



J. Serb. Chem. Soc. 81 (6) 661–672 (2016)
JSCS–4875

Interpretative optimization of the isocratic ion chromatographic separation of anions

ŽAKLINA N. TODOROVIĆ^{1*}, LJUBINKA V. RAJAKOVIĆ² and ANTONIJE E. ONJIA¹

¹Vinča Institute of Nuclear Sciences, University of Belgrade, P. O. Box 522, 11001 Belgrade, Serbia and ²Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia

(Received 27 September 2015, revised 10 January, accepted 29 February 2016)

Abstract: Interpretive retention modeling was utilized to optimize the isocratic ion chromatographic (IC) separation of the nine anions (formate, fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate and oxalate). The carbonate-bicarbonate eluent was used and separation was done on a Dionex AS14 ion-exchange column. The influence of combined effects of two mobile phase factors, the total eluent concentration (2–6 mM) and the carbonate/bicarbonate ratio from 1:9 to 9:1 (which correspond to pH range 9.35–11.27), on the IC separation was studied. The multiple species analyte/eluent model that takes into account ion-exchange equilibria of the eluent and sample anions was used. In order to estimate the parameters in the model, a non-linear fitting of the retention data, obtained at two-factor three-level experimental design, was applied. To find the optimal conditions in the experimental design, the normalized resolution product as a chromatographic objective function was employed. This criterion includes both the individual peak resolution and the total analysis time. A good agreement between experimental and simulated chromatograms was obtained.

Keywords: anions; ion chromatography; isocratic; multiple species analyte/eluent; resolution product.

INTRODUCTION

Ion chromatography (IC) is a well-established technique for the separation of inorganic and low-molecular weight organic anions. Separation of anions can be performed by either isocratic or gradient elution. Optimization of gradient IC elution is well-favored for separating complex mixtures with a wide retention range,^{1,2} whereas with isocratic elution a compromise has to be found that leads to a good separation in an acceptable time.³ There are several advantages to the use of isocratic elution: relatively simple equipment is required, without the need

* Corresponding author. E-mail: zaklina@vinca.rs
doi: 10.2298/JSC150927022T

for column re-equilibration after every IC run, low baseline drift, and relatively few parameters need to be optimized.⁴

One of the widely used mobile phase in IC analysis is $\text{CO}_3^{2-}/\text{HCO}_3^-$ buffer. Even though with recently invented eluent generators⁵ OH^- appeared to be the standard eluent with gradient elution, a large number of isocratic analysis is still being done by using the $\text{CO}_3^{2-}/\text{HCO}_3^-$ eluent. The elution power and selectivity of this eluent can be adjusted over a wide range solely by the concentration ratio of CO_3^{2-} and HCO_3^- .

In practical IC separations, finding an optimal mobile phase composition is usually the most difficult and time-consuming task. The traditional approach to this task is to perform an experiment by “trial-and-error” or by changing one control variable at time while holding the rest constant. Such methods can frequently require a very large number of experiments to identify the optimal conditions.⁶ Recently, the computer-assisted IC separation has addressed this problem, using factorial design (FD),⁷⁻⁹ simplex method¹ or neural network approach.^{10,11} Artificial neural network (ANN) modelling has ability to predict separation with no prior knowledge of the retention model. However, without the input of preliminary data, ANN will provide little information useful in improving the quality of separation. On the other hand, interpretive optimization strategy by using factorial designs requires a clear understanding of retention mechanism.¹²⁻¹³

In this article, an approach to estimate the retention behavior of nine common anions (formate, fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate and oxalate) and to optimize their isocratic elution using a carbonate-bicarbonate mobile phase is presented.

EXPERIMENTAL

Chemicals and reagents

The individual stock solutions (1.0 mg mL^{-1}) of nine anions listed above, obtained from AccuStandard (New Haven, CT, USA) and Merck (Darmstadt, Germany), were used to prepare the working mixture. In cases of formate and oxalate, 1.0 mg mL^{-1} standards were prepared by dissolving the appropriate amounts of the corresponding mass of analytical reagent grade chemicals in deionized water. This mixture was diluted appropriately in mobile phase to prepare the solution of different concentration for each anion: fluoride, formate and bromide 0.5 mg L^{-1} , chloride 5.0 mg L^{-1} , nitrite 0.25 mg L^{-1} , phosphate, oxalate and nitrate 1.0 mg L^{-1} and sulfate 5.0 mg L^{-1} . This difference in concentration levels are common in environmental samples, *i.e.*, atmospheric aerosol samples as an example. The reagent grade carbonate and bicarbonate (glac.) were purchased from Merck (Darmstadt, Germany). Milli-Q system (Millipore Co., Bedford, MA, USA) processed water ($18 \text{ M}\Omega \text{ cm}^{-1}$) was used for these experiments.

