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RESOLUTION OF OPTICAL ISOMERS BY THIN LAYER CHROMATOGRAPHY

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Abstract. A process for the separation of enantiomers by TLC is described. Reversed-phase plates, pre-treated with a copper II complex of N,N-di-n-propyl-L-alanine separate all the dansyl protein amino acids, except proline, each to its D and L enantiomers.

The HPLC separation of enantiomers on reversed phase columns using chiral metal complexes in the mobile phase was reported by several groups.[1] In a systematic study aimed at the elucidation of the mechanistic aspects of the resolutions, we have applied copper II complexes of N,N-dialkyl-α-amino acids as mobile phase additives.[2] As a result, the complex with N,N-di-n-propyl-L-alanine (Cu-DPA) was introduced as an efficient and versatile additive for the resolution of several classes of compounds into enantiomers. It resolves all the free protein amino acids,[3] amino acid esters, dipeptides, lactam of lysine, ß-amino acids and other compounds.[4,5] Recently, Weinstein and Weiner[6] resolved into enantiomers all the dansyl protein amino acids, except proline, using Cu-DPA in the mobile phase.

A considerable amount of the chiral complex is adsorbed on the reversed phase particles in the HPLC column, namely, selective interactions with the enantiomers occur at the interface between the solid and the mobile phases. This finding led us to apply the method in an analogous manner to TLC: we impregnated reversed phase plates with the chiral complex and separated the dansyl amino acid enantiomers by developing the plates with water-acetonitrile buffers, preferably containing Cu-DPA. All the dansyl protein amino acids, except proline, were separated into enantiomers.

Preparation of Plates. Reversed-phase TLC plates (Merck, Darmstadt) 10x20 cm (Whatman, Kent, England) 5x20 cm, were developed (prior to application of the dansyl amino acids), in 0.3 M sodium acetate in 40% acetonitrile and 60% water, adjusted to pH 7 by acetic acid (Buffer A). After fan drying, the plates were immersed in a solution of 8 mM N,N-di-n-propyl-L-alanine and 4 mM cupric acetate in 97.5% acetonitrile and 2.5% water for one hour and up to overnight, or sprayed with the solution, and left to dry in the air. The plates are stable and can be stored for further use.

Separation of Enantiomers. Dansyl amino acids in aqueous solution were applied to the plate and developed in Buffer A with or without N,N-di-n-propyl-L-alanine (4 mM) and cupric acetate (1 mM) dissolved in it. The enantiomers were detected by irradiating U.V. light (360 nm) to yield fluorescent yellow-green spots. L dansyl amino acids gave one spot, corresponding to the slower-running enantiomer. Figure 1 depicts the resolution of some dansyl amino acids into enantiomers on TLC plates, photographed under U.V. irradiation. Dansylic acid obtained in the derivatization step is distinguished by its blue fluorescence (photographed with a green filter it is not seen). Acetonitrile concentration may be varied, according to the amino acid resolved: 25% acetonitrile is preferred for glutamic and aspartic acids, serine and threonine, 40% for the

others. The high salt content prevents the layer from peeling, a common problem in reversed phase TLC, and the plates can be re-used.

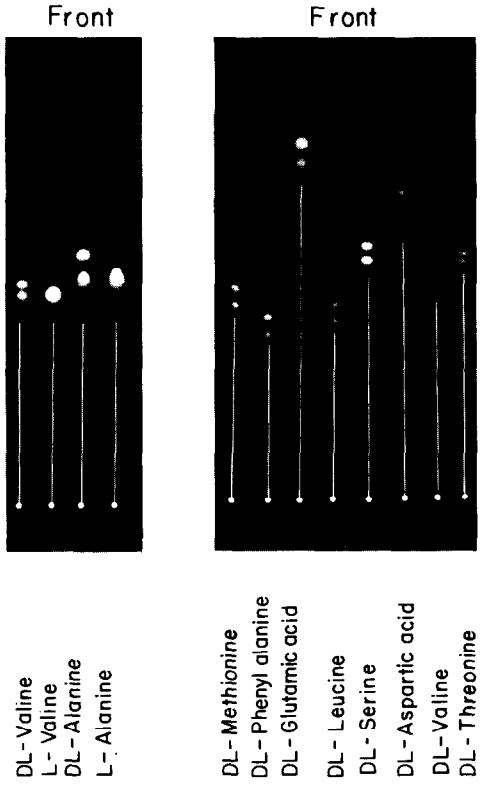

Figure 1. Dansyl amino acids resolved to enantiomers by treated reversed-phase TLC plates, spotted with 1 µl of ∿5 10^{-4}M solution. Plate length is 20 cm. Photographed under U.V. illumination.

The TLC method is sensitive, fast, and very simple. The plates are commercially available and the chiral ligand is easily synthesized.[3] Quantitative results may be obtained by densitometry or by measuring the fluorescence or U.V. absorption of the extracted spots.

We are currently studying the application of TLC resolutions to additional compounds, especially those which we resolved by HPLC, using the N,N-dialkyl-α-amino acid ligands.

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