3). In most cases, a strong EOF flow is favorable and positive, neutral, and negatively charged species are all swept in the same direction past the detector. McLaughlin et al. [34,35] described the theory and practical effects of optimizing voltage, ionic strength, capillary dimensions, temperature, addition of organic modifiers, and numerous other FSE parameters using drugs and peptides. Neutral solutes do not separate by FSE, but migrate together at the velocity of the EOF. Terabe et al. [36] and Otsuka and Terabe [37] addressed the problem with the development of MECC. In MECC, a surfactant such as sodium dodecyl sulfate (SDS) is added to the buffer at a concentration above its critical micelle concentration (CMC). The micelles have a net negative charge and migrate in the opposite direction from the EOF; however, because the EOF is stronger, the micelles are dragged past the detector. The neutral solutes partition themselves between the micelle and the buffer, depending upon their hydrophobic nature. The longer a solute remains in the micelle, the later it will elute. Due to the hydrophobic nature of many molecules commonly encountered in industrial and environmental chemistry, MECC is frequently the most attractive choice.

The most common approach in CE for the detection of ionic and non-ionic compounds containing chromophores is direct UV detection. In situations where the concentration is extremely low, many alternative methods of enhancing UV–Vis detector sensitivity have developed [36–40]. Szücs et al. [41] used hop bitter acids to study sample stacking effects.

Indirect detection using a chromophoric electrolyte has been used for analytes without chromophores. Field amplified stacking combined with indirect detection with has proven to be the best method for increasing sensitivity of inorganic ions using a low ionic strength buffer [42,43].

However, a more straight forward detection technique for non-chromophoric analytes is via their conductance in a low conductive medium. The first conductivity detectors [44–46] revealed the following serious problems in the detection of ions: (a) background noise from the high voltage prevents high-sensitivity detection, and (b) sensitivity depends on the difference in conductivity between the analyte and the electrolyte. To determine the levels of solute ions in the presence of vast amounts of bulk buffer ions, it is necessary to convert the buffer ions to a weakly conducting form.

The most efficient and highly successful approach to the suppression of buffer ions has been adapted from ion chromatography (IC) [47]. An ion-exchange membrane positioned between the separator column and conductivity cell converts the buffer ions to their weakly conductive form. The suppressor is placed in the outlet vial, which contains the regenerant and the ground electrode. This combination, called suppressed conductivity detection (CE–SC), eliminates, or at least greatly decreases, conductivity due to the bulk ions, while simultaneously enhancing the detectability of the analyte ions. Although suppressed conductivity is a well established technique in IC, it was not until 1995 that the first report of suppressed conductivity detection for CE was published [48]. Avdalovic et al. [49] and Stillian et al. [50] reported on the improved determination of cations and anions by CE combined with suppressed conductivity detection.

The main goals of this paper are (a) present a

unified methods development strategy for the separation of industrial and environmental analytes; (b) to demonstrate CE methods development optimization for key parameters that can be manipulated to improve separation with cause and effect overlay illustrations; and (c) offer alternative methods to enhance sensitivity of analytes with poor chromophores or at trace level concentration. A variety of analytes of industrial significance have been selected to illustrate the flexibility of CE. In some cases, the electropherograms are used to illustrate an effect, and are not necessarily the completely optimized separation.

2. Experimental

2.1. CE Instrumentation

Some experiments were performed using either the CES-I Capillary Electrophoresis System (Dionex, Sunnyvale, CA, USA; indicated by use of temperature=ambient) with a prototype ED40 detector (suppressed conductivity or amperometric detection, Dionex) or on a prototype instrument (Peltier-controlled liquid temperature control) with AD20 (Dionex) UV absorbance detection. Data analysis and control were accomplished using the Dionex PeakNet Chromatography Workstation (software version 4.20) and UI20 analog interface (Dionex). All data were translated into ASCII format and electronically transferred for comparison. Fused-silica (FS) capillaries (Polymicro Technologies, Phoenix, AZ, USA) ranged from 75 μm I.D. and 360–375 μm O.D. Proprietary hydrophilic coated capillaries (Dionex) were prepared in-house.

2.2. Chemicals

All buffers were prepared from reagent-grade salts, filtered through 0.45 μm membrane filters, and degassed prior to use. Hexamethonium hydroxide, sodium octanesulfonate, and anion regenerant solution (5 m*M* sulfuric acid) were obtained from Dionex. CAPS (3-[Cyclohexylamino]-1-propanesulfonic acid) was obtained from Sigma (St. Louis, MO, USA). HPLC grade methanol, isopropanol, and acetonitrile were obtained from EM Science (Gibbstown, NJ, USA). All other inorganic salts/acids/bases used to prepare buffers were obtained from Sigma, Eastman Kodak (Rochester, NY, USA), Pierce (Rockford, IL, USA), Fisher (Fair Lawn, NJ, USA), Aldrich (Milwaukee, WI, USA), Fluka (Buchs, Switzerland), and E. Merck (Darmstadt, Germany). The 500 000 molecular mass dextran polymer was acquired from Polysciences (Warrington, PA, USA).

