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Reproducibility of Retention and Identification Data in Gas-Liquid Chromatography

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The elementary sorption processes taking place in a chromatographic column are discussed. The reasons for the lack of reproducibility of retention data in GLC are analysed. The basic requirements for a chromatographic apparatus capable of giving high-precision retention data are formulated, and conditions suitable for the measurement of retention quantities for identification are recommended. 243 references.

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I. INTRODUCTION

Gas chromatography was developed as a method of quantitative microanalysis of complex mixtures. In the early stages of its development the retention volume V_g was used[1-3] as the basic parameter for identifying the components of complex mixtures. However it was found that discrepancies in the measured values of V_g between different laboratories could be as high as 10–15%, and this led Adlard and coworkers[4] to recommend the use of the more reproducible Kovats retention indices for identification. Even this recommendation failed to solve the identification problem, because retention indices (I) measured in different laboratories were found to vary by 0.7 to 7.9 units, corresponding to an error of 1.6–12% in the measurement of retention volume. These discrepancies have focussed attention on the reasons for the unsatisfactory reproducibility of retention data obtained in different laboratories.

Careful studies of the sorption mechanism under GLC conditions have shown[5,6] that the retention data are affected not only by the gas–liquid separation coefficient K_l, but also by adsorption at interfaces and by the extent of curvature of the sorption isotherms: as a result, the retention data are a function of the amount of stationary phase deposited on the carrier (w_l) and of the sample size (q_S), i.e. of parameters which do not appear explicitly in the classical expression for calculating retention data. These conclusions raised doubts on the validity of retention data as a source of qualitative GLC information: in particular, it was stressed[7] that only in the most favourable circumstances can retention data be used to specify the order in which components will emerge from a column.

Yet the increasing precision of the measurement of retention data and the simplicity of the procedure are very attractive features of the use of these quantities for identification. The basic reasons for the lack of reproducibility of retention data measured in different laboratories have been investigated fairly fully, but the results have not yet been critically discussed in review articles, and therefore it is difficult at present to standardise methods of determining retention data †. This review aims to analyse the reasons underlying the lack of reproducibility of retention quantities, to assist the standardisation of experimental conditions, and to examine possible ways of presenting retention data for identification.

The general equation relating to net retention volume V_N to the parameters of the sorbent is[8]

$$V_N = \sum_i^k \frac{\partial V_N}{\partial V_i} V_i + \sum_j^m \frac{\partial V_N}{\partial S_j} S_j, \qquad (1)$$

where V_i is the volume of the i-th type (or state) of stationary phase, and S_j is the surface area of the j-th type of interface of the sorbent. Usually, with $w_l > 0{,}5\%$, the stationary phase on the surface of the solid carrier is present as a capillary liquid (index "c") or as an adsorbed layer (index "a")[9]. The following types of interface can be identified on the sorbent: solid carrier–stationary phase (index sl), capillary liquid–gas (index clg), and adsorbed liquid layer–gas (index alg). If all the distribution coefficients are constant Eqn.(1) can be written

$$V_N = K_{cl}V_{cl} + K_{al}V_{al} + K_{sl}S_{sl} + K_{clg}S_{clg} + K_{alg}S_{alg}, \qquad (2)$$

† The use of retention data for identification is discussed in the following recently published books: M.S.Vigdergauz, L.V.Semenchenko, V.A.Ezrets, and Yu.N.Bogoslovskii, "Kachestvennyi Gazokhromatograficheskii Analiz" (Qualitative Gas-chromatographic Analysis), Izd.Nauka, Moscow, 1978; Yu.N.Bogoslovskii, B.I.Anvaer, and M.S.Vigdergauz, "Spravochnik Khromatograficheskikh Velichin Uderzhivaniya" (Handbook of Chromatographic Retention Quantities), Izd.Standarty, Moscow, 1978; M.S.Vigdergauz, "Raschety v Gazovoi Khromatografii" (Calculations in Gas Chromatography), Izd.Khimiya, Moscow, 1978.

where K are the distribution coefficients of the sorbate between phases. With most of the sorbents commonly used in GLC ($w_l = 10-15\%$) over 90% of the stationary phase is in the form of a capillary liquid[9]: the contribution of the other four terms on the right-hand side of Eqn.(2) varies with the amount of stationary phase and with the nature of the carrier, and this is one of the main reasons for the lack of reproducibility of retention data between laboratories.

II. SOLUBILITY IN THE STATIONARY PHASE

When the stationary phase is first added to the carrier an adsorbed layer of liquid is formed: in the case of white diatomaceous carriers the formation of this layer is complete at $w_l = 0.3\%$, as shown by the existence of an optimum amount of modifier on the surface of the carrier[10-12]. Further additions of liquid phase produce two simultaneous effects: an increase in the thickness of the adsorbed layer, and the filling of the pores of the carrier by the "capillary liquid". The properties of the adsorbed layer of liquid are affected by the presence of the carrier surface, and they differ from those of the pure (or of the capillary) liquid, as shown by a decrease in the vapour pressure of the liquid[13,14]. There are reports[15] of differences in properties between the capillary and the pure liquid, though the theoretical analysis[16] suggests that substantial differences can be expected only for high-molecular stationary phases. The phase equilibrium constants for the pure and the capillary liquid (sorbates and sorbents of different polarity) have been found to be the same within experimental error (~1%).[4,17-20].

The effect of the carrier surface on the properties of the adsorbed liquid layer is discussed in Refs.21–26. It is found[27] that 5 monolayers of water and 10 monolayers of nitrogen are adsorbed on titanium dioxide. The effect of the solid surface on the properties of the adsorbed liquid can be detected at distances of up to 100 Å.[24] The upper temperature limit for the use of an adsorbed layer of stationary phase is found[28-31] to be higher in capillary columns than in packed columns. The thermodynamic properties of adsorbed and capillary liquids were found to be different[32,33], and an increase in density of a liquid in the adsorbed layer has been demonstrated by direct measurements[34]. Detailed studies[35-41] of the properties of layers of stationary phase adsorbed on diatomaceous carriers, silica gel, and graphite have established that the melting point of the compound increases by 4– K upon adsorption.

The retention volume (referred to the stability in the stationary phase, V_{Nl}) can be expressed as

$$V_{Nl} = K_{al}V_{al} + K_{cl}V_{cl};$$

and therefore, since $V_l = V_{al} + V_{cl}$, we can write

$$V_{Nl} = K_{cl}(V_l - V_{al}) + K_{al}V_{al}.$$

If the measurement is carried out at a sufficiently large value of w_l (such that only V_{Cl} is affected in changes in w_l) we can extrapolate the plot of V_{Nl} against V_l to $V_l = 0$ and obtain the extrapolated value V_N^{ext}:

$$V_N^{ext} = V_{al}(K_{al} - K_{cl}).$$

Evidently, $V_N^{ext} = 0$ for $K_{al} = K_{cl}$. It has been found experimentally[25] that $V_N^{ext} < 0$, i.e. $K_{cl} > K_{al}$.

Let us examine the case of a sorbate whose solubility depends on its concentration in solution. Liquid solutions usually have isotherms of the anti-Langmuir type[42-44], the only exceptions being solutions in which the activity coefficient is much less than unity[45]. The concentration dependence of the activity coefficient γ for non-electrolyte solutions is given by the Margules equation[46]

$$\ln\gamma = (1-x^2)\ln\gamma^{\infty},$$

where γ^{∞} is the activity coefficient of the solution at infinite dilution, and x is the mole fraction of solute. We can also use the Krichevskii equation[47], which is expressed in terms of the distribution coefficients $\tilde{K} = p/x$:

$$RT\ln\tilde{K} = RT\ln\tilde{K}^{\infty} - A_1x(2-x),$$

where A_1 is the concentration solubility coefficient and p is the vapour pressure in Torr. Replacing the parameter $\tilde{K}$ by the chromatographic distribution coefficient K_l, which characterises the ratio of the bulk concentrations, we obtain the equation

$$RT\ln K_l = RT\ln K_l^{\infty} + A_2x(2-x),$$

from which it follows that K_l increases with the concentration of the solution. A_2 is also a concentration coefficient. The concentration coefficients increase with the difference in polarity between the stationary phase and the sorbate[48]. It has been shown[20,49] that K_l varies with the concentration of the solution even if the solution obeys Henry's law: concentration-independent K_l values are found only in some solutions showing negative deviations from Raoult's law.

