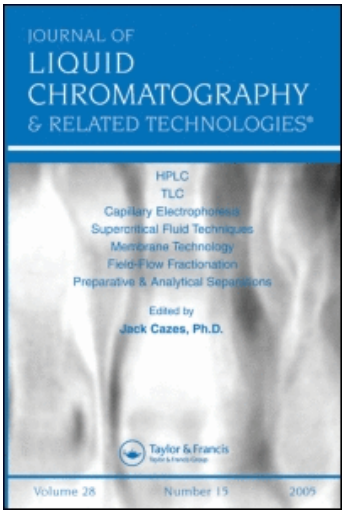

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Solute Retention in Column Liquid Chromatography: IX. Comparison of Methods of Determination of the Void Volume in Liquid-Liquid Chromatography

R. A. Djerki[a]; R. J. Laub[a]

[a] Department of Chemistry, San Diego State University, San Diego, California

To cite this Article Djerki, R. A. and Laub, R. J.(1987) 'Solute Retention in Column Liquid Chromatography: IX. Comparison of Methods of Determination of the Void Volume in Liquid-Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 10: 8, 1749 — 1767

To link to this Article: DOI: 10.1080/01483918708066797

URL: http://dx.doi.org/10.1080/01483918708066797

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(8&9), 1749-1767 (1987)

SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. IX. COMPARISON OF METHODS OF DETERMINATION OF THE VOID VOLUME IN LIQUID-LIQUID CHROMATOGRAPHY

R. A. Djerki and R. J. Laub*
Department of Chemistry
San Diego State University
San Diego, California 92182

ABSTRACT

It is shown in the case of true liquid-liquid chromatography (LLC) (OV-1 polysiloxane stationary phase; methanol, water, or blended mobile phases) that no one method of determination of the column void volume gives unambiguous results. A generalized convention regarding definition of the column and system void volumes is therefore developed. It is stipulated that, barring multiple retention mechanisms, the former, V_M, is comprised of the space within a column to which solutes have access, yet which is not otherwise occupied by the stationary phase, the packing matrix, or immobilized carrier. V_M should thereby be no greater than the retention volume of any solute; and should also be invariant with mobile-phase composition as well as with temperature. The method of self-consistent fitting of the retentions of homologous series of alcohol solutes as a function of homolog number is said overall to provide the column void volume that most closely satisfies these criteria, although several other techniques give results that agree to

*Author to whom correspondence may be addressed.

within close to experimental error with this value. An alternative thermodynamic-based convention wherein the V_M that yields an activity coefficient of unity for the mobile phase, irrespective of its composition, is also said to have merit.

INTRODUCTION

Modern-day strategies employed for the prediction of column liquid chromatographic (LC) separations (1,2) are routinely formulated in terms of solute capacity factors, which require in turn that the column void ("dead") time t_M or volume V_M be known or determined; where $k' = (t_R - t_M)/t_M = K_R V_S/V_M$, V_S is the volume of stationary phase, and K_R is the concentration-based solute partition coefficient. However, it is not necessary to know V_M in order to effect separations by quantitative adjustment of the LC system and conditions, for example, the Jones-Wellington (3) separation factor $S_f = (t_{R,i} - t_{R,j})/(t_{R,i} + t_{R,j})$ can be employed to the same end as relative retentions (alpha values, where $\alpha_{i/j} = k'_i/k'_j$) in optimizing chromatographic analyses via window diagrams (4) of various sorts (5,6).

Even so, the system void time (or volume) remains indispensible to the calculation of equilibrium and other physicochemical properties from purely chromatographic data, e.g., solute infinite-dilution activity and partition coefficients as well as finite-concentration sorption isotherms. In addition, retentions cast in terms of capacity factors form the basis of all quantitative models of the sorption process. For example, the additivity of terms implicit in ln k' for a particular homologous series of solutes with a column system of constant phase ratio β ($= V_M/V_S$) implies serial additivity in the free energy of sorption.

As a result, the formulation of a theoretical definition of the void volume in column liquid chromatography has generated considerable interest in recent years, as has the practical means of its assessment (7-22), as described and discussed in the recent review by Smith, Nieass, and Wainwright (23). However, no consensus has yet been reached on an appropriate description, let alone means of measurement, of either t_M or V_M.

In this work, formalisms appropriate for the void volume in the conceptually-simple case of liquid-liquid chromatography (LLC) are considered, for which the mechanisms(s) of solute retentions are presumed

to be less ambiguous than the adsorption- or reverse-phase modes of LC. The results are nevertheless extended, where possible, to liquid chromatography generally.

