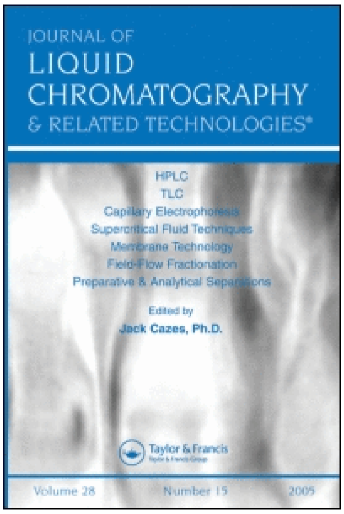

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PREDICTION OF NONLINEAR GRADIENT SIGNAL IN ION CHROMATOGRAPHY BASED ON "EXPERIMENT-FREE" METHODOLOGY

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☐ *One of the strategies that might be applied in ion chromatographic (IC) multi-segment gradient optimization is the simplification of gradient profile and its approximation by nonlinear elution. This work describes the IC signal modeling strategy for linear and nonlinear gradients. The performance characteristics of developed models do not depend on the linearity of gradient profile, but rather on initial gradient of eluent competing ion concentration. Four different IC models were developed and tested, requiring three, two, or single experimental data points. Moreover, a model based on "experiment-free" methodology using standard column manufacturer quality assurance documents was developed. It is shown that all the developed models have good predictive ability that decreases by reducing the model complexity and number of experimental data required. However, even the "experiment-free" methodology offers reasonable performance characteristics (average of relative error 4.99%) and might be recommended for IC method development purposes.*

Keywords ion chromatography, nonlinear gradient, signal prediction

INTRODUCTION

It is common practice to use step or linear gradient profiles of eluting counter ion concentration for the optimization of separation in ion chromatography (IC).[1–4] However, for some complex samples (e.g., series of homologues), this methodology may prove unsatisfactory resulting in complex multi-segment gradient profiles.[5] Such profiles are extremely difficult to optimize due to too many adjustable parameters; namely, the complexity of optimization, demand for experimental data, and computing time all increase exponentially with the number of segments employed. In such cases, a simple curved gradient approach might be more promising for achieving the optimal separation. Therein, the number of parameters that needs to be optimized is diminished; a single-step optimization

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problem is constructed that may be solved easily. In the subsequent real analysis of a complex sample, it is straightforward to approximate the solution by a series of linear gradient profiles, if necessary. However, this approach has not been investigated thoroughly so far.

A lot of effort was put forth in finding retention models capable of predicting gradient IC signal accurately. An ideal model would exert excellent performance characteristics. At the same time, it must be both time and money-saving particularly when related to routine applications. Artificial neural networks (ANN) retention models proved to be a very accurate and fast tool for linear gradient retention modeling. However, they are useful in a strictly limited domain of parameters (gradient slope, gradient starting time, and/or gradient starting concentration) that is explored in the network training process.[6–9] An approach that overcomes this drawback is based on the theoretical description of gradient retention. Examples are found in research;[10–13] the models are extensively tested and compared with some other well-known retention models,[14–17] showing good predictive ability both in gradient or isocratic IC. In our previous papers,[18,19] we suggested a very useful alternative based on transfer of information from the simple (isocratic) elution mode to a more complex (gradient) one. In all the mentioned cases, several (more than one) experiments are needed for creating the model with good predictive ability. This can be a severe limitation if a complex sample available in low amount needs to be analyzed. Moreover, the optimal method cannot be transferred safely to another IC column, which makes the global optimization process repetitive and costly.

The aim of this work was the development of retention model that was able to predict the IC signal of nonlinear gradient profiles. In the first instance, we used our own experimental data for the model development; this was the classical approach. In the second instance, we tried to use manufacturer quality assurance documents provided with the column in an attempt to make IC signal prediction free of any additional experiment. Retention models based on two or three experimental IC runs were, therefore, developed and compared with the models based on “experiment-free” methodology to provide a better insight into their performance characteristics.

