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ARTICLE *in* ANALYTICAL CHEMISTRY · AUGUST 1998

Impact Factor: 5.64 · DOI: 10.1021/ac9803582

Anal. Chem. **1998,** *70,* 3940−3945

Role of Barium Ions in the Anion-Exchange Chromatographic Separation of Carbohydrates with Pulsed Amperometric Detection

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Barium ion is shown here as an effective component of alkaline mobile phases for improving the separation of carbohydrates in anion-exchange chromatography with pulsed amperometric detection. We demonstrate that the introduction of barium ensures a complete removal of carbonate, making it possible to carry out a large number of separations without column reequilibration after each run. This is particularly helpful when diluted alkaline eluents (i.e., <20 mM NaOH) are employed. Under such experimental conditions, the analysis time of carbohydrates is drastically reduced and the reproducibility of the chromatographic data greatly improved. For example, the normalized capacity factor (k'/k'_0) of lactulose, which represents the most retained compound investigated using 16 mM NaOH as the eluent, exhibits only a slight decrease from 1.00 to 0.94 after 7.5 h of chromatographic run. The net improvement in the peak shape of D-ribose, D-allose, and D-talose has been interpreted by considering the complex formation between barium and sugar molecules having an axial−equatorial−axial sequence of three OH groups on the six-membered ring. The presence of Ba(II) (0.5−0.7 mM) in alkaline eluents has been demonstrated by ion chromatography with conductivity detection.

The appearance of high-performance anion-exchange chromatography (HPAEC) irrevocably changed the way carbohydrate analysis is performed. Columns packed with poly(styrene−divinylbenzene)-based stationary phases functionalized with alkyl quaternary ammonium groups, using either macroporous resins (∼10 μm) or pellicular microbead (∼50−530 nm) latex, have been widely employed for many different applications and are especially suitable for the separation of carbohydrates.[1−5] Such rugged stationary phases have become very popular owing to the high inertness and good selectivity using alkaline mobile phases. In conjunction with HPAEC, there has been also the development of pulsed amperometric detection (PAD); so together these techniques have substantially improved the determination of sugars and nonreducing sugars at low levels.[6−9]

According to the manufacturer's protocol,[10] the use of comparably low concentrated mobile phases, i.e., <20 mM NaOH, is particularly designed for the separation of closely related compounds such as D-galactose, D-glucose, and D-mannose, despite similarities in their overall molecular size and number of hydroxyl groups. Using alkaline eluents, the key issue that must be addressed is related to the presence in solution of carbonate ion. At normal conditions (25 °C and 1 atm), the saturation concentration in pure water of dissolved atmospheric CO_2 is 33 mM.[11] Carbon dioxide can produce carbonate, a divalent anion at pH ≥12, which will bind strongly to the column and interfere with carbohydrate binding. The problem is insidious because carbonate in sodium hydroxide eluents imparts a progressive decrease of retention on successive injections, making the carbohydrate determinations very frustrating and tedious. In fact, even though the eluents are continuously sparged with inert gases, there is the need to flush the anion-exchange column with a more concentrated hydroxide solution (e.g., 200 mM NaOH) after each run to exchange the CO_3^{2-} from the stationary phase and to put the anion-exchange resin in the hydroxide form (i.e., column regeneration).[10,12] Since the column has to be subsequently equilibrated by the desired eluent (e.g., 16 mM NaOH), with long run times up to 50−60 min, there would be a considerable advantage in being able to circumvent the regeneration step. The relevance of the issue is particularly felt in the ion-chromatographic field so that Dionex Corp. has very recently launched a novel system based on the on-line electrochemically generated alkaline eluent, which seems to be very useful for eliminating carbonate interference.

