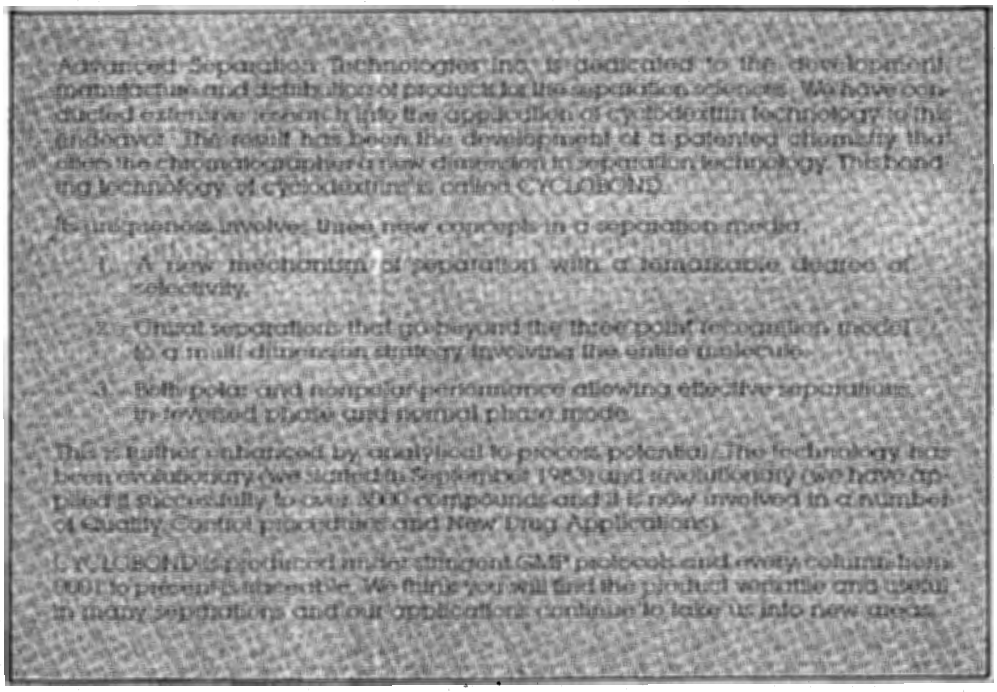
CYCLOBOND™

HANDBOOK

A GUIDE TO USING CYCLODEXTRIN BONDED PHASES

astec



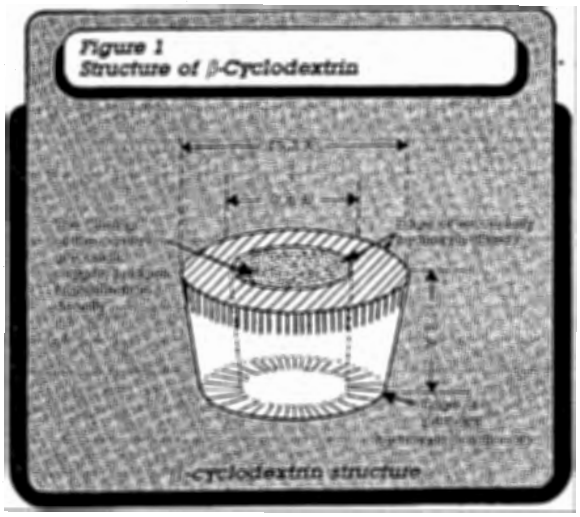
INTRODUCTION

This booklet has been prepared for the users of CYCLOBOND columns to aid them in developing and optimizing separations of their compounds. Since CYCLOBOND columns perform their separation via a new and unique primary mechanism, and a handful of secondary mechanisms, these will be briefly described so they might be invoked to get the most out of any CYCLOBOND column. Varying the conditions of the separation to aid in the optimization will be discussed as will the care of the column to ensure its long and reproducible use. Please take time to familiarize yourself with the use of the columns by reading this text thoroughly. By doing so you will get better and faster results. If you still have questions, do not hesitate to call us to obtain help.

