High-Speed Electrochemically Modulated Liquid Chromatography

Lisa M. Ponton[†] and **Marc D. Porter**[*]
*Ames Laboratory–USDOE, Department of Chemistry, and Institute of Combinatorial Discovery,
Iowa State University, Ames, Iowa 50011*

Abstract

The performance advantages of carrying out electrochemically modulated liquid chromatography (EMLC) at elevated temperatures and mobile-phase flow rates are investigated. EMLC has the unique ability to manipulate analyte retention and enhance separation efficiencies through changes in the potential applied to a conductive stationary phase. Operation of high-performance liquid chromatography systems at elevated column temperatures also provides pathways to improve chromatographic performance by enhancing analyte diffusivity and facilitating the use of higher mobile-phase flow rates than conventionally attainable. The results show that performing EMLC separations at elevated temperatures (e.g., 100 °C) reduces the analysis time of a mixture of aromatic sulfonates in a mixed mobile phase by more than a factor of 20. Moreover, use of higher operating temperatures enables the separation of this mixture with an entirely aqueous mobile phase in less than 2 min.

This paper investigates the advantages of performing separations using electrochemically modulated liquid chromatography (EMLC) at elevated column temperatures. EMLC is a unique chromatographic technique that manipulates retention through changes in the potential applied (E_{app}) to a conductive stationary-phase-like porous graphitic carbon (PGC). This capability is realized by fashioning a high-performance liquid chromatography (HPLC) column into an electrochemical cell and utilizing the packing as both a chromatographic stationary phase and as a working electrode. As a consequence, a change in E_{app} alters the effective surface composition of the stationary phase and, in turn, retention.[1] Several laboratories,[2-5] including our own,[6-10] have demonstrated that EMLC can be utilized for the separation of a wide range of mixtures (e.g., aromatic sulfonates,[10] monosubstituted benzenes,[9] protonated pyridines and anilines,[11] corticosteroids,[7] benzodiazepines,[6] short-chain alkanoic acids,[12] and metal ion complexes[13]).

Recent studies have shown that elevated column temperatures can also be used to manipulate retention in HPLC by significantly reducing analysis times while maintaining an effective separation.[14-16] This observation was theoretically predicted several years ago[17,18] and comes about because of three different temperature-dependent phenomena. First, retention in most reversed-phase separations is an exothermic process.[19-21] An increase in column temperature therefore leads to a decrease in elution time. Second, the viscosity of most mobile phases decreases with increasing temperature. As a consequence, the back pressure of the HPLC system decreases, enabling operation at higher flow rates. Third, higher column temperatures increase analyte diffusivity and desorption kinetics. Enhanced diffusivity increases the efficiency of a separation by lowering the *C*-term in the van Deemter equation,[22] which counters the loss in efficiency that arises at higher mobile-phase flow rates.

[*]Corresponding author. E-mail: mporter@porter1.ameslab.gov.
[†]Present address: Department of Chemistry, Elon University, Elon, NC 27244.

Another notable advantage of operation at elevated column temperatures is the decrease in the dielectric constant of water. That is, water behaves more like a hydrophobic solvent with increasing temperature.[23] For example, the dielectric constant for water is ~55 at 55 °C and is close to that of methanol (~33) at 200 °C.[24] This behavior leads to the possibility of carrying out separations using an entirely aqueous mobile phase, with associated reductions in toxicity, flammability, and waste disposal costs.[23]

Using elevated column temperatures in EMLC also has a practical electrochemical advantage: a decrease in the resistivity of electrolyte solutions due to an increase in electrolyte diffusivity. [25] Because EMLC is a hybrid of HPLC and electrochemistry, the column design must address the divergent requirement of the two techniques, namely, the ratio of the column dead volume to the surface area of the stationary phase. The resolution of a separation is enhanced by minimizing this ratio, i.e., a large stationary-phase surface area and a small dead volume. However, to avoid high resistance in the mobile phase and the resulting challenges of controlling E_{app}, this ratio should be maximized, i.e., a small electrode surface area and a large solution volume. Strategies to decrease mobile-phase resistance, such as operation at elevated column temperatures, therefore have the potential to enhance the electrochemical characteristics (e.g., time to equilibrate upon a change in E_{app}) of an EMLC column while maintaining effective chromatographic performance.

Herein, we describe the findings from an investigation aimed at determining whether operation at elevated column temperatures improves the chromatographic and electrochemical performance of EMLC columns. The following sections detail the requisite hardware for such experiments and the results from assessments based on separations of a mixture of aromatic sulfonates (ASFs). The feasibility of separating such mixtures in an entirely aqueous mobile phase is also investigated, along with a brief examination of issues related to the thermal stability of the column packing.