Chromatographic instrumentation and conditions

A Dionex DX-300 chromatographic system consisting of: gradient pump, chromatography module, conductivity detector, ASRS suppressor and Peaknet 5.1 chromatography workstation was used. The injected volume was $50 \mu\text{L}$. The separation of anions was carried

out on a Dionex AS14 column, at ambient temperature, with flow rate of 1.0 mL min⁻¹. The mobile phases comprised of 2.0–6.0 mM total amount of carbonate/bicarbonate within the pH range from 9.35 to 11.27 were applied to make a full two-factor experimental design and to study the retention behavior.

Software

The estimation of model parameters were performed using Mathcad 2000 software (MathSoft Inc., USA), applying the iterative Levenberg–Marquardt algorithm.¹⁴ To make the Levenberg–Marquardt method more effective on actual calculations, the basic method was modified as described elsewhere.¹⁵ For the simulation of chromatograms, a method for the fitting of the skewed and Gaussian peaks taken from Ref.¹⁶ was employed. In general, a chromatographic peaks shape originates from the Gaussian distribution function, although, tailings and frontings are common in ion chromatography. This can be easily drawn by exponentially modified Gaussian (EMG) function. The EMG distribution is the convolution of a Gaussian distribution and an exponential distribution, which are independent on each other. It is defined by three parameters: retention time and standard deviation of the parent Gaussian function and the time constant of the exponential decay function providing that asymmetrical peaks are shaped in a chromatogram.

RESULTS AND DISCUSSION

Interpretative optimization

To study the IC separation of a mixture of anions, retention behavior of each anion in the mixture is to be investigated. First step in interpretative optimization is the choice of a proper retention model. The model should provide adequate precision in estimation of the variable effect with a minimum of experiments. In general, there should be more experiments than variables.¹⁷

In the recent survey^{2,18,19} several retention models were used in the prediction power of anions separation using the CO₃²⁻/HCO₃⁻ or OH⁻ eluent. Madden and Haddad²⁰ compared the seven retention models. The series of models comprised of the linear solvent strength model, the dual eluent species model, the Kuwamoto model, the Hoover model, the extended dual eluent species model and the multiple species eluent/analyte model, together with an empirical end-points model, are used.

The most complex of the theoretical models is multiple species eluent/analyte model, which is studied in this work. This model takes into consideration the interaction of all eluent species with all analyte species, as well as the effects of varying eluent pH. Following equation²¹ describes this model for the case of a triprotic analyte, H₃A:

$$k = K_{A/HCO_3} \left(\frac{\sqrt{p^2 + q} - p}{4K_{CO_3/HCO_3} [CO_3^{2-}]} \right)^3 \Phi_A +$$

$$\begin{aligned}
& +K_{\text{HA}/\text{HCO}_3} \left(\frac{\sqrt{p^2 + q} - p}{4K_{\text{CO}_3/\text{HCO}_3} [\text{CO}_3^{2-}]} \right)^2 \Phi_{\text{HA}} + \\
& +K_{\text{H}_2\text{A}/\text{HCO}_3} \left(\frac{\sqrt{p^2 + q} - p}{4K_{\text{CO}_3/\text{HCO}_3} [\text{CO}_3^{2-}]} \right)^2 \Phi_{\text{H}_2\text{A}} \quad (1)
\end{aligned}$$

where:

$$\begin{aligned}
p &= [\text{HCO}_3^-] + K_{\text{OH}/\text{HCO}_3} [\text{OH}^-] \\
q &= 8K_{\text{CO}_3/\text{HCO}_3} Q
\end{aligned}$$

and

$$Q = 2([\text{CO}_3^{2-}] + [\text{HCO}_3^-]) + [\text{OH}^-]$$

The symbols Φ_A , Φ_{HA} and $\Phi_{\text{H}_2\text{A}}$ denote the partial mole fractions of deprotonated and partially protonated forms of the analyte ions H_3A .