Chemical standards used as analytes were obtained from various sources: chlorinated phenols and phenoxy acid herbicides; nitroaromatic and nitramine compounds were obtained from Chem Services (West Chester, PA, USA); aromatic acids/reaction intermediates from Sigma, Fluka, and Aldrich; dyes from Eastman Kodak, Aldrich and Fluka; linear benzene alkyl sulfonates (LAS) from Monsanto (St. Louis, MO, USA); and inorganic anions/cations from Aldrich and Sigma. The polyacrylic acid polymers were a gift from L. Lépine of Hydro Québec (Québec, Canada).

2.3. Capillary electrophoresis conditions

Experimental conditions (e.g., buffer system, separation voltage, injection technique, detection wavelength) are given with individual figures. Pressure injections were made using 3.45 kPa (0.5 psi).

3. Results and discussion

3.1. Unified methods development strategy for FSE and MECC

This section is offered in an attempt to offer the reader a unified strategy to develop a CE method for the separation of industrial and environmental analytes using FSE and MECC.

3.1.1. Ensure sample solubility in the separation solution

Some industrial analytes are soluble in aqueous buffers, however more hydrophobic solutes may require the use of additives. The need for high percentages (>20%) of an organic solvent to dissolve the sample indicates use of MECC. Surfactants can help.

3.1.2. Choose a capillary length and diameter

A good first choice is a 50 cm×50 μm I.D. fused-silica capillary. Separation complexity dictates capillary length (35–40 cm for 2–10 analytes, 50–60 cm for 11–50 analytes, 70–80 cm for 50–80 analytes, and 90–100 cm for >80 analytes). Efficiency, resolution, detection limits, and mass loading requirements dictate capillary diameter. For best efficiency use 25 or 50 μm I.D. capillaries; for best UV detection limits use 50–100 μm I.D. capillaries; and, for best mass loading use 100–200 μm I.D. capillaries. Coated capillaries are recommended for difficult separations, better reproducibility, and for fast MECC with reversed polarity.

3.1.3. Choose a capillary temperature

A good first choice temperature is 20–25 °C. For chiral separations, and high concentration buffers use 15–20 °C. For faster separations, or if required to solubilize analytes, use 30–60 °C. Vary the set temperature from 20–60 °C in 5 °C increments to optimize solubility, selectivity, or conformational stability.

3.1.4. Optimize buffer pH

Select a buffer that gives good pH control in the region of interest. Investigate pH above and below the pK_a of the analyte of interest. Use small pH changes (i.e., 0.1–0.5 pH units) to optimize the separation. If the pK_a values for a sample are unknown, conduct initial separations in appropriate buffers at or near pH 2.5, 4.0, 5.5, 7.0, 8.5, and 10 to find promising pH range.

3.1.5. Optimize buffer concentration

A good first choice for buffer concentration is 50–100 m*M* with a 50 μm I.D. capillary or 25–50 m*M* with a 75 μm capillary. Select buffer concentration depending on separation requirements. Use lower ionic strengths for speed, relatively few analytes, or when separation selectivity is high. Use higher ionic strengths for closely related analytes, numerous analytes, or micropreparative scaleup. To stack samples, maximize ionic strength differences between the sample and the buffer. Zwitterionic and denaturing agents can be used at high concentrations (>1 *M*).

3.1.6. Optimize separation voltage

Produce an Ohm's Law Plot (voltage versus current) with the desired buffer from 0 to 30 kV in 2.5 kV. For a given buffer and capillary the Ohm's law plot indicates the voltage that will give the fastest separation with optimum efficiency and resolution. Use the highest voltage possible within the relatively linear range of the plot.

3.1.7. Use additives to maximize differences or mask interactions

Ion pair reagents (e.g., 10–100 m*M* pentanesulfonic acid) are effective in promoting the separation of short, hydrophilic solutes. Use MECC with 25–200 m*M* SDS for hydrophobic and neutral species. SDS works well at neutral and basic pH, while bile salts such as cholates are effective at acidic pH. Add non-ionic surfactants (up to 50 m*M*) or organic modifiers (1–25% by volume) to modify partitioning and change selectivity. Use dibasic amines (0.1–5 m*M*) to modify EOF and reduce interactions with the capillary wall at low pH. Use zwitterions (25 m*M*–1.5 *M*), denaturants (50 m*M*–8 *M*), or ethylene glycol to minimize hydrophobic interactions among solutes and with the capillary wall. Use sieving buffers (e.g., 1–15%, w/v of dextrans) for size–based separations of analytes with large differences in size.

3.2. pH optimization of aromatic acids and reaction intermediates

Buffer pH plays an important role in CE because it affects both the overall charge of the analyte and the EOF. Furthermore, pH is the most important parameter used to optimize selectivity, resolution, and peak shape; even small differences in the pK_a value of the compounds can be the basis of separating closely related molecules by CE. Effective resolution of mixtures may involve titration across the pK_a of the compounds. The effect of the pH was studied for a mixture of eight aromatic acids by varying a 100 m*M* sodium tetraborate electrolyte from pH 8.55 to 9.3 with 400 m*M* boric acid (Fig. 1A–D). In general, migration times increased as the pH increased. Even though the EOF increased as the pH increased, the more highly charged analytes moved to longer migration times because they are attempting to move

