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THE USE OF POLY(4-VINYLBENZENEBORONIC ACID) RESINS IN THE FRACTIONATION AND INTERCONVERSION OF CARBOHYDRATES

S. A. BARKER, B. W. HATT, P. J. SOMERS, AND R. R. WOODBURY

Department of Chemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (Great Britain)

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ABSTRACT

Poly(4-vinylbenzeneboronic acid) resins have been prepared and used as chromatographic packings in the fractionation of carbohydrates with water as the eluent. The effect of the pH and temperature of the eluent on the fractionations was investigated. These resins have also been used to displace the pseudo-equilibrium established in aqueous alkali between D-glucose, D-fructose, and D-mannose to give high yields of D-fructose, and the use of a model reactor for the preparation of D-fructose is described.

INTRODUCTION

The interaction of carbohydrates with dihydroxyborono groups is well known, and Böeseken[1] and others[2,3] have investigated the complexes formed by borate and boric acid with sugars and alditols. Areneboronic acids form similar complexes with monosaccharides[4–7], and it has been shown[8] that they may be used to displace the pseudo-equilibrium which is established, in the presence of alkali, between D-glucose, D-fructose, and D-mannose to give high yields of D-fructose. The use of an insoluble resin matrix containing covalently bound dihydroxyborono groups would provide (*a*) a chromatographic packing for the separation of sugars and alditols, and (*b*) an easily recoverable reagent which could be used to provide high yields of D-fructose from the action of alkali on D-glucose and D-mannose. This paper describes the use and evaluation of cross-linked poly(4-vinylbenzeneboronic acid) resins for these purposes.

DISCUSSION

It has been shown[9,10] that 4-vinylbenzeneboronic acid and its derivatives may be polymerized and copolymerized with styrene. The use of iminodiethyl 4-vinylbenzeneboronate as the monomer in this work has the advantage that the dihydroxyborono group is blocked during the polymerization and that the blocking group may be subsequently removed by treatment with dilute acid. Various methods were used to

prepare the resin, and the main difficulty was the low solubility of the derivative in most solvents. Chloroform, benzyl alcohol, and *N,N*-dimethylformamide were used effectively, and free-radical polymerization was initiated by the thermal decomposition of azobisisobutyronitrile. Copolymerization with divinylbenzene and styrene gave resins only when all components had been carefully purified. Generally, only a small amount of divinylbenzene was added to give a lightly cross-linked gel which would swell in alkaline, aqueous solutions to allow monosaccharides to enter the matrix.

TABLE I

ELUTION[a] OF COMPOUNDS FROM A COLUMN OF POLY(4-VINYLBENZENEBORONIC ACID) RESIN

Compound	*Retention factor*	*Shape of peak*[b]	*Compound*	*Retention factor*	*Shape of peak*[b]
D-Glucose	0.73	S	L-Arabinose	0.84	T
D-Fructose	1.44	T	D-Xylose	0.82	T
D-Mannose	0.75	S	D-Ribose	—	very B
D-Galactose	1.16	T	Maltose	0.70	S
D-Lyxose	0.84	T	Ethylene glycol	1.07	S

[a]With deaerated, distilled water at 20° (pH 6.1). [b]S, sharp; B, broad; T, tailed.

The elution of a range of carbohydrates from a column of poly(4-vinylbenzeneboronic acid) resin is summarized in Table I, the eluent being water (pH 6.1) at 20°. The intention of this part of the research was to produce a packing which would fractionate sugars when water was the sole eluent. Several methods for the analysis of carbohydrate mixtures are based on the technique developed by Khym and Zill[11] in which the sample is eluted by a borate buffer from an anion-exchange resin. These methods have the disadvantage that recovery of components necessitates the removal of borate, and thus a method which used only water as eluent would be advantageous. It is apparent from the retention factors (peak elution-volume/total bed-volume) given in Table I that peak separations have been obtained between certain sugars but many of the peaks were tailed (unsymmetrical), suggesting a non-linear absorption isotherm for the system. When a sugar complexes with dihydroxyborono groups, the acidity of the solution rises[5] and this would give rise to tailing unless the solution is buffered. Since this work was instigated, Weith *et al.*[12] have reported the use of carboxymethylcellulose containing benzeneboronic acid groups for the fractionation of nucleic acid components, sugars, and other polyols using buffered eluents, but again recovery would involve removal of the buffering molecules from the eluate. A half-neutralised solution of an areneboronic acid will act as a buffer, and thus it is possible for the resin to be self-buffering if the eluent pH is equal to the pK_a value for the acid. For the resin used, this self-buffering effect would occur at about pH 9.3 (pK_a for 4-methylbenzeneboronic acid[13] is 9.30) which would require the eluent to be alkaline.

