Coupling temperature control with electrochemically modulated liquid chromatography: fundamental aspects and applications

by

Lisa M. Ponton

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Program of Study Committee:
Marc D. Porter, Major Professor
Victor Lin
Andrew Hillier
Thomas Greenbowe
Richard Seagrave

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Graduate College

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This is to certify that the doctoral dissertation of

Lisa M. Ponton

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Major Professor

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Achieving great things is not done alone; it is done by relying on others. This dissertation is the result of the efforts of many people, though only a few of them will understand what is written here.

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ABSTRACT

The primary focus of the doctoral research presented herein has been the integration of temperature control into electrochemically modulated liquid chromatography (EMLC). The combination of temperature control and the tunable characteristics of carbonaceous EMLC stationary phases have been invaluable in deciphering the subtleties of the retention mechanism. The effects of temperature and E_{app} on the retention of several naphthalene disulfonates were therefore examined by the van't Hoff relationship. The results indicate that while the retention of both compounds is exothermic at levels comparable to that in many reversed-phase separations, the potential dependence of the separation is actually entropically affected in a manner paralleling that of several classical ion exchange systems. Furthermore, the retention of small inorganic anions at constant temperature also showed evidence of an ion exchange type of mechanism. While a more complete mechanistic description will come from examining the thermodynamics of retention for a wider variety of analytes, this research has laid the groundwork for full exploitation of temperature as a tool to develop retention rules for EMLC.

Operating EMLC at elevated temperature and flow conditions has decreased analysis time and has enabled the separation of analytes not normally achievable on a carbon stationary phase. The separation of several aromatic sulfonates was achieved in less than 1 min, a reduction of analysis time by more than a factor of 20 as compared to room temperature separations. The use of higher operating temperatures also facilitated the separation of this mixture with an entirely aqueous mobile phase in less than 2 min. This methodology was extended to the difficult separation of polycyclic aromatic hydrocarbons on

PGC. This study also brought to light the mechanistic implications of the unique retention behavior of these analytes through variations of the mobile phase composition.

CHAPTER 1. GENERAL INTRODUCTION

This dissertation describes the application of electrochemically modulated liquid chromatography (EMLC) to a variety of separation challenges and advances a fundamental understanding of the processes driving retention on carbon stationary phases. In recent years, EMLC has emerged as a powerful separation technique applicable to different categories of analytes using the same stationary phase,[1, 2] the development of which is described below. To date, this research has primarily focused on the utility of EMLC as a separation technique[3-8] and on improvements to column design.[9] Very little has been done to advance a thorough mechanistic understanding of EMLC-based retention.[10-13]

Several of the projects described in this dissertation revolve around incorporating temperature control into the function of EMLC. Temperature is used in high performance liquid chromatography (HPLC) both as a means of determining the thermodynamics of the separation process and of enhancing the speed of the separation. The work described herein employs temperature in EMLC for both purposes. Each chapter following the general introduction is a manuscript submitted for publication.

Literature Review

HPLC has become one of the most used techniques in chemical analysis because a variety of complex mixtures can be separated based on subtle differences in interactions between mobile and stationary phases.[14, 15] However, separations of different categories of analytes typically require compositionally different stationary phases. For example, resolution of aliphatic or aromatic compounds can be achieved on a reversed-phase silica

packing, but inorganic ions require an ion exchange material. Once an appropriate stationary phase is selected, changes in mobile phase composition, either with isocratic or gradient elution strategies are often used to enhance resolution and decrease elution time.

Development of EMLC. In EMLC, conductive stationary phases like glassy carbon (GC) and porous graphitic carbon (PGC) are packed into an HPLC column that is also configured to function as a three-electrode electrochemical cell. As such, the packing acts both as a chromatographic stationary phase and a high surface area working electrode. This dual function results in the unique ability to manipulate the surface charge density of the conductive packing through changes in applied potential (E_{app}), which in turn, alters analyte retention. EMLC can therefore be viewed as an approach for fine tuning separations by changes in the effective composition of the stationary phase.

The design of the EMLC column is shown in Figure 1.[9] A conductive stationary phase, such as GC or PGC, is packed inside a Nafion™ cation-exchange membrane that has been inserted in tubular form into a porous stainless steel column. In addition to serving as a rigid column support, the porous stainless steel housing acts as a high surface area auxiliary electrode. The Nafion™ tubing functions as: (1) a container for the carbon stationary phase, (2) an electronic insulator between working and auxiliary electrode, and (3) a salt bridge for ion transport. An Ag/AgCl (saturated NaCl) electrode, a reference for E_{app}, is placed in a reservoir surrounding the auxiliary electrode.

The development of EMLC can be traced back to the 1960s where flowing electrolytic cells were used to separate metal cations in water.[16-20] These separations relied on the deposition and subsequent stripping of metals from the working electrode,

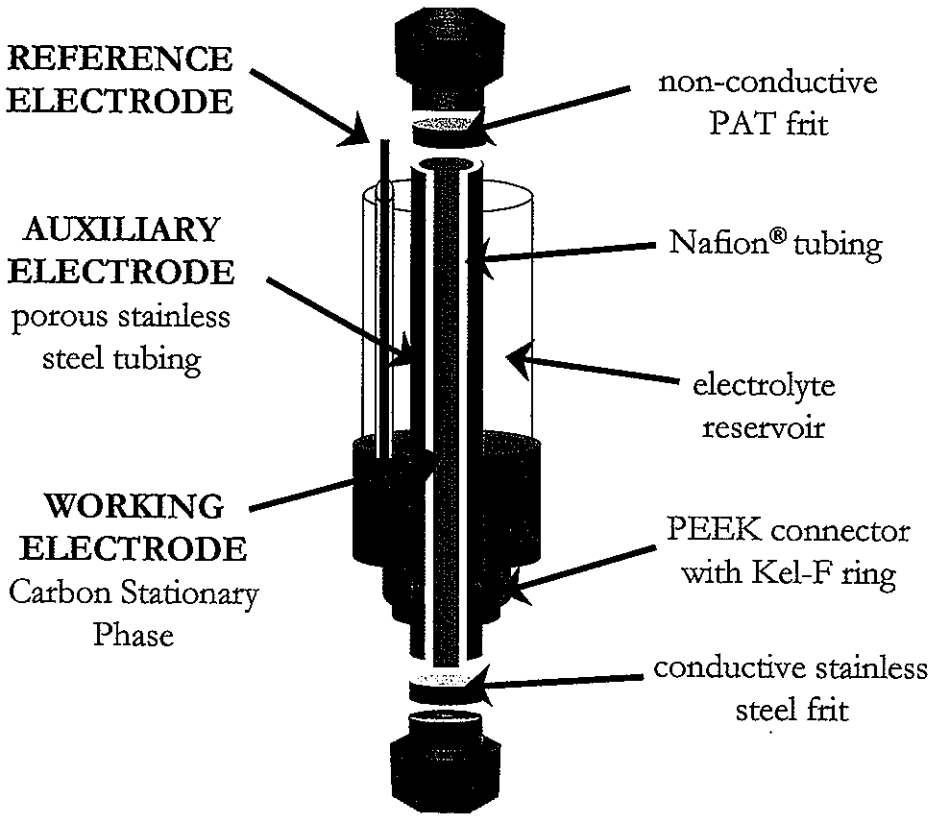

Figure 1. Column design for electrochemically modulated liquid chromatography.

an on-off mechanism rather than an equilibrium partitioning mechanism. The metal ions were deposited (reduced) at one potential and then stripped by stepping the potential to remove (oxidize) each metal individually. The deposition/stripping method was extended in the 1970s to the separation of organic compounds via electrostatic control of analyte adsorption, i.e., electrosorption, rather than electron transfer.[21-23]

A major advancement was developed by Antrim et al.[24, 25] through the use of a stainless steel column housing that was capable of withstanding higher pressures, making the separation more akin to HPLC. Using this column design, they examined different fixed potentials and monitored the change in the retention of neutral organic compounds. By functioning at different values of E_{app}, the equilibrium distribution of the analyte is altered, which thereby affects retention. The retention described in this example is then, a manipulation of a partitioning mechanism, unlike the previous examples based on deposition/stripping concepts. A significant drawback of this design was the extremely poor chromatographic efficiencies found in the separation (2-10 theoretical plates).[24, 25]

Another direction in the development of EMLC was the use of electroactive ionomers (polymers with both electroactive and ion exchange functionalities) coated onto carbon particles. In the reduced form, the electroactive site on the ionomer is neutral, therefore the ion-exchange site will uptake mobile phase cations. In the oxidized form, the electroactive site is positive and there is charge compensation between the electroactive site and ion-exchange site, resulting in the release of the cations. This retention mechanism is another example of an on-off methodology.[26]

Conducting polymers[27] were also used for potential-controlled separations. For example, through electrochemical control, the oxidation state of polypyrrole, a conducting

polymer, can be manipulated. In its reduced form, the polymer exists in its uncharged, nonconductive state. Oxidizing the polymer drives the reincorporation of a counterion, which results in a positively charged conductive phase. In this example, analyte retention will be different for the reduced and oxidized form of the stationary phase, resulting in a partitioning mechanism in which retention is manipulated via changes in E_{app}.[28-31]

Several interesting stationary phases have been used to examine potential-controlled retention such as: carbon,[32, 33] crown ether coated conductive particles,[34] polyaniline coated particles[34-37] polypyrrole-coated stationary phases,[35-38] a heme-agarose sorbent,[39] a heme-derivitized stationary phase,[40] and an unmodified mercaptohexanol stationary phase.[40] A ℓ-glutamate doped polypyrrole has also been coated onto carbon fibers as a molecular imprinted stationary phase.[41] Despite the extensive research to this point, chromatographic efficiencies of potential controlled separations comparable to conventional HPLC had not yet been achieved. EMLC methodology has also been utilized for the development of electrochemically controlled solid phase microextraction by taking advantage of the deposition/stripping nature described in some of the earlier studies on EMLC.[42-45]

A major advancement in EMLC came with the redesign of the column to withstand the chromatographic pressures necessary for the use of a high pressure slurry packer and the high pressures needed when using high performance packings. This first truly chromatographic column was used for the separation of a series of aromatic sulfonates.[10] Further examination of the effect of E_{app} (fixed and gradient) by analysis of a variety of aromatic sulfonates began to delineate some of the important features of the retention mechanism.[11, 46] These early studies used glassy carbon beads as the chromatographic stationary phase. The development of PGC by Knox as a high efficiency HPLC packing

material, described below,[47-50] allowed significantly improved chromatographic efficiency in EMLC.[11, 46]

While chromatographic efficiencies were improved, there were still limitations in the electrochemical performance (large charging currents and long equilibration times). Further column design improvements used a porous stainless steel housing as the auxiliary electrode, resulting in a three-fold improvement in equilibration time.[9] Re-examination of the aromatic sulfonates yielded a clear linear dependence of the natural log of the capacity factor (ln k') on E_{app}.[9] Using the new column design, a series of different compounds were separated: corticosteroids,[8] neutral monosubstituted benzenes,[12] benzodiazepines,[6] and aromatic anions and cations.[51] Through electrosorption-based modification of PGC with β-cyclodextrin, the enantiomeric separation of hexobarbital and mephenytoin[5] and benzodiazepines[7] were achieved. EMLC has also been coupled with electrospray mass spectrometry[3] and the chemical modification of the carbon stationary phase with diazonium salts[4] and via the Kolbe reaction[52] have been explored. Using an alternate column design that also allowed the use of a slurry packer, several short-chain carboxylic acids were separated on GC.[53]

Development of a High Efficiency Carbon Stationary Phase. Prior to the development of EMLC was the synthesis of a high efficiency carbon phase for HPLC. Kiselev's studies on the heats of adsorption of organic compounds onto graphitized carbon black[54-57] and the use of graphitized carbon black in gas chromatography[58, 59] led to the idea of using carbon in the HPLC separation of both nonpolar and polar compounds.[60] However, the carbon phases at the time were too fragile to withstand the pressures required by HPLC. The first HPLC experiment using carbon employed silica gel particles coated with pyrolytic carbon for the separation of organic compounds of varying polarity.[61, 62] Shortly after this

first demonstration, Guiochon and coworkers published several articles using pyrolytic carbon coated onto carbon black particles for the separation of organic compounds of varying polarity.[63-65] While demonstrating the utility of carbon as a solid stationary phase for the separation of nonpolar and polar compounds, this work suffered from a high degree of band broadening, yielding separations inferior to the chromatographic efficiency typical of bonded stationary phase counterparts.

The use of carbon in HPLC became a realistic option with the development of PGC.[47, 66] PGC is fabricated by the pyrolysis, in an inert environment, of a phenol-formaldehyde resin on a mesoporous silica gel, which serves as a dissolvable template to define particle and pore size.[47, 48, 50, 66] The extent of graphitization (size of intraplanar microcrystallites) of the resulting structure is strongly dependent on pyrolysis temperature.[48, 67] PGC is viewed as having a significant (but not quantified) amount of intertwined ribbons with a basal plane structure.[47, 48] The intertwined ribbons give PGC its mechanical strength as well as its high graphitic nature. Because of its stability over a large pH range and its unique ability to retain both nonpolar and polar compounds, PGC has found utility in a wide variety of applications, including the separation of geometric isomers, enantiomers, sugars and carbohydrates, polychlorinated biphenyls, ionized compounds, and other highly polar compounds.[50] Recent applications of PGC include the separation of biological compounds,[68, 69] pharmaceuticals,[70, 71] and environmental contaminants.[72, 73]

Virtually all EMLC work has employed either PGC or GC. While PGC is the more efficient packing, there are several key differences between PGC and GC that make them both interesting to investigate as a stationary phase. One structural difference is that PGC is a porous stationary phase (high surface area), while GC is a nonporous stationary phase

(lower surface area), resulting in a higher chromatographic efficiency for PGC. Another important surface structural difference is the ratio of edge plane (sp^3 hybridized) to basal plane (sp^2 hybridized) carbon. Based on Raman and AFM studies, it is a currently held belief that the GC surface is comprised of a small fraction (< 10%) of highly ordered domains.[74, 75] In other words, the GC surface has very little electronically pure edge or basal planes; these regions exist, rather, as electronically perturbed domains that have a partial charge character that enhances interactions with aromatic systems. PGC on the other hand, as previously mentioned, is viewed as intertwined ribbons with a highly pronounced basal plane structure; that is, a 2-D graphite structure.[48]

Another critical feature of a carbon surface is the concentration of surface oxygen. X-ray photoelectron spectroscopy (XPS) studies in our laboratory have confirmed that as-received PGC particles have negligible levels of surface oxygen (< 1%), while GC spheres have a substantial surface oxygen concentration (~5%). In addition to the concentration of oxygen on the carbon surface, the nature of these groups (e.g., phenol, carbonyl, lactone, carboxylic acid, *o*-quinone, and *p*-quinone) may impart different sorptive properties and may be present in differing amounts depending on material processing.[67] Moreover, it has been shown that the surface carbonyl species (benzoquinones and ketones) are confined to edge plane regions.[76]

It is also important to realize that the slow oxidation of surface groups on these phases can result in a change in retention.[77] After treating a PGC column with an oxidizing agent, no clear peak for a negatively charged analyte was observed in the resulting chromatogram. However, after equilibrating the column for several days, a band for the eluent appeared with close to the same retention time as a non-treated columns.[77] Based on

this evidence, it appears that the surface was slowly reduced to its original state by the mobile phase. Results in our laboratory, which are presented in Appendix A, have found that a change in the surface oxygen content of the PGC strongly correlates with a change in analyte retention.

Retention on Carbon Stationary Phases. Today's separation theories for carbon phases are founded on a combination of intermolecular forces including donor-acceptor, dispersive, and solvophobic interactions.[47, 78, 79] Donor-acceptor interactions occur as a result of the aromatic nature of the carbon surface (interactions between the n and/or π electrons of an analyte and the delocalized π electrons at the carbon surface). The high polarizability of the surface leads to a dipole or "mirror charge" beneath the carbon surface in the presence of polar analytes.[48, 80] This induced dipole leads to an electrostatic attraction.

In EMLC, electrostatic attractions appear to dominate the potential dependence of the retention of aromatic sulfonates.[6, 9] However, due to the control of the charge density of the carbon surface in EMLC, it is unclear whether an induced dipole will be caused by the presence of a polar analyte. Some types of carbon phases have varying amounts of oxygen-containing groups that may lend a significant polar character to the stationary phase. This polarity can lead to dipole and H-bonding interactions with analytes. Dispersive and solvophobic forces can also act to increase analyte retention as a function of hydrophobicity, as recently modeled by several groups.[81-83] Solvophobic interactions, which reflect the thermodynamics of the solvation of an analyte in the mobile phase, are a consequence of how an analyte disrupts local solvent structure.[7, 84] In an HPLC separation, changes in solvophobicity are affected by a change in the organic content of the mobile phase. The situation with EMLC is notably different. We will conceptually examine possible

solvophobic effects in EMLC within the context of changes in the structure (e.g., charge distribution) of the electrical double layer with E_{app}. Determining both the retention dependencies of highly aromatic nonpolar compounds, polycyclic aromatic hydrocarbons (Chapter 5), and small inorganic anions (Chapter 3) with E_{app} will add to this understanding.

The electrical double layer is viewed as an arrangement of charged species and oriented dipoles at the electrode-solution interface.[85, 86] The solution side of the double layer consists of several tiers: the inner Helmholtz plane (IHP), the outer Helmholtz plane (OHP), and the diffuse layer. The IHP consists of specifically adsorbed ions and highly ordered solvent molecules. The OHP is composed of solvated ions and solvent molecules that are less ordered than in the IHP. The diffuse layer is a region of excess charge that balances the charge on the substrate, and extends from the OHP into the bulk solution.[85, 86] The thicknesses of these layers, especially the diffuse layer, depend upon the charge on the substrate and electrolyte concentration. The implications of the dependence of the diffuse layer thickness on charge density as manipulated by changes in E_{app}, will need to be addressed as we work to develop an accurate picture of the volume of the stationary phase (V_{sp}). The importance of V_{sp} is elucidated in the next section.

Thermodynamic Determinations in HPLC. Retention in HPLC is often expressed as the capacity factor, k', which is the ratio of the number of moles of analyte in the stationary phase (n_{sp}) to the number of moles of analyte in the mobile phase (n_{mp}).

$$k' = \frac{n_{sp}}{n_{mp}} \qquad [1]$$

According to this definition, the capacity factor is directly proportional to the equilibrium constant (K) for the interaction governing retention.

$$k' = K\phi \quad [2]$$

The term ϕ is the phase volume ratio,

$$\phi = \frac{V_{sp}}{V_{mp}} \quad [3]$$

where the volume of the stationary phase is given by V_{sp} and the volume of the mobile phase is represented by V_{mp}.

Based on these definitions, an analysis of retention can then be carried out via equilibrium thermodynamics,

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad [4]$$

$$\Delta G^0 = -RT \ln K \quad [5]$$

where ΔG^0 is the change in the standard Gibbs free energy, ΔH^0 is the change in standard enthalpy, ΔS^0 is the change in standard entropy, T is the temperature, and R is the gas constant.

Combination of equations 2, 4, and 5 allows the extraction of the thermodynamic characteristics (ΔH^0 and ΔS^0) of chromatographic systems from the temperature-dependence of k', as given by the van't Hoff relationship in eqn. 6.

$$\ln k' = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \quad [6]$$

If ΔH^0 is a constant over the target temperature range, then the slope of a plot of ln k' against the inverse of T is directly proportional to ΔH^0. By extension, the linearity of the van't Hoff plot also implies that other thermodynamic quantities, such as heat capacity of the mobile phase, are independent of temperature in the same range. A positive slope in the van't Hoff

plot is indicative of an exothermic process, whereas a negative slope is indicative of an endothermic process.

If a value for ϕ can be determined, the standard entropy change can be calculated from the y-intercept of the plot, which equals $\Delta S^0/R + \ln \phi$. A negative value for ΔS^0 indicates a decrease of disorder in the system, whereas a positive ΔS^0 indicates an increase in disorder in the system upon adsorption of the analyte onto the stationary phase. If both ΔH^0 and ΔS^0 are known, the overall change in standard Gibbs free energy (ΔG^0) of the interaction can then be calculated. Since chromatographic retention is a spontaneous process, ΔG^0 should be negative.

The application of the van't Hoff equation to HPLC has a long history.[87] Several laboratories have used this approach to define the thermodynamic basis of retention mechanisms in many systems, such as reversed-phase liquid chromatography (RPLC)[87-92] and ion-exchange chromatography (IEC).[93-95] The similarities or differences in the values of both ΔH^0 and ΔS^0, as determined under the same separation conditions, are often valuable in assessing whether or not the retention mechanism of a group of analytes is the same or different. The principle focus in the cited work was on delineating the enthalpic contribution to retention.

In RPLC, retention generally decreases with increases in temperature,[88-92] indicating that the overall retention mechanism between an analyte and the reversed-phase packing is exothermic (negative values of ΔH^0). Retention at carbon stationary phases is also typically exothermic.[96-98] This situation arises from the polarity difference between the stationary and mobile phases. For a given mobile phase and stationary phase, the greater the similarity

between the polarity of the analyte and the stationary phase, the stronger the interaction; hence, ΔH^0 is more exothermic. Conversely, the closer in polarity the analyte is to the mobile phase, the less exothermic the interaction. When comparing the retention across different mobile phase compositions, a larger polarity difference between the mobile and stationary phases corresponds to a more exothermic interaction. As such, the magnitude of exothermicity can reflect the type and strength of the interaction between the analyte and stationary phase.

While RPLC separations are generally exothermic, ΔS^0 can have a significant role in selectivity changes as a function of temperature[90] as well as in shifts in retention with a variety of mobile phase polarities.[88, 89] In the separation of benzodiazepines, ΔS^0 increased with increased mobile phase polarity (decreased methanol content) due to changes in water ordering around the analyte in the mobile phase (i.e., the hydrophobic effect).[89] Furthermore, it was found that while ΔH^0 dominated the retention, ΔS^0 played an increasing role with a decrease in mobile phase polarity.[89] Similarly, systematic studies on the retention of neutral aromatic compounds at carbon phases showed that the greater the contact area of the organic compound with the surface (i.e., how flat the molecule sits on the surface), the lower the entropy of adsorption due to lower surface mobility resulting from local order surrounding the analyte on the mobile phase.[96] Nevertheless, ΔH^0 is often the overriding energy driving the separation in most cases.[88, 89, 96]

The situation with IEC, however, can be dramatically different in that the ΔH^0 can actually be positive (i.e., endothermic), particularly for organic ions.[93-95, 99] Since the measured retention is spontaneous, entropy must play the governing role in the retention.

The hydrophobic nature of the organic ions leads to increased disorder upon adsorption due to extensive short-range ordering of the water molecules surrounding the organic ions in the mobile phase.[93-95, 99, 100] The impact of entropy is particularly notable in the ion exchange of ionic aromatic compounds (e.g., benzene sulfonates and naphthalene sulfonates).[101] These compounds have both ionic and hydrophobic character. Here again, the hydrophobic interactions can lead to a retention process that is entropically and not enthalpically controlled due to the breakup of structured water in the mobile phase as the analyte is adsorbed onto the ion exchanger.[101]

High Speed Chromatography. Up to this point, the use of temperature has been discussed from a fundamental perspective. Temperature can also be employed as a means of optimizing a separation. While seldom enhancing the resolution of the separation or the asymmetry of an elution band, elevating the column temperature has several distinct advantages that have been known for some time.[102, 103] As discussed above, increasing the column temperature in RPLC generally decreases the retention time due to the exothermic nature of the interaction.[88-92] In addition, the mobile phase viscosity decreases significantly at elevated temperature, which is evident in a drop in the column back pressure. With a drop in column back pressure, higher flow rates can be achieved without exceeding instrumental pressure limits, thereby increasing the speed of the separation, generally without loss of resolution.

Early work postulated that fast liquid chromatography could be achieved at high flow rates using small particles in a long column.[104] The larger particles commonly used in HPLC require slower flow than the optimum linear velocity due to the pressure limits of the instrument. Slower flow results in an increase in longitudinal diffusion as evident in

increased band broadening.[105] Conversely, higher flow rates would decrease longitudinal diffusion due to the analyte spending less time on the column. The more time the analyte spends on the column, the more the extent of longitudinal diffusion.

Only recently, however, have the advantages of increased temperature been fully realized in the performance of fast and ultra-fast liquid chromatography.[106, 107] By functioning at elevated column temperatures, the flow rate or linear velocity of the mobile phase can be increased due to the drop in mobile phase viscosity. Increasing the flow through the column can decrease the efficiency of the separation if the optimum linear velocity is exceeded. However, an increase in temperature increases the efficiency by improving analyte diffusivity. Careful instrumental design must balance these two effects, as well as address the issues related to the temperature limits of commercial hardware, in order to fully exploit this opportunity. Implementation of such a design by Carr and coworkers has resulted in separations in well under 1 min.[106, 107]

Investigating both the thermodynamic and mobile phase viscosity effects of temperature, as well as furthering the understanding of the retention mechanism in EMLC through changes in mobile phase conditions and careful choice of analytes, is the focus of this dissertation.

Dissertation Organization

Chapter 2 describes the use of varied temperatures in EMLC to calculate the standard enthalpy and entropy of the retention mechanism on GC. Chapter 3 describes the first demonstration of the potential-controlled separation of inorganic anions on carbon at constant temperature. Chapter 4 discusses the use of elevated temperature and flow rate to

achieve EMLC separations in less than 1 min. Chapter 5 describes the unique behavior of polycyclic aromatic hydrocarbons with different mobile phase conditions and at elevated temperature and flow rate. Finally, a general conclusion is presented in Chapter 6. In the process of completing the above described experiments some interesting effects of elevated temperature on carbon were discovered. Specifically, operation at higher temperatures accelerates the oxidation of the packing surface, as discussed in Appendix A.

And now for something completely different: a project involving the analysis of spacecraft water is briefly described in Appendix B. This work is the start of efforts to improve the archiving of trace organic compounds in spacecraft water onboard the Shuttle and International Space Station for ground analysis upon return to Earth.

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CHAPTER 2. THERMODYNAMICS OF RETENTION IN ELECTROCHEMICALLY MODULATED LIQUID CHROMATOGRAPHY

A paper in preparation for publication

Lisa M. Ponton,[1,2] David W. Keller,[2] and Marc D. Porter[2,3]

Abstract

The temperature dependence of retention was investigated to examine the on-column thermodynamics for separations based on electrochemically modulated liquid chromatography (EMLC). EMLC couples electrochemistry and liquid chromatography for the facile manipulation of analyte retention through changes in the potential applied (E_{app}) to conductive stationary phases like glassy carbon (GC). A detailed understanding of the EMLC-based separation mechanism would therefore be beneficial for the development of predictive retention rules, while also yielding insights into phenomena at electrified interfaces. The work herein details an investigation in which the dependence of retention as a function of column temperature for two nathphalene difulfonates (1,5- and 2,6-naphthalene disulfonate) was determined at several values of E_{app}. In each case, the van't Hoff relationship was employed to calculate the enthalpic and entropic contributions to the retention process (i.e., the transfer of an analyte from the mobile to stationary phase). The results show that the retention for both compounds is exothermic at a level comparable to many reversed phase separations. However, the overall dependence of retention by E_{app} is

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[1] Primary researcher and author

[2] Institute of Combinatorial Discovery, Chemistry Department, Iowa State University, and Ames Laboratory-USDOE Ames, IA 50011

[3] Author for correspondence

actually entropically controlled in a manner paralleling that for organic anions at resin-based ion exchange materials. Possible origins of the observed entropic control are examined, and its implications to EMLC-based separations are discussed.

Introduction

Electrochemically modulated liquid chromatography (EMLC) is an intriguing variant of high performance liquid chromatography (HPLC).[1] In EMLC, conductive stationary phases like glassy carbon (GC) and porous graphitic carbon (PGC) are packed in a HPLC column that also functions as a three-electrode electrochemical cell. The packing therefore acts as a chromatographic stationary phase and a high surface area working electrode. This dual usage results in the unique ability to manipulate the donor-acceptor properties (e.g., surface charge density) of the conductive packing through changes in applied potential (E_{app}), which, in turn, alters retention. Reports from several laboratories,[2-7] including our own,[8-16] have shown that EMLC can be utilized for the separation of a wide range of mixtures (e.g., aromatic sulfonates,[12] monosubstituted benzenes,[11] pyridines and anilines,[13] corticosteroids,[10] benzodiazepines,[8] and short chain alkanoic acids[5]).

To date, interpretations of how E_{app} affects retention have employed structural parameters like local dipole moment and hydrophobicity, which have been coupled to electrical double layer theory and molecular adsorption models to include contributions from the supporting electrolyte and mobile phase.[11] The work reported herein is the first in a series of investigations that takes a different tact in furthering a mechanistic understanding of EMLC by examining the effects of temperature on retention through the van't Hoff relationship. Several laboratories have used this approach to develop a thermodynamic

insights into retention mechanisms for a number of separation formats, including reversed-phase liquid chromatography (RPLC)[17-22] and ion-exchange chromatography (IEC).[23-25] Moreover, comparisons of the enthalpy (ΔH^0) and entropy (ΔS^0) for the transfer of an analyte from the mobile to stationary phase can be valuable in assessing whether there are mechanistic similarities for the retention for different analytes.

The retention of small molecules in RPLC almost always decreases with increases in temperature,[18-22] which is diagnostic of an enthalpically driven process. Retention at carbonaceous stationary phases is also typically exothermic.[26-28] The magnitude of the exothermicity can be qualitatively assessed by comparisons of the polarity of the analyte with respect to the stationary and mobile phases. In general, the closer the polarity of the analyte and stationary phase, the stronger the interaction and the greater the exothermicity of retention. Conversely, the closer the polarity of the analyte and mobile phase, the lower the exothermic contribution to retention.

There are a few instances in which small molecule-retention in RPLC undergoes an increase with temperature.[22, 29-32] One of the most intriguing examples involves the temperature dependence of retention for ethanol, isopropanol, and butanol at a packing composed of hydrophobic porous beads (i.e., a reversed phase packing devoid of charged groups or residual silanols).[29] Using water as the mobile phase, the retention of the three analytes exhibited an increase with temperature. This dependence was attributed to the hydrophobic effect,[29, 33] which is indicative of a retention process under entropic control. Results also showed that the gradual addition of an organic modifier (i.e., methanol) in the mobile phase decreased the observed endothermicity, and in the case of butanol actually lead

to an exothermic retention process. Other examples[19] found that while ΔH^0 dominated the retention, ΔS^0 increased with increased mobile phase polarity.

Retention in IEC can also be endothermic, particularly for organic ions,[23-25, 34] which translates to a process controlled by entropy. The impact of entropy is particularly notable in the ion exchange of ionic aromatic compounds (e.g., benzene sulfonates and naphthalene sulfonates).[35] Again, hydrophobic interactions lead to a retention process that is entropically and not enthalpically controlled, reflecting the breakup of the more structured water clusters surrounding these hydrophobic anions when retained by the ion exchanger.[35]

The work reported herein employs a similar set of tactics to gain insights into the on-column thermodynamics of retention in EMLC. This paper therefore describes: 1) the findings from an investigation of the temperature dependent retention in EMLC separations of two naphthalene disulfonates (i.e., 1,5- and 2,6-naphthalene disulfonate) at GC stationary phases; and 2) the implications of these findings to fundamental issues in adsorption phenomena at electrified interfaces. By the extraction of the enthalpic (ΔH^0) and entropic (ΔS^0) contributions to EMLC-based retention and comparisons with relevant data in the literature on RPLC and IEC, further insights into factors central to EMLC-based retention are detailed.

