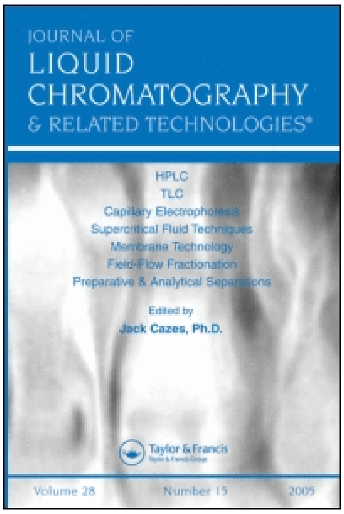

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Selective Separation of Polar Compounds Using an Electric Field Coupled Normal Phase HPLC Column

W. -X. Yao[a]; C. -P. Pau[ab]; G. Patonay[ac]; I. M. Warner[a]; C. -N. Ho[d]
[a] Department of Chemistry Emory, University Atlanta, Georgia [b] Centers for Disease Control, Atlanta, Georgia [c] Department of Chemistry, Georgia State University, Atlanta, Georgia [d] Department of Chemistry, East Tennessee State University, Johnson City, Tennessee

To cite this Article Yao, W. -X. , Pau, C. -P. , Patonay, G. , Warner, I. M. and Ho, C. -N.(1988) 'Selective Separation of Polar Compounds Using an Electric Field Coupled Normal Phase HPLC Column', Journal of Liquid Chromatography & Related Technologies, 11: 13, 2751 — 2769

To link to this Article: DOI: 10.1080/01483918808076759

URL: http://dx.doi.org/10.1080/01483918808076759

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(13), 2751-2769 (1988)

SELECTIVE SEPARATION OF POLAR COMPOUNDS USING AN ELECTRIC FIELD COUPLED NORMAL PHASE HPLC COLUMN

WEI-XI YAO[1], CHOU-PONG PAU[1+],
GABOR PATONAY[1@], ISIAH M. WARNER[1*] AND
CHU-NGI HO[2]
[1]*Department of Chemistry*
Emory University
Atlanta,Georgia 30322
[2]*Department of Chemistry*
East Tennessee State University
Johnson City, Tennessee 37614-002

ABSTRACT

An electric field coupled method for separation of polar compounds using normal phase high performance liquid chromatography is described. The method, which is not based on electrophoresis or electrochromatography, selectively changes the column capacity for polar analytes. The use of different columns showed improved column efficiency and up to a 62% increase in the number of theoretical plates. The theory for the improvement is presented. The significance and utility of the method for selective analysis of polar fractions in environmental samples is discussed.

+ Present address: Centers for Disease Control, Atlanta, Georgia
@ Present address: Department of Chemistry, Georgia State University, Atlanta, Georgia
* To whom correspondence should be addressed

INTRODUCTION

Successful high performance liquid chromatogaphy (HPLC) separation requires a well balanced compromise between resolution, sample capacity and analysis time. Thus, a large amount of effort has gone into the characterization of each of these variables. Although reverse phase liquid chromatography continues to increase in use, normal phase liquid chromatography (NPHPLC) also continues to be important. Moreover, in spite of the complexity, the retention mechanism in normal phase has been better characterized than in reversed-phase HPLC.