Comparison of the experimental designs

In Fig. 1a comparison of the experimental designs for modeling the retention behavior as a function of pH values and concentration of CO_3^{2-} and HCO_3^- is presented. Because the two-level two-factor design (Fig. 1a) has only four experimental points, the response surface can be described by a flat plane and is not able to account for any curvature that might be found, so only first-order single factor effect can be calculated.

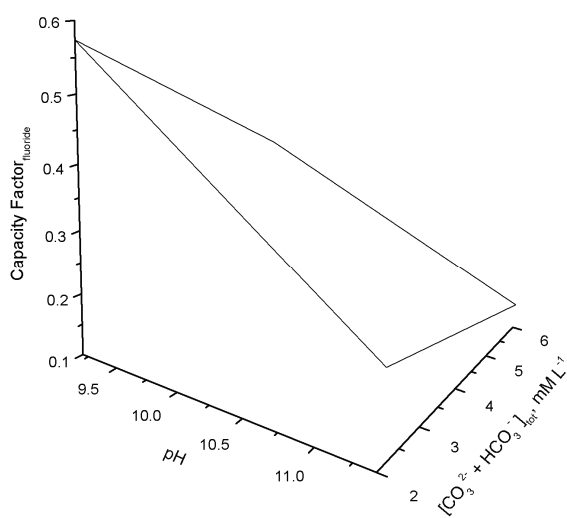
The star design, containing five experimental points, takes into account single-factor curvature effect and it could be used to estimate second-order single-factor effects. However, this design is unable to estimate interaction between pH and the total amount of the eluent.

To estimate interaction, the effect of one factor has to be determined at two or more level of the other factor, which is allowed by the three-level two-factor design (Fig. 1b).

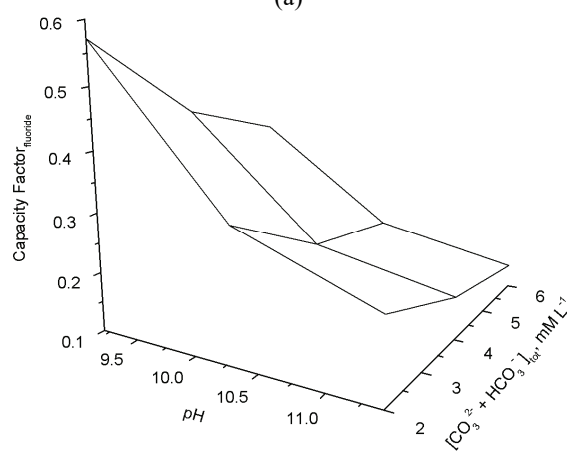
Distribution of the species in the eluent

Separation of inorganic anions especially in the presence of low molecular weight organic acid can be difficult with isocratic elution. Good retention of fluoride and formate, phosphate, sulfate and oxalate depend on the concentration of carbonate and bicarbonate as well as the pH value of the eluent. The effect of

pH is important in anions separation effecting their ionization. When weak acids anions (formate, phosphate, carbonate and oxalate) ions are present in mixtures with other ions that show no pH dependency, the control of mobile phase pH becomes an important variable to be manipulated in the optimization of the IC separation.



(a)



(b)

Fig. 1. Comparison of the experimental designs for modeling the retention behavior as a function of pH values and total concentration of CO_3^{2-} and HCO_3^- : a) two-level two-factor and b) three-level two-factor.

In this study, $\text{CO}_3^{2-}/\text{HCO}_3^-$ is used as eluent and the pH value of the eluent is from 9.35 to 11.27. As shown in Fig. 2, the phosphates mostly exist in the secondary high charge forms in the eluent at this pH values. This means that it will be eluted after the singly charged ions and before sulfate ions. At pH higher than 11.27 phosphates exist in the highest charge and will be eluted after sulfate ions.