Experimental Methods

Chemicals and Reagents. The analytes, 1,5-naphthalene disulfonate (1,5-NDS) and 2,6-naphthalene disulfonate (2,6-NDS), were purchased from Aldrich Chemical (Milwaukee, WI). Both were used at a concentration of 12 μM after dissolution in Milli-Q water (Millipore, Bedford, MA). The mobile phase consisted of 40 mM (aqueous) sodium

hexafluorophosphate (Aldrich) with 5% HPLC grade acetonitrile (Fisher Scientific, Pittsburgh, PA) in Milli-Q water, which was passed through a 0.5-μm filter (GE Osmonics Inc., Minnetonka, MN) prior to use.

The GC particles were obtained from Alfa Aesar (Ward Hill, MA) having a size range of 0.4–12 μm and further sieved by air classification to 5–10 μm.[12] Base on BET characterizations, these particles have a surface area of 2.39 m^2/g. Prior to packing, the particles were washed in sulfuric acid and Milli-Q water to remove calcium and fluoride from the surface, which are attributed to residues from particle preparation. Characterizations by X-ray photoelectron spectroscopy revealed, as previously reported,[12] that as-received GC has a surface oxygen content of ~6% (atomic), which is largely distributed among phenol, carboxylic acid, and quinone groups.

Instrumentation. The design and construction of the EMLC column[9] and hardware for temperature control[16] have been previously described. In brief, the EMLC column uses a conductive stationary phase, such as GC, that is packed inside a Nafion™ (Perma Pure Inc., Toms River, NJ) cation-exchange membrane that has been previously inserted in tubular form into a porous stainless steel column (Mott Corp., Farmington, CT). The Nafion™ tubing functions as: 1) an electronic insulator between the conductive packing and auxiliary electrode; and 2) a salt bridge between the packing and auxiliary and reference electrodes. The porous stainless steel housing also serves as a high surface area auxiliary electrode. All values of E_{app} are reported with respect to an Ag/AgCl (saturated NaCl) electrode, which was placed in an electrolyte-filled reservoir that surrounds the porous stainless steel column at the same temperature as the column. The temperature coefficient for the potential for this type of reference electrode is -0.71 mV/°C in the temperature range of 20-55 °C.[36] We note that

the 35 °C increase in temperature would result in a decrease in E_{app} of only 25 mV with respect to a reference electrode at 20 °C. The resulting effect on retention at most contributes to a -2 kJ/mol systematic error in the determination of ΔH^0.

The experimental system for temperature dependence investigations largely duplicated the designs described by Carr and co-workers.[37, 38] The temperature was controlled to ±0.2 °C by the immersion of key system components (i.e., EMLC column and 20-cm length of stainless steel tubing connected to the column inlet), both of which were surrounded in a latex bag for protection against reservoir leakage and to insulate the column from contact with the water in the in the thermostated water bath (Polyscience, Niles, IL). The 20-cm length of stainless steel tubing was used to temperature-equilibrate the mobile phase and sample before entering the column. The lack of any observable band broadening, a diagnostic of thermal mismatch, supports the adequate pre-heating of the liquid before entering the column;[39] tests also showed that the column efficiency at 22 °C (8200 plates/m) was similar to that at 55 °C (8400 plates/m), which further supports adequate thermal equilibration. The column was allowed to equilibrate for 30 min after each alteration in temperature, noting that the associated change in system backpressure required ~10 min to stabilize.

The column was attached to an Agilent Technologies model 1050 HPLC 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). The elution profiles were monitored at 226 nm. The value of E_{app} was controlled by an Amel potentiostat to ±1 mV.

Data Analysis. The void time for the column was measured by the injection of water for calculations of the capacity factor, k', i.e., n_{sp}/n_{mp}, where n_{sp} is the moles of analyte on the stationary phase and n_{mp} is the moles of analyte in the mobile phase. Retention times were determined by using first moment statistical analysis in order to compensate for band assymetry.[40] The first moment (M_1), defined as

$$M_1 = \frac{\int_{-\infty}^{\infty} th(t)dt}{\int_{-\infty}^{\infty} h(t)dt} \qquad [1]$$

where $h(t)$ is the height at time t, represents the centroid of the chromatographic peak.

On-column thermodynamics, i.e., ΔH^0 and ΔS^0, were extracted from the temperature-dependence of the capacity factor (k') that is given by the van't Hoff relationship in Equation 2,

$$\ln k' = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \qquad [2]$$

where R is the gas constant and T is the column temperature. The term ϕ is the phase volume ratio and is defined by Equation 3,

$$\phi = \frac{V_{sp}}{V_{mp}} \qquad [3]$$

with the volume of the stationary phase given by V_{sp} and the volume of the mobile phase by V_{mp}. If ΔH^0 is a constant over the tested temperature range, then the slope from a plot of ln k' against the inverse of T is directly proportional to ΔH^0. By extension, the linearity of the van't Hoff plot often, but not always, implies that related thermodynamic quantities, such as the heat capacity of the mobile phase, are invariant over the same temperature range.

The y-intercept of a van't Hoff plot can be used in the determination of ΔS^0, if the value of ϕ is known. However, the development of an exacting definition and determination of ϕ is a long standing issue in most chromatographic systems.[41] The value of V_{mp} can be determined in several ways, including the minor disturbance method which uses an "unretained" component of the mobile phase as a void marker.[42] Other methods (e.g., pyconometry)[42] were ruled out as possibilities because of the use of Nafion™ and porous stainless steel in the EMLC column design, both of which may retain liquid and compromise the measurement. We therefore determined V_{mp} from the pseudo peak for water, which is used for analyte dissolution and injected into the mixed mobile phase. From 194 replicate injections, the elution time for the pseudo peak equaled 0.570 ± 0.004 min, which coupled with the 0.499 ± 0.002 mL/min flow rate of the mobile phase, translated to a V_{mp} of 0.284 ± 0.002 mL. Determinations were also carried out in which the sample was dissolved in acetonitrile:water mixtures of varied composition (0-5% acetonitrile) with and without supporting electrolyte. There were no detectable changes in the elution time for the void maker when the acetonitrile level was less than 3%; the void marker was not detectable when the acetonitrile level was greater than 3%.

The functional definition and determination of V_{sp} are equally, if not more, problematic in many chromatographic systems.[43] In RPLC, for example, the uncertainty in the free volume of the bonded phase and the extent in which it interacts with an analyte has major implications in terms of the value of V_{sp}. In our case, however, the GC stationary phase is an uncoated and nonporous solid. We have therefore made the reasonable assumption that V_{sp} defined by the surface area of the packing and the thickness of the compact component of the electrical double layer, both of which are invariant with changes

in E_{app}. As such, V_{sp} can be calculated as the product of the surface area of the packing loaded into the column and the thickness of the compact layer. The total surface area of the stationary phase can be determined from the mass of GC loaded in the column (i.e., 0.49 g), whereas the thickness of the compact layer is the sum of the thicknesses of the inner and outer Helmholtz layers (i.e., 5.4 Å).[44, 45] Based on this model, V_{sp} equals 0.63 μL, with ϕ then calculated to be 2.2×10^{-3}. We note that the linearity of the data in the inset in Figure 1 (see below) supports the invariance of ϕ with E_{app}. The other component of the electrical double layer is the diffuse layer, the thickness of which is potential dependent. Any variance in the determination of V_{sp} would add a systematic error to the determination of ΔS^0, which would have no impact on the trends in the data observed. For example, for every additional 1 Å of stationary phase thickness, ΔS^0 would decrease by 1 J/molK and increase ΔG^0 by 0.3 kJ/mol.

If both ΔH^0 and ΔS^0 are known, the standard Gibbs free energy (ΔG^0) for retention can be readily calculated. Since retention is a spontaneous process, calculation of ΔG^0 can be used as a check for the determination of ΔH^0 and ΔS^0.

Results and Discussion

Retention as a function of E_{app}. As previously reported,[9] the change in retention as a function of E_{app} for a variety of aromatic sulfonates at a carbonaceous packing follows predictions based on electrostatic interactions. Figure 1 shows the effect of E_{app} on the retention of 1,5- and 2,6-NDS. Both analytes exhibit a marked decrease in retention as E_{app} becomes more negative, with 1,5-NDS eluting before 2,6-NDS in each instance. The effect

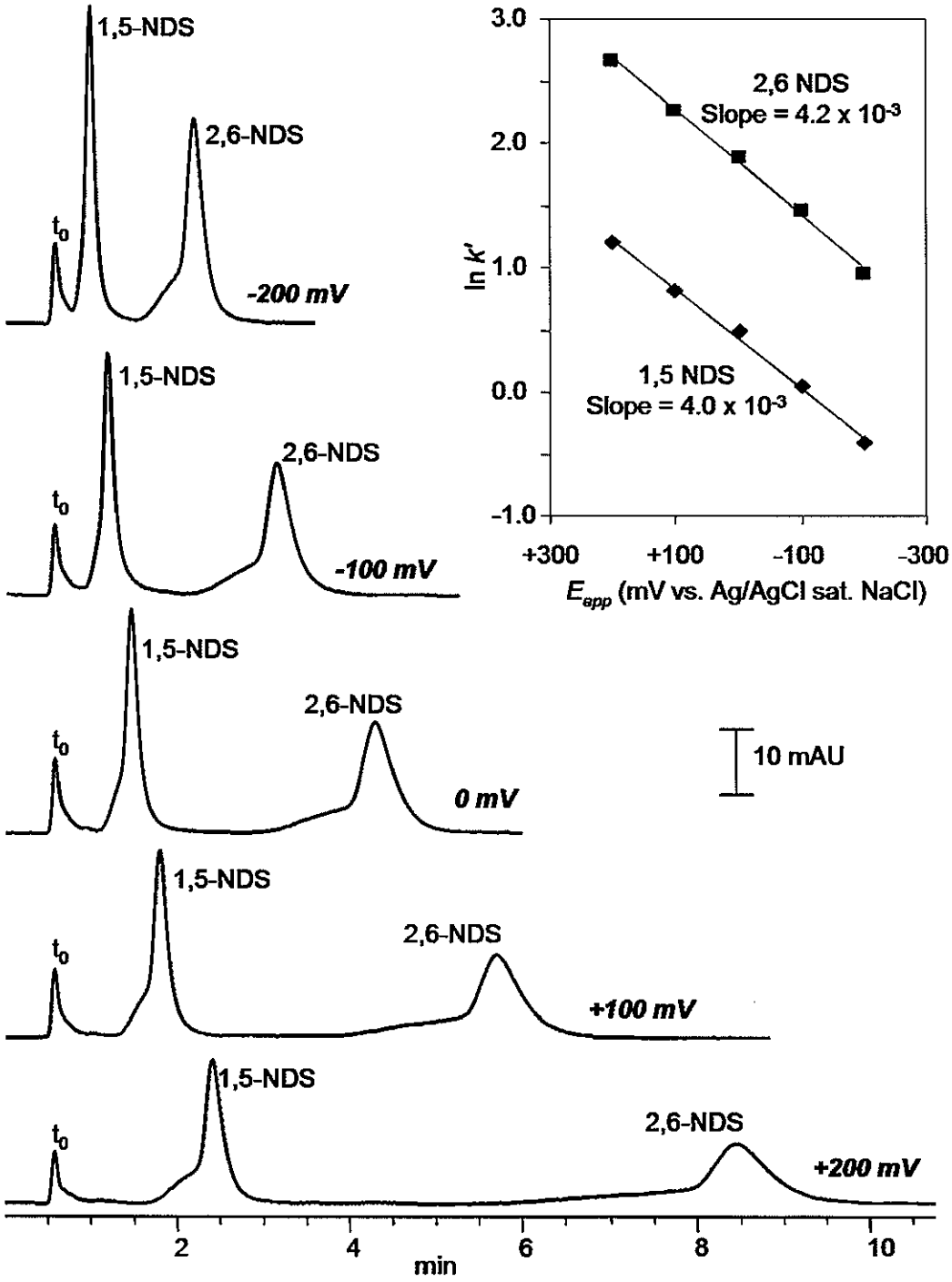

Figure 1. Chromatograms for a mixture of 1,5- and 2,6-NDS as a function of E_{app} using a GC stationary phase. Mobile phase: 0.5 mL/min 40 mM $NaPF_6$ (5% acetonitrile in water). The open circuit potential under this mobile phase was +300 mV. Inset: Dependence of ln k' vs E_{app} for 1,5- (♦) and 2,6-NDS (■). Error bars are smaller than the size of the data points.

of E_{app} on retention is particularly evident when comparing the chromatograms collected at +200 mV and -200 mV. The retention of 2,6-NDS decreases from 8.4 to 2.2 min, whereas that for 1,5-NDS decreases from 2.4 to 1.1 min.

The inset in Figure 1 summarizes these dependencies through plots of ln k' vs. E_{app}. In both cases, ln k' is linearly dependent on E_{app}. These observations can be qualitatively understood by consideration of the ion distribution law, which relates the influence of a potential difference on the equilibrium concentration of ions within the region bound by the potential difference.[46] Assuming that E_{app} modulates only the electrostatic interaction between a charged organic analyte and stationary phase,[13] the ion distribution law can be written for our purposes as,

$$\left[A^{z-}\right]_{sp} = \left[A^{z-}\right]_{mp} \exp\left(\frac{-zeE_{app}}{kT}\right) \qquad [4]$$

where $[A^{z-}]_{sp}$ is the concentration of an anionic analyte at the surface of the stationary phase, $[A^{z-}]_{mp}$ is the concentration of the same analyte in the mobile phase, z is the charge on the ion, e is the charge of an electron, k is the Boltzmann constant, and T is the absolute temperature. By substituting the definition of ϕ (Equation 3) and that of k',

$$k' = \frac{[A^{z-}]_{sp}}{[A^{z-}]_{mp}}\phi \qquad [5]$$

into Equation 5, we can then write Equation 6.

$$\ln k'_{A^{z-}} = \left(\frac{-ze}{kT}\right)E_{app} + \ln\phi \qquad [6]$$

Equation 5 predicts that the retention of anions will increase linearly as E_{app} becomes more positive. A parallel treatment shows that the converse applies to the retention of cations.

The dependencies for 1,5- and 2,6-NDS in Figure 1 are consistent with the prediction of Equation 6, a result that also holds for a number of monosubstituted aromatic sulfonates.[11] Linear dependencies have also been found for several protonated anilines and pyridines,[13] recognizing that ln k' for positively charged analytes increases linearly with a decrease in E_{app}. Moreover, Equation 6 applies to all the ions present in the system, including those of the supporting electrolyte. The resulting slopes in the Figure 1 inset therefore reflect how changes in E_{app} affects the competition between the doubly charged anions 1,5- and 2,6-NDS and the singly charged hexafluorophosphate. The next two sections include discussions of the importance of the supporting electrolyte.

Temperature Dependence of EMLC Retention – Determination of Enthalpy. Figure 2 presents a portion of the obtained retention dependence data for 1,5- and 2,6-NDS. It shows results at four different temperatures (22, 30, 45, and 55°C) and three different values of E_{app} (+200, 0, and -200 mV). At each E_{app}, the retention of both analytes decreases as column temperature increases. The retention, for example, of 2,6-NDS at -200 mV decreases from 2.4 min at 22 °C to 1.0 min at 55 °C, and from 7.1 to 3.7 min at +200 mV for the same two temperatures. This trend is also evident for 1,5-NDS, which together with that for 2,6-NDS, indicate that the manipulation of retention by changes in E_{app} for 1,5- and 2,6-NDS is exothermic. The exothermic nature of retention can be further exploited by taking advantage of the increase in solvent viscosity to allow the speed of the separation to be dramatically increased, as has been done with in a recent publication.[16]

A workup of the retention data for the two analytes in terms of van't Hoff plots is shown in Figure 3 at five different values of E_{app} (+200, +100, 0, -100, and -200 mV) and eight different temperatures spanning from 22 to 55 °C. From an enthalpic perspective, the

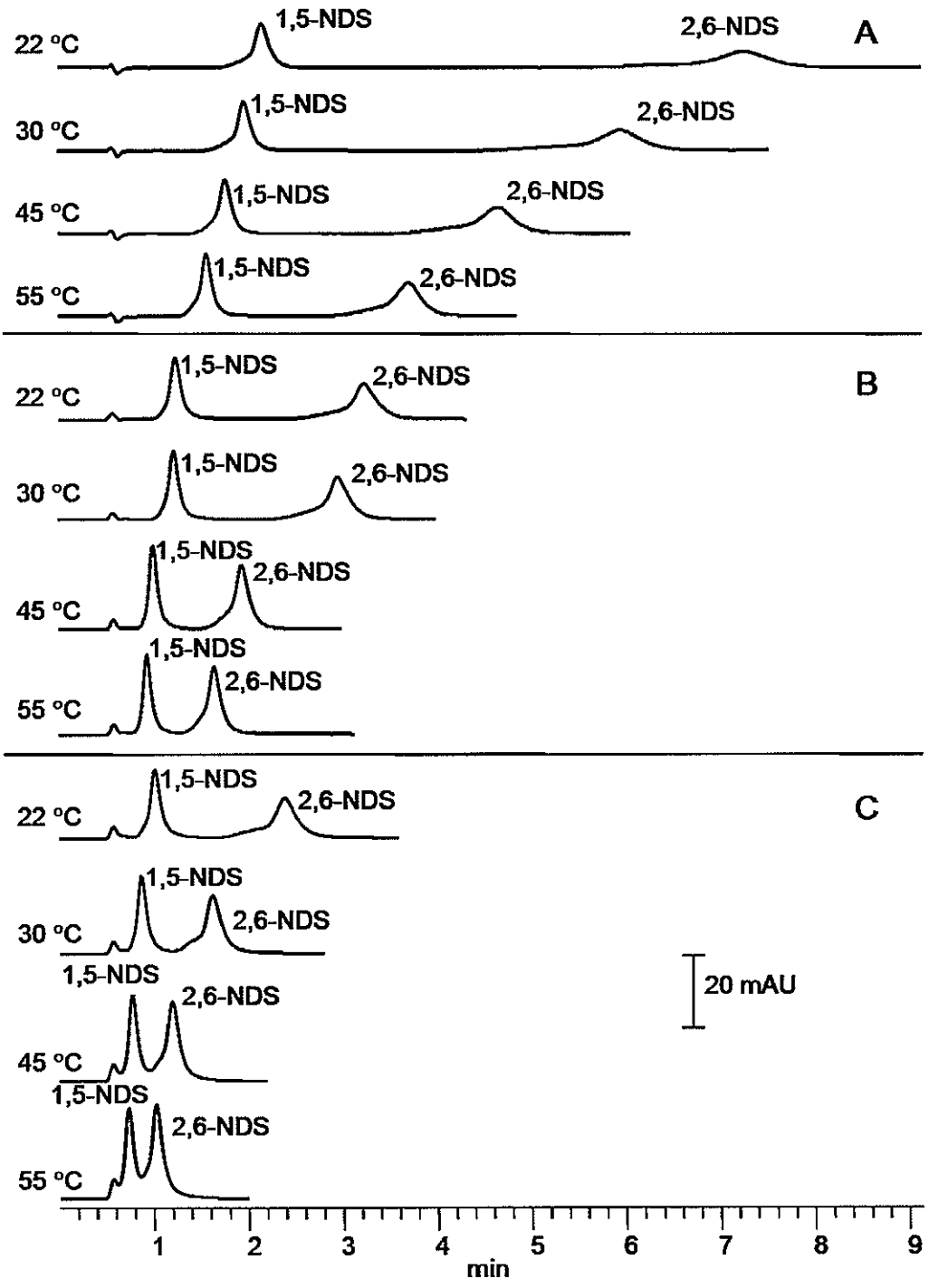

Figure 2. Chromatograms for a mixture of 1,5- and 2,6-NDS at +200 (A), 0 (B), and -200 mV (C) collected at 22, 30, 45, and 55°C using a GC stationary phase. Mobile phase: 0.5 mL/min 40 mM $NaPF_6$ (5% acetonitrile in water).

plots reveal that: 1) ln k' exhibits a linear dependence for both analytes at all five values of E_{app}; 2) the slopes are all positively valued (i.e.; a linear increase in ln k' with respect to $1/T$); and 3) the magnitudes of the slopes for both analytes increase as E_{app} moves negatively. Temperature therefore has a more notable impact on retention the more negative the value of E_{app}. Moreover, retention becomes an increasingly exothermic at the more negative values of E_{app}.

The calculated values of ΔH^0 for both analytes are summarized in a portion of Table 1. The values for ΔH^0 range from -11 to -31 kJ/mol over the spread of E_{app}, with 2,6-NDS more exothermic than 1,5-NDS at each E_{app}. These values are comparable to those reported in RPLC (-6 to -31 kJ/mol),[19-22] and are qualitatively consistent with the reversed phase character exhibited by PGC due to the easily polarized graphitic surface.[47]

These data also show that the retention of 1,5- and 2,6-NDS becomes increasingly exothermic as E_{app} becomes more negative. The exothermicity for both analytes increases by 12 kJ/mol when E_{app} changes from +200 to -200 mV. As mentioned earlier, many RPLC and IEC processes are characterized by an increase in exothermicity and a concomitant increase in retention. However, these analytes undergo a decrease exothermicity as retention increases, which is in direct opposition to most RPLC and IEC process.

Temperature Dependence of EMLC Retention – Determination of Entropy. Since ΔH^0 does not fully explain the trend in retention as a function of E_{app}, ΔS^0 must play a significant role. As observed in Figure 3, the y-intercepts of the van't Hoff plots become increasingly positive as E_{app} moves positively. Calculating ϕ as described earlier, the entropy of the interaction can be calculated from the intercepts of the van't Hoff plot using Equation 2, as given in Table 1 and Figure 4B. The more positive the value for ΔS^0, the more favored

Table 1. Thermodynamic parameters in the EMLC-based retention of 1,5- and 2,6- naphthalene disulfonates.

	ΔH^0 (kJ/mol)		$T\Delta S^0$ (kJ/mol)		ΔG^0 (kJ/mol)	
E_{app} (mV)	1,5 NDS	2,6 NDS	1,5 NDS	2,6 NDS	1,5 NDS	2,6 NDS
+200	-11 ± 1	-19 ± 1	6 ± 1	2 ± 1	-17 ± 1	-21 ± 1
+100	-13 ± 1	-20 ± 1	4 ± 1	0 ± 1	-16 ± 1	-20 ± 1
0	-16 ± 1	-24 ± 1	-1 ± 1	-5 ± 1	-16 ± 1	-19 ± 1
-100	-18 ± 1	-27 ± 1	-3 ± 1	-8 ± 1	-15 ± 1	-19 ± 1
-200	-23 ± 1	-31 ± 1	-9 ± 1	-12 ± 1	-15 ± 1	-18 ± 1

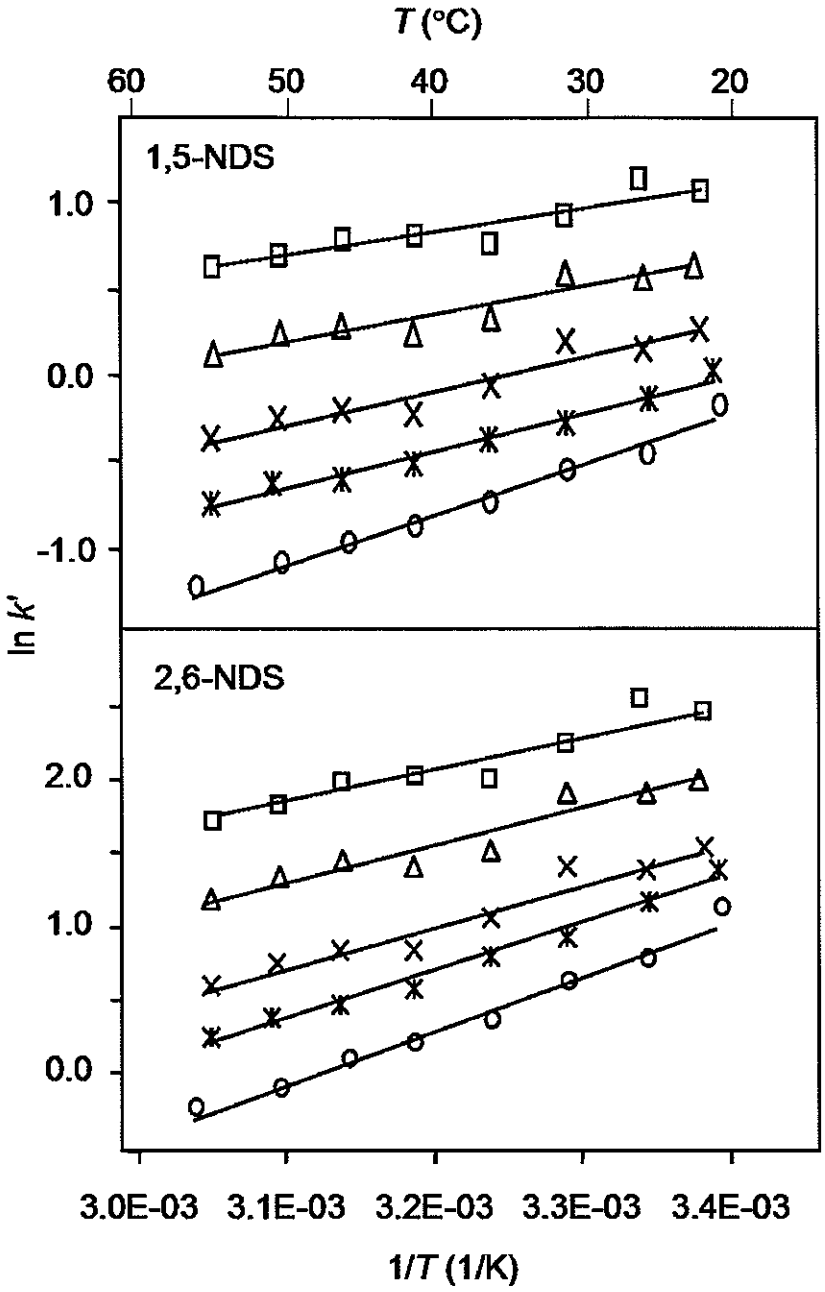

Figure 3. van't Hoff plots for 1,5- and 2,6-NDS at a GC stationary phase at several different values of E_{app}: +200 (□), +100 (△), 0 (×), -100 (✱), and -200 mV (○). Mobile phase: 0.5 mL/min 40 mM $NaPF_6$ (5% acetonitrile in water). Error bars are smaller than the data points.

the equilibrium process. Therefore, as the entropy becomes more positive, the system becomes more disordered as a result of the equilibrium process. This disorder could be due to the presence of the analyte in the mobile phase resulting in a more disordered mobile phase or the presence of the supporting electrolyte causing more disorder in the stationary phase. In either case, retention should increase as ΔS^0 becomes more positive because the interaction becomes more favorable. This trend is consistent with the retention trend observed in Figure 1, suggesting that in retention as a function of potential is likely governed by entropy.

In ion exchange processes the dominance of entropy has been attributed to the solvation/desolvation of the analyte.[35, 48, 49] The importance of ΔS^0 is ascribed to the classical "structure-forming action" exerted by the nonpolar moiety of 1,5-NDS on adjacent water molecules (i.e., the hydrophobic interaction).[20, 33, 48] The adsorption of 1,5-NDS therefore entails a collapse of these structured water clusters, and if their collapse is the overriding contributor to ΔS^0, the overall entropy of the system will increase. The energy consumed in breaking up these clusters, which form by an increase in the degree of hydrogen bonding between water molecules,[50] will also have an impact on ΔH^0. The results for 2,6-NDS lead to the same interpretation, and have particular precedence in the literature on ion exchange chromatography.[35, 49]

Overall then, the adsorption of a charged organic analyte onto the surface requires the desorption of an electrolyte ion. The measured change in enthalpy is then a combination of the energetics of both events. Since the measured change in enthalpy is exothermic, the adsorption of the organic ion is favored over the adsorption of the electrolyte ion. However some energy is required to remove the electrolyte ion thereby decreasing the observed

exothermicity of adsorption. As the E_{app} becomes more negative, the interaction of all ions to the surface decreases. In other words, the increase in the exothermicity of retention as E_{app} moves to more negative values arises from the inherently stronger interaction of the organic anions, over the supporting electrolyte ion, with the PGC surface. A parallel argument that describes the observed trend in ΔS^0, recognizes that the system is more disordered when there is more supporting electrolyte in the mobile phase.

As a final check on the balance of ΔH^0 and ΔS^0, the Gibbs free energy (ΔG^0) can be calculated by

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad [7]$$

as shown in Table 1 and Figure 4C. All values of ΔG^0 are negative indicating that the retention mechanism is spontaneous. Comparison of the ΔG^0 value for 1,5-NDS and 2,6-NDS shows that more retained 2,6-NDS is more spontaneous, as expected. Furthermore, the retention process is slightly less spontaneous for each analyte at more negative potentials. Thus, the equilibrium constant would be smaller at -200 mV than it is at +200 mV, which is consistent with the observed trend in retention as a smaller equilibrium constant correlates to a smaller capacity factor, i.e., lower retention.

The thermodynamic analysis of this EMLC-based separation indicates that while retention is in part favored by a RPLC mechanism as evident in the enthalpy of interaction, retention is also strongly governed by an IEC mechanism as evident in the entropy of the interaction. The enthalpic contribution leads to the strong adsorption commonly observed when PGC is employed as a stationary phase and its trend is dominated by the effect of electrolyte in the system. The entropic contribution to retention gives rise to the observed

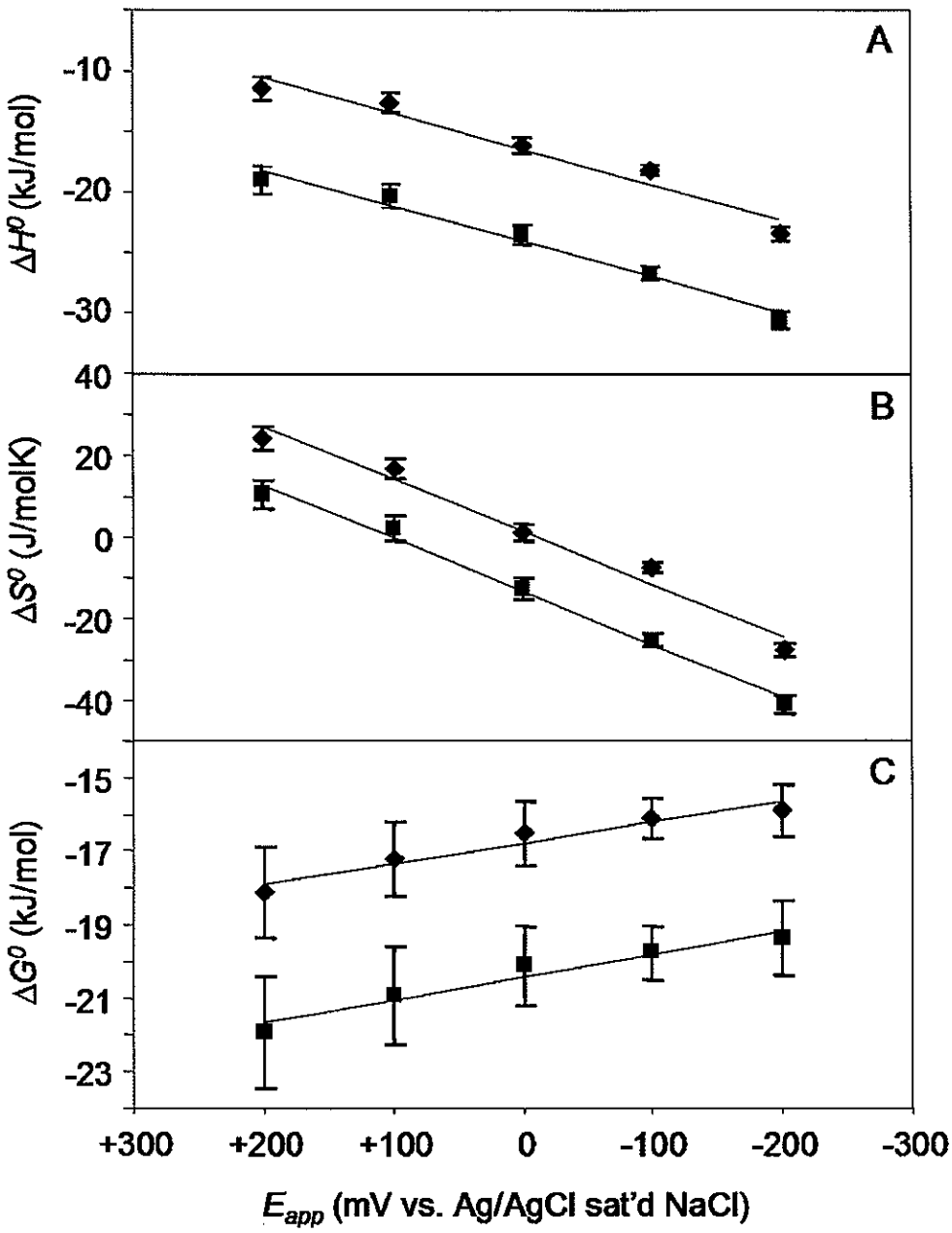

Figure 4. Dependence of ΔH^0 (A), ΔS^0 (B), and ΔG^0 (C) on E_{app} for 1,5- (♦) and 2,6-NDS (■) at a GC stationary phase. Mobile phase: 0.5 mL/min 40 mM $NaPF_6$ (5% acetonitrile in water).

dependence of retention on E_{app} and is dominated by the effect of analyte in the system. The indication of multiple factors in the retention mechanism supports the power of EMLC in separating a wide range of analytes.

