Journal of Chromatography, 251 (1982) 17–34
Chromatographic Reviews
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHREV. 155

CYCLODEXTRINS AS STATIONARY PHASES IN CHROMATOGRAPHY*

E. SMOLKOVÁ-KEULEMANSOVÁ
Department of Analytical Chemistry, Charles University, Prague (Czechoslovakia)
(Received August 30th, 1981)

CONTENTS

1. Introduction 17
1.1. Cyclodextrins 17
1.2. Cyclodextrin polymers 20
1.3. Chromatographic analysis of cyclodextrins 20
2. Cyclodextrins as stationary phases in chromatography 20
2.1. Liquid chromatography 20
2.1.1. Gel inclusion chromatography 21
2.1.2. Affinity chromatography 26
2.1.3. Evaluation of the use of cyclodextrins in liquid chromatography 26
2.2. Gas chromatography 27
2.2.1. Cyclodextrin derivatives in gas chromatography 27
2.2.2. Gas–solid chromatography 28
2.2.3. Evaluation of the use of cyclodextrins in gas chromatography 30
3. Conclusion 32
4. Acknowledgements 33
5. Summary 33
6. Note 33
References 33

1. INTRODUCTION

The exceptional properties of cyclodextrins (CD) have been described in many papers, books and reviews[1–10] and increasing attention has been paid to their study and use in recent years. This paper is intended to show, on the basis of the literature data and the results of our research, the possibilities of using chromatographic methods for the study of CD and the use of the selective formation of inclusion compounds (IC) of cyclodextrins for separation and analytical purposes using various chromatographic procedures.

1.1. Cyclodextrins

Cyclodextrins, which are cyclic oligosaccharides composed of D(+)-glucopyranose units interconnected by α-(1,4) bonds, are interesting chiefly for their inclusion properties, which are exhibited both in the solid state and in aqueous solutions. Inclusion complexes are formed inside the CD cavity, the geometry and chemical composition of which determine the selectivity of the inclusion process.

* Presented as plenary lecture at the International Microsymposium on Clathrates and Molecular Inclusion Phenomena, Stará Lesná, September 7–11, 1981.

0378-4355/82/0000–0000/$4.50

On laboratory and industrial scales, CD are produced by enzymatic degradation of starch, a polysaccharide containing glucose units interconnected to form a laevorotatory helix. The action of the enzyme disrupts the helix and the two ends of the fragment are connected to form a cyclic molecule (Fig. 1). As the enzymes used cut the helix not completely specifically, CD with various numbers of glucose units are formed; α-, β- or γ-CD, formed by 6, 7 and 8 glucose units, respectively, are present in the greatest amounts. The CD structure has a special arrangement of the functional groups, which has a great effect on the difference in the properties of α-, β- and γ-CD. For example, the structure of β-CD is given in Fig. 2. The secondary hydroxyl groups on the C(2) and C(3) atoms, O(2)H and O(3)H, are localized on one side of the ring, whereas the primary hydroxyl groups on C(6), O(6)H, are on the opposite side. The interior of the ring contains only a circular configuration of hydrogen atoms and glycoside oxygen atoms; therefore, the ring interior is apolar relative to water. From a side view, the shape of the ring molecule is conical. The wider side contains the secondary hydroxyl groups and the opposite opening is occupied by the primary hydroxyl groups. The glucose units assume the chair conformation, the C(6)–O(6) groups deviating out of the ring. However, during interaction with a guest (hydrogen bonding) these groups can deviate into the ring. Intramolecular hydrogen bonding, O(3)–O(2), occurs between the secondary hydroxyl groups of the neighbouring glucose units and makes the CD ring more rigid.

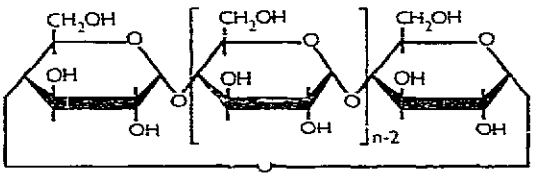

Fig. 1. Structural formula of CD.

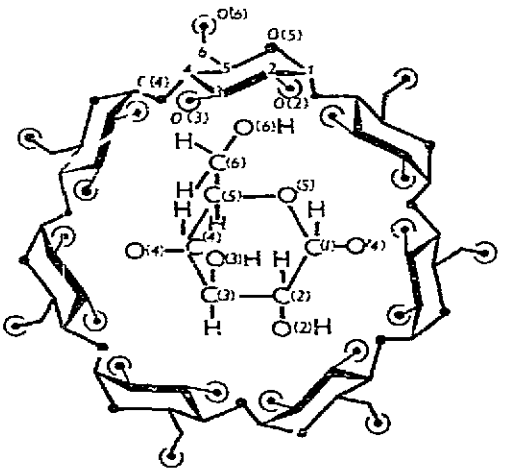

Fig. 2. Structure of β-CD.

α-, β- and γ-CD form cavities with a size of 5–8 Å that permit inclusion of molecules (or their parts) of corresponding dimensions (Fig. 3)[11]. An inclusion process between CD and a guest can be described by a dissociation constant, K_d, with values of the order of 10^{-3} mol/l characteristic of weak intermolecular interactions; there is no dependence on the guest chemical properties.

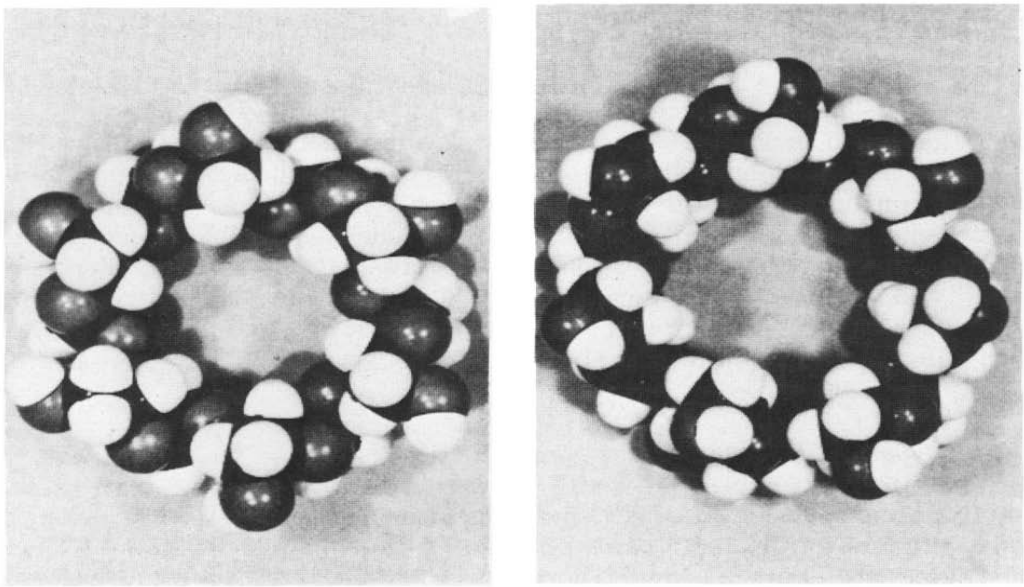

Fig. 3. Models of α-CD (left) and β-CD (right) molecules.

