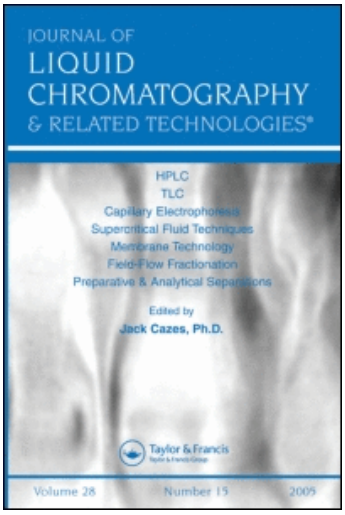


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# Retention of Neutral Solutes in Capillary Electrochromatography

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# RETENTION OF NEUTRAL SOLUTES IN CAPILLARY ELECTROCHROMATOGRAPHY

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## ABSTRACT

The retention mechanisms of neutral solutes were compared between electrically driven chromatography and pressure driven chromatography. The entropies of solute transfer from a mobile phase to a stationary phase were more negative in electrically driven chromatography than in pressure driven liquid chromatography. These differences can be attributed, in part, to the generation of heat which occurs during electro-driven chromatography and which causes significant differences between the set and the actual column temperature. Retention factors in electrochromatography are affected significantly by changes in electric field strength. These variations can be minimized by performing the experiments at elevated column temperatures. For example, when the column temperature was increased from 293 K to 333 K, the RSD for pentachlorobenzene was lowered from 7.3 % to 4.3 %. In addition the analysis time was shortened by approximately 45 %. Due to the critical effect of temperature on separation in electrochromatography, it is imperative that the column temperature be kept constant and measured accurately.

## INTRODUCTION

Optimal chromatographic performance is required for the analysis of complex samples or when only minute amounts of sample are available. Capillary electrochromatography (CEC) is emerging as a new micro separation technique, which combines the high efficiency of capillary electrophoresis with the versatile selectivity found in liquid chromatography. Theory[1-3] and applications[4-10] of CEC have been discussed in earlier papers. In CEC the capillary tubes are coated with stationary phase, or packed with octadecyl silanized silica gels. CEC differs from packed capillary and open tube capillary HPLC in that the eluent is driven thorough a column by an electric field rather than by pressure. The electrically generated flow produces minimal contribution to peak broadening.[1] Previous investigations[11,12] have shown that CEC offers better efficiency of separation than capillary LC. The two modes should offer equivalent selectivities for separation of a neutral compound. The separation of neutral analytes in CEC is based primarily on the solutes different partition ratios between a stationary phase and a mobile phase. For ionized analytes, electromigration as well as partitioning contribute to CEC selectivity. Electromigration of the solute in the mobile phase contributes to the overall k' as has been pointed out by Knox[1].



For analytes whose elution in CEC depend on partitioning and electromigration, the overall k' is given by [9]

$$k'_{CEC} = [(t_R - t_0)/ t_0] + (t_R / t_{eff}) \quad (1)$$

where $t_R$ and $t_0$ stand for the elution time and the electroosmotic time (elution time of a neutral unretained marker), respectively. The effective retention time of the analyte, $t_{eff}$, represents solute elution disregarding any contribution of solute partitioning between the stationary and mobile phase. It can be determined using equation 2

$$t_{eff} = t't_0/(t`-t_0) \quad (2)$$

The values of solute migration time, t', can be obtained from the CZE experiments.

The first term in eq. 1 can be approximated by the retention factor (k') determined by measuring the analyte retention time in pressure driven LC. The second term in eq. 1 is the contribution of the electrophoretic mobility to the overall solute retardation without any contribution of solute partitioning between the stationary and mobile phase. Recently, an equation was proposed[3] in which $k'_{CEC}$ was expressed as a combination of the solute retention factor (k') and the electrophoretic velocity factor ($k'_{CE}$)

$$k'_{CEC} = k' + k'_{CE}\,k' + k'_{CE} \quad (3)$$

where the product $k'_{CE}k'$ reflects simultaneous presence of chromatography and electrophoresis. The separate contributions of partitioning and of electromigration to overall solute retardation can be determined by performing parallel LC and CEC experiments under the same experimental conditions (stationary phase, mobile phase composition, and column temperature). The LC experiment provides values for the contribution of solute partitioning to the overall retention factor, $k'_{CEC}$. For neutral solutes (the limit of $k'_{CE} = 0$) the retention factor, $k'_{CEC}$, is determined by equation 4, and solute retention is solely a partition dependent process.