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(1) Rocklin, R. D.; Pohl, C. A. *J. Liq. Chromatogr.* **1983**, *6*, 1577.
(2) Olechno, J.; Carter, S. R.; Edwards, W. T.; Gillen, D. G. *Am. Biotech. Lab.* **1987**, *5*, 38.
(3) Johnson, D. C.; Dobberpuhl, D. A.; Roberts, R. A.; Vandeberg, P. J. *J. Chromatogr.* **1993**, *640*, 79.
(4) Johnson, D. C.; LaCourse, W. R. In *Carbohydrate Analysis. High Performance Liquid Chromatography and Capillary Electrophoresis*; El Rassi, Z., Ed.; Elsevier: Amsterdam, 1995; Chapter 10.
(5) Lee, Y. C. *J. Chromatogr., A* **1996**, *720*, 137.
(6) Hughes, S.; Johnson, D. C. *Anal. Chim. Acta* **1981**, *132*, 11.
(7) Johnson, D. C. *Nature* (*London*) **1986**, *321*, 541.
(8) Johnson, D. C.; LaCourse, W. R. *Anal. Chem.* **1990**, *62*, 589A.
(9) LaCourse, W. R.; Johnson, D. C. *Anal. Chem.* **1993**, *65*, 50.
(10) Dionex Corp. *Installation, Instructions and Troubleshooting Guide*; 1991; Document 034441.
(11) Hammond, C. R., Ed. *Handbook of Chemistry and Physics*, 71st ed.; CRC Press: Boca Raton, FL, 1990; Section IV.
(12) Dionex Corp., Technical Note 20, 1994.

S0003-2700(98)00358-8 CCC: $15.00
Published on Web 08/08/1998

In a series of recent publications,[13−15] we demonstrated the feasibility of performing anion-exchange chromatographic separations with strongly alkaline mobile phases (500−600 mM NaOH) containing divalent metal ions such as Ca(II), Sr(II), or Ba(II) at millimolar concentrations. The use especially of barium, or strontium ions has offered an improved means for the determination of carbohydrates and alditols in real samples, using a macroporous column with high anion-exchange capacity (i.e., 1.45 mequiv/column).[12,15] In this article, we shall report in detail on the role of barium in HPAEC using diluted NaOH eluents (e.g., 16 mM) and employing a pellicular column with a relatively low exchange capacity (i.e., 0.1 mequiv/column). Such conditions are of great interest for the separation of closely related mono- and disaccharides. Besides this, the analytical implications of selective carbohydrate complexation with barium have been also addressed. Excellent reproducibility of retention during repetitive chromatographic runs was obtained, allowing reliable carbohydrate analysis. The results indicated the efficacy of this novel approach for minimizing carbonate interference in the carbohydrate quantitation by HPAEC−PAD.

EXPERIMENTAL SECTION

Chemicals. Sodium hydroxide, 50% (w/w) solution in water ($d = 1.515$ g mL^{-1}), methanesulfonic acid 99%, $Ba(OH)_2 \cdot 8H_2O$ 98%, $Ba(CH_3COO)_2$ 99%, D-(+)-galactose 97%, D-ribose 98%, and α-D-lactose monohydrate 97% were purchased from Aldrich Chemical Co. (St. Louis, MO); lactulose >98% was from Fluka Chemie (Buchs, Switzerland); and α-L-rhamnose 98%, D-arabinose 99%, D-fructose 99%, α-D-(+)-glucose 99.5%, D-(+)-mannose 99%, β-D-allose, α-D-talose, and sucrose were from Sigma Chemical Co. (Steinheim, Germany); these chemicals were used as received. Stock solutions of sugars were prepared in pure water containing 0.1% sodium azide to prevent microbial growth. Working solutions of carbohydrates were prepared fresh daily by dilution of the stock solutions. Other chemicals employed were of analytical grade and were used without further purification. Doubly distilled, deionized water was used throughout for preparing solutions. Sodium hydroxide solutions used as the mobile phases of the desired concentration were prepared by diluting of carbonate-free 50% (w/w) NaOH solution in pure water, which had been purged with N_2 gas. The exact concentration of the hydroxide ion in the mobile phase was determined by titration against a standard solution of hydrochloric acid.

