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SENSITIVITY OF RETENTION INDEX TO VARIATIONS IN COLUMN LIQUID LOADING AND SAMPLE SIZE

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SUMMARY

The reliability of retention index values in gas–liquid chromatography has been investigated. The liquid phase loading and sample size were varied on non-polar *n*-octadecane and highly polar 3,3′-oxydipropionitrile columns. It was found that these parameters greatly influenced the Kováts retention index for ethyl methyl ketone and for diisopropyl ether on *n*-octadecane columns, the variation being as great as 300 units for the former and 140 units for the latter. For the 3,3′-oxydipropionitrile columns the variation of retention index with sample size was small for all solutes except ethanol, where it was significant. Variation of the column loading had large effects on the Kováts retention indices for all solutes. It was concluded that both column loading and sample size ought to be high in order to keep the variation in retention indices as small as possible.

INTRODUCTION

Many articles dealing with the retention of a substance relative to one or more reference solutes have appeared since the 1950s. Recent surveys have been published by Ettre[1–3] and Haken[4]. Published retention data obtained under apparently the same conditions often differ considerably, *e.g.*, results for the Kováts retention index for benzene on squalane columns at 373°K differ by about 60 units in different investigations[5,6]. These differences have often been considered to depend on errors in the measurements of variables such as carrier gas flow-rate, retention time or gas hold-up time. Impurities in the stationary phase and the use of different batches of the stationary phase have also been mentioned as causes of divergent retention index values.

However, little attention has been paid to differences in retention indices as a result of mixed retention mechanisms. Retention indices are influenced by sample size, liquid phase loading and type of support if the retention of either the investigated solutes or the reference compounds is not due entirely to bulk solution. Under these conditions, all correlations between increments in retention indices and structural differences in investigated solutes are dubious.

We have investigated polar solutes on non-polar columns and non-polar solutes on polar columns, where, according to Martin[7], Martire[8], Pecsok and Gump[9] and others, mixed retention mechanisms can be expected. Our aim was to estimate the importance of variations in sample size and liquid phase loading on the retention values. The Kováts retention index system[10], based on the retention of homologous *n*-alkanes, was used for the calculation of retention indices.

EXPERIMENTAL

All measurements were made with a high-precision gas chromatograph coupled on-line with an Alpha LSI-2 mini-computer described elsewhere[11–13].

n-Octadecane and 3,3'-oxydipropionitrile (ODPN) were used as stationary phases on Supasorb (40–60 mesh), acid-washed and treated with hexamethyldisilazane (BDH, Poole, Great Britain), as the support. V-shaped glass columns (1000 × 4 mm I.D.) containing *ca.* 4 g of packing were used. The temperature was 333.15 ± 0.03°K. Hydrogen was used as the carrier gas and methane was used for the determination of the void volume. The pressure drop across the column varied between 50 and 70 mmHg and the carrier gas flow-rate between 54 and 56 ml/min with the *n*-octadecane columns, and 60–85 mmHg and 56–58 ml/min with the ODPN columns. The number of injections for each solute was about 100–150 on the *n*-octadecane columns and 40–60 on the ODPN columns. The amount of packing material and the percentage of stationary phase were carefully measured during the preparation of the columns.

Vapour samples (containing methane) were automatically injected using the injection system described earlier[14]. The amount injected for each solute varied over 3–4 decades within the range $5 \cdot 10^{-5}$–$5 \cdot 10^{-10}$ mole.

Ethyl methyl ketone, diisopropyl ether, *n*-pentane and *n*-heptane were used as solutes on the *n*-octadecane columns, except that with a 5% loading, where *n*-hexane and *n*-octane were used instead of *n*-pentane and *n*-heptane. *n*-Nonane, *n*-undecane, benzene, toluene, trifluorobenzene, acetonitrile, ethanol, ethyl methyl ketone, di-*n*-butyl ether and 1-chlorobutane were used on the ODPN columns. The detector response was calibrated by injection of 2 μl of dilute solutions in carbon disulphide with a Hamilton syringe. Corrections for bleeding of the stationary phase during the experiments were made as described earlier[15]. The column loadings used are given in Table I.