Conclusions

Determination of the thermodynamic contributions to retention allows comparisons of the retention mechanism under a variety of conditions. Here we examined the retention behavior of two naphthalene disulfonates as a function of applied potential by calculating the enthalpic and entropic contributions to retention. Interestingly, while the retention mechanism is exothermic at all potentials, the trend in the exothermicity of the interaction is opposite the observed retention trend as a function of potential. Further calculation of the entropic contribution and overall Gibbs free energy shows that the dependence on potential is entropically controlled rather than enthalpically controlled. Entropically driven separations are commonly found in IEC suggesting a similarity in the retention mechanism between EMLC- and IEC-based separations. However, most IEC entropically driven separations are accompanied by a positive enthalpy rather than the observed negative enthalpy found here, therefore retention in EMLC is likely a combination of both RPLC and IEC characteristics.

Acknowledgments

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CHAPTER 3. ELECTROCHEMICALLY MODULATED LIQUID CHROMATOGRAPHIC SEPARATIONS OF INORGANIC ANIONS

A paper submitted for publication in *Journal of Chromatography A*[1]

Lisa M. Ponton[2,3] and Marc D. Porter[3,4]

Abstract

Inorganic anion retention on a porous graphitic carbon (PGC) stationary phase is investigated by electrochemically modulated liquid chromatography (EMLC). Through various combinations of the potential applied (E_{app}) to the PGC packing and/or changes in the composition (sodium salts of tetrafluoroborate, sulfate, and fluoride) and concentration (10, 25, and 50 mM) of an aqueous mobile phase, conditions for the separation of two different inorganic anion mixtures (iodate, bromide, nitrite, and nitrate or iodate, bromate, and chlorate) are developed. Results show that retention was affected by both variables. Moreover, plots of ln k' are linearly dependent on both E_{app} and ln [E], where k' is the analyte capacity factor and [E] is the supporting electrolyte concentration. Based on these findings, insights into the retention mechanism are briefly discussed by drawing on the theory for ion exchange chromatography.

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[2] Primary researcher and author
[3] Institute of Combinatorial Discovery, Chemistry Department, Iowa State University, and Ames Laboratory-USDOE Ames, IA 50011
[4] Author for correspondence

Introduction

Several reports have described the use of carbonaceous packings like porous graphitic carbon (PGC) for the separation of inorganic anions.[1-9] One set of strategies, takes advantage of the hydrophobic character of PGC to control retention by either the dynamic modification of the stationary phase or the addition of ion-interaction reagents to the mobile phase. In the former, modification of PGC with the weak anion exchanger polyethyleneimine enabled the separation of several inorganic anions.[1] With the latter, tetrabutylammonium hydroxide[2] and alkylamines[3] were added to the mobile phase to affect the separation.

Another pathway to these separations exploited the semi-metal character of PGC. This strategy relied on the formation of a "mirror-charge" at the packing surface, which lead to retention by a charge-induced dipole.[4, 5] The separation of the oxo-anions TcO_4^- and ReO_4^- was proposed to arise from this mechanism.[6, 7] A recent report has extended this concept by the use of electronic competitors.[8] This study found that adsorptive competition between carboxylic acids and inorganic anions for PGC dictated the analyte retention. Inorganic anions were also separated on PGC with dilute aqueous sodium sulfate as the eluent,[9] work that also demonstrated a retention mechanism with ion exchange characteristics.

This paper examines the separation of inorganic anions by electrochemically modulated liquid chromatography (EMLC).[10] In EMLC, a conductive stationary phase, such as PGC, is packed into an LC column that is also configured to function as a three-electrode electrochemical cell. The packing therefore acts as both a chromatographic stationary phase and a high surface area working electrode. Through changes in applied potential (E_{app}), the donor-acceptor properties (e.g., surface charge density) of the conductive packing can be

manipulated, which subsequently alters analyte retention. EMLC can therefore be viewed as an approach for fine tuning separations by controlling the effective composition of the stationary phase.

Utilizing this dimension for affecting retention, reports from several laboratories,[11-15] including our own,[16-24] have shown that changes in E_{app} can be exploited for the separation a wide range of mixtures. Early reports examined the effect of E_{app} on the separation of monosubstituted benzenes,[17] aromatic sulfonates,[20] and pyridines and anilines.[24] More recent studies explored the effectiveness of EMLC for manipulating the retention of corticosteroid[16] and benzodiazepine[19] mixtures. An investigation that evaluated the ability to enhance performance by operation at elevated temperatures has also appeared,[23] along with efforts to detail the mechanistic basis of EMLC separations.[15, 20-22, 24]

The work presented herein explores the effects of E_{app} and different aqueous electrolytes in the mobile phase on the separation of various inorganic anion (iodate, bromide, nitrite, and nitrate or iodate, bromate, and chlorate) mixtures by using an EMLC column packed with PGC. The following sections therefore examine: 1) the ability of E_{app} to manipulate retention for optimization of such separations; and 2) the dependence of retention on the identity and concentration of sodium tetrafluoroborate, sodium sulfate, and sodium fluoride as supporting electrolytes. Both sets of findings are then examined to gain insights into the retention mechanism, drawing in particular on the theoretical underpinnings of ion exchange chromatography.[25-27]

Experimental Methods

Chemicals and Reagents. The analytes, i.e., the potassium salts of iodate, bromate, chlorate, bromide, and nitrate and the sodium salt of nitrite were purchased from Aldrich Chemical (Milwaukee, WI). All were used at a concentration of 20 mM after dissolution in Milli-Q purified water (Millipore, Bedford, MA). Most of these analytes were chosen due to their compatibility with UV absorbance detection.[28] Chlorate ion, however, was detected because of a change in the refractive index of the mobile phase, which gave rise to a very weak pseudo peak in the absorbance signal.

The mobile phase consisted of varied concentrations of either sodium fluoride, sodium sulfate, or sodium tetrafluoroborate (Aldrich), which were dissolved in Milli-Q purified water. The mobile phases were passed through a 0.5-μm filter (GE Osmonics, Minnetonka, MN) prior to use. The PGC particles, 7-μm diameter (Hypercarb), were obtained from Thermo Hypersil (Bellfonte, PA). Characterizations of the as-received PGC by X-ray photoelectron spectroscopy agreed with previous results,[20] which showed a very low surface oxygen content (0.14 atomic %) that was largely distributed among phenol, carbonyl, carboxylic acid, lactone, and quinone groups.[29]

Instrumentation. The design and construction of the EMLC column has been described in detail elsewhere.[18] In brief, the PGC stationary phase is packed inside a Nafion™ (Perma Pure Inc., Toms River, NJ) cation-exchange membrane, in tubular form, that has been inserted into a porous stainless steel column (Mott Corp., Farmington, CT). The Nafion™ tubing functions as: 1) a container for the PGC stationary phase, 2) an electronic insulator between working and auxiliary electrode, and 3) a salt bridge for ion transport. The porous stainless steel housing also serves as a high surface area auxiliary

electrode. An Ag/AgCl (saturated NaCl) electrode acts as the reference electrode and was placed in an electrolyte-filled reservoir surrounding the auxiliary electrode; all values of E_{app} are reported with respect to this electrode.

The column was attached to an Agilent Technologies (Palo Alto, CA) model 1050 HPLC equipped with an autosampler, quaternary pump, and a diode array detector. The samples were injected at a 1.0-μL. The elution profiles were monitored at 200 nm. The value of E_{app} was controlled by an Amel high power potentiostat (Milan, Italy) to ±1 mV. All data were collected at 24 ± 1 °C. A water blank was employed to determine the void time in calculations of the capacity factor (k'). The resolution (R_s) was estimated based on the 5 sigma method resident in the HP Chemstation software. By way of a working definition, a separation of neighboring components that has an R_s value of 1.5 or greater is termed "baseline resolution", whereas "effective resolution" is used to describe values of R_s in the range of 1.0 to 1.5.[30]

Results and Discussion

Retention as a Function of E_{app}. The change in retention as a function of E_{app} for a large number of aromatic sulfonates at carbon packings follows predictions based on electrostatic forces.[18] In line with these earlier studies, the retention of the inorganic anions is also dependent on E_{app}. Figure 1 presents an example of these findings, using a mixture of iodate, bromide, nitrite, and nitrate, four different values of E_{app} (0, +200, +400, and +600 mV), and 25 mM aqueous sodium fluoride as the supporting electrolyte. As is evident, increases in E_{app} result in longer retention times, which is consistent with the increase in the positive surface charge density on the PGC packing. Elution requires ~2.5 min at the lowest

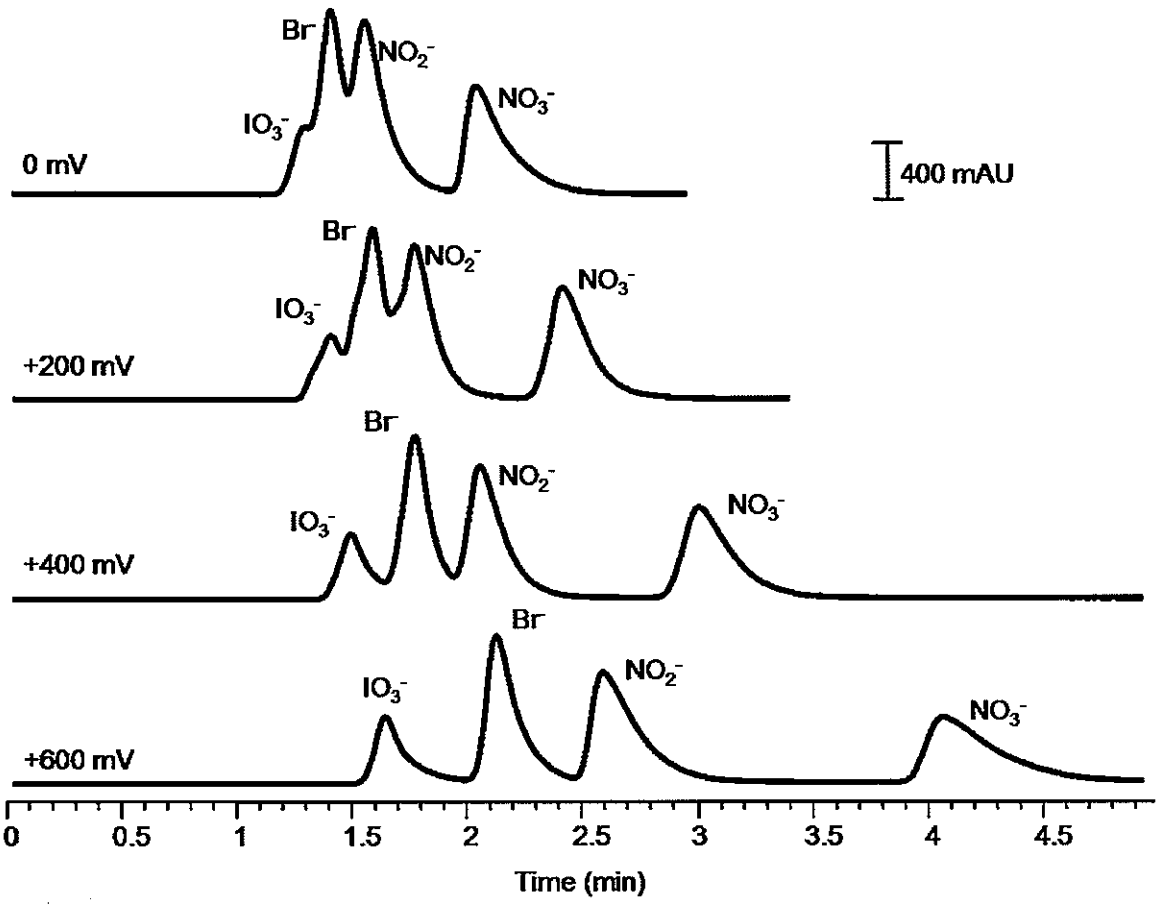

Figure 1. Chromatograms for a mixture of iodate, bromide, nitrite, and nitrate as a function of E_{app} using a PGC stationary phase. The mobile phase was composed of 25 mM aqueous NaF at a flow rate of 0.5 mL/min.

value of E_{app} (0 mV), and ~4.7 min at the highest value of E_{app} (+600 mV). The elution bands are also characterized by a notable level of tailing, which is often observed with PGC as a stationary phase.[4]

In addition to affecting retention, the change in E_{app} also improves the resolution of the separation. At 0 mV, the first three analytes (iodate, bromide, and nitrite) are poorly resolved from one another ($R_s < 1.0$), with only nitrate effectively resolved ($R_s = 1.2$). Increasing E_{app} to more positive values gradually improves the resolution of the separation. All four anions are effectively resolved ($R_s \geq 1.0$) at +400 mV and elute in less than 3.5 min. Near baseline resolution is realized at +600 mV with the smallest resolution between bromide and nitrite ($R_s = 1.1$) at an elution time of ~4.7 min. Higher values of E_{app} were not examined to avoid triggering the rapid oxidation of the PGC surface.[20, 31]

We also examined the separation of a mixture composed of the oxo-anions iodate, bromate, and chlorate. These results are shown in Figure 2 and were obtained under the same set of conditions used for Figure 1. Again, retention undergoes an increase as E_{app} becomes more positive. At 0 mV, iodate and bromate are nearly resolved ($R_s = 0.8$), but chlorate is virtually undetectable in the tail of the bromate elution band. As before, the movement of E_{app} to more positive values gradually increases the retention and resolution of the three components in the mixture. Baseline resolution is realized for iodate and bromate ($R_s = 1.9$), with near baseline resolution for bromate and chlorate ($R_s = 1.3$) at +600 mV.

Figure 3 summaries the retention data in Figures 1 and 2 through plots of ln k' vs. E_{app}. As observed for several sample types in EMLC,[18] each plot has a linear dependence. These dependencies can be qualitatively understood by applying the ion distribution law,[24]

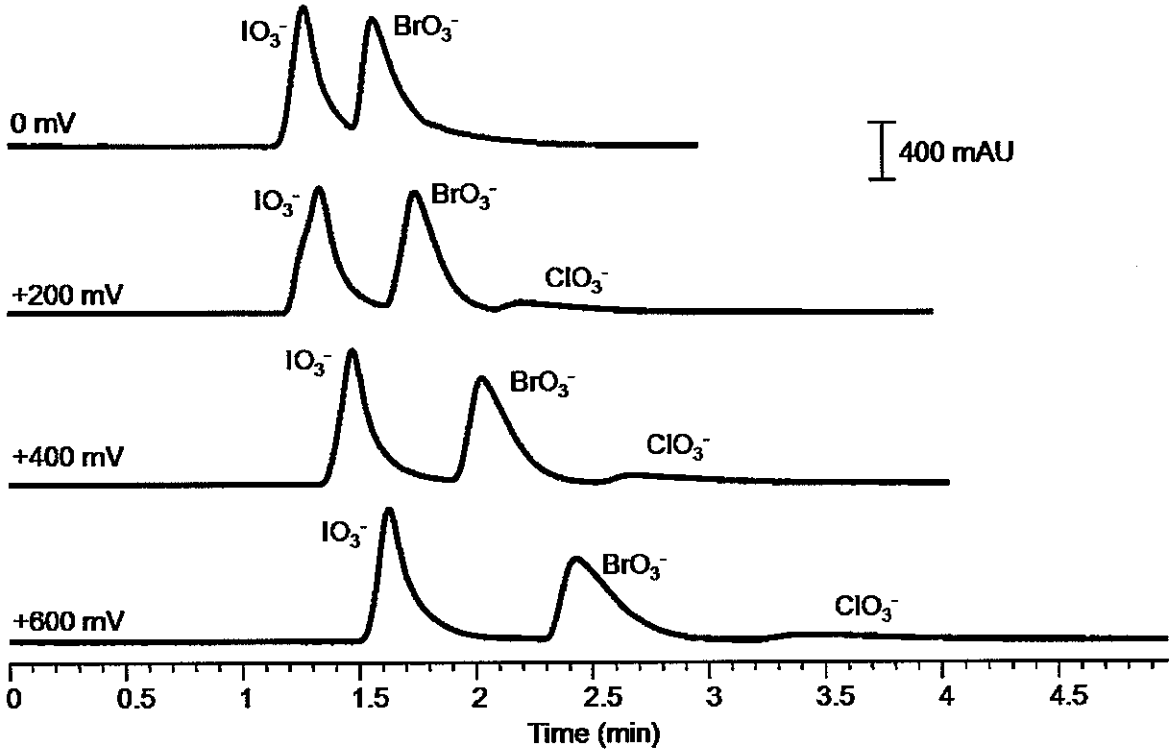

Figure 2. Chromatograms for a mixture of iodate, bromate, and chlorate as a function of E_{app} using a PGC stationary phase. The mobile phase was composed of 25 mM aqueous NaF at a flow rate of 0.5 mL/min. At 0 mV, ClO_3^- elutes in the tail of the BrO_3^- band.

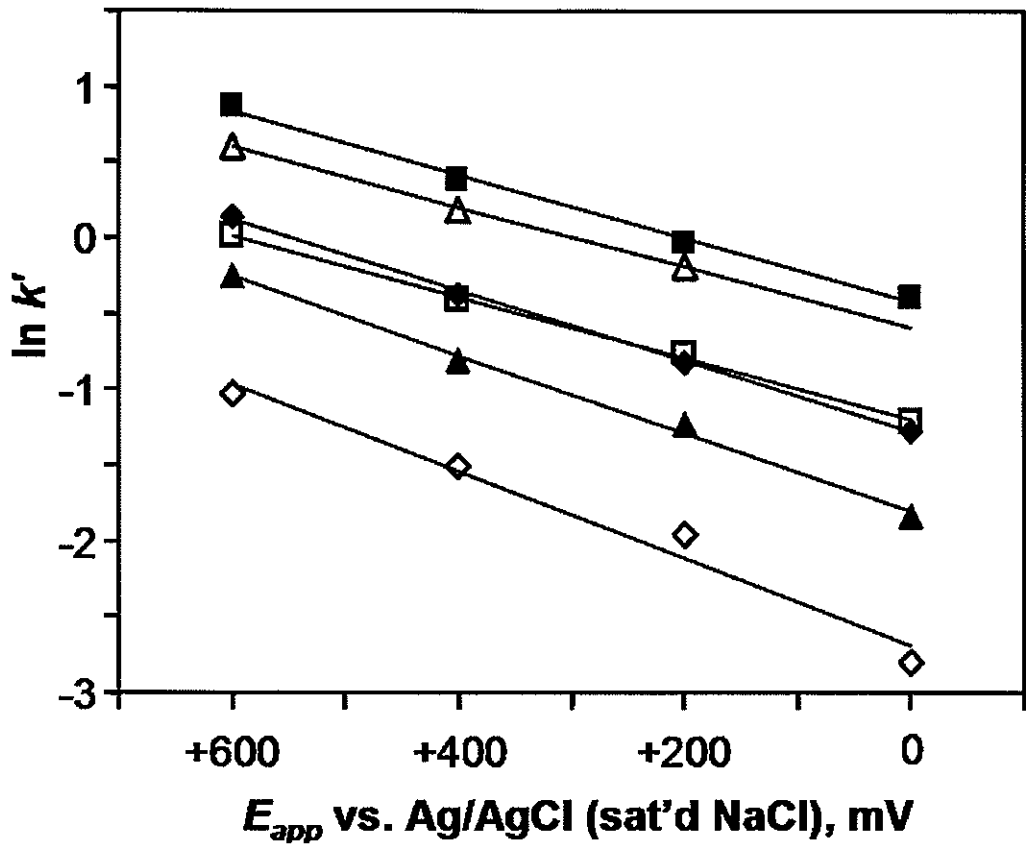

Figure 3. Dependence of ln k' vs E_{app} for iodate (◇), bromate (□), chlorate (△), bromide (▲), nitrite (♦), and nitrate (■). Error bars are smaller than the size of the data points. Data from Figures 1 and 2.

which details the influence of a potential difference on the equilibrium concentration of ions within the region between the potential difference.[32]

The results in Figure 3 also reveal that the EMLC-based retention sensitivity, *S*, which is defined as the slope of the plot, for the six analytes is comparable. This conclusion is supported by the data presented in Table 1, which includes the R^2 value of each plot. The Table shows a maximum difference in *S* of less than 50%. This agreement lends support to the argument that the dependence of inorganic anion retention with respect E_{app} is controlled primarily by the stationary phase surface charge density. In other words, ions with the same charge should be affected in the same manner via the ion distribution law.[32] The slight variations in the sensitivities for ions of the same charge are attributed to differences in their specific (i.e., chemical) interactions with PGC (see last section). Experiments using 50 mM NaF as the eluent (data not presented) also showed a linear dependence, though with much shorter retention times. The next section examines the importance of the supporting electrolyte in more detail.

Retention as a Function of Electrolyte Identity and Concentration. Three different salts were examined as mobile phase additives: sodium tetrafluoroborate, sodium sulfate, and sodium fluoride. These salts served as the supporting electrolyte in the mobile phase for control of E_{app} and were tested for their ability to function as an electronic competitor for retention manipulation. Each electrolyte was used at a concentration of 10, 25, and 50 mM. Figure 4 shows the chromatograms for the separation of iodate, bromide, nitrite, and nitrate as a function of the identity and concentration of the different supporting electrolytes. These data were collected with E_{app} set at +600 mV, as guided by the data in Figure 1. These results yield three immediate observations. First, fluoride is the most

Table 1. Sensitivities[a] (S) of retention for a series of inorganic anions to changes in E_{app}.[b]

Analyte	S (x 10^3)	R^2
NO_2^-	2.34 (0.05)[c]	0.996
ClO_3^-	1.98 (0.04)	0.997
IO_3^-	2.89 (0.12)	0.961
NO_3^-	2.10 (0.05)	0.994
BrO_3^-	2.03 (0.03)	0.997
Br^-	2.59 (0.06)	0.992

[a]The sensitivity is defined as the slope of a plot of ln k' vs. ln [E].
[b]Data from Figures 1 and 2.
[c]Standard error from the linear regression listed in parentheses.

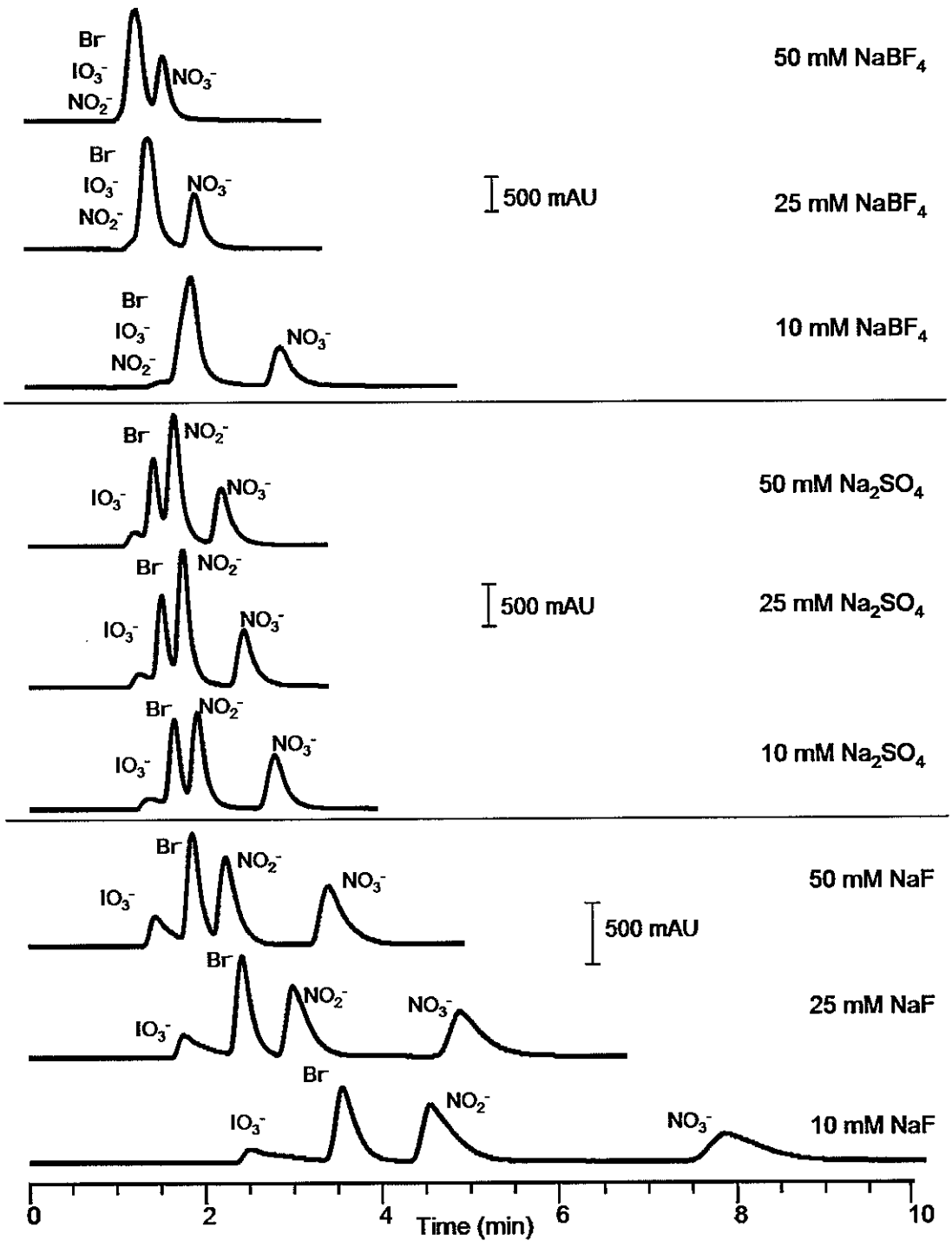

Figure 4. Chromatograms for a mixture of iodate, bromide, nitrite, and nitrate as a function of eluent identity and concentration using a PGC stationary phase at E_{app} of +600 mV. The mobile phase was composed of aqueous sodium tetrafluoroborate, sodium sulfate, or sodium fluoride at either 10, 25, or 50 mM. The flow rate was 0.5 mL/min.

effective of the three anionic eluents in resolving the mixture. Second, tetrafluoroborate is the strongest eluting anion, followed by sulfate and then fluoride. Third, analyte retention in all three electrolytes increases with decreasing concentration.

With respect to resolution, Figure 4 shows that three (iodate, bromide, and nitrite ions) of the four analytes co-elute with sodium tetrafluoroborate; only nitrate is effectively resolved ($R_s = 1.0$). With sodium sulfate, the separation approaches, but does not quite reach baseline resolution for the four analytes. The mixture is effectively resolved when carrying out the separation with fluoride as the eluent ($R_s \geq 1.0$ for all concentrations). Unfortunately, the tailing commonly found with PGC as a stationary phase[4] is particularly evident in these separations. We attribute this observation to the weaker elution strength of fluoride relative to the two anionic eluents. These results also suggest that running separations at lower supporting electrolyte concentrations may further improve sample resolution, recognizing, however, that too low of an electrolyte concentration may compromise the ability to control E_{app}.

Mechanistic Insights. In addition to serving as a basis for the design of protocols for manipulating the separation, the data in the last two sections provide mechanistic insights into the retention of these analytes at PGC. At a conventional anion exchange column,[9] the elution order is $IO_3^- < NO_2^- < Br^- < NO_3^-$. The elution order, for example, in Figure 4 is $IO_3^- < Br^- < NO_2^- < NO_3^-$. Although not in complete agreement, a comparison of elution orders suggests that the retention of these analytes at PGC has mechanistic similarities to that in ion exchange chromatography.

Takeuchi et al.[9] also recently concluded that the retention of inorganic anions by PGC exhibits ion-exchange characteristics. This assertion was based on an investigation of the

dependence of ln k' on ln [E], where [E] is the concentration of an ionic eluent added to the mobile phase. A plot of ln k' vs. ln [E] at a resin-based ion exchange packing generally follows a linear dependence with a slope that often, but not always, correlates with the ratio of the analyte-to-eluent charge.[27, 28] A classic example of this dependence was reported by Rocklin et al.[33] Rocklin and coworkers investigated the effect hydroxide ion concentration has on inorganic anion retention at an anion exchange resin, and found slopes for plots of ln k' vs. ln [E] for chloride, nitrate, sulfate, and fumarate equal to –1.03, –0.95, –2.10, and –2.03, respectively. Takeuchi et al.[9] determined that the elution of iodate and iodide at PGC vs. sulfate concentration resulted in slopes close to the expected value of –0.5, i.e., –0.58 for iodate and –0.50 for iodide.

An analysis of the data in Figure 4 also shows the existence of a linear relationship between ln k' and ln [E] for each analyte-eluent combination. The results of this analysis are presented in Figure 5, with each plot having a R^2 value greater than 0.99. These dependencies further confirm the importance of electrostatic interactions to the EMLC-based separation of these compounds. Interestingly, the slopes with fluoride and tetrafluoroborate, the two singly charged eluents, are roughly the same (Figure 5A). Moreover, the slopes for bromide, nitrite, and nitrate with the doubly charged sulfate as the eluent are all notably lower than those the tetrafluoroborate and fluoride eluents, and can also be loosely grouped together (Figure 5B). Iodate, however, has a much steeper slope than those for bromide, nitrite, and nitrate. Importantly, the slopes in Figure 5A are generally steeper than those in Figure 5B, which is qualitatively consistent with the correlations expected for analyte:eluent charge ratios. These results support the claim that the EMLC-based separation of these analytes has ion exchange characteristics.

