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APPLICATIONS OF CYCLODEXTRINS IN CHROMATOGRAPHIC SEPARATIONS AND PURIFICATION METHODS

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A. INTRODUCTION

Cyclodextrins have been utilized as biomimetic models for a wide variety of processes ranging from enzymatic catalysis and membrane transport to protein active-site—substrate binding interactions.[1-21] As a consequence, numerous articles characterizing cyclodextrins as well as delineating their unique properties pervade the chemical literature.[22-148] Even though the results of those studies promise great potential in terms of analytical chemistry, especially as they pertain to chromatographic separations, relatively few articles concerning their applications have been published. In view of the present inadequacy, this article is dedicated to the systematic reviewing of the use of cyclodextrins in chromatography and chemical separations. The topics to be covered include the use of cyclodextrins as selective isomeric precipitating agents, their use and role in chromatographic stationary or mobile phases, and their ability to enhance chromatographic detections. As a prerequisite to this discussion, a description of the structure of cyclodextrins, their properties, and their selective substrate binding ability will be presented. This background information is vital if one is to rationalize and to understand the role of cyclodextrins in these chromatographic applications. The goal is to give a broad, general picture of the different possible uses of cyclodextrins in the field of separation science and to stimulate further work in this area.

B. STRUCTURE AND PROPERTIES OF CYCLODEXTRINS

A number of books[1-3] and review articles[4-21] have been devoted to the detailed descriptions of the structure and properties of natural and modified cyclodextrins. To be consistent with the purpose of this paper, only those structural aspects and properties of cyclodextrins that have a bearing on their use or application in chromatographic separations will be discussed.

1. Natural Cyclodextrins.

Cyclodextrins (also known as cycloamyloses, cycloglucans, Schardinger dextrins, or cycloglucopyranoses), abbreviated CD, are a series of oligosaccharides produced by the action of Bacillus

macerans amylase on starch.[214] They are natural macrocyclic polymers of glucose that contain from six to twelve D-(+)-glucopyranose units which are bonded via α-(1,4) linkages, with all glucose units in a C1 (D) chair conformation (Figure 1). The cyclodextrin's conformations in solution are nearly identical with those observed in the crystalline state. The overall structural features of CDs are shown in Figure 2. As can be seen, they are not cylindrical molecules but have the shape of a toroid or hollow truncated cone. The side of the torus with the larger circumference contains the secondary hydroxyl groups (on carbons 2 and 3 of the glucose units) while the primary hydroxyl groups (on carbon 6 of the glucose units) are on the other smaller side. The interior of the cavity thus defined contains two rings of C-H groups with a ring of glycosidic oxygens in between. As a result, the cavity is relatively hydrophobic (compared to polar solvents like water) while the external faces are hydrophilic.[1,9,21]

The number of glucose units determines the name of each specific CD, which is designated by a Greek letter: α- for six (cyclohexaamylose) or α-CD; β- for seven (cycloheptaamylose) or β-CD; γ- for eight (cyclooctaamylose) or γ-CD; and so on. Although CDs containing as many as twelve glucose units have been identified, only the first four members of the series have been studied in detail. The dimensions and the size of the cavity are determined by the number of these glucose moieties present. Table I summarizes some of the more important physical properties of the four most commonly used CDs, including their molecular dimensions.[1,21]

The CDs are stable under normal experimental conditions and are nontoxic.[1,21,22] "All toxicity tests have shown that orally administered [pure] cyclodextrin is harmless."[21,22] Except for possible deprotonation of their hydroxyl protons (pK_a = 12.1 - 12.6),[1,9,23] CDs are fairly stable in alkaline media. However, they are susceptible to acidic hydrolysis.[1,21] For instance, in the presence of 1.15 N HCℓ, the rate constant for acid hydrolysis of β-CD at 40° C is approximately 10^{-5} min^{-1}.[24]

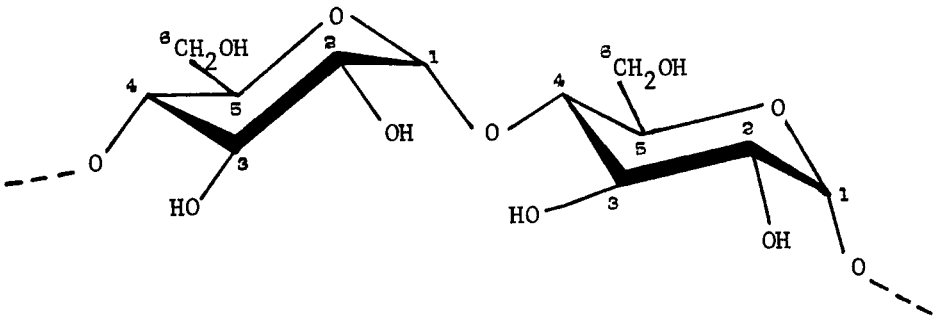

FIGURE 1

Structural diagram of two glucopyranose moieties of a cyclodextrin that illustrate details of the α-(1,4) glycosidic linkage, their C1 (D) chair conformation, and the numbering system employed to describe the ring system.

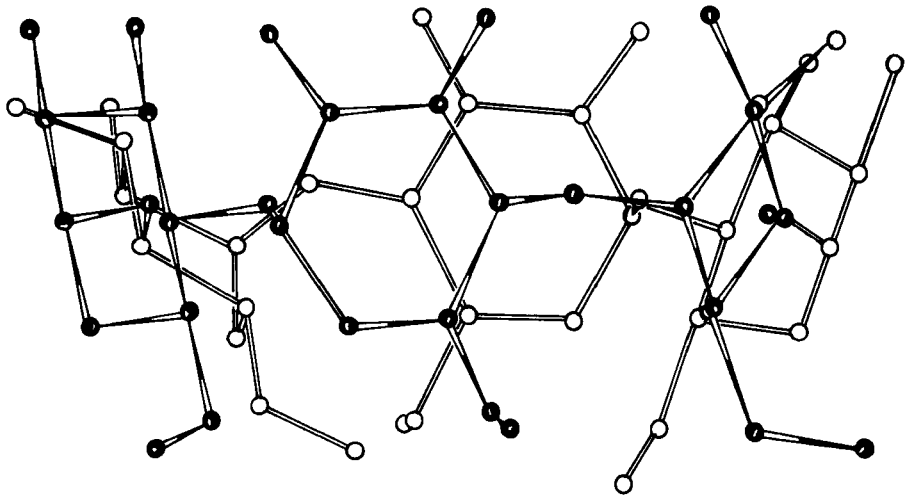

FIGURE 2

Side view of an α-cyclodextrin molecule. The top of the torus corresponds to the more open side that contains the secondary hydroxyl groups (on carbon 2 and 3 of each glucose unit).

TABLE I

Selected Physical Properties of Cyclodextrins[1,9,21]

Cyclodextrin	Number of Glucose Units	Molecular Weight	Cavity Dimensions, Å			Specific Rotation $[\alpha]_D^{25}$	Melting Point, °C	Solubility in Water, M
			External Diameter[a]	Internal Diameter[b]	Depth[c]			
α-CD*	6	972.86	14.6±0.4 (13.7)[d]	4.5-6.0 (5.7)[d]	4.5 (7.8)[d]	150.5± 0.5	278° (dec)	0.114[e] 0.138[f]
β-CD*	7	1135.01	15.4±0.4 (15.3)[d]	6.0-8.0 (7.8)[d]	~7.0 (7.9-8)[d]	162.5± 0.5	298-300° (dec)	0.016[e] 0.019[f] (0.038)[g]
γ-CD*	8	1297.15	17.5±0.4 (16.9)[d]	~8-10 (9.5)[d]	~7.0 (7.9-8)[d]	177.4± 0.5	267° (dec)	0.179[e]
δ-CD	9	1459				191±3		v.s.

a) Taken from reference 21 (originally measured on CPK models).
b) Taken from reference 21 (as determined from CPK models) unless otherwise stated. The smaller value of the range is for the ring of H atoms bonded to C(5) while the larger value is for the ring of H atoms bonded to the C(3) of the glucose rings.
c) Taken from reference 1 unless otherwise indicated.
d) Taken from reference 17.
e) Calculated from data in reference 25 at 25° C.
f) Calculated from data in reference 25 at 30° C.
g) Solubility in glycerol at 25°C; taken from reference 21.

*Commercial suppliers of these cyclodextrins are listed under entries 215 and 216 of the References and Notes section.

2. Modified Cyclodextrins.

In attempts to design better enzymatic catalysts, many modified (derivatized) CDs have been prepared.[1,9,14,25-61] One may note that the hydroxyl groups on carbons 2, 3, and 6 are available as starting points of structural modification without any danger of eliminating the central cavity which is the key to their chemistry. Despite the fact that the successful selective modification of CDs must overcome rather formidable synthetic difficulties (owing mainly to the multiplicity of the potentially reactive hydroxyl groups present), a large number of mono-substituted CDs, specifically bifunctionalized or multi-functionalized CDs, and polymeric CD-containing resins have been synthesized.[1,9,14,21,61] Various functional groups (including the -amino, -alkyl, -alkoxy, -azido, -polyamino, -halo, -phosphate, -sulfonate, -tosylate, -imidazolyl, -acetohydroxamic, -polyamino/metal cation, -ammonium, etc.) have been incorporated into the CD moiety. Additionally, ester and ether derivatives have been made [such as the carboxymethyl ether (abbreviated α-CME or β-CME),[42] sulfonatopropyl ether (α- or β-SPE), the respective acetate, propionate, butyrate, valerate, or cinnamate esters[54,146] designated β-CDA, β-CDPr, β-CDB, β-CDV, and β-CDC), and a cationic quaternary ammonium ether[55]]. The dimeric CDs, bis(β-cyclodextrin)succinate or glutarate [abbreviated $(\beta\text{-CD})_2$-S or $(\beta\text{-CD})_2$-G] have been reported.[41] Table II summarizes the structure and nomenclature for selected, functionalized CDs.

A number of CD polymers (in the form of powders, films, resins, or beads) have been synthesized.[21,61] The most popular one seems to be an O-alkylated polymeric resin (abbreviated α- or β-ECP, Figure 3) produced by reaction of CDs with epichlorohydrin.[40,43-45] CDs have been polymerized in solutions of poly(vinyl alcohol) using ethylene glycol bis(epoxypropyl)ether as the cross linking agent to give CD co-polymers (abbreviated α-, β-, and γ-CDP).[46-48] Poly(acryloyl-β-cyclodextrin), abbreviated PA-β-CD, is a water soluble CD polymer.[41] Compound 7 (Table II) has been coupled either to succinylhydrazide to yield a gel product (designated β-en-Bio-Gel) or to agarose using 1,4-butanediol diglycidyl ether to give another

TABLE II

Structure and Nomenclature for Selected Cyclodextrin Derivatives

Name (Compound number)[a]	n[b]	X[c]=	Y[c]=	Z[c]=
Tetradecakis-2,6-O-dimethyl-β-CD (1)[26,28]	7	$-OCH_3$	-OH	$-OCH_3$
Dodecakis-2,6-O-dimethyl-α-CD (2)[26,28,40]	6	$-OCH_3$	-OH	$-OCH_3$
Cyclohepta(amylose-6-toluenosulfate) (3)[27,34]	7	-OH	-OH	-OTs
Cyclohepta(6-N-methylformamido)amylose (4)[34]	7	-OH	-OH	$-N(CH_3)CHO$
Heptakis-2,3,6-tri-O-methyl-β-CD (5)[26,57]	7	$-OCH_3$	$-OCH_3$	$-OCH_3$
Hexakis-2,3,6-tri-O-methyl-α-CD (6)[26,57]	6	$-OCH_3$	$-OCH_3$	$-OCH_3$
Mono-(6-O-aminoethylamino-6-deoxy)-β-CD (7)[30]	1	-OH	-OH	$-NH(CH_2)_2NH_2$
Mono-(6-O-diethylenetriamino-6-deoxy)-β-CD (8)[31]	1	-OH	-OH	$-NH(CH_2)_2NH-(CH_2)_2NH_2$
Cycloheptaamylose-2-monophosphate (9)[32]	1	$-OPO_3H_2$	-OH	-OH
Cycloheptaamylose-6-monophosphate (10)[32]	1	-OH	-OH	$-OPO_3H_2$
β-Cyclodextrin-6-monotosylate (11)[33]	1	-OH	-OH	-OTs
Mono[6-(t-butylthio)]-β-CD (12)[33]	1	-OH	-OH	$-SC(CH_3)_3$
α-Cyclodextrinyl-6,6'-bisimidazole (13)[29]	1	-OH	-OH	-Im
Monometallo[6-O-diethylenetriamino]-β-CD; metal = Cu(II) or Zn(II) (14)[31]	1	-OH	-OH	$-NH(CH_2)_2NH-(CH_2)_2NH_2$
Triamino-per-O-methyl-α-CD (15)[37]	6	$-OCH_3$	$-OCH_3$	----
	3	----	----	$-NH_3^+$, $-OCH_3$
Monoamino-per-O-methyl-α-CD (16)[37]	5	$-OCH_3$	$-OCH_3$	$-OCH_3$
	1	$-OCH_3$	$-OCH_3$	$-NH_3^+$
Hepta[6-(9-adenyl)-6-deoxy]-β-CD (17)[58]	7	-OH	-OH	-adenyl
6,6',0,0'-(4,4'-Azobenzenedicarbonyl)-cycloheptaamylose; cis or trans isomers (18)[35,36]	2	-OH	-OH	-azobenzene-dicarbonyl
6,6',0,0'-(4,4'-Diphenyldisulfonate)-cycloheptaamylose (19)[29,33,34,38,39]	2	-OH	-OH	-diphenyl-disulfonate

a) Superscript indicates literature reference(s).

b) The n refers to the number of glucose units of the CD that have been modified with the indicated substituents.

c) The letters X, Y, and Z denote the particular substituents present on carbons 2, 3, and 6, respectively, of the glucopyranose unit.

gel (β-en-agarose).[50] A number of polyurethane resins (symbolized by α- or β-CDPU) were prepared by reaction of CD with diisocyanate in either pyridine or N,N-dimethylformamide.[51-53] A β-CD poly(vinylidene chloride-allyl chloride) copolymer has been characterized.[59] Lastly, CD have also been immobilized on or with polymeric materials. For instance, α-CD was immobilized on Sepharose 6B which had been activated with 1,4-bis(2,3-epoxypropoxy butane);[49] β-CD was fixed on porous, crosslinked 2-hydroxyethylmethacrylate-glycidylmethacrylate polymers;[60] and β-CD has been impregnated in hydroxypropylmethyl cellulose[58] and other cellulosic materials.[62-4] A number of these CD polymers have been utilized as the stationary phase in chromatographic separations. This will be discussed in detail in a later section (section D-3).

The dimensions (internal and external diameter) of the CD cavity are largely retained in all of these modified or polymeric CDs. However, the depth (height) of the cavity can be somewhat altered depending on the type(s) of substituents present.[27,33-35] Naturally, various other physical properties can be changed substantially compared to those of the parent CDs. For example, tri-O-methyl-β-CD (compound 5, Table II) has a melting point of 89°

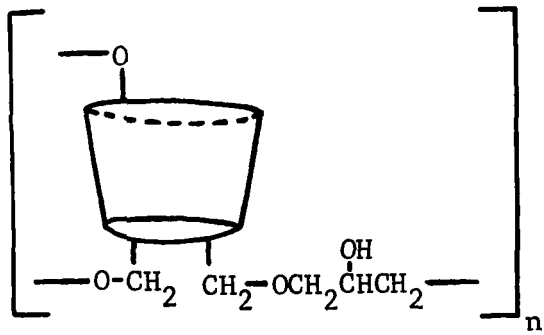

FIGURE 3

Proposed structure of the epichlorhydrin-cyclodextrin polymer.[40]

[compared to 298 - 300° (dec) for β-CD] and is roughly fifteen times more soluble in cold water.[28,57] The solubility of the cyclodextrin polymers depends upon their degree of polymerization.[21] Those CD derivatives possessing ionizable group(s) will have additional characteristic ionization constants. Thus, compounds 9 and 10 (Table II), which have ionizable phosphate substituents, have pK_a values of 2.51 and 5.89; and 2.58 and 6.86; respectively,[32] while the triamino-α-CD derivative (compound 15) has values of 7.42, 8.02, and 8.79.[37] Some modified CDs, such as C-2 aminated-β-CD, are much more resistant to acid hydrolysis compared to the parent β-CD itself.[1,147] The toxicological properties of the modified or polymeric CDs are determined by the nature of the substituents incorporated.

C. COMPLEXING ABILITY OF CYCLODEXTRINS

1. Inclusion Complex Formation.

The most remarkable property of CDs (and the one that is the basis for their use in chemical separations) is their ability to selectively include a wide variety of guest organic and inorganic molecules or ions into their hydrophobic cavity. The resultant included molecule-CD ensembles are usually termed inclusion compounds (also referred to as inclusion complexes, adducts, or clathrates). The sizes of these guest molecules can range from the noble gases to fatty acyl coenzyme A compounds.[1, 65,66] Although the stoichiometry of the inclusion complexes formed in aqueous solution is usually 1:1; 2:1, 1:2, or nonstoichiometric complexes have also been observed, particularly in the crystalline state.[1,5,21] In the solid state, these complexes crystallize in either "cage-", "channel-", or "layer-type" structures depending on the nature and size of the guest molecule.[1,70] Complex formation between the CD host (usually present in the solid state or dissolved in solution) and guest substrate molecules (present in solution or in the gas phase) is a dynamic equilibrium process[1,67,68] that can be represented by equation 1. The stability of the 1:1 CD·S inclusion

$$CD + S \underset{}{\overset{K_f}{\rightleftharpoons}} CD \cdot S \qquad (1)$$

complex formed can be quantitatively described in terms of a formation (stability or binding) equilibrium constant, K_f, as defined in equation 2:

$$K_f = \frac{[CD \cdot S]}{[CD]\ [S]} \qquad (2)$$

where [CD·S] represents the concentration of the substrate complexed with the CD while [CD] and [S] represent the free equilibrium concentrations of the uncomplexed cyclodextrin and substrate, respectively. The greater the K_f value, the more stable is the CD inclusion complex.

The formation of an inclusion complex with CD can alter some of the physical and/or chemical properties of the guest molecule. Among the properties that can be modified are chemical reactivity,[1,9,21,23] pK value,[71,72] half-wave potential,[73,74] solubility,[1,21,75] vapor pressure,[1] as well as various spectral parameters.[1,21,65,76-81] Hence, inclusion complex formation can be easily detected and monitored by a variety of instrumental methods, e.g. ESR,[77,82] NMR,[67,83,84] UV-visible absorption,[13,23,76-70] fluorescence,[65,80,81] Raman,[85] CD or ORD,[86,87] X-ray analysis,[88-91] polarographic,[73,74] potentiometric,[71] calorimetry,[92,93] position annihilation,[94] MSR,[95] and thermoanalytical methods (DSC, TG, EGA).[96] Equilibrium constants for the binding of various guest molecules to α-, β-, and/or γ-CD thus determined are summarized in Table III.

