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CARBOHYDRATE–BORATE ELUENTS FOR ANION CHROMATOGRAPHY

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SUMMARY

The chromatographic efficiencies of four different carbohydrate–borate eluents at pH values between 8.0 and 9.5 were compared. The carbohydrates studied were gluconate, mannonic acid, glucose and mannitol. The mannonic acid–borate eluent was as efficient as the original gluconate–borate eluent but the glucose–borate and mannitol–borate eluents gave poor results. For each eluent several carbohydrate borate complexes were responsible for the elution of anions.

INTRODUCTION

Single column, or non-suppressed, ion chromatography is now a well established technique for anion analysis[1]. The use of a dilute low conductance eluent, together with a low-capacity anion-exchange column, makes it possible to detect anions conductometrically without the need to suppress eluent conductivity. Eluents that have been used in this technique include phthalic acid and other aromatic acids[1–4]. Single column chromatography, however, does not provide the sensitivity that can be achieved with the two-column suppressed system[5].

In 1983 a new eluent for single column anion chromatography was introduced that was very different from those previously used[6]. It consisted of a gluconate–borate mixture that, when used with a Toyo Soda polymethacrylate column, offered increased sensitivity with excellent resolution. The chemistry and elution mechanism of this useful eluent have not been fully explained but Schmuckler *et al.*[7] concluded that since both gluconate and borate when used separately as eluents gave very poor results, the efficiency of the eluent depended on the formation of a gluconate–borate complex.

The present work was undertaken for two purposes: (1) to atempt to elucidate further the nature of the gluconate–borate complex and determine the driving force, or developing ion, of the eluent and (2) to determine if carbohydrates other than gluconate, when mixed with borate, would form efficient eluents. The gluconate that was used in the original eluent was replaced in turn by three other polyhydroxy compounds (POHC) —mannonic acid, mannitol and glucose— and each new eluent was tested for chromatographic efficiency at several pH values and several different

borate–carbohydrate concentrations. ^{13}C NMR spectra of the carbohydrates were taken before and after the addition of boric acid and borate.

EXPERIMENTAL

Instrumentation
        The chromatographic system consisted of a Waters M-45 pump used at a constant flow-rate of 1.2 ml/min, a Rheodyne 7010 injector with a 100-μl sample loop, a Waters IC-PAK anion column (50 × 4.6 mm I.D., 10 μm particle diameter, 0.03 mequiv./ml capacity) and a Wescan 213 (10 mV output) conductivity detector coupled to a Hewlett-Packard 3390A integrator.

Reagents
        All reagents used were analytical grade and solutions were prepared in Milli-Q water. Borate buffer eluents were prepared with (a) D-gluconic acid, potassium salt, (b) α-D-glucose, (c) D-mannitol, and (d) L-mannonic acid (γ-lactone). The composition of the initial eluents was as follows: carbohydrate 1.48 mM, boric acid 5.82 mM, sodium tetraborate decahydrate 1.30 mM, acetonitrile 12% (v/v), glycerol 0.25% (v/v). The pH was adjusted with 0.1 M potassium hydroxide or 0.1 M hydrochloric acid. For the more dilute eluents, carbohydrate, boric acid and borate concentrations were made (a) 2/3 and (b) 1/2 of their initial concentrations, concentrations of acetonitrile and glycerol were not changed. Standard anion solutions were prepared from the sodium salts.

NMR measurements
        ^{13}C NMR spectra were obtained in ^{2}H$_2$O with 1,4-dioxane as internal reference, using a Bruker WP-80 FT spectrometer (20.1 MHz, broad band decoupled).

