DIFFERENT BASES FOR THE GAS CHROMATOGRAPHIC RETENTION INDEX SYSTEM

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SUMMARY

The concept of a generalized retention index is discussed. The potential usefulness of nine homologous series as a reference for a retention index system is investigated. The functional constants — the additive terms for interconversion between systems — are given, together with their confidence intervals. *n*-Alkanes are not suitable reference compounds for high polarity stationary phases. Compounds of medium polarity, such as the *n*-aldehydes, give more precise values for stationary phases of low and high polarity. A polarity scale may be based on these more reproducible indices. This is shown by comparing *n*-alkane with *n*-aldehyde polarities. A polarity scale based on the variance of the retention indices can compare indices referred to different homologues.

INTRODUCTION

The Kovats' retention index system[1] has been widely accepted in the chromatographic literature as a means of comparing retention data and characterizing stationary phases. Kovats' index compares the retention behaviour of a compound with that of *n*-alkanes measured under identical conditions. The retention index is approximately independent of the gas flow-rate. The temperature dependence of the retention index is usually less than 1 index unit per degree[2]. If adsorption effects are negligible, *i.e.* the support surface is deactivated and Gibbs adsorption is of minor importance because the polarity difference between solutes and stationary phase is small, then the Kovats' index is also independent of the liquid phase loading[3]. Under high resolution conditions the reproducibility from laboratory to laboratory is about 1 index unit[4] for low polarity stationary phases. For high polarity columns the capacity ratio of the *n*-alkanes is very sensitive to impurities, ageing, solute concentration and the surface to volume ratio of the stationary phase. In these cases the reproducibility of the classical Kovats' retention index leaves much to be desired, and from a practical point of view it is inconvenient to inject solutes together with their reference *n*-alkanes having a boiling point of 150° or higher[5]. To remedy these deficiencies it has frequently been suggested that a more polar homologous series than the *n*-alkanes be employed[5–7]

as reference series. Primary alcohols[5,8,9], methyl-*n*-alkyl ketones[6,8], *n*-alkyl esters[2,7,8,10], *n*-alkylbenzenes[11] and *n*-alkyl iodides[12] have been suggested for this purpose. Lipid chemists are using the ECL (equivalent chain length) system and the CN (carbon number) system, where the methyl esters of linear saturated acids are performing the same function as the *n*-alkanes in the Kovats' retention index system. Generally speaking, it is desirable to use reference compounds that are chemically similar to those under analysis.

This paper is an application of the generalized retention index system of Novák and Ružičková[8]. Nine homologous series of reference compounds on four stationary phases of widely different polarities are compared.

EXPERIMENTAL

Measurements were made using an F & M 810 gas chromatograph (F & M, Avondale, Pa., U.S.A.) equipped with a flame ionization detector. Retention times were obtained using a D26 computing integrator (Bodenseewerk Perkin-Elmer, Überlingen, G.F.R.) measuring to 0.01 min. The column temperature was kept at $100 \pm 0.2°$. The columns (2.5 mm I.D.) contained 4–8% of stationary phase on a Chromosorb G AW DMCS (80–100 mesh) support. The stationary phases were (1) squalane (Applied Science, State College, Pa., U.S.A.), (2) di(2-ethylhexyl)sebacate (DOS) (Heyden, München, G.F.R.), (3) Carbowax 20M (Regis, Chicago, Ill., U.S.A.), (4) 1,2,3-tris(2-cyanoethoxy)propane (TCEP) (Merck, Darmstadt, G.F.R.).

The flow-rate of the nitrogen carrier gas was 30 ml/min. The column length was varied between 2 and 5 m to keep the retention times above 1 min. The dead time was determined by injecting methane[13], as the iterative procedure using data of homologous series[14] can be subject to larger errors. The injected sample sizes were kept as small as possible, usually under 0.02 μl. At least three columns were prepared of each stationary phase.

The retention times for each solute investigated were determined on each column at least three times. The 30 substances suggested by Rohrschneider[15] and members of the following homologous series were selected as solutes: (1) *n*-alkane, (2) *n*-alk-1-ene, (3) 1-chloro-*n*-alkane, (4) *n*-aldehyde, (5) *n*-alkan-2-ol, (6) *n*-alkan-1-ol, (7) 1-amino-*n*-alkane, (8) *n*-alkylbenzene, (9) *n*-alkylcyclohexane.

