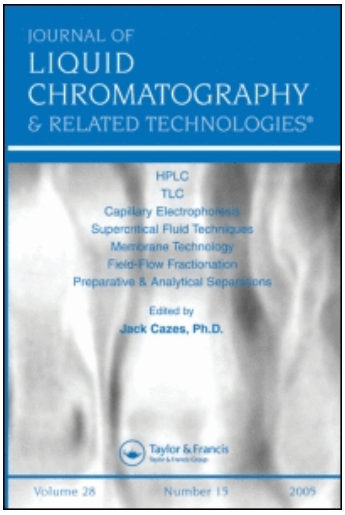

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Co- and Counter-Electroosmotic Flow Capillary Electrophoresis of Anionic Phenols Using UV and MS Detection

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Abstract: Co- and counter-electroosmotic flow (EOF) capillary electrophoresis (CE) of anionic phenols, using UV and mass spectrometry (MS) detection were investigated. The co-EOF CE-UV detection was performed by using hexadimethrine bromide (HDB) as an EOF modifier, which provided the separation in 6.2 min. The counter-EOF CE-MS was obtained without HDB and coupling was achieved by sheath liquid interface. The CE-UV provided the better linearity ($r^2 > 0.99$) than the CE-MS ($r^2 > 0.81$), however, both methods gave comparable detection limits (5 μg/mL). CE-UV is efficient for the routine quantitative analysis, whereas CE-MS is useful for identification of the phenols.

Keywords: Anionic phenols, Co-electroosmotic, Counter-electroosmotic, MS detection, UV detection

INTRODUCTION

Capillary electrophoresis (CE) is now a well established method, which is applicable for analyses of various classes of analytes. In addition to the electrophoretic migration, EOF is a main driving force in CE that enables the migration of analytes along the capillary. Controlling of EOF can

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significantly influence the efficiency and selectivity of a separation. Modification of the capillary wall can alter the EOF and enhance precision and resolution of a CE separation.[1] Three methods are currently available for capillary wall modifications, including dynamic and permanent coating and covalent bonding and/or cross-linking of the EOF modifiers to the capillary wall.[2] Among them, dynamic coatings are favorable since it can be easily performed by adding EOF modifiers into the background electrolyte (BGE) or by flushing during a preconditioning step. Regeneration or washing between runs are recommended to maintain the reproducibility for the dynamic coating.[1] Coating the capillary wall surface with long-chain alkylammonium salts (e.g., cetyltrimethylammonium bromide (CTAB), tetradecyltrimethyl ammonium bromide (TTAB), etc.), other cationic surfactants, polycations (e.g., hexadimethrine bromide (HDB)) and other alkylamines can reverse the EOF direction and facilitate the migration of anions. The technique is known as the high speed co-electroosmotic CE (the co-EOF CE).[3]

Reversal of EOF to generate the co-EOF CE has been applied for analyses of various compounds such as phenolic acids and derivatized amino acids, organic acids in beers and wines, phenolic acids in olive oil, inorganic anions, arsenic species, amino acid enantiomers, protease and reverse transcriptase inhibitors.[2–11] Pancorbo and coworkers determined fourteen phenolic acids in olive oil sample using co-EOF CE, dynamically coated capillary with HDB, with UV detection.[5] The same research group used *N,N*-dimethylacrylamide-ethylpyrrolidine methacrylate for capillary coating for the separation of phenolic and amino acids.[2] Bianchi and coworkers employed HDB as a capillary coating for the analysis of water soluble organic acids in wine using indirect UV detection.[3] Ramirez and coworkers also utilized HDB to obtain co-EOF CE for the determination of nineteen low molecular mass organic acids in beer samples.[4] Diress and coworkers used cationic surfactants (e.g., CTAB and didodecyldimethylammonium bromide (DDAB) to reverse the EOF for the separation of inorganic anions.[6] Sun and coworkers determined arsenic species by CE, using poly(diallydimethylammonium) chloride (PDDAC) as a capillary coating.[7,8] Kang and coworker used vancomycin as a chiral selector and HDB for the capillary coating to obtain the enantiomeric separation of amino acids.[9] Gutleben and coworkers achieved the separation of eleven protease and reverse transcriptase inhibitors using poly-anionic surfactants to generate the co-EOF CE.[10] Vrouwe and coworkers applied the co-EOF CE to a microchip format to provide the rapid separation of inorganic ions.[11]

Presently, the counter-EOF and the co-EOF CE of phenols (i.e., 4-hydroxybenzoic acid (4-H), vanillin (V) vanillic acid (VA), syringaldehyde (S), and ferulic acid (F) (Figure 1) in their anionic forms were investigated. Simultaneous separation of these phenols is of interest due to

