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p-VINYLBENZENEBORONIC ACID POLYMERS FOR SEPARATION OF VICINAL DIOLS

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SUMMARY

A relatively simple procedure was developed for preparing poly(*p*-vinylbenzeneboronic acid)-coated porous polystyrene beads which were shown to sorb and desorb vicinal diols reversibly through complex formation and to reject other hydroxy compounds. The complexing is highly pH dependent. By suitable adjustment of operating conditions, columns can be used for quantitative recovery of vicinal diols or can be used to separate mixtures of different vicinal diols chromatographically.

INTRODUCTION

In studies on the conversion of L-tyrosine to L-DOPA by a tyrosine hydroxylase enzyme obtained from the red beet *Beta vulgaris*, we encountered difficulties in separating and recovering L-DOPA from starting material. Although we were able, through spectral absorption measurements, to demonstrate separations for analytical determinations on alumina columns and, using boric acid complexes, on ion-exchange resins or carbon columns, quantitative recovery of L-DOPA after elution with acid was not practical. Alumina was taken up in, and contaminated, the acidic eluates and the solubilities of boric acid and L-DOPA and their salts were too similar.

To overcome these problems we prepared polymers containing covalently bound dihydroxyboryl groups to which L-DOPA could be complexed through its vicinal hydroxyl groups.

Separation of mixtures of ribo- and deoxyribonucleic acids by complex formation with dihydroxyboryl-containing polymers was demonstrated by Weith *et al.*[2] and Schott[3]. In the former system the support matrix was cellulose, in the latter it was a polymethacrylic acid gel.

Letsinger and Hamilton[4] first reported making boronic acid containing polymers prepared from vinyl monomers either as homopolymers or as copolymers with either *p*-chlorostyrene or acrylamide. Further reports of polymers of this type were made by Pellon *et al.*[5], Hoffman *et al.*[6-9], and Lennarz and Snyder[10].

We have investigated four systems for polymerizing *p*-vinylbenzeneboronic

acid, *viz.* (a) copolymerization with styrene, (b) copolymerization with N,N′-methyl-ene-bis(acrylamide), (c) copolymerization with acrylamide and N,N′-methylene-bis(acrylamide), and (d) interstitial homopolymerization on porous polystyrene beads. Of these, the interstitial homopolymerization was considered to give the most useful column packing material with sufficient capacity and sorption and desorption charac-teristics for our needs.

Preparation of *p*-vinylbenzeneboronic acid resembled the method of Lennarz and Snyder[8]. By avoiding inverse addition of Grignard reagent we have simplified the procedure with no loss in yield.

EXPERIMENTAL[*]

Preparation of p-vinylbenzeneboronic acid

In a 100-ml flask equipped with stirrer, condenser, pressure-relieved dropping funnel, and inert atmosphere inlet, were placed 3.0 g (125 mM) magnesium turnings, 25 ml tetrahydrofuran (THF; distilled from lithium aluminum hydride), and one crystal of iodine. A solution of *p*-bromostyrene, 18.3 g (100 mM) in 20 ml THF, was added slowly with stirring until reaction commenced. The volume of the reaction mixture was increased by adding 125 ml of THF and addition of *p*-bromostyrene was continued at a rate sufficient to maintain slight reflux for a period of about 10 min. Stirring was continued for 1 h at room temperature. The mixture was cooled to −80° and 36.5 g (40 ml, 350 mM) of trimethyl borate were added in one portion as rapidly as possible. The mixture was allowed to warm to room temperature during a period of 1 h and was then worked up by addition of 25 ml HCl in 100 ml water followed by removal of most of the solvent on a rotary evaporator. The aqueous residue was extracted with three 150-ml portions of diethyl ether and the combined ether phases were extracted with two 150-ml portions of 1 *N* NaOH and the aqueous phases combined and washed with 150 ml ether. The basic extract was acidified and extracted with three 150-ml portions of ether. The ether phases were combined, dried over magnesium sulfate and the ether evaporated to recover 11.0 g of crude *p*-vinyl-benzeneboronic acid which, on crystallization from 450 ml of boiling water, yielded 9.90 g (67 mM, 67% yield) of colorless crystals.

Polymerization of p-vinylbenzeneboronic acid

(a) Copolymerization with styrene. In a 50-ml flask were placed 5.0 g of styrene (previously passed through Woelm Activity I neutral alumina), 2.5 g of *p*-vinylbenzeneboronic acid, 5 ml of bis(2-methoxyethyl) ether, and 0.10 g azobis(iso-butyronitrile). The flask was purged with argon and then sealed with a wired-on stopper. The mixture was warmed in an oven to 80–90° for about 1 h to give a foamy gelatinous mixture, and heating was then continued at 100° for an additional hour. The reaction mixture was allowed to cool to room temperature and the rubbery product transferred with reagent solution to a mortar and ground gently with 50 ml of added chloroform. The finely divided polymer was triturated two more times with

chloroform, collected by suction on a sintered funnel, and dried at room temperature under vacuum.

(b) Copolymerization with N,N'-methylene-bis(acrylamide). In a 200-ml flask fitted with a stirrer were placed 2.0 g of *p*-vinylbenzeneboronic acid, 100 ml of distilled degassed water, and 0.10 g of N,N'-methylene-bis(acrylamide). The mixture was warmed under argon atmosphere with stirring to 80–90° and 0.10 g of ammonium persulfate added in one portion. The mixture became turbid and lumps of polymer formed as the reaction proceeded. Heating was continued for 3 h, during which time additional 0.10-g quantities of ammonium persulfate were added at hourly intervals. The mixture was allowed to cool to room temperature and most of the supernatant emulsion and suspended fine particles were decanted off. The lumps of polymer remaining were broken up, washed with several portions of water, collected by suction filtration and dried under vacuum.