THEORY

The generalized retention index $I_y^x(i)$ of a substance i on the stationary phase x refers to the retention of the homologous series y[8]:

$$I_y^x(i) = 100 \frac{\log V_N^x(i) - \log V_N^x(yn)}{\log V_N^x(yn+a) - \log V_N^x(yn)} + 100n + K_y \tag{1}$$

where $V_N^x(yn)$ is the net retention volume on the stationary phase x of the reference compound with n methylene groups, $V_N^x(yn+a)$ is the net retention volume of the reference compound with $n+a$ methylene groups, and K_y is a constant, chosen to avoid negative indices (for $y = n$-alkane, $K_y = 0$).

When y is the *n*-alkane series, the Kovats' retention index is a special case.

If the slope of log $V_N^x(yn)$ over $I_y^x(yn)$ is the same for all reference series, the difference between all retention indices measured in the index system $y1$ and the one measured in the index system $y2$ reduces to a constant[8]. In a computationally appropriate form one can write:

$$I_{y1}^x(i) - I_{y2}^x(i) = A_{y2}^x/B_{y2}^x - A_{y1}^x/B_{y1}^x = FU_{y1,y2}^x \tag{2}$$

where B_y^x is the slope of log $V_N^x(yn)$ over $I_y^x(yn)$, and A_y^x is the ordinate intercept of line log $V_n^x(yn)$ over $I_y^x(yn)$. In this case the difference for all substances is equal to the functional constant $FU_{y1,y2}^x$ which depends on the reference compounds $y1$ and $y2$ and the stationary phase x.

The differences between Kovats' indices of certain standard substances on the phase in question and on squalane are used as a polarity measure of the stationary phase[16]. If one employs the generalized retention index system in the same way, the difference between the two polarity scales thus obtained is of interest. This difference between the indices referred to as $y1$ and measured on the stationary phases $x1$ and $x2$ and the indices referred to as $y2$ is, if the slopes are the same for each column, given by:

$$[I_{y1}^{x1}(i) - I_{y1}^{x2}(i)] - [I_{y2}^{x1}(i) - I_{y2}^{x2}(i)] = ([A_{y1}^{x2} - A_{y2}^{x2}]/B_{y1}^{x2}) + ([A_{y2}^{x1} - A_{y1}^{x1}]/B_{y1}^{x1}) \tag{3}$$

The polarity values will differ by a constant factor. Their difference will be a function of the retention volumes if the assumption about the slopes does not hold.

The slopes B_y^x are equal if log $V_N^x(yn+1)/V_N^x(yn)$ is the same for the higher members of all homologous groups. In terms of an additivity model for the free energy of retention[8], this means that the molar Gibbs free energy of the methylene group on a given stationary phase is the same for all homologues.

RESULTS AND DISCUSSION

Table I lists the mean value of the measured n-aldehyde retention indices for the four columns investigated. The retention indices of the references have assigned values. The indices in the respective index systems have been chosen in such a way that the following substances have index values of 1000:

(1)n-Decane; (2) Hept-1-ene; (3) 1-Chlorohexane; (4) Hexylaldehyde; (5) Octan-2-ol; (6) Hexan-1-ol; (7) 1-Aminohexane; (8) Methylbenzene; (9) Methylcyclohexane. This means that, for example, in the aldehyde index system an index of 1150 signifies a substance eluting between n-heptyl and n-octyl aldehyde, whereas the same index in the alkylbenzene system denotes a substance between ethylbenzene and n-propylbenzene. This choice avoids negative retention indices.

The gas hold-up time has been experimentally determined. Assuming a linear relationship between the adjusted retention times and the carbon number of the reference series, one linear least square fit was calculated using all reference points. In this way the measured indices of the references differ by up to four retention index units from their assigned values, since the relationship is in fact not strictly linear (compare Table I). One can force the experimental values to coincide with their assigned values by fitting piecewise or using a non-linear relationship. However, we consider these latter methods less accurate in cases where one has to extrapolate.