DEFINITION OF THE COLUMN VOID VOLUME

Defining V_M simply as the total column volume occupied by the mobile phase is clearly unsatisfactory, because the carrier may well dissolve in, or otherwise solvate, liquid stationary phases. Moreover, the manner in which this occurs can conceivably be quite complex, for example, the extent of solvation will more than likely vary with eluent composition. In addition, not only will the selectivity of the stationary phase be altered, but also, its effective volume V_S can vary as well, for example, surface-bonded phases can be made to collapse or extend (24,25) or, indeed, swell (26). The situation is therefore analogous to liquid-solid chromatography (LSC), including silica, alumina, and reverse-phase systems, where it is now widely recognized and accepted that multicomponent carriers can be immobilized neat or in admixture on the surface of the stationary phase in a variety of ways.

In contrast, we consider that, in the instance of nonionic solutes, solvents, and packings, the **column** void volume V_M is comprised of the space within a column to which solutes have access, yet which is not occupied by, or otherwise under the influence of, the stationary phase or the packing matrix. The **system** void volume V_{sys} is then V_M, taken together with connective volumes V_{con} between the injector and the column and the column and the detector. [We assume that in properly-designed apparatus the contribution of V_{con} to V_M is negligible, or can at least be taken account of. Also, we take the packing to be totally porous, i.e., such that size-exclusion phenomena (27-29) do not contribute to solute retentions.]

Two additional parameters can be defined that clarify the roles of the mobile and stationary phases. First, V_{max} is the total volume that any given mobile-phase component can occupy within the column; and, secondly, V_{imm} is taken as the sum of the volumes of all mobile-phase components that are immobilized by the stationary packing, including any stagnant pools of carrier contained within support pores. The true column void volume V_M is then the difference between V_{max} and V_{imm}. This

definition thus makes any portion of the column part of the stationary phase if, barring diffusional phenomena, solutes cease to move longitudinally through the system as a result of interacting with it. Further, V_M is considered solely in terms of the volume of moving phase within a column, all other space being assumed to be occupied either by stationary phase, immobile carrier, or packing matrix.

Two boundary conditions can additionally be stipulated. First, the void volume should be smaller than (or at best equal to) the retention volume of any solute. Secondly, V_M should be independent of the temperature of the system as well as the composition of the mobile phase.

EXPERIMENTAL METHODS OF DETERMINING V_M

Broadly speaking, methods for determining the column void volume can be divided into two groups. The first includes techniques whereby V_M is determined indirectly, for example, by reiterative fitting of raw retentions to mathematical models, e.g., the supposed linearity of semilogarithmic plots of retention volumes of homologous series against the homolog number, n. In the second type, the void volume is measured directly from the retention time of a "marker" (i.e., presumed nonretained) solute, such as a mobile-phase component that may be labeled isotopically, or an inorganic salt. Another direct-observation method is measurement of the difference in column weights obtained before and after filling the system with one or another liquids of known density.

The Method of Tare Weight

In this technique, each of two bulk mobile-phase constituents is pumped separately through the column, following which it is capped and weighed. The column void volume, which actually corresponds to V_{max}, is then calculated from the relation:

$$V_{max} = \frac{m_1 - m_2}{\rho_1 - \rho_2} \tag{1}$$

where m_1 and m_2 are the weights of the column containing each of the two different solvents, and where ρ_1 and ρ_2 are their densities at the temperature at which the measurements were carried out.

The Method of Homologous Series

Several methods of assessing the void volume are based upon the use of homologous series of solutes. In essence, each makes use of the regression of plots of ln (V_R') (the solute adjusted retention volume, $V_R - V_M$) against homolog number n, where linearity is expected so long as the free energy of sorption of the solutes varies incrementally from one homolog to the next. Generally, linear behavior also implies a singular mechanism responsible for the retention of each member of the series.

Numerical Computation. When the above-mentioned homologous-series plots are in fact linear, the true column void volume V_M can be calculated by solving simultaneous equations for several member-homolog solutes, where the pertinent expression is (7):

$$V_R = V_M + \exp(a\ n + b) \tag{2}$$

or, taking logarithms,

$$\ln V_R' = a\ n + b \tag{3}$$

where a and b are constants that are characteristic of a specific homologous series. The method thus amounts to self-consistent fitting of eqns. 2 or 3.