Polar components of environmental pollutants have been established as more mutagenic and carcinogenic than their non-polar counterparts.(1,2) Consequently, it is important to quantitatively and qualitatively characterize the chemical constituents of polar fractions of environmental samples. Currently, normal phase high performance liquid chromatography is widely used for analysis of polar fractions.(3-10) This technique affords 1) the use of less corrosive solvents 2) lower inlet pressure and 3) the capability for sample class separation.(4) The stationary phase material used in NPHPLC consists of polar adsorbents such as silica or alumina or moderately polar packing materials obtained by chemically bonding polar components on the surface of the silica. Thus, solute-stationary phase interactions in NPHPLC systems are polar interactions. Such interactions often require a specific topographical arrangement for the solute molecule at the surface of the stationary phase. Consequently, a specific orientation of the solute molecule relative to the surface normal of the stationary phase is always favored for maximum adsorption. This favorable orientation is randomly achieved due to the basic structure of the packing material and the random orientation of the solute. In other words, there is no preferred orientation of the solute when compared to the macroscopic column dimensions, e.g. column wall normal. Such is also true for all packing materials used in NPHPLC systems. To enhance selectivity, gradient elution is often required. It has been suggested that retention of a polar compound on normal phase columns is a function of hydrogen (H) bonds between the solute molecules and the polar stationary phase.(11) Hence, changes in separation with changes in solvent polarity is often observed.

Another approach to studying the retention properties of polar compounds on normal phase columns is to influence the probability of forming H bonds between the solute and stationary phase. Such effects can be achieved by limiting the free rotation of the polar solute molecules through use of an external force, e.g. with use of an external electric field. In this paper, we introduce a new method that increases the selectivity of NPHPLC for polar compound analysis. The method is based on the application of a high voltage external electric field. This electric field does not induce an electrophoretic migration, as in electrophoresis or as in electrochromatography, since the polar compounds of interest are not ionic. Rather, the process can best be explained in terms of preferred orientation in the presence of an external electric field. One can predict that due to lack of electophoretic migration, reversing the direction of the electric field should not have any effect. This can be verified by examining the effects of the retention time changes with the direction of the electric field. In addition, the use of non-polar solvents in normal phase separations reduces the number of possible oxidation - reduction processes as well as allows operation at much higher voltages before reaching the solvent breakdown voltage.

THEORY

If an electric field is applied to the column, the random distribution of apolar solute molecules will not be affected or only slightly affected. However, the isotropic behavior of polar solute molecules may be influenced significantly by an external electric field. Depending on their dipole moments, polarizability, temperature of the environment, and the viscosity of the mobile phase, such molecules will exhibit a more or less oriented adsorption. Consequently, the number of optimum sites available for adsorption should decrease with increasing electric field. In order to explore the separation characteristics of a NPHPLC column in the presence of an external electric field, it is necessary to examine the influence of the electric field upon the equilibrium distribution of the analyte molecules between the stationary and mobile phases. If X_s and X_m refer to the concentrations of the analyte in the stationary and mobile phases, respectively, the distribution constant can then be written as

$$K^e = \frac{(X_s)^e}{(X_m)^e} \quad \text{and} \quad K^o = \frac{(X_s)^o}{(X_m)^o} \tag{1}$$

where e and o superscripts denote the respective presence and absence of an external electric field. Since the adsorption characteristics of a nonpolarizable, non-polar molecule does not appreciably change in the external electric field, and since the concentration of the non polarizable, non-polar analyte in the stationary phase does not change, it necessarily follows that

$$(X_{sn})^o = (X_{sn})^e \tag{2}$$

or

$$(K_n)^o = (K_n)^e \tag{3}$$

where the subscript n denotes a non-polarizable non-polar molecule.

For polar molecules

$$(X_{sp})^{o} > (X_{sp})^{e} \tag{4}$$

resulting in a decreased distribution constant, i.e.

$$(K_{p})^{o} < (K_{p})^{e} \tag{5}$$

where the subscript p denotes a polar molecule. This equation is obvious since polar molecules cannot freely orient in the presence of an electric field to achieve an optimum alignment for adsorption. This decreased distribution constant will result in a decreased column capacity for polar analytes.

If ΔX_{sp} and ΔK_{p} are defined as the concentration change of the polar solute in the stationary phase and the change of the distribution constant, respectively, we can write the capacity factor K'_{p} for the polar fraction as

$$(K'_{p})^{o} = (K_{p})^{o} \frac{(V_{s})^{o}}{(V_{m})^{o}}$$

and

$$(K'_{p})^{e} = (K_{p})^{e} \frac{(V_{x})^{o}}{(V_{m})^{e}} \tag{6}$$

or

$$\Delta K'_{p} = \Delta K_{p} \frac{V_{s}}{V_{m}} \tag{7}$$

where V_{s} and V_{m} are the respective volumes of the stationary and mobile phases. The volumes V_{s} and V_{m} are virtually independent of the applied external electric field.

