Journal of Chromatography A, 1218 (2011) 2995–3001

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comparison of void volume, mobile phase volume and accessible volume determined from retention data for oligomers in reversed-phase liquid chromatographic systems

Mei Wang[a], Jennifer Mallette[b], Jon F. Parcher[a,*]

[a] National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA
[b] Chemistry Department, University of Mississippi, University, MS 38677, USA

ARTICLE INFO

Article history:
Received 11 November 2010
Received in revised form 27 February 2011
Accepted 16 March 2011
Available online 7 April 2011

Keywords:
Void volume
Accessible volume
Tracer pulse chromatography

ABSTRACT

The experimental technique of mass spectrometric tracer pulse chromatography was used to determine the void volume, i.e., the total volume of eluent in the column, and the volume of eluent moving freely through the column, i.e., mobile phase volume, for a series of eluents with a C_{18}-bonded RPLC column. The interstitial volume of the column was determined by size exclusion chromatography. In order to evaluate the utility of the accessible volumes determined from the retention volumes of homologous solutes, the accessible volume of the column was determined as a function of eluent composition and temperature with polystyrene and polyethylene glycol samples using Martin's Rule. Comparison of these four measured volumes indicated that the experimentally measured accessible volumes did not correspond to either the void volumes, mobile phase volumes or interstitial volumes.

1. Introduction

The void volume, V^0, of liquid chromatographic columns is usually defined as the total volume of eluent in a column [1]. Although this definition of void volume is neither theoretically nor thermodynamically meaningful, such a void volume definition does allow the determination of an experimentally accessible quantity. The measured void volume is often combined with the interstitial volume of a column determined by size exclusion chromatography to estimate the pore volume of chromatographic packings. The change in pore volume with temperature, eluent composition, or sample type can then be used to investigate retention mechanisms and pore-filling phenomena [2]. This approach, however, is only valid if all eluent components reside either in the pore volume or the interstitial volume, not within or on the stationary phase.

The most commonly reported retention parameter for LC systems is the retention factor, k_i. The retention factor is defined as the ratio of the number of moles of component i in the stationary phase, n_i^S, to the number of moles in the mobile phase, n_i^M. This parameter is determined experimentally from the retention volume of component i, $V_{R,i}$, and the volume of the mobile (freely flowing) phase, V^M.

$$k_i = \frac{n_i^S}{n_i^M} = \left\{ \frac{V_{R,i} - V^M}{V^M} \right\} \quad (1)$$

Defined thusly, the quantities k_i and V^M are both thermodynamically sound. However, because accurate determination of the volume of mobile phase in an LC column is difficult, the total volume of eluent, V^0, or the accessible volume, V_0^*, is often substituted for the volume of mobile phase, V^M, in the calculation of the retention factor. The validity of such a substitution is determined by the soundness of the assumption that none of the eluent is held fixed either in or on the stationary phase.

The calculated retention factor can be used in a variety of ways. This is a dimensionless parameter that is employed primarily for method development and for comparison of LC columns. If it is assumed that the phase ratio is constant, the temperature dependence of the retention factor can be used to determine thermodynamic parameters, such as enthalpy and entropy change, for the transfer of a solute between the stationary and mobile phases in a chromatographic column [3–5]. The thermodynamic information is often subsequently used for the elucidation of chromatographic retention mechanisms. Likewise, the composition dependence of the retention factor can lead to the determination of equilibrium isotherms for solutes in or on the stationary phase [6–8]. The main objective of such studies is usually the investigation of retention mechanisms along with the prediction of elution profiles of solutes as a function of the concentration of solute and the

* Corresponding author. Tel.: +1 601 232 7301; fax: +1 601 232 7300.
E-mail address: chjfp@olemiss.edu (J.F. Parcher).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.03.037

composition of the eluent. Furthermore, retention factor data are often used to determine the selectivity of various columns and the optimization of chromatographic methods and columns. Thus, the accurate measurement of the retention factors for RP-HPLC systems is indispensable for the development of new separations and the optimization of existing applications.

Given the inherent significance of the exactitude of column void volume data [9,10] in the accurate determination of retention factor data, it is somewhat surprising that there is currently no widely accepted experimental method for the determination of this vital quantity. Myriad experimental methods have been suggested for the measurement of void volumes in liquid chromatographic systems and a wide variety of reviews have appeared in the literature on this subject [11–17]. In 2002, Rimmer et al. [18] reviewed existing methods which included pycnometry, the minor disturbance and tracer pulse methods, unretained markers and homologous series of solutes.