EXPERIMENTAL METHODS

Chemicals and Reagents

Benzenesulfonic acid (BS), methylbenzenesulfonic acid (MBS), *p*-chlorobenzenesulfonic acid (CBS), sodium 1,5-naphthalenedisulfonate (1,5-NDS), and sodium 2,6-naphthalenedisulfonate (2,6-NDS) were purchased from Aldrich Chemical (Milwaukee, WI). Analyte solutions were prepared with 20 μM concentrations in 0.1 M aqueous lithium perchlorate (Aldrich).

Two mobile phases were employed in this study. Mobile phase A consisted of 0.1 M lithium perchlorate in a 95:5 v/v mixture of high-purity water (Milli-Q system, Millipore, Bedford, MA) and HPLC grade acetonitrile (Fisher Scientific, Pittsburgh, PA). Mobile phase B consisted of 0.1 M lithium perchlorate in high-purity water. Both mobile phases were passed through a 0.5-μm filter (GE Osmonics Inc., Minnetonka, MN) prior to use.

Hypercarb, 7-μm porous graphitic carbon particles, were obtained from Thermo Hypersil (Bellfonte, PA). Characterizations by X-ray photoelectron spectroscopy agreed with previous results[10] and showed that as-received PGC exhibits a very low surface oxygen content (0.14 atom %), which is largely distributed among phenol, carbonyl, carboxylic acid, lactone, and quinone groups.[26]

Instrumentation. (i) EMLC Column

The design and construction of the EMLC column has been described in detail elsewhere.[8] Briefly, a stationary phase such as PGC is packed inside a Nafion (Perma Pure Inc., Toms River, NJ) cation exchange membrane fitted into a porous stainless steel column (Mott Corp.,

Farmington, CT). The porous stainless steel housing serves as a high surface area auxiliary electrode. The Nafion membrane serves three functions: (1) a container for the GC stationary phase, (2) an electronic insulator between working and auxiliary electrode, and (3) a salt bridge for ion transport. An Ag/AgCl (saturated NaCl) reference electrode was placed in a reservoir surrounding the auxiliary electrode. This assembly is represented in Figure 1 as the EMLC column.

(ii) EMLC System

The column was attached to an Agilent Technologies (Palo Alto, CA) model 1050 HPLC system equipped with a quaternary pump and a diode array detector. The samples were injected via a Rheodyne model 7125 injector with a 5.0-μL loop (Cotati, CA), and the elution profiles were monitored at 220 nm. The potential applied to the stationary phase was controlled by an Amel (Milan, Italy) potentiostat to ±1 mV.

(iii) Temperature Control Hardware

The experimental setup for the control of temperature is illustrated in Figure 1 and parallels several of the design elements devised by Carr and coworkers.[14,16] The temperature (±0.2 °C) was controlled by immersion of the EMLC column and a 20-cm length of stainless steel tubing connected to the column inlet, all of which were surrounded by a latex bag (not shown), in a thermostated water bath (Polyscience, Niles, IL) filled with 50:50 (v/v) ethylene glycol/water. The 20-cm length of stainless steel tubing was placed in the water bath in order to temperature equilibrate the mobile phase and sample before entering the column. There was no detectable evidence of band broadening due to thermal mismatch between the entering solution and the EMLC column. This result indicates the preheating setup performed adequately.[27] Upon leaving the column, the mobile phase passed through a circulating cooling bath held at room temperature prior to entering the detector to prevent thermal noise in the detector. An additional 20 bar of back pressure was applied in-line after the detector through a restrictor to avoid boiling the mobile phase while in the column.

With this setup, the highest accessible column temperature was 100 °C, a limit defined by the evaporative loss of the electrolyte solution in the reservoir that houses the reference electrode. This temperature limitation, coupled with the pressure cutoff of the chromatographic pumping system, placed an upper limit of 2.0 mL/min for the flow rate of both mobile phases. That is, increasing the column temperature markedly reduced the system back pressure. At 25.0 °C, for example, the back pressure for the system was 240 bar, whereas that at 100.0 °C was only 125 bar. This result is qualitatively in line with tabulations of the observed decrease in mobile-phase viscosity with increases in temperature.[24] Higher temperatures, and therefore higher flow rates, would be accessible if the reservoir were designed to enable modest pressurization to prevent boil-off of the electrolyte solution. Work to this end is underway.