Use of an eluent at a pH (9.6) such that the dihydroxyborono groups on the resin are half-neutralized allows compounds which are absorbed to be eluted as symmetrical peaks (Table VII). The D-fructose peaks (for low-concentration samples) did not tail at pH 9.6 but the peaks were broad with respect to those of D-glucose and D-mannose. When the loading was increased from 250 μg to 250 mg for the individual sugars, the retention factor for D-fructose was considerably decreased and the tailing reappeared. This can be explained by the fact that a very high concentration of D-fructose will give a considerable local increase in acidity, due to complex formation, that cannot be buffered by the resin, and this view is confirmed by noting that the pH of the eluate went from 9.6 to $\sim$7 as the D-fructose was eluted. It should be noted that comparison of retention factors at different pH values is not valid, since the total bed-volume is pH dependent due to swelling of the resin in alkali. Also, retention factors would be expected to vary between batches of resins as the specific volume of the resin will depend on the amount of cross-linking agents and will be affected by impurities in the polymerization mixture.

The self-buffering would occur at lower pH values by the substitution of electron-withdrawing groups in the aromatic ring containing the dihydroxyborono group, for instance, nitro groups (pK_a for 4-nitrobenzeneboronic acid[13] is 7.15); investigation into the use of matrices containing such groups is planned.

The retention factors (Table I) are in general agreement with the degree of complex formation between the sugars and benzeneboronic acid[5,14]. The retention of ethylene glycol appears anomalous on consideration of the equilibrium constant of its complex but, since it is a smaller molecule than the other compounds, it is probably undergoing molecular sieving. The effect of temperature and pH on the retention factors of D-glucose and D-fructose are shown in Tables II and III, respectively. The separation of the two compounds would obviously be favoured at higher temperature and this is borne out in practice (Fig. 1). It is also apparent that D-

TABLE II

EFFECT OF TEMPERATURE ON RETENTION FACTORS (pH 6.1)

Compound	*20°*	*25°*	*50°*
D-Glucose	0.73	0.75	0.83
D-Fructose	1.34	1.80	2.61

TABLE III

EFFECT OF pH ON RETENTION FACTORS (20°)

Compound	*pH: 3.0*	*5.1*	*6.1*	*10.0*
D-Glucose	0.76	0.73	0.73	0.78
D-Fructose	1.34	1.42	1.44	4.30

fructose interacts with the resin even at pH 3.0 but, as expected, it is more strongly absorbed at higher pH values.

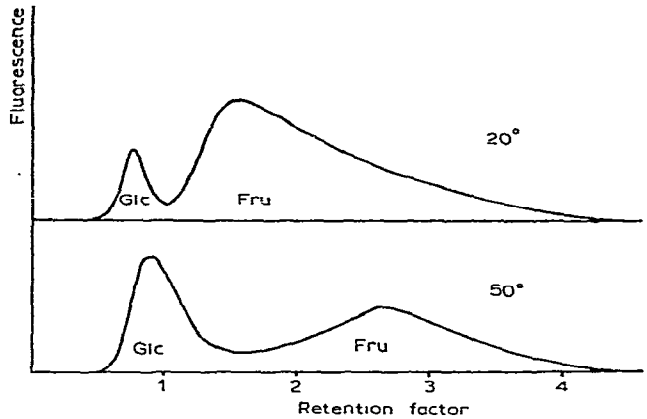

Fig. 1. Separation of D-glucose and D-fructose on a column of poly(4-vinylbenzeneboronic acid) resin; elution with water (pH 6.1) at 20 and 50°.