Controversy still surrounds the exact nature of the binding forces involved in the complex formation. Typically, CD inclusion complex formation is associated with a favorable enthalpy and an unfavorable (or only slight favorable) entropy change.[1,21] Consequently, the binding forces between the guest and the CD molecule in solution have been discussed in terms of several possible factors:[1,9]

(i) Van der Waals-London dispersion forces (so-called "hydrophobic effect")[211] due to favorable dipole-dipole interactions between the guest and host CD molecules;[1,9,21,23,65,70,78,94,102,103,116,117]

(ii) Hydrogen bonding between the guest molecule and the secondary hydroxyl groups of the CD;[1,21,76,94,118,119]

TABLE III

Formation Equilibrium Constants for CD-Guest Inclusion Complexes

Guest Molecule	K_f, M^{-1} [a]			Ref.
	α-CD Complex	β-CD Complex	γ-CD Complex	
1. KCl	0[b]	0[c]-2.6[d]	----	97-99
2. KBr	3.5[b]	1[c]-6.6[d]	----	97-99
3. KI	12.4[b]	18.2[d]	----	97-99
4. KNO_3	1.4[b]	0.7[c]-5.5[d]	----	97-99
5. KSCN	18.7[b]	5.7[c]-9.9[d]	----	97-99
6. $KClO_4$	28.9[b]	9[c]-27[d]	----	97-99
7. Acetate ion	<1.0	----	----	13,100
8. Propionate ion	1.8	----	----	13,100
9. Isobutyrate ion	4.6	----	----	13,100
10. Benzoate ion	12.3	----	----	13,100
11. Cyclohexane carboxylate ion	52.6	----	----	13,100
12. Dodecylsulfate ion	111	356	----	101
13. Methanol	0.9[e]	0.3[f]	----	102
14. Ethanol	5.6[e]	0.9[f]	----	102
15. 1-Propanol	23.4[e]	3.7[f]	----	102
16. 1-Butanol	89.1[e]	16.6[f]	----	102
17. 1-Pentanol	323[e]	63.1[f]	----	102
18. 2-Methyl-2-propanol	4.4[e]	47.9[f]	----	102
19. 3-Methyl-1-butanol	74.1[e]	177.8[f]	----	102
20. 2,2-Dimethyl-1-propanol	29.5[e]	575.4[f]	----	102
21. 1-Heptanol	2290[e]	708[f]	----	102
22. 1-Octanol	6309[e]	1479[f]	----	102
23. 3,3-Dimethyl-2-butanol	19.9[e]	563.3[f]	----	102
24. Benzoic acid	700-1100[g]	125[g]	----	92,104
25. o-Hydroxybenzoic acid	63[h]	----	----	103

TABLE III (Con'd)

Guest Molecule	K_f, M^{-1} [a] α-CD Complex	β-CD Complex	γ-CD Complex	Ref.
26. m-Hydroxybenzoic acid	172-708[h]	----	----	71,103
27. p-Hydroxybenzoic acid	1032-2000[h]	----	----	71,103
28. o-Nitrobenzoic acid	83[h]	----	----	94,103
29. m-Nitrobenzoic acid	153[h]	----	----	94,103
30. p-Nitrobenzoic acid	153[h]	----	----	94,103
31. o-Methylbenzoic acid	416[h]	----	----	103
32. m-Methylbenzoic acid	666[h]	----	----	103
33. p-Methylbenzoic acid	1587[h]	----	----	103
34. Phenol	18.9[i] (15,800)[g]	(2511)[g]	----	23,92
35. o-Nitrophenol	8[j] (200)[k]	(357)[k]	----	73,94
36. m-Nitrophenol	54[j] (500)[k]	(147)[k]	----	73,94
37. p-Nitrophenol	341[j] (2439)[k]	(1020)[k]	----	73,94
38. 3-Methyl-4-nitrophenol	N.B.[l]	----	----	78
39. 3,5-Dimethyl-4-nitrophenol	N.B.[l]	----	----	78
40. o-Tolyl acetate	52.6[m]	----	----	23
41. m-Tolyl acetate	58.8[m]	----	----	23
42. p-Tolyl acetate	91[m]	----	----	23
43. m-t-Butylphenyl acetate	500[m]	7692[m] (55.5)[u]	----	23
44. Prostaglandin E_1	1200-1439[n]	1450-1700[n]	----	105,106
45. Prostaglandin E_2	560-760[n]	1240-1270[n]	----	106
46. Prostaglandin A_1	970-1300[n]	1160-1400[n]	----	105,107
47. Prostaglandin A_2	720-840[n]	1280-1560[n]	----	106,107
48. Prostaglandin B_1	970-1200[n]	410-780[n]	----	105,106

TABLE III (Con'd)

Guest Molecule	K_f, M^{-1} [a]			Ref.
	α-CD Complex	β-CD Complex	γ-CD Complex	
49. Prostaglandin B_2	790[n]	420-430[n]	----	106
50. Prostaglandin $F_{2\alpha}$	250-390[n]	740-1240[n]	----	106
51. Prostacyclin	461[o]	902[o]	157[o]	108
52. Chlorpromazine	200[p]	12000[p]	1000[p]	108,109
53. L-Phenylalanine	12600[g] (15.8)[o]	----	----	92,110
54. D-Phenylalanine	(20.6)[o]	----	----	110
55. L-Tryptophan	31[g]	----	----	92
56. R-(-) Sarin	25[q]	----	----	111
57. S-(+) Sarin	166[q]	----	----	111
58. (+)-CTPONPE	52.6[r]	1481[s]	----	82,112
59. (-)-CTPONPE	76.9[r]	1481[s]	----	82,112
60. Pyridine	158[g]	1.6[u]	----	92,113
61. Anisole	----	(200)[a] (2.5)[u]	----	113
62. Ferrocene	----	(50)[u] (66)[v]	----	113
63. 2-Methoxy-5-t-butylcinnamate	----	(100)[w]	----	34
64. p-Nitrophenyl-1-adamantane formate	76.9[x]	(2778)[x] (213)[y]	1220[x]	114,115

a) Values at 25° C in aqueous medium unless otherwise indicated.
b) Ionic strength, I = 0.01 - 0.03 M.
c) pH = 10.5.
d) pH = 5.7, I = 0.10 M.
e) pH = 1.2, I = 0.50 M.
f) pH = 6.4, I = 0.05 M.
g) In near neutral pH.
h) In 0.08 M HCl.
i) pH = 2.2, I = 0.06 M.
j) pH = 3.5 at 22.0° C.
k) pH = 10.0 at 20.0° C.
l) No apparent binding observed.
m) pH = 10.6, I = 0.20 M, 0.5% CH_3CN.
n) pH = 6.0, I = 0.20 M.
o) pH = 11.0.
p) pH = 7.0, I = 0.30 M.
q) pH = 9.0.
r) pH = 8.62.
s) pH = 5.75 or 9.70.
t) Dipolar aprotic solvent.
u) In DMSO solvent.
v) In DMF solvent.
w) In 60% DMSO/40% Water, "pH" = 6.8 at 30° C.
x) In 0.10 N NaOH, 1% CH_3CN, at 16.0° C.
y) In 0.10 N NaOH, 34% CH_3CN, at 16.0° C.

(iii) Decrease of the strain energy in the ring frame system of the CD (true in the case of α-CD only[21])[120,121] and/or

(iv) Release of high energy CD cavity water molecules during complex formation.[1,17,21,122]

In most cases, a combination of these factors seems to be operative, with the first two usually dominant.[1,21]

Regardless of the binding forces involved, the most important parameters that determine whether an inclusion complex can be formed are the relative size and geometry of the guest molecule in relation to the dimensions of the host CD cavity.[1,9,21] Data presented in Table III clearly show that the stability of the inclusion complexes formed varies with the sizes of both the guest molecule and the host CD. If the substrate is too small, it will easily pass in and out of the CD cavity with little or no binding (i.e. low K_f value, refer to entries 1-9, Table III). However, if the guest molecule is too large or possesses bulky substituents, it will not fit the cavity and thus not bind to the host CD (refer to entries 38,39 of Table III).

In the formation of the complex, the guest molecule approaches and penetrates the CD cavity from the more open and accessible secondary hydroxyl side[1,9,21] (Figure 4). The guest is included in such a manner as to allow its nonpolar portion(s) maximum contact with the hydrophobic CD cavity, while its polar portion(s) is simultaneously oriented so as to be near the hydrophilic surface of the CD which allows for maximum contact with the bulk solvent (usually water) and/or favorable interaction with the CDs secondary hydroxyl groups. In the absence of adverse steric effects, the particular hydrophobic portion(s) of guest compounds capable of penetrating in this fashion include either substituted or unsubstituted alkyl and aromatic groups, among which the substituents present can be: -COOH,[67,71,83] $-CCl_3$,[123] $-NO_2$,[67,71,78] -CN,[67,71] -I,[1,67,71] -R,[1,67,71] -phenyl,[1,104] -naphthyl,[124] and -H.[67,71] On the other hand, inclusion of polar groups (whether formally charged or uncharged) into the cavity is not favored. For instance, in many circumstances,[1] the -OH,[67,71] $-O^{\ominus}$,[71,78] $-NH_2$,[71,91] $-NH_3^{\oplus}$,[67] $-SO_3^{\ominus}$,[89]

and $CO_2^{\ominus}$,[71,104] functional groups of compounds do not seem to be able to penetrate the CD cavity very well. These findings can be explained by solvation considerations and/or by the strong dipole orientational preferences of the substrate molecule.[125] Along these lines, it has been noted that the greater the substrate molecule's dipole moment becomes, the smaller its binding ability will be. This is consistent with the view that the more polar the substrate molecule gets, the less its tendency to partition into the CD cavity will be.[125]

2. Binding Trends Exhibited by Families of Compounds Towards Natural CDs.

Qualitatively, the complexing tendencies of a given family of guest molecules can be correlated with the fit of the substrate to the CD cavity,[1,9,21,23,78,126] although no such correlations are evident when comparing the complexing abilities of different families of compounds.[1] It is rather remarkable that there is no obvious correlation between any physical or chemical property (of the compound or its functional group(s) present) of different fam-

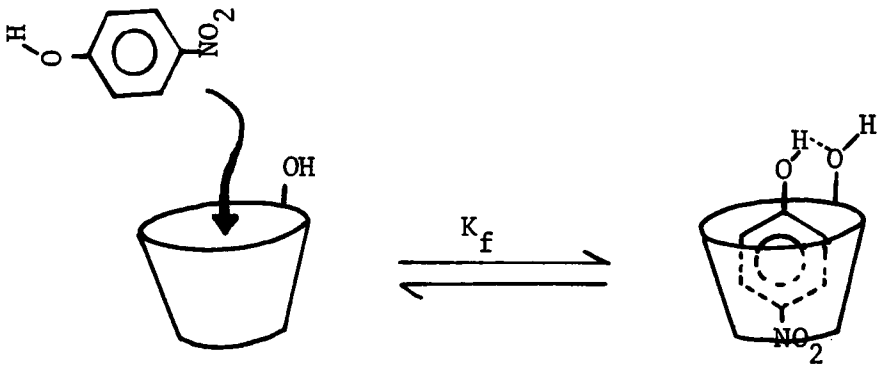

FIGURE 4

A schematic represenation showing the approach and binding of p-nitrophenol from the wide, 2,3-hydroxyl side of the cavity, nitro group first, to CD.

ilies of guest molecules and the inclusion complex formation equilibrium constant.[21] Within certain families of compounds, however, some correlations exist between the strength of binding and some measure of the guest molecule's hydrophobicity (such as polarizability,[1,9,23] molar volume,[23] molar refraction,[23,102] Hammett or Taft substituent constants,[1,9,102,103] Charton's ν parameter,[102] and/or the partition coefficient of the substrate in an apolar-water solvent system[102]).[1,9,21] For example, for a series of structurally related phenyl ester substrates, a fairly good correlation exists between the molar refraction (or molecular volume) of the substrate and the respective binding constants of the CD complex.[23] Similarly, the binding constants of a series of para-substituted benzoic acids correlated well to their Hammett substituent constants which may be related to the polarizability of the guest molecule.[103] For a series of cinnamates, the binding constants of their α-CD complex correlated well with the substrate's dipole moment.[125] An excellent linear correlation was also observed between the binding constants of a series of alcohols to α- or β-CD and the partition coefficient (between diethylether and water) of the alcohol.[102] Additionally, a quantitative correlation between the association constants for CD complex formation of inorganic ions and some water structure sensitive parameter has been reported.[98] However, even within the same family of compounds, such correlations many times do not exist.[127]

Specific examples of some of the generalizations outlined in the previous three paragraphs can be gleaned from the data presented in Table III. As can be seen, small inorganic ions bind to β-CD to varying degrees as they do to α-CD depending on the size of the anion.[99] The magnitude of the binding reflects their polar nature, and the general order of stability (K_f values) seem to parallel their respective size and ability to fit the CD cavity (refer to entries 1-6, Table III). The same sort of stability trend with respect to size is observed in organic alkyl carboxylates or sulfate anions (entries 7-12, Table III).

Another interesting example concerns the complexing tendencies of alcohols to α- or β-CD. It appears that the linear (unbranched)

alcohols can more closely fit the relatively small α-CD cavity, whereas they are too small to be in intimate contact with the relatively larger β-CD cavity.[102] The non-linear, branched alcohols, on the other hand, are too bulky to be included deeply within the α-CD cavity and can therefore better fit the larger β-CD cavity instead. The binding constants (entries 13-23, Table III) for the alcohol-CD inclusion complexes formed reflect this dependence on size and the ability of the substrate to fit the CD cavity. It was postulated that Van der Waals forces (hydrophobic interactions) were mainly responsible for the binding of these alcohols to CD.[102] As the bulkiness of the alcohol increases, the stability of the α-CD complexes decreases due to Van der Waals repulsion while that of the β-CD complexes increases due to favorable Van der Waals interactions.[102] Similar trends in the binding of aliphatic hydrocarbons to CDs have been observed.[28,150,208] Thus, depending upon the CD employed, different selectivities in the binding ability of structural isomeric alcohols or alkanes to CDs are observed.

A similar discrimination in the binding of positional ortho, meta, and para-disubstituted aromatic isomers to CDs has been observed.[1,9,21] In general, the order of stability for binding to α-CD follows this sequence: para ≥ meta ≥ ortho.[48] Typically, the greater stability of the para isomers is a result of their "almost perfect fit" to the α-CD cavity.[1,9,21] In some cases, some extra stability can be attained as the other appropriate substituent (Y) of the para isomer is in a position whereby it can effectively interact, via hydrogen-bond formation, with one of the secondary hydroxyl groups on the rim of the α-CD cavity[70,94] (Figure 5). The lower stability of the meta isomer has been attributed to the more shallow penetration, (due to steric constraints), of that isomer into the α-CD cavity which results in an off-axis alignment of the meta substrate in the α-CD cavity[86] (Figure 5); to the lower polarizability of the meta substrate compared with the para;[71] and, in some cases, to the reduced ability of the meta Y substituent to hydrogen-bond to the secondary hydroxyl groups of the CD rim.[94] The reduced stability of the ortho isomer (compared to the meta and para) is mostly caused by steric hindrance which results in a

greater off-axis alignment (i.e. less penetration into cavity).[86] In addition to the geometric size restriction, the binding capacity of some ortho isomers might be even further reduced, due to the inactivation of the substituents present, by intramolecular hydrogen-bonding between the two groups. Comparison of the data in Table III (entries 24-43) substantiate the outlined generalities concerning the order of stability of o-, m-, and p-disubstituted isomers to α-CD.

The stability of the inclusion complexes formed between monosubstituted or para-disubstituted aromatics and β-CD is usually diminished compared to that observed for α-CD (refer to entries 24, 34, and 37, Table III). This is the consequence of the fact that these types of molecules, whose dimensions are such that they better fit the smaller α-CD cavity, bind the larger β-CD cavity much less tightly. On the other hand, the ortho-disubstituted isomers, which do not fit the α-CD cavity very well due to steric hindrance, can better bind to the larger β-CD cavity. This results in an enhanced binding constant for the β-CD complexes compared to those of α-CD (refer to entry 35, Table III). The binding strengths of meta isomers to β-CD (compared to α-CD) depend on the bulkiness of the

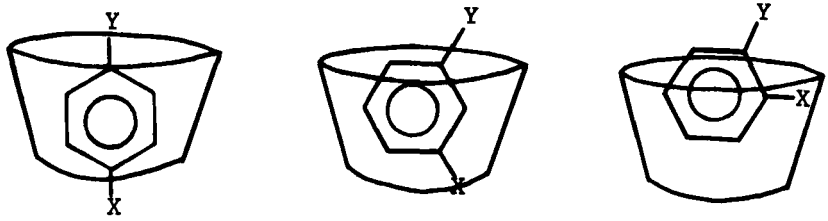

FIGURE 5

A simplified comparison of how ortho-, meta-, and para-disubstituted benzenes sit in the cyclodextrin cavity. The X represents the more hydrophobic substituent while Y represents the more hydrophilic one.

substituents present and/or on their ability to hydrogen-bond to the secondary hydroxyl groups of the CD. Consequently, either increases or decreases in stability are observed for the inclusion complexes formed (refer to entries 36, 43, Table III). It should be noted here that the trends outlined concerning the relative binding abilities of o-, m-, or p-isomers to α- or β-CD are only simplified generalizations and not without some notable exceptions.[1,9,21]

A vast amount of data has recently been published regarding the factors, forces, modes, and trends involved in the binding of a wide variety of pesticides (pyrethroids),[128] pharmaceutical compounds (such as prostaglandins,[108,109,129,130] phenothiazines,[108,109] barbiturates,[131] sulfonamides,[84,109,132] sulfonylureas,[132] anthranilic acids,[79,109] dinucleoside phosphates,[133] vitamins,[134] and cinnamic acids[125,135],[109,136] and amino acids[209,210] to CDs. In most cases, the stability of the complexes formed (within each of these families of compounds) can be correlated to the ability of the drug molecule to fit the appropriate CD cavity. Limitations of space preclude a description of the binding trends observed within each of these families of compounds, thus only the complexes formed with prostaglandins (entries 44 to 50, Table III) will be detailed as an illustration. Prostaglandins are a series of twenty-carbon poly-unsaturated fatty acids that contain one five-member ring and two side-chains which differ from one another only in the number and position of double bonds and hydroxyl groups present. Figure 6 gives the structures for the prostaglandins listed in Table III.

Studies have shown that α-CD includes these molecules in a different fashion than does β-CD. In the presence of α-CD, the C_{16}-C_{20} alkyl portion of the prostaglandin side-chain is predominantly included within the α-CD cavity. In this configuration, the terminal carboxyl group can favorably interact via hydrogen-bond formation with the primary hydroxy groups of the α-CD (Figure 7). The substituted five-member ring system of prostaglandin is apparently too large to be included within the α-CD cavity.[105,106,109,136] In contrast, the larger β-CD cavity is capable of including this substituted ring system along with its immediate neighbors

(see Figure 7). In this case, the terminal carboxylic group can hydrogen-bond to the secondary hydroxyl groups of the β-CD. In most cases, the β-CD prostaglandin inclusion complexes are more rigid and have greater stability constants than those of α-CD[109, 136] (refer to entries 44-50, Table III).

The prostaglandins of type 1 (say PGE_1) differ from those of type 2 (PGE_2) only in the nature of one of the two side-chains present. Namely, the type 1 members all have a R = $(CH_2)_6COOH$ group present while those of type 2 possess a site of unsaturation, R = $CH_2CH{=}CH(CH_2)_3COOH$. The different type 1 prostaglandins (PGE_1, PGA_1, PGB_1, etc.) differ from each other only in the substituents and degree of unsaturation present in the five-member ring system. Thus, for the α-CD inclusion complexes, there are substantial dif-

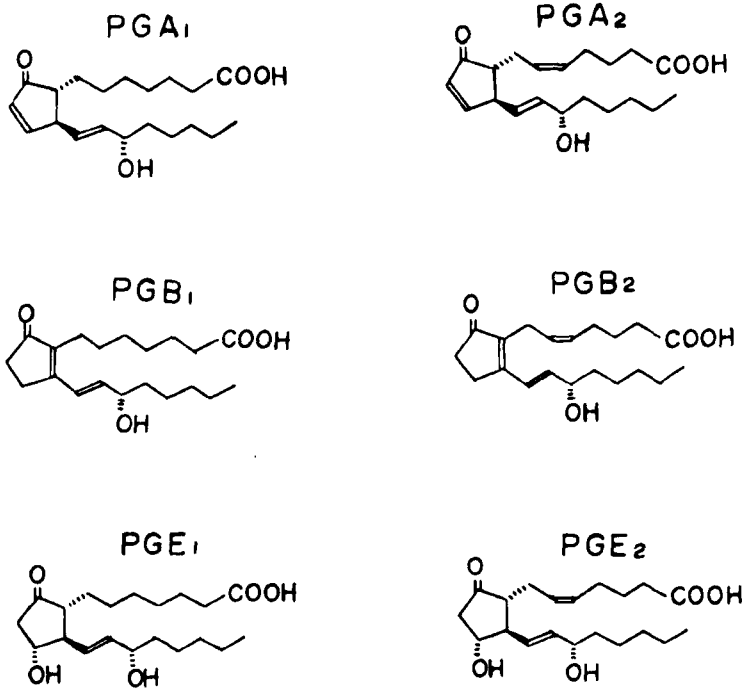

FIGURE 6

Structures of the A-, B-, and E-type prostaglandins.

ferences between the binding constants of the type 1 and type 2 prostaglandins (PGE_1 compared to PGE_2), which reflects the structural differences between the side-chains present. In contrast, there should not be much of a difference for the β-CD complexes since inclusion is not occurring near the dissimilar side-chains. Additionally, the stability constants of all type 1 prostaglandin-(PGE_1, PGA_1, PGB_1) or all type 2 prostaglandin-(PGE_2, PGA_2, PGB_2)-α-CD complexes should be similar whereas those of β-CD should be different since the β-CD includes the ring system which is different in each instance. The results in Table III (entries 44-50) corroborate these expectations.

