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Use of combined sodium hydroxide and carbonate–bicarbonate eluents with various anion-exchange columns

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Abstract

Retention characteristics of various anion-exchange columns were studied, using combinations of sodium hydroxide and carbonate–bicarbonate as eluents. A Dionex 4000i unit was utilized for this work. Dionex AS4A and AS11 columns for determining inorganic anions were evaluated, both using suppressed conductivity detection. Also investigated were CarboPac PA1/AS6 columns, which are used with pulsed amperometric detection to separate saccharides. Eluent components were proportioned via the mixing capabilities of the gradient pumps.

1. Introduction

Dionex anion-exchange columns are used with a variety of eluents, including sodium hydroxide and sodium carbonate–sodium bicarbonate. The AS11 and CarboPac PA1/AS6 columns are designed primarily for use with the first solution, while the AS4A often is used with the second. When sodium hydroxide is used, it is traditionally kept free of carbonates, as they act as "pushers" [1,2]. Any leakage of carbon dioxide into the eluent will lead to irreproducible retention times and altered resolution of analytes.

Some chromatographers have separated anions on the AS4A using mixtures of these three solutions. To optimize chromatography of various samples, Gros and Gorenc [3] studied a wide range of carbonate–bicarbonate eluents, but they never introduced hydroxide. Balconi and Sigon [4] combined sodium hydroxide and sodium bicarbonate, but their main goal was to separate chloride from the "water dip", while eluting sulfate in ten minutes or less. Talmage and Biemer [5] used a specific ratio of sodium hydroxide and sodium carbonate to optimize resolution of fluoride, nitrate, monofluorophosphate, sulfate, and phosphate in toothpaste samples. None attempted to determine the general behavior of the column under a wide range of eluent mixes. For the AS11 and PA1/AS6 columns, no evidence was found to suggest that these mixed eluents have been tried at all.

The goal of this work was to determine if controlled mixing of the above eluents could give desirable retention characteristics on these columns (*e.g.*, faster run times, better resolution). Parameters monitored were: (1) retention time, (2) retention order, and (3) peak shape. Also, the ability to remove carbonate from the columns was tested.

2. Experimental

2.1. Materials

The chemicals used in preparing standard solutions and eluents were obtained from various

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suppliers. Sodium hydroxide was purchased in carbonate-free solution form from Fisher Scientific (Pittsburgh, PA, USA); all other chemicals were the highest purity available. All water was from the in-house system, available at a resistivity of 18 MΩ. In varying concentrations as needed, separate solutions of sodium hydroxide, sodium carbonate, and sodium bicarbonate were prepared for use as eluents. Water for eluents was sparged with helium before solutions were prepared; sparging continued for the life of the mixtures. Analyte solutions were prepared in concentrations that provided an adequate response on the chromatogram. For the conductivity work, values ranged from 1 to 6 ppm (w/w); sugars were made up at 50 ppm each.

2.2. Apparatus and columns

A Dionex (Sunnyvale, CA, USA) Series 4000i ion chromatograph was utilized for all work. All columns were from Dionex and were 4 mm I.D. In analyses of inorganic anions, post-column eluent suppression was accomplished with a Dionex Anion Self-Regenerating Suppressor (ASRS-I); detection was via a Dionex CDM-2 conductivity detector at an output range of 1 μS. Column sets employed were: (1) IonPac AG11 guard and AS11 analytical, and (2) IonPac AG4A guard and AS4A analytical columns. In both cases, the eluent flow-rate was 2.0 ml/min. A 50-μl sample loop was used.

Sugar separations were effected using: (1) HPIC-AG6 guard and HPIC-AS6 analytical, or (2) CarboPac PA1 guard and PA1 analytical column sets. In both cases, the flow-rate for the mobile phase was 1.0 ml/min. A Dionex PAD-2 pulsed amperometric detector with a gold electrode was used at an output range of 30 000 nA; applied potentials were 0.05 V for 420 ms, 0.80 V for 180 ms, and −0.10 V for 360 ms. Sample loop size was 10 μl.

To remove carbonate–bicarbonate from the columns, 500 m*M* sodium hydroxide was pumped through (bypassing any suppressor and the detector) for 30 min, followed by deionized water for 30 min. Flow-rates were the same as for the applicable eluent. For all work, instrument control and data collection were performed with a personal computer and the Dionex AI-450 software.