Garegg and Lindberg have shown[6] that sulphonated benzeneboronic acid may be used to separate carbohydrates and related alditols by electrophoresis. An anion-exchange resin was converted into the sulphonated benzeneboronic acid form, and various compounds were eluted from a column of the resin at room temperature with water as sole eluent; the retention factors are given in Table IV. In this case it is obviously essential to use water as any added anions would displace the anionic counter-ions. It is seen that many of the compounds were strongly absorbed, some to such an extent that they were not eluted within a reasonable time, and that again some of the peaks eluted were tailed.

TABLE IV

ELUTION OF COMPOUNDS FROM DOWEX-AG1 X2 RESIN[a]

Sample	*Retention factor*	*Shape of peak*[b]	*Sample*	*Retention factor*	*Shape of peak*[b]
D-Glucose	0.45	S	Threitol	2.25	T
D-Galactose	0.50	S	D-Mannose	2.80	B
Ethylene glycol	0.60	S	D-Arabinitol	~13.5	very B
Propane-1,2-diol	0.65	S	Mannitol	>7.0	—
Glycerol	0.75	S	Xylitol	>8.0	—
L-Rhamnose	0.85	T	D-Xylose	>10.0	—
Erythritol	1.40	T	D-Fructose	>10.0	—

[a]In sulphonated benzeneboronic acid form; elution with water (pH 6.1) at 20°. [b]S, sharp; B, broad; T, tailed.

Attention was then turned to the use of poly(4-vinylbenzeneboronic acid) resin in the preparation of D-fructose, a sugar of commercial importance. It has been shown[8] that some areneboronic acids increase the yield of D-fructose from the action of alkali on D-glucose from <30% to >80%, and this resin could obviously be used to perform the dual function of displacing the pseudo-equilibria in favour of D-fructose and in the separation of the D-fructose from the other isomeric products. First, the ability of a batch of the poly(4-vinylbenzeneboronic acid) resin to absorb D-fructose in preference to D-glucose was demonstrated (Table V, Expt. *A*). The resin was briefly stirred in a solution at pH 12 (the intended pH for the transformation) containing D-glucose and D-fructose, each sugar in equimolar amounts with respect to the calculated content of dihydroxyborono groups in the resin. The results show that D-fructose and D-glucose are absorbed in the ratio 3.9:1 and that they may be recovered from the resin by washing with acid. In Experiment *B* (Table V), the resin was heated in the presence of a solution of D-glucose at pH 12 for 4 h (the time shown to give the optimal yield of D-fructose[8] with benzeneboronic acid). The supernatant liquid was removed, the absorbed sugars were washed from the resin by acid, and both fractions were analysed for D-fructose. The yield was 50.1%, less than that expected but still over 20% more than that which would be obtained in the absence of the resin, and 88% of the D-fructose produced was absorbed by the resin. The amounts of sugars other than D-fructose were only estimated, since D-glucose and D-mannose do not give the same specific response in the assay used. However, it was shown that D-mannose is produced in only 2–3% yield[8], and thus the amounts given in Table V (Expt. *B*) correspond closely to the amounts of D-glucose. The analysis of the sugars in the supernatant liquid shows that D-fructose is present as ~21.5% of all the sugars present in solution, a value that would be expected in the absence of areneboronic acids. It is apparent that the resin may be used to perform both the functions mentioned earlier. It is probable that the characteristics of the resin will

TABLE V

USE OF POLY(4-VINYLBENZENEBORONIC ACID) RESIN TO ABSORB SUGARS

	Weight of sugar in supernatant liquid (mg)	*Weight of sugar absorbed on resin (mg)*	*Total recovery of sugars*
Expt. A (Standard mixture)			
D-Glucose	265	63	
D-Fructose	83	245	94%
Expt. B (Alkaline equilibration mixture)			
D-Fructose	21	155	91.3%
Other hexoses, calculated as D-glucose	77	68	(yield of D-fructose, based on original D-glucose, 50.1%)

be affected by the degree of cross-linking and by the presence of hydrophobic monomers, such as styrene, in the matrix.

