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## BENZENEPOLYCARBOXYLIC ACID SALTS AS ELUENTS IN ANION CHROMATOGRAPHY

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### SUMMARY

Several benzenepolycarboxylate salts were used as eluents for separating common inorganic anions on a highly efficient anion-exchange column. Eluents containing a  $-3$ -charged benzenetricarboxylate (BTA) anion or a  $-4$  pyromellitate anion gave better separations than  $-2$  phthalate eluents of samples containing both  $-1$  and  $-2$  anions. Compared with phthalate, a lower concentration of BTA or pyromellitate is used in the eluent, with a consequent improvement in the sensitivity of detection by indirect spectrophotometry. With BTA and pyromellitate eluents, sulfate elutes much earlier than nitrate and other monovalent anions than it does with phthalate. However, this effect may depend partly on the type and capacity of the ion-exchange resin in addition to the eluent.

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### INTRODUCTION

A number of eluents have been used for the ion-chromatographic separation of anions<sup>1</sup>. Almost all eluents utilized to date have contained displacing anions with a  $-1$  or  $-2$  charge. In general, eluent anions of  $-2$  charge are more effective for late-eluting sample anions than eluent anions of  $-1$  charge. Jardy *et al.*<sup>2</sup> found salts of 1,2,4,5-benzenetetracarboxylic acid (pyromellitic acid) to be effective for eluting several sample anions. Pyromellitate can assume various charges up to  $-4$ , depending on the pH at which the eluent is buffered.

The purpose of this work was to evaluate aromatic compounds that form  $-3$  and  $-4$  anions as eluents for ion chromatography and to compare these with the widely used phthalate eluents. The new eluents were found to provide excellent resolution of anion mixtures with better sensitivity than eluents containing displacing anions of lower charge. They also showed some interesting effects with regard to the order of elution of sample anions.

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## EXPERIMENTAL

### *Apparatus*

The ion-chromatographic system consisted of the following components: a Milton-Roy mini-pump (Laboratory Data Control, Rivera Beach, FL, U.S.A.), a Rheodyne Model 7000 injection valve (Ranin, Waburn, MA, U.S.A.) equipped with 20-, 100- and 500- $\mu$ l sample loops, a Milton-Roy pulse damper (Laboratory Data Control) placed between the pump and sample injector, a model 970 A variable-wavelength detector (Tracor Instruments, Austin, TX, U.S.A.), a Model 213 conductivity detector (Wescan Instruments, Santa Clara, CA, U.S.A.), a Curkin recorder (Curkin Scientific, Hawleyville, CA, U.S.A.) and glass-lined stainless-steel columns (15 cm  $\times$  4 mm I.D. and 25 cm  $\times$  4 mm I.D.) (Scientific Glass Engineering, Austin, U.S.A.). A Shandon high-performance liquid chromatographic column packer (Shandon Southern Instruments, Bason, PA, U.S.A.) was used to pack the resin into the columns at a packing pressure of 3500 p.s.i.

### *Resins*

A 4.2- $\mu$ m spherical non-porous polystyrene resin and a 0.09- $\mu$ m latex suspension 76% functionalized with quaternary ammonium groups were supplied by Rohm and Haas (Spring House, PA, U.S.A.). The latex was coated onto the polystyrene resin from an aqueous slurry containing sodium chloride by a published procedure<sup>3</sup>. The coated resin had an exchange capacity of 0.027 mequiv./g. The coated resin was packed into the columns as described above.

### *Reagents and solutions*

1,3,5-Benzenetricarboxylic acid (BTA) (97%, Aldrich) and pyromellitic acid (96%, Aldrich) were purified as follows. A saturated solution in boiling water was filtered rapidly through a 0.2- $\mu$ m membrane filter with suction. On cooling, the precipitate was filtered by suction and washed. All other chemicals were of analytical-reagent grade and were used without further purification. Distilled, deionized water was used throughout.

Aqueous solutions of phthalate, BTA and pyromellitate were prepared by dissolving the acid in water and adjusting the pH to the desired value with 0.1 *M* ammonia solution. These eluents were then filtered through a 0.2- $\mu$ m membrane filter and vacuum degassed. Stock solutions of sample anions were prepared from the sodium or potassium salts and diluted as required.