Data Treatment

A water blank was employed to determine the void time for calculations of the capacity factor, k'. Retention times were determined from the first statistical moment of the elution profile in order to compensate for band asymmetry.[28] Three to seven replicate injections were used for the calculation of k'. Retention times typically exhibited less than a 2% relative standard deviation (RSD) over the course of 2–3 h of experimentation. The resolution of a separation was calculated based on the retention time of an elution band and its width at 4.4% of the height by using the 5σ method resident in the software of the HP Chem-Station. All values for E_{app} are reported with respect to the potential of Ag/AgCl (saturated NaCl) at 25 °C by accounting for the temperature dependence of its formal reduction potential, which is −0.83 mV/°C between 25 and 95 °C.[29]

RESULTS AND DISCUSSION

Effects of E_{app}

For comparison to the temperature-dependence studies that follow, Figure 2 demonstrates the ability of EMLC to manipulate the retention characteristics of PGC at 25.0 °C through changes in E_{app}. These results were obtained at values of E_{app} from +100 to −500 mV, using the mixed-solvent mobile phase A. The mixture contained three monovalent anions (BS, MBS, CBS) and a divalent anion (1,5-NDS). As is evident, all four analytes exhibit a marked decrease in retention as E_{app} becomes more negative. This change is particularly apparent when comparing the chromatogram obtained at +100 mV with that observed at −500 mV. At +100 mV, the mixture requires ~23 min for complete elution. The separation at −500 mV, however, is complete in less than 9 min. This decrease translates to a reduction in overall run time by a factor of 2.4.

There are also notable differences in the resolution and elution order of the separations. BS and MBS are baseline resolved at +100 mV, whereas CBS and 1,5-NDS coelute. All four components are baseline resolved at −100 mV. At −300 mV, however, MBS and 1,5-NDS coelute. The separation again improves at −500 mV; BS and CBS are baseline resolved, with a resolution of 1.2 for 1,5-NDS and MBS.

The inset in Figure 2 summarizes the retention dependence of all four analytes through plots of ln k' versus E_{app}. The error bars for the values of ln k' are smaller than the data points and represent one standard deviation calculated from three or more replicate injections. These results demonstrate two key aspects of the EMLC-based separation of these compounds. First, the retention of each analyte exhibits a linear dependence with respect to E_{app}. Such a dependence reflects how E_{app} affects the electrostatic interactions between an ionic species and a charged stationary phase via the ion distribution law.[8, 30]

Second, the slope of such a plot represents the sensitivity of retention to changes in E_{app}. The plots show that the divalent anion 1,5-NDS is roughly twice as sensitive to changes in E_{app} when compared to the three monovalent anions: BS, MBS, and CBS. These differences are quantified by the sensitivity summary presented in column A of Table 1. In this case, the difference results from the valency term in the ion distribution law,[30] which predicts that the effect of an electric field on the concentration distribution of a dianion will be twice as large as that on a monoanion. The differing sensitivity is reflected by the change in elution order. At +100 mV, the order of increasing elution time is BS < MBS < CBS ≈ 1,5-NDS. The elution order, however, changes as E_{app} becomes more negative due to the greater sensitivity of 1,5-NDS. As a result, the elution order at −500 mV is BS < 1,5-NDS < MBS < CBS. The sensitivity plots also *suggest* that a baseline separation with a run time that is ~50% less than the run time observed at −100 mV may be possible at more negative values of E_{app} (e.g., −700 mV). Taken together, these results demonstrate that the analytical figures of merit in a separation (i.e., resolution and retention time) can be effectively and easily manipulated by EMLC.[8]

Effects of Temperature

Figure 3 presents a series of chromatograms that were obtained using the same analyte mixture while elevating the column temperature. Chromatograms A–C were collected using mobile phase A at 25.0, 61.5, and 100.0 °C with E_{app} set at +100 mV with respect to the Ag/AgCl (saturated NaCl) reference electrode at each column temperature. Correction for the temperature dependence of the reference electrode yields values for E_{app} of +100, +70, and +40 mV for the above temperatures, respectively. The flow rate for the mobile phase was 0.5 mL/min. We note that chromatogram A in Figure 3 was obtained at the same temperature and

E_{app} as chromatogram A in Figure 2 but was collected after 48 h of continuous operation over a range of elevated temperatures (see below).

As is evident in chromatograms A–C, increasing temperature clearly reduces the overall elution time. The change from 25.0 to 61.5 °C decreases the run time from ~20 to ~12 min, which is further reduced to less than 7 min at 100.0 °C. The decrease in run time at 100.0 °C represents a reduction by a factor of 3.1 with respect to that at 25.0 °C.

