

the use of sulfuric acid charring techniques, and sample zones can be easily scraped from the glass support for subsequent elution of compounds from the sorbent. Binder-free silica gel plates containing a small amount of colloidal silica to aid layer adherence are also available. For detection of zones by fluorescence quenching, plates are impregnated with indicator compounds (e.g., manganese-activated zinc silicate) that cause the layer to fluoresce uniformly when exposed to 254 or 366 nm UV light. Glass is the most inert support material, and its planarity is advantageous when the layer will be scanned for quantitative analysis. Procedures and devices for preparing homemade plates are described in Chapter 3 of the third edition of Fried and Sherma (1). Homemade plates, the quality of which is almost never equivalent to that of commercial plates, are rarely made except when a needed layer is not available or cost is a major consideration.

To remove extraneous materials that may be present due to manufacture, shipping, or storage conditions, it is advisable to preclean plates before use. This has often been done by predevelopment to the top with dichloromethane–methanol (1:1) or the mobile phase to be used for the analysis. The following two-step HPTLC plate cleaning method has been proposed (90) for surface residue removal in critical applications when optimum sensitivity is required for detection and quantification: Develop the plate to the top with methanol, air dry for 5 min, totally immerse the plate in a tank filled with methanol, air dry for 5 min, oven dry for 15 min at 80°C, and cool in a desiccator before use. The routine activation of adsorbents at 70–80°C for 30 min, or at a higher temperature, is often proposed in the literature, but this treatment is not usually necessary for commercial plates unless they have been exposed to high humidity. RP plates do not require activation prior to use. Suggestions for initial treatment, prewashing, activation, and conditioning of different types of glass- and foil-backed layers have been published (91).

A. Adsorbents

Silica gel is by far the most frequently used layer material for adsorption TLC. Some characteristic properties, including porosity, flow resistance, particle size, optimum velocity, and plate height, have been tabulated for three popular brands of silica gel TLC and HPTLC plates (38). Separations take place primarily by hydrogen bonding or dipole interaction with surface silanol groups by using lipophilic mobile phases, and analytes are separated into groups according to their polarity. Typical properties of TLC silica gel are a silanol group level of approximately 8 $\mu\text{mol}/\text{m}^2$; pore diameter of 40, 60, 80, or 100 Å; and specific pore volumes of 0.5–2.0 mL (89). Specific differences in the types and distributions of silanol groups for individual sorbents may result in selectivity differences, and separations will not be exactly reproducible on different brands of silica gel layers (25). Other TLC adsorbents include aluminum oxide (alumina), magnesium oxide [used mostly for carotenoid pigment separations (92)], magnesium silicate (Florisil) (93), polyamide, and kieselguhr (94).

Alumina (95) is a polar adsorbent that is similar to silica gel in its general chromatographic properties, but it has an especially high adsorption affinity for carbon–carbon double bonds and better selectivity toward aromatic hydrocarbons and their derivatives. The alumina surface is more complex than silica gel, containing hydroxyl groups, aluminum cations, and oxide anions, and pH and hydration level alter separation properties (25). It is available in basic (pH 9–10), neutral (7–8), and acid (4–4.5) forms. The specific surface area of aluminas range from 50 to 250 m^2/g (89). The high density of hydroxyl groups ($\sim 13 \mu\text{mol}/\text{m}^2$) leads to a significant degree of water adsorption, and alumina layers are usually activated by heating for 10 min at 120°C before use (89).

Polyamides 6 (Nylon 6; polymeric caprolactam) and 11 (polymeric undecanamide) have surface —CO—NH— groups and show high affinity and selectivity for polar compounds that can form hydrogen bonds with the exposed carbonyl groups. However, depending on the type of analyte and mobile phase, three separation mechanisms can operate with polyamide: adsorption, partition (normal- and reversed-phase), and ion exchange. This has led to separations of compounds from a wide array of chemical classes such as amino acids, phenols, phenolic compounds, carboxylic acids, cyclodextrins (96), coumarins, and flavonoids (97). Polyamide has been impregnated with various metal salts to improve the separation of sulfonamides (98). Separation