## RESULTS AND DISCUSSION

### *Selection of eluent acids*

*o*-Phthalic acid has  $pK_a$  values of  $pK_1 = 2.95$  and  $pK_2 = 5.41$ , which means that in an eluent containing phthalic acid, buffered to pH *ca.* 6 or above, mostly the  $-2$  phthalate anion is present. The equivalent conductance of  $-2$  phthalate is sufficiently lower than that of most common anions for use in single-column ion chromatography with a conductivity detector. Phthalate eluents absorb fairly strongly in the UV spectral region so that indirect spectrophotometric detection of common ions is also feasible.

Salts of a number of other aromatic acids with multiple acidic groups were evaluated as eluents for ion chromatography. Of those tested, salts of 1,3,5-benzenetricarboxylic acid (BTA) and pyromellitic acid were selected for further study. BTA has  $pK_a$  values of  $pK_1 = 3.01$ ,  $pK_2 = 3.71$  and  $pK_3 = 4.49$  and pyromellitic acid has values of  $pK_1 = 1.92$ ,  $pK_2 = 2.87$ ,  $pK_3 = 4.49$  and  $pK_4 = 5.63$ . Thus, when buffered at pH 6–7, BTA eluents exist as the  $-3$  anion and pyromellitate eluents primarily as the  $-4$  anion.

#### *Comparison of ion chromatograms using eluents of different charge*

*Effect of eluent concentration.* The exchange reaction in ion chromatography between an eluent anion, E, of charge  $a$  and a sample anion, A, of charge  $b$  can be written as



where R represents an exchange site in the ion-exchange resin. By manipulation of eqn. 1, it can easily be shown<sup>4</sup> that the adjusted retention time ( $t'_R$ ) or the capacity ratio ( $k'$ ) of a sample anion is proportional to the concentration of the eluent anion, E. A plot of either  $\log t'_R$  or  $\log k'$  versus  $\log [E]$  should have a slope of  $-b/a$ .

Several common inorganic anions were separated chromatographically on an anion-exchange column using various eluents. The pH of each eluent was adjusted so that the eluent anion would attain its maximum charge of  $-2$  for phthalate,  $-3$  for BTA and  $-4$  for pyromellitate. Retention times were measured and the logarithm of the adjusted retention time for each ion was plotted against the logarithm of the eluent concentration. All of the plots were linear, as shown in Fig. 1 for TBA eluent.

In every instance the plot of  $\log t'_R$  against  $\log [E]$  was very close to the theoretical line. The theoretical slope for phthalate ( $E = -2$ ) is  $-1/2$  for monovalent anions and  $-1$  for divalent anions. The theoretical slope for BTA ( $E = -3$ ) is  $-1/3$  for  $A^-$  and  $-2/3$  for  $A^{2-}$ . The theoretical slope for pyromellitate ( $E = -4$ ) is  $-1/4$  for  $A^-$  and  $-1/2$  for  $A^{2-}$ .

*Comparison of eluents.* The three eluents, phthalate, BTA and pyromellitate, were compared by separating mixtures of several common anions on an efficient anion-exchange column. The concentration of each eluent was adjusted to give optimum resolution for the anion mixture. Indirect spectrophotometric detection of the sample anions was used in each case.

Fig. 2 shows a typical chromatogram obtained with a phthalate eluent. The low eluent concentration required to resolve the early eluting anions causes the retention times of the nitrate and sulfate to be long.

Fig. 3A shows the separation of the same anions except phosphate with a BTA eluent. Fig. 3B shows the separation of all seven anions on a longer column. The time required for the separation is much shorter than in Fig. 2. The peaks are much sharper and higher even though the concentrations of the sample anions are lower than in Fig. 2. The  $-3$  BTA anion is a more powerful eluent than  $-2$  phthalate so that a much lower concentration is needed for the separation ( $0.2$  mM BTA compared with  $1.2$  mM phthalate).