$$k'_{CEC} = k' = (t_R - t_0)/t_0 \quad (4)$$



## EXPERIMENTAL

The CEC and CZE experiments were performed on an HP$^{3D}$CE system Hewlett-Packard (Waldbronn, Germany) with built-in diode array detector. The system incorporates an HP$^{3D}$CE ChemStation for instrument control, data acquisition, and data analysis. External supply of air was obtained from Carba Gas (Basle, Switzerland) and was required to pressurize the outlet and the inlet vial (approximately 12 bar). The packed capillary columns used in this work had a 33.5 cm total column length, a 25 cm packed bed length, and 100 μm i.d. Columns packed with Hypersil C18 material, particle size 3 μm, were obtained from Hewlett-Packard (Waldbronn, Germany) and the columns packed with Grom-Sil 100 ODS1 were obtained from Stagroma (Wallisellen, Switzerland). The average particle size was 3 μm.

A SB-methyl-100 column (total column length 30.0 cm, effective column length 21.5 cm, 50 μm i.d., film thickness $d_f$ = 0.25 μm) was purchased from Lee Scientific (Salt Lake City, UT, USA). In CZE experiments, a column with 33.5 cm total column length and 50 μm i.d. Hewlett-Packard (Waldbronn, Germany) was used. The packed capillary columns were electroosmotically flushed with the run buffer (15-20 kV) for circa 30 min prior to the first injection, or after altering the experimental conditions. The open tubular columns were pressure flushed (10 mbar) with running buffer for 5 min prior to use and after changing the experimental conditions.

Throughout this work, electrokinetic (10 kV) sample injection was utilized, with an injection time of 3 seconds. The elution time of thiourea was used as a marker for the electroosmotic time ($t_0$). The mobile phase (1.5 mM phosphate buffer pH 7/acetonitrile, 7/3 v:v) was prepared from 50 mM phosphate buffer

**Table 1**

**Calculated Retention Factors (k') at Different Temperatures in CEC and LC Mode with Corresponding RSD (%) for n=3***

| Solute | 303K | | 308K | | 313K | | 318K | | 323K | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CEC | LC | CEC | LC | CEC | LC | CEC | LC | CEC | LC |
| Monochlorobenzene | 0.31 | 0.75 | 0.29 | 0.72 | 0.26 | 0.70 | 0.27 | 0.64 | 0.26 | 0.61 |
| RSD(%) | 0.83 | 0.36 | 0.75 | 0.31 | 0.66 | 0.83 | 0.80 | 0.10 | 0.47 | 0.23 |
| 1,4-Dichlorobenzene | 0.41 | 1.09 | 0.39 | 1.04 | 0.35 | 1.00 | 0.37 | 0.92 | 0.34 | 0.86 |
| RSD(%) | 0.91 | 0.60 | 0.80 | 0.23 | 0.57 | 0.78 | 0.81 | 0.10 | 0.56 | 0.30 |
| 1,3,5 Trichlorobenzene | 0.74 | 2.14 | 0.70 | 1.99 | 0.64 | 1.93 | 0.65 | 1.78 | 0.61 | 1.67 |
| RSD(%) | 0.67 | 2.00 | 1.22 | 0.27 | 0.31 | 1.98 | 1.07 | 0.10 | 0.81 | 0.25 |
| 1,2,4,5 Tetrachloro- | 0.86 | 2.63 | 0.82 | 2.44 | 0.75 | 2.35 | 0.76 | 2.20 | 0.71 | 2.06 |
| RSD(%) | 0.50 | 1.50 | 1.40 | 0.28 | 0.25 | 1.84 | 1.10 | 0.11 | 0.95 | 0.35 |
| Pentachlorobenzene | 1.17 | 3.71 | 1.22 | 3.54 | 1.02 | 3.35 | 1.03 | 3.25 | 0.95 | 3.02 |
| RSD(%) | 0.20 | 0.92 | 1.69 | 0.52 | 0.17 | 0.51 | 1.38 | 0.13 | 1.13 | 0.40 |
| Hexachlorobenzene | 1.53 | 5.31 | 1.48 | 4.97 | 1.33 | 4.68 | 1.34 | 4.38 | 1.23 | 4.05 |
| RSD(%) | 0.28 | 0.56 | 1.91 | 0.39 | 0.19 | 0.34 | 1.72 | 0.05 | 1.33 | 0.34 |