The thermodynamic parameters enthalphy (ΔH) and entropy (ΔS) can be found from the temperature dependence of K_d. The ΔH values are always negative, *i.e.*, the inclusion compounds dissociate on increasing the temperature, whereas ΔS can be either positive or negative depending on the force type.

The dependence of K_d on the guest polarizability indicates that Van der Waals forces predominate during the inclusion[12,13]; it can be shown from the crystallographic data that hydrogen bonding can also occur between the guest and (primarily) the O(6)H group of the CD. Moreover, hydrophobic interaction can also occur during the inclusion[14,15]. The question is which mechanism predominates in the inclusion of the guest molecule into the CD ring. The character of the interaction between CD and a guest has been studied many times, but an unambiguous picture has not yet been obtained. It seems that Van der Waals interactions often predominate, including interactions of permanent and induced dipoles and London dispersion forces. These forces are approximately proportional to the reciprocal of the sixth power of the distance between the guest and host and to the polarizability of the two components. In view of the internal dimensions of CD, where the distance between the guest and the host is small, the magnitude of these interactions can be significant.

1.2. Cyclodextrin polymers

The specific properties of CD are retained even if they are used in the form of polymers which are less soluble in water than the orginal CD. This is important, because the ability of CD to form water-soluble inclusion compounds is often a limiting factor, especially when CD is to be used as a stationary phase in liquid chromatography. Homopolymers are formed by polymerization of CD derivatives[16]. Copolymers are formed by reaction of the CD hydroxyl groups with bi- or polyfunctional molecules[17]. These polymers are often termed resins. As CD are polyfunctional molecules, cross-linking can occur and from a certain molecular weight resins with gel structures are formed, which are insoluble in water. Immobilized CD on a polymeric support has also been prepared (*e.g.*, on Sepharose), either chemically bonded[18] or obtained by mixing the CD with a water-insoluble polymeric gel [poly(vinyl acetate), polyacrylamide, nitrocellulose][19]. Block polymerization was originally used for the preparation of a water-insoluble resin and the product had to be mechanically ground[19,20]. During the pearl preparation[21,22] in which the heat is removed during spatial cross-linking, a product with a uniform grain size can be obtained. Research carried out in this direction has permitted the use of cyclodextrin polymers as chromatographic materials.

1.3. Chromatographic analysis of cyclodextrins

In addition to detection of CD by paper chromatography based on a characteristic coloration of the complexes with iodine and iodide[23] (α-CD yields a blue-black colour, whereas the analogous complexes of β- and γ-CD are brown-yellow), chromatographic procedures have been developed which are less tedious and time consuming than the classical separations. The oldest method for the separation of individual CDs based on paper chromatography still required 18 h for a single analysis. Thin-layer chromatography on cellulose has been found to be more advantageous and applicable also to preparations. Column liquid and gas chromatography can also be employed for the separation and determination of α-, β- and γ-CD. However, gas chromatography requires prior derivatization to cyclodextrin dimethylsilyl ethers. The best results in the shortest time were obtained by using high-performance liquid chromatography, when the analysis time decreased to 16 min. The methods of chromatographic separation, experimental conditions and references are summarized in Table 1.

2. CYCLODEXTRINS AS STATIONARY PHASES IN CHROMATOGRAPHY

The fact that CD can form inclusion complexes preferentially with certain types of compound, depending primarily on the molecular shape, has led to studies of their use as stationary phases in chromatography.

2.1. Liquid chromatography

The course of the inclusion process, which has been described in most papers as a process proceeding in the aqueous phase, agrees with the considerations about the

TABLE I

CHROMATOGRAPHIC SEPARATION OF CYCLODEXTRINS

Chromatography	*Chromatographic conditions*	*Ref.*
Paper	Paper, Schleicher & Schüll 2045; mobile phase, butanol–pyridine–water (1:1:1).	24, 25
	Paper, Whatman No. 1; mobile phase, butanol–dimethylformamide–water (2:1:1); detection, alcoholic solution of I_2.	26
Thin-layer	Microchromatoplates with silicic acid; mobile phases. (a) butanol–acetic acid–water–pyridine–dimethylformamide (6:3:1:2:4). (b) butanol–acetic acid–water (6:3:1); detection, H_2SO_4–$K_2Cr_2O_7$.	26
	Microcrystalline cellulose; mobile phase, butanol–ethanol–water (4:3:3); detection, I_2 solution.	27
Liquid column	Cellulose; gradient elution, water–ethanol–butanol.	28
	Cellulose; elution, butanol–ethanol–water (42:29:29); flow-rate, 75–90 ml h; column length, 63 cm; detection, polarimeter.	29
	Active carbon; gradient elution, butanol–water; flow-rate, 130–170 ml h; detection, polarimeter.	30
	Sephadex G-15; elution, water–sodium azide (0.2 %).	31
	Molselect G-25, G-15, cross-linked dextran; elution, water; detection, polarimeter.	32
High-performance liquid	μBondapak–carbohydrate; mobile phase, acetonitrile–25–30 % water; column length, 30 cm; I.D., 1 mm; flow-rate, 2 ml min; time of analysis, 18 min.	33
Gas	Dimethylsilyl ethers of cyclodextrins; column, 3 % SXR on Chromosorb W AW DMCS (80–100 mesh); temperature, 325°C, programmed at 20°C min up to 405°C; carrier gas, helium; flow-rate, 45–50 ml min.	34

use of the selective character of inclusion in liquid chromatography. The requirement that the host be insoluble in aqueous media is met by cyclodextrin polymers (CDP) which, as stated above, retain the inclusion properties. These substances have become suitable chromatographic materials and have been used chiefly in gel inclusion chromatography[35].

2.1.1. Gel inclusion chromatography

Water-insoluble CD polymers in the form of gels can interact with various compounds according to various mechanisms[36]. They involve (a) interactions in the cavities, *i.e.*, the formation of inclusion compounds, (b) interactions in the internal pores of polymeric pearls and (c) interactions on the surface (which can be neglected).

These mechanisms, compared with Sephadex gels as non-inclusion analogues, have become the basis of gel inclusion chromatography (GIC).