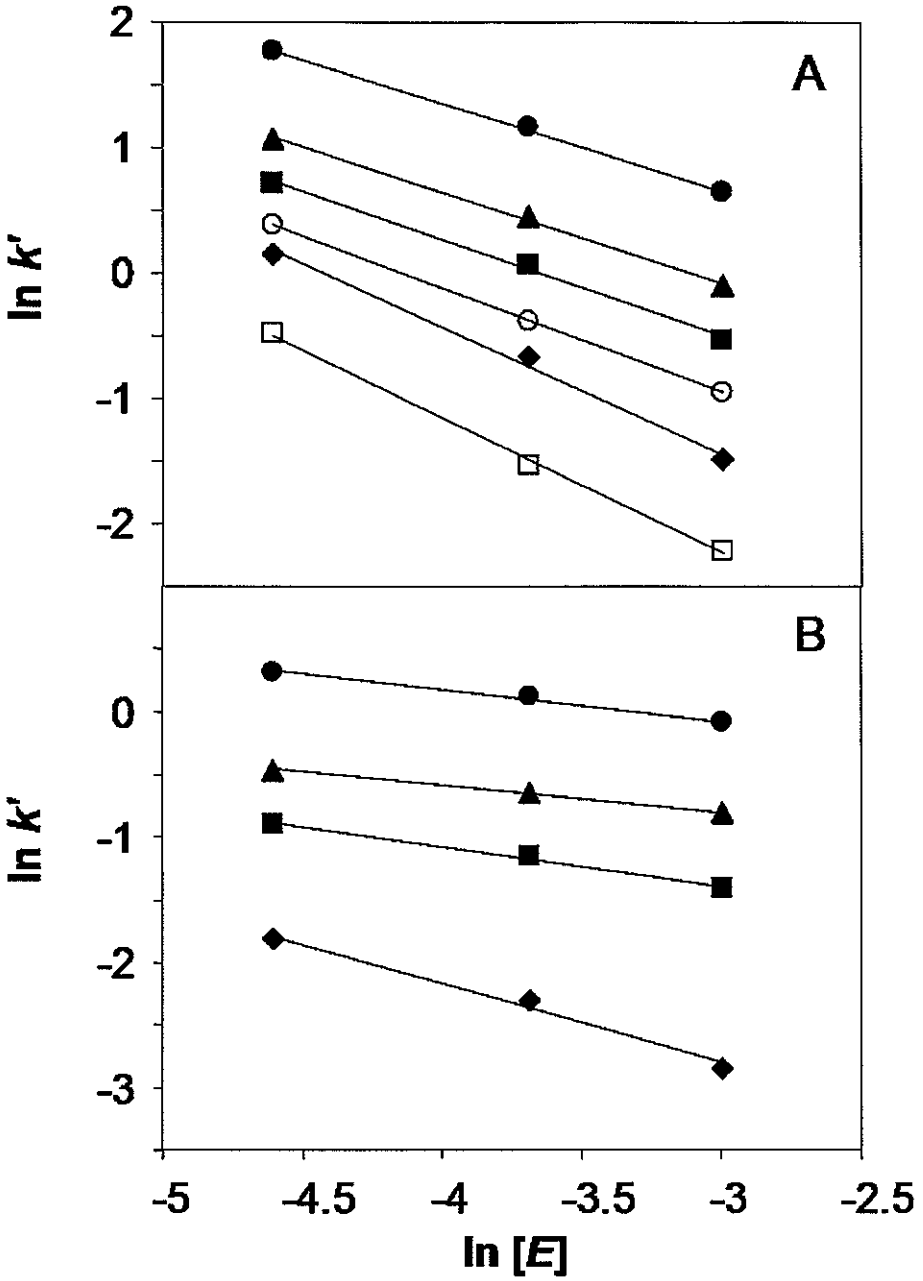

Figure 5. Dependence of ln k' vs. ln [E]. Plot A: iodate (♦), bromide (■), nitrite (▲), and nitrate (●) with NaF; and iodate (□), bromide (□), nitrite (□), and nitrate (○) with $NaBF_4$ (iodate, bromide, and nitrite coelute with $NaBF_4$). Plot B: iodate (♦), bromide (■), nitrite (▲), and nitrate (●) with Na_2SO_4. All eluent concentrations are molar. Error bars are smaller than the size of the data points. Data from Figure 4.

Two additional observations develop from a more exacting analysis of the data in Figure 5. This analysis is presented in Table 2 as the slope of the plots, which are listed in increasing analyte elution order for each supporting electrolyte. First, one of the singly charged analyte-eluent combinations (iodate-fluoride) has a slope that is experimentally identical to the analyte:eluent charge ratio. The other slopes for fluoride as the eluent, the bromide-, nitrite-, and nitrate-fluoride combinations, deviate by as much as –31%. The iodate-, bromide-, and nitrite-tetrafluoroborate combinations also have slopes within +10% of expectations; the deviation for the nitrate-tetrafluoroborate combination, however, is –17%. Interestingly, the data with the doubly charged sulfate as the eluent shows both significant positive (+26% with iodate) and negative (e.g., –58% for nitrite) deviations. Takeuchi et al[9] also found a positive deviation (+16%) for iodate with sulfate as the eluent.

Second, the magnitude of the deviation in the plots of ln k' vs. ln [E] largely correlate with the extent of analyte retention. For example, nitrate is the most strongly retained analyte when fluoride is used as the supporting electrolyte. Nitrate also has the largest departure from the slope based on the analyte:eluent charge ratio. Nitrite follows nitrate in elution order with fluoride as the eluent, and ranks second in terms of the departure from the charge ratio expectation. An examination of the data for bromide and iodate completes the trend. Most of the data with the other two eluents exhibit the same trends. Only the data with nitrate and nitrite as the analytes and sulfate as the eluent falls outside of this correlation, but only by an amount slightly greater than the uncertainty in the data.

Deviations from charge ratio expectations in plots of ln k' vs. ln [E] can in some cases be accounted for by the dependence of the activity coefficients of ions in the mobile phase on eluent concentration.[26, 33] An analysis to correct for this effect uses the slope from a plot of

Table 2. Slopes from plots of ln k' vs ln [E] with E_{app} set at +600 mV.[a]

	Supporting electrolyte/eluent		
Analyte	NaF	Na_2SO_4	$NaBF_4$
IO_3^-	-1.01 (0.02)[b]	-0.63 (0.03)	-1.09[c] (0.03)
Br^-	-0.77 (0.01)	-0.31 (0.01)	-1.09[c] (0.03)
NO_2^-	-0.72 (0.01)	-0.21 (0.01)	-1.09[c] (0.03)
NO_3^-	-0.69 (0.01)	-0.25 (0.01)	-0.83 (0.01)
theoretical prediction	-1	-0.5	-1

[a]Data from Figure 4.
[b]Standard error from the linear regression listed in parentheses.
[c]Since all three analytes coeluted, k' was calculated based on peak height of the combined peak.

$\ln\left(\gamma_A / \gamma_E^{-z_A/z_E}\right)$ vs. ln [E], where γ_A and γ_E are the respective activity coefficients for the analyte and eluent, and z_A and z_E are the respective analyte and eluent charges.[33] Nevertheless, the results from corrections to the data in Figure 5, which used literature activity coefficients,[34] only marginally improve the correlations between the expected and observed slopes. For example, the activity coefficient correction calculated for fluoride as the eluent equals –0.07 and that with sulfate as the eluent is –0.10. Thus, the slopes corrected for differences in activity coefficients with fluoride as the eluent continue to show large negative deviations (e.g., –24% for nitrate), whereas both large positive (+44% for iodate) and negative (–30% for nitrate) deviations persist in the data with sulfate as the eluent.

There is, however, an important difference in separations using ion-exchange resins and those based on EMLC that should be considered in this analysis. In ion exchange chromatography, the fixed charge groups on the resin define the surface charge density, which dictates the surface potential and therefore the potential gradient within the interphase between the resin and bulk solution. Importantly, the fixed charge density limits changes in the activity coefficients of both the eluent and analyte in the stationary phase when the eluent concentration in the mobile phase is low (less than ~100 mM)[27] with respect to the concentration of fixed charges (~0.1 meq/g for low capacity resins and ~4 meq/g for high capacity resins)[28] on most ion exchange resins.

The surface charge density of the packing in EMLC develops differently. In EMLC, it is the potential applied to the packing, and not the surface charge density, that is directly controlled by the potentiostat. The charge density on the packing surface and the gradient in

the potential in the interphase reflect the response of the interphase to the applied potential. Estimates to be reported elsewhere,[22] which extract the surface charge density at PGC as a function of E_{app} between +50 and −150 mV, yield a value of ~1.3 μC/cm^2 when extrapolated to +600 mV. This value is comparable to that at electrified mercury surfaces with fluoride as the electrolyte anion. Based on the surface area of PGC (~120 m^2/g),[4] this charge density translates to ~20 μeq/g. The concentration of charges at PGC is therefore much less than that found in exchange resins,[27] arguing that the basis for the assumption applied to ion exchange resins cannot reliably be applied to EMLC.

Such an analysis becomes even more suspect if an anion interacts with PGC by both nonspecific (electrostatic) and specific (chemical) interactions. The correlation between the magnitude of the deviation with elution time lends support to this concern as specific interactions often alter the charge density of an adsorbate. In other words, the theoretical framework behind the mechanistic analysis of the plots of ln k' vs. E_{app} or ln [E] in the earlier Figures is based solely on electrostatic interactions. However, if both electrostatic and specific interactions contribute to retention, then factors such as the desolvation of an analyte when specifically adsorbing on the surface can result in a dramatic change in the structure and electrostatics at the interphase of the packing, complicating an objective treatment of the activity coefficients of the species in the interphase.

Complex retention mechanisms are not uncommon occurrences in ion exchange chromatography and can be elucidated, for example, by examining the temperature dependence of retention under varying mobile phase compositions.[35, 36] By employing temperature as an adjustable parameter, a van't Hoff-type analysis can be utilized to quantify

both the enthalpic and entropic contributions to retention, paralleling our recent study on the separation of organic dianions.[21] Work to this end is underway.

Conclusion

The manipulation and optimization of inorganic anion retention on PGC through changes in both the mobile phase composition and the E_{app} of the stationary phase is demonstrated. The choice of an electronic competitor and its concentration in the mobile phase has a profound impact on the resolution of the separation, with fluoride proving the most effective eluent of the three electronic competitors tested. Results also showed that these separations could be manipulated by changes in E_{app}. From a mechanistic perspective, plots of ln k' were found to be linearly dependent on both E_{app} and ln [E]. This behavior can be described in the context of an ion-exchange mechanism between analyte ions in the mobile phase and eluent ions in the interphase formed on the surface of PGC. Deviations from the expectations for the slopes of the ln k' vs. ln [E] plots were attributed to effects specific interactions, in addition to electrostatic interactions, on retention. Efforts to test this conclusion are presently being designed.

Acknowledgments

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CHAPTER 4. HIGH SPEED ELECTROCHEMICALLY MODULATED LIQUID CHROMATOGRAPHY

A paper accepted for publication in *Analytical Chemistry*[1]

Lisa M. Ponton[2,3] and Marc D. Porter[3,4]

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.

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[2] Primary researcher and author

[3] Institute of Combinatorial Discovery, Chemistry Department, Iowa State University, and Ames Laboratory-USDOE Ames, IA 50011

[4] Author for correspondence

Introduction

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 allows for the manipulation of analyte retention through changes in the potential applied (E_{app}) to conductive stationary phases, such as 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, which, in turn, affects analyte 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 analyte 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 analyte retention in HPLC by significantly reducing analysis times while maintaining the effectiveness of a 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.

Another notable advantage that derives from operation at elevated column temperatures is the decrease in the dielectric constant for water with increasing temperature. 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 diffusion.[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, in order to avoid high resistance in the mobile phase and resulting challenges of controlling E_{app}, this ratio should be maximized, i.e., a small electrode surface area and a large solution volume. Strategies that provide a means to decrease mobile phase resistance, like operation at elevated column temperatures, therefore have the potential to optimize the electrochemical behavior (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 if 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 using 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 (PGC) 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 atomic %), 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 co-workers.[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

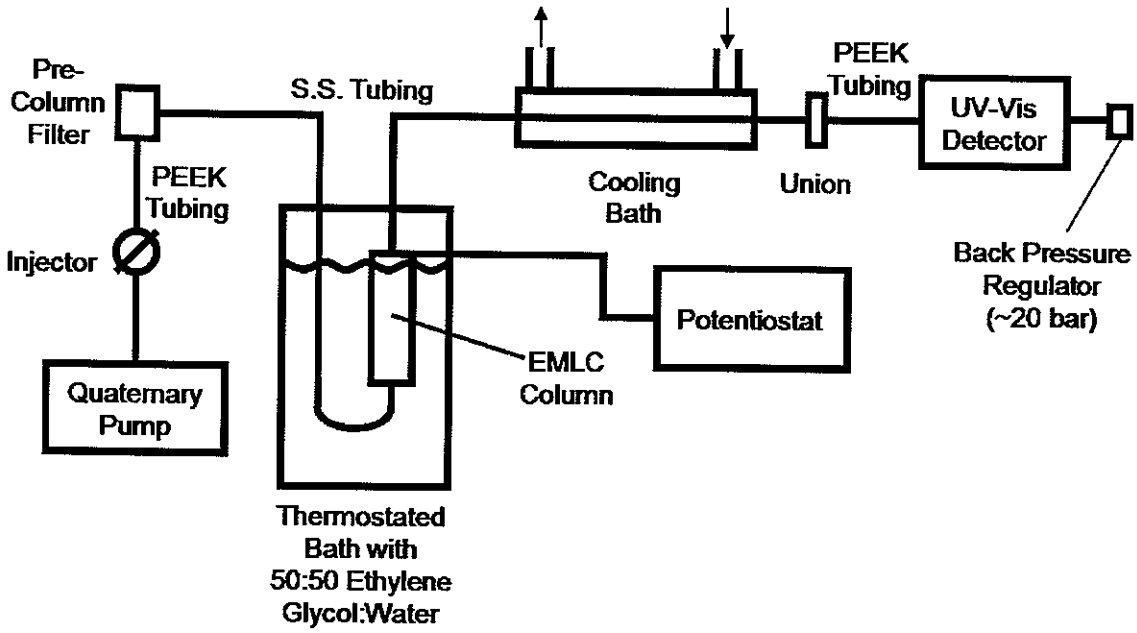

Figure 1. Instrument schematic for high speed EMLC.

(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 cut-off 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 hrs 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 5 sigma method resident in the software of the HP ChemStation. 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, and 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 runtime 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,

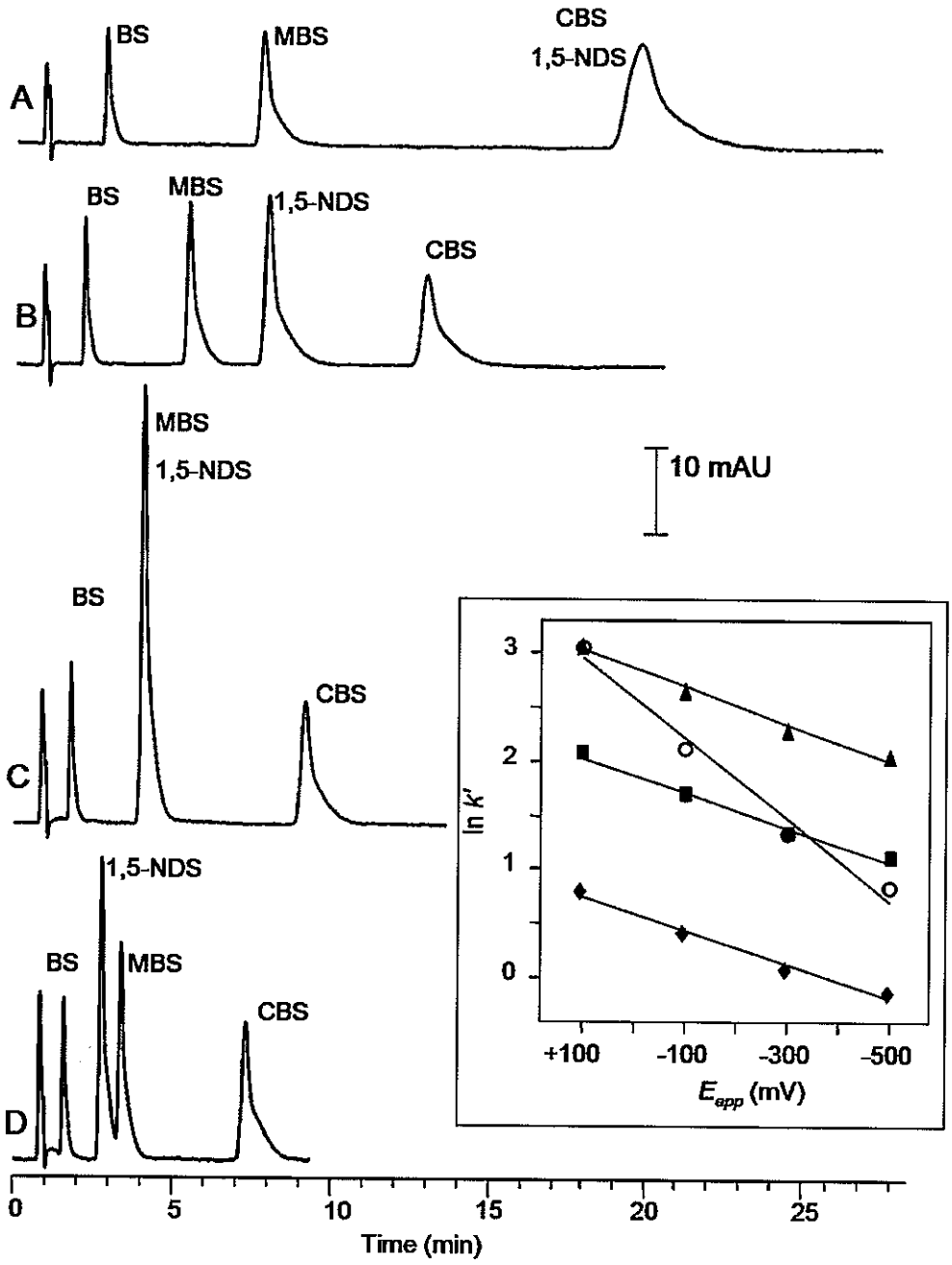

Figure 2. Dependence of the retention of BS, MBS, CBS, and 1,5-NDS on E_{app} at a flow rate of 0.5 mL/min, and a mobile phase composed of 0.1 M lithium perchlorate and 5% acetonitrile in water, and a temperature of 25.0 °C. (A) +100 mV; (B) -100 mV; (C) -300 mV; and (D) -500 mV. Inset: Plot ln k' vs. E_{app} for BS (♦), MBS (■), CBS (▲), and 1,5-NDS (**O**).

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' vs. 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 runtime that is ~50% less than the runtime observed at –100 mV may be possible at more negative values of E_{app} (e.g., –700 mV). Taken together, these results demonstrate that

Table 1. Slopes from the plots of ln k' vs. E_{app} (10^{-3} mV^{-1}).

Experimental Conditions	A	B	C
Flow Rate Mobile Phase Temperature	0.5 mL/min A 25 °C	2.0 mL/min A 100 °C	2.0 mL/min B 100 °C
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*	4.19 ± 0.08	ND*

*ND = not determined

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 hrs 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 runtime from ~20 min to ~12 min, which is further reduced to less than 7 min at 100.0 °C. The decrease in runtime 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' vs. 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

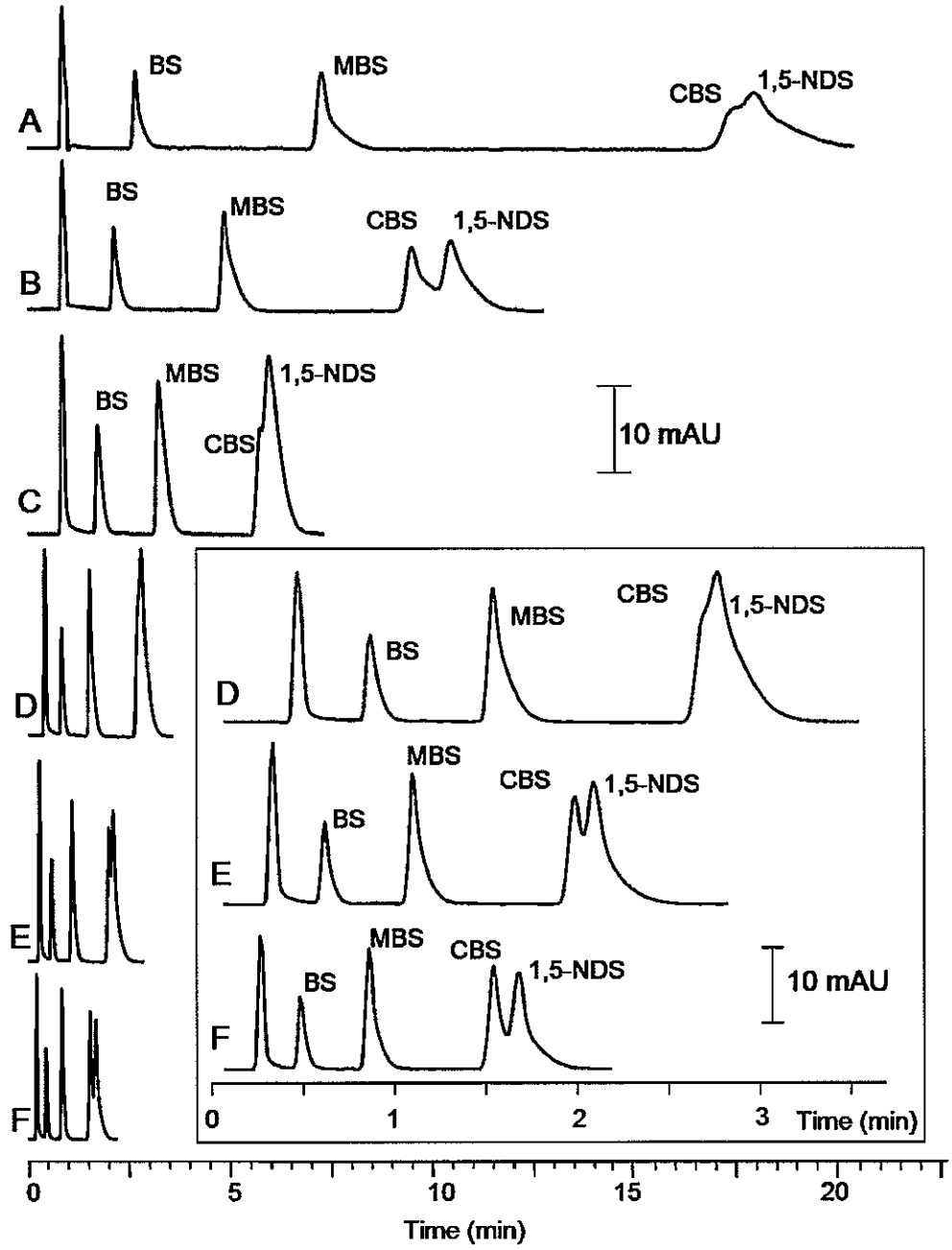

Figure 3. Temperature dependence for the EMLC separation of a mixture of BS, MBS, CBS, and 1,5-NDS with a mobile phase composed of 0.1 M lithium perchlorate and 5% acetonitrile in water. Chromatogram (A): 0.5 mL/min at 25.0 °C, and +100 mV; (B): 0.5 mL/min at 61.5 °C, and +70 mV; (C): 0.5 mL/min at 100.0 °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.

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 changing the flow rate from 0.5 to 2.0 mL/min at 100.0 °C. The improvement at 2.0 mL/min represents a reduction in runtime 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, and 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 runtime to ~3 min by changing E_{app} to –160 mV. Further decreases in runtime 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 runtime reduces to ~1.6 min. Decreasing E_{app} to –560 mV further reduces the runtime 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' vs. E_{app} for the five analytes at 100.0 °C. Like the data in Figure 2, these plots reveal: 1) the existence of a linear relationship between ln k' and E_{app}; and 2) the sensitivity of the dianions 1,5-NDS and

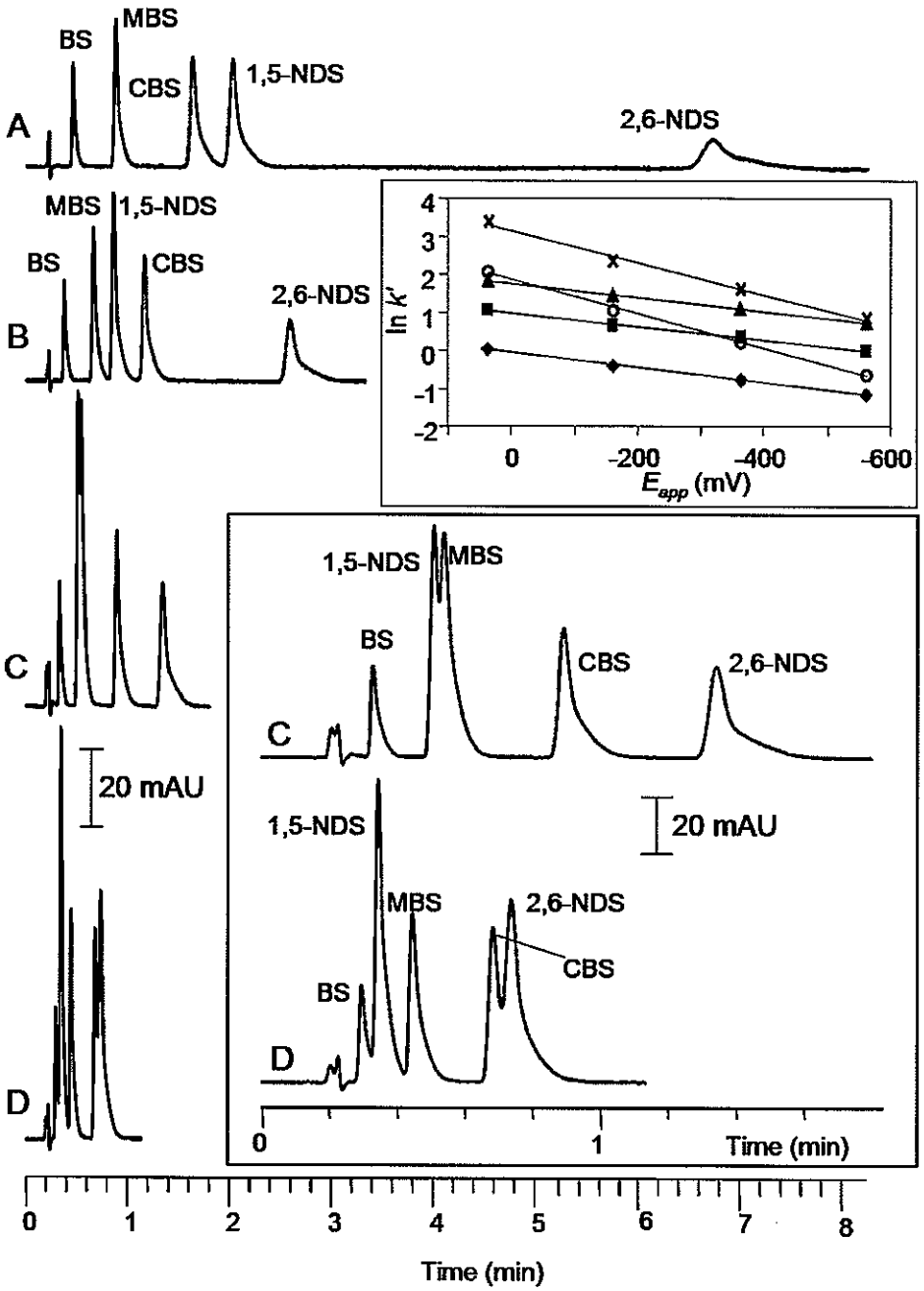

Figure 4. Dependence of retention of BS, MBS, CBS, 1,5-NDS, and 2,6-NDS on E_{app} at a flow rate of 2.0 mL/min with a mobile phase composed of 0.1 M lithium perchlorate and 5% acetonitrile in water and a temperature of 100.0 °C. (A) +40 mV; (B) -160 mV; (C) -360 mV; and (D) -560 mV. Inset: ln k' vs.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

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 runtime, 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 towards 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' vs. 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 runtime 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 if 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 runtime. At +40 mV, the separation in mobile phase B (chromatogram A) required a runtime 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 runtime can be markedly reduced while maintaining an effective separation. Both BS and MBS are baseline resolved at –760 mV, whereas the resolution between 1,5-NDS and CBS equals 1.4; importantly the runtime 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

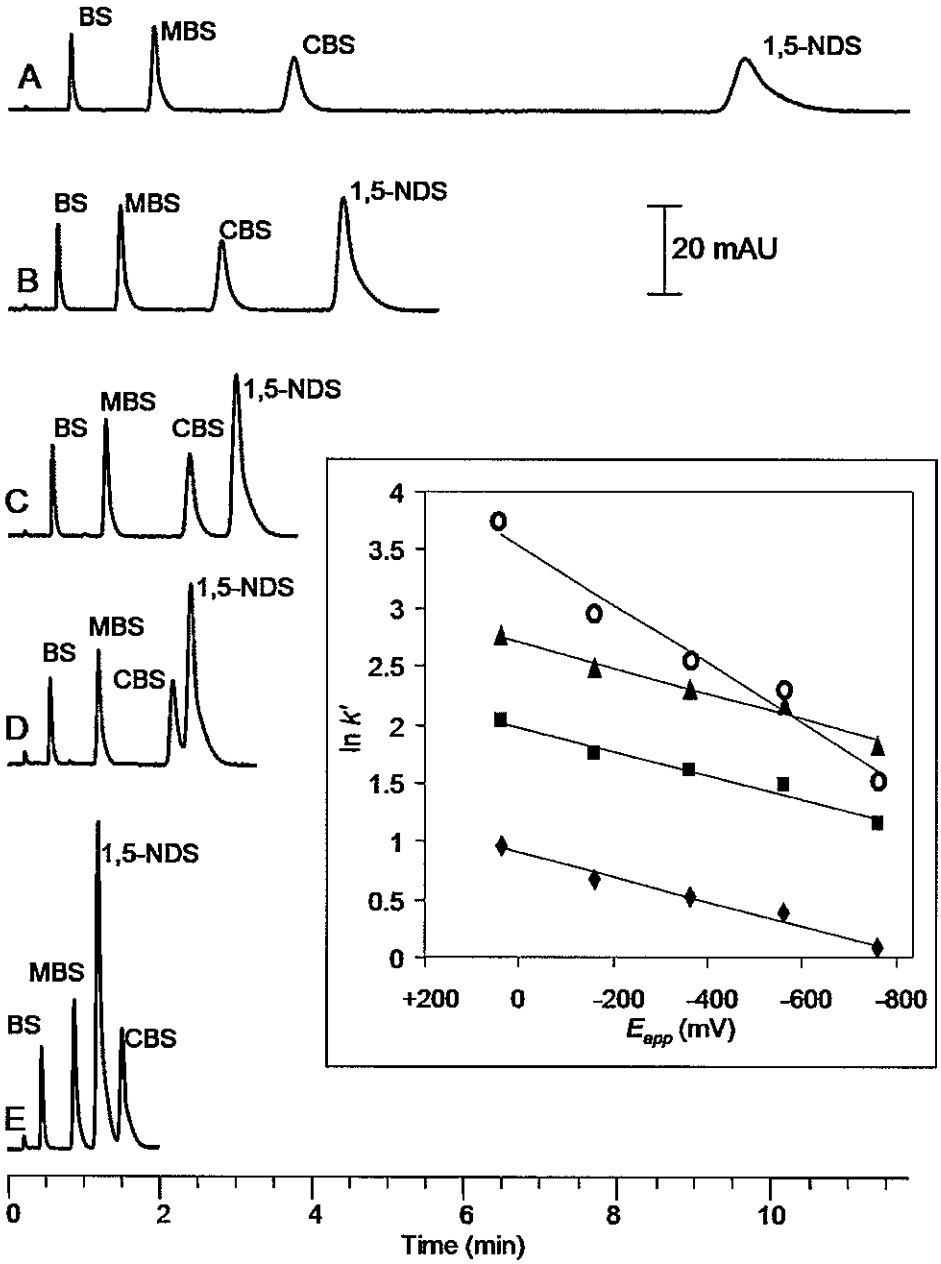

Figure 5. Dependence of retention of BS, MBS, CBS, and 1,5-NDS on E_{app} at a flow rate of 2.0 mL/min with a mobile phase composed of 0.1 M lithium perchlorate in water and a temperature of 100.0 °C. (A) +40 mV; (B) -160 mV; (C) -360 mV; (D) -560 mV; and (E) -760 mV. Inset: Plot ln k' vs. E_{app} BS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

interactions induced by E_{app}. In other words, the linear dependencies for the plots of ln k' vs. 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 if the decrease in solution resistivity and/or increase in 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 three 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 two-fold decrease in the time for the column to equilibrate to a change in applied potential. Together, these finding 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.