Assuming a constant flow rate, equation (7) reduces to

$$\Delta K'_p = \frac{(t_r)^o{}_p - t_o}{t_o} - \frac{(t_r)^e{}_p - t_o}{t_o} = \frac{(t_r)^o - (t_r)^e{}_p}{t_o} = \frac{\Delta(t_r)_p}{t_o} \qquad (8)$$

where t_o is the retention time of a non-adsorbing component, i.e., a component that spends 100% of its time in the mobile phase.

Similarly, we obtain for a non polarizable, nonpolar component

$$\Delta K'_n = 0 \qquad (9)$$

In other words, in the presence of an external field, the retention time of the polar component varies and the retention time of the nonpolar component does not. The magnitude of the effect is primarily dependent on the strength of the electric field, the dipole moment of the molecule, as well as the polarizability of the molecule. This is apparent since the stronger the field, the less likely the probability that the polar molecule can optimally orient to accommodate the randomly organized surface. However, a quantitative treatment of this effect is beyond the scope of this manuscript and is not necessary to explain the experimental results. According to equation (8), the retention time change is inversely proportional to the flow rate, (FR), i.e.

$$\Delta(t_r)_p = t_o \Delta K'_p = \frac{V_m}{FR} \Delta K'_p \qquad (10)$$

If equation (10) is compared to the usual equation for retention time, i.e.

$$t_r = t_o\,(1+K'), \qquad (11)$$

we see that Equation (10) describes a modified chromatogram where only the polar fraction is detected. The parameter, $\Delta(t_r)_p$, can then be described as the reduced retention time of the polar component.

A larger retention time change is expected if the external electric field is stronger or if the solute molecule is more polar. Since the random motion of a dipole increases with increased temperature, a lower reduced retention time is expected for polar molecules at higher temperatures. The decreased capacity factor of a polar molecule in the presence of an external electric field results in increased migration rate. One of the more important advantages of the electric field assisted NPLC (ENPLC) has already been mentioned, i.e. the selective determination of polar components in the presence of coeluting nonpolar components. Finally, it should be noted that due to the lowered K' value of the polar molecule in the presence of an external electric field, the molecule in theory spends less time in the stationary phase resulting in an increased mass transfer rate and decreased height equivalent to a theoretical plate. Restricted diffusion of the analyte molecules in the presence of an external electric field may be one of the possible explanations for this increased column efficiency.

EXPERIMENTAL

Materials

The compounds α-naphthol and naphthalene were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.), naphthalene from Fischer Scientific Company (Fair Lawn, NJ, U.S.A.), 1-naphthaleneethanol, methyl benzoate, 9-anthraldehyde, indole, benzaldehyde dimethyl acetal and 1-aminonaphthalene from Aldrich

Chemical Company (Milwaukee, WI, U.S.A.). The HPLC grade cyclohexane, and methylene chloride were obtained from American Burdick and Jackson (Muskegon, MI, U.S.A.) Nucleosil-NH_2 (10 µm) was purchased from Alltech Associates (Deerfield, IL, U.S.A.) and Spherisorb-alumina (5µm) and Spherisorb-silica (5 µm) from Phenomenex (Rancho Pales Verdes, CA, U.S.A.).