* Column, 33.5 cm (total length) x 100 μm i.d., Hypersil C18, $d_p$ = 3mm. Mobile phase: 1.5mM phosphate buffer pH = 7/ACN (7:3 v/v). Temperature range from 303K to 323K.



pH 7 from Hewlett-Packard (Waldbronn, Germany) and HPLC grade acetonitrile from Merck (Darmstad, Germany). The detection wavelength was 210 nm. The chlorobenzenes were obtained from Aldrich-Chemie (Steiheim, Germany) or Fluka (Buchs, Switzerland). The instrumental set-up utilized for capillary LC has been described previously[14].

## DISCUSSION

Table 1 shows the retention factors for the chlorobenzene solutes obtained with a Hypersil C18 column, mobile phase: 1.5 mM phosphate buffer pH = 7/acetonitrile (7:3 v/v) in LC and CEC mode, and in the temperature range from 303 K to 323 K. In the CEC mode, the applied electric field strength was kept constant at 0.9 kV/cm. In these experiments, the relative standard deviation for the solute retention factors in pressure and electrically driven chromatography never exceeded 2.0 per cent (n =3).

The Gibbs free energy $\Delta G$ of solute transfer from a mobile phase to a stationary phase in electrically driven chromatography or pressure driven chromatography can be calculated using eqn. 5

$$\Delta G = -RT \log k' \qquad (5)$$

**Table 2**

**Retention Enthalpies, ΔH (kJ/mol), and the Free Energies at 303K, ΔG (kJ/mol), in CEC and LC Mode**

| Solute | $-\Delta H_{CEC}$ (kJ/mol) | $-\Delta H_{LC}$ (kJ/mol) | $\Delta G_{CEC}$ (kJ/mol) | $\Delta G_{LC}$ (kJ/mol) |
|---|---|---|---|---|
| Monochlorobenzene | 7.0 ± 2.0 | 9.2 ± 0.8 | 2.96 ± 0.02 | 0.72 ± 0.02 |
| 1,4 Dichlorobenzene | 7.0 ± 1.8 | 10.2 ± 0.8 | 2.21 ± 0.02 | -0.21 ± 0.01 |
| 1,3,5 Trichlorobenzene | 7.2 ± 1.4 | 10.3 ± 0.6 | 0.77 ± 0.05 | -1.91 ± 0.62 |
| 1,2,4,5 Tetrachlorobenzene | 7.7 ± 1.3 | 10.3 ± 0.6 | 0.38 ± 0.02 | -2.43 ± 0.61 |
| Pentachlorobenzene | 8.3 ± 1.3 | 9.4 ± 0.7 | -0.40 ± 0.01 | -3.34 ± 0.31 |
| Hexachlorobenzene | 8.6 ± 1.4 | 11.4 ± o.5 | -1.08 ± 0.03 | -4.20 ± 0.21 |



where R is the universal gas constant, T is column temperature and k' is retention factor gathered by CEC or LC. The values for Gibbs free energy at 303 K, obtained in CEC and LC experiments ($\Delta G_{CEC}$ and $\Delta G_{LC}$, respectively) are listed in Table 2. For neutral solutes, whose retardation is primarily dependent on partitioning, the retention factors obtained from CEC experiments should be the same as the retention factors calculated by measuring analyte retention time in pressure driven LC under the same experimental conditions. In LC experiments, the $\Delta G_{LC}$ values were negative for all solutes excluding monochlorobenzene. In the CEC mode, the solutes showed a positive change in free energy of transfer. The only exceptions were pentachlorobenzene and hexachlorobenzene, which exhibited a small negative ΔG. Therefore, solute transfer from the mobile phase to the stationary phase in CEC mode is less favorable than in LC mode. Independent CZE experiments provided the only means of determining the contribution of solute electromigration to the free energy of solute transfer in CEC. In the CZE experiments, all six chlorobenzenes were migrating with electroosmotic flow ($t_o$), rendering $k'_{CE}$ equal to zero and transposing equation 3 into equation 4. In view of these results, solute electromigration can be dismissed as an additional mechanism acting in solute retardation in CEC. Solute partitioning between the mobile and stationary phase is the only mechanism responsible for the retention of neutral solutes in CEC. Retention mechanisms in CEC and HPLC were examined in greater detail by estimating increment per chlorine atom in the homologous series of chlorobenzenes. The mean selectivity for the homologous series, $\bar{\alpha}$ was calculated using equation 6,

