tioning of initial zones is critical when measurements are to be made by scanning. Automated sample application is preferred for best results in quantitative TLC.

A. Choice of the Sample Solvent

Samples and standards are best prepared in a solvent that dissolves the analytes completely, is volatile, has low viscosity, wets the sorbent layer, and is a weak chromatographic solvent for the analytes. In practice, it may be impossible to find a solvent with all of these properties. For silica gel TLC, it is important to use the weakest (least polar) solvent that allows quantitative dissolution and spotting of the sample, so that preliminary development and separation within the initial spot at the origin does not occur, resulting in significant loss in separation efficiency. The R_f of the compounds of interest should be <0.1 in this solvent. The choice of a weak solvent is more difficult for RP layers because solvents that wet the layer (e.g., acetonitrile, methanol) may be strong (nonpolar) enough to cause predevelopment of the spot. If all or part of the sample is solidified or adsorbed onto the layer surface, a slow dissolution effect can cause significant tailing of the spots.

B. Application of Spots

Instruments and techniques for a sample application are described in Chapter 5 of this Handbook and Chapter 5 of Ref. 1.

Samples and standards are applied to the layer as small round spots by using one of a variety of application devices, for example, a wooden stick with flattened end, glass capillary pipet, or syringe with a 90° needle tip. Drummond microcap micropipets, available in virtually any size between 0.1 and 200 μL (Fig. 3), and 10–50 μL digital microdispensers (Fig. 4) are highly recommended for manual applications for both qualitative and quantitative TLC. For linear or circular HPTLC, initial zone diameter should not exceed 1.5 mm for maximum resolution. Spots for HPTLC can be applied to an exact layer position using a 100 or 200 nL Pt-Ir pipet held in a mechanical device that electromagnetically brings the pipet into reproducible contact with the layer without surface damage (Camag Nanomat). Camag and Desaga also supply completely automated devices with which selectable volumes of samples and standards are applied from vials

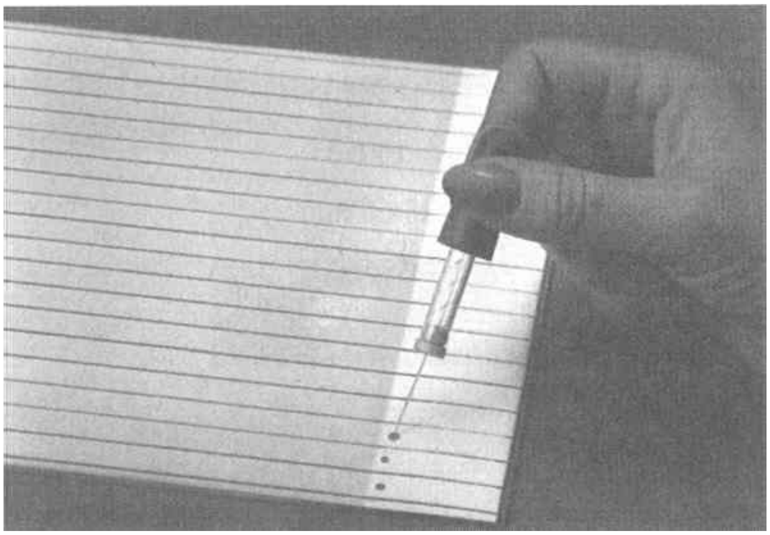

Figure 3 Application of initial zones to a glass-backed 20 × 20 cm channeled preadsorbent silica gel plate using a Microcap micropipet. (Photograph supplied by Analtech.)