HPAEC with PAD. Carbohydrates analysis was performed using a metal-free isocratic pump (Dionex, Sunnyvale, CA), model IP20, a Dionex pulsed amperometric detector (model ED40), and a Dionex metal-free rotary injection valve with 10-μL injection loop. A relatively low capacity anion-exchange column (100 μequiv), Dionex CarboPac PA1 (250 mm × 4 mm i.d.) coupled with a guard CarboPac PA1 column (50 mm × 4 mm i.d.) was used for the separations. The flow-through detection cell (Dionex) contained a 1.4-mm-diameter gold working electrode and a Ag|AgCl reference electrode with the titanium cell body serving as the counter electrode. Acquisition and processing of chromatographic data were done by a personal computer equipped with the Kontron PC Integration Pack software (Kontron Instruments, Milan, Italy). Sodium hydroxide eluents were kept in plastic bottles, and a nitrogen headspace was maintained on the solutions with a Dionex eluent organizer (EO1). Typical experiments consisted of using first a conventional alkaline eluent and to subsequently modify the same solution with barium ions. The pulsed amperometric detector settings were as follows: (i) $E_{OX} = +800$ mV ($t_{OX} = 180$ ms), $E_{DET} = +200$ mV ($t_{DEL} = 200$ ms, $t_{INT} = 240$ ms), and $E_{RED} = -300$ mV ($t_{RED} = 360$ ms) in the case of 100 mM NaOH with and without barium ions; and (ii) $E_{OX} = +800$ mV ($t_{OX} = 180$ ms), $E_{DET} = +250$ mV ($t_{DEL} = 200$ ms, $t_{INT} = 240$ ms), and $E_{RED} = -250$ mV ($t_{RED} = 360$ ms) when 16 mM NaOH with and without barium ions was used as eluent. The response time was set to 1 s. All experiments were carried out at ambient temperature in isocratic elution using a flow rate of 1.0 mL min^{-1}. It is strongly recommended to make the addition of barium a few hours before using the eluent solution. We wish to emphasize that excellent results can be obtained over the ensuing day of barium addition to the freshly prepared alkaline eluent, without the need of filtering the solution. Besides, we found that thoroughly washing the pump system with 0.5 M HCl at the end of each working day is a good rule, to avoid any problem with wear of the piston and piston seals. We have been using such a procedure for months and no mechanical problems due to barium carbonate precipitation were experienced. The capacity factor, k', was calculated according to the expression $k' = (t_R - t_M)/t_M$, where t_R is the retention time and t_M is the column dead time, measured from the front disturbance in the chromatogram.

Ion Chromatography with Conductivity Detection. The ion-exchange chromatographic experiments were performed with a Dionex apparatus composed of a pressurized (He) eluent organizer, a GP40 programmable gradient pump, a 10-μL Rheodyne injector, and the Dionex conductance detector (ED40) equipped with a temperature-compensated conductivity cell. The anions were eluted with an aqueous mobile phase of 20 mM NaOH, at a flow rate of 1.5 mL min^{-1}, using an IonPac AG12A (50 mm × 4 mm i.d.) guard column in series with an IonPac AS12A (250 mm × 4 mm i.d.) column. A Dionex anion self-regenerating suppressor (ASRS-I, 4 mm) operating at 300 mA converted the mobile phase to weakly conducting water, suitable for conductivity detection. The determination of barium in solutions was performed with an IonPac CG12A (50 mm × 4 mm i.d.) guard column in series with an IonPac CS12A (250 mm × 4 mm i.d.) column, followed by a cation self-regenerating suppressor (CSRS-I, 4-mm) operating at 100 mA. The mobile phase was 18 mM CH_3SO_3H at a flow rate of 1.0 mL min^{-1}. The flow-through conductivity cell has a cell constant of 200 cm^{-1} as the nominal value. Data acquisition was computer controlled through a Chrom-Card for Windows (TermoQuest, Milan, Italy).

RESULTS AND DISCUSSION

Effect of Ba^{2+} in Alkaline Eluents. Chromatograms resulting from two separate injections of L-rhamnose, D-arabinose, D-glucose, D-ribose, lactose, and D-cellobiose, using the conventional 100 mM NaOH mobile phase and 100 mM NaOH + 1 mM $Ba(OH)_2$ under the same experimental conditions, are presented in parts a and b of Figure 1, respectively. The capacity factor, k', evaluated from both chromatograms, along with the values of D-fructose, D-allose and D-talose, are compared in Table 1. The

(13) Cataldi, T. R. I.; Centonze, D.; Casella, I. G., Desimoni, E. *J. Chromatogr., A* **1997**, *773*, 115.
(14) Cataldi, T. R. I.; Centonze, D.; Margiotta, G. *Anal. Chem.* **1997**, *69*, 4882.
(15) Cataldi, T. R. I.; Margiotta, G.; Zambonin, C. G. *Food Chem.* **1998,** *62*, 109.