Fig. 4 depicts the separation of the same anions as in Fig. 3 using a pyromellitate eluent. The anions are fairly well resolved, although not as well as in Fig. 3 with

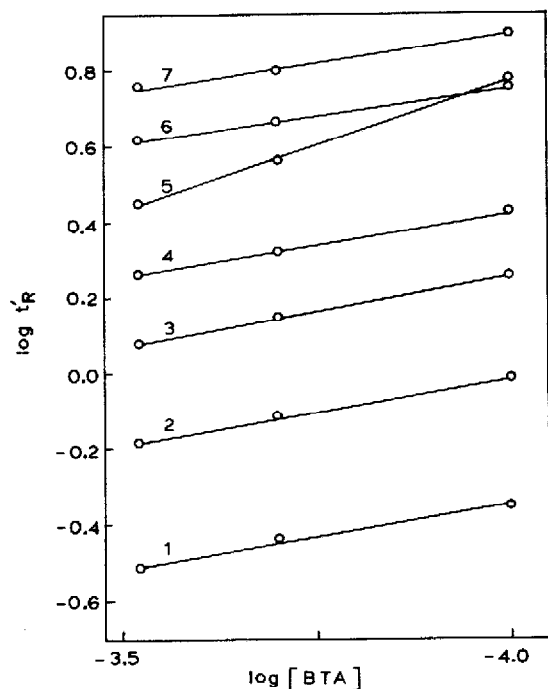


Fig. 1. Logarithm of adjusted retention times of anions vs. logarithm of different concentrations of BTA (pH 6.0). Flow-rate, 0.5 ml/min; detection at 250 nm; 20- $\mu$ l sample; 25-cm column. 1 =  $F^-$ ; 2 =  $H_2PO_4^-$ ; 3 =  $Cl^-$ ; 4 =  $NO_2^-$ ; 5 =  $SO_4^{2-}$ ; 6 =  $Br^-$ ; 7 =  $NO_3^-$ .

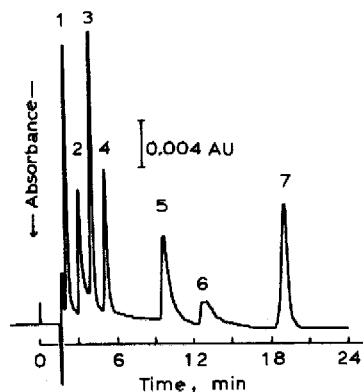


Fig. 2. Separation of seven anions with 1.2 mM phthalate eluent (pH 6.0), flow-rate 0.5 ml/min, detection at 272 nm, 20- $\mu$ l sample and 15-cm column. Peaks: 1 =  $F^-$  (5 ppm); 2 =  $H_2PO_4^-$  (40 ppm); 3 =  $Cl^-$  (10 ppm); 4 =  $NO_2^-$  (10 ppm); 5 =  $Br^-$  (25 ppm); 6 =  $NO_3^-$  (15 ppm); 7 =  $SO_4^{2-}$  (20 ppm).

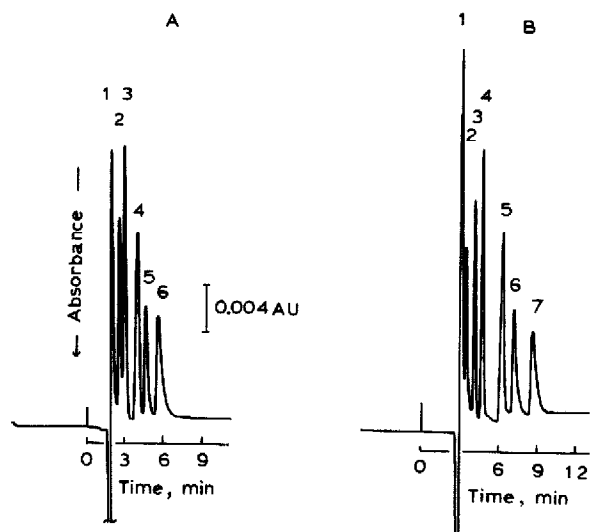


Fig. 3. Separation of anions with 0.2 mM BTA eluent (pH 6.0) and detection at 250 nm. (A) 15-cm column: 1 =  $F^-$  (5 ppm); 2 =  $Cl^-$  (5 ppm); 3 =  $NO_2^-$  (10 ppm); 4 =  $SO_4^{2-}$  (10 ppm); 5 =  $Br^-$  (10 ppm); 6 =  $NO_3^-$  (10 ppm); (B) 25-cm column: 1 =  $F^-$ ; 2 =  $H_2PO_4$  (20 ppm); 3 =  $Cl^-$ ; 4 =  $NO_2^-$ ; 5 =  $SO_4^{2-}$ ; 6 =  $Br^-$ ; 7 =  $NO_3^-$ .