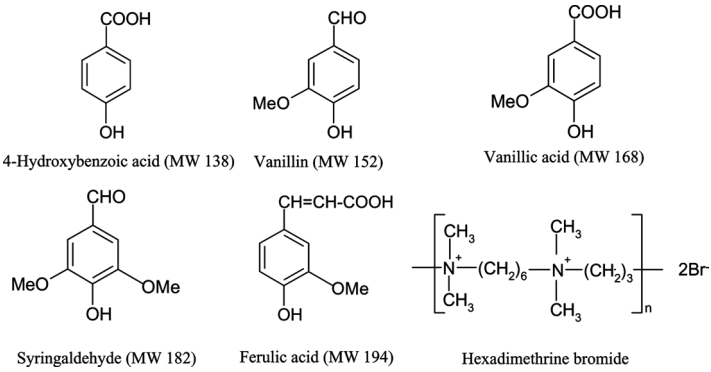


Figure 1. Structures of the investigated phenols and hexadimethrine bromide (HDB).

their relevance as constituents in food, plant and lignins. Unlike other works, the separation of the anionic phenols was performed in both co-EOF and counter-EOF under various CE conditions (i.e., temperature, organic solvents, EOF modifiers, and capillary wall coating techniques). Influences of these factors on the migration behavior, electrophoretic mobilities, and analytical parameters of the anionic phenols were thoroughly evaluated. An optimum condition that provided the high speed analysis of the anionic phenols could be determined based on their analytical parameters and percent relative standard deviations (%RSDs) on the migration time, electroosmotic flow, and electrophoretic mobilities. Furthermore, the proper CE conditions, which were compatible with UV and MS detection, were investigated. MS parameters, such as drying gas flow rate and sheath liquid composition were optimized to obtain good signal to noise (S/N) ratios of the anionic phenols.

EXPERIMENTAL

Reagents and Chemicals

Standards, including 4-hydroxybenzoic acid, vanillic acid, syringaldehyde, ferulic acid and vanillin, ammonium acetate, hexadimethrine bromide and thiourea were from Fluka (Buchs, Switzerland). Methanol was from Merck (Darmstadt, Germany). Deionized water was generated from ELGA Purelab*Ultra* system (Celle, Germany).

The initial BGE composition was adopted from Ref. 12. The buffer containing 30 mM ammonium acetate was prepared by dissolving an

appropriate amount of ammonium acetate in deionized water and adjusting the pH to 10.0 with ammonium hydroxide. The buffer was used to prepare different working BGE (i.e., BGE with and without 10% v/v methanol, with and without 0.001% w/v HDB). A stock solution of 1.0% w/v HDB was made by dissolving an appropriate amount of HDB in deionized water and the diluted HDB solution (0.1% w/v) was prepared by dilution from the stock HDB solution with deionized water. The concentration of HDB was indicated in %w/v since HDB has no defined molecular weight.[4]

A working standard mixture of the five phenols (25 μg/mL) was prepared by diluting the stock standard mixture (100 μg/mL) with deionized water. Thiourea solution (50 μg/mL) was prepared by diluting the stock solution (1,000 μg/mL) with deionized water and was used as an EOF marker.

CE-UV Instrument and Conditions

CE measurements were performed on a ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector, an automatic injector, an autosampler, and a power supply. Separations were carried out using fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 88.5 cm (80 cm to UV detector) and an inner diameter of 75 μm. The polyimide at both ends of the capillary was removed for 4 cm to prevent the swelling of the polyimide in the partly organic buffer liquids. The detection wavelength was at 214 nm with a bandwidth of 4 nm. Experiments were carried out in a positive mode (anode at the inlet and cathode at the outlet) for the counter-EOF CE and a negative mode for the co-EOF experiments. Sample injections were achieved by hydrodynamic injection at 40 mbar for 5 s, followed by pressure at 25 mbar for 3 s of the BGE to improve the injection reproducibility. Data handling was performed by ^{3D}CE Chemstation Rev. A.10.01 software (Hewlett Packard, Waldbronn, Germany). A new capillary was washed with 1 N NaOH (10 min), deionized water (10 min), and BGE (10 min), respectively. Between runs, the capillary was washed with 1 N NaOH (3 min), deionized water (2 min), and BGE (3 min), respectively.

Various CE conditions (Table 1) were investigated for the separation of the anionic phenols. These conditions are classified as the counter-EOF (conditions 1–3) and the co-EOF CE (conditions 4–11). Dynamic coating of the capillary wall was achieved by adding 0.001% w/v HDB directly into the BGE (conditions 4–7) or in the flushing solution (0.1% w/v) during the preconditioning step (conditions 8–11). The optimum condition was determined from the selectivity (α), resolution (R_S), number of theoretical plate (N), percent relative standard deviations (%RSDs), and migration time of nine injections. These parameters were

Table 1. Various CE conditions and total migration times[a]

Condition	MeOH (% v/v)	HDB (% w/v)[b]	Voltage[c]	Temp (°C)	Total t_m (min)
1	10	0	+	25	40.8
2	10	0	+	40	25.7
3	0	0	+	40	13.5
4	10	0.001	−	25	9.3
5	10	0.001	−	40	6.2
6	0	0.001	−	25	7.3
7	0	0.001	−	40	5.3
8	10	0.1	−	25	8.5
9	10	0.1	−	40	6.5
10	0	0.1	−	25	7.5
11	0	0.1	−	40	5.6