$$\bar{\alpha} = \sum_{1}^{N} \alpha_n / N \qquad (6)$$

where N is the number of series tested (in our case 6) and $\alpha_n$ is the contribution of each additional chlorine atom in the successive homologues series of chlorobenzenes. $\alpha_n$ can be calculated by the following equation[15]

$$\alpha_n = [(k'_n/k'_{n-1})(k'_{n+1}/k'_n)]^{1/2} \quad (7)$$

where $k'_n$, $k'_{n-1}$ and $k'_{n+1}$ represent retention factors of chlorobenzenes in the homologous series (n = number of chlorine atoms). For the chlorobenzene solutes, partitioning should determine separation in both CEC and LC modes and the values for $\overline{\alpha}$ should be the same in both modes. When the experiments were carried out at 303K, $\overline{\alpha}$ of 1.5 and 1.2 were calculated for LC and CEC modes, respectively. This difference in selectivity between the two chromatographic modes can be attributed to a temperature effect in CEC mode and will be discussed later.

The temperature dependence of k' can be expressed by van't Hoff equation

$$\log k' = -\Delta H/RT + \Delta S/R + \log \Phi \quad (8)$$

where R is the universal gas constant, $\Phi$ is the phase ratio, and $\Delta H$ is the standard enthalpy change, and $\Delta S$ is the standard entropy change of a solute transfer from a mobile phase to a stationary phase. The $\Delta H$ values for six chlorobenzenes in pressure driven and electroosmotically driven chromatography were calculated from the linear regression of the plots log k' vs. 1/T. If the heat capacity change upon transfer is zero, and the phase ratio is independent of temperature the relationship of log k' versus the reciprocal of absolute temperature is linear. For the temperature range used in these experiments, it can be assumed that both the phase ratio, $\Phi$, and the heat capacity are essentially independent of temperature. Table 2 lists the calculated enthalpies along with the corresponding relative standard deviations in percent. The latter were calculated using standard error propagation techniques from the uncertainties of the slope provided by linear regression. The entropies for all analytes were negative in both CEC and LC modes. It is reasonable to expect that pressure- and electrically-driven chromatography will give the same values for solute enthalpy.

In chromatography, the partitioning process should be independent of the means by which the solvent is moved through the column (pressure or electrically driven flow). We found that a small difference in entropy (for all solutes) exists in the two chromatographic modes. The $\Delta H$ values were more negative in LC experiments. The change in stationary phase under strong electric field[18,19] cannot be ruled out as one of the sources of the discrepancy in the thermodynamic functions determined by LC and CEC. Tsuda[19] postulated that in electrochromatography the reproducibility of the solute retention time and its

peak height depend on the length of time the voltage is applied. He assumed that the time delay to achieve a constant retention may be linked to the time it takes the applied electric field to modify the stationary phase.

**Temperature Effect**

Solute electrophoretic mobility and solute partitioning in CEC are temperature dependent processes. Temperature affects the pH of the buffer, the viscosity of the mobile phase, and the equilibrium constants of partitioning processes. It has been shown that temperature has a strong influence on solute retention in pressure driven chromatography.[21] Wheeler et al.[22] reported a second order phase transition in octadecylsilica, a commonly used stationary phase in LC and CEC. Phase transitions of this sort occur over a wide temperature range and do not affect all solutes the same way.