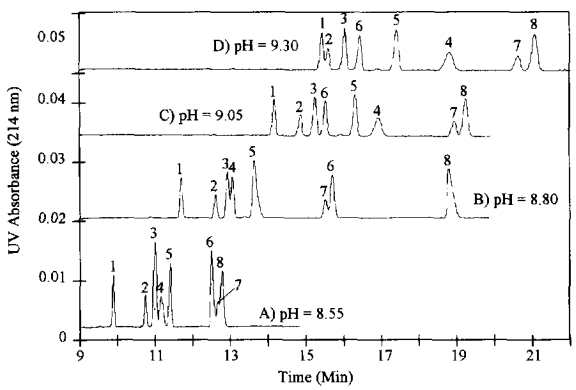

Fig. 1. (A–D) pH optimization of aromatic acids/chemical reaction intermediates. Capillary, 55 cm (L_d)×60 cm (L_t)×50 μm I.D. FS; buffer, 100 m*M* sodium tetraborate adjusted to pH 8.55–9.3 with 400 m*M* boric acid, (A) pH 8.55, (B) pH 8.80, (C) pH 9.05, (D) pH 9.30; voltage, (A–E) 25 kV, (F) 30 kV; temperature, ambient (forced air cooling); detection, UV (200 nm); injection, gravity (10 s at 150 mm); concentration, 40 μg/ml in water; peaks, (1) 4-hydrophenylacetic acid, (2) phenoxyacetic acid, (3) 2-bromobenzoic acid, (4) salicylic acid, (5) 2-chlorobenzoic acid, (6) 4-hydroxybenzoic acid, (7) nicotinic acid, (8) benzoic acid.

against the EOF. Most compounds maintained the same elution order. However, peaks 4 and 6 dramatically changed their migration behavior. Some pairs (e.g., 1, 2) achieved only weaker resolution as the pH increased, while others (e.g., 7, 8) were better resolved.

3.3. Separation of phenoxy acid herbicides using an EOF modifier

In some cases, it is necessary to modify or even reverse the EOF to achieve the desired separation when resolution is either inadequate or excessive. The EOF is reversed by adding controlled amounts of a long chained amine modifier. While one end of the molecule binds strongly to the acidic silanol moieties on the wall of the separation capillary, the alkyl chain protrudes into solution and associates with other hydrophobic tails, thus generating a positive layer reaching into the inner lumen of the capillary. Examples of additives used in this manner include additives such as 1,4-diaminobutane [51], piperazine [52], cetyltrimethylammonium bromide (CTAB) [53], tetradecyltrimethylammonium bromide (TTAB) [54], hexamethonium bromide [55,56] and diethylenetriamine (DETA) [42,43] at levels of 0.5–5 m*M*. Eliminating capillary wall surface interactions by using an appropriate amine modifier at low pH results in better recovery and peak shape improvement. Fig. 2 shows an example of the use of hexamethonium hydroxide to reverse the EOF for the separation of eight chlorinated phenoxy acid herbicides. Baseline resolution of all components was achieved in less than 6 min.

3.4. Ionic strength optimization of chlorinated phenols

Ionic strength of the buffer is another tool that an investigator can use to improve efficiency, sensitivity, and resolution [34,35] in FSE. In this study, improvement of the separation of chlorinated phenols was achieved by optimizing the ionic strength of the electrolyte (Fig. 3), The total sodium concentration of the buffer was varied: (3A) 49.8 m*M*, (3B) 60.9 m*M*, (3C) 72 m*M*, (3D) 84 m*M*, (3E) 95 m*M*. As ionic strength increased lower mobilities were observed (mobility is directly related to zeta potential and inversely to time). Peak height also increased as ionic strength differences between the sample (water) and run buffer were increased. This effect, known as focusing, is caused by the higher electric field strength in the sample injection plug (water) com-

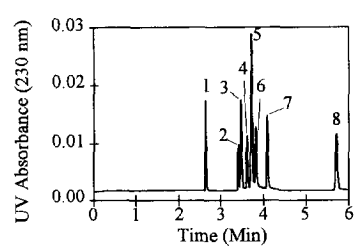

Fig. 2. Separation of environmentally important chlorinated phenoxy acid herbicides using EOF modifier. Capillary, 45 cm (L_d)×50 cm (L_t)×50 μm I.D. FS; buffer, 19 m*M* disodium monohydrogenphosphate–19 m*M* sodium dihydrogenphosphate–0.75 m*M* hexamethonium hydroxide (adjusted pH to 2.9 with phosphoric acid); voltage, −20 kV; temperature, ambient; detection, UV (230 nm); injection, gravity (10 s at 50 mm); concentration, 5–100 μg/ml in water; peaks, (1) Dicamba, (2) 2,4,5-T, (3) 2,4-D, (4) Silvex, (5) Dichlorprop, (6) MCPP, (7) MCPA, (8) 2,4-DB.

