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Prediction of peptide retention time in normal-phase liquid chromatography

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Abstract

Peptide separation by normal-phase liquid chromatography was studied in my two previous papers. The present study shows a linear relationship between the logarithm of the capacity factor and the logarithm of the volume fraction of modifier in the mobile phase. This permitted the use of a model to predict isocratic and gradient retention times following two initial gradient runs. An algorithm for predicting was based on a modification of the ion-exchange model reported in the previous paper. Observed retention times were in reasonably good agreement with those predicted.

Keywords: Retention prediction; Peptides

1. Introduction

A new combination for peptide separation by normal-phase liquid chromatography (NPLC) has been reported [1], in which a TSK gel Amide-80, carbamoyl groups bonded to silica gel matrix, is used with an acetonitrile (ACN)–water mixed solution containing 0.1% trifluoroacetic acid (TFA). This combination was able to retain and separate hydrophilic peptides with no retention on an octadecyl silica (ODS) column in reversed-phase liquid chromatography (RPLC). Separation selectivities in normal-phase and reversed-phase methods differed significantly [1,2]. Peptide recovery from the Amide-80 column exceeded 80% and repeatability and reproducibility were satisfactory. This form of NPLC is often referred to as hydrophilic interaction chromatography [3–7].

There have been various investigations [6,8–29] of the relationship between retention time and composition of mobile phase. Jandera [8] treated the relationships theoretically in the distribution model for RPLC [8–12,14–19,22,23] and adsorption model for non-aqueous NPLC [8,18,26–29], and expressed the relationship with certain simplifying assumptions in the form of two simple equations. To the author's knowledge, no such equations have been applied to peptide separations in NPLC. This prospect was investigated in the present study. A modification of the ion-exchange model [30] was used to predict retention times [14,30–36] following two initial gradient runs [14,30,36].

2. Theoretical

2.1. Relationship between k' and ψ

Several models have been proposed to explain the

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retention mechanism in NPLC [5,6,13,21,22,37–46]. Retention in NPLC [8,9,24–29] as a function of the composition of binary mobile phases can be described using theoretical models of adsorption. In 1968, Snyder [39] proposed the first retention model in adsorption chromatography. Lately, this model has become widely known as the Snyder–Soczewinski displacement model [25,26,40–47] which is based on the displacement [43,45,46] of solvent molecules from the stationary surface by solute molecules. However, this model is difficult to use because of the introduction of the several physical parameters. The relationship between capacity factor and composition of the mobile phase is considered theoretically and expressed in a simple equation by Jandera:

$$\log k'(\psi) = \log k'(0) - S \cdot \log \psi \quad (1)$$

where $k'(0)$ is the capacity factor at the ψ equal to or very close to zero, S is equal to $-\mathrm{d}[\log k']/\mathrm{d}[\log \psi]$ for a given solute and polar solvent, and ψ is the concentration (volume fraction) of the polar solvent (which is water in this study) in the mobile phase.

On the other hand, in a reversed-phase system, Eq. (2) is widely used to describe the dependence of the capacity factor on the composition of binary mobile phase [8–12,14–19,21–23]:

$$\log k'(\psi) = \log k'w - S' \cdot \psi \quad (2)$$

where $k'w$ is the capacity factor for water as mobile phase, S' is equal to $-\mathrm{d}[\log k']/\mathrm{d}\psi$ for the given solute and organic solvent, and ψ is the concentration (volume fraction) of the organic solvent in the mobile phase.

2.2. Gradient elution equation

The gradient elution equation was based on the algorithm of a modification of the ion-exchange (IEX) model reported in the previous paper [30].