Graphical Variant. An innovative graphical method of determining the best-fit V_M has also been described by Laub and Madden (22). They plotted the linear least-squares correlation coefficients calculated with assumed void volumes against V_M, and obtained smooth curves that exhibited maxima. The overall best V_M was then found by inspection as that giving the highest r. A modest advantage of their method is that the temperature-dependence of such curve maxima (hence that of V_M) can easily be assessed when the data are plotted on a common graph. [Barring significant expansion coefficients for the column tube and packing, solutes that

do yield temperature-dependent V_M (e.g., esters) are of course unsuitable for determination of the column void volume by the homologous-series method, since there must then be operative more than one mechanism of retention for the probe compounds. The extent of temperature-invariance of V_M is therefore an important test of the veracity of dead-volume data determined in this way and, indeed, although the criterion is rarely invoked, by any other technique as well.]

The Method of Minor Disturbance

Injection of a small amount of one or the other components of binary carrier liquids results in a pulse of mobile phase enriched with that compound, which often gives a peak ("disturbance") upon emergence from the column. The band maximum is then claimed by advocates of this method to correspond to the true void volume, that is, each component of the mobile phase is treated as a nonsorbed solute.

According to some (8,10,21), the "disturbance" band maximum is in fact a measure of the first derivative of what amounts to the carrier-component sorption isotherm. Thus, the method is said to give a realistic estimate of the void volume only if the data are taken over the linear region of the isotherm.

Values of V_M determined from injection of this or that mobile-phase component might be thought to depend upon the eluent composition. However, several studies have shown that disturbance peaks resulting from injections of solutions of water in various organic carriers give peak-maximum retention volumes that are identical to those observed with organic compounds injected as solutions in aqueous mobile phases, irrespective in either instance of whether the mobile phase is predominantly aqueous or organic.

The Use of Inorganic Salts

The suitability of a particular material as a void-volume "marker" can be judged in terms of several factors. First, the compound should not be retained by the stationary phase, yet it must not pass through the column more quickly than the average linear velocity of the mobile phase. Also, there should be no intraparticle exclusion, i.e., the compound must be free

to penetrate the pores of the support. In addition, the compound should not influence what distribution of aqueous or organic modifier there may be in (or on) the stationary phase.

Inorganic salts, especially sodium nitrate and sodium nitrite, have been said by a number of workers to meet these criteria (9,14,15,17,19); $NaNO_3$ has also been claimed by some (9,14) overall to be superior, although there are as yet no fundamental-based criteria for choice of one material over another. However, as pointed out elsewhere (17,19), inorganic salts often give void volumes that are dependent upon the amount injected as well as the eluent composition (particularly its pH).

The Use of Isotopically-Labeled Compounds

Isotopically-labeled materials have been used for determining the column void volume in two ways. Most commonly, the elution volume of deuterated mobile-phase component is monitored, for which a refractive-index detector is prerequisite. Alternatively, radioactive labels can be used, e.g., ^3H and ^{14}C. A scintillation counter is then required for detection of these species.

Deuterated Species. Methods utilizing marker solutes such as D_2O have been highly recommended by several workers (14,17). However, in determining V_M with this or that deuterated species (e.g., D_2O in water-methanol mobile phases with reverse-phase column systems), it must be presumed that the extent of its interaction with the stationary phase is as negligibly small as is supposed to be the case for the nondeuterated compound. There can also arise isotope effects, that is, differences in retentions due to molecular size. Moreover, if the mobile phase is some combination of methanol and water, the retention of deuterated water will vary depending on the isotopic exchange between labeled and unlabeled water molecules and, in addition, on isotopic exchange between labeled water and methanol, as pointed out by Zhu (20) and as commented upon at length by others (8,10).

Of equal importance, deuterated species presumably have access to stagnant pools of carrier. Also, the materials will be sorbed by the stationary phase virtually as readily as nondeuterated compounds. Thus, the void volume determined by this technique will actually be $V_M + V_{imm} = V_{max}$.

Radioactive Species. The use of radioactive materials has been investigated by several (10,21), and has been claimed in particular by Knox and Kaliszan (21) overall to be the most accurate method of determining the void volume. It must be recognized, however, that isotopic exchange occurs e.g., with tritiated hydroxyl groups as well as with deuterated species and, in fact, exchange will occur with all protons that are bound to oxygen atoms. The consequence of this is that the retention volume of a labeled marker will be a weighted average of all of the species involved in exchange. Thus, measurements must be made of the retention volumes of each of the isotopically-labeled components, and the void volume then calculated (21) as:

$$V_M = \phi_1 V_1 + \phi_2 V_2 + \ldots + \phi_n V_n \quad (4)$$

where V_1 through V_n are the elution volumes of the isotopically-labeled components 1 through n, of volume fractions ϕ_1 through ϕ_n, present in the eluent feed. However, as with deuterated species, even if such measurements were carried out, the resultant void volume would in fact amount to the sum of V_M and V_{imm}.