Although it has been shown that the carbon–boron bond in areneboronic acids is suprisingly stable in the presence of alkali at elevated temperatures[15], degradation of the resin would be minimised if it was used at temperatures lower than those used for the conversion. To this aim, a system was designed and used (Fig. 2) where the resin was maintained at room temperature and the alkali-catalysed interconversion was carried out in a heated coil. The solution was recycled by a peristaltic pump through the two modules, thus allowing the D-fructose-depleted solution eluted from the column of resin to be heated, forming more D-fructose which is removed on a re-pass through the resin. Four typical runs are summarized in Table VI, the reactor-coil being maintained at 37° and 50°. The optimal recycle-time was calculated from the estimated volume of liquid in the system and the optimal heating times found earlier[8] for benzeneboronic acid in solution. A large series of runs under standardized conditions would be necessary to find the optimal time for this system. The results show that similar yields are obtained by this system as when the resin and sugars are heated together. The yield fell to 42.3% of D-fructose when an excess of D-glucose with respect to the capacity of the resin was used.

TABLE VI

USE OF POLY(4-VINYLBENZENEBORONIC ACID) RESIN IN THE CONVERSION OF D-GLUCOSE INTO D-FRUCTOSE

	Weight of D-*fructose in supernatant liquid* (*g*)	*Weight of* D-*fructose absorbed by resin* (*g*)	*Yield of* D-*fructose* (%)	*Temperature of reactor coil* (*degrees*)	*Recycle time* (*h*)
Run 1	0.161	0.224	56.5	37	22
Run 2	0.219	0.348	56.7	37	46
Run 3	0.018	0.342	52.8	50	6
Run 4	0.652	0.406	42.3	37	21.5

It has thus been shown that a resin containing dihydroxyboronophenylene groups may be used in the chromatography of sugars and alditols at both neutral and alkaline pH values, and in the production of high yields of D-fructose from D-glucose in the presence of alkali. The investigation will continue into the effect of the degree of cross-linking of the resin, the use of substituted dihydroxyboronophenylene groups, and into new methods of attaching the functional groups to preformed inert matrices.

EXPERIMENTAL

Materials and general methods. — 4-Vinylbenzeneboronic acid was prepared by the method of Letsinger and Hamilton[9], and the crude product was treated with diethanolamine by the method of Hoffmann and Thomas[10] to give iminodiethyl 4-vinylbenzeneboronate (overall yield 46.7%, after two recrystallisations from

acetone). Sulphonated benzeneboronic acid was prepared, as its sodium salt, by the method of Garegg and Lindberg[6].

The automated analytical methods used Technicon AutoAnalyzer modular equipment. Hexoses were determined by the automated cysteine–sulphuric acid assay[16], and D-fructose by an automated resorcinol assay[14]. Formaldehyde obtained by the periodate oxidation of carbohydrates was determined by the automated spectrofluorimetric periodate–pentane-2,4-dione assay[17].

Synthesis and use of poly(4-vinylbenzeneboronic acid) resin. — Iminodiethyl 4-vinylbenzeneboronate (25.0 g), divinylbenzene–ethylvinylbenzene mixture (53:47 w/w, 1.0 g), and azobisisobutyronitrile (70 mg) were dissolved in dry, distilled benzyl alcohol (80 ml), and the solution was deaerated with nitrogen. The mixture was maintained at 70°, under nitrogen, for 20 h. The resultant gel was removed, washed with chloroform, and dried. The crushed resin was washed with M hydrochloric acid until the effluent no longer gave a yellow colour in the periodate–pentane-2,4-dione assay. It was then washed with water and dried over phosphorus pentaoxide (overall yield 81%). The resin was dry-sieved to give a fraction 60–120 mesh (12.0 g) which was slurried with water and packed into a jacketed column (122 × 0.6 cm). The column (20°) was pumped (0.16 ml/min) with distilled, deaerated water (pH 6.1). Solutions (0.2 ml) of various carbohydrates (20–200 μg) were eluted separately from the column and their elution positions monitored with the automated periodate–pentane-2,4-dione assay. Table I gives the retention factors (elution volume/total bed-volume) for the compounds and the shapes of the elution peaks.

Effect of temperature and pH on the elution of D-*glucose and* D-*fructose.* — D-Glucose and D-fructose were eluted from the column in a similar manner to the last experiment, maintaining the column at 25° and at 50°. The column was then returned to 20° and, after equilibration with distilled water (pH 5.1), samples of D-glucose and D-fructose were again eluted. This experiment was then repeated by using a dilute solution of formic acid (pH 3.0) and a solution of sodium hydroxide (pH 10.0). Tables II and III give the results obtained.