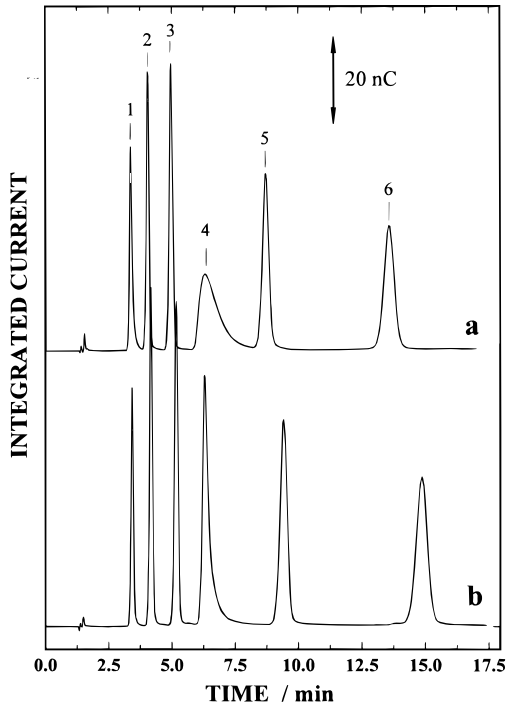

Figure 1. Separation of carbohydrates in HPAEC with integrated PAD using 100 mM NaOH (a) and 100 mM NaOH + 1 mM $Ba(OH)_2$ (b) as the mobile phase. Peak and concentration: (1) L-rhamnose, 50 μM; (2) D-arabinose, 50 μM; (3) D-glucose, 50 μM; (4) D-ribose, 100 μM; (5) lactose, 50 μM; and (6) D-cellobiose, 50 μM. The eluent was prepared by carbonate-free 50% NaOH using degassed water and was continuously sparged with purified N_2. Column, CarboPac PA1 plus guard column; flow rate, 1.0 mL min^{-1}. The working electrode was gold.

Table 1. Effect of Ba(II) (1 mM) on the Capacity Factors of Carbohydrates in HPAEC–PAD[a]

	k'		
carbohydrate	100 mM NaOH	100 mM NaOH + 1 mM $Ba(OH)_2$	$\Delta k'$
L-rhamnose	1.39	1.49	+0.10
D-arabinose	1.87	2.04	+0.17
D-glucose	2.52	2.75	+0.23
D-fructose	2.99	3.25	+0.26
D-ribose	3.48	3.56	+0.08
D-allose	3.61	3.54	−0.07
D-talose	5.51	5.40	−0.11
lactose	5.19	5.85	+0.66
D-cellobiose	8.65	9.82	+1.17

[a] Isocratic elutions with a dead time, t_M, of 1.36 min. See experimental conditions stated in Figure 1.

difference in capacity factors, $\Delta k'$, is given in the fourth column of the same table as well. By examining these data, it appears that using the barium-containing mobile phase, the retention of some compounds is increased with D-cellobiose being more affected, passing from 13.2 to 14.8 min. As will be proved below, we explain the above behavior as the consequence of carbonate removal. The depletion of such a strong counterion from the mobile phase, indeed, should lead to longer retention times. Note that the addition of barium ions to a less concentrated alkaline mobile phase (i.e., 16 mM NaOH), which is very useful for the separation of mono- (e.g., D-galactose, D-glucose, and D-mannose) and disaccharides (e.g., lactose and lactulose), led to similar results (not shown). It is worthwhile mentioning that whatever the eluent concentration employed, a singular behavior of some sugar molecules was noted; their retention times were comparatively shorter in the presence of barium. Referring to the values listed in Table 1, the increase in k' is not followed by all compounds investigated; especially D-ribose, D-allose, and D-talose exhibit about the same capacity factors, with $\Delta k'$ equal to 0.08, −0.07, and −0.11, respectively. By taking these findings into account, the presence of Ba(II) provides additional indications of what we had already anticipated in a previous paper regarding the ability of this divalent ion to influence the anion-exchange chromatographic separation of alditols and carbohydrates in strongly alkaline solutions (i.e., 500−600 mM NaOH).[14]

