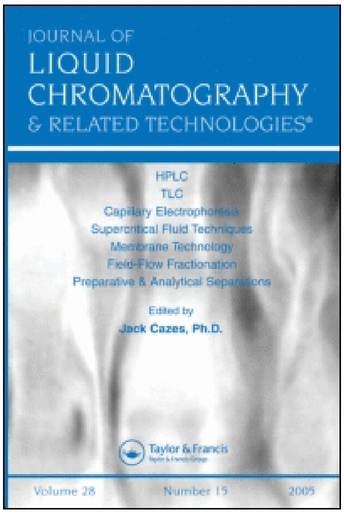

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Application of a Gradient Retention Model Developed by Using Isocratic Data for the Prediction of Retention, Resolution, and Peak Asymmetry in Ion Chromatography

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Abstract: In this work a model was developed for the prediction of retention time, resolution, and peak asymmetry in gradient elution mode by using isocratic experimental data. The predictive performance and generalization ability of the developed model was extensively tested by using an external experimental data set. The analysis of errors was performed in order to discuss and explain characteristics of the model. It was shown that the model performed satisfactorily and that it could be used for a modeling procedure in the optimization part of the ion chromatography method development.

Keywords: Gradient elution, Ion chromatography, Peak asymmetry, Resolution, Retention model

INTRODUCTION

A major challenge in the development of ion chromatographic separation methods is the rational selection of optimal experimental conditions that can provide an adequate separation and a reasonable run time.

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The reliability of an optimization procedure depends on two factors: the quality of the retention description for all compounds present in the mixture and the selection of the criterion function used to measure the separation between each pair of chromatographic peaks.

Several theoretical and empirical retention models have been proposed and tested for isocratic[1–13] and gradient predictions.[14–16] Artificial neural network (ANN) retention models are the most suitable choice for retention modeling of isocratic elution due to their speed and accuracy, as well as to their simple implementation in a global optimization process.[17–19] However, artificial neural network retention models may correlate only a limited number of parameters describing variable gradient curves (i.e., retention time and slope of linear gradient elution curve[14]). The inclusion of other parameters would drastically increase the number of experiments needed for the modeling procedure. This problem can be overcome by using gradient retention prediction based on isocratic retention experimental data.[20,21]

Usually, the separation is globally optimized by taking into account exclusively, the retention times of solutes, which enables a fast and simple calculation. However, this is suitable for modeling chromatograms that include symmetrical peaks and show similar plate counts.[22] Inclusion of the peak size and shape in the optimization process might be of interest when asymmetric or low efficiency peaks are involved, or when poorly separated symmetrical peaks are observed. There is no universal theoretical model for the exact description of the shape of chromatographic peaks. A number of empirical mathematical functions have been reported in the literature. Commonly, the elution profiles of symmetrical chromatographic peaks are described by the Gaussian model.[23–27] However, the use of this model for skewed peaks may produce large errors. Several functions have been recommended in the literature to avoid this problem.[28,29] One of them is a Gaussian based equation whose variance is a combined parabolic Lorentzian function (parabolic Lorentzian modified Gaussian model, PLMG).[30] The parabola accounts for the non-Gaussian shaped peak, whereas the Lorentzian function cancels the variance growth out of the peak region. The PLMG model is, however, not adequate for prediction purposes due to its complexity. It has too many parameters (seven), which have no direct meaning in terms of peak shape characteristics.

The aim of this work was the development of a gradient elution retention model that could be applied for the simultaneous modeling of resolution and peak asymmetry in ion chromatography. The investigations resulted in the gradient elution retention model that was based on isocratic experimental data, which allowed for the fast and simple modeling of various gradient profiles. This study includes the extensive error analysis of the developed gradient elution model in order to verify its performance characteristics.

MODEL DESCRIPTION

Generally, an asymmetric chromatography peak may be described by model functions that include at least three parameters, one related to the peak position, the other to its variance (broadness), and the last one to the skewness. The fourth parameter is normally added to account for the peak area or its height, which is related to the concentration of the solute, but this one should remain constant on switching from the isocratic to gradient elution mode.

The three parameters require at least three points to be read from an isocratic peak for its hopefully complete description. The choice of the first point is straightforward:

- retention time of the peak maximum (k denotes the corresponding retention factor).

The selection of other points is not as simple. The points have to be picked in the non-overlapping region of the peeks in case of crowded chromatograms. We assumed that peaks would overlap commonly only in their basal region, so that the half-height of the peaks would be in principle "clear" for the picking. This in accordance with the common definition of peak asymmetry that reads as the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope, with all measurements made at 50% of the maximum peak height. The other two points are, therefore:

- retention time of the half-height point at the fronting side of the peak ($k_{-50\%}$ denotes the corresponding retention factor),
- retention time of the half-height point at the tailing side of the peak ($\mathrm{k}_{+50\%}$ denotes the corresponding retention factor).