The optical isomers of most chiral guest molecules have very similar binding constants to the CDs.[1,110] However, appreciable selectivity in the binding of the enantiomeric isomers of certain guest molecules to CD have been reported (refer to entries 53, 54, 56-59, Table III).[82,110,111,112,137-141] The S-(+)-Sarin and (-)-

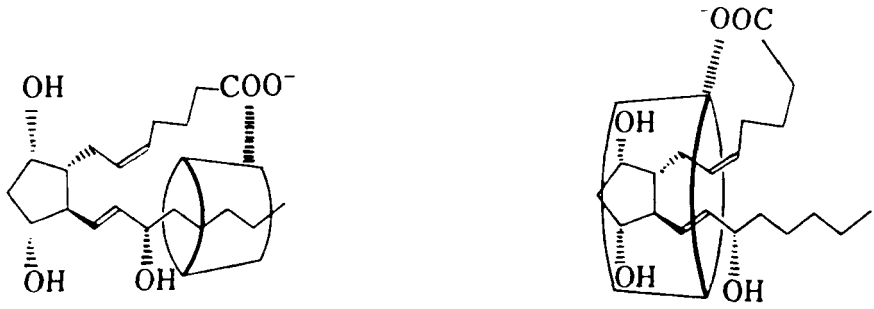

FIGURE 7

Illustration of the proposed binding modes for the formation of prostaglandin (specifically $PGF_{2\alpha}$) inclusion complexes with α- and β-cyclodextrin. Reprinted with permission from F. Hirayama, K. Uekama, and H. Koinuma, Chem. Pharm. Bull., 28, 1979 (1980).

CTPONPE-α-CD inclusion complexes, for example, are roughly 7 and 1 1/2 times as stable, respectively, than the corresponding R-(-)-Sarin and (+)-CTPONPE complexes;[82,111,112] [Sarin = isopropylmethylphosphonofluoridate and CTPONPE = 3-carboxy-2,2,5,5-tetramethyl-pyrrolidin-1-oxy-m-nitrophenyl ester]. In contrast to the stereoselective binding of CTPONPE to α-CD, no enantiomeric specificity was observed for the inclusion of this compound to β-CD.[112] That there should be chiral discrimination in the binding of enantiomeric molecules to CDs is not surprising since all CDs are themselves chiral molecules. It has been noted that there is a pronounced correlation between the stereospecificity of inclusion of chiral sulfoxides into β-CD and the steric bulk and spatial arrangement of the substituents connected to the chiral sulfur atom.[141] Other than this novel work, there have not been any investigations into the origins of the chiral selectivity exhibited upon inclusion complex formation.

In summary, this section has clearly shown that the natural CDs are very discriminating in their inclusion complexing tendencies towards different structural-, positional-, or stereo-isomeric molecules as well as between very similar structurally related compounds.

3. Binding to Modified Cyclodextrins.

The selective complexing ability and trends exhibited by the parent CDs are largely retained in the structurally modified or polymeric CDs.[1,21,40,44,45] In fact, their binding selectivity is, in many instances, enhanced.[1,21,41,144,145] The binding constants for the formation of inclusion complexes between various substrates and some of the modified CDs which are listed in Table II are summarized in Table IV. Compared to the binding behavior shown by the parent CDs, the binding constants for inclusion to these modified CDs can be diminished (entries 4, 6, 14, 15, Table IV), remain roughly the same (entries 1, 3, 11, 12), or be greatly enhanced (entries 5, 16-19, 35-38, Table IV).

Just as in the case for the parent CDs, these results for the CD derivatives can be rationalized in terms of the ability of the guest molecule to fit the modified CD cavity. In addition to the

TABLE IV

Binding Constants for Selected Guest-Modified CD Complexes

Guest-Cyclodextrin System[a]	K_f, M^{-1}[b] for Modified CD complex	K_f, M^{-1}[b] for Parent CD complex	Ref.
1) p-Nitrophenolate-(1)-β-CD	806[c]	628[c]	26
2) p-Nitrophenolate-(2)-α-CD	7874[c]	2000[c]	26
3) m-Nitrophenylacetate-(4)-β-CD	196[d]	189[d]	27,34
4) m-Nitrophenylacetate-(4)-β-CD[e]	40[d]	189[d]	27,34
5) 2-Methoxy-5-t-butylcinnamate ester-(4)-β-CD	833[f]	100[f]	34
6) p-Nitrophenylacetate-trans-(18)-β-CD	43[g]	136[g]	35
7) p-Nitrophenylacetate-cis-(18)-β-CD	526[g]	136[g]	35
8) L-Phenylalanine-cis-(18)-β-CD	45[h]	32[h]	36
9) L-Tryptophan-cis-(18)-β-CD	54[h]	----	36
10) L-Valine-cis-(18)-β-CD	19[h]	----	36
11) p-Nitrophenylacetate-(8)-β-CD	555[i]	476[i]	31
12) p-Nitrophenylacetate-(14)-β-CD	1205[i]	476[i]	31
13) Cyclohexane carboxylate-(14)-β-CD	1887[i]	141[i]	31
14) p-t-Butylphenacylalcohol-(9)-β-CD	333[j]	833[j]	32
15) p-t-Butylphenacylalcohol-(10)-β-CD	333[j]	833[j]	32
16) m-Nitrophenylacetate-(12)-β-CD	1200[k]	163[k]	33
17) m-Nitrophenylacetate-(19)-β-CD	9100[k]	163[k]	33
18) p-Nitrophenylacetate-(12)-β-CD	3000[k]	208[k]	33
19) p-Nitrophenylacetate-(19)-β-CD	83000[k]	208[k]	33
20) Benzyl alcohol-(15)-α-CD	41.2[l]	----	37
21) Benzylphosphate-(15)-α-CD	32000[l]	----	37
22) 2,4-Dinitrophenol-(15)-α-CD	1010[l]	----	37
23) Methyl orange-(6)-α-CD	4466[m]	776[m]	28,71
24) Methyl orange-(5)-β-CD	63[m]	----	28,71
25) Hexane-α-SPE	2.7[n]	----	42
26) Hexane-β-SPE	0.7[n]	----	42
27) Hexane-α-CME	1.3[n]	----	42

TABLE IV (Con'd)

Guest-Cyclodextrin System[a]	K_f, M^{-1} [b] for Modified CD Complex	K_f, M^{-1} [b] for Parent CD Complex	Ref.
28) Hexane-β-CME	0.5[n]	----	42
29) Hexane-α-ECP	4.0[n]	----	42
30) Hexane-β-ECP	1.7[n]	----	42
31) 2,3-Dimethylbutane-α-SPE	0.5[n]	----	42
32) 2,3-Dimethylbutane-β-SPE	2.7[n]	----	42
33) 2,3-Dimethylbutane-α-CME	0.2[n]	----	42
34) 2,3-Dimethylbutane-β-CME	2.5[n]	----	42
35) Potassium 2-p-toluidinyl-naphthalene-6-sulfonate-$(\beta\text{-CD})_2$-G	8300[o]	20[o]	41
36) Potassium 2-p-toluidinyl-naphthalene-6-sulfonate-$(\beta\text{-CD})_2$-S	17400[o]	20[o]	41
37) Potassium 2-p-toluidinyl-napthtalene-6-sulfonate-PA-β-CD	10000[o]	20[o]	41
38) Anisole-(2)-α-CD	1200	271	40

a) Compound numbers in parenthesis correspond to the modified CDs listed in Table II.
b) Values determined at 25.0° C in water unless otherwise specified.
c) pH = 11.0, I = 0.50 M.
d) pH = 9.0, I = 0.20 M, in presence of 0.5 - 0.75 % CH_3CN.
e) The N-ethyl derivative of compound (4).
f) In 60 % (v/v) DMSO-Water at 30.0° C, "pH" = 6.8.
g) pH = 8.7.
h) Equilibrium constant for formation of the 1:2 host-guest complex.
i) pH = 10.0.
j) pH = 9.70 at 37.0° C.
k) pH = 10.60, I = 0.15 M, in the presence of 0.5 - 0.75 % CH_3CN.
l) pH = 7.00, I = 0.10 M, at 30.0° C.
m) pH = 2.10.
n) At 20.0° C.
o) For the 1:2 host-substrate complex formed at pH = 5.9.

considerations already outlined for the natural CDs, the following factors can become important when trying to explain the relative binding ability of substances to modified CDs:

(i) Alterations of the cavity height (depth)-- In most of the modified CD systems, the net effect of the added substituents results in a more hydrophobic cavity whose depth can be either shallower or deeper than that of the parent CD.[33-36] Consequently, guest molecules that better fit the "deeper" modified cavity will have larger binding constants due to enhanced favorable hydrophobic interactions[28,33-36,40] (refer to entries 2, 5, 7, 12, 13, 16-19, 23, Table IV). Those substrates that can't be sufficiently included in a "shallower" cavity will, on the other hand, exhibit similar or somewhat diminished binding abilities[27,31,35,36] (entries 3, 4, 6, 11, Table IV).

(ii) Diminished accessibility-- In some cases, the position and bulk of the added substituents obstruct the approach and binding ability of a guest molecule to the modified CD[32] (entries 14, 15, Table IV).

(iii) Electrostatic effects-- Incorporation of charged substituents [such as the cationic ammonium group (compounds 15, 16, Table II) or coordinated metal cations (compound 14, Table II)][31,37] in the modified CD means that additional favorable electrostatic (both coulombic and/or coordination) interactions are possible, which will enhance the binding ability of anionic hydrophobic substrates[31,37,142] (entries 12, 13, 21, Table IV).

(iv) Cooperative binding-- In the binding of some larger guest molecules to dimeric or polymeric CDs, it was found that 2:1 rather than 1:1 host-guest stoichiometry prevailed and that these complexes were extremely stable[21,41] (refer to entries 35-37, Table IV). It was proposed that the close proximity between two adjacent CD moieties and the high local concentration of CD within the polymer allowed for cooperativity in binding between adjacent CD molecules.[41,143,147] Most polymeric CD-guest inclusion complexes also assume a more rigid conformation than is possible for the parent CD complexes.[41] This fact also helps to account for their increased stability.

In conclusion, although predictions concerning the binding trends expected within or among families of compounds to the different parent CDs are possible, such generalizations become much more difficult for the CD derivatives or polymers. In both instances, the use of space-filling molecular models allows one to better visualize the potential size and steric constraints involved for a particular guest-CD host system and thus greatly aids prediction of the expected relative binding abilities.[34]

4. Other Factors that Influence CD-Inclusion Complex Stability.

Various experimental conditions can affect the binding ability of guest molecules to CDs. Among these, the more important factors are temperature and pressure, the solvent or cosolvent present, and the pH of the solution. Both the parent and modified CDs are similarly affected. Each of the three listed factors will be briefly described.

(i) Temperature and Pressure-- The stability of CD inclusion complexes in the solid state is greater than that in solution and is dependent upon the temperature, pressure, and nature of the guest molecule.[1,3,21,28] The thermal decomposition of such complexes is usually accompanied by diffusion of the guest molecule away from the CD matrix or melt. Typically, the thermal stability of the crystalline complexes is quite high since most complexes do not decompose until around 100° C or higher (in vacuo).[3,21,28] The data presented in Table V show that the stability constants for various CD-inclusion complexes in solution decrease significantly as the temperature increases. As a consequence, most of these complexes will be completely dissociated in water once the temperature reaches the 60-70°C range.

The effect of changes in vapor pressure upon the stability constants has been infrequently investigated. One report showed that the stability constants for 1:1 complexes did not significantly vary as a function of the vapor pressure whereas those of the 1:2 complexes in solution did (i.e. K_f decreased as the vapor pressure increased).[147]

Table V

Temperature Effects on the Binding Constants for Formation of Various CD-Guest Inclusion Complexes

Guest-CD Inclusion Complex	K_f, M^{-1} [a] at the indicated temperature (°C)									
	1°	15°	20°	25°	30°	35°	40°	45°	50°	55°
p-Hydroxybenzoic acid-α-CD[71]	---	3710	---	1032	752	---	416	---	262	---
Hexane-α-SPE[42]	10	---	2.7	---	1.3	---	0.8	---	---	---
m-Ethylphenyl acetate-β-CD[23]	---	---	---	462	---	---	351	---	270	---
Cetyltrimethylammonium bromide-β-CD[101]	---	---	---	2240	---	1850	---	1560	---	1020
Methyl orange-α-CD[71]	---	1091	926	800	---	---	---	---	---	---
Benzoic acid-α-CD[104]	---	1397	---	751	583	---	325	---	203	---
Benzoate anion-α-CD[104]	---	14.4	---	10.5	9.7	---	8.1	---	6.6	---
Diisopropylphosphorofluoridate-α-CD[116]	(12.8)[b]	6.3	---	4.8	---	3.6	---	2.2	---	---
c-Nitrobenzimidazole-α-CD cinnamate[146]	---	---	182	---	93	---	---	---	48	---
2,3-Dimethylbutane-α-ECP[42]	14.7	5.7	---	4.1	---	2.9	---	---	---	0.8

a) In an aqueous medium.

b) Value given for 5.0° C.

(ii) Effect of Solvent and Cosolvent-- Inclusion complexes can be formed either in solution or in the crystalline state.[1] It is important to note that inclusion complexes in solution are apparently formed only in the presence of aqueous, mixed aqueous (water-DMSO, -DMF, -acetonitrile, or -alcohol), or dipolar aprotic solvents.[1,9,101,113,148] No interaction between aromatic substrates and CDs were observed in nonpolar solvents (such as benzene, chloroform, ether, dioxane, or carbon tetrachloride).[1,9,75] In fact, when crystalline CD inclusion complexes are dissolved in such solvents (methylene chloride, trichloroethylene), they completely dissociate.[137-139] Although complexes can be formed in DMSO, DMF, or mixed aqueous solvent systems, the binding of substrates to CDs in these circumstances is greatly reduced compared to that in water alone.[1,113-115] For instance, the binding constant for the inclusion complex formed between hexadecyltrimethylammonium bromide and β-CD is 2240 M^{-1} (at 25° C) in water whereas it is only 450 M^{-1} in the presence of 23.3% (v/v) ethanol-water[101] (refer also to entries 61, 62, 64 for β-CD in Table III).

(iii) pH Effects-- The stability of CD inclusion complexes has been shown to be dependent upon the charge of the guest molecule.[9,100,127] Hence, the pH will affect only the binding of those guest molecules that possess ionizable acidic or basic functional groups. In almost all cases, the binding ability of the formally charged species (either cationic or anionic) is smaller than that of the corresponding neutral species (see Table VI). This is presumably due to diminished hydrophobic interactions between the charged guest molecule and the nonpolar CD cavity. The binding of substituted phenols to CD is an exception to this rule in that the ionized phenolate anions better bind CD than do the corresponding neutral phenols (refer to the last 3 entries, Table VI). The exact reason(s) for this remain unclear,[73,78] although it may be due in part to favorable interactions between the secondary hydroxyl groups of the CD and the charged oxygen atom of the phenolate anion.

Figure 8 shows the pH profile for the stability constant, K_f, of the sulfathiazole-β-CD complex.[127] The maximum binding observed

TABLE VI

A Comparison of Stability Constants for the Binding of Neutral and Charged Species to Cyclodextrins

Substrate-CD System	K_f, M^{-1} a		Ref.
	Neutral Species	Charged Anionic Species[b]	
Perchloric acid-α-CD	39.8	20.0	92
Propionic acid-α-CD	31.1[c]	1.8	23,71
trans-Cinnamic acid-α-CD	2260	110	125
Benzoic acid-α-CD	751	14.5	104
p-Nitrobenzoic acid-α-CD	153-172[c]	27.4[c]	71,94
p-Hydroxybenzoic acid-α-CD	1032	11.5	71
m-Nitrobenzoic acid-α-CD	105[c]	42.4[c]	71
m-Hydroxybenzoic acid-α-CD	353[c]	3.4[c]	71
p-Cyanophenol-α-CD	140	632	71
p-Nitrophenol-α-CD	20-341	2500	73,78
2,6-Dimethyl-4-nitrophenol-α-CD	568	1062	73,78, 94

a) In water at 25.0° C unless otherwise specified.

b) Refers to the appropriate corresponding acid anion or phenolate ion of the listed neutral species.

c) At 30° C.

around pH 5.50 reflects the fact that the sulfathiazole is unionized at this pH and hence, maximum hydrophobic interactions between it and the CD cavity are possible. As the pH increases or decreases from this value, the fraction of charged, ionized species present increases with a concurrent decrease of the binding constant[127] [the pK values for the amide proton and the amino group of sulfathiazole are 7.12 and 2.30, respectively]. Similar type profiles

would be expected for other guest molecules possessing ionizable groups.

In natural CDs, the ionization of the CD itself usually presents no problem since the ionizable primary and secondary hydroxyl groups present have relatively high pK_a values (12.0 - 12.6). However, some of the modified CDs possess other substituents that are ionizable under less drastic conditions. In these cases, the ionization of both the guest and CD molecules must be taken into account when attempting to anticipate the pH effects. It is possible that either

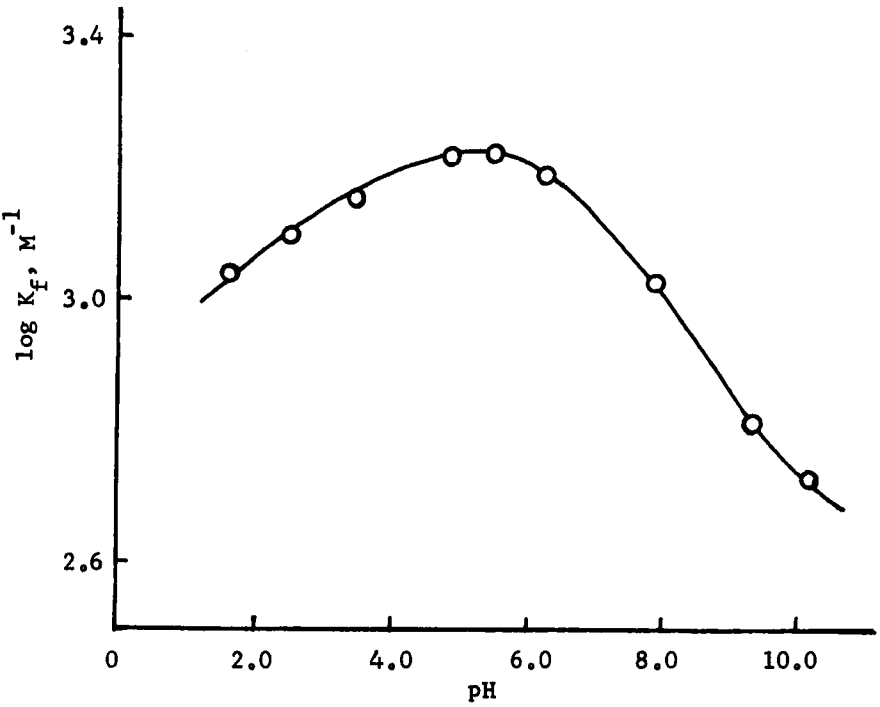

FIGURE 8

Plot of the log of the formation binding constant of the sulfathiazole-β-CD inclusion complex vs. pH at 25.0° C. Reprinted with permission from K. Uekama, F. Hirayana, M. Otagiri, and K. Ikeda, Chem. Pharm. Bull., 26, 1162 (1978).

favorable (or unfavorable) electrostatic interactions can occur between the guest and CD molecules in these type of systems (in addition to the other interactions mentioned, section C-1, supra vide).

In the binding of benzyl phosphate to the modified CD, triammonio-per-O-methyl-α-CD (compound 15, Table II), for instance, the binding constants are 500 M^{-1}, 32,000 M^{-1}, and < 10 M^{-1} at pH values 5.50, 7.00, and 9.50, respectively.[37] These data merely mirror the degree of ionization of both the guest and host CD and the electrostatic interactions possible. The guest benzyl phosphate molecule is essentially present in the monoanionic form at pH 5.50 and is 70% in its dianionic state at pH 7.00, while the modified CD host is predominantly present in its cationic state (all ammonio groups protonated) at both pH values. Consequently, due to the added favorable electrostatic effects, the binding constant is over 60 times greater at pH 7.00 than at pH 5.50.[37] At pH 9.50, the host CD is neutral and the benzyl phosphate is still in its dianionic form. Consequently, the drastically diminished binding observed at this pH is due to elimination of the favorable electrostatic interactions that were possible at the lower pHs. Similar type considerations are required to analyze the pH effects on the binding of any ionizable substrate to modified CDs that also possess an ionizable substituent.

As can be seen from this section, there are a variety of factors that can be varied if one wants to control the relative binding order of several compounds of interest to the CDs.