Competitive Interactions of Carbohydrates. Interestingly, in the chromatographic separation of carbohydrates, advantage can be taken of the complex formation between metal ions present in the eluent and the sugar molecules to be separated. Such a mechanism resembles that of ligand-exchange chromatography (LEC), in which metal ions are immobilized on the stationary phase and the separations depend on the complex formation between carbohydrates and the resin counterions.[16−18] Angyal has studied at length sugar−metal ion complexes by thin-layer LEC and proton magnetic resonance.[19] He concluded that, for cyclic carbohydrates, the most effective arrangement for the complexation is a sequence of an axial (ax), an equatorial (eq), and an axial (ax) hydroxyl group on a six-membered ring or three consecutive cis hydroxyl groups on a five-membered ring. Among the carbohydrates studied, only D-ribose, D-allose, and D-talose present at least one anomeric conformation having sequences of hydroxyl groups suitable for interactions with Ba(II). While no data are available about the complexation of metal ions with sugars in alkaline solutions, the stability constants in water relative to complexes of β-D-ribofuranose and α-D-allopyranose with Ba(II) are 4.3 and 2.9 M^{-1}, respectively.[19,20] We suggest that the possible formation of such weak complexes in the alkaline solution could screen, at least in part, the negative charge on the dissociated sugar molecules, leading to the observed reduction of retention (Table 1).

In Figure 2, only the chromatographic profile around the D-ribose peaks (A) and D-allose plus D-talose peaks (B) are displayed. The asymmetrical peak profile, concerning these three sugars, is clearly more pronounced in 100 mM NaOH (solid lines), while peak intensity and shape are distinctly enhanced when the running mobile phase contained barium ions (dashed lines). For D-ribose, D-allose, and D-talose, there was a general increase in peak height (peak area) amounting to 230 (20%), 205 (25%), and 75% (20%), respectively. More specific details, concerning the influence of barium on the amperometric detection at gold electrodes, have been discussed previously.[21] Results similar to the barium hydroxide addition were obtained with barium acetate (1 mM) and are not given here. The major substantial difference, however, is related to the presence of acetate ion (2 mM) in the

(16) Goulding, R. W. *J. Chromatogr.* **1975**, *103*, 229.
(17) Honda, S.; Suzuki, S.; Kakehi, K. *J. Chromatogr.* **1984**, *291*, 317.
(18) Stefansson, M., Westerlund, D. *J. Chromatogr., A* **1996**, *720*, 127.
(19) Angyal, S. J. *Adv. Carbohydr. Chem. Biochem.* **1989**, *47*, 1.
(20) Angyal, S. J. *Aust. J. Chem.* **1972**, *25*, 1957.
(21) Cataldi, T. R. I.; Casella, I. G.; Centonze, D. *Anal. Chem.* **1997**, *69*, 4849.

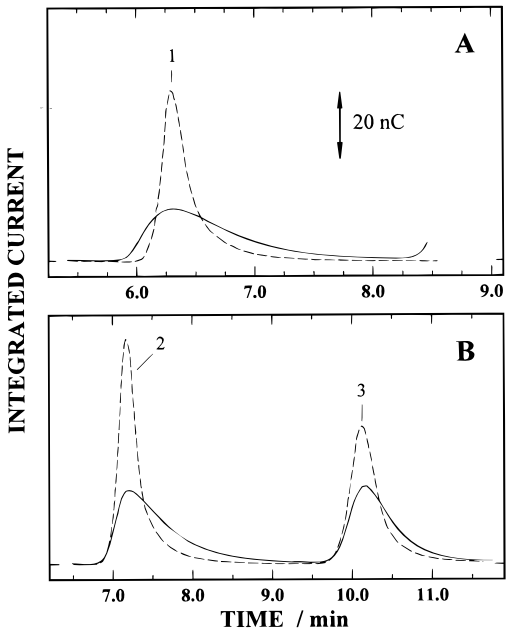

Figure 2. Comparison of the chromatograms of D-ribose (1) (A) and D-allose (2) plus D-talose (3) (B) using conventional alkaline mobile phase, 100 mM NaOH (solid line), and upon addition of $Ba(OH)_2$ at a concentration of 1 mM (dashed line). The concentration of carbohydrates was 100 μM. Other conditions as in Figure 1.

hydroxide mobile phase, which concurrently imparts to all compounds a decrease in retention.[2,22,23]