Acknowledgments

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CHAPTER 5. ELECTROCHEMICALLY MODULATED LIQUID CHROMATOGRAPHIC SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS

A paper in preparation for publication

Lisa M. Ponton[1,2] and Marc D. Porter[2,3]

Abstract

The retention behavior of several small polycyclic aromatic hydrocarbons (PAHs) on porous graphitic carbon (PGC) has been examined by electrochemically modulated liquid chromatography (EMLC) through changes in the composition and concentration of the supporting electrolyte and applied potential (E_{app}). Both solvophobic and donor-acceptor interactions were found to effect retention. The solvophobic component of the retention mechanism was revealed through changes in supporting electrolyte identity and concentration, while the role of donor-acceptor interactions were reflected by the sensitivity of elution to changes in E_{app}. Of particular interest are the retention shifts with changes in the concentration of tetrabutylammonium perchlorate, which alters both the ionic strength and polarity of the mobile phase. Improvements in the speed of the analysis through operation at an elevated column temperature and flow rate, allowed a more detailed investigation into the details of donor-acceptor retention interactions through comparisons of the elution order and sensitivity of retention to changes in E_{app}.

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[1] Primary researcher and author
[2] Institute of Combinatorial Discovery, Chemistry Department, Iowa State University, and Ames Laboratory-USDOE Ames, IA 50011
[3] Author for correspondence

Introduction

Polycyclic aromatic hydrocarbons (PAHs) have adverse health effects and their extraction and detection continues to be of major importance.[1] Post extraction, the most common methods of PAH analysis are gas chromatography[2, 3] and high performance liquid chromatography (HPLC).[2, 4] While gas chromatography is often the method of choice, only PAHs with less than 24 carbons have sufficient volatility for analysis. HPLC has been used to overcome this limitation.

PAHs are commonly separated in HPLC with reversed-phase packings (e.g., C18-modified silica).[4] These separations are generally accomplished by using a mobile phase that contains a high percentage of an organic component to decrease its polarity.[4] Porous graphitic carbon (PGC) has also proven to be a versatile and stable stationary phase for the use in HPLC and has strong reversed-phase retention characteristics.[5] Separations of PAHs with PGC, however, have had limited success.[6, 7] The highly aromatic nature of both the analytes and stationary phase yield extremely long retention times and severe band broadening. Even employing extremely strong eluents (e.g., methylene chloride) has done little to improve the situation.[6] However, this strong attraction makes PAHs an interesting class of probe molecules to explore the mechanism of retention on carbon in more detail.

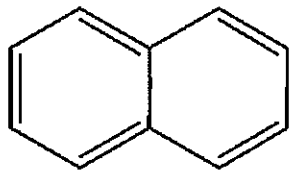
Electrochemically modulated liquid chromatography (EMLC) is a technique that takes advantage of some of the unique characteristics of PGC.[8] In EMLC, conductive stationary phases like PGC also act as a high surface area working electrode. The unique ability to manipulate the donor-acceptor properties of the conductive packing through changes in applied potential (E_{app}) therefore provides an alternative means to control analyte retention. Several reports from our laboratory[9-13] and others[14-17] have demonstrated that a

wide range of mixtures can be effectively separated with EMLC. Earlier reports examined the separation of aromatic sulfonates,[13] monosubstituted benzenes,[10] protonated pyridines and anilines,[18] corticosteroids,[9] and benzodiazepines.[12] Improvements in the column design, aimed at reducing the time required for equilibration of the packing after changes in E_{app}, were also described.[11] More recently, we have incorporated temperature as an additional control parameter in EMLC separations, aimed at delineating the thermodynamic contributions to retention[19] and at improving analysis times through operation at elevated flow rates.[20]

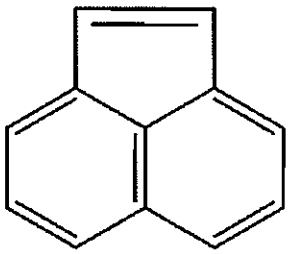
The work presented herein applies EMLC to the separation of several small PAHs. Some interesting chromatographic behavior was observed both as a function of E_{app} and supporting electrolyte concentration, which sheds some light into the retention mechanism of highly aromatic nonpolar compounds at carbon. It is also demonstrated that elevating column temperature and flow rate can improve the speed of the separation.

Experimental Methods

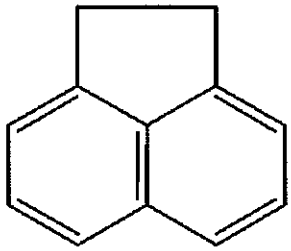
Chemicals and Reagents. The analytes: naphthalene, acenaphthene, acenaphthylene, and fluorene, shown in Chart 1, were purchased from Aldrich Chemical (Milwaukee, WI). All were used at a concentration of 10–20 μg/mL after dissolution in HPLC grade acetonitrile (Fisher Scientific, Pittsburgh, PA). The mobile phase consisted of various concentrations of lithium perchlorate and tetrabutylammonium perchlorate (Aldrich), in acetonitrile. The mobile phases were passed through a 0.5-μm filter (GE Osmonics Inc., Minnetonka, MN) prior to use. The 7-μm PGC (Hypercarb) particles were obtained from Thermo Hypersil (Bellfonte, PA).



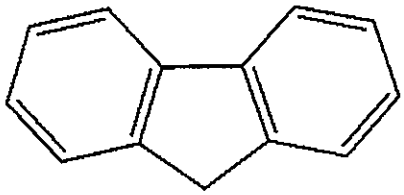
(1) Naphthalene



(3) Acenaphthylene



(2) Acenaphthene



(4) Fluorene

Chart 1. Polycyclic aromatic hydrocarbons

Instrumentation. The design and construction of the EMLC column has been described in detail elsewhere.[11] An Ag/Ag^+ reference electrode was used for potential control of the stationary phase and all values of E_{app} are reported with respect to this electrode. The column was attached to an Agilent Technologies model 1050 HPLC equipped with an autosampler, quaternary pump, and a UV-Vis diode array detector. The samples were injected using a 5.0-μL injection volume. The elution profiles were monitored at 240 nm. The potential applied to the stationary phase was controlled by an Amel potentiostat to ±1 mV.

The experimental setup for the control of temperature has also been detailed recently[20] and parallels several of the design elements devised by Carr and co-workers.[14, 16] The temperature was controlled to ±0.2 °C in a thermostated water bath (Polyscience, Niles, IL) filled with 50:50 (v/v) ethylene glycol:water. A 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. 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. A maximum temperature of 75 °C was used to limit the evaporative loss of the electrolyte solution in the reservoir that houses the reference electrode.

Data Treatment. An acetonitrile 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.[21] Three or more replicate injections were used for the calculation of k'. The resolution (R_s) of a separation was calculated based on statistical moment analysis resident in the HP Chemstation software, where the first moment represents the peak centroid and the second

moment is the peak variance.[21] By way of a working definition, an R_s value of 1.5 or greater for a separation of neighboring components is termed "baseline resolution", whereas "effective resolution" is used to describe values of R_s in the range of 1.0 to 1.5.[22]

Results and Discussion

Separations with a Lithium Perchlorate Mobile Phase. Figure 1 demonstrates the ability of EMLC to manipulate the retention of naphthalene and acenaphthene at PGC through changes in E_{app}. These results were obtained at four different values of E_{app} (+200, –100, –400, and –700 mV) using 100 mM lithium perchlorate as the mobile phase at a 1.0 mL/min flow rate. The column temperature was 24 °C. Both analytes exhibit a decrease in retention as E_{app} is moved to more negative values. For example, acenaphthene decreases from a retention time of 12.4 min at +200 mV (Chromatogram A) to 6.0 min at –700 mV (Chromatogram D). This behavior is similar to that observed for neutral monosubstituted benzene compounds in EMLC separations,[10, 11] and is attributed to changes in the donor-acceptor interactions between the π-systems of the analyte and the PGC surface. In other words, PGC becomes a stronger acceptor with increases in E_{app}, which, in turn increases the interactions with the π-systems of these analytes.

The results in Figure 1, along with those not shown for 10 and 50 mM concentrations of lithium perchlorate, were further analyzed by the plots of ln k' vs. E_{app} shown in the inset. 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. The first key observation in the inset is that the retention of each analyte exhibits a linear dependence on E_{app}. This behavior illustrates the effect on donor-acceptor interactions between the

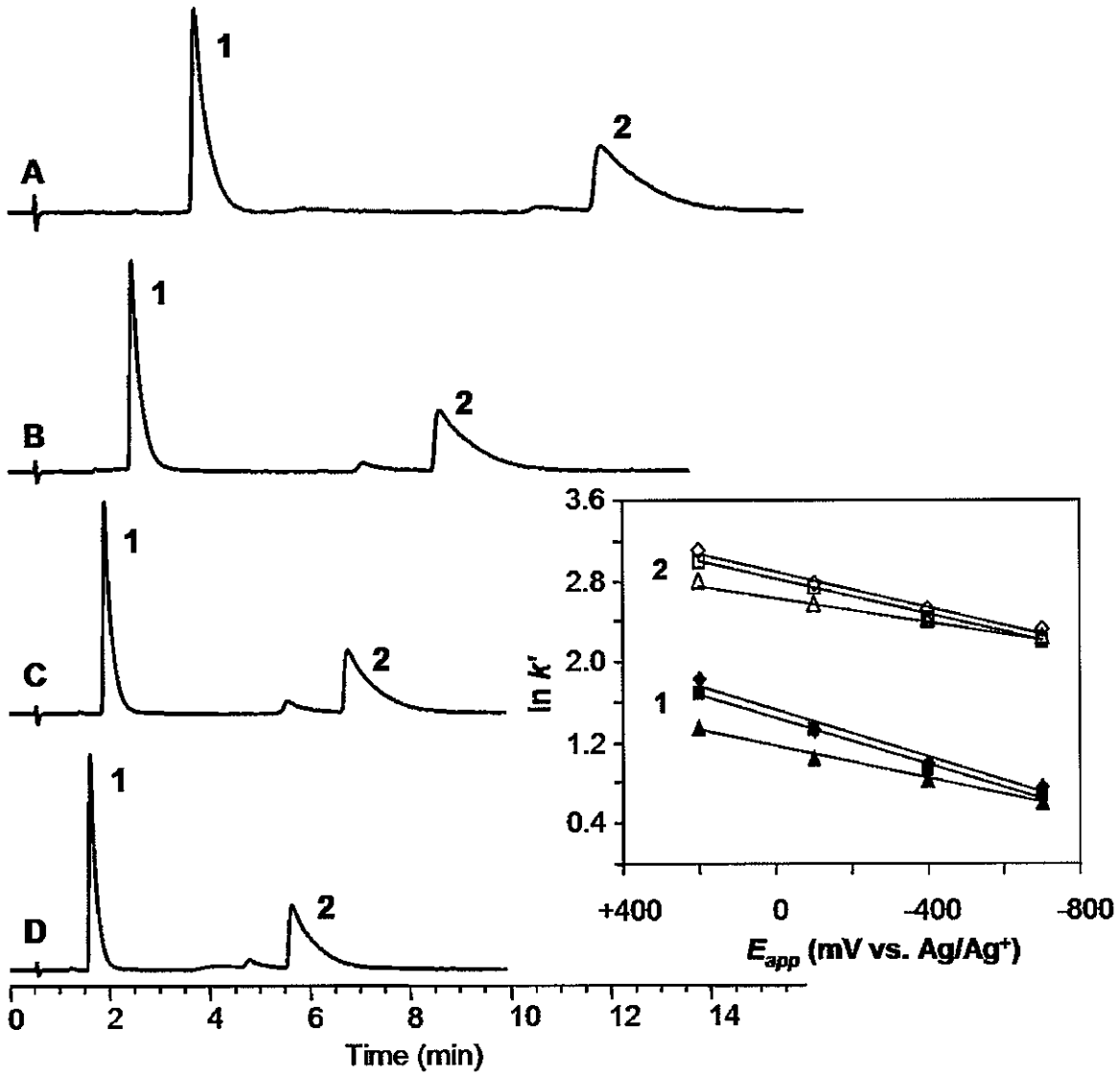


Figure 1. Dependence of the retention of naphthalene (1) and acenaphthene (2) on E_{app} at a flow rate of 1.0 mL/min, and a mobile phase composed of 100 mM $LiClO_4$ in acetonitrile, and a temperature of 24 °C. (A) +200 mV; (B) -100 mV; (C) -400 mV; and (D) -700 mV. Inset: Plot ln k' vs. E_{app} for naphthalene (solid symbols) and acenaphthene (open symbols) with 100 mM (◆, ◇), 50 mM (■, □), and 10 mM (▲, △) $LiClO_4$. Error bars are smaller than the size of the data points.

π-systems in the analyte and those in PGC. Linearity of these types of plots has also been observed for a series of neutral monosubstituted benzenes.[10, 11]

The slopes of these plots can be viewed as the sensitivity of retention (*S*) to changes in E_{app}, which are summarized in Table 1. With that in mind, there are several further observations. First, the slopes for acenaphthene are smaller than those for naphthalene, indicating that E_{app} has a larger impact on the retention of naphthalene. Interestingly, acenaphthene has a lower sensitivity than naphthalene, despite being more retained. As extensively reported in the PGC literature,[23] retention of aromatic compounds is dictated first by the degree of unsaturation and second by an increase in the number of carbon atoms. As evident in Chart 1, naphthalene and acenaphthene have the same number of π-electrons (i.e., degree of unsaturation), but have different numbers of carbon atoms. Naphthalene, with 10 carbons, is planar. The nonplanar acenaphthene has two additional sp^3 hybridized carbons. The presence of two more carbons apparently leads to the longer retention of acenaphthene over naphthalene. We suspect that the planarity of naphthalene allows a more intimate interaction between the two π-systems, resulting in a greater sensitivity to changes in the donor-acceptor strength of PGC.

Examining the changes in retention as the in supporting electrolyte concentration varies also shows some interesting behavior. The retention of each analyte decreases as the concentration of $LiClO_4$ decreases (100, 50, and 10 mM), as shown in Figure 2. Increasing the ion concentration in the mobile phase will decrease the solubility (i.e., increase in solvophobicity) of the PAHs, due to their neutral nonpolar characteristics. In addition, the PAHs are strongly attracted to the carbon stationary phase due to their aromatic nature. Therefore, at a higher ionic strength mobile phase, the relative difference of characteristics

Table 1. Slopes from the plots of ln k' vs. E_{app} (10^{-3} mV^{-1}).

Mobile Phase	Conc (mM)	(1) Naphthalene	(2) Acenaphthene
$LiClO_4$	100	1.18	0.87
	50	1.14	0.88
	10	0.80	0.61
$TBAClO_4$	100	1.35	1.07
	50	1.35	1.05
	10	1.10	0.90

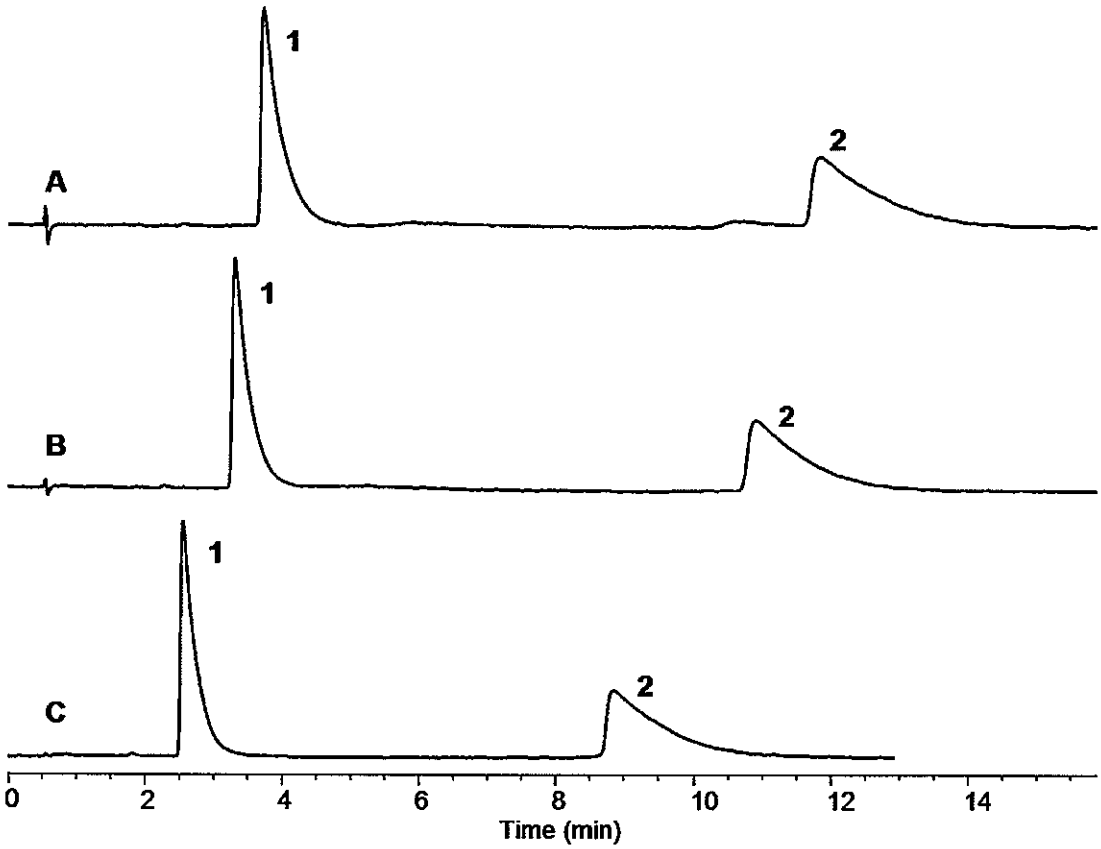


Figure 2. Dependence of the retention of naphthalene (1) and acenaphthene (2) on $LiClO_4$ concentration at a flow rate of 1.0 mL/min, and a temperature of 24 °C. (A) 100 mM; (B) 50 mM; and (C) 10 mM.

between the mobile and stationary phase is the largest. Coupling these factors would result in stronger retention in a higher ionic strength mobile phase (i.e., 100 mM). Nevertheless, this effect is relatively small. For example, acenaphthene decreases in retention from 12.4 to 9.3 min as the supporting electrolyte is decreased from 100 to 10 mM (Figure 2, Chromatograms A and C).

Another interesting trend is evident in comparing the values of S for each analyte with changes in concentration of $LiClO_4$ (Table 1). The value of S decreases with decreasing supporting electrolyte concentration, suggesting that retention is manipulated more readily with a higher ionic strength mobile phase. Mechanistic implications of this observation are discussed with the trends found in the data described in the following section.

Separations with a Tetrabutylammonium Perchlorate Mobile Phase. A similar set of experiments were performed using tetrabutylammonium perchlorate (100 mM) as the supporting electrolyte. In comparing the chromatograms for naphthalene and acenaphthene in Figure 1 with that in Figure 3, it is evident that $TBAClO_4$ is a stronger eluting agent. The runtime for the separation with $LiClO_4$ takes nearly 14 min, while that with $TBAClO_4$ is just under 9 min. Moreover, the retention behavior of naphthalene and acenaphthene with changes in E_{app} in $TBAClO_4$ mimics that in $LiClO_4$. The linear dependence of the plots of ln k' vs. E_{app}, shown in the Figure 3 inset, again indicates the importance of donor-acceptor interactions to retention.

In further comparing S of the two supporting electrolytes, it is observed that the values of S with $TBAClO_4$ are greater than those with $LiClO_4$. This is an interesting comparison because the observed shift in retention is a convolution of the sensitivity of the interaction of the analyte and supporting electrolyte with PGC to changes in E_{app}.

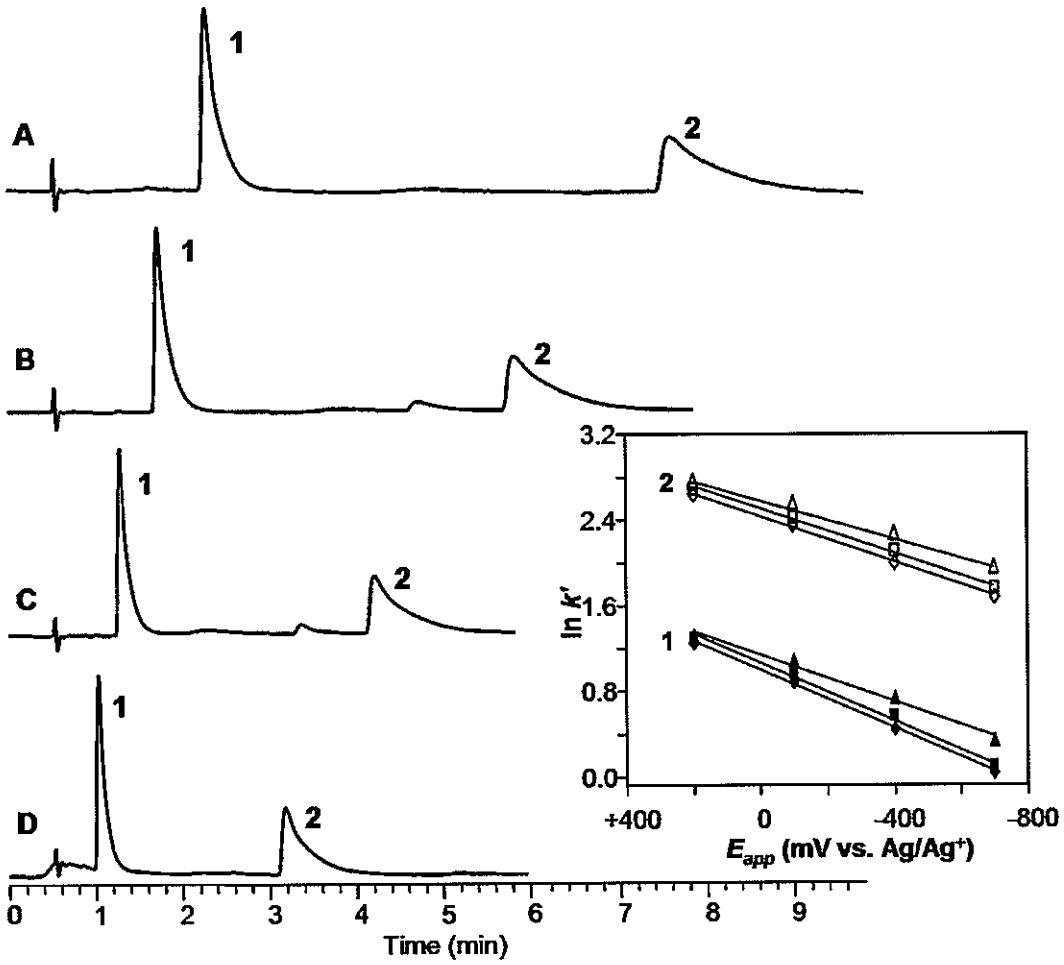


Figure 3. Dependence of the retention of naphthalene (1) and acenaphthene (2) on E_{app} at a flow rate of 1.0 mL/min, and a mobile phase composed of 100 mM $TBAClO_4$ in acetonitrile, and a temperature of 24 °C. (A) +200 mV; (B) -100 mV; (C) -400 mV; and (D) -700 mV. Inset: Plot ln k' vs. E_{app} for naphthalene (solid symbols) and acenaphthene (open symbols) with 100 mM (◆, ◇), 50 mM (■, □), and 10 mM (▲, △) $TBAClO_4$. Error bars are smaller than the size of the data points.

Comparing the measured *S*-values for the same analyte in mobile phases with two different supporting electrolytes allows conclusions to be made about the effect of E_{app} on the interactions of the supporting electrolyte with PGC. The data suggest that $TBAClO_4$ is itself more sensitive to E_{app} than $LiClO_4$, implying that $TBAClO_4$ is a stronger competitor for the surface. This conclusion is supported by the lower retention times observed for naphthalene and acenaphthene with the $TBAClO_4$ mobile phase.

In comparing the sensitivities of the analytes for a given condition, the value of *S* for acenaphthene are smaller than those for naphthalene. As stated above, this trend is likely due to the higher planarity of naphthalene over acenaphthene, which results in a closer interaction between the π-electrons of naphthalene and the surface and thus a greater sensitivity to changes in E_{app}.

In examining retention as a function of electrolyte concentration for $TBAClO_4$ (Figure 4), a surprising trend was observed. As the concentration of supporting electrolyte was decreased, the retention increased slightly. For example, the retention time of acenaphthene is seen to increase from 7.9 to 8.8 min as the $TBAClO_4$ concentration decreases from 100 to 10 mM. This dependence is opposite of that observed for retention with the $LiClO_4$ mobile phase. There are, however, a different set of factors to consider with $TBAClO_4$. Tetrabutylammonium itself is a fairly large organic cation with nonpolar characteristics. Therefore, increasing the supporting electrolyte concentration not only increases the ionic strength of the mobile phase, but decreases the polarity of the mobile phase as well. The first factor would tend to increase retention, whereas the second factor would have the opposite effect. Since retention decreases with increasing concentration, the

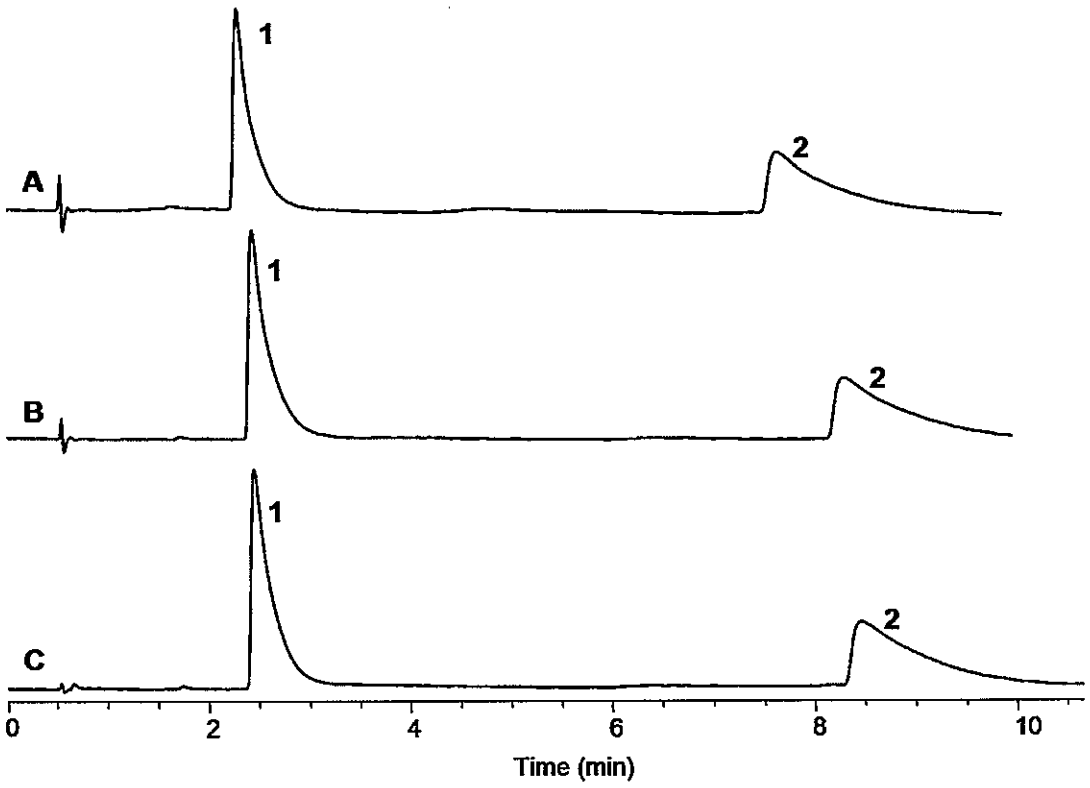


Figure 4. Dependence of the retention of naphthalene (1) and acenaphthene (2) on $TBAClO_4$ concentration at a flow rate of 1.0 mL/min, and a temperature of 24 °C. (A) 100 mM; (B) 50 mM; and (C) 10 mM.

lower polarity of the mobile phase appears to have a slightly greater effect than the increased ionic strength.

The sensitivities of the analytes to E_{app} are larger for the higher concentrations of $TBAClO_4$, as was observed for $LiClO_4$ (Table 1). This differences suggests that the decrease in mobile phase polarity due to the presence of the tetrabutylammonium cation counters the increase in ionic strength to result in an increase in retention. In other words, solvophobicity dictates the change in retention time, while donor-acceptor retention interactions determines the sensitivity of the separation to changes in E_{app}.

High Speed Separation of PAHs. As demonstrated in a recent report from our laboratory,[20] elevating the column temperature can dramatically improve the speed of an EMLC analysis without significant loss in chromatographic efficiency. This section describes the application of this methodology to the separation of a mixture of naphthalene, acenaphthene, acenaphthylene, and fluorene. Using a 10 mM $LiClO_4$ mobile phase and elevating the column temperature to 75 °C enabled the flow rate to be increased to 3.0 mL/min without exceeding the maximum pump pressure (400 bar).