TABLE I

COLUMN LOADINGS

Column	Stationary phase	Approx. loading (%)	Liquid volume, V_L (ml)
1	*n*-Octadecane	5	0.2020
2		10	0.4022
3		15	0.6491
4		20	0.8560
5		25	1.1874
6	3,3'-Oxydipropionitrile	10	0.3100
7		15	0.5082
8		20	0.6828
9		25	0.9936

RESULTS

Kováts retention indices (I) were calculated with the equation

$$I = 100 \left(n \cdot \frac{\log V_{R_x} - \log V_{R_z}}{\log V_{R_{z+n}} - \log V_{R_z}} + z \right) \tag{1}$$

where V_{R_x}, V_{R_z} and $V_{R_{z+n}}$ are the retention volumes of the substance x of interest and n-alkanes with z and $z+n$ carbon atoms, respectively.

The retention volume corresponding to the peak maximum was used for the retention index calculations as it can be measured with better precision than the centre of gravity or the median of skewed peaks.

Octadecane columns

The alkanes showed no significant variation in retention volume with sample size. Therefore, an average value of the retention volume for each alkane could be used in further calculations. The retention volume of ethyl methyl ketone varied widely and that of diisopropyl ether moderately with sample size. The ethyl methyl ketone peaks showed very bad tailing, which became worse as the size of the injected samples became smaller. The diisopropyl ether peaks also showed some tailing. Fig. 1 shows the variation in retention volume for diisopropyl ether on the 15% n-octadecane column.
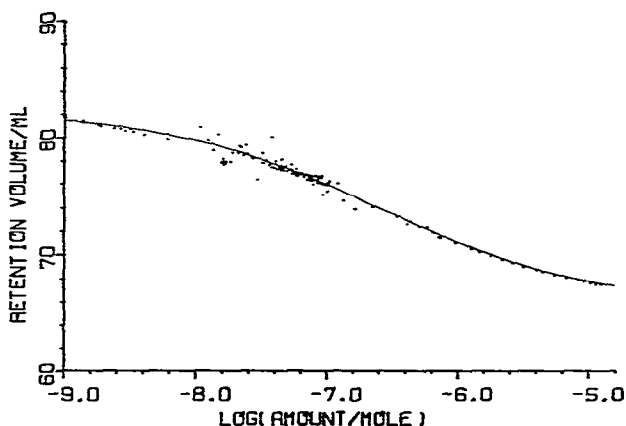

Fig. 1. Retention volume *versus* amount of sample for diisopropyl ether on 15% n-octadecane column.

The function

$$V_R = A + \frac{C}{\left(1 + B \cdot \sqrt{\dfrac{A_r}{V_R + V_g}} \right)^2} \tag{2}$$

has been fitted to the experimental points. A_r is the peak area, V_R the retention volume, V_g the void volume and A, B and C are constants.

Eqn. 2 was derived under the assumption that the retention volume is composed of contributions of bulk partition and adsorption at only one of the liquid interfaces. The first term, A, is equal to $K \cdot V_l$, where K is the partition coefficient, which, in the actual concentration range, can be considered to be constant, and V_l is the volume of the stationary phase. Thus, A can be interpreted as the contribution of bulk partition to the retention volume. The second term is derived from the Langmuir adsorption isotherm and expresses the influence of adsorption at different sample sizes. The constant C is the contribution from adsorption at infinite dilution $(A_r = 0)$.

Eqn. 2 was found empirically to give a good representation of the experimental results for ethyl methyl ketone and diisopropyl ether on all of the n-octadecane columns. The theoretical consideration leading to this equation is beyond the scope of this paper and will be published elsewhere[16].

The constants A, B and C can be determined by linear regression and simplex optimization[17]. The solid line in Fig. 1 was obtained with eqn. 2.