[a]BEG = 30 mM ammonium acetate buffer (pH 10.0), CE capillary = 88.5 cm (effective length: 80 cm) × 75 µm, 30 kV.
[b]for condition 4–7, HDB was added into the BGE, for condition 8–11, HDB was flushed through the capillary during pre-conditioning step.
[c]+ = positive polarity, − = negative polarity.

calculated by the following equations:

$$\alpha = \mu_2/\mu_1 \tag{1}$$

$$R_S = 1.18(t_2 - t_1)/(w_{0.5\ (1)} + w_{0.5\ (2)}) \tag{2}$$

$$N = 5.56\ (t/w_{0.5})^2 \tag{3}$$

where μ_1, μ_2 are the apparent mobilities, t_1, t_2 are the migration times, and $w_{0.5\ (1)}$, $w_{0.5\ (2)}$ are the peak width at half height of peak 1 and 2, respectively.

CE-UV-MS Instrument and Conditions

In the CE-UV-MS experiments, the CE apparatus was equipped with a diode array detector set at 214 nm and coupled to the MS detector by an orthogonal electrospray interface (ESI), using a commercial coaxial sheath flow interface (Agilent Technologies, Waldbronn, Germany). A fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 80 cm and 75 µm was used and UV and MS detection was performed at 25 and 80 cm, respectively, from the inlet of the

capillary. The polyimide at the inlet was removed for 4.0 cm and for 9.7 cm at the outlet side going into the MS interface, to prevent the swelling of the polyimide in the partly organic buffer liquids, and then inserted into the spray needle. Since the effective length for UV detection was shortened, a lower voltage of 25 kV and temperature of 25°C were adopted to provide sufficient separation of the phenols by CE-UV-MS. A new capillary was washed as described above without insertion of the outlet into the spray needle. Between runs, the capillary was washed with BGE (3 min). To obtain a stable CE current, the nebulizer gas from the MS had to be switched off during the preconditioning and injecting steps and switched on again after applying the separating voltage.

MS detection was performed on an Esquire LC ion trap (Bruker Daltonics GmbH Bremen, Germany) equipped with an orthogonal electrospray interface (model G1607A from Agilent Technologies, Palo Alto, CA, USA). Electrical contact at the electrospray needle tip was established via a flow of conductive sheath liquid, delivered at a flow rate of 1 μL/min by a microsyringe pump (Cole-Parmer, Vernon Hills, Illinois, USA). Electrospray ionization was operated in a negative mode with a voltage of 3,200 V, nebulizer gas of 20 psi at 150°C. Nitrogen was used as both electrospray nebulizer gas and drying gas. ESI-MS operating conditions were optimized by adjustment of the ESI spray needle and the CE capillary, CE BGE compositions, drying gas flow rates, and sheath liquid compositions. The mass spectrometer was scanned from m/z 100 to 200 at a rate of 2 s/scan. The maximum accumulation time for the ion trap was set at 5.00 ms, the target count at 20,000, and the trap drive level at 100%. The instrument was controlled by a PC running Esquire v. 3.3 software from Bruker Daltonics.

Optimization of ESI conditions was investigated using a test mixture of the phenols dissolved in the BGE. The test solution (10 μg/mL) was directly introduced into the ESI interface at a rate of 5 μL/min with a by a microsyringe pump (Cole-Parmer, Vernon Hills, Illinois, USA). The conditions were optimized to obtain the highest signal intensity of vanillic acid with the molecular ion peaks at $m/z = 167$.

RESULTS AND DISCUSSION

CE-UV

Effects of CE Conditions on the Separation of the Anionic Phenols

Typical electropherograms of the anionic phenols in the counter-EOF and the co-EOF conditions are shown in Figure 2. Under the investigated BGE, which was at pH 10.0, the phenols were ionized and yielded anions.

