The Use of Linear Expressions of Solute Boiling Point Versus Retention to Indicate Special Interactions with the Molecular Rings of Modified Cyclodextrin Phases in Gas Chromatography

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Abstract

The boiling points (°C, 1×10) of diverse C_{10} polar solutes from volatile oils are set against their relative retention times versus *n*-undecane to calculate linear equations for 12 commercial modified cyclodextrin (CD) capillary phases. Ten data points are considered for each CD, then solutes are rejected until 5 or more remain that give an expression with a correlation coefficient of at least 0.990 and a standard deviation of less than 5.5. Three phases give almost perfect correlation, and 3 other CDs have difficulty complying. Solutes involved in the equations (most frequently cuminal, linalol, and carvone) are presumed to have a 'standard' polar transient interaction with the molecular rings of the CDs concerned. Several remaining solutes (mostly citral, fenchone, and menthol) exhibit extra retention over the calculated standard (up to 772%), which is believed to indicate a firm 'host' CD or 'guest' solute molecular fit in some cases. Other solutes show less retention than calculated (mostly citronellal, citronellol, estragole, and pulegone). This suggests rejection by the CD, which behaves merely as a conventional stationary phase to them. The intercept constant in the equation for each phase is suggested to be a numerical relative polarity indicator. These *b* values indicate that 3 hydroxypropyl CDs show the most polarity with values from 28 to 43; and CDs that are fully substituted with inert groups fall in the range of 15 to 20.

Introduction

The first modified cyclodextrins (CD) that offered their molecular rings for the aid of separations were four β-CD esters, very soon after the invention of gas chromatography (GC). Schlenk, Gellerman, and Sand (1) in 1962 used packed columns of acetate, propionate, butyrate, or valerate to find that the shifts of the retention times of a compound (e.g., fatty acid ester) from phase to phase facilitate its classification because of its differing interactions with the various CD molecular ring-obstructing ester groups. Over 30 years later,

commercially available capillaries containing films of CD esters (2) or other modifications (3) were used for the classification of volatile oil constituents. However, since then, a major attraction of modified CD phases has been their potential to resolve certain enantiomeric pairs. In attempts to overcome such reluctant separations, many variations have been synthesized that have had diverse substitutions of the 2-, 3-, and 6-hydroxy groups for each of the α-glucose units that make up the CD molecular rings. If these had high melting-point solids, they were used by dissolving them in moderately low-polarity polysiloxanes (4), although this influenced some separations (5). In 1990, Armstrong and co-workers (6) started to prepare Chiraldex, which was substituted CDs that were liquid enough to be used alone in capillaries.

In 1993, Smith and Simpson (7) were using a γ-CD ester phase and commented that the mechanisms of separation by CD-based phases were not understood. They postulated possible reasons for their selectivity after observing the behavior of C_8 alcohol isomers, and they took into consideration that there may be more than one mechanism. These mechanisms are suggested here to be: (*a*) a transient entry/exit of solute molecules into or from the rings giving a 'standard' CD retention; (*b*) relatively firm interaction with the interior of the 'host' CD molecular rings because of a matching 'guest' solute shape, which produces considerably extra retention over the anticipated standard; or (*c*) no interaction with the ring so the CD-phase behaves just as a normal gas–liquid chromatographic medium producing separations by partition (and possibly others such as adsorption), which exhibits less retention than standard for a CD.

These equations can be studied mathematically after the style of Saura-Calixto and co-workers (8) who obtained linear plots for retention indices (I_R) as long ago as 1982 of such diverse solutes as alcohols, ketones, and esters against their boiling points and on conventional high- and low-polarity phases. Their expression was:

$$I_R = at_b + b \qquad \text{Eq. 1}$$

where t_b (°C) is the boiling point of the solute and a and b are constants, with b being partly related to the stationary phase. Heberger (9) agreed in 1988 that solute boiling-point dependence for moderately polar phases could describe the retention data of just 3 alkylbenzenes (linearly and with more complex equations) and be used for prediction. Recently in 1999, he concluded that retention indices for alkylbenzenes and their boiling points were not accidental but bear a firm physico-chemical link, and (for prediction purposes) the quadratic equations were suitable and description by the linear model seemed to be reasonably good (10). The present study has taken previously published relative retention values against *n*-alkane (a substance used as a standard for retention index work), and confirmed such a linear relationship using 9 low-polarity solutes on 6 modified CDs (11). Any expression of this relationship should give a high correlation coefficient of at least 0.995 for the solutes involved, but may only apply precisely (± 3%) to a limited number of low-polarity solutes (from 4 to 7) in which the CD is only behaving to them as a conventional phase. A generalized form of the expression used is:

$$t_{R\ rel.}c_{11} = aT - b \qquad \text{Eq. 2}$$

where $t_{R\ rel.}c_{11}$ is the relative retention against *n*-undecane and T (°C) is the boiling point of the solute (1×10^{-2}). The solutes involved always included the monoterpene hydrocarbons acyclic, myrcene, and monocyclic α-terpinene, but the non-compliant low-polarity solutes must have been exhibiting some extra retention (as mentioned above). Using a dipentyl γ-CD phase at 100°C when a was calculated to be 3.446 and b was 5.03 (11), monocyclic limonene gave 108% of the calculated retention, which suggests that its molecules only showed a transient CD-ring interaction, whereas 4 bicyclics showed greatly increased retention of 53–422% (with the largest value from camphene), which indicates a firm host/guest molecular bonding. Subsequent studies of a liquid crystal capillary (12,13) indicated the need for 2 linear expressions for both polar or low-polar solutes for each phase.

The present work attempted to determine polar solute expressions for 12 commercially available modified CD phases (including two γ-CD esters and one β-CD ester) with diluting polysiloxanes in some cases. Only two of these modified CDs could be considered to be of moderately high polarity (as will be discussed). Unlike nonpolar solutes, a transient molecular ring retention should be normal for these polar substances so that a retention of less than calculated may be seen when the CD is behaving as a conventional phase for a particular solute. Solutes whose molecular shapes fit a given CD cavity particularly well should still be apparent by their considerable extra retention.

From preliminary consideration of approximately 20 molecularly diverse C_{10} substances that were found in various volatile oils, 10 relatively simple compounds (7) were selected as likely to provide data for the required polar linear expressions. Their boiling points ranged from approximately 50 to 194°C. The aromatic cuminal and the acyclic monoterpenoid linalol appeared suitable for the determination of linear expressions for most of the 12 CD phases, and the monoterpenoids

bicyclic fenchone and acyclic citral were needed for only 2 CDs.

Experimental

Apparatus

Hewlett-Packard (Palo Alto, CA) GC models 5790A and 5890II were used at 125°C, with flame ionization detectors set at 215°C. The splitter injection ports were maintained at 200°C.

A 30-m Cyclodex-B capillary was purchased from J&W Scientific (Folsom, CA) and 20 m of it was used, having a 0.25-mm i.d. and requiring a helium flow rate at the capillary exit of approximately 1.5 mL/min. This capillary had a 0.25-μm film of heptakis permethyl-β-CD. A Cydex-B capillary was obtained from SGE International (Ringwood, Victoria, Australia) (25-m × 0.22-mm i.d.) that also required helium flow of approximately 1.5 mL/min. This capillary also had a 0.25-μm film of heptakis permethyl-β-CD, but in a methyl polysiloxane made fairly polar by 7% each of cyanopropyl and phenyl substituents (BP-10).

A Beta-Dex 225 capillary was obtained from Supelco (Bellefonte, PA) (30-m × 0.25-mm i.d.), requiring a fast flow of helium of more than 2.1 mL/min. It had a 0.25-μm film of heptakis diacetyl 6-*tert*-butyl dimethylsilyl (TBDMS)-β-CD in which 25% was embedded in a methyl polysiloxane with 20% phenyl substituents ("SPB-20"). Purchased from the same source and with the same properties was a Beta-Dex 325 capillary (Supelco), which differed significantly in being a dimethyl 6-TBDMS-β-CD instead of a diester phase. The inert bulky molecular TBDMS group was -O-Si$(CH_3)_2$–C$(CH_3)_3$. The Chiraldex modified CD phases in capillaries were obtained from ASTEC (Whippany, NJ). Two capillaries were donated, the remaining 6 were purchased. All were 10-m × 0.25-mm i.d. with a film thickness of 0.25 μm. The Chiraldex letters A-, B-, and G- indicate α-, β-, or γ-CDs, respectively, with 6, 7, or 8 glucose units forming the ring molecules, also respectively. The suffix "-DA" indicates a 2,6-dipentyl-CD; these were the only CDs with an unmodified 3-hydroxy group remaining on each α-glucose unit present. The suffix "-PH" indicates a 2-hydroxypropyl 3,6-dimethyl-CD; these were the most polar CDs presented (as will be discussed). G-BP indicates a 3-butyryl dipentyl-β-CD, and G-PN indicates a 3-propionyl dipentyl-β-CD. These two are monoesters, unlike the CDs originally used in 1962 (1). Helium flow rates of 0.6 to 1.6 mL/min were required. All phases were heated and cooled at 8°C/min or less to avoid damaging them.

