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Electrochemically modulated liquid chromatography: an electrochemical strategy for manipulating chromatographic retention

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I. Introduction

High performance liquid chromatography (HPLC) is one of the most widely used techniques in the analytical sciences.[1] By relying on the subtle interplay of analyte interactions with mobile and stationary phases, HPLC is capable of separating a variety of complex mixtures, including neutrals,[2] charged species,[3] enantiomers,[4,5] and biological materials.[6,7] However, to separate a wide range of sample types, different stationary phases are often required. For example, a reversed phase silica support is used to resolve a mixture of aromatic hydrocarbons, but an ion-exchange material is generally more effective in separating a mixture of inorganic anions. Along with changes in stationary phases, many types of separations require optimization of the mobile phase composition, with gradient elution strategies exploited to both enhance resolution and decrease elution time.

One of the latest additions to the HPLC toolbox is its hybridization with electrochemistry in a technique termed electrochemically modulated liquid chromatography (EMLC).[8] EMLC works by altering retention through changes in the potential applied (E_{app}) to a conductive stationary phase [*e.g.*, porous graphitic carbon (PGC)]. An EMLC column is constructed by fashioning a LC column into an electrochemical cell. The packing is therefore dual-purposed, serving as a chromatographic stationary phase and as a working electrode. This strategy provides a means to manipulate the interfacial properties (*e.g.*, surface charge, double layer structure, and oxidation state) of the stationary phase through changes in E_{app} which, in turn, alters analyte retention. The stationary phase can therefore be viewed as a compositionally tunable material with

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retention characteristics that can be adjusted to enhance the efficiency of a separation both prior to as well as during the elution process. The latter capability provides an approach that can be exploited to direct improvements in separation efficiency by dynamically altering the effective composition of the stationary phase. This mode of operation has been termed a compositional stationary phase gradient. As such, EMLC represents a novel strategy for manipulating the separation of a wide range of complex samples, including aromatic sulfonates,[9] corticosteroids,[10] and barbiturates.[11]

This review examines the development and variety of EMLC[8] as an addition to the many intriguing variants of HPLC. As a starting point, the construction of an EMLC column is detailed by identifying the design challenges inherent in converting a HPLC column into an electrochemical cell, and then describing the construction of the column in use today. The next section examines fundamental issues related to the retention mechanism, discussing the roles of the stationary phase and mobile phase, and how E_{app} affects the partitioning of analytes between the two phases. The remainder of the review presents examples of the application of EMLC to the separation of anionic species and various pharmaceutical agents using both uncoated carbonaceous stationary phases as well as those coated with chiral selectors. This section closes with a discussion of a coupling EMLC and electrospray mass spectrometry (ES-MS) and of the use of an EMLC column in preparing a new family of chemically stable reversed phase materials. The review closes with a brief examination of issues related to improvements in column performance, emerging challenges in the development of a mechanistic description of the retention process, and other areas for the application of EMLC as a separation technique.

II. Apparatus and accessories

II.1. Column construction

Like any hybrid method, the merger of two techniques like HPLC and electrochemistry poses a significant challenge. The challenge rests with constructing a column that addresses how the performance of the two techniques is affected by the ratio of the column dead volume to the surface area of the stationary phase. Chromatographically, this ratio must be small in order to have the requisite column capacity for high resolution separations. Electrochemically, this ratio must be large in order to minimize complications from high levels of solution resistance. It is the difficulty in overcoming these two obstacles that plagued the emergence of EMLC as a viable separation technique.

Historically, the origins of EMLC can be traced back to the 1950s and 1960s when concepts for the desalination of water and for the removal of heavy metal ions from contaminated water supplies explored a union of liquid chromatographic and electrochemical techniques. Unfortunately, these and many subsequent investigations (see 1991 review by Wallace and coworkers[12]) suffered from poor chromatographic efficiency (10–200 plates m^{-1}). This limitation reflected the complexity in constructing columns that successfully merged the aforementioned design criteria required for high chromatographic efficiencies and effective potential control of the working electrode/stationary phase. The situation is complicated by the need to incorporate placement of a reference electrode, which is required for precise control of the potential applied to the stationary phase, into the design. In addition, a high surface area auxiliary electrode is needed to collect the current that flows from the high surface area working electrode. Finally, the components of the column must be electrochemically as well as chemically inert and an inert supporting electrolyte may need to be added to the mobile phase in order to achieve the requisite solution conductivity. This modification is necessary to insure that the solution resistance of the mobile phase does not impair the ability to control the potential of the stationary phase.

Effective control of the potential applied to the stationary phase is further challenged by the need for phases that are not only conductive but also embody the characteristic size (2–10 µm), shape (spherical), and surface area (150–200 $\mathrm{m}^2\ \mathrm{g}^{-1}$) required for high separation efficiencies using packed columns.[2] As a consequence, the small voids between the particles and within the pores of the particles that are present in most high capacity stationary phases translate to high solution resistance paths for a packed column under potential control. These high resistance paths increase the time required to charge the electrical double layer at the stationary phase/mobile phase interface, and therefore the time needed for the column to equilibrate between changes in E_{app}. This process is analogous to equilibrating a conventional LC column after a change in the composition of the mobile phase. Taken together, an EMLC column must be designed to merge the demands of two methodologies that are in direct opposition in terms of their design specifications for effective performance.

Fig. 1 is a schematic diagram illustrating the basic design of an EMLC column that incorporates the key components required by both techniques.[9] The column is constructed by first inserting a tubular Nafion® ion-exchange membrane into a porous stainless steel cylinder. The stainless steel cylinder functions as: (1) a high surface area auxiliary electrode that can carry the large current flow generated at the high surface area working electrode; (2) an inflexible container that can withstand the high pressures generated during column packing and chromatographic flow; and (3) a container that defines the length and diameter of the column. The ion-exchange membrane also performs three important functions. First, it electrically isolates the stationary phase from the auxiliary electrode. Second, it acts like a salt bridge and connects the circuit between the stationary phase and two other electrodes. Third, it acts as a barrier to minimize loss of analyte through the porous stainless steel cylinder and to reduce the possible contamination of the stationary phase by any electrolysis products generated at the auxiliary electrode.