(c) Copolymerization with acrylamide and N,N'-methylene-bis(acrylamide). Polymerization was carried out in a 250-ml flask as described in *b* using 1.50 g of *p*-vinylbenzeneboronic acid, 1.50 g of acrylamide, 0.15 g of N,N'-methylene-bis-(acrylamide), 150 ml of distilled water, and 0.10-g portions of ammonium persulfate. The product was worked up similarly.

(d) Interstitial homopolymerization on porous polystyrene beads. A solution of 1.00 g of *p*-vinylbenzeneboronic acid in 25 ml of bis(2-methoxyethyl) ether containing 0.01 g of azobis(isobutyronitrile) was added to 5.0 g of macroporous polystyrene beads (Chromosorb 102, 80–100 mesh) and the mixture placed under vacuum to aid in the removal of air entrapped within the beads. The vacuum was released and the excess solution, about 10 ml, was removed from the beads with a pipette. The flask containing the moist material was placed on a rotary evaporator where more solvent, about 10 ml, was removed during 20 min, while the bath temperature was gradually raised to 90°. The residual free-flowing powder was then warmed to 120° in vacuum for 1 h and allowed to cool. The product was then suspended with stirring in 50 ml of dimethylformamide containing 50 % water and warmed to 80° for 1 h. The coated beads, now free of unentrained polymer, were collected by suction filtration and washed on the filter successively with methanol and ether followed by drying under vacuum at room temperature. The uptake of polymer under these conditions was 0.36 g on the 5 g of microporous beads.

COLUMN PERFORMANCE TESTS ·

Determination of breakthrough capacity

A column (21 × 1.0 cm I.D.) of boronic acid resin was prepared in 0.1 M, pH 8.0 phosphate buffer using 5.36 g of coated polystyrene beads of which 0.36 g represented poly(*p*-vinylbenzeneboronic acid) (example *d* of the polymer preparations). The column was equilibrated with buffer for 1 h and a solution of 10^{-3} M D,L-DOPA, 1×10^{-5} M in sodium bisulfite, and in pH 8.0 buffer was pumped through the column at 60 ml/h. DOPA concentration in the effluent was monitored at 280 nm. DOPA appeared in the effluent when 120 ml of solution had passed through the column. When 130 ml had passed, the concentration of DOPA in the eluate was essentially the same as the concentration in the input solution. The breakthrough capacity was 130 μM/g. Under the same conditions the tyrosine breakthrough volume

TABLE I

RETENTION VOLUMES OF PHENOLIC COMPOUNDS AT pH 8.0

Compound	Elution volume (ml)
L-Tyrosine	20–30
p-Hydroxycinnamic acid	25–100
2,4-Dihydroxycinnamic acid	20–30
3-Methoxy-4-hydroxycinnamic acid	20–30
3,4-Dihydroxycinnamic acid	250–600
D,L-DOPA	250–450

TABLE II

RETENTION VOLUMES OF PHENOLIC ACIDS AT pH 6.8

Compound	Elution volume (ml)
3,4-Dihydroxycinnamic acid	45–150
D,L-DOPA	30–100

was approximately 10 ml for a 7×10^{-4} M solution in pH 8.0 buffer. Elution of the complexed DOPA and regeneration of the column was effected with 0.1 N acetic acid followed by equilibration with buffer.

Affinity for aromatic hydroxy acid

The column used in the previous experiments, after equilibration at pH 8.0, was loaded in successive determinations with approximately 15 μM solutions of selected phenolic compounds using a flow-rate of 60 ml/h. The results are summarized in Table I. Elution volume data for two of the strongly retained compounds were also
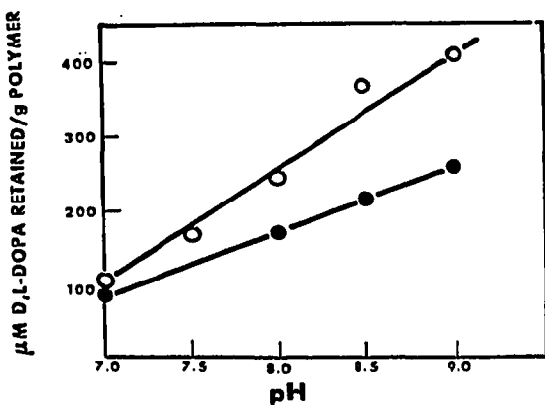

Fig. 1. Effect of pH on retention capacity of columns containing poly(p-vinylbenzeneboronic acid). ●, Co-polymer with polystyrene (preparation a); ○, interstitial homopolymerization on polystyrene beads (preparation d). Columns, 20 × 1 cm; flow-rate, 60 ml/h, effluent monitored at 280 nm.

determined at pH 6.8. The data are given in Table II. Clearly only the *o*-dihydroxy compounds are held by the complexing action of the column and binding is strongly pH dependent.

Effect of pH

The effect of pH on the capacity of two of the boric acid polymer columns to complex DOPA is shown in the graph in Fig. 1. A straight-line relationship is indicated. Although there is greater complexing above pH 9, it is not practical to go beyond pH 9 because of the increased sensitivity of DOPA to oxidation at high pH. The interstitial homopolymer on porous polystyrene beads (*d*) had a higher capacity (47% at pH 8) for adsorbing DOPA than the styrene copolymer.

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