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Note

Chromatography of tea polyphenols on Sephadex columns as a method of estimation of molecular size

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Sephadex LH-20, which is swollen by organic solvents, has been shown to interact with simple phenols to give predominantly sieving, adsorption or partition, depending on the eluent. Thus in methanol, acetonitrile and tetrahydrofuran (THF) adsorption predominated, while sieving was observed in dimethylformamide (DMF)[1]. Partition effects were important with lignin model compounds in aqueous dioxan, while with DMF sieving again occurred[2]. Little retention of tea pigments was noted with Sephadex LH-20 in aqueous DMF[3], but in alcohols[4–9], aqueous acetone[3,10], aqueous alcohols[11] and methanol–chloroform[12] adsorption and/or partition effects predominated with a variety of polyphenols.

In the case of G-type Sephadex, lignin model compounds showed adsorption as the predominant process in water, while sieving predominated in aqueous dioxan and dimethyl sulphoxide[2]. Although molecular sieving predominated in the chromatography of tannic acid constituents[13] and some flavanols[14] in aqueous acetone, adsorption played a major role with phenolic glucosides in aqueous methanol[15].

The estimation of the molecular weights of polyphenols from wine[16], apple peel[17], tea[18] and carob pods[19] has been carried out with aqueous alcohols on Sephadex G-25. It was assumed in these instances that the exclusion limit of the columns could be estimated from the degree of swelling of the gel, but this assumption has been shown to be erroneous[13,14]. Hawthorn polyphenols appear to have been separated by sieving in aqueous methanol on Sephadex LH-20 (ref. 20).

This paper deals with the behaviour of several tea polyphenols on Sephadex LH-20 under conditions reported to favour molecular sieving. The behaviour of a model polyphenol mixture in four solvent systems on Sephadex LH-20 and G-25 has also been observed.

EXPERIMENTAL

The isolation of tea flavanols has been described[21]. Bisflavanol A was prepared by enzymic oxidation of (−)-epigallocatechin gallate[22]. Benzotropolone derivatives were supplied by Dr. P. D. Collier. Dextran blue, tannic acid, (+)-catechin, pyrogallol and quercetin were obtained commercially.

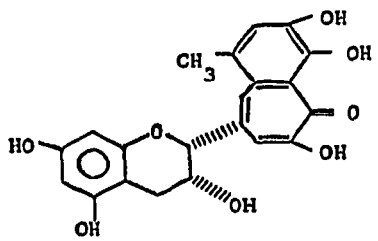
All columns (diameter 1.6 cm) consisted of 10 g (dry weight) of gel, which was equilibrated overnight in the appropriate solvent. Fractions of 0.6–0.8 ml were col-

lected. Sample sizes were 5 mg for single compounds and 10 mg for mixtures. The elution volume is defined here as the volume of eluent preceding and including that of the first fraction containing the compound. The fractions were subjected to TLC in order to determine their composition. Tannic acid and compounds I–V (see Table I) were chromatographed on Polygram Cel-300 sheets (Macherey, Nagel & Co., Düren, G.F.R.) with 4% acetic acid and compounds VI–XII (see Table I) on Polyamid-6 UV 254 sheets (Macherey, Nagel & Co.) with butanone-2–methanol–acetic acid (5:5:1). Tannic acid and compounds I–V (see Table I) were rendered visible with $Fe^{3+}/Fe(CN)_6^{3-}$.

TABLE I

ELUTION VOLUMES (V_e) AND MOLECULAR WEIGHTS OF POLYPHENOLS

Sephadex LH-20 in 70% (v/v) aqueous DMF.

Compound	*Code*	$V_e \pm 0.8$ (*ml*)	*Mol. wt.*
Dextran blue	DB	14.0	*ca.* 10^6
Tannic acid	TA	14.0	1600–1700
Bisflavanol A	I	19.5	914
(−)-Epigallocatechin gallate	II	23.5	458
(−)-Epicatechin gallate	III	24.8	442
(+)-Catechin	IV	29.0	290
Pyrogallol	V	34.0	126
Theaflavin digallate	VI	21.5	868
Theaflavin monogallate	VII	24.5	716
Theaflavin	VIII	27.0	564
Epitheaflavic acid	IX	23.5	430
	X	35.0	400
3′,4′-Dihydroxy-6,7-benzotropolone	XI	50.0	216
Quercetin	XII	48.5	302