RESULTS AND DISCUSSION

        Typical chromatograms obtained using the four different carbohydrate–borate eluents, at pH 8.5, for the separation of a group of anions are shown in Fig. 1. Based on previous results with gluconate–borate eluent[7], and ^{13}C NMR studies that are described later, it is concluded that in each case a carbohydrate–borate complex was responsible for the elution of the anions. With mannonic acid–borate eluent, retention times were slightly shorter than with the original gluconate–borate eluent and resolution was equally good. When mannitol–borate or glucose–borate eluents were used, however, retention times increased considerably, particularly for the glucose–borate eluent. These findings suggest that a carboxyl group in the carbohydrate increases the eluting power of the carbohydrate–borate eluent.
        Boric acid and borate complexation with POHC has been studied for many years using a variety of techniques including conductivity, pH, optical rotation, and refractive index measurements, ionophoresis, potentiometric titrations and, more recently, ^{1}H, ^{13}C and ^{11}B NMR[8–23]. Equilibria between boric acid (B^0), borate (B$^-$), and diol functions (L), are summarized in Fig. 2 (eqns. 1–4). In addition to complexes of type B^{0}L, B$^-$L and B$^-$L$_2$, many POHCs, including gluconate, mannonate, glucose and mannitol, can, depending on conditions, also form complexes of type (B$^-$)$_2$L (Fig.
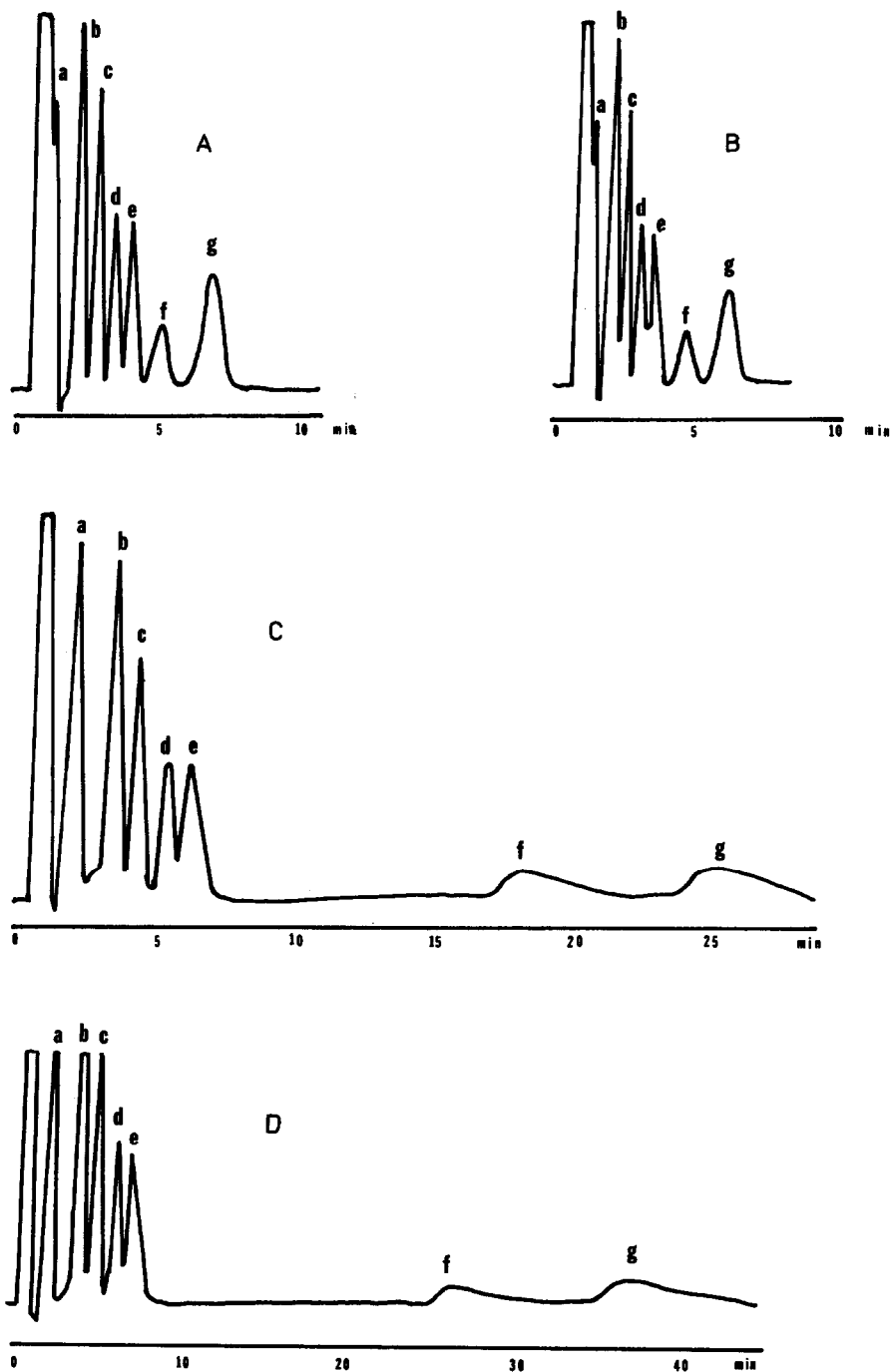
Fig. 1. Separation of seven anions with (A) gluconate–borate, (B) mannonic acid–borate, (C) glucose–borate and (D) mannitol–borate eluents. Flow-rate, 1.2 ml/min; sample concentration, 50 ppm of each anion. Peaks: a = F^-, b = Cl^-, c = NO_2^-, d = Br^-, e = NO_3^-, f = HPO_4^{2-}, g = SO_4^{2-}.