Two additional observations can be drawn from these data. First, the temperature-induced decreases in the retention time of all four analytes are greater in magnitude than can be accounted for by the temperature dependence of E_{app}. For instance, the ln k' versus E_{app} plot for the separations at 25.0 °C (Figure 2) predicts a decrease in k' by a factor of 1.06 for CBS, based on a change in E_{app} from +100 to +70 mV. The observed decrease in k' at 61.5 °C and +70 mV (chromatogram B in Figure 3), however, is a factor of 1.84. A comparable analysis of the data for 1,5-NDS yields an expected decrease in k' of 1.17 for a 30-mV drop in E_{app} at 25.0 °C, whereas the retention at 61.5 °C shows a decrease for k' of 1.73. These differences in expected decrease of k' are attributed primarily to the impact of an increased column temperature on an exothermic retention process,[19-21] which would further decrease the time required for elution.[31]

Second, the separation at 61.5 °C has marginally better resolution than those at either 25.0 or 100.0 °C. This observation suggests that temperature may provide a useful means to manipulate the selectivity of an EMLC-based separation in a manner analogous to that in classical ion exchange chromatography, where differences in the enthalpy of retention result in changes in resolution as temperature is increased.[32,33] Further studies are nevertheless required in order to fully explore this phenomenon.

Next, the effects of the mobile-phase flow rate on performance were examined. These results are shown in chromatograms C–F in Figure 3, which were obtained at an E_{app} of +40 mV, a column temperature of 100.0 °C, and flow rates of 0.5, 1.0, 1.5, and 2.0 mL/min, respectively. The chromatograms show that an increase in flow rate clearly reduces the time required for the separation. That is, the total separation time is reduced from ~7 to ~2 min when the flow rate is changed from 0.5 to 2.0 mL/min at 100.0 °C. The improvement at 2.0 mL/min represents a reduction in run time by a factor of 10 in comparison to the chromatogram collected under a more typical set of operational conditions (i.e., a column temperature of 25.0 °C and mobile-phase flow rate of 0.5 mL/min, chromatogram A). There are also some interesting, but subtle increases in the resolution of the separation in the elution of CBS and 1,5-NDS, shown in chromatograms D–F, that we do not have an explanation for at this time.

Effects of Temperature in Conjunction with E_{app}

The last two sections have demonstrated that E_{app} and temperature can each be exploited to improve EMLC performance. This section investigates how the manipulation of E_{app} at elevated temperatures can be utilized to enhance chromatographic effectiveness. This study therefore examined the influence of E_{app} on a separation performed at 100.0 °C using a flow rate of 2.0 mL/min.

Figure 4 presents the resulting chromatograms that were obtained for a five-component mixture of aromatic sulfonates (BS, MBS, CBS, 1,5-NDS, 2,6-NDS) using mobile phase A at four different values of E_{app}: +40, −160, −360, and −560 mV. Chromatogram A is the separation of the mixture at +40 mV. All five components are baseline resolved, with complete elution requiring less than 8 min. Chromatogram B reveals that baseline resolution can be maintained, while reducing the run time to ~3 min by changing E_{app} to −160 mV. Further decreases in run time are realized by lowering E_{app} to −360 and −560 mV, as shown more clearly in the lower

Figure 4 inset. At −360 mV, the run time reduces to ~1.6 min. Decreasing E_{app} to −560 mV further reduces the run time to ~0.9 min. The resolution of the separation, however, has degraded in both cases. At −360 mV, 1,5-NDS strongly overlaps with MBS, while CBS and 2,6-NDS are only partially resolved at −560 mV.

The upper inset in Figure 4 shows plots of ln k' versus E_{app} for the five analytes at 100.0 °C. Like the data in Figure 2, these plots reveal the following: (1) there exists a linear relationship between ln k' and E_{app}; and (2) the sensitivity of the dianions 1,5-NDS and 2,6-NDS is roughly twice that of the monoanions BS, MBS, and CBS (Table 1, column B). The influence of E_{app} on retention at 100.0 °C therefore continues to follow the predictions based on electrostatic interactions. Again, the reduction in run time, which in this case is a factor of ~9, reflects the high sensitivity of the divalent anion 2,6-NDS to changes in E_{app}.

These data also reveal that the sensitivity of analyte retention toward changes in E_{app} at 100.0 °C is slightly greater than that at 25.0 °C. In other words, the slopes of the sensitivity plots for the analytes tested in the experiments at both 25.0 and 100.0 °C are ~20% steeper at 100.0 °C (Table 1, columns A and B). This disparity is attributed to the inherent difference in the interactions between the packing and analyte at the two temperatures. As evident from the insets in Figures 2 and 4, retention is lower at 100.0 °C, which arises because of an increase in analyte solubility and a decrease in retention at higher temperatures due to the exothermicity of the interaction. A change in E_{app} should therefore have greater impact on retention at 100.0 °C, which is reflected by the differences in Table 1.

