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Note

Gel filtration of flavonoids on Fractogel PGM 2000

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The separation of flavonoid aglycones and glycosides by gel filtration is normally carried out on Sephadex LH-20 (Pharmacia, Sweden), a modified dextran[1]. We have found that the application of gel filtration on Sephadex LH-20 can also be advantageous in several particular separations, especially in the clean-up of flavonoids isolated from paper, polyamide and other supports, on the preparative scale with crude plant extracts[2,3]. Sephadex LH-20 swells in aqueous and organic solvents and has the ability to perform separations by at least three mechanisms: sieving, π-bonding and hydrogen bonding[4]. Which of these mechanism (sieving or adsorption) predominates is determined by the eluents employed. This paper reports the gel filtration of a range of flavonoids on Fractogel PGM 2000, and compares the latter with Sephadex LH-20.

MATERIALS AND METHODS

The matrix of Fractogel PGM 2000 developed by Merck and Heitz and Winau[5] consists of a copolymer of ethyleneglycolmethacrylate ($n = 1$) and a polyethyleneglycoldimethacrylate (Fig. 1), and is particularly suitable for chromatography in aqueous and organic solvents. This gel has an exclusion limit of 2000 for polyethylene in water. Since methanol is a good solvent for flavonoid aglycones and glycosides, we used it in our work as a swelling medium and eluent. The relative swelling volume of PGM 2000 in methanol is 4.3 ml/g dry gel. In our investigations we used columns (80 × 2.5 I.D.) packed with gel to a height of 60 cm. After the swelling of the gel in methanol, the slurry was poured into the column and left to settle. The gel was continuously washed with methanol. An adapter was connected to the top of the column by which the samples (flavonoids dissolved in methanol, 2 mg per 5 ml each) were applied and immediately eluted at a flow-rate of 2 ml/min. Eluents were monitored spectrophotometrically (flow-cell 350 and 254 nm) and controlled by thin-layer

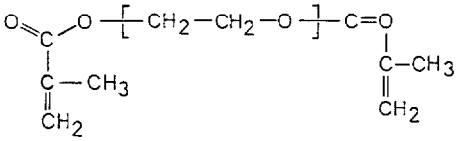

Fig. 1. Matrix of Merckogel PGM 2000.

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chromatography[6,7]. The results of the separation on PGM 2000 are reported as K_{av} values.

RESULTS AND DISCUSSION

The behaviour of flavonoids on PGM 2000 indicates a definitive correlation between K_{av} values and structure (Table I and Figs. 2 and 3). On interpretation of the K_{av} values it was found that the adsorption effect dominates the sieving effect. The degree of adsorption depends on the number and position of the free hydroxyl groups. The higher the number of the hydroxyl groups, the stronger the degree of

TABLE I

K_{av} VALUES OF FLAVONOIDS ON PGM 2000 AND SEPHADEX LH-20 IN METHANOL

Compound	*Substituents*	K_{av}	
		PGM	*LH-20*
Flavone			
	7-OH	1.9	1.1
	4′-OH	2.0	1.1
	2′-OH	2.2	1.1
	6-OH	2.4	1.2
	5-OH	2.6	1.3
	3-OH	3.0	1.4
Apigenin	5,7,4′-OH	3.0	1.6
Luteolin	5,7,3′,4′-OH	5.3	2.2
Kaempferol	3,5,7,4′-OH	7.4	3.2
Kaempferide	3,5,7-OH,4′-OCH_3	6.4	3.0
Robinetin	3,7,3′,4′,5′-OH	7.9	3.3
Quercetin	3,5,7,3′,4′-OH	9.6	3.7
Morin	3,5,7,2′,4′-OH	36.1	1.8
Myricetin	3,5,7,3′,4′,5′-OH	14.5	4.2
Rhamnetin	3,5,3′,4′-OH,7-OCH_3	9.4	3.2
Isorhamnetin	3,5,7,4′-OH,3-OCH_3	6.9	2.9
Tamarixetin	3,5,7,3′-OH,4′-OCH_3	7.3	3.0
Cosmosiin	5,4′-OH,7-Glucoside	3.0	1.7
Luteolin-7-glucoside	5,3′,4′-OH,7-Glucoside	4.0	2.3
Luteolin-5-glucoside	7,3′,4′-OH,5-Glucoside	2.2	1.8
Isoquercitrin	5,7,3′,4′-OH,3-Glucoside	3.2	1.9
Quercitrin	5,7,3′,4′,3-Rhamnoside	3.4	2.0
Hyperoside	5,7,3′,4′,3-Galactoside	3.0	1.8
Rutin	5,7,3′,4′,3-Rutinoside	2.4	1.3
Myricitrin	5,7,3′,4′,5′-OH,3-Rhamnoside	3.9	2.4
Kaempferol-rhamnoglucoside	5,7,4′-OH,3-Rhamnoglucoside	1.0	0.5
Rhoifolin	5,4′-OH,7-Rhamnoglucoside	2.6	1.7
Robinin	5,4′-OH,3-Rhamnogalacto-side,7-Rhamnoside	1.1	0.5
Flavanone			
Naringenin	5,7,4′-OH	4.7	2.3
Hesperetin	5,7,3′-OH,4′-OCH_3	4.8	2.2