Column Preparation

A schematic of our column design is shown in Figure 1. It consists of a 4.6 mm x 50 mm stainless steel tube (A), two three-way fittings (B) and two stainless steel rods (d = 1.5 mm) as electrodes (C). The electrodes were situated at both ends of the column and insulated from the column and end fittings using Teflon plugs (D). Stainless steel frits (E) located at the side-arm of the three-way fittings were used as bed supports. Newly packed columns were washed with 30% methylene chloride in cyclohexane for 30 hours at a flow rate of 0.5 ml/min to remove possible electroactive contaminents. Then, a voltage of 15 kV was applied to the electrodes while solvent was flowing. The current was carefully monitored until it decreased to 0.01mA or less.

Procedure

A Beckman system (Beckman Instruments, San Ramon, CA, U.S.A.), including a model 420 controller, a 112M solvent delivery system, a 160 detector and a 210 injector with a 20 µL sample loop, was used for all HPLC measurements. A DEL Electronics (Mount Vernon, NY, U.S.A.) model 875A high voltage power supply was used to generate the electric field. Three types of packing materials, Nucleosil-NH_2, Spherisorb-alumina and

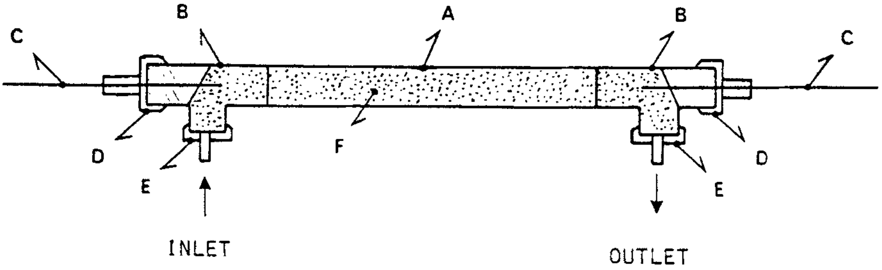

Figure 1: A schematic diagram showing an HPLC column with electrodes for application of electric fields

(A). Stainless steel column (4.6 x 50mm)
(B). Three way fitting
(C). Stainless steel electrode
(D). Teflon plug
(E). Stainless steel frit
(F). Packing materials

Spherisorb-silica were examined. The chromatographic columns were packed using a slurry method.(11)

Calculations

Column efficiency is a consideration in the studies described here. The number of theoretical plates (N) was calculated using the usual equation, i.e.

$$N = 5.54 \left[\frac{t_r}{W_{1/2}} \right]^2 \qquad (12)$$

where t_r is the retention time of the compound and $W_{1/2}$ is the peak width at half peak height.

RESULTS AND DISCUSSION

Retention time is the parameter which is usually used to indicate the extent of interaction between the solute and the

TABLE 1

Chromatographic Retention Time of Naphthalene, Indole and 1-Aminonaphthalene Separated by Three Columns Under the Influence of Various External Electric Fields

Column	Mobile Phase	Sample	Retention time (min.)					RRT*
			0.0 kV	5.0 kV	7.5 kV	10.0 kV	12.5 kV	12.5 kV
Nucleosil-NH_2	60%A	Naphthalene	2.00	2.00	2.00	2.00	1.99	0.01
	40%B	Indole	5.23	5.18	5.15	5.10	5.11	0.12
		1-Aminonaphthalene	8.13	7.97	7.86	7.72	7.69	0.44
Spherisorb silica	65%A	Naphthalene	2.24	2.24	2.24	2.25	2.25	-0.01
	35%B	Indole	4.12	4.12	4.12	4.11	4.09	0.03
		1-Aminonaphthalene	15.2	14.9	14.9	14.8	14.6	0.60
Spherisorb-alumina	45%A	Naphthalene	2.30	2.30	2.30	2.30	2.29	0.01
	55%B	Indole	5.97	5.96	5.95	5.85	5.84	0.13
		1-Aminonaphthalene	11.9	11.8	11.7	11.6	11.6	0.30

(1) A = cyclohexane, B = methylene chloride and flow rate = 1.0 ml/min.

