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Note

Gluconate–borate eluent for anion chromatography

Nature of the complex and comparison with other eluents

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Single-column ion chromatography has become a widely used technique, many different columns and a variety of eluents being employed. In chromatographic separations of ions on resin containing ion-exchange functionalities electrostatic interaction between the sample ion and the fixed ion-exchange site is the main factor influencing analyte retention. There is, therefore, a constant search for new eluents capable of overcoming that strong interaction with a view to obtaining shorter retention times, better resolution and higher sensitivity. Fritz *et al.*[1] have studied a number of organic acid eluents and have shown that on the polystyrene–divinylbenzene (PS–DVB) stationary phase non-ionized organic acids are much more strongly adsorbed than their acid salts, and that some organic acids, such as nicotinic acid, can serve as efficient eluents for a mixture of anions.

More recently a Japanese research group has developed a new eluent for anions, which consists of a gluconate–borate mixture in an aqueous acetonitrile solution[2,3]. Using this eluent, the pH of which is 8.5, they achieved a rapid and sharp separation of anions on a short Toyo Soda polyacrylate (PA) anion-exchange column.

The gluconate–borate eluent is radically different from the aromatic acids, such as phthalic, benzoic and salicylic acids, which are presently used for single column ion chromatography. This fact prompted us to investigate the nature of the gluconate–borate eluent in order to understand better the chemical properties which contribute to its success as an eluent. The present work describes structural features of the gluconate–borate eluent and compares its chromatographic performance, on two different columns, with that of other eluents commonly used for the separation and quantitation of monovalent anions.

EXPERIMENTAL

Instrumentation

The chromatographic system included a Waters M-45 pump and a Rheodyne 7010 injector with 100-μl sample loop. A Wescan 213 conductivity detector (10 mV output) was used for the detection of anions, and a Varian Model VUV-10 UV detector (210 nm, 10 mV output) was used for the detection of gluconate–borate, phthalate and benzoate. Both detectors were used with a Hewlett Packard 3390A integrator.

The functionalized columns used were (1) a Waters IC-PAK anion column (50 × 4.6 mm I.D.) packed with porous PA anion-exchange resin (particle diameter 10 μm, capacity 0.030 mequiv./ml), used with a flow-rate of 1.2 ml/minute, and (2) a Hamilton PRP-X100 column (250 × 4.1 mm I.D.) packed with PS–DVB anion-exchange resin (particle diameter 10 μm, capacity 0.20 mequiv./g), used with a flow-rate of 2.0 ml/min. Both columns were functionalized with quarternary ammonium groups. The unfunctionalized columns used were (1) a TSK guard column ICA (50 × 4.6 mm I.D., 12-μm unfunctionalized PA) from Waters and (2) an identical size column packed with Hamilton PRP-1 10 μm unfunctionalized PS–DVB. Both columns were used with a flow-rate of 1.2 ml/min.

Potentiometric titrations

The titrations of the gluconate–borate mixtures were monitored using a Corning-135 pH meter with an Orion 91-62 combination pH electrode.

Nuclear magnetic resonance measurements

^{13}C NMR spectra were obtained in $^{2}H_2O$ with 1,4-dioxane as internal reference, a Bruker WP-80 FT spectrometer being used (20.1 MHz, broad band decoupled).

Standard electrolyte solutions

Stock solutions of 1000 ppm each of the injected anions were prepared by dissolving analytical grade sodium salts in Milli Q water.

Eluents

(1) The gluconate–borate eluent had the following composition: sodium gluconate 1.48 m*M*, boric acid 5.82 m*M*, sodium tetraborate decahydrate 1.30 m*M*, acetonitrile 12% (v/v), glycerol 0.25% (v/v).

(2) Benzoic acid was 2 m*M*, and neutralized with potassium hydroxide to pH 6.50.

(3) Potassium hydrogen phthalate was 2 m*M*, pH 4.30.

(4) The phosphoric acid eluents were prepared as follows: 90 m*M* phosphoric acid (pH 1.78), 90 m*M* phosphoric acid–acetonitrile (9:1 v/v) (pH 1.78), 15 m*M* phosphoric acid (pH 6.77) and 15 m*M* phosphoric acid–acetonitrile (9:1, v/v) (pH 6.77). The ionic strengths were adjusted $u = 0.1$ with sodium chloride.

RESULTS AND DISCUSSION

The gluconate–borate eluent consists of a mixture of sodium gluconate and borax buffer at pH 8.5. It can only be used with anion-exchange columns based on polymeric matrices such as PS–DVB and PA. It is unsuitable for silica ion-exchange columns.