the BTA eluent. The  $-4$  pyromellitate is a more powerful eluent than either of the previous eluents. The very low concentration of the pyromellitate needed for the separation (0.02 mM) permits an excellent detection of the sample anions, even at very low concentrations. Jardy *et al.*<sup>2</sup> also obtained extremely low detection limits for several anions using pyromellitate eluents with indirect spectrophotometric detection.

With each of the three benzenepolycarboxylate eluents, indirect spectrophotometric detection of the sample anions was always better than with a conductivity detector. With BTA eluent, both fluoride and phosphate gave peaks of lower conductance whereas the other anions gave peaks of higher conductance than the background. At the very low concentration levels used in Fig. 4 with pyromellitate eluent, no sample peaks were obtained with a conductivity detector.

*Effect of eluent on anion retention times.* Comparison of the chromatograms in Figs. 2-4 shows that sulfate elutes earlier than chloride as the charge on the eluent anion increases. The retention times of several anions with the various eluents are compared in Table I. The separation factors ( $\alpha$ ) are larger for the early eluting anions as the charge on the eluent anion becomes larger. However, the drastic change in the retention of sulfate with respect to nitrate is undoubtedly the most interesting result in Table I. It is worth noting that Jardy *et al.*<sup>2</sup> reported that sulfate eluted *after* bromide and nitrate using pyromellitate at pH 4. However, the exchange capacity of their resin and the concentration of the eluent were different from those used in this work.

The selectivities of commercial ion-chromatographic columns for various anions should be similar to those obtained with the columns used in this work. However, with any column the retention times of divalent anions are more affected by changes in resin capacity and eluent concentration than the retention times of mono-

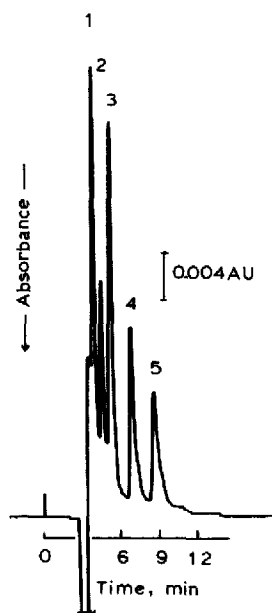


Fig. 4. Separation of anions with 0.02 mM pyromellitate eluent (pH 7.0), detection at 220 nm, 25-cm column. Peaks: 1 =  $\text{Cl}^-$  (1.25 ppm); 2 =  $\text{NO}_2^-$  (2 ppm); 3 =  $\text{SO}_4^{2-}$  (2 ppm); 4 =  $\text{Br}^-$  (2 ppm); 5 =  $\text{NO}_3^-$  (2 ppm).  $\text{F}^-$  and  $\text{H}_2\text{PO}_4^-$  were eluted earlier than  $\text{Cl}^-$ .

valent anions. Therefore, the position of divalent anions relative to monovalent anions may change.

#### Applications of benzenepolycarboxylate eluents

*Determination of nitrate.* Nitrate pollution of drinking water and water supplies has become a matter of increasing concern. A high detector sensitivity setting is often necessary to detect low concentrations of nitrate in water. However, nitrate elutes between chloride and sulfate in conventional ion chromatography, and the large