*Reduced Retention Time (min.)

stationary phase and thus we will use as such in our discussion. Table 1 summarizes the retention time of naphthalene, indole and 1-aminonaphthalene separated on Nucleosil-NH_2, Spherisorb-alumina, and Spherisorb-silica columns in the presence and absence of an external electric field. In general, we note that the retention time of a given compound decreases with increasing voltage. Furthermore, as predicted in our theoretical discussion, the retention time of the polar compound 1-aminonaphthalene decreases more than that of a non-polar compound (e.g. naphthalene). We also note that after reversing the electrode polarity, the retention times of these compounds decreases in the same manner (data not shown), indicating that electrophoresis, is not present or at least negligible.

Since the probability of hydrogen bonding is greatly dependent upon the polarity of the solvent system, different mixtures of cyclohexene (solvent A) and methylene chloride (solvent B) were also used in our study. Since methylene chloride is more polar than cyclohexane, an increase in methylene chloride concentration should increase the polarity of the mobile phase. As predicted, the results in Table 2 indicate a reduced effect of the electric field on the solute with increasing solvent polarity. This phenomenon is due to two important factors. First, the intensity of the effective electric field is inversely proportional to the dielectric constant of the material between the electrodes. Our material consists of two major components, i.e., the stationary phase and the mobile phase. Since, the dielectric constant of methylene chloride (ε = 9.08) is much greater than that of the cyclohexane (ε = 2.03) the resulting dielectric constant of the mobile phase - stationary phase system increases when the methylene chloride content of the mobile phase increases. A second important phenomenon can be described as a shielding effect of the polar solute molecules by the polar solvent molecules. This also decreases the effect of the external electric field. Consequently, one can expect the best solvent system for ENPLC applications to be a non-polar solvent with low dielectric constant. Cyclohexane (ε = 2.03) is one of the logical candidates, but other saturated hydrocarbons may be

TABLE 2

Retention Time Changes and Mobile Phases using a Nucleosil - NH_2 Column

Mobile (1) Phase	Sample	Retention time (min.)			RRT*
		0.0kV (tr_0)	12.5kV ($tr_{12.5}$)	change(%)$^{(2)}$ (Dtr/tr_0)	12.kV
80%A	Naphthalene	2.06	2.06	0.0	0.0
20%B	Indole	12.68	11.90	6.2	0.78
	1-Aminonaphalene	19.58	18.06	7.8	1.52
60%A	Naphthalene	2.00	1.99	0.5	0.01
40%B	Indole	5.23	5.11	2.3	0.12
	1-Aminonaphalene	8.13	7.69	5.4	0.44
40%A	Naphthalene	1.97	1.96	0.5	0.01
60%B	Indole	3.83	3.75	2.1	0.13
	1-Aminonaphalene	5.71	5.43	4.9	0.28
20%A	Naphthalene	1.91	1.91	0.0	0.0
80%B	Indole	2.92	2.89	1.0	0.0
	1-Aminonaphalene	4.13	4.02	2.7	0.09
0%A	Naphthalene	1.90	1.89	0.5	0.01
100%B	Indole	2.61	2.60	0.4	0.01
	1-Aminonaphalene	3.62	3.59	0.8	0.03

(1) A = cyclohexane, B = methylene chloride, flow rate = 1.0 ml/min. and column temperature = 20.0°C

(2)

$$\text{change } (\%) = \frac{tr_{12.5} - tr_0}{tr_0} \times 100\%$$

*Reduced Retention Time (min.)

equally suitable. The column temperature is also an important factor in ENPLC. At higher temperatures, the thermal energy of the solute molecule is greater. This should result in lowered adsorption of the stationary phase. The rotational motion of the polar molecule is also faster at higher temperatures which decreases the number of aligned molecules in the presence of an applied electric field. Accordingly, a reduced effect of the external voltage is expected with increasing temperatures. Table 3 is a summary of results obtained at different column temperatures. The relative retention time change ($\Delta t_r/\Delta t_{ro}$) or the reduced retention time is significantly higher at lower temperatures. These results suggest that the electric field induced selective determination of polar solutes is much more favorable at lower temperatures.