Thus, with a few exceptions, K_l is a function of the concentration of the solution, and therefore V_N also varies accordingly. Concentration effects are particularly strong in the chromatography of polar substances on non-polar stationary phases, as shown also by the molar heats of dissolution[20,50].

Differences in properties between the adsorbed and the capillary liquid are most conveniently revealed by comparing V_N values measured in capillary and in packed column (note that the adsorbed layer of liquid is more substantial in a capillary column than in a packed column). This type of comparison has shown[51-53] that the Kovats indices for hydrocarbons, their chloro-substituted derivatives, ketones, esters, alcohols, nitro compounds, and nitriles in squalane are identical for the two types of column, though the reproducibility of the retention data is unsatisfactory in the case of polar sorbates[52]. When polar stationary phases are used some differences between the retention data in capillary and in packed columns are observed[53].

III. THE SOLID CARRIER–STATIONARY PHASE INTERFACE

Though inertness towards adsorption is one of the main requirements for a chromatographic carrier, this requirement is never fully satisfied because all surfaces have an adsorption potential. Quantitative allowance for the effect of the carrier on the retention data was first made by Keller and Stewart[54], and the effect is discussed in detail in Refs.55–57. If adsorption at the *sl* interface is predominant the parameter V_N is given by the equation[58]

$$V_N/V_l = K_l\frac{K_{sl}S_{sl}}{V_l}. \qquad (3)$$

Extrapolation of plots of V_N/V_l against $1/V_l$ to $1/V_l = 0$ gives the constant K_l and the contribution of interfacial adsorption to V_N. A reference point method had been proposed[8] for calculating the adsorption contribution to V_N, but its precision is not high[59].

The effect of adsorption on V_N is especially marked when non-polar stationary phases are used to separate polar sorbates, and in this case it is very important to choose the carrier correctly. White and pink diatomaceous carriers have

different adsorption activities. Replacement of a pink carrier by a white carrier was found[60] to lower the adsorption contribution to V_N 2.5–3-fold. The lowest adsorption activity towards polar compounds is found in silanised white diatomites, which yield the most inert carriers (HP type) after heat treatment. A good example of such a carrier is Chromation N Super. The adsorption activity of diatomaceous carriers decreases in the following sequence of sorbates: amines > n-alcohols > iso-alcohols > carbonyl compounds > nitriles > nitro compounds > ethers > chloro-hyrocarbons > aromatic hydrocarbons > olefins > saturated hydrocarbons.

V_N for polar sorbates in non-polar sorbents is significantly affected by the nature of the carrier surface, and therefore the reproducibility of retention data in these systems can be problematic, since neither adsorbents nor carriers have fully reproducible properties. Evans and Smith[61] prepared a squalane and dinonyl phthalate sorbent with three different batches of silanised Chromosorb G: the retention indices for polar sorbates on these sorbents failed to show any differences arising from the different adsorption activities of the solid carriers. The effect of differences in the carrier on the retention data has been studied also by other workers[62–67].

Teflon has been recommended[68–70] as the ideal inert carrier, with the smallest possible adsorption effects: however, later work[61,71,72] revealed a high adsorption activity in Teflon towards low-polarity compounds, and Conder[73] showed that with the optimum amount (8%) of stationary phase on this carrier the contribution from adsorption to V_N is significant for all classes of sorbate.

Diatomaceous carriers have been found[74] to have a smaller contribution from adsorption to V_N than glass micro-spheres coated with n-octadecane.

Because of the adsorption effects at the *sl* interface fixed values of w_l must be used to ensure reproducible retention data from different laboratories. However the poor reproducibility of retention data for diatomaceous carriers arises from the curvature of the adsorption isotherm[54] as well as from adsorption. A typical manifestation of this effect is the "tailing" of peaks, and also the observed dependence of V_N on the size of the sample[75–78]. Obviously, this dependence may completely invalidate V_N as a source of information for qualitative analysis, and therefore the achievement of reproducible retention data in non-linear GLC has become a very important problem.

The effect of non-linearity in the adsorption isotherm for the *sl* interface is most marked in the separation of polar sorbates on non-polar or weakly polar stationary phases. In this case the molecules of the stationary phase are competing with those of the sorbate for adsorption on the carrier surface. Since diatomaceous carriers are polar, the molecules of the polar sorbate (in spite of their lower molecular weight) can penetrate close to the carrier surface and be adsorbed, the inhomogeneity of the carrier surface resulting in a non-linear adsorption isotherm[79–82]. When a polar stationary phase is used the sorbate molecules cannot penetrate the layer of adsorbed stationary phase, and the adsorption isotherm of the sorbate molecules on the energetically uniform adsorbed layer of stationary phase is approximately linear. With other (non-diatomaceous) types of carrier the general behaviour is very similar. Thus the adsorption isotherm of alcohols on Teflon coated with squalane is found to be curved[75].

The strong effect of sample size on V_N in polar sorbate–non-polar stationary phase systems can be ascribed to the curvature of the isotherm describing the adsorption at the *sl* interface (since the effect disappears when polar stationary phases are used). This prompted a search for methods of modifying the carrier surface to make it homogeneous towards interfacial adsorption. The first attempts were made in the early days of GLC [1,83] by adding a polar modifier (a fatty acid) to a weakly polar stationary phase used to separate a mixture of fatty acids. However the modifier did not prove to be universally effective. More recent attempts at modifying the carrier surface include the use of an inert polymer[84], silver[85], and copper[86], but even these were not fully successful in screening the active surface of the carrier.

The first successful results were obtained by chemically modifying the surface of the carrier (silanation[87,88] and polymerisation on the carrier[10,12,89]). Convincing results were obtained also by physical modification of the carrier with polar substances[31,90–92]. It was shown that not all polar liquids are suitable for physical modification: for example, poly(vinyl alcohol) is less successful than diglycerol as a modifier[11], and polyglycols (of different molecular weight) are now the most commonly used physical modifiers. When the carrier surface is coated with a thin layer of physical modifier the polar sorbate molecules are unable to reach the carrier surface, and adsorption is confined to the interface between the adsorbed layer of modifier and the capillary liquid.

"Self-modification" of the carrier surface under the influence of the polar sorbate can be important in the separation of polar sorbates on non-polar sorbents. In this case the sorbate peak has a highly extended trailing edge, and the carrier surface remains exposed to the modifying effect of the polar component (which influences its sorption properties) until that component has been completely eluted from the column. Therefore if the next sample is added to the column before complete elution of the previous polar sample grossly erroneous retention data may be obtained: thus in the case of ethyl methyl ketone on a squalane column the discrepancy can be as high as 50% in the retention volume and 1 kcal mole^{-1} in the relative molar heat of dissolution. Hence the retention data of these systems should be measured only by injecting pure compounds into the column, and the possibility of mutual effects of the sorbates on the individual retention volumes should be borne in mind when mixtures of polar substances are analysed.

Chemical modification by grafted polymers offers a useful increase in the maximum working temperature of sorbents. Thus sorbents modified with grafted Carbowax 20 M have been used (in conjunction with a flame-ionisation detector) at temperatures up to ~260 °C, i.e. well above the maximum working temperature of Carbowax 20 M.