The gradient retention time (t_g) can be calculated using Eq. (3) for any gradient, provided that $k'(\psi)$ is known [23,30,31]:

$$\int_0^{t_g - t_0 - t_D} \mathrm{d}[f^{-1}(\psi)]/k'(\psi) = t_0 - t_D/k'(0) \quad (3)$$

where t_0 is the retention time of an unretained sample, t_D is the delay time of the gradient, $k'(0)$ is the capacity factor at the ψ equal to or very close to zero and ψ is a function of time, t, i.e., $\psi = f(t)$. Given two linear gradient runs with different gradient slope (bi; $i = 1,2$), and with the same initial modifier concentration (a): the gradient becomes of the form:

$$\psi = bi \cdot t + a \quad (4)$$

The gradient time $t_g i$ ($i = 1, 2$) is given by:

$$t_g i = 1/bi\{[bi \cdot (S+1) \cdot (t_D \cdot k'(0) - t_D) + a^{S+1}]^{1/(S+1)} - a\} + t_0 + t_D \quad (5)$$

Eq. (5) involves two unknowns, $k'(0)$ and S, that can be solved by numerical means. If the following gradient conditions can be assumed [30]:

$$k'(0) \gg \frac{a^{S+1} + t_D}{bi \cdot (S+1) \cdot t_D} \quad (6)$$

Eqs. (5) and (6) allow an explicit solution for $k'(0)$ and S:

$$S + 1 = \log(b1/b2)/\log\{[a + b2(t_g 2 - t_0 - t_D)]/[a + b1(t_g 1 - t_0 - t_D)]\} \quad (7)$$

$$k'(0) = \{[a + b1(t_g 1 - t_0 - t_D)]^{1/(S+1)}/[b1 + (S + 1)] + t_D\}/t_0 \quad (8)$$

The best-fit values of $k'(0)$ and S allow the prediction of both isocratic and gradient retention times under a variety of experimental conditions.

3. Experimental section

3.1. Materials

HPLC-grade acetonitrile (ACN) was obtained from Nacalai Tesque, (Kyoto, Japan), and trifluoroacetic acid (TFA) and formic acid from Wako Pure Chemical Industries, (Osaka, Japan). Milli-Q (Japan Millipore, Tokyo, Japan) was used for water purification. Most of the peptides were purchased from Sigma (St. Louis, MO) and The Peptide Institute, (Osaka, Japan) and the others obtained by cyanogen bromide degradation of myoglobin. The TSK gel Amide-80 (25×0.46 cm I.D.) column was from Tosoh (Tokyo, Japan).

3.2. Apparatus

The HPLC system was a Tosoh liquid chromatograph equipped with a SC-8020 microcomputer, CCPM-II pump, UV-8020 detector, AS-8020 autosample injector and CO-8020 column oven.

3.3. Methods

Eluent A (initial eluent) was 0.1% TFA in ACN–water (97:3) and eluent B, 0.1% TFA in ACN–water (55:45). The peptides were dissolved in 5 μl of formic acid, followed by the addition of 40 μl of ACN and separation by linear gradient from eluent A to eluent B. The flow-rate was 1.0 ml/min. Elution was monitored by UV absorption at 215 nm. The temperature in the column oven was 40°C. The void volume (t_0) of the TSK gel Amide-80, determined by the retention of ACN, was 2.55 ml. The dwell volume (t_D) of the gradient was 2.30 ml.

Simulations and calculations were carried out on an NEC PC-9801 personal computer. Software for calculations were programmed in C language.

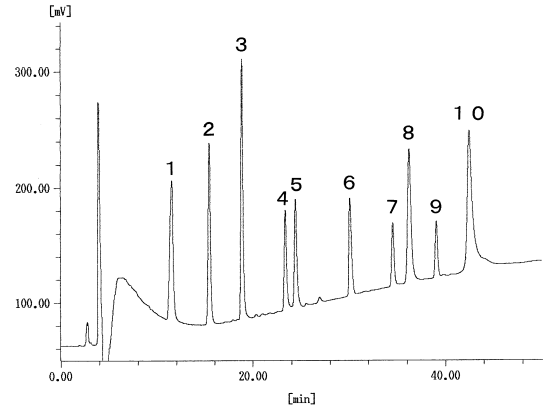

Fig. 1. Chromatograms of peptides separated on TSK gel Amide-80. The peptide mixture was separated with 70-min linear gradients of water from 3 to 45% in 0.1% TFA (0.6% water per min.). Peak identification: 1=FY; 2=FGGF; 3=FLEEI; 4=DYMGWMDP-NH2; 5=NFTYGGF; 6=AGSE; 7=WAGGDASGE; 8=YGGFMTSQKSQTPLVT; 9=ASTTTNYT; 10=VLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPETLEKFDRFKHLKTEAE.

4. Results and discussion

4.1. Relationship between k' and ψ

Fig. 1 shows the separation of hydrophilic and hydrophobic peptides in the present NPLC. Peptides retained by the amide column were eluted by increasing the proportion of water and the polarity of the mobile phase. This elution pattern is analogous to non-aqueous NPLC [8,18,24–29]. Therefore, the model equations used for NPLC were applied here. The relationship between retention time and composition of mobile phase was investigated. The retention data obtained for peptides were listed in Table 1 and plotted against the logarithm of water concentration in Fig. 2. Fig. 2 shows a linear decrease of capacity factors with increasing volume fraction, ψ, of water in the ACN in the mobile phase. As expected from Eq. (1), the data points fall on straight lines. The corresponding correlation coefficients were all above 0.99. Eq. (1) is obeyed more often in the usual NPLC system. Therefore, from this linear relationship, it might be assumed that the retention mechanism of the present method would be involved in that of the usual NPLC. This linear relationship would be useful for explaining the mechanism mathematically in the present method in the near future. However, it is not possible to establish what the mechanism is here.

4.2. Prediction of retention time

On the basis of the present prediction model, the prediction of retention time of isocratic and gradient retention times [14,30–36] from two initial gradient runs [14,30,36] was attempted. First, gradient retention time (t_g1, t_g2) of the same set of peptides was measured on the same column with three gradient times (70, 90, 120 min). The results were subjected to calculation of $k'(0)$ and S values using the gradient data for the 70- and 90-min gradients and Eqs. (7) and (8), and are summarized in Table 2. The calculation results are in close agreement with the experimentally determined data from Table 1.

The isocratic retention times were calculated by Eq. (1) using gradient-derived $k'(0)$ and S values from Table 2. The results are summarized in Table 3. The agreement between the calculated and ex-

Table 1
Isocratic retention times of peptides

Sample	Retention time (min)									*S*	Log′(0)	*r*
	Water (v/v%)											
	15.925	12.400	10.050	7.700	6.530	5.350	4.175					
1				6.47		8.91	10.96			1.25	3.00	0.999
2			6.47	9.23		15.56	21.73			1.81	4.64	0.997
3		5.88	8.58	16.30	24.00					2.93	7.64	0.998
4	5.84	10.43	19.48	48.91						3.64	10.31	0.999
5	6.92	12.13	21.41	49.82						3.27	9.58	0.999
	Water (v/v%)											
	27.675	22.975	19.450	17.100	15.925	14.750	13.575	12.400	10.050			
6		6.67	8.67	10.90			17.12	20.69	35.575	2.49	8.27	0.999
7	5.46	7.76	11.64	16.85			36.23			3.44	11.51	0.999
8		7.24	13.13	23.03	32.17	48.39				5.12	16.64	0.999
9	6.86	10.65	17.31	33.39						3.57	12.35	0.999
	Water (v/v%)											
	25.325	22.975	21.800	20.625	19.450							
10	6.27	12.75	20.48	36.59	70.68					11.01	35.91	0.999