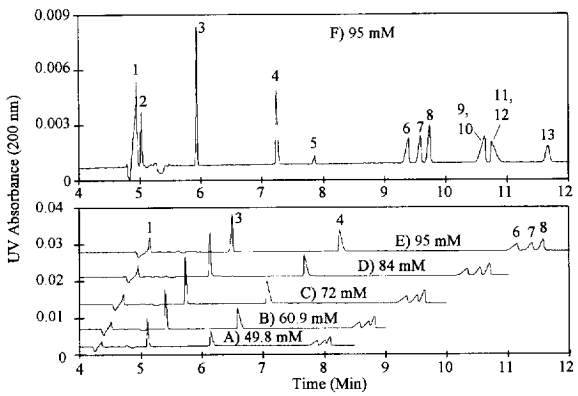

Fig. 3. (A–F) Ionic strength optimization of chlorinated phenolic solutes and (G) optimized FSE separation of chlorinated and other environmentally related phenols. Capillary: 52.8 cm (L_d)×60 cm (L_t)×50 μm I.D. FS; buffer (all pH 8.0), (A) 16.8 m*M* sodium tetraborate–33 m*M* sodium dihydrogenphosphate, (B) 21.4 m*M* sodium tetraborate–39.5 m*M* sodium dihydrogenphosphate, (C) 26 m*M* sodium tetraborate–46 m*M* sodium dihydrogenphosphate, (D) 31 m*M* sodium tetraborate–53 m*M* sodium dihydrogenphosphate, (E–F) 35 m*M* sodium tetraborate–60 m*M* sodium dihydrogenphosphate; voltage, (A–E) 25 kV, (F) 30 kV; temperature, 25 °C; detection: UV (200 nm); injection, pressure (5 s at 3.45 kPa); concentration, 5–100 μg/ml in water; peaks: (1) phenol, (2) 4-chloro-3-methylphenol, (3) 2-chlorophenol, (4) 2,4-dichlorophenol, (5) 4-cresol (impurity), (6) 2,4,6-trichlorophenol, (7) 2,3,4,6-tetrachlorophenol, (8) pentachlorophenol, (9) 2-nitrophenol, (10) 2,4-dinitrophenol, (11) 4-nitrophenol, (12) 2-cresol, (13) 3-cresol (impurity).

pared to the low electric field strength in the electrolyte (m*M* as indicated). The solutes in the sample migrate rapidly until they reach the lower field in the run buffer, where they stack. Since the current increased in direct proportion to the ionic strength (e.g., >5 W/m), good temperature control was essential to realize the gain in efficiency and resolution obtained.

A more complicated mixture of the same analytes plus other environmentally significant phenols (Fig. 3F) were separated at 30 kV on the same capillary using a high ionic strength buffer. Most compounds were baseline resolved in less than 12 min. A few compounds coeluted because of their similar charge to mass ratios under the conditions used. They are easily resolved using MECC with the addition of 50 m*M* sodium dodecyl sulfate (SDS, data not shown).

3.5. Optimization of SDS concentration of isomerized α- and β-acids from hop extracts

Increasing the concentration of the micelle can increase resolution, efficiency, and selectivity in MECC [3,34,36,37]. Vindevogel and Sandra [6] investigated many parameters to optimize the MECC separation of isomerized α- and β-acids from hop extracts. They stated that resolution is not further improved upon increasing the SDS concentration (max. 40 m*M*). This observation is probably the result of internal capillary joule heating at the elevated levels of SDS. In this study the optimization of the SDS concentration was investigated at much higher levels to produce much stronger stacking conditions. The effect of SDS concentration on the separation of the α-acids was studied over the range of 25–200 m*M* SDS (Fig. 4A–D) using a sodium tetraborate electrolyte at pH 8.55. The hydrophobic samples were initially dissolved in 100% methanol

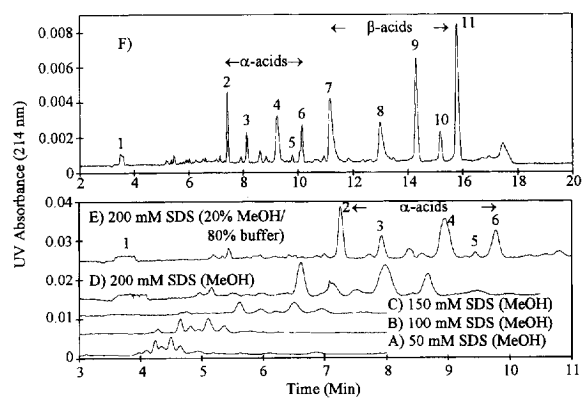

Fig. 4. (A–E) MECC SDS concentration optimization of isomerized α-acids and (F) optimized separation of isomerized α-acids and β-acids from hop extracts: (A) 50 m*M* SDS, (B) 100 m*M* SDS, (C) 150 m*M* SDS, (D-F) 200 m*M*. Capillary: 55 cm (L_d)×60 cm (L_t)×50 μm I.D. FS; buffer, 25 m*M* sodium tetraborate adjusted to pH 8.55 with 100 m*M* boric acid–with 50–200 m*M* SDS (as indicated); voltage, 30 kV; temperature, ambient (forced air cooling); detection, UV (214 nm); injection: gravity (50 s at 150 mm); concentration, (A–D) 500 μg/ml dissolved in methanol, (E–F) 500 μg/ml dissolved in methanol–buffer (20:80); peaks, (1) formamide (marker), (2) *cis*-isocohumulone, (3) *trans*-isocohumulone, (4) *cis*-isoadhumulone, (5) *cis*-isohumulone, (6) *trans*-isohumulone, (7) colupulone, (8) lupulone, (9) adlupulone, (10) adhumulone, (11) humulone.