The retention behavior of weak acid anions is controlled mainly by the retention of the analyte anions with the highest charge and secondary high charge.²²

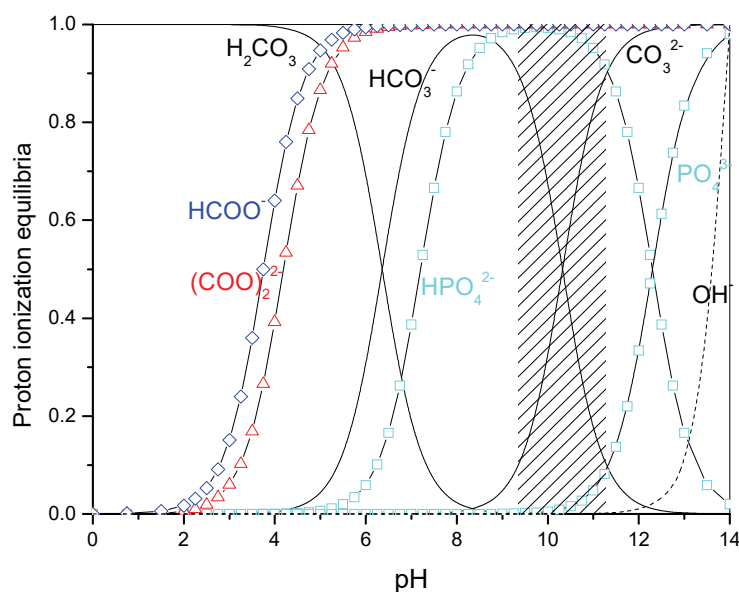


Fig 2. Proton ionisation equilibria of the bicarbonate/carbonate eluent and partially protonated anions.

Bicarbonate can alter anions retention according to the ion interaction mechanism.²³ Thus, the amount of bicarbonate in the eluent affects the interaction of carbonate with the stationary phase. A high degree of interaction between the two factors, concentration of carbonate and bicarbonate, can be described by an appropriate model.²⁴ The parameters in Eq. (1) were estimated by the non-linear least squares method.²⁴

Predicted and calculated data

Experimental data, which have been acquired according to two-factor three-level experimental design, are used to solve the intereluent constant, k , and the sum of squares of deviation (SSE) for each anion. The results are summarized in Table I. Non-linear regression fitting was employed for the parameter estimation. Comparison between estimated and experimentally retention data show good agreement.

A pseudo-three dimensional plot of the estimated capacity factor of phosphate as a function of the total bicarbonate/carbonate concentrations and carbonate percentage is shown in Fig. 3. The effect of pH is higher along the edge of

the low carbonate concentration while the effect is reduced at high carbonate percent.

TABLE I. Summarized experimentally obtained and calculated *k* values for selected analytes

Analyte	<i>k</i>										<i>SSE</i>
Fluoride	a	0.593	0.395	0.321	0.321	0.210	0.173	0.247	0.173	0.136	0.055
	b	0.714	0.357	0.238	0.285	0.143	0.095	0.168	0.084	0.056	
Formate	a	0.963	0.654	0.519	0.531	0.358	0.296	0.420	0.284	0.222	0.105
	b	1.132	0.566	0.377	0.557	0.278	0.186	0.345	0.173	0.115	
Chloride	a	2.111	1.481	1.198	1.235	0.852	0.691	0.963	0.667	0.556	0.581
	b	2.491	1.245	0.830	1.338	0.669	0.446	0.855	0.428	0.285	
Nitrite	a	3.099	2.148	1.827	1.741	1.210	0.975	1.370	0.951	0.790	1.161
	b	3.533	1.767	1.178	2.052	1.026	0.684	1.354	0.677	0.451	
Bromide	a	6.062	4.099	3.296	3.296	2.321	1.889	2.580	1.827	1.519	1.096
	b	5.942	4.227	3.097	3.937	2.226	1.541	2.633	1.411	0.962	
Nitrate	a	7.210	4.852	3.926	4.000	2.790	2.259	3.099	2.210	1.815	1.503
	b	7.060	4.996	3.656	4.752	2.695	1.867	3.206	1.722	1.174	
Phosphate	a	30.037	13.296	8.432	12.123	6.000	3.667	8.679	4.012	2.506	1.729
	b	30.024	13.156	8.525	12.781	6.155	4.070	7.667	3.772	2.505	
Sulfate	a	46.778	21.333	13.728	14.173	6.580	4.160	8.926	4.025	2.580	1.104
	b	46.777	12.293	13.730	14.413	6.952	4.578	8.074	3.952	2.616	
Oxalate	a	63.864	28.704	18.481	19.049	8.889	5.667	11.605	5.457	3.494	1.136
	b	63.851	28.720	18.435	19.317	9.270	6.094	10.772	5.257	3.476	
Carbonate, %		10		50		90				–	