Generation of heat during the analysis is a serious drawback in CEC [16]. Heat is generated within the column during the run by the flow of current through the electrolyte in the column, and is given as[20]

$$Q=E^2\lambda c\varepsilon \qquad (9)$$

where E is the applied electric field strength, $\lambda$ is the equivalent conductivity, c is the molar concentration of electrolyte, and $\varepsilon$ is the total porosity of the packed bed. According to equation 9, the heat generated is proportional to the square of the applied electric field. In CEC, E should be in the range of 1 kV/cm to generate a high electroosmotic flow and produce short run times, though a high electric field will generate a significant amount of heat in the column. To keep the temperature constant and uniform throughout the column, the rate of heat generation within the column must equal the total amount of energy leaving the column at the column-coolant interface. Due to slow radial heat transfer through the column and the cooling medium, the heat generated during the CEC run causes the column temperature to rise above the set temperature.[16,17,23,24] The thermal gradient (radial and axial temperature gradient) depends on the inner radius of the column, the thickness of the tube wall, the nature of the packed bed inside the tube, the thickness of the polyimide coating, and the heat transfer to the surroundings. The excess temperature at the center of the tube and tube walls can be expressed as[20]

$$\Delta T = Qd_c^2/16K = E^2\lambda c\varepsilon d_c^2/16K \qquad (10)$$

where Q is the rate of heat generated per unit volume within the cylinder and K is the thermal conductivity of the medium surrounding the separation column. In the presently available commercial CE instruments only a part of the

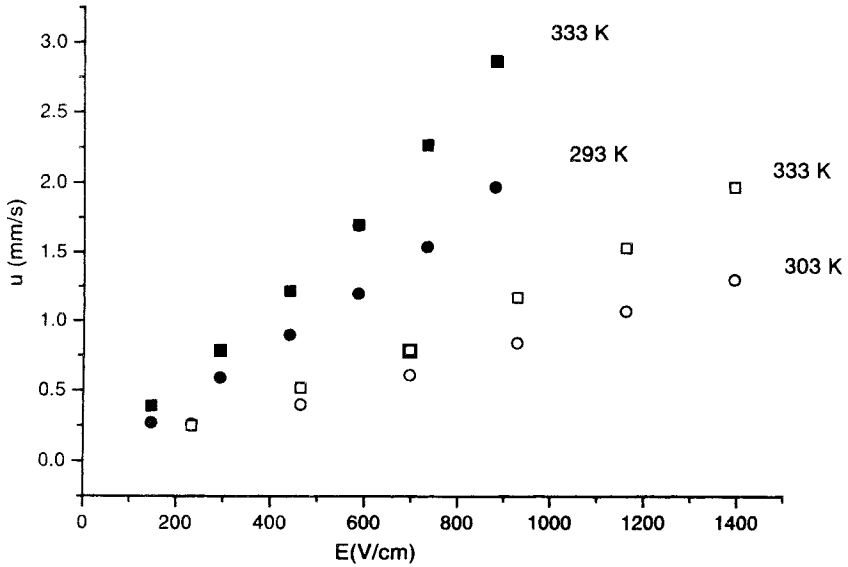


**Figure 1**. The dependence of linear velocity (mm/s) on applied electric field strength (V/cm). Column: Grom-Sil 100 ODS1, particle size 3 μm, total column length = 33.5 cm, packed bed length = 25 cm, i.d. = 100 μm; ); Mobile phase: 1.5 mM phosphate buffer pH 7/acetonitrile (7/3 v:v); Column set temperature 333 K (■) and 293 K (●). Column: SB-methyl-100, total column length = 30.0 cm, effective column length = 21.5 cm, i.d. = 50 μm, $d_f$ = 0.25 μm; Mobile phase: 1.5 mM phosphate buffer pH 7/acetonitrile (6/4 v:v); Column set temperature 333 K (□) and 303 K (O).



separation column is thermostated, i.e. the part of the separation column within the cartridge. The ends of the capillary which stick out of the cartridge and are immersed in the running buffer reservoirs are not thermostated. In the case of 25 cm packed capillary approximately 8 cm of the packed bed (33 % of the total packed bed length) on the injection side of the instrument is not temperature controlled. This part of the capillary column will not be affected by a change in set temperature on the CEC instrument. A non-uniform temperature profile in the separation column contributes to the discrepancy between thermodynamic data obtained in CEC and LC experiments.

Accurate temperature control of the capillary and precise measurement of column temperature are an important issues in CEC, because of their influence on the reproducibility of the retention factors. In order to examine the interrelationship between k' and electroosmotic flow with the variation of applied electric field, a set of CEC experiments were done using a packed capillary column and a coated capillary column.