In Table 2 are summarized the peak asymmetry factors, A_s, evaluated using 100 and 16 mM NaOH as the eluents, before and after the addition of barium. We wish to emphasize that, in the presence of barium, despite a noticeable improvement of tailing, the A_s values are still comparably high. A possible explanation of this phenomenon should also account for the pronounced tailing observed for D-ribose, D-allose, and D-talose, and to a lesser extent for L-rhamnose and D-fructose, using conventional alkaline eluents. Indeed, after column regeneration with hydrochloric acid first and then with water, which ensures the almost complete absence of CO_3^{2-} accompanied by the maximum ion-exchange column capacity, the asymmetry factors were surprisingly elevated. We infer from this that upon barium addition there are two contrasting effects on the anion-exchange separation of carbohydrates. The first is due to carbonate depletion, which leads to a general increase of retention because almost all the ion-exchange sites of the column are free to interact. Those sugar molecules having favorable complexing ability of some conformers, however, tail more strongly owing to selective interactions with the positively charged stationary phase. The second concurrent phenomenon is related to the above suggested complexation of sugar with barium in solution, through a competitive mechanism that imparts a lowering of retention and an improvement of tailing, as reported in Table 2.

Determination of Carbonate and Barium Ions in the Alkaline Eluents. To prove whether the carbonate ion was effectively removed from the alkaline mobile phase, its content was evaluated by ion chromatography (IC) with conductivity detection in suppressed conductivity mode. Figure 3, shows a comparison between the IC of a sample of alkaline eluent (i.e., 100 mM NaOH) before and after addition of 1 mM $Ba(OH)_2$ using the same conditions of flow rate and mobile phase, chromatograms a and b, respectively. The significantly absence of carbonate is quite well demonstrated in chromatogram b. For these experiments, a flow rate of 1.5 mL min^{-1} and a 20 mM NaOH solution as the mobile phase was used. In the same figure chromatogram c referred to a mobile-phase sample treated with $Ba(CH_3COO)_2$, which illustrates that the efficient removal of carbonate is exactly the same as with $Ba(OH)_2$. Of note is also the appearance, in the last chromatogram, of the acetate peak at a retention time of 3.0 min. The small peak at ~6.4 min is due to chloride present as an impurity. Control experiments carried out with standard mixtures of inorganic anions confirmed the identity of the peaks and agreed with the evaluation of the amount of carbonate in the unmodified eluents. Its concentration was found to be variable but generally never higher than 0.5 mM. Equivalent results were obtained when the samples were 16 mM NaOH before (a) and after (b) the addition of barium ions, as shown in Figure 3B. The small peak of carbonate in chromatogram a, which corresponds to ~0.2 mM CO_3^{2-}, is due to the relatively low NaOH concentration. Yet the good agreement between these findings, in terms of sharp suppression of the carbonate peak, is evident. Hence, if the alkaline eluent is modified with barium ions, then the resultant solution can be really considered as carbonate-free.

To confirm the interpretation given for the complexation of sugar molecules by barium ions, the presence in the modified alkaline mobile phase of Ba(II) was verified by ion chromatography using a cation-exchange column (see Experimental Section). Using 18 mM CH_3SO_3H as an eluent at a flow rate of 1.0 mL min^{-1}, several alkaline solutions modified with 1 mM $Ba(CH_3COO)_2$ were analyzed (not shown). Conductivity suppression was achieved with a cation self-regenerating suppressor. As confirmed by a standard solution, the barium ion peak was observed at a retention time of 22.6 min. Though the concentration of free barium ions in the alkaline eluents depends on the amount of carbonate present, we normally obtained values not higher than 0.5−0.7 mM. The choice of 1 mM as the barium concentration allows both the effective removal of carbonate and concomitant complexation of some sugar molecules. The low solubility of $Ba(OH)_2$ prevents the use of higher concentrations of barium in the alkaline solutions.[14]

Validation of Carbonate-Free Alkaline Eluents. Currently, the major problem for the carbohydrate separation when 10−20 mM NaOH eluents are used in HPAEC is the need for flushing the column after each run with a more concentrated NaOH eluent to remove CO_3^{2-} impurities. As mentioned above, such a divalent ion is electrostatically attracted to the positively charged stationary phase leading to a severe decrease in column selectivity and loss of resolution. This also limits the reliability of quantitative measurements based on peak (area) intensity. To compare the inherent advantage of using barium ion as an eluent additive, in Figure 4 are plotted the normalized capacity factors (k'/k'_0) of D-arabinose, D-galactose, D-glucose, D-ribose, lactose, and lactulose evaluated over several hours during which the sample mixture was repetitively injected. The k'_0 represents the value of each compound evaluated by the initial run of a comprehensive set of

(22) Townsend, R. R.; Hardy, M. R.; Hindsgaule, O.; Lee, Y. C. *Anal. Biochem.* **1988**, *174*, 459.
(23) Lee, Y. C. *Anal. Biochem.* **1990**, *189*, 151.