Knox and Kaliszan (21) claimed that, in practice, the simplest way to determine what they took to be V_M was to flush the column with one eluent, neat, and then determine the retention volume of its isotopically-labeled counterpart. They also pointed out that uncertainties could arise if the results were extrapolated to multicomponent carriers, since there might be excess mixing volumes. It might also be the case that different components of the mobile phase could be sterically excluded to different degrees by some packings.

EXPERIMENTAL

Apparatus and Equipment

The liquid chromatograph employed in this work was a Varian Model 8500 equipped with a Knauer differential refractive index detector. The injection valve, which was fitted with a 10-mm^3 external sample loop, was from Valco.

The column was 50 cm by 4.6 mm i.d.; while the packing was comprised of Johns-Manville Chromosorb G support (120/140-mesh, AW DMCS-treated) that was coated with 10% by weight liquid phase, here, OV-1 poly(dimethylsiloxane) from Ohio Valley Specialty Chemical Co.

Constant system temperature was maintained to within ± 0.05 K by enclosing the column in a glass jacket through which was circulated water from a Neslab Exacal thermostat. The temperature was monitored with a Hewlett-Packard Model 2802A platinum resistance system, accurate to ± 0.02 K. The eluent was brought to the column temperature by thermostating the solvent feed line (a length of ca. 1 m) connecting the outlet of the pump to the heavily insulated injection valve. Equilibration with each new mobile phase at a given temperature (usually allowed to take place overnight) was judged acceptable when solute retention times were reproducible to better than ± 2% over at least three sequential injections.

The flow rate was monitored continuously during the course of each set of retention measurements by passing the column eluent into a thermostated graduated pipet.

Materials and Supplies

Two homologous series of solutes were employed. The first set was comprised of the aromatic hydrocarbons: toluene, ethylbenzene, n-propylbenzene, and n-butylbenzene. The second consisted of the aliphatic alcohols: methanol, ethanol, n-propanol, n-butanol, n-pentanol, n-hexanol, and n-heptanol. All were from Chem Services except methanol, which was Fisher HPLC grade that was obtained in bulk for use also as a mobile phase. The water that was employed as a solute as well as mobile phase was doubly-distilled. Inorganic salts were ACS reagent-grade. All solutes were injected as solutions in the particular mobile-phase composition being examined at the time.

All aqueous-organic carrier mixtures were made up by volume. Each was prepared fresh, and was also degassed in an ultrasonic bath (L&R Model T-9) for at least 15 min just prior to use.

Procedures

Prior to the evaluation of V_M by each of the methods detailed below, the extra-column void volume was assessed independently by measuring the

retention volumes of the test solutes with the column removed from the system, but with the injector and detector connecting tubes joined by a zero-dead-volume union. The apparent column void volume was then obtained by subtracting the small extra-column volume V_{con} from the observed (or calculated) system volume V_{sys}.

Methanol and water were employed as the neat test solvents for the method of tare weight: after equilibration of the column with each, it and its contents, together with the connecting tubes from the injection valve and detector, were weighed to the nearest mg. The system void volume was then calculated from eqn. 1, where the requisite density data were taken from ref. 30.

For the homologous-series phase of the work, the retentions of the aromatic hydrocarbon solutes were determined at 293, 298, and 303 K with pure methanol mobile phase; and at 298 K with 90% methanol/10% water. The retentions of the alcohol solutes were also measured at these temperatures with pure methanol carrier. Calculation of the parameters a and b of eqn. 2, thence the apparent column void volume, was carried out by the procedure described by Purnell and coworkers (7) and Laub and Madden (22), and amounted essentially to solving the simultaneous equations generated with the retention data for at least four solutes. The graphical method devised by Laub and Madden (22) was also employed, where a void volume was first assumed and the corresponding correlation coefficient then calculated for the solute-probe retention data regressed in accordance with eqn. 2. A perpendicular dropped to the abscissa from the curve-maximum of the plot of r (the correlation coefficient) against the assumed void volume was taken to give the best-fit V_M.

Studies of the "disturbance" method were carried out with 100, 90, 70, 50, 30, 10, and 0% methanol at 298 K. Solutions consisting of methanol or water in slight excess (ca. 0.1%) over the carrier composition gave disturbance peaks that were clearly evident even at modest sensitivity settings of the detector.

Sodium nitrate was chosen as representative of inorganic salt markers on the basis of its ready availability as well the strong recommendations made on its behalf by others (9,14). Its retention volumes were determined with pure methanol mobile phase at 293, 298, and 303 K, and for 90, 70, 50, 30, 10, and 0% methanol at 298 K.

