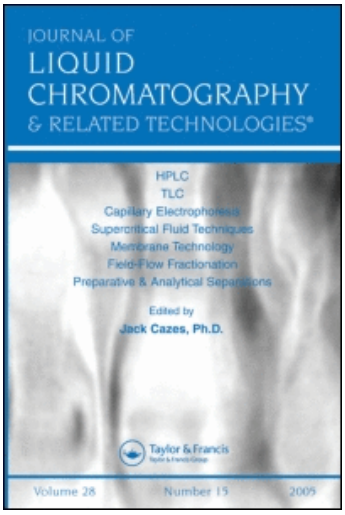

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THE MULTIMODAL CYCLODEXTRIN BONDED STATIONARY PHASES FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The use of α-, β-, and γ-cyclodextrin bonded stationary phases as multimodal support in HPLC is discussed. These supports are being used as reversed-phase and normal phase for the separation of widely different groups of compounds. Also, they are used as for the separation of optical, geometrical, positional and structural isomers in addition to ions and organo-metallic compounds. A brief discussion of separation mechanisms and application and their use with other stationary phases to improve selectivity are also presented.

INTRODUCTION

High performance liquid chromatography (HPLC) is an excellent separation technique for small as well as large molecules. The last decade witnessed numerous advances in instrumentation and column support materials. In instrumentation new injection devices, auto samplers and pumping systems have been introduced which are controlled, in most cases, by a computer. New detectors included the photodiode array detector which was first introduced by Hewlett-Packard and now available from at least half a dozen

companies. Also, HPLC/MS interfaces can be purchased. Many column packing materials have been introduced, some with base silica, others with polymeric base which are suitable for use with acidic and basic mobile phases from pH 1 to 14. However, the wealth of material is in the introduction of specific modified silica, such as cyano-, phenyl-, amino-, chiral, etc. It is becoming apparent that it would be not only helpful but economical if the column can be used in more than one chromatographic mode (multimodal) so that the chromatographer does not require a column for each separation problem. Recently, in ion exchange (IEC) HPLC a trend has developed whereby a column is used in more than one chromatpgrahic mode, IEC or hydrophobic interaction (HIC) depending on the mobile phase used (1,2). Mixed supports of different properties where also used as multimodal stationary phases (1,3,4). The mixed supports can be made by placing two different ligands on the same silica (3), by mixing two different derivatized silica in a certain ratio (1,4) or by connecting two different property columns in series to achieve difficult separations (1,4). Nahum and Horvath (5) and Kennedy, et al (2) observed that in reversed-phase (RP) chromatography, two different modes may be employed, depending on whether the mobile phase has a high or low content of water. This is due to silanol contributions. It has also been observed in protein as well as biomolecule separations that a weak cation exchange column can be used to separate a mixture of proteins by either IEC or HIC modes depending on whether the salt concentration increased or decreased in the gradient mobile phase (2,3). Rassi and Horvath (6) used a mixed anion/cation supports column for the separation of proteins. Floyd et al (7) simultaneously bonded both ionic and hydrophabic silanes to form a mixed ligand phase, which was used for the separation of oligodeoxyribonucleotides by both IEC and HIC. Also, the same type of mixed supports was used for ion pair separation (3). Colmsjo and Ericsson (8) synthesized packings with two different functional groups bonded to the same silica, C_{18}/cyanodecyl and C_{18}/cyanopropyl, for the selective separation of

some polycyclic aromatic hydrocarbons. Packings with both polar and nonpolar groups on the same particle were also synthesized (9). The mixing of alkylsilanes of various chain lengths (10,11); of alkylsilane phases of different carbon load (12); and of C_{18} and cyclodextrin bonded phases (4,13) have been used to improve the selectivity. An excellent discussion of mixed-interaction stationary phases was presented by Floyd and Hartwick (14).