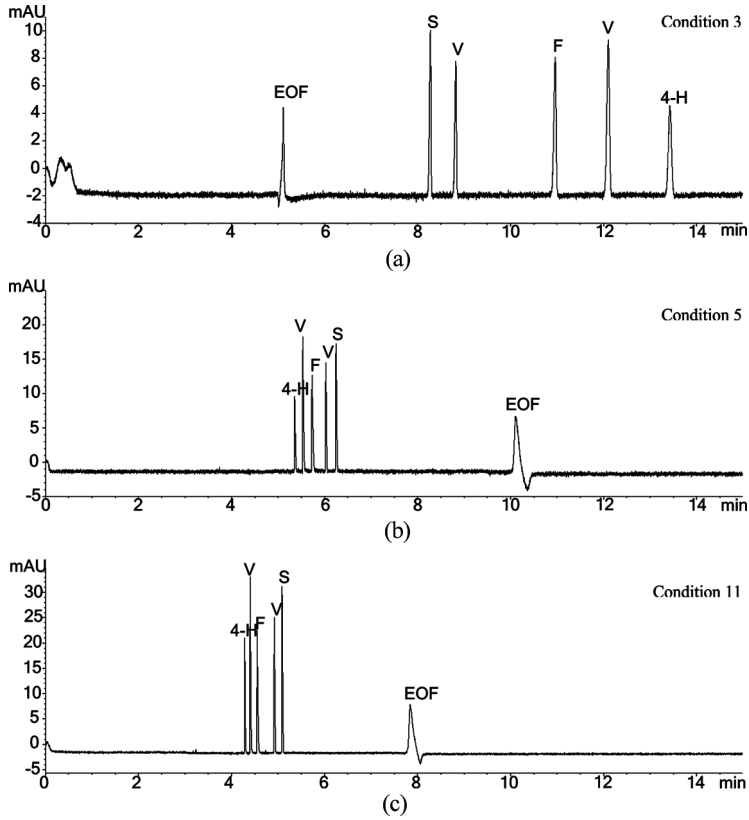


Figure 2. Typical electropherograms of the anionic phenols (25 μg/mL in BGE) under the counter-EOF and co-EOF CE conditions. Identification: 4-H = 4-hydroxabenzoic acid, VA = vanillic acid, F = ferulic acid, V = vanillin, S = syringaldehyde.

In the positive polarity (conditions 1–3), they moved in the opposite direction to the EOF and migrated after the EOF. According to their charge to mass (z/m) ratios, the migration order of the anionic phenols under the counter-EOF CE was syringaldehyde, vanillin, ferulic acid, vanillic acid, and 4-hydroxybenzoic acid, respectively. The total migration of the anionic phenols under the counter-EOF condition varied from 13–40 min, depending upon the temperature and the organic solvent. The counter-EOF mode using the BGE without MeOH at 25°C was not performed since it is obvious that at a lower temperature, the migration time would be lengthened without much improvement on the separation efficiency.

To further shorten the migration time, the co-EOF condition (conditions 4–11) was investigated to encourage the migration of the anionic phenols in the same direction with the EOF. The co-EOF conditions provided the high speed separation of the anionic phenols in less than 10 min (Table 1). The migration order in the co-EOF conditions were merely reversed of that of the counter-EOF condition (i.e., 4-hydroxybenzoic, vanillic acid, ferulic acid, vanillin, and syringaldehyde, respectively).

The temperature, organic solvents, EOF modifiers greatly affected the migration time of the anionic phenols. The higher the temperature, the shorter the migration time was obtained (Table 1) due to the reduction of the BGE viscosity. In the presence of the organic solvent (i.e., 10% v/v MeOH), the migration time was lengthened due to the increase of the BGE viscosity, the decrease of zeta potential, and the reduction of the EOF. The EOF modifier, which generated the co-EOF migration, significantly reduced the migration time from 40 to less than 10 min. Different capillary wall coating techniques (addition of HDB in the BGE or in the flushing solution during the preconditioning step) gave comparable migration times, and a stable EOF reversal could be achieved; the HDB was applied, even in a preconditioning step without being present in the BGE. However, the baseline noise from the BGE containing HDB was higher than its absence from the BGE.

Effects of the CE Conditions on the Analytical Parameters of the Anionic Phenols

Electrophoretic Mobility. Figure 3 shows the electrophoretic mobility of each anionic phenol under various CE conditions. The negative values of the electrophoretic mobilities under conditions 1–3, which were the counter-EOF CE, indicated that the analytes migrated in the opposite direction to that of the EOF. The negative values of the mobilities both of the EOF and of the analytes under conditions 4–11, which were the co-EOF CE, were stemmed from the negative polarity of the power supply. In the counter-EOF, the anionic phenols moved against the direction of the EOF, thus all migrated after the EOF. In the co-EOF CE, the anionic phenols moved in the same direction with the EOF and they migrate before the EOF.

At a higher temperature (40°C), the electrophoretic mobility of each anionic phenol was higher than at a lower temperature (25°C). Addition of organic solvents (e.g., methanol) into the BGE reduced the electrophoretic mobility of the analytes due to the reduction of the zeta potential, thus slow down the EOF in bare capillaries. Moreover, methanol increased the BGE viscosity and decreased the analytes' mobility. Electrophoretic mobilities of the anionic phenols under the co-EOF