For the statistical operations, a Casio *fx-570s* calculator was used.

Methods

Ten substances found in volatile oils (C_{10} monoterpenoids and aromatics) that were obtained from various commercial sources were used as probe solutes by injecting their trace residues repeatedly from an 'emptied' microsyringe. These compounds are given with summaries of their chemical natures in Table I. Solute relative retentions were determined

with respect to *n*-undecane, as was previously done (2,3). Holdup-time was deducted from observed retention times, which was determined by extrapolating the retentions of *n*-heptane and *n*-hexane to methane on semilogarithmic graph paper.

Calculations were made for each of the 12 CD phases using the relative retentions of the 10 probe solutes, gradually deleting no more than 5 solutes until the correlation coefficient of those remaining was at least 0.990. This should result in a percentage observing relative retention error compared with that calculated of no more than ± 7.5% for solutes involved in deriving the linear expression and a standard deviation of less than 5.5 for this percentage. These requirements were not fully obtained with 3 phases. An example graph of the results for Chiraldex G-PN is shown in Figure 1, with the 5 solutes in heavy boxes being those that yielded a linear expression (given in Table I) with a correlation coefficient of 0.997. The line for all 10 solutes is also shown, but it has a smaller correlation coefficient of 0.927. Plots for the 5 noncomplying solutes are scattered on either side of the best fit line.

Results and Discussion

The average observed relative retentions against *n*-undecane (C_{11}) and calculations resulting from their application with equation 2 are given in Table I; the 10 solutes studied are also listed. They are given in decreasing sequence of their use in characterizing linear expressions determined for the various CD phases. The second, third, and fourth most-used solutes were interestingly those selected 18 years ago to investigate the practical polarities of conventional phases in packed columns (14). The most-used solute in this study, cuminal, was also selected as one of 2 solutes to compare the polarities of CD and conventional phases (15). For each of the phases, the per-

Table I. Average RR* Versus *n*-Undecane at 125°C of 10 Volatile Oil Constituents (Lower Part) on 12 Modified Cyclodextrin Phases

Phase name			BetaDex-325		Chiraldex G-PH		Chiraldex A-PH	
Modified cyclodextrin			heptakis (β) 6-TBDMS di-methyl		octakis (γ) 2-hydroxypropyl di-methyl		hexakis (α) 2-hydroxypropyl di-methyl	
Diluent			20 phenyl, 80 methyl siloxane		none		none	
Film thickness (µm)			0.25		0.25		0.25	
Capillary dimensions			30-m × 0.25-mm i.d.		10-m × 0.25-mm i.d.		10-m × 0.25-mm i.d.	
Calculated RR* versus *n*-undecane			8.470T – 15.262		21.095T – 37.113		15.856T – 28.150	
Correlation coefficient (*n* solutes)			0.999 (7)		0.998 (6)		0.993 (6)	
Standard deviation (*n* solutes) %RR observed versus calculated			0.92		2.17		3.33	