A comparison of the interaction isotherms for two types of gel, Sephadex and a CD polymer[17] (prepared from a mixture of α-, β- and γ-CD by cross-linking with epichlorohydrin) with aniline, pyridine, benzaldehyde, butyric acid and *o*- and *p*-nitrophenol (Fig. 4), is very illustrative. Great differences have been found in all these instances, confirming the inclusion character of interactions with the CD polymer. It

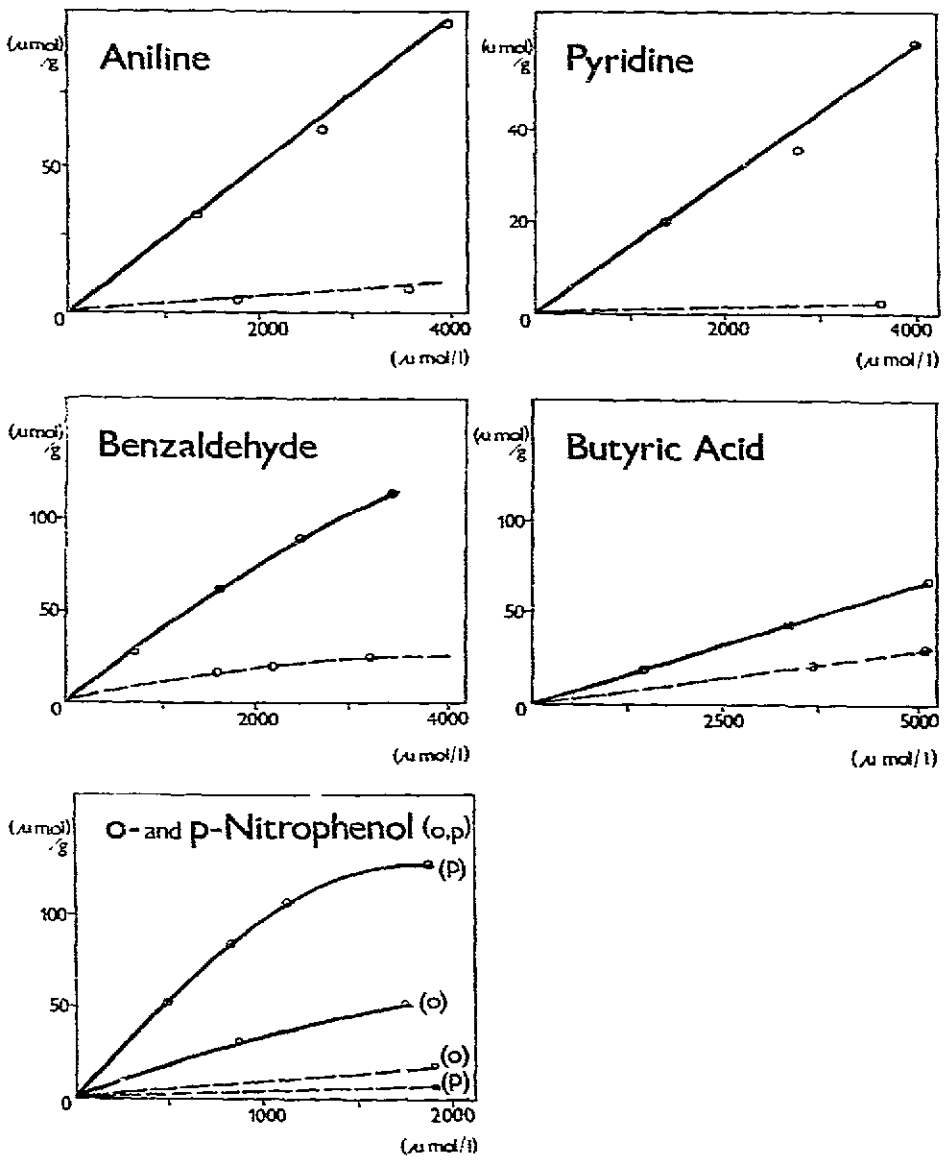

Fig. 4. Interaction isotherms of various organic compounds on a mixture of α, β and γ-CD resin (solid lines) and epichlorhydrin–dextran resin (broken lines) (from ref. 17).

follows from the shape of the isotherms of *o*- and *p*-nitrophenol that these substances cannot be separated on Sephadex, whereas the different inclusion interactions on the CD polymer can be used to advantage for their chromatographic separation[19].

Similar conclusions can be drawn from the interaction isotherms for the isomers of chlorobenzoic acid with α- or β-CD polymer (Fig. 5)[37]. It follows from the slopes of the isotherms that *m*-chlorobenzoic interacts most strongly, whereas *p*- and *o*-chlorobenzoic acids interact differently, but both substantially less. The stronger interaction with β-CD than with α-CD corresponds to the inclusion mechanism which is governed by the size of the cavity in the CD ring. The elution data given in Table 2 demonstrate the different behaviour of the substances studied on a β-CD polymer and Sephadex[35]. The data confirm a strong interaction with the β-CD polymer and the fact that *o*-chlorobenzoic acid can be separated chromatographically from benzoic acid, whereas the peaks of the two components overlap on Sephadex.

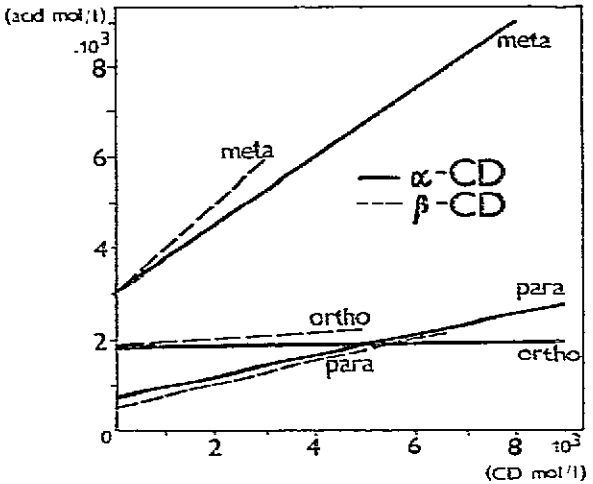

Fig. 5. Interaction isotherms of chlorbenzoic acids with α-CD (solid lines) and β-CD (broken lines) at 30°C (from ref. 35).

TABLE 2

ELUTION VOLUMES (V_e) ON SEPHADEX G-25 COARSE AND β-E25 RESIN

Compound	V_e *(ml)*	
	Sephadex G-25	*β-E25 resin*
Aniline · HCl	150	511
Benzoic acid	100	400*
Phenol	133	—**
o-Chlorobenzoic acid	83	98
m-Chlorobenzoic acid	83	400*
Dextran blue	55	40

* Tailing.

** Does not leave the column.

A general conclusion can be made on the basis of the above data about an increased affinity of CD towards aromatic molecules and a steric specificity towards their isomers. This property has been examined in detail with amino acids as model substances on CD polymers prepared for chromatographic purposes by polymerization with ethylene glycol diepoxypropyl ether in the presence of poly(vinyl acetate)[38–40]. Comparative experiments performed on α-, β- and γ-CD polymers in weakly acidic solutions (pH 5–6) showed the greatest differences in the retention data on the β-CD gel. This polymer has been found most suitable for separations of aromatic amino acids (phenylalanine, tyrosine, tryptophan). A high separation efficiency (H = 0.7–0.8 mm) was attained for these substances at laboratory temperature and a flow-rate of 10–20 ml/h. Under these conditions aromatic acids could be separated from non-aromatic acids (lysine, alanine), as well as from one another. The behaviour of these substances on α-, β- and γ-CD polymers can be demonstrated by chromatograms obtained under the same conditions (Fig. 6). The results obtained for aromatic acids, especially on β-CDP, correspond to the assumption of the extent of inclusion in the total interaction, whereas the mechanism of the separation of lysine and alanine depends primarily on adsorption. This conclusion is also confirmed by comparing the chromatograms obtained with β-CDP and Sephadex under identical conditions (Fig. 7).