The effect of adsorption at the *sl* interface on the retention indices can be observed also in capillary columns[93]. Thus it is found that the effect of the surface of a stainless steel capillary on the adsorption of the substances to be separated depends on the method of production of the capillary and on the treatment applied to its surface before deposition of the stationary phase: for example, thorough washing of the capillary surface with various solvents can increase its adsorption properties very markedly[94], though the optimum amount of solvent has to be used for the greatest effect. For this reason workers have turned their attention to glass capillaries[95], but the adsorption activity of glass was found to depend on the presence of sodium, boron, and hydroxyl groups[96,97]. The presence of OH groups causes irreversible adsorption of several polar compounds as well as introducing curvature into the adsorption isotherm[10,96,98–101]. Silanation[102,103], etching with hydrogen fluoride, and physical or chemical modification have been used to lower the adsorption activity of the surface of glass capillaries. One of the best methods is the glass surface deactivation procedure described in Refs. 10 and 12 and applied to capillary columns[104,105] in conjunction with etching with hydrogen fluoride[106]. After

being etched the capillary is coated with Carbowax 20 M, its ends are sealed, and the column is heated in a nitrogen atmosphere for 24 h at 280 °C. The physisorbed Carbowax 20 M is then washed out of the column with dichloromethane and methanol. However there is at least one report[102] of unsatisfactory reproducibility of the retention data after this treatment.

The extent of adsorption on the *sl* interface of capillary columns depends also on the purity of the stationary phase. For example it was shown[104] that "tailing" of the peaks of polar components occurs with SF-96 silicone oil but not with a high-purity grade of silicone oil (OV-101).

Thus the results discussed in this Section lead to the following recommendations for improving the reproducibility of retention data: a) only glass columns modified by Carbowax 20 M (or silanised) should be used in capillary chromatography; b) in packed columns, high-grade carriers of the Chromaton N Super type should be used, and non-silanised carriers should be chemically modified with Carbowax 20 M (amines and alkalis for the separation of amines, phosphoric acid for the analysis of acids); c) stationary phases of the highest possible purity should be used. These precautions should minimise the effects of adsorption at the *sl* interface (especially those associated with the curvature of the adsorption isotherm) on the effective value of V_N.

IV. ADSORPTION AT THE LIQUID-GAS INTERFACE

Adsorption of the compounds to be separated at the free surface of the stationary phase is possible[107]. A combination of static and chromatographic experiments[108,109] has shown that the value of V_N for the benzene–thiodipropionitrile and cyclohexane–thiodipropionitrile systems is significantly affected by adsorption at the *gl* interface.

Since the surface tension of non-polar stationary phases is relatively low we cannot expect large adsorption effects at the *gl* interface with non-polar or weakly polar stationary phases. Thus effects of adsorption at the *gl* interface on V_N were not detected in studies[110] of the retention of isooctane, cyclohexane, benzene, toluene, acetone, ethyl methyl ketone, and methanol on squalane and tricresyl phosphate: this result is consistent with other similar work[60,111,112]. However some workers consider[113–116] that V_N is affected by adsorption at the *gl* interface for polar sorbates in non-polar stationary phases, though their conclusions seem to be based on an incorrect treatment of the experimental results. Thus the change in $\ln\gamma$ brought about by an increase in the mole fraction of sorbate is treated[115] as a manifestation of interfacial adsorption, in spite of the anti-Langmuir shape of the adsorption isotherm (which suggests a concentration effect). Similarly erroneous conclusions are drawn from measurements[116] of sorption in dinonyl phthalate at high solution concentrations.

Polar stationary phases have a high surface tension, and adsorption at the *gl* interface is very marked on sorbents coated with stationary phases of this type, especially in the separation of hydrocarbons. For example, the adsorption contribution to V_N in the separation of hydrocarbons with thiodipropionitrile varies from 10 to 70%.[60] Similar effects were observed[117] for poly(ethylene glycol succinate).

A detailed study[118] of interfacial adsorption on a Carbowax 20 M column was based on the equation

$$V_g = V_g^{\infty} + k_1/w_l + k_2(1/w_l - 1/10);$$

where k_1 and k_2 are constants describing the contribution of adsorption at the *gl* and *sl* interfaces respectively. The coefficient 1/10 is introduced to allow for the authors' assumption[118] that when $w_l \geq 10\%$ adsorption at the *sl* interface is negligible.

The results of that study[118] lead to the following conclusions: a) the parameter k_1 increases along a homologous series, i.e. the contribution of adsorpton to V_N increases with the length of the alkyl radical, and it is negligibly small in the case of cyclic molecules; b) the k_2 values for isomeric ketones differ by ~10–15%, and this can affect the selectivity of stationary phases towards ketones (as compared to the solubility of ketones in the pure stationary phase).

The dependence of adsorption at the surface of the stationary phase upon the number of methylene groups in the alkyl radical of the sorbate can be expressed at[119]

$$K_{gl}^{p}/K_{gl}^{x} \approx (K_{gl})^{\Delta C},$$

where ΔC is the difference between the numbers of carbon atoms in the molecules of two compounds with the same retention volume, one of which is a n-paraffin (superscript *p*); the subscript *gl* denotes the gas-liquid interface.

Since the effect of adsorption at the surface of the stationary phase on V_N is usually smaller than the effect of adsorption at the *sl* interface it is useful to distinguish the systems in which the contribution to V_N of interfacial adsorption on the stationary phase surface is less than 1% by using the criterion[120]

$$K_{gl}/K_l < V_l/100S_{gl}.$$

As a rule the contribution to V_N of adsorption at the surface of the stationary phase can be ignored in systems with $\gamma < 5$, i.e. in the chromatography of substances of comparable polarity.

Liquid surfaces are energetically far more uniform than solid surfaces: for this reason the curvature of the adsorption isotherm is detectable only after completion of the first monolayer in the case of adsorption at the *gl* interface. Indeed, effects arising from non-linearity of the adsorption isotherm are observed[48] only at very low vapour pressures (~10^{-3} mmHg).

In GLC the stationary phase can be in two different energy states on the carrier, as was pointed out above. Hence it is correct to consider two types of adsorption at the stationary phase–gas interface: capillary liquid–gas adsorption and adsorbed liquid–gas adsorption. The properties of the two liquid surfaces are different, and as w_l is increased the area of the adsorbed liquid layer decreases while the area of the capillary liquid correspondingly increases. These differences can give rise to discrepancies between the retention data for polar stationary phases obtained with capillary and with packed columns.

V. EFFECT OF CHANGES IN EXPERIMENTAL CONDITIONS ON THE RETENTION VOLUME

Allowing for all possible sorption mechanisms we can write the following equation for real GLC situations (i.e. assuming normal analytical values of w_l):

$$V_N = \int_0^{V_{cl}} \left(\frac{\partial c_{cl}}{\partial c_g}\right) \mathrm{d}V_{cl} + \int_0^{V_{al}} \left(\frac{\partial c_{al}}{\partial c_g}\right) \mathrm{d}V_{al} + \\ + \int_0^{S_{sl}} \left(\frac{\partial q_{sl}}{\partial c_g'}\right) \mathrm{d}S_{sl} + \int_0^{S_{alg}} \left(\frac{\partial q_{alg}}{\partial c_g'}\right) \mathrm{d}S_{alg} + \int_0^{S_{clg}} \left(\frac{\partial q_{clg}}{\partial c_g'}\right) \mathrm{d}S_{clg},$$

where c_g is the volume concentration of sorbate in the gas phase, c_g' is the weight concentration of sorbate in the gas

phase (g ml^{-1}), c_l is the volume concentration of sorbate in the liquid, and q is the amount of adsorbed material (g cm^{-2}).

To a first approximation the terms of this equation can be evaluated separately as follows.