TABLE I

MEASURED *n*-ALDEHYDE RETENTION INDICES

Substances	Stationary phase			
	Squalane	DOS	Carbowax 20M	TCEP
n-Aldehyde				
C_3H_7CHO	—	700	704	701
C_4H_9CHO	790	797	794	793
$C_5H_{11}CHO$	897	901	899	901
$C_6H_{13}CHO$	1000	1003	1001	1004
$C_7H_{15}CHO$	1103	1101	1102	1104
$C_8H_{17}CHO$	1202	1197	1201	1201
$C_9H_{19}CHO$	1301	—	1300	1299
$C_{10}H_{21}CHO$	1397	—	1399	1397
Rohrschneider substances				
Ethanol	604	701	818	793
$CH_3COC_2H_5$	781	800	820	864
Nitromethane	698	833	1064	1160
Pyridine	952	976	1100	1174
2,4-Dimethylpentane	880	789	512	211
2-Ethylhex-1-ene	1031	953	752	507
Cyclohexane	923	836	650	399
Benzene	900	878	870	794
Toluene	1010	982	970	902
Styrene	1127	1118	1176	1158
Acetone	658	698	721	781
Crotonaldehyde	834	876	962	1041
t-Butanol	713	772	776	730
Chloroform	836	872	927	775
Thiophene	905	898	949	885
n-Butylacetate	1002	991	986	958
Allylalcohol	713	822	1004	1002
Dioxane	906	914	986	1063
Acetonitrile	615	735	921	1021
Nitroethane	811	904	1073	1187
Methyl iodide	763	742	730	600
n-Butylether	1113	1042	879	690
Ethyl bromide	743	724	672	454
CCl_4	905	859	796	574
Propan-2-ol	671	742	806	768
Phenylacetylene	1089	1114	1270	1234
1,1-$C_2F_2Cl_4$	715	880	790	559
Cyclopentanol	987	1048	1206	1221
n-Alkane				
C_5H_{12}	736	—	—	—
C_6H_{14}	852	—	—	—
C_7H_{16}	953	856	—	—
C_8H_{18}	1054	954	—	—
C_9H_{20}	1154	1052	815	—
$C_{10}H_{22}$	1252	1149	912	—
$C_{11}H_{24}$	1351	1245	1011	—
$C_{12}H_{26}$	1449	1340	1109	—
$C_{13}H_{28}$	—	—	1206	—
$C_{14}H_{30}$	—	—	1303	—
$C_{15}H_{32}$	—	—	1402	—

TABLE I *(continued)*

Substances	Stationary phase			
	Squalane	DOS	Carbowax 20M	TCEP
n-Alkan-1-ol				
C_3H_7OH	—	814	927	897
C_4H_9OH	—	919	1037	1007
$C_5H_{11}OH$	—	1023	1143	1113
$C_6H_{13}OH$	—	1124	1247	1216
$C_7H_{15}OH$	—	1222	1347	1315
$C_8H_{17}OH$	—	1320	1448	1413
$C_9H_{19}OH$	—	—	—	1513
$C_{10}H_{21}OH$	—	—	—	1608
n-Alkan-2-ol				
$CH_3CH_2OHCH_3$	681	743	812	762
$C_2H_5CH_2OHCH_2$	799	851	913	864
$C_3H_7CH_2OHCH_3$	901	950	1010	958
$C_4H_9CH_2OHCH_3$	1003	1049	1113	1061
$C_5H_{11}CH_2OHCH_3$	1104	1147	1214	1161
$C_6H_{13}CH_2OHCH_3$	1204	1245	1314	1260
$C_7H_{15}CH_2OHCH_3$	1303	1340	1408	1358
1-Amino-n-alkane				
$C_4H_9NH_2$	—	—	804	—
$C_5H_{11}NH_2$	—	—	911	—
$C_6H_{13}NH_2$	—	—	1010	—
$C_7H_{15}NH_2$	—	—	1114	—
$C_8H_{17}NH_2$	—	—	1214	—
$C_9H_{19}NH_2$	—	—	1312	—
$C_{10}H_{21}NH_2$	—	—	1411	—
1-Chloro-n-alkane				
C_3H_7Cl	776	744	—	—
C_4H_9Cl	881	847	761	—
$C_5H_{11}Cl$	987	948	866	—
$C_6H_{13}Cl$	1087	1047	966	—
$C_7H_{15}Cl$	1187	1145	1066	—
$C_8H_{17}Cl$	1287	1243	1166	—
n-Alkylbenzene				
$C_2H_5C_6H_5$	1102	1067	—	—
$C_3H_7C_6H_5$	1191	1152	1135	1058
$C_4H_9C_6H_5$	1290	1249	1232	1155
$C_5H_{11}C_6H_5$	1384	1341	1327	1251
$C_6H_{13}C_6H_5$	—	—	1421	1346
$C_7H_{15}C_6H_5$	—	—	1517	1447
n-Alk-1-ene				
$C_3H_7CHCH_2$	727	654	—	—
$C_4H_9CHCH_2$	834	757	—	—
$C_5H_{11}CHCH_2$	936	858	655	—
$C_6H_{13}CHCH_2$	1036	956	755	—
$C_7H_{15}CHCH_2$	1136	1054	856	—
$C_8H_{17}CHCH_2$	1235	1150	955	—
n-Alkylcyclohexane				
$C_2H_5C_6H_{11}$	1101	1006	826	—
$C_3H_7C_6H_{11}$	1192	1096	915	—
$C_4H_9C_6H_{11}$	1288	1190	1013	—
$C_5H_{11}C_6H_{11}$	1386	1286	1110	—