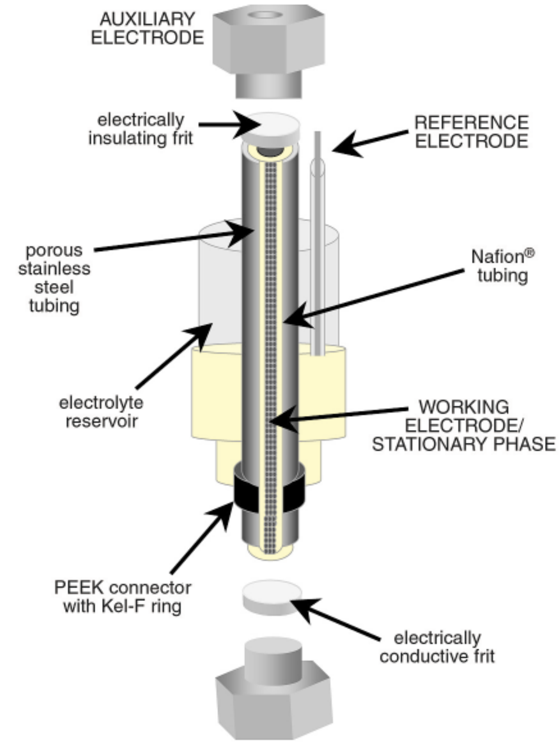


Fig. 1 Schematic diagram of an EMLC column.

The Nafion®-lined column is then packed with high surface area carbonaceous particles (*e.g.*, PGC), which function as both the stationary phase and as the working electrode. Electrical contact with the stationary phase/working electrode combination is made at the bottom of the column through a stainless steel frit. After packing with the stationary phase, the column is fitted with an electrolyte-filled reservoir that surrounds the stainless steel cylinder. The electrical circuit is then completed by placing a reference electrode (*e.g.*, Ag/AgCl (saturated NaCl)) inside the reservoir.

With this design, the column equilibrates to changes in E_{app} on a time scale comparable to that needed to condition a conventional column when changing the composition of the mobile phase (~20 min). Moreover, chromatographic efficiencies approach those attainable with many conventional columns (*e.g.*, 15000 plates m^{-1}).[9]

In general, the reproducibility of a separation using these columns rivals that for conventional HPLC, provided that the column is periodically flushed with acetonitrile or another strongly eluting solvent.[9] With careful maintenance, columns have functioned effectively for as long as 6 months.

II.2. Stationary phases

There are several types of conductive supports that can be used for EMLC, including metal particles, metal-coated glass particles, stainless steel microbeads, uncoated graphitic particles, and graphitic particles coated with a conductive polymer like polyvinylferrocene or polypyrrole.[13–17] Of the many options, carbon-based particles (*e.g.*, PGC)[18] are the most widely used stationary phases in EMLC. These phases are electrochemically stable over a wide potential window,[19] although care must be taken to avoid oxidizing the material at anodic potentials greater than ~+0.5 V (*vs.* Ag/AgCl (saturated NaCl)). These materials are also resistant to hydrolysis in alkaline and acidic mobile phases,[18] which has been a longstanding problem with many bonded phases.[20] In addition, these phases possess the mechanical strength necessary to retain their shape under high packing pressures (~8000 psi). Materials like PGC also possess both the surface area (~150 $m^2 g^{-1}$) necessary to perform effectively as a chromatographic stationary phase and the inherent electrical conductivity ($\geqslant 0.01\ \Omega$ cm) to function as a working electrode. Just as importantly, this type of material is commercially available as mono-dispersed, micron-sized particles (*e.g.*, Hypercarb™).

II.3. Instrumentation

Other than the aforementioned column design specifications, EMLC uses conventional HPLC instrumentation (*e.g.* pump, injector, and detector) along with a standard potentiostat to control the applied potential. In other words, an EMLC column can simply be connected to any of the HPLC systems available today *via* the same types of fittings used for a standard HPLC column.

III. Fundamentals of operation and retention mechanisms

LC separations are achieved by the partitioning of an analyte between mobile and stationary phases. The partitioning can be mathematically described by a distribution coefficient (K_d), which is the ratio of concentrations of an analyte sorbed on the stationary phase (C_s) and dissolved in the mobile phase (C_m), and is given by eqn. (1).

$$K_d = C_s/C_m \tag{1}$$

EMLC separations are also fundamentally linked to K_d. However, EMLC adds another dimension to manipulating the separation by using E_{app} to manipulate K_d, thus altering the retention time of analytes.

Two principal formats have been used. In its earliest format,[12,21] EMLC was applied to the separation of metal ions (M^{n+}) by their reductive deposition onto a conductive stationary phase as zero-valent metals (M^0). The generic form of this reaction is

$$M^{n+} + ne^- \rightarrow M^0 \tag{2}$$

where n represents the electron (e^-) stoichiometry of the reaction. The concentrations of M^{n+} and M^0 can then be defined by the difference in the standard reduction potential for the reaction (E^0) and E_{app} through the Nernst equation:

$$E_{app} = E^0 - (0.059/n)(\log[M^0]/[M^{n+}]) \tag{3}$$

Combining eqn. (1) and (3), after equating M^0 and M^{n+} to C_s and C_m, respectively, yields

$$\log K_d = (n/0.059)(E^0 - E_{app}) \tag{4}$$

Eqn. (4) shows that K_d changes by a factor of ten for every 59 mV alteration in E_{app} for processes where $n = 1$, and by a factor of five for those where $n = 2$. However, while providing a means for the quantitative deposition of metal ions, the process suffers from poor selectivity. That is, metal ions that have standard reduction potentials more positive than E_{app} are exhaustively deposited onto the column, whereas those that have standard reduction potentials more negative than E_{app} pass through the column without being retained. Strategies that take advantage of this general concept have recently been reviewed,[8,12] including those which exploit the uptake of metal ions and other charged species by ion exchange mechanisms that can be manipulated by the electrochemical transformation of stationary phases coated with redox polymers.[13–17]

