Journal of Chromatography, 485 (1989) 533–540
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 866

PREDICTION OF RETENTION TIMES IN ION-EXCHANGE CHROMATOGRAPHY

TATSURU SASAGAWA*, YASUHIRO SAKAMOTO, TSUKASA HIROSE, TATSUNARI YOSHIDA, YOSHIMI KOBAYASHI and YOSHIHIRO SATO

Scientific Instrument Division, Tokyo Research Centre, Tosoh Co. Ltd., 2743-1, Hayakawa Ayase, Kanagawa 252 (Japan)

and

KYOKO KOIZUMI

Faculty of Pharmaceutical Science, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663 (Japan)

SUMMARY

A method for predicting ion-exchange isocratic capacity factors from two initial gradient runs is developed. This does not assume so-called linear solvent strength (LSS) conditions, which cause significant errors in $k'(C)$ *vs.* C relationships in ion-exchange chromatography. The errors associated with this approach and the LSS model are examined. The present approach allows a more accurate prediction of isocratic capacity factors for ion-exchange chromatography. Experimental application of the method to a variety of compounds, including peptides, polynucleotides and polysaccharides, separated by ion-exchange chromatography is described.

INTRODUCTION

Gradient elution is an indispensable technique for the separation of compounds that have very different retentions. The conditions for gradient elution are usually selected by a trial-and-error method. More recently, Dolan *et al.*[1] developed computer software that predicts how various changes in chromatographic conditions affect the resolution in gradient elution. This procedure assumes a linear solvent strength (LSS) behaviour of solute retention, which is approximately the case in reversed-phase separations, but is far from true for ion-exchange chromatography. It was noted that significant errors in capacity factor (k') *vs.* salt concentration (C) relationships can result. The aim of this study was to develop another method for deriving k' *vs.* C functions from gradient retention data for ion-exchange systems.

THEORY

The capacity factors of chromatographed solutes remain constant during elution under isocratic conditions. However, in gradient elution chromatography they change

0021-9673/89/$03.50

with time in accordance with increasing salt concentration in the mobile phase. The capacity factor of a solute is related to the salt concentration in the mobile phase by the equation[2,3]

$$k'(C) = k'(C_0)(C/C_0)^Z \tag{1}$$

where $k'(C_0)$ is the capacity factor when $C = C_0$.

The gradient retention time (t_g) can be calculated using eqn. 2 for any gradient, provided that $k'(C)$ is known[4]:

$$\int_0^{t_g - t_0 - t_D} \mathrm{d}[f^{-1}(C)]/k'(C) = t_0 - t_D/k'(C_0) \tag{2}$$

where t_0 is the retention time of an unretained sample, t_D is the delay time of the gradient, $k'(C_0)$ is the capacity factor at the initial salt concentration (C_0) and C is a function of time, t, *i.e.*, $C = f(t)$.

If two linear gradient runs are carried out with different gradient slopes (b_1, b_2), and with the same initial salt concentration C_0, the gradients become of the form

$$C = b_1 t + C_0 \tag{3}$$

$$C = b_2 t + C_0 \tag{4}$$

The gradient retention times t_{g1} and t_{g2} are given by

$$t_{g1} = C_0/b_1(\{(Z + 1)[t_0 k'(C_0) - t_D](b_1/C_0) + 1\}^{1/(Z+1)} - 1) + t_0 + t_D \tag{5}$$

$$t_{g2} = C_0/b_2(\{(Z + 1)[t_0 k'(C_0) - t_D](b_2/C_0) + 1\}^{1/(Z+1)} - 1) + t_0 + t_D \tag{6}$$