The upper inset in Figure 4 also enables identification of conditions that may yield a more effective separation than those obtained. That is, the differences in analyte retention predicted by the plots of ln k' versus E_{app} suggest that the mixture of ASFs can be fully resolved at an E_{app} of ~−460 mV. Though not tested, an analysis of these data also indicates a possible run time of ~1 min and an elution order of BS < 1,5-NDS < MBS < CBS < 2,6-NDS at −460 mV. More importantly, the results in this section show that the chromatographic performance of EMLC can be enhanced by the combination of elevated column temperature and E_{app}.

Aqueous Mobile Phase

The next set of chromatographic experiments examined the ability to carry out EMLC separations at PGC in a purely aqueous mobile phase, i.e., mobile phase B. The intent was to determine whether the increase in the hydrophobicity of water that results from an elevation in temperature would raise ASF solubility to a level sufficient to perform an EMLC-based separation in an entirely aqueous mobile phase. We add that the use of a purely aqueous mobile phase is particularly challenging to the separation of aromatic compounds on carbonaceous packings, such as PGC, because the strong interactions between extended π-electron systems often lead to exceedingly long elution times and strong band tailing.[34] Attempts in our laboratory to separate ASFs using PGC with mobile phase B and a column temperature of 25.0 °C yielded intolerably long retention times. MBS, for example, required nearly 60 min to elute at +100 mV. Decreasing E_{app} to −500 mV reduced the elution time to ~40 min but was still deemed unacceptably long.

Figure 5 shows the separation of BS, MBS, CBS, and 1,5-NDS in mobile phase B with the column held at 100.0 °C and a flow rate of 2.0 mL/min. Five different values of E_{app} were used: +40, −160, −360, −560, and −760 mV. As is evident from comparisons to the separations with mobile phase A shown in Figure 4, elimination of acetonitrile sharply increases the run time. At +40 mV, the separation in mobile phase B (chromatogram A) required a run time of ~11 min. The analogous experiment with mobile phase A (chromatogram A, Figure 4) yielded a total elution time of ~2.5 min. Interestingly, by moving E_{app} to more negative values, the run time can be markedly reduced while an effective separation is maintained. Both BS and

MBS are baseline resolved at −760 mV, whereas the resolution between 1,5-NDS and CBS equals 1.4; importantly the run time is only ~1.9 min.

The sensitivity plots presented in the inset of Figure 5 again show that the ability of EMLC to fine-tune separations of these analytes arises from changes in the electrostatic interactions induced by E_{app}. In other words, the linear dependencies for the plots of ln k' versus E_{app}, coupled with greater sensitivity of the dianion 1,5-NDS with respect to the monoanions, BS, MBS, and CBS (Table 1, column C), are consistent with an electrostatic interaction model.

We also note that the sensitivities at 100.0 °C in the mobile phase B are lower than those in mobile phase A at 100.0 °C, a disparity that can be explained in a manner similar to that used to interpret the differences in the slopes when using mobile phase A at the two temperatures. That is, since retention is inherently greater in mobile phase B, which is a water-only supporting electrolyte (0.1 M LiClO$_4$), the sensitivity to E_{app} will be less than that in mobile phase A, which contains 5% acetonitrile.

Equilibration Time

Experiments were also performed to determine whether the decrease in solution resistivity or solution diffusivity that occurs with increasing temperature[25] would reduce the time required for the column to equilibrate to a change in E_{app}. These tests were conducted using mobile phase A at either 20.0 or 60.0 °C. First, BS was injected at a preset value of E_{app} in order to determine its retention time. After elution, BS was again injected, with E_{app} immediately stepped to and held at a different value. Injections were repeated continuously until no further changes in retention were observed. The first test employed a step from −50 to +50 mV. At 20.0 °C, retention of BS reached a constant value in 44 min. The time for stabilization at 60.0 °C, however, was only 22 min. Increases in the magnitude of the step yielded longer equilibration times. For example, a step from −100 to +100 mV at 20.0 °C required 62 min before reaching a limiting value (2% RSD for at least three consecutive injections) for the retention of BS, but only 33 min at 60.0 °C. These results demonstrate a clear reduction of the time required to equilibrate the column when operated at elevated temperatures, and tests to more fully assess the scope of this capability are planned.

Retention Stability at Elevated Temperatures

We also briefly examined the effects of temperature on the stability of the PGC packing. At room temperature, our EMLC columns exhibit a day-to-day reproducibility of ~2% RSD for k'. Typically, the columns are stored with a continuous passage of water at 0.1 mL/min between uses. Occasionally, a wash with acetonitrile or methanol for a few hours is needed to recondition the column, presumably to remove the buildup of material that slowly accumulates on the packing surface.