$$\text{HO-B}\overset{\text{OH}}{\underset{\text{OH}}{}} \; + \; \text{OH}^- \; \overset{Ka}{\rightleftharpoons} \; \text{HO}\overset{\text{OH}}{\underset{\text{OH}}{\overset{}{\text{B}}}}\overset{\text{OH}}{\underset{\text{OH}}{}} \qquad\qquad (1)$$

B°   B⁻

$$\text{B}^- \; + \; \text{L}\left[\overset{-\text{OH}}{\underset{-\text{OH}}{}}\right] \; \overset{K_1}{\rightleftharpoons} \; \text{B}^-\text{L} \; + \; 2\text{H}_2\text{O} \qquad\qquad (2)$$

$$\text{B}^-\text{L} \; + \; \text{L}\left[\overset{-\text{OH}}{\underset{-\text{OH}}{}}\right] \; \overset{K_2}{\rightleftharpoons} \; \text{B}^-\text{L}_2 \; + \; 2\text{H}_2\text{O} \qquad\qquad (3)$$

$$\text{B}^\circ \; + \; \text{L}\left[\overset{-\text{OH}}{\underset{-\text{OH}}{}}\right] \; \rightleftharpoons \; \text{B}^\circ\text{L} \; + \; 2\text{H}_2\text{O} \qquad\qquad (4)$$
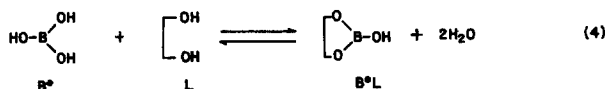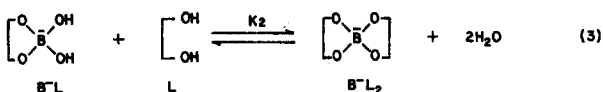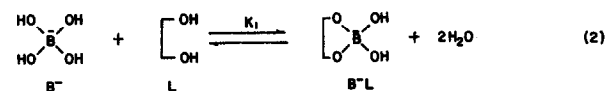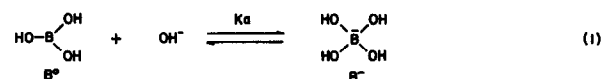
Fig. 2. Equilibria between boric acid (B°), borate (B⁻) and a diol function (L).