(indicated as MeOH in Fig. 4A–D). As the concentration was increased from 50 to 200 m*M* SDS, the peaks were better resolved and moved to longer migration times; however, efficiency was sacrificed. Reinjection of the sample dissolved in a buffer–methanol mixture (80:20, v/v) showed improved peak shapes and resolution of a number of peaks not seen previously (Fig. 4E). Fig. 4F is an expanded view of the optimized separation of the isomerized α- and β-acids from hop extracts. Good peak symmetry and resolution of more than 30 peaks and shoulders was obtained in less than 18 min. This method makes it relatively easy to qualitatively determine the hop acids in beer samples. However, the hop acid concentrations in beer samples are relatively low, making quantitation a problem. Other stacking techniques to improve detection limits of hop acids in beers are being investigated.

3.6. Voltage optimization for the MECC separation of acidic dyes

Voltage mainly affects speed, theoretical efficiency, resolution, and internal joule heating in the capillary [34]. Brinkshaw et al. [7] and Hinks et al. [14] have investigated the application of FSE and MECC to a number of acidic, neutral, and basic dyes. Acidic, basic disperse, and dyes of the Sudan series were also investigated in this study. Solubility experiments indicated probable use of MECC for most of the dyes studied. After initial pH, ionic strength, and SDS concentration optimization; a sodium tetraborate buffer (pH 8.8) containing 75 or 125 m*M* SDS was chosen. Voltage was varied from 15 to 30 kV (data shown for 17.5–25 kV in Fig. 5A-D). Calculated efficiency and resolution increased from 15 to 20 kV, plateaued at 22.5 kV, and decreased at values higher than 22.5 kV. The separation time decreased as voltage was increased. The separation was still adequate at 25 kV to determine the relative purity of the dye mixtures. Ten acidic dyes were separated in less than 10 min (Fig. 5D) with fairly good peak symmetry at 25 kV. Some dyes in the mixture were found to be relatively impure; Methyl Red produced 4–5 peaks and Acid Green 25 showed three major peaks that partially coeluted with Orange II. A second example shows a CE method

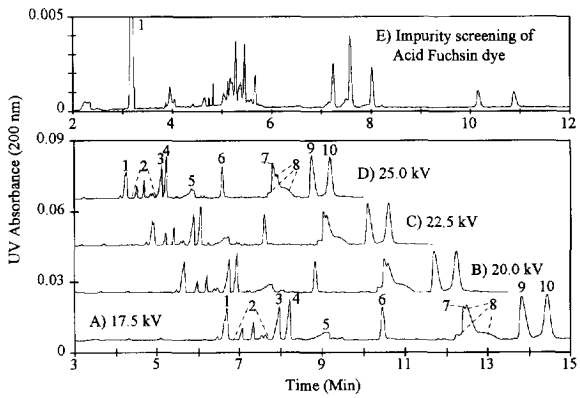

Fig. 5. (A–D) Voltage optimization for MECC separation of acidic dyes and (E) impurity screening of acid fuchsin dye by MECC. Capillary, 42.8 cm (L_d)×50 cm (L_t)×75 μm I.D. FS; buffer, 15 m*M* Sodium tetraborate adjusted to pH 8.8 with 15 m*M* boric acid with, (A) 125 m*M* SDS, (B) 75 m*M* SDS; voltage, (A) 17.5 kV, (B) 20 kV, (C) 22.5 kV, (D–E) 25 kV; temperature, 25 °C; detection, UV (210 nm); injection, pressure (5 s, 3.45 kPa); concentration, 250 μg/ml in water; peaks, (1) benzyl alcohol (marker), (2) Methyl Red, (3) Bromophenol Blue, (4) Phenol Red, (5) Alizarin Red S, (6) Methyl Orange, (7) Orange II, (8) Acid Green 25, (9) Acid Blue 113, (10) Acid Red 114.

for determining the purity of chemically synthesized dyes or dyes extracted from natural substances. Impurity screening of acid fuchsin dye (Acid Violet 19) by MECC was accomplished in less than 12 min (Fig. 5E). These results show that CE can resolve complicated mixtures of dyes found in foods, textiles, pharmaceuticals, and other commercial products.

3.7. Use of organic solvents in the MECC separation of nitroaromatic/nitramine compounds

Using organic modifiers as additives to the electrolyte selectivity, resolution and peak symmetry may improve by a change in the zeta potential, viscosity, dielectric constant, permittivity, and net charge of the micelles [34]. We investigated the effect of different organic modifiers on the MECC separation of 12 nitroaromatic and nitramine compounds (Fig. 6 A–D). As the organic modifiers were added, all the peaks moved to later migration times. A careful inspection of the enlarged portions (Fig. 6