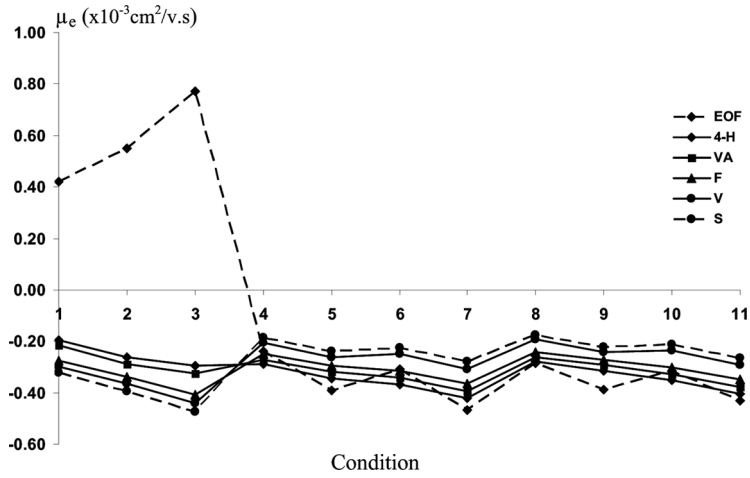
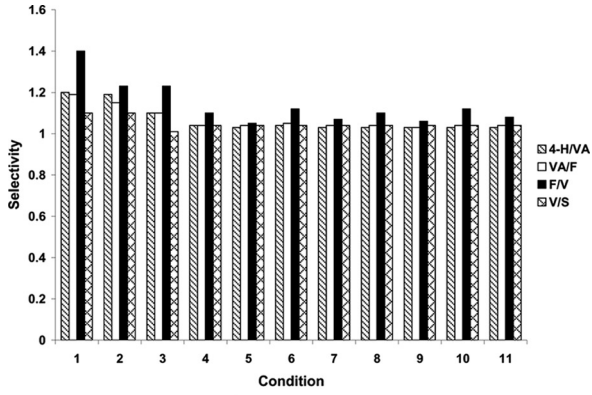


Figure 3. Electroosmotic flow and electrophoretic mobilities of the anionic phenols under various CE conditions. Abbreviation: same as Figure 2.

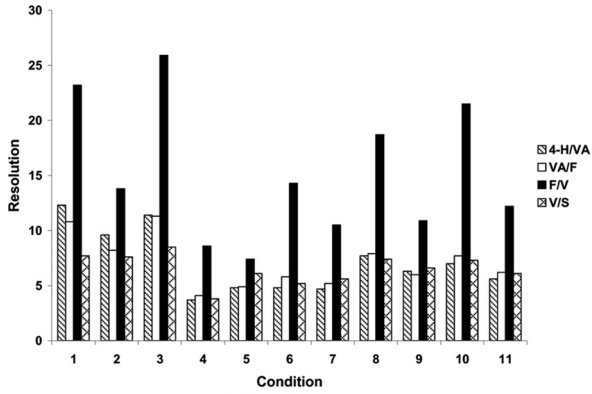
condition either in the presence of HDB in the BGE or in the flushing solution were comparable (Figure 3), although they were slightly higher in the former case. Different coating techniques did not affect the electrophoretic mobilities of the anionic phenols since both could effectively generate the co-EOF separation.

Selectivity

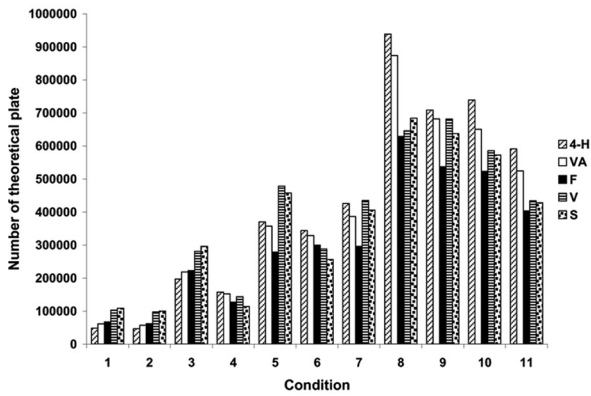
Selectivity of the anionic phenols under various CE conditions is shown in Figure 4a. Generally, selectivity of the anionic phenols in the counter-EOF CE (conditions 1–3) was higher than the co-EOF CE (conditions 4–11). In the former technique, the anionic phenols moved against the EOF and reached the detector after the EOF, which allowed them to travel along the capillary longer and they were more separated apart from each other. For the counter-EOF CE, selectivity was more than 1.10 with the maximum of 1.40 for ferulic acid/vanillin (condition 1). In the counter-EOF CE, selectivity of 4-hydroxybenzoic acid/vanillic acid, vanillic acid/ferulic acid, and vanillin/syringaldehyde was comparable ($\alpha = 1.10$–1.20) under various conditions, whereas selectivity of ferulic acid/vanillin was influenced by temperature. Lowering the temperature normally gave higher selectivity than at the higher temperature. In the counter-EOF CE, the selectivity of the anionic phenols in the BGE containing organic solvent was slightly greater than the BGE without organic solvents, since organic solvents influenced the electroosmotic and electrophoretic mobility, hence, the selectivity.



(a) Selectivity



(b) Resolution



(c) Number of theoretical plate

Figure 4. (a) selectivity, (b) resolution, and (c) number of theoretical plates of the anionic phenols under various CE conditions. Abbreviation: same as Figure 2.

In the co-EOF CE, the selectivity was higher than 1.03 with the maximum of 1.12 for ferulic acid/vanillin (condition 10). Under the co-EOF influence (conditions 4–11), selectivity of 4-hydroxybenzoic acid/vanillic acid, vanillic acid/ferulic acid, and vanillin/syringaldehyde remained unchanged by the effects of temperature, organic solvents, and the coating techniques ($\alpha = 1.03$–1.04). However, the selectivity of ferulic acid/vanillin was highly affected by the temperature (Figure 4a). The selectivity of ferulic acid/vanillin increased when lowering the temperature.