Figure 5 shows the separation of the PAHs under these conditions at four different values of E_{app}: +200, –100, –400, and –700 mV. At +200 mV, the analytes elute in less than 4 min with baseline resolution. Like the room temperature separations, all four PAHs show a decrease in retention as E_{app} becomes more negative. The separations at –100 and –400 mV exhibit a decrease in retention while maintaining baseline resolution (R_s >1.5). At –700 mV, the runtime has decreased to ~2.5 min, with near baseline resolution (R_s = 1.3) for acenaphthene and acenaphthylene. While these operational conditions effectively resolved this mixture, PAHs with three or more aromatic rings were still so strongly retained that

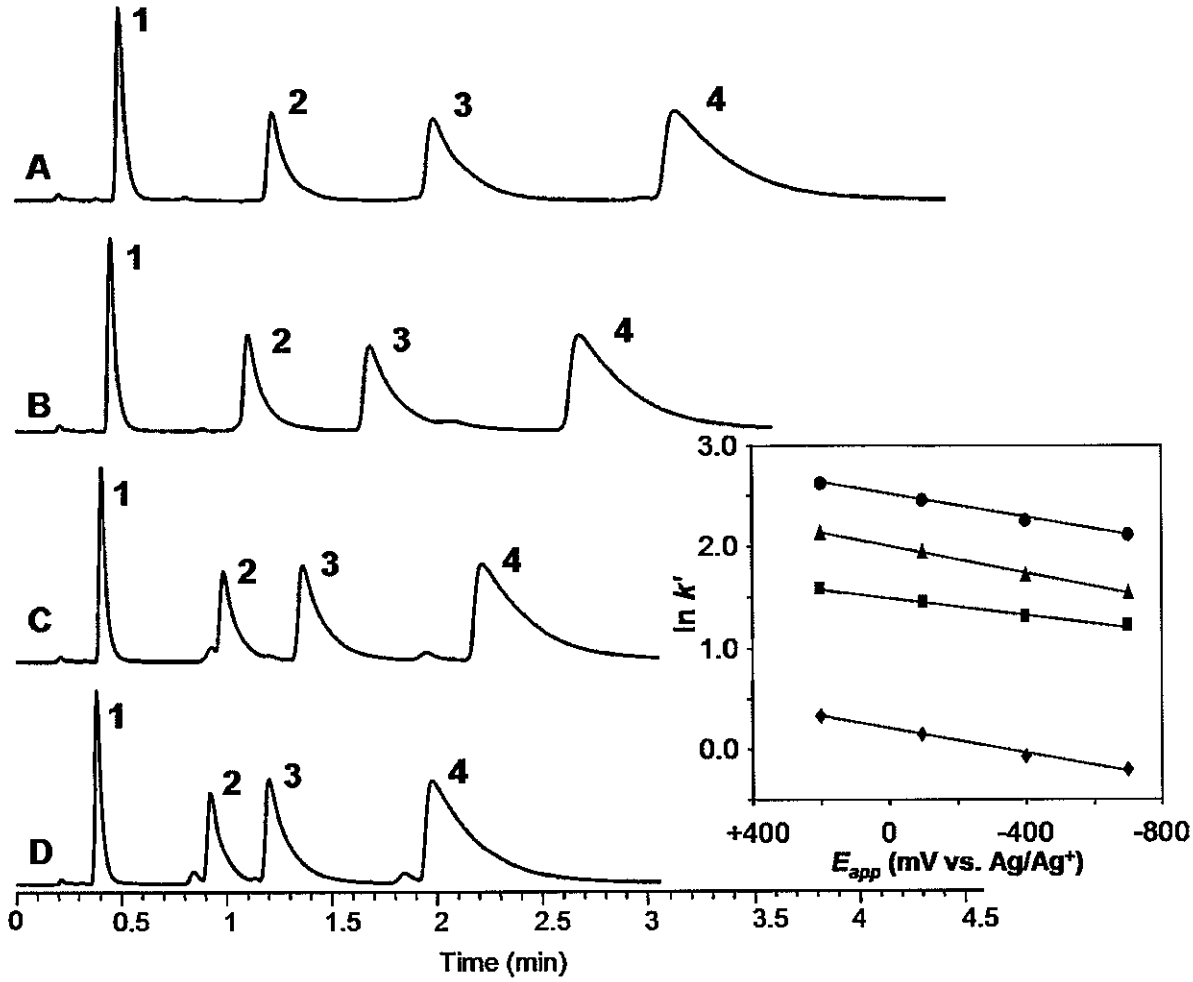


Figure 5. Dependence of the retention of naphthalene (1) and acenaphthene (2) on E_{app} at a flow rate of 3.0 mL/min, and a mobile phase composed of 10 mM $LiClO_4$ in acetonitrile, and a temperature of 75 °C. (A) +200 mV; (B) -100 mV; (C) -400 mV; and (D) -700 mV. Inset: Plot ln k' vs. E_{app} for naphthalene (◆), acenaphthene (■), acenaphthylene (▲), and fluorene (●). Error bars are smaller than the size of the data points.

severe band broadening was observed. Though not shown, the elution of anthracene required 12.4 min at –700 mV, but had a 6.2 min-wide baseline. Clearly, further studies need to explore ways to improve the elution of larger PAHs, with the use of stronger eluting mobile phases (e.g., methylene chloride or tetrahydrofuran) and/or a column redesigned to operate at elevated temperature representing intriguing possibilities.

The elution order observed for these high speed separations follows the trend established in previous studies on smaller aromatic hydrocarbons.[23] Elution order is first determined by the number of double bonds that define the π-systems of the analytes, and second by the number of carbon atoms on the molecule. The PAHs with more double bonds have longer elution times. For PAHs with the same number of double bonds, the compound with the fewer number of carbon atoms elutes first. Naphthalene and acenaphthene both have five double bonds, and therefore elute first. Acenaphthene has two additional sp^3 hybridized carbon atoms giving it a total of 12 carbon atoms in the molecule, resulting in longer retention than naphthalene, which has only 10 carbon atoms. Acenaphthene is followed by acenaphthylene (six double bonds, 12 carbon atoms), fluorene (six double bonds, 13 carbon atoms), and anthracene (seven double bonds, 14 carbon atoms).

The sensitivity plots presented in the inset of Figure 5 again show the ability of EMLC to fine tune separations of these PAHs by manipulating donor-acceptor interactions via changes in E_{app}. The decreasing resolution between acenaphthene and acenaphthylene as E_{app} becomes more negative is evident by the convergence of these plots.

The *S*-values are quantified in Table 2. There are two interesting observations in the values for *S*. First, the order of decreasing *S* is acenaphthylene, naphthalene, fluorene, and acenaphthene. While the order of *S* of does not follow the elution order, there is an

Table 2. Slopes from the plots of ln k' vs. E_{app} (10^{-3} mV^{-1}) under high speed conditions.

	PAH	Slope
1	Naphthalene	0.60
2	Acenaphthene	0.42
3	Acenaphthylene	0.67
4	Fluorene	0.58

interesting trend. The first level of discrimination for *S*, as was observed above, is the planarity of the compound followed by the number of double bonds. Acenaphthylene and naphthalene are both planar with acenaphthylene having more double bonds than naphthalene. It therefore follows that acenaphthylene would be more sensitive to changes in E_{app} than naphthalene. Though not tested, this trend suggests that anthracene would be even more sensitive to changes in E_{app}. Unfortunately, the severe band broadening in the retention of anthracene prohibited testing this hypothesis.

The second interesting observation is that the *S*-values for naphthalene and acenaphthene are lower at elevated temperature and flow rates than at room temperature. While the observed decrease in retention is primarily governed by the exothermicity of the interaction and the increased flow rate, the sensitivity is governed by the change in the solvophobicity of the interaction. As temperature increases, the dielectric constant of acetonitrile decreases,[24] making the mobile phase more nonpolar. Therefore, at elevated temperature the solubility of the PAHs in the mobile phase has increased, decreasing the solvophobicity of the interaction. These weaker solvophobic interactions allow the retention of the PAHs to be less affected by changes in the stationary phase, resulting in the decreased manipulation of the donor-acceptor interactions via changes in E_{app}.

Conclusion

The unique retention behavior of PAHs on PGC to changes in mobile phase composition and E_{app} has been described. The sensitivity of retention to E_{app} highlights the donor-acceptor component of the retention mechanism, while changes in supporting electrolyte concentration demonstrate the solvophobic component of the retention

mechanism. Of particular interest is the change of retention with changes in the concentration of $TBAClO_4$, where retention shifts are dictated by both ionic strength and polarity issues. It has also been demonstrated that elevating column temperature and flow rate improves the separation time of the PAHs examined here. The improvement in the speed of the analysis allowed a more detailed investigation into the details of the donor-acceptor retention parameter through comparisons of the retention of four PAHs. Further studies into the thermodynamics of retention of these and other PAHs on PGC will aid in the elucidation of the different aspects of the retention mechanism in more detail.

Acknowledgment

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CHAPTER 6. GENERAL CONCLUSIONS

Research Overview

The primary focus of this doctoral research has been the integration of temperature control into electrochemically modulated liquid chromatography (EMLC). The addition of temperature as a controllable variable to the tunable characteristics of carbonaceous EMLC stationary phases (i.e., porous graphitic (PGC) and glassy (GC) carbon) has allowed insights into the subtleties of the retention mechanism. Furthermore, operating EMLC at elevated temperature can markedly reduce analysis times.

Chapter 2 describes the determination of thermodynamic contributions to retention, enabling comparison of retention mechanisms under a variety of conditions. The elution behavior of two naphthalene disulfonates as a function of applied potential (E_{app}) was examined by calculating the enthalpic and entropic contributions to retention. As expected for anions, the retention time decreases as E_{app} becomes more negative. Interestingly, while the retention mechanism is exothermic at all potentials, the exothermicity of the interaction increases as analyte retention decreases. Calculations of the entropic contribution to retention and overall Gibbs free energy show that the retention dependence of these analytes on E_{app} is entropically rather than enthalpically controlled. Since entropically driven separations are commonly found in ion exchange chromatography (IEC), this suggests a level of similarity in the retention mechanism between EMLC- and IEC-based separations. However, most IEC entropically driven separations are accompanied by a positive change in enthalpy rather than the observed negative change in enthalpy found here. Retention on PGC has a strongly reversed-phase liquid chromatography (RPLC) mechanism,[1] where negative

changes in enthalpies are commonly found. The retention of aromatic ions in EMLC is therefore a combination of both RPLC and IEC mechanisms.

Further support for an ion-exchange mechanism at carbon packings is presented in Chapter 3, in which the manipulation and optimization of inorganic anion retention on PGC through changes in both the mobile phase composition and the E_{app} of the stationary phase is described. The choice of an electronic competitor and its concentration, [E], in the mobile phase have a profound impact on the resolution of the separation, with fluoride proving the most effective eluent of the three (tetrafluoroborate, sulfate, and fluoride) electronic competitors tested. Results also showed that these separations could be manipulated by changes in E_{app}. From a mechanistic perspective, plots of ln k' (capacity factor) were found to be linearly dependent on both E_{app} and ln [E]. This behavior can be described in the context of an ion-exchange mechanism between analyte ions in the mobile phase and eluent ions in the interphase formed on the surface of PGC. Deviations from the expectations for the slopes of the ln k' vs. ln [E] plots were attributed to the effects of specific interactions on the retention mechanism, which are not readily accounted for by correct theories of solvation thermodynamics.

Temperature-controlled EMLC has also been useful in the optimization of analysis time by taking advantage of the drop in mobile phase viscosity at higher temperatures, which allows the use of faster flow rates. Chapter 4 discussed the application of elevated temperatures and flow rates in EMLC-based separations resulting in a reduction in analysis time by a factor of more than 20, yielding an effective separation in less than 1 min for several aromatic sulfonates. Enhancement in the speed of the separation also allowed the elimination of the organic component of the mobile phase, resulting in the separation of the

aromatic sulfonates in less than 2 min in an entirely aqueous mobile phase. Together, these findings have clear implications for the improvement of EMLC by increasing sample throughput as well as reducing mobile phase toxicity, flammability, and waste disposal costs.

Chapter 5 describes the retention of polycyclic aromatic hydrocarbons (PAHs) on PGC with changes in E_{app} for both lithium perchlorate and tetrabutylammonium perchlorate mobile phases. Retention of PAHs is dominated by both donor-acceptor properties, as highlighted by the effect of E_{app} on retention, and solvophobic properties, evident in examining the changes in supporting electrolyte identity and concentration. Elevated temperature and flow rate, similar in design to the experiment described in Chapter 4, were also employed for the improvement in the speed of the analysis. Moreover, these results provide further insight into the details of the donor-acceptor based retention through comparisons of the retention of four PAHs.

Prospectus

The successful integration of temperature into EMLC separations will allow further studies on a wide variety of analytes to probe different aspects of retention. For example, small inorganic anion analysis will yield insight into the details of the ion-exchange mechanism. Expanding these ions to incorporate nonpolar and polar functionalities, as well as aromaticity, will further test the impact of the hydrophobic effect via thermodynamic analysis. In addition, the examination of neutral compounds, such as polycyclic aromatic hydrocarbons and other smaller aromatic compounds will provide insight into the reversed-phase aspect of retention. Together, these studies will yield a more detailed mechanism of EMLC-based retention.

In addition to new analyte probes, an extension of the thermodynamic analysis described in the General Introduction and Chapter 2 is the use of extra-thermodynamic values to determine the similarity of retention mechanism within and throughout several classes of analytes. One of the more common thermodynamic approaches used in chromatography is enthalpy-entropy compensation (EEC).[2, 3] To avoid statistical artifacts in data processing, EEC is given by:

$$\ln k'_T = \frac{-\Delta H^0}{R}\left(\frac{1}{T}-\frac{1}{\beta}\right)-\frac{\Delta G^0}{R\beta}+\ln\phi \qquad [1]$$

where β is the compensation temperature, ΔG^0_β is the Gibbs free energy at β, and k'_T is the capacity factor at temperature T.[4-6] This approach has been used to examine mechanisms for a wide range of analytes, mobile phases, and stationary phases.[3, 7-9] When the retention mechanism is the same, the compensation temperatures are comparable. Therefore, comparisons of EEC behavior have proven valuable in identifying key differences, for example, between reversed and normal phase separations.[3] Using this means of analysis with EMLC will aid in the differentiation of the different retention mechanisms involved.

A key hurdle to overcome in thermodynamic analyses is accurate determination of the phase volume ratio (ϕ). The *y*-intercept of a van't Hoff plot can only be used in the determination of ΔS^0 if the value of ϕ is known. However, the development of an exact definition and determination of ϕ is a long standing issue in chromatographic systems. The value of V_{mp} can be determined in several ways.[10] For EMLC, using the retention time of a pseudo peak (i.e., a mismatched matrix between the sample and mobile phase) is the most effective method. The functional definition and determination of V_{sp} is more problematic, as it is in many chromatographic systems.[11]

For many solid stationary phases (those without a bonded phase), it is common to treat retention as a surface adsorption phenomenon.[12] However, this is not necessarily a valid assumption, because it ignores the effect of the electrical double layer. The compositional details of the double layer may have a significant impact on the retention of analytes. Therefore, we believe that this layer needs to be considered as part of the stationary phase and impacts the value for V_{sp}. The solution side of the electrical double layer consists of two components: the compact layer and the diffuse layer. The thickness of the compact layer is defined by the diameter of ions specifically adsorbed on the surface of the stationary phase, and therefore is independent of the charge density of the stationary phase. On the other hand, the thickness of the diffuse layer is dependent on both the charge density of the stationary phase and the mobile phase composition. As a result, the diffuse layer thickness will vary when different conditions are employed. The double layer structure is even more complex in flowing systems because a stagnant solution layer is formed at the surface, the thickness of which depends on particle size and flow rates. The stagnant layer potentially decreases the effective thickness of the diffuse layer. Since the data in Chapter 2 was interpreted to indicate a constant stationary phase thickness over a wide range of E_{app}, V_{sp} can be defined by the surface area of the packing and the thickness of the compact component of the electrical double layer. In light of this discussion, more detailed investigations into the determination of an accurate value for V_{sp} are necessary. In particular, it would be valuable to determine what components of the electrical double layer reside within the stagnant solution layer in these systems.

In utilizing elevated temperatures in EMLC for high speed separations, the upper limit on temperature was 100 °C, as described in Chapter 4, due to the current EMLC column

design. The external reservoir (pictured in Chapter 1, Figure 1) is exposed to the atmosphere. Taking temperatures above 100 °C would result in severe evaporative loss of the supporting electrolyte in the reservoir. The ability to approach much higher operating temperatures (> 250 °C) would not only further reduce analysis times, but potentially extend the breadth of analytes that can be separated in an aqueous mobile phase by EMLC.

As mentioned in Chapter 4, elevated temperature has several effects on the mobile phase. First, the solvent viscosity decreases as temperature is elevated resulting in a higher maximum flow rate attainable, thus reducing analysis time. Second, there is a decrease in the dielectric constant for water with increasing temperature, resulting in water behaving more like a hydrophobic solvent with increasing temperature.[13] For example, the dielectric constant for water is ~80 at 20 °C and decreases to ~33 at 200 °C, which is close to that of methanol.[14] By making the mobile phase more "methanol-like", hydrophobic analytes (e.g., the PAHs examined in Chapter 5) can be analyzed as a result of operating the EMLC column under these conditions.

Attainment of this goal will require a redesigned EMLC column, where the external reservoir is closed to the atmosphere and pressurized to the level necessary to prevent boiling of the supporting electrolyte. Several possibilities have been explored and the solution will likely reside in designing a sealed collar around the top and bottom of the reservoir with an extended contact for connection of the reference electrode to the potentiostat. The pressure requirement for the reservoir is slightly above the vapor pressure of the mobile phase at the maximum temperature reached, for example, 40 bar at 250 °C for water.[14]

In the process of examining the use of elevated temperatures in EMLC, the time to equilibrate the column to changes in E_{app} was also examined. As briefly described in

Chapter 4, the elevation of column temperature from 20 to 60 °C resulted in the reduction of equilibration time to changes in E_{app} by ~50%. Further studies are needed to explore this phenomenon in more detail. If equilibration times can be significantly reduced, operating the EMLC column in a potential gradient format rather than a fixed potential format can further improve sample analysis in a manner similar to how mobile phase gradients affect separations.

During the process of examining the thermodynamics of retention in EMLC and execution of high speed EMLC separations, an irreversible change in the stationary phase was observed. After operation of our EMLC columns at elevated temperatures, retention times of the analytes were found to decrease. Characterization of the stationary phase by X-ray photoelectron spectroscopy (XPS) after using the columns showed that the oxygen content of the stationary phase had increased, as is described in Chapter 4 and in more detail in Appendix A. Based on this evidence, it is clear that the stability of the packing must be improved in order to fully exploit the advantages of operation at elevated temperatures. One possible approach to stabilize the carbon stationary phase is through hydrogen plasma pre-treated. Recent reports have shown that compared to polished glassy electrodes, hydrogenating glassy carbon electrodes stabilizes the electrochemical response.[15, 16] Similar treatment may prove valuable in enhancing the long term performance of PGC and GC packings when operated at elevated temperature. Clearly, future studies need to be performed to not only stabilize the chromatographic behavior of carbon stationary phases, but also to determine the degree of instability.

In summary, the successful use of temperature as an additional control parameter in EMLC has enabled the determination of the thermodynamics of retention of naphthalene

disulfonates and the rapid analysis of several aromatic sulfonates. The groundwork has been laid for full exploitation of temperature to develop retention rules for EMLC through a clearer understanding of the retention mechanism for a wide range of analyte classes. Furthermore, the use of elevated temperature and flow conditions will enable the separation of analytes not normally achievable on a carbon stationary phase.

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APPENDIX A. EFFECTS OF ELEVATED TEMPERATURE ON THE OXIDATION OF CARBON IN ELECTROCHEMICALLY MODULATED LIQUID CHROMATOGRAPHY

Lisa M. Ponton,[1,2] James Anderegg,[2] and Marc D. Porter[2,3]

During the examination of the thermodynamics of retention in electrochemically modulated liquid chromatography (EMLC) and execution of high speed EMLC separations, a change in the stationary phase was observed. This irreversible change is a consequence of the increased susceptibility to oxidation of both the porous graphitic carbon (PGC) and glassy carbon (GC) packings at elevated temperatures. It was found that the retention times of the analytes examined decreased during operation of our EMLC columns at elevated temperatures and in some cases changed to a degree sufficient to complicate data assessment. To assess the basis of this change, when possible, columns were unpacked after usage and characterized using X-ray photoelectron spectroscopy (XPS). All columns exhibited an increase in the oxygen content after usage, as evident in Table 1. For some columns examined by XPS, chromatograms were collected at the same conditions shortly after packing the column and shortly before unpacking the column. The results are listed in Table 1 as the percent loss in retention time (t_r) for the analytes. These findings show a correlation between the decrease in retention observed and an increase in the percent atomic oxygen present on the surface.

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[1] Primary researcher and author
[2] Institute of Combinatorial Discovery, Chemistry Department, Iowa State University, and Ames Laboratory-USDOE Ames, IA 50011
[3] Author for correspondence

It is important to note that the after completion of the van't Hoff experiments presented in Chapter 2, no degradation of retention was observed, as was evidenced by comparing chromatograms collected at the same conditions at the start and at the completion of the study. Furthermore, the separation of inorganic anions (Chapter 3) and polycyclic aromatic hydrocarbons (Chapter 5) also did not show a change in retention after completion of the experiments. However, as discussed in Chapter 4, there was a slight change in retention throughout the duration of the experiment. This point is brought out in the discussion of the data in the chapter.

To further illustrate the degradation of retention, several detailed examples where this behavior was observed follow. The first detailed example is the separation of 1,5- and 2,6-naphthalenedisulfonate (1,5- and 2,6-NDS, respectively) on a conventional column, i.e., no potential control (Conv-GC-4), packed with glassy carbon, as shown in Figure 1. Chromatograms A and B were collected on the same day at 20.2 °C and 25.3 °C respectively. These two chromatograms were part of a van't Hoff analysis. Using the slope of the van't Hoff plot (as described in Chapter 2) the retention times for 1,5- and 2,6-NDS at 22 °C would be 1.5 and 4.9 min respectively. Chromatogram C shows the separation of the same analytes under the same conditions at 22.3 °C. In the 9 days between the collection of Chromatograms A and C, three van't Hoff experiments were conducted, each reaching a maximum temperature (T_{max}) of 55 °C. As is evident, retention has decreased to 1.1 min for 1,5-NDS and 2.5 min for 2,6-NDS, correlating to a decrease in retention of 32% and 48%, respectively.

Figure 2 shows the XPS spectra for the as-received GC particles used to pack the column used in Figure 1. The top spectrum is the survey spectrum with the high resolution

spectrum for each component of interest below. The largest peak observed is for carbon, as expected. There are also several other interesting features. First, there are measurable quantities of O, Ca, and F on the surface. The calcium and fluorine are attributed to the manufacturing process. With respect to carbon, this sample of GC showed 6.6 atomic % O prior to use. After completion of the above described chromatographic experiments, the carbon was unpacked from the column as re-examined with XPS. The results in Figure 3 show that after use at elevated temperatures the oxygen content has risen to 8.8 % with respect to carbon. Several of the other observed elements are traceable to the analyte, supporting electrolytes, and mobile phases employed during the van't Hoff experiments.

Figure 4 shows the separation of several monosubstituted benzene sulfonates with an EMLC column packed with GC (EMLC-GC-8). The fronting observed in the chromatograms is attributed to a small void in the column packing. Both Chromatograms A and B were collected under the same conditions at 25.0 °C with an applied potential of +200 mV. Chromatogram B was collected 11 days and 2 van't Hoff analyses (T_{max} = 60 °C) after Chromatogram A. As is evident, retention has decreased from 1.0 min to 0.7 min for methylbenzene sulfonate (MBS) and 1.8 min to 1.0 min for chlorobenzene sulfonate (CBS). These reductions in retention time correlate to a decrease in retention of 30 % for MBS and 44 % for CBS. Benzene sulfonate (BS) was not examined in Chromatogram B due to the decreased retention times. The GC used in these experiments had been pre-washed with sulfuric acid to remove the calcium and fluorine observed in the XPS spectra shown in Figure 2. Figure 5 shows the XPS spectra for the "cleaned" GC prior to use in the EMLC column. The oxygen content in this case is 2.1 %. After chromatographic experiments, Figure 6, there is a marked increase in the oxygen content to 9.4 %.

This phenomenon was not isolated to GC; it was also observed for PGC. Figure 7 shows the chromatograms for the monosubstituted benzene sulfonates on an EMLC column packed with PGC (EMLC-PGC-16). All three chromatograms were collected at the same conditions at room temperature (21 °C) and +100 mV. Chromatogram A was collected prior to a high speed experiment, similar to that described in Chapter 3. Chromatogram B was collected five days later after extended use of the column at 100°C. Chromatogram C was collected three days later, only being flushed with solvents at room temperature during that time. CBS decreased in retention from 20.9 to 19.3 to 16.7 min, an overall 20 % decrease in retention. MBS and BS both exhibited an 18 and 14 % decrease in retention respectively. Figure 8 shows the XPS spectra for as received PGC. PGC is very clean with only 0.1 % O measurable. After use, the PGC showed an oxygen content of 2.0 %, Figure 9.

Finally, Table 2 consists of data examining the relative effect of the components of the mobile phase through a batch experiment. A small amount of PGC (~0.5 g) was placed in a vial with various mobile phase components for 5 days at 60 °C. After this treatment the samples were characterized by XPS. These results show that all mobile phase compositions examined oxidize the surface to some degree, with the exception of pure water. The increase due to acetonitrile and methanol, however, is minimal. Introduction of electrolyte significantly increased the oxygen content in all cases.

Similar chromatographic evidence for the redox activity of PGC has been documented by other authors.[1-3] Shibukawa et al.[2] describe using the redox property of PGC to their advantage in an on-column derivatization method. They found that by introduction of a reducing agent, this redox activity could be manipulated between having oxidative capability to reductive capability for some metal complexes. In another example, Tornkvist

et al.[3] documented the chromatographic impact of passing oxidizing agents and reducing agents through the column and found evidence for a slow oxidation of PGC with time.

Based on the above, it is apparent that retention can be profoundly impacted by the changing presence of surface oxygen throughout the duration of elevated temperature experiments. Therefore, the stability of the packing needs to be improved in order to fully exploit the advantages of operation at elevated temperatures. One possible approach is pre-treating the carbon stationary phase in a hydrogen plasma. Recent reports have shown that hydrogenating glassy carbon electrodes stabilizes their electrochemical response in comparison to polished electrodes.[4, 5] Similar treatment may prove valuable in enhancing the long term performance of the PGC and GC packing when operated at elevated temperature. Clearly future studies need to be performed to not only stabilize the chromatographic behavior of carbon stationary phases, but to determine the degree of instability.

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Table 1. Chromatographic evidence for oxidation of PGC as a result of elevated temperature.

Carbon Sample	**% O**	**% loss in t_r**
Unused GC (sieved)	6.6	
Conv-GC-1	12.5	14 %
Conv-GC-2	3.8	
Conv-GC-4	8.8	32 – 48 %
Unused GC (washed with H_2SO_4)	2.1	
EMLC-GC-2	4.1	39 – 51 %
EMLC-GC-3	6.5	
EMLC-GC-4	10.0	
EMLC-GC-5	9.5	5 – 10 %
EMLC-GC-6	11.1	12 – 32 %
EMLC-GC-7	5.9	
EMLC-GC-8	9.4	30 – 44 %
As received PGC	0.1	
EMLC-PGC-16	2.0	14 – 20 %
EMLC-PGC-19	0.9	7 – 14 %
EMLC-PGC-20	0.5	
EMLC-PGC-21	0.7	
EMLC-PGC-23	0.4	
EMLC-PGC-24	0.4	
EMLC-PGC-25	0.3	
EMLC-PGC-27	1.8	
EMLC-PGC-28	0.8	
EMLC-PGC-29	0.9	

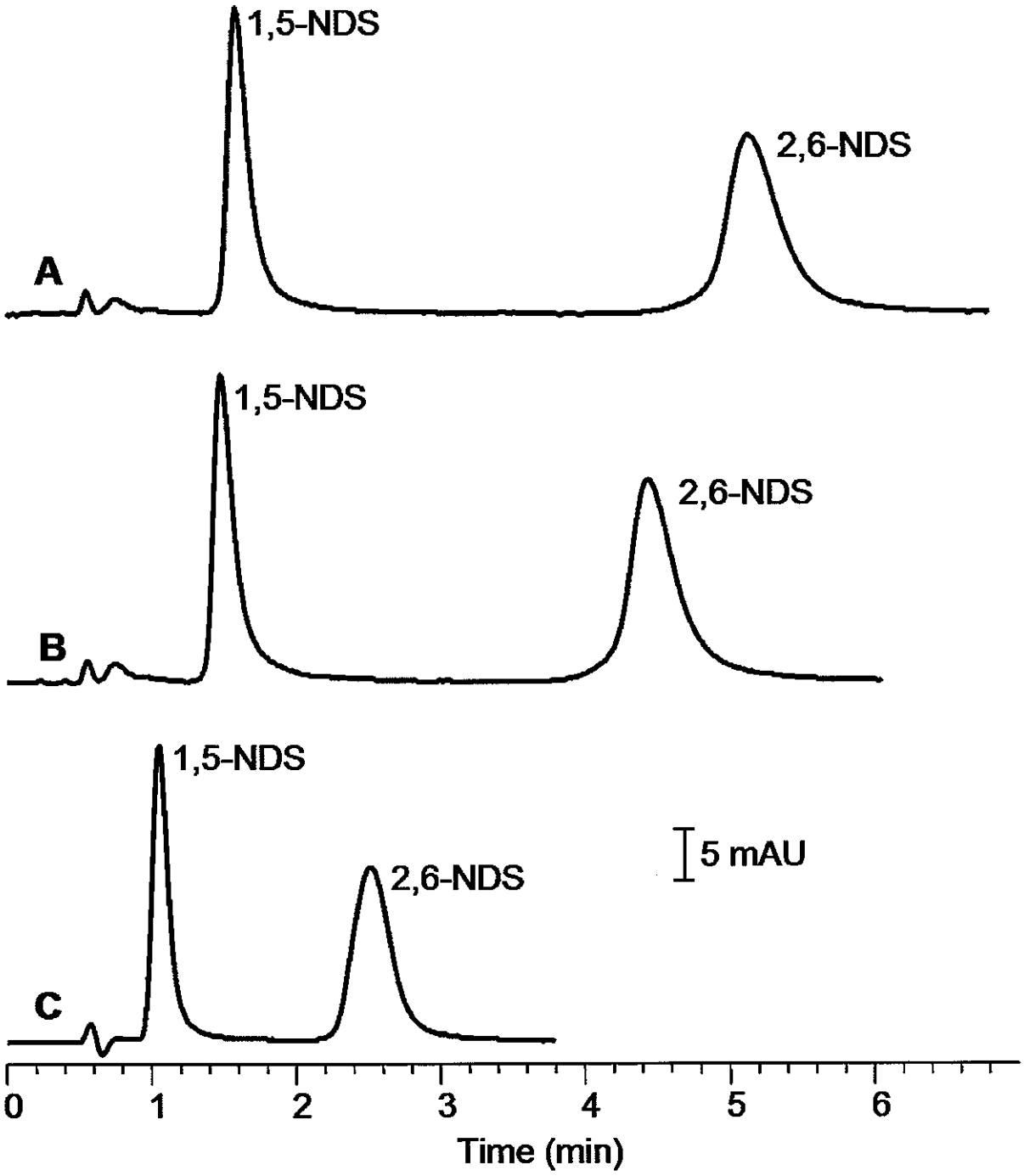

Figure 1. Chromatograms for a mixture of 1,5- and 2,6-NDS with column Conv-GC-4. The mobile phase was composed of 95 mM lithium perchlorate in 95:5 v:v water:acetonitrile. The flow rate was 0.5 mL/min. Chromatograms A and B were collected on the same day at 20.2 °C and 25.3 °C respectively. Chromatogram C was collected 9 days later at 22.3 °C after 3 van't Hoff analyses (T_{max} = 55.0 °C).

