particle size and pore diameter), and the developing technique. Relatively good resolution of some common sugars can be obtained by fast-migrating solvent systems such as mixtures of acetonitrile and water. Single development of an HPTLC plate is usually finished in less than 10 min. This enables fast routine analyses and also permits the extensive use of the multiple development technique for enhancing the efficiency of separation (55). To minimize the influence of oxidized binders on detection and quantitative evaluation, it is advisable to preclean the commercially available TLC plates by developing them in a mixture of chloroform and methanol (1:1) or in pure methanol, followed by drying and reactivating. Surface-active silica gel thin layers tend to absorb water molecules from the surrounding atmosphere, leading to a reduction in their activity and in their chromatographic retention capacity. Therefore, it is also advisable to standardize these surface activities to obtain reproducible chromatographic results. This can be done by activating the plate with heat and storing it in a desiccator until it is needed. Carbohydrates can also be on silica gel plates with a sample concentration zone, but such plates are not very effective when solvent systems with a high water content are used. The resolution of sugars on silica TLC plates, although not as robust as with other systems such as HPLC, is sufficient for a number of applications. For example, Cline et al. (3) were able to determine, using TLC, that maltose, not trehalose as had been previously reported, was a host sugar utilized by parasitic flukes (since confirmed by GC-MS).

Silica 50,000 (Si 50,000) is a synthetically prepared inactive silicon dioxide with chromatographic properties comparable to those of the naturally occurring kieselguhrs or diatomaceous earth (a natural product based on the cell walls of diatoms, which consist mainly of silicic acid). Silica 50,000 sorbent has a uniform large pore size of 5000 nm and was originally used as a concentration zone on silica gel 60 TLC plates. Because this wide-pore material has a very low surface area and low activity, it can also be applied as a stationary-phase support for normal-phase partition chromatography. It is very suitable for separation of polar compounds such as carbohydrates, and the time required for analysis is shorter and the resolution achieved better than for analyses based on paper chromatography (42a). Separation of different types of carbohydrates can be attained with water-based solvent systems such as those commonly used with more typical silica gel sorbents.

3. Aminopropyl-Bonded Silica

Amino groups added as aminopropyl groups bonded to silica gel, or simply as amino-silica gel (NH_2-silica gel), are particularly useful as sorbent modifications for carbohydrate analysis (32,56). As is the case with other polar bonded phases, such sorbents can be used in either the normal-phase or reversed-phase mode. An anion-exchange mechanism can also influence the separation. Chromatographic properties of amino-silica gel layers are similar to those of nonmodified silica gel, and some identical solvent systems can be used with both sorbents (Table 6). The main advantage of amino-bonded silica is that it affords simple detection of separated sugars by a thermal in situ reaction (56–59). Sugars are readily converted, leading to stable, intensely fluorescing derivatives. The thermal treatment, after development, does not lead to a discoloration of the chromatograms as is often the case with chemical postchromatographic derivatization (56). A disadvantage of the aminopropyl-modified silica gel layer is the tendency for glycosylamine to form between reducing sugars and the amino groups on the stationary phase (32). The separation of sugars on aminopropyl-bonded thin layers is usually done with water-containing solvent systems such as acetonitrile–water mixtures. Due to the basicity of the layer, the pH of the aqueous mobile phase is high, exceeding pH 9 (16). This is a favorable condition for interactions between reducing sugars and the aminopropyl groups of the bonded silica. Sugar residues that are especially apt to interact covalently with the aminopropyl groups are those that contain appreciable levels of the acyclic (aldehydic) forms in tautomeric equilibrium with their ring (furanose and pyranose) structures. Examples of such sugars are 2-deoxyglucose, xylose, rhamnose, galactose, and mannose. The result of this glycosylamine formation, which is common with sugars containing more than 0.05% of the aldehydic form (in solution), is that these sugars show practically no mobility after being spotted and thus remain in their original positions on the plate. The reaction can also influence spot or band shapes of even those sugars that have very low levels of acyclic forms such as glucose and fructose (32).