3. Results and discussion

3.1. CarboPac PA1 (formerly AS6)

This research centered on lactose and sucrose, two of the saccharides this column was designed to separate (see Table 1 for a summary of retention times *vs.* eluent composition). With the typically used eluent of 100 m*M* sodium hydroxide, lactose eluted before sucrose (the "normal" order), with a run time of 17 min (Fig. 1a). Hydroxide mobile phases between 100 and 500 m*M* were tried, and all gave this same sequence. When the eluent concentration was dropped below 100 m*M*, retention times lengthened, as expected. By 60 m*M*, though, the two sugars essentially coeluted in around 20 min. A further reduction to 40 m*M* resolved the pair again, but in *reverse* order. However, sucrose and lactose were retained for 25 and 28 min, respectively.

Carbonate solutions of 0.5 and 1.0 m*M* were

Table 1
Retention times *vs.* eluent composition for the PA1 column

Eluent composition		Retention times (min)	
NaOH (m*M*)	Na_2CO_3 (m*M*)	Lactose	Sucrose
100	–	13.8	16.0
80	–	15.2	16.6
60	–	19.6	19.6
40	–	28.0	24.7
100	0.5	6.7	7.4
80	0.5	6.6	6.8
60	0.5	5.9	5.8
40	0.5	4.9	4.4
100	1.0	5.2	5.7
80	1.0	5.0	5.2
60	1.0	4.6	4.6
40	1.0	3.7	3.4

Experimental conditions are those detailed in the Experimental section.

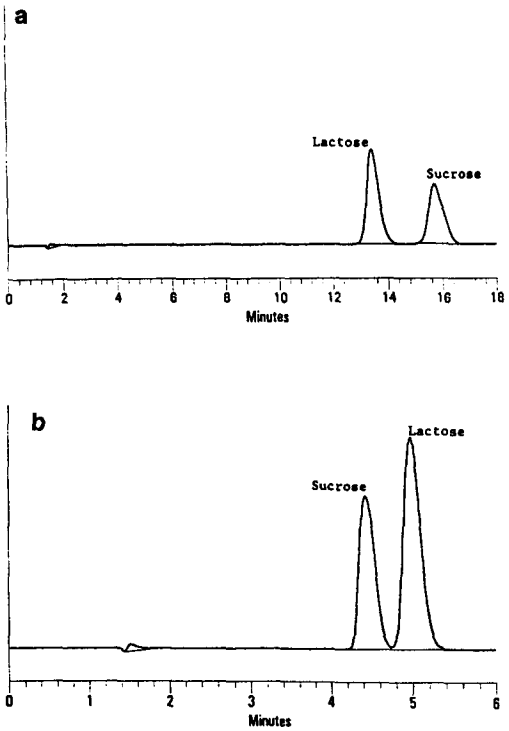

Fig. 1. (a) shows the normal retention order of lactose and sucrose (both at 50 ppm, w/w) on the CarboPac PA1 or AS6 column, using 100 m*M* NaOH as the eluent. In (b), the order is reversed with a mobile phase of 40 m*M* NaOH–0.5 m*M* Na_2CO_3. This sequence was seen for pure 40 m*M* NaOH as well, but run times were about 30 min.

added to various levels of sodium hydroxide (between 40 and 100 m*M*). The retention order was always the same as seen for the sodium hydroxide alone, but the retention times were reduced. (Fig. 1b illustrates the results for 0.5 m*M* sodium carbonate with 40 m*M* sodium hydroxide.) In addition, the resolution often was not altered significantly. For a given concentration of carbonate, though, the retention times increased as the sodium hydroxide molarity increased, presumably because less carbonate "pusher" could remain on the column with higher amounts of hydroxide.

When carbonate–bicarbonate eluents were tried in various ratios (total molarity: 1.5 to 5.5 m*M*), the two sugars coeluted in approximately 1.5 min. Also, the response was poor, since the PAD requires a high pH for maximum sensitivity [6].

3.2. AS11 and AS4A

Ten anions were chromatographed on the AS11. They always eluted in the order: fluoride (off first), acetate, formate, chlorite, bromate, chloride, nitrite, bromide, nitrate, and chlorate. The first seven of these were tested on the AS4A; elution order was the same. All reported concentration values for eluents are net of the reaction: $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$. This process appeared to be essentially instantaneous and complete in the gradient pump.

