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The separation of condensed tannins on Sephadex G-25 eluted with 50% aqueous acetone

The use of dextran gels for the separation of polymeric polyphenols has aroused much interest since SOMERS' initial work on condensed tannins from wine[1,2]. Subsequent applications have included the separation of condensed tannins from hawthorn[3], tea[3], apple peel[5], oak leaf[6], and carob pod[7]. Also *Eucalyptus*[8] and tannic acid[9] hydrolysable tannins have been fractionated.

SEIKEL AND HILLIS[8] found that *Eucalyptus delegatensis* hydrolysable tannins could be successfully separated on Sephadex G-10–G-50 columns using water as eluent. However KING AND PRUDEN[9] found that water was unsatisfactory for fractionating tannic acid on Sephadex G-25 and used water–acetone mixtures for the eluting solvent. They noted that the latter solvent decreased the tendency of the tannin to be adsorbed on dextran. These effects are even more pronounced for condensed tannins which possess a higher proportion of phenolic hydroxyl groups, a higher molecular weight and resulting lower water solubility. RUSSELL *et al.*[10] have shown that the mobility of mimosa condensed tannins on both cellulose and collagen is high for 50–70% acetone, whereas the mobility is virtually zero for either of the pure solvents. Similarly we have shown that *Pinus radiata* bark condensed tannins have a maximum solubility in 60% acetone[11]. The mobility of condensed tannins in acetone–water mixtures is only rivalled by dipolar aprotic solvents such as dimethyl formamide or dimethyl sulphoxide[10].

Application of Sephadex chromatography to the separation of tannins from *Pinus radiata* bark, using 50% acetone as eluting solvent, leads us to conclude that the exclusion limit for Sephadex G-25 is much higher than that determined by KING AND PRUDEN[9] using dyestuffs as mol.wt. standards. This leads us to redetermine the exclusion limit of G-25 for condensed tannins.

Experimental

Sephadex G-25 (Fine, Pharmacia) was equilibrated in acetone (analytical reagent grade)–water (1:1) and the swollen gel was packed into a calibrated glass column 2.5-cm I.D., bed volume 220–230 ml. For separations the flow-rate was maintained at 60 ml/h and samples were collected in 2.2-ml fractions. Absorbance *versus* volume profiles were constructed by measuring the absorbance of each fraction at 350 nm. The dead volume (V_0) was determined with either blue dextran or tannin samples which were excluded from the gel.

Swelling determination. The results in Table I were obtained by weighing 1-g samples of Sephadex into 10 ml measuring cylinders and making the volume up to 10 ml with the appropriate acetone–water mixtures. The volume occupied by the gel was determined after two weeks swelling.

Chemicals. (+)-Catechin and (+)-dihydroquercetin were commercial (Koch-Light) samples. "Avocado dimer" is a 4,8-linked dicatechin isolated from avocado seed by the method of GEISSMAN AND DITTMAR[12]. "Mimosa trimer" is a group of trimeric 4,6-linked flavans present in the methanol solubles of *Acacia mearnsii* heart-

TABLE I

SWELLING OF SEPHADEX G-25 IN ACETONE–WATER MIXTURES

Acetone (%)	*Mole fraction*	*Bed volume/g (± 0.1 ml)*
0	0	5.4
10	0.03	5.4
20	0.06	5.0
30	0.09	4.9
40	0.14	4.2
50	0.20	3.2
60	0.27	2.7
70	0.36	2.5
80	0.50	2.3
90	0.69	1.8
100	1.00	1.3

wood[13]. The crude flavanoid mixture from this source was fractionated to determine the retention volume of the trimeric flavans.

Results and discussion

Fig. 1 shows a plot of elution volume/column bed volume (V_e/V_b) *versus* log molecular weight for a number of flavanoid components of known molecular weight. Results indicate an exclusion limit of mol.wt. 1070±200 for condensed tannins (the point of intersection of V_e/V_b with V_0/V_b).

This result is supported by the work of SOMERS on wine tannins. Neither anthocyanins (mol.wt. 500–530) nor acylated anthocyanins (mol.wt. near 700) were excluded from Sephadex G-25 using 50% aqueous acetone (HCl)[2]. It is clear that the exclusion limit is dependent on the class of compounds being separated. The gel cannot be operating ideally as a molecular sieve for titan yellow (mol.wt. 695) which was found to be excluded from the gel[9]. A further example we have found is the exclusion

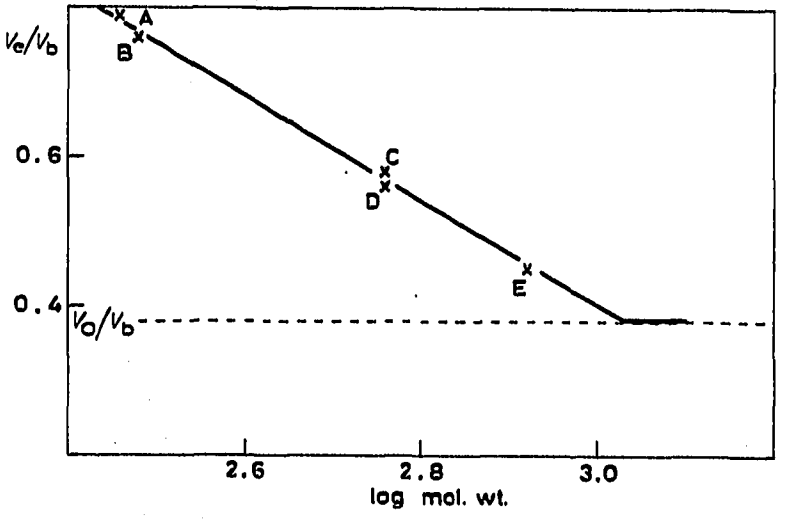

Fig. 1. Plot showing determination of the exclusion limit of Sephadex G-25 (Fine) with 50% aqueous acetone as eluent. A, Catechin (mol. wt. 290); B, dihydroquercetin (mol. wt. 304); C, naringin (mol. wt. 580); D, "Avocado dimer" (mol. wt. 578); E, "Mimosa trimers" (mol. wt. 834).

of chlorophyll (mol.wt. 956) from the gel, not only in 50% acetone but also from Sephadex G-25 eluted with 60% and 70% aqueous acetone.

It should be noted, however, that the dextran is probably behaving "ideally" for the condensed tannin separations. The degree of swelling of Sephadex G-25 in 50% acetone (see Table I) is 3.2 ml/g of dry gel. This value is similar to the swelling of Sephadex G-15 in water, and the exclusion limit of this gel for the separation of dextrans and peptides is mol.wt. < 1500 (ref. 14), a value similar to that determined for Sephadex G-25 in 50% acetone.

However, on the basis of the above work the earlier assumption of SOMERS[1] and FORREST AND BENDALL[4] that the exclusion limit is directly proportional to the percentage swelling of the gel is not valid.

Finally it may be concluded, that Sephadex G-25 eluted with 50% aqueous acetone, will exclude condensed tannins with mol.wt. near 1100 and will separate flavanoids in the mol.wt. range 300–900.

Chemistry Division,
D.S.I.R., Petone
(New Zealand)

L. J. PORTER
R. D. WILSON

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