Resolution

Resolution of the anionic phenols under various CE conditions is illustrated in Figure 4b. R_s of the anionic phenols in all cases were ≥ 3.7. In general, at a lower temperature the resolution was higher than at a higher temperature. Among the investigated anionic phenols, resolution of ferulic acid/vanillin was significantly influenced by the temperature effect (Figure 4B). Under the counter-EOF conditions (conditions 1–3), resolution of the anionic phenols was larger than the co-EOF conditions (conditions 4–11). In the counter-EOF CE, the resolution improved in the BGE without organic solvent (condition 3 vs 2) since the peak dispersion and peak widths of the phenols were greatly reduced. In the co-EOF CE, organic solvent did not influence the resolution except in condition 6 vs 4. Condition 6 (without MeOH) provided the higher resolution than condition 4 (with MeOH). This might be due to the lower peak dispersion in the BGE without organic solvent. Coating the capillary by flushing HDB during the precondition step gave higher resolution for all anionic phenols than adding it into the BGE (condition 8 vs 4, 9 vs 5, 10 vs 5, 10 vs 6 and 11 vs 7). Moreover, addition of HDB into the BGE caused more baseline noises than flushing it during the preconditioning step.

Efficiency

The number of theoretical plates (N) of each anionic phenol under various CE conditions is presented in Figure 4c. The co-EOF condition provided the higher number of theoretical plate than the counter-EOF for most anionic phenols because of the smaller peak width at half-height. In the co-EOF CE, N was obtained in a range of 113,000–738,000 while in the counter-EOF CE, N varied from 46,000–108,000 for a capillary with a total length of 88.5 cm. In the counter-EOF condition, N was reduced at elevated temperature (condition 1 vs 2) and in the BGE containing MeOH (condition 2 vs 3). Under the co-EOF condition, temperature and organic solvent influenced the number of theoretical plates differently, depending on the coating technique. In the BGE

containing HDB, N increased at elevated temperature and in the BGE without MeOH. In contrast, flushing HDB during the preconditioning step provided higher N at a lower temperature and in the BGE containing MeOH.

Percent Relative Standard Deviations

In addition to the analytical parameters, percent relative standard deviations (%RSDs) of migration times, electroosmotic flow, and electrophoretic mobilities of the anionic phenols from nine injections under the investigated conditions were calculated (Figure 5). Coating the capillary wall surface by the addition of HDB into the BGE (conditions 4–7) yielded the smaller %RSDs than by flushing it during the preconditioning step (conditions 8–11). We reasoned that HDB requires more frequent application of the coating material for reproducible separation, thus addition of HDB into the BGE gave lower %RSDs than flushing it during the preconditioning step.

Among the eleven conditions, an optimum CE condition for the separation of the anionic phenols could be justified based on their migration time, resolution, selectivity, number of theoretical plates, and the %RSDs of migration times, EOF and electrophoretic mobilities. Although condition 6 gave the smallest %RSDs for the migration time, electroosmotic flow, and electrophoretic mobilities of the anionic phenols, the baseline noise in condition 6 was higher than in condition 5. The signal to noise ratio (S/N) of condition 5 ($S/N = 20$) was larger than condition 6 ($S/N = 11$). Thus, condition 5 (see Table 1) was optimized providing excellent analytical parameters ($R_s > 4.8$, $\alpha > 1.03$, and

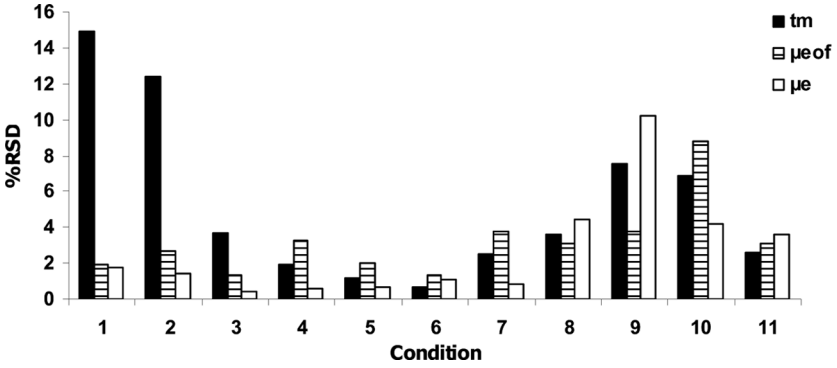


Figure 5. Percent relative standard deviations ($n = 9$) of migration time, electroosmotic flow, and electrophoretic mobilities obtained from various CE conditions in Table 1.

N > 278,000) with low %RSDs (<2.0%), high signal to noise ratio, and short analysis time (6.2 min).