Solute	Type	Boiling point (°C)	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %
Cuminal	Aco	235.5	4.70	100.4	13.10	104.2	9.54	103.8
(±) Linalol	Nol	199	1.61	101.3	4.92	101	3.31	97.4
			1.64	103.1				
(±) Carvone	Mco	231	4.33	100.5	11.50	99	7.32	86.4
Estragole	Aet	215	2.90	98.3	7.57	91.8	5.76	97
(+) Pulegone	Mco	224	3.71	100	8.88	87.6	6.11	82.9
(+) Citronellol	Nol	244.5	3.71	68.1	14.3	98.9	10.16	95.7
(+) Citronellal	Nco	207.8	1.97	84.2	4.07	60.6	3.01	62.7
(–) Menthol	Mol	216.4	3.06	99.7	9.10	106.3	6.20	100.6
c-Citral	Nco	229	4.48	108.2	10.9	97.4	8.50	104.2
(–) Fenchone	Bco	193.5	1.44	127.4	3.68	99.2	1.87	73.9

* RR, relative retention. Calculated RRs are linear relationships using the solute boiling point (°C, 1×10^{-2}). Percentage observed versus calculated RR (set to the right) was not used to calculate its linear expression.
† A is aromatic. Bicyclic, B; monocyclic, M; and acyclic, N are monoterpenoids.
‡ Boiling points are from Merck Index (Merck, Rahway, NJ) and *Handbook of Chemistry and Physics* (18).

centage of observed relative retention against that calculated is shown for each solute, with the 4 or 5 not used in determining the linear expression set against those used. The 12 modified CD phases are given in descending sequence of correlation coefficients for their linear expressions, for the 7- and 6-solute expressions (first 3), and then for those obtained using only 5 solutes. The β-CDs BetaDex-325, Cyclodex-B, and Cydex-B each gave almost perfect correlation. The γ-CDs Chiraldexes, G-PH, and G-PN were near perfect, (5 phases showed correlation coefficients of 0.997 or better), which substantiates the method used. On the right-hand side of Table I, the CD-phases showing the most reluctance to yield precise linear expressions can be seen. Correlation coefficients below 0.990 or standard deviations above 5.5 (as percentages of observed relative retentions) for 3 quite different Chiraldex phases or both of these conditions had to be accepted. Under the conditions used (125°C), enantiomeric resolutions were only observed for carvone on BetaDex-225 and linalol on BetaDex-325, although possibly

they could occur in other phases at lower temperatures with slower gas flow rates. Fenchone, menthol, and pulegone were used as single enantiomers, and the aromatics cuminal and estragole did not form chiral isomers.

As can be seen from Table I, the partly flat molecules of cuminal (with a rotating isopropyl group on the opposite side of the aromatic ring to the polar aldehyde) were involved in determining nearly all the linear expressions and showed what must be standard polar/CD retention of the transient type on all the phases except the very polar hydroxypropyl Chiraldex B-PH. Even here, its extra retention was only 22%; therefore, cuminal did not correspond to any specific CD cavities. The other aromatic probe, estragole, was a less polar ether and showed standard polar/CD retention with 8 of the phases. It displayed retention less than that calculated by 83–92% on the other 4 CDs, which should have behaved as conventional phases to it. These aromatic probes were of no diagnostic value for CD structures, but were valued for determining their reten-

Table I. (continued)

Phase name			Cyclodex B		Cydex B		Chiraldex G-PN	
Modified cyclodextrin			heptakis (β) tri-methyl		heptakis (β) tri-methyl		octakis (γ) 3-propionyl, di-pentyl	
Diluent			none		7 cyanopropyl, 7 phenyl, 86 methyl siloxan		none	
Film thickness (μm)			0.25		0.25		0.25	
Capillary dimensions			20-m × 0.25-mm i.d.		25-m × 0.22-mm i.d.		10-m × 0.25-mm i.d.	
Calculated RR* versus *n*-undecane			$10.898T - 19.499$		$10.516T - 18.818$		$11.357T - 20.346$	
Correlation coefficient (*n* solutes)			0.999 (5)		0.999 (5)		0.997 (5)	
Standard deviation (*n* solutes) %RR observed versus calculated			1.10		1.19		3.43	