The above mentioned materials are made of either mixed ligands on the same silica or by physically mixing different selectivity materials and packing them in the same column. The synthesis of mixed ligand materials may pose a problem of lot-to-lot reproducibility. Also, it may not be easy to obtain the required ratio of ligands accurately, due to ligand preference or ligand-ligand interaction. A 1:1 ratio may not be 1:1 but 0.9:1.1. On the other hand it is easier to control the ratios of physically mixed supports. However, other aspects should be controlled such as homogeniety of mixing and packing the column. Supports should have the same particle size and pore dimensions, surface area, carbon loading and compatibility with the mobile phase. All the above means that care should be exerted in synthesizing, packing and using mixed ligands and physically mixed supports. An easier approach and a less demanding one would be to produce materials with a single ligand. A ligand which possesses different properties (hydrophobic, hydrophilic, ionic...etc) and can be used with a wide variety of mobile phases (polar and nonpolar with or without water or buffer in the mobile phase mixture). Some of the available bonded silica stationary phases, for example the amino-, and the cyanoalkylsilanes can be used in the normal and reversed phase modes. However, the most versatile silica bonded materials are the cyclodextrin bonded phases which have been used as reversed-, normal-, ionic- and chiral phases for HPLC separations. This study is not a review of the properties and applications of cyclodextrin bonded silicas (CyD), but a discussion of the CyD as a multimodal stationary

phase. Selected examples are presented. For a review the reader should consult references (15,16,17).

Recently, in a discussion session on multidimensional chromatography (Ninth International Symposium on Capillary Chromatography, Monterey, California, May 16-19, 1988) it became apparent that the terms multidimensional and multimodal chromatography are being misused, and confusion in nomenclature exists. This author's opinion is that both terms apply to different aspects of the chromatographic process. In one case, both terms apply to the same system. For example, a TLC plate can be developed in a normal phase mode solvent, taken out, dried, turned 90° and developed in a reversed-phase mode solvent. This means that the plate was developed in two chromatographic modes (multimodal) and in two dimensions (multidimensional). Therefore, it is multimodal because we have more than one mode of chromatography and multidimensional because the resolved spots on the plate have dimension or distance, i.e. points in space as defined by Drs. Giddings and Huber, which I agree with. Multidimensional also will apply to the coupling of two chromatographic systems HPLC/GC; GC/SFC; etc. or the coupling of a chromatographic system with a spectroscopic technique.

In this manuscript multimodal stationary phase applies to a material that can be used in different chromatographic modes, where the only thing that changes is the mobile phase. By changing the mobile phase we change the chromatographic mode (adsorption, partition, IEC, HIC, inclusion...etc). If the chromatographic mode (process) does not change it is multidimensional, otherwise it is multimodal. For example, when a silica gel plate is developed in a normal phase solvent, taken out, dried, turned 90° and developed in another normal phase solvent (same chromatography mode) we talk of multidimensional development. The Journal of Liquid Chromatography will be pleased to publish any commends regarding this terminology.

THE CYCLODEXTRINS

The alpha, beta and gamma cyclodextrins are cyclic oligosaccharides which contain 6-, 7-, and 8- glucose units, respectively. These compounds which have the shape of a truncated cone have a hydrophobic inner cavity and hydrophilic outer surface. In addition, they are chiral molecules where each glucose unit contains five chiral atoms. This means that the β-CyD contains thirty-five chiral atoms. These properties, hydrophobic, hydrophilic and chiral can be used to effect different separation problems. In addition, CyD forms inclusion complexes with compounds in solution, which means that compounds are separated by their geometrical fit into the CyD cavity. Depending on the size of the molecules to be separated, the chromatographer can select the right cavity size. For example, α-CyD is most useful for molecules smaller than benzene ring such as amino acids, inorganic ions...etc. The β-CyD has an inner diameter of 7.8A and is used for molecules larger than a benzene ring but smaller than benzo(a)pyrene. The γ-CyD is used for large molecules. The most widely used one is the β-CyD. The available bonded CyD-silica phases are made by bonding the CyD to a silica (5μ, spherical) by a specific nonhydrolytic silane linkage which forms a stable bond, which preserves the properties of the CyD.

