2. Method

Six to eight standard dilutions in an appropriate concentration range for each amino acid are prepared; 2 mL of amino acid solution and 2 mL of buffered ninhydrin are mixed in a test tube, heated in a boiling water bath for 15 min, and cooled to room temperature, and 3 mL of 50% ethanol is added. The extinction is read at 570 nm (or 440 nm for proline) after 10 min. Standard plots of concentration versus absorbance are drawn for each amino acid. The scraped layer corresponding to each spot is extracted with 70% ethanol in a known minimum volume, and ninhydrin reaction is performed followed by spectrophotometry. The concentration of unknown samples is read from the standard plots. TLC with densitometry was used to determine 0.5 mg/L of phenylalanine in blood serum as an indicator of phenylketonuria (181).

B. PTH Amino Acids

The quantification of PTH amino acids is carried out in situ or after elution. For in situ determination, the fluorescence-quenching areas of PTH derivatives are usually measured, against the fluorescent background, at 254 nm. Pataki and coworkers (182,183) used a Turner fluorimeter fitted with a door for scanning chromatoplates and found that the position of the scanner, the standardization of time between scanning and the end of chromatography, the loading volume, the developing distance, and the layer thickness were the important influencing factors for reproducibility. The quantification of PTH amino acids is also carried out by measuring their UV absorbance after they have been eluted from the layer (184). The scraped layer is extracted with methanol overnight and centrifuged for 30 min at 300 rpm, and the spectra of the extracts are recorded in the range from 320 nm to about 230 nm. To obtain reproducible UV absorbances the layers must by washed with methanol prior to development and with chloroform after the separation has been carried out. The quantification of PTH amino acids in our lab during sequence determination of certain plant proteins (96,97) has been practiced as follows. The developed chromatograms are exposed to iodine vapors, and the brownish spots of PTH amino acids are scraped off and thoroughly eluted with 95% ethanol or ethyl acetate, and the iodine is removed by warming the sample tubes in a warm water bath. The optical densities are read at 269 and 245 nm, appropriate blank determinations are carried out, standard plots are drawn, and concentrations of unknown samples are calculated.

C. DNP Amino Acids*

The layer is scraped off the plate and extracted for 5 min with 1 mL of 0.05 M Tris buffer of pH 8.6 at room temperature. Then the slurry is removed by centrifugation, and the clear liquid is evaluated by measuring the optical density at 360 or 385 nm for DNP-proline. For a blank, a similar extract is obtained form a clear spot on the same layer. Pataki and Wang (183) recommended the use of direct fluorimetric quantification (fluorescence quenching) in situ. Silica gel G plates were developed in chloroform–benzyl alcohol–acetic acid (70:30:3) and *n*-propanol–ammonia (7:3), and polyamide plates were developed in benzene–acetic acid (4:1). The spots were scanned by using a Camag/Turner scanner, after being dried in a stream of air, at the scanning speed of 20 mm/min and an excitation wavelength of 254 nm.

VIII. AMINO ACIDS AS CHIRAL SELECTORS

As already noted above, impregnation of adsorbent of thin layers with various types of compounds has resulted in improved separation of various classes of compounds. One of the most interesting features of impregnation is the enantiomeric resolution of a variety of compounds using optically pure selectors as the impregnating reagents. Because the focus of this chapter is on TLC of amino acids, it was considered worthwhile to draw the reader's attention to the application of optically pure isomers of amino acids as suitable chiral selectors for direct enantiomeric separation and

*Based on Ref. 185.