Table 2. Asymmetry Factors Evaluated before and after Addition of Ba(II) to Alkaline Eluents[a]

	A_s			
	100 mM NaOH[b]	100 mM NaOH + 1 mM Ba(II)[b]	16 mM NaOH	16 mM NaOH + 1 mM Ba(II)
L-rhamnose	1.85	1.10	1.70	1.10
D-fructose	1.50	1.20	1.60	1.10
D-ribose	3.25	2.60	2.50	2.00
D-allose	4.25	2.35	3.30	2.50
D-talose	2.50	1.80	2.30	1.90

[a] Peak asymmetry factors (±0.05) calculated as the ratio of the trailing to leading half-width at 10% of the peak height. [b] See experimental conditions stated in Figure 1.

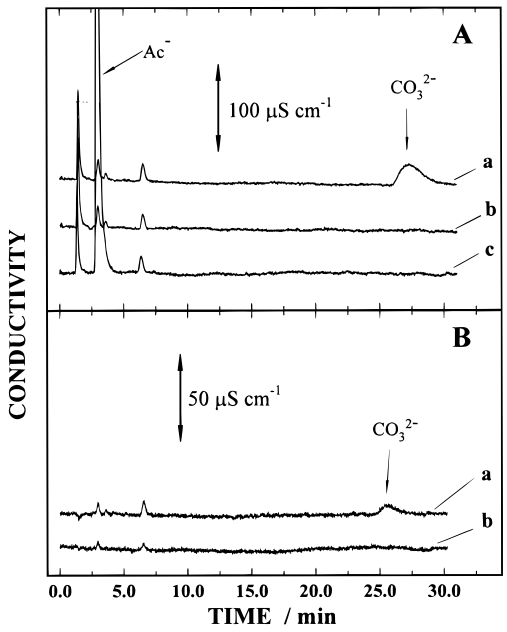

Figure 3. Anion-exchange chromatographic separation with conductivity detection of CO_3^{2-} in the following samples: (A) 100 mM NaOH solution before (a) and after addition of 1 mM $Ba(OH)_2$ (b) or 1 mM $Ba(CH_3COO)_2$ (c); (B) 16 mM NaOH solution before (a) and after addition of 1 mM $Ba(OH)_2$ (b). Eluent, 20 mM NaOH at a flow rate of 1.5 mL min^{-1}. Column, IonPac AS12A plus guard column. Conductivity suppression was achieved with an anion self-regenerating suppressor.

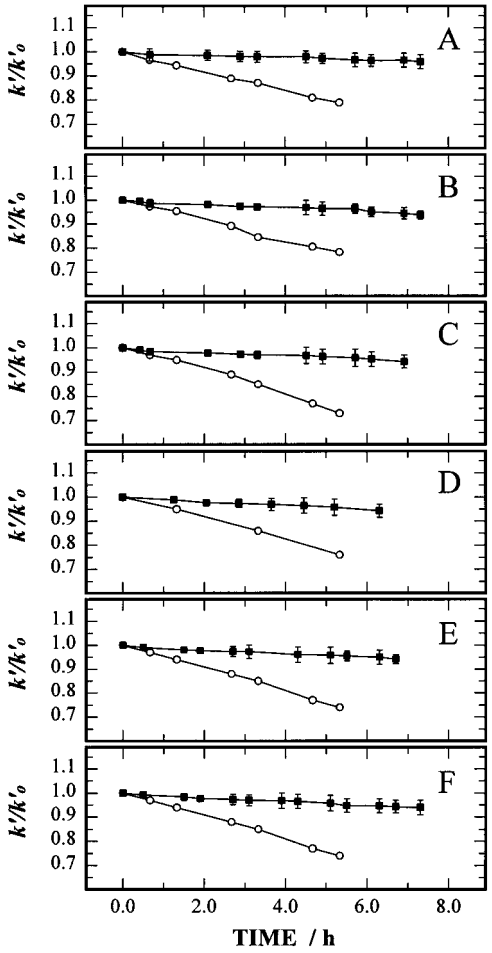

Figure 4. Time dependence of the normalized capacity factor (k'/k'_0) of D-arabinose (A), D-galactose (B), D-glucose (C), D-ribose (D), lactose (E), and lactulose (F) using conventional 16 mM NaOH (solid circles) and 16 mM NaOH + 1 mM $Ba(CH_3COO)_2$ (open circles) as the eluents. The error bars represent the difference between the highest and lowest value obtained.