Throughout the set of isocratic experiments, the quadratic polynomial dependence was used to establish relationship between the logarithm of the retention factor and logarithm of KOH concentration, $c(\mathrm{KOH})$, in mobile phase:

$$\log k = a_0 + a_1 \log c(\mathrm{KOH}) + a_2[\log c(\mathrm{KOH})]^2 \tag{1}$$

$$\log k_{-50\%} = a_0 + a_1 \log c(\mathrm{KOH}) + a_2[\log c(\mathrm{KOH})]^2 \tag{2}$$

$$\log k_{+50\%} = a_0 + a_1 \log c(\mathrm{KOH}) + a_2[\log c(\mathrm{KOH})]^2 \tag{3}$$

where a_i are regression coefficients with characteristic values for a given ion chromatographic system, which were determined by using the common regression methodology.

Mathematical treatment that yields an analytical expression for the retention for a particular gradient profile was based on a previously described procedure.[21] The integral gradient elution equations of the general form:

$$F\left(t_{\mathrm{g}}, k, t_0\right) = 0 \tag{4}$$

$$F\left(t_{\mathrm{g}-50\%}, k_{-50\%}, t_0\right) = 0 \tag{5}$$

$$F\left(t_{\mathrm{g}+50\%}, k_{+50\%}, t_0\right) = 0 \tag{6}$$

were used to describe the final retention times of peak maxima and peak half-heights. In Equations 4, 5, and 6, t_{g}, $t_{\mathrm{g}-50\%}$ and $t_{\mathrm{g}+50\%}$ represent the final (integral) retention times of the solute and t_0 is the column void time.

The general form of the integral gradient equation is converted to the form suitable for numeric integration by allowing for the temporal variation of c. The detailed procedure was elaborated before,[21] and here we present the final results:

$$t_0 = \int_0^{t_{\mathrm{g}}-t_0} \frac{dt}{k[c(t)]} \tag{7}$$

$$t_0 = \int_0^{t_{\mathrm{g}-50\%}-t_0} \frac{dt}{k_{-50\%}[c(t)]} \tag{8}$$

$$t_0 = \int_0^{t_{\mathrm{g}+50\%}-t_0} \frac{dt}{k_{+50\%}[c(t)]} \tag{9}$$

The gradient elution time (the variable to be calculated, t_{g}, $t_{\mathrm{g}-50\%}$, $t_{\mathrm{g}+50\%}$) of a solute can be found by splitting the integral in small isocratic time steps:

$$t_0 = \int_0^{t_g-t_0} \frac{dt}{k[c(t)]} = \int_0^{t_1} \frac{dt}{k[c(t)]} + \int_{t_1}^{t_2} \frac{dt}{k[c(t)]} + \cdots + \int_{t_{i-1}}^{t_1} \frac{dt}{k[c(t)]} + \int_{t_i}^{t_{i+1}} \frac{dt}{k[c(t)]} \tag{10}$$

$$t_0 = \int_0^{t_{g-50\%}-t_0} \frac{dt}{k_{-50\%}[c(t)]} = \int_0^{t_1} \frac{dt}{k_{-50\%}[c(t)]} + \int_{t_1}^{t_2} \frac{dt}{k_{-50\%}[c(t)]} + \cdots + \int_{t_{i-1}}^{t_1} \frac{dt}{k_{-50\%}[c(t)]} + \int_{t_i}^{t_{i+1}} \frac{dt}{k_{-50\%}[c(t)]} \tag{11}$$

$$t_0 = \int_0^{t_{g+50\%}-t_0} \frac{dt}{k_{+50\%}[c(t)]} = \int_0^{t_1} \frac{dt}{k_{+50\%}[c(t)]} + \int_{t_1}^{t_2} \frac{dt}{k_{+50\%}[c(t)]} + \cdots + \int_{t_{i-1}}^{t_1} \frac{dt}{k_{+50\%}[c(t)]} + \int_{t_i}^{t_{i+1}} \frac{dt}{k_{+50\%}[c(t)]} \tag{12}$$

Furthermore, $k[c]$ can be assumed constant for each step by taking the mean value of $k[c]$ values at the boundaries of the integral. By using this assumption, the approximate cumulative integral I on the right-hand side of Equations 10–12 can be easily solved yielding the final solution for gradient retention:

$$t_{\mathrm{g}} = t_0 + t_i + (t_0 - I_{0,i})k(c)_{i,i+1} \tag{13}$$

$$t_{\mathrm{g}-50\%} = t_0 + t_i + (t_0 - I_{(-50\%)0,i})k_{-50\%}(c)_{i,i+1} \tag{14}$$

$$t_{\mathrm{g}+50\%} = t_0 + t_i + (t_0 - I_{(+50\%)0,i})k_{+50\%}(c)_{i,i+1} \tag{15}$$

EXPERIMENTAL

Instrumentation

A Dionex DX600 chromatography system (Sunnyvale, CA, USA), equipped with quaternary gradient pump (GS50), eluent generator module (EG40), degas unit on eluent generator, trap column (CR-TC), chromatography module (LC30), and detector module (ED50A) was used in all the experiments. A Dionex IonPac AG19 (4 × 50 mm) guard column, an IonPac AS19 (4 × 250 mm) separation column, and an ASRS – ULTRA II 4 mm electrolytic suppressor (working in recycle mode) were used, respectively. The sample loop volume was 25 µL; eluent flow rate was 1.0 mL/min.

