(B) Two dimensional cellulose TLC :
H_2O, 5% CH_3OH in H_2O, n-BuOH–HOAc–H_2O (4:1:5)
(C) Cellulose mixed with 3% by weight of polyamide is useful for a wide range of flavones and flavonols : 15% acetic acid as solvent.
Aurones and chalcones : n–PrOH–HOAc-water (1:1:1), i-PrOH–Me_2CO-water (5:1:4)
Flavylium Salts : HCO_2H–HCl–H_2O (10:1:3), Amyl alc-HOAc–H_2O (2:1:1), Forestal (HOAc–HCl–H_2O 30:3:6), 60% HOAc
(D) Using SiO_2-cellulose mixture
Anthocyanins isolated in .mg quantities : Me_2CO-0.5N-HCl (1:3), n-BuOH–2N–HCl (1:1), H_2O–HCl–HCO_2H (8:4:1)
(E) Addition of 10% polyamide (PVP) to cellulose
Anthocyanins : n-BuOH–HOAc–H_2O (4:1:5), AcOH–H_2O–HCl (15:82:3)

1.5.3 Separations on polyamide

An excellent polyamide powder may be prepared from polyamide pellets, which is described by Myler et al.[94] Polyamide separates flavonoids either by partition or adsorption processes depending upon the solvent type used.[18] The adsorption process is favoured with water-alcohol mixtures and is not suitable for flavonoids aglycones. However, when Lipophilic solvents such as $CHCl_3$–CH_3OH-butanone (60:26:14, water saturated) are used, partition processes are operative. Hence flavonoid aglycones are resolved. Two-dimensional separations on polyamide must use an aqueous solvent in one direction and a lipophilic solvent in the other.
(A) Hydroxyflavones and flavonols : C_6H_6–MeEtCO–MeOH (3:1:1) or (4:3:3), $CHCl_3$–C_2H_5OH–MeEtCO-acetyl-acetone (16:10:5:1).
(B) Polyacetylated or polymethylated flavones and flavonols : Light petroleum-C_6H_6–MeEtCO–MeOH (50:40:5:5) (30:60:5:5) or (60:30:5:5) or (60:28:3:7)
(C) Hydroxy flavonoids and flavonols : 90% HCO_2H, HOAc–H_2O–HCl (30:10:3)
(D) Flavones from liverworts and algae[50] : MeOH–HOAc–H_2O (18:1:1)
(E) Catechins, chalcones, flavonones and flavylium salts : H_2O–C_2H_5OH-acetylacetone (4:2:1)

The removal of compounds has been made by Markham[50] by using a plate, one half of which is spread with polyamide, and the other with cellulose. The compound to be isolated was chromatographically separated on the polyamide, run through to the cellulose powder with the same solvent and then eluted from the cellulose powder with methanol.

1.5.4 Separations with High Performance Thin Layer Chromatography (HPTLC)

This technique provides better separations than TLC of complex flavonoid mixtures. HPTLC is a development of TLC carried out using small particles, usually about 5 μm in diameter, all of a closely similar size. Rapid separation can be achieved horizontally by linear, circular and anticircular development.[37,40,41] An application is reported by Hiermann and Kartnig[31] who used benzene-ethyl acetate-formic acid (40:10:5) for separation of numerous flavonoids on HPTLC silica gel plates.

The material of widest application is the octadecyl bonded reverse-phase silica gel or RP-18. In certain cases, RP-2, and RP-8 materials have been applied to the separation of flavonoids.[4,87] Methanol-water mixtures containing an acid (formic acid or acetic acid) are used for flavonoid aglycones.[32] For example,

CH_3OH–H_2O-formic acid (28:10:5)[32]
CH_3OH–H_2O-formic acid (28:4:5)[32]
CH_3OH–H_2O-Acetic acid (70:28:2)[4]
EtOH–H_2O (55:44)[87]

1.6 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is one of the most powerful methods for the analysis of flavonoids. It has become more popular as a method for their isolation on a semiprepararive or preparative scale. An excellent and very useful review is that of Kingston[44] and other reviews are of Van Sumere et al,[88] Pryde and Gilbert[63] and Engelhardt.[20] In the flavonoid field, HPLC has been mainly used as an analytical technique, e.g., for quantitative determination of plant constituents, for checking the purity of natural samples and for chemo-taxonomic comparison. It has also been used for the isolation of flavonoids on a preparative scale.

HPLC is somewhat skin to GLC except that the carrier gas is replaced by a solvent or solvent mixture. In principle, it is ideally suited to the chromatographic analysis (both quantitative and qualitative) of more volatile compounds. Advantages claimed for HPLC analysis include (i) short analysis time, (ii) high resolution, (iii) no derivatization required, (iv) no risk of thermal decompositions and (v) easy quantification.

1.6.1 Separation on silica gel columns

This column packing material, rarely used but has been recommended for the separation of nonpolar or weakly polar flavonoids. HPLC using Lichrosorb Si 60 as adsorbent and a mixture of heptane-propan-2-ol (60:40)