The second of the most used retention formats in EMLC employs a much different tactic by taking advantage of the effect of E_{app} on the acceptor–donor properties and double layer structure of the surface of uncoated carbon-based stationary phases. The basis of this format is depicted in Fig. 2. At the

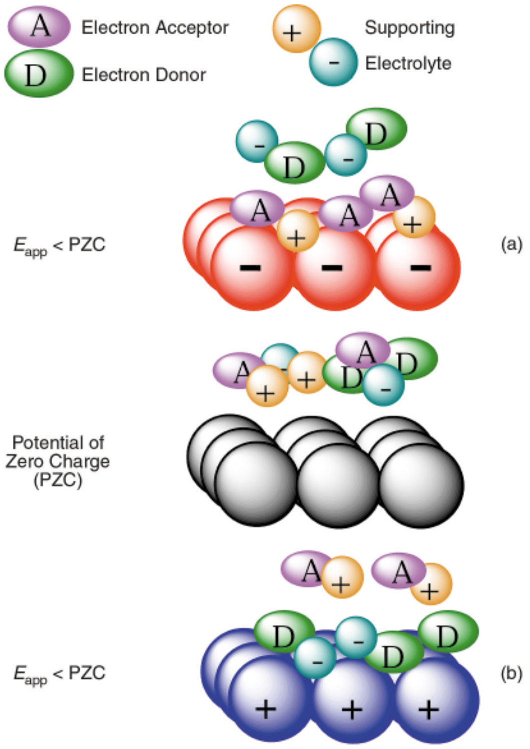


Fig. 2 Schematic representation of the effect of applied potential on analyte retention using a conductive stationary phase.

potential of zero charge (PZC), the interactions between an analyte and stationary phase qualitatively reflect those inherent in carbon particles.[22] In other words, the charge on the surface of the stationary phase at the PZC is zero, and solution located at the stationary phase/mobile phase interface is structurally indistinguishable from that in bulk mobile phase. Movement of E_{app} negative of the PZC results in a stationary phase with a net negative surface charge, and the formation of a compact double layer. Under these conditions, the electrostatic interactions of acceptor analytes are enhanced, leading to an increase in their retention time.[23] However, the concomitant enhancement of interactions with the stationary phase by the cations that are part of the supporting electrolyte may compete with the interactions of acceptor analytes, diminishing the overall effect of E_{app} on retention.

The opposite situation applies to instances in which E_{app} is moved to a value that is positive of the PZC. Above the PZC, the interactions of both donor analytes and supporting electrolyte anions with the stationary phase increase as a consequence of the excess positive charge that develops on the stationary phase surface. Again, the competitive sorption of anions from the supporting electrolyte will modulate the overall effect of E_{app} on retention.

These scenarios qualitatively describe the concept underlying the ability of EMLC to fine-tune analyte retention. As E_{app} becomes more positive, the conductive packing becomes a stronger acceptor. As such, analytes with a predominant donor character will be more strongly retained, whereas those with a stronger acceptor character will be more weakly retained. The converse applies to the impact of E_{app} on tuning the retention of acceptor analytes. The following concludes this section by describing a first-order treatment of the retention mechanism from purely coulombic arguments.

As a starting point in developing a generalized formulization for this type of retention process, the dependence of the capacity factor (k') on E_{app} can be described in terms of the influence of an electric field on a Boltzmann distribution of ions.[24] At a given E_{app}, the concentrations of cation A^{z+} and anion B^{z-} can be represented as follows:

$$[A^{z+}]_0 = [A^{z+}]_\infty \exp(-zeE_{app}/kT) \qquad (5)$$

$$[B^{z-}]_0 = [B^{z-}]_\infty \exp(zeE_{app}/kT) \qquad (6)$$

where $[A^{z+}]_0$ and $[B^{z-}]_0$ are concentrations of the ions at the surface of the stationary phase, $[A^{z+}]_\infty$ and $[B^{z-}]_\infty$ are concentrations of the ions in the bulk mobile phase, z is the valency of the ion, and e is the charge of an electron. Since k' is defined by the relative concentrations of a species partitioned between the stationary phase and mobile phase, as modified by the ratio of the stationary phase to mobile phase volumes (ϕ), we can write for A^{z+}

$$k'_{A^{z+}} = \phi \frac{[A^{z+}]_0}{[A^{z+}]_\infty} \qquad (7)$$

Combining eqn. (5) and (7) yields,

$$\log k'_{A^{z+}} = \frac{1}{2.303}(-ze/kT)E_{app} + \log\phi \qquad (8)$$

Similarly, for B^{z-}

$$\log k'_{B^{z-}} = \frac{1}{2.303}(ze/kT)E_{app} + \log\phi \qquad (9)$$

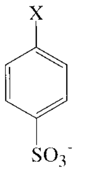
Neglecting possible contributions from other types of interactions that may affect retention (*e.g.*, solvophobicity and competition from supporting electrolyte anions and cations), these formulizations can be used to predict the dependence of k' on E_{app}. If log k' is plotted as a function of E_{app}, cations would then produce negative slopes and anions would produce positive slopes. Furthermore, doubly charged ions would generate slopes twice as large as those for singly charged ions. This treatment, although simplistic, nonetheless serves as an effective starting point for predicting the effects of E_{app} on retention. The framework for accounting for contributions from specifically adsorbed ions as well as for organic mobile phase modifiers has recently been described,[25] but is only beginning to be verified. Issues related to the separation of uncharged analytes and their interactions with a charged stationary phase are discussed at the end of the review. The fundamental aspects of EMLC are now beginning to emerge, and these properties can be exploited in resolving the many separation challenges faced in the analytical laboratory, as demonstrated in the following section.

IV. Applications

This section presents a few examples of the capabilities of the technique, including the separation of aromatic compounds, pharmaceutical agents, and chiral compounds. These examples were selected to demonstrate the ability to separate particularly challenging sample mixtures based solely on changes in the effective composition of the stationary phase. This section also describes the hybridization of EMLC with electrospray mass spectrometry. This union takes advantage of the ability of EMLC to manipulate analyte retention solely by changes in E_{app}, which minimizes variation of ionization efficiencies that are commonly encountered when gradient elution conditions are used in HPLC. This section closes by describing the application of an EMLC column to the covalent modification of PGC, which leads to the creation of a new, chemically stable family of reversed phase materials.