Eqns. 5 and 6 involve two unknowns, $k'(C_0)$ and Z, that can be solved by numerical means. If the following gradient conditions can be assumed:

$$k'(C_0) \gg (t_D/t_0) + C_0/[b_1 t_0(Z + 1)] \tag{7}$$

then eqns. 5 and 6 become much simpler:

$$t_{g1} = C_0/b_1\{[(Z + 1)t_0 k'(C_0)(b_1/C_0) + 1]^{1/(Z+1)}\} + t_0 + t_D \tag{8}$$

$$t_{g2} = C_0/b_2\{[(Z + 1)t_0 k'(C_0)(b_2/C_0) + 1]^{1/(Z+1)}\} + t_0 + t_D \tag{9}$$

Eqns. 8 and 9 allow explicit solution for $k'(C_0)$ and Z:

$$1 + Z = \frac{\log(b_2/b_1)}{\log[(t_{g2} + C_0/b_2 - t_0 - t_D)b_2/(t_{g1} + C_0/b_1 - t_0 - t_D)b_1]} \tag{10}$$

$$k'(C_0) = \frac{(t_{g1}b_1/C_0)^{1+Z}C_0}{b_1 t_0(1 + Z)} \tag{11}$$

Using these as initial values, eqns. 5 and 6 converge fairly rapidly. The best-fit values of $k'(C_0)$ and Z allow the prediction of both isocratic and gradient retention times under a variety of experimental conditions.

EXPERIMENTAL

Equipment

The HPLC system was a Tosoh (Tokyo, Japan) liquid chromatograph equipped with a CCPM pump, UV 8000 detector and heated column compartment. The dwell volume of this system was 1.5 ml unless stated otherwise. The column dead volume or t_0 of a TSKgel CM3SW column was determined by the retention time of sodium nitrite (1.7 ml). For other columns, column dead volumes were calculated assuming a porosity of 0.4.

Simulations and calculations were carried out either on an NEC PC-9801 personal computer or an IBM PS/2 personal computer. A computer program for calculating reversed-phase retention parameters (S and k'_0 in eqns. 5 and 10 in ref. 2) was written based on the algorism of Quarry *et al.*[2]. Software for gradient simulations as described here were programmed in C language. DryLab G was purchased from LC Resources (Lafayette, CA, U.S.A.).

Peptides were separated on a TSKgel CM3SW carboxymethyl cation-exchange column (7.5 cm × 0.75 cm I.D.) (Tosoh) at a flow-rate of 1.0 ml/min. A synthetic polynucleotide mixture was separated on a TSKgel DEAE-NPR (2.5 μm) column (3.5 cm × 0.46 cm I.D.) (Tosoh) at a flow-rate of 1.5 ml/min unless stated otherwise. Polysaccharides were separated on a CarboPac PA-1 column (25 cm × 0.46 cm I.D.) (Dionex, Sunnyvale, CA, U.S.A.) at a flow-rate of 1.0 ml/min. Other details of chromatographic system were as described in ref. 5.

Mobile phases

For peptide separation, buffer A was 10 mM NaH_2PO_4 (pH 6.0) containing 10 mM NaCl and buffer B was 10 mM NaH_2PO_4 (pH 6.0) containing 0.75 M NaCl. For nucleotide separation, buffer A was 20 mM Tris–HCl (pH 9.0) containing 0.25 M NaCl and buffer B was 20 mM Tris–HCl (pH 9.0) containing 1.0 M NaCl. For polysaccharide separation, buffer A was 0.15 M NaOH containing 75 mM sodium acetate and buffer B was 0.15 M NaOH containing 0.25 M sodium acetate.

Reagents

A Milli-Q system was used for water purification. Peptides were purchased from the Peptide Institute (Osaka, Japan). Synthetic polynucleotide was purchased from Pharmacia (Uppsala, Sweden). Homologous series of linear α(1→6)-D-glucans were obtained by acid hydrolysis of dextran[5].

RESULTS AND DISCUSSION

The isocratic $k'(C)$ values of three peptides were measured on a cation-exchange column at different sodium chloride concentrations. The results are given in Table I and are plotted against logarithm of salt concentration in Fig. 1. As expected from eqn. 1, the data points fall on straight lines (not shown; correlation coefficients all above 0.99).