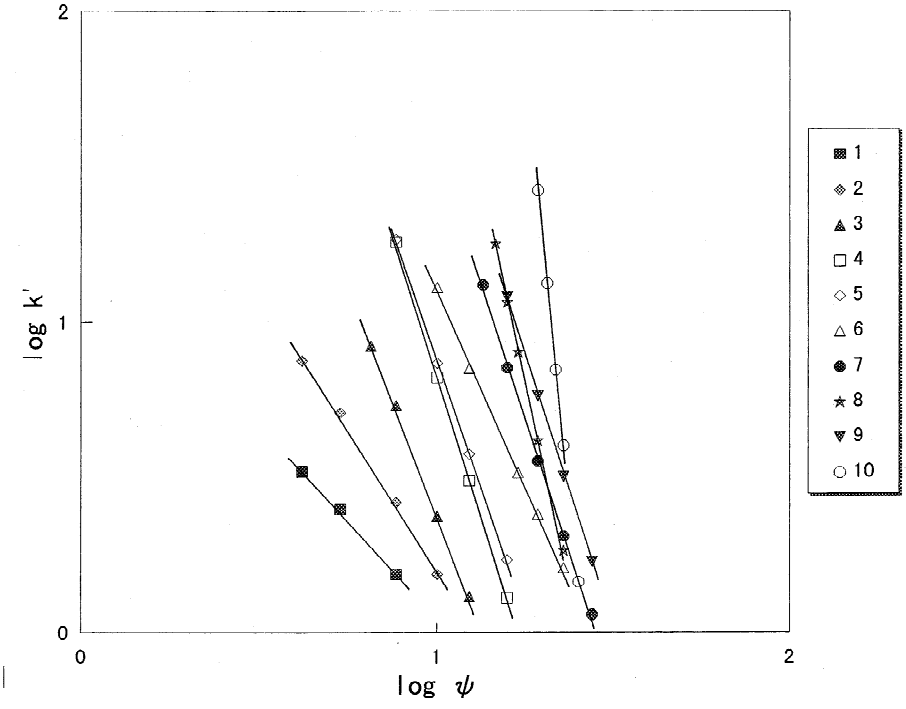

Fig. 2. Dependence of capacity factor, $k'(\psi)$, of peptides on the concentration of water, ψ(%, v/v), in ACN. Isocratic data (Table 1) were plotted. Peak identification was shown in Fig. 1.

Table 2
Gradient retention times of peptide

Sample	Gradient time (min)		S	Log $k'(0)$
	70	90		
1	10.90	11.50	1.65	3.50
2	14.66	15.93	1.88	4.76
3	18.28	20.67	2.99	7.76
4	23.32	27.06	3.86	10.84
5	24.10	27.83	3.34	9.73
6	28.18	32.34	2.55	8.36
7	33.88	39.96	3.88	12.71
8	35.89	42.99	5.52	17.72
9	37.66	44.62	3.97	13.46
10	41.64	51.11	12.01	38.91

perimental retention data was good (average deviation 2~6%).

The gradient time of the 120-min gradient was predicted using Eq. (5) and calculation of $k'(0)$ and S values from Table 2. The results are summarized in Table 4. The mean deviation between the predicted and experimental gradient retention times was only 0.64%.

From Tables 3 and 4, it could be shown that the approach using the gradient-derived best-fit values of $k'(0)$ and S on the basis of the present prediction model allows good prediction of isocratic and gradient retention time for the present NPLC of peptides.

Table 3
Comparison of predicted and observed isocratic retention times of peptides

Sample		Retention time (min)									Mean deviation (%)
		Water (v/v%)									
		15.925	12.400	10.050	7.700	6.530	5.350	4.175			
1	Observed				6.47		8.91	10.96			
	Predicted				5.81		8.19	10.86			6.39
2				6.47	9.23		15.56	21.73			
				6.79	9.32		15.62	23.18			3.25
3			5.88	8.58	16.30	24.00					
			6.14	8.95	16.30	24.83					3.05
4		5.84	10.43	19.48	48.91						
		5.88	10.69	20.42	51.85						3.49
5		6.92	12.13	21.41	49.82						
		6.99	12.33	21.92	49.22						1.57
		Water (v/v%)									
		27.675	22.975	19.450	11.100	15.925	14.750	13.575	12.400	10.050	
6			6.67	8.67	10.90			17.12	20.69	35.58	
			6.58	8.51	10.69			16.93	20.57	33.10	2.30
7		5.46	7.76	11.64	16.85			36.23			
		5.04	7.28	11.24	16.65			36.56			3.89
8			7.24	13.13	23.03	32.17	48.39				
			6.83	12.72	22.87	32.46	48.02				2.24
9		6.86	10.65	17.31		33.39					
		6.35	10.09	16.81		33.60					4.05
		Water (v/v%)									
		25.325	22.975	21.800	20.625	19.450					
10		6.27	12.75	20.48	36.59	70.68					
		5.71	11.91	19.81	35.77	69.39					4.58