IV.1. Analytical separations

IV.1.a. Aromatic sulfonates. One of the first demonstrations of the utility of EMLC was performed by using PGC as the stationary phase for separating the six-component mixture of the substituted aromatic sulfonates that are listed in Scheme 1.[9] Fig. 3 presents the EMLC separations for this mixture of environmentally-significant compounds at three different values of E_{app}: (A) +0.50 V, (B) open circuit (+0.15 V), and (C) −0.60 V. At the open circuit potential, only four of the six components in the mixture are reasonably resolved. Two of the components, benzenesulfonate and 4-hydroxybenzenesulfonate, coelute. As shown in Fig. 3A, applying +0.50 V to the stationary phase increases the retention time of all six analytes with respect to that at the open circuit potential. For example, the retention time of 4-chlorobenzenesulfonate increases from 2.4 min at the open circuit potential to 5.2 min at +0. 50 V. 4-sulfobenzoic acid undergoes an even greater relative increase, with a retention time of 2.7 min at the open circuit potential and 9.1 min at +0.5 V. Moreover, all six analytes are fully separated, demonstrating the capability of manipulating the retention of this class of compounds solely through changes in E_{app}.



Compound	Acronym	X
1	ABS	NH_2
2	HBS	OH
3	BS	H
4	TBS	CH_3
5	CBS	Cl
6	BAS	COOH

Scheme 1

In sharp contrast to the separation at +0.50 V, the retention times for all six analytes are dramatically reduced at −0.60 V with respect to those at the open circuit potential. At −0.60 V, the mixture completely elutes in less than 2 min. Furthermore, the separation at −0.60 V is remarkably effective in resolving five of the six components in the original mixture (4-hydroxybenzenesulfonate was electroreduced between −0.10 and −0.20 V, and therefore excluded from the injected mixture). These data collectively show that the key analytical figures of merit in a separation (resolution and retention time) can be effectively and easily manipulated by EMLC.

A more detailed summary of these data is presented in Fig. 4, which plots log k' as a function of E_{app} for all six analytes. Three important points can be drawn from these plots. First, the retention for the six analytes increases as E_{app} becomes more positive. Second, the plots of log k' for all analytes are linearly dependent on E_{app}. Third, the magnitude of the change in retention differs between analytes. The first two points follow the predictions based on eqn. (9), *i.e.*, a plot of log k' for an anionic species should increase linearly as E_{app} becomes more positive. Perhaps the more interesting aspect of these data, however, is that the slopes of the plots for each of the analytes are different. This observation suggests that the predictability of EMLC-based retention is more complex than simply using changes in mobile phase composition. For example, an increase in the amount of organic modifier to the mobile phase increases the retention of the polar compounds in a sample when using a reversed phase material. The data in Fig. 4 argues that developing a set of useful predictive ‘retention rules’ for EMLC may prove more difficult.

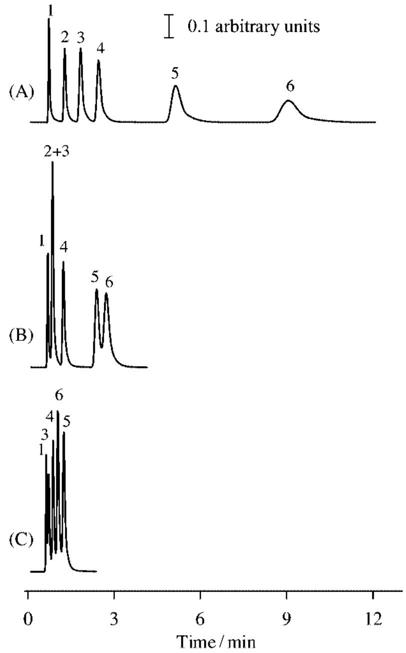


Fig. 3 Separation of a six-component mixture of aromatic sulfonates (see Scheme 1) on a PGC stationary phase at: (A) +0.50 V, (B) open circuit (+0.15 V), and (C) −0.60 V (*vs.* Ag/AgCl/saturated NaCl). The mobile phase consisted of 88 : 12 (v/v) aqueous (0.1 M $LiClO_4$, 0.1% TFA): acetonitrile (0.1 M $LiClO_4$) at a flow rate of 0.90 mL min^{-1}. Reproduced with permission from *Anal. Chem.*, 1998, **70**, 94. Copyright 1997 American Chemical Society.

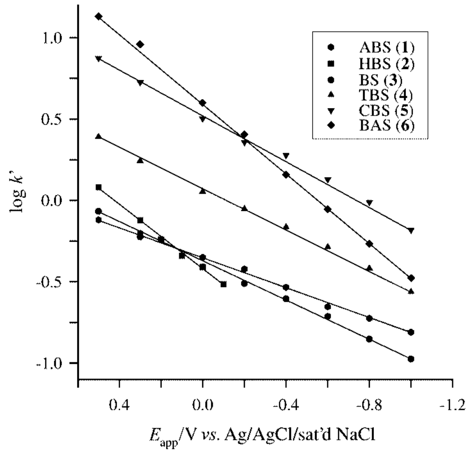


Fig. 4 Plots of log k' *vs.* E_{app} for separations of the six-component mixture of arenesulfonic acids between +0.50 V and −1.00 V using a PGC stationary phase. The data for 4-hydroxybenzene sulfonate extends only from +0.50 to −0.10 V because it is reduced below −0.10 V. Reproduced with permission from *Anal. Chem.*, 1998, **70**, 94. Copyright 1997 American Chemical Society.

Separations of this type of mixture can also be manipulated by changes in E_{app} during analyte elution, serving as a means to induce a compositional stationary phase gradient. This approach conceptually parallels temperature programming in gas chromatography. Fig. 5 demonstrates the utility of this approach by imposing a linear voltage ramp upon a stationary phase composed of glassy carbon particles from +0.3 to −1.0 V at 0.010 V s^{-1} immediately upon sample injection.[26] The early stages of this ramp increase the resolution of the more weakly retained analytes when separated at the open circuit potential by increasing their retention. The later stages of the ramp, in contrast, are designed to decrease the retention and narrow the elution bands for those analytes that were more strongly retained in the open circuit separation. Thus, a stationary phase gradient offers a facile pathway for the optimization of separations.

