C. Chromatographic Techniques

1. Adsorbents and Thin Layers

A variety of adsorbents such as silica gel, alumina, polyamide, and cellulose are available commercially for use in TLC work. Alumina and silica gel are used with or without suitable binder such as gypsum or starch. Mixtures of two adsorbents or adsorbents impregnated with certain reagents such as 8-hydroxyquinoline and various metal ions have also been used successfully to improve resolution. By far the most thin-layer work has been done on layers prepared from water-based slurries of the adsorbents. Even with the same amount and type of binder, the amount of water that is used for a given slurry varies among the different brands of adsorbents. For example, in the case of cellulose the amount of powder to be mixed with water varies depending on the supplier; Serva, Camag, and Whatman have recommended the use of 60–80 mL, 65 mL, and 25 mL of water, respectively, for 10 g of their cellulose powders. These slurries may be prepared by shaking a stoppered flask or by homogenizing for a few seconds with a mechanical mixer. On the other hand, for the preparation of an aluminum oxide slurry (acid, basic, or neutral) it is recommended that one use 35 g of aluminum oxide with 30 mL of water with spreading equipment, and 6 g of adsorbent in 15 mL of ethanol–water (9:1) mixture for pouring directly onto the plate without a spreading apparatus. Korzun et al. (33) used a slurry of 120 g of alumina G in 110 mL of water to prepare 1 mm thick layers for preparative TLC. Cellulose powders in general contain impurities that are soluble in water or organic solvents, which should be removed by washing the cellulose powder several times with acetic acid (0.1 M), methanol, and acetone and drying before use. The layer is made by "turbo mixing" MN (Macherey-Nagel) cellulose-300 (15 g) for 10 min in distilled water (90 mL) and then spreading it to give a 0.25 mm thick layer. The layers are left overnight to dry.

The cellulose layers have several advantages; e.g., they are stable, they can be used with various specific reagents, and they give reproducible data. They are recommended particularly for quantitative evaluation by densitometry. The drawbacks of cellulose layers are that corrosive reagents cannot be used and the sensitivities of detection reactions of certain amino acids are lower than on silica gel layers.

The best known and most widely used adsorbents for TLC purposes are from Merck, but products of other firms can be used satisfactorily. Precoated plates are widely known, and an increasing number of workers use them for the investigation of amino acids and their derivatives. For example, ready-made cellulose layers from Macherey-Nagel (Germany) containing MN cellulose-300 in appropriately bound form are one of the best-known products. Chiralplate from the same firm, for the separation of enantiomers of amino acids and their various derivatives, contains a coating of reversed-phase silica gel impregnated with a chiral selector and copper ions. Use of homemade thin-layer plates has been found to be more convenient in our laboratory, and it is recommended that one not change the brand of adsorbent during a particular set of experiments.

2. Preparation of Thin Plates

A slurry of silica gel G (50 g) in distilled water (100 mL) is prepared and spread with the help of a Stahl-type applicator on five glass plates of 20 × 20 cm to obtain 0.5 mm thick layers. The plates are allowed to set properly at room temperature and are then dried (activated) in an oven at an appropriate temperature (60–90°C) for 6 h or overnight. The plates are cooled to room temperature before the samples are applied.

The same method has been used successfully to prepare plates with silica gel, silica gel–polyamide, and cellulose and with these adsorbents impregnated with a variety of reagents including HDEHP, TOPO, 8-hydroxyquinoline, dibenzoyl methane, and several metal salts (13–30). Brucine (20) and tartaric acid (22) were also mixed in slurries of silica gel as impregnating reagents to resolve enantiomers of amino acids and their PTH derivatives. Mixtures of H_2O–EtOH or other organic solvents can also be used depending on the nature of the impregnating reagents. Citrate (31) and phosphate (32) buffers have also been used for slurrying silica gel in place of water. It is customary to use 0.25 or 0.50 mm thick layers in activated form, but for preparative purposes 1–2 mm thick layers are best (33).