Although not yet systematically studied, operation at elevated temperatures led to a slow decrease in retention time. One set of data showed that 3 days of continuous experimentation, which spanned both mobile phases, a range of temperatures, and various values of E_{app}, led to a decrease in capacity factors by 4–10%. We suspect that this decrease arises, at least in part, from an increase in the concentration of oxygen groups on the packing surface.[35] Characterization of the packing, after use in temperature experiments for several days, by X-ray photoelectron spectroscopy indicated that the concentration of surface oxygen groups had reached a level as high as 0.9% (atomic) as a consequence of extended use. It is therefore evident that the stability of the packing at elevated temperatures needs to be improved in order to fully exploit the advantages of operation at elevated temperatures. Along these lines, recent reports have shown that hydrogenating glassy carbon electrodes stabilizes their electrochemical response in comparison to polished electrodes.[36,37] We believe that such

processing may also prove valuable in enhancing the long-term performance of the PGC packing when operated at elevated temperature.

CONCLUSIONS

This work has demonstrated the power of combining changes in applied potential via EMLC with elevated operational temperatures in markedly enhancing the separation of ASFs. With this integration, the analysis time of such a mixture at a PGC stationary phase was reduced by more than a factor of 20, yielding an effective separation in less than 1 min. The use of higher operation temperatures (i.e., 100 °C) also enabled (1) the effective separation of this mixture with a water-only mobile phase in under 2 min and (2) a 2-fold decrease in the time for the column to equilibrate to a change in applied potential. Together, these findings have clear implications with respect to improvements for EMLC in sample throughput as well as to reductions in mobile-phase toxicity, flammability, and waste disposal costs. We are also exploring ways to enhance the extended performance of this packing through the use of hydrogen plasma treatments.

References

1. Harnisch JA, Porter MD. Analyst 2001;126:1841–1849. [PubMed: 11763053]

2. Ge H, Teasdale PR, Wallace GG. J. Chromatogr 1991;544:305–316.

3. Nagaoka T, Fugimoto M, Nakao H, Kakuno K, Yano J, Ogura K. J. Electroanal. Chem 1994;364:179–188.

4. Ge H, Wallace GG. J. Liq. Chromatogr 1990;13:3245–3260.

5. Mitakos A, Panderi I. Anal. Chim. Acta 2004;505:107–114.

6. Ting EY, Porter MD. J. Chromatogr., A 1998;793:204–208.

7. Ting E-Y, Porter MD. Anal. Chem 1997;69:675–678.

8. Ting E-Y, Porter MD. Anal. Chem 1998;70:94–99.

9. Ting E-Y, Porter MD. J. Electroanal. Chem 1997;443:180–185.

10. Deinhammer RS, Ting E-Y, Porter MD. Anal. Chem 1995;67:237–246.

11. Takano, H.; Porter, MD. New Directions in Electroanalytical Chemistry II. Leddy, J.; Vanysek, P.; Porter, MD., editors. 99-5. The Electrochemical Society, Inc.; Seattle, WA: 1999. p. 50-60.

12. Knizia MW, Vuorilehto K, Schrader J, Sell D. Electroanalysis 2003;15:49–54.

13. Shibukawa M, Unno A, Miura T, Nagoya A, Oguma K. Anal. Chem 2003;75:2775–2783. [PubMed: 12948149]

14. Li J, Hu Y, Carr PW. Anal. Chem 1997;69:3884–3888. [PubMed: 9322427]

15. Yan B, Zhao J, Brown JS, Blackwell J, Carr PW. Anal. Chem 2000;72:1253–1262. [PubMed: 10740867]

16. Thompson JD, Carr PW. Anal. Chem 2002;74:4150–4159. [PubMed: 12199587]

17. Antia FD, Horvath C. J. Chromatogr 1988;435:1–15.

18. Colin H, Diez-Masa JC, Guiochon G, Czajkowska T, Miedziak I. J. Chromatogr 1978;167:41–65.

19. Guillaume Y, Guinchard C. J. Liq. Chromatogr 1994;17:2809–2820.

20. Cole LA, Dorsey JG. Anal. Chem 1992;64:1317–1323. [PubMed: 1503212]

21. Cole LA, Dorsey JG, Dill KA. Anal. Chem 1992;64:1324–1327. [PubMed: 1503213]

22. Skoog, DA.; Holler, FJ.; Nieman, TA. Principles of Instrumental Analysis. 5th ed.. Harcourt Brace College Publishers; Philadelphia: 1998.