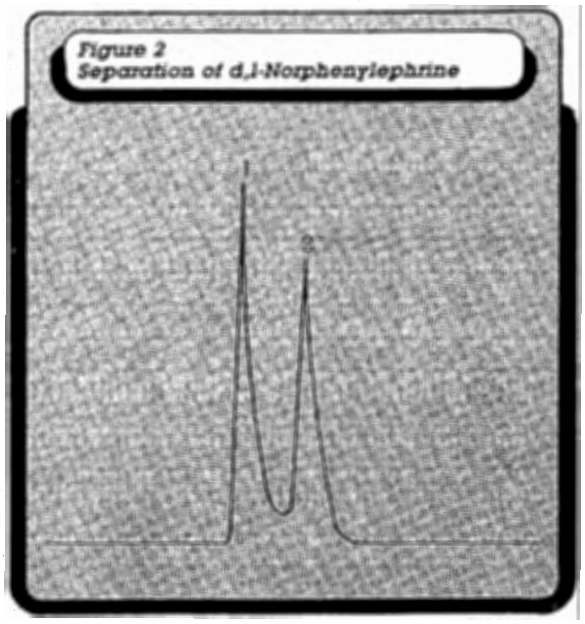
CYCLOBOND COLUMNS

CYCLOBOND columns are packed with unique stationary phases of chemically bonded cyclodextrins to a high purity, 5 micron, spherical silica gel. The cyclodextrins are linked through a $-CH_2-$ chain of optimum length via a patented process which yields a stable non-hydrolytic, non-nitrogen containing bond. Five CYCLOBOND columns are now avialable called CYCLOBOND I, CYCLOBOND I ACETYLATED, CYCLOBOND II, CYCLOBOND III and CYCLOBOND III ACETYLATED.

CYCLOBOND I is bonded with beta-cyclodextrin (β-CD) and from available separations performed thus far, appears to be the most versatile of the products. The β-CD is a macrocyclic molecule that contains 7 glucopyranose units arranged in the shape of a hollow truncated cone in which the *interior* cavity is relatively *hydrophobic* being comprised of essentially methylene and 1,4-glucoside linkages producing a high electron density for extensive interaction with analyte. The *exterior* faces, on the other hand, are *hydrophilic*. The larger opening is surrounded by the secondary hydroxyls. The 2-hydroxyls are rotated clockwise and the 3-hydroxyls are rotated counterclockwise. The primary hydroxyls constitute the smaller end of the cone. The functional structure and dimensions of beta-cyclodextrin are given in Figure 1.



CYCLOBOND I ACETYLATED is formed by acetylating the fixed secondary hydroxyls on the rim of the β-CD cavity. This has an effect of changing the size of the cavity and/or changing the interaction between the hydrogen bonding site of the cyclodextrin and the specific functional group on the asymmetric carbon. Figure 2 shows the separation of d,l-Norphenylephrine on this column. The acetyl group has shown a high degree of selectivity for geometric separations based on the position of double bonds.



ANALYTES:

1. l-Norphenylephrine
2. d-Norphenylephrine

CONDITIONS:

COLUMN:	CYCLOBOND I ACETYLATED
SIZE:	250x4.6mm
MOBILE PHASE:	0.5% Acetic Acid, pH 6.1
FLOW RATE:	0.5 ml/min
PRESSURE:	600 psi
CHART SPEED:	0.5 cm/min
DETECTION:	254 nm
CONCENTRATION:	1.0 μg/μl

CYCLOBOND II is the γ-CD form consisting of 8 glucopyranose units arranged in the same truncated cone shape and is useful for isomeric compounds based on anthracene, chrysene and pyrene ring structures. CYCLOBOND III is the α-CD form consisting of 6 glucopyranose units, also a truncated shape with a smaller internal diameter. The smaller diameter of the CYCLOBOND III allows it to be most useful for molecules smaller than benzene, many underivatized amino acids, prostaglandins, and inorganic ions. CYCLOBOND III ACETYLATED bridges the cavity sizes between α-CD and β-CD, and in addition offers specific functional group interaction as in CYCLOBOND I ACETYLATED.

POSSIBLE MECHANISMS WITH CYCLOBOND COLUMNS

The structures of these cyclodextrin bonded columns have been discussed since they constitute the basis on which the separation mechanism operates. The selectivity of each of these phases is very different and as with any liquid chromatographic separation, the exact mechanism changes as a result of the structure of the solute, their solubility, the mobile phases, and all of these in relationship to the phase in the column. These columns are particularly effective for molecules having a hydrophilic portion which can fit the cyclodextrin cavity reasonably well.