TABLE I

RELATIVE RETENTION TIMES ( $\text{Cl}^- = 1.00$ ) AND SEPARATION FACTORS ( $\alpha$ ) WITH DIFFERENT ELUENTS

Ion(s)	Parameter	Phthalate, 1.2 mM	BTA, 0.2 mM	Pyromellitate, 0.1 mM
$\text{NO}_2^-$	$t_R$	1.42	1.56	1.61
$\text{Br}^-$		3.21	3.60	4.25
$\text{NO}_3^-$		4.35	4.92	5.65
$\text{SO}_4^{2-}$		6.96	3.00	1.78
$\text{NO}_2^-/\text{Cl}^-$		$\alpha$	1.42	1.56
$\text{Br}^-/\text{NO}_2^-$		2.26	2.31	2.70
$\text{NO}_3^-/\text{Br}^-$		1.36	1.37	1.30
$\text{SO}_4^{2-}/\text{NO}_3^-$		1.60	0.61	0.32

chloride and sulfate peaks at a high sensitivity setting may obscure the smaller nitrate peak. By using a BTA or pyromellitate eluent, nitrate is eluted after both chloride and sulfate as a well resolved peak. As a very low concentration of either of these eluents can be used, the detection sensitivity of nitrate is also very good.

Figure 5 shows a chromatogram for the determination of small amounts of nitrate in drinking water. Almost all drinking-water samples contain much more chloride and sulfate than nitrate, yet the nitrate peak is well resolved. The baselines of this and other water samples were initially very curved in the early part of the chromatogram. This was believed to be caused by the high concentrations of hydrogencarbonate in the hard-water samples. Treatment of the samples with acid, followed by sonication or purging with an inert gas to remove most of the carbon dioxide, gave chromatograms with a better baseline. Results for determination of nitrate in various water samples are summarized in Table II.

*Determination of organic acids.* The ability of BTA eluents to achieve fast and complete separations of inorganic anions suggested the use of this eluent to separate

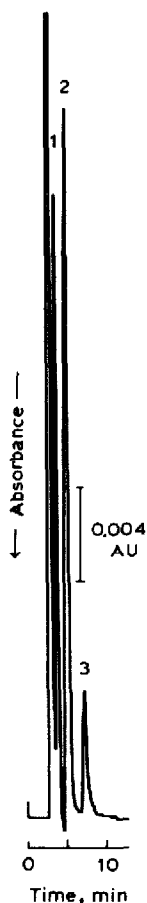


Fig. 5. Chromatogram of Des Moines tap water (diluted 50-fold) with 0.02 *M* pyromellitate eluent (pH 7.0) and detection at 240 nm. Peaks =  $\text{Cl}^-$ ; 2 =  $\text{SO}_4^{2-}$ ; 3 =  $\text{NO}_3^-$ .

TABLE II

## DETERMINATION OF NITRATE CONCENTRATION IN WATER SAMPLES

Conditions: coated 25-cm columns; 0.03 mM pyromellitate (pH 7.0); flow-rate 0.5 ml/min; spectrophotometric detector, 240 nm.

Sample	Dilution (fold)	Injection volume ( $\mu$ l)	Found in original sample (ppm) <sup>a</sup>
Ames tap water (A)	10	100	0.45
Ames tap water (B)	20	500	0.60
Pella tap water	10	100	12.3
Amana tap water	400	500	13.2
Des Moines River	50	100	7.15
Des Moines tap water	50	100	18.0
Skunk River	50	100	9.85
Squaw Creek River	100	100	26.8
George, Iowa City, well 14	100	100	59.4

<sup>a</sup> Average of two determinations.

anions of organic acids. Fig. 6 shows a good separation of four organic acids with excellent detection sensitivity using a BTA eluent. At the detection wavelength used (250 nm), ascorbic acid absorbs more strongly than the eluent and appears as a positive peak (increased absorbance). The other acid anions appear as negative peaks (decreased absorbance) owing to exchanging with the more highly absorbing BTA anion.

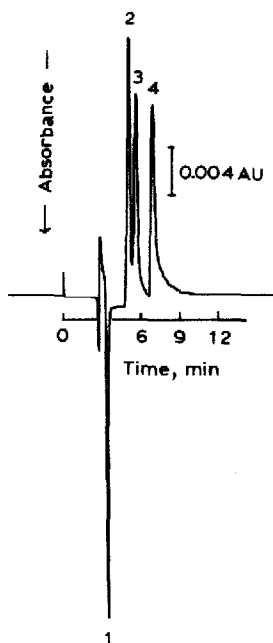


Fig. 6. Separation of four organic anions under the same conditions as in Fig. 3B. peaks 1 = ascorbate (10 ppm); 2 = malonate (20 ppm); 3 = tartrate (20 ppm); 4 = oxalate (20 ppm).



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