Reagents and Solutions

Standard solutions of bromate, bromide, nitrite, iodide, and perchlorate (1.0000 g/L) were prepared from air dried (at 105°C) salts of individual anions of p.a. grade (Merck, Darmstadt, Germany). Appropriate amounts of individual salts were weighed into a volumetric flask (100 mL) and dissolved with Milli-Q water. Mixed stock standard solutions of bromate, bromide, nitrite, and perchlorate (100.00 mg/L) were prepared by measuring the appropriate volume of standard solutions into a 100 mL volumetric flask, which was later filled to the mark with Milli-Q water. Working eluent solutions were prepared online by appropriate dilution of KOH with Milli-Q water. In all cases, 18 MΩcm^{-1} water (Millipore, Bedford, MA, USA) was used for dilution.

Table 1. Experimental data used for development of isocratic retention model

	(KOH)/mmol/L	BrO_3^-	Br^-	NO_2^-	I^-	ClO_4^-
Retention behavior at the point of maximum peak height/min	73.18	3.15	3.69	4.15	7.60	12.20
	64.66	3.20	3.79	4.29	8.15	13.42
	56.14	3.32	4.01	4.58	8.93	14.95
	47.61	3.46	4.27	4.94	10.78	17.44
	39.09	3.66	4.61	5.42	11.65	20.36
	30.57	4.04	5.25	6.27	14.17	25.34
	22.05	4.71	6.36	7.76	18.52	33.96
	13.52	6.14	8.82	11.09	28.57	54.10
	5.00	12.64	19.76	25.77	72.12	139.97
Retention behavior at the point of 50% of maximum peak height at the fronting side of the peak/min	73.18	3.12	3.66	4.11	7.54	12.08
	64.66	3.17	3.75	4.25	8.07	13.28
	56.14	3.29	3.97	4.54	8.85	14.80
	47.61	3.43	4.22	4.90	10.68	17.26
	39.09	3.62	4.56	5.37	11.53	20.15
	30.57	4.00	5.19	6.22	14.03	25.09
	22.05	4.66	6.30	7.69	18.33	33.58
	13.52	6.08	8.73	11.00	28.33	53.56
	5.00	12.51	19.56	25.52	71.46	138.42
Retention behavior at the point of 50% of maximum peak height at the tailing side of the peak/min	73.18	3.20	3.75	4.20	7.71	12.42
	64.66	3.24	3.85	4.34	8.26	13.65
	56.14	3.37	4.07	4.63	9.05	15.21
	47.61	3.51	4.33	5.00	10.92	17.72
	39.09	3.70	4.68	5.48	11.81	20.72
	30.57	4.08	5.32	6.34	14.37	25.78
	22.05	4.76	6.45	7.85	18.79	34.56
	13.52	6.21	8.94	11.22	29.00	54.98
	5.00	12.77	20.02	26.06	73.25	142.70

Experimental Design

In isocratic elution mode the retention time is monitored in relation with the concentration of KOH in eluent. The concentration of KOH used for

Table 2. Experimental data used for validation of isocratic retention model. Retention behavior at the point of maximum peak height

(KOH)/mmol/L	BrO_3^-/min	Br^-/min	NO_2^-/min	I^-/min	ClO_4^-/min
80	3.07	3.57	3.99	7.21	11.55
78.3	3.16	3.71	4.18	7.47	11.74
76.59	3.06	3.56	3.99	7.27	11.65
74.89	3.16	3.68	4.11	7.47	12.00
71.48	3.10	3.64	4.12	7.67	12.30
69.77	3.16	3.74	4.22	7.86	12.75
68.07	3.15	3.73	4.22	8.05	12.94
66.36	3.15	3.73	4.22	7.98	13.06
62.95	3.25	3.89	4.42	8.34	13.82
61.25	3.20	3.83	4.35	8.41	13.91
59.55	3.25	3.93	4.49	8.63	14.34
57.84	3.26	3.93	4.49	8.82	14.69
54.43	3.36	4.05	4.63	9.18	15.45
52.73	3.37	4.13	4.76	9.41	15.89
51.02	3.37	4.11	4.73	9.54	16.19
49.32	3.41	4.19	4.84	9.94	16.80
45.91	3.48	4.31	5.00	10.33	17.75
44.20	3.54	4.39	5.11	10.64	18.44
42.50	3.57	4.45	5.19	10.95	19.04
40.8	3.62	4.54	5.32	11.36	19.63
37.39	3.69	4.67	5.50	11.92	20.95
35.68	3.81	4.86	5.74	12.54	22.10
33.98	3.91	5.00	5.92	13.04	23.09
32.27	3.90	5.05	6.01	13.43	23.91
28.86	4.15	5.43	6.51	14.84	26.72
27.16	4.21	5.56	6.74	15.46	28.01
25.45	4.35	5.80	7.02	16.45	29.93
23.75	4.51	6.07	7.37	17.47	31.89
20.34	4.87	6.67	8.20	19.93	36.86
18.64	5.22	7.11	8.73	21.48	39.91
16.93	5.41	7.55	9.36	23.33	43.52
15.23	5.72	8.11	10.13	25.70	48.29
11.82	6.70	9.74	12.31	32.08	61.04
10.11	7.45	11.00	13.99	37.11	71.01
8.41	8.47	12.73	16.31	43.96	84.78
6.7	10.03	15.36	19.86	54.61	105.59

isocratic elution mode experimental data collection was varied from 6.70 mmol/L to 80.00 mmol/L. In principle, each experimental data point should have an equal influence on the retention model, if the