Electroosmotic flow is proportional to applied field strength (E)

$$u_{EOF} = \varepsilon_r \varepsilon_0 \xi E/\eta \quad (11)$$

where $\varepsilon_0$ is the permittivity of a vacuum, $\varepsilon_r$ and $\eta$ are the dielectric constant and viscosity of the mobile phase and $\xi$ is the zeta potential. Figure 1 depicts the increase in electroosmotic flow when the applied electric field strength is increased in a packed capillary (at 293 K and 333 K) and in a coated tube (at 303 K and 333 K). If fast separation is required, one should not only work at high field strengths, but also at elevated temperature. In equation 11, the ratio $\varepsilon_r/\eta$ is temperature dependent. The $u_{EOF}$ increases with decrease of mobile phase viscosity. When the column temperature was increased (at the same applied electric fields strengths, 0.9 kV/cm) from 293 K to 333 K, the $u_{EOF}$ increased approximately 50 %. Most of the observed increase can be ascribed to the decrease in viscosity of the mobile phase.



It was expected that only the solute retention times would change as a consequence of a change of electroosmotic flow, and that the solute retention factors would be independent of applied electric field strength. Tables 3 and 4 show the k' values for the chlorobenzene solutes obtained at several electric field strengths and at two temperatures in coated capillaries (SB-Methyl-100) and in packed capillaries (stationary phase Grom-Sil 100 ODS1). The retention factors were reproducible, (RSD $\leq$ 2.5 %) over a series of injections ($3 \leq n \leq 6$) at all electric field strengths and at all temperatures. Significantly higher RSDs were obtained when the k' values were averaged over the whole range of different applied electric field strengths (from 147 V/cm to 882 V/cm for a packed capillary column and 233 V/cm to 1395 V/cm for a coated capillary column, tables 3 and 4, respectively). For the packed capillary column the following values were calculated: 5.5 RSD % at 293 K and 3.44 RSD % at 333 K for the early eluting monochlorobenzene, RSD 7.3 % (at 293 K) and RSD 4.29 % (at 333 K) for the late eluting pentachlorobenzene. In the CEC experiments with coated capillary, the following values were calculated for monochlorobenzene 5.1 RSD % at 303 K, 2.2 RSD % at 333 K, while for pentachlorobenzene the RSDs were 8.4 % at 303 K and 3.2 % at 333 K. The values for the solute retention factors in the packed capillary column decreased about 15 % (column temperature 293 K) and 7% (column temperature 333 K) when the values for electric field strengths of 147 V/cm and 882 V/cm were taken into consideration.

It was difficult to determine the general trend of the change in retention factor versus applied electric field strength for coated capillaries. It is highly probable that at 303 K, the stationary phase is not completely liquid-like (the lower limit of the application range of SB-methyl 100 phase is around 303 K). An increase in the applied electric field raises the temperature inside the column,

