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CHIRAL STATIONARY PHASES FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS: A MINI-REVIEW

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ABSTRACT

There has been a proliferation of papers on the use of chiral stationary phases (CSP's) to separate optical isomers in high performance liquid chromatography. The chemistry, mechanism and stability of these CSP's can vary widely. Furthermore, the applicability, availability and cost of a CSP can mean the difference between its being of passing academic interest as opposed to a technique that could have a significant impact on science and technology. Six different classes of chiral stationary phases are examined and discussed including the new chiral cyclodextrin bonded phases. The separation mechanism, strengths and limitations of the CSP's are also considered whenever such information is available.

INTRODUCTION

In the last decade there has been a tremendous impetus to develop efficient liquid chromatographic techniques for the separation of racemates. There are several reasons for this. For example, an efficient method for determining optical purity would be highly beneficial in many scientific disciplines in-

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cluding: organic and inorganic synthesis, kinetics, pharmacology, geochronology (i.e., using the degree of amino acid racemization to date organic articles of archaeological importance) and so forth. The pharmaceutical industry obviously needs effective analytical and preparative separations for a variety of enantiomeric compounds which are known to have different physiological activities (1-4). The fact that the traditional method of resolving racemic mixtures (i.e., fractional recrystallization of diastereomeric salts) is relatively difficult, inefficient and limited in applicability (5,6) has greatly increased the interest in alternative techniques. The efficiency, speed, wide applicability and reproducibility of the modern liquid chromatograph have made it the instrument of choice for most of the recently reported enantiomeric separations. Be this as it may, it is clear that recent HPLC techniques have evolved and/or benefited from classic column chromatographic methods (6). It also appears that the most interesting research in this area involves the development of new highly selective stationary phases. In this review the chemistry, applicability and limitations of six classes of chiral stationary phases will be examined. This work is not intended to be a comprehensive review of enantiomeric separations. For example, work on the use of chiral mobile phase additives and ligand exchange LC will not be covered. Readers interested in these particular areas are refered to the

the many fine reviews and papers that have recently been published (7-16). The six classes of chiral stationary to be considered in this work are: cyclodextrin bonded phases, π-complex/hydrogen bonding stationary phades, polymeric stationary phases, charge transfer stationary phases, protein bonded phases, and crown ether bonded phases.

I. Chiral Cyclodextrin Bonded Phases

Cyclodextrins are chiral, toroidal shaped molecules formed by the action of Bacillus macerans amylase on starch (see Figure 1)(17-19). These macrocyclic polymers contain from six to twelve glucose units bonded through α-(1,4)linkages. The three smallest homologs, α-cyclodextrin (cyclohexaamylose), β-cyclodextrin (cycloheptaamylose) and γ-cyclodextrin (cyclooctaamylase) are available commercially while larger homologues must be individually produced and isolated. Cyclodextrins have several structural features that make them highly useful in separations (Figure 1). First of all, the interior of the cyclodextrin cavity contains no hydroxyl groups and is relatively hydrophobic. Consequently they are able to complex a variety of water insoluble or sparingly soluble molecules, see Figure 2. This property led to their use as mobile phase modifiers in the TLC separation of a variety of structural isomers (20-22). Traditional column chromatography on polymerized cyclodextrin was investigated as well (23-25).

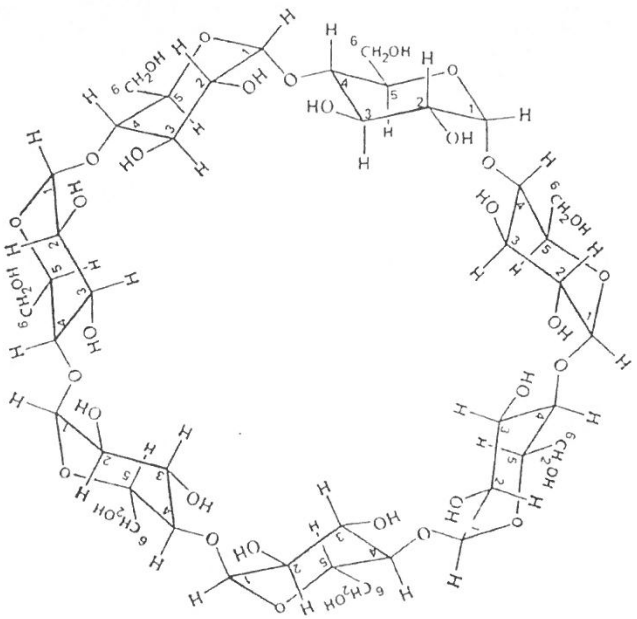

Figure 1. A schematic showing the structure of β-cyclodextrin. The cavity is hydrophobic and is 7 to 8 Å deep. Note that all glucose units are locked in a chair conformation and joined by a stable α-(1,4)glycosidic linkage. All hydroxyl groups are on the outer edges of the molecule with the primary 6-hydroxyls restricting the "bottom side" of the molecule.

Most importantly, each glucose unit contains five chiral atoms and the 2-hydroxyl groups at the entrance of the cyclodextrin cavity project in a clockwise direction (Figure 1). Chiral recognition has been shown to be optimal on a β-cyclodextrin column for compounds the size of biphenyl or a little larger (26,27). If, in addition, the chiral solute also contains a substituent that can hydrogen bond with the 2-hydroxyl

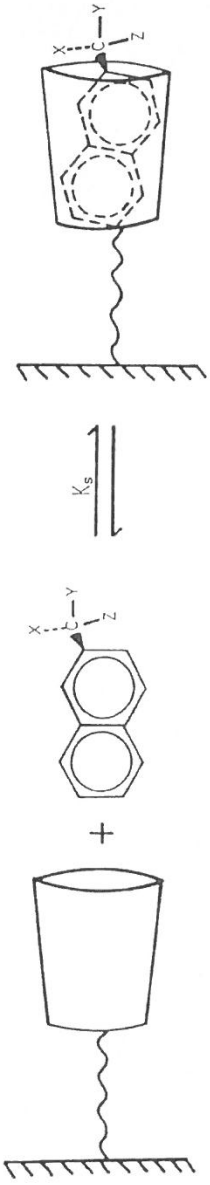

Figure 2. A schematic of cyclodextrin bonded to a silica gel support and reversibly forming an inclusion complex with a chiral molecule. Neither the linkage nor the cyclodextrin contain nitrogen (e.g., amines or amides) in any form.

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