1) The distribution coefficient K_{cl} is the parameter which most directly determines the value of V_N: the smaller the activity coefficient, the greater is the contribution of solubility in the capillary liquid to V_N. If the system contains an average amount of stationary phase, and $\gamma \leq 1$, we can ignore adsorption effects on the retention volume (to a precision of 1%). This behaviour is observed in non-popar and weakly polar systems.

2) The distribution coefficient K_{al} has a substantial effect on V_N for low values of w_l. The contribution of this term to the relative retention quantities is small for $w_l > 10\%$.

3) The distribution coefficient K_{sl} can have a powerful influence on the retention, especially in systems consisting of a non-polar or weakly polar stationary phase and a polar sorbate. The numerical value of this distribution coefficient is significantly influenced by the size of the sample.

4) The distribution coefficients K_{alg} and K_{clg} can affect V_N when the stationary phase is polar and the sorbate is weakly polar or non-polar. These effects can be ignored (to a precision of 1%) for $\gamma < 5$ and normal values of w_l.

Since in a real chromatographic system the relative contributions of these sorption mechanisms depend on the experimental conditions it is interesting to consider the way in which the basic solubility characteristic (V_g) varies as w_l increases. As the stationary phase is first added to the carrier a film of adsorbed liquid is formed on the solid surface, but further additions of stationary phase cause a shrinkage of the *gl* interface due to the filling of capillaries and pores. The increased amount of capillary liquid on the carrier lowers the adsorption contributions to V_N: the combined result of all these effects is to lower V_g to ~10–15% as stationary phase is added to the carrier. Still further additions of stationary phase to the sorbent has practically no effect on the surface area of the *gl* interface, and the amount of adsorbed liquid also remains constant. Only the amount of capillary liquid on the carrier increases. Since the components of the mixture are more soluble in the capillary liquid than in the adsorbed layer, a small (~3–8%) increase in V_g is observed[25,121] as w_l increases beyond 15%, but V_g becomes independent of w_l when $w_l > 30\%$. As a result, reproducible inter-laboratory comparisons are obtained only with sorbents for which $w_l \cong 12$ to 15% (i.e. sorbents for which the dependence of V_g on w_l is a minimum).

There are various recommendations[18,122] on methods for obtaining invariant qualitative retention data by extrapolating plots of V_N/w_l against $1/w_l$ to $1/w_l = 0$ [see Eqn.(3)], and the K_l values obtained by this method are affected only by the solubility in the capillary liquid. However the method is laborious and the measurement of w_l has a low precision, and therefore the results are of doubtful value. Furthermore the non-linear dependence of V_g on w_l at fairly high values of w_l limits the validity of extrapolations from experimental data. However if we wish to identify the components of a complex mixture we are unlikely to achieve satisfactory separation over a wide range of w_l values. Therefore it is preferable to adopt a fixed value of w_l (~12–15%) in order to achieve reproducible measurements of retention data.

1. Size of Sample

The effect of sample size on V_N is usually caused by the non-linearity of the adsorption isotherm. The concentration of sorbate at the maximum of the chromatographic band c_g (in the gas phase) falls as the band travels along the column [Eqn.(49)]. The parameters of this equation are the effective diffusion coefficient D, the column length L, the velocity of the carrier gas u, and the sorption coefficient per unit length of column K' [123]:

$$c_g = \frac{q_s}{3.54K'\sqrt{DL/u}}. \qquad (4)$$

The steepest fall of c_g occurs near the inlet of the chromatographic column, where deviations from Henry's law can be expected; at large enough values of L the retention is affected more markedly by the curvature of the isotherm which describes the adsorption at the interface. Allowing for both dissolution and adsorption gives a complex picture of the dependence of V_g on L: as the column length is increased V_g initially decreases, before going through a shallow minimum. This minimum occurs at a column length of ~1 m, and therefore it is desirable to use columns with $L \geq 1$ m when the aim is identification of the components[48].

For a constant column length the variation of V_N with q_s also goes through an extremum, with a much sharper minimum in the region of mutual compensation of the dissolution and the adsorption isotherm, which creates an apparent independence of V_N on q_s.[48] This region can be very narrow (as in the alcohol–squalane system) or relatively broad (five orders of magnitude of q_s for the benzene–squalane system). For practical reasons it is preferable to measure retention data in the region of the minimum of the V_N–q_s relationship.

However in a real GLC apparatus it may be impossible (for a number of reasons) to measure retention data in the region of the minimum. This is the case, for example, in the analysis of trace impurities (especially, in the atmosphere) when the size of the sample is insufficient to bring the measurement to the region of the minimum and the retention volume is affected by the curvature of the adsorption isotherm. In these circumstances we can resort to one of the following procedures: a) measure V_N at a fixed value of q_s or at a fixed peak height h, or b) extrapolate V_N to infinite dilution of the solution. The latter variant implies adsorption and dissolution in the Henry's law region: it is very attractive, but in the majority of non-linear systems it has to be operated at the limit of sensitivity of the detector, with signals not much above noise level, and therefore it may be impossible in practice to work with sufficiently low sorbate vapour pressures. However for theoretical work the second variant is undoubtedly very important.

The first variant is by far the more important in practical work. In order to use it we must know the dependence of V_N on q_s or on h: the V_N values for fixed values of h or q_s are found by interpolation from experimental data. Retention data obtained at a fixed value of q_s lack physicochemical meaning, because (at different column temperatures) they correspond to different vapour pressures of sorbate and different degrees of column loading (or solution concentration), i.e. these data are neither isobaric nor isosteric[58]. Values referred to a fixed value of h are more useful, because they correspond to isobaric experimental conditions[45].

Various ways of obtaining invariant retention data in non-linear GLC have been proposed. One method[74] involves extrapolation of the concentration-dependent part of the dependence of V_N on q_s to infinite dilution. Thus it was shown experimentally[74] that the V_g values for n-propanol and diethylamine in heptadecane and dioctyl ether are identical for 10 and 15% concentrations of the stationary phase on the carrier when referred to infinitely dilute solutions. Other workers[124] allowed for adsorption effects by correcting the retention data extrapolated to infinite dilution for the amount of stationary phase in the column, as recommended in Ref.45.

Though the data were processed on a computer, there is some uncertainty in the extrapolation of the non-linear relationships, leading to an error of at least 1–1.5%. The method is laborious, and it gives a low precision in the measurement of w_l.

The measurement of retention data under isosteric conditions is described in Ref.45: the value of h at which V_N is measured is chosen by using the expression

$$h_j/h_0 = V_{Nj}/V_{N0} = V_{lj}/V_{l0}. \quad (5)$$

The measurements are made by using a set of columns [from zero to j, see subscripts in Eqn.(5)] containing different amounts of stationary phase. Since fairly high concentrations of solution are permitted, a significant proportion of the sorbate may be in the gas phase. We allow for this effect by introducing the concentration of sorbate in the gas phase (y) and the Martin compressibility factor (J). Isosteric solubility data are obtained by extrapolating the plot of $V_N/V_l(1-Jy)$ against $1/V_l$ to $1/V_l = 0$. The value of V_N at the given value of h is obtained from the trailing, diffuse edge of the peak, but this procedure causes substantial errors arising from the diffusion processes in the column. On the whole the method can be very useful in physicochemical research but it is difficult to exploit in practical analytical GLC.

Reproducible retention data in the adsorption region of the dependence of V_N on q_S (i.e. for sample sizes more typical of analytical GLC) can be obtained by using the equation[125,126]

$$V_N = A_3/\lg h + B, \quad (6)$$

where A_3 and B are constant. The region over which this equation is valid is limited to 2–5 orders of magnitude of h, but within this region we can evaluate V_N at any value of h by interpolation of plots of V_N against $1/\lg h$. Eqn.(6) was derived on the assumption that the interfacial adsorption in GLC (which makes the greatest contribution to adsorption at the *sl* boundary) is described by the Temkin isotherm equation: its validity has been demonstrated with a large number of systems, including a polar sorbate in a non-polar (or weakly polar) stationary phase.