Several data sets were obtained using two directions of the electric field, i.e. with the electric field vector parallel with the direction of flow and when the electric field vector was antiparallel with the direction of the flow. Results were indicating little or no electrophoretic phenomena. Since the presence of trace amounts of water or other contaminants could not be ruled out in the mobile phase, we repeated the experiments with freshly distilled solvents and found no significant changes in the results. The electric current through the electrodes was also carefully monitored to examine the possibility of electrophoresis and significant oxidation-reduction processes. The current increased with the applied voltage, but never exceeded 10 µA. This small current is likely due to imperfect insulation of the electrodes and possible trace amounts of ionic species, e.g. water in the mobile and stationary phase. However, this small current does not interfere with the operation of the ENPLC column since the strength of the electric field does not change. Nevertheless, one of our future goals is to further reduce this current by examining the variables that contribute to this current.

Column efficiency was calculated according to Equation 12. Table 4 is a summary of the results for three different columns.

TABLE 3

Retention Time Changes and Column Temperature using a Nucleosil- NH_2 Column

Temperature - (C)	Sample	Retention time (min.) 0.0kV (tr_0)	12.5kV ($tr_{12.5}$)	change(%)$^{(2)}$ (Dtr/tr_o)	RRT* 12.kV
1.0	Naphthalene	2.07	2.07	0.0	0.0
	Indole	8.39	8.17	2.6	0.22
	1-Aminonaphalene	12.80	11.99	6.3	0.81
10.0	Naphthalene	2.05	2.05	0.0	0.0
	Indole	7.80	7.70	1.3	0.10
	1-Aminonaphalene	11.87	11.55	2.7	0.32
20.0	Naphthalene	2.05	2.04	0.5	0.01
	Indole	7.30	7.24	0.8	0.06
	1-Aminonaphalene	11.22	11.00	2.0	0.22
30.0	Naphthalene	2.01	2.01	0.0	0.0
	Indole	6.82	6.77	0.7	0.05
	1-Aminonaphalene	10.30	10.12	1.7	0.18
40.0	Naphthalene	1.98	1.98	0.0	0.05
	Indole	6.24	6.23	0.2	0.01
	1-Aminonaphalene	9.10	9.15	0.5	0.05

(1) mobile phase: cyclohexane/methylene chloride = 70/30
flow rate = 1.0 ml./min.

(2)

$$\text{change } (\%) = \frac{tr_{12.5} - tr_0 \times 100\%}{tr_0}$$

*Reduced Retention Time (min.)

TABLE 4

Number of Theoretical Plates of Three Columns Examined Under the Influence of an Electric Field

Column	Mobile Phase (1)	Sample	Number of theoretical plates (N) per meter		
			0.0 kV	12.5 kV	Change (%)(2)
Nucelosil-NH$_2$	60%A 40%B	Naphthalene	1560	1560	0
		Indole	1790	2415	35
		1-Aminonaphthalene	2010	2879	43
Spherisorb-silica	65%A 35%B	Naphthalene	2250	2270	1
		Indole	2940	4340	48
		1-Aminonaphthalene	5890	9570	62
Spherisorb-Alumina	45%A 55%B	Naphthalene	2370	2490	5
		Indole	3480	4440	28
		1-Aminonaphthalene	3700	4960	34

(1) A = cyclohexane, B = methylene chloride and flow rate = 1.0 ml/min.

(2)

$$\text{change (\%)} = \frac{N_{12.5} - N_0 \times 100\%}{N_0}$$

These results indicate that N increases for all three samples, naphthalene, indole, and 1-aminonaphthalene on all three columns examined except for naphthalene on a silica column, where no significant change was observed. Also, the polar compound 1-aminonaphthalene has a higher change (62%) under the same electric field strength, indicating that polar solutes interact with the electric field stronger than non-polar solutes as predicted in our earlier discussion.