D. GENERAL APPLICATIONS OF CYCLODEXTRIN INCLUSION COMPLEXES IN THE ART OF SEPARATION SCIENCE

CDs and their inclusion compounds have found amazingly diverse and practical uses.[1,8,16,21] These range from their application in the pharmaceutical to the agricultural industries. CD inclusion complex formation has been used to stabilize volatile or sensitive compounds, modify physiochemical properties of guest molecules, solubilize water insoluble or slightly soluble materials, etc.[1,16,21] The same properties and advantages of CDs in these applications can be applied to chemical separation and purification. As has been

detailed and illustrated in section C, selectivity in the binding of certain structural, geometric, positional, and optical isomers of compounds to CDs is possible. Likewise, discrimination in the binding of compounds that are very similar in structure is observed. It should be obvious, then, that this selectivity in binding to CDs forms the basis for their use in chemical separations.[149]

It should be mentioned here that there are other host materials [such as Dianin's compound (4-p-hydroxyphenyl-2,2,4-trimethylchroman), urea, thiourea, hydroxy-aromatic compounds (i.e. phenol, hydroquinone), and Werner complexes] capable of selectively forming inclusion complexes with suitable guest molecules.[16,21,149] However, in general, the CDs enjoy a number of advantages over these other potential hosts. The CDs, for example, exhibit host-guest chemistry both in the solid state and in solution (whereas most of the other host-inclusion complexes generally decompose into their components on dissolution in water); CDs have a permanent structure and its inclusion-complexes show fairly high heat stability (whereas these are not the case for most of the other hosts); CDs are less toxic;[21] and CDs can interact selectively with a wider variety of guest molecules.[1,9,21] For these reasons, the use of CDs in chromatographic methods seems more promising and potentially significant. The five general applications of CDs in purification methods and chromatographic separations will be surveyed next.

1. Resolution of Enantiomeric, Positional, and Structural Isomers by Selective Precipitation with Cyclodextrins.

Structural recognition in complexation is an important requirement in the resolution of isomeric compounds. Hosts that are ideally suited for enantiomeric, positional, geometric, or structural isomer separations by selective precipitation should exhibit the following properties:

(i) They should be relatively strong complexing agents that can discriminate between the isomers of a variety of guest molecules by rather large factors;

(ii) the hosts should be easily preparable and resolvable; and

(iii) the host should be stable, recoverable, and reusable.

It is not surprising then that CDs have been employed in such precipitation separations since they do satisfy the above requirements.[150]

One of the most important applications of CD, first described in 1959,[137] concerns the resolution of racemates by means of stereoselective inclusion-complex formation. Since CD molecules are themselves chiral, they can interact with the racemic R,S (+,-) isomers of a guest molecule to form a diastereomeric pair of inclusion complexes with each included racemate (equation 3).

$$\text{D-(+)-CD} + \begin{cases} \text{R-isomer} \overset{K_f^R}{\rightleftharpoons} \text{D-(+)-CD·R isomeric complex} \\ \text{S-isomer} \overset{K_f^S}{\rightleftharpoons} \text{D-(+)-CD·S isomeric complex} \end{cases} \qquad (3)$$

The two complexes formed exhibit different physical properties, including the magnitude of their respective formation equilibrium constants, K_f[21,110,137] and solubility product constants, K_{sp}. The fact that the R,S-optical isomers of many guest molecules do have appreciable differences in their binding ability to CD means that stereospecific inclusion into CDs can be applied as a general method for the resolution of racemic molecules. Consequently, when a racemic substrate (usually present in a 5-10 fold excess) is added to an aqueous ~1.5% CD solution, one of the diastereomeric complexes preferentially precipitates out, permitting a partial resolution of the optical isomers. Subsequent decomposition of the resulting complex (by distillation or via trituration in an organic solvent such as methylene chloride or trichloroethylene at 25-60° C) gives substrates that are rich in one enantiomer. Likewise, distillation of the filtrate (from the original precipitation solution) yields substrates rich in the other optical isomer.

The first reported use of this method concerned the resolution of chiral carboxylic acid esters.[137] Optical purities of 3-10% were achieved. Subsequently, racemic mixtures of O-alkyl alkylphosphinates,[138] sulfoxides,[139] O-alkyl alkylsulfinates,[140] and thiosulfi-

nate-S-esters[141] were reportedly resolved. In these latter examples, optical purities in the range of 24-84%, 4-14%, 4-70%, and 2-68% respectively were usually obtained after only one separation step.[138-141] Maximum optical purities in the 70-85% range can be achieved for these compounds after several precipitation cycles or after fractional crystallization of the inclusion complex.[21,141] Some of the results obtained (optical purities, absolute configurations, etc.) regarding the partial resolution of these compounds with CDs are summarized in Table VII.

In a similar fashion, other isomeric or structurally related compounds can also be separated through the use of CDs. This is evinced by the fact that within a similar series of guest molecules, the complexing tendency toward a particular CD can be qualitatively correlated to the size and spatial disposition of the substituents on the guest substrate.[1,9,126] For example, while benzene can be effectively precipitated from aqueous solutions of α-, β-, or γ-CD, anthracene is only precipitated in the presence of γ-CD.[9] Likewise, bromobenzene is more effectively precipitated from an aqueous solution of β-CD than is benzene while the reverse situation is true in the presence of α-CD. The use of such discriminating CD inclusion precipitates has been incorporated into schemes for the separation of various fatty acids and substituted aromatic or aliphatic structural isomers from one another.[152-158] Additionally, such selective precipitation schemes have been employed to separate and purify mixtures of the different CDs themselves.[1,9,126,151]

This general procedure of purification consists of adding the organic mixture containing the isomeric components to a concentrated aqueous solution of the appropriate CD (typically present in a 4-fold weight excess). A precipitate usually appears in 30-60 minutes, which corresponds to the CD inclusion complex formed due to the preferential binding of only one of the possible isomers present. The separated component in the precipitate can then be regenerated from the inclusion complex by either a steam distillation or extraction (using diethyl ether, methylene chloride, etc.) procedure.

TABLE VII

Resolution of Chiral Compounds via CD-Inclusion Formation

Chiral Guest Compound			Host CD	Optical[a] Purity,%	Absolute Configuration	Ref.
2,3-Dibromo-3-phenylpropionic acid			β-CD	11.3	----	137
2,3-Dibromosuccinic acid			β-CD	8.2	----	137
Ethyl mandelate			β-CD	3.3	----	137
Ethyl atrolactate			β-CD	8.4	----	137
Ethyl α-bromophenylacetate			β-CD	5.8	----	137
Menthol			β-CD	4.9	----	137
1-Bromo-1-chloro-2,2,2-trifluoroethane			α-CD	< 1.0	----	217
O-alkyl alkylphosphinates, $(R'O)(R^2)P(X)H$						
R'=	R^2=	X=				
i-prop	methyl	O	β-CD	66-84	----	138
i-prop	ethyl	O	β-CD	60.0	----	138
ethyl	ethyl	O	α-CD	23.8	----	138
ethyl	phenyl	O	α-CD	28.8	----	138
Sulfinyl Compounds, $(R')S(O)(R^2)$						
R'=	R^2=					
benzyl	methyl		β-CD	8.0	R	139,141
benzyl	ethyl		β-CD	4.7	R	139,141
benzyl	t-butyl		β-CD	14.5	R	139,141
phenyl	methyl		β-CD	4.4	R	139,141
tolyl	methyl		β-CD	8.1	R	139,141
methyl	O-i-prop		β-CD	68-70	S	140,141
methyl	O-t-butyl		β-CD	12.4	S	140,141
methyl	O-neopentyl		β-CD	4.2	R	140,141
i-prop	O-methyl		β-CD	12.8	R	140,141
t-butyl	S-t-butyl		β-CD	13.6	----	140,141
p-tolyl	S-t-butyl		β-CD	2.5	R	140,141

a) After one precipitation cycle.

The types of isomeric compounds that have been separated in this manner include: cresols (o-, m-, p-methylphenols),[152] the ethylphenols,[152] xylenols (the variously substituted dimethylphenols),[152] various p-alkyl(C_2-C_5)toluenes,[153] o-, m-, p-cymenes (isopropyltoluenes),[154] trimethylbenzenes (1,2,4-; 1,3,5-; or 1,2,3-substituted isomers),[155] as well as the ortho, meta, and para isomers of nitrotoluene, chlorotoluene, nitrophenol, and dichlorobenzene.[156] Separation of straight-chain from branched-chain aliphatic hydrocarbons has also been reported.[157] Table VIII summarizes some of the results obtained in these separations. The data indicate that α-CD preferentially binds and precipitates the p-disubstituted benzene isomers rather than the meta or ortho. This selectivity reflects the usual order of stability expected for the binding of these isomers to α-CD (refer to section C-2). The fact that α-CD precipitates n-octane and not isooctane reflects its ability to better bind linear, unbranched aliphatic organic molecules rather than their branched analogues. β-CD, on the other hand, better binds the more bulky, branched isomers. Thus, the separation of fatty acids according to their degree of unsaturation with β-CD demonstrates that as the number of carbon-carbon double bonds in the fatty acid increase, so does their relative degree of bulkiness or branching. Consequently, in a mixture that contains saturated and unsaturated fatty acids, the most unsaturated compound will better bind to β-CD and be preferentially precipitated (refer to the last entries in Table VIII).[150,156,158]

In a variation of general technique just discussed, a mixture of presumably α- and β-CD was used to remove free fatty acids (both saturated and unsaturated) from crude corn oil. In this procedure, the CDs were emulsified with oil and after separation of the phases, the oil was recovered. Hence, oil originally containing a total of 7.50% free fatty acid contained, after the treatment, only 1.13%.[158] Lastly, petroleum has been recovered from oil sand via use of β-CD. Addition of β-CD to the oil sand resulted in formation of the corresponding β-CD-petroleum inclusion complex, from which, after separation from the sand, the petroleum can be regenerated and collected.[160]

TABLE VIII

Separation of Structural and Positional Isomers via Selective CD-Inclusion Complex Precipitation

Composition of the Original Mixture System	Host CD	Composition of the Precipitated CD-Inclusion Complex	Ref.
50% m-cresol	α-CD	44%	152
50% p-cresol		56%	
35% o-cymene	α-CD	1%	153
27% m-cymene		2%	
38% p-cymene		97%	
30% hemimellitene[a]	α-CD	78%	154
32% pseudocumene[b]		21%	
38% mesitylene[c]		1%	
51% p-bromotoluene	β-CD	99.5%	157
49% o-bromotoluene		0.5%	
50% n-octane	α-CD	100%	155
50% isooctane		0%	
47.3% stearic acid	β-CD	24.2%	156,
52.7% linolenic acid[f]		75.8%	158
4.9% palmitic acid	β-CD	3.7%	150
3.2% stearic acid		0.0%	
16.5% oleic acid[d]		8.0%	
15.5% linoleic acid[e]		9.5%	
59.8% linolenic acid[f]		78.7%	

a) 1,2,3-trimethylbenzene
b) 1,2,4-trimethylbenzene
c) 1,3,5-trimethylbenzene
d) contains one double bond
e) contains two double bonds
f) contains three double bonds

As can be seen, the selective formation of CD inclusion complex precipitates has led to a variety of useful isomeric separation schemes. In many cases, the separation schemes devised are as good (in terms of simplicity, time factor, and isomeric purity obtained) as other existing schemes. In other instances, particularly those involving optical isomer resolution, the CD procedure many times is the only one available that will affect the desired isomeric separation.[138,139] By the use of this CD method, it now becomes possible to resolve compounds that do not possess the necessary acidic and/or basic functional groups that were required in the classical resolution schemes (which are based on formation of diastereomeric salts after addition of optically active acids or bases).[139-141] However, these precipitation techniques are probably not easily adapted to resolutions on a preparative scale because they are incomplete and laborious.

Future work in this area should be directed towards devising similar separation schemes for other important isomeric compounds (such as the substituted isomeric cyclohexanes, cyclopentanes, or cyclopentanones, for instance).[161] The use of modified or polymeric cyclodextrins in this area should be investigated. Some exciting recent work along these lines has already been reported. Namely, CD polymeric beads were used to separate organic compounds from water.[162] The use of this approach has potential advantages in terms of convenience and efficiency.

2. Enantiomeric Enrichment Through Enantioselective CD Catalysis of Racemic Substrate Reactions.

The ability of CD to catalyze a variety of chemical reactions has been well documented.[1,9,21,212] They catalyze certain reactions, particularly hydrolyses and transacylations, with some specificity and thus can, to some extent, differentiate between enantiomers (or positional isomers for that matter).[23,110,163-170] A common kinetic scheme can be written to describe these reactions (equation 4):

$$\text{D-(+)-CD} + \begin{matrix} \text{(R)-Substrate} \rightleftharpoons \underset{\text{complex}}{\text{CD}\cdot\text{(R)}} \xrightarrow{k_r} \text{(R)-Product} \\ \text{(S)-Substrate} \rightleftharpoons \underset{\text{complex}}{\text{CD}\cdot\text{(S)}} \xrightarrow{k_s} \text{(S)-Product} \end{matrix} + \text{CD} \qquad (4)$$

In many instances, the specific rate constant for the reaction of one enantiomer is sufficiently greater than that of the other.[1,21] In the basic hydrolysis of isopropyl-S-2-dimethylaminoethylmethylphosphonthioate, for example, the rate constant for the (-)-enantiomer is 110 times greater than that for the (+)-enantiomer (at 25° C in the presence of α-CD.)[163] Consequently, partial racemate resolution occurs as a result of this preferential reaction of one of the substrates' optical isomers present.

Table IX summarizes some of the data available concerning the rate differentials between enantiomers as well as some of the enrichments obtainable in these type of systems. Except for the systems involving the hydrolytic cleavage of organophosphates, the enantiomeric excess obtained is hardly high enough to render the technique very useful for most deracemization applications.[21,165]

3. Utilization of Cyclodextrin-Containing Stationary Phases in Gas and Liquid Chromatography.

The broadest range of CD applications in separation science seems to be in their use as stationary phase materials in various liquid-solid, gas-solid, or gas-liquid chromatographic separations. Since the natural CDs are soluble in water and other polar or dipolar aprotic solvents, they must be converted to an insoluble matrix via modification or polymerization in order to be employed as a stationary separating phase. Many of the modified or polymeric CDs mentioned in section B-2 have been employed as stationary phase materials. Their use has led to separations of a wide variety of structural, positional, and optical isomeric compounds. The basis of the separations observed stems directly from the selectivity in binding that the different isomers and compounds have towards a particular CD present in the stationary phase. Those compounds that interact strongly with CD (i.e. have large K_f values) will be retained longer and thus exhibit relatively long retention times (or volumes). Compounds that weakly bind CD (e.g. small K_f values) will be eluted relatively rapidly. With the knowledge of the stability of the CD inclusion complexes formed, accurate predictions can be made concerning the expected elution behavior of those com-

TABLE IX

Summary of Enantiomeric Enrichments Possible in CD Stereoselective Catalyzed Reactions[a]

Reaction System	Host CD	Rate Differential	Product Enantiomer Enriched	Enantiomer Ratio, L/D
Hydrolysis of Sarin[b,111]	α-CD	R-/S+ = 36	R-(-)	-----
Hydrolysis of isopropyl-p-nitrophenylmethylphosphonate[c,163]	α-CD	R-/S+ = 14	R-(-)	-----
Hydrolysis of isopropyl-S-2-dimethylamino-ethyl-methylphosphonothioate[d,163]	α-CD	-/+ = 110	(-)	-----
Hydrolysis of (E)-(carboxymethylene)-1,2-ferrocenocyclohexane-p-nitrophenylester[e,166]	β-CD	+/- = 20	(+)	-----
Hydrolysis of 2-phenyl-4-benzyl oxazolone[165]	α-CD[f]	-----	(L)	76/24
	β-CD[g]	-----	(L)	80/20
Hydrolysis of 2-methyl-4-benzyl oxazolone[165]	α-CD[h]	-----	(L)	51/49
	β-CD[f]	-----	(D)	33/67
Hydrolysis of 2,4-dimethyl oxazolone[165]	α-CD[f]	-----	(D)	25/75
	β-CD[f]	-----	(L)	58/42
Hydrolysis of 2-phenyl-4-methyl oxazolone[165]	α-CD[i]	-----	(L)	80/20
	β-CD[i]	-----	(L)	80/20
Hydrolysis of CTPONPE[j,82]	α-CD	+/- = 6.9	(+)	-----
Hydrolysis of acetyl-phenylalanine-m-nitrophenyl acetate[164]	β-CD	L/D = 2	(L)	-----
Hydrolysis of p-(and m-) nitrophenyl acetate or benzyl acetate[168]	β-CD[k]	m/p > 1	meta isomers	-----

a) In aqueous media at 25.0° C unless otherwise stated.
b) pH = 9.0.
c) In 0.0950 N NaOH.
d) In 0.0944 N NaOH.
e) At 30.0° C, pH = 10.17.
f) pH = 7.86.
g) pH = 11.0.
h) pH = 11.7.
i) pH = 10.0.
j) pH = 8.6.
k) The 6-(2-hydroxyethylthiol) derivative of β-CD.

pounds on a particular CD stationary phase (refer to section C-2 for some general rules that aid in the prediction of the relative stabilities of certain types of CD inclusion complexes).

One of the first described and most commonly used CD stationary phases is a polymeric CD-epichlorohydrin resin, abbreviated ECP, (refer to Figure 3).[43-45,172-175,179-181] It has been utilized to separate various natural products (vitamins, amino acids), perfumes,[44,181] aromatic amino acids,[44,45,181] o- or p-nitrophenol,[45] substituted chlorobenzoic acids,[44] nucleic acids,[172,173] enantiomeric mandelic acid derivatives,[175] and the diastereomers of $Co(NH_3)_4$-glucose-6-phosphate ADP.[179,180]

Table X summarizes the elution behavior exhibited by some organic compounds on α-ECP, β-ECP, and Sephadex G-25 stationary

TABLE X

Summary of the Elution Behavior of Organic Compounds on ECP Resins

Compound	Elution Volume, V_e[a]		
	α-ECP	β-ECP	Sephadex G-25
Benxoic acid	200[b]	200[b] (400)[c]	(100)[c]
o-Chlorobenzoic acid	45[b]	50[b] (98)[c]	(83)[c]
m-Chlorobenzoic acid	200[b]	200[b] (400)[c]	(83)[c]
p-Chlorobenzoic acid	d	d	----
Glycyl tyrosine	----	(142)[c]	(120)[c]
L-Tryosine	----	(105)[c]	(135)[c]
Aniline hydrochloride	----	(511)[c]	(150)[c]

a) Data taken from reference 44.

b) Mobile phase is 20% ethanol-water, flow rate = 125 ml/hr, column packed with 11 g ECP resin.

c) Mobile phase is 20% ethanol-water, flow rate = 125 ml/hr, column packed with 21 g ECP resin; (or 21 g of Sephadex G-25 resin).

d) Solubility too low to obtain any significant peaks.

phases (the mobile phase was 20% ethanol-water). As expected, the elution behavior of these substances on the CD stationary phases parallels their relative binding ability to α- or β-CD in solution.[44] Figure 9 shows that the complete baseline separation of benzoic and o-chlorobenzoic acid is possible on α-ECP. In contrast, such separation of these two compounds on the Sephadex G-25 phase was not possible.[44] In addition to the higher separation efficiency, the ECP resins appear to be more heat resistant than is the Sephadex G-25. The adsorption and desorption behaviors observed on these α- or β-ECP stationary phases can be described by Langmuir or Freudlich isotherms depending upon the particular compound involved.[174]

Gel chromatography on a column containing β-ECP as the stationary phase has been used successfully for the separation of nucleosides and nucleotides.[172,173] Solution studies showed that nucleic acid components with adenine bases form inclusion complexes with β-CD.[133,173] The order of stability of the CD-inclusion complexes of some of these nucleic acids is:

3'-AMP > 5'-AMP > ATP > 2', 3'-cyclic AMP > ADP > 2'-AMP.