CE-UV-MS

A major concern of transferring CE-UV methods to CE/ESI-MS is incompatibility of the BGE and additives with the CE-MS interface. The optimum CE-UV condition for the separation of the anionic phenols could not be directly applied for CE-MS. For MS detection, BGE containing HDB should be omitted to avoid ion suppression and contamination of the ion source, thus the counter-EOF CE was used instead of the co-EOF CE. Re-optimization of the CE condition was performed by varying the vacuum at the inlet vial and the BGE composition. MS parameters, including drying gas flow rate and sheath liquid composition, were also investigated.

When 0 or 50 mbar of pressure was applied at the CE inlet vials, the anionic phenols were overlapped as one (at 2 min) and two (at 2.5 and 3.5 min) peaks, respectively. This is not only attributed to the shortened effective length of the capillary (UV detection after 25 cm) as the electrophoretic mobilities obtained in this experimental setup are much higher than in the CE-UV mode. Separation efficiency was greatly improved when vacuum (−30 and −50 mbar) was applied and baseline separation was obtained at −50 mbar. Application of vacuum was necessary to overcome the effect from the nebulizer gas (20 psi) of the MS. BGE composition greatly influenced CE separation and S/N in MS detection. Increasing the amount of methanol in the BGE from 10 to 50%, reduced the CE current from 40 to 13 μA, but the total migration time was lengthened due to the increase of the BGE viscosity. Importantly, the S/N from the MS significantly decreased (up to 11 folds) when 50% methanol was added into the BGE (Figure 6a). In contrast, increasing the drying gas flow rates from 3 to 5 L/min improved the S/N up to 9.4 folds (Figure 6b). Drying gas affects the stability and signal intensity and high drying gas flow rates accelerates the buffer desolvation, thus, the sensitivity improved. Sheath liquid (SL) compositions also played an important role on the stability and ionization efficiency of the anionic phenols. SL is necessary to make up the flow from a capillary and to close the CE electrical circuit. SL compositions and flow rates can significantly influence the sensitivity and electrical contact between CE and ESI. Normally, SL consists of organic solvents to improve the ionic evaporation efficiency. Increasing SL flow rates can decrease S/N due to the dilution of samples, whereas lowering the flow rates can cause instability of the spray. Increasing the amount of isopropanol in the sheath liquid improved the S/N of the anionic phenols (Figure 6c). The more viscous

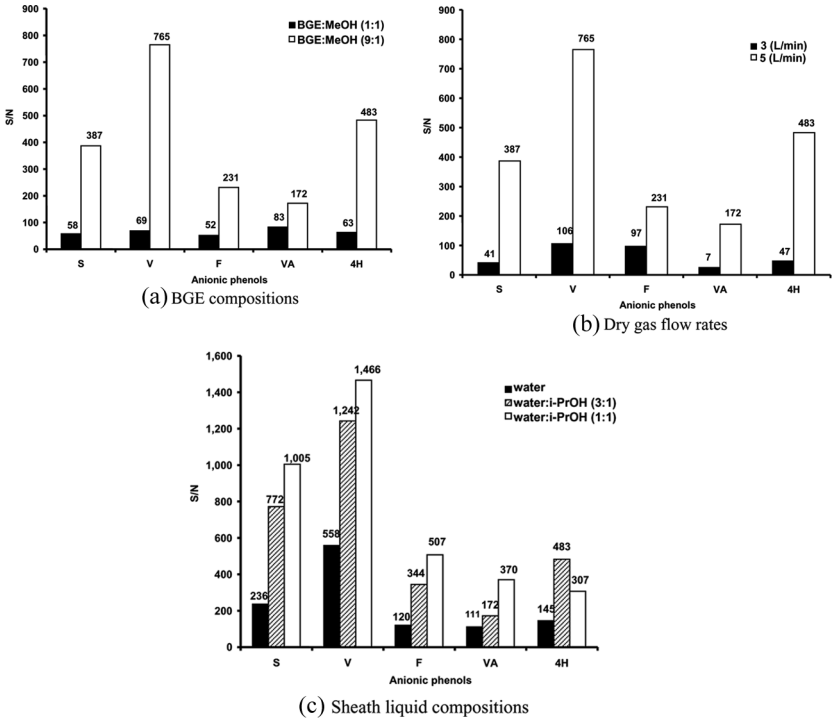


Figure 6. Effects of (a) BGE compositions, (b) drying gas flow rates, and (c) sheath liquid compositions on the S/N of the anionic phenols from MS detection.

sheath liquid (i.e., water:*i*-PrOH (1:1, v/v)) gave a stable spray compared to the sheath liquid consisting of water:*i*-PrOH (3:1, v/v) or pure water. Pure isopropanol was also applied as the sheath liquid, but the baseline noise was so high that it overlapped with the signals.

The optimum CE-UV-MS condition was in the BGE containing 30 mM ammonium acetate (pH 10.0) and methanol (10% v/v) using a voltage (positive polarity) and temperature of 25 kV and 25°C, respectively, the drying gas flow rates of 5 L/min and sheath liquid consisting of water and isopropanol (1:1). Under this condition, baseline separation of the anionic phenol was achieved in 7 min (UV detection at 25 cm, Figure 7a) and 22 min (MS detection at 80 cm, Figure 7b). Method linearity and sensitivity of the phenols were tested in a range of 5–50 μg/mL. Linearity for the UV detection was excellent with the correlation coefficients (r^2) of greater than 0.99 for all compounds, whereas the r^2 from MS detection varied from 0.81–0.97 (Table 2). Detection limits calculated from S/N = 3 were 5 μg/mL for both detectors.