Table 4
Comparison of predicted and observed gradient retention times of peptides

Sample	Observed	Predicted
1	11.68	12.24
2	17.39	17.55
3	23.87	23.89
4	32.20	32.26
5	32.93	32.98
6	37.96	37.99
7	48.45	48.48
8	53.12	53.12
9	54.35	54.39
10	64.95	64.97

Mean deviation (%) 0.64%
The data of observed and predicted are for the 120-min gradient.

5. Conclusion

The elution pattern in the present NPLC was analogous to non-aqueous NPLC. Now, there was the linear relationship between log k' and log ψ. Therefore, from this linear relationship, it might be assumed that the retention mechanism of the present method would be involved in the usual NPLC. This linear relationship would be useful for explaining the mechanism mathematically in the present NPLC in the future. This permitted the use of a model to predict isocratic and gradient retention times following two initial gradient runs. An algorithm for prediction was used as a modification of the ion-exchange model reported in the previous paper. Observed retention times were in reasonably good agreement with those predicted.

6. Symbols

ψ	volume fraction of modifier in the mobile phase.
a	value of ψ at the beginning of the gradient.
bi	gradient steepness parameter, defined by Eq. (3).
$b1$, $b2$	value of b for two gradient runs differing only in gradient times.
$f(t)$	shape of gradient program as a function of time.
$k'(\psi)$	solute capacity factor.
$k'(0)$	capacity factor at the ψ equal to or very close to zero for the present NPLC.
$k'w$	capacity factor at the water for RPLC.
t	time (min).
$t_g i$	retention time in gradient elution (min).
t_0	time required for a nonretained solute to elute from the column (min).
t_D	dwell time for gradient elution (min); equal to the time it takes a change in the mobile phase composition to pass from the gradient mixer to the column inlet (min).
S	equal to $-\mathrm{d}[\log k'(0)]/\mathrm{d}[\log \psi]$.
S'	equal to $-\mathrm{d}[\log k'(0)]/\mathrm{d}\psi$.

