8. Polychromatic reagents: Moffat and Lyttle (42) developed a polychromatic ninhydrin reagent. It consisted of (a) ninhydrin (0.2%) in ethanol (50 mL) + acetic acid (10 mL) + 2,4,6-collidine (2 mL) and (b) a solution of copper nitrate (1.0%) in absolute ethanol. The two solutions are mixed in a ratio of 50:3 before use. Krauss and Reinbothe (43) replaced ethanol by methanol and also achieved polychromatic amino acid detection by joint application of ninhydrin and primary, secondary, or tertiary amines. The layers were first sprayed with diethylamine, dried for 3 min at 110°C, cooled, and then sprayed with 0.2% methanolic ninhydrin and heated for 10 min at 110°C, when the spots of amino acids appeared on a pale blue background. Use of ninhydrin (0.27 g), isatin (0.13 g), and triethylamine (2 mL) in methanol (100 mL) gave spots of amino acids on a yellow background.

Several other reactions have also been used for the detection of specific amino acids (Table 2). Oxalic acid (ethanolic 1.25%), dithiooxamide (ethanolic saturated), or dithizone followed by ninhydrin was used to aid identification and detect amino acids with various specific colors (54a). Acetyl acetone–formaldehyde detected amino acids as yellow spots under UV (54b). By using isatin–ninhydrin (5:2) in aqueous butanol (54c) or by modifying ninhydrin detection reagent by addition of D-camphor (54d) and various acids (54e), identification of amino acids was improved. Spraying of layers with 1,3-indanedione or *o*-mercaptobenzoic acid prior to ninhydrin improved sensitivity limits and color differentiation in amino acid detection (54f). 3,5-Dinitrobenzoyl chloride was used for detecting amino acids at a 3–4 μg level (54g), and synchronization of timing was achieved by coupling pneumatic nebulization with optical fiber–based detection in a chemiluminescence TLC system to detect dansyl amino acids (54h). A new spray reagent, *p*-dichlorodicyanobenzoquinone, detected amino acids with 0.1–1 μg detection limits and produced various distinguishable colors that facilitate identification (54i). Chromatograms sprayed with ninhydrin (0.3 g ninhydrin in 100 mL of *n*-butanol plus 3 mL of glacial acetic acid), air-dried for 5 s, resprayed, and heated in an oven at 110°C for 10 min gave the best sensitivity, stability, and color differentiation in comparison to different recipes of ninhydrin and fluorescamime sprays (72a).

D. TLC Systems for Amino Acids

An extensive bibliography of literature references from 1974 to 2000 on the TLC separation of amino acids has been provided by Sherma (55a–f), Sherma and Fried (56), and Zweig and Sherma (57). Silica gel and cellulose have been the major choice of adsorbents for one- or two-dimensional resolution of amino acids. These have been used as is (untreated) or impregnated with some other reagent employing a large number of solvents. Some of the successful systems for one- and two-dimensional resolution of amino acids are given in Tables 3 and 4, respectively. Sleckman and Sherma (69) compared the separation of amino acids on silica gel, cellulose, and ion-exchange thin layers using *n*-butanol–acetic acid–water (3:1:1) and discussed advantages and disadvantages

Table 2 Detection Reactions for Specific Amino Acids

Amino acid	Reagent	Reference
Arg	8-Hydroxyquinoline	44
Arg	α-Naphthol, urea, Br_2	45
Arg, His, Lys	BiI_3	46
Asp	Ninhydrin, borate soln, HCl	47
Cys, Met	NaN_3, iodine	48
Gly	*o*-Phthalaldehyde, KOH	49
His	Sulfanilic acid	50, 51
Ser, Thr, Tyr	Sodium metaperiodate, Nessler reagent	53
Trp	*p*-Dimethylaminobenzaldehyde	54