^aRetention factors from experimentally obtained chromatograms; ^bretention factors obtained from calculated chromatograms

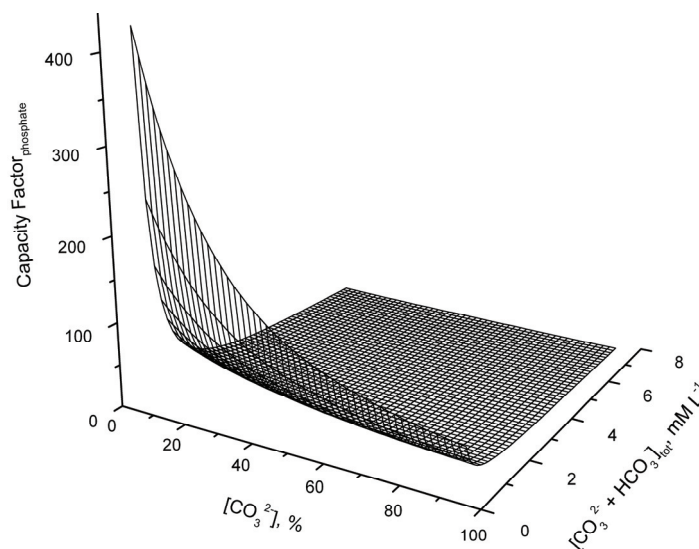


Fig. 3. Predicted capacity factor for phosphate as function of carbonate/bicarbonate ratio and total concentration of eluent.

The applicability of the integrated retention model can be checked by the comparison of the differences between the calculated and the measured retention coefficients^{Error! Bookmark not defined.} as can be seen from the Fig. 4. Calculated retention coefficients as function of the measured retention coefficients is linear with slope and intercept close to one and zero ($y = -0.1322x + 1.00395$), respectively. This confirms the agreement between theoretical and experimental values of retention coefficients. The average absolute difference between the calculated and observed values is generally less than 5%, which is within the margins of the experimental precision.

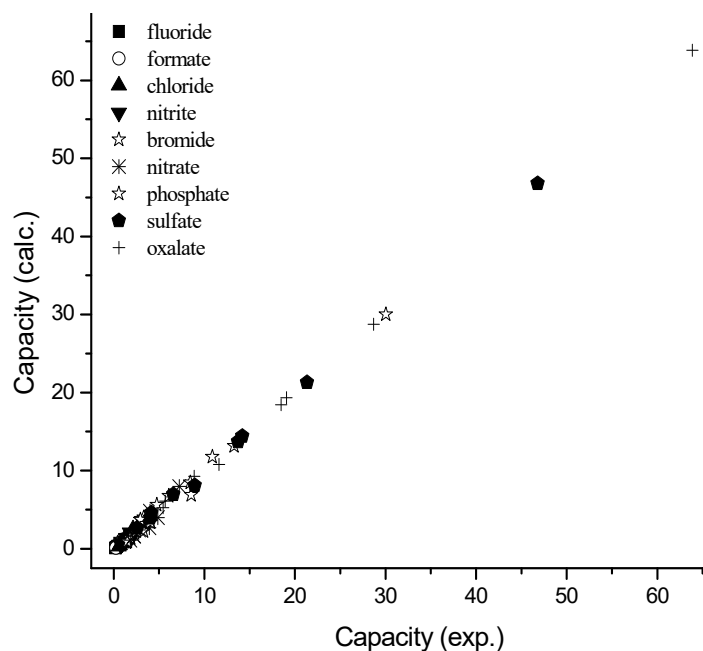


Fig. 4. Relationship between the measured and calculated capacity factors for nine anions.

