ELSEVIER

Journal of Chromatography A, 922 (2001) 277–282

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Migration of neutral solutes by double stepwise gradient elution in capillary electrochromatography

Weibing Zhang[a], Lihua Zhang[a,b], Guichen Ping[a], Yukui Zhang[a,*], A. Kettrup[b]

[a]*National Chromatographic R. and A. Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116011, China*

[b]*Institute of Ecological Chemistry, GSF-National Research Center for Environment and Health, D-85764 Neuherberg, Germany*

Received 4 December 2000; received in revised form 10 April 2001; accepted 11 April 2001

Abstract

Characteristics of electroosmotic flow (EOF) and the migration of neutral solutes under double stepwise gradient elution in capillary electrochromatography were studied systematically. EOF velocity proved to be the function of operation time changing with the introduction of the second mobile phase. Accordingly, the retention of components also changed. The migration of neutral solutes was studied under the following three situations; A, components eluted when the column was filled only with the first kind of mobile phase; B, solutes eluted still in the first kind of mobile phase while at that time two kinds of mobile phase coexisted in the column and C, samples eluted in the second kind of mobile phase. Equations to describe the retention times of components under these three kinds of conditions were deduced and applied to predict the retention times of 12 aromatic compounds. Relative errors between experimental and calculated values were below 5.0%, which proved the reliability of the equations. In addition, parameters that might affect the retention time of solutes, such as the transferring time of mobile phase vials, the capacity factors of components and EOF velocities two steps were studied systematically

Keywords: Electrochromatography; Gradient elution; Electroosmotic flow

1. Introduction

Capillary electrochromatography (CEC), as the hybrid of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), has the advantages of high resolution, high efficiency, short analysis time, low consumption of solvent and samples and so on [1–5]. Consequently, in the last 10 years, it has attracted more and more attention from scientists in environmental science [6–8], pharmaceutical industry [9–11] and biological chemistry [12–15].

Up to now, isocratic elution of CEC has commonly been used in the analysis of neutral compounds [16–18]. However, in order to separate complex samples in a short time without losing resolution, different kinds of gradient elution, such as gradient elution of mobile phase [19–22], operation voltage [23–25] and temperature [26], were proposed. Among them, stepwise gradient elution of mobile

*Corresponding author. Tel.: +86-411-369-3427; fax: +86-411-369-3427.

E-mail address: ykzhang1@online.ln.cn (Y. Zhang).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00876-7

phase on commercial CE instruments was proved to be easily realized with high accuracy, which had been successfully applied to the analysis of pharmaceuticals [27], DNA additives [28], aromatic compounds [29] and so on. However, theoretical study of component migration of under stepwise gradient mode has not been reported.

In this paper, the deduction of equations to describe EOF velocity and the retention times of neutral solutes under double stepwise gradient elution and different situation is given. In addition, based on these equations, the prediction of the retention times of components was realized and the effects of parameters studied.

2. Experimental

2.1. Instruments

All experiments were performed on a P/ACE 5510 system with System Gold software (Beckman, Fullerton, CA, USA). An SP-8800 pump (Spectra-Physics, San Jose, CA, USA) was used to prepare CEC columns.

2.2. Materials and chemicals

Spherisorb of 3 μm ODS and silica gel of 5 μm were obtained from Phase Separations (Norwalk, NJ, USA). Fused-silica capillaries (75 μm I.D.×365 μm O.D.) were purchased from Yongnian Optic Fiber Plant (Hebei, China).

Methanol and acetonitrile (Yuwang Chemical Plant, Shandong, China) were of chromatographic grade. Trihydroxymethylaminomethane (Tris) (Shanghai No. 1 Chemical Plant, Shanghai, China), thiourea (Shenyang Chemical Plant, Liaoning, China) and all aromatic compounds were of analytical grade. Double-deionized water was purified by Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Column preparation and separation conditions

CEC columns were prepared by slurry methods as reported previously [30,31].

The length of packed bed of CEC columns was 20 cm with the total length of 27 cm. Two mobile phases were used: methanol–water (60:40, v/v) (mobile phase 1) and acetonitrile–water (80:20, v/v) (mobile phase 2); the concentration of Tris was 4 m*M* with a pH of 8.70. Electrokinetic injection was utilized at 1 kV, 1 s.

Separation voltage was set at 20 kV and UV detection wavelength was 200 nm.