3, eqns. 5 and 6)[20–23]. In strongly alkaline conditions very little complex of typ B°L would be present (Fig. 2, eqn. 4). Tridendate complexes also exist but require polyols with very specific configurations[21,24]. 6-Polyhydroxy compounds because of their many diol functions can, theoretically, form a large number of different borate esters. Polyhydroxycarboxylic acids can form both diol and α-hydroxycarboxylic acid esters with borate[20]. In aqueous solution at pH $> pK_a$ polyhydroxycarboxylic acid but $< pK_a$ boric acid ($pK_a = 9.07$), B⁻L$_A$ type esters are formed according to eqn. 7 in Fig. 4. At pH above 9 more B⁻ is formed and the equilibrium can be written as shown in eqn. 8. With increasing pH the B⁻L$_A$ esters dissociate and formation of diol esters, B⁻L⁻, is favored (eqn. 9).

Experiments in which the eluent pH was changed from 8.0 to 9.5 showed that, for each of the four carbohydrate–borate eluents studied, anion retention times were pH dependent and decreased as pH increased (Table I). It has been shown, using model dihydroxy compounds, that the amount of borate esterified increases with increasing pH[20,22]: the equilibria in eqns. 1, 2 and 3 (Fig. 2) lie increasingly to the right thus increasing the concentration of charged species in the eluent. This would be expected to increase the efficiency of the eluent in displacing anions from their sites on the ion exchange resin, as was found experimentally. In the carbohydrate–borate eluents that were used total boron concentration always exceeded carbohydrate concentration (molar ratio B/carbohydrate = 7.4) and, therefore, monoesters (B⁻L) rather than the diesters (B⁻L$_2$) would be the main species formed.

$$\text{B}^- \; + \; \text{L}\left[\overset{-\text{OH}}{\underset{-\text{OH}}{\overset{-\text{OH}}{\underset{-\text{OH}}{}}}}\right] \; \rightleftharpoons \; \text{B}^-\text{L} \; + \; 2\text{H}_2\text{O} \qquad\qquad (5)$$

$$\text{B}^- \; + \; \text{B}^-\text{L} \; \rightleftharpoons \; (\text{B}^-)_2\text{L} \; + \; 2\text{H}_2\text{O} \qquad\qquad (6)$$
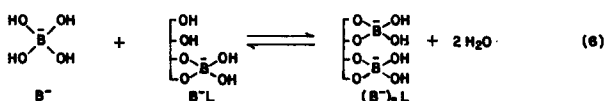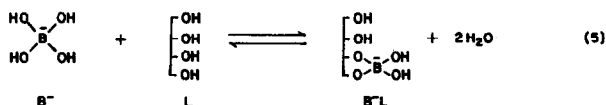
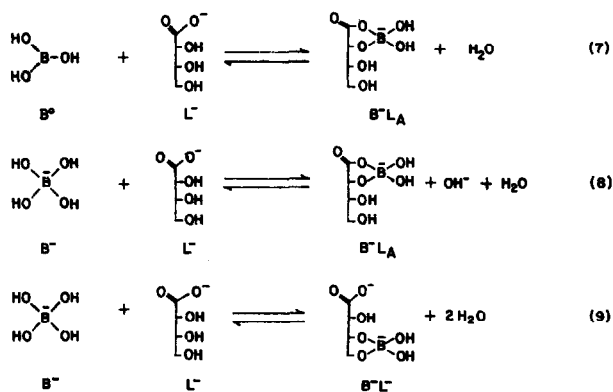Fig. 3. Equilibria between borate and a polyol function: borate in excess.

Fig. 4. Borate ester formation by polyhydroxy carboxylates.