Response surface methodology

The next step in the optimization of chromatographic analytical procedures is the selection of an appropriate response function. The choice of the most relevant function depends on the overall goal of the separation.²⁵ A commonly used criterion for numerical quantification of chromatogram is normalized resolution product.²¹ In this separation criterion, all peaks of interest are considered.²⁶ The normalized resolution product is related to the average resolution taken over all the peaks in the chromatogram. It reflects the regularity of peak spacing over the chromatogram and varies from 0 to 1, it is equal to 0 when one or more peaks totally co-elute and one when all the pairs of peaks show the same resolution in

the chromatogram. The normalized resolution product (r) is described by the following equation:

$$r = \prod_{i=1}^{n-1} \{R_{S_i, i+1} / [(n-1)^{-1} \sum_{i=1}^{n-1} R_{S_i, i+1}]\} \quad (2)$$

where n is the number of peaks and $R_{S_i, i+1}$ is the resolution between peaks i and $i+1$. The normalized resolution product for the separation of nine anions is shown in Fig. 5. The point corresponding to the total bicarbonate/carbonate content of 1.5 mM and 70 % carbonate was selected as an optimum. The value of 0.36 for r is the maximum in the factor space as shown in the contour graph (Fig. 5). At these conditions, the retention times are still not too long and the resolution of fluoride and formate is acceptable. An alternative optimum with r value of 0.30 at the 80 % carbonate and 3.0 mM of the total bicarbonate/carbonate content, which provides faster elution but with not so good resolution, is obtained. There is a valley between these two optima (as seen in Fig. 5) with very bad resolution. By examination of the simulated chromatograms, it was revealed that there is the peak reversal phenomenon in the system. The only anion that undergoes to the peak reversal is phosphate. At the studied range of the total bicarbonate/carbonate content and the percentage of carbonates, phosphates exchange the elution order with the sulfates. The reason for this behavior is the fact that pH value, which is governed by the percentage of carbonate content, has a great effect on

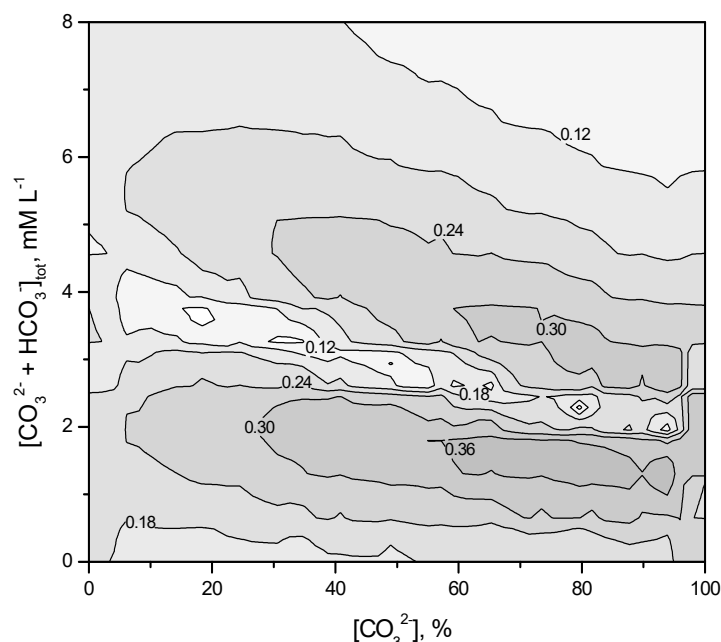


Fig. 5. Normalized resolution product for the separation of nine anions.

the form of phosphate in the eluent. When there are no PO_4^{3-} in the eluent, which occurs at lower pH values, in the shaded area in Fig. 4, sulfates are more retained on the column. In case when there is significant content of PO_4^{3-} in the eluent, the elution order of phosphate and sulfate is reversed. The minimum in total resolution corresponds to overlapping of these peaks.

Simulated chromatogram

Following to above results, the simulation of chromatograms is the next step in the interpretative optimization strategy. The separation of anions was done by using the eluent composition of 70 % carbonate and 1.5 mM of the total bicarbonate/carbonate content from Fig. 5. The comparison of the experimental and the calculated chromatogram for any point on the response surface (Fig. 5) using simplified form of EMG function²⁷ is shown in Fig. 6. It can be seen that the retention times and resolutions of peaks are approximately the same for the predicted and observed chromatogram.

