dimension. The sheet is dried after the second run and exposed to HCl vapors until all yellow spots turn red or blue. For discrimination between DABTH-Ile and DABTH-Leu, one-dimensional separation on polyamide (143) using formic acid–ethanol (10:9) or one-dimensional separation on silica gel (Merck) using (144) chloroform–ethanol (100:3) is carried out. The successful identification of DABTH amino acids relies on the skillful running of the small polyamide sheet and interpretation of the pattern of spots (141,145).

D. Dinitrophenyl Amino Acids

Use of dinitrophenyl (DNP) amino acids, formed by condensation of 1-fluoro-2,4-dinitrobenzene (FDNB) with the free amino group of an amino acid, was first described by Sanger in 1945 (83). Sanger identified DNP amino acids by paper chromatography. Since then many modifications to the methods of obtaining derivatives of amino acids for sequence analysis and to the identification of such derivatives have been reported, and the use of DNP amino acids for sequencing purposes is rapidly going out of date. Nevertheless, the importance of DNP amino acids is not yet lost. In view of the limited applications of DNP amino acids at present, the methods of preparing these derivatives from standard amino acids or peptides are not described here. However, the details of those procedures can be obtained from Rosmus and Deyl (88) and Bailey (146).

In addition to the references cited previously (83–91), Kirchner (147) presented considerable information on TLC analysis of DNP amino acids based on the literature available up to 1970. Grant and Wicken (148) prepared thin layers (5 plates of 20 × 20 cm × 0.25 mm) from a mixture of 10 g of cellulose MN-300 and 4 g of silica gel H (Merck), homogenized in 80 mL of water. The plates were dried overnight at 37°C and developed in the first dimension in two solvents successively: isopropanol–acetic acid–H_2O (75:10:15) for 15 min, then *n*-butanol–0.15 N ammonium hydroxide (1:1, upper phase). The dried chromatograms were developed in 1.5 M sodium phosphate buffer (pH 6.0) in the second dimension.

In almost all the methods reported, the separation was carried out in groups of water-soluble and ether-soluble DNP amino acids, and for each group mostly two-dimensional TLC was performed. In 1988, Bhushan and Reddy (29) reported a few solvent systems for one-dimensional resolution of DNP amino acids on silica gel plates (Table 14).

The DNP amino acids have been visualized by UV light (360 nm with dried plates; 254 nm with wet ones) or by their yellow color, which deepens upon exposure to ammonia vapors. Thin layers of silica gel usually give an intense purple fluorescence for DNP amino acids under UV light, which masks the presence of very faint spots and decreases the color contrasts. The cellulose–silica mixed layers (148) gave much lower fluorescence and preserved the color contrasts among the various derivatives.

Because of the photosensitivity of these derivatives, it is advisable to carry out their preparation and chromatography in the absence of direct illumination.

IV. RESOLUTION OF AMINO ACIDS AND DERIVATIVES ON IMPREGNATED LAYERS

Thin-layer chromatography of amino acids and derivatives on impregnated plates was reviewed by Bhushan and Parshad (30c) and Bhushan and Martens (30e), and chromatography on thin layers impregnated with organic stationary phases was reviewed by Gasparic (150a); see also Chapter 17 on enantiomer separations in this handbook. The reagents and methods used for impregnation are not to be confused with locating or spray reagents, because the latter are required for identification even on impregnated plates. The various methods used for impregnation include mixing the impregnation reagent with the inert support, spraying it onto the plate, exposing the layer to the vapors of the impregnating reagent, immersing or dipping the plate in the solution of reagent, or allowing the solution to ascend or descend in a normal manner of development. Chemical reaction between the inert support and a suitable reagent can also be considered impregnation. The various explanations given to the role of the impregnating agent in the resolution process include ion pairing, complex formation, ligand exchange, coordination bonds, charge transfer, ion exchange, and hydrogen bonding.