Pycnometry is an accurate experimental method for the determination of void volumes; however, it requires off-line manipulation of the column. As a result, this is not a very popular method for the determination of void volumes. Moreover, void volumes determined by pycnometry appear to represent a lower limit since chromatographically measured void volumes are usually higher than the pycnometric values.

Void volumes can be accurately determined from minor disturbance techniques by measuring the retention volumes of concentration pulses over the full eluent composition range. The void volume can then be determined by integration [16,19]:

$$V^0 = \int_0^1 V_R \, d\,\theta_i^M \tag{2}$$

where V_R is the retention volume of the concentration pulse at a fixed eluent composition θ_i^M. This particular method yields only a single, integrated value of the void volume.

Another experimental method involves the use of isotopically labeled components of the eluent. This method, commonly referred to as tracer pulse chromatography, provides a value of the void volume at any eluent composition from the following relation [1,20,21]

$$V^0 = \sum^{i} V_{R,i}^{*} \theta_i^M \tag{3}$$

where $V_{R,i}^{*}$ is the retention volume of a detectable isotope of eluent component i. This experimental technique was recently evaluated by Fornstedt [22,23].

These three techniques viz., pycnometry, tracer pulse and minor disturbance methods, produce comparable data representing the total volume of eluent in a column without regard to the distribution of eluent between the stationary and mobile phases or between the interstitial and pore volumes.

Several additional methods have been proposed for the estimation of column void volumes. Unretained markers represent the most popular method for the determination of column void volume. Unfortunately, no solute has yet been identified that is unretained at all eluent compositions. The retention volume of a injection solvent peak is frequently used as a measure of the void volume. A similar method involves the use of a very strong eluent such as pure acetonitrile to prevent retention of any solute. In this case, the retention volume of any unexcluded solute would yield a measure of the void volume; whereas, the retention of any completely excluded solute will equal the interstitial volume. The major problem with the last three techniques is the difficulty of ensuring that the probe solutes or eluent components are truly unretained by the bonded stationary phase. The use of the retention time of an injection solvent is particularly questionable in view of the many investigations indicating that most common reversed phase liquid chromatography eluents are sorbed by RPLC bonded packings [1,14,24–30].

2. Theory

Recently, polymer chemists have used the retention of oligomers with Martin's rule to determine a characteristic volume of chromatographic columns. This volume is often used as a void volume for subsequent determination of retention factors. This idea is based on the observed logarithmic relationship between the retention volume of oligomers and the number of repeat units, n, within a homologous series. This relation is known as Martin's Rule [31]:

$$\ln\left(\frac{V_{R,n} - V^M}{V^M}\right) = A + Bn \tag{4}$$

where A and B are adjustable parameters. In early work, the retention factors for each oligomer were calculated from the retention volumes and an estimated value of the mobile phase volume. The "best" linear fit was obtained by adjusting the mobile phase volume to give the maximum value of the correlation coefficient for the regression.

In 1980, Berendsen et al. [11] developed a method to determine the *mobile phase* volume by considering the retention volumes of a homologous series of alcohols. The mobile phase volume can be obtained from the slope and intercept of a plot of the retention volume of consecutive homologues (V_{n+1} vs. V_n) using the following relationship

$$V_{n+1} = (1 - e^B)V^M + e^B V_n \tag{5}$$

where e^B represents the separation factor, α, between two neighboring homologues assuming the separation factor is constant and independent of the number of repeat units. Berendsen's experimental results indicated that the volumes measured from pycnometry, tracer pulse chromatography, and a homologous series were equivalent in pure water. However, the volume measured from Eq. (5) varied with eluent composition. The change in mobile phase volume was attributed to the solvation of the bonded phase by the eluent. In pure methanol, only the volumes determined from pycnometry and tracer pulse chromatography agreed. The principle disadvantage of this method is the uncertainty in the calculations of the volume due to its indirect determination from both the slope and intercept. It is not necessary to know the value of n, but the homologues must differ consistently by one unit.

More recently, Trathnigg and Skvortsov [32] developed a slightly different data analysis scheme for the determination of a volume they designated as the accessible volume, V_0^*. In this method, a volume was determined that produced a linear relationship between the retention volumes of consecutive homologues and the difference in the elution volumes of neighboring homologues, $\Delta V_n = V_{n+1} - V_n$. From the following equation, it was shown the intercept will give a value for the accessible volume, V_0^*, by extrapolation to $\Delta V_n = 0$.

$$V_{n+1} = V_0^* + \left\{\frac{e^B}{e^B - 1}\right\} \Delta V_n \tag{6}$$

One advantage of this method is the fact that the accessible volume can be obtained directly from the intercept only. Often, however, this technique may involve extensive extrapolation which, in turn, lowers the accuracy of the measured volume. The volume, V_0^*, represents an adjustable parameter used to produce a linear relation between $\ln\{V_{R,i} - V_0^*\}$ and $\ln k$ and the degree

of polymerization of the polymeric solutes. The physical meaning of V_0^* is uncertain.