Because of the unusual shape of the cyclodextrin molecules (whether α, β, or Υ) and the different polarities of the various surfaces, different interactions with solutes are possible. One of the first observed phenomenon with cyclodextrins was their ability to form inclusion complexes. In order for a solute to be able to do this it must meet certain requirements. It must have a hydrophobic segment that will fit snugly into the CD cavity. The complex can also be more stable by having polar groups that hydrogen bond to the secondary hydroxyls at the edge of the cone. Structural differences greatly effect retention of the analyte. If, for instance, oxygen atoms are in the structure of an analyte, retention is decreased (see ref. 18), due to electrostatic repulsion. The position of the oxygen can also effect the retention of the analyte. Increasing the number of methylene groups enhances retention so that 4, 5, 6, 8, etc. carbon rings can easily be separated. Chlorine atoms have a high affinity for the cavity and their position and number greatly effect retention.

If the diameter of the solute is too large, no complex can form because the hydrophobic segment cannot enter into the mouth of the CD cavity. If too small, the solute can enter, but the complex is not as stable (smaller formation constant). As general rules, single ring compounds such as benzene or smaller, fit well into α-CD (CYCLOBOND III); compounds such as substituted benzenes, naphthalene or biphenyl derivatives fit well into β-CD (CYCLOBOND I); and, larger compounds such as pyrene fit well into Υ-CD (CYCLOBOND II). Figure 3 illustrates the retention of these compounds on the various CYCLOBOND columns.

In Figure 3 the decreased retention observed for chrysene, phenanthrene and anthracene on CYCLOBOND II is thought to be the result of less interaction due to the linear nature of these aromatic structures. The more bulky pyrene shows expected behavior. Further, due to the bulky nature of Υ-CD, less is bonded to the silica surface. The naphthalene structure enters the gamma cavity horizontally and the beta cavity vertically so the total bonding energy is equivalent. The slight difference in retention is the result of diminished Υ-CD on the CYCLOBOND II.

It should be noted that not all of the solute structure has to fit into the cyclodextrin cavity for the separation to occur, but at least a portion of it must fit well enough to form a stable complex.

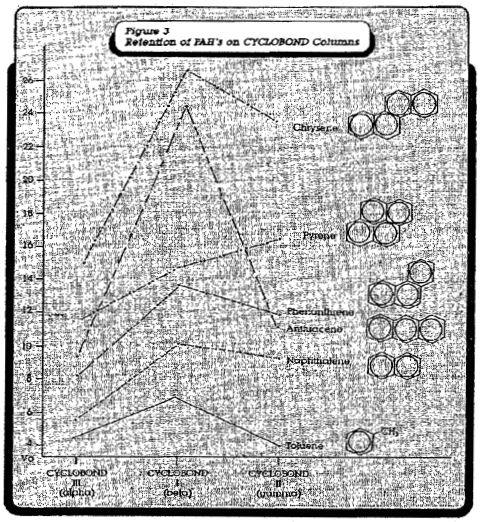
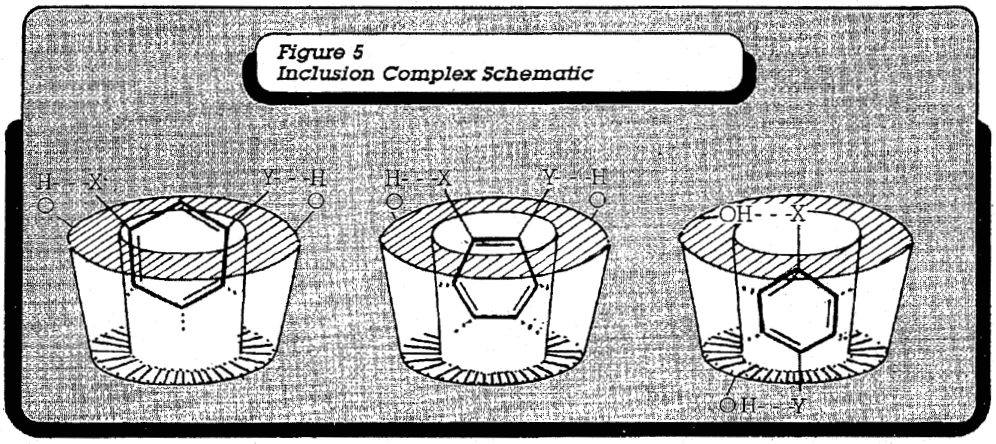


Figure 4 shows o-, m-, and p-nitroanilines separated on each of the CYCLOBOND I, II and III columns with MeOH/H_2O; 40/60. These separations of structural isomers are based primarily on the fit of these molecules into the CD cavity. The same elution order of m-, o-, and p- isomers results regardless of the substituents. With CYCLOBOND II

CONDITIONS.