Solute	Type	Boiling point (°C)	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %
Cuminal	Aco	235.5	6.13	99.4	5.86	98.5	6.55	101.7
(±) Linalol	Nol	199	2.17	99.1	2.07	98.6	2.42	105.7
(±) Carvone	Mco	231	5.74	101	5.54	101.5	5.98	100.8
Estragole	Aet	215	3.39	86.3	3.28	86.5	3.93	95.6
(+) Pulegone	Mco	224	4.84	98.6	4.74	100	5.04	98
(+) Citronellol	Nol	244.5	5.31	74.2	4.97	72.1	6.54	87.6
(+) Citronellal	Nco	207.8	2.57	81.6	2.51	83.2	2.46	74.8
(–) Menthol	Mol	216.4	4.15	101.4	3.97	100.8	5.54	129.7
c-Citral	Nco	229	6.24	114.5	6.13	116.7	6.33	110.9
(–) Fenchone	Bco	193.5	2.08	130.9	2.00	130.7	2.59	156

* RR, relative retention. Calculated RRs are linear relationships using the solute boiling point (°C, 1×10^{-2}). Percentage observed versus calculated RR (set to the right) was not used to calculate its linear expression.

† A is aromatic. Bicyclic, B; monocyclic, M; and acyclic, N are monoterpenoids.

‡ Boiling points are from Merck Index (Merck, Rahway, NJ) and *Handbook of Chemistry and Physics* (18).

tion expressions.

The contrastingly flexible acyclic molecules of the monoterpenoid alcohol linalol gave what seemed to be standard polar/CD retention on 10 of the phases, but with greatly increased retention on 2 of the 6 β-CDs. The 156% extra retention on Chiraldex B-PH and the 95% extra on B-DA suggested a very close host/guest fit for their CD molecular rings to the molecules of linalol. Although there could be an interaction between the hydroxyl groups of both CDs with this alcohol, this was not seen with the other acyclic alcohol citronellol. This latter gave standard polar/CD retention with just half the phases, including Chiraldexes B-PH and B-DA. No extra retention was seen by it with any CD, but the only sign that these others were behaving as conventional phases was by showing 60–88% of the calculated retention.

The partly rigid monocyclic monoterpenoid, carvone, had a rotating chiral C_3 isopropylene chain at an angle on its ring to the polar carbonyl group. Nine phases showed standard polar/CD retention with it, and the 2 α-CDs behaved as conventional phases and rejected this solute with 86 and 82% of the calculated retention. Only BetaDex-225 had a slightly extra retention of carvones (being 10 and 13% for the 2 enantiomers), but this did not indicate very close molecular fit. The other monocyclic ketone, pulegone, was a fully rigid molecule because of the C_3 chain being attached to its ring by a double bond, which is adjacent to the carbonyl group. It exhibited standard polar CD response to 8 of the phases and no extra retention. It behaved with the 2 α-CDs and 2 other solutes as though they were conventional phases, with 74–91% of the calculated retention. These 2 solutes also failed to indicate CD-ring shapes.

The 2 flexible acyclic aldehyde solutes only showed a standard polar/CD response on 2–3 phases. Citronellal responded as though the remaining 9 CDs were conventional phases, with 61–84% of the calculated retention. In contrast, citral always exhibited extra retention (Figure 1), which must be the con-

Table I. (continued)								
Phase name			Betadex-225		Chiraldex A-DA		Chiraldex B-DA	
Modified cyclodextrin			heptakis (β) 6-TBDMS, di-acetyl		hexakis (α) 2,6-di-pentyl, 3-unsubs.		heptakis (β) 2,6-di-pentyl, 3-unsubs.	
Diluent			20 phenyl, 80 methyl siloxane		none		none	
Film thickness (μm)			0.25		0.25		0.25	
Capillary dimensions			30-m × 0.25-mm i.d.		10-m × 0.25-mm i.d.		10-m × 0.25-mm i.d.	
Calculated RR* versus *n*-undecane			9.287T – 16.750		8.305T – 14.866		12.138T – 22.960	
Correlation coefficient (*n* solutes)			0.992 (5)		0.991 (5)		0.990 (5)	
Standard deviation (*n* solutes) %RR observed versus calculated			4.50		5.39		4.54	
Solute	Type	Boiling point (°C)	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %
Cuminal	Aco	235.5	5.13	100.2	5.05	107.6	5.90	104.9
(±) Linalol	Nol	199	1.71	98.8	1.57	94.6	2.32	195
(±) Carvone	Mco	231	5.17	109.8	3.54	82	5.31	104.5
			5.31	112.7				
Estragole	Aet	215	3.49	108.3	3.10	103.6	2.95	94
(+) Pulegone	Mco	224	3.91	96.5	2.77	74.1	3.83	90.6
(+) Citronellol	Nol	244.5	3.55	59.6	5.11	93.9	6.40	95.2
(+) Citronellal	Nco	207.8	2.44	95.7	1.81	75.7	2.26	100
(–) Menthol	Mol	216.4	2.78	83	3.73	119.9	5.92	179
c-Citral	Nco	229	6.64	146.8	5.93	142.9	5.27	108.9
(–) Fenchone	Bco	193.5	1.51	123.8	1.16	96.7	2.05	387