The nature of the base, degree of polymerization, and position of the phosphate group in the nucleotide were found to influence their complexing ability.[173] As shown in Figure 10, the order of elution of the three adenosine monophosphates (2'-AMP, 5'-AMP, and 3'-AMP) on β-ECP is exactly what is expected based on the above order of stability for their β-CD inclusion complexes (i.e. 3'-AMP which best binds β-CD, is eluted last). Figure 11 illustrates the separation obtained on β-ECP for four RNA-derived-3'-nucleotides at 0° C and at 25.0° C. The compounds are only partially resolved at 25° C whereas they are practically completely resolved at 0.0° C.[172,173] The better separation at the lower temperature is due to the fact that CD inclusion complex stability is enhanced at lower temperatures (refer to section C-4-i). This also explains why the elution volumes are increased at 0.0° C.[173] The effect of pH was also briefly examined[172] and appears to adhere to the rules outlined in Section C-4-iii (pH effects). In addition to the nucleic acids already mentioned, the epimeric compounds, 2'-O-methylarabino-

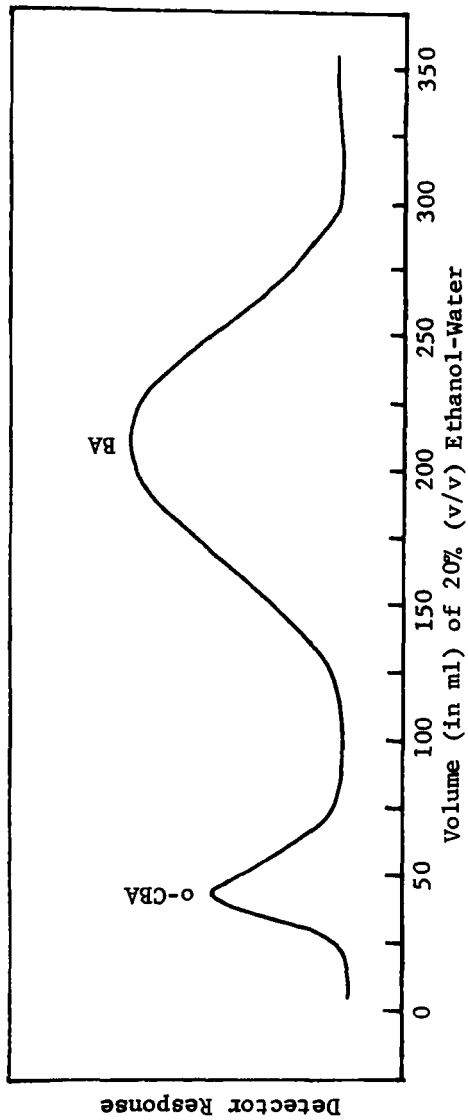

FIGURE 9

Separation of benzoic acid (BA) and o-chlorobenzoic acid (o-CBA) on α-ECP gel (30.0° C, flow rate =125 ml/hr using the indicated mobile phase solution). Reproduced with permission from N. Wiedenhof, Stärke, 21, 164 (1969).

furanosyladenine and 2'-O-methyladenosine;[173] the 3'-O-methyl nucleosides;[172,173] RNA-derived-5'-ribonucleotides; RNA-derived-3'-ribonucleotides; DNA-5'-nucleotides;[173] $Co(NH_3)_4$(Gly-6-P)ADP and $Co(NH_3)_4$ATP;[180] as well as the diastereomers of $Co(NH_3)_4$ADP or $Cr(NH_3)_4$ADP[179,180] have been separated in a similar fashion. The results indicated that the separation of these compounds on the β-ECP stationary phase is a general technique very useful in the field of nucleic acid chemistry and has some important advantages over other potential methods.[173]

Stationary phases of ECP gels have also been utilized for the liquid chromatographic separation of enantiomeric mandelic acid derivatives.[175] The β-ECP exhibited preferential binding of the L-(+) isomers over the D-(-) isomers in all cases. The opposite trend was followed if α-ECP was used as the stationary phase (i.e.

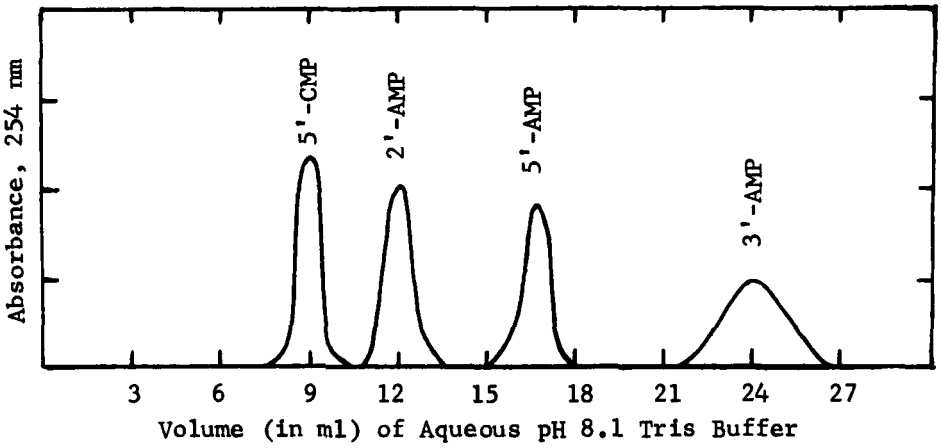

FIGURE 10

Chromatographic separation of three adenosine monophosphates and cytidine-5'-monophosphate on a β-ECP gel (25.0° C, flow rate = 2.8 ml/hr using the indicated mobile phase).[182] Reproduced with permission from J. L. Hoffman, Anal. Biochem., 33, 209 (1970).

binding of the D-(-) isomer was preferred). The mandelic acid derivatives more strongly bind to the β- than α-ECP gel. Hence its use gave the best optical resolution of the enantiomers.[175] The adsorption behavior of mandelic acid derivates on β-ECP obeys the Langmuir equation and indicates that the observed resolution was mainly accomplished through the formation of inclusion complexes.[175]

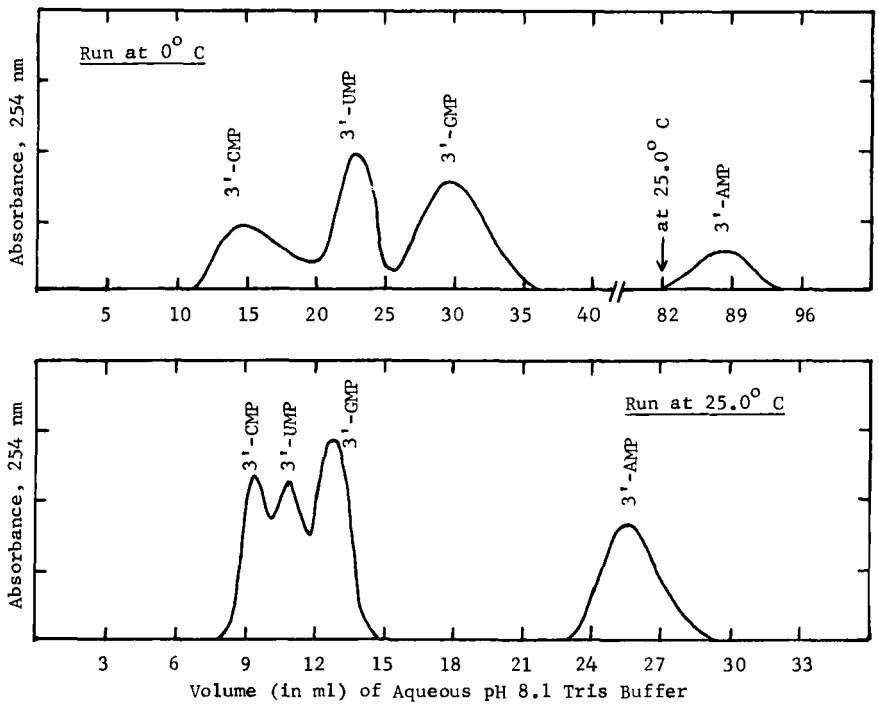

FIGURE 11

Chromatographic separation of four major RNA-derived 3'-nucleotides on a β-ECP gel [the top chromatogram was run at 0.0° C until 82.0 ml elution volume when the temperature was changed to 25.0° C at a flow rate of 3.7 ml/hr using the indicated mobile phase; the bottom chromatogram was run as 25.0° C at a flow rate of 4.0 ml/hr using the indicated mobile phase].[182] Reproduced with permission from J. L. Hoffman, Anal. Biochem., 33, 209 (1970).

Table XI summarizes some of the data for the resolution of the D-(-) isomers on β-ECP.

CD polymer gels, obtained from their polymerization in solution with poly(vinyl alcohol) using ethylene glycol-bis(epoxypropyl) ether as a cross-linking agent[45-48,162,176] (abbreviated α-, β-, or γ-CDP), have also been employed as the stationary phase in some chromatographic separations.[46,176,178] The chromatographic behaviors of some twenty natural amino acids[46,176] and nine alkaloids [178] have been characterized on such gels. Some of these data are compiled in Table XII. As can be seen, the aromatic amino acids can be easily separated from the non-aromatic ones and, under appropriate conditions, even from each other using either the α-, β-, or γ-CDP gels.[46] However, best resolution is obtained on the β-CDP gel. Figure 12 shows that the complete baseline separation of lysine, alanine, phenylalanine, tyrosine, and tryptophan was possible on β-CDP whereas only incomplete separation was achieved on conven-

TABLE XI

Amount of D-(-) Isomer Eluted in the First Two Fractions on β-ECP

Racemic Compound[a]	% D-(-) Isomer in Fraction[b]	
	# 1	# 2
Mandelic acid	67	57
Methyl mandelate	100(69)[c,d]	80
Ethyl mandelate	75	73
O-Methyl mandelic acid	83	79

a) The data were taken from reference 175. All separations were done using the β-ECP stationary phase and water as the mobile phase, flow rate = 10 ml/min.

b) The D-(-) isomers are eluted first since they bind β-ECP the poorest.

c) Value obtained using the α-ECP stationary phase.

d) Refers to amount of the L-(+) isomer eluted.

TABLE XII

Relative Elution Volumes (V_e/V_t) of Selected Compounds on Columns Packed with Cyclodextrin-Polymer Gel Stationary Phases

Compound	Relative Elution Volume on			Ref.
	α-CDP	β-CDP	γ-CDP	
Tryptophan[a]	2.20	1.90	1.35	46,176
Tyrosine[a]	1.05	1.15	0.95	46
Phenylalanine[a]	1.05	0.95	0.85	46
Alanine[a]	0.77	0.74	0.72	46
Aspartic acid[a]	----	0.69	----	46
Glutaric acid[a]	----	0.69	----	46
Histidine[a]	----	0.69	----	46
Lysine[a]	0.72	0.63	0.68	46
Other amino acids[a,b]	----	0.75	----	46
Tabersomine[c]	----	1.8-1.9	----	178
Vincamine[c]	----	1.3-1.4	----	178
Ethyl apovincaminate[c]	----	3.0-3.1	----	178
(-)-Quebrachamine[c]	----	2.9-3.0	----	178
(-)-Vincadine[c]	----	1.4-1.5	----	178
(-)-N-Methyl-quebrachamine[c]	----	2.2-2.3	----	178

a) At 25° C, mobile phase was aqueous pH 5-6 phosphate buffer, flow rate 40 ml/hr.
b) Other amino acids were cysteine, glycine, leucine, isoleucine, methionine, proline, serine, threonine, valine, asparagine, and glutamine.
c) At 25° C, mobile phase was aqueous pH 4 citrate buffer, flow rate 30 ml/hr.

tional stationary phases, like Sepahadex G-25, under the same conditions.[46] Likewise, the use of the β-CDP gel also allowed the separation of alkaloids which could not be separated on the Sepahadex gel. Most significantly, the partial resolution of the (±) enantiomers of vincadifformine was achieved (see Figure 13).[178] These CDP gels, in addition, have been used as adsorbents in cigarette filters and as supports for removing organic compounds from water.[162]

Several α- and β-CD containing polyurethane resins (abbreviated CDPU), cross-linked with different diisocyanates, have been used with success in both liquid and gas chromatographic separations.[51-53,177] For example, the separations of a series of alcohols, ke-

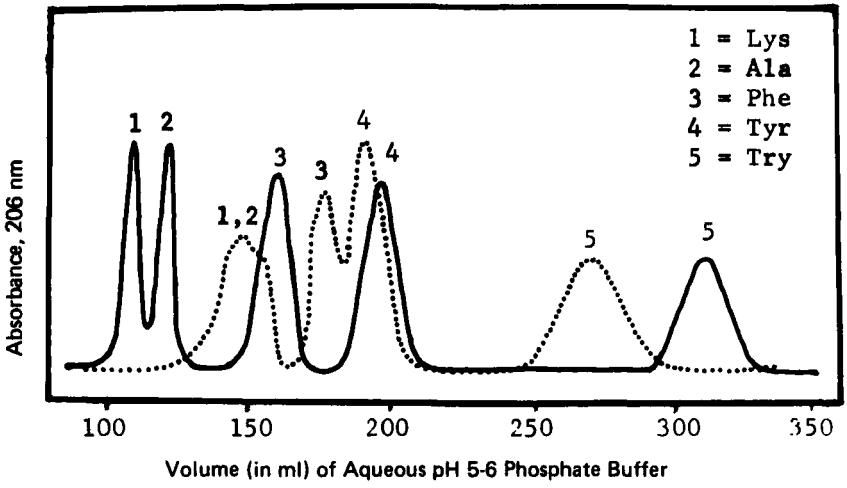

FIGURE 12

Separation of amino acids on colums packed with β-CDP (solid line) or sephadex G-25 (dotted line) [at 25.0° C on a 1.6 X 88 cm column, flow rate = 10 ml/hr with the indicated mobile phase]. Adapted with permission from B. Zsadon, M. Szilasi, K. H. Otta, F. Tudos, E. Fenyvesi, and J. Szejtli, Acta. Chim. Acad. Sci. Hung., 100, 271, 273 (1979).

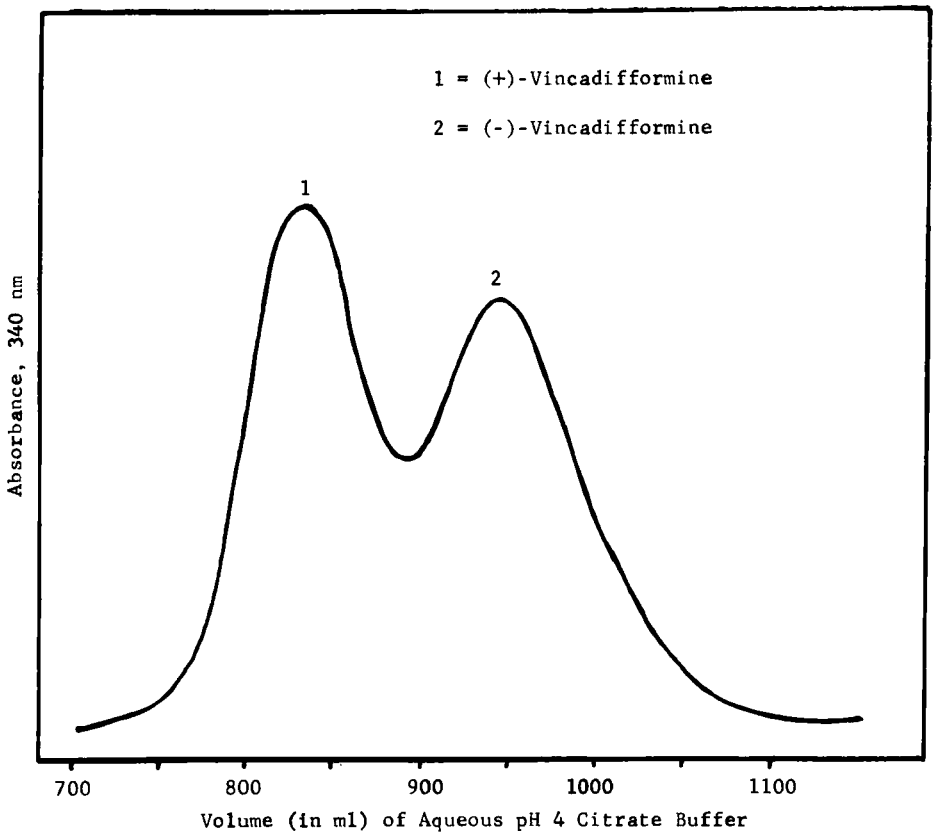

FIGURE 13

Liquid chromatographic separation of the (±) enantiomers of vicadifformine on β-CDP stationary phase [at 25.0° C on a 1.6 X 28 cm column, flow rate = 30 ml/hr with the indicated mobile phase]. Reproduced with permission from B. Zsadon, M. Szilasi, and F. Tudos, J. Chromatogr., 208, 112 (1981).

tones, esters, isomeric xylenes, picolines, and lutidines by gas chromatography were reported using either α- or β-CDPU stationary phases.[51] Table XIII summarizes part of the published data. Within each family of compounds, the elution observed on α- or β-CDPU follows the same order as was expected based on considerations of the particular molecules's size (and shape) and its ability to fit the respective CD cavity of the polymer. For example, the order of the stability constants for the formation of α- and β-CD-(C_1-C_4) primary alcohol inclusion complexes in solution followed this order: methanol < ethanol < propanol < butanol (refer to entries 13-16, Table III). Hence, the elution order observed, in which methanol is eluted first and butanol eluted last, on the α- and β-CDPU resins should be fully expected. Likewise, the elution behavior of the positional o-, m-, and p-xylene isomers (as well as the other aromatic isomers studied) seem to reflect accurately their expected binding ability to the respective CD cavity present in the α- or β-CDPU resins.[51]

The liquid chromatographic separations of some aromatic amino acids on these resins have also been recently reported.[177] Whereas good separation of the aromatic amino acids was achieved on the β-CDPU resins, no such separation was apparent when using either the α-CDPU resin or a polyurethane resin that contained no CD[177] (Table XIII). Although the same type of amino acid retention behavior was exhibited on different kinds of β-CDPU resins tested, it was found that the retention times were dependent upon the specific type of isocyanate cross-linking agent used.[51,177] This suggests that some type of secondary interactions may be present between the amino acids and the cross-linking agent; however, the results (i.e. elution order) still seem to parallel the ability of the different amino acid molecules to fit and bind (primarily via hydrophobic and hydrogen-bonding interactions) to the CD cavity present in the CDPU resin.[177] In general, the retention times increased as the pH was raised from 5.5 to 8.2. This fact was attributed to the increased hydrogen-bonding interactions possible between the CD hydroxyl groups and the amino acid due to dissociation of the amino acid carboxylic acid group at the higher pH.[177]

TABLE XIII
Relative Retention Times of Organic Compounds on CDPU Resins

Compound	Relative Retention Times			
	α-CDPU[a,b]	α-CDPU[c,d]	β-CDPU[e,b]	β-CDPU[f,d]
Phenylglycine	1.00(17.7)[g]	-----	1.00(28.5)[g], 1.00(45.3)[h]	-----
Tyrosine	1.07[g]	-----	2.23[g],1.91[h]	-----
Tryptophan	1.36[g]	-----	6.28[g],5.43[h]	-----
Phenylalanine	1.06[g]	-----	25.82[g],11.03[h]	-----
Kynuremine	1.00[g]	-----	7.84[g],7.00[h]	-----
β-(3,4-dihydroxy-phenyl)alanine	0.84[g]	-----	1.15[g]	-----
Benzene	-----	1.00(6.59)[i] 1.00(3.88)[j]	------	1.00(16.7)[i] 1.00(7.25)[j]
Hexane	-----	0.16[i]	------	0.04[i]
Heptane	-----	0.24[i]	------	0.06[i]
Octane	-----	0.29[i]	------	0.10[i]
Methanol	-----	0.53[i]	------	0.36[i]
Ethanol	-----	0.83[i]	------	0.78[i]
Propanol	-----	2.03[i]	------	1.96[i]
Butanol	-----	6.01[i]	------	4.63[i]
Toluene	-----	1.20[i],1.25[j]	------	2.71[i],1.11[j]
o-Xylene	-----	0.60[i],1.53[j]	------	0.89[i],1.00[j]
m-Xylene	-----	1.07[i],2.20[j]	------	2.22[i],1.09[j]
p-Xylene	-----	1.87[i],4.93[j]	------	6.53[i],1.43[j]

a) Specifically, the α-HDI-P-4.9-A resin.[177]
b) Retention times relative to phenylglycine, whose actual retention time is given in parenthesis.
c) Specifically, the α-HDI-DMF-5.9-A resin.[177,51]
d) Retention times relative to benzene, whose actual retention time is given in parenthesis.
e) Specifically, the β-H6XDI-P-6.0-A resin.[177]
f) Specifically, the β-HDI-DMF-5.5-A resin.[51]
g) The mobile phase was aqueous pH 5.5 phosphate buffer, flow rate = 20 ml/hr, on a 35 x .52 cm column.
h) Same as in (g) except that the pH was 8.2.
i) Gas chromatographic determination using N_2 as the carrier gas, flow rate = 30 ml/min, column and detector temperature = 150° C.
j) Same as in (i) except that the temperature was 170° C.

