(1:3). Flavonols from the fern *Pityrogramma chrysoconica* were separated using bezene with increasing quantities of butanone (and methanol) as solvent.[91] Aurones and chalcones and flavonols have been purified on a PVP column using methanol as eluting solvent. However, water-methanol combinations are required for the elution of biflavones from a polyamide-celite column[57] and for the separation of flavanones from chalcones.[56]

### *1.3.5 Sephadex gel*

Sephadex gel is an excellent stationary phase. It is a highly cross-linked dextran on which separations are obtained on the basis of molecular size[22] and the substances are eluted in order of decreasing molecular size. The gel must be swollen in water prior to use (12 hr) and the extent to which individual gels swell (water regain) determines the molecular weight range of compounds which can be separated on that gel (Sephadex G series). Commercially available products include: G-10 (for MW 0-700) and G-25 (for mol. wt. 100-1500). However, compounds other than dextran have different affinities for the gel. The hydroxypropylated dextran gel, Sephadex LH-20 (Pharmacie sephadex LH-20) is designed for use with organic solvents or water/solvent mixtures and the exclusion limit-for this gel is MW 2000 and 10,000. Both of these Sephadex types have been used in the separation of flavonoids.

Adsorption of flavonoids is associated with free phenolic hydroxyl groups. Thus, Johnston et al[39], using sephadex LH-20 with methanol as solvent, observed a general correlation between the elution volume ($V_e$) of the flavonoid aglycons and the number (but not the acidity) of the free phenolic hydroxyls present (see Table 1). Here relative elution rates are presented as $V_e/V_o$ where $V_e$ = elution volume or total amount of solvent required to elute sample and $V_o$ = void volume or the column.

In Table 1, the high $V_e/V_o$ values for quercetin and myricetin (8.3 and 9.2) when compared with the low $V_e/V_o$ values for their methyl ethers (3.4 and 3.1) clearly indicate that adsorption is a major factor in the retention of these aglycones on the column. Some specific examples of separations based on adsorption are given in Table 2. The solvent systems that have been used for elution of Sephadex columns for different flavonoids are given in Table 3.

**Table 1 :** $V_e/V_o$ of Flavonoids on Sephadex LH-20 in $CH_3OH$

| Compound[a] | Substituents | $V_e/V_o$[b] |
|---|---|---|
| **1) Flavones and Flavonols** | | |
| Apigenin | 5,7,4′-OH | 5.3 |
| Luteolin | 5,7,3′,4′ | 6.3 |
| Resokaempferol | 3,7,4′-OH | 5.9 |
| Fisetin | 3,7,3′,4′-OH | 6.6 |
| Robinetin | 3,7,3′,4′5′-OH | 7.4 |
| Kaempferol | 3,5,7,4′-OH | 7.5 |
| Quercetin | 3,5,7,3′,4′-OH | 8.3 |
| Morin | 3,5,7,2′,4′-OH | 4.4 |
| Myricetin | 3,5,7,3′,4′,5′-OH | 9.2 |
| 3-*O*-Methylquercetin | 5,7,3′,4′-OH-3-OMe | 5.6 |
| Azaleatin | 3,7,3’,4′-OH, 5-OMe | 5.9 |
| Isorhamnetin | 7,5,7,4′-OH, 3′-OMe | 7.0 |
| Tamarixetin | 3,5,7,3′-OH, 4′-OMe | 7.3 |
| Rhamnetin | 3,5,3′,4′-OH, 7′-OMe | 7.7 |
| Penta-*O*-methylquerecetin | 3,5,7,3′,4′-OMe | 3.4 |
| Hexa-*O*-methylmyricetin | 3,5,7,3′,4′,5′-OMe | 3.1 |
| Quercetin pentaacetate | 3,5,7,3′,4′-OMe | 2.7 |

| | | |
|---|---|---|
| **2) Flavanones & Dihydroflavonols** | | |
| Naringenin | 5,7,4′-OH | 5.4 |
| Eriodictyol | 5,7,3′,4′-OH | 5.8 |
| Taxifolin | 3,5,7,3′,4′-OH | 5.5 |
| **3) (+)-Catechin** | 3,5,7, 3′,4′-OH | 5.2 |

[a] Sample : 2.5 mg/0.5 ml ; flow rate, 3.5 ml min-1.
[b] under these conditons : $V_e/V_o = 2.2\ K_D + 1$.

**Table 2**

| Flavonoid | Sephadex | Solvent | Reference |
|---|---|---|---|
| 1) Anthocyanins | G-25 | 60% aq. alcohol (+ acid) | Somers[71] |
| | | aq. acetone (+ acid) | Somers[72] |
| | LH-20 | Methanol (+ acid) | Aslen et al[3] |
| 2) Flavonol digallates | LH-20 | $CH_3OH$-$CHCl_3$ (1 : 1) | Coxon et al[13] |
| | | $CH_3OH$-$CHCl_3$-light petroleum (2 : 1: 1) | |
| 3) Molybdate complexes of flavonols, dihydroflavonols and flavanones | G-25 | 0.001M Sodium molybdate | Woof & Pierce[93] |
| 4) Flavan-3-ols procyanidin dimers & procyanidin oligomer | LH-20 | EtOH or EtOH-propan-1-ol (1 : 1) | Thompson[80] |

**Table 3**

| Sephadex type | Solvent system | Ref. |
|---|---|---|
| G-10 | Water | 9 |
| G-10 | $H_2O$ + $CH_3OH$ (6:4) | 85 |
| G-25 | $H_2O$-acetone (8 : 2 → 6.4) | 59a |
| LH-20 | $CH_3OH$-$H_2O$ (3 :7) | 54 |
| LH-20 | $CH_3OH$-$H_2O$ (8 :2) | 33 |
| LH-20 | $CHCl_3$-$CH_3OH$ (9 :1) | 42 |
| LH-20 | $CH_3OH$-$H_2O$ (3 :1) | 25 |
| LH-20 | $Me_2CO$-$CH_3OH$-$H_2O$ (2 : 1 : 1) | 24 |

Approximate MW values may be obtained in cases where separation is based primarily on molecular size (Fischer[22]). These are established by measuring elution volume of the unknown and by applying this figure to the straight line graph of elution volumes versus log MW, predetermined for a range of chemically similar compounds (For dihydroflavonoids, see Porter and Wilson[62]).

Sephadex gels have been used for semimicro scale separation of flavonoids. For example, Johnston et al[39] separated 250mg mixtures of quercetin and rutin on LH-20; Porter[62] separated a 2g mixture of catechin and dihydroquercetin on G-25 (column dimensions 30 cm × 2 cm) using water saturated sec-butanol; Thompson et al[80] separated procyanidins on LH-20.

## *1.4 Paper Chromatography*

This technique which has occupied a dominant position in flavonoid analysis and separation has been reviewed by Mabry et al.[48] It is suitable for the separation of complex mixtures of all types of flavonoids and their glycosides. It is highly convenient for isolating both small and relatively large amounts of flavonoids and for preliminary analysis of a plant extract for the presence of flavonoids. Above all, it is cheap because of the low cost of the necessary equipment and materials.

Paper chromatographic analysis is comm-only carried out on Whatman No.1, No.3 or 3MM paper for optimum resolution. It is folded so that the paper is secured in a trough for descending chromatography. A solution of the sample in acetone is then applied (spotted) to the paper at a point about 8 cms in from the side edge and 3 cm in from the last fold. The solution should be applied at this centre; drying of the spot being helped by the use of a hand dryer. The amount should be applied sufficiently so that no smearing results. Chromatographic tank such as Shandon Pan Glass chromato-tank should be used.