Analogously, separation of indole alkaloids could be achieved on β-CDP, whereas on other carbohydrate type gels such as Sephadex, high and different retentions of alkaloids have been observed[41]. This suggests that the mechanism of the

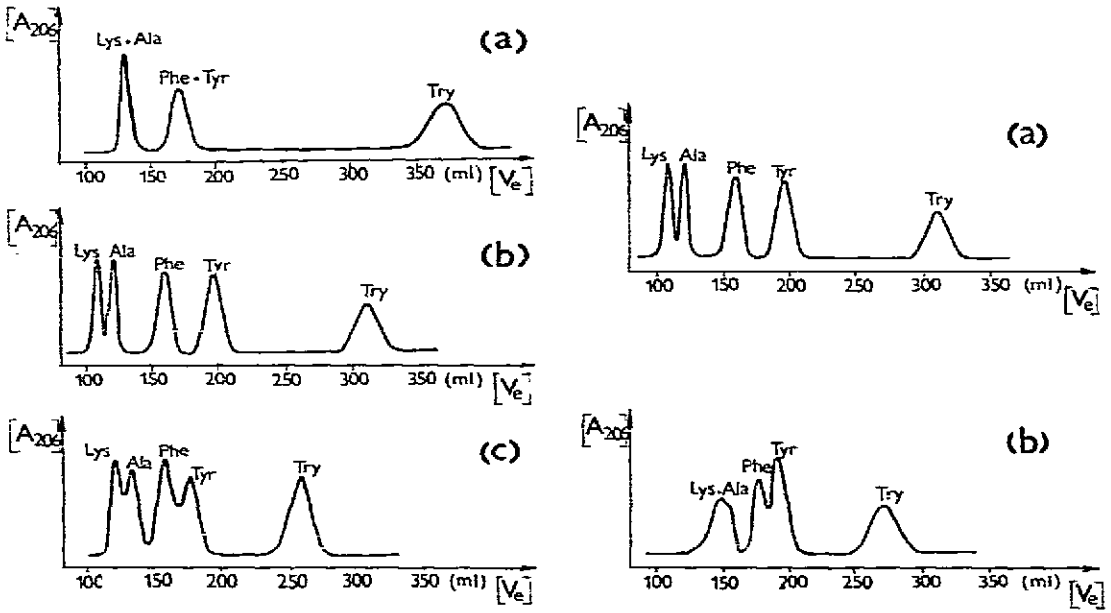

Fig. 6. Gel inclusion chromatographic separation of amino acids (a) on α-CDP, (b) on β-CDP and (c) on γ-CDP (from ref. 39).

Fig. 7. Gel inclusion chromatographic separation of amino acids (a) on β-CDP and (b) on Sephadex G-25 gel (from ref. 39).

interaction is governed by inclusion complex formation or a combined effect of inclusion and adsorption, rather than by adsorption only.

Recently separations of some aromatic amino acids on α- and β-CD polyurethane resins have been reported[42]. Good separation was achieved on the β-CD polyurethane resin, whereas no separation was observed on the α-CD resin and on polyurethane without CD. The retention behaviour on different kinds of β-CD polyurethane resin was found to be dependent upon the specific type of isocyanate cross-linking agent used. This suggests that some type of secondary interaction could be present. However, the elution order of the compounds separated confirms a host–quest interaction between the CD present in the resin and the different amino acid molecules.

The ability of some components of nucleic acids, especially those with an adenine base, to form compounds with β-CD, can also be readily used for chromatographic separations of various nucleotides and nucleosides[43,44]. In parallel experiments with solutions of CD and a CD gel it has been shown that the inclusion bond is affected by some factors, such as pH and the position of the phosphate group in the nucleotide. The latter effect with various adenosine monophosphates (AMP) and β-CD can be readily demonstrated on the shapes of the differential spectra (Fig. 8). As nucleotides with the adenine base have the same absorbance, the magnitude of the spectral shift should be proportional to the magnitude of the interaction with CD.

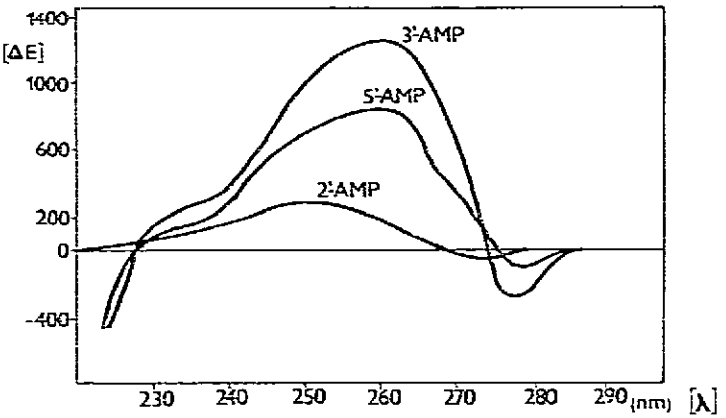

Fig. 8. Interaction of β-CD with adenosine monophosphates (from ref. 44).

The shape of the spectrum of 3′-AMP (adenosine-3-monophosphate) and 5′-AMP indicates that the strongest interaction occurs when the phosphate group remains outside the CD cavity (owing to ionization). Compared with these isomers, 2′-AMP, which contains the base and the phosphate group on the neighbouring carbon atoms of ribose, reacts substantially less with CD. From these spectral measurements conclusions can also be drawn about the chromatographic behaviour of these substances. Components with the largest change in the spectrum should be retained most on the chromatographic column. The order of elution of isomeric adenine monophosphates (Fig. 9) justifies these conclusions. The inclusion process, as stated above, is also

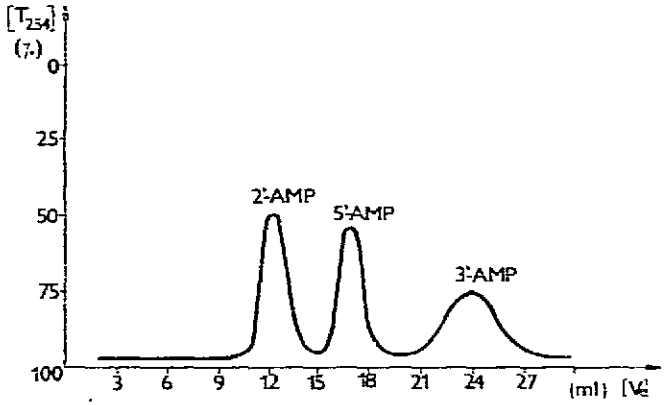

Fig. 9. Chromatogram of adenosine monophosphates on β-CD gel (from ref. 44).

affected by the pH. For successful separation the pH should be 7–8; at lower values the nucleotides–β-CD interaction decreases considerably.