SEPARATION MECHANISM

The primary mode of separation by CyD is the formation of inclusion complexes (18) between the guest molecules to be separated and the host CyD hydrophobic cavity. These separations are effected in general by methanol/water or acetonitrile/water and in certain cases by the addition of a buffer. (These will be discussed later). Other separations, normal phase type, are carried out in hydrocarbon or hydrocarbon/alcohol mixtures. The mechanism of separation by normal phase mode is not well understood. However, it is not through an inclusion complex formation (19). It may be the result of the interaction between the guest compounds and the outside

hydrophilic surface of the CyD. Other factors such as Vander Waals, dipole-dipole interaction and hydrogen bonding may play a role in the separation process. The mechanism of separation of a wide variety of isomers (optical, geometrical and structural) is carried out by inclusion complex formation. The isomer separations are readily achieved due to different strengths of the inclusion complexes formed in the hydrophobic cavity, the better the fit the stronger the complex.

APPLICATIONS

A review of the literature reveals that cyclodextrin bonded phases have been used for widely different applications. As mentioned earlier this review deals with the CyD from the point of their use as multimodal phases. Examples are selected for illustration of the points raised. The most widely used column is the β-CyD. This may be due to the fact that it was the first commercially available material in the United States, due to its applicability to compounds of pharmaceutical interest. As a result most of the published work was on columns packed with this material. Cyclodextrins were first used as additive to the mobile phase to effect separation or to the stationary phase to form a gel (20). In 1983 Fujimura et al (21) reported the bonding of cyclodextrins to silica and used it for the separation of some aromatic compounds such as benzenes derivatives (o-, m-, p-nitrotoluene, xylene nitroaniline, cresol...etc) and naphthalene derivatives. The separations looked impressive in most cases. However, the column was not widely used nor gained acceptance among chromatographers due to the amine and diamine type linkage which are hydrolytically unstable, placing limitations on the use of aqueous phases (16). Armstrong (16) developed the first high efficiency bonded α-, β- and Y-cyclodextrin phases for use in TLC, LC and HPLC. (These columns are commercially available from Advanced Separation Technologies, Inc., Whippany, NJ). The bonding was done through a nonamine or amide spacer (an 8 aliphatic carbon chain) which proved to be stable and easier to produce than the previous attempt (21).

The separations reported using CyD stationary phases may be divided into the following groups. Selected examples are presented:

a) Reversed-phase type separations: These separations are achieved by using a mobile phase of methanol/water or acetonitrile/water. The separation is carried out by inclusion complex formation. The better the fit of the molecule in the cavity the stronger the complex formed. The separation of widely different groups of compounds was carried out including polycyclic aromatic hydrocarbons (22), mycotoxins (22), quinones (22), cyclic (23) and acyclic nitrosoamines (24), barbiturates (19), dipeptides (25,26), and a mixture of short chain peptides (27), see Figure 1, to mention some. Also, the separation of aspartame (Nutrasweet) from diet soft drinks (28), vitamins B_2 and K_5 and other organic compounds (29) were reported. The above separations were carried out on β-CyD using a mobile phase of methanol/water, acetonitrile/water or with the addition of 0.1-0.01 M triethylammonium acetate (TEAA) at pH 4-5. Addition of the buffer improved the shape of the resolved peaks and gave better resolution.

b) Normal phase type separations: These separations are carried out by using an organic (non-aqueous) mobile phase. Chang and Wu (30) were able to separate a mixture of substituted anilines (positional isomers) using γ-CyD column and a mobile phase of 2-propanol/heptane (7.5:92.5). Using a β-CyD column and a mobile phase of isopropanol/hexane (31) we were able to separate a mixture of pyrazole and 4-hydroxypyrazole, Figure 2.

c) Chiral and geometrical isomers: CyD columns have been widely used for the separation of optical, geometrical, positional isomers in addition to enantiomers, epimers and conformers (16,19,24,26,29,32-36). The CyD cavity and the inclusion complex formation are well suited for the separation of such compounds. Armstrong et al (32) reported the separation of drug stereoisomers such as propranolol, metoprolol,