RESULTS AND DISCUSSION

The Method of Tare Weight

The system void volume V_{sys} was found by this technique to be 18.0_6 cm^3, while the extra-column volume of connecting tubes V_{con} was determined to be less than 0.01 cm^3. Thus, the tare-weight column void volume V_M was calculated to be 18.0_5 cm^3.

Methods Utilizing Homologous Series

Both sets of probe-solutes were utilized in this portion of the work, since Laub and Madden (22) reported that while aromatic hydrocarbons were suitable for reverse-phase systems, alcohols were not because of the considerable temperature-dependence of the resultant V_M.

Aromatic Hydrocarbons. The raw retention volumes for the aromatic hydrocarbon solutes with 100% methanol at 293, 298, and 303 K, and with 90% methanol/10% water at 298 K, are provided in Table 1.

Numerical Computation. The solute V_R gave erratic results when used to calculate V_M from eqns. 2 and 3. This was found to be a consequence of the small differences between the retention data and the column V_M, where even trivial errors in the former resulted in large errors in the latter. For example, the average of all of the data in Table 1 with pure methanol mobile phase is 18.9 cm^3, less than 0.9 cm^3 removed from the tare-weight value of 18.1 cm^3. Indeed, the values are in fact identical if the error on the two volumes is assumed to be ± 2%, i.e., ± 0.4 cm^3.

Graphical Variant. The aromatic hydrocarbon data were next reduced in accordance with the graphical variant method, that is, in terms of the regression of eqn. 3 against the assumed V_M. The data are plotted in Fig. 1 and, in fact, extrapolate to the highest r at $V_M = 0$ ($r = 0.9992$).

Aliphatic Alcohol Solutes. The homologous-series method of determination of V_M with the set of normal aliphatic alcohol solutes was carried out next. The raw retention volumes with pure methanol mobile phase at three temperatures are presented in Table 2. The data are as expected insofar as the retention volumes are lower at higher temperatures. However, the average for all values is 17.5_2 cm^3, that is, to within experimental error, the V_R are virtually invariant with temperature.

TABLE 1

Retention Volumes for Aromatic Hydrocarbon Solutes with Methanol Mobile Phase at 293, 298, and 303 K; and with 90% Methanol/10% Water Carrier at 298 K

	V_R/cm^3			
	293 K	298 K		303 K
Solute	100% MeOH	100% MeOH	90% MeOH	100% MeOH
Benzene		18.6_4		
Toluene	18.1_2	18.7_6	18.5_4	18.5_7
Ethylbenzene	18.5_3	18.9_8	18.7_9	19.9_3
n-Propylbenzene	18.9_9	19.1_6	19.0_1	21.3_8
n-Butylbenzene	19.4_2	19.3_8	19.2_7	22.9_1
Ave.	18.7_7	18.9_8	18.9_0	20.7_0

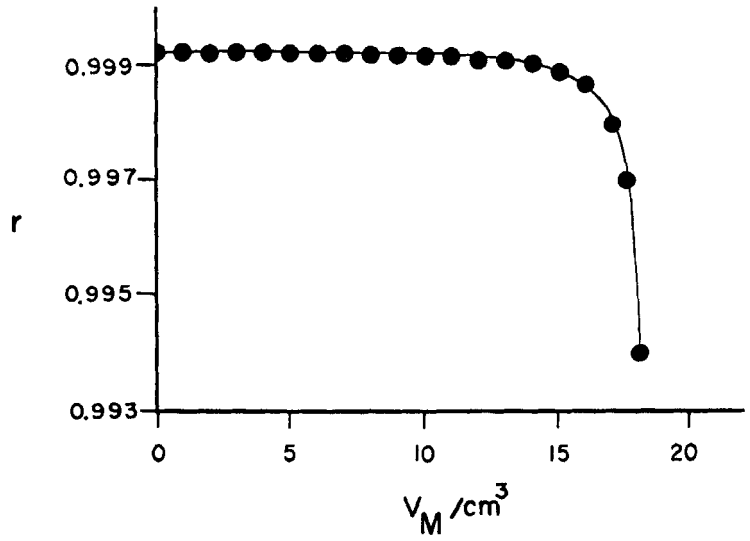

FIGURE 1. Plot of linear least-squares correlation coefficient r against assumed V_M for regression (eqn. 3) of homologous series of aromatic hydrocarbon solutes at 298 K with methanol mobile phase.