TABLE I

ISOCRATIC RETENTION TIMES OF PEPTIDES

NaCl (M)	*Retention times (min)*[a]								
	Neurotensin			*Angiotensin III*			*ACTH*		
0.5							8.1	(9.70)	[10.5]
0.375							14.8	(17.34)	[19.39]
0.338							19.0	(21.62)	[23.39]
0.3							26.0	(27.99)	[28.45]
0.263	3.2	(3.26)	[2.59]	8.2	(9.64)	[12.13]	38.0	(37.42)	[34.28]
0.225	3.3	(3.45)	[2.95]	9.2	(10.93)	[12.63]			
0.188	3.4	(3.70)	[3.43]	10.6	(12.68)	[14.52]			
0.150	3.7	(4.05)	[4.13]	13.2	(15.36)	[16.79]			
0.113	4.0	(4.59)	[5.07]	17.2	(19.66)	[19.39]			
0.06	5.0	(6.27)	[7.09]	30.0	(34.8)	[23.92]			
Z	0.54			1.01			2.69		
Log $k'(C_0)$	1.63			4.63			11.81		
Mean deviation (%)		10.8	18.5		17.1	30.4		11.97	20.59

[a] The numbers in parentheses and brackets are retention times predicted by the present method and by the reversed-phase model, respectively.

Gradient retention times (t_{g1}, t_{g2}) of the same set of peptides were measured on the same column with three different gradient times (20, 30 and 45 min). The results are summarized in Table II and were subjected to calculation of $k'(C_0)$ and Z values both by the present method and by using a reversed-phase model (LSS model). The resulting

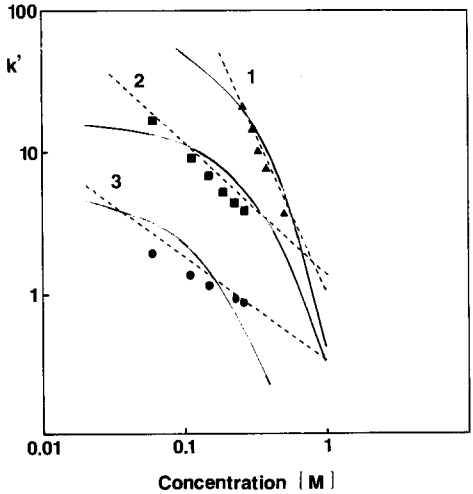

Fig. 1. Comparison of $k'(C)$ *vs.* C values for peptides from isocratic and gradient measurements. Isocratic data (Table I) were plotted: ● = neurotensin; ■ = angiotensin III and ▲ = ACTH. Gradient data were derived from Table II. $k'(C)$ *vs.* C values were calculated from eqns. 5 and 6 and the corresponding equations of the LSS model as discussed in the text. Dashed lines and solid curves correspond to the present and LSS model, respectively.

TABLE II

GRADIENT RETENTION TIMES OF PEPTIDES

Buffer A, 0.01 *M* NaCl; buffer B, 0.75 *M* NaCl.

Compound	*Time (min)*[a]			*Z*	*Log k'(C₀)*
	20	*30*	*45*		
Neurotensin	6.8	7.4	8.0 (8.06) [8.00]	0.73	2.30
Angiotensin III	13.5	15.7	18.2 (18.31) [18.00]	0.97	4.71
ACTH	19.5	24.8	31.8 (31.76) [31.48]	2.32	10.66
Mean deviation (%)			0.5 0.7		

[a] The numbers in parentheses and brackets are retention predicted times by the present method and by the reversed-phase model, respectively.

log $k'(C_0)$ *vs.* log C functions are plotted in Fig. 1 (dashed lines) and are in close agreement to the experimentally determined data. Using the reversed-phase model, however, the plots (solid curves) deviate from the experimental data points significantly at both high and low salt concentrations. The non-linearity is most pronounced for small values of Z, as expected from ref. 2.