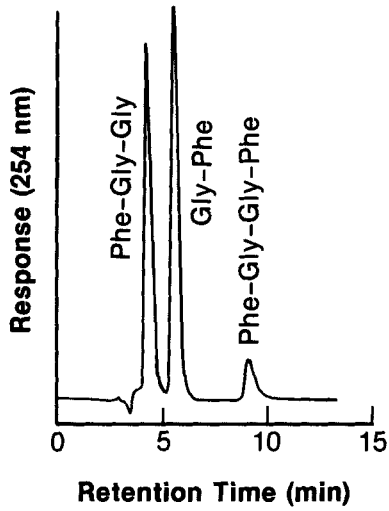

Figure 1. Separation of di-, tri-, and tetrapeptides using a β-CyD column, 250 x 4.6 mm, 5 μ, spherical using a mobile phase of 80% methanol/20% TEAA (0.1%, pH 4.0) at a flow rate of 1.0 ml/min, a chart speed of 0.5 cm/min and detection of 254 nm.

verpamil, chlorthalidone, hexobarbital, ketoprofen, methadone and methylphenidate, to name a few. Others (16,19,33,34) reported the separation of d- and l- isomers of amino acids and their dansyl derivatives. The separation of structural isomers (o-, m-, p-xylene, o, m-, p-cresol, o-, m-, p-nitroaniline...etc), geometrical isomers (cis-clomiphene and trans-clomiphene...etc) and steroid epimers were reported (35). Issaq, et al reported the separation of the conformers (E and Z isomers) of some acyclic nitrosamines (24) and nitrosamino acids (36). Ridlon and Issaq (26) reported the separation of selected dipeptide stereoisomers, Table 1.

The effect of temperature (37) and of pH, type of buffer and organic modifier concentration in the mobile phase on the separation of stereoisomers using a β-CyD column were studied (26,36,37). Table 2 show the effect of the concentration of acetonitrile in the mobile

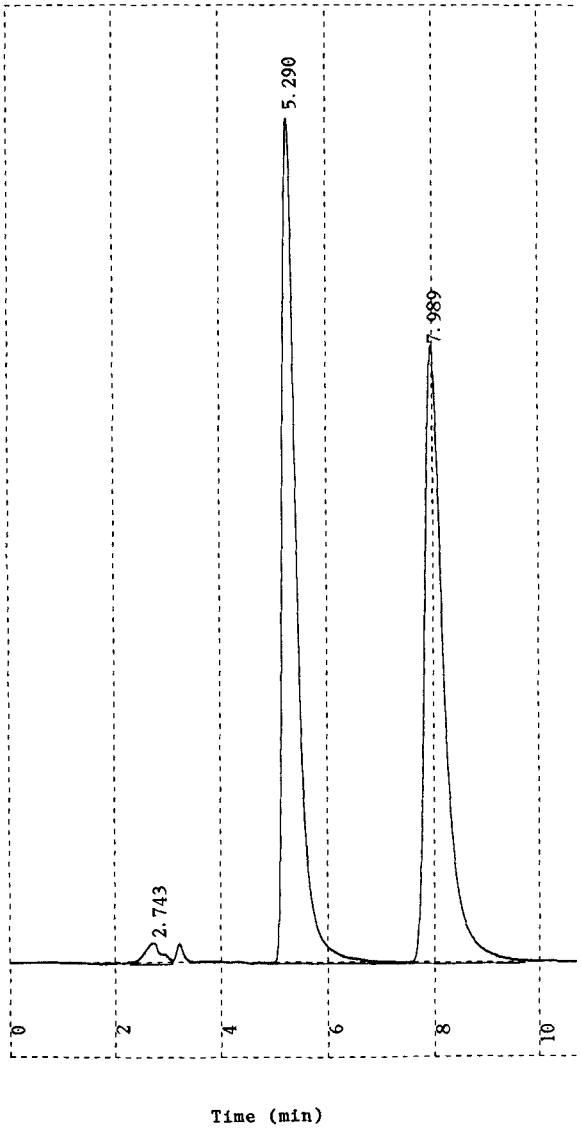

Figure 2. Separation of a mixture of pyrazole and 4-hydroxypyrazole using an α-CyD column and a mobile phase of 50% isopropanol in hexane at a flow rate of 1 ml/min, and detection at 224 nm.