* RR, relative retention. Calculated RRs are linear relationships using the solute boiling point (°C, 1 × 10⁻²). Percentage observed versus calculated RR (set to the right) was not used to calculate its linear expression.
† A is aromatic. Bicyclic, B; monocyclic, M; and acyclic, N are monoterpenoids.
‡ Boiling points are from Merck Index (Merck, Rahway, NJ) and *Handbook of Chemistry and Physics* (18).

sequence of its second double bond. Values of over 40% extra retention on Chiraldex A-DA and BetaDex-225 suggested that their CD molecular cavities both closely corresponded to the molecules of citral, despite being an α- and a β-CD, respectively.

This argument applied to monocyclic menthol, whose rigid ring and rotating isopropyl group adjoining its polar alcohol must fit well into the CD cavities of the differing Chiraldexes B-DA (79% extra retention), B-PH (67%), G-DA (58%) and possibly G-PN (30% extra). These cavities were β- and γ-CDs, but BetaDex-225 rejected menthol molecules, which is the only phase to behave conventionally to them.

The rigid molecules of bicyclic fenchone only showed standard polar/CD interaction with 2 quite different Chiraldexes, A-DA and G-PH. With the remaining CDs, there was often considerable extra retention. An impressive 772% extra with Chiraldex B-PH suggested a near-perfect fit between its CD rings and fenchone molecules. Similar reasoning applied to

Chiraldex B-DA (287% extra), G-DA (120%), G-PN (56%, see Figure 1), and possibly 4 other CDs with approximately 30% extra retention. Fenchone responded quite differently to the 3 PH phases, with standard reaction to the γ-CD, but with vast excess retention on the β-equivalent (given previously) and the α-PH behaving as a conventional medium with only 74% of calculated retention.

With appropriate molecular modeling, it should be possible to define the CD cavity shapes of Chiraldex B-PH from its extra retentions of fenchone (772%), linalol (156%), and menthol (67%) even though these were bi-, non-, and monocyclic monoterpenoids, respectively, with carbonyl or alcohol polar groups. This was the case also for Chiraldex B-DA from extra retentions of the same 3 probe solutes, which suggested the β-CD ring size was dominant for these 2 phases. Chiraldex G-DA retained only fenchone (120% extra) and menthol (58%) strongly; therefore, its standard polar/CD response to linalol should have been significant in revealing its cavity shape. The

Table I. (continued)

Phase name			Chiraldex G-BP		Chiraldex B-PH		Chiraldex G-DA	
Modified cyclodextrin			octakis (γ) 3-butyryl, dipentyl		heptakis (β) 2-hydroxypropyl, di-methyl		octakis (γ) 2,6-di-pentyl, 3-unsubs.	
Diluent			none		none		none	
Film thickness (μm)			0.25		0.25		0.25	
Capillary dimensions			10-m × 0.25-mm i.d.		10-m × 0.25-mm i.d.		10-m × 0.25-mm i.d.	
Calculated RR* versus *n*-undecane			10.300T – 18.365		22.556T – 43.286		11.188T – 19.277	
Correlation coefficient (*n* solutes)			0.989 (5)		0.988 (5)		0.984 (5)	
Standard deviation (*n* solutes) %RR observed versus calculated			4.37		6.12		6.05	