Table 3. Experimental data used for validation of isocratic retention model. Retention behavior at the point of 50% of maximum peak height at the fronting side of the peak

(KOH)/mmol/L	BrO_3^-/min	Br^-/min	NO_2^-/min	I^-/min	ClO_4^-/min
80	3.04	3.53	3.95	7.14	11.44
78.3	3.12	3.67	4.14	7.40	11.63
76.59	3.02	3.52	3.95	7.20	11.53
74.89	3.13	3.64	4.08	7.40	11.88
71.48	3.07	3.60	4.08	7.60	12.18
69.77	3.13	3.69	4.18	7.79	12.62
68.07	3.12	3.69	4.18	7.98	12.81
66.36	3.12	3.69	4.18	7.90	12.92
62.95	3.21	3.84	4.38	8.26	13.68
61.25	3.17	3.78	4.31	8.33	13.77
59.55	3.21	3.88	4.45	8.55	14.19
57.84	3.23	3.89	4.45	8.73	14.54
54.43	3.32	4.00	4.59	9.09	15.29
52.73	3.34	4.08	4.72	9.32	15.72
51.02	3.34	4.07	4.69	9.45	16.02
49.32	3.38	4.14	4.79	9.84	16.61
45.91	3.45	4.26	4.95	10.23	17.56
44.2	3.50	4.34	5.06	10.54	18.24
42.5	3.53	4.40	5.14	10.84	18.83
40.8	3.59	4.49	5.27	11.25	19.43
37.39	3.65	4.62	5.45	11.80	20.69
35.68	3.78	4.81	5.69	12.41	21.86
33.98	3.87	4.94	5.86	12.91	22.84
32.27	3.86	4.99	5.95	13.30	23.65
28.86	4.11	5.37	6.45	14.71	26.42
27.16	4.16	5.49	6.67	15.31	27.67
25.45	4.31	5.74	6.96	16.28	29.60
23.75	4.47	6.00	7.30	17.29	31.57
20.34	4.82	6.60	8.12	19.73	36.44
18.64	5.17	7.03	8.66	21.28	39.53
16.93	5.35	7.47	9.27	23.04	42.99
15.23	5.66	8.02	10.03	25.46	47.83
11.82	6.63	9.63	12.19	31.80	60.40
10.11	7.37	10.88	13.86	36.71	70.25
8.41	8.38	12.59	16.17	43.57	83.84
6.7	9.93	15.20	19.67	54.02	104.61

Table 4. Experimental data used for validation of isocratic retention model. Retention behavior at the point of 50% of maximum peak height at the tailing side of the peak

(KOH)/mmol/L	BrO_3^-/min	Br^-/min	NO_2^-/min	I^-/min	ClO_4^-/min
80	3.10	3.62	4.03	7.31	11.74
78.3	3.20	3.77	4.23	7.56	11.94
76.59	3.09	3.61	4.03	7.37	11.84
74.89	3.20	3.74	4.16	7.57	12.20
71.48	3.13	3.69	4.17	7.77	12.51
69.77	3.20	3.79	4.27	7.96	12.97
68.07	3.19	3.79	4.27	8.15	13.16
66.36	3.19	3.79	4.27	8.09	13.28
62.95	3.29	3.94	4.47	8.45	14.05
61.25	3.24	3.88	4.40	8.52	14.15
59.55	3.29	3.98	4.54	8.75	14.59
57.84	3.30	3.99	4.54	8.94	14.94
54.43	3.40	4.11	4.68	9.30	15.72
52.73	3.41	4.19	4.81	9.54	16.16
51.02	3.41	4.17	4.78	9.67	16.46
49.32	3.45	4.25	4.89	10.07	17.08
45.91	3.52	4.37	5.05	10.47	18.06
44.2	3.58	4.45	5.16	10.79	18.75
42.5	3.61	4.51	5.25	11.10	19.38
40.8	3.66	4.61	5.37	11.51	19.98
37.39	3.73	4.74	5.57	12.09	21.29
35.68	3.86	4.93	5.80	12.71	22.48
33.98	3.95	5.07	5.98	13.22	23.49
32.27	3.95	5.12	6.08	13.63	24.33
28.86	4.20	5.51	6.58	15.06	27.16
27.16	4.25	5.63	6.82	15.69	28.48
25.45	4.40	5.88	7.10	16.68	30.44
23.75	4.56	6.15	7.45	17.72	32.44
20.34	4.93	6.76	8.29	20.21	37.48
18.64	5.27	7.20	8.83	21.79	40.64
16.93	5.47	7.65	9.47	23.64	44.20
15.23	5.78	8.22	10.24	26.06	49.16
11.82	6.77	9.87	12.45	32.58	62.09
10.11	7.52	11.15	14.17	37.69	72.18
8.41	8.56	12.90	16.51	44.63	86.17
6.7	10.14	15.57	20.09	55.31	107.44

modeling and validation set is to be the representative of data from the whole design area. For that purpose, the design space was divided into nine equidistant subspaces. The modeling set (Table 1) was constructed by randomly choosing one experimental data point from each of the