It is of interest to note that at pH 8.0 anion retention times obtained with the mannonic acid–borate eluent were considerably longer than those obtained with the gluconate–borate eluent, whereas at higher pH the mannonic acid–borate retention times were nearly all slightly shorter than the corresponding gluconate–borate retention times (Table I). This may be due to the very complex equilibria that exist in the eluents, particularly at pH below 9, and the fact that with increasing pH the relative concentrations of the various charged species in solution will not necessarily change at

TABLE I

ADJUSTED RETENTION TIMES (min) OF ANIONS SEPARATED AT DIFFERENT pH VALUES BY THE FOUR CARBOHYDRATE–BORATE ELUENTS

Anion concentrations: 50 ppm.

Anion injected	Carbohydrate in eluent	pH			
		8.0	8.5	9.0	9.5
Cl^-	Gluconate	2.15	1.45	0.94	0.75
	Mannonic acid	2.60	1.24	0.89	0.70
	Mannitol	–	2.60	1.45	1.18
	Glucose	–	2.95	1.74	1.07
NO_3^-	Gluconate	3.92	3.20	1.98	1.61
	Mannonic acid	5.93	2.73	1.94	1.53
	Mannitol	–	5.19	2.91	2.37
	Glucose	–	5.75	3.36	2.20
HPO_4^{2-}	Gluconate	5.66	4.22	2.16	1.65
	Mannonic acid	11.70	3.87	2.33	1.61
	Mannitol	–	17.01	5.78	4.25
	Glucose	–	22.91	8.12	3.65
SO_4^{2-}	Gluconate	8.51	5.98	3.03	2.19
	Mannonic acid	18.09	5.31	3.11	2.10
	Mannitol	–	24.06	7.85	5.66
	Glucose	–	32.04	10.73	4.69

the same rate for both gluconate and mannonate. As will be discussed later, the association constants, k_1 and k_2 (Fig. 2), for gluconate and mannonate differ considerably[21].

It can also be seen in Table I that at all pH values tested, gluconate–borate and mannonic acid–borate were stronger eluents than glucose–borate and mannitol–borate eluents and that this difference in elution strength became less marked as pH was increased. This again suggests that the carboxylate group is a factor in determining eluent strength.

The association constants, k_1 and k_2 (Fig. 2), have been calculated for a wide variety of diols and POHC using refractive index/optical rotation data[10] and, more recently, ^{11}B NMR measurements[17,20–23]. ^{11}B and ^{13}C NMR[14–23] studies of boric acid–borate complexation with a variety of POHC provided direct evidence for the formation of both B^-L and B^-L_2 type complexes. Association constants have been found to vary greatly with the nature of the POHC[17,20–22]. Steric hindrance, stabilization as a result of substitution in the parent POHC, relative positions of the hydroxyl groups in the molecule, and possibilities for hydrogen bonding between the POHC and borate, were all found to be determining factors. Van Duin et al.[21] determined that the stability of the borate–carbohydrate complexes increased as the number of hydroxyl groups in the molecule increased, and that *threo* complexes were more stable than the corresponding *erythro* complexes. They observed that the introduction of a negatively charged carboxylate group into the carbohydrate generally decreased the stability of the complex as a result of electronic repulsion between the carboxylate group and borate (B^-).

^{11}B NMR studies by Van Duin et al[21] showed that for both gluconate and mannonate in strongly alkaline solution, the main borate complex formed was a B^-L^- *threo*-complex. Association constants, measured at pH 11, were 240 and 1200 l/mol, respectively. Smaller amounts of *erythro* (association constants 72 and 140 l/mol, respectively) and α,γ-complexes (association constants 19 and 54 l/mol, respectively) were also formed. Only a 3,4-*threo* complex can be formed by mannonate but for gluconate a 2,3-*threo* complex is also possible. ^{13}C NMR[23] showed that the 2,3-*threo* complex for gluconate made up only 10% of the total *threo* signal, probably because in this position repulsion between the borate and carboxylate ions would be stronger and this would decrease the stability of the complex. It is evident from the values of the association constants that the mannonate–borate complexes were more stable than the corresponding gluconate–borate complexes. ^{11}B NMR is unable to distinguish between borate esters of type B^-L, $(B^-)_2L$ and $(B^-)_3L$ (where L may be charged or uncharged), all of which can be formed, but with ^{13}C NMR it was possible to show that, at pH 11, $(B^-)_2L^-$ complexes as well as B^-L^- complexes, were formed by both gluconate and mannonate[23]. Association constants were also determined[21] for the diesters, $B^-(L^-)_2$, and were reported to be 31 and 48 l/mol, respectively.