A number of acylated cyclodextrins [α-CD acetate,[171] β-CD acetate,[54,171] β-CD propionate,[54] β-CD butyrate,[54] β-CD valerate,[54] and the 2,3,6-tri-O-methylated α- or β-CDs[28] (compounds 5,6, Table II)] have been used as stationary phases for gas-liquid chromatography. A series of α-olefins, alcohols, aldehydes, aldehyde-esters, diesters, and esters (such as the ten isomeric heptadecanoates, unsaturated esters, saturated fatty acid methyl esters) have been separated on phases of the five former acylated CDs (the stationary phases consisted of 20% acylated CD on either Chromosorb R or W).[54,171] Preparative gas-liquid chromatographic work using these phases was also possible.[171,183] The excellent heat stability exhibited by these stationary phases permitted the use of a hydrogen flame detector which, in turn, allowed the detection limits for the above mentioned compounds to be decreased roughly 100 times compared to that possible on conventional phase columns.[54,171] It was not reported whether or not the retention times of the compounds chromatographed on these phases were affected by the CD inclusion complex formation phenomenon.

The methylated CD phases, on the other hand, affected the retention times of the organic compounds in a manner which suggested that CD inclusion complex formation was operative.[28] These stationary phases consisted of 5% of the methylated CD (compounds 5, 6, Table II) on silanized Chromosorb W or of silicone oil that contained 10% methylated CD. The gas-liquid chromatographic separation of hydrocarbon mixtures on these phases was possible (above 100° C under isothermal conditions with a nitrogen flow rate of 25-30 ml/min).[28] The order of elution follows the one that was expected from examination of the CD inclusion complex stability of the involved compounds. Isooctane, for example, had a larger retention time on the β-methylated CD phase than on the α-methylated one. This merely reflects the fact that the branched-chain hydrocarbons bind the larger cavity better than the smaller α-CD cavity.[28] It was also noted that in the 100-130° C temperature range (which is well above the boiling points of the hydrocarbons separated), increases in temperature caused increases in resolution. This trend

is opposite to that typically observed with ordinary, conventional stationary phases and substantiates the fact that CD inclusion complexes are being formed.[28]

Two chemically bonded β-CD gels, β-en-Bio-Gel and β-en-agarose, were prepared by coupling mono-(6-β-aminoethylamino-6-deoxy)-β-CD to either succinylhydrazide Bio-Gel P-2 or 1,4-butanediol diglycidyl ether.[50] Both have been utilized as stationary phases in the liquid chromatographic separations of the o-, m-, and p-isomers of nitroaniline, dinitrobenzene, toluidine, cresol, ethyl phenol, and aminobenzoic acid (Table XIV).[50] A typical chromatogram, obtained for the separation of o-, m-, and p-nitroaniline, is shown

TABLE XIV

Retention Times of Disubstituted Benzene Isomers on the β-en-Bio-Gel and β-en-Agarose Phases

Compound	Retention Time (min) for Indicated Isomers[c]		
	ortho-	meta-	para-
Nitroaniline	476[a], 116[b]	298[a], 103[b]	1338[a], 163[b]
Dinitrobenzene	481[a], 104[b]	187[a], 83[b]	224[a], 83[b]
Toluidine	272[a], 86[b]	307[a], 86[b]	454[a], 86[b]
Cresol	349[a], 102[b]	425[a], 102[b]	636[a], 102[b]
Aminobenzoic acid	48[a], ----	48[a], ----	60[a], ----
Ethylphenol	450[a], 111[b]	765[a], 123[b]	1275[a], 139[b]

a) Data taken from reference 50 for the β-en-Bio-Gel stationary phase, flow rate 9 ml/hr on a 50 cm X 5.2 mm column using water as the mobile phase.

b) Data taken from reference 50 for the β-en-agarose stationary phase, flow rate 18 ml/hr on a 90 cm X 5.2 mm column using water as the mobile phase.

c) The apparently long retention times can be diminished by appropriate variation of the experimental conditions (i.e. temperature, solvent composition, % CD in stationary phase, etc.).[50]

in Figure 14. As can be seen, a complete baseline separation of the isomers was obtainable, whereas if a non-CD containing succinylhydrazide Bio-Gel was used, only a partial resolution of these isomers was possible. In general, the order of elution of the positional isomers of the six disubstituted benzenes was the same on both the β-en-Bio-Gel and β-en-agarose phases and followed the trend: p > m, o.[50] These results can be rationalized considering the spatial arrangement of the substituents on the benzene ring and the ability of the isomers to fit the β-CD cavity. Meanwhile, it is interesting to point out that the order of stability for the native β-CD-nitroaniline inclusion complexes in solution is p > o > m (refer to Table III, entries 35-37 under β-CD). Hence, the observed order for the retention times of these isomers (p > o > m) accurately

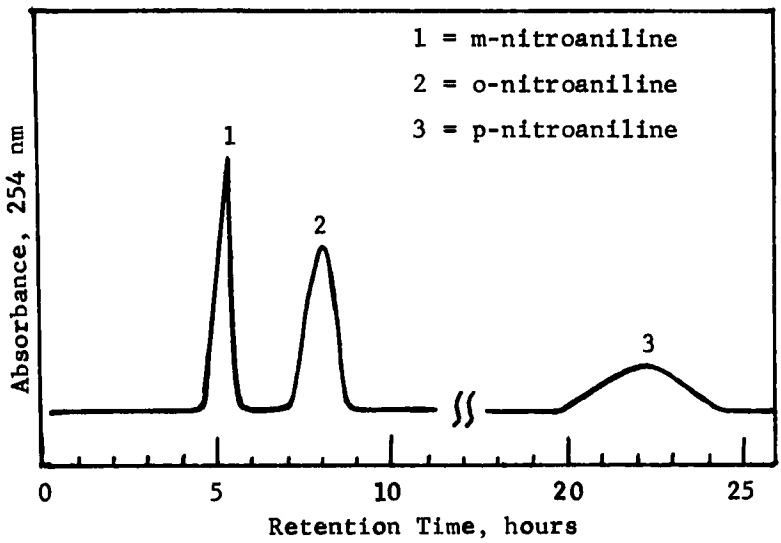

FIGURE 14

Liquid chromatographic separation of the isomeric nitroanilines on the β-en-Bio-Gel stationary phase (flow rate 9 ml/hr using water as the mobile phase). Reproduced with permission form M. Tanaka, Y. Mizobuchi, T. Sonoda, and T. Shano, Anal. Lett., 14, 281 (1981).

reflect their ability to interact with and bind the β-CD cavity. These β-CD stationary phases, as have been noted, are able to provide selectivity in the separation of positional disubstituted benzene isomers that is not obtainable with the most widely used conventional systems.[50]

Another stationary phase, prepared by immobilization of α-CD on Sepharose 6B, has been employed to separate β-amylase from albumin (and α-amylase).[49] The β-amylase is retarded on a column of this phase whereas the ablumin (and α-amylase) is not. The β-amylase was subsequently eluted from the column by using an aqueous solution of α-CD as the mobile phase. This same separation was not possible when using a column with just Sepharose 6B.[49]

The use of cellulose acetate supports impregnated with β-CD has been reported to separate (i.e. filter out) tar and nicotine in cigarettes.[62-64] While a regular cigarette filter (without CD) trapped only 13.2 mg tar and 0.9 mg nicotine per cigarette, the β-CD-cellulose filter trapped 68.6 mg and 2.0 mg of these components respectively.[62]

The permeation characteristics of disubstituted benzenes (dichlorobenzene, chloronitrobenzene, nitrotoluene, and xylene) through hydroxypropyl methyl cellulose membranes containing various amounts of α- and β-CD have been reported.[58] In the presence of the CDs, the permeation rates decreased and the membrane's selectivity increased. These results are explicable in terms of inclusion complex formation.

As should be obvious from the material reviewed in this section, CD containing stationary phases show specific (untypical) adsorption based on CD-inclusion complex formation with the components being separated. The elution behaviors shown by the compounds on these phases are directly related to the stability of the respective inclusion complexes that are formed. The physical properties of the various types of CD resins and polymers prepared are comparable to those of the usual commercial stationary phases typically employed.[51] Table XV summarizes a few of those properties for the CD phases mentioned in this section.

The use of CD-containing stationary phases has some important advantages over the more traditional phases. Some of these include: (i) The gels are easily and reproducibly prepared in stable form; (ii) they exhibit excellent heat stability;[54,173] (iii) the elution volumes are well suited to analytical scale runs and there is no problem in scaling up the runs to preparative levels;[173,183] (iv) usually, gradient elution is not required to affect separations; (v) many times, there is neither need to regenerate the column be-

TABLE XV

Physical Properties of Some of the CD Polymers and Resins used as Stationary Phases in Chromatography

CD Polymer System	% CD Content	Number of Free -OH Groups/CD Molecule	% PVA[a] Content	Water Retention, g/g	Gel Bead Volume, ml/g	Surface area, m^2/g
α-CDP[45,46]	46	---	0.3	1.8	4.0	---
β-CDP[45,46]	48	---	0.4	1.7	4.2	---
γ-CDP[45,46]	50	---	0.4	1.6	4.1	---
α-ECP[44]	---	---	---	2.6	---	---
β-ECP[44]	---	---	---	2.1-3.7	---	---
α-CDPU[b,c177]	78.1	---	---	---	1.9	180
β-CDPU[d,c177]	63.9	15.5	---	---	2.3	260
α-CDPU[e,c51]	59.3	10.1	---	---	---	180
β-CDPU[f,c51]	---	13.5	---	---	---	170

a) Polyvinyl alcohol content.

b) Specifically α-HDI-P-4.5-A.

c) These resins exhibit high temperature stability (up to 200-230° C).

d) Specifically β-H6XPI-P-6.0A.

e) Specifically α-HDI-DMF-5.9A.

f) Specifically β-HDI-DMF-5.5A.

tween runs--if necessary, the columns can be easily regenerated by washing with distilled water[175]--nor to repack them. Additionally, the chromatographic technique is another alternative approach which allows for the study of the inclusion complex formation process. This is particularly important when attempting to measure the CD's interactions with compounds that otherwise do not give easily measurable changes in their physical properties upon such complexation.[174]

In view of all of the results just described, it is evident that the use of CD stationary phases is a valuable addition to the art and practice of modern gas and liquid chromatography. Future work should be directed towards refining the general technique and applying it to a greater variety of separation problems. The use of CD thin-layer chromatographic stationary phases seems to be a very promising new research area.[184]

4. Use of Cyclodextrins as the Mobile Phase in TLC and HPLC Separations.

More recently, the focus of work involving CDs in chromatography has shifted to their utilization as a unique mobile phase in TLC and HPLC separations.[105,185-191] The basis for the observed separations stems again from the ability of the CD to selectively bind different guest molecules. This observed selectivity in binding is a consequence of the size of the guest molecule and the spatial disposition of its substituents (refer to section C-2). The acronym "pseudo-phase liquid chromatography" was coined to describe this type of separation where significant partitioning of a solute occurs to discrete CD molecules dissolved in the mobile phase rather than to the bulk solvent of the mobile phase.[188] The novel use of micellar systems as the mobile phase can also be used in this manner.[188]

Aqueous solutions of CD proved to be an effective mobile phase when chromatographing a wide variety of aromatic compounds on polyamide thin-layer sheets.[187-190] Table XVI summarizes some of the obtained results (R_f values). As can be seen, no appreciable separation of the listed compounds was possible if a distilled

water mobile phase was used. However, the separations of a variety of mono-substituted; isomeric ortho-, meta-, and para-disubstituted; and some tri- and quadri-substituted phenols,[189] nitriles,[190] anilines,[190] and benzoic acids[187] as well as vitamin K's were achieved if aqueous α-CD was used.[187-190] Figure 15 shows the typical thin-layer chromatogram obtained for some of these compounds. In most cases, the compounds moved as distinct spots and their R_f value was dependent on the concentration of the CD in the mobile phase. In a given family of compounds, (o-, m-, and p-nitrophenols, for example), the isomer with the largest stability constant for α-CD complex formation had the larger R_f value. In general, the para substituted isomers have larger R_f values than the meta isomers, which in turn have larger R_f values than the ortho substituted isomers (Table XVI). This order is exactly what is expected in view of the binding ability of these isomers to α-CD[187-190] (refer to section C-2). One can also distinguish between different series of para (or meta) substituted isomers (for example, p-X-phenols, where X = H, Cl, Br, I, or COOH; refer to Table XVI). Among these compounds, the general trend of R_f values again seems to parallel that of the stability constants of the α-CD complexes formed.[187,189,190] Preliminary results indicate that the similar use of an α-CD mobile phase will allow for the HPLC separation of these aromatic compounds.[191] For instance, using a 0.10 M α-CD mobile phase and a C_{18} reverse phase column, the HPLC separation of the isomeric o-, m-, and p-bromobenzoic acids was possible.[191]

The HPLC separation of some prostaglandins--namely PGE_1, PGA_1, and PGB_1--using an aqueous 0.5% solution of α- or β-CD as the mobile phase and a stationary phase column packed with AV-02-500 anion-exchange pellicular support has been reported.[105,109] Due to their hydrophobic nature, the prostaglandins usually exhibited relatively long retention times with incomplete separation on the anionic-exchange support when using only an aqueous mobile phase. However, via use of the aqueous CD mobile phase, the retention times were greatly diminished (Figure 16). As can be seen, the use of β-CD gave much better separation than α-CD in the mobile phase. These

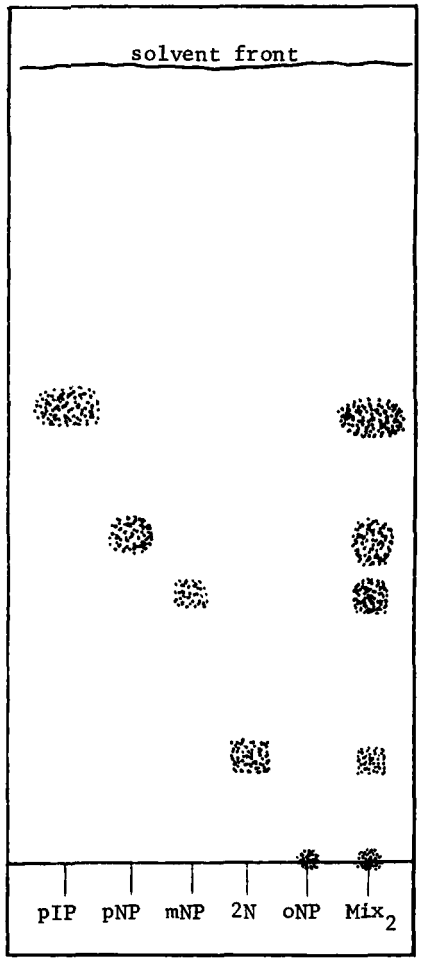

FIGURE 15

Tracing of a typical polyamide thin-layer chromatogram developed with 0.10 M α-CD at 25.0° C. The compounds separated were: p-iodophenol (pIP), p-nitrophenol (pNP), m-nitrophenol (mNP), 2-naphthol (2N), o-nitophenol (oNP), and a mixture of these five compounds (Mix_2).

TABLE XVI

R_f Values of Substituted Aromatic Compounds on Polyamide TLC Sheets Using Aqueous Solutions of α-CD as the Mobile Phase[a]

Aromatic Compound	R_f Values in		Ref.
	0.00M α-CD	0.10M α-CD	
o-Cyanophenol	0.06	0.15	190
m-Cyanophenol	0.09	0.38	190
p-Cyanophenol	0.08	0.50	190
o-Nitroaniline	0.06	0.17	190
m-Nitroaniline	0.09	0.38	190
p-Nitroaniline	0.06	0.67	190
o-Aminobenzoic acid	0.09	0.15	187
m-Aminobenzoic acid	0.15	0.27	190
p-Aminobenzoic acid	0.12	0.79	187
o-Nitrophenol	0.00	0.00	189
m-Nitrophenol	0.04	0.34	189
p-Nitrophenol	0.03	0.39	189
o-Bromobenzoic acid	0.11	0.16	187
m-Bromobenzoic acid	0.04	0.41	187
p-Bromobenzoic acid	0.02	0.67	187
p-Hydroxybenzoic acid	0.11	0.64	189
p-Iodophenol	0.02	0.56	189
p-Bromophenol	0.01	0.40	189
p-Chlorophenol	0.03	0.29	189
Phenol	0.17	0.25	189
1-Naphthol	0.01	0.02	189
2-Naphthol	0.00	0.14	189
o-Nitrobenzonitrile	0.21	0.23	190
p-Nitrobenzonitrile	0.20	0.41	190
2,4,6-Trinitrophenol	0.00	0.02	189
2,4,6-Trichlorophenol	0.00	0.20	189
3,5-Dinitroaniline	0.10	0.64	190
2,4-Dinitroaniline	0.04	0.19	190
1,4-Naphthoquinone	0.02	0.14	190
Vitamin K_5[b]	0.05	0.41	190
Vitamin K_1[c]	0.00	0.00	190

a) At 25.0° C.
b) 4-Amino-2-methyl-1-naphthol.
c) 2-Methyl-3-phytyl-1,4-naphthaquinone.

results are explicable in terms of the binding ability of these prostaglandins to α- or β-CD.[105,109] The stability constants for the PGE_1-, PGA_1-, and PGB_1-α-CD complexes are 1430 M^{-1}, 1300 M^{-1}, and 1200 M^{-1} respectively, while those of the β-CD complexes are 1700 M^{-1}, 1400 M^{-1}, and 780 M^{-1} respectively (refer to section C-2 for more detailed discussion of the binding of prostaglandins to CD). Thus, the inefficient separation observed for the α-CD phase is due to the great similarity of the binding constants of the

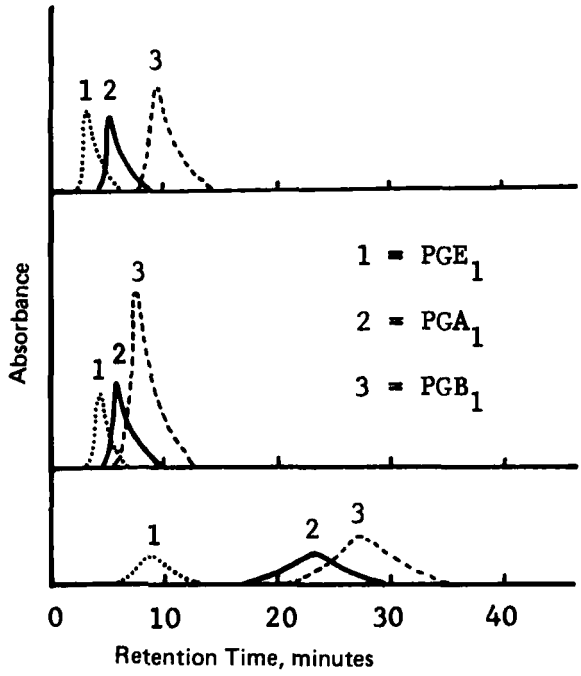

FIGURE 16

Liquid chromatographic separation of PGE_1 (dotted lines), PGA_1 (solid lines), and PGB_1 (dashed lines) on a strong anion-exchange pellicular stationary phase using an aqueous pH 6.0 phosphate buffer mobile phase without CD (bottom chromatogram), with 0.5% α-CD (middle chromatogram), and with 0.5% β-CD present (top chromatogram) [flow rate = 0.5 ml/min]. Reprinted with permission of the copyright owner from K. Uekama, F. Hirayama, K. Ikeda, and K. Inaba, J. Pharm. Sci., 66, 706 (1977).

three prostaglandins involved. For both CDs, the order of elution follows that of the magnitude of the stability constants. For instance, in β-CD, PGE_1 has the greatest binding ability, hence it is eluted first, while PGB_1, which has a much lower ability to bind to the β-CD in the mobile phase, is eluted last.[105] Consequently, a very simple, rapid, and sensitive method for the separation and quantitation of prostaglandins was developed based upon the use of an aqueous β-CD mobile phase.