The isocratic retention times were calculated using gradient-derived Z and $k'(C_0)$ values and the results were summarized in Table I. The agreement between the calculated and experimental data was acceptable (average deviation 13%) and was better than that with the reversed-phase model (average deviation 23%). The gradient-derived best-fit values of $k'(C_0)$ and Z allow the prediction of experimental values of gradient retention times. As shown in Table II, using the data for the 20- and 30-min gradients, the gradient retention time of the 45-min gradient was predicted. The deviation between the experimental and predicted gradient retention times was only 0.5%, compared with 0.7% with the reversed-phase model.

To test the prediction power of the present method for other classes of compounds, gradient retention times of polynucleotides and polysaccharides were predicted by the present method and the results were compared with those obtained by using DryLab, based on the LSS model.

Retention times of the hydrolysate of polyadenylic acid containing the 17-mer up to the 100-mer (only 20- and 30- to the 80-mer were identified) were measured on an anion-exchange column with three different gradient times (60, 120 and 240 min)[6]. The retention parameters of each of the 32 polynucleotides were calculated from the data for the 60- and 240-min gradients (columns 2 and 3, Table III). The retention times with a 120-min gradient were predicted using these parameters and are also given in Table III with the observed retention times. The mean deviation of the predicted and observed retention times with the present method was only 2.77% (column 5) which is better than that obtained by DryLab (2.83%) (column 6).

The data in columns 7 and 8 in Table III were obtained with two columns connected in series and a flow-rate of 1.0 instead of 1.5 ml/min. It is obvious that the present method can also predict precise retention times under different elution conditions from those of the initial two runs (the mean deviation is only 0.57%).

TABLE III

COMPARISON OF PREDICTED AND OBSERVED RETENTION TIMES OF POLYNUCLEOTIDES

The dwell volume of this system is 1.6 ml.

No. of structural repeat	*Gradient time (min)*[a]				*Z*	*Log k' (C_0)*
	60	*240*	*120*	*60*		
20	5.40	12.16	8.30 (8.09) [8.10]	7.22 (7.49)	20.80	5.91
30	9.15	28.46	16.26 (15.95) [15.94]	10.93 (10.91)	32.89	12.66
31	9.43	29.72	16.90 (16.55) [16.53]	11.20 (11.16)	34.37	13.46
32	9.66	30.78	17.38 (17.04) [17.09]	11.44 (11.37)	35.77	14.20
33	9.95	31.92	18.08 (17.61) [17.60]	11.66 (11.65)	36.25	14.73
34	10.19	32.96	18.51 (18.11) [18.10]	11.91 (11.88)	37.20	15.37
35	10.42	33.82	18.95 (18.56) [18.54]	12.12 (12.13)	36.84	15.54
36	10.64	34.78	19.46 (19.02) [19.00]	12.32 (12.33)	37.82	16.18
37	10.87	35.78	19.94 (19.50) [19.49]	12.51 (12.54)	38.99	16.92
38	11.05	36.56	20.37 (19.88) [19.86]	12.69 (12.70)	39.89	17.50
39	11.23	37.34	20.79 (20.26) [20.24]	12.89 (12.88)	40.66	18.04
40	11.41	38.16	21.21 (20.64) [20.62]	13.05 (13.03)	42.16	18.87
41	11.59	39.10	21.62 (21.05) [21.04]	13.21 (13.16)	45.17	20.31
42	11.76	39.74	22.01 (21.38) [21.37]	13.38 (13.34)	45.01	20.50
43	11.92	40.40	22.43 (21.71) [21.69]	13.52 (13.49)	45.57	20.97
44	12.05	41.14	22.78 (22.02) [22.01]	13.66 (13.58)	48.63	22.43
45	12.23	41.96	23.13 (22.41) [22.39]	13.76 (13.74)	50.64	23.56
46	12.39	42.60	23.44 (22.73) [22.71]	13.96 (13.90)	50.97	23.96
47	12.53	43.20	23.73 (23.02) [23.00]	14.10 (14.03)	51.93	24.60
48	12.67	43.90	24.11 (23.34) [23.32]	14.23 (14.15)	54.04	25.77
49	12.82	44.52	24.46 (23.64) [23.62]	14.37 (14.30)	54.74	26.34
50	13.00	45.26	24.74 (24.00) [23.99]	14.49 (14.46)	55.94	27.18
51	13.11	45.80	25.02 (24.25) [24.23]	14.63 (14.55)	57.78	28.21
52	13.24	46.32	25.30 (24.51) [24.49]	14.76 (14.69)	57.71	28.42
53	13.37	46.84	25.58 (24.77) [24.75]	14.89 (14.81)	58.50	29.00
54	13.49	47.42	25.88 (25.03) [25.02]	15.03 (14.91)	60.93	30.35
55	13.67	48.17	26.10 (25.40) [25.37]	15.14 (15.09)	61.51	30.97
56	13.73	48.66	26.37 (25.59) [25.57]	15.26 (15.10)	67.82	34.06
57	13.85	49.16	26.64 (25.83) [25.81]	15.39 (15.21)	68.52	34.64
58	13.97	49.66	26.89 (26.08) [26.06]	15.52 (15.33)	69.32	35.29
59	14.08	50.14	27.13 (26.31) [26.29]	15.62 (15.43)	70.70	36.18
60	14.19	50.61	27.42 (26.54) [26.52]	15.74 (15.54)	71.81	36.97
Mean deviation (%)			2.77 2.83	0.57		