Table 1

Separation of selected dipeptide stereoisomers on a 250 x 4.6 mm β-CyD column and a mobile phase of 10% methanol/90% 0.1% triethylammoniumacetate pH 4.0

Stereoisomers	α
Gly-Phe/Phe-Gly	1.37
Val-Phe/Phe-Val	1.35
Ala-Phe/Phe-Ala	1.39
Tyr-Phe/Phe-Tyr	1.13
Trp-Phe/Phe-Trp	1.37

Table 2

Effect of % acetonitrile (ACN) in the mobile phase on α of nitroso sarcosine (NSAR), nitrosothiazolidine carboxylic acid (NTCA), nitrosoproline (NPRO) and nitrosohydroxyproline (NHPRO), syn and anti conformers, using α-CyD column (150 x 4.6 mm, 5 μ spherical), and a mobile phase of acetonitrile/0.01 M triethylammonium acetate pH 5

Compound	%ACN 60	70	80	90	95
NSAR	1.14	1.14	1.23	1.25	1.32
NTCA	1.09	1.11	1.16	1.20	1.29
NPRO	1.16	1.12	1.21	1.39	1.51
NHPRO	1.13	1.18	1.24	1.41	1.58

phase on the retention of syn and anti isomers of selected nitrosamino acids. The results show that increasing the concentration of acetonitrile in the mobile phase gave a better separation factor (α). This is due to the decreased solubility of the nitrosamino acids in the rich acetonitrile mobile phase. Feitsma et al (38) studied the separation of aromitic carboxylic acid enantiomers using β-CyD column.

d) Acids and bases: The separation of amino acids and their dansyl derivatives by HPLC (16,29) and micro HPLC (34), nitrosamino acids (36) and carboxylic acids (38) were mentioned in the previous section. For details the reader is advised to read the original papers. Abidi (39) was able to resolve a mixture of n-alkyldimethylammonium chlorides into their homalogous components using a β-CyD and a mobile phase of methanol/water (50:50) or acetonitrile/water (30:70). It was found that resolution and selectivity were susceptible to changes in the mobile phase, capacity factors decreased as organic modifiers increased. For example a four component mixture was well resolved with 15% acetonitrile, but coeluted at 55% acetonitrile in the mobile phase (39). The same when methanol was used. 50% methanol gave good resolution of the same amine mixture but the mixture coeluted at 70% methanol. This is the opposite of what was observed earlier, Table 2.

e) Separation of ions: Armstrong et al (29) reported the separation of chloride ion from bromide ion (α = 4.2) and bromide ion from iodide ion (α = 1.4) using a mobile phase of methanol/water.

Issaq and Williams (40) used an α- and a β-CyD columns for the separation of nitrate ion from nitrite ion, Figure 3. No differences in retention were observed in using the α- or the β-CyD columns, which may suggest that the mechanism of separation of these ions is not through inclusion complex formation but by other forces. Also, formate and sulphate ions were separated under the above (40) experimental conditions using a conductivity detector.

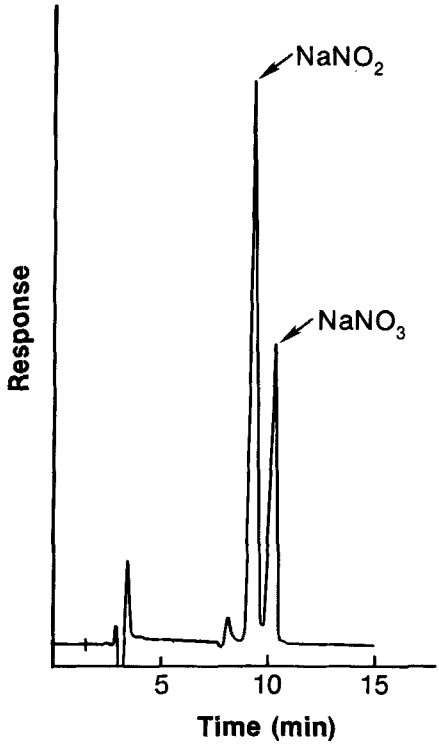

Figure 3. Separation of nitrate ion from nitrite ion on a β-CyD column, 250 x 4.6 mm, 5 μ spherical using a mobile phase of 60% methanol/40% TEAA (0.01%, pH 4.0) at a flow rate of 1.5 ml/min, a chart speed of 0.5 cm/min and detection at 218 nm.