With oligonucleotides containing adenine the guest–host interaction is not as strong. Therefore, anion-exchange groups were built into a CD gel (dimethylaminoethyl groups. The gel thus obtained exhibits both ion-exchange and inclusion properties and has also been used to separate tRNA[44].

CD polymers can also be used for the separation of racemic mandelic acid and its derivatives[45]. The β-CD polymer preferentially forms inclusion compounds with L-(+) isomers, permitting the separation of the DL methyl ester of mandelic acid; the first fraction contains pure D-(−) isomer. The α-CD polymer has the opposite effect and binds D-(−) isomers preferentially; however, the separation of racemates is then not complete.

2.1.2. Affinity chromatography

A competitive action of α-CD on the activity of β-amylase has been used for the solution of special analytical problems[46]. If α-CD is bound to epoxy-Sepharose 6B a gel is formed that retains β-amylase, whereas α-amylase is not retained. After elution of β-amylase by a highly specific eluent with competitive counter ligand, in this case α-CD, a highly pure enzyme with a high activity is obtained[18].

Alternatively β-CD has been used as immobilized ligand on epoxy-Sepharose 6B in biospecific affinity chromatography[47]. In this case α-amylase was selectively retained and separated from proteins and then selectively eluted by a buffer containing β-CD. By this chromatographic procedure a recovery of 90% was obtained with a purification of up to 180 fold compared to crude extracts.

β-CD with other oligosaccharides has also been used for selective retardation of phosphorylases by affinity electrophoresis on polyacryl gel. On the base of the mobility changes of phosphorylase in dependence on the concentration of the oligosaccharides in the gel, the dissociation constants of complexes have been calculated[48].

2.1.3. Evaluation of the use of cyclodextrins in liquid chromatography

The results obtained have shown that the application of CD and CD polymers in liquid chromatography, especially in gel inclusion chromatography, has been very

successful. A number of concepts have been confirmed and the knowledge of the inclusion mechanism has been widened on the basis of data obtained by various instrumental methods. In addition, the use of CD as stationary phases has made possible many important analytical applications. The selectivity of the inclusion process is the main factor that makes these substances so attractive as chromatographic materials. However, only relatively low efficiencies have been attained so far, which also follow from the conditions of liquid chromatography. Therefore, efforts have been made in a few instances to study the character of the inclusion process with CD using guests in the gaseous phase. This process, observed, *e.g.*, during the inclusion of odorous compounds[49], does not permit more general conclusions to be drawn at present.

2.2. Gas chromatography

The present knowledge of the inclusion character has so far not enabled the inclusion of guests present in the gaseous phase into the CD cavity to be described in greater detail. In a similar manner to urea, CD also exhibit various affinities, *e.g.*, towards linear- and branched-chain alkanes[50–52]. In contrast to urea, where the inclusion structure is only formed in contact with the guest, the CD cavity is present before the inclusion process. Analogous to other inclusion compounds, molecular dimensions play a predominant role; too small guest molecules are not accepted by the relatively large CD cavity because of the short-range character of the forces operative. For example, helium, neon and argon do not form inclusion compounds with α-CD, whereas krypton and xenon do[53]. Similarly, α-CD forms very stable inclusion compounds with oxygen, carbon dioxide and chlorine at high pressure[11].

2.2.1. Cyclodextrin derivatives in gas chromatography

Acetylated cyclodextrins (α- and β-CD acetate, β-CD propionate, butyrate and valerate) have been used[54,56] as polar gas chromatographic stationary phases; however, the separation process was unaffected by inclusion. This use was based on the finding that polyesters used for chromatographic separations of fatty acids have C:O ratios similar to those of saccharide esters, the applicability of which, however, is limited by temperature. Acylated cyclodextrins have a relatively high thermal stability (220–236°C) and a good separation efficiency for various polar compounds (α-olefins, aldehydes, alcohols, esters, aldehyde-esters and diesters).

Different results have been obtained with methylcyclodextrins phases[57], where the retention of organic compounds could be influenced by inclusion processes. The methylated CD was either deposited on silanized Chromosorb W or was part of mixed phase of 10% methylated CD in silicone oil. The elution of the hydrocarbons studied was in agreement with the stability of the inclusion compounds. Isooctane had a larger retention time on methylated β-CD than on methylated α-CD, which corresponds to the larger β-CD cavity.

The requirement of thermal stability (especially important for gas chromatography) is also met by the original CD. A thermogravimetric study of β-CD[58] (Fig. 10) shows that at 80–100°C a decrease in weight of about 8% occurs owing to the loss of moisture. A further weight decrease takes place at about 300°C, when CD begins to decompose. A necessary condition for gas chromatographic use is the presence of inclusion process even at high temperatures.

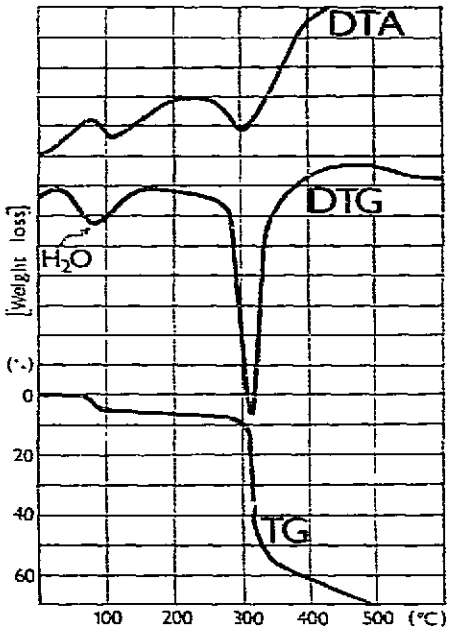

Fig. 10. Thermoderivatogram of β-CD (from ref. 58).

Recent results have shown that these requirements can be satisfied by macroporous polymers with inbuilt CD molecules[59] and by CD deposited on chromatographic supports[60].

2.2.2. Gas–solid chromatography

Cyclodextrin–polyurethane resins have been used as stationary phases in gas–solid chromatography (GSC)[59] and their sorption properties have been studied. It was found that specific interactions governed by the dimensions and configuration of the host molecules take place. The retention data for many organic substances are correlated with the inclusion phenomena, *i.e.*, with the size of the α- and β-CD cavities, and with the effect of π-electrons and heteroatoms in the guest molecule. Compared with common organic polymers (Amberlite, Porapak Q, Tenax GC), an advantage of polymers containing CD is their selectivity, which could also be utilized in trace analysis for the pre-concentration of test substances.

The formation and properties of the inclusion compounds of α- and β-CD can also be studied in the GSC system if CD (10%, w/w) is deposited on Chromosorb W from a dimethylformamide solution[60]. The sorbates were chosen to include organic molecules of various structural types and geometries (hydrocarbons, hydrocarbon halogeno derivatives, alcohols, ethers and aromatic substances).