IV.1.b. Corticosteroids. One of the challenges frequently encountered when using PGC stationary phases is the separation of structurally similar compounds. A particularly interesting example is found in the separation of a mixture containing the four corticosteroids depicted in Fig. 6. In this case, the subtle structural differences are manifest in the presence or absence of a double bond in the A-ring of the steroid (*e.g.*, prednisolone (**2**) *vs.* cortisone (**3**)) and the ketonic- or hydroxy-oxygen (*e.g.*, prednisone (**1**) *vs.* prednisolone (**2**)). Separations using bonded reversed phases are generally more effective for compounds with small differences in chemical functionality,[27] while carbon-based phases are typically more successful in discriminating against compounds with differences in π-electron character.[28] As a consequence, the separation of this mixture at

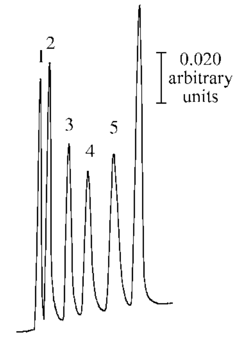
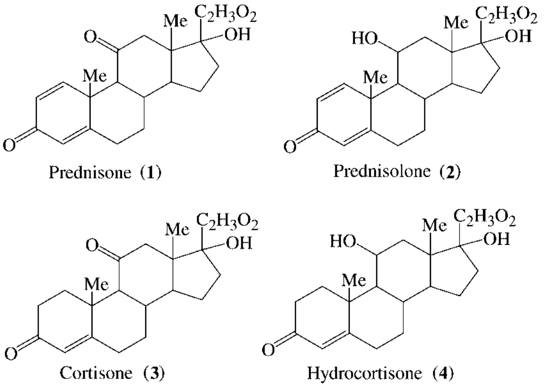


Fig. 5 Separation of a six-component mixture of arenesulfonates (1, 1,2-benzenedisulfonate; 2, benzenesulfonate; 3, 4-hydroxybenzenesulfonate; 4, 4-ethylbenzenesulfonate; 5, 4-chlorobenzenesulfonate; and 6, 1,5-naphthalenedisulfonate) by application of a linear voltage ramp from +0.30 to −1.00 V at 0.010 V s^{-1} (*vs.* Ag/AgCl/saturated NaCl). The ramp was initiated immediately upon sample injection, glassy carbon particles were used as the stationary phase. The mobile phase consisted of aqueous 0.1 M $LiClO_4$ containing 1.5% CH_3CN at a flow rate of 0.75 mL min^{-1}. Reproduced with permission from *J. Electroanal. Chem.*, 1995, **362**, 295. Copyright 1995 Elsevier Science SA.

PGC requires extensive optimization of the mobile phase composition in order to achieve an effective separation.[28] By taking advantage of the ability to manipulate the donor–acceptor character of PGC through changes in E_{app}, EMLC provides a platform that readily surmounts this difficulty.[10]

Fig. 7 presents the EMLC separations at PGC for the mixture at six different values of E_{app}: (A) +0.50 V, (B) open circuit (+0.44 V), (C) +0.30 V, (D) 0.00 V, (E) −0.20 V, (F) −0.40 V, and (G) −0.70 V. At the open circuit potential, the separation is only marginally effective. Prednisone (**1**) and prednisolone (**2**) are partially resolved, and cortisone (**3**) and hydrocortisone (**4**) virtually coelute. However, changes in E_{app} result in marked differences in retention and, more interestingly, in elution order. This difference, which translates to a nonlinear dependence in plots of log k' *vs.* E_{app}, is attribute in part to competition for adsorption sites on PGC by the supporting electrolyte. Specifically, as E_{app} becomes more negative, the retention of both prednisone and prednisolone increases, but with prednisone increasing to a notably greater extent. This difference transposes the elution order of the two compounds at ~ −0.20 V. As such, prednisolone is retained more strongly than prednisone at the more positive extremes of E_{app}, the two compounds coelute at 0.00 V, and prednisone is more strongly retained than prednisolone at more negative E_{app}.



Prednisone (1)

Prednisolone (2)

Cortisone (3)

Hydrocortisone (4)

Fig. 6 Chemical structures and numerical labeling scheme of corticosteroid analytes.

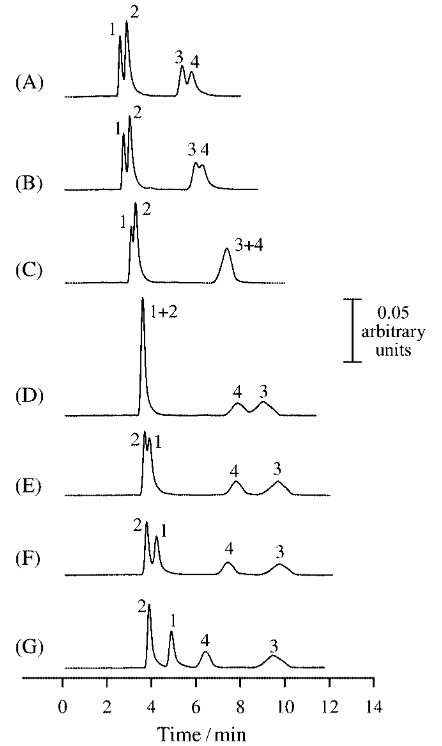


Fig. 7 Separation of a four-component corticosteroid mixture (Fig. 6) on a PGC stationary phase as a function of E_{app}: (A) +0.50 V, (B) open circuit (+0.44 V), (C) +0.30 V, (D) 0.00 V, (E) −0.20 V, (F) −0.40 V, and (G) −0.70 V (*vs.* Ag/AgCl/saturated NaCl). The mobile phase consisted of 50 : 50 aqueous (0.1 M $HClO_4$)–acetonitrile (0.1 M $LiClO_4$). The flow rate was 0.90 mL min^{-1}. Reproduced with permission from *Anal. Chem.*, 1997, **69**, 675. Copyright 1997 American Chemical Society.