Solute	Type	Boiling point (°C)	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %	Observed RR	Observed RR/calculated %
Cuminal	Aco	235.5	6.19	105	11.98	121.8	6.99	98.9
(±) Linalol	Nol	199	2.25	105.6	4.09	256	2.72	91
(±) Carvone	Mco	231	5.32	97.9	9.47	107.4	6.76	102.9
Estragole	Aet	215	3.75	99.2	5.53	106.2	3.98	83.3
(+) Pulegone	Mco	224	4.43	94.1	6.63	91.6	6.28	108.7
(+) Citronellol	Nol	244.5	5.99	87.8	11.65	98.2	7.73	95.7
(+) Citronellal	Nco	207.8	2.30	76.7	3.42	95.5	2.60	65.5
(–) Menthol	Mol	216.4	4.66	118.9	9.22	167	7.80	158
c-Citral	Nco	229	5.83	111.7	9.24	110.5	6.95	109.6
(–) Fenchone	Bco	193.5	2.09	133.1	3.14	872	5.21	220

* RR, relative retention. Calculated RRs are linear relationships using the solute boiling point (°C, 1 × 10⁻²). Percentage observed versus calculated RR (set to the right) was not used to calculate its linear expression.
† A is aromatic. Bicyclic, B; monocyclic, M; and acyclic, N are monoterpenoids.
‡ Boiling points are from Merck Index (Merck, Rahway, NJ) and *Handbook of Chemistry and Physics* (18).

same applied to Chiraldex G-PN to a lesser extent in which the γ-CD ring size dominated these two. The 47% and 43% extra retention with citral may help in defining the cavities of BetaDex-225 and Chiraldex A-DA, respectively. The data in this study were of no help with Chiraldexes G-PH and A-PH, and probably not at all with the remaining 4 CDs.

The effect of a diluting polysiloxane present in Cydex-B was compared with the undiluted permethyl-β-CD of Cyclodex-B. This is very small, and both phases had near perfect correlation for their very similar expressions determined with the same 5 solutes. Relative retentions were slightly bigger on the undiluted CD. The monoester γ-CDs, Chiraldexes G-BP and G-PN, behaved similarly to all 10 solutes. The longer chain butyryl phase gave lower relative retentions. The di-ester β-CD BetaDex-225 differed from them in its extra retention of carvones, its rejection of menthol, and its standard response to citronellal.

If the intercept b in equation 2 is related to the phase studied (8), it should be a numerical indicator of their relative polarities. Listed here is a decreasing sequence of negative b values from Table I: 43.3 Chiraldex B-PH {928}, 37.1 Chiraldex G-PH {945}, 28.2 Chiraldex A-PH {884}, 23.0 Chiraldex B-DA {802}, 20.4 Chiraldex G-PN {843}, 19.5 Cyclodex-B {843}, 19.3 Chiraldex G-DA {837}, 18.8 Cydex-B, 18.4 Chiraldex G-BP {858}, 16.8 BetaDex-225, 15.3 BetaDex-325, and 14.9 Chiraldex A-DA {788}. The bracketed numbers are for comparison and are the average McReynolds' indices of 8 solute probes previously determined (15). The 3 Chiraldex PH phases were obviously the most polar here by both b values and McReynolds' indices (with a polar 25% cyanopropyl polysiloxane having an average McReynolds' index of 965, for comparison), although there is dispute over which of the two is the top one. Chiraldex A-DA
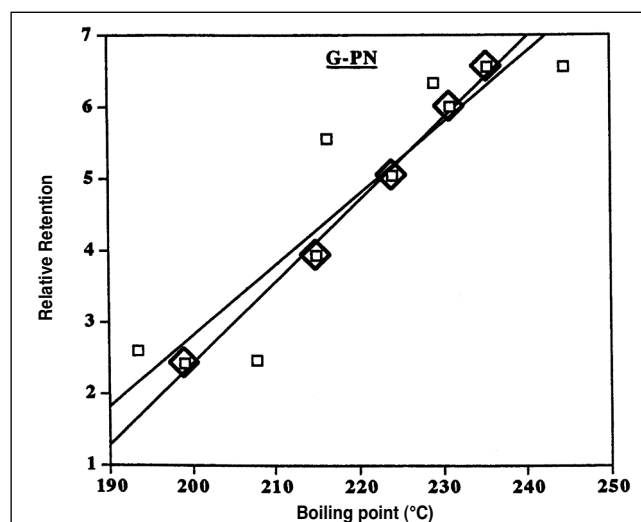
was less polar, but not as low in polarity as methyl polysiloxane, which had an index of 724. The positions of the Chiraldexes B-DA and G-BP were reversed in sequence in the 2 ratings above. The CDs that had their hydroxyls fully substituted by inert methyl groups (pentyls, TBDMS, and esters) appeared in a range of b values from 15.3 to 20.4, and they corresponded when both values were available.