Eqn.(6) can be used to obtain isobaric or isosteric retention data under given column operating conditions. For example, isobaric data could be obtained for $1/\lg h = 0.3$ (flame-ionisation detector; full-scale deflection 10^{-11} A; all values of h recalculated for this sensitivity and expressed in mm; specified column length, amount of stationary phase on the carrier, and velocity of carrier gas). In this way the experimental conditions can be standardised sufficiently to give reproducible retention data in different laboratories for non-linear GLC.

Isosteric retention quantities are often useful for calculating thermodynamic values: these can be calculated by inserting into Eqn.(5) the V_N values for given values of h obtained by interpolation from Eqn.(6).

2. Time-changes of the Sorption Properties of Columns

At high column temperatures the low-molecular fractions of the stationary phase can evaporate from the first parts of the column[21,127–130], causing a change in the selectivity of the column with time. Data on the separation of alcohols on a poly(ethylene glycol) column have shown[21] that the slope of the $\ln V_g - 1/T$ plot is not affected by evaporation of the stationary phase from the first part of the column, though the straight lines are shifted along the ordinate axis: this suggests a constant molar heat of sorption and a varying molar entropy of sorption, which is typical of an adsorbed liquid layer[48]. Thus the adsorbed layer has a greater temperature stability than the capillary layer, and initially only the capillary liquid evaporates from the first part of the column while the adsorbed layer remains on the carrier. The evaporation of some of the stationary phase from the first part of the column causes a local increase in concentration of the solution and a significant influence of interfacial adsorption on the retention data, which increases the dependence of V_N on q_S. This phenomenon sets a limit to the range of column temperatures consistent with reproducible retention results: the highest temperature at which a stationary phase is used should be 30–60 K below the usually recommended value. The use of large samples can also contribute to the loss of stationary phase from the first part of the column[130].

The time-changes in the properties of the column due to evaporation of the stationary phase are relatively slight. Far more serious obstacles to the acquisition of reproducible retention data arise from changes in the chemical composition of the stationary phase. Thus prolonged heating of squalane at 80 °C is found[131] to increase the concentration of lower hydrocarons from 6.1 to 14.7%: this effect is accompanied by changes in the relative retention volume of n-pentane, n-hexane, and benzene by 1.0, 2.5, and 1.5% respectively.

The most serious problems in the acheivement of reproducible retention data arise from the formation of polar compounds, which modify the surface of the carrier. These reactions usually occur at high temperatures in the presence of traces of oxygen (introduced by the carrier gas). Thus the passage of oxygen through a squalane column at 100 °C has been shown[132] to lower the retention index of polar compounds by more than 40 units, and similar effects have been observed in columns containing octadecanol. This behaviour is consistent with the formation of polar compounds as a result of the oxidation of squalane. The retention of polar (or readily polarisable) compounds is very sensitive to the concentration of impurities belonging to other classes in the squalane[133]: for example, in the presence of 0.1% of squalene the Kovats index for aromatic hydrocarbons is altered by 0.15 units.

Many commercial stationary phases consist of a mixture of polymers of different molecular weight (for example, polyglycols). The influence of molecular weight distribution on the selectivity of the stationary phase has been studied[134,135] in the poly(ethylene glycol) system (PEG). A comparison of three different PEG of average molecular weight 400 but prepared from individual PEGs taken in different proportions revealed an arithmetic mean deviation of the Kovats indices on these sorbents varying from 1.4 to 4 units. Alcohols are especially sensitive to changes in composition of the PEG, because of the change in the number of free hydroxyl groups in the stationary phase. However after prolonged operation at the highest permissible working temperatures, which cause partial evaporation of the lower fractions from the column, the spread of retention indices of PEG columns operated with different sorbents was only 0.4–0.8 units (i.e. of the same order as the experimental error).

The selectivity of the stationary phase is affected not only by the number of polar (active) functional end-groups per unit volume, but also by the molecular weight. The latter effect is observed also in stationary phases of the paraffin type, which do not contain functional groups[136–138]. It is stressed[136] that the polarity of the paraffin stationary phase increases with its molecular weight. These observations underline the importance of careful purification of the stationary phase when reproducible retention data are required, and the disadvantages of using stationary phases consisting of mixtures of polymers.

The water content of the sorbent can also influence the retention of polar compounds on non-polar stationary phases[81], and also of all classes of sorbate on polar stationary phases[134]. When non-polar stationary phases are used the *sl* interface is modified by the presence of water in the sense of decreasing the retention of polar compounds. Moisture in PEG produces a sharp fall in V_g for all sorbates, but the original properties of the sorbent can be recovered by drying.

The column history (i.e. its length of service, and the nature of the mixtures which have been analysed on the column) can have a significant effect on the retention properties, especially in columns with non-polar or weakly polar stationary phases. It has been pointed out[139] that in stainless steel capillary columns the retention index for hydrocarbons changes after prolonged operation, and adsorption effects increase at the same time. Thus after 1200 h operation of the column using Apiezon L the Kovats indices for cyclic and aromatic hydrocarbons increased by 2–3 units, an effect ascribed[139] to the catalytic activity of stainless steel towards the oxidation of Apiezon oil. No such effects were observed on glass capillaries.

After 3 years' operation between 50 and 70 °C with hydrocarbon mixtures the Kovats index for aromatic hydrocarbons of a stainless steel capillary column containing squalane changed by less than 0.1 unit[140], but when the same column was operated between 80 and 95 °C for several weeks the Kovats index for aromatic hydrocarbons rose by 0.2 units. The highest permissible operating temperature of stainless steel columns containing squalane can be inferred from these results. Operation of the same column for 6 years at temperatures between 86 and 130 °C produced a 10 unit change in the Kovats indices for aromatic hydrocarbons, and a 2 unit change in the indices for alkenes.

If polar stationary phases are used the retention data are unaffected by adsorption at the *sl* interface, but the role of adsorption at the *gl* interface becomes much more marked (depending on the concentration of impurities in the stationary phase, since these impurities become concentrated at interfaces). The impurity content can vary during the operation of the column: thus after three months' operation of a column containing acetyl tributyrate the relative retention of paraffins varied by 0.5%, whereas that of aromatic hydrocarbons (which are less sensitive to this type of adsorption) varied by only 0.1%.[140] On a highly polar stationary phase (1,2,3-*tris*-(2-cyanoethoxy)propane) at 94 °C the retention indices of aromatic hydrocarbons vary by 0.3 units per day as a result of differences in the adsorption of n-paraffins (introduced as standards) on the stationary phase[140].

Some interesting observations on the ageing of sorbents in packed columns have been reported[81]. After treatment of a squalane sorbent for 35 h at 110 °C in a stream of nitrogen the V_g value for n-propanol falls off sharply, and similar effects are observed after prolonged heating in vacuum or refluxing of the sorbent under hexane[141,142].

Thus the basic reason for the lack of reproducibility of retention data in analytical GLC is interfacial adsorption, coupled with the non-linearity of the isotherm describing this adsorption. We must stress that interfacial adsorption effects are most marked in systems consisting of molecules of different polarity, which form non-ideal solutions (with $\gamma > 5$). Under these conditions it is most important to choose and standardise the experimental conditions carefully in order to optimise the reproducibility of the retention data. In other types of system these requirements are less stringent unless the highest precision is required (errors of less than 0.1% in the relative retention and 0.05 units in the retention indices).

VI. METHODS OF PRESENTING RETENTION DATA FOR IDENTIFICATION

In this Section we shall discuss the "apparatus" errors in the measurement of retention parameters and we shall propose rational methods of calculating retention quantities for later use in inter-laboratory identifications and comparisons.