The same general dependencies for the retention of cortisone and hydrocortisone are observed between +0.50 V and ~ −0.20 V. However, at more negative values of E_{app}, the retention of both compounds begins to decrease, with hydrocortisone exhibiting a greater change than cortisone. Based on the combination of these dependencies, all four components are fully resolved within ~10 min at −0.70 V. This result represents a distinct improvement over that reported for an isocratic separation of these compounds at PGC using a tertiary mobile phase,[28] which was ineffective in fully resolving all of the components in the mixture. Furthermore, this improvement in efficiency was realized along with ~30% reduction in analysis time. Thus, the ability to fine-tune the retention properties of PGC through changes in E_{app} serves as an efficient means to overcome the complications presented by the separation of this set of structurally similar compounds.

IV.1.c. Enantiomers. Separations of enantiomeric compounds are often performed in HPLC by using a stationary phase that is functionalized with a chiral selector or by having a chiral selector that is present as an additive in the mobile phase.[5] These types of separations are of major importance to pharmaceutical and clinical laboratories because therapeutic efficacy is often enantiomerically specific. However, the high level of structural discrimination required to resolve compounds based solely on the difference in their 'handedness' generally mandates the use and optimization of a wide range of separation formats. The following example shows that EMLC can be adapted to address the challenges inherent in such separations.

The strategy for imparting enantiomeric selectivity to an EMLC separation derives from the ability to modify the surface of a PGC stationary phase by the electrosorption of a chiral selector.[11] Electrosorption phenomena encompass adsorption processes that are dependent on E_{app},[29] *i.e.*, the underlying mechanism exploited by EMLC. The concept for transforming an achiral stationary phase to a chiral stationary phase is illustrated in Fig. 8, which depicts the expected dependence of the electrosorption of the chiral selector β-cyclodextrin (β-CD) onto PGC. The choice of this selector reflects the strong adsorptive interactions between hydroxyl functionalities and carbonaceous packing as E_{app} becomes increasingly positive.[19] Thus, β-CD strongly, but not irreversibly, electrosorbs onto the PGC surface by interactions with the seven primary hydroxy moieties that are located on the bottom of the β-CD cavity.

Results demonstrating this concept are presented in Fig. 9, using the enantiomeric pair of *d,l*-hexobarbital as the analyte mixture, β-CD as the mobile phase additive, and PGC as the stationary phase. In the absence of β-CD as a mobile phase additive (Fig. 9(I)), there is no detectable enantiomeric resolution of the sedative. Rather, the retention of the analytes increases as E_{app} becomes more negative. This trend is consistent with the composition of the mobile phase (pH ~ 2), which transforms the analytes into acceptors because of their protonation. However, when β-CD is added as a selector (Fig. 9(II)), the *d,l* pair is resolved at −1.0, 0.0, and +0.5 V, with the highest resolution at +0.5 V. There is no observable enantiomeric separation for −0.5 V. Experiments in which an excess of one enantiomer was added to the mixture reveal that the separation is controlled at the more negative values of E_{app} by the chiral additive in the mobile phase and at the more positive values of E_{app} by the electrosorbed chiral selector. The difference in elution order for the two enantiomers supports this interpretation.

Experiments have also shown that the electrosorption-based modification of PGC is reversible. This observation suggests that electrosorption may serve as a versatile means for altering the surface composition of conductive stationary phases in that a chiral column could, for example, be readily converted to a column with reversed phase character by the electrosorption of a different mobile phase additive (*e.g.*, a long alkyl chain alcohol). This intriguing strategy offers the possibility of requiring only one column for the separation of a wide range of complex mixtures.

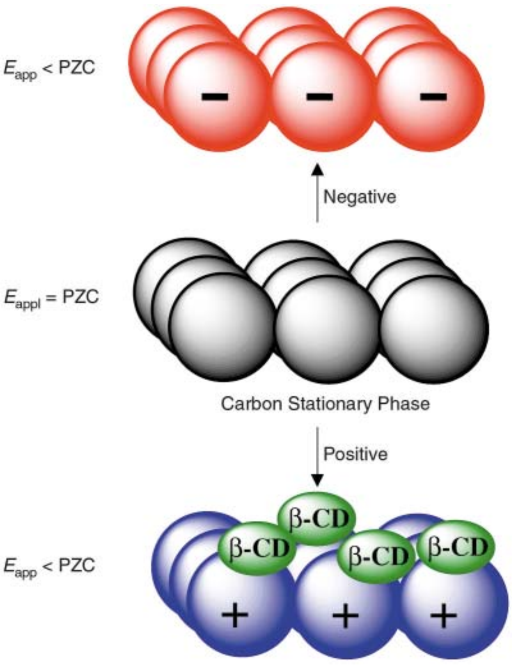


Fig. 8 Schematic representation of the electrosorption of β-cyclodextrin to a PGC stationary phase.

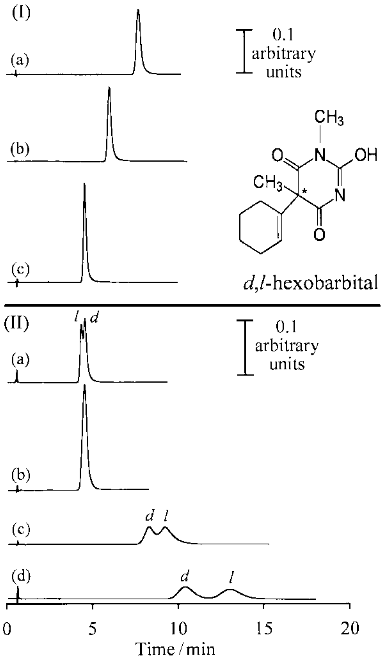


Fig. 9 Chromatography of *d,l*-hexobarbital at several applied potentials on porous graphitic carbon using a mobile phase comprised of 80 : 20 aqueous 0.1 M $LiClO_4$, 20 mM phosphate buffer (pH ~ 2)–acetonitrile, and a flow rate of 0.90 mL min^{-1}. (I) Separations without β-cyclodextrin in the mobile phase at (a) $E_{app} = -1.0$ V, (b) $E_{app} = 0$ V, and (c) $E_{app} = +0.50$ V (*vs.* Ag/AgCl/ saturated NaCl). (II) Separations with 15 mM β-cyclodextrin as a mobile phase additive at (a) $E_{app} = -1.0$ V, (b) $E_{app} = -0.5$ V, (c) $E_{app} = 0$ V, and (d) $E_{app} = +0.5$ V. Reproduced with permission from *Anal. Chem.*, 1998, **70**, 4314. Copyright 1998 American Chemical Society.