23. Smith RM, Burgess RJ. J. Chromatogr., A 1997;785:49–55.

24. CRC Handbook of Chemistry and Physics. 72nd ed.. CRC Press Inc.; Boca Raton, FL: p. 1991-1992.

25. Bockris, JOM.; Reddy, AKN. Modern Electrochemistry: An Introduction to an Interdisciplinary Area. Plenum Press; New York: 1970.

26. Kinoshita, K. Carbon: Electrochemical and Physicochemical Properties. John Wiley & Sons; New York: 1988.

27. Thompson JD, Brown JS, Carr PW. Anal. Chem 2001;73:3340–3347. [PubMed: 11476234]

28. Foley JP, Dorsey JG. Anal. Chem 1983;55:730–737.

29. Ives, DJG.; Janz, GJ., editors. Reference Electrodes: Theory and Practice. Academic Press; New York: 1961.

30. Oldham, KB.; Myland, JC. Fundamentals of Electrochemical Science. Academic Press; San Diego: 1994.

31. Ponton LM, Keller D, Porter MD. Recent results show that entropy plays a major role in governing the EMLC-based retention of these compounds, paralleling the findings from several studies that examined the separation of organic anions by ion exchange chromatographyIn preparation

32. Hatsis P, Lucy CA. Analyst 2001;126:2113–2118. [PubMed: 11814187]

33. Hatsis P, Lucy CA. J. Chromatogr., A 2001;920:3–11. [PubMed: 11453014]

34. Knox, JH.; Ross, P. Advances in Chromatography. Brown, PR.; Grushka, E., editors. 37. Marcel Dekker; New York: 1997. p. 73-119.

35. Tornkvist A, Markides KE, Nyholm L. Analyst 2003;128:844–848.

36. Chen Q, Swain GM. Langmuir 1998;14:7017–7026.

37. DeClements R, Swain GM, Dallas T, Holtz MW, Herrick RD III, Stickney JL. Langmuir 1996;12:6578–6586.
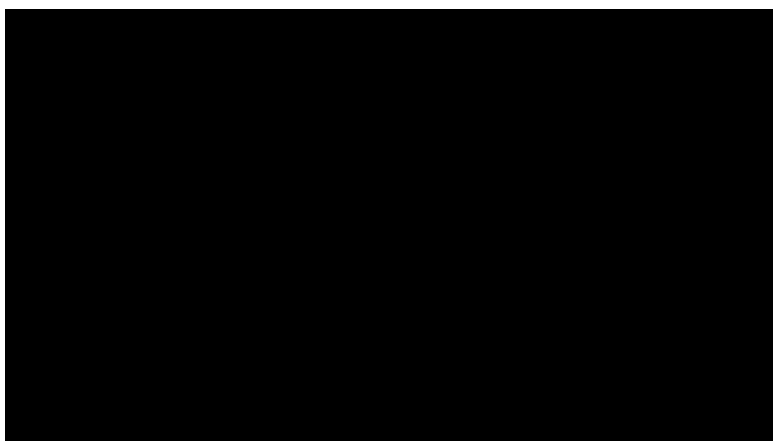
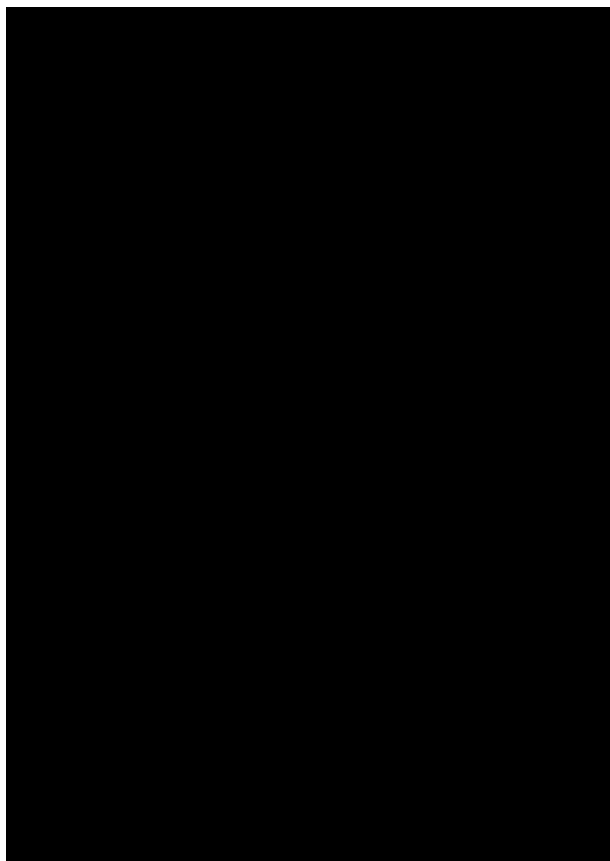**Figure 1.**
Instrument schematic for high-speed EMLC.