To evaluate the character of the interaction, the dependence of the retention data on the polarizability of the sorbate was primarily followed, as a measure of the action of the dispersion forces in the interior of the cyclodextrin ring[13,60]. However, the effect of surface forces during inclusion cannot be excluded, as the sorbate molecules are in equilibrium not only with the inside of the CD ring, but also with its

surface. Therefore, the retention time can also be determined by the time of deposition on the surface prior to the interaction in the CD cavity. In Table 3 some of the measured data are summarized[60]. With aliphatic hydrocarbons only dispersion forces are operative and hence the great differences in the retention on α- and β-CD can be explained only by the inclusion process. In view of the smaller cavity of α-CD, this process is more pronounced, as demonstrated by the retention data, where for *n*-pentane the difference is *ca.* 490 sec for α- and β-CD; with higher hydrocarbons the interaction is so strong that they are completely retained in the α-CD cavity.

TABLE 3

RETENTION DATA OF VARIOUS SORBATES ON α-CD AND β-CD

Column temperature, 80°C; column length, 120 cm; I.D., 3 mm; carrier gas, nitrogen (30 ml/min); detector, FID.

Sorbate	*B.p. (°C)*	t'_R *(sec)*	
		β-CD	*α-CD*
n-Pentane	36.07	5	496
n-Hexane	68.7	25	*
n-Heptane	98.42	75	*
Cyclohexane	80.7	81	1588
Benzene	80.1	133	1628
Toluene	110.8	191	1379
1,2-Dichloroethane	83.7	154	1429
Trichloroethylene	87.0	111	1730
1,1,2,2-Tetrachloroethane	146.0	228	850
Tetrachloromethane	76.8	105	254
Chlorobenzene	132.1	501	3328
Bromobenzene	156.2	1008	*
Methanol	64.7	686	437
Ethanol	78.4	348	790
Propanol	97.8	1119	2169
Isopropanol	82.4	487	1577
Diethyl ether	34.51	914	723
Diisopropyl ether	67.8	107	232

* Does not leave the column.

A comparison of the retention data for hydrocarbon halogeno derivatives shows pronounced differences, from which it can be assumed that during inclusion into α-CD the forces inside the CD ring play a greater role, because the dispersion forces in the α-CD cavity act at a shorter distance. The especially large difference between the retention data for trichloroethylene and 1,2-dichloroethane on α- and β-CD is made even more important by the fact that the boiling points of the substances are 40–60°C lower than those for the tetrahalogeno derivatives studied, which have substantially lower retentions.

The results of measurements on aromatic and cyclic compounds again indicate

a greater interaction with α-CD. A comparison of the data for benzene and toluene is interesting; the retention order is reversed as, in agreement with the model of the α-CD inclusion compound with benzene (see Fig. 11), benzene is more retained than toluene by α-CD.

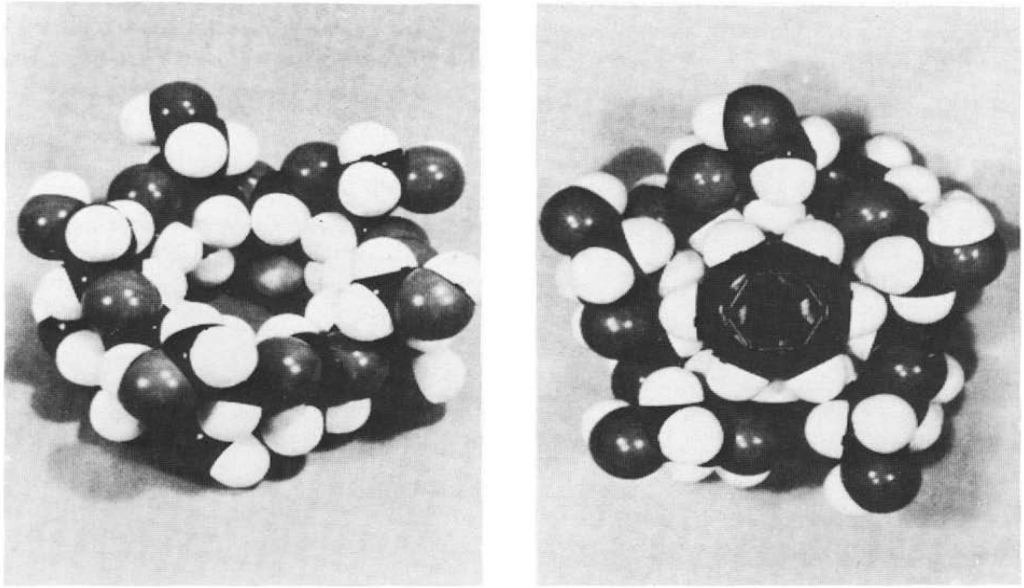

Fig. 11. Models of α-CD and α-CD inclusion compounds with benzene.

The effect of the geometric structure of the sorbates was very pronounced with diethyl ether and diisopropyl ether. The retention of the more volatile diethyl ether is more than three times higher for α-CD compared with β-CD and almost nine times higher compared with diisopropyl ether (see Table 3). This fact can be explained by the steric arrangement; analogous to urea, the branched-chain ether is less retained, in view of the cavity dimensions.

The results of the study of the interactions of α- and β-CD with various alcohols do not show great differences in retention times. This fact can be explained by the effect of the surface hydroxyl groups, which contribute significantly to the retention of polar substances on the two cyclodextrins. In addition to these polar forces, an inclusion process also takes place and is more marked for α-CD. In view of the relatively small cavity, α-CD binds the alcohols into more stable complexes, as a result of closer contact. Matsui and Mochida[61] arrived at the same conclusion and confirmed the greater stability of the α-CD inclusion compound with an aliphatic chain on the basis of the calculation of the association constants for the α- and β-CD inclusion compounds with various alcohols in aqueous solution. The difference found in the association constants again confirms the great effect of the volume of the guest molecule on the stability of the inclusion compounds of cyclodextrins with alcohols.

2.2.3. Evaluation of the use of cyclodextrins in gas chromatography

In the overall evaluation and comparison of the experimental results

TABLE 4

PHYSICAL PROPERTIES AND RETENTION DATA ON CD–POLYURETHANE RESINS

Column temperature, 150°C; column length, 120 or 80 cm; I.D., 3 mm; carrier gas, nitrogen (30 ml/min); detector, FID. Data from ref. 58.

Property	*Resin**		
	β; HDI–DMF–5.5–A	*α; HDI–DMF–5.9–A*	*α; HDI–DMF–13.3–A*
Temperature limit (°C)	200	230	230
Specific surface area (m^2 g)	170	180	280
OH residues per CD molecule	13.5	10.1	0.9
Retention times of various sorbates**:			
n-Hexane	0.04	0.16	0.27
n-Heptane	0.06	0.24	0.33
Cyclohexane	0.05	0.07	0.12
Benzene	1.00 (16.68***)	1.00 (6.59***)	1.00 (4.16***)
Toluene	1.20	2.73	1.96
Methanol	0.36	0.53	1.06
Ethanol	0.78	0.83	1.55
Propanol	1.96	2.03	2.80

* Resins obtained by polymerization of CD with hexamethylenediisocyanate (HDI) in N,N-dimethylformamide (DMF). Precipitant: acetone (*A*) (feed composition: β-CD 10 g + 5.5 g HD; α-CD 8.6 g + 5.9 g HDI; α-CD 8.6 g + 13.3 g HDI).