The eluent was shown to be effective for the separation of weak-acid anions, such as NO_2^-, HCO_3^-, $H_2PO_4^-$, as well as for long-retained ions, such as CNS^- and I^-. As can be seen in Fig. 1, six ions can be effectively separated on a PA ion-exchange column with this eluent, within 16 min. Retention times for CNS^- (15 min) and iodide (9.1 min) are shorter than those obtained with most common eluents used with a PS–DVB ion-exchange column. Also, well resolved peaks are obtained for the weak-acid anions injected. If gluconate and borate are used individually as eluents, at pH values between 3.5 and 8.5, to separate the same sample ions, very poor results are obtained. In both cases, for example, it takes more than 60 min for chloride to be eluted, even at pH 8.5. It is, therefore, evident that a synergism exists between gluconate and borate which is responsible for the efficiency of the eluent.

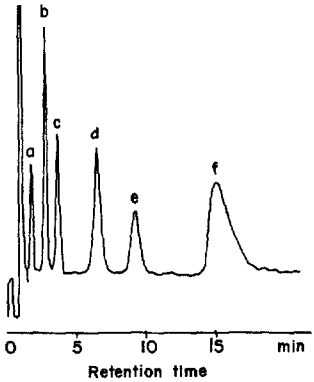
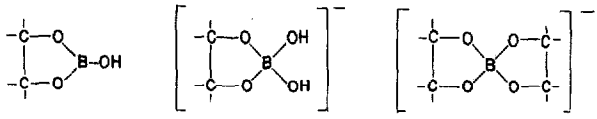


Fig. 1. Separation of six ions on the Waters IC-PAK anion column with borate–gluconate eluent. Flow-rate: 1.2 ml/min. a = HCO_3^-; b = NO_2^-; c = ClO_3^-; d = $H_2PO_4^-$; e = I^-; f = SCN^-.

It is well known that boric acid tends to form complexes with polyhydroxy compounds[4] and examples of such complexes are those formed with glycerol, mannitol, glucose, etc. Formulae that have been suggested for these complexes include the following ring-type species (I)[5].



In order better to understand the function of this unique eluent, some potentiometric titrations of the aqueous eluent solutions with 0.1 *M* hydrochloric acid were carried out, and ^{13}C NMR studies of gluconate and the gluconate–borate mixture were made.

The titration curves are illustrated in Fig. 2, and may be interpreted as follows. The titration of borax ($Na_2B_4O_7$) with standard hydrochloric acid is of the strong base-strong acid kind (curve A). Titrating a mixture of borax and H_3BO_3 with hydrochloric acid causes only a small change in the titration pattern (curve B), and the titration curve of a mixture of borax and sodium gluconate with hydrochloric acid is also in the same pH region (curve C). A drastic change, however, is brought about when a mixture of all three components *viz.* borax, boric acid and sodium gluconate, is titrated with hydrochloric acid (curve D). A slight further shift in the pK_a is observed when the mixture also contains 12% acetonitrile and a small amount of glycerol (approx. 1%) (curve E).

^{13}C NMR has proved useful in elucidating the structure of complexes formed by the interaction of boric acid and borate ions with hydroxy compounds[6]. A comparison of the ^{13}C NMR spectrum of gluconate with that of a gluconate–boric acid–sodium tetraborate mixture confirmed the formation of a borate–gluconate