1. Specific Retention Volumes

Theoretically the parameter V_g is independent of the amount of stationary phase in the column, but it is influenced by variations in the velocity of the carrier gas and the temperature of the column. Both temperature fluctuations and temperature gradients can affect V_g. In commercial apparatus the temperature of the column thermostat is controlled to give fluctuations of less than 0.1 K and local temperature differences of less than 0.5 K along the column. In many of the currently available models the flow rate of the carrier gas is automatically controlled, but nevertheless some modifications to the gas control part of the apparatus are usually needed for the highest precision in the measurement of V_g.

The first high-precision chromatograph for the measurement of retention is described in Ref.143. Subsequently several papers on high-precision chromatography were published, with emphasis on achieving a constant flow rate of the carrier gas and a constant column temperature, and on accurate measurement of the main parameters of the chromatographic experiment. The most successful development was an apparatus with manual (semi-automatic) control[131], in which the input pressure was measured with a standard deviation of 0.24 mmHg, the output pressure with a standard deviation of 0.1 mmHg, and the column temperature to ±0.05 K. An *a-priori* calculation of the possible standard deviation s^0 in the measurement of V_g gave a value of 0.19%.

According to error theory, the overall error of the experiment is determined mainly by the two or three experimental parameters which introduce the largest errors. In the measurement of V_g the precision is limited by the error in w_l: in packed columns the standard deviation of this parameter is $s^0 = 0.16–0.32\%$, and in capillary column $s^0 = 0.24–0.42\%$.[131] An additional error in the measurement of V_g in capillary columns arises from the difficulty of measuring small gas-flow rates accurately.

We can measure w_l by weighing the sorbent before and after filling the column, and extracting the stationary phase from the sorbent after removing it from the column[131], but it is important to allow for the loss of stationary phase during activation of the column. The "burning off" and extraction of the stationary phase from the sorbent could be incomplete if some of the stationary phase becomes chemically bound to the surface of the carrier by the high-temperature activation[10]. The error of the measurement of w_l in the column determines the overall error of the measurement of V_g (0.15%), even if the chromatographic apparatus is ideal in all respects. These problems explain the large scatter of V_g values reported from different laboratories[144]. Thus we cannot recommend the use of V_g as a reliable criterion for identifying the components of a mixture from retention data.

2. Absolute Retention Volume

This parameter does not require measurement of w_l, and therefore its measurement error is lower than that of V_g. For qualitative analysis the parameter has a serious drawback: its dependence on w_l. Hence it cannot be recommended for

identification in GLC. However it is useful to analyse the sources of error in the determination of V_N, because they explain the shortcomings of various methods of measuring relative retention characteristics widely used in qualitative analysis.

The errors in the measurement of V_N with a soap-bubble gas flow-meter are discussed in detail in Ref.145. Before analysing these errors we should stress the requirement for high efficiency of the chromatographic column used to measure the retention data, since this parameter does not appear in the classical equations for calculating the errors of the measurement of V_N. The efficiency of the column affects the precision of the measurement of retention quantities through two parameters: a) it is essential to work with a sharp, narrow, steep-fronted peak in order to obtain an accurate measurement of the time of emergence of the peak from the column, and b) in the measurement of retention data for mixtures it is important to achieve separation of the components as early as possible in order to avoid mutual interactions. In order to locate the peak maximum with a precision of 0.1% the efficiency of the column should be at least 500 theoretical plates[145]. However it has been suggested[146] that a column efficiency an order higher is needed to achieve this level of precision, but the arguments used are not convincing.

When retention data are determined simultaneously for several components displacement effects and interactions between incompletely resolved bands are possible. The displacement effect consists in the replacement of the less strongly sorbed by the more strongly sorbed components, which decreases the retention time of the less strongly sorbed components. It has been shown[145] that in the case of two poorly resolved bands the appearance time of the first peak is shorter, and that of the second peak is longer, than the corresponding times for the pure components, but this phenomenon does not appear to involve the mutual solubility of the components[147]. In general the separation criterion should not be less than 3 to ensure that the mutual effect of the components on their measured retention is less than 0.1%.[145]

a) Measurement of the retention time of a non-sorbed gas (t_0). The method of measuring this quantity depends mainly on the type of detector used. With a katharometer it is convenient to measure t_0 by the appearance time of helium or other inert gas. However inorganic gases are not detected by a flame-ionisation detector, and therefore in this case it is convenient to use methane: at a column temperature of 100 °C the appearance times of air and methane are indistinguishable, but at lower temperatures the sorption of methane can cause a systematic error in the measurement of t_0.[148]

A more correct method of measuring t_0 with a flame-ionisation detector is to determine the retention time of helium against a background provided by the diffuse trailing edge of any other peak (vacancy chromatography method) at a high setting of the detector sensitivity, or by injecting a sample of helium into the column with nitrogen containing a trace of methane as the carrier gas (the function of the methane being to provide a steady base-line detector current[149]). A base-line detector current can also be produced by raising the column temperature until the vapour of the stationary phase or of its decomposition products appears at the column outlet. In every case the helium sample is detected as a negative peak.

Many of the recommended methods of calculating t_0 [150-153] rely on linear approximations of the retention data for n-paraffins. However a comparison[117] of calculated and experimental values of t_0 clearly shows the greater precision of the values obtained experimentally.

b) Measurement of the retention time of the component under study. The appearance time of the peak maximum of a component can be measured as the distance (on the chromatogram) between the sample injection and the appearance of the peak, or it can be measured directly with a stop-watch. If the former method is used the measurement error can be considerable[154]: a 2 s error in the sample injection marker, a 30 s error due to variations in chart speed, an additional error of 2% due to the effect of mains frequency fluctuations on the chart speed, and a 10 s error in locating the peak maximum. It is usually easier to measure the retention time with a stop-watch, when the error is less than 0.02% for retention times of >10 min. In this method the overall error is at least four times smaller than in the determination of retention times from measurements of length on the chromatogram[155]. However the latter method is convenient because it does not require the presence of the experimenter at the appearance time of the peak: it is often used, therefore, in approximate measurements.

Electronic chronometers offer a high precision for the measurement of the appearance time of individual peaks, but this precision cannot be exploited to the full because of the limitations of the experimenter's reaction times (0.1–0.3 s). By using a computer to detect the injection of the sample and to measure the appearance time of the peak we can reduce the error of the measurement of retention time 5–7-fold in the case of a non-polar stationary phase, but if the sorbent is coated with a polar stationary phase the precision is not significantly improved by using a computer[94].

The absolute error of the measurement of retention time is proportional to the retention time, and for very long retention times the relative error of the measurement is constant[156].

In addition to the errors associated with the measurement of retention time, which can be minimised, we need to consider errors in the determination of retention time arising from instability of the experimental parameters. These errors are mainly responsible for the overall experimental error of measured retention times[157-167].

Stable conditions for the measurement of retention times are most easily obtained by using a computer-controlled chromatograph, especially in the separation of complex mixtures of many components[22,160-164]. Conventional electronic proportional temperature controllers can stabilise the temperature of the column thermostat to within 0.05 K, and this uncertainty can be lowered to 0.02 K by using computer control[165,166]. The fluctuations in the flow rate of the carrier gas can be less than 0.1 ml min^{-1} with computer control. Some workers recommend the use of liquid-filled thermostats[167], which offer a temperature stability ranging from 0.0025 to 0.01 K, but these thermostats also have a number of shortcomings (corrosion of the apparatus, high thermal inertia, and large temperature gradients along the column). Air thermostats can give temperature differences as low as 0.02 K in the vertical direction and 0.2 K in the radial direction[168].