As already reported[7], ^{13}C NMR confirmed that, on adding boric acid and borate to potassium gluconate in the same proportions as in the initial eluent, a gluconate–borate complex was formed. A similar result was obtained for mannonate. In both cases, the main change in the spectrum on adding boric acid and borate was a broadening and overlapping of the C-2, C-3, C-4 and C-5 signals so that one broad resonance (approximately 10 ppm wide) with two unresolved peaks was observed. Based on the work of Van Duin et al.[21,23] it can be concluded that the two

unresolved peaks represent the 3,4-*threo* complex. In both the gluconate–borate and mannonate–borate spectra slight broadening of the C-1 signal was observed, suggesting the formation of an α-hydroxycarboxylic acid ester with formula B^-L_A. The absence of a peak at approximately 80 ppm, and the similarity of the mannonic acid–borate spectrum to the gluconate–borate spectrum, indicated that in its borate complexes mannonic acid was in the straight chain form[25].

Makkee *et al.*[22] used ^{11}B and ^{13}C NMR spectroscopy to investigate glucose–borate complexes in aqueous solution at pH 6–12. They found that when excess borate was present several borate monoesters were formed. When the B/L ratio was 10, the main complex formed at pH 12 was of type $(B^-)_2L$ and at this pH complexation reached a maximum. They concluded that an equilibrium was formed between α-D-glucofuranose 1,2:3,5-diborate (5- and 6-membered rings) and α-D-glucofuranose 1,2:5,6-diborate (two 5-membered rings). D-Glucose on forming borate complexes was transformed from the pyranose to the furanose form.

The ^{13}C NMR glucose–borate spectrum that we obtained was complex and difficult to interpret but clearly showed that complexation had occurred. Marked downfield shifts in the C-1 and C-6 resonances and the appearance of peaks between 80 and 90 ppm indicated that at least one furanoid complex was formed[25]. However, as a result of line broadening it was not possible to make definite assignments.

^{11}B and ^{13}C NMR spectra of mannitol–borate complexes obtained by Makkee *et al.*[22] showed that, as was the case for glucose when excess borate was present, in addition to B^-L complexes, $(B^-)_2L$ and possibly $(B^-)_3L$ complexes, were also formed, complexation increasing as pH was raised. The preferred position for complex formation was at the 3- and 4-hydroxyl groups. Mannitol very readily forms a B^-L_2 complex but, as already mentioned, at the B/L ratios used in our study this complex would not be expected to be the main product. However, the ^{13}C NMR spectra that we obtained on adding boric acid and borate to mannitol showed a considerable broadening of both the C-2/C-5 and C-3/C-4 signals with a downfield shift of 2 ppm for both signals which suggests formation of a B^-L_2 complex. Makkee *et al.*[22] reported that association constants for borate complexes with mannitol were approximately two orders of magnitude higher than those for glucose.

Our results and the work of Van Duin *et al.*[20,21,23] and Makkee *et al.*[22] suggest that, under the conditions that we used, the main borate complex formed by gluconate and mannonic acid was a diol ester of type B^-L^-. Smaller concentrations of a $(B^-)_2L$ complex and the α-hydroxycarboxylic acid ester, B^-L_A, also appear to be formed. For glucose and mannitol the complexes formed would be of types B^-L and $(B^-)_2L$, with possibly some formation of B^-L_2 in the case of mannitol. The superior performance of the gluconate–borate and mannonic acid–borate eluents, as compared to the glucose–borate and mannitol–borate eluents, would at first appear to be due to the charge contributed by the carboxylate group. However, as shown by the experiment described in the following section, this cannot be the complete explanation.