References

[1] T. Yoshida, Anal. Chem. 69 (1997) 3038.
[2] T. Yoshida, J. Chromatogr. A 808 (1998) 105.
[3] L.A.Th. Verhaar, B.F.M. Kuster, J. Chromatogr. 234 (1982) 57.
[4] P. Orth, H. Engelhardt, Chromatographia 15 (1982) 91.
[5] Z.L. Nikolov, P.J. Reilly, J. Chromatogr. 352 (1985) 287.
[6] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[7] A.J. Alpert, P.C. Andrews, J. Chromatogr. 443 (1988) 85.
[8] P. Jandera, J. Churacek, J. Chromatogr. 91 (1974) 207.
[9] P. Jandera, L. Petranek, M. Kucerova, J. Chromatogr. A 791 (1997) 1.
[10] M.T.W. Heam, B. Grego, J. Chromatogr. 255 (1983) 125.
[11] M.A. Stadalius, H.S. Gold, L.R. Snyder, J. Chromatogr. 296 (1984) 31.
[12] W.S. Hancock, D.R. Knighton, J.R. Napier, D.R.K. Harding, R. Venable, J. Chromatogr. 367 (1986) 1.
[13] X. Geng, F.E. Regnier, J. Chromatogr. 296 (1984) 15.
[14] Y. Sakamoto, N. Kawakami, T. Sasagawa, J. Chromatogr. 442 (1988) 69.
[15] J.W. Dolan, J.R. Gant, L.R. Snyder, J. Chromatogr. 165 (1979) 31.
[16] M. Hsieh, J.G. Dorsey, J. Chromatogr. 631 (1993) 63.
[17] L.R. Snyder, M.A. Quarry, J. Liq. Chromatogr. 10 (1987) 1789.
[18] P. Jandera, Chromatographia 26 (1988) 417.
[19] H.A. Cooper, R.J. Hurtubise, J. Chromatogr. 360 (1986) 313.
[20] P. Jandera, J. Urbanek, J. Chromatogr. A 689 (1995) 255.
[21] J. Fischer, P. Jandera, J. Chromatogr. A 681 (1996) 3.
[22] P. Jandera, J. Kubat, J. Chromatogr. 500 (1990) 281.
[23] P.J. Schoenmakers, H.A.H. Billiet, L.D. Galan, J. Chromatogr. 185 (1979) 179.
[24] L.R. Snyder, J.L. Glajch, J. Chromatogr. 214 (1981) 1.
[25] E.L. Weiser, A.W. Salotto, S.M. Flach, L.R. Snyder, J. Chromatogr. 303 (1984) 1.
[26] P. Jandera, J. Rozkosna, J. Chromatogr. 362 (1986) 325.
[27] T. Hamoir, D.L. Massart, J. Chromatogr. A 673 (1994) 1.

[28] M. Lubke, J.L. Quere, D. Barron, J. Chromatogr. A 690 (1995) 41.
[29] P. Jandera, M. Kucerova, J. Holikova, J. Chromatogr. A 762 (1997) 15.
[30] T. Sasagawa, Y. Sakamoto, T. Hirose, T. Yoshida, Y. Kobayashi, S. Sato, J. Chromatogr. 485 (1989) 533.
[31] P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen, L.D. Galan, J. Chromatogr. 149 (1978) 519.
[32] L.R. Snyder, in: Cs. Horvath (Ed.), High-performance liquid Chromatography – Advances and Perspectives, vol. 1, Academic Press, New York, 1980, ch. 4.
[33] J.P. Larmann, J.J. Destefano, A.P. Goldberg, R.W. Stout, L.R. Snyder, M.A. Stadalius, J. Chromatogr. 255 (1983) 163.
[34] M.A. Quarry, R.L. Grob, L.R. Snyder, J. Chromatogr. 285 (1984) 1.
[35] R.W. Stout, S.I. Sivakoff, R.D. Ricker, L.R. Snyder, J. Chromatogr. 353 (1986) 439.
[36] M.A. Quarry, R.L. Grob, L.R. Snyder, Anal. Chem. 58 (1986) 907.
[37] R.P.W. Scott, P. Kucera, Anal. Chem. 45 (1973) 749.
[38] R.P.W. Scott, P. Kucera, J. Chromatogr. 112 (1975) 425.
[39] L.R. Snyder, Principles of Adsorption Chromatography, Marcel Dekker, New York, NY, 1968.
[40] L.R. Snyder, Anal. Chem. 46 (1974) 1384.
[41] L.R. Snyder, J.J. Kirkland, Introduction to Modern Liquid Chromatography, 2nd ed., Wiley-Interscience, New York, 1979.
[42] L.R. Snyder, H. Poppe, J. Chromatogr. 184 (1980) 363.
[43] L.R. Snyder, T.C. Schunk, Anal. Chem. 54 (1984) 1764.
[44] E. Soczewinski, Anal. Chem. 41 (1969) 179.
[45] E. Soczewinski, J. Chromatogr. 130 (1977) 23.
[46] E. Soczewinski, J. Chromatogr. 388 (1987) 91.
[47] C.A. Chang, C.S. Huang, Anal. Chem. 57 (1985) 997.