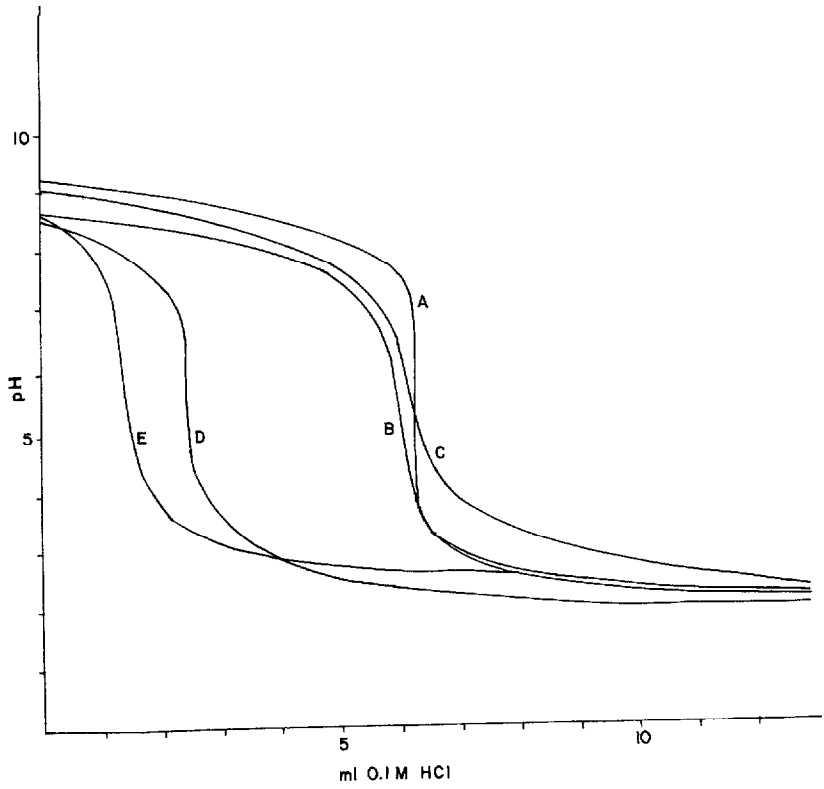


Fig. 2. Potentiometric titrations of borate–boric acid–gluconate mixtures with 0.10 *M* HCl. A = $Na_2B_4O_7$ (0.01 *M*); B = $Na_2B_4O_7$ (0.01 *M*) + H_3BO_3 (0.05 *M*); C = $Na_2B_4O_7$ (0.01 *M*) + sodium gluconate (0.01 *M*); D = $Na_2B_4O_7$ (0.01 *M*) + H_3BO_3 (0.05 *M*) + sodium gluconate (0.01 *M*); and E is same as D but in 12% acetonitrile.

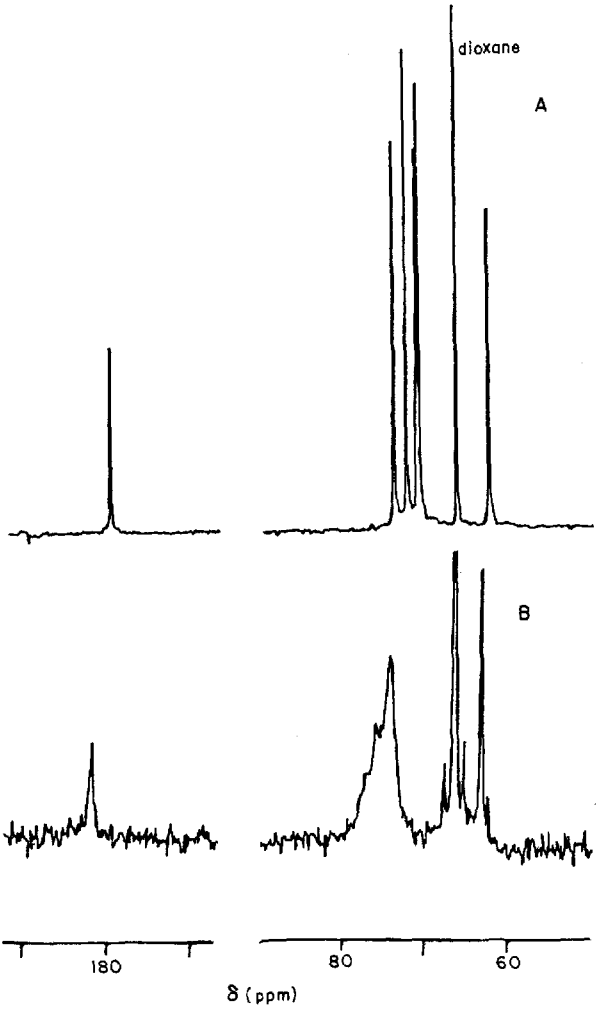


Fig. 3. ^{13}C NMR spectra (20 MHz, FT) of (A) 1 *M* potassium gluconate (2586 scans) and (B) potassium gluconate (0.148 *M*)–boric acid (0.582 *M*)–sodium tetraborate (0.130 *M*) mixture (30 411 scans).

complex (Fig. 3). For the ^{13}C NMR measurements gluconate, boric acid and sodium tetraborate were used in the same ratio as in the eluent but at concentrations 100 times as great (0.148 *M*:0.582 *M*:0.130 *M*). As a result of complex formation the four–CHOH carbons observed between 71 and 74 ppm could no longer be resolved. This does not seem to be due entirely to overlap resulting from band broadening, because more than four merged peaks appear to be present. The existence of several shortlived species could explain this finding. The carbonyl carbon at 180 ppm, and the $-CH_2OH$ carbon at 62 ppm, were broadened and shifted slightly downfield as a result of complex formation. These changes in the spectrum were not related to pH. The ^{13}C NMR evidence thus supports the formation of a ring-type anionic complex (I) between gluconate and borate.