TABLE II

95% CONFIDENCE INTERVAL OF THE MEAN RETENTION INDICES

Column	Reference								
	n-Alkane	n-Alk-1-ene	1-Chloro-n-alkane	n-Aldehyde	n-Alkan-2-ol	n-Alkan-1-ol	1-Amino-alkane	n-Alkyl-benzene	n-Alkyl-cyclohexane
Squalane	3.2	1.7	3.5	3.1	3.7			3.2	4.1
DOS	2.2	2.6	2.0	2.6	2.4	3.2		3.1	4.1
Carbowax 20M	3.8	4.4	3.4	3.2	3.7	3.2	5.3	3.4	4.1
TCEP				4.9	7.1	5.8		8.2	

Table II gives the confidence intervals of the mean retention data. These values are high compared with what can be achieved in terms of reproducibility on high resolution capillary columns[4]. However, in our experience reproducibility is appreciably better only if data obtained on the same column are compared or in cases where the polarity difference between solutes and stationary phase is small. In the present study the polarity of the solutes ranged from the n-alkanes to the n-alkanols. In these cases one can get more precise data only by measuring the retention at equal concentrations[17] and not simply via the maximum of the elution peak, as is common practice.

The polar columns in particular show marked ageing effects[4]. Because the retention ratio of two successive homologues decreases as the polarity of the stationary phase increases, the precision of the retention indices on polar columns will be inherently smaller than on non-polar columns. The retention on polar columns is also more sensitive to small amounts of impurities[18]. The listed confidence intervals are fairly typical for retention indices determined for solutes covering a wide range of polarities on more than one packed column prepared from the stationary phase.

The precision depends also on the reference compounds used. The confidence interval of the classical Kovats' indices on TCEP was as high as 20 index units, whereas the n-alkan-1-ol indices on these columns could be reproduced within 6 index units. For squalane the opposite holds true. The n-aldehydes (reference compounds of a median polarity) show a fair precision on all columns investigated.

Table III compares the measured Kovats' indices with published data. The agreement for squalane is fair. For the more polar Carbowax 20M a systematic error, well beyond the limits of experimental error, is observed.

TABLE III

COMPARISON OF MEASURED WITH PUBLISHED KOVATS' RETENTION INDICES FOR n-ALKYLBENZENES AT 100°

Reference compound	Squalane				Carbowax 20M		
	Measured	Ref. 22	Ref. 4	Ref. 15	Measured	Ref. 22	Ref. 15
Benzene	647	650	649	649	956	947	971
Methylbenzene	757	760	757	757	1059	1043	1066
Ethylbenzene	850	850	847		1147	1127	
n-Propylbenzene	939	938	936		1227	1205	
n-Butylbenzene	1038	1037	1035		1327	1302	
n-Pentylbenzene	1133	1133	1134		1424	1394	
n-Hexylbenzene		1231			1519	1489	

Table IV shows the relationship between Kovats' indices and the other retention indices. The latter can be transformed into Kovats' indices by adding a constant factor, the functional constant FU. These factors and the standard deviation between the thus obtained and the measured Kovats' indices are shown in Table IV. Usually the standard deviation is smaller than the combined confidence intervals. The assumptions underlying eqns 2 and 3 hold within the experimental error. Only the slopes of the n-alkan-1-ols and the n-alkylbenzenes differ more than allowed for by the experimental uncertainties.