RESULTS AND DISCUSSION

Table I and Fig. 1 show the chromatographic data for several tea polyphenols on Sephadex LH-20 in 70% DMF. The compounds can be divided into three groups: a group including compounds I–V and tannic acid, which give a linear relationship for the plot of elution volume (V_e)/column bed volume (V_b) *versus* the logarithm of the molecular weight; compounds VI, VII, VIII, X and XI, which give a curve; and compounds IX and XII, which do not fit in either of the first two groups. In the first two groups, the compounds possess the same acidic functional groups; the linear group (compounds I–V) contains only phenolic and alcoholic hydroxyl groups, while the group that generates the curve are all benzotropolone derivatives with phenolic

and alcoholic hydroxyl groups. The concavity of the curve indicates that factors such as adsorption and/or partitioning are more important with the members of lower molecular weight. The exclusion limit of this chromatographic system for compounds containing phenolic and alcoholic groups appears to be between molecular weight 1000 and 1600. Compounds IX and XII, which do not fit in either group, contain functional groups with different acidities: the carboxyl group and flavanol moiety, respectively. Thus, as has been implied[14], the estimation of molecular size is possible only if a calibration curve is available that includes compounds containing the same functional groups.

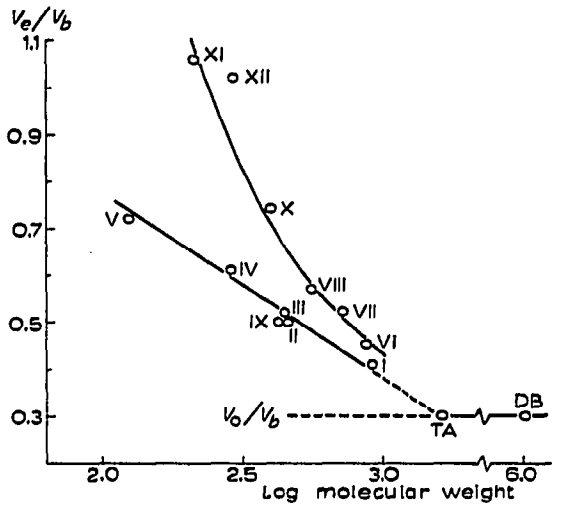


Fig. 1. Plot of elution volume (V_e)/column bed volume (V_b) *versus* the logarithm of the molecular weights of polyphenols. Sephadex LH-20 in 70% (v/v) aqueous DMF. $V_b = 47.3$ ml; V_o = void volume. For identification of compounds, see Table I.

Since for isolation purposes more volatile solvent mixtures are convenient, the behaviour of tea polyphenols in aqueous volatile organic solvents was studied. A binary mixture was utilized that consisted of (−)-epicatechin (mol. wt. 290) and (−)-epigallocatechin gallate (mol. wt. 455), and the behaviour of the mixture was examined in 50% (v/v) aqueous acetone, THF, acetonitrile and methanol on Sephadex LH-20 and G-25. It has been demonstrated that (−)-epicatechin is eluted first from adsorption columns and (−)-epigallocatechin gallate first from partition columns[23]. In the four solvent systems studied, (−)-epicatechin was eluted first in all instances on Sephadex LH-20 and the elution volumes were greater than the total column volume. Thus it appears that adsorption is the predominant process on Sephadex LH-20 in these solvent systems and that molecular-weight estimations carried out under these conditions must be viewed with scepticism[20].

On Sephadex G-25, retention of the polyphenols occurred in aqueous acetonitrile and no separation was evident, suggesting that adsorption played an important role. Aqueous methanol was also retained and the elution of (−)-epicatechin first clearly indicated the predominance of adsorption processes. In aqueous THF and acetone, (−)-epigallocatechin gallate was eluted first, showing that sieving or partition

effects predominate. Chromatography of a binary mixture of bisflavanol A (mol. wt. 914) and (−)-epigallocatechin gallate in aqueous THF or acetone showed that sieving is the predominant process, since bisflavanol A was eluted first (at approximately the void volume) and it is known that on partition columns (−)-epigallocatechin gallate is eluted first[23]. Thus on Sephadex G-25 the predominant process in the chromatography of polyphenols is solvent-dependent; aqueous acetone or THF are suitable for sieving. The estimation of molecular size in aqueous alcohols[16–19] must be regarded with caution.

The chromatographic behaviour of polyphenols on Sephadex columns is solvent-dependent, and hence the conclusion that sieving predominates under a given set of conditions must be supported by calibration of the column with compounds that contain the same functional groups as those to be studied.

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