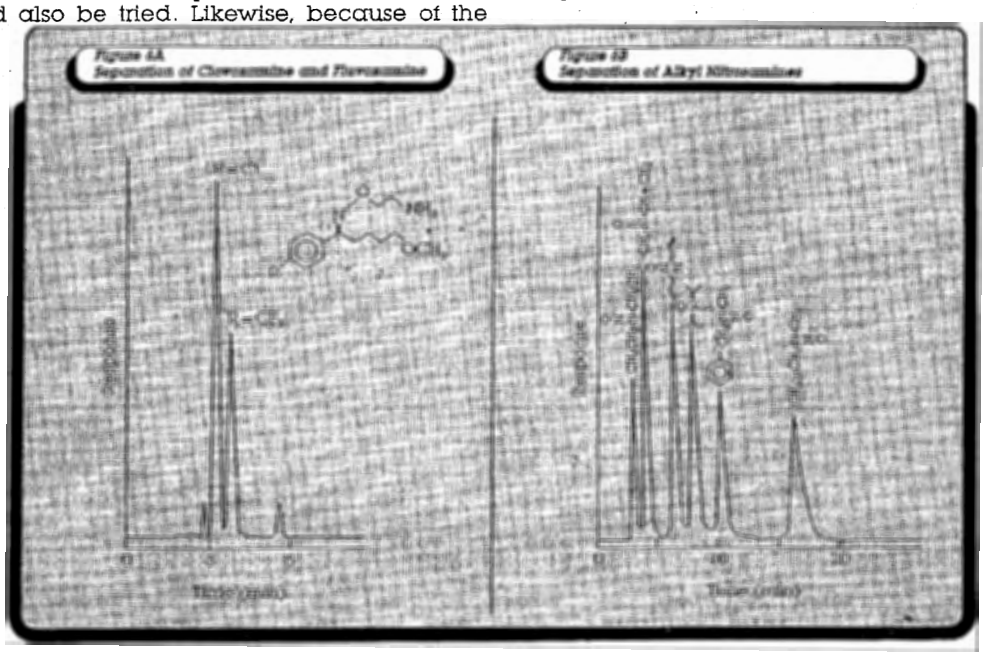
COLUMN.	CYCLOBOND III, I and II
SIZE.	250x4.6mm
MOBILE PHASE.	MeOH/H_2O; 40/60
FLOW RATE.	1.0 ml/min
CHART SPEED.	1.0 cm/min
DETECTION.	254 nm
CONCENTRATION.	0.5μg/μl

(c), the Y-CD cavity is too large to allow sufficient interaction of the aromatic ring with the internal surfaces of the CD so little difference is seen between ortho and meta isomers. With CYCLOBOND I (b) the best overall resolution is seen because there is good interaction of the aromatic ring with the CD internal surface and the p- isomer fits very well into the β-CD cavity allowing for stable hydrogen bonding of the nitro group with the primary hydroxyls, thereby increasing its retention. The m- isomer is too bulky for a good fit, hence the earliest resolution. This is shown diagrammatically in Figure 5. With CYCLOBOND III (a), longer retention times are observed in this case due to tighter fit for that portion of the aromatic group interacting with the cavity.



---- Hydrogen bonding
• • • Electrostatic association
X Hydrophobic groups that can penetrate cavity, ie, -NO_2, -Cl, -OH
Y Polar groups that can hydrogen bond -COOH, -NH_2

It is also possible to operate the CYCLOBOND column as a reversed phase column using only the nonpolar character of the internal surface of the CD and the solvated surfaces of the entire structure. It can be considered a rather unique reversed phase, very different from anything in the LC market today. When attempting new separations via reversed phase, a CYCLOBOND column should also be tried. Likewise, because of the prevalence of the primary and secondary hydroxyl groups, it can be used as a high density, very efficient diol column. These latter types of columns have found wide use in the separation of proteins and peptides. CYCLOBOND columns can be used as all purpose columns as seen in the typical reversed phase separations shown in Figure 6A and 6B.



CONDITIONS,
COLUMN; CYCLOBOND I
SIZE; 100x4.6mm
MOBILE PHASE, CH_3CN/0.1% TEAA pH 4.0, 15/85
FLOW RATE; 1.0 ml/min

CONDITIONS,
COLUMN; CYCLOBOND I
SIZE; 250x4.6mm
MOBILE PHASE, CH_3OH/H_2O, 30/70
FLOW RATE; 1.0 ml/min

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.