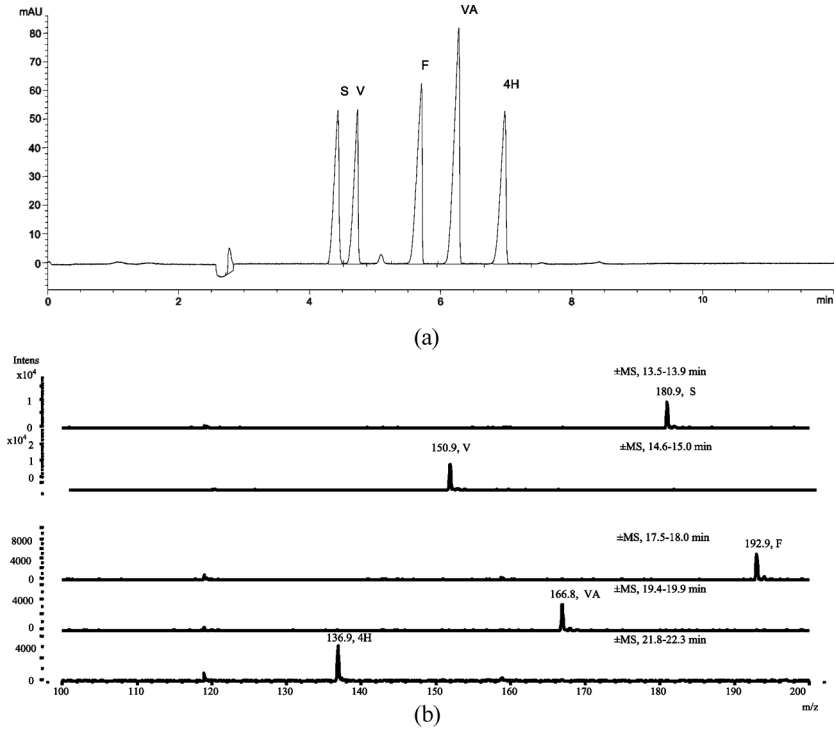


Figure 7. CE-UV-MS of the anionic phenols. (a) Electropherograms of a phenol mixture (50 μg/mL in water). Condition: BGE, 30 mM NH_4OAc (pH 10.0): MeOH, 9:1; capillary, 80 cm total length (25 cm to UV detector), 75 μm ID; hydrodynamic injection at 50 mbar for 10 s; voltage 25 kV; temperature, 25°C; detection by UV at 214 nm. (b) Ion chromatograms of a phenol mixture (50 μg/mL in water). Condition: BGE, 30 mM NH_4OAc (pH 10.0): MeOH (9:1 v/v); capillary, 80 cm total length, 75 μm ID; hydrodynamic injection at 50 mbar for 10 s; voltage 25 kV; temperature, 25°C; sheath liquid, water: i-PrOH (1:1); sheath flow, 1 μL/min. Identification: same as Figure 2.

Table 2. Regression of the anionic phenols in a range of 5–50 μg/mL

	CE-UV	CE-MS
4-H	$6.089x + 3.285$ ($r^2 = 0.9979$)	$4032.1x + 9514.7$ ($r^2 = 0.8154$)
VA	$9.526x + 0.275$ ($r^2 = 0.9992$)	$4130.7x - 2915.7$ ($r^2 = 0.9632$)
F	$7.124x + 1.008$ ($r^2 = 0.9978$)	$5539.8x - 6236.9$ ($r^2 = 0.9445$)
V	$4.906x + 2.204$ ($r^2 = 0.9984$)	$1281.5x + 1673.7$ ($r^2 = 0.9793$)
S	$5.471x + 1.823$ ($r^2 = 0.9980$)	$7512.1x + 4308.3$ ($r^2 = 0.9376$)

CONCLUSIONS

Separation of vanillic acid, ferulic acid, syringaldehyde, 4-hydroxybenzoic acid, and vanillin could be achieved by the counter-EOF and co-EOF CE, but both techniques influenced the separation of the anionic phenols differently. Temperature, organic solvent, EOF modifiers, and the capillary coating techniques greatly affected the migration time, electrophoretic mobilities, selectivity, resolution, and separation efficiency of these compounds. Counter- and co-EOF CE were compatible with UV detection, but co-EOF CE provided the high efficiency and high speed for the analysis of the anionic phenols. CE-UV-MS separation was achieved using the counter-EOF CE to avoid the non-volatile EOF modifiers, which might risk the MS source contamination. The proposed co-EOF CE-UV and counter-EOF CE-UV-MS methods can be applied for analysis of other anionic phenols or structural related compounds. The CE-UV method is useful for routine analysis where time is a major concern, whereas the CE-UV-MS method is valuable for positive identification of unknowns.

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