Several investigators have measured V^M (Eq. (5)) or V_0^* (Eq. (6)) as a function of temperature or eluent composition. The experimental results are somewhat contradictory. Berendsen et al. [11] found that the volumes calculated from the application of Martin's rule decreased as the proportion of methanol in methanol/water eluents increased. Trathnigg et al. [2] found that the calculated volumes increased as the percentage of THF in THF/water eluents increased. The same group [32] found that the calculated volume only varied slightly with eluent composition for aqueous acetone or methanol eluents. Kim et al. [5] measured V_0^* from polystyrene samples in a RPLC system with THF/methanol eluents and found that the measured volume decreased significantly as the percentage of THF in the eluents increased. In the same study, it was determined that V_0^* decreased slightly with increasing temperature. Similar results were obtained by Möckel [33] in an earlier investigation. However, direct comparison of these diverse experiments is dubious because different columns, eluents and samples were used by the various investigators.

Some general conclusions can, however, be derived from the literature. In most cases, (1) there does exist a volume that will produce a linear plot of $\ln k$ vs. n (Martin's rule), (2) this volume changes only slightly with temperature, (3) it varies significantly with the type and composition of eluent and (4) the calculated volume is usually less than the void volume measured by some of the other experimental procedures discussed previously. This last point is also questionable because results have also been reported [34] where $V_0^* > V^0$ and even instances where $V_0^* < 0$ [35]. The relation between the accessible volume and the interstitial volume measured from size exclusion chromatography with excluded solutes is also uncertain. However, in the few reported comparisons, the measured accessible volume was always greater than the interstitial volume [2,34]. Thus, a fifth general conclusion could be posited, viz., the exact physical significance of the accessible volume calculated for the retention of polymeric oligomers has not yet been established. It is possible that the accessible volume does not accurately or consistently provides a measure of either void volume, interstitial volume or the volume of mobile phase.

The objective of the present investigation was to clarify the uncertainties in the meaning of measured accessible volumes determined from the retention of polymeric solutes. The experimental approach was to compare the accessible volumes measured with different polymers and various eluents with void volumes determined by tracer pulse chromatography, interstitial volumes determined by size exclusion chromatography and mobile phase volumes determined from excess sorption data. A secondary goal was to determine the validity of retention factor data obtained by substituting the accessible volume for the mobile phase volume.

3. Experimental

3.1. Chemicals

The HPLC grade eluents methanol, acetonitrile, water and optima grade tetrahydrofuran were purchased from Fisher Scientific. Polyethylene glycol 300, 400, and 600 were obtained from Sigma–Aldrich. Polystyrene samples with masses of 820 and 100,000 were purchased from Aldrich and Fluka, respectively. Isotopic samples of methanol (methanol-d_3, 99.8 atom % D), acetonitrile (acetonitrile-d_3, 99.8 atom % D), tetrahydrofuran (tetrahydrofuran-d_8, 99.8 atom % D), and water (water-d_2, 99.8 atom % D) were obtained from Aldrich.

3.2. Column

The HPLC column used in this work was an Agilent ZORBAX Eclipse Plus C18 column. The column dimensions were 4.6 mm × 250 mm. The ZORBAX Rx-SIL support has a nominal surface area of 160 m^2/g and a controlled pore size of 95 Å. The particle size was 5 μm. The column was thermostated in a water jacket.

3.3. Instrumentation

3.3.1. HPLC

The liquid chromatography system was a Hewlett-Packard 1050. This instrument included a single pump with a proportioning valve, an autosampler with a 25 μL sample loop, a multi-wavelength UV–Visible detector, and ChemStation software.

3.3.2. MS

The mass spectrometer was the detector in an Agilent 6120 Single Quad LC/MS instrument. This system included a multimode ionization source and a single quadrupole mass analyzer. The ionization source was capable of either atmospheric pressure electro-spray ionization (APESI) or atmospheric pressure chemical ionization (APCI).