AS11

Typically, a sodium hydroxide gradient [7] is used with this column. The above ten anions are well resolved within 9 min. However, a gradient pump is required and reequilibration time (5 to 10 min) is necessary. In this work, isocratic eluent mixtures of hydroxide and carbonate were tested to see if similar chromatography could be achieved (see Table 2). When 0.48 m*M* sodium hydroxide was tried, all ten ions were resolved quite nicely (Fig. 2a), but chlorate eluted only after 29.5 min. An increase to 1.1 m*M* gave run times similar to the gradient run, but fluoride and acetate essentially coeluted. However, when a hydroxide–carbonate ratio of 0.43 m*M*:0.05 m*M* was used, chlorate's retention time was 14.9 min; separation of fluoride and acetate suffered somewhat (Fig. 2b). Further reductions in the eluent ratio continued to decrease retention times, but at the expense of resolution (fluoride and acetate, bromate and chloride). Also, a "water dip" eventually appeared right before fluoride.

Bicarbonate–carbonate eluents alone were not able to achieve the chromatography shown in Fig. 2b. As seen in Fig. 2c, an eluent ratio of 0.12 m*M*:0.36 m*M* (bicarbonate–carbonate) could not resolve fluoride and acetate, or bromate and chloride. Ratios of 0.24 m*M*:0.24 m*M* and 0.36 m*M*:0.12 m*M* did not improve resolution, even though retention times lengthened.

Table 2
Retention times *vs.* eluent composition for the AS11 column

Eluent composition			Retention times of peaks[a] (min)									
NaOH (m*M*)	$NaHCO_3$ (m*M*)	Na_2CO_3 (m*M*)	1	2	3	4	5	6	7	8	9	10
[b]			2.2	2.4	3.3	4.6	5.7	5.9	6.4	8.2	8.3	8.5
0.48	–	–	2.1	2.4	3.1	4.4	7.6	8.6	11.4	24.8	27.0	29.5
1.10	–	–	1.4	1.4	1.8	2.4	3.7	4.2	5.4	11.1	12.0	13.1
0.43	–	0.05	1.4	1.6	1.9	2.5	4.1	4.4	5.9	12.2	13.5	14.9
0.33	–	0.10	1.3	1.4	1.7	2.2	3.6	3.8	5.1	10.4	11.5	12.7
0.24	–	0.24	1.2	1.2	1.4	1.8	2.9	2.9	3.8	7.6	8.4	9.3
–	0.12	0.36	1.1	1.1	1.4	1.8	2.8	2.8	3.6	7.1	7.9	8.7
–	0.24	0.24	1.2	1.2	1.5	2.0	3.1	3.1	4.2	8.1	9.1	10.2
–	0.36	0.12	1.4	1.5	1.8	2.4	4.0	4.0	5.5	10.5	13.0	14.1
–	0.48	–	2.7	3.0	4.3	6.8	12.2	12.2	17.6	36.6	41.0	46.3
–	–	0.48	1.1	1.1	1.3	1.7	2.6	2.6	3.3	6.4	7.0	7.8

Experimental conditions are those detailed in the Experimental section.
[a] Peak identification: 1 = fluoride, 2 = acetate, 3 = formate, 4 = chlorite, 5 = bromate, 6 = chloride, 7 = nitrite, 8 = bromide, 9 = nitrate, and 10 = chlorate.
[b] These retention times are for the typical eluent scheme (a sodium hydroxide gradient) on this column; see Ref. 7 for gradient details.

In addition, peak shapes for bromide, nitrate, and chlorate often were poor. Similarly poor chromatography was seen with either 0.48 m*M* carbonate or 0.48 m*M* bicarbonate eluents.

Eluents with total molarity less than 0.3 m*M* gave excellent separations in most cases, but retention times became quite long (*e.g.*, chlorite did not elute for at least 9 or 10 min). Once the total concentration exceeded 0.5 m*M*, retention times became very short for all species and coelution was a problem (fluoride with acetate, chloride with bromate).

AS4A

The standard eluent conditions on this column are 1.7 m*M* sodium bicarbonate–1.8 m*M* sodium carbonate. The run time for the first seven anions listed above was only two min, but fluoride and acetate coeluted and were not resolved from the "water dip" (see Table 3). When 0.48 m*M* sodium hydroxide was used, chromatography was excellent, but nitrite did not elute until 22.2 min (Fig. 3a). However, if 0.05 m*M* sodium carbonate was added to 0.43 m*M* sodium hydroxide, resolution remained high and nitrite eluted in 7.1 min (Fig. 3b). In neither case was the "water dip" a problem.