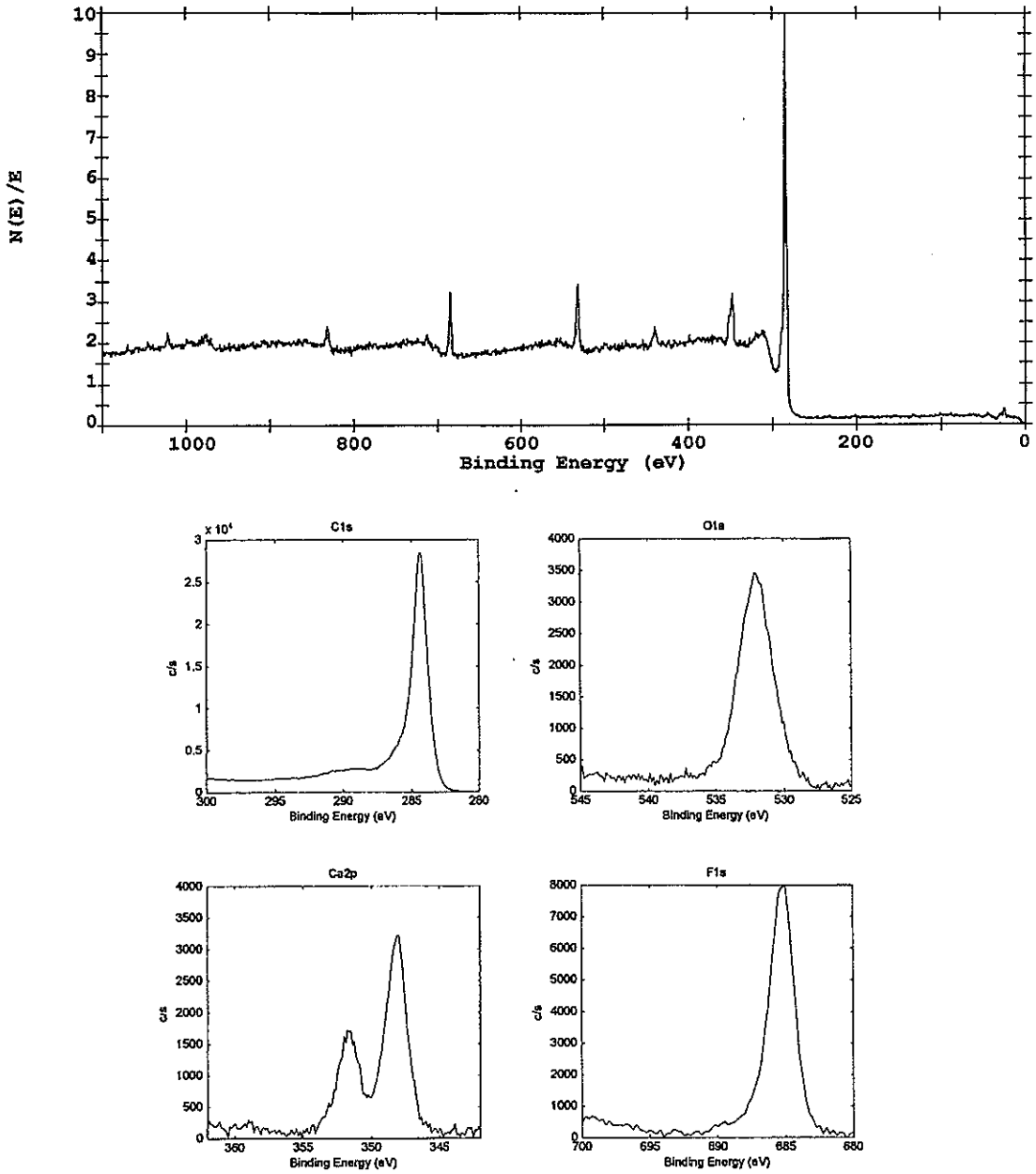

Figure 2. XPS data for sieved GC used to pack column used in Figure 1.

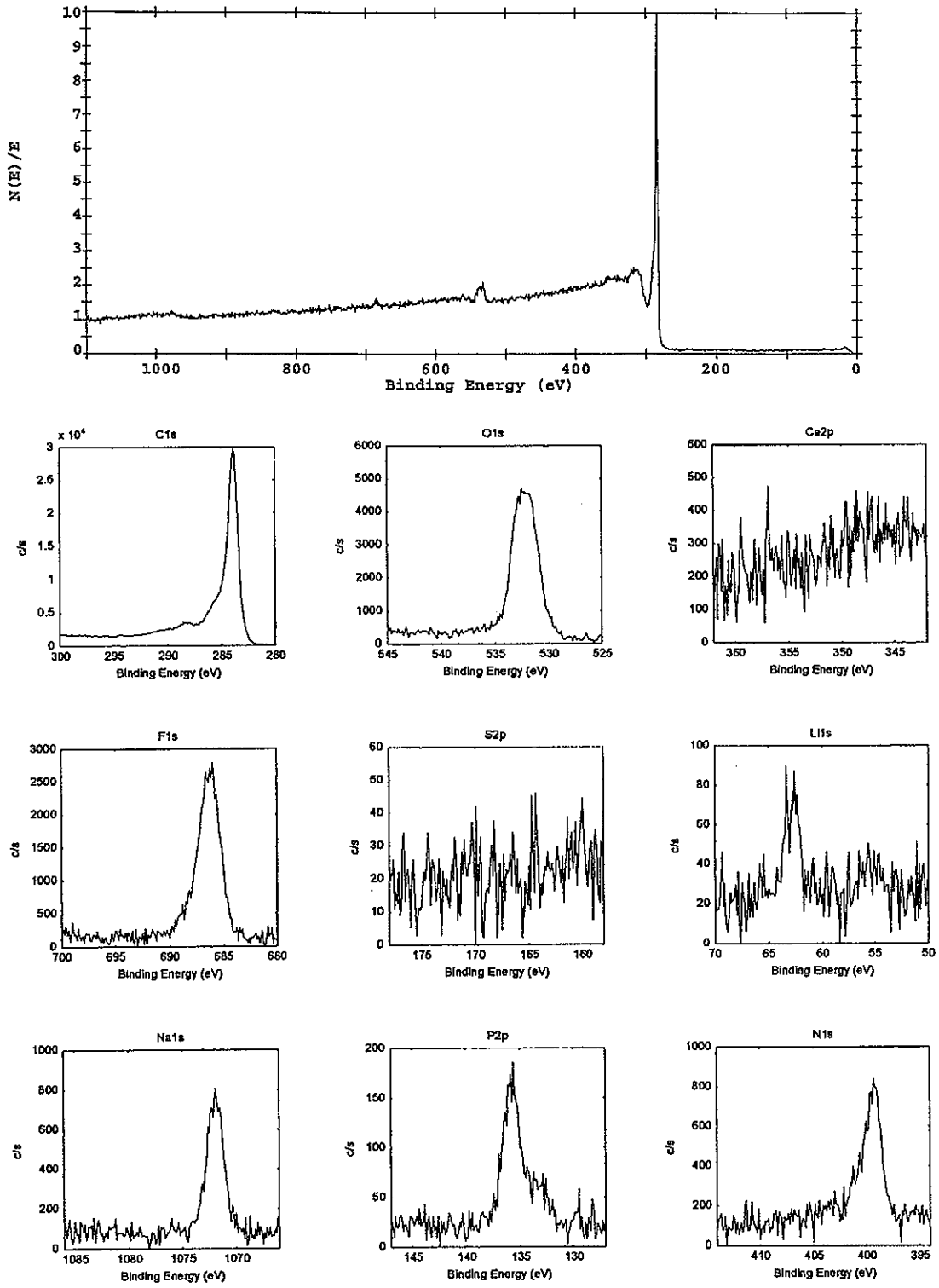

Figure 3. XPS data for GC after use in column from in Figure 1.

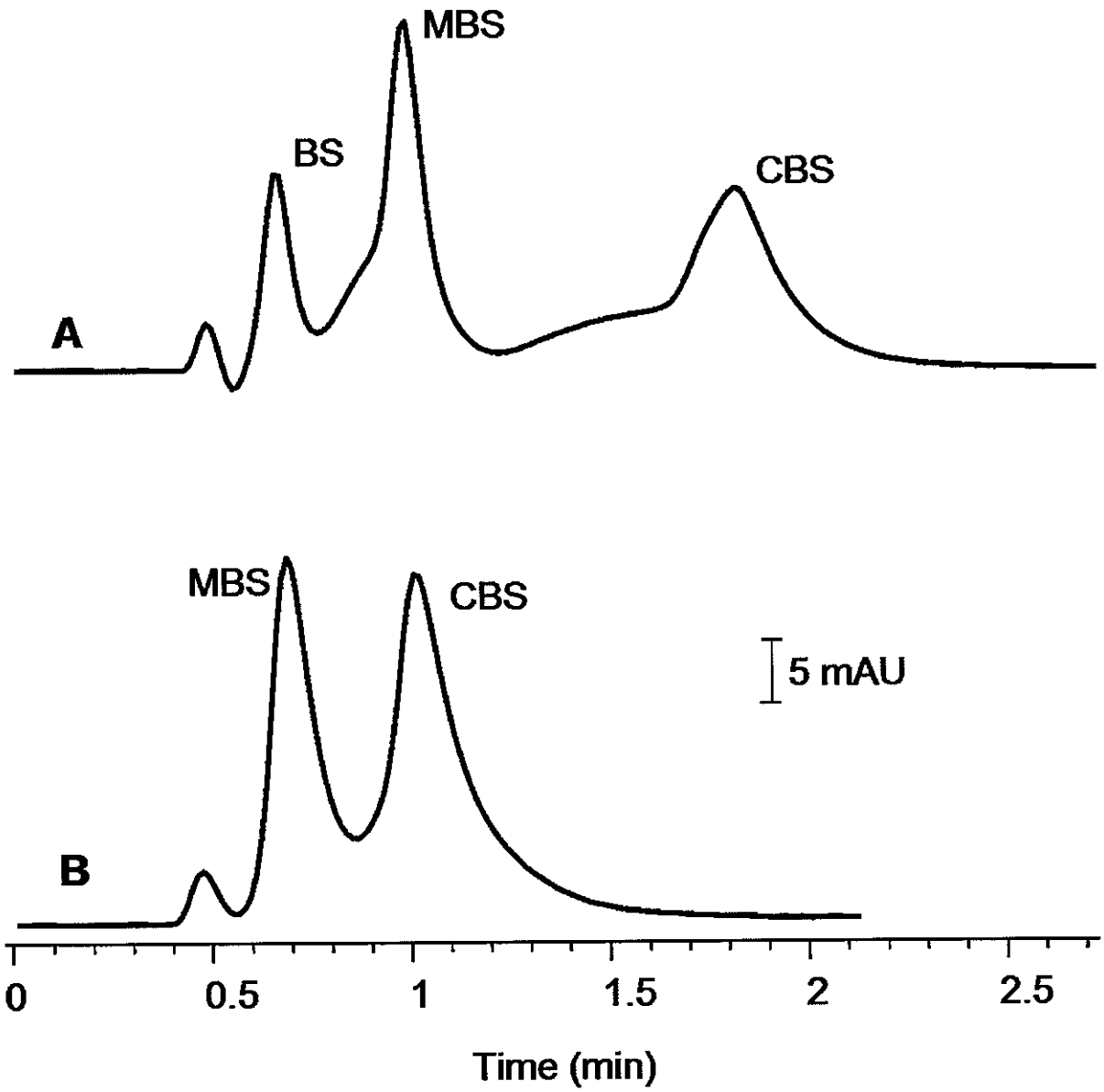

Figure 4. Chromatograms for a mixture of BS, MBS, and CBS with column EMLC-GC-8. The mobile phase was composed of 5 mM sodium hexafluorophosphate in 95:5 v:v water:acetonitrile. The flow rate was 0.5 mL/min. Both chromatograms were collected at a temperature of 25.0 °C and an applied potential of +200 mV vs. Ag/AgCl (sat'd NaCl). Chromatogram B was collected 11 days later after 2 van't Hoff analyses (T_{max} = 60.0 °C). The observed fronting is due to a small void in the column packing.

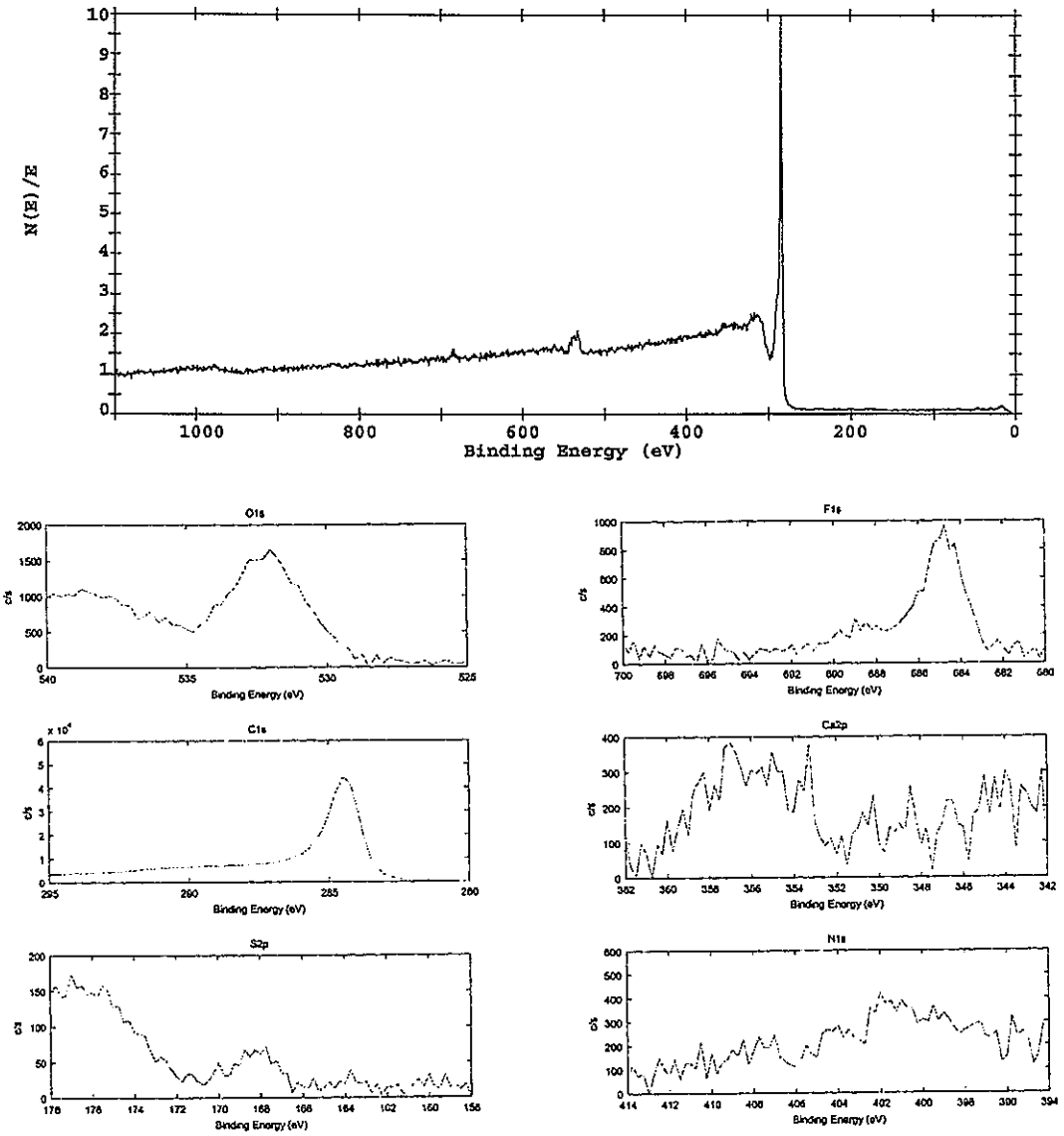

Figure 5. XPS data for cleaned GC used to pack column used in Figure 4.

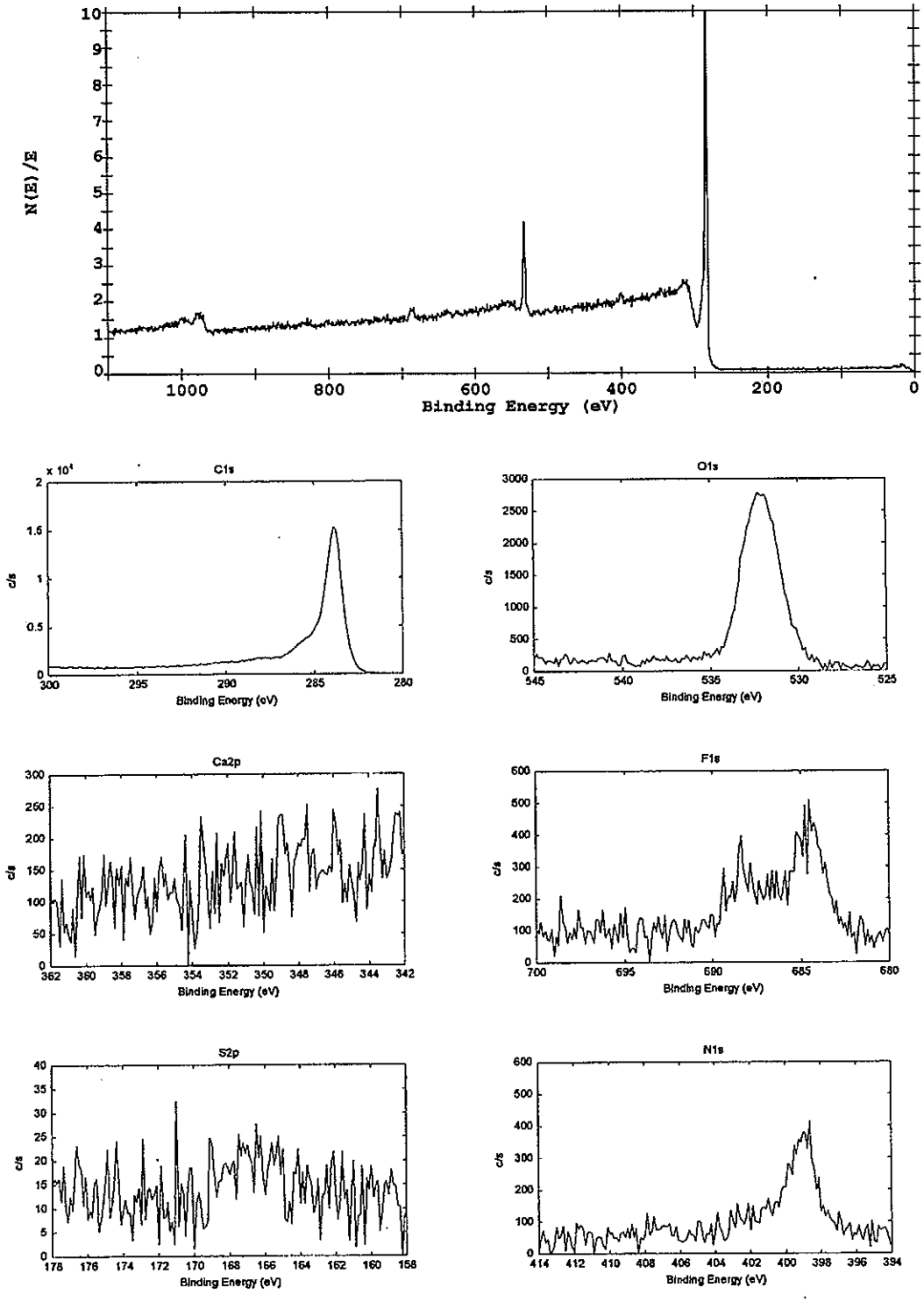

Figure 6. XPS data for GC after use in column from Figure 4.

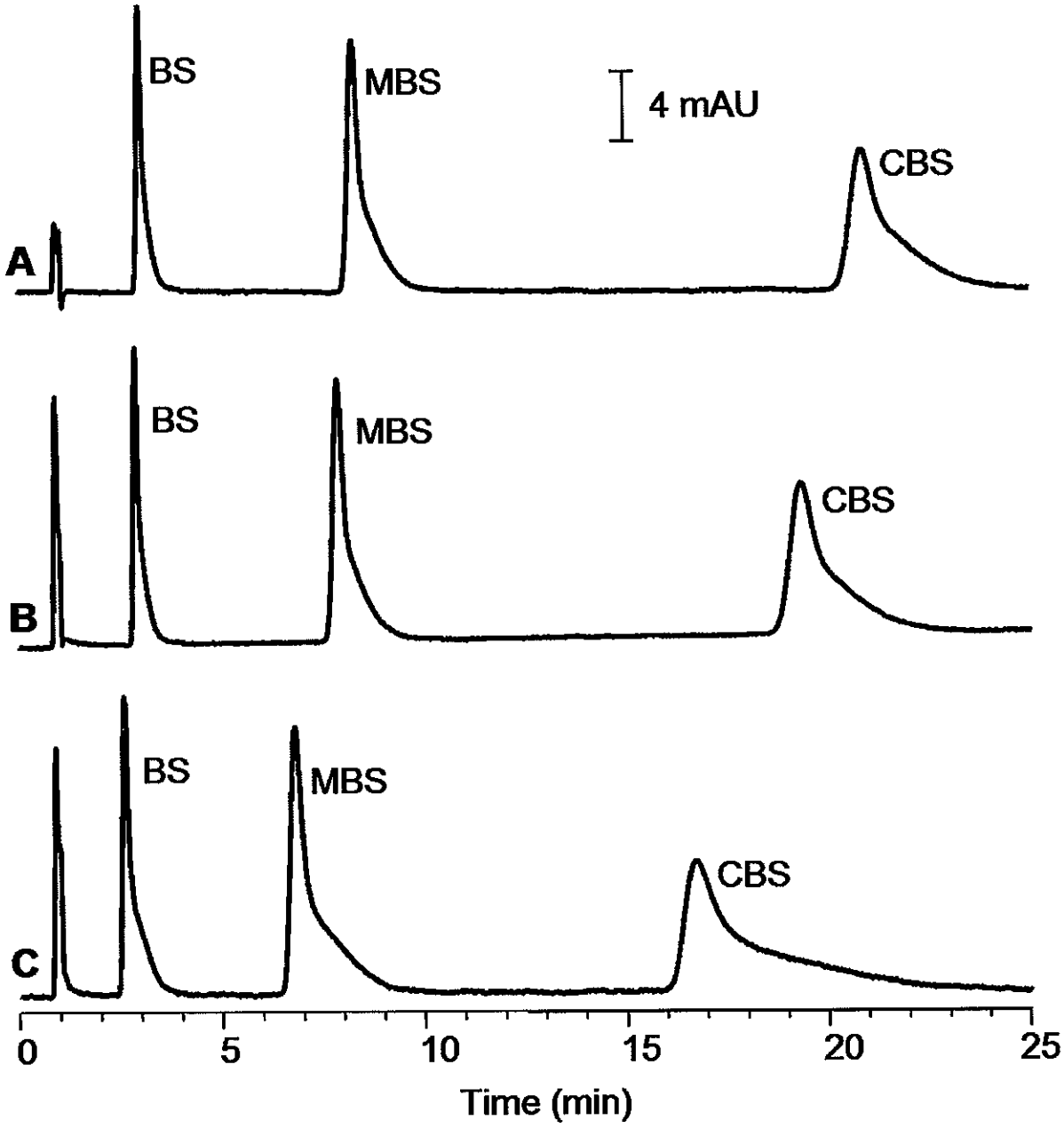

Figure 7. Chromatograms for a mixture of BS, MBS, and CBS with column EMLC-PGC-16. The mobile phase was composed of 100 mM lithium perchlorate in 95:5 v:v water:acetonitrile. The flow rate was 0.5 mL/min. All chromatograms were collected at room temperature (21 °C) and an applied potential of +100 mV vs. Ag/AgCl (sat'd NaCl). Chromatogram B was collected 5 days later than Chromatogram A after taking the column to 100 °C for high speed EMLC. Chromatogram C was collected another 3 days later after flushing the column at room temperature.

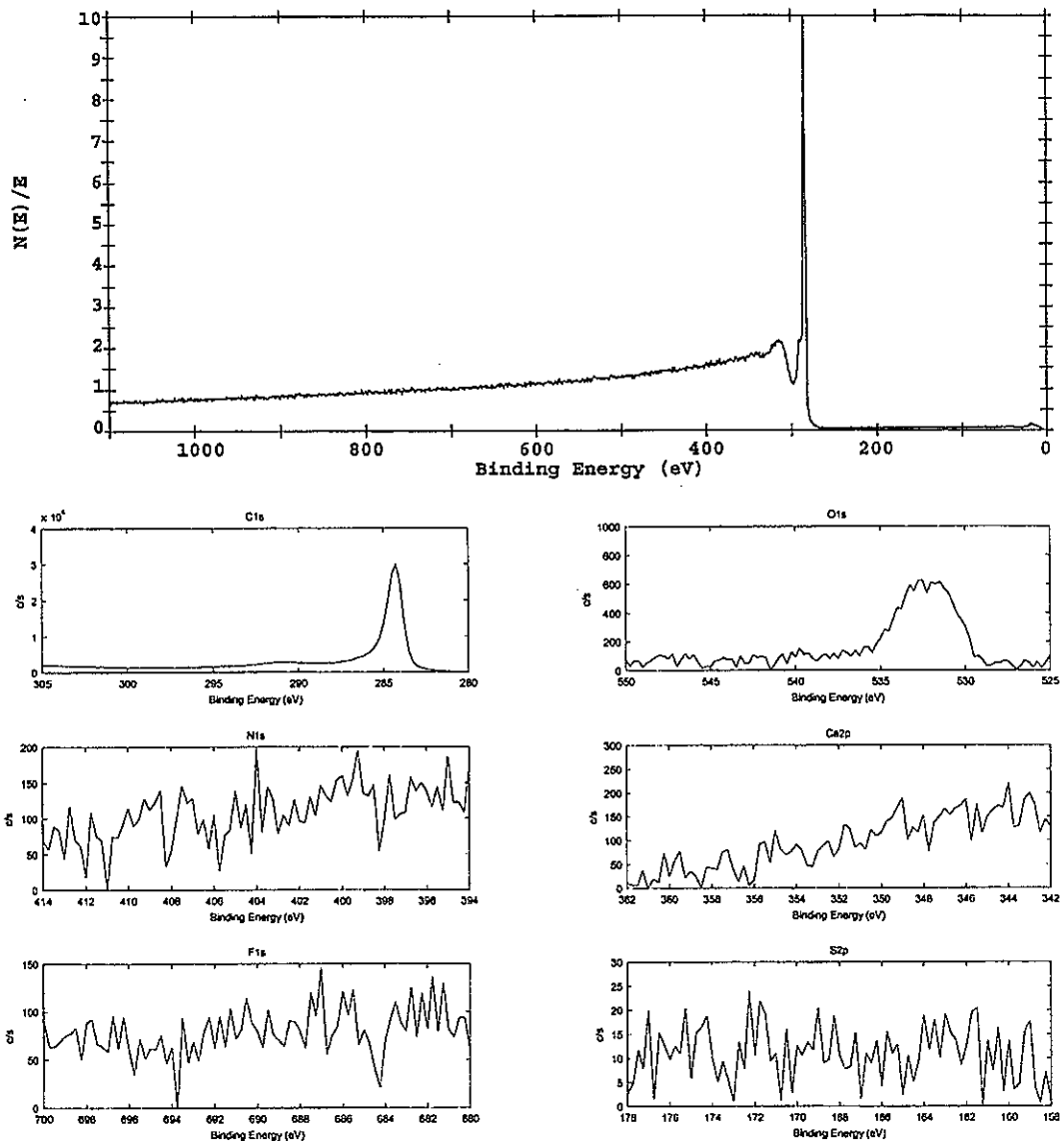

Figure 8. XPS data for as received PGC used to pack column used in Figure 7.

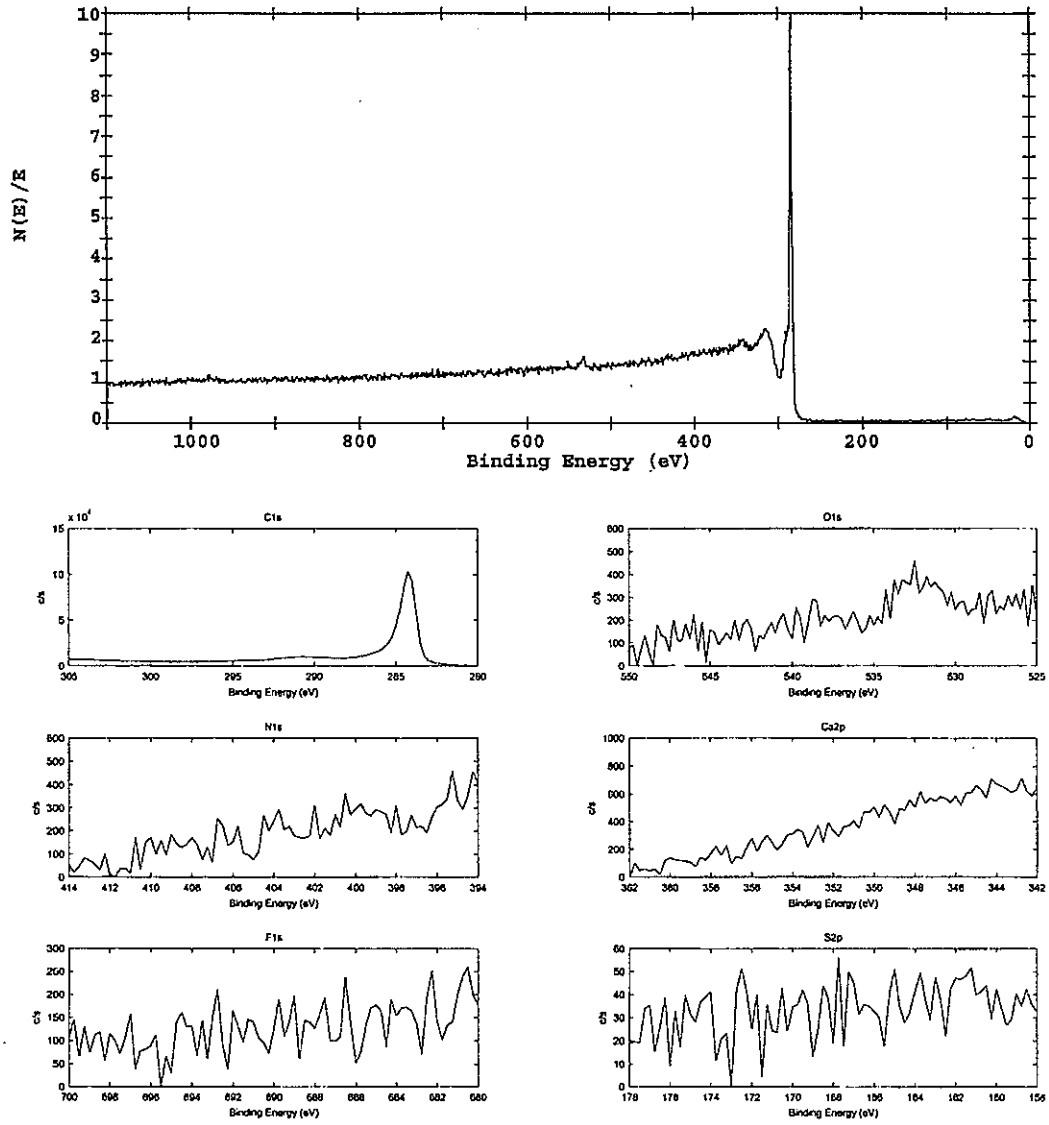

Figure 9. XPS data for PGC after use in column from Figure 7.

Table 2. Batch oxidation of PGC.

Solvent	% O
as received PGC	0
dried PGC	0.08
water	0.1
acetonitrile	0.6
95:5 water:acetonitrile	0.45
methanol	0.59
0.1 M $LiClO_4$ in water	1.2
0.1 M $LiClO_4$in actetonitrile	1.19
0.1 M $LiClO_4$in 95:5 water:acetonitrile	1.56
0.1 M $LiClO_4$in 50:50 water:acetonitrile	7.71
0.1 M $NaPF_6$ in water	1.05
0.1 M $NaPF_6$ in 75:25 water:acetonitrile	1.38
0.1 M NaF in water	2.1
0.1 M NaF in 75:25 water:acetonitrile	2.14
0.1 M $TBAClO_4$* in acetonitrile	1.04

*$TBAClO_4$ is tetrabutylammonium perchlorate

APPENDIX B. ARCHIVING TRACE ORGANIC CONTAMINANTS IN SPACECRAFT WATER

A paper published by SAE International

Lisa M. Ponton,[2,3] Daniel Gazda,[3] Robert J. Lipert,[3] James S. Fritz,[3] and Marc D. Porter[3,5]
Jeff Rutz,[4] Paul Mudgett,[4] Dawn Dungan,[4] and John Schultz[4]

Abstract

One of the long-standing concerns in space exploration is the presence of trace organic contaminants in recycled spacecraft water supplies. At present, water samples on the International Space Station (ISS) are collected at regular intervals, stored in Teflon™-lined containers, and returned to Earth for characterization. This approach, while effective in defining water quality, has several notable problems. First, this method of archiving removes a significant volume of the ISS water supply. Second, the archived water consumes valuable cargo space in returning Shuttle and Soyuz vehicles. Third, the organic contaminants present in the collected samples may degrade upon extended storage. The latter problem clearly compromises sample integrity. Upon return to Earth, sample degradation is minimized by refrigeration. Due to present resource constraints, however, refrigeration is not a viable option in space.