f) Organometallic compounds: Armstrong et al (41) reported the separation of thirteen enantiomeric pairs of ferrocenes, ruthenocenes and osmocenes using the β-CyD column and a mobile phase of methanol/water. The separation mechanism in this case is through inclusion complex formation. However, they found that enantiomers of compounds that formed weaker or fewer hydrogen bonds with the CyD were better separated. Chang et al (42) found that the mechanism of separation, of several arene tricarbonylchromium (0) compounds

together with the arene ligands, on a β-CyD column was a mixed one, where both inclusion and solubility or solvophobic interactions were involved. The chromatograms of these separations showed sharp and symmetrical peaks which were resolved from each other.

ß) Mixed CyD/other phases: CyD supports are well suited, due to their chemical properties, to be used with other supports as mixed stationary phases to improve the selectivity of separation. Issaq et al (4) studied the selectivity of a mixed β-CyD/C_{18} materials in the same column. Also, a β-CyD column was used in series with a C_{18} reversed phase column (4) and SCX column (43). In both cases (4,43) separations were achieved which were not possible with either column.

The CyD stationary phases are good candidates for use in a mixture or in series with other columns because widely different mobile phases can be used. When mixed phases or columns of different selectivities are coupled in series, the stationary phases selected should be compatible with the mobile phase.

CONCLUSION

It is clear that cyclodextrin bonded stationary phases can be truly called multimodal supports reversed phase, normal phase and ion exchange which offer a high degree of selectivity for a widely different groups of compounds. They can be used for the separation of acids, bases ions, and a wide range of isomers (optical, geometrical, conformers, etc) in addition to other organic and organo metallic compounds. In this manuscript a brief discussion of the mechanism of separation was reported. Also, the effect of organic modifiers on α was touched-upon. For those interested in the effect of temperature, pH and other parameters on resolution using CyD stationary phases the following references are recommended (26,34,37-39).

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REFERENCE

1. Cs. Horvath, W.R. Melander and Z. El Rassi, 9th Int. Symp. on Column Liq. Chromatogr., Edinburgh, UK, July 1985.

2. L.A. Kennedy, W. Kapaciewicz and F.E. Regnier, J. Chromatogr, 359, 73 (1986).

3. J.B. Crowther and R.A. Hartwick, Chromatographia 16, 349 (1982).

4. H.J. Issaq, D.W. Mellini and T.E. Beesley, J. Liq. Chromatogr. 11, 333 (1988).

5. A. Nahum and Cs. Horvath, J. Chromatogr. 203, 53 (1981).

6. Z. El Rassi and Cs. Horvath, J. Chr. 359, 255 (1986).

7. T.R. Floyd, L.W. Yu and R.A. Hartwick, Chromatographia 21, 402 (1986).

8. A.L. Colmsjo and M.W. Ericsson, Chromatographia 21, 392 (1986).

9. W.E. Hammers, A.G.M. Theeuwes, W.K. Brederode and C.L. DeLingy, J. Chromatogr. 234, 321 (1982).

10. C. Gonneyt, D. Morel, E. Ramamonjinina, J. Serptinet, P. Claudy and J.M. Letoffe, J. Chromatogr. 330, 227 (1985).

11. K. Ogan and E. Katz, J. Chromatogr. 188, 115 (1980).

12. S.A. Wise, L.C. Sander, W.E. May, J. Liq. Chromatogr. 6, 2709 (1983).

13. H.J. Issaq, D.W. Mellini and T.E. Beesley, Mixed C_8/cyano and C_8/amino columns. Paper in preparation.

14. T.R. Floyd and R.A. Hartwick "Mixed-Interaction Stationary Phases in HPLC" in High Performance Liquid Chromatography, Cs. Horwath, ed., Vol. 4, 1986, Academic Press, Inc., Orlando, Florida, 1986, p. 45-90.