Mixing carbonate and bicarbonate (each at 0.24 m*M*) shortened nitrite's retention time to 4 min, but at the expense of resolution of the first four peaks. Pure carbonate produced a poor separation as well. Bicarbonate alone could resolve all seven ions, but the last one remained on the column for 15.7 min.

It should be noted that with the pure sodium hydroxide (Fig. 3a), two to three days were needed to bring the retention times to equilibrium. Seven hours after the system was changed from the post-cleaning rinse to the hydroxide eluent, nitrite still was being retained for 40 min. By equilibrium, though, the anion was eluting in half that time.

As with the AS11, long retention times were seen with eluents totalling less than 0.3 m*M* (nitrite at 10 min or greater). Coelution (fluoride

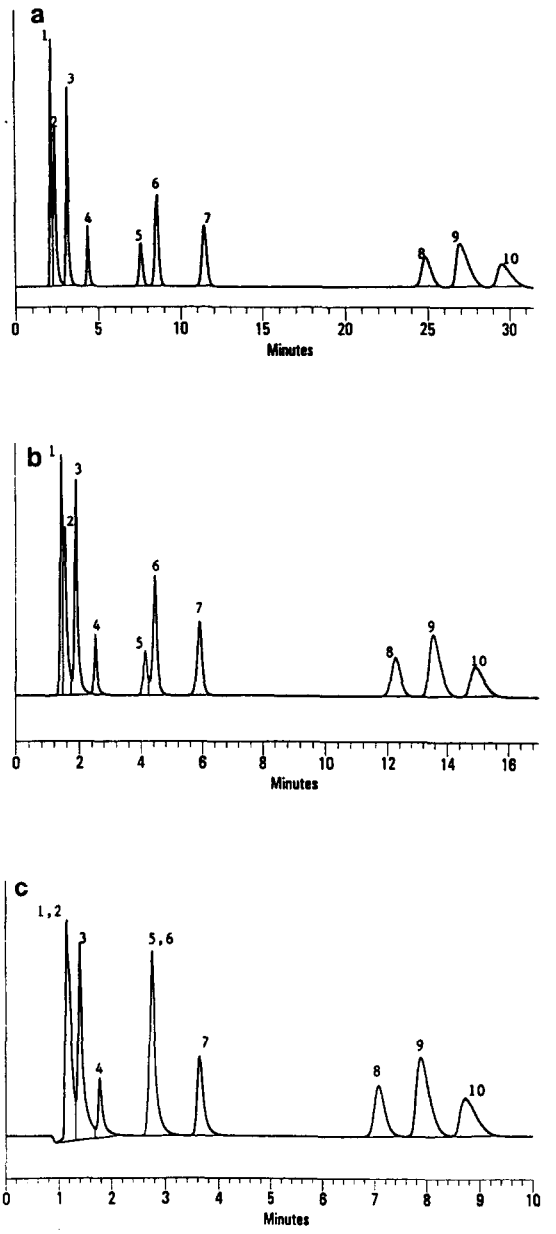

Fig. 2. Chromatograms showing the retention characteristics of inorganic anions on the AS11 column, using mixtures of NaOH (A), $NaHCO_3$ (B), and Na_2CO_3 (C) as the mobile phase. Eluent ratios (A–B–C, all values in m*M*) are: (a) 0.48:0:0; (b) 0.43:0:0.05; (c) 0:0.12:0.36. Peaks: 1 = fluoride, 1 ppm; 2 = acetate, 5 ppm; 3 = formate, 6 ppm; 4 = chlorite, 2 ppm; 5 = bromate, 3 ppm; 6 = chloride, 1 ppm; 7 = nitrite, 2 ppm; 8 = bromide, 3 ppm; 9 = nitrate, 4 ppm; and 10 = chlorate, 4 ppm (all ppm values are w/w ratios).

with acetate) again was a factor if total molarity was above 1.0 m*M*.

4. Conclusions and summary

On all three columns, results show that *controlled* proportioning of hydroxide, carbonate, and bicarbonate mobile phases can affect retention characteristics beneficially. Retention times are stable and reproducible for any given eluent mix. Also, run times can be reduced, usually without sacrificing resolution.