This paper describes the first findings from an investigation of solid phase extraction (SPE) as an effective approach to sample archiving. With SPE, the organic contaminants in

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[2] Primary researcher and author
[3] Microanalytical Instrumentation Center, Chemistry Department, Iowa State University, and Ames Laboratory-USDOE Ames, IA 50011
[4] Wyle Laboratories, Houston, TX 77058
[5] Author for correspondence

the spacecraft water can be trapped and concentrated on a thin membrane or other extraction medium, with the resulting effluent recycled back into the water supply as opposed to being stored and returned to Earth. This approach, therefore, has the potential to: (1) dramatically reduce the amount of water removed for sampling, (2) minimize the stowage needed to return the samples to Earth, and (3) mitigate sample degradation. Results from an evaluation of this concept using synthetic spacecraft water and a range of extraction materials are described, along with the findings from a KC-135 flight evaluation of the effectiveness of and experimental challenges in implementing this concept in microgravity. Plans for the next generation of experiments, based on these results, are also briefly discussed.

Introduction

To date, on-board equipment for monitoring spacecraft water quality has been developed for determining total organic carbon,[1] pH, conductivity, and bacteria levels. More in-depth characterizations rely on archival sampling. For the Shuttle-Mir Program, 100-800 mL samples of potable water and 25-700 mL samples of humidity condensate were periodically collected and returned to Earth on Shuttle and Soyuz vehicles.[2, 3] Over 40 potable water samples and 30 condensate samples were returned from Mir during this program.[4] These samples represented a significant amount of return vehicle payload capacity (a combined mass of 36.4 kg) and constituted a considerable portion of the Mir water supply (the equivalent of 10 person-days consumption).

The sampling program for the International Space Station (ISS) water system also exemplifies the necessity for an improved approach to archiving. Since the beginning of this

program in 2000 (Expeditions 1-5), NASA has collected 66 samples (25 potable water samples, 14 stored ground-supplied water samples, 4 filter reactor effluent samples, and 23 humidity condensate samples), each at a volume of ~800 mL.[5, 6] The importance for an alternative archival strategy is further magnified when considering the need to sample the contingency water supplied to the ISS by Shuttle missions. To date, 49 contingency water containers transferred from the Shuttle to the ISS had an average volume of 116 mL removed from each container for archiving purposes.[7] In total, this process has removed 58.5 L from the ISS. As of February 10, 2003, the inventoried volume of water on the ISS totaled 986 L, indicating that 5.9% of the water was employed for archival sampling.

In addition to removing a significant volume of water from the ISS, this traditional approach to sample archiving raises concerns about sample integrity due to possible degradation and contamination during storage, transportation, and handling. For the ISS, archived samples are returned to Earth every two to six months, as dictated by Shuttle or Soyuz launch schedules. If, however, an efficient means to return only the chemical contaminants, concentrated and stabilized from a defined volume of water, can be devised, then the complications presented by sample archiving would be greatly reduced.

Solid phase extraction (SPE) is an effective means to remove and concentrate contaminants from water for storage and analysis upon return to the analytical laboratory.[8-10] In its simplest form, SPE consists of loosely-packed extractant particles, similar to those employed in liquid chromatography, that are loaded into a small column or cartridge. Resin-loaded membrane disks that can be used in conventional filtration systems have also been developed and provide a more effective and compact means of extraction.[8] In each case, the

analytes are extracted by either pushing the sample through the extraction media using a syringe or pulling the sample through the media with vacuum. After extraction, the cartridges or disks can then be either archived or immediately processed for water quality assessment. Importantly, past studies have shown that samples archived by SPE are stable for extended periods of storage.[10-12]

The long-range goal of the work described herein is the development of a methodology using SPE for archiving organic contaminants in spacecraft water. Examples of the contaminants found in spacecraft water are listed in Table 1, along with their respective octanol-water partition constants (K_{ow}), which is a measure of hydrophobicity.[13-15] This list, while not exhaustive, is representative of the classes of compounds (hydrophobic, hydrophilic, neutral, charged, etc.) identified in earlier characterizations of archived spacecraft water,[16, 17] and will be used to prepare synthetic water samples for development of a SPE-based archival method. If effective, this approach would not only significantly decrease the amount of water removed from the ISS system, but also dramatically lower the burden on return vehicle payload capacity.

Three important conditions must be met in order for this strategy to be successful. First, the extraction must be effective in the removal of water system contaminants. Second, the archival contaminants must be stable for up to six months when impregnated in the extraction material until returned to Earth for analysis. Third, procedures for extraction must be easily and rapidly carried out by crewmembers. This final condition is necessary in order to minimize the time required to complete the archiving procedure.

Table 1. Test compounds for SPE Development[a] and their respective octanol-water partition constants (K_{ow})[b]

Acetone -0.24[c]	Diethyl phthalate 2.51[e]	Methylamine -0.57[d]
Acetophenone 1.63[c]	Dodecanoic acid 4.6[c]	1-Methyl-2-pyrrolidinone NA[g]
Benzyl alcohol 1.05[c]	Di(ethylene glycol) butyl ether 0.40[d]	Nonadecane NA[g]
n-Butanol 0.84[c]	n-Ethyl-p-toluene sulfonamide NA[g]	Squalene NA[g]
Ethylene glycol butyl ether NA[g]	Formaldehyde 0.35[c]	1-Tetradecanol NA[g]
Propylene glycol NA[g]	Hexanoic acid 1.92[c]	Methanol -0.74[c]
3-t-Butylphenol ~3[c,f]	Iodoform ~2[c,f]	Ethanol -0.30[c]
Caprolactam -0.19[d]	Indole 2.14[d]	Isopropanol 0.05[c]

[a]These compounds, which are only a small portion of the organic contaminants found in spacecraft water samples, are representative of the many chemical classes of the contaminants.[8]

[b]The degree of hydrophobicity is described by the octanol-water partition coefficient (K_{ow}) and is defined as the equilibrium ratio of the concentration of the analyte in octanol and the concentration of the analyte in water.

[c]From ref.13.

[d]From ref.14.

[e]From ref.15.

[f]Value approximated based on similar compound.

[g]NA=Value not available.

This paper reports on the first stage of an ongoing investigation into the use of Empore™ extraction membranes as a media for potentially meeting the above criteria. This stage of the investigation had three phases. First, SDB-XC Empore™ extraction membranes (described in Table 2) were used with little deviation from manufacturer preparation recommendations to establish a baseline methodology for assessing the effects of microgravity on SPE performance. Based on the literature,[10] this type of membrane (SDB-XC) proved effective in the uptake of several polar organic compounds, such as those found in past samples of spacecraft water.[16, 17] Second, this methodology was employed for extraction of a synthetic humidity condensate sample on a recent KC-135 microgravity flight to assess the potential implementation of SPE in a microgravity environment. Finally, to assess the broad-based potential of employing SPE as an effective archival methodology, the performance of four other compositionally different membrane materials was briefly investigated.

Experimental Methods

Reagents and Materials. Hexanoic acid, acetophenone, benzyl alcohol, indole, 3-*t*-butylphenol, caprolactam, *n*-ethyl-*p*-toluene sulfonamide, diethyl phthalate, di(ethylene glycol) butyl ether, 1-methyl-2-pyrrolidinone, and ethylene glycol butyl ether were obtained from Aldrich Chemical (Milwaukee, WI). Ethyl acetate, acetone, and isopropyl alcohol were purchased from Fisher Scientific (Pittsburgh, PA). The synthetic water samples were prepared using Milli-Q water (Millipore, Bedford, MA) and their make up and use are

described below. Teflon™ bags (1000 mL) were obtained from American Fluoroseal Corporation (Gaithersburg, MD) and were fitted with either reflux valves (B. Braun Medical, Bethlehem, PA) or Luer check valves (Qosina, Edgewood, NY) equipped with a tethered cap (Qosina). These bags are currently being used in ISS archival sampling. The 3M Empore™ extraction disks (Fisher Scientific), listed in Table 2, were cut to 13 mm diameters in order to fit into conventional filter cartridges (Fisher Scientific). Caps used to seal the filter cartridges were obtained from Qosina.

Gas Chromatography/Mass Spectrometry (GC/MS). Analysis of the resulting eluents after SPE archiving was performed on one of two Agilent Technologies Model 6890 Gas Chromatographs, each equipped with a Model 5973 Mass Selective Detector. One instrument, located at Iowa State University in Ames, Iowa, had a Hewlett Packard 5MS column (crosslinked 5% phenyl-methylpolysiloxane), 30 m x 0.25 mm x 0.25 μm film thickness. The Ames instrument was used in all three phases of this investigation. The second instrument located at the Johnson Space Center (JSC) had a J&W DB-5.625 column (equivalent to 5% phenyl-methylpolysiloxane), 50 m x 0.20 mm x 0.33 μm film thickness. The JSC instrument was employed in Phase 2 for the analysis of the KC-135 flight samples immediately upon completion of the flight experiments. In both cases, the splitless injection volume was 1 μL.

Water Samples. *Phase 1: Preliminary Ground-Based Experiments.* The synthetic water sample used for pre-flight ground experiments contained six organic compounds often observed in the humidity condensate from the ISS or other types of spacecraft. These compounds, all at a concentration of 10 ppm, were: hexanoic acid, acetophenone, benzyl

Table 2. Empore™ Extraction Membrane Characteristics.[18]

	Functional Group	**Retention Mechanism**	**Pore Size (Å)**	**Average Particle Size (mm)**	**Surface Area (m^2/g)**	**pH range**	**Advantages**
SDB-XC	Poly(styrene divinyl benzene)	Reversed Phase	80	15	350	1 - 14	Retains aliphatic and aromatic compounds
SDB-RPS	Poly(styrene divinyl benzene) sulfonate	Reversed Phase and Cation Exchange	80	15	350	1 - 14	Same as SDB-XC plus organic analytes with polar character
C18-HD	Silica with octadecyl (endcapped)	Strong Reversed Phase	60	12	NA	2 - 7.5	Approved for use with numerous EPA Methods
C8-HD	Silica with octyl (endcapped)	Moderate Reversed Phase	60	12	NA	2 - 7.5	Approved for use with numerous EPA Methods
MPC-HD	Silica with octyl and benzene sulfonic acid	Moderate Reversed Phase and Cation Exchange	60	NA	NA	2 - 7.5	Ion exchange 0.03 mEq/g

alcohol, indole, 3-*t*-butylphenol, and caprolactam. Although spacecraft water contains organic constituents at a much lower concentration, a higher concentration was employed in order to enable the ability to carry out both the Phase 1 and Phase 2 experiment within the time span of several parabolas of a KC-135 flight (20-40 s each). Future studies will be extended to include concentrations.

Phase 2: KC-135 Flight Experiments. The flight experiments were performed using a synthetic water sample containing the same six organic compounds used in Phase 1. The concentration of each analyte was 10 ppm with the exception of hexanoic acid, which was increased to 20 ppm due to the low detector sensitivity for hexanoic acid. This sample was stored in a 1-L Teflon™ bag during the KC-135 flight. Prior to flight, the sample was refrigerated.

Phase 3: Ground-Based Performance of Alternative Membrane Materials. Two synthetic water samples were prepared with ~20 ppm concentrations for each analyte and used in testing the extraction characteristics of the membranes listed in Table 2. The first sample consisted of indole, caprolactam, hexanoic acid, acetophenone, benzyl alcohol, 3-*t*-butylphenol, and *n*-ethyl-*p*-toluene sulfonamide. The second sample consisted of diethyl phthalate, di(ethylene glycol) butyl ether, 1-methyl-2-pyrrolidinone, and ethylene glycol butyl ether.

SPE Extraction Methods. *Phase 1: Preliminary Ground-Based Experiments.* The SDB-XC extraction membranes were cut to a 13 mm diameter and loaded into filter cartridges (shown in Figure 1), wetted with 1-mL acetone and 2-mL isopropyl alcohol, and used immediately. Triplicate extractions were performed with either 5.0 or 10.0 mL sample

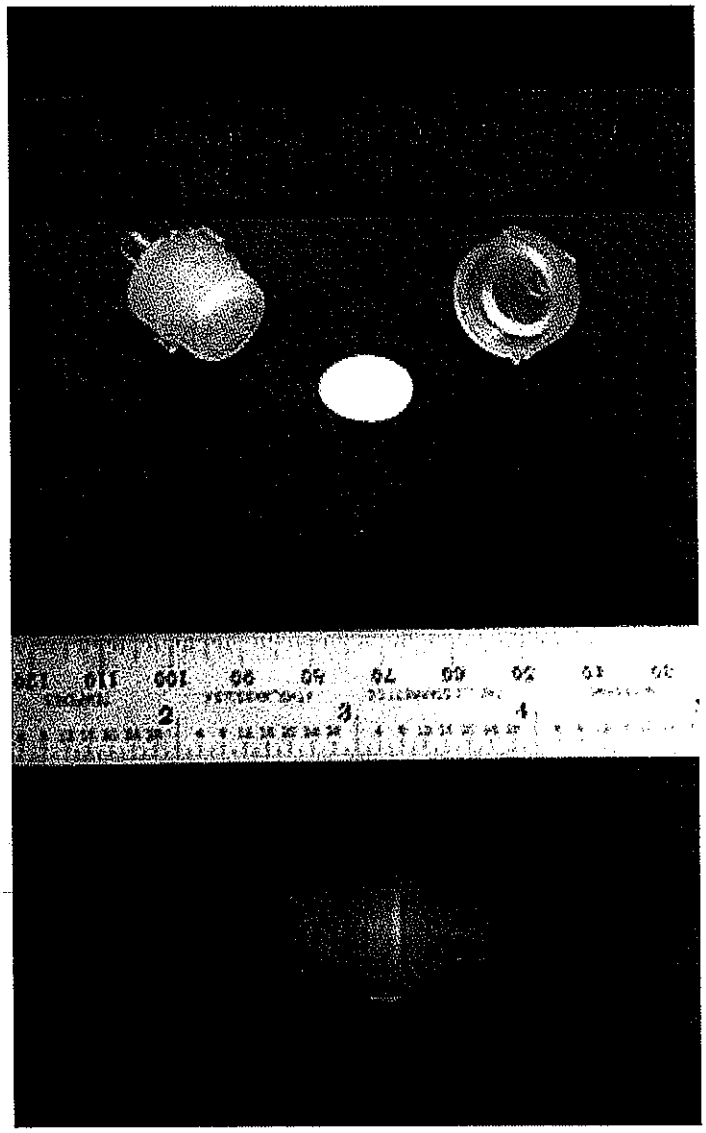

Figure 1. a. Empore™ extraction membrane and separated filter cartridge. b. Coupled filter cartridge containing Empore™ extraction membrane.

volumes. The extracts were then immediately eluted off the disks with 5.0 mL of ethyl acetate. The resulting eluent was analyzed by GC/MS.

Phase 2: KC-135 Flight Experiments. The same extraction procedure was performed during the KC-135 flight with the cartridges loaded with SDB-XC extraction membranes. The membranes were wetted with 1-mL acetone and 2-mL isopropyl alcohol, and then capped and stowed one day prior to flight. This deviation from wetting immediately prior to use, reflects NASA safety regulations against the use of organic solvents during KC-135 flights. The membranes were still visibly wetted when used in-flight. Ten replicate extractions were performed using both 5- and 10-mL sample volumes, with the effluent collected in a 1-L waste bag. These cartridges were then capped (Figure 2) and stowed for ground-based laboratory analysis. Half of the archived samples were eluted upon landing and the other half of the archived samples were sealed in plastic bags for one week and then eluted as part of the effort to characterize the stability of the analytes during storage on Earth. Elution employed 4.0 L of ethyl acetate, a level reflecting availability of vial sizes. The resulting eluents were analyzed by GC/MS. A companion set of experiments was performed in the laboratory as a control in order to assess any effects of microgravity on extraction efficiency.

Phase 3: Ground-Based Performance of Alternative Membrane Materials. The five Empore™ disks examined (SDB-XC, SDB-RPS, C8-HD, C18-HD, and MPC-HD) are described in Table 2, along with their respective characteristics as extraction media. Two sets of tests were conducted. The first set employed membranes wetted first with 1-mL of acetone and then with 2-mL of isopropyl alcohol, reflecting vendor protocols. The second set

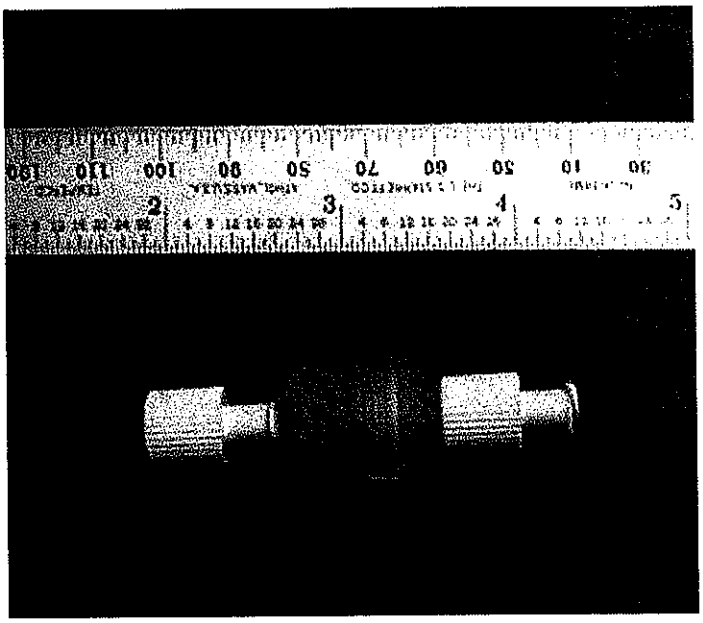

Figure 2. Capped filter cartridge containing Empore™ extraction membrane.

used "as received" membranes and was carried out to assess whether membrane wetting was a requisite to extraction performance. Small (5.0 mL) aliquots of each water sample were passed through the membranes. The extracted compounds were then eluted off the membrane with 5.0 mL of ethyl acetate and analyzed by GC/MS.

Results and Discussion

Phase 1: Preliminary Ground-Based Experiments. The Empore™ SDB-XC extraction membranes were used as a starting point in the development of a fast and efficient SPE method for sample archiving and in the establishment of a baseline from which to assess any effects of microgravity on SPE extraction performance. These membranes were selected because reversed phase medium has characterizations that should prove effective in extracting both aliphatic and aromatic compounds from water. Of the compounds listed in Table 1, the six chosen have a range of chemical characteristics (listed in order of decreasing hydrophobicity): 3-*t*-butylphenol, indole, hexanoic acid, acetophenone, benzyl alcohol, and caprolactam. Two sample volumes, 5.0 and 10.0 mL, of the synthetic humidity condensate water were employed, with extractions performed in triplicate. The percent recoveries of each compound in the eluent from these experiments are given in Table 3. For the 5-mL sample volume experiments, hexanoic acid, acetophenone, 3-*t*-butylphenol, and indole exhibited the best overall extraction efficiencies and low percent relative standard deviation (%RSD). For the 10-mL sample volume experiments, the extraction efficiencies of hexanoic acid and benzyl alcohol, however, were considerably lower. Caprolactam was not extracted in sufficient quantities to be detected by the Ames GC/MS system for either the 5- or 10-mL

Table 3. Pre-Flight Ground Results from SPE Cartridges.[a]

5 mL Sample Volume	Hex[b]	Acet[b]	Benz[b]	Ind[b]	But[b]	Cap[b]
% Recovery	88.5	102.2	55.1	93.3	80.9	ND[c]
Error	6.3	5.7	9.9	4.2	4.8	
% RSD	7.2	5.6	18.0	4.5	5.9	

10 mL Sample Volume						
% Recovery	58.4	96.8	27.8	86.9	77.6	ND[c]
Error	10.0	19.3	4.8	18.3	15.4	
% RSD	17.1	19.9	17.3	21.1	19.9	

[a]Data generated at Iowa State University.

[b]Hex=Hexanoic Acid, Acet=Acetophenone, Benz=Benzyl Alcohol, Ind=Indole, But=3-*t*-Butylphenol, Cap=Caprolactam.

[c]ND=Not Detected

experiments. Despite the less than ideal recoveries for this test mixture, the results were judged to be sufficiently reproducible that this combination of extraction media and synthetic space water could be reliably employed in a first evaluation of SPE as a microgravity archiving technique. The next section describes the finding from these tests, with preliminary efforts to improve extraction efficiencies by the use of different SPE media briefly detailed in the last section of this paper.

Phase 2: KC-135 Flight Experiments. Based on the high recoveries for acetophenone, indole, and 3-*t*-butylphenol in the preliminary ground-based experiments and the precisions for the extraction-elution of all five retained test analytes, the same sample-membrane combination was used in the KC-135 microgravity performance assessment. For comparison purposes, a companion set of extractions and analyses were performed using the same synthetic water sample immediately after flight. Tables 4 and 5 contain the results of the GC/MS analyses of the ethyl acetate extracts from the flight and ground based experiments. Each result is the average of five replicates. The standard deviation and percent relative standard deviation (%RSD) are also listed.

Table 4 shows the results for the KC-135 flight and ground-based extractions with analyte elution and GC/MS analysis performed upon landing (i.e., elution within a few hours after the extractions in KC-135 microgravity simulations). The results show that the recoveries for the flight and ground extractions are in strong agreement. For example, the extraction of 3-*t*-butylphenol differs by less than 3% regardless of sample volume or location (KC-135 flight extraction vs. ground-based extraction). Moreover, this analyte exhibited a %RSD as low as 1.6. The precision for the extraction-elution of caprolactam was also

Table 4. Results from SDB-XC Cartridges Eluted Upon Landing.[a]

Flight Results

5 mL Sample Volume	Hex[b]	Acet[b]	Benz[b]	Ind[b]	But[b]	Cap[b]
% Recovery	187.9	77.2	34.8	76.3	89.1	63.5
Error	7.8	3.0	4.5	3.5	3.3	0.9
% RSD	4.2	3.9	12.8	4.6	3.7	1.4

10 mL Sample Volume

% Recovery	152.1	76.0	23.4	74.0	87.0	31.3
Error	8.6	2.1	2.4	1.9	1.9	0.6
% RSD	5.6	2.7	10.4	2.6	2.1	1.9

Ground Results

5 mL Sample Volume

% Recovery	185.9	79.7	38.9	75.8	88.9	63.6
Error	8.7	2.2	3.9	1.9	1.4	1.2
% RSD	4.7	2.7	9.9	2.5	1.6	1.8

10 mL Sample Volume

% Recovery	143.0	77.3	23.9	70.4	86.3	31.7
Error	5.1	3.0	3.1	3.7	2.7	0.5
% RSD	3.5	3.9	12.8	5.3	3.1	1.7

[a]Data generated at Johnson Space Center. The analysis system at the Johnson Space Center was able to detect lower concentrations of Caprolactam than the analysis system at Iowa State University.

[b]Hex=Hexanoic Acid, Acet=Acetophenone, Benz=Benzyl Alcohol, Ind=Indole, But=3-*t*-Butylphenol, Cap=Caprolactam.

relatively high. However, the same amount of caprolactam was recovered for both the 5- and 10-mL sample volumes. This discrepancy, though to a lesser extent, is also evident for several of the other compounds, suggesting that possible overloading of the membranes needs to be examined. Nevertheless, the excellent agreement between the flight and ground-based extractions strongly support the potential use of this strategy as an archival method in the microgravity environment.

The second goal of the KC-135 flight experiment was to initiate a ground-based examination of analyte stability during archival storage. Table 5 shows the percent recoveries for the experiments where elution was delayed until after a one-week storage of both in-flight and ground based extractions. Again, because the KC-135 flight- and ground-based extractions have similar recoveries, the results indicate that the archiving procedure is not dependent on gravity. However, comparing the recoveries in Table 4 to those in Table 5, there is clear evidence for analyte loss over the one-week ground-based storage period. Inspection revealed that the membranes were noticeably drier after storage, suggesting that sample evaporation due to poorly sealed cartridges may be a problem. The larger %RSD values for the stored samples support this assertion. For example, 3-*t*-butylphenol has an overall 11.1 %RSD, which is considerably higher than the 2.9 %RSD observed in the samples eluted immediately post flight (Table 4). To correct this problem, the archival hardware will be refitted with a more airtight capping system that will also reduce the headspace in the cartridge holding the membrane. Further work will assess ground-based storage stability over extended times (i.e., more than six months), with the goal to carry out paralleled studies on ISS as its capacity develops with the addition of new modules.

Table 5. Results from SDB-XC Cartridges Eluted 1 Week After Landing.[a]

Flight Results

5 mL Sample Volume	Hex[b]	Acet[b]	Benz[b]	Ind[b]	But[b]	Cap[b]
% Recovery	27.0	44.3	19.3	51.2	64.0	ND[c]
Error	1.8	4.9	4.9	7.7	7.6	
% RSD	6.6	11.0	25.2	15.0	11.9	

10 mL Sample Volume

% Recovery	19.8	45.8	12.0	49.1	63.3	ND[c]
Error	5.2	7.1	3.6	6.3	8.2	
% RSD	26.4	15.4	30.0	12.9	12.9	

Ground Results

5 mL Sample Volume

% Recovery	28.2	44.7	19.3	53.3	62.4	ND[c]
Error	2.6	3.5	3.8	4.9	6.8	
% RSD	9.2	7.8	19.9	9.3	10.9	

10 mL Sample Volume

% Recovery	29.3	45.7	12.2	52.6	66.5	ND[c]
Error	6.2	4.7	1.9	5.6	7.6	
% RSD	21.1	10.2	15.1	10.6	11.4	

[a]Data generated at Iowa State University.

[b]Hex=Hexanoic Acid, Acet=Acetophenone, Benz=Benzyl Alcohol, Ind=Indole, But=3-*t*-Butylphenol, Cap=Caprolactam.

[c]ND=Not Detected

As to the inability to obtain 100% recoveries for the six organic analytes, we note that methods adopted by regulating agencies like the EPA have recoveries similar to those reported in Table 3. Using EPA Method 62,[19] a multilaboratory study yielded recoveries ranging from 36% for phenol to 184% for 3,3'-dichlorobenzidine. Our findings are, therefore, reasonably in-line with those for recently mandated protocols. Nevertheless, a more detailed investigation of the types of extraction media available (Table 2) is warranted to ensure that all possible forms of contaminants can be reliably extracted. As Table 1 shows, the contaminants span a wide range of chemical properties (i.e., hydrophobic, hydrophilic, neutral, charged, etc.). It is therefore of interest to examine the effectiveness of membranes and other materials with differing extraction properties. A first step in this direction is a screening of the available Empore™ extraction membranes, and our preliminary findings to this end are described in the next section.

Phase 3: Ground-Based Performance of Alternative Membrane Materials. Since there is a wide range of compositionally different SPE materials readily available, it may be possible to develop an archival system that can effectively extract a large majority of space water contaminants. Table 6 shows the results from a preliminary screening of several Empore™ extraction membranes. The membrane characteristics are described in Table 2. Because minimized membrane preparation is desired, these tests were conducted employing both "as received" membranes and those prepared following the above wetting procedure. Relative peak heights were used to compare the extraction efficiency with respect to membrane types and pretreatments by normalizing the absolute peak height for each compound to that of the least efficient membrane (i.e., the membrane with the lowest

Table 6. Relative Peak Heights for various Empore™ Membranes.[a]

	"As Received" Membranes Relative Peak Height[b]					Wetted Membranes Relative Peak Height[b]				
Compound	SDB-XC	SDB-RPS	C18-HD	C8-HD	MPC-HD	SDB-XC	SDB-RPS	C18-HD	C8-HD	MPC-HD
Indole	3.2	7.6	1.4	1.1	1.9	6.0	6.2	2.1	2.3	1.0
Caprolactam	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]
Hexanoic acid	-[c]	3.0	-[c]	-[c]	2.1	5.3	-[c]	1.0	1.0	-[c]
Acetophenone	5.8	9.8	4.3	3.7	5.7	6.4	6.0	1.7	1.7	1.0
Benzyl alcohol	-[c]	-[c]	-[c]	-[c]	-[c]	1.0	-[c]	-[c]	-[c]	-[c]
3-*t*-Butylphenol	1.5	2.6	2.1	1.9	2.3	1.7	1.9	1.8	1.0	1.0
n-Ethyl-*p*-toluene sulfonamide	1.0	23.4	4.2	2.4	18.9	22.8	24.0	20.0	13.3	11.6
Diethyl phthalate	2.7	3.8	3.6	3.3	3.0	3.3	3.3	3.1	1.0	2.5
Di(ethylene glycol) butyl ether	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]
1-Methyl-2-pyrrolidinone	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]
Ethylene glycol butyl ether	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]

[a]Work completed at Iowa State University.

[b]The peak heights for each compound are normalized to the lowest peak height obtained.

[c]Compound not detected.

peak height for the compound). For example, indole yielded its lowest effective recovery on the wetted MPC-HD membrane; therefore, all the peak heights for indole were normalized to this value. Comparison of the relative peak heights for indole reveals that the "as received" SDB-RPS membrane gave the best performance.

Interestingly, the results for the "as received" membranes compared favorably to those for the wetted membranes, and for SDB-RPS and MPC-HD were better. The "as received" SDB-RPS membrane proved the most efficient of those examined. The other four types of membranes had mixed performance after wetting, and functioned less effectively than the "as received" SDB-RPS membrane. This finding is particularly encouraging because the use of organic solvents is prohibited on the ISS. Any membrane preparation will need to be completed prior to launch, and storage time may be up to six months before use. Ongoing experiments are examining the reproducibility of employing the SDB-RPS membrane without wetting as a component in an archiving method.

Table 6 also shows that several of the compounds examined were not extracted by any one membrane. Moreover, there are other contaminants in spacecraft water (e.g., methanol and ethanol), which would not be concentrated based on the chemical properties of these extraction materials. Therefore, the screening of extraction media will be expanded to include other types of SPE materials. Since the media examined to date were largely reversed phase (non-polar) in nature, the search will include normal phase media (polar, e.g., Florisil, magnesium silicate), zeolite adsorbants (e.g. Silicalite), as well as other ion-exchange materials.

Conclusions

The results from these preliminary experiments demonstrate the potential for the development of a SPE-based sample archiving method for spacecraft water by using a small array of SPE materials. The flight and ground-based extractions for KC-135 experiments yielded similar recoveries, indicating that the performance of SPE as an archival method is not gravity dependent. Furthermore, comparison of results for samples eluted upon KC-135 landing to those done one week later indicate clear evidence for analyte loss, likely due to sample evaporation during the one-week storage period. Subsequent ground-based experiments with alternate membranes showed promising results for an off-the-shelf membrane used "as-received" versus wetted. Near-term objectives for the development of this method include: identifying extraction media that effectively trap and stabilize more of the compounds listed in Table 1, improving hardware to limit sample evaporation without the use of refrigeration, and extending storage stability studies to times comparable to that expected for storage on the ISS. Studies are therefore ongoing to examine different extraction materials and effective storage conditions. Successful implementation of such a SPE sample archiving method would reduce the amount of water removed from the ISS water system and lower the payload and storage space needed on return vehicles.

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