Separation of a mixture of D-*glucose and* D-*fructose.* — A solution (0.2 ml) of D-glucose (200 μg) and D-fructose (2 mg) was eluted from the column with distilled water (20°, pH 5.1, 0.17 ml/min), the eluate being monitored after a dilution stage. A solution (0.2 ml) of D-glucose (200 μg) and D-fructose (200 μg) was then eluted from the column under similar conditions at 50°. Fig. 1 shows the separation obtained.

Use of an anion-exchange resin in its sulphonated benzeneboronic acid form to separate polyols. — A sample (~10 ml) of the Dowex-AG1 x2 (200–400 mesh, carbonate form) resin was regenerated by allowing an aqueous solution of the sodium salt of sulphonated benzeneboronic acid (10 g in 150 ml) to slowly percolate the resin. The resin was then washed with water, packed into a column (29 × 0.4 cm), and pumped with distilled, deaerated water (pH 6.1, 0.18 ml/min). The eluent was monitored with the automated cysteine–sulphuric acid and periodate–pentane-2,4-dione assays. With the column maintained at 20°, solutions (0.1 ml) of various carbohydrates and diols (50 μg) were eluted separately from the column (Table IV).

Use of poly(4-vinylbenzeneboronic acid) resin in the conversion of D-*glucose into* D-*fructose.* — Iminodiethyl 4-vinylbenzeneboronate (1.060 g), divinylbenzene–ethylvinylbenzene (53:47 w/w, 0.175 g), styrene (0.763 g), and azobisisobutyronitrile (45 mg) were dissolved in chloroform (10 ml), and the solution was deaerated with nitrogen and heated in a sealed phial for 4 h at 70°.

The gel was treated in the manner described above and the dried poly(4-vinylbenzeneboronic acid) resin (0.68 g) was placed in a jacketed tube fitted with a sintered-glass disc and a tap at the lower end. A solution (18.6 ml) containing D-glucose (0.35 g), D-fructose (0.35 g), and sodium hydroxide (0.121 g) was added to the resin, and the slurry (pH 12.0) was stirred at room temperature for 15 min. The supernatant liquid was filtered from the resin; the resin was then washed with M hydrochloric acid (3 × 10 ml) and the washings were combined. The two samples were analysed, after suitable dilution, by the resorcinol assay for D-fructose and the cysteine–sulphuric acid assay for total hexose content (Table V, Expt. *A*).

The resin was washed with M sodium hydroxide (100 ml) and distilled water (500 ml), and the moist resin was placed in the jacketed tube. A solution (16.2 ml) containing D-glucose (0.35 g) and sodium hydroxide (24 mg) was added to the resin, and the slurry, maintained at 50° under nitrogen, was stirred for 4 h. The supernatant liquid was then filtered from the cooled slurry and the resin washed with 0.1M hydrochloric acid (4 × 20 ml). These two fractions were analysed as before for D-fructose, and the amount of D-glucose and D-mannose present was estimated (Table V, Expt. *B*).

Use of poly(4-vinylbenzeneboronic acid) resin in a reactor. — Iminodiethyl 4-vinylbenzeneboronate (2.00 g) and a mixture of divinylbenzene–ethylvinylbenzene (53:47 w/w, 0.612 g) were dissolved in *N*,*N*-dimethylformamide (8 ml) with warming. The solution was deaerated with nitrogen and, after the addition of azobisisobutyronitrile (0.01 g), it was heated in a sealed phial for 6 h at 60°. The gel was removed and washed in the manner described previously. The resin (2.0 g) was washed with 0.1M sodium hydroxide (500 ml) and packed into a column (7 × 1.3 cm). The column, maintained at 19°, was pumped with an aqueous solution of sodium hydroxide (pH 12.0, 0.23 ml/min) for 6 h until the eluent also had pH 12.0.

