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Note

Liquid chromatographic separation of plant phenolics using polyethylene glycol dimethacrylate gel

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Polyethylene glycol dimethacrylate gel (EM Gel Type OR-PGM 2000) was developed by Heitz and Winau[1] and Heitz[2] as an improved medium for gel chromatography and has previously been applied to the separation of polyethylene glycols[3], proteins[3], and dihydroxybenzenes[4], and to the characterization of beers[3]. Because of a continuing need for improved methods of analysis of very complex mixtures of compounds such as found in plant extracts, the use of this gel for the separation of plant phenolics was investigated.

EM Gel Type OR-PGM 2000, 120–230 mesh (EM Labs., Elmsford, N.Y., U.S.A.) was swelled in an excess of eluting agent overnight. A 90 × 1 cm column was packed by pouring a thick slurry of gel into the column and allowing it to sediment. Each compound, or mixture of compounds, was dissolved in the eluent and 0.1 ml injected on the column by means of a sample injection valve. The ultraviolet absorbance of the eluent at 254 nm was monitored. Plant extracts were prepared by extracting approximately 100 g of plant material with 0.5 *M* HCl in methanol–water (1:1). Extracts were lyophilized for storage and redissolved in 50 ml of eluent for chromatography. The injection volume was also 0.1 ml.

Several eluents were tested in the course of this work. Those containing methanol tended to give bubble problems and were abandoned. With eluents containing HCl, some material in plant extracts was strongly adsorbed on the column and would appear in later chromatograms. The mixture finally arrived at, 0.1 *M* acetic acid in isopropanol–water (1:1), is a good solvent for plant extracts, is convenient to use, and gives reproducible chromatograms. At elevated column temperature (62°) solutes eluted earlier and in sharper bands than at ambient temperature, and the operating pressure —in this case about 20 p.s.i.— was lower. Columns could be operated at fast flow-rates and at high pressures but tended to compact for a long period of time and chromatograms were not reproducible during that time. There was also some loss in resolution.

Chromatograms of the compounds tested are shown in Fig. 1. Elution volumes for both benzoic and cinnamic acid derivatives were greater than the total column volume (about 70 ml) and they are evidently retained on the gel and separated by

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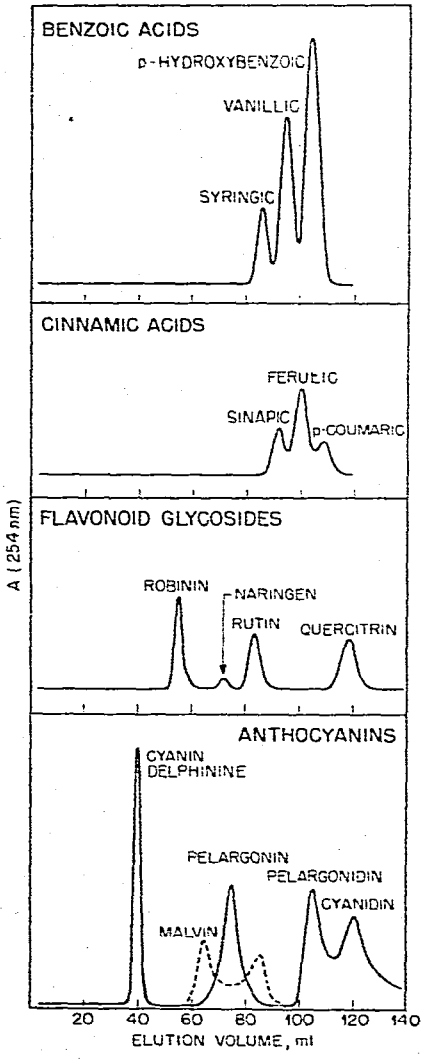
adsorption effects, as reported by Seki[4] for dihydroxybenzenes. Phenolic acids having more than one free phenolic group are even more strongly retained and have much greater elution volumes. Gallic acid, for example, was irreversibly adsorbed. Several flavonoid aglycones tested were also strongly adsorbed on the gel and no elution peaks were seen. Flavonoid glycosides, however, did elute in a reasonable time and could be separated. The elution order was the same as that observed with Sephadex LH-20[5]. With the possible exception of robinin, which eluted in the included volume, these compounds were also adsorbed. All the anthocyanidins and the anthocyanidin diglucosides, except cyanin, were decolorized in the column. However, they did give



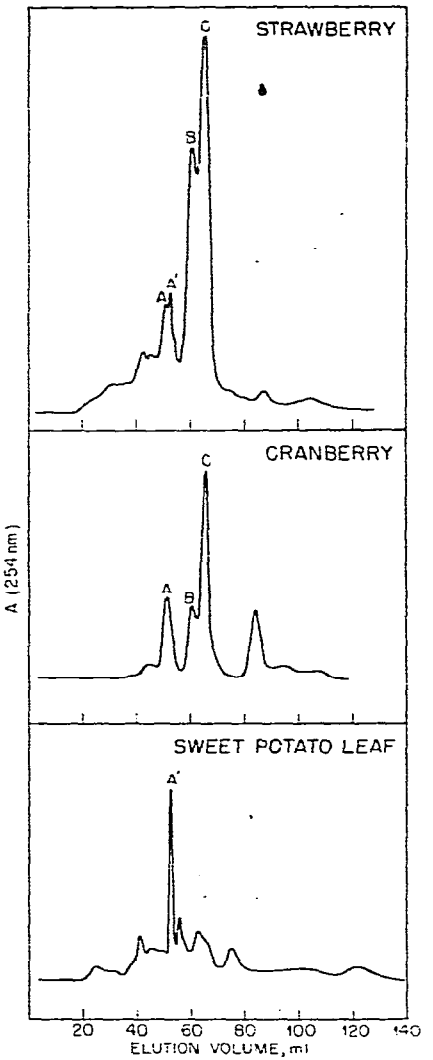


Fig. 1. Separation of plant phenolics. Column, 90 × 1 cm, packing, polyethylene glycol dimethacrylate gel; eluent, 0.1 *M* acetic acid in isopropanol–water (1:1); flow-rate, 20 ml/h; temperature, 62°.

Fig. 2. Chromatography of plant extracts. Conditions as in Fig. 1.

entirely reproducible elution peaks. The diglucosides eluted before the aglycones. Cyanidin and delphinidin 3,5-diglucosides eluted together. Malvidin 3,5-diglucoside gave two peaks, probably because the material tested was an impure technical grade. It is uncertain which of the peaks represents the authentic compound, and the impurity has not been identified, nevertheless both are reasonably well separated from the other anthocyanins. Of the anthocyanidins, pelargonidin and cyanidin were eluted and separated but elution peaks for delphinidin and malvidin did not appear in less than 150 ml.

Chromatograms of three plant extracts —cranberry and strawberry fruits and a sweet potato leaf extract— are shown in Fig. 2. Most of the ultraviolet-absorbing material in these samples eluted within the included volume of the column and molecular sieving may be an important part of the separation mechanism. Chromatography of several sweet potato leaf extracts suggested that the material eluting between 20 and 30 ml, close to the column void volume, contains the high-molecular-weight plant tannins. Good separations were achieved of several components in each extract. Although none of the major peaks in these chromatograms has as yet been identified, the peaks labelled A, B, and C in the cranberry and strawberry chromatograms have identical elution volumes and are probably the same compound, or ones very similar. The sharp peak, A′, appearing as a shoulder of A in the strawberry chromatogram has the same elution volume as an equally sharp peak in the sweet potato leaf chromatogram.

From these results, it appears that polyethylene glycol gels may be a useful tool in the analysis of complex plant-derived materials.

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