The use of computer control of the operating parameters of the column can greatly reduce the lack of reproducibility of the experimental conditions but it cannot eliminate it completely. Therefore allowance for the residual fluctuations of the experimental parameters, and appropriate corrections to the measured quantities, are as important as control[163,169,170]. All the experimental parameters were measured at intervals varying from 0.05 s to several seconds and the retention volume was obtained as the sum of micro-elementary volumes corresponding to the measured column temperatures and carrier gas flow rates[171]. The detectors were thermo-anemometers, manometers, and resistance thermometers: for a column temperature stability of 0.01 K and a pressure measurement precision of 0.05 mmHg the standard deviation of

the measurement of retention volume of n-heptane in octadecane at 60 °C was 0.43% with manual processing of the data and 0.02% with computer processing[171].

Thus by using commercial apparatus with appropriate modifications to the gas handling system the standard deviation of the measurement of V_N is 0.1–0.2%: an order of magnitude improvement in precision is possible by using computer control and data processing methods.

3. Relative Retention Volume

This parameter was first proposed for the identification of components in 1952 [172]: its value is independent of the amount of stationary phase (if interfacial adsorption is negligible), but it is affected by changes in the flow rate of the carrier gas and by changes in column temperature.

The precision of the measurement of relative retention volume (r) can be raised by correctly choosing the reference standard. With high-grade apparatus the method of introducing the standard does not affect the error of the measurement of r: it can be introduced with the components of the mixture to be analysed, or after each analysis, or even after several analyses[156]. A 20–50% improvement in precision in the measurement of r by introducing the standard as part of the unknown mixture (rather than separately) has been reported[139]. However adding the standard as part of the mixture is legitimate only when the peak of the standard is completely resolved from those of the unknown components, and therefore the retention times of the components are unaffected by the presence of the standard. The best compromise is to add the standard to the column once every 2–4 h, depending on the stability of the control of experimental conditions.

The standard substance should appear at the outlet of the column in 5–15 min, its adsorption at the interfaces should be negligibly small, and it should be readily available. For these reasons aromatic hydrocarbons are often used as standards, since their adsorption at the *sl* and *gl* interfaces makes a negligible contribution to V_N, and the compounds are easily obtained. For example, at low temperatures (up to 100 °C) benzene can be used as a standard, naphthalene at temperatures up to 200 °C, and anthracene or phenanthrene at still higher temperatures.

In general r can be measured with any arbitrarily chosen standard, but if the results are to be published and used in other laboratories for qualitative analysis they should be recalculated on the basis of one of the above standards. This involves determining the r value of the arbitrary standard with respect to one of the aromatic hydrocarbons. In the R_{x9} system of r values with a single standard[101,173,174] n-nonane is used as the standard compound: the reproducibility of the measurement of retention indices in this system is given as 10. An important disadvantage of the use of a n-paraffin as the standard is the large contribution to V_N from adsorption at the surface of the stationary phase when polar liquids are used.

In the early experiments[175] the error in the measurement of r was 0.5%, but the use of computers has lowered this error to 0.02% [168,176–180]. With manual processing of the data the error of the measurement of r in glass capillary columns was 0.16–0.19% for OV-17 silicone oil, 0.26–0.28% for Carbowax 20M, and 0.38–0.55% for SF-96 silicone oil[181]; the increase in error caused by the last two stationary phases is probably due to poor wetting of the capillary surface by the liquid and hence to adsorption at the glass–gas interface[182]. In stainless steel capillary columns the error in the measurement of r is slightly higher[183].

4. Systems of Retention Indices

A detailed mathematical analysis of the errors in the calculation of retention indices has been published[156,184–186]. The first report of high reproducibility in the measurement of retention indices was made in 1970 [187], and it was shown that the error in the measurement of retention indices can be decreased by an order of magnitude by using a computer. The conditions for precise measurement of the retention indices are formulated in Refs. 131, 188, and 189, and an error of 0.03 units was claimed[94]. Sojak and Rijks[140] describe the conditions which allow the retention indices of hydrocarbons on squalane to be measured with an error of 0.02 units. Attempts to calculate retention from the centre of gravity rather than from the apex of the peak[190,191] failed to reduce the error below 0.05 units.

The reliability of the determination of retention indices on capillary columns is discussed in detail in Ref. 140. Changes in the input pressure of the carrier gas from 2.5 to 4 atm produced changes of 0.08–0.13 units in the retention indices of hydrocarbons when hydrogen was used as the carrier gas, whereas with nitrogen as the carrier gas the retention indices varied by 0.27–0.33 units when the pressure at the column inlet was varied from 1 to 4 atm. Within the limits of experimental error (0.05 units is the retention index) there was no effect from changes in helium pressure. The response to changes in carrier gas pressure increases in the sequence paraffins < alkenes < cycloparaffins < aromatic hydrocarbons[140].

In Kovats' conventional system of retention indices the standard components are n-paraffins, for which the retention data on polar stationary phases are strongly affected by interfacial adsorption (leading to poor reproducibility). It was pointed out[119] that the error in the calculation of the retention indices increases with the polarity of the stationary phase. According to Bonastre and coworkers[118] a change from 10 to 30% in the w_l value of Carbowax 20M on a sorbent increases the retention indices from 14 to 22 units because of the decreased retention of n-paraffins. In order to overcome this difficulty the use of a homologous series of n-alcohols as standards is proposed[192] when polar stationary phases are used. This suggestion is not incompatible with the concept of a system of retention indices, based on the assumption of direct proportionality between $\lg V_N$ and C (where C is the number of carbon atoms in the molecule) for a homologous series[193,194].

The desirability of choosing polar standard substances when polar stationary phases are used has been stressed by many workers[122,133,195–203], with a tendency to use as standard the homologous series whose members are being studied. However there are objections to the use of a homologous series as standard on polar sorbents, because interfacial adsorption increases sharply with the length of the alkyl radical and this lowers the reliability of the results. Another important shortcoming of the retention index system is the assumed linear dependence of $\lg V_N$ on C, which does not apply in a number of systems[193,204–206]. Deviations from linearity arise from the parity rule[206], and from the interaction of methyl groups[207], which led to the principle of non-calculability of the indices (according to which the linear dependence of $\lg V_N$ on C is only approximate, with a confidence interval of two units). When the molecules of the stationary phase contain aromatic rings the relationship is no longer linear[204].

The consistency of retention indices obtained in different laboratories has been analysed in several papers. The error in the measurement of the retention index from data reported by different laboratories is stated[140] to be 0.2 unit, but we should note that recalculation of the quoted results[140] gives

an error varying from 0.6 to 1.5 unit. The discrepancies between retention indices obtained in different laboratories may be due to effects from the material of the capillary column or to changes in composition or in concentration of the stationary phase during operation of the column. Thus it has been shown[208] that after high-temperature activation of a non-polar capillary column the retention index of aromatic hydrocarbons varies by 8 units. Standardisation of the capillary surface is said[94] to lower inter-laboratory discrepancies (to 0.2 unit of index). The work of Schomburg (quoted in Ref.155) has shown that if the capillary columns are prepared in one laboratory and distributed to other laboratories the discrepancy between the results is less than 0.05 unit, whereas if the columns are prepared in the different laboratories the reproducibility falls to 1 unit.

A careful comparison of the retention data for n-paraffins on squalane obtained in different laboratories under a satisfactorily controlled experimental conditions is given in Ref.209: the error varies from 0.09 to 0.28 unit whereas the reproducibility within each laboratory is 0.025 unit. For an internal reproducibility of 0.2 unit[19,95] an inter-laboratory comparison gave a discrepancy of 1 unit[95,205-213]. Hence the consistency of the retention quantities between laboratories is 4–5 times worse, on average, than the measurement error within one laboratory, though we should note that all these results apply to systems in which the retention of the components is almost unaffected by adsorption effects. When the influence of interfacial adsorption is not negligible the above recommendations for achieving reproducible retention data must be strictly observed.