TABLE IV

RELATIONSHIP BETWEEN KOVATS' INDICES AND ALTERNATIVE RETENTION INDICES. THE FUNCTIONAL CONSTANT $FU_{y1,y2}$

a = functional constant $FU_{y1,y2}$; b = standard deviation between Kovats' indices calculated via the functional constant and determined directly;
c = combined confidences intervals of the means on a level of significance of 95%.

Column		Reference: 1	2							
		n-Alkane	n-Alk-1-ene	1-Chloro-n-alkane	n-Aldehyde	n-Alkan-2-ol	n-Alkan-1-ol	1-Amino-alkane	n-Alkyl-benzene	n-Alkyl-cyclohexane
Squalane	a	0	−319	−170	−253	−51			−247	−248
	b	0	4	1	1	3			12	10
	c	(4)	(4)	(6)	(6)	(6)			(6)	(6)
DOS	a	0	−299	−107	−153	96	−34		−181	−246
	b	0	5	5	5	2	8		10	7
	c	(3)	(4)	(4)	(4)	(4)	(5)		(5)	(6)
Carbowax 20M	a	0	−255	56	90	401	325	100	41	−197
	b	0	5	7	5	5	13	7	12	6
	c	(4)	(7)	(6)	(6)	(7)	(6)	(8)	(6)	(7)

Comparing the merits of the other retention index systems, it is evident that n-alkylbenzenes and n-alkylcyclohexanes are not very practical for low-molecular-weight substances. The lowest applicable members, ethylbenzene and ethylcyclohexane, have retention times at least as high as that of octane. For high-temperature chromatography, however, they might have advantages over homologues with a low-molecular-weight functional group. The n-alkanes are not to be recommended for highly polar phases, whereas the n-alkan-1-ols are unsuitable for non-polar phases. The n-alk-1-enes show similar behaviour to the n-alkanes. The first member of the n-alkan-2-ols showing a linear methylene increment is n-butan-2-ol. Ketones, which have been suggested as reference compounds[6,8], should show a similar behaviour.

Neither of the series is a practical reference for solutes with volatilities comparable to hexane or less. The 1-aminoalkanes are not to be recommended as references for an index system. The 1-chloro-n-alkanes may be used except for high polarity phases. The aldehydes give reproducible data on all phases. They are superior in this respect to the n-alkanes. Even propionaldehyde shows, with the exception of squalane, a constant methylene increment. The odour and the instability of aldehydes towards oxygen are not serious problems if aldehydes are kept in appropriate vials[7]. The data show that within a confidence interval of 2 index units the difference between even and odd members of the homologous series can be neglected[19].

Polarity values based on Kovats' indices and the aldehyde indices are compared in Table V. The aldehyde polarities do not increase as much as the n-alkane polarities. The aldehyde polarity of benzene decreases with increasing polarity of the stationary phase. The aldehydes make the effect of phase polarity on relative retention more obvious[16]. Their values for highly polar phases are more accurate than those based on n-alkanes.

TABLE V

POLARITY SCALE BASED ON KOVATS' (1) AND THE ALDEHYDE RETENTION INDICES (4)

$(1) = I^x_{alkane} - I^{squalane}_{alkane}$; $(4) = I^x_{aldehyde} - I^{squalane}_{aldehyde}$.

Probe (x)	Squalane		DOS		Carbowax 20M		TCEP	
	(1)	(4)	(1)	(4)	(1)	(4)	(1)	(4)
Benzene	0	0	74	−22	309	−30	491	−106
Ethanol	0	0	190	97	554	214	782	189
Butan-2-one	0	0	115	19	379	39	665	64
Nitromethane	0	0	232	135	711	366	988	462
Pyridine	0	0	123	24	492	148	747	222
Total	0	0	734	253	2445	737	3673	831

If aldehydes are not available or precluded from use for other reasons, one should quote retention data by referring to homologous which are similar to the solutes of interest. These data are on the whole proportional to Kovats' indices. However, in using the latter for polar stationary phases, one of the main advantages of retention indices is lost, namely reproducibility and insensitivity towards experimental parameters. This is especially important for analytical work.

Polarity scales using the index differences of certain probes on two phases have to be based on the same reference. If one employs a polarity scale based on the variance of a set of standard solutes, as has been proposed[18], one may compare retention indices referred to different reference systems. The variance is not effected by the choice of reference provided eqns. 2 and 3 hold. Attempts to analyse retention indices for the basics of the retention mechanism by means of factor analysis or pattern recognition[20,21] are also independent of the reference chosen.

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