

mol/L) as the mobile phase showed excellent results for a variety of ortho-, meta-, and para-substituted diphenylmethyl alcohols.

#### IV. ENANTIOMERIC SEPARATIONS ON CELLULOSE THIN-LAYER PLATES

##### A. Resolution Mechanism

Cellulose is a linear macromolecule composed of optically active D-glucose units with its chains arranged on a partially crystalline fiber structure with helical cavities. Separation of enantiomers is made possible by differences in the way they fit into the lamellar chiral layer structure of the support.

##### B. Survey of Applications of Racemic Separations

Thin-layer chromatography on cellulose can be considered a continuation of classical paper chromatography. The first investigations during the mid-1970s concentrated on a transfer of paper chromatographic racemate separations to cellulose layers with the aim of shortening development times and improving separation efficiencies. Thus, Contractor and Wragg (84) needed only 1 h for the resolution of racemic tryptophan and hydroxy analogs on cellulose CC 41 with a solvent system already known from the paper chromatographic separation of these compounds. Antipode separation of kynurenine and derivatives can be achieved on Avicel SF-coated glass plates in about 2.3 h (90).

The research listed in Table 2 (91–108) deals mainly with separation problems concerning amino acids, amino acid derivatives, and dipeptides, focusing on the influence of the structure of the chiral support and the eluent temperature on the separation behavior of the racemates. Separation of the aromatic amino acids phenylalanine,  $\beta$ -2-thienylalanine, 4-fluorophenylalanine, and tyrosine could not be achieved on microcrystalline or amorphous cellulose; tryptophan isomers, however, could be reproducibly resolved on microcrystalline cellulose layers (93). Lowering the eluent temperature from 30°C to 0°C enhances enantiomeric resolution. However, developing times of  $10 \pm 1$  h (0°C),  $7.5 \pm 0.5$  h (10°C),  $5 \pm 0.5$  h (20°C), and 3.5 h (30°C) have to be tolerated; hydrophobic eluent combinations further enhance separation, because they improve formation of the helical cellulose conformation (97). Separation of racemic 3,4-dihydroxyphenylalanine, tryptophan, and 5-hydroxytryptophan can be achieved in only 2 h on a cellulose HPTLC plate (99); these experiments are described in detail in Section IV.C.

Lederer and coworkers (100,101,104–108) investigated the influence of various salt concentrations in the mobile phase on the separation of tryptophan, methyltryptophan, and fluorotryptophan. In these experiments lithium chloride, sodium chloride, and ammonium sulfate solutions were used for the separation on native and microcrystalline cellulose, and also separations with aqueous copper sulfate and sodium chloride solutions containing  $\alpha$ -CD were also described.

Roomi and Tsao (110) described a method for the separation of isomers of ascorbic acid and their oxidation product dehydroascorbic acid on sodium borate-impregnated silica gel and cellulose plates. Studies of additives such as metaphosphoric acid were done. The mobile phase was acetonitrile-acetone-water-acetic acid (80:5:15:2). This procedure was adopted to separate and identify ascorbic acid and its oxidation product in food products, pharmaceutical preparations, and biological tissues and fluids.

Mixtures of microcrystalline cellulose and cellulose derivatives were used by Suedee and Heard (111). For the mixtures they used triphenylcarbamates and tested the separation of *rac*-propranolol and *rac*-bupranolol. The best resolution of propranolol was obtained on cellulose tris(3,5-dimethylphenylcarbamate) and that of bupranolol, on cellulose tris(3,4-dichlorophenylcarbamate) with the mobile phase hexane-propan-2-ol (80:20).

Cyclohexylcarbamates of cellulose and amylose were prepared by Okamoto and coworkers (112) and were tested as the chiral stationary phase for thin-layer chromatography, showing good separation factors for various compounds such as 1-(9-anthryl)-2,2,2-trifluoroethanol, Tröger's base, and benzoin.