2.4. Procedure of gradient elution

Gradient elution was realized by changing the mobile phase vials automatically under the control of System Gold software. Compounds were first separated in mobile phase 1 for 23.5 min at 20 kV. Then the operation voltage was decreased to zero and both ends of the column were transferred to vials with the mobile phase 2. After a pause of 0.17 min, the voltage was resumed to 20 kV and the separation was continued.

3. Theory

3.1. Variation of the EOF velocity under gradient elution

Different from that in HPLC, the velocity of the mobile phase changed in stepwise gradient elution in CEC with the change of permittivity and viscosity of the mobile phase.

Due to the plug-like flow velocity profile of CEC, it could be considered that the two kinds of mobile phase did not mix at their interface. Furthermore, since the sectional area of mobile phase in the open tubular part of a packed column was larger than that in the packed bed, the resistance distribution in the open part was negligible. So only the contribution of two mobile phase zones to the velocity of EOF was taken into consideration in our work.

According to the principle of resistance distribution, EOF velocity in a CEC column with the coexistence of two kinds of mobile phase could be described by Eq. (1):

$$u_{eo} = (1 - l/l_0)u_{eo1} + l/l_0 \cdot u_{eo2} \quad (1)$$

where u_{eo1} and u_{eo2} are the EOF velocities in the two kinds of mobile phase; l_0 is the length of packed bed;

($l_0 - l$) and l are the zone lengths of mobile phase 1 and 2 under gradient elution, respectively.

In our experiments, the column was first filled with mobile phase 1, and then mobile phase 2 was introduced. After the entering time of t, the zone length of mobile phase 2 in the column (l) should be as follows:

$$l = \int_0^t u_{eo}\, d\tau \tag{2}$$

where τ is integral constant, t is the duration when the mobile phase was introduced into the capillary column.

With the combination of Eqs. (1) and (2), the following results could be obtained:

$$u_{eo} = u_{eo1} \exp(t/t)^* \tag{3}$$

$$l = u_{eo1}\, t^* [\exp(t/t^*) - 1] \tag{4}$$

$$t^* = l_0/(u_{eo2} - u_{eo1}) = t_{02}/(1 - t_{02}/t_{01}) \tag{5}$$

where t_{01} and t_{02} are the dead time in mobile phases 1 and 2.

From Eq. (3), it can be seen that the EOF velocity was a function of the time when mobile phase 2 was introduced. When the whole column was filled with mobile phase 2, that means $l = l_0$, the time when mobile phase 1 was completely replaced by 2 (t_f) could be obtained:

$$t_f = t^* \ln\frac{t_{01}}{t_{02}} \tag{6}$$

Accordingly, an integrative equation to describe the EOF velocity under double stepwise gradient elution of CEC is obtained as follows:

$$\begin{aligned} u_{eo} = {} & H(t - t_1)u_{eo1} - [H(t - t_1) \\ & - H(t - t_1 - t_f)]u_{eo1} \exp[(t - t_1)/t^*] \\ & + [1 - H(t - t_1 - t_f)]u_{eo2} \end{aligned} \tag{7}$$

where t_1 is the operation time of the first step. $H(\Delta t)$ is Heaviside function, a special function, $H(\Delta t) = 1$, when $\Delta t < 0$ and $H(\Delta t) = 0$, when $\Delta t > 0$.

3.2. Migration of neutral solutes under gradient elution

3.2.1. Migration of components in the first step of gradient elution

In the first step, the column was completely filled with mobile phase 1 and the migration of solutes was the same as under isocratic elution. The velocity of solutes (u) could be calculated according to Eq. (8):

$$u = \frac{u_{eo1}}{1 + k_1'} \tag{8}$$

where k_1' was the capacity factor of the component in mobile phase 1.

If the capacity factor of the component was small, it could be eluted in t_1 with the retention time of t_R:

$$t_R = t_{01}(1 + k_1') \tag{9}$$

If the component could not be eluted in mobile phase 1, the distance it passed (l_1) could be calculated by Eq. (10):

$$l_1 = \frac{u_{eo1} t_1}{1 + k_1'} \tag{10}$$

3.2.2. Migration of components in the second step of gradient elution

From the beginning of the second step of gradient elution, mobile phase 2 was introduced into the column. According to Eq. (7), the velocity of EOF kept on changing till all the column was filled with mobile phase 2.