TABLE 2

Retention Volumes for Aliphatic Alcohol Solutes with Methanol Mobile Phase at 293, 298, and 303 K

	V_R/cm^3		
Solute	293 K	298 K	303 K
n-Propanol	17.4_0	17.2_6	17.2_2
n-Butanol	17.5_2	17.3_7	17.3_3
n-Pentanol	17.6_5	17.4_9	17.4_2
n-Hexanol	17.7_8	17.5_9	17.5_6
n-Heptanol	17.9_0	17.7_0	17.6_4

As a result, the solute retentions were measured at 298 K over the mobile-phase compositional range: pure water, followed by 10, 30, 50, 70, and 90% v/v methanol. The results are presented in Table 3. For mobile phases comprised of 50% or more of methanol, the retention volumes did not change significantly with composition. However, for mobile phases of less than 30% methanol, the retentions increased substantially with homolog number.

Numerical Computation. The void volumes calculated from the data for n-pentanol through n-heptanol at 0 and 10°C were 17.6_9 and 17.6_6 cm^3, respectively, in good agreement with the average of all of the data of Table 2 and well as those for 50-90% methanol in Table 3. In addition, this value agrees to within experimental error with the tare-weight V_M, 18.1 cm^3. The alcohol solutes therefore appear to be better suited than the aromatic hydrocarbons for determination of the column void volume with the stationary and mobile phases to hand in this work.

Graphical Variant. The plots of r against assumed V_M are shown in Fig. 2 for the alcohol retention data with water (open circles) and 10% methanol (filled circles) mobile phases. Each clearly provides a maximum at ca. 18.0_3 cm^3, again in good agreement with the tare-weight void volume as well as with the raw V_R for the alcohol solutes with mobile phases of 50% or greater methanol content.

TABLE 3

Retention Volumes for Aliphatic Alcohol Solutes at 298 K with Mobile Phases Comprised of Indicated Percentages of Methanol in Water

	V_R/cm^3					
Solute	90%	70%	50%	30%	10%	0%
Ethanol	17.2_5	17.3_3	17.5_2	17.6_7	17.8_1	17.8_3
n-Propanol	17.3_6	17.4_4	17.5_5	17.7_2	17.8_5	18.0_8
n-Butanol	17.4_5	17.5_5	17.6_7	18.0_0	18.2_3	18.3_0
n-Pentanol	17.5_4	17.6_6	17.9_2	18.5_4	18.8_2	19.2_1
n-Hexanol	17.6_3	17.8_1	18.2_5	19.7_3	21.6_5	23.3_4
n-Heptanol	17.7_3	17.9_6	18.5_4	22.7_1	31.3_8	38.7_1

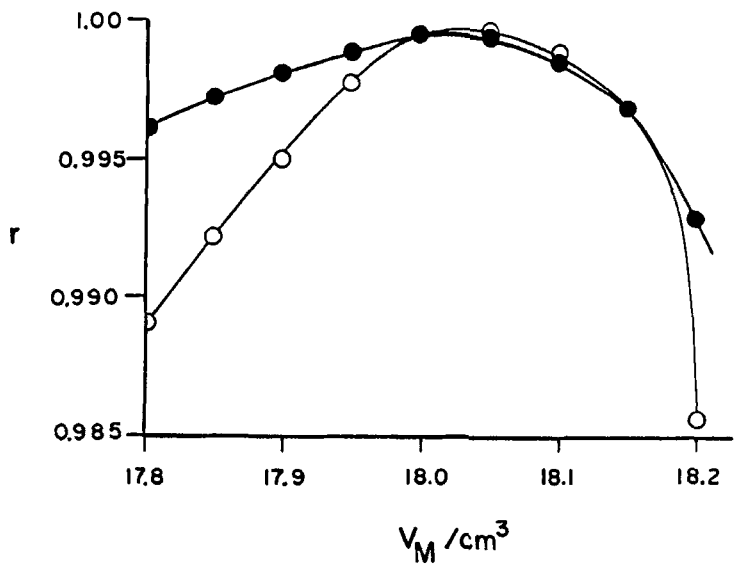

FIGURE 2. As in Fig. 1; aliphatic alcohol solutes. Filled circles: 10% methanol/90% water mobile phase; open circles: water carrier.

TABLE 4

Retention Volumes for Methanol and Water at 298 K with Mobile Phases Comprised of Indicated Percentages of Methanol in Water

	V_R/cm^3						
Solute	100%	90%	70%	50%	30%	10%	0%
Methanol		17.5_2	17.3_3	17.5_9	17.7_6	17.7_9	17.7_9
Water	17.8_0	17.9_1	17.8_7	17.6_5	17.6_5	17.6_0	
Ave.		17.7_2	17.6_0	17.6_2	17.7_1	17.7_0	

The Method of Minor Disturbance

The observed disturbance peak-maximum retention volumes at 298 K are presented in Table 4. We found, first, that the averages of the data were in substantial agreement with the values of V_M found by the methods previously described. Also, the void volumes derived from the band maxima of individual carrier components were coincident to better than ± 2%. However, when the mobile phase was comprised mostly of methanol, the retention volumes for methanol were in fact marginally smaller than those for water; while with less methanol in the mobile phase the retention volumes for water were the smaller of the two.