By inserting V_R from eqn. 2 into eqn. 1, the variation in Kováts retention index with sample size was calculated. Fig. 2 shows this variation for diisopropyl ether
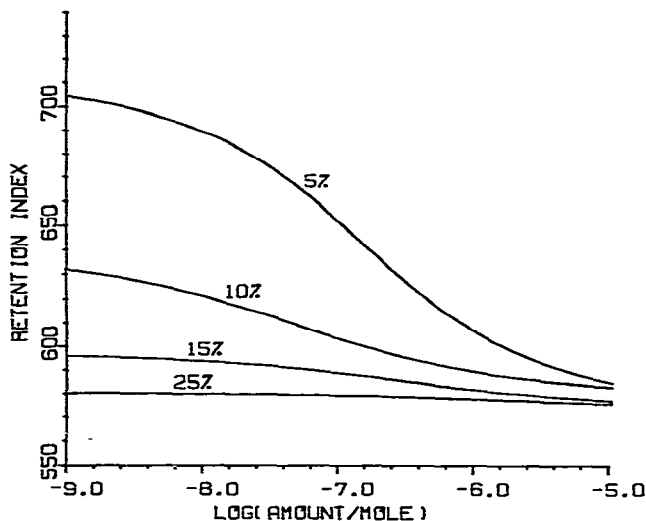

Fig. 2. Kováts retention index *versus* amount of sample for diisopropyl ether on n-octadecane columns.

and Fig. 3 for ethyl methyl ketone for the n-octadecane columns. It is apparent that the liquid phase loading strongly influences the Kováts retention index. The variation in retention index with stationary phase volume (V_L) and amounts of ethyl methyl ketone and diisopropyl ether injected is shown in Fig. 4. The upper curve for each solute corresponds to $A + C$ in eqn. 2 (infinite dilution) and the lower to A (large amounts injected). It is clear that the use of a high column loading and large amounts injected gives smaller variations in retention index. Hence the recommendations from several workers that samples as small as possible should be injected can be misleading[1,18].
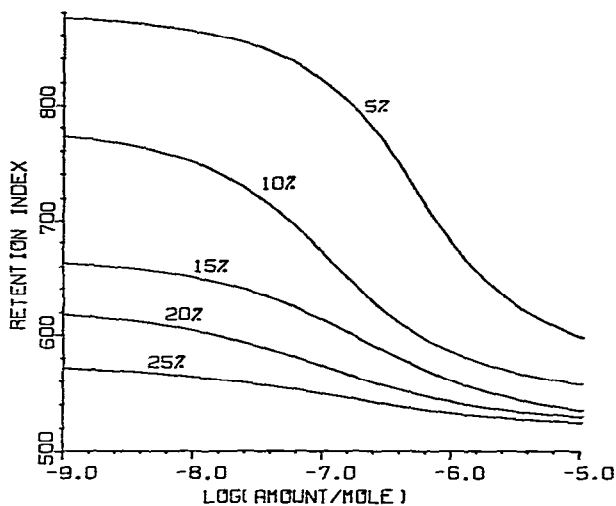
Fig. 3. Kováts retention index *versus* amount of sample for ethyl methyl ketone on *n*-octadecane columns.
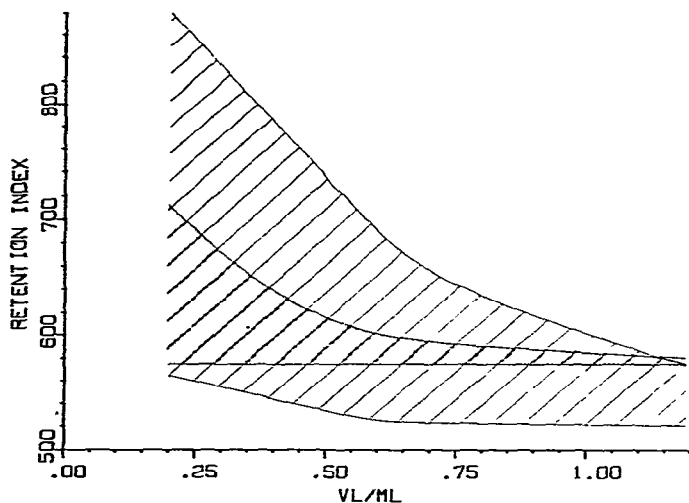

Fig. 4. Kováts retention index *versus* stationary liquid volume for diisopropyl ether (shaded) and ethyl methyl ketone (hatched) on *n*-octadecane columns.