If the time it took for mobile phase 2 to catch up with sample zone was set to be t_c, when $t_1 < t_R < (t_1 + t_c)$, the component was still eluted in mobile phase 1. Different from the situation discussed above, at this time two kinds of mobile phase coexisted in the column. The retention time of solutes should fit the following equation:

$$\int_{t_1}^{t_R} \frac{u_{eo}}{1 + k_1'}\, dt + \frac{u_{eo1} t_1}{1 + k_1'} = l_0 \tag{11}$$

According to Eq. (7), we could obtain the retention time of components with Eq. (12):

$$t_R = t_1 + t^* \ln\{[(1 + k_1')t_{01} - t_1]/t^* + 1\} \tag{12}$$

If the component had not been eluted before it was caught up by mobile phase 2, its position in the column (l_2) should be equal to the distance that mobile phase 2 passed. Therefore,

$$l_2 = \int_{t_1}^{t_1=t_c} u_{eo}\,dt = \frac{u_{eo1}t_1}{1+k_1'} + \int_{t_1}^{t_1+t_c} \frac{u_{eo}}{1+k_1'}\,dt = \frac{u_{eo1}t_1}{k_1'} \quad (13)$$

that means that the distance that the component passed was independent of the nature of the second mobile phase.

According to Eq. (13), t_c could be obtained:

$$t_c = t^* \ln\left(1 + \frac{t_1}{k_1' t^*}\right) \quad (14)$$

When the component was caught up by mobile phase 2, it would be eluted in that phase. Since the velocity of solute in the column was less than that of EOF, it would not be eluted before the column was filled with mobile phase 2. Accordingly, the position of the uneluted component (l_3) when $t > (t_1 + t_f)$ could be calculated with Eq. (15):

$$l_3 = \frac{u_{eo1}t_1}{k_1'} + \int_{t_1+t_c}^{t_1+t_f} \frac{u_{eo}}{1+k_2'}\,dt \quad (15)$$

The velocity of solutes should be:

$$u = \frac{u_{eo2}}{1+k_2'} \quad (16)$$

and the retention times of components eluted in mobile phase 2 could be obtained:

$$t_R = t_1 + t_f - t^* + (1+k_2')t_{02} + \frac{t_{02}(k_1' t^* - k_2' t_1)}{t_{01}k_1'} \quad (17)$$

4. Results and discussion

Based on the discussion above, the retention times of solutes under double stepwise gradient elution of CEC could be calculated.

Twelve aromatic compounds were separated, respectively, under isocratic elution with 60% methanol and 80% acetonitrile in the mobile phase. Under the former situation, baseline separation of all components was obtained in 114.96 min, while under the latter one, although the total analysis time was shortened to 16.55 min, some of the components were coeluted. So double stepwise gradient elution was utilized in order to shorten the analysis time without losing resolution (as shown in Fig. 1).

From the results under isocratic elution, t_{01} and t_{02} were obtained to be 8.00 and 3.50 min, respectively. t^* and t_f could be calculated according to Eqs. (5) and (6) with the results of 6.22 and 5.14 min, respectively. At the same time, capacity factors of each compound in mobile phase 1 and 2 could be obtained and t_c of each component could be calculated according to Eq. (14). The results are shown in Table 1.

Since the mobile phase vials were transferred at 23.5 min, from Table 1 it could be concluded that the first five components were eluted before mobile phase 2 was introduced into the column. Their retention times could be calculated according to Eq. (9). It should be pointed out that after the mobile phase vials were transferred, the pause time of 0.17 min should be considered when retention of the rest were studied. Since the components of 6 and 7 were eluted between the time t_1 (23.5 min) and ($t_1 + t_f +$ 0.17), their retention times should be calculated

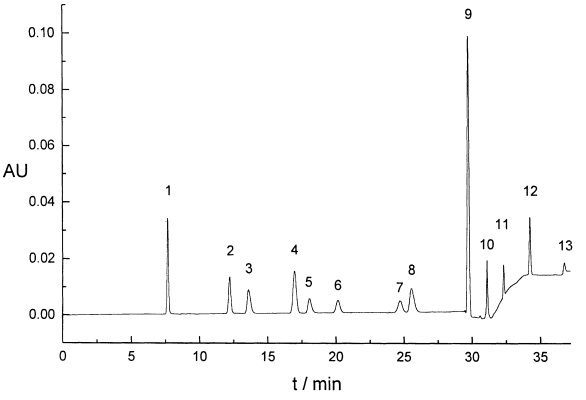

Fig. 1. Separation of 12 aromatic compounds under double stepwise gradient elution. For conditions see the Experimental section. Peaks: 1 = thiourea; 2 = aniline; 3 = phenol; 4 = phenylethyl alcohol; 5 = benzonitrile; 6 = acetophenone; 7 = phenylpropyl alcohol; 8 = nitrobenzene; 9 = benzene; 10 = toluene; 11 = ethylbenzene; 12 = *n*-propylbenzene; 13 = *n*-butylbenzene.