[a] The numbers in parentheses and brackets are retention times predicted by the present method and DryLab, respectively.

The retention times of α(1→6)-linked homologous α-D-gluco-polysaccharides (containing 10–43 structural units) were measured on an anion-exchange column with three different gradient times (60, 80 and 100 min) and the results with predicted retention at a gradient time of 80 min are summarized in Table IV. The mean deviation of the predicted and observed retention times was only 0.63%, compared with 0.66% with DryLab.

TABLE IV

COMPARISON OF OBSERVED AND PREDICTED RETENTION TIMES OF POLYSACCHARIDES

No. of structural repeat	*Gradient time (min)*[a]					*Z*	*Log k' (C_0)*
	60	*100*	*80*	*80*	*80*		
10	15.59	17.02	16.19	(16.43)	[16.43]	1.59	2.45
11	17.77	20.12	18.86	(19.12)	[19.13]	2.04	2.76
12	19.88	23.24	21.48	(21.77)	[21.79]	2.39	3.06
13	21.84	26.33	24.03	(24.33)	[24.35]	2.77	3.37
14	23.69	29.25	26.44	(26.74)	[26.76]	3.04	3.64
15	25.38	31.97	28.70	(28.97)	[28.98]	3.29	3.90
16	26.91	34.59	30.80	(31.05)	[31.07]	3.59	4.18
17	28.38	37.08	32.77	(33.04)	[33.06]	3.85	4.45
18	29.75	39.47	34.64	(34.93)	[34.94]	4.12	4.72
19	31.03	41.69	36.41	(36.68)	[36.69]	4.36	4.98
20	32.25	43.73	38.06	(38.31)	[38.33]	4.52	5.20
21	33.39	45.61	39.62	(39.83)	[39.84]	4.65	5.39
22	34.45	47.44	41.00	(41.27)	[41.29]	4.83	5.61
23	35.44	49.18	42.47	(42.64)	[42.65]	5.02	5.84
24	36.37	50.86	43.75	(43.94)	[43.95]	5.25	6.09
25	37.25	52.42	44.95	(45.15)	[45.17]	5.44	6.31
26	38.09	53.90	46.09	(46.32)	[46.33]	5.62	6.52
27	38.90	55.29	47.19	(47.42)	[47.42]	5.75	6.71
28	39.68	56.60	48.25	(48.46)	[48.47]	5.85	6.87
29	40.41	57.85	49.27	(49.45)	[49.46]	5.97	7.04
30	41.11	59.07	50.26	(50.41)	[50.42]	6.11	7.22
31	41.78	60.24	51.19	(51.33)	[51.34]	6.24	7.40
32	42.40	61.34	52.07	(52.18)	[52.19]	6.39	7.59
33	42.99	62.41	52.89	(53.01)	[53.02]	6.55	7.78
34	43.55	63.46	53.65	(53.82)	[53.82]	6.75	8.01
35	44.10	64.45	54.40	(54.58)	[54.58]	6.91	8.20
36	44.62	65.39	55.13	(55.31)	[55.31]	7.05	8.39
37	45.14	66.28	55.82	(56.01)	[56.02]	7.15	8.53
38	45.62	67.11	56.49	(56.67)	[56.67]	7.25	8.67
39	46.09	67.93	57.13	(57.31)	[57.31]	7.35	8.82
40	46.53	68.72	57.75	(57.92)	[57.97]	7.47	8.98
41	46.96	69.46	58.37	(58.51)	[58.51]	7.56	9.10
42	47.37	70.20	58.95	(59.08)	[59.08]	7.67	9.25
43	47.80	70.94	59.54	(59.63)	[60.82]	7.86	9.46
Mean deviation (%)				0.63	0.66		