In all of the reported TLC or HPLC work, the R_f values or retention times exhibited a curvilinear dependence upon the concentration of CD in the mobile phase (refer to Figure 17). That is, when the concentration of CD increased, the R_f values in the TLC work increased whereas the retention times in the HPLC work decreased. These observations may be ascribed to an increase in the solubility and partitioning of the solutes by complexation with the the CD molecules present in the mobile phase.[105,187] In fact, a basic partition treatment which can account for the chromatographic behavior (in HPLC) of many solutes eluted with aqueous CD mobile phases has been developed.[185,186] This quantitative treatment explains the observed elution behavior of the solute in terms of certain stationary phase parameters (such as the volume of the stationary phase and the void volume of the column) and three partition coefficients (refer to Figure 18). The partition coefficients involved include those of the solute between bulk water and the stationary phase (K_{ws}), between the stationary phase and the cyclodextrin (K_{cs}), and between the cyclodextrin and water (K_f).[185,186] The final form of the relationship is give by equation (5):

$$\frac{1}{[T_o' - T_{obs}]} = \frac{1}{[T_o' - T_c]} + \frac{1}{[CD]}\left(\frac{1}{K_f[T_o' - T_c]}\right) \qquad (5)$$

where T_{obs} equals the retention time of the solute at a given CD concentration, T_c equals the retention time of the CD·solute complex, and T' represents the retention time of the solute itself.[186] Equation 5 would predict a decreasing retention time with increasing CD concentration if the solute partitions to the CD. Indeed, this

equation successfully accounts for the observed HPLC behavior exhibited by prostaglandins in the presence of an aqueous CD mobile phase.[105,185] Additionally, a plot of the left-hand side of equation 5 vs. the reciprocal of the CD concentration allows for the calculation of the complex stability constant, K_f, from the slope and intercept. The values thus obtained for some 52 compounds

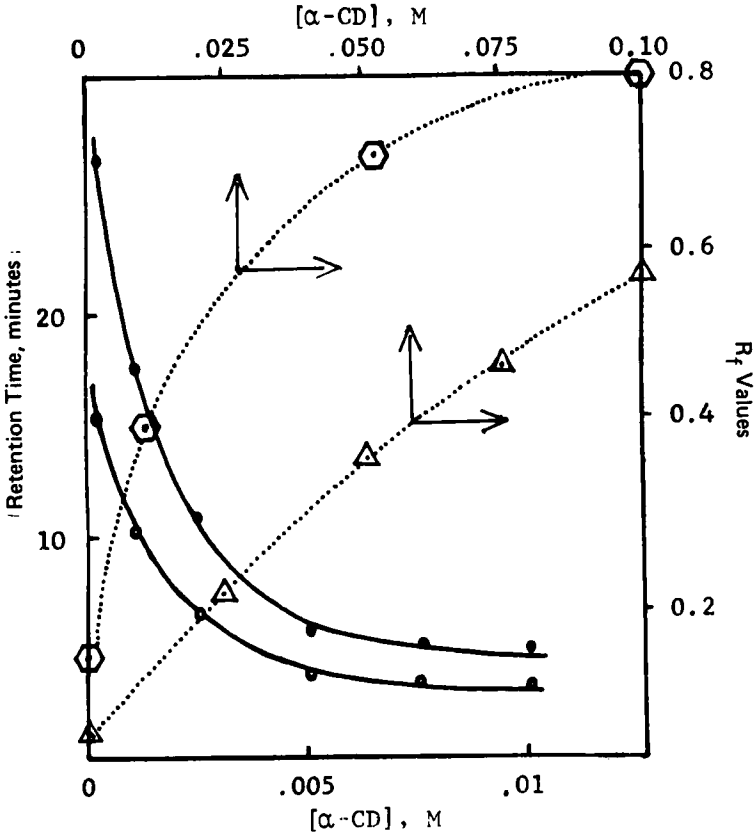

FIGURE 17

Effects of α-CD concentration present in the aqueous mobile phase on the retention times of PGA_1 (—o—) and PGB_1 (—●—) on an anion-exchange column[105] and on the R_f values of p-aminobenzoic acid (·⊙·) and p-iodophenol (··▲··) on a polyamide TLC sheet.[187,189]

(substituted barbiturates, phenothiazines, sulfonamides and sulfonylureas) agreed well with those determined by other methods.[185,186]

The successful derivation of this equation is important in several respects. First, it allows for a greater understanding and utilization of the chromatographic technique itself. Secondly, the equation has important ramifications outside the field of chromatography in that it allows for the quick, accurate, and unambiguous evaluation of the binding ability of various solutes to CDs. Indeed, this method is applicable under conditions where other

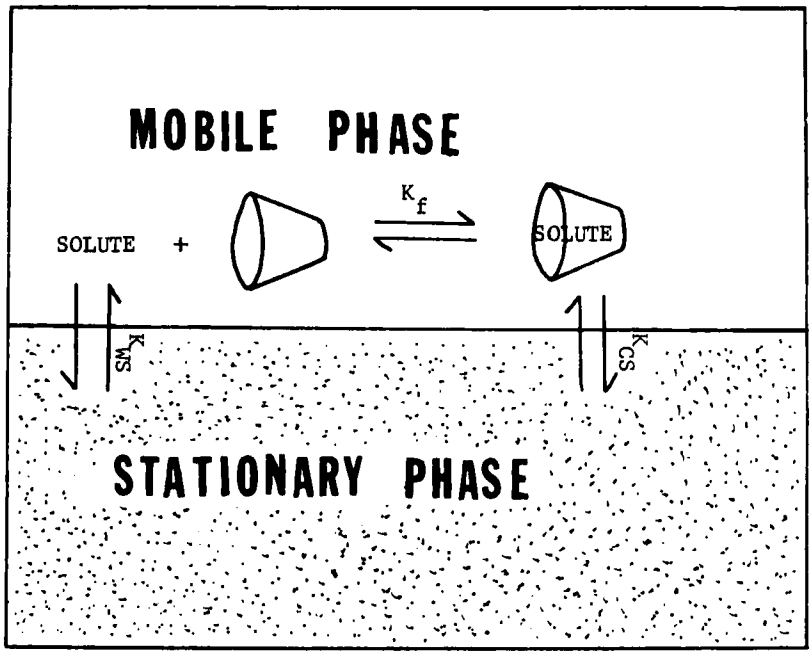

FIGURE 18

A schematic representation of the three "phase" model for CD chromatography. The elution behavior of a solute would be dependent upon the combined effects of three partition coefficients (i.e. that of a solute between water and the CD, K_f; that of the solute between water and the stationary phase, K_{ws}; and that of the CD complexed solute between water and the stationary phase, K_{cs}).

methods fail to yield stability constants for the CD inclusion complexes.[185,186] Thus, this chromatographic method will be of significance in many fields of CD chemistry (CD catalysis, pharmaceutical and agricultural applications, etc.).

For many TLC and HPLC separations, it is believed that the use of aqueous CD mobile phases can offer significant advantages over the more traditional organic solvent or mixed solvent systems that have been previously employed to affect separations of the aforementioned aromatic compounds.[187-191] Possible advantages include:

(i) High selectivity. The partitioning and binding of many hydrophilic, amphiphilic, and hydrophobic organic molecules to the CD cavity can be highly selective, much more so than partitioning to a single solvent or mixed solvent systems. In fact, no single traditional mobile phase is capable of separating the wide variety of organic compounds listed in Table XVI.[187-190]

(ii) Safety. Aqueous CD solutions are nontoxic and much less volatile or flammable than many of the currently employed organic or mixed solvent mobile phase systems.

(iii) It is generally easier to purify the water and crystalline CDs that comprise the CD mobile phase than to eliminate trace impurities from organic solvents.

(iv) With aqueous CD mobile phases, one can similtaneously chromatograph both nonpolar and polar solutes, whereas an aqueous-organic gradient would be required to affect the same separation in most traditional mobile phase systems.

(v) The use of an aqueous CD phase eliminates most of the solubility problems typically associated with the use of organic solvents in terms of one's ability to add solutes in order to control or vary the experimental conditions (such as pH, ionic strength, buffer capacity, etc.).

(vi) There are several advantages concerning detection. Since aqueous CD solutions are nonabsorbing in the near ultraviolet, they will not interfere with UV detection. Also, the compressibility of organic solvents will sometimes result in fluctuating baselines when using a refractive index detector. The use of relatively im-

compressible aqueous CD mobile phases can obviate this problem. Also, solutes included in CDs often exhibit enhanced absorbance or fluorescence. Thus, the detections limits can be considerably lowered as compared to those with conventional mobile phases (refer to next section).

The main disadvantage in using CD mobile phases is that no separation is possible for those compounds that do not partition or bind the CD (such as many of the ortho di-substituted benzenes, refer to Table XVI). In these instances, the conventional mobile phase systems would be required or the use of specifically modified CDs. Additionally, the cost of using such CD mobile phases is, at present, relatively high. However, with the increasing widespread use of CDs in so many other fields of applications, it is only a matter of time before it will become profitable to manufacture them on an industrial scale, thus greatly reducing their cost.[21,45]

Future work in this area should focus on the expansion and further utilization of this new technique. The use of a wider variety of stationary phases and modified CDs is bound to yield many interesting results on the uses and limitations of this method.

5. Enhanced Chromatographic Detection via Use of Cyclodextrins.

The success of any chromatographic separation ultimately relies on the location (in planar methods) or detection (in liquid methods) of the separated substances. CDs have been successfully employed to aid in the detection of several compounds separated by thin-layer chromatography.[193-200,206] In general, the detection of colored substances is not a problem in TLC. However, in cases where the compounds are colorless or have only weak UV-visible absorption, a spray reagent is many times necessary to locate the components and/or to increase the sensitivity of the detection method. A CD spray reagent has proven very useful in the visualization and detection of a number of functional type compounds (including lipids, saturated and unsaturated monoglycerides;[193-195] fatty alcohols, acid, methyl or ethyl esters;[193] and long-chain n-hydrocarbons[193, 198]).

In the specific detection procedure for these compounds, the developed chromatograms are sprayed with the CD reagent (i.e. a solution of 1% α-CD in 30% ethanol-water), dried in air, and kept for one hour in a humidity chamber whose atmosphere is saturated with water vapor. After subjecting the moist chromatograms to iodine vapors, saturated compounds appear as white spots which, with prolonged exposure, can turn yellow or brown, while unsaturated compounds form light blue to dark brown spots on a bluish-purple background.[193,198,199] The detection sensitivity is about 20 μg for most of these substances.[193] Also, the di- and tri-glycerides (at ≧ 200 μg levels) can be revealed by the same visualization procedure after prior enzymatic hydrolysis with 1% pancreatin.[199] In most cases, the color fades after several days but may be regenerated after repeated exposures to iodine vapors. Faint spots are more easily seen under blue or mercury light.[193,194]

This visualization technique is, of course, based upon CD inclusion complex formation. That is, inclusion complexes with the separated components can be formed on the developed TLC stationary phase after being sprayed with α-CD. In these in situ generated CD inclusion complexes, the α-CD cavity is already occupied by a guest molecule and, consequently, is unable to react with the iodine molecule. The uncomplexed α-CD molecules are, on the other hand, unoccupied and will react with the iodine to form the deep bluish-purple-colored α-CD-iodine inclusion complex (more details concerning the nature, structure, and color of α-CD-polyiodide complexes are given in reference 90). Accordingly, the separated, complexed compounds are detected as white to yellow spots upon a bluish-purple background.[193,194] As was pointed out in the original article, the CD reagent can be considered an universal one in that it is certainly applicable to the detection of many other compounds regardless of their functional groups.[193] The only requirement is that the molecule be able to fit and bind to the CD cavity. Some preliminary results indicate that mono-substituted, meta and para di-substituted, as well as some tri-substituted aromatic compounds can be similarly detected.[184]

Furthermore, spraying β-CD on silica gel thin-layer chromatograms enhanced (up to ten-fold) and stabilized the fluorescence of separated dansylated amino acids, thus facilitating their detection.[206] In this procedure, the thin-layer sheet, after the developing of the dansylated amino acids, is first sprayed with an aqueous solution of 1.8% β-CD and then dried over P_2O_5 for 30 minutes under reduced pressure. The spots, subsequently, can be detected under visible light (365nm) or quantitatively determined using a fluorometer.[206] The typical detection limits are in the range of 0.1 - 0.5 pmole/spot and a linear calibration curve (of fluorescence intensity versus concentration) is obtained in the 5 - 30 picomole region.[206] The enhanced fluorescence observed in the presence of CD is a result of the fact that the dansylated amino acids form inclusion complexes with the β-CD. The dansylated derivatives are somewhat protected from quenchers and are much more rigid in the hydrophobic environment of the CD cavity.[201,202,206]

Similar enhancements of the fluorescence intensity of solutes (such as 2-methylnaphthoquinone-1,4,[204] fluorescamine,[206] α-naphthyloxyacetic acid,[35] 2-p-toluidinylnaphthalene-6-sulfonate,[41] pyrene,[213] and various benzene derivatives[205]) upon formation of CD inclusion complexes in solution have been reported.[1,21] In view of these results, it is somewhat surprising that more applications have not as yet been reported in which CD are used to enhance chromatographic detections. Hence, much work remains to be done in this area.

In addition to the lower detection limits possible, the use of CD inclusion complex formation has some advantages over the typical chemical derivatization schemes that are carried out in order to enhance detection. First, the substances are not chemically changed and can be readily recovered by decomposing the CD inclusion complex. Secondly, aqueous solutions of CD, unlike the typical organic solvents and reagents required for chemical derivatization, are relatively nontoxic and safe to work with. Thirdly, many of the solute's properties are, upon inclusion, altered which in many cases can facilitate its detection.

E. CONCLUSION

The separation of a variety of compounds and isomers, made possible by the selective formation of CD inclusion complexes, has been employed in some multi-stage chromatographic separation processes (GLC, GSC, LLC, HPLC, and TLC) and precipitation purification schemes. Additionally, inclusion complex formation has been utilized to effect certain chromatographic detections. The work published so far indicates that the chromatographic application of CD inclusion processes allows the solution of specific analytical problems and that chromatography in the future will also become a very effective and valuable method for the continuing study of such inclusion phenomenon.

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G. REFERENCES AND NOTES

1. M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry", Springer-Verlag, New York, 1978.
2. J. H. Fendler and E. J. Fendler, in "Catalysis in Micellar and Macromolecular Systems", Academic Press, New York, 1975, p. 451.
3. F. Carmer, "Einschlussverbindungen", Springer-Verlag, Berlin, 1954.
4. D. French, Adv. Carbohyd. Chem., 12, 189 (1957).

5. F. R. Senti and S. R. Erlander, in "Non-Stoichiometric Compounds", L. Mandelcorn, ed., Academic Press, New York, 1964, p. 588.

6. J. A. Thoma and L. Stewart, in "Starch, Chemistry and Technology", R. L. Whistler and E. F. Paschall, eds., Academic Press, New York, 1965, p. 209.

7. F. Cramer and H. Hettler, Naturwissenschaften, 54, 625 (1967).

8. T. Kuge, Hyomen, 10, 422 (1972).

9. D. W. Griffiths and M. L. Bender, Adv. Cat., 23, 209 (1973).

10. K. H. Frömming, Pharm. Unserer. Zeit., 2, 109 (1973).

11. S. G. Frank. J. Pharm. Sci., 64, 1585 (1975).

12. W. Saenger, in "Environmental Effects on Molecular Structure and Properties", B. Pullman, ed., D. Reidel Publishing Co., Dordrecht-Holland, 1976, p. 265.

13. R. J. J. Bergeron, J. Chem. Educ., 54, 204 (1977).

14. A. Mifune and A. Shima, J. Synth. Org. Chem. Japan, 35, 116 (1977).

15. M. L. Bender and M. Komiyama, in "Bioorganic Chemistry", E. E. Van Tamelen, ed., Academic Press, New York, 1977, Vol. 1, Chapter 2.

16. D. D. MacNicol, J. J. McKendrick, and D. R. Wilson, Chem. Soc. Rev. (London), 7, 65 (1978).

17. J. Szejtli, Stärke, 30, 427 (1978).

18. S. Kobayashi, Denpun, 24, 19 (1979).

19. K. Horikoshi, Nippon Jozo, Zasshi., 74, 721 (1979).

20. R. Breslow, Adv. Chem. Ser., 191, 1 (1980).

21. W. Saenger, Angew. Chem. Int. Ed. Engl., 19, 344 (1980).

22. FAO Nutrition Meetings, Series No. 46, A. WHO/Food AAD/70.36.

23. R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender J. Amer. Chem. Soc., 89, 3242 (1967).

24. J. Szejtli, Stärke, 29, 410 (1977).

25. N. Wiedenhof and J. N. J. J. Lammers, Carbohyd. Res., 4, 318 (1967); 7, 1 (1968).

26. R. J. Bergeron, D. M. Pillor, G. Gibeily, and W. P. Roberts, Bioorg. Chem., 7, 263 (1978).

27. J. Emert and R. Breslow, J. Amer. Chem. Soc., 97, 670 (1975).

28. B. Casu, M. Reggiani, and G. R. Sanderson, Carbohydr. Res., 76, 59 (1979).

29. R. Breslow, P. Bovy, and C. L. Hersh, J. Amer. Chem. Soc., 102, 2115 (1980).

30. I. Tabushi, Y. Kuroda, and K. Shimokawa, J. Amer. Chem. Soc., 101, 4759 (1979).

31. I. Tabushi, N. Shimizu, T. Sugimoto, M. Shiozuka, and K. Yamamura, J. Amer. Chem. Soc., 99, 7100 (1977).

32. B. Siegel, A. Pinter, and R. Breslow, J. Amer. Chem. Soc., 99, 2309 (1977).

33. K. Fujita, A. Shinoda, and T. Imoto, J. Amer. Chem. Soc., 102, 1161 (1980).

34. R. Breslow, M. F. Czarniecki, J. Emert, and H. Hamaguchi, J. Amer. Chem. Soc., 102, 762 (1980).

35. A. Ueno, K. Takahashi, and T. Osa, J. C. S. Chem. Comm., 94 (1981).

36. A. Ueno, R. Saka, and T. Osa, Chem. Lett. (Japan), 841 (1979).

37. J. Boger and J. R. Knowles, J. Amer. Chem. Soc., 101, 7631 (1979).

38. I. Tabushi, Y. Kuroda, K. Yokota, and L. C. Yuan, J. Amer. Soc., 103, 711 (1981).

39. I. Tabushi, K. Shimokawa, and K. Fujita, Tetrahedron Lett., 1527 (1977).

40. R. Breslow, H. Kohn, and B. Siefel, Tetrahedron Lett., 1645 (1976).

41. A. Harada, M. Furue, and S. Nozakura, Polymer J., 12, 29 (1980).

42. J. N. J. J. Lammers, J. L. Koole, and A. J. G. Van Diemen, Recl. Trav. Chim., 91, 483 (1972).

43. N. Wiedenhof, J. N. J. J. Lammers, and C. L. V. P. Van Eck, Stärke, 21, 119 (1969).

44. N. Wiedenhof, Stärke, 21, 163 (1969).

45. J. Solms and R. H. Egli, Helv. Chim. Acta., 48, 1225 (1965); N. Wiedenhof, C. L. Van Panthaleon, B. Van Eck, and J. N. J. J. Lammers, Brit. Patent, 1,244,990 (1971); E. Fenyvesi, B. Zsadan, J. Szejtli, and F. Tüdos, Ann. Univ. Sci. Budap., Sect. Chim., 15, 13 (1979).

46. B. Zsadon, M. Szilasi, K. H. Otta, F. Tüdos, E. Fenyvesi, and J. Szejtli, Acta. Chim. Acad. Sci. Hung., 100, 265 (1979).

47. J. Szejtli, E. Fenyvesi, and B. Zsadon, Stärke, 30, 127 (1978).

48. J. Szejtli, E. Fenyvesi, S. Zoltan, B. Zsadon, and F. Tüdos, Gen. Offen., 2,927,733 (1980); C. A., 92, 181902 p (1980).

49. P. Vretblad, FEBS Lett., 47, 86 (1974).

50. M. Tanaka, Y. Mizobuchi, T. Sonoda, and T. Shono, Anal. Lett., 14, 281 (1981).

51. Y. Mizobuchi, M. Tanaka, and T. Shono, J. Chromatogr., 194, 153 (1980).

52. L. C. Case and L. K. Case, U. S. Patent, 3,502,601 (1970); C. A., 72, 112255e (1970).

53. T. Hayakawa, T. Yamada, S. Hidaka, M. Yamagishi, K. Takeda, and F. Toda, Polym. Prepr., Amer. Chem. Soc., Div. Polym. Chem., 20, 530 (1979).

54. H. Schlenk, J. L. Gellerman, and D. M. Sand, Anal. Chem., 34, 1529 (1962).

55. S. M. Parmerter, E. E. Allen, and G. A. Glenn, U. S. Patent, 3,453,257 (1969); C. A., 71, 91821y (1969).

56. H. Kurita, M. Kawazu, and K. Takashima, Japan, Kokai, 7485, 014 (1974); C. A., 81, 169767p (1974).

57. J. Szejtli, A. Liptak, I. Jodal, P. Fugedi, P. Nanasi, and A. Neszmelyi, Stärke, 32, 165 (1980).

58. C. H. Lee, J. Appl. Polym. Sci., 26, 489 (1981).

59. M. Maciejewski and Z. Durski, J. Macromol. Sci., A16, 441 (1981); M. Maciejewski, Polish Patent, 100828 (1979); C. A., 92, 42886y (1980); Polish Patent 103,276 (1979); C. A. 92, 147826g (1980).