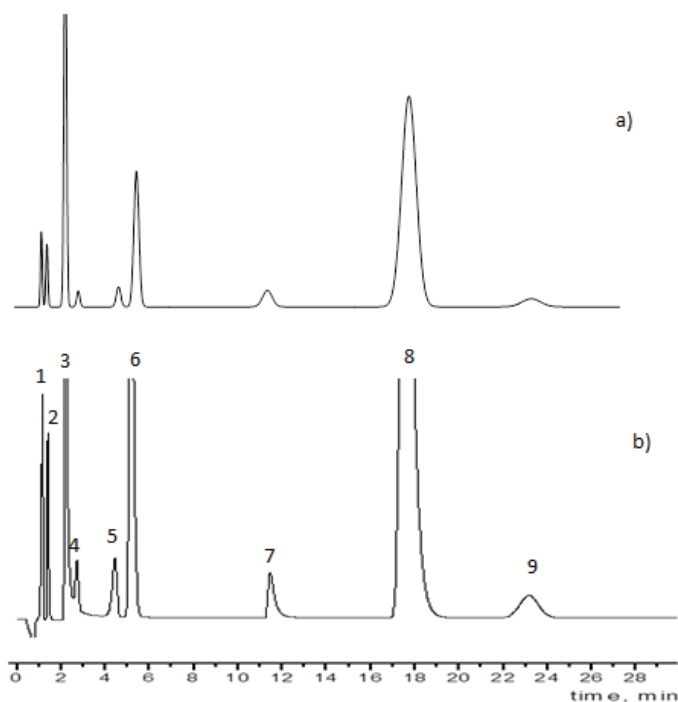


Fig. 6. Predicted (a) and obtained (b) chromatograms of nine anions at 70 % carbonate and 1.5 mM of the total bicarbonate/carbonate content: (1) formate, (2) fluoride, (3) chloride, (4) nitrite, (5) bromide, (6) nitrate, (7) phosphate, (8) sulfate and (9) oxalate.

In the predicted chromatogram, the difference in the concentration levels were included. However, the peak skewness was not taken into account. This was

reason why the separation of chloride from nitrite differs in the simulated chromatogram from the experimental one. In this retention modeling, the formate/fluoride peak pair is the one that governs the total resolution. At the first sight it seems that the chloride/nitrite resolution is the most problematic in this IC separation. In most environmental samples, there is a large difference in concentrations, *i.e.*, chloride concentration is much higher than nitrite concentration, which makes IC determination of nitrite quite complex.

Main resulting novelty in this study is an advancement in the IC optimization through an integration of several different steps: analyte speciation in the mobile phase, retention models analysis, experimental designs evaluation and chromatographic peaks simulation. This approach relies on both simultaneous and sequential investigation of the variables in the system, which affect the overall performances of the IC analysis of inorganic anions and low molecular weight organic acids.

CONCLUSIONS

A good prediction of the combined effects of two mobile phase factors, the eluent composition and total eluent concentration on the isocratic separation of nine common anions were obtained by fitting the multiple species analyte/eluent model to experimental data at two-factor three-level experimental design. The multiple species analyte/eluent model was found to be very suitable because it requires minimal input data but provides reliable prediction of retention factors. Good agreement between the experimentally obtained and the calculated retention times and peak resolution confirms the high predictive power of the used model.

Acknowledgements. This work was financially supported by the Research Project of the Ministry of Education, Science and Technological Development of the Republic Serbia, III43009.

ИЗВОД

ИНТЕРПРЕТАТИВНА ОПТИМИЗАЦИЈА ИЗОКРАТСКОГ РАЗДВАЈАЊА АНЈОНА ЈОНСКОМ ХРОМАТОГРАФИЈОМ

ЖАКЛИНА Н. ТОДОРОВИЋ¹, ЉУБИНКА В. РАЈАКОВИЋ² И АНТОНИЈЕ Е. ОЊИА¹

¹Институт за нуклеарне науке „Винча“, Универзитет у Београду, б.бр. 522, 11001 Београд и

²Технолошко-металуришки факултет, Универзитет у Београду, Карнегијева 4, б.бр. 494, 11120 Београд

У раду је коришћена интерпретативна оптимизациона стратегија за изократско раздвајање девет анјона (формијата, флуорида, хлорида, нитрита, бромид, нитрата, фосфата, сулфата и оксалата) јонском хроматографијом (IC). Као мобилна фаза коришћена је смеша карбонат–бикарбонат а раздвајање је урађено на Dionex AS14 јоноизмењивачкој колони. Проучаван је утицај комбинованих ефеката два фактора на IC раздвајање, укупне концентрације мобилне фазе (2–6 mM) и однос карбонат/бикарбонат од 1:9 до 9:1 (што одговара pH опсегу 9,35–11,27). У раду је коришћена теорија мултиком-

петицијског елуенса која узима у обзир јоноизмењивачку равнотежу између елуента и анализираног анјона. Нелинеарно фитовање експерименталних параметара, добијених у 2^3 експерименталном дизајну, примењено је за процену параметара модела. За проналажење оптималних услова експерименталног дизајна коришћен је нормализовани резолуциони продукт. Овај критеријум укључује и резолуцију пикова и укупно време анализе. Добијено је добро слагање између експериментално добијеног и симулираног хроматограма.