Finally, a standard sample was prepared containing eight compounds dissolved in cyclohexane; naphthalene (13.4 µg/ml), indole (16.1 µg/ml) methyl benzoate (32.9 µg/ml), 9-anthraldehyde (5.8 µg/ml), benzaldehyde dimethyl acetal (77.7 µg/ml), α-naphthol (35.5 µg/ml), 1-aminonaphthalene (40.9 µg/ml and 1-naphthaleneethanol (115.4 µg/ml). The sample (20 µL) was injected and separated on a silica column, with and without an electric field (12.5 kV). Figure 2 provides the observed chromotograms; Curve I is with an electric field (12.5 kV) and II is without an electric field. With the electric field, unresolved peaks (3,4 and 5,6) were better separated and an unidentified peak (9) appeared. These results also showed that column efficiency was improved in this case with the electric field. The reproducibility of the ENPLC separation was also examined using an alumina column. Different standards at different concentrations (0.0, 5.0, 4.0 µg/ml of naphthalene, 175.0 µg/ml of methyl benzoate, 14.4 µg/ml of 9-anthraldehyde and 48.4 µg/ml of indole) were injected 11 times at 0.0 kV and 5 times at 5.0 - 15.0 kV repeatedly (20 µl each). The average retention time and average peak height of each compound in the presence of the same electric potential were calculated. An overall relative standard deviation for all 4 compounds was 0.23% and 1.3% for retention time and peak height, respectively. Therefore, this column was stable under the experimental conditions outlined. However, long-term column stability was not satisfactory. After several days of operation, the shift of retention time for a given sample was decreased, indicating that the column was less sensitive to the electric field. We noticed that a brownish-yellow color developed on the packing materials around the anode. A qualitative test on this colored packing materials with the addition

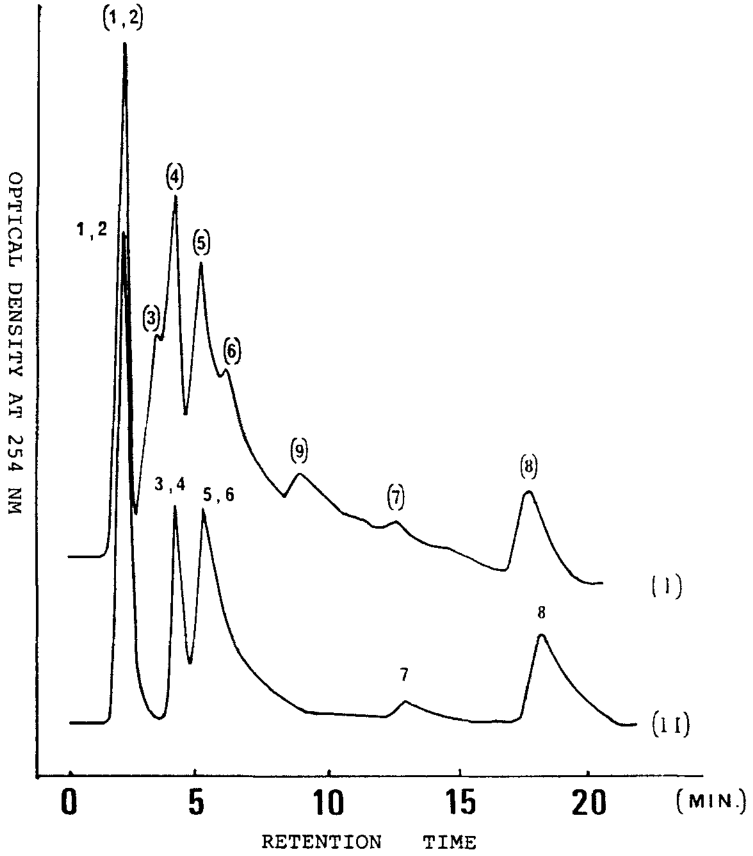

Figure 2: Separation of a standard mixture with (I) and without (II) an electric field (12.5 kV) on a silica column. Flow rate 1.0 ml/mm. Mobile phase: 35% cyclohexane, 65% methylene chloride. Peak identity as follows:

(1). Naphthalene
(2). Indole
(3). Methyl benzoate
(4). 9-Anthraldehyde
(5). Benzaldehyde dimethyl-acetal
(6). α-Naphthol
(7). 1-Aminonaphthalene
(8). 1-Naphthaleneethanol

of $K_4(FeCN_6)$ gave a blue color, indicating the presence of Fe(III). The sample was then analyzed by mass-spectrometry using negative ion fast atom bombardment in a thioglycerol matrix. A major fragment of 107 mass-units was observed, suggesting the presence of $Fe(OH)_3$. The most probable explanation is that the formation of $Fe(OH)_3$ decreased the effective electric field. This problem can probably be circumvented by using a more inert electrode, e.g. platinum.

Although the feasibility of the electric field coupled normal phase liquid chromatography has been demonstrated, several aspects need to be explored further. First, the column should be redesigned to achieve higher electric fields, possibly with lower electrode voltage. Second, computer aided data acquisition also can enhance the usefulness of the system by comparing chromatograms acquired with and without electric field and subsequently eliminating peaks which are not affected by the electric field. Finding the optimum mobile phase-stationary phase system is also an important goal. Finally, it should be noted that the ENPLC method described in this manuscript is not an electrophoretic method nor an electrochromatographic method. Electrochromatography was introduced by Antrim et al.(13) In this electrochemical method, the carbonceuous stationary phase was modified by applying small (less than 1V) potentials on the conductive stationary phase. Electrochromatography is an electrophoretic procedure where sorptive interactions are also present. The polarity of applied voltage in electrochromatography is important since the solute is accelerated or retarded according to the polarity of the external voltage. Electrochromatography using high external voltages has been also described in the literature.(14)

ACKNOWLEDGEMENTS

This work was supported by a U.S. Department of Energy grant (#DE-AS05-82ER60100). I.M. Warner is also grateful for support from a Presidential Young Investigator Award (#CHE-8351675). We acknowledge Dr. Judy Sophianopoulos for technical assistance.

REFERENCES

1. D.A. Haugen, M.J. Peak, K.M. Suhrbier and V.C. Stamoudis, Anal. Chem., 54 (1982) 32.
2. G. Grimmer, H. Brune, R. Deutsch-Wenzel, G. Dettbam, J. Misfeld, U. Abel and J. Timm, Cancer Lett, 23 (1984) 167.
3. D. Karlesky, D.C. Shelly and I.M. Warner, J. Liquid Chromatog., 6 (3) (1984) 471.
4. A.M. Siouffi, M. Righezza and G. Guiochon, J. Chromatog., 18 (1986) 189.
5. A Matsunaga, Anal. Chem., 55 (1983) 1375.
6. S.M. Staraverov and G.V. Lisichkin, Chromatog., 21 (3) (1986) 165.
7. W. Ecknig, B. Trung and R. Radeglia, Chromatog., 16 (1982) 178.
8. P.G. Amateis and L.T. Taylor, Anal. Chem., 56 (1984) 966.
9. G. Felix, C. Bertrand and F.V. Gastel, Chromatog., 20 (3) (1985) 155.
10. A.H.T. Maria, S.L. John and G.D. John, Anal. Chem., 58 (1986) 744.
11. E.L. Johnson and R. Stevenson, Basic Liquid Chromatography, Varian, Palo Alto, CA (1978).
12. L.R. Synder and J.J. Kirkland, Introduction to Modern Liquid Chromatography (Second Edition), John Wiley and Sons, Inc. (1979).
13. R.F. Antrim, R.A. Scherer, A.M. Yacynych, Anal. Chim. Acta, 164 (1984) 283.
14. T. Tsuda, Anal. Chem. 59 (1987) 523.