Figure 2.
Dependence of the retention of BS, MBS, CBS, and 1,5-NDS on E_{app} at a flow rate of 0.5 mL/min and a mobile phase composed of 0.1 M lithium perchlorate in a 5% acetonitrile, 95% water mixture, and a temperature of 25.0 °C. (A) +100, (B) −100, (C) −300, and (D) −500 mV. Inset: Plot ln k' vs E_{app} for BS (◆), MBS (■), CBS (▲), and 1,5-NDS ([unk]).
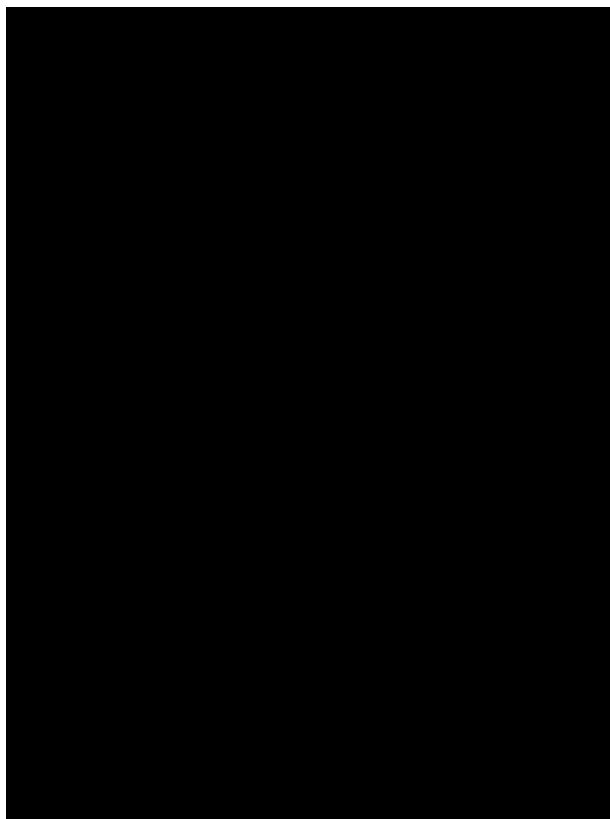
Figure 3.
Temperature dependence for the EMLC separation of a mixture of BS, MBS, CBS, and 1,5-NDS with a mobile phase composed of 0.1 M lithium perchlorate in a 5% acetonitrile, 95% water mixture. Chromatograms: (A) 0.5 mL/min at 25.0 °C, and +100 mV; (B) 0.5 mL/min at 61.5 °C, and +70 mV; (C) 0.5 mL/min at 100.0 °C, and +40 mV; (D) 1.0 mL/min at 100.0 °C, and +40 mV; (E) 1.5 mL/min at 100.0 °C, and +40 mV; and (F) 2.0 mL/min at 100.0 °C, and +40 mV.

Figure 4.
Dependence of retention of BS, MBS, CBS, 1,5-NDS, and 2,6-NDS on E_{app} at a flow rate of 2.0 mL/min with a mobile phase composed of 0.1 M lithium perchlorate and 5% acetonitrile in water and a temperature of 100.0 °C. (A) +40, (B) −160, (C) −360, and (D) −560 mV. Inset: ln k' vs E_{app} BS (◆), MBS (■), CBS (▲), 1,5-NDS (○), and 2,6-NDS (×).

Figure 5.
Dependence of retention of BS, MBS, CBS, and 1,5-NDS on E_{app} at a flow rate of 2.0 mL/min with a mobile phase composed of 0.1 M lithium perchlorate in water and a temperature of 100.0 °C. (A) +40, (B) −160, (C) −360 , (D) −560, and (E) −760 mV. Inset: Plot ln k' vs E_{app} BS (◆), MBS (■), CBS (▲), and 1,5-NDS (○).

Table 1

Slopes from the Plots of ln k' versus E_{app} (10^{-3} mV^{-1})

	A	B	C
mobile phase flow rate (mL/min)	0.5	2.0	2.0
temperature (°C)	25	100	100
analyte			
BS	1.58 ± 0.05	2.00 ± 0.03	1.06 ± 0.05
MBS	1.62 ± 0.05	1.80 ± 0.02	1.03 ± 0.05
CBS	1.71 ± 0.05	1.87 ± 0.02	1.01 ± 0.05
1,5-NDS	3.76 ± 0.12	4.46 ± 0.07	2.57 ± 0.12
2,6-NDS	nd[a]	4.19 ± 0.08	nd[a]

[a] nd, not determined.