Flavone aglycones have been separated with $CHCl_3$–MeOH-mixture.[42] Good separations were obtained with $CHCH_3$–MeOH–H_20 in the proportions (80:20:1)[33] or in the proportions (65:20:2) and (80:18:2) or EtOAc–Me_2CO-water (25:5:1),[83] or C_6H_6–$CHCl_3$ (1:1)– or $CHCl_3$–Et-OAc for less polar aglucones.

1.3.2 Alumina (Al_2O_3)

***Eluotropic series* (Woelm):** Light petroleum– CCl_4–C_6H_6–$CHCl_3$ (alcohol free)–$(C_2H_5)_2O$–CH_3CO_2Et–pyridine–Me_2CO–n–PrOH–EtOH–CH_3OH–water.

Alumina according to 'Brockman scale' of activities ranges from I to V and these categories correspond to water contents of 1,4,7,10 and 19% (w/w) respectively. Alumina is marketed in three types: acidic (pH 4), neutral (pH 7.5) and basic (pH 10).

Alumina finds little use in the separation of flavonoids because it forms complex, it forms complex with 4–keto–5–hydroxyl and 4-keto-3-hydroxy system which are present in many flavonoids[61] and also with *o*-dihydroxy group[49] on the flavonoids nucleus. Neutral alumina (activity I) has been used successfully for the separation of completely methylated and fully substituted flavonols.[55,17] Some biflavonoids have also been purified. In general, alumina is rarely used for separation of flavonols.

1.3.3 Cellulose [Microcrystalline cellulose (Merck) and Whatman CF-11]

Cellulose column chromatography is a scaled-up form of paper chromatography, and is suited to the separation of all classes of flavonoids, specially glycosides from one another or from aglycones and for the separation of less-polar aglycones. It is used for separations based on both adsorption and partition (depending upon the solvent used), although a distinction between the two is often difficult to make. In principle, the full range of solvents developed for use in paper partition chromatography is available for partition column chromatography and many have been used with success. Cellulose powder has a low capacity and limited resolving power. Therefore, there has been a noticeable trend away from cellulose to polyamides which offer equivalent versatility but much higher capacity and resolving power.

The solvents for cellulose column are of aqueous alcohols and acid types. Some examples are as follows

1) Water-saturated butanol : Flavones
2) 2% aqueous acetic acid : Flavan-3,4-diols, dihydroflavonols
3) 5% aq. MeOH-H_2O-General : Flavanones, 3-methoxyflavones
4) H_2O-15% HOAc (v/v) : Glycosides, flavonols, chalcones

Cellulose column chromatography has not been suggested for the separation of flavylium salts.

1.3.4 Polyamide [Polyclar AT, Polypenco 66D, Polyamide (Woelm)]

Elutropic Series : Water-methanol-acetone-aq-NaOH-formamide-DMF-aqurea (Anderson & Sowers,[2] Endres[19])

Polyamides are mainly of the Perlon-type (Polycaprolactam), Nylon-type (polyhexamethylene-diamine adipate) or Polyclar-type (Polyvinylpyrroli-one, PVP). They must be thoroughly prewashed with MeOH and H_2O. All have a high capacity for phenolic hydroxyl groups via their amide carbonyl functions.[2,19] The elution of phenolics from columns of polyamide depends either on the ability of the solvent to replace the phenol on the H-bonding site, in which case hydrophilic solvents are used, or the ability of the solvent to form even stronger bonds with the phenol than does the polyamide (e.g. urea). The strength of attachment of phenolic compounds depends to a large extent on the number and type of H-bonds formed.[19]

Like cellulose, polyamide is suitable for the separation of all types of flavonoids.[48] However, it has the advantages over cellulose of higher capacity and higher resolution. In addition, solvents different from those commonly used in paper chromatography are employed and hence the two methods complement, rather than duplicate each other.

The current literature abounds with examples in which column chromatography has been used for the separation of flavonoids. Excellent separation of anthocyanins has been achieved. Using an acid-washed PVP, Van Teeling et al[89] separated anthocyanins from co-occurring flavonols and anthocynidins, all of which moved more slowly than the anthocyanins when 0.25N HCl was used as solvent.

For the separation of flavonoid aglycones, non-aqueous solvents seem to be the most satisfactory. For instance, a mixture of mono-, di- and trimethylated kaempferol and quercetin derivatives form *Larrea cuneifolia* was resolved on polyamide with Egger's solvent ($CHCl_3$: CH_3OH : MeEtCO : 2,4-pentanedione, 20:10:5:1) as eluent.[85] Generally this solvent $CHCl_3$: EtOH : MeEtCO : Me_2CO (40:20:5:1) is used for aglycones with decreasing level of $CHCl_3$. Methylated luteolin and quercitin derivatives were separated[1] by the use of $CHCl_3$-ethyl acetate (3:1) followed by increasingly more polar solvent such as $CHCl_3$-CH_3OH-MeEtCO-acetone (20:10:5:1) and (10:10:5:1) and $CHCl_3$-MeOH