## Table 3

### k' Values at Various Applied Electric Fields (kV/cm)*

| E (V/cm): | 233 | | 465 | | 698 | | 930 | | 1163 | | 1395 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T (K): | 333 | 303 | 333 | 303 | 333 | 303 | 333 | 303 | 333 | 303 | 333 | 303 | 333 | 303 |
| U (mm/sec): | 0.25 | 0.26 | 0.52 | 0.40 | 0.79 | 0.61 | 1.17 | 0.84 | 1.53 | 1.07 | 1.97 | 1.30 | | |
| | n = 3 | n = 3 | n = 5 | n = 3 | n = 6 | n = 3 | n = 4 | n = 3 | n = 4 | n = 3 | n = 3 | n = 4 | RSD (%) | RSD (%) |
| Solute | | | | | | | | | | | | | n = 25 | n = 19 |
| Monochloro-benzene | 0.17 | 0.21 | 0.18 | 0.20 | 0.17 | 0.20 | 0.17 | 0.19 | 0.17 | 0.19 | 0.17 | 0.20 | 2.16 | 5.14 |
| | 1.24% | 3.91% | 1.70% | 0.72% | 2.58% | 0.97% | 0.62% | 1.36% | 0.96% | 0.50% | 1.64% | 0.62% | | |
| 1,4-Dichloro-benzene | 0.29 | 0.39 | 0.31 | 0.37 | 0.30 | 0.35 | 0.29 | 0.35 | 0.29 | 0.34 | 0.30 | 0.36 | 2.42 | 5.87 |
| | 2.17% | 4.40% | 1.42% | 1.78% | 1.67% | 1.39% | 0.57% | 0.77% | 1.03% | 0.53% | 1.78% | 1.10% | | |
| 1,3,5-Trichloro-benzene | 0.71 | 0.96 | 0.76 | 0.95 | 0.71 | 0.88 | 0.71 | 0.87 | 0.71 | 0.84 | 0.72 | 0.90 | 2.54 | 6.49 |
| | 2.35% | 4.91% | 1.61% | 3.50% | 1.05% | 1.70% | 0.67% | 0.44% | 1.39% | 0.57% | 1.56% | 0.97% | | |
| 1,2,4,5-Tetrachloro-benzene | 0.85 | 1.19 | 0.91 | 1.18 | 0.86 | 1.08 | 0.85 | 1.07 | 0.85 | 1.04 | 0.86 | 1.10 | 2.82 | 6.89 |
| | 2.41% | 5.28% | 1.96% | 4.37% | 1.10% | 1.29% | 0.81% | 1.04% | 1.71% | 0.95% | 1.50% | 1.02% | | |
| Pentachloro-benzene | 1.31 | 1.94 | 1.42 | 1.94 | 1.32 | 1.72 | 1.31 | 1.72 | 1.30 | 1.64 | 1.33 | 1.74 | 3.21 | 8.39 |
| | 2.72% | 5.40% | 2.19% | 6.08% | 1.14% | 1.43% | 0.52% | 2.22% | 1.42% | 0.71% | 1.95% | 0.65% | | |

* Effective column length, 21.5 cm, stationary phase, SB-Methyl 100, $d_f$ = 0.25μm, i.d. 50 μm, o.d. 375 μm, column set temperature 303 K, mobile phase: 1.5 mM phosphate buffer pH 7.0/ACN (6:4 v/v), detection 210 nm.

## Table 4

### k' Values at Various Applied Electric Fields (kV/cm)*

| E (V/cm): | 147 | | 294 | | 441 | | 588 | | 735 | | 882 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T (K): | 333 | 293 | 333 | 293 | 333 | 293 | 333 | 293 | 333 | 293 | 333 | 293 | 333 | 293 |
| U (mm/sec): | 0.39 | 0.27 | 0.79 | 0.59 | 1.22 | 0.90 | 1.70 | 1.20 | 2.27 | 1.54 | 2.88 | 1.97 | | |
| | n = 3 | n = 3 | n = 3 | n = 4 | n = 3 | n = 4 | n = 3 | n = 3 | n = 4 | n = 3 | n = 6 | n = 3 | RSD (%) | RSD (%) |
| Solute | | | | | | | | | | | | | n = 22 | n = 20 |
| Monochloro-benzene | 1.05 | 1.45 | 0.98 | 1.31 | 1.02 | 1.35 | 1.00 | 1.40 | 0.95 | 1.48 | 1.00 | 1.28 | 3.44 | 5.55 |
| | 1.20% | 1.19% | 2.92% | 1.81% | 0.07% | 0.90% | 0.66% | 1.45% | 1.26% | 0.53% | 1.35% | 1.67% | | |
| 1,4-Dichloro-benzene | 1.43 | 2.06 | 1.33 | 1.83 | 1.38 | 1.87 | 1.36 | 1.99 | 1.27 | 2.09 | 1.35 | 1.77 | 3.68 | 6.26 |
| | 0.65% | 0.48% | 2.59% | 1.31% | 0.17% | 0.35% | 0.79% | 2.05% | 1.39% | 0.33% | 0.64% | 1.71% | | |
| 1,3,5-Trichloro-benzene | 2.44 | 3.58 | 2.27 | 3.16 | 2.36 | 3.22 | 2.31 | 3.44 | 2.15 | 3.63 | 2.28 | 3.07 | 3.97 | 6.55 |
| | 0.62% | 0.95% | 2.20% | 0.88% | 0.34% | 0.46% | 0.79% | 2.19% | 1.32% | 0.81% | 0.49% | 1.30% | | |
| 1,2,4,5-Tetrachloro-benzene | 2.92 | 4.39 | 2.71 | 3.80 | 2.82 | 3.92 | 2.76 | 4.22 | 2.56 | 4.43 | 2.72 | 3.73 | 4.12 | 6.99 |
| | 0.88% | 0.46% | 2.13% | 0.24% | 0.39% | 0.50% | 0.82% | 2.33% | 1.42% | 0.85% | 0.53% | 1.48% | | |
| Pentachloro-benzene | 3.98 | 6.16 | 3.69 | 5.33 | 3.84 | 5.44 | 3.75 | 5.88 | 3.46 | 6.20 | 3.70 | 5.16 | 4.29 | 7.29 |
| | 0.84% | 0.45% | 1.85% | 0.70% | 0.55% | 0.35% | 0.87% | 2.60% | 1.49% | 1.41% | 0.54% | 1.50% | | |