3.4. Experimental procedures

The flow rate for all experiments was 0.5 mL/min, and the sample size was 5 μL. Mass spectrometric tracer pulse experiments were used to determine the void volume and excess isotherms in all eluent systems [24]. The volume of the mobile phase in the column was estimated from the measured excess volume of the eluent components. The interstitial volume of the column was determined by size exclusion chromatography with high molecular weight polystyrene samples with pure tetrahydrofuran as the eluent. The extra column volume was measured from the retention time of any solute without the column in the system. All retention data reported in this work were corrected for this contribution. The sample solvents used in this study were always the chromatographic eluent. The eluents were used as the injection solvent in order to avoid the potential influence of the injection solvent on the retention of the oligomeric analytes.

Accessible volumes were determined with samples of polyethylene glycol and polystyrene. Polyethylene glycol 300, 400, and 600 were detected with the mass spectrometer in binary aqueous eluents of methanol and acetonitrile because the samples did not absorb in the UV–Vis range. Polystyrene was detected with the UV–Vis detector in a tetrahydrofuran/methanol eluent because the non-polar polystyrene sample could not be ionized in either the APCI or APESI sources.

The excess volume of eluent taken up by the bonded packing was determined from tracer pulse experimental data using the relation [24,36]:

$$V_i^{XS} = \theta_i^M [V_{R,i}^* - V^0] \quad (7)$$

where V_i^{XS} represents the total volume of component i in the system in excess of the volume of component i that would be in the same system (V^0) with a totally inert adsorbent [36,37]. This definition corresponds to the \vNA convention proposed by Riedo and Kovats [37]. The mobile phase volume, V^M, is related to the excess volume by the relation

$$V_i^{XS} = V_i^s - (V^0 - V^M)\theta_i^M \quad (8)$$

where V_i^s represents the volume of eluent component i held static by the bonded stationary phase. Excess isotherms usually display a non-stationary inflection point in the range $0.4 \le \theta_i^M \le 0.8$. In this

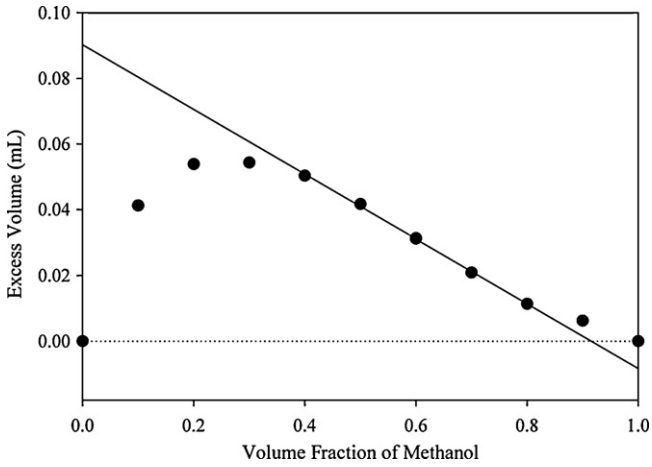

Fig. 1. Excess volume isotherm for methanol on reversed-phase liquid chromatography bonded packing at 30 °C. The linear regression results were: slope = $V^0 - V^M$, intercept = 0.090 = V_i^s.

range, the value of V^M can be determined from linear regression of the excess isotherm [36,38].

4. Results and discussion

4.1. Polyethylene glycols (PEGs) with methanol/water eluents

The results of the tracer pulse and polymer experiments with all eluents are given in Table S-1. Fig. 1 illustrates the excess sorption isotherm for methanol in or on the C_{18}-bonded phase. The value of $V^s = (V^0 - V^M)$, the volume of eluent in the bonded stationary phase, determined from the linear portion of the excess isotherm was 99 μL in the range $0.4 \leq \theta_i^M \leq 0.8$. For water-rich eluents ($\theta_i^M \leq 0.4$), it was assumed that water was unretained by the hydrophobic bonded phase [26] and the mobile phase volume was thus equal to the retention volume of deuterated water, i.e., $V^M = V^*_{R,H_2O}$. The interstitial volume of the column was determined to be 1.64 mL.

For the determination of accessible volumes from oligomeric solutes, a mass spectrometer operated in the selected ion monitor mode was used as the detector. One of the advantages of this detection system was it's ability to "resolve" oligomer peaks that were not actually resolved in the chromatographic column.

Fig. 2 illustrates the relation between the various volumes measured for this system. The void volume was relatively constant over the full composition range. The mobile phase volume diminished slightly as the amount of methanol taken up by the stationary phase increased.

The accessible volume was calculated from three PEG samples with different nominal molecular weights (300, 400 and 600). Overlapping oligomer sets were used to assess the reproducibility of the retention volumes of the same oligomer in different samples as well as to extend the range of oligomer numbers available