Table 1
t_0 of each component under double stepwise gradient elution

Component	Capacity factor		t_c (min)
	60% Methanol	80% Acetonitrile	
Aniline	0.67	0.26	11.80
Phenol	0.75	0.34	11.16
Phenylethyl alcohol	1.18	0.47	8.91
Benzonitrile	1.32	0.52	8.41
Acetophenone	1.59	0.63	7.55
Phenylpropyl alcohol	2.16	0.80	6.29
Nitrobenzene	2.27	1.29	6.09
Benzene	3.12	1.35	4.94
Toluene	3.69	1.75	4.38
Ethylbenzene	7.24	2.17	2.61
n-Propylbenzene	10.47	2.52	1.91
n-Butylbenzene	12.88	3.43	1.59

For conditions, see the Experimental section.

according to Eq. (12). In addition, the rest of the components were eluted later than $(t_1 + t_f + 0.17)$ (28.91 min), Eq. (17) should be taken to calculate their elution times.

Based on the discussion above, the retention time of each component was predicted (shown in Table 2). From the results it could be seen that the relative errors of the retention times of components between the experimental and calculated values were below 5%, that means the equations deduced above, prediction of the retention of neutral solutes under double stepwise gradient elution could be realized by the equations deduced above with good accuracy.

Table 2
Prediction on the retention times of each component under double stepwise gradient elution

Component	t_r(exp)	t_r(cal)	Relative error (%)
Aniline	12.53	12.58	−0.40
Phenol	14.00	14.05	−0.36
Phenylethyl alcohol	17.75	17.52	1.30
Benzonitrile	18.62	18.69	−0.38
Acetophenone	20.76	20.84	−0.39
Phenylpropyl alcohol	25.23	25.28	−0.20
Nitrobenzene	25.87	26.24	−1.43
Benzene	29.00	29.93	−3.21
Toluene	29.54	30.93	−4.71
Ethylbenzene	31.44	31.93	−1.56
n-Propylbenzene	32.61	32.93	−0.98
n-Butylbenzene	34.60	33.93	1.94

For conditions see the Experimental section.

In addition, since in the first step of gradient elution components were eluted in a manner similar to that under isocratic elution, and the peak capacity under the situation that components eluted before they were caught up by mobile phase 2 was small, a further discussion about the effects of parameters on retention times of solutes eluted with mobile phase 2 was carried out according to Eq. (17).

From Eq. (7) it can be seen that there is a quite good linear relationship between retention of components and the transferring time of mobile phase vials. In order to shorten the analysis time of samples, it was necessary to select the transferring time properly. With faster EOF velocity in mobile phase 2 and less capacity factors of components, total analysis time would also be less. Eq. (7) also indicates that the selection of mobile phase 2, which has great effects on capacity factors of solutes in the second step, plays an important role in determining the analysis time of gradient elution since retention of components increases linearly with the increasing of capacity factors in mobile phase 2. It could be seen that the capacity factors of components and the velocities of EOF in the two steps also affected the elution time of samples.