For many anions there is a linear relationship between the logarithm of their retention times and the logarithm of the eluent concentration[26,27]. If a monovalent eluent is used, the slope of the line will be equal to one for a monovalent anion and two for a divalent anion. Similarly, if a divalent eluent is used, the slopes will be 0.5 and one, for monovalent and divalent anions, respectively. Therefore, theoretically, if anions of known charge are separated at different eluent concentrations, it should be possible to deduce the charge on the eluent.

TABLE II

ELUENT CHARGE VALUES CALCULATED FROM THE SLOPES OF THE PLOTS OF LOG
RETENTION TIME *VERSUS* LOG ELUENT CONCENTRATION[26,27]

Three different eluent concentrations were used to determine the slopes. Correlation coefficients were 0.99
or greater except where noted.

	Cl^-	NO_2^-	Br^-	NO_3^-	HPO_4^{2-}	SO_4^{2-}	Average
Gluconate–borate	1.30	1.37	1.43	1.51	1.39	1.36	1.39
Mannonic acid–borate	1.08**	1.02*	1.05*	1.13**	1.16	1.18*	1.10
Glucose–borate	1.25	1.25	1.25	1.20	1.14	1.09	1.20
Mannitol–borate	1.32	1.37	1.37	1.32	1.29	1.29	1.33

     * Correlation coefficient, 0.98.
   ** Correlation coefficient, 0.97.

     The relationship between eluent concentration and retention times for six anions
was determined for the four carbohydrate–borate eluents at three different concentra-
tions: the initial concentration and two lower concentrations. In each case, as the
carbohydrate concentration was changed the ratio of carbohydrate concentration to
buffer (boric acid and borate) concentration was kept constant. From the slopes of the
plots the charge on the eluent was calculated (Table II) and found to range from 1.02 to
1.51, with the mannonic acid–borate data giving charge values that were closest to one.
Charge values that deviated furthest from one were obtained from the gluconate–
borate data. The difference between the eluent charge as calculated from the
gluconate–borate data and from the mannonic acid–borate data is surprising since the
elution properties of these two eluents have been shown to be very similar. Assuming
that the relationship between eluent concentration and anion retention times holds
true for the complex eluents being studied, the difference between the charge found for
the gluconate–borate eluent and the charge found for the mannonic acid–borate eluent
must be due to a difference in the ratio of mono- to divalent complexes in the two
eluents. For the gluconate–borate eluent, presumably doubly charged B^-L^- is the
main driving ion with sufficient contribution from singly charged B^-L_A to give an
eluent charge of approximately 1.4. Erkelens *et al.*[28] have indicated that for this eluent
triply charged $B^-(L^-)_2$ acts as a driving ion but, as already mentioned, formation of
this complex appears unlikely when borate is present in excess. The results obtained for
the mannonic acid–borate eluent suggests that in this case the B^-L_A complex is the
main driving ion. The comparable efficiency of the gluconate–borate and mannonic
acid–borate eluents may depend on the fact that mannonic acid complexes more
readily with boric acid and borate than does gluconate, and the higher concentration
of the singly charged mannonic acid–borate complex in the eluent may outweigh the
advantage of the greater charge on the gluconate–borate complex.
     For glucose and mannitol it can be concluded that the B^-L complex is the main
driving ion, with some contribution from the $(B^-)_2L$ complex to give the observed
values for the eluent charge. With mannitol B^-L_2 may also be a factor. The low
efficiency of the glucose–borate and mannitol–borate eluents may be due, in part, to
structural considerations. The ring structure of glucose in its borate complexes may
prevent the glucose–borate eluent from being as efficient as those eluents in which the

carbohydrate is in the straight chain form. Large spcies such as $(B^-)_2L$ and B^-L_2 would further decrease eluent efficiency.

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