In conclusion we shall briefly dwell on some systems of retention indices which have been developed to remedy the shortcomings of the Kovats system. In view of the non-linearity of the dependence of $\lg V_N$ or C Matukuma[214] proposed a new form of index in which three successive homologues of the n-paraffin series are used as standards:

$$I_w = 100C + \frac{\lg(1 + i/a - i/b)}{\lg(b/a)} 100,$$

where $i = t_{Nx} - t_{Nc}$, $a = t_{Nc} - t_{N(c-1)}$, $b = t_{N(c+1)} - t_{Nc}$, t_{Nx} is the appearance time of the unknown component, and t_{Nc}, $t_{N(c+1)}$, $t_{N(c-1)}$ are the appearance times of the n-paraffins with the same number of carbon atoms as the unknown, one carbon atom fewer, and one carbon atom more respectively. Though this system of indices yields more accurate results it suffers from a loss of generality in comparison with the Kovats system.

The arithmetical indices proposed by Vigdergauz[215] are the simplest form of indices:

$$I_a = \frac{t_{Rx} - t_{Rc}}{t_{R(c+1)} - t_{Rc}},$$

where t_R are the appearance times of the components uncorrected for the dead volume of the column (the unknown components and the n-paraffins with the same number of carbon atoms). Expressions relating the arithmetic indices to the Kovats indices have been obtained[216-218], and some applications of the arithmetic indices have been described[219,220]. Though the calculation of the arithmetic indices is extremely simple the indices are not particularly useful because they contain no physicochemical information and they lack the generality of the Kovats indices for identification.

The differences between different systems of retention indices are not fundamental in principle, and the choice of a particular type of index is usually dictated by tradition or by convenience. Evidently, the Kovats index was historically the first and it will continue to be widely used in analytical practice.

5. Comparison of Different Methods of Presenting Retention Data

The basic characteristics of the four most widely used retention parameters are compared in the Table. The specific retention volume V_g has some important advantages over the other parameters because it contains physicochemical information, but it cannot be recommended as an identification parameter because the precision of its measurement is low.

The relative retention volume r and the Kovats index I are similar in many ways, and the choice between them is often a matter of convenience or tradition. The parameter I lends itself particularly well to calculations by the additivity principle: any n-paraffin with an appearance time comparable to that of the unknown component can be used as standard for the calculation of this parameter. However with polar stationary phases we must use homologous series of polar compounds as standards, and even this procedure does not exclude interfacial adsorption effects on the retention parameters of the standards. Another shortcoming of the Kovats indices is the need to assume a linear dependence of $\lg V_N$ on C, which is often not appropriate. Furthermore the error in the measurement of I is almost twice as great as the error in the measurement of r.

Characteristics of the retention parameters.

Parameter	Reproducibility, %		Advantages	Disadvantages
	within laboratory	between laboratories		
Specific retention volume V_g	0.2	4.5	A thermodynamic parameter, describing the gas–liquid equilibrium; can be used to calculate all the thermodynamic characteristics of the phase equilibrium; independent of the amount of stationary phase in the column	Laborious measurement of the amount of stationary phase in the column, and associated errors
Net retention volume V_N	0.1 (0.02)	–	–	**
Relative retention volume r	0.1 (0.02)	0.8	Simple to calculate, consistent with the order of appearance of the peaks, few parameters to be measured. No assumptions needed	The need to recalculate the results to a single standard from secondary standards
Kovats index I	0.2 (0.05)	2.3	Rational presentation of the results as a sum of increments associated with the atomic groups in the sorbate molecule; any member of the homologous series can be used as standard	The need to postulate linearity of the logarithm of the retention volume as a function of number of carbon atoms in the molecule for members of a homologous series; the need to use at least two standards

*The errors obtained in computer-controlled experiments are shown in brackets.

**Not recommended for identification, not reproducible between laboratories.

Though the equivalence of I and r as identification parameters has been convincingly demonstrated[184] the r values are more convenient to use because they are simpler. They are also more accurate because no assumptions are made in their derivation. Since the choice between I and r does not raise

any fundamental questions, it is desirable to standardise on one of these quantities: the choice of r (with aromatic hydrocarbons as standards) appears to be the best overall approach to a standard identification procedure in GLC.

Because of the complexity of sorption processes in chromatographic columns the following experimental parameters should be stated in publications to ensure reproducibility of the retention data in other laboratories: 1) the column temperature (to the nearest 0.1 K), 2) a full description of the stationary phase (purity, origin, amount of liquid phase on the carrier, details of the activation process), 3) a full description of the carrier, 4) length, diameter, and material of the column, and (in the case of capillary columns) pretreatment conditions, 5) size of injected sample and dependence of the appearance time on sample size, 6) method of measuring the dead time of the system, 7) nature, input pressure, and flow rate of the carrier gas, 8) sensitivity of the detector and chart recorder, 9) type and model of the chromatograph, 10) errors in the determination of retention data.

It is also desirable to state the operating life of the column, and to point out any changes in retention behaviour observed after lengthy operation of the column, as well as the role of adsorption effects. Lastly, the results being reported should be compared with published data if possible.

6. Choice of Parameters for Identification in GLC

Reliable identification of a component requires the coincidence of two independent retention parameters for the unkown peak and for the peak of the standard. We shall discuss two variants of the method of identification from retention data, though non-retention methods (spectral, chemical, etc.) are also widely used.

a) Chromatographic spectra. Coincidence of the retention parameters for the unknown and the standard component on two or three different stationary phases is a good criterion of identify of the substances being compared. Stationary phases of very different polarity and chemical nature are often used for comparison. The following can be recommended for identifications: squalane (up to 100 °C), the C_{87} hydrocarbon[221] (up to 250 °C), oxydipropionitrile (up to 80 °C), *hexakis*(2-cyanoethoxy)hexane (up to 180 °C), polyphenyl ether from 6 rings (up to 250 °C).

The separation of not very complex mixtures on two or three columns with different stationary phases yields "chromatographic spectra" which can be used to identify the components[222-228]. Analogous to the chromatographic spectrum is the parameter ΔI, calculated as the difference between the Kovats indices for the unknown component on two stationary phases. Both these methods are useful for the identification of the components in mixtures of 5–10 substances present at different concentrations. The main difficulty in identification from chromatographic spectra is the "recognition" of the peaks of a particular component when the mixture is separated on different columns, since the peaks appear in different order at the column outlet and they can only be recognised by their relative height. Another drawback of the chromatographic spectra is the difficulty of obtaining an acceptably complete resolution of all the peaks for a complex mixture on a number of stationary phases. Often only one stationary phase is available for identifying the components of a complex mixture. In this case we need another independent parameter determined on the same stationary phase—the temperature dependence of the retention quantities—in addition to the indices r or ΔI for reliable identification.

b) Temperature dependence of the relative retention volume and of the Kovats index. These temperature dependences are determined directly by the enthalpic selectivity of the stationary phase (the relative molar heat of dissolution ΔH_S°, whose value can be calculated relatively simply by using the additivity principle). The quantity ΔH_S° can be used directly for identification[229,230], because it is much less sensitive to experimental conditions (such as interfacial adsorption) than the parameter r. It can be evaluated very simply from experimental data by using the expression

$$\Delta H_s^0 = \frac{4.58\,(\lg r_1 - \lg r_2)}{1/T_1 - 1/T_2},$$

where the subscripts 1 and 2 refer to different column temperatures.

The temperature dependence of the retention can be described also by indirect parameters (e.g. the constants of an equation of the Antoine type[231,232]) or by the temperature dependence of the Kovats index δI. The latter type of dependence is widely used to identify substances of different classes[24,233-243,209-212], but we should note that δI can vary with temperature as a result of non-linearity of the relationship between I and the column temperature, and this effect must be allowed for when δI is used for identification. Since neither the constants of the Antoine equation nor δI contain as much physicochemical information as ΔH_S° we recommend the two quantities r and ΔH_S° as the basic independent parameters to be used for identification.

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