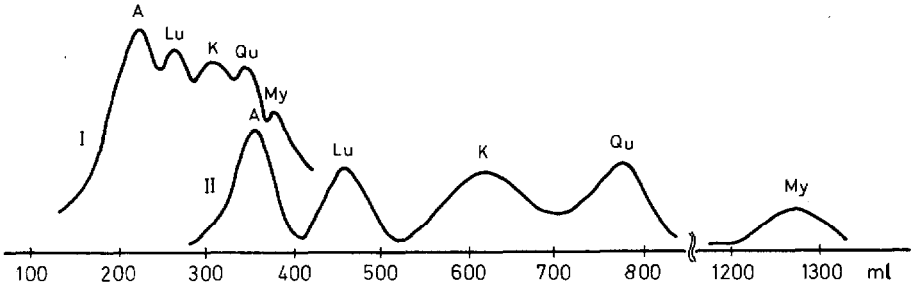

Fig. 2. Separation of flavonoid aglycones on Sephadex LH-20 (I) and Merckogel PGM 2000 (II) in methanol: apigenin (A), luteolin (Lu), kaempferol (K), quercetin (Qu) and myricetin (My); flow-rate, 2 ml/min; detection, 350 nm.

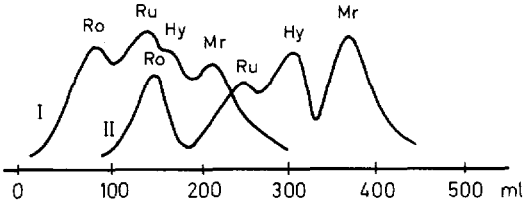

Fig. 3. Separation of flavonoid glycosides on Sephadex LH-20 (I) and Merckogel PGM 2000 (II) in methanol: robinin (Ro), rutin (Ru), hyperoside (Hy) and myricitrin (Mr); flow-rate, 2 ml/min; detection, 350 nm.

adsorption. The values in Table I, especially for the monohydroxyflavones, indicate the dependence of the K_{av} on the acidity (position) of the hydroxyl groups. The higher the acidity of the flavonoids, except 5-hydroxyflavone, the faster their elution from the column. The acidity increases from 5-OH (pK_a 11.56), to 3-OH (pK_a 9.6), to 6.OH (pK_a 9.09), to 2-OH (pK_a 8.85), to 4-OH (pK_a 8.28) to 7-OH (pK_a 7.39)[8]. In comparison with Sephadex LH-20, PGM 2000 generally retards flavonoids more and hence a longer elution time is required (with the exception of Morin, which possessed a distinct retarding effect on PGM 2000). For most of the flavonoids a better separation was achieved. The K_{av} values of several flavonoids on Sephadex LH-20 and PGM 2000 were compared under the same conditions (Table I). PGM 2000 could be employed for the separation of flavonoids from different plant extracts. The gel is regenerated by washing it thoroughly with 0.05 *N* sodium hydroxide.

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