** Relative to benzene = 1.00.

*** Actual retention time (min).

with CD–polyurethane resin and CD deposited on a chromatographic support as stationary phases, it is appropriate to quote some data that can help in explaining the character of the interaction. With the CD–polyurethane resin it has been found that the preparation conditions affect their physico-chemical properties (Table 4). The differences in the specific surface area can affect, as follows from the data given below, the magnitude of the retention data and make unambiguous interpretation difficult. On the other hand, the retention on the material with the lowest content of residual hydroxyl groups demonstrates that with polar substances the interaction is not markedly affected by these processes and the results can be interpreted on the basis of the inclusion process.

Although the measurements were carried out at various temperatures, *viz.*, 150–170°C with CD-polyurethane resin and 50–80°C with CD on an inert support, certain correlations can be made (see Tables 3 and 4 and Fig. 12) and some more general conclusions can be drawn, which can be supported by the results of measurements in aqueous solutions of α- or β-CD.

(1) The differences of several orders of magnitude in the retention of aliphatic hydrocarbons between α- and β-CD confirm the inclusion character of the interaction.

(2) With benzene, toluene and cyclohexane, an increased retention of benzene was found on α-CD, whereas with CD-polyurethane resin a stronger interaction with

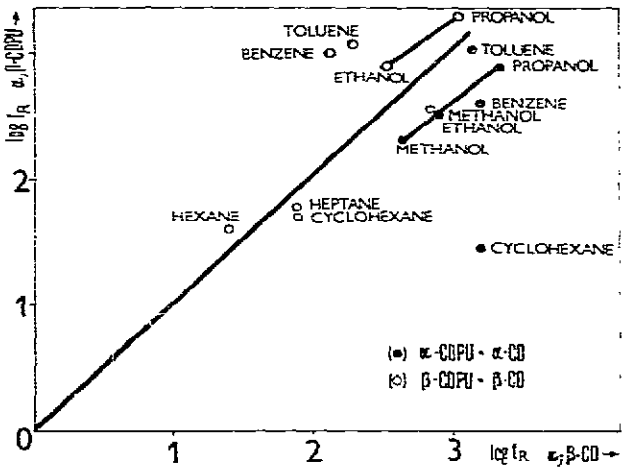

Fig. 12. Correlation diagram of retention data for various compounds measured on cyclodextrin–polyurethane resins and on cyclodextrins deposited on Chromosorb W.

β-CDP was found and explained by the existence of π-bonds. The greater interaction with α-CD, however, fully corresponds to the model in which the dimensions of the benzene molecule better match those of the cavity of α-CD as the host.

(3) The great differences in the retention of the hydrocarbon halogeno derivatives indicate that with α-CD the bonding forces can differ from those encountered with β-CD. The difference in the retention data of trichloroethylene and 1,2-dichloroethane, compared with tetrachloro derivatives, is especially marked in view of the boiling points.

(4) The experimental results for benzene halogeno derivatives, chloro- and bromobenzene, on α- and β-CD agree with the measurements in aqueous solutions. The effect of the bulky molecule of the bromo derivative is reflected in increased retention.

(5) The increased stability of the inclusion compounds of α-CD with unbranched aliphatic alcohols compared with the interaction with β-CD agrees with the association constants calculated for aqueous solutions.

(6) The interaction of unbranched and branched-chain compounds (alcohols, ethers) shows a lower retention for branched-chain compounds, the geometry of which does not correspond to the dimensions of the CD cavity.

Although the results obtained are rather qualitative, it is clear that both with macroporous polymers with inbuilt CD molecules and CD deposited on a chromatographic support, the forces operative inside the CD ring also play a role under GSC conditions, *i.e.*, the inclusion compounds are formed even when cyclodextrin as the host is in contact with sorbates in the gaseous state.

3. CONCLUSION

It can be concluded that the inclusion processes of cyclodextrins can be studied successfully by chromatographic methods and that chromatography will become an effective method for studying inclusion phenomena in general. On the other hand, it is

expected that, similar to gel inclusion chromatography, the selective formation of cyclodextrin inclusion compounds will be much more widely applied to other chromatographic techniques and will be used to solve many specific analytical problems.

4. ACKNOWLEDGEMENTS

The author thanks Dr. S. Krýsl for great help in collecting the literature data, M. Procházka for careful drawing of the figures and M. Rusová for technical help during preparation of the manuscript.

5. SUMMARY

Cyclodextrins and cyclodextrin polymers have been studied and applied in chromatography in recent years. Research carried out in this field is critically reviewed. The advantages of using the selective properties of these compounds for chromatographic separations and the possibilities of studying inclusion processes of different cyclodextrins with various types of substances, in the liquid as well as in the gaseous phase, are demonstrated and discussed.

6. NOTE

After presentation of this review, I have become acquainted with an interesting preprint[62] dealing with a similar subject, which confirms my conclusions. In addition, the possibilities of using cyclodextrins as mobile phase in TLC and HPLC are mentioned. More recently, papers have been published demonstrating that aqueous solutions of cyclodextrins have some important advantages over common mobile phases, based on the high selectivity of inclusion processes. Chromatographic separation of a variety of aromatic compounds such as monosubstituted isomers, isomers of di-, tri- and tetra-substituted phenols, nitriles, anilines and isomers of benzoic acid in TLC as well as of prostaglandins in HPLC have been reported[63–67].

REFERENCES

1 F. Cramer, *Einschlussverbindungen*, Springer Verlag, Berlin, Göttingen, 1954.
2 L. Mandelcorn, *Non-stoichiometric Compounds*, Academic Press, New York, 1964.
3 F. Cramer and H. Hettler, *Naturwissenschaften*, 54 (1967) 625.
4 J. A. Thoma and L. Stewart, in R. F. Whistler and E. F. Paschall (Editors), *Starch, Chemistry and Technology*, Vol. 1, Academic Press, New York, 1965.
5 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer Verlag, Berlin, Heidelberg, New York, 1978, p. 290.
6 W. Saenger, *Angew. Chem.*, 92 (1980) 343.
7 R. J. Bergeron, *J. Chem. Educ.*, 54 (1977) 204.
8 K. H. Fromming, *Pharm. Unserer Zeit*, 2 (1973) 109.
9 S. G. Frank, *J. Pharm. Sci.*, 64 (1975) 1585.
10 J. Szejtli, *Stärke*, 30 (1978) 427.
11 J. N. J. J. Lammers, *PhD Thesis*, Technische Hogeschool Eindhoven, 1970.
12 R. L. Van Etten, J. F. Sebastian, G. A. Clowers and M. L. Bender, *J. Amer. Chem. Soc.*, 89 (1967) 3242.
13 R. J. Bergeron, M. A. Channing, G. J. Gibeily and D. M. Pillor, *J. Amer. Chem. Soc.*, 99 (1977) 5146.
14 C. Nemethy and H. A. Schegara, *J. Chem. Phys.*, 36 (1962) 3401.
15 M. Komiyama and M. L. Bender, *J. Chem. Phys.*, 100 (1978) 2259.
16 M. Furue, A. Harada and S. J. Nozakura, *J. Polym. Sci., Polym. Lett. Ed.*, 13 (1975) 357.