The eluent under discussion is, therefore, seen to be an alkaline buffer of pH 8.5 containing a borate–carbohydrate anionic complex, to which acetonitrile has been added in order to facilitate phase transfer, and produce sharper peaks and shorter retention times.

A comparison was made of the performance of the eluent and some other eluents commonly used in single column ion chromatography, on both the Waters IC-PAK anion column and a Hamilton PRP-X100 column. The results obtained with potassium hydrogen phthalate, potassium benzoate and the gluconate–borate eluent are shown in Table I. It can be seen that the gluconate–borate eluent outperforms phthalate on the PA resin, but it is an even weaker eluent than benzoate on the PS–DVB resin.

It was of interest to determine if the excellent performance of the gluconate–borate eluent on the PA column was due to the gluconate–borate complex acting as a driving ion, or if this eluent was adsorbed on the PA resin to a greater extent than on the PS–DVB resin. Fritz *et al.*[1] observed that on PS–DVB those organic acids which adsorb on to the polymer, are generally more effective eluents.

The retention times of gluconate–borate, benzoic acid (or benzoate) and phthalic acid (or phthalate) on unfunctionalizeed PA and PS–DVB resins, obtained using a phosphoric acid (or phosphate) eluent at pH 1.78 and at pH 6.77, and with and without 10% acetonitrile, were compared (Table II). At pH 1.78, but not at pH 6.77, benzoic acid and phthalic acid were considerably retained on both resins, retention being stronger in the absence of acetonitrile. Adsorption was much greater on the PS–DVB resin than on PA, and on both resin columns benzoic acid was more strongly retained than phthalic acid. Gluconate–borate, however, was not appreciably retained on either resin under any of the conditions studied. Adsorption on the resin cannot, therefore, explain the superior performance of gluconate–borate on the PA resin.

The capacities of the two resins are quite different, and are 0.2 mequiv./g for

TABLE I

RETENTION TIMES (min) OF ANIONS ON TWO COLUMNS

For IC-PAK anion column, flow-rate 1.2 ml/min; for PRP-X100 column, flow-rate 2.0 ml/min.

Sample anion	*IC-PAK Anion column*			*PRP-X100 column*		
	Gluconate–borate (pH 8.5)	*Benzoate (pH 6.5)*	*Phthalate (pH 4.3)*	*Gluconate–borate (pH 8.5)*	*Benzoate (pH 6.5)*	*Phthalate (pH 4.3)*
F^-	0.79	2.98	–	0.94	3.38	–
Cl^-	1.37	5.63	1.56	2.89	5.31	1.71
Br^-	2.62	10.00	2.88	6.02	8.39	2.85
I^-	7.87	30.41	9.52	29.73	24.76	8.67
NO_3^-	3.16	12.09	2.92	7.87	10.49	3.26
NO_2^-	2.01	7.97	2.38	3.66	6.74	2.15
NO_3^-	2.81	10.13	2.93	11.55	12.53	4.14
BrO_3^-	1.09	5.27	1.30	3.38	5.89	1.93
IO_3^-	1.14	3.15	0.66	1.44	3.66	–

TABLE II

RETENTION TIMES (min) OF GLUCONATE–BORATE, BENZOIC ACID (OR BENZOATE) AND PHTHALIC ACID (OR PHTHALATE) ON UNFUNCTIONALIZED PS–DVB AND PA RESINS

Eluents: A = 15 m*M* phosphate (pH 6.77); B = 15 m*M* phosphate–acetonitrile (9:1) (pH 6.77); C = 90 m*M* phosphoric acid (pH 1.78); D = 90 m*M* phosphoric acid–acetonitrile (9:1) (pH 1.78).

Sample injected	*PS–DVB*				*PA*			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Benzoic acid	3.2	1.00	>60	16.24	0.67	0.65	7.99	5.56
Phthalic acid	1.07	1.08	11.79	3.49	0.78	0.77	2.71	2.09
Gluconate–boronate	0.65	0.62	0.72	0.70	0.63	0.60	0.64	0.75

PS–DVB and 0.03 mequiv./g for PA. If this difference were the main factor in determining elution behavior, benzoic acid and phthalic acid eluents would be expected to function like gluconate–borate, and elute anions more rapidly from PA than from PS–DVB, but this does not occur (Table I).

It must be concluded that other factors, such as geometry and steric effects, are important in explaining the performance of the gluconate–borate eluent. Further work is being done to elucidate these factors.

ACKNOWLEDGEMENT

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