Ethyl methyl ketone and ethyl acetate have been used as solutes in earlier investigations of complexation in binary stationary phase systems with a non-polar component (squalane) and a weakly polar component (dodecyl laurate or 1-chloro-octadecane)[19,20]. In these systems a variation in retention volume with sample size was also found.

3,3'-Oxydipropionitrile (ODPN) column
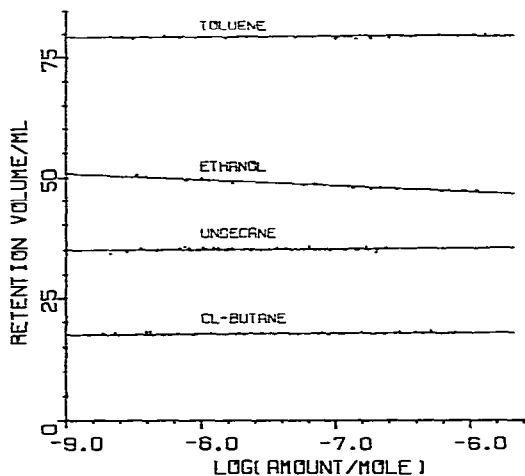   Fig. 5 shows retention volume as a function of amount injected for some

Fig. 5. Retention volume *versus* amount of sample for some solutes on 10% ODPN column.

solutes on an ODPN column with 10% (w/w) liquid phase loading. Similar plots were obtained on the other ODPN columns. Only for ethanol was the retention volume significantly influenced by the sample size.

When plots of retention volume *versus* liquid phase volume are made (Fig. 6), the regression lines for all solutes except the alkanes pass through the origin.The regression lines for the *n*-alkanes have significant positive intercepts. The retention volume for ethanol varies with sample size and therefore no definitive regression line can be drawn in this instance. This means that the adsorption effects for all solutes except the alkanes and to some extent ethanol are small on polar ODPN columns.
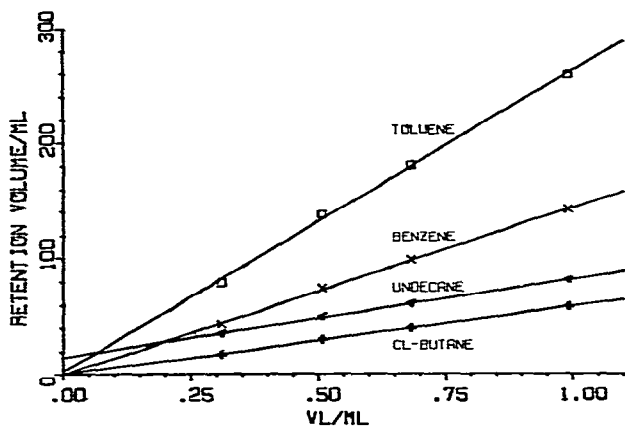

Fig. 6. Retention volume *versus* stationary liquid volume for some solutes on ODPN columns.