ION CHROMATOGRAPHIC SIGNAL MODELING

Peak Shape Modeling

In this work, the generalized logistic function (GLF) was used for the peak shape modeling;[20] its general form is:

$$f(t) = \frac{C}{B}\frac{\mathrm{Exp}\left(\frac{A-t}{B}\right)}{\left[1+\mathrm{Exp}\left(\frac{A-t}{B}\right)\right]^{C+1}} \qquad (1)$$

Parameter A is associated with the distribution median; parameter B characterizes the distribution width; and parameter C bears information about the distribution skewness. GLF is a three parameter function requiring three experimental parameters to be completely defined. For that reason, three retention times (t_R), i.e., peak t_R, left half-width t_R, and right half-width t_R, were modeled. Additionally, peak area was selected as the fourth measurable parameter, due to its constancy for a defined eluent flow rate. It is used for scaling the chromatographic peaks to normalized GLF distributions.

Gradient Retention Modeling

Gradient elution chromatography may be described in principle either by differential or by integral equations. In this work the final (integral) retention times of solutes, t_g are described in terms of measurable properties (retention factor, k, and void time of a column, t_0). Hence, the integral equation seemed to be the reasonable choice. The final integral equation takes the following general form:

$$F(t_g, k, t_0) = 0 \tag{2}$$

although it may be given in any arrangement suitable for numerical solving. The numerical integration procedure includes splitting of the integral in small isocratic time steps. Within those steps, the capacity factor can be assumed constant and estimated by the isocratic retention model. This approach is used for modeling of peak left half-width and right half-width retention times.

Isocratic Retention Modeling

The well-known linear solvent strength isocratic retention model[21–23] is the simplest and most applied model in the ion chromatography. It can be described by the Eq. (3); (referred as *MODEL 2* in this work):

$$\log k = a_0 + a_1 \log(c(KOH)) \tag{3}$$

$c(KOH)$ denotes concentration of eluent competing ion (OH^- from KOH), a_0 is a constant for a given analyte, fixed eluent composition and stationary phase, and a_1 is associated with the negative of the analyte/eluent charge ratio. Since linear solvent strength retention model considers only electrostatic effects leading to ion exchange retention, the presence of other mechanisms or occurrence of factors influencing the retention will influence the predictive ability of the model. The presence of additional retention mechanism and/or factors in IC system can be identified as deviation from linearity. One possibility to overcome this problem is to include additional factor into the polynomial equation as a correction for nonlinearity (referred as *MODEL 3* in this work):

$$\log k = a_0 + a_1 \log(c(KOH)) + a_2 \log^2(c(KOH)) \tag{4}$$

The minimal number of experimental data points for *MODEL 2* and *MODEL 3* is two and three, respectively. Constant a_1, representing the negative of the ratio between analyte (x) and eluent charge (y) in *MODEL 2*, is *a priori* known for a given system. This leaves the intercept a_0 as the only unknown (*MODEL 1*):

$$\log k = a_0 + \frac{x}{y} \log(c(KOH)) \tag{5}$$

This implies that only one experiment is sufficient for modeling the retention. The documents related to the quality assurance of IC column and provided by the manufacturer normally include a test chromatogram

TABLE 1 Theoretical and Calculated Coefficients of the Isocratic Elution Retention Models Equations for Retention Time, Retention Time of the Left Half Width, and Retention Time of the Right Half Width