IV.2. EMLC and mass spectrometry

One of the important developments in LC over the past several years has been its merger with mass spectrometry. This merger provides a means to identify compounds upon their elution from the column as well as to lower their limits of detection. Unfortunately, the use of elution gradients that are often necessary to achieve an effective chromatographic separation degrades detection by MS because of changes in analyte ionization efficiencies. Interfacing MS with EMLC can notably reduce the complications posed by a solvent gradient by taking advantage of the ability to manipulate isocratically chromatographic resolution by changes in E_{app}.

In exploring this possibility, EMLC has recently been coupled to an electrospray mass spectrometer (ES-MS) to create a new hyphenated technique (EMLC/ES-MS).[30] The interest in ES-MS stems from its value in analyzing nonvolatile, polar compounds as well as high molecular weight polymers. Fig. 10 is a schematic diagram of the EMLC/ES-MS system. It is composed of a HPLC pump and mobile phase/supporting electrolyte reservoir, sample injector, EMLC column, potentiostat, ES-MS instrument, and UV detector. A grounded flow splitter allows the simultaneous collection of both UV and MS data during elution. Importantly, the development of this hybridized analysis system was achieved simply by replacing the conventional LC column already part of the integrated analysis system with an EMLC column and connecting the column to a potentiostat. No other changes in hardware were required.

As one of the first examples, EMLC/ES-MS was applied to the separation of the corticosteroid mixture described earlier in this section. This set of experiments was performed not only to test the utility of coupling the two techniques, but also to evaluate the feasibility of reliably operating an EMLC column with the solvent/electrolyte combinations requisite for effective ES-MS operation. The latter reflects problems associated with the use of nonvolatile electrolytes (*e.g.*, lithium perchlorate), which may plug the ES nozzle and subsequently suppress the analytical signal. ES-MS functions best when using volatile electrolytes (*e.g.*, ammonium acetate) at concentrations less than ~ 20 mM.[31]

Fig. 11 presents the summed ion current chromatograms obtained for the mixture at two extremes in E_{app} (Fig. 11a: +0.50 V and Fig. 11b: −1.0 V) using selected ion monitoring. To adapt this separation for use with ES-MS, the concentration of the organic component of the mobile phase was increased (20 : 80 (v/v) H_2O–CH_3CN) and the relatively nonvolatile $LiClO_4$ electrolyte was replaced with a much more volatile electrolyte combination [*i.e.*, 10 mM ammonium acetate (NH_4OAc) and 0.8% by volume acetic acid (HOAc)]. In previous ES-MS investigations with organic–H_2O solvent mixtures, concentrations up to 10 mM NH_4OAc were found to only marginally suppress analyte signal. Furthermore, the addition of up to ~ 1% (v/v) HOAc enhanced the solution conductivity as well as the analytical signal for basic analytes.[32]

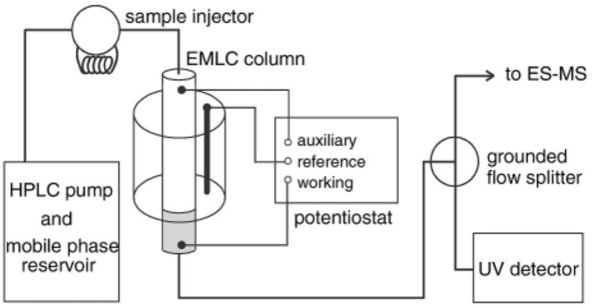


Fig. 10 Schematic diagram of the EMLC/ES-MS system. Reproduced with permission from *Anal. Chem.*, 2000, **72**, 2641. Copyright 2000 American Chemical Society.

With this mobile phase, the corticosteroids were detected by ES-MS as their protonated analogs. That is the mass to charge ratio (*m*/*z*) for prednisone $(\mathbf{1} + \mathrm{H})^+$ is 359, 361 for prednisolone $(\mathbf{2} + \mathrm{H})^+$ and cortisone $(\mathbf{3} + \mathrm{H})^+$, and 363 for hydrocortisone $(\mathbf{4} + \mathrm{H})^+$.

These findings demonstrate a critical attribute of EMLC as a separation technique when coupled to ES-MS. That is, EMLC has the ability to manipulate separation efficiencies without employing gradient elution techniques. These chromatograms, along with those not shown for the separations at intermediate values of E_{app}, also indicate that all four corticosteroids are electrochemically stable throughout most of the tested range of E_{app}. However, at +0.50 V, a broad elution band at *m*/*z* 363 (*i.e.*, the same *m*/*z* as for $(\mathbf{4} + \mathrm{H})^+$) superimposes itself across the elution bands for **1** and **2**. We do not as yet understand the origin of this band. Nonetheless, this finding argues that coupling EMLC and ES-MS may be particularly well suited for investigating mechanistic aspects of electrode reactions. Indeed, a companion study used this capability to examine the electroreduction of the anticonvulsant nitrazepam.[30] These results set the stage for more detailed investigations of various types of electrokinetic processes (*e.g.*, electropolymerization and electrocatalysis) which can take advantage of the ability to detect and quantify low level, high mass products by ES-MS.

IV.3. Electrosynthesis of bonded phases on carbon supports

One of the more recent applications of EMLC involves its use, not as a chromatographic column, but as an electrochemical reactor for the chemical derivatization of carbonaceous stationary phases.[33] The goal was to create PGC phases that would function more like the widely used bonded phases on silica supports that are produced by silane-coupling chemistry, but with increased stability in strongly acidic or strongly basic solution.[20] To address this possibility, PGC and glassy carbon (GC) phases were covalently modified with a series of substituted aryl-groups *via* the on-column electroreduction of arenediazonium salts. The mechanism for this electrochemical process is illustrated in Fig. 12.[34–36] In effect, the carbon packing is used to generate an aryl-radical by the one-electron reduction of the diazonium moiety, with the radical subsequently inserting into the carbon–carbon framework of the stationary phase by forming a carbon–nitrogen bond.

