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Note

Chromatographic separation of dihydroxybenzenes on a column of Merckogel PGM 2000

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The adsorption of phenolic compounds on Sephadex gels is well known and has been ascribed to the ether linkages present in the gel[1]. As Merckogel PGM 2000 contains ether linkages in its gel matrix[2], the use of this gel for the adsorption chromatography of dihydroxybenzenes was investigated.

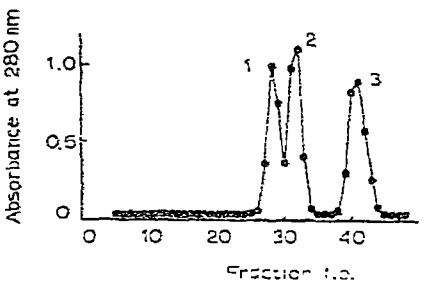
The gel (particle size range 63–100 μm) was washed and poured into a chromatographic tube with the buffer to be used as the eluent, and a column 42 cm in height and 0.5 cm in diameter was prepared. The dihydroxybenzenes were dissolved in the eluent to be used for the chromatographic separation and 0.3 ml of the solution obtained was applied to the column. Elution was performed with the same eluent, and the eluate was collected in fractions of 1.0 ml using a timer-operated fraction collector, the receptacle being changed every 20 min. Conditions for the chromatographic separation are given in Table I. Each fraction was diluted with 2.0 ml of 1 *N* hydrochloric acid and the absorbances of the resulting solutions were measured at 280 nm.

TABLE I

CONDITIONS OF THE CHROMATOGRAPHIC SEPARATION

Figure	*Eluent*	*Column size (cm)*	*Temperature (°C)*
1	Phosphate buffer, 0.05 M, pH 6.0	42 × 0.5	22
2	Phosphate (0.05 *M*)–borate (2/3 *M*) buffer, pH 6.0	42 × 0.5	22

As shown in Figs. 1 and 2, the three isomers of dihydroxybenzene could be separated from each other, and when a buffer containing boric acid was used as the eluent the elution volume of pyrocatechol decreased, resulting in the complete separation of the three isomers of dihydroxybenzene (Fig. 2). The decrease in the adsorption of pyrocatechol on Merckogel PGM 2000 in the presence of borate ion may be due to the negative charge of the borate complex of pyrocatechol, because the adsorption on Merckogel PGM 2000 of ionized compounds, *e.g.*, benzoate anions at pH 6.0,



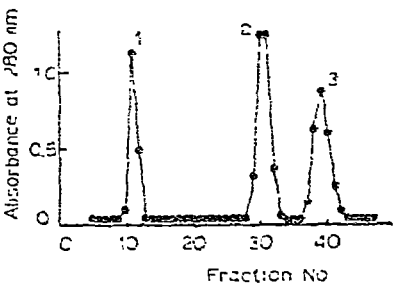


Fig. 1. Elution of the three isomers of dihydroxybenzene under the conditions given in Table I. The compounds in order of elution from the column are pyrocatechol (1), hydroquinone (2) and resorcinol (3).

Fig. 2. Elution of the three isomers of dihydroxybenzene under the conditions given in Table I. The compounds in order of elution from the column are pyrocatechol (1), hydroquinone (2) and resorcinol (3).

TABLE II

RECOVERY OF DIHYDROXYBENZENES FROM THE COLUMN

Figure	*Dihydroxybenzene*	*Added (μg)*	*Recovered (μg)*	*Recovery (%)*
1	Pyrocatechol	391	412	105
	Hydroquinone	409	401	98
	Resorcinol	877	898	102
2	Pyrocatechol	314	305	97
	Hydroquinone	473	492	104
	Resorcinol	733	770	105

was much weaker than that of the non-ionized benzoic acid at pH 3.0. As shown in Table II, the recovery of dihydroxybenzenes from the column was quantitative and the column could be used repeatedly.

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