It is noteworthy that several of the retention volumes presented in Table 3 are smaller than those shown in Table 4, for example, the retentions for ethanol, n-propanol, and n-butanol with 90% methanol as well as those for ethanol and n-propanol with 50% methanol are each less than the void volume determined by the minor disturbance method for mobile phases of the same respective compositions.

The Use of Inorganic Salts

The retention volumes of $NaNO_3$ with the indicated mobile phases at 293, 298, and 303 K are presented in Table 5. We found, first, that the reproducibility of the retention data was better than ± 1.5%. Secondly, the

TABLE 5

Retention Volumes for Sodium Nitrate at 293, 298, and 303 K with Methanol Mobile Phase; and with Pure Methanol to Pure Water Mobile Phases at 298 K

	V_R/cm^3						
T/K	100%	90%	70%	50%	30%	10%	0%
293	17.6_1						
298	17.9_4	18.0_3	17.4_6	17.7_5	17.7_4	18.0_5	18.0_4
303	17.4_9						

sample size did not seem to matter, i.e., all retentions for the salt agreed to within experimental error. However, the void volumes determined by this method were larger than those found with some other solutes, e.g., methanol and water with the minor disturbance technique.

Comparison of Methods

Although close to experimental error, the methods of tare weight, inorganic salts (here, $NaNO_3$), and minor disturbance, all gave V_M that were somehat higher than several of the measured retention volumes and, consequently, must be said to be accordingly less accurate for the systems to hand in this work. In contrast, the numerical computational method (or its graphical counterpart) with the aliphatic alcohols solutes appears to yield reasonable results that are at least self-consistent. However, it is clear that this method requires that the retention volume of the last-retained solute be ca. twice the dead volume in order to minimize the effects of experimental scatter. That is, the smaller the difference of the probe-solute retentions from the true V_M the more precise will the measurements of V_R have to be. Also, utilizing long-retained members of an homologous series will obviate the difficulty often encountered with small molecules, namely, that the latter often exhibit specific energetic interactions with the stationary phase that do not obtain for higher members of the same series.

ACKNOWLEDGMENTS

We gratefully acknowledge support received for this work in part from the Department of Energy Office of Basic Energy Sciences, and from the National Science Foundation.

REFERENCES

1. Schoenmakers, P. J., Optimization of Chromatographic Selectivity: A Guide to Method Development, Vol. 35, Journal of Chromatography Library, Elsevier, Amsterdam, 1986.