(Примљено 27. септембра 2015, ревидирано 10. јануара, прихваћено 29. фебруара 2016)

REFERENCES

1. V. Drgan, D. Kotnik, M. Novič, *Anal. Chim. Acta* **705** (2011) 315
2. E. Tyteca, S.H. Park, R.A. Shellie, P.R. Haddad, G. Desmet, *J. Chromatogr. A* **1381** (2015) 101
3. P. Zakaria, G.W. Dicoski, B.K. Ng, R.A. Shellie, M. Hanna-Brown, P.R. Haddad, *J. Chromatogr. A* **1216** (2009) 6600
4. A.P. Schellinger, P.W. Carr, *J. Chromatogr. A* **1109** (2006) 253
5. M. Novič, Y. Liu, N. Avdalovic, B. Pihlar, *J. Chromatogr. A* **957** (2002) 165
6. A. Onjia, T. Vasiljević, Dj. Čokeša, M. Laušević, *J. Serb. Chem. Soc.* **67** (2002) 745
7. M. Achilli, L. Romele, *J. Chromatogr. A* **770** (1997) 29
8. Š. Ukič, T. Bolanča, M. Rogošić, *J. Sep. Sci.* **34** (2011) 780
9. J. Zirojevic, Z. Jovic, A. Djurdjevic, A. Ciric, P. Djurdjevic, *Acta Chromatogr.* **27** (2015) 215
10. J.E. Madden, N. Avdalović, P.R. Haddad, J. Havel, *J. Chromatogr. A* **910** (2001) 173
11. P.E. Morgan, D.J. Barlow, M. Hanna-Brown, R.J. Flanagan, *Chromatographia* **75** (2012) 693
12. J.R. Torres-Lapasio, M. Roses, E. Bosch, M.C. Garcia-Alvarez-Coque, *J. Chromatogr. A* **886** (2000) 31
13. S. Pous-Torres, J.R. Torres-Lapasio, M.J. Ruiz-Angel, M.C. Garcia-Alvarez-Coque, *J. Sep. Sci.* **32** (2009) 2793
14. W. Press, W. Flannery, S. Teukolsky, B. Vetterling, *Numerical Recipes in C*, Cambridge Univ. Press, New York, 1992, p. 683
15. J. More, B. Garbow, K. Hillstrom, *User's Guide to Minpack I*, Argonne National Lab. publ. ANL-1980, p. 80
16. J.R. Torres-Lapasio, J.J. Baeza-Baeza, M.C. Garcia-Alvarez-Coque, *Anal. Chem.* **69** (1997) 3822
17. S. Sremac, A. Popović, Ž. Todorović, Dj. Čokeša, A. Onjia, *Talanta* **76** (2008) 66
18. V. Drgan, M. Novič, M. Novič, *J. Chromatogr. A* **1216** (2009) 6502
19. V. Drgan, M. Novič, B. Pihlar, M. Novič, *J. Chromatogr. A* **1185** (2008) 109
20. J.E. Madden, P.R. Haddad, *J. Chromatogr. A* **850** (1999) 29
21. J. Madden, P.R. Haddad, P. Hajos, *Trend. Anal. Chem.* **15** (1996) 531
22. F. Houtun, L. Yaoping, X. Minjie, *J. Chromatogr. A* **945** (2002) 97
23. B.A. Bidlingmeyer, S.N. Deming, W.P. Price, B. Sachok, M. Petrussek, *J. Chromatogr. A* **186** (1979) 419
24. P. Hajos, O. Horvath, V. Denke, *Anal. Chem.* **67** (1995) 434
25. E. Klein, S. Rivera, *J. Liq. Chromatogr. Rel. Tech.* **23** (2000) 2097
26. P. Haddad, A. Drouen, H. Billiet, L. De Galan, *J. Chromatogr.* **282** (1983) 71
27. V.B. Di Marco, G.G. Bombi, *J. Chromatogr. A* **931** (2001) 1.