Another method of polarity rating for phases was also applied from the same source (15). The elution of 3 selected McReynolds' probe solutes at 100°C only gave the 'high polarity' sequence of 2-octyne first, then n-butanol and pyridine for Chiraldex B-PH, thus agreeing with its highest b value. Chiraldex G-PH was rated as 'intermediate polarity' with the elution sequence of butanol, octyne in the middle, then pyridine, as also did G-BP, which corresponded to its average McReynolds' index. All other CD phases gave the 'low-polarity' sequence of butanol first, pyridine, and then octyne last, including Chiraldex A-PH. Heberger (16) has recently applied principal component analysis (PCA) noting that at least 2 polarity indicators were needed, because there was no polarity scale that characterized the stationary phases well enough on its own. He also noted that there was no unambiguous sequence among the phases according to any of the polarity indicators, even if they were highly correlated. Although the 3 polarity scales were so similar that they could be used interchangeably, if the 30 phases were ordered by increasing polarity according to Castello et al. (ΔC, a plot against them), the polarity descriptors by McReynolds' or Kovats' coefficients would be a rather jagged zigzag pattern. Thus, the inconsistencies in our various attempts to mathematically sequence CD phases in comparative polarities were to be expected.

The PCA of the data in this study by Morgan (17) confirmed the idea that more than one dimension was needed to represent the variability among these CD phases. Two principal components account for 95.9% of the variation in the data about its mean, and 3 account for 98.2%. A space projection of these first 3 PCs confirmed the 3 Chiraldex PH phases as being apart from the others; B-DA is also apart in a different orientation. These spatial projections corresponded to the McReynolds' indices given previously, rather than the negative b values of

Figure 1. Plots by Morgan (17) of relative retentions versus n-undecane on Chiraldex G-PN phase for 10 probe solutes against each Boiling Point (°C, 1×10^{-2}). From left to right the data points represent fenchone, linalol, citronellal, estragole, menthol, pulegone, citral, carvone, cuminal and citronellol. Several solutes (linalol, estragole, pulegone, carvone, and cuminal) were selected to determine the linear expression for G-PN given in Table I ($r = 0.997$). The line for this is shown together with that for all 10 solutes.
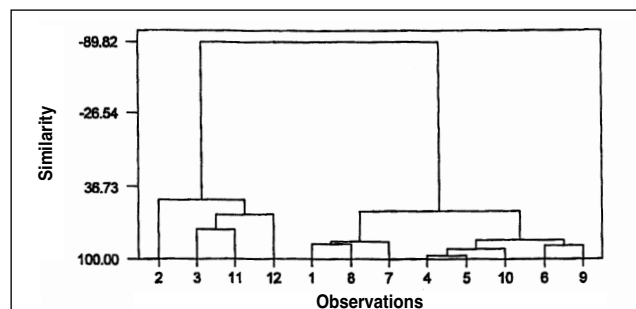

Figure 2. Hierarchical cluster analysis (standardized variables, Euclidean distance, and Ward's clustering method) by Morgan (17) of the data in Table I. Observations displayed left to right from the CD phases Chiraldex G-PH (2), A-PH (3), B-PH (11), and G-DA (12); Beta-Dex 325 (1); Chiraldex A-DA (8); Beta-Dex 225 (7); Cyclodex-B (4); and Cydex-B (5); and Chiraldex G-BP (10), G-PN (6), and B-DA (9).

the phases. Hierarchical cluster analysis (Figure 2) also grouped the 3 Chiraldex PH phases together at one side, with B-DA at the other. This confirmed that the 3 Chiraldex-DA phases behave quite differently to each other, and that Cydex-B and Cyclodex-B were very similar. A selection of 3 different CD phases for a chromatographic laboratory can be made from these results (i.e., Chiraldexes A-PH and B-DA with Beta-Dex 225).

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