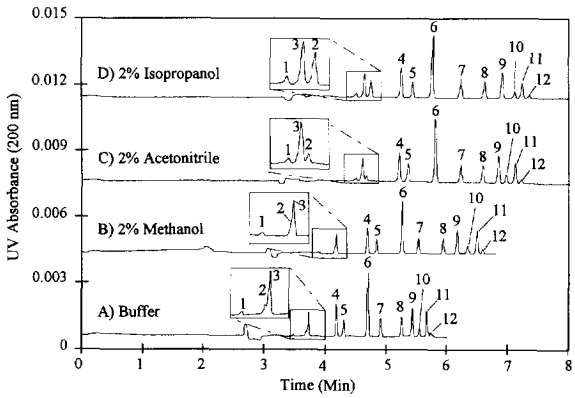

Fig. 6. MECC separation of nitroaromatic and nitramine compounds using organic modifiers. Capillary, 45 cm (L_d)×50 cm (L_t)×50 μm I.D. FS; buffer, 10 m*M* sodium borate–50 m*M* boric acid–50 m*M* SDS in (A) 100% water (B) water–methanol (98:2, v/v), (C) water–acetonitrile (98:2, v/v), (D) water–isopropanol (98:2, v/v); voltage, 20 kV; temperature, ambient; detection, UV (230 nm); injection, gravity (10 s at 50 mm); (1) HMX, (2) 1,3,5-trinitrobenzene, (3) RDX, (4) 1,3-dinitrobenzene, (5) nitrobenzene, (6) 2,4,6-trinitrotoluene, (7) Tetryl, (8) 2,4-dinitrotoluene, (9) 2,6-dinitrotoluene, (10) *o*-nitrotoluene, (11) *p*-nitrotoluene, (12) *m*-nitrotoluene.

insets) indicates that buffer alone does not resolve 1,3,5-trinitrobenzene and RDX (peaks 2 and 3, Fig. 6A). Adding 2% (v/v) methanol causes peak 2 to move directly under peak 3 (Fig. 6B). Adding 2% (v/v) acetonitrile allows almost complete resolution of peaks 2 and 3 as well as a selectivity change, causing a reversal of migration order (Fig. 6C). The effect is more clearly seen using 2% (v/v) isopropanol, where the peaks are now clearly resolved to baseline (Fig. 6D). An additional benefit of adding the organic modifiers is that several other compounds are better resolved than with buffer alone (see peaks 10–12). In general, small amounts of organic modifiers (1–20%, v/v) may be useful to achieve selectivity changes, while concentrations higher than 20% (v/v) can disrupt micelle formation in MECC buffers and thus should be avoided.

3.8. MECC separation of linear benzene alkyl sulfonates (LAS) with a coated capillary

Recently Janini et al. [18] demonstrated the separation of hydrophobic species using MECC on a coated capillary (eliminates EOF). After reversal of the polarity, extremely fast separations can be achieved of analytes incorporated into the micelles. The relatively hydrophobic LAS surfactants contain variable alkyl chain lengths (e.g., less than C_{10} to C_{16}) which can also be highly branched. HPLC methods can only achieve separations with low efficiencies and long run times. Zweigenbaum [34] used FSE with a TRIS–glycine buffer containing 50% (v/v) acetonitrile to produce a rapid, highly efficient separation that showed 10–12 LAS related peaks in 13 min. Using Triton-X treated capillaries the MECC separation of LASs resolved more than 20 peaks and shoulders, however, due to the EOF decrease, the analysis took almost 70 min [17]. A similar buffer was used but using a coated capillary which provided a reversed polarity compared to normal MECC separations. Here, a neutral, hydrophilic coated capillary was used in conjunction with a CAPS–SDS–sodium octylsulfonate–20% acetonitrile buffer to separate a number of LAS samples (Fig. 7A–E). The light LAS sample contains shorter alkyl chains (Fig. 8A) and the heavy sample reveals longer alkyl side chains (Fig. 8D). The LAS mixture contains both the light and the heavy chains (Fig.

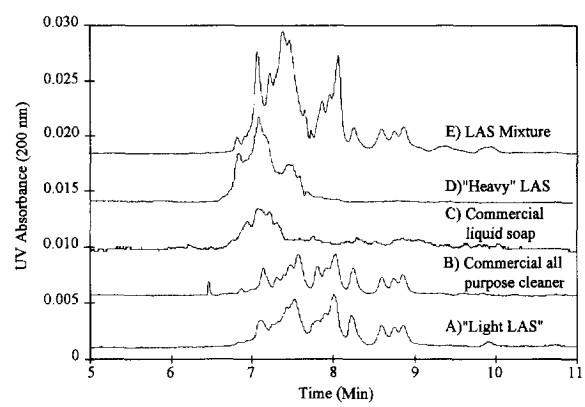

Fig. 7. Separation of LAS samples and commercial cleaners using MECC, coated capillary, and reversed polarity. Capillary, 42.8 cm (L_d)×50 cm (L_t)×75 μm I.D. hydrophilic coated FS; buffer, 8 m*M* CAPS–20 m*M* SDS–40 m*M* sodium octylsulfonate in water–acetonitrile (80:20, v/v); voltage, 25 kV; temperature, 22.5 °C; detection, UV (224 nm); injection, pressure (5 s, 3.45 kPa); concentration, (A, D, E) 2 mg/ml total in water, (B, C)100 mg/ml commercial all purpose cleaner and liquid soap total in water; peaks, (A) Light LAS mixture, (B) commercial all purpose cleaner, (C) commercial liquid soap, (D) Heavy LAS mixture (E) LAS formulation A235.