The contribution of adsorption to the retention volumes for *n*-nonane and *n*-undecane will greatly affect the Kováts retention index and the use of different liquid phase loadings leads to differences in retention index (see Fig. 7). The variations are smaller at high loadings. For ethanol, the variation in retention index with phase
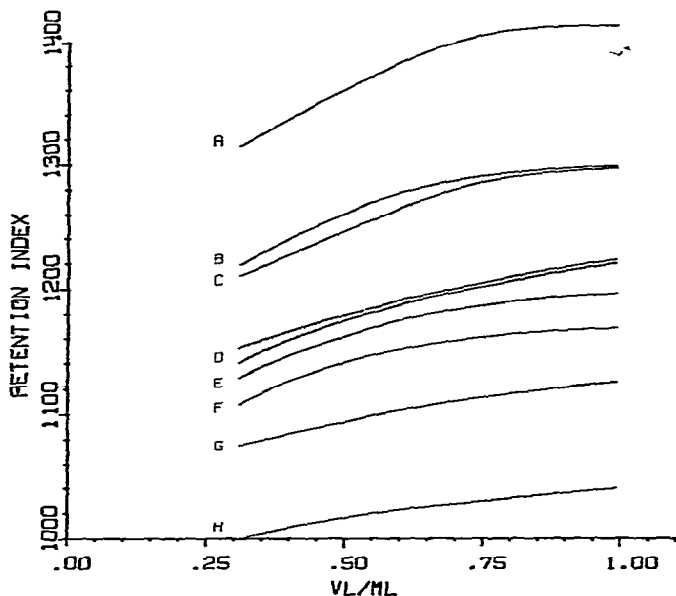
Fig. 7. Kováts retention index *versus* stationary liquid volume on ODPN columns. Solutes: A = acetonitrile; B = toluene; C = ethyl methyl ketone; D = ethanol; E = benzene; F = trifluorobenzene; G = di-*n*-butyl ether; H = 1-chlorobutane.

volume is described as an area between two curves. These curves give the limits of the variation of the retention index with amount injected, the upper curve corresponding to about $5 \cdot 10^{-9}$ mole and the lower curve to $5 \cdot 10^{-6}$ mole.

DISCUSSION

The results show that variations in Kováts retention indices, due to adsorption effects, are most pronounced on non-polar stationary phases with polar solutes. The Kováts retention index varies for polar solutes on non-polar columns because of adsorption contributions to the retention volume for the polar solutes. On polar columns, the variation in Kováts retention index is essentially due to adsorption effects for the alkane reference compounds.

For systems in which adsorption effects occur, it is obviously desirable to use conditions such that the adsorption is minimized and bulk partition is the main retention mechanism. For the *n*-octadecane system, eqn. 2 is in good agreement with the experimental points. The equation clearly shows that two measures can be taken in order to minimize the relative effect of the second term, which corresponds to adsorption effects. The first term can be increased by using a high liquid phase loading. The second term can be reduced by the injection of large samples, which means working in a range where the adsorption sites are saturated. Of course, the sample size must be small enough not to give appreciable effects on the partition constant due to changes in the bulk activity coefficients. In our experiments, an injection of about 10^{-6} mole per gram of stationary phase was found to be suitable. Figs. 2, 3 and 4 show clearly that it is advisable to use high liquid phase loadings and

large sample sizes. For the ODPN columns, the sample size is not important (Fig. 5) but here also high liquid phase loadings are desirable (Fig. 7).

It would be preferable if the same set of reference compounds could be used for several stationary phases. Instead of *n*-alkanes as reference compounds, homologous series of propyl ethers[21], methyl esters[22] and alkanols[23] have been suggested for polar stationary phases. Straight-chain aliphatic 2-ketones[24] have also been suggested as suitable for both polar and non-polar columns. Our experience suggests that *n*-alkanols are unsuitable on polar columns because of the variation in retention volume with amount injected and, for the same reason, 2-ketones are unsuitable on non-polar columns.

It would be interesting to try a set of alkylbenzenes as reference compounds, as they seem to behave almost ideally on several stationary phases of different polarities, *e.g.*, ODPN, alkyl ester[19], phthalate esters[25], haloalkanes[20] and alkanes such as squalane[19] and octadecane. Retention indices were calculated with eqns. 1 and 2 using benzene and toluene as reference compounds on the ODPN columns. For all solutes except the alkanes, the variation in retention index with stationary phase volume showed a 5–10-fold decrease compared with the Kováts retention indices. However, a variation of 5–10 retention index units still remains when the liquid phase loading is varied between 10 and 25% (w/w). These results again reflect the severe limitations of all retention index measurements. If the reference compounds and the other solutes investigated are not very similar with respect to functional groups, one can usually expect variations of all types of retention indices with liquid phase loading and with the amount injected.

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