[a] The numbers in parentheses and brackets are retention times predicted by the present method and DryLab, respectively.

CONCLUSION

This investigation has shown that approximate ion-exchange isocratic capacity factors can be predicted from two gradient runs in the same HPLC system. This procedure does not assume linear solvent strength (LSS) behaviour of solute retention,

which is not an appropriate model for ion-exchange chromatography. Application of the model to peptide, polysaccharide and polynucleotide data resulted in very good agreement between the predicted and experimental gradient retention times. The agreement is better than that obtained by the LSS model. The differences in precision, however, are generally not very great. For Z and >1, there is not much difference expected in the final predictions by DryLab, as discussed in ref. 2.

SYMBOLS

b	gradient steepness parameter, defined by eqn. 3
b_1, b_2	value of b for two gradient runs differing only in gradient times
C	concentration of salt (M) in the mobile phase
C_0	value of C at the beginning of an ion-exchange gradient
f(t)	shape of gradient programme as a function of time t
$k'(C)$	solute capacity factor
$k'(C_0)$	isocratic $k'(C)$ value for the solute in the initial mobile phase
S	equal to $-\mathrm{d}(\log k')/\mathrm{d}\varphi$ for a given solute and organic solvent
t	time (min)
t_g	retention time in gradient elution (min)
t_D	dwell time for gradient elution (min); equal to the time it takes a change in mobile phase composition to pass from the gradient mixer to the column inlet (min)
t_0	time required for a non-retained solute to elute from the column
Z	equal to $-\mathrm{d}[\log k'(C)]/\mathrm{d}(\log C)$
φ	volume fraction of organic solvent in the mobile phase in reversed-phase chromatography

ACKNOWLEDGEMENT

We thank manager M. Matsuzaki for permitting this paper to be published.

REFERENCES

1 J. W. Dolan, L. R. Snyder and M. A. Quarry, *Chromatographia*, 24 (1987) 261.
2 M. A. Quarry, R. A. Grob and L. R. Snyder, *Anal. Chem.*, 58 (1986) 907.
3 P. Jandera, M. Janderová and J. Churáček, *J. Chromatogr.*, 148 (1978) 79.
4 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
5 K. Koizumi, T. Tanimoto and Y. Okada, *J. Chromatogr.*, 464 (1989) 365.
6 Y. Kato, T. Kitamura, A. Mitsui, Y. Yamasaki, T. Hashimoto, T. Murotsu, S. Fukushige and K. Matsubara, *J. Chromatogr.*, 447 (1988) 212.