60. Mitsubishi Chemical Industries Co., Ltd., Japan Kokai Tokkyo Koho, 80 75,402 (1980); C. A., 93, 134146f (1980).

61. M. Kojima and F. Toda, Kagaku Seibutsu, 18, 702 (1980); C. A., 94, 140051h (1981).

62. K. Miwa and Y. Tanaka, Japan. Koaki, 75,125,100 (1975); C. A., 84, 14843y (1976).

63. K. Anakawa, Ger. Offen., 2,527,234 (1976); C. A., 84 147993u (1976).

64. K. Miwa and Y. Tanaka, Japan. Kokai, 76 32,799 (1976); C. A., 84 176893s (1976).

65. F. Cramer, W. Saenger, and H. Spatz, J. Amer. Chem. Soc., 89, 1, 14 (1967).

66. R. A. Bergerson, Y. Machida, and K. Bloch, J. Biol. Chem., 250, 1223 (1975).

67. D. J. Wood, F. E. Hruska, and W. Saenger, J. Amer. Chem. Soc., 99, 1735 (1977).

68. M. Komiyama and H. Hirai, Chem. Lett. (Japan), 1467, 1471 (1980); Polym. J., 13, 171 (1981).

69. K. Uekama, Kagaku Yakugaku Kyoshitsu, 60, 73 (1978).

70. K. Harata, Bull. Chem. Soc. Japan, 49, 2066 (1976); 52, 2451 (1979).

71. R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson, and D. A. Laufer, J. Amer. Chem. Soc., 103, 1750 (1981).

72. N. Yoshida and M. Fujimoto, Chem. Lett. (Japan), 231 (1980).

73. T. Osa, T. Matsue, and M. Fujihira, Heterocycles, 6, 1833 (1977).

74. S. Yamaguchi and T. Tsukamoto, Nippon Kagaku Kaishi, 1856 (1976).

75. J. L. Lach and T. F. Chin, J. Pharm. Sci., 53, 29, 924 (1964).

76. J. L. Hoffman and R. M. Brock, Biochem., 9, 3542 (1970).

77. N. M. Atherton and S. J. Strach, J. Magn. Reson., 17, 134 (1975).

78. R. J. Bergeron, M. A. Channing, G. J. Gibeily, and D. M. Pillor, J. Amer. Chem. Soc., 99, 5146 (1977).

79. K. Ikeda, K. Uekama, and M. Otagiri, Chem. Pharm. Bull., 23, 201 (1975).

80. C. J. Seliskar and L. Brand, Science, 171, 799 (1971).

81. A. Ueno, K. Takahashi, and T. Osa, J. C. S. Chem. Comm., 921 (1980).

82. K. Flohr, R. M. Paton, and E. T. Kaiser, J. Amer. Chem. Soc., 97, 1209 (1975).

83. D. D. MacNicol, Tetrahedron Lett., 38, 3325 (1975).

84. K. Uekama, F. Hirayama, and H. Koinuma, Chem. Lett. (Japan), 1393 (1977).

85. H. Sato, S. Higuchi, N. Teramae, and S. Tanaka, Chem. Lett. (Japan), 299 (1979).

86. H. Shimizu, A. Kaito, and M. Hatano, Bull. Chem. Soc. Japan, 52, 2678 (1979); 54, 513 (1981).

87. D. A. Rees, J. Chem. Soc., B., 877 (1970).

88. B. Hingertz and W. Saenger, Nature, 255, 396 (1975).

89. K. Harata, Bull. Chem. Soc. Japan, 49, 2066 (1976).

90. M. Noltemeyer and W. Saenger, J. Amer. Chem. Soc., 102, 2710 (1980).

91. K. Harata, Bull. Chem. Soc. Japan, 53, 2782 (1980).

92. E. A. Lewis and L. D. Hansen, J. C. S. Perkin Trans. II, 2081 (1973).

93. G. E. Hardee, M. Otagiri, and J. H. Perrin, Acta. Pharm. Succ., 15, 188 (1978).

94. E. S. Hall and H. J. Ache, J. Phys. Chem., 83, 1805 (1979).

95. Y. C. Jean, B. W. Ng, Y. Ito, T. Q. Nguyen, and D. C. Walker, Hyperfine Interact., 8, 351 (1981).

96. J. Sztatisz, S. Gal, J. Komives, A. Stadler-Szoke, and J. Szejtli, Therm. Anal. [Proc. Inter. Conf. Therm. Anal.], 2, 487 (1980).

97. A. Buvari and L. Barcza, Inorganica Chimica Acta., 33, L179 (1979).

98. J. F. Wojcik and R. P. Rohrbach, J. Phys. Chem., 79, 2251 (1975).

99. R. P. Rohrbach, L. J. Rodriguez, E. M. Eyring, and J. F. Wojcik, J. Phys. Chem., 81 944 (1977).

100. R. J. Bergeron, D. M. Pillor, G. Gibeily, and W. P. Roberts, Bioorg. Chem., 7, 263 (1978).

101. T. Okubo, H. Kitano, and N. Ise, J. Phys. Chem., 80, 2661 (1976).

102. Y. Matsui and K. Mochida, Bull. Chem. Soc. Japan, 52, 2808 (1979).

103. B. Casu and L. Rava, Ric. Sci., 36, 733 (1966).

104. R. I. Gelb, L. M. Schwartz, R. F. Johnson, and D. A. Laufer, J. Amer. Chem. Soc., 101,1869 (1979).

105. K. Uekama, F. Hirayama, K. Ikeda, and K. Inaba, J. Pharm. Sci., 66, 706 (1977).

106. K. Uekama, F. Hirayama, and T. Irie, Chem. Lett. (Japan), 661 (1978).

107. K. Uekama, F. Hirayama, S. Yamasaki, M. Otagiri, and K. Ikeda, Chem. Lett. (Japan), 1389 (1977).

108. K. Uekama, T. Irie, and F. Hirayama, Chem. Lett. (Japan), 1109 (1978); K. Uekama, F. Hirayama, T. Wakuda, and M. Otagiri, Chem. Pharm. Bull., 29, 213 (1981).

109. K. Uekama, Japan, J. Antibiotics, XXXII Suppl., S-103 (1979).

110. A. Cooper and D. D. MacNicol. J. C. S. Perkin II, 760 (1978).

111. C. van Hooidonk and J. C. A. E. Breebaart-Hansen, Recl. Trav. Chim. Pays.-Bas., 89, 289 (1970).

112. R. M. Paton and E. T. Kaiser, J. Amer. Chem. Soc., 92, 4723 (1970).

113. B. Siegel and R. Breslow, J. Amer. Chem. Soc., 97, 6869 (1975).

114. M. Komiyama and S. Inoue, Bull. Chem. Soc. Japan, 53, 3266 (1980).

115. R. I. Gelb, L. M. Schwartz, and D. A. Laufer, Bioorg. Chem., 9, 450 (1980).

116. C. van Hooidonk and J. C. A. E. Breebaart-Hansen, Recl. Trav. Chim. Pays.-Bas., 90, 680 (1971).

117. I. Tabushi, Y. Kiyosuke, T. Sugimoto, and K. Yamamura, J. Amer. Chem. Soc., 100, 916 (1978).

118. F. Cramer and W. Kampe. J. Amer. Chem. Soc., 87, 1115 (1965).

119. Y. Matsui, H. Naruse, K. Mochida, and Y. Date, Bull. Chem. Soc. Japan, 43, 1909 (1970).

120. B. Hingerty and W. Saenger, J. Amer. Chem. Soc., 98, 3357 (1976).

121. P. C. Manor and W. Saenger, J. Amer. Chem. Soc., 96, 3630 (1974).

122. R. J. Bergeron and M. P. Meeley, Bioorg. Chem., 5, 197 (1976).

123. J. Szejtli, Stärke, 29, 26 (1977).

124. K. Harata, Bull. Chem. Soc. Japan, 52, 1807 (1979).

125. T. W. Rosanske and K. A. Connors, J. Pharm. Sci., 69, 173, 564 (1980).

126. F. Cramer and F. M. Henglein, Chem. Ber., 90, 2561 (1957).

127. K. Uekama, F. Hirayama, M. Otagiri, Y. Otagiri, and K. Ikeda, Chem. Pharm. Bull., 26, 1162 (1978).

128. I. Yamamoto and Y. Katsuda, Pestic. Sci., 11, 134 (1980).

129. F. Hirayama, K. Uekama, and H. Koinuma, Chem. Pharm. Bull., 28, 1975 (1980).

130. M. Hayashi and A. Ishihara, S. African Patent, 74 00,295 (1974).

131. M. Otagiri, K. T. Miyaji, K. Uekama, and K. Ikeda, Chem. Pharm. Bull., 24, 1146 (1976).

132. K. Uekama, F. Hirayama, N. Matsuo, and H. Koinuma, Chem. Lett. (Japan), 703 (1978).

133. C. Formoso, Biopolym., 13, 909 (1974).

134. J. Szejtli and E. Bolla, Stärke, 32, 386 (1980).

135. K. Uekama, M. Otagiri, Y. Kanie, S. Tanaka, and K. Ikeda, Chem. Pharm. Bull., 23, 1421 (1975); K. Uekama, F. Hirayama, K. Esaki, and M. Inoue, Chem. Pharm. Bull., 27, 76 (1979).

136. K. Uekama, N. Matsuo, F. Hirayama, T. Yamaguchi, Y. Imamura, and H. Ichibagase, Chem. Pharm. Bull., 27, 398 (1979).

137. F. Cramer and W. Dietsche, Chem. Ber., 92, 378 (1959).

138. H. P. Benschop and G. R. Van den Berg, J. C. S. Chem. Commum., 1431 (1970).

139. M. Mikolajczyk, J. Drabowicz, and F. Cramer, J. C. S. Chem. Commun., 317 (1971).

140. M. Mikolajczyk and J. Drabowicz, Tetrahedron Lett., 2379 (1972).

141. M. Mikolajczyk and J. Drabowicz, J. Amer. Chem. Soc., 100, 2510 (1978).

142. R. Breslow and L. E. Overman, J. Amer. Chem. Soc., 92, 1075 (1970).

143. A. Harada, M. Furue, and S. Nozakura, Macromolecules, 9, 701, 705 (1976); 10, 676 (1977).

144. Y. Murakami, Yuki Gosei Kagaku Kyokaishi, 38, 361 (1980); C. A., 93, 167728a (1980).

145. A. Ueno, R. Saka, K. Takahashi, and T. Osa, Heterocycles, 15, 671 (1981); I. Tabushi, Y. Kuroda, and Y. Sakata, Heterocycles, 15, 815 (1981).

146. Y. Kurono, V. Stamoudis, and M. L. Bender, Bioorgan. Chem., 5, 393 (1976); M. Komiyama and M. L. Bender, Proc. Natl. Acad. Sci., USA, 73, 2969 (1976).

147. J. N. J. J. Lammers and A. J. G. Van Diemen, Recl. Trav. Chim., 91, 733 (1972).

148. K. Yamada, J. Morimoto, and H. Iida, Chiba Daigaku Kogakubu Kenkyu Hokoku, 26, 85 (1975); C. A., 83, 121753v (1975).

149. E. Smolkova-Keulemansova and S. Krysl, J. Chromatogr., 184, 347 (1980).

150. J. Szejtli, E. Banky-Elod, A. Stadler, P. Tetenyi. I. Hethelyi, and L. Kernoczy, Acta. Chim. Acad. Sci. Hung., 99, 447 (1979).

151. D. French, M. L. Levine, J. H. Pazur, and E. Norberg, J. Amer. Chem. Soc., 71, 353 (1949).

152. Y. Suzuki, T. Maki, and K. Mineta, Japan. Kokai, 75,151, 833 (1975); C. A., 84, 105201d (1976).

153. Y. Suzuki, T. Maki, and K. Mineta, Japan. Kokai, 75 96,530 (1975); C. A., 83, 205896g (1975).

154. Y. Suzuki and T. Maki, Japan. Kokai, 75,151,827 (1975); C. A., 84, 105179c (1976).

155. Y. Suzuki and T. Maki, Japan. Kokai, 75,151,804 (1975); C. A., 84, 135074g (1976).

156. J. Szejtli, E. Banky, A. Stadler, P. Tetenyi, I. Hethelyi, and L. Kernoczy, Hung. Teljes, 16,602 (1979); C. A. 92, 22061x (1980).

157. K. Yamada, Japan. Kokai, 77 42,825 (1977); C. A., 87, 134490q (1977).

158. J. Szejtli and E. Banky-Elod, Stärke, 27, 368 (1975).

159. R. A. Reiners and F. J. Birkhaug, U. S. Patent, 3,491,132 (1970); C. A., 72, 89056e (1970).

160. I. Shibanai, K. Horikoshi, and N. Nakamura, Eur. Pat. Appl., 16,855 (1980); C. A., 94, 87013u (1981).

161. M. Otagiri, K. Ikeda, K. Uekama, O. Ito, and M. Hatano, Chem. Lett. (Japan), 679 (1974).

162. J. Szejtli, E. Fenyvesi, S. Zoltan, B. Zsadon, and F. Tudos, Ger. Offen., 2,927,733 (1980); C. A., 92, 181902p (1980).

163. C. Van Hooidonk, Recl. Trav. Chim., 89, 845 (1970); 91, 1103 (1972).

164. Y. Kitaura and M. L. Bender, Bioorg. Chem., 4, 237 (1975).

165. V. Daffe and J. Fastrez, J. Amer. Chem. Soc., 102, 3601 (1980).

166. G. L. Trainor and R. Breslow, J. Amer. Chem. Soc., 103, 154 (1981).

167. M. Komiyama and M. L. Bender, J. Amer. Chem. Soc., 99, 8021 (1977).

168. K. Fujita, A. Shinoda, and T. Imoto, Tetrahedron Lett., 21, 1541 (1980).

169. R. Dyllick-Brenzinger and J. D. Roberts, J. Amer. Chem. Soc., 102, 1166 (1980).

170. I. Nakamura, T. Sugimoto, J. Oda, and Y. Inouye, Agric. Biol. Chem., 45, 309 (1981).

171. D. M. Sand and H. Schlenk, Anal. Chem., 33, 1624 (1961); H. Schlenk and D. M. Sand, Anal. Chem., 34, 1676 (1962).

172. J. L. Hoffman, J. Macromol. Sci.-Chem., A7, 1147 (1973).

173. J. L. Hoffman, Anal. Biochem., 33, 209 (1970).

174. N. Wiedenhof and R. G. Trieling, Stärke, 23, 129 (1971).

175. A. Harada, M. Furue, and S.-I. Nozakura, J. Polym. Sci., 16, 189 (1978).

176. B. Zsadon, M. Szilasi, F. Tüdos, E. Fenyvesi, and J. Szejtli, Stärke, 31, 11 (1979).

177. Y. Mizobuchi, M. Tanaka, and T. Shono, J. Chromatogr., 208, 35 (1981).

178. B. Zsadon, M. Szilasi, F. Tüdos, and J. Szejtli, J. Chromatogr., 208, 109 (1981).

179. R. D. Cornelius and W. W. Cleland, Abstr. Amer. Chem. Soc. Meeting, Biol. Chem. 13, Sept., 1977.

180. R. D. Cornelius and W. W. Cleland, Biochemistry, 17, 3279 (1978).

181. Societe des Produite Nestle S. A., Chem. Abstr., 64, 16687a (1966).

182. In the abbreviations for the compounds, C = cytidine, U = uridine, G = guanosine, A = adenosine, P = phosphate, and M = mono.

183. T. H. Haines, S. Aaronson, J. L. Gellerman, and H. Schlenk, Nature, 194, 1282 (1962).

184. W. L. Hinze and W. G. Burkert, unpublished results.

185. K. Uekama, F. Hirayama, S. Nasu, N. Matsuo, and T. Irie, Chem. Pharm. Bull., 26, 3477 (1978).

186. K. Uekama, F. Hirayama, and T. Irie, Chem. Lett. (Japan), 661 (1978).

187. W. L. Hinze and D. W. Armstrong, Anal. Lett., 13, 1093 (1980).

188. D. W. Armstrong, J. Liq. Chromatogr., 3, 895 (1980).

189. W. G. Burkert, C. N. Owensby, and W. L. Hinze, J. Liq. Chromatogr., 4, 1065 (1981).

190. W. G. Burkert, C. N. Owensby, B. Bayliss, and W. L. Hinze, unpublished results, 1981.

191. D. W. Armstrong, F. Lafranchise, and W. L. Hinze, preliminary results, 1981.

192. L. Szente and J. Szejtli, Herba Hung., 19, 57 (1980); Chem. Abstr., 93, 225496m (1980).

193. H. K. Mangold, B. G. Lamp, and H. Schlenk, J. Amer. Chem. Soc., 77, 6070 (1955).

194. J. L. Gellerman and H. Schlenk, Experientia, 12, 342 (1956).

195. H. Schlenk, J. L. Gellerman, J. A. Tillotson, and H. K. Mangold, L. Amer. Oil Chemists' Soc., 34, 377 (1957).

196. H. K. Mangold, J. L. Gellerman, and H. Schlenk, Fed. Proc., 17, 269 (1958).

197. D. C. Malins and H. K. Mangold, J. Amer. Oil Chemists' Soc., 37, 576 (1960).

198. K. Randerath, "Thin-Layer Chromatography," Academic Press, New York, 1963, p. 126.

199. I. M. Hais and K. Macek, ed., "Paper Chromatography," Academic Press, New York, 1963, p. 343, 797.

200. E. Stahl, "Thin-Layer Chromatography--A Laboratory Handbook," Springer-Verlag, New York, 1969, p. 865.

201. T. Kinoshita, F. Iinuma, and A. Tsuji, Biochem. Biophys. Res. Commun., 51, 666 (1973).

202. T. Kinoshita, F. Iinuma, and A. Tsuji, Chem. Pharm. Bull., 22, 2413, 2735 (1974).

203. H. Kondo, H. Nakatani, and K. Hiromi, J. Biochem. (Japan), 79, 393 (1976).

204. I. Tabushi, K. Yamamura, K. Fujita, and H. Kawakubo, J. Amer. Chem. Soc., 101, 1019 (1979).

205. M. Hoshino, M. Imamura, K. Ikehara, and Y. Hama, Kokagaku Toronkai Koen Yoshishu, 93 (1979); M. Hoshino, M. Imamura, K. Ikehara, and Y. Hama, J. Phys. Chem., 85, 1820 (1981).

206. T. Kinoshita, F. Iinuma, K. Atsumi, Y. Kanada, and A. Tsuji, Chem. Pharm. Bull., 23, 1166 (1975).

207. K. Nakaya, M. Yabuta, F. Iinuma, T. Kinoshita, and Y. Nakamura, Biochem. Biophys. Res. Commun., 67, 760 (1975).

208. A. Wshnia and S. J. Lappi, J. Mol. Biol., 82, 77 (1974).

209. Y. Inoue, Y. Katono, and R. Chujo, Bull. Chem. Soc. Japan, 52, 1692 (1979).

210. Y. Inoue and Y. Miyata, Bull. Chem. Soc. Japan, 54, 809 (1981).

211. C. J. van Oss, D. R. Absolom, and A. W. Neumann, Colloid Polym. Sci., 258, 424 (1980).

212. M. Komiyama and H. Hirai, Kagaku no Ryoiki, 35, 29 (1981).

213. K. Kano, I. Takenoshita, and T. Ogawa, Chem. Lett. (Japan), 1035 (1980).

214. Other enzymes, such as Bacillus magaterium, Krebsiella pneumoniae M 5 al, and Bacillus stereothermophilus, can be used in a similar fashion.[21]

215. The indicated cyclodextrins can be obtained from the following companies (in the U.S.A.):[216] Accurate Chemical and Scientific Corporation, 28 Tec Street, Hicksville, N. Y. 11801 (α- and β-CD); Aldrich Chemical Company, 940 W. St. Paul Avenue, Milwaukee, WI 53233 (α-, β-, and γ-CD); Polysciences, Inc., Paul Valley Industrial Park, Warrington, PA 18976 (α- and β-CD); Research Plus Labs, Inc., P. O. Box 324, Bayonne, N. J. 07002 (α- and β-CD); and Sigma Chemical Company, P. O. Box 14508, St. Louis, MO 63178 (α-, β-, and γ-CD). A number of other companies supply one of the cyclodextrins (usually only α- or β-CD) and are listed in reference 216.

216. "Chem Sources-U. S. A., 1981," Directories Publishing Company, Inc., Ormond Beach, Florida, 1981, pp. 176-177.

217. J. Knabe and N. S. Agarwal, Deut. Apoth.-Ztg., 113, 1449 (1973); Chem. Abstr., 80, 63817u (1973).