measurements. The capacity factors obtained with a conventional 16 mM NaOH eluent after column regeneration and those obtained upon addition of 1 mM $Ba(CH_3COO)_2$ are summarized in Table 3. The distinct difference between these results is immediately apparent for all compounds investigated. While the conventional eluent leads to a gradual diminution of the retention, with k'/k'_0 ratios decreased to about 0.8−0.7 after 4−5 h for all compounds (open circles), excellent reproducibility was obtained with barium as a mobile phase component (solid circles). As shown in the plots of Figure 4, each data point represents the mean value of at least three measurements obtained in different and even nonconsecutive days. The precision of the capacity factor ratios was for all investigated compounds between 0.8 and 2.5% RSD. Capacity factors after ~4 h decreased only up to 2−4% of the initial values, when the barium-containing eluent was used. Interestingly, the k'/k'_0 ratio of lactulose, which represents one of the most retained compounds investigated, exhibits a slight decrease from 1.00 to 0.94 after 7.5 h of chromatographic runs (Figure 4F). This means that the introduction of Ba(II) guarantees almost identical elution conditions because complete and prolonged carbonate depletion is attained during the anion-exchange chromatographic separations. We wish to emphasize that the above results were obtained by adopting the strategy of using the modified alkaline eluent over the ensuing day of barium addition. Under such experimental conditions, rapid chromatographic analyses are possible because no extensive column reequilibration after each run is required. Yet such a step has to be performed at least at the beginning of each working session.

Table 3. Effect of the Eluent Composition on the Capacity Factors (k'_0) of Carbohydrates in HPAEC−PAD[a]

carbohydrate	k'_0 16 mM NaOH	k'_0 16 mM NaOH + 1 mM $Ba(CH_3COO)_2$	carbohydrate	k'_0 16 mM NaOH	k'_0 16 mM NaOH +1 mM $Ba(CH_3COO)_2$
L-rhamnose	5.22	3.76	D-allose	12.83	7.82
D-arabinose	5.80	4.03	D-ribose	13.22	8.68
D-galactose	7.78	5.20	lactose	21.29	12.72
D-glucose	8.50	5.82	lactulose	24.25	13.93
D-mannose	10.19	6.79	D-talose	27.15	17.22
D-fructose	12.46	7.64			

[a] Column, CarboPac PA1 plus guard column; eluent flow rate, 1.0 mL min^{-1}. The column was flushed for ~1 h with the carbonate-free 200 mM NaOH solution before equilibration.

These results on standard mixtures provide strong incentive to apply such a strategy to real samples in which the presence of several carbohydrates may require, for their separation, the use of diluted alkaline eluents.

CONCLUSIONS

New evidence has resulted from this study to demonstrate the action of barium ion in alkaline eluents, which is related to both a very effective removal of carbonate and the ability to complex carbohydrates. Although to eliminate carbonate interference an on-line system using disposable cartridges for producing KOH eluents is now on the market, no comparably straightforward and inexpensive procedure is available, which ensures thoroughly carbonate-free mobile phases flowing through the column. An application of the beneficial effects related with the introduction of Ba(II) in diluted alkaline mobile phases is demonstrated. The enhanced separation performances resulted in reduced analysis time. Likewise, the present results strongly support that barium binding to sugar molecules occurs also in alkaline solutions, especially with those aldoses having a favorable sequence of three hydroxyl groups. While the idea has been illustrated in the context of HPAEC, it could be readily extended to other separation schemes. For instance, barium ions in solution may hold the key to successful separation of complex carbohydrate mixtures by capillary electrophoresis with electrochemical detection.

ACKNOWLEDGMENT

We acknowledge with thanks the comments and suggestions of William R. LaCourse. The Regione Basilicata provided partial funding through “LaMI”. This work was also supported by the National Research Council of Italy (CNR, Rome) and Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, Rome).

Received for review March 30, 1998. Accepted June 18, 1998.

AC9803582