2. Berridge, J. C., Techniques for the Automated Optimization of HPLC Separations, Wiley, New York, 1986.

3. Jones, P., and Wellington, C. A., J. Chromatogr., 213, 357 (1981).

4. Laub, R. J., and Purnell, J. H., J. Chromatogr., 112, 71 (1975).

5. Hsu, A.-J., Laub, R. J., and Madden, S. J., J. Liq. Chromatogr., 7, 615 (1984).

6. Laub, R. J., J. Liq. Chromatogr., 7, 647 (1984).

7. Al-Thamir, W. K., Purnell, J. H., Wellington, C. A., and Laub, R. J., J. Chromatogr., 173, 388 (1980).

8. McCormick, R. M., and Karger, B. L., Anal. Chem., 52, 2249 (1980).

9. Wells, M. J. M., and Clark, C. R., Anal. Chem., 53, 1341 (1981).

10. Slaats, E. H., Markovski, W., Fekete, J., and Poppe, H., J. Chromatogr., 207, 299 (1981).

11. Krstulovic, A. M., Colin, H., and Guiochon, G., Anal. Chem., 54, 2438 (1982).

12. Colin, H., Krstulovic, A. M., Guiochon, G., and Bounine, J. P., Chromatographia, 17, 209 (1983).

13. Mockel, H. J., and Freyholdt, T., Chromatographia, 17, 215 (1983).

14. Jinno, K., Ozakai, N., and Sato, T., Chromatographia, 17, 341 (1983).

15. Vit, I., Popl, M., and Fahnrich, J., J. Chromatogr., 281, 293 (1983).

16. Melander, W. R., Erard, J. F., and Horvath, Cs., J. Chromatogr., 282, 211 (1983).

17. Engelhardt, H., Muller, H., and Dreyer, B., Chromatographia, 19, 240 (1983).

18. Gutnikov, G., and Hung, L., Chromatographia, 19, 260 (1984).

19. Sadek, P. C., Carr, P. W., and Bowers, L. D., LC Mag. 3, 590 (1984).

20. Zhu, P. L., Chromatographia, 20, 425 (1985).

21. Knox, J. H., and Kaliszan, R., J. Chromatogr., 349, 211 (1985).

22. Laub, R. J., and Madden, S. J., J. Liq. Chromatogr., 8, 173 (1985).

23. Smith, R. J., Nieass, C. S., and Wainwright, M. S., J. Liq. Chromatogr., 9, 1387 (1986).

24. Boehm, R. E., and Martire, D. E., J. Phys. Chem., 84, 3620 (1980).

25. Martire, D. E., and Boehm, R. E., J. Phys. Chem., 87, 1045 (1983).

26. Pretorius, V., and Lawson, K., J. High Resolut. Chromatogr. Chromatogr. Commun., 9, 335 (1986).

27. Scott, R. P. W., and Kucera, P., J. Chromatogr., 125, 251 (1976).

28. Lai, S.-T., Sangermano, L., and Locke, D. C., J. High Resolut. Chromatogr. Chromatogr. Commun., 7, 158 (1984).

29. Unger, K. K., Jilge, G., Kinkel, J. N., and Hearn, M. T., J. Chromatogr., 359, 61 (1986).

30. Noda, K., Ohashi, M., and Ishida, K., J. Chem. Eng. Data, 27, 32 (1982).

31. Huber, G. A., and Kovats, E. sz., Anal. Chem., 45, 1155 (1973).

32. Fritz, D. F., and Kovats, E. sz., Anal. Chem., 45, 1175 (1973).

33. Laub, R. J., Anal. Chem., 56, 2110, 2115 (1984).

34. Locke, D. C., and Martire, D. E., Anal. Chem., 39, 921 (1967).

35. Laub, R. J., in Chromatography and Separations Chemistry, ACS Symposium Series No. 297, Ahuja, S., ed., American Chemical Society, Washington, D.C., 1986, Ch. 1.

APPENDIX. DEFINITION OF V_M FOR NEAT MOBILE PHASES, A SPECIAL CASE

As an alternative, in the limiting case of pure mobile phases, it would seem reasonable to define the column void volume as that which gives an activity coefficient of unity for the carrier in itself (e.g., methanol in methanol), that is, force a kind of thermodynamic "normalization". To evaluate this approach, we measured the fully-corrected molality-based (31,32) activity coefficient $\eta_1^{S,\infty}$ of methanol in OV-1 by GLC:

$$V_g^T(\mathrm{GLC}) = \frac{10^{-3}RT}{\eta_1^{S,\infty} p_1^o} \exp\left(-\frac{p_1^o B_{11}}{RT}\right) \qquad (A.1)$$

where $V_g^T(\mathrm{GLC})$ is the solute specific retention volume at the column temperature T, R is the gas constant, p_1^o is the bulk-solute vapor pressure, and B_{11} is its second-interaction virial coefficient (33). V_M was then adjusted so as to yield a $\gamma_1^{M,\infty}$ of unity in the expression for the specific retention volume of methanol solute with pure methanol carrier (34):

$$V_g^T(\mathrm{LLC}) = \frac{V_R - V_M}{w_S} = \frac{10^{-3}\,\gamma_1^{M,\infty} M_M}{\eta_1^{S,\infty} \rho_M} \qquad (A.2)$$

where $\gamma_1^{M,\infty}$ is the solute mole-fraction based activity coefficient in the mobile phase M of density ρ_M and molecular weight M_M. The result was 17.0_4 cm^3, which is reasonably close to the values derived by the methods mentioned earlier, particularly that with the homologous series of alcohols, 17.5 cm^3.

Presumably, "normalization" of the mobile-phase activity coefficient cannot be applied to multicomponent carriers because it is not possible to define an activity coefficient for the blend. However, this is not so, for example, Laub (35) found that a mean activity coefficient $\bar{\gamma}_M$ can be defined for mixed solvents B + C (= M) of mole-fraction composition $x_B = 1 - x_C$ that takes the general form:

$$\ln \bar{\gamma}_M = \frac{g^E}{2RT} = \frac{1}{2}\left[x_B \ln \gamma_{B(M)} + x_C \ln \gamma_{C(M)}\right] \qquad (A.3)$$

The column void volume might therefore be calculable from some weighted average of carrier-component retention data and eqns. A.2 and A.3, the further study of which we hope soon to report.