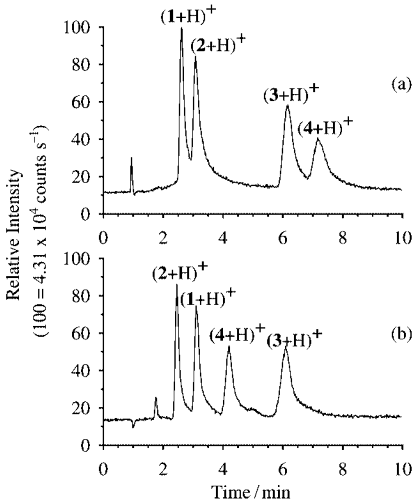


Fig. 11 Summed ion current chromatograms for a mixture of corticosteroids (Fig. 6) on PGC as a function of E_{app}: (a) +0.50 V, and (b) -1.0 V (*vs.* Ag/AgCl/saturated NaCl). The mobile phase consists of 20 : 80 (v/v) water–acetonitrile containing 10 mM $\mathrm{NH_4OAc}$ and 0.8% vol. HOAc with a flow rate of 1.0 mL min^{-1}. These data were recorded in selected ion monitoring mode for monoprotonated prednisone (**1**, *m*/*z* 359), prednisolone (**2**, *m*/*z* 361), cortisone (**3**, *m*/*z* 361), and hydrocortisone (**4**, *m*/*z* 363). Reproduced with permission from *Anal. Chem.*, 2000, **72**, 2641. Copyright 2000 American Chemical Society.

The effect of the modification on retention was tested using a mixture of the aromatic analytes anisole and fluoranthene. This test mixture was selected in view of the inherent difficulty in eluting polynuclear hydrocarbons from carbon-based stationary phases.[37] This mixture therefore provides a basis for evaluating the effectiveness of the modification on masking interactions with the underlying stationary phase. Fig. 13a shows the separation of the mixture on unmodified GC phase. In this case, anisole elutes from the column in ~30 s. Fluoranthene, on the other hand, fails to detectably elute within the 2 h test period. However, after modification by the one-electron reduction of 4-nitrobenzenediazonium tetrafluoroborate, both compounds elute from the column in less than 1 min. These results demonstrate that the coating effectively masks the strong interactions that lead to the irreversible adsorption of polynuclear aromatic compounds like fluoranthene. Subsequent studies also found that, as expected, the type of linkage formed between the modifier and stationary phase is stable upon extended exposure to mobile phases that readily degrade silica-based bonded phases.[20] Ongoing efforts are exploring the utility of this approach for the creation of a new family of reversed phase materials.

V. Conclusions

EMLC has emerged as a viable new tool for addressing the many challenges encountered in the day-to-day operations in today's analytical and clinical laboratories. Advances in this

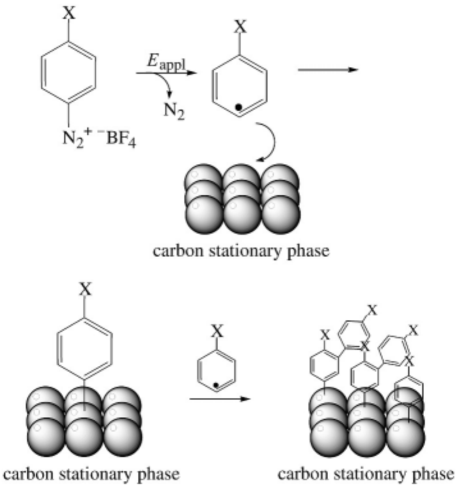


Fig. 12 Schematic representation of the functionalization of carbon stationary phases *via* the on-column electroreduction of arenediazonium salts.

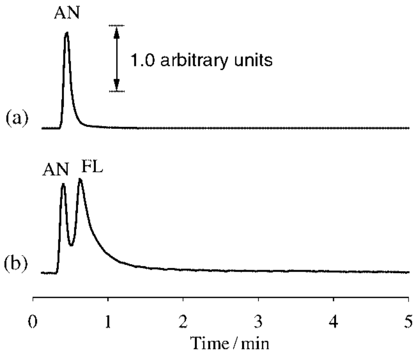


Fig. 13 Comparison of the separation of a mixture of anisole and fluoranthene on (a) unmodified PGC stationary phase and (b) 4-nitrobenzene-modified PGC. The mobile phase was comprised of 50 : 50 aqueous (0.1 M $\mathrm{LiClO_4}$)–acetonitrile at a flow rate of 0.50 mL min^{-1}.

area will continue with improvements in column design that reduce the equilibration time to changes in E_{app}, and lead the way to applications using a voltage gradient (rapid change of E_{app}) during elution. There are also several intriguing opportunities in the creation of separation formats using new types of stationary phases. Other types of conducting phases that use, for example, electrochemically transformable coatings[12–17] offer pathways to manipulate separations by variations in the porosity of a coating that can be affected by the size of the dopant ion used in electrochemical coating process. Variations in porosity may add size discrimination to the selectivity of the separation process. In addition, the incorporation of functional groups as substituents on the monomer precursors used for polymer formation can be exploited to design coatings with differences in hydrophobicity–hydrophilicity.

Issues related to the development of a fundamental understanding of the retention mechanism need to be addressed in order to advance the application of EMLC as a separation tool. These issues not only include advancing the general aspects of the mechanism already noted, but also furthering the descriptions of the heterogeneity of the surface sites on stationary phases like PGC and GC. The surface of both GC[38] and, to a lesser extent, PGC[18] is composed of a distribution of edge and basal planes, amorphous and defect regions, and surface functional groups. Gaining further insights into how the different sites affect the retention process is required in order to develop the 'retention rules' required for EMLC to emerge as a predictive tool for the many types of separations routinely encountered in the analytical laboratory. Systematic investigations along these lines promise to stimulate the wide spread interest in this new mode of LC separations.

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