From Fig. 2 it could also be seen the total analysis

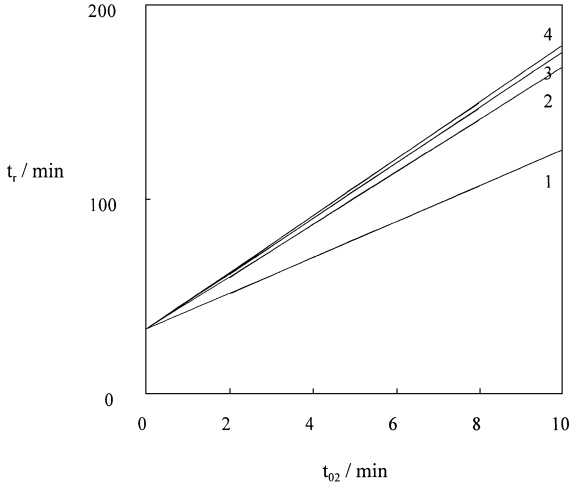

Fig. 2. Effect of the velocity of EOF in mobile phase 2 on retention times of components in mobile phase 2. For conditions see the Experimental section. Lines: (1) $k_1' = 20$, $k_2' = 10$, $t_{01} = 10$, $t_1 = 30$; (2) $k_1' = 20$, $k_2' = 15$, $t_{01} = 10$, $t_1 = 30$; (3) $k_1' = 30$, $k_2' = 15$, $t_{01} = 10$, $t_1 = 30$; (4) $k_1' = 20$, $k_2' = 10$, $t_{01} = 12$, $t_1 = 30$.

time of samples under gradient elution also increased with the velocity of EOF in the second step.

5. Conclusion

Equations to describe the migration of neutral solutes under double stepwise gradient elution of CEC and different situation were deduced and applied for the prediction of the retention of 12 aromatic compounds, which were obtained with high accuracy. In addition, parameters that might affect the analysis time of samples, such as the transferring time of mobile phase vials, capacity factors of solutes and velocities of EOF under two step gradient were studied carefully. All the work established a solid foundation for the further optimization of operation conditions in gradient elution of CEC.

Acknowledgements

Authors are greatly thankful for the financial support from the National Natural Science Foundation of China.

References

[1] V. Pretorious, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[2] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[3] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[4] Cs. Horváth, LC–GC 10 (1990) 622.
[5] L.S. Ettre, Chromatographia 51 (2000) 7.
[6] A. Karcher, Z. El Rassi, Electrophoresis 20 (1996) 3280.
[7] D. Li, H.H. Knobel, V.T. Remcho, J. Chromatogr. B 95 (1997) 169.
[8] N.P. Cassells, C.S. Lane, M. Depala, Chemosphere 40 (2000) 609.
[9] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[10] I.S. Lurie, T.S. Conver, V.L. Ford, Anal. Chem. 70 (1998) 4563.
[11] J. Reilly, M. Saeed, J. Chromatogr. A 829 (1998) 175.
[12] S. Li, D.K. Lloyd, J. Chromatogr. A 666 (1994) 321.
[13] B. Behnke, E. Bayet, J. Chromatogr. A 680 (1994) 93.
[14] A. Palm, M.V. Novotny, Anal. Chem. 69 (1997) 4499.
[15] S. Zhang, X. Huang, J. Zhang, Cs. Horváth, J. Chromatogr. A 887 (2000) 465.
[16] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 255.
[17] D.A. Stead, R.G. Reid, R.B. Taylor, J. Chromatogr. A 798 (1998) 259.
[18] A. Dermaux, P. Sandra, Electrophoresis 20 (1998) 3027.
[19] C. Yan, R. Dadoo, R.N. Zare, D.S. Anex, Anal. Chem. 68 (1996) 2726.
[20] M.R. Taylor, Ph. Teak, S.A. Underwood, Anal. Chem. 69 (1997) 4429.
[21] B. Behnke, J.W. Metzger, Electrophoresis 20 (1999) 80.
[22] C. Ericson, S. Hjerén, Anal. Chem. 71 (1999) 1621.
[23] A.S.C.A. Rimmer, J.G. Dorsey, J. Chromatogr. A 828 (1998) 105.
[24] M.R. Euerby, C.M. Johnson, M. Cikalo, K.D. Bartle, Chromatographia 47 (1998) 135.
[25] B.M. Xin, M.L. Lee, J. Microcol. Sep. 4 (1999) 271.
[26] N.M. Djordjevic, E. Fitzpatrik, F. Hondiere, G. Lerch, G. Rozing, J. Chromatogr. A 887 (2000) 245.
[27] M.R. Euerby, D. Gilligan, C.M. Johnson, K.D. Bartle, Analyst 122 (1997) 1087.
[28] J. Ding, J. Szeliga, P. Vorous, J. Chromatogr. A 802 (1997) 327.
[29] L. Zhang, W. Shi, H. Zou, Y. Zhang, J. Cap. Electrophor. 15 (1997) 201.
[30] B.J. Boughtflower, T. Underwood, C.J. Paterson, Chromatographia 40 (1995) 329.
[31] Y. Zhang, W. Shi, L. Zhang, H. Zou, J. Chromatogr. A 802 (1998) 59.