Spot tailing can be minimized or prevented by impregnating the amino-bonded layer with a buffer. Impregnation can be done by immersing the plates in a 0.2 M aqueous solution of monosodium dihydrogen phosphate for 15 min. After draining, the plates should be dried in a vacuum oven at 70°C (32). The result of this procedure is neutralization of the aminopropyl groups, which consequently drops the pH of the layer to about pH 6.2. An additional advantage of impregnating the aminopropyl-bonded silica HPTLC plates with monosodium dihydrogen phosphate is that the sugars are visualized more readily after derivatization because the background is cleaner. The impregnation of the layer influences the reaction of some postchromatographic derivatization reagents. Some reagents for visualizing sugars were found to be ineffective when impregnated aminopropyl-bonded silica plates were used. With regard to detection using the aniline–diphenylamine reagent, all sugars can be detected on these phosphate-impregnated aminopropyl-bonded plates if phosphoric acid is substituted for sulfuric acid (32). Amino-bonded layers can also be impregnated with other phosphate buffers. A potassium dihydrogen phosphate solution, at concentrations between 0.2 and 1 M, was more satisfactory for multiple developments owing to its lower solubility in the mobile phases (16). Precleaning of amino-modified silica plates can be achieved by successively developing the plates in pyridine–ethyl acetate–water–glacial acetic acid–propionic acid (50:50:10:5:5) and 1-propanol–nitromethane–water–glacial acetic acid (30:30:10:1) (59).

4. Other Layers

Kieselguhr and combinations of silica gel and kieselguhr have also been used for separation of sugars for a number of years (8a). Better results can now be obtained with the newer commercially available plates using sorbents such as Si 50,000. The same is valid for traditional polyamide layers, because one can usually obtain better results with other supports.

Alumina is less suitable for the separation of most carbohydrates. Owing to the presence of oxide ions, the surface of alumina is quite basic (pH approximately 12). Acids with pK_a lower than 13 transfer protons to this surface, producing charged conjugate bases that are strongly absorbed.

Chemically bonded layers, with the exception of the amino-bonded silica gel, are not suitable for carbohydrate analysis. Cyano- and diol-modified silica gel sorbents are not typically used for thin-layer analysis of carbohydrates. These bonded layers exhibit low retention and are generally inferior to the polar amino-modified silica for separation of carbohydrates (60). Retention of carbohydrates is even lower on reversed-phase bonded layers. Although these layers are less suitable for carbohydrate separation, they can be used for separation of some sugar derivatives. Reversed-phase bonded layers have been used for separation of aminoglycoside antibiotics (46), and silanized silica gel has been used for thin-layer electromatography (electrophoresis) of some sugars (61,62).

B. Solvent Systems

Because of the complexity of carbohydrates as a class, there is no universal solvent system optimized to give a complete profile of carbohydrate content in every situation. Various solvent systems using mixtures of water with acetonitrile, alcohols (methanol, ethanol, 1-propanol, 2-propanol, 1-butanol), acetone, acetic acid, ethyl acetate, and pyridine are efficient in the separation of some sugars. The mobility of carbohydrates on polar layers depends primarily on their molecular weight and on the number of hydroxyl groups; disaccharides, for example, show higher retention than monosaccharides. Separation of oligosaccharides and lower polysaccharides can usually be achieved through multiple development of plates with solvent systems containing high proportions of water. For example, mixtures of D-glucose-containing oligo- and polysaccharides (with degrees of polymerization up to 35 glucose units) can be separated on silica gel 60 TLC or Si 50,000 HPTLC plates utilizing such methodology (63) (Tables 2, 3, and 6).

Solvent systems based on a mixture of acetonitrile and water show short developing times (55) and can be used on silica gel, Si 50,000, and amino-bonded layers (20). These developing systems are frequently used and give excellent results, especially when used in combination with multiple developing techniques. With a simple variation in the water or buffer content of the