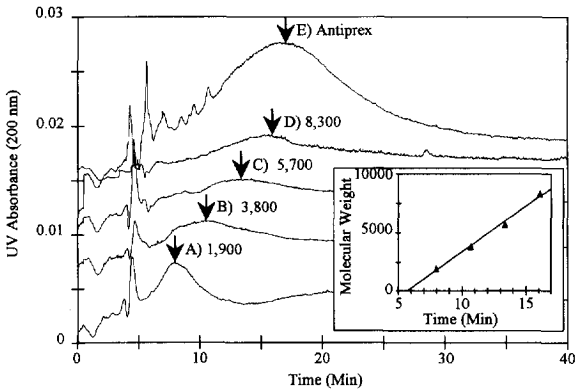

Fig. 8. (A–E) Sieving separation of polyacrylate (PAA) polymers, inset shows molecular mass (arrow indicates center of a particular molecular mass distribution) versus time calibration plot. Capillary, 42.8 cm (L_d)×50 cm (L_t)×75 μm I.D. hydrophilic coated FS; buffer, 50 m*M* Tris, 100 m*M* boric acid, 0.1% SDS, 12.5% Dextran (M_r 500 000); voltage, 30 kV; temperature, 25 °C; detection, UV (200 nm); injection, electrokinetic (60 s, 10 kV); concentration, (A–D) 500 ppm in water, (E) 1–10 dilution with water; Peaks, (A) 1930 M_r PAA, (B) 3800 M_r PAA, (C) 5700 M_r PAA, (D) 8300 M_r PAA (E) Antiprex polyacrylic acid polymer solution.

7E). Two examples of commercial products were chosen to illustrate the use of MECC. The all purpose cleaner and the liquid soap appears to contain light LASs (Fig. 7B) and heavy LASs (Fig. 7C), respectively. With no EOF the hydrophobic analytes move rapidly through the capillary with good efficiency to partially resolve the branched chains. Although the LASs are not completely resolved, the ability to recognize a pattern was adequate to identify LASs in commercial products.

3.9. Size separation of polyacrylates

Sieving polymers are frequently used to separate proteins and other biomolecules using the dynamic sieving electrophoresis (DSE) mode of CE. In DSE a dilute polymer (e.g., dextran) is incorporated into the buffer at a concentration to induce a sieving mechanism [57]. Rapid determination of the apparent size distribution of industrial polymers is frequently required. Therefore DSE was applied to improve the separation of polyacrylates using a neutral hydrophilic coated capillary and a Tris–borate buffer containing 12.5% dextran (M_r 500 000) and 0.1% SDS (Fig. 8A–E). A calibration curve of M_r versus migration time for four standard compounds (Fig. 8 inset) was relatively linear, indicating a sieving mechanism. The Antiprex sample contains a diversity of polyacrylates with a molecular mass ranging from 2000 to approximately 15 000 (Fig. 8E), indicating that CE can be used to fingerprint polyacrylates with an acceptable separation time. Other sieving media, however, should be tested to generate a higher efficiency.

3.10. Sensitivity enhancement of non-chromophoric inorganic anions using CE with suppressed conductivity detection (CE–SC)

A large group of ionic compounds, mainly inorganic anions and cations, were analyzed in a variety of matrices including waste water, chemical products, and aerosols. Many either have poor UV chromophores or are at trace level concentrations. A refinement was made to the CE–SC design originally proposed by Avdalovic et al. [49] and Dasgupta and Bao [48]. CE–SC is a highly sensitive and selective method for the determination of UV-transparent ionic species using direct injection techniques without sample preconcentration (no electrostacking). A CE–SC separation of 11 inorganic anions is shown in Fig. 9. Minimum detection limits for common organic and inorganic ions are in the range of 1–10

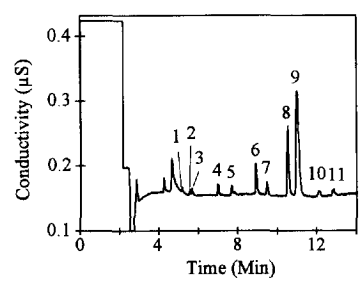

Fig. 9. Detection of inorganic anions at trace levels using suppressed conductivity detection. Capillary, 55 cm (L_d)×60 cm (L_t)×75 μm I.D. FS; buffer, 2 m*M* sodium tetraborate, pH 9.2; voltage, 24 kV; temperature, ambient (forced air cooling); detection, suppressed conductivity; suppressed conductivity regenerant, 10 m*M* sulfuric acid at 1 ml/min; injection, gravity (3 s at 30 mm); concentration, 1.9–12 μg/l (or ppb); peaks, (1) chlorite, (2) fluoride, (3) phosphate, (4) chlorate, (5) perchlorate, (6) nitrate, (7) nitrite, (8) sulfate, (9) chloride, (10) bromide, (11) chromate.

ppb. The major benefit of CE–SC is that it is 100× more sensitive than non-suppressed conductivity detection, thus representing a complementary technique to the classical ion chromatography with regards to selectivity, efficiency, and analysis time.

3.11. High ionic strength buffer field amplification capillary electrophoresis (HISFACE)

In many cases CE seems to lack sufficient sensitivity to detect trace level amounts of chromophoric analytes. High ionic strength focusing allows concentration of analytes by stacking dilute samples during separation in a high ionic strength buffer. Maximizing the ionic strength differences between the sample and the buffer yields the best focusing (5–50× increase in sensitivity). Previously, detection limits were determined for three aromatic acids using high ionic strength focusing of a normal injection (5 s at 3.45 kPa dissolved in water) using a 60 cm×75 μm fused-silica capillary. The detection limits at 200 nm (S/N=3, data not shown) were: 2-bromobenzoic acid (103 μg/l, 0.51 μM), benzoic acid (180 μg/l, 1.47 μM), and benzenesulfonic acid (65 μg/l, 0.41 μM).