Table 5. Experimental data used for validation of gradient retention model. Retention behavior at the point of maximum peak height

Gradient starting time/min	Initial concentration of KOH/mmol/L	Slope of the gradient profile/degrees	BrO_3^-/min	Br^-/min	NO_2^-/min	I^-/min	ClO_4^-/min
0.00	10.00	53.00	6.48	8.62	10.21	19.22	28.09
0.00	10.00	71.00	5.85	7.47	8.63	15.02	21.10
0.00	10.00	90.00	3.16	3.64	4.03	7.09	11.15
0.00	15.00	53.00	5.46	7.25	8.62	16.88	25.36
0.00	15.00	71.00	5.14	6.59	7.66	13.76	19.68
0.00	15.00	90.00	3.15	3.63	4.03	7.08	11.13
0.00	20.00	53.00	4.82	6.32	7.51	14.99	23.01
0.00	20.00	71.00	4.62	5.90	6.87	12.62	18.35
0.00	20.00	90.00	3.16	3.63	4.03	7.09	11.13
10.00	10.00	53.00	7.53	11.11	13.99	25.81	35.71
10.00	10.00	71.00	7.51	11.08	13.82	22.60	29.42
10.00	10.00	90.00	7.50	11.03	12.74	15.74	19.81
10.00	15.00	53.00	5.85	8.25	10.26	21.95	31.87
10.00	15.00	71.00	5.82	8.21	10.22	20.14	27.21
10.00	15.00	90.00	5.80	8.18	10.18	15.09	19.18
10.00	20.00	53.00	4.99	6.79	8.31	18.74	28.44
10.00	20.00	71.00	4.96	6.76	8.27	17.84	25.08
10.00	20.00	90.00	4.97	6.76	8.27	14.46	18.56
20.00	10.00	53.00	7.55	11.31	14.21	31.77	43.14
20.00	10.00	71.00	7.51	11.07	14.07	29.70	37.64
20.00	10.00	90.00	7.50	11.04	14.02	24.44	28.57
20.00	15.00	53.00	5.85	8.25	10.27	25.45	37.91
20.00	15.00	71.00	5.65	7.94	9.86	19.83	26.95
20.00	15.00	90.00	5.81	8.18	10.17	23.15	27.31
20.00	20.00	53.00	4.98	6.79	8.30	20.01	33.10
20.00	20.00	71.00	4.97	6.76	8.27	19.88	31.16
20.00	20.00	90.00	4.98	6.77	8.28	19.85	26.07

subspaces. The rest of the experimental data points were used for validation procedures (Tables 2–4).

The gradient elution mode was defined by using permutation of the following conditions: initial concentrations used for gradient elution were 10.00 mmol/L, 15.00 mmol/L, and 20.00 mmol/L; gradient starting times of gradient elution were 0.00 min, 10.00 min, and 20.00 min; slopes of gradient elution linear curves were 53.00, 71.00, and 90.00. The gradient elution experimental data set is presented in Table 5, and it was used for validation of the gradient elution model only.

Modeling Calculations

The homemade retention model was created in MATLAB environment (MATLAB 6.0, MathWorks, Sherborn, MA, USA). The following routines were written: splitting of experimental data set onto modeling and validation sets, isocratic elution retention modeling routine, gradient elution retention modeling routine, calculation of resolution and peak asymmetry, and comparison of isocratic and gradient prediction performance.

RESULTS AND DISCUSSION

The modeling set of isocratic experiments (Table 1) was used for the determination of coefficients of the relation between capacity factor and concentration of the eluent competing ion (Equations 1–3). The rest of the experimental data set was used for validation procedures (Tables 2–4). This allowed for the estimation of generalization potential of the proposed isocratic retention model, as well as for the calculation of correlation coefficients between measured and predicted data by using validation data set only. Table 5 summarizes the calculated regression coefficients for each particular ion; thus the isocratic part of the retention model is completely defined. Moreover, the table shows that the correlation coefficients (calculated by using validation experimental data set only) are almost identical for the peak maximum case and for both of the half-height cases. This result can lead to the conclusion that the prediction of retention time for a particular ion is not significantly affected by the exact place at the peak for which it is calculated. On the other hand, calculated correlation coefficients for particular ions are ranged between 0.9998 for bromide and 0.9703 for perchlorate. Thus, a considerable difference between prediction performances for various ions is observed. Nevertheless, even the worst case can be considered good enough, keeping in mind that correlation coefficients were calculated by using a validation experimental data set only. This result indicates the great potential of the model to be applied for gradient elution calculations.

The comparison between prediction performance for peak maximum retention time in both isocratic and gradient elution mode for a particular ion is shown in Figure 1. The calculated errors for bromide, nitrite, and iodide are randomly distributed around zero, indicating that there is no systematic error present in the model. In the case of bromate prediction, errors are mainly distributed above zero for both isocratic and gradient retention model. It can also be observed that gradient elution errors exhibit the same trend as isocratic ones. Probably, the isocratic elution retention model performs with a systematic error, which is later on propagated through the gradient model; the gradient model does not seem to bring

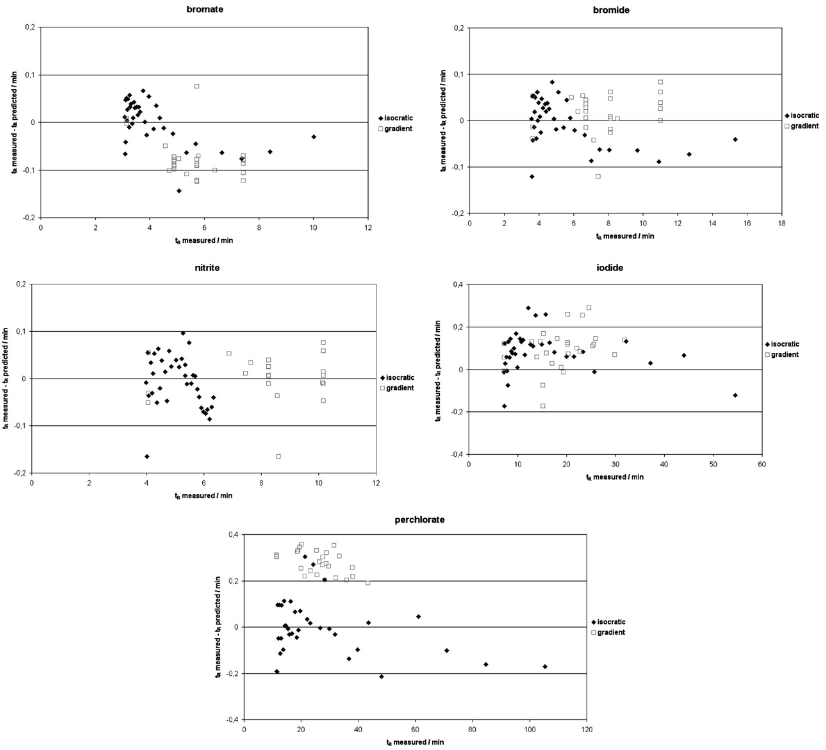

Figure 1. Prediction of isocratic and gradient retention: absolute error vs. measured retention time.

forth the systematic error in the case of bromate prediction. The predictive ability test for perchlorate shows that the gradient model performs with a significantly larger error then the isocratic elution retention model; the majority of gradient errors are distributed above zero. This feature points to a systematic error in the gradient elution retention model, since the isocratic errors are randomly distributed around zero. One may ask if the origin of the systematic error is due to the numerical integration procedure incorporated in the model. However, Figure 1 shows that the errors of late eluted anions are considerably larger than the errors of fast eluting anions in both isocratic and gradient elution mode. This means that the number of steps used for numerical integration does not influence, significantly, the predictive ability of the model. Finally, it can be observed that the magnitude of all errors for both isocratic and gradient retention model and for all anions are generally small and that the models have the potential for prediction of resolution and peak asymmetry.

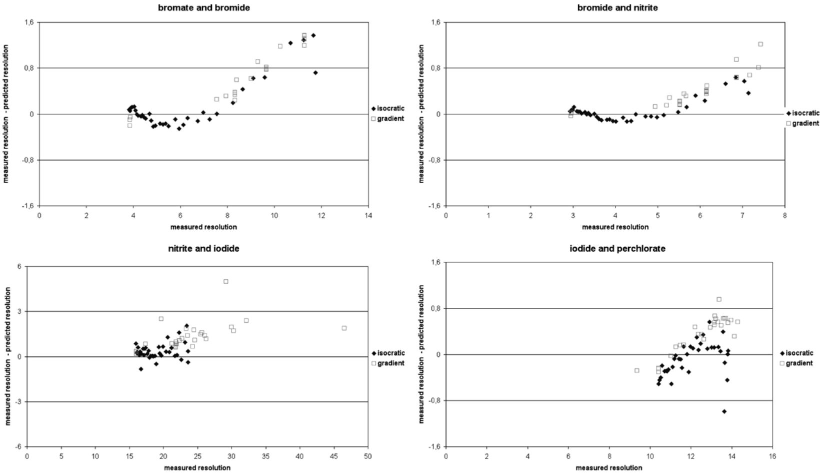

Figure 2. Prediction of resolution in isocratic and gradient elution mode: absolute error vs. measured resolution.

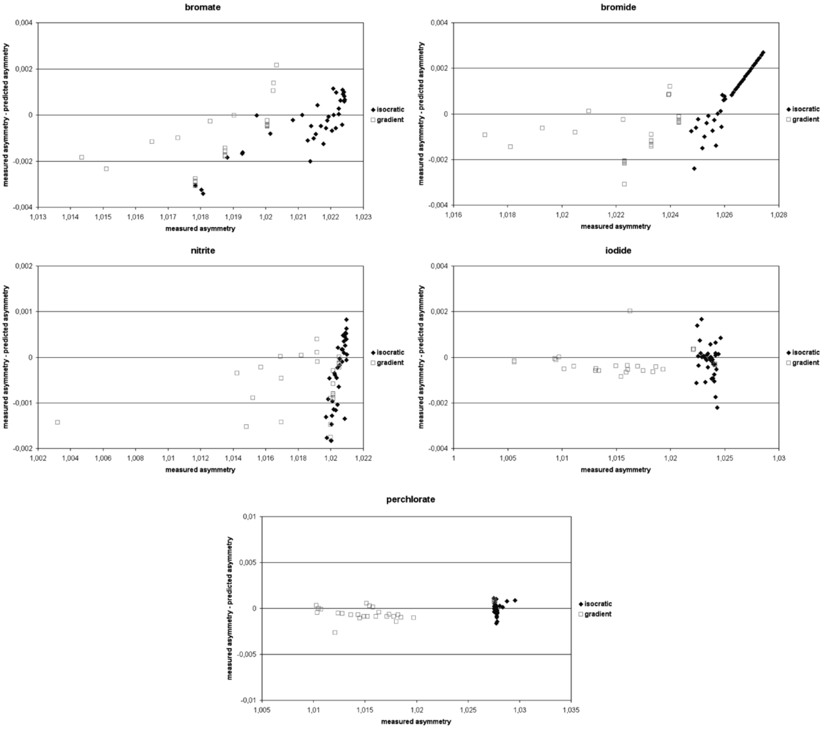

Figure 3. Prediction of peak asymmetry in isocratic and gradient elution mode: absolute error vs. measured peak asymmetry.

Figure 2 presents the performance characteristics results for modeling of resolution between neighboring peaks of investigated anions. It can be seen that both isocratic and gradient elution resolution model errors are distributed above zero for all anions and show the same trend. This indicates that the gradient elution resolution model merely propagate the characteristic systematic error of the isocratic model. Furthermore, the observed trend of errors can be explained by the increased number of parameters, which needs to be modeled and incorporated in the final resolution value. This also explains the fact that the prediction

Table 6. Experimental data used for validation of gradient retention model. Retention behavior at the point of 50% of maximum peak height at the fronting side of the peak

Gradient starting time/min	Initial concentration of KOH/ mmol/L	Slope of the gradient profile/ degrees	BrO_3^-/ min	Br^-/ min	NO_2^-/ min	I^-/ min	ClO_4^-/ min
0.00	10.00	53.00	6.42	8.55	10.13	19.08	27.89
0.00	10.00	71.00	5.81	7.41	8.57	14.92	20.96
0.00	10.00	90.00	3.14	3.60	4.00	7.03	11.03
0.00	15.00	53.00	5.41	7.18	8.56	16.75	25.16
0.00	15.00	71.00	5.09	6.53	7.60	13.66	19.53
0.00	15.00	90.00	3.13	3.59	3.99	7.02	11.01
0.00	20.00	53.00	4.78	6.26	7.44	14.87	22.81
0.00	20.00	71.00	4.58	5.85	6.81	12.53	18.21
0.00	20.00	90.00	3.13	3.59	3.99	7.02	11.01
10.00	10.00	53.00	7.46	11.00	13.87	25.65	35.50
10.00	10.00	71.00	7.44	10.95	13.71	22.50	29.27
10.00	10.00	90.00	7.42	10.91	12.71	15.68	19.70
10.00	15.00	53.00	5.79	8.16	10.17	21.81	31.65
10.00	15.00	71.00	5.76	8.11	10.12	20.03	27.05
10.00	15.00	90.00	5.75	8.09	10.08	15.03	19.07
10.00	20.00	53.00	4.94	6.71	8.23	18.60	28.23
10.00	20.00	71.00	4.92	6.68	8.19	17.72	24.93
10.00	20.00	90.00	4.92	6.69	8.19	14.40	18.44
20.00	10.00	53.00	7.47	11.19	14.08	31.58	42.92
20.00	10.00	71.00	7.43	10.95	13.94	29.58	37.49
20.00	10.00	90.00	7.43	10.92	13.88	24.38	28.45
20.00	15.00	53.00	5.79	8.15	10.17	25.24	37.67
20.00	15.00	71.00	5.59	7.85	9.77	19.71	26.79
20.00	15.00	90.00	5.75	8.09	10.08	23.09	27.20
20.00	20.00	53.00	4.93	6.71	8.23	19.80	32.86
20.00	20.00	71.00	4.92	6.69	8.19	19.67	30.96
20.00	20.00	90.00	4.94	6.70	8.20	19.65	25.95

ability of the model, with respect to resolution, is somewhat lower than with respect to retention (the retention calculations are the core of resolution calculations). Nevertheless, the magnitude of errors observed proves very good predictive ability in both isocratic and gradient elution mode for all anions. Therefore, the model can be used for optimization purposes in the development of ion chromatography methods.

Figure 3 presents the performance characteristics results for modeling of peak asymmetry for all the investigated anions. The peak asymmetry in gradient elution is predicted with lower absolute errors

Table 7. Experimental data used for validation of gradient retention model. Retention behavior at the point of 50% of maximum peak height at the tailing side of the peak

Gradient starting time/min	Initial concentration of KOH/ mmol/L	Slope of the gradient profile/ degrees	$\mathrm{BrO_3^-}$/ min	$\mathrm{Br^-}$/ min	$\mathrm{NO_2^-}$/ min	$\mathrm{I^-}$/ min	$\mathrm{ClO_4^-}$/ min
0.00	10.00	53.00	6.54	8.71	10.30	19.40	28.39
0.00	10.00	71.00	5.90	7.54	8.70	15.15	21.31
0.00	10.00	90.00	3.19	3.68	4.08	7.18	11.32
0.00	15.00	53.00	5.51	7.34	8.70	17.06	25.65
0.00	15.00	71.00	5.18	6.66	7.72	13.89	19.88
0.00	15.00	90.00	3.19	3.67	4.07	7.17	11.31
0.00	20.00	53.00	4.87	6.40	7.58	15.16	23.28
0.00	20.00	71.00	4.66	5.97	6.93	12.74	18.56
0.00	20.00	90.00	3.19	3.68	4.07	7.17	11.31
10.00	10.00	53.00	7.61	11.27	14.13	26.01	36.03
10.00	10.00	71.00	7.59	11.22	13.93	22.75	29.65
10.00	10.00	90.00	7.58	11.18	12.77	15.82	19.99
10.00	15.00	53.00	5.91	8.36	10.38	22.17	32.19
10.00	15.00	71.00	5.88	8.31	10.33	20.30	27.44
10.00	15.00	90.00	5.87	8.29	10.29	15.17	19.36
10.00	20.00	53.00	5.04	6.88	8.40	18.95	28.75
10.00	20.00	71.00	5.02	6.85	8.36	18.01	25.32
10.00	20.00	90.00	5.03	6.85	8.36	14.54	18.73
20.00	10.00	53.00	7.63	11.47	14.37	32.01	43.50
20.00	10.00	71.00	7.59	11.22	14.24	29.89	37.89
20.00	10.00	90.00	7.58	11.19	14.18	24.52	28.74
20.00	15.00	53.00	5.91	8.35	10.38	25.74	38.28
20.00	15.00	71.00	5.71	8.04	9.97	19.99	27.19
20.00	15.00	90.00	5.87	8.29	10.29	23.23	27.49
20.00	20.00	53.00	5.03	6.88	8.40	20.29	33.50
20.00	20.00	71.00	5.02	6.85	8.36	20.16	31.44
20.00	20.00	90.00	5.04	6.86	8.37	20.13	26.24

Table 8. Regression coefficients of the isocratic retention model. Retention behavior at the maximum peak height (t_R). at the point of 50% of maximum peak height at the fronting ($t_{R-50\%}$) and tailing ($t_{R+50\%}$) side of the peak

	Coefficient	t_R	$t_{R-50\%}$	$t_{R+50\%}$
Bromate	a_0	1.3734	1.3351	1.3539
	a_1	−1.1073	−1.0443	−1.0746
	a_2	0.0309	−0.0051	0.0121
	Correlation	0.9994	0.9994	0.9994
Bromide	a_0	1.5884	1.5645	1.5742
	a_1	−1.0870	−1.0559	−1.0680
	a_2	0.0343	0.0147	0.0229
	Correlation	0.9998	0.9998	0.9998
Nitrite	a_0	1.7070	1.6899	1.6977
	a_1	−1.0695	−1.0522	−1.0602
	a_2	0.0296	0.0188	0.0239
	Correlation	0.9989	0.9989	0.9989
Iodide	a_0	2.1555	2.1423	2.1465
	a_1	−1.0254	−1.0202	−1.0211
	a_2	0.0207	0.0176	0.0186
	Correlation	0.9743	0.9746	0.9739
Perchlorate	a_0	2.4479	2.4296	2.4359
	a_1	−1.0075	−0.9973	−1.0002
	a_2	0.0071	0.0026	0.0039
	Correlation	0.9703	0.9703	0.9703

than in the isocratic elution, although, the isocratic model and prediction is a starting point for stepwise numerical integration of the gradient model. However, the retention times of the anions are much longer in the isocratic elution mode and the observed peak asymmetries are generally larger. The magnitude of gradient asymmetry error clearly shows the increasing trend with the increase of absolute asymmetry value. These results indicate that both of the models possess a proportional systematic error. Still, the magnitude of errors remains rather small, pointing to the conclusion that the developed model can be successfully used in the optimization process in ion chromatography method development.

CONCLUSION

This work describes the application of the gradient elution retention model developed by using isocratic data for the prediction of retention, resolution, and peak asymmetry in ion chromatography. The results show that the isocratic elution retention model generalizes the behavior of the ion chromatographic system in an adequate manner, and that it can be used for the prediction and analysis of gradient elution. The mode of the propagation of errors in the bromate retention case indicates that the numerical integration of the gradient elution retention model does not affect the prediction ability of the model. The performance characteristics of the model, with respect to resolution prediction, are somewhat lower than with respect to retention prediction due to the fact that more parameters need to be calculated and incorporated in the prediction. The prediction of peak asymmetry in gradient elution mode is more accurate than in the isocratic elution mode, as a result of the faster elution and lower peak asymmetry characteristic for the gradient mode. Finally, the magnitude of all errors proves the very good predictive ability in both isocratic and gradient elution mode for all the investigated anions. Therefore, the model can be used for optimization purposes in the ion chromatography method development.

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