15. T.J. Ward and D.W. Armstrong, J. Liq. Chromatogr. 9, 407 (1986).

16. D.W. Armstrong, Bonded phase materials for chromatographic separations, U.S. Patent #4539399, (1985).

17. Cyclobond Handbook, a guide to using cyclodextrin bonded phases, compiled by Advanced Separation Technologies, Inc., Whippany, NJ (1987).

18. J. Szejtli, Cyclodextrins and their inclusion complexes, Adademiai Kiado, Budapest (1982).

19. D.W. Armstrong and W. DeMond, J. Chromatogr. Sc. 22, 411 (1984).

20. W.L. Hinze, Sep. Purif. Methods 10, 195 (1981).

21. K. Fujimura, T. Ueda and T. Ando, Anal. Chem. 55, 446 (1983).

22. D.W. Armstrong, A. Alak, W. DeMond, W.L. Hinze and T.E. Riehl, J. Liq. Chromatogr. 8, 261 (1985).

23. H.J. Issaq, J.H. McConnell, D.E. Weiss, D.G. Williams and J.E. Saavedra, J. Liq. Chromatogr. 9, 1783 (1986).

24. H.J. Issaq, M. Glennon, D.E. Weiss, G.N. Chmurny and J.E. Saavedra, J. Liq. Chromatogr. 9, 2763 (1986).

25. H.J. Issaq, J. Liq. Chromatogr. 9, 229 (1986).

26. C.D. Ridlon and H.J. Issaq, J. Liq. Chromatogr. 9, 3377 (1986).

27. H.J. Issaq, unpublished results.

28. H.J. Issaq, D.E. Weiss, C.D. Ridlon, S.D. Fox and G.M. Muschik, J. Liq. Chromatogr. 9, 1791 (1986).

29. D.W. Armstrong, A. Alak, K. Bui, W. DeMond, T. Ward, T.E. Richl and W.L. Hinze, J. Incl. Phenomena 2, 533 (1984).

30. C.A. Chang and Q. Wu, j. Liq. Chromatogr. 10, 1359 (1987).

31. H.J. Issaq, unpublished results.

32. D.W. Armstrong, T.J. Ward, R.D. Armstrong and T.E. Beesley, Science 232, 1132 (1986).

33. W.L. Hinze, T.E. Riehl, D.W. Armstrong, W. DeMond, A. Alak and T. Ward, Anal. Chem. 57, 237 (1985).

34. T. Takenchi, H. Asai and D. Ishii, J. Chromatogr. 357, 409 (1986).

35. D.W. Armstrong, W. Demond, A. Alak, W.L. Hinze, T.E. Riehland, K.H. Bui, Anal. Chem. 57, 234 (1985).

36. H.J. Issaq, D.W. Williams, N. Schultz and J.E. Saavedra, J. Chromatogr. (in press).

37. H.J. Issaq, M.L. Glennon, D.E. Weiss and S.D. Fox, In ordered media in chemical separations, W.L. Hinze and D.W. Armstrong, eds., ACS Symposium Series 342, Washington, D.C., 1986, pp. 260-271.

38. K.G. Feitsina, J. Bosman, B.F.H. Drenth, and R.A. DeZeeuw, J. Chromatogr. 333, 59 (1985).

39. S.L. Abidi, J. Chromatogr. 362, 33 (1986).

40. H.J. Issaq and D.W. Williams, manuscript in preparation.

41. D.W. Armstrong, W. DeMond and B.P. Czech, Anal. Chem. 57, 481 (1985).

42. C.A. Chang, H. Abdel-Aziz, N. Melchor, Q. Wu, K.H. Pannell and D.W. Armstrong, J. Chromatogr. 347, 51 (1985).

43. H.J. Issaq and G.M. Muschik, American Laboratory, May 1988, pp. 23-31.