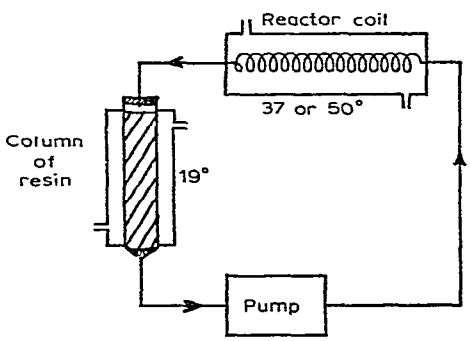

Fig. 2. Schematic diagram of reactor system using poly(4-vinylbenzeneboronic acid) resin.

Run 1. The column was connected at the top to a small jacketed coil (2 ml) and at the bottom to a peristaltic pump to give a closed flow-system (Fig. 2). D-Glucose (0.682 g) was dissolved in M sodium hydroxide (1.9 ml) and the solution (pH 12) was loaded on to the column. The column eluent was recycled through the reactor-coil (37°) and the column (19°) for 22 h. The eluate was then collected from the column until the rest of the system was empty. The resin was washed with dilute hydrochloric acid (M 2 vol.; 0.1M 6 vol.) and the washings were combined. Both solutions were analysed, after dilution, by the resorcinol assay for D-fructose (Table VI). The initial concentration of D-glucose in the system was ~6.8% w/w.

Run 2. The reactor and column were prepared as before, and a sample (2.9 ml) of an aqueous solution of D-glucose (1.00 g) at pH 12.0 (NaOH) was loaded on to the column. The eluate was recycled (0.23 ml/min) for 46 h with the reactor coil at 37°. The eluate was collected and the resin washed with 0.5M hydrochloric acid (100 ml). The two samples were analysed for D-fructose (Table VI). The initial concentration of D-glucose was ~10% w/w.

Run 3. Run 1 was repeated, except that the coil was maintained at 50° and the eluate was recycled for 6 h. The results of the analysis of the two fractions collected are given in Table VI.

Run 4. The reactor and column were prepared as before, and a solution (4.15 ml) of D-glucose (2.50 g) at pH 12.0 (NaOH) was loaded on to the column. The eluate was recycled (0.23 ml/min) for 21.5 h with the reactor-coil maintained at 37°. Analysis of the supernatant liquid and of the acid washings of the resin is given in Table VI. The initial concentration of D-glucose was ~25% w/w.

TABLE VII

SEPARATION OF SUGARS ON A COLUMN OF POLY(4-VINYLBENZENEBORONIC ACID) RESIN

Eluent	*Sample*	*Weight of sample (μg)*	*Retention factor of peak*	*Shape of peak*[a]
7.3	D-Glucose	250	1.02	S
	D-Mannose	310	0.98	S
	D-Fructose	250	1.46	S
	D-Glucose	100	1.04	S
	D-Fructose	100	1.42	S
	D-Mannose	180	1.08	S
	D-Fructose	100	1.42	S
9.6	D-Glucose	250	0.48	S
	D-Mannose	250	0.60	S
	D-Fructose	250	5.25	B
	D-Glucose	250	0.48	S
	D-Fructose	750	4.25	B
	D-Mannose	250 mg	0.71	S
	D-Glucose	250 mg	0.81	S
	D-Fructose	250 mg	0.92	T

[a]S, sharp; B, broad; T, tailed.

Separation of D-*glucose,* D-*fructose, and* D-*mannose on poly(4-vinylbenzeneboronic acid) resin.* — Iminodiethyl 4-vinylbenzeneboronate (2.01 g) and a mixture of divinylbenzene–ethylvinylbenzene (53:47 w/w, 0.626 g) was dissolved, with warming, in *N,N*-dimethylformamide (10 ml). Azobisisobutyronitrile (2 mg) was added and the solution was sealed, under nitrogen, in a phial. The phial was heated for 96 h at 68°. The washed resin was packed in a jacketed column (11 × 0.8 cm) and maintained at 18 ± 1°. The column was pumped (0.23 ml/min) with distilled water and with dilute solutions of sodium hydroxide. With each new eluent, when the pH of the eluate was the same as that of the eluent, solutions (0.1–0.5 ml) of D-glucose, D-mannose, and D-fructose (100 μg–250 mg) were eluted from the column, the elution position of the sugars being monitored with the automated resorcinol and cysteine–sulphuric acid assays. Table VII gives the retention factors (peak elution-volume/total bed-volume) for these fractionations.

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