of low viscosity also have a great influence on mobile-phase velocity. Mobile phases based on mixtures of higher alcohols and water show very slow migration rates.

Most laboratories use commercially available precoated TLC plates. A variety of materials and qualities of layers are available, and there is no need to prepare plates in the laboratory. In addition, there are several ways to adjust the selectivity of commercially available layers by impregnation with a modifier, generally achieved by immersing the layer into a solution of the modifier and allowing the solvent to evaporate. Common impregnating reagents used in the TLC of carbohydrates are phosphates (16,32–34), borates (35), and boric acid (36,37). Boric and boronic acids can form reversible complexes and are often used to differentiate isomers with vicinal hydrogen-bonding functional groups (38). Other impregnating reagents include bisulfite, known for its characteristic addition reactions with aldoses and ketoses (24,37), molybdate, tungstate, and other metal salts (39,40). The highest separation efficiency can be obtained by using precoated high-performance TLC (HPTLC) plates (41).

The complexity of carbohydrates and the limited separation capacity of TLC can cause the overlap of some spots of the reference standard mixtures. This is not always a disadvantage, because it is uncommon for some sugars to occur together in a sample. For instance, L-fucose arises from animal glycoproteins, L-arabinose from plant polysaccharides, and D-fructose from specific enzyme inversions of D-glucose.

A. Layers

The most frequently used layers for separation of carbohydrates are cellulose, silica, silica 50,000, and amino-bonded silica.

1. Cellulose

Precoated TLC plates with both native and microcrystalline cellulose layers have been used for the separation of carbohydrates and other very polar compounds for several years (8a) (Table 1). An advantage of these low surface area sorbents is that most of the separations previously achieved by paper chromatography can be obtained by TLC using the same solvent systems (8a). It is generally assumed that a partition mechanism is responsible for retention on these materials. Advantages of cellulose plates over paper chromatography include a shorter development time, less spot diffusion, and less background staining with some spray reagents. Cellulose thin-layer plates are sometimes modified by impregnation with buffers or salts. The best results can be obtained on commercially available HPTLC plates coated with a special grade of microcrystalline cellulose (42a). One cellulose HPTLC application involves inositol phosphate analysis (42b).

At present, cellulose layers are often replaced by synthetically prepared wide-pore silica (Si 50,000). This very low surface area material has been recommended for the separation of substances similar to those normally separated on cellulose (42a). Compared to cellulose, the silica gel thin-layer sorbents do not swell in organic solvents, and the plates can be used with aggressive visualizing reagents (18).

2. Silica

Silica gel thin layers are the most commonly used sorbents in TLC of carbohydrates. These layers are suitable for most classes of sugars and sugar derivatives, the major exception being intact complex polysaccharides. Numerous solvent systems provide relatively good separation of monosaccharides, disaccharides, and lower oligosaccharides (Tables 2 and 3); malto-oligosaccharides (14,16,43,44); amino sugars (27,40) (Table 4); aminoglycoside antibiotics (44,46); sugar alcohols (48,49) (Table 4); uronic acids (50,51); derivatives (52,53) (Table 4); and cyclodextrins (54). Another advantage of TLC and HPTLC silica gel layers is their chemical stability against almost all solvents, strong acids, and other corrosive reagents used in postchromatographic derivatization procedures. To obtain better selectivity of some sugars of interest, these layers are sometimes modified by pretreatment with a suitable buffer or with inorganic salts. Alternatively, sugars may be derivatized prior to analysis to improve separation or visualization (Table 5).

Separation time required for a single development of silica gel layers varies from a few minutes to a few hours and depends on the solvent system composition, the layer quality (i.e.,