17 J. Solms and R. H. Egli, *Helv. Chim. Acta*, 48 (1965) 1225.
18 P. Vretblad, *FEBS Lett.*, 47 (1974) 86.
19 Societe des Produits Nestlé, *Dutch Pat.*, 6,505,361 (1964).
20 L. C. Case, *U.S. Pat.*, 3,510,471 (1970).
21 N. Wiedenhof, J. N. J. J. Lammers and C. L. Panthaleon van Eck, *Stärke*, 21 (1969) 119.
22 N. Wiedenhof, C. L. Panthaleon van Eck and J. N. J. J. Lammers, *Brit. Pat.*, 1,244,990 (1971).
23 D. French, A. O. Pulley, J. A. Effenberger and M. Abdulah, *Arch. Biochem. biophys.*, 111 (1965) 153.
24 F. Cramer and D. Steinle, *Justus Liebigs Ann. Chem.*, 595 (1955) 81.
25 F. Cramer and F. M. Henglein, *Chem. Ber.*, 91 (1957) 308.
26 N. Wiedenhof, *J. Chromatogr.*, 15 (1964) 100.
27 K. Takeo and Y. Kondo, *Agr. Biol. Chem.*, 34 (1970) 954.
28 J. A. Thoma, H. B. Wright and D. French, *Arch. Biochem. Biophys.*, 82 (1959) 85.
29 J. N. J. J. Lammers, *Stärke*, 19 (1967) 70.
30 J. N. J. J. Lammers, *J. Chromatogr.*, 41 (1969) 462.
31 J. H. Carter and E. Y. C. Lee, *Anal. Biochem.*, 38 (1971) 521.
32 B. Zsadon, M. Szilasi, J. Szejtli, G. Seres and F. Tüdös, *Stärke*, 30 (1978) 276.
33 B. Zsadon, K. H. Otta, F. Tüdös and J. Szejtli, *J. Chromatogr.*, 172 (1979) 490.
34 J. B. Beadle, *J. Chromatogr.*, 42 (1969) 201.
35 N. Wiedenhof, *Stärke*, 21 (1969) 163.
36 J. Szejtli, E. Fenyvesi and B. Zsadon, *Stärke*, 30 (1978) 127.
37 T. F. Chin, *PhD Thesis*, 1962; Univ. Microfilms Inc., Ann Arbor, MI, U.S.A., see ref. 35.
38 E. Fenyvesi, B. Zsadon, J. Szejtli and F. Tüdös, *Ann. Univ. Sci. Budap. Rolando Eötvös Nominatae*, 15 (1979) 13.
39 B. Zsadon, M. Szilasi, K. H. Otta, F. Tüdös, E. Fenyvesi and J. Szejtli, *Acta Chim. Acad. Sci. Hung.*, 100 (1979) 265.
40 B. Zsadon, M. Szilasi, F. Tüdös, E. Fenyvesi and J. Szejtli, *Stärke*, 31 (1979) 11.
41 B. Zsadon, M. Szilasi, F. Tüdös and J. Szejtli, *J. Chromatogr.*, 208 (1981) 109.
42 Y. Mizobuchi, M. Tanaka and T. Shono, *J. Chromatogr.*, 208 (1981) 35.
43 J. L. Hoffman, *Anal. Chem.*, 33 (1970) 209.
44 J. L. Hoffman, *J. Macromol. Sci. Chem.*, 7 (1973) 1147.
45 A. Harade, M. Furuke and S. I. Mozakura, *J. Polym. Sci.*, 16 (1978) 189.
46 J. A. Thoma, J. D. E. Koshland, J. Rusica and R. Baldwein, *Biochem. Biophys. Res. Commun.*, 12 (1963) 184.
47 M. P. Silvanovich and R. D. Hill, *Anal. Biochem.*, 73 (1976) 430.
48 K. Takeo and S. Nakamura, in O. Hoffmann-Ostenhof, H. Breitenbach, F. Koller, D. Kraft and O. Scheiner (Editors), *Affinity Chromatography*, Pergamon Press, Oxford, New York, 1978, p. 67.
49 J. L. Hoffman and R. M. Bock, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 26 (1967) 501.
50 J. N. J. J. Lammers, J. L. Koole and A. J. G. van Diemen, *Rec. Trav. Chim. Pays-Bas*, 91 (1972) 483.
51 J. N. J. J. Lammers and A. J. G. van Diemen, *Rec. Trav. Chim. Pays-Bas*, 91 (1972) 733.
52 J. Szejtli, E. Fenyvesi and B. Zsadon, *Stärke*, 30 (1978) 127.
53 F. Cramer and F. M. Henglein, *Chem. Ber.*, 90 (1957) 2572.
54 D. M. Sand and H. Schlenk, *Anal. Chem.*, 33 (1961) 1624.
55 H. Schlenk and D. M. Sand, *Anal. Chem.*, 34 (1962) 1676.
56 H. Schlenk, J. L. Gellerman and D. M. Sand, *Anal. Chem.*, 34 (1962) 1529.
57 B. Casu, M. Reggiani and C. R. Sanderon, C. R., *Carbohydr. Res.*, 76 (1975) 59.
58 J. Szejtli and Zs. Budai, *Acta Chim. Acad. Sci. Hung.*, 94 (1977) 383.
59 Y. Mizobuchi, M. Tanaka and T. Shono, *J. Chromatogr.*, 194 (1980) 153.
60 E. Smolková, H. Králová, S. Krýsl and L. Feltl, *J. Chromatogr.*, in press.
61 Y. Matsui and K. Mochida, *Bull. Chem. Soc. Jpn.*, 52 (1979) 2808.
62 W. L. Hinze, *Sep. Purif. Methods*, submitted for publication.
63 K. Uekama, F. Hirayma, K. Ikeda and K. Inaba, *J. Pharm. Sci.*, 66 (1977) 706.
64 K. Uekama, F. Hirayma, S. Nasu, N. Matsuo and T. Irie, *Chem. Pharm. Bull.*, 26 (1978) 3477.
65 W. L. Hinze and D. W. Armstrong, *Anal. Lett.*, 13 (1980) 1098.
66 D. W. Armstrong, *J. Liq. Chromatogr.*, 3 (1980) 895.
67 W. G. Buckert, C. N. Owensby and W. L. Hinze, *J. Liq. Chromatogr.*, 4 (1981) 1065.