Elution order of the inorganic anions remains unchanged with these mixes. However, on the AS11, mixing of carbonate and hydroxide offers the possibility of isocratically separating early-eluting anions in reasonable lengths of time. It also permits broader application of the widely used AS4A. With proper ratios on this latter column, fluoride can be resolved completely from both the "water dip" and from acetate, an achievement impossible with the standard carbonate–bicarbonate eluent.

Lactose and sucrose *can* be eluted in reverse order, depending on the sodium hydroxide concentration. However, with pure hydroxide, having sucrose first requires a run time of 30 min. Adding between 0.5 and 1.0 m*M* carbonate maintains separation, but reduces retention times to around 4 min. This phenomenon will be beneficial in cases where one sugar is present in much greater concentration than the other. By controlling the eluent mix, the minor constituent can be eluted first and thereby be resolved from the large peak that follows. Also, retention times can be adjusted as desired.

Carbonate can be removed easily from all exchangers by using strong sodium hydroxide, thereby restoring original selectivity. However, if any formerly used mixture then is reintroduced, the previously seen chromatography again is obtainable. All of the above results indicate that combining carbonate and hydroxide in specific proportions gives the chromatographer another useful means of managing his separations.

Table 3
Retention times *vs.* eluent composition for the AS4A column

Eluent composition			Retention times of peaks[a] (min)						
NaOH (m*M*)	$NaHCO_3$ (m*M*)	Na_2CO_3 (m*M*)	1	2	3	4	5	6	7
–	1.7	1.8	1.0	1.0	1.1	1.2	1.4	1.6	1.9
0.48	–	–	5.0	5.8	7.6	9.4	13.6	15.9	22.2
0.43	–	0.05	2.0	2.3	2.8	3.3	4.5	5.2	7.1
–	0.24	0.24	1.4	1.6	1.8	2.0	2.7	3.1	4.0
–	–	0.48	1.3	1.4	1.6	1.8	2.3	2.6	3.3
–	0.48	–	3.7	4.2	5.4	6.7	9.6	11.2	15.7

Experimental conditions are those detailed in the Experimental section.
[a] Peak identification: 1 = fluoride, 2 = acetate, 3 = formate, 4 = chlorite, 5 = bromate, 6 = chloride, and 7 = nitrite.

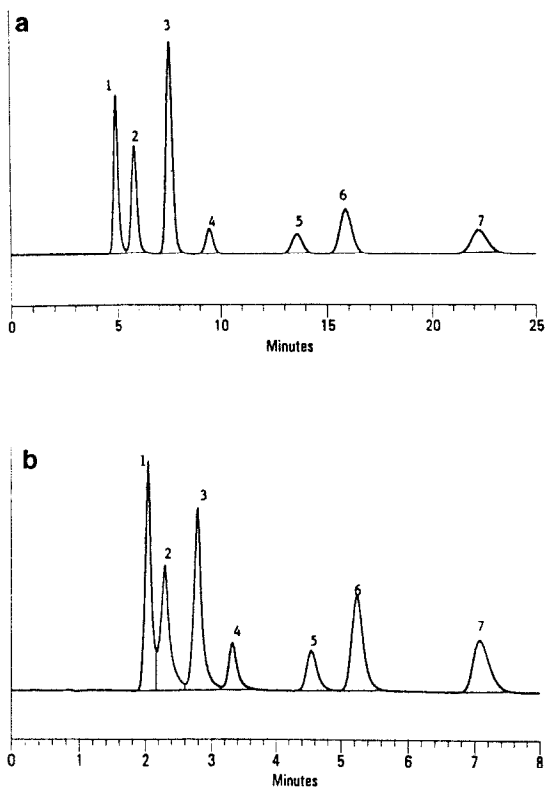

Fig. 3. Chromatograms showing the retention characteristics of inorganic anions on the AS4A column, using mixtures of NaOH (A), $NaHCO_3$ (B), and Na_2CO_3 (C) as the mobile phase. Eluent ratios (A–B–C, all values in m*M*) are: (a) 0.48:0:0; (b) 0.43:0:0.05. Peaks: 1 = fluoride, 1 ppm; 2 = acetate, 5 ppm; 3 = formate, 6 ppm; 4 = chlorite, 2 ppm; 5 = bromate, 3 ppm; 6 = chloride, 1 ppm; and 7 = nitrite, 2 ppm (all ppm values are w/w ratios).

5. Acknowledgement

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