filtered samples may still contain large amounts of interfering compounds such as salts, urea, lipids, or proteins that influence the application and separation. In some cases, these compounds can be removed simply by adding washed resin of an appropriate chromatographic packing material to a sample in a flask, shaking the mixture for 10 min, and filtering the sample or passing it through a column of the mixed bed resin using an appropriate eluent. More commonly, sample preparation involves passing samples, using an appropriate water-based eluent, through disposable, ready-to-use columns packed with various resins (C-18, amino, ion-exchange, size exclusion, or other resins as appropriate). For example, free carbohydrates in aqueous solution can be separated from gangliosides and other polar glycolipids by passing samples repeatedly through C-18 cartridges or minicolumns. Carbohydrates pass through the cartridges or columns. Lipids are retained by the C-18 gel but can easily be obtained, if desired, by elution with methanol. To evaluate the performance of a given cleanup procedure, five to 10 different standard mixtures of sugars should be submitted to the identical procedure. In most instances, this type of solution pretreatment is sufficient for application and separation on TLC plates.

Extraction and sample preparation methods may result in some dilution of the carbohydrates. Bandwise application of samples at the origin using spray-on techniques and modern instrumental applicators enables application of relatively large volumes of solutions (up to approximately 100 μL).

More typically, especially for spotwise application on HPTLC plates, Hamilton syringes or micropipets are used to apply much smaller volumes of sample. To obtain sufficient concentrations, samples may have to be concentrated. However, if the concentration is too high, sample solutions will have relatively high viscosity and surface tension, which prevents the filling and emptying of syringes or micropipets by capillary forces. Therefore, it may be necessary to adjust sample concentration by dilution with methanol until a suitable combination of concentration and viscosity is obtained. Solid samples should be dissolved in distilled water (pH 5.5), dilute acid, or alcohol and water mixtures and treated as solutions (with proper pretreatment, as appropriate).

B. Plant Material

Analysis of free sugars in fruits and vegetables is usually done by extraction of fresh plant tissue with ethanol or methanol or with mixtures of methanol and water. This is followed by concentration of the extract, which removes all the alcohol, and filtration through Celite or centrifugation of the concentrated aqueous extract. The clear solution can be spotted directly on the TLC plate. In some instances it is necessary to dry a sample of a fresh material to constant weight at elevated temperature. The appropriate amount of dry material, usually 1–5 g, is then blended with aqueous ethanol (about 10 mL/g), using an appropriate homogenizer, for 3–5 min at room temperature. The slurry is centrifuged and the clear supernatant decanted. The residue is treated as before, supernatants are combined, the solvent is evaporated, and the dry residue is dissolved in an exact volume of water or aqueous methanol. It may be useful to include, at some stage of the sample preparation, a washing of the extract with light petroleum to remove lipids. However, this is not always necessary. Fruit acids can also influence the separation. They can be removed with disposable solid-phase extraction columns packed with silica gel or ion-exchange resins or by using the following classical procedure: addition of 10% aqueous solution of lead acetate to the clean supernatant to precipitate the fruit acids, centrifugation, removal of the excess Pb ions in the clear solution by H_2S, neutralization of the clear solution with an anion exchanger, and sample cleanup using gel permeation chromatography.

C. Samples with Traces of Sugars

Sometimes samples contain only traces of the sugars of interest or small amounts of disaccharides or oligosaccharides together with the main sugars such as glucose, fructose, and sucrose. The separation and detection of such sugars in complex mixtures by TLC is a difficult and time-consuming task. Purification and enrichment of samples for TLC or for any other chromatographic technique can be accomplished by gel permeation chromatography or by passing the sample through a prepared disposable aminopropyl or polyamide column for sample preparation. These