Chien and Burgi [42,43] proposed concentrating large volumes of dilute samples using field amplification. Field amplified stacking was applied to separation of anions using relatively dilute buffers (e.g., 2–10 mM) resulting in better than a 100× increase in sensitivity. In this study the techniques of high ionic strength focusing and field amplified capillary electrophoresis (HISFACE) were combined. The schematic in Fig. 10 shows the steps of HISFACE. (A) The capillary is initially filled with high ionic strength buffer and a large sample (e.g., 75% of capillary volume) is loaded using low pressure. (B) The water plug is removed and the sample concentrated by applying voltage with the polarity reversed (EOF forces cations and water plug to exit capillary, anions concentrate). (C) The voltage is stopped before the compounds of interest (anions) exit the capillary. (D) The separation voltage is reapplied (after reversing polarity again) and anions are further concentrated using high ionic strength stacking.

Fig. 11 shows the effect of using the HISFACE technique to concentrate a very dilute mixture of the same three anions. The concentration for all three compounds range from 256–512 ppt (Fig. 11A).

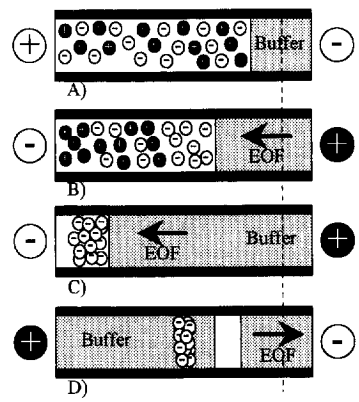

Fig. 10. Representation of high ionic strength buffer field amplification capillary electrophoresis (HISFACE), (A) Large sample (prepared in water) injected (225 s, 3.45 kPa, 75 μm×60 cm), no voltage is used; (B) high voltage (−10 kV) is applied to capillary with polarity reversed (outlet positive); (C) water and positive ions are removed while the capillary retains the (−) analytes, when current reaches 95–98% of the original value, voltage is stopped; (D) Polarity is switched to normal (outlet negative), separation voltage (25 kV) applied, further stacking occurs to improve sensitivity.

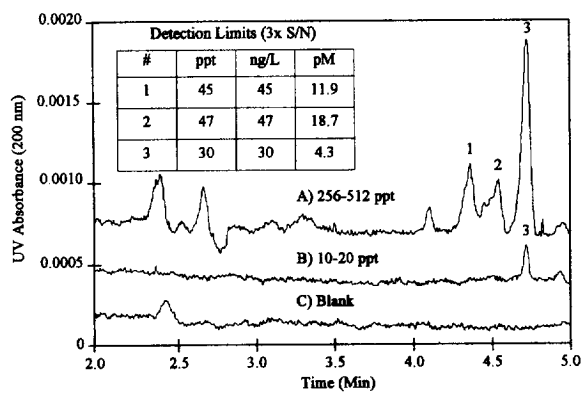

Fig. 11. Concentration of a large volume-low concentration sample using HISFACE. Capillary, 52.8 cm (L_d)×60 cm (L_t)×75 μm I.D. hydrophilic coated FS; buffer, 125 mM sodium tetraborate adjusted to pH 8.3 with 125 mM boric; voltage, 25 kV; temperature, 25 °C; detection, UV (200 nm); injection, pressure (225 s, 3.45 kPa); field amplification, water plug removal step, −10 kV for 155 s, concentration, (A) water blank, (B) 10.2–20.4 ng/l (or ppt) in water, (C) 256–512 ng/l (or ppt) in water; peaks, (1) 2-bromobenzoic acid, (2) benzoic acid, (3) benzenesulfonic acid.

After the anions are further diluted (10–30 ppt), only benzenesulfonic acid is visible (Fig. 11B). The water blank shows no peaks in the area of interest (Fig. 11C). Using HISFACE the detection limits at 200 nm ($S/N=3$) were: 2-bromobenzoic acid (45 ng/l, 11.9 pM), benzoic acid (47 ng/l, 18.7 pM), and benzenesulfonic acid (30 ng/l, 4.3 pM). These results indicate an improvement in detection limits of more than 2000× compared to simple ionic stacking. HISFACE techniques also offer the additional advantage of selectively removing most of the interferences that would normally migrate prior to the analytes of interest.

4. Conclusions

CE can be applied to the separation of a wide variety of industrial and environmental analytes including phenolics, herbicides, aromatic acids, isomerized α-and β-acids, nitroaromatics, nitramines, basic and acidic dyes, LASs, polyacrylates, and inorganic ions. The proposed unified approach to methods development frequently lead to fast, highly efficient separations of a wide variety of analytes. Suppressed conductivity can achieve the sensitivity required to detect low levels of conductive solutes. Concentration of low level chromophoric analytes can be achieved using HISFACE and UV–Vis detection. It is anticipated that CE will find wide applicability in industrial and environmental laboratories in the future.

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