		MODEL 0		*MODEL 1*		*MODEL 2*		*MODEL 3*		
	Ion	a_1	a_2	a_1	a_2	a_1	a_2	a_1	a_2	a_3
$t_{50\%\ \mathrm{left}}$	Fluoride	0,9921	−1	1.0329	−1	0.9808	−0.9669	1.0633	−1.1004	0.0507
	Chlorite	1,3009	−1	1.3366	−1	1.2302	−0.9198	1.3706	−1.1459	0.0823
	Chloride	1,4461	−1	1.4787	−1	1.3832	−0.9331	1.4492	−1.0385	0.0388
	Chlorate	1,6978	−1	1.7320	−1	1.6523	−0.9523	1.6130	−0.8870	−0.0228
	Nitrate	1,8152	−1	1.8317	−1	1.7689	−0.9536	1.8437	−1.0728	0.0432
	Sulfate	3,3341	−2	3.2822	−2	3.3201	−2.0132	3.5220	−2.3394	0.1183
t_{max}	Fluoride	1,0067	−1	1.0504	−1	0.9599	−0.9347	1.0976	−1.1573	0.0824
	Chlorite	1,3061	−1	1.3470	−1	1.2257	−0.9078	1.3832	−1.1614	0.0921
	Chloride	1,4507	−1	1.4865	−1	1.3812	−0.9249	1.4622	−1.0545	0.0474
	Chlorate	1,7043	−1	1.7382	−1	1.6505	−0.9451	1.6292	−0.9089	−0.0124
	Nitrate	1,8199	−1	1.8375	−1	1.7668	−0.9470	1.8544	−1.0868	0.0506
	Sulfate	3,3380	−2	3.2879	−2	3.3116	−1.9998	3.5514	−2.3873	0.1402
$t_{50\%\ \mathrm{right}}$	Fluoride	1,0469	−1	1.0686	−1	0.9328	−0.8967	1.1388	−1.2294	0.1216
	Chlorite	1,3245	−1	1.3576	−1	1.2184	−0.8931	1.4005	−1.1864	0.1063
	Chloride	1,4652	−1	1.4948	−1	1.3774	−0.9148	1.4754	−1.0719	0.0572
	Chlorate	1,7120	−1	1.7456	−1	1.6500	−0.9378	1.6489	−0.9342	−0.0007
	Nitrate	1,8265	−1	1.8447	−1	1.7694	−0.9424	1.8713	−1.1055	0.0589
	Sulfate	3,3466	−2	3.2961	−2	3.3094	−1.9892	3.5867	−2.4374	0.1619

with identified IC parameters (retention of particular component). If such a document is available, additional saving in experimental work is possible practically leading to an "experiment-free" IC retention modeling (*MODEL 0*). This model is basically identical with *MODEL 2*; however, the theoretical slope (a_1) is used and literature IC data serve for the intercept (a_0) estimation.

All the described models were used for peak left half-width and right half-width t_R modeling. Theoretical and calculated coefficients of isocratic retention models are presented in Table 1.

EXPERIMENTAL

Instrumentation

Experiments were performed on a Dionex DX600 ion chromatography system (Sunnyvale, CA, USA). The system was equipped with a quaternary gradient pump (GS50), eluent generator module (EG40), degas unit on eluent generator, trap column (CR-TC), chromatography module (LC30), and detector module (ED50A). Hydroxide-selective AS19 (4 mm × 250 mm) anion separation column and AG19 (4 mm × 50 mm) anion guard column were used for separation of inorganic anion species. For decreasing of background noise and increasing of outgoing analytical signal, an ASRS ULTRA II electrolytic suppressor (working in recycle mode) was used. All experimental measurements were performed using constant sample loop volume (25 μL), eluent flow rate (1.0 mL/min) and temperature (35°C). The whole system was computer-controlled by Chromeleon 6.70, Build 1820 software.

Reagents and Solutions

Mixed stock standard solution of fluoride (30.00 mg/L), chlorite (100.00 mg/L), chloride (60.00 mg/L), chlorate(250.00 mg/L), nitrite (250.00 mg/L), and sulfate (250.00 mg/L) was prepared from the salts of individual anions of p.a. grade (Merck, Darmstadt, Germany); the salts were dried at 105°C, weighed into volumetric flask (1000 mL) and dissolved by Milli-Q water (18 MΩcm^{-1} water, Millipore, Bedford, MA, USA). Working standard solutions of fluoride (3.00 mg/L), chlorite (10.00 mg/L), chloride (6.00 mg/L), chlorate (25.00 mg/L), nitrite (25.00 mg/L), and sulfate (25.00 mg/L) were prepared by diluting mixed stock standard solution by the factor of 10. Working eluent solutions were prepared on-line by appropriate dilution of KOH cartridge solution (Dionex) with Milli-Q water (Millipore, Bedford, MA, USA; 18 MΩcm^{-1}).