* Column: Chrom-Sil 100, ODS1 PE, 3μm, 25 cm packed bed, 34 cm total length, i.d. 100 μm, o.d. 375 μm, column set temperature 293 K, mobile phase: 1.5 mM phosphate buffer pH 7.0/ACN (6:4 v/v), detection 210 nm.

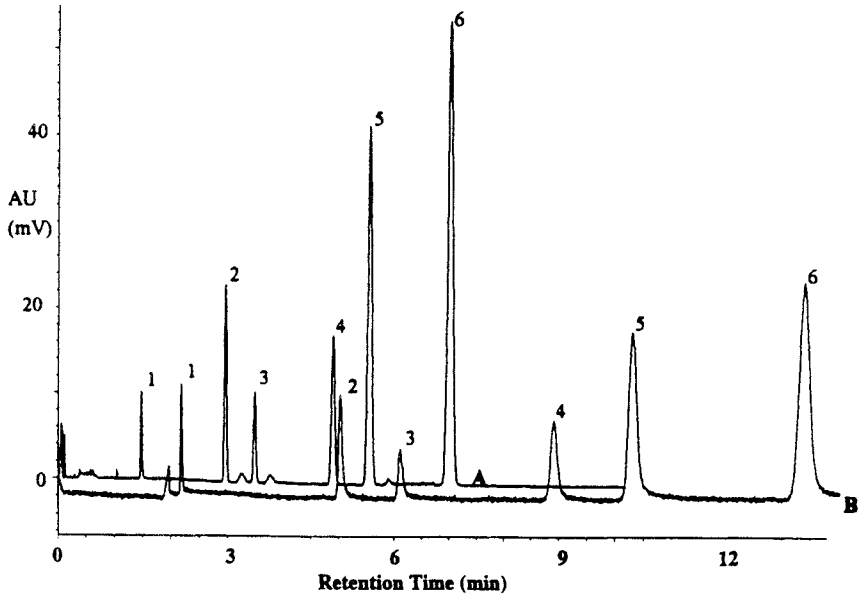


**Figure 2**. Separation of a mixture of chlorobenzenes in CEC at 333 K (trace A) and 293 K (trace B). Column: Grom-Sil 100 ODS1, particle size 3 μm, total column length = 33.5 cm, packed bed length = 25 cm, i.d. = 100 μm. Mobile phase: 1.5 mM phosphate buffer pH 7/acetonitrile (7/3 v:v). UV detection at 210 nm. Peak identification: 1) thiourea, 2) monochlorobenzene, 3) 1,4 dichlorobenzene, 4) 1,3,5 trichlorobenzene, 5) 1,2,4,5 tetrachlorobenzene, 6) pentachlorobenzene.



causing an increase in solute retention. Induced temperature increase within the column may liquify the thin film stationary phase (total film thickness 0.25 μm), thus further increasing the amount of the stationary phase available to the solutes. For both packed and coated columns, the relative standard deviation is significantly lower at higher temperatures.

The primary purpose of thermostatically controlling capillary temperature in CEC is the removal of Joule heat, but temperature control can also be used as a parameter to optimize CEC separation.

Figure 2 depicts the separation of five chlorobenzenes at 333 K (trace A) and at 293 K (trace B). The degree of solute retention was easily adjusted by increasing the column temperature. At 333 K, the run time was 45 per cent shorter than at 293 K.

## CONCLUSION

Our results indicate that solute retention in CEC is easily altered by changing the temperature. Precise adjustment of the column temperature can be used as a parameter to tune selectivity in CEC separations. Accurate temperature control of the total column length and precise temperature measurement are vital for obtaining reproducible retention factors in CEC mode.

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