Experimental Design

The concentration of KOH in eluent for isocratic elution was varied from 5 to 80 mmol/L to provide 45 equidistant isocratic experimental data points. The measurements were performed in triplicate; thus, a total of 135 experimental isocratic data points was obtained. Average values of each triplicate were used for calculations. In order to create the representative isocratic data set, the experimental space was divided into three logarithmically equidistant subspaces. This was due to the fact that experimental data were aimed for modeling the logarithmic relations. The way of selecting the data required for modeling varied depending on the model used. When modeling required three experimental data points each data point was picked from a different subspace. For the two experimental data point case two lateral subspaces were used for picking the data. The central subspace was sampled for the single experimental data point case. Random function was used consistently for picking. Dionex Quality Assurance Report, Ion Pac AS19 Analytical column (4 × 250 mm), Product No. 062885 was used for "experiment-free" IC retention modeling.

The following mathematical formulae describe the gradient curve for concave (Eq. 6) and convex (Eq. 7) profiles generated by Dionex GS50 gradient pump:

$$V_e = V_f + (1 - k)(V_t - V_f)\left(1 - 2^{\frac{-10(T_e - T_f)}{(T_t - T_f)}}\right) + \frac{k(V_t - V_f)(T_e - T_f)}{(T_t - T_f)} \tag{6}$$

$$V_e = V_f + (1 - k)(V_t - Vf)\left(2^{\frac{-10(T_e - T_f)}{(T_t - T_f)}}\right) + \frac{k(V_t - V_f)(T_e - T_f)}{(T_t - T_f)} \tag{7}$$

V_e is an instantaneous eluent concentration; V_f is eluent concentration at the end of previous time step; V_t is eluent concentration at the beginning of next time step; T_e is current elapsed time; T_f is time at the beginning of the gradient step and T_t time at the ending of the gradient step; k is a parameter that describes the degree of curvature. In this work $k = 0.25$ was used both for convex and concave gradient profiles; linear gradient profile was tested as well. Furthermore, three values of onset gradient time were used: 0, 10 and 20 minutes; the gradient rates used were 1.33 mM/min and 2.90 mM/min. The gradient starting concentrations were 10.00, 15.00, and 20.00 mM KOH. All the gradients were halted at 80.00 mM KOH. Gradient experiment was designed by using full-factorial methodology; total of 54 gradient experiments were performed.

All calculations were performed in MATLAB environment (MATLAB 7.8.0. R2009a, MathWorks, Sherborn, MA, USA).

RESULTS AND DISCUSSION

All the deviations presented in Table 2 and Figure 1 were calculated as an average of relative error (%). Table 2 presents the predictive ability of developed models in dependence on the curvature of gradient profiles. One can observe that the highest predictive ability was obtained for a concave curvature profile followed by the straight line profile. The poorest prediction was obtained for convex curvature profile for all the investigated anions. This implies that the prediction ability did not depend on the linearity of gradient profile (if the nonlinear profile is described by an adequate model). On the other hand, predictive ability of all the models increased by using gradient profiles that deliver lower average concentration of eluent at the beginning of the separation process. The explanation was attributed to low initial eluent concentration gradients associated with a convex function that promoted the completion of equilibrium at the early stages of separation. Low gradients at the beginning of separation

TABLE 2 Prediction Ability of Linear and Non-Linear Gradient Profiles Obtained by Using Different Curvatures, Slopes, Retention Models, and Required Number of Experimental Data Points

		Relative Error/%					
		Slope = 53°			Slope = 71°		
Ion	Model	Concave	Straight Line	Convex	Concave	Straight Line	Convex
Fluoride	0	3.35	1.09	1.34	2.56	1.98	0.75
	1	2.41	2.11	0.90	1.86	2.36	0.89
	2	2.83	0.84	1.40	2.55	2.69	1.39
	3	2.82	1.45	1.91	2.62	3.56	2.15
Chlorate	0	7.87	2.96	3.00	7.10	4.27	4.09
	1	8.28	6.00	3.56	7.34	5.93	4.96
	2	6.47	2.15	2.36	5.58	3.53	2.04
	3	6.77	1.69	2.43	5.89	3.09	2.36
Chlorite	0	5.09	1.48	1.55	4.08	2.67	1.74
	1	5.11	3.50	1.83	3.94	3.52	2.18
	2	5.29	1.70	1.95	4.17	2.91	1.65
	3	4.32	0.98	1.85	3.51	2.85	1.25
Chloride	0	6.43	2.19	2.11	5.39	3.39	2.82
	1	6.39	4.27	2.36	5.19	4.26	3.17
	2	5.91	1.81	2.01	4.59	3.14	1.76
	3	5.22	0.89	1.78	3.92	2.49	1.07
Nitrate	0	9.06	3.13	3.15	7.52	4.61	4.16
	1	7.14	5.34	2.68	5.56	5.24	2.37
	2	7.75	1.41	2.89	6.14	3.31	2.35
	3	6.70	0.69	2.98	5.04	2.48	1.15
Sulfate	0	19.11	7.25	8.86	14.22	10.34	9.16
	1	12.00	7.52	12.17	7.33	7.24	3.39
	2	13.91	1.45	9.29	9.65	7.34	4.43
	3	12.19	2.00	11.65	7.66	6.48	3.11

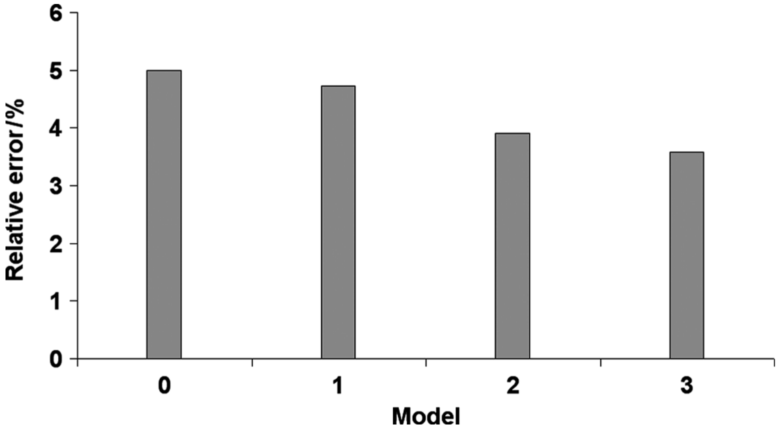

FIGURE 1 Average relative error of developed ion chromatographic signal prediction models.

were created by the pump with lower absolute error that additionally contributed to the better definition of the IC separation process. The small deviations from the described phenomenon were observed (e.g., fluoride, sulfate). However, the observed deviations occurred randomly, and they may be attributed neither to the elution order (fluoride) nor to the valence (sulfate) of analyte. Moreover, it is obvious that the predictive ability was far less influenced by the slope of gradient curve than by the general shape of the curve.

Table 2 and Figure 2 (presenting average relative error of model predictions) show that the increase of model complexity and amount of experimental information results in the higher predictive ability as expected. This can be discussed by examining Table 1 showing theoretical and calculated coefficients of retention model equations. The intercept characterized by parameter a_1 remained practically intact. However, the general slope as characterized by a_2 was affected by the addition of a square term in the model—in fact, it loses some of its physical significance. The a_3 remain at relatively low values, as compared to a_1 and a_2, proving the ion exchange as the principal separation mechanism. Figure 2 also shows that prediction error of the *MODEL 0, MODEL 1, MODEL 2,* and *MODEL 3* decreases almost linearly with the increase of number of adjustable parameters. This observation has to be taken cautiously and applied only on models using very few data for modeling procedure. Models that used more than a few data for modeling show only slight improvement (more or less logarithmic dependence between experimental information and prediction ability) by increasing modeling experimental information.[8,9,18,19] One may suspect that the lowest predictive ability of *MODEL 0* might be attributed to the incompatibility of the experimental information provided

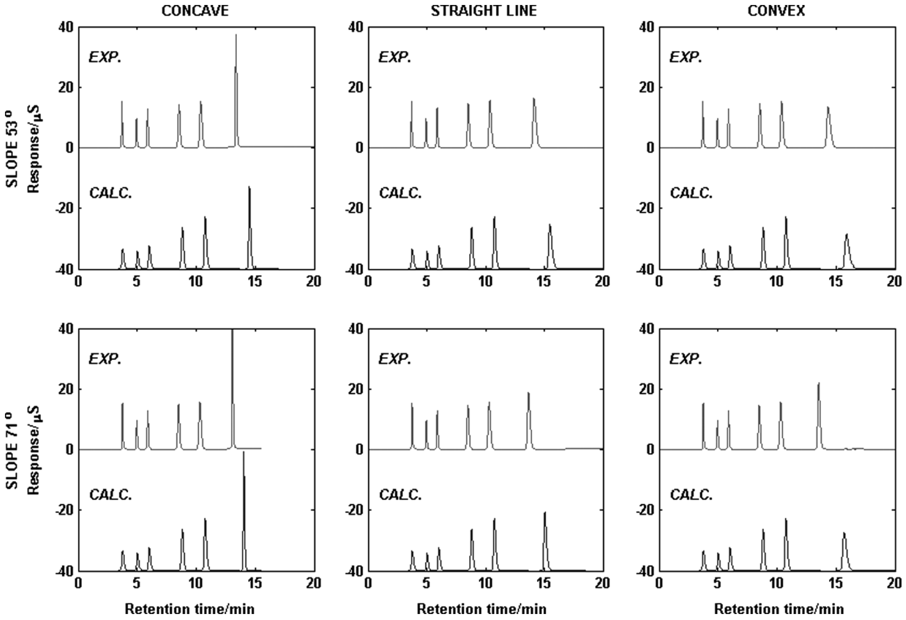

FIGURE 2 Measured and predicted chromatograms obtained by using "experiment-free" modeling methodology.

by the column manufacturer and the real ion chromatographic separation process studied. However, one must emphasize that it is not correct to ascribe the uncertainty to the disbalance between ion chromatographic column and documentation provided. The uncertainty may arise from contributions of any other part of the overall analytical system used for particular analysis (equipment, atmosphere, wet chemistry, etc.). Still, *MODEL 0* shows reasonably good performance characteristics for modeling both nonlinear and linear gradient profiles.

Figure 2 compares measured and predicted chromatograms obtained by "experiment-free" modeling methodology for different linear and nonlinear gradient profiles. The retention data predictions by *MODEL 0* are used for peak shape modeling followed by recalculation of the complete chromatograms. One observes that the obtained virtual chromatograms can be applied successfully for routine optimization purposes. They can be particularly useful for fast screening of IC conditions, selection of suitable stationary phase, and in cases where limited amounts of sample are available for IC analysis. The combination of here presented "experiment-free" methodology with multi-criteria decision-making approach[24] offers a valuable tool for the IC method development that is both efficient and economically justified.

CONCLUSIONS

This work compares several retention models that can be used for nonlinear and liner gradient elution signal prediction. It is shown that prediction ability of the developed models does not depend on the linearity of gradient profiles. Gradient profiles that create higher eluent concentration gradients in the early stage of separation can be predicted with lower accuracy, due to incomplete equilibration, and/or larger absolute pump uncertainty. The increase of number of adjustable parameters and, consequently, number of experimental data points used for model development decrease the relative error of prediction almost linearly, but only in a very limited domain (0–3 data parameters/points). Nevertheless, the "experiment-free" model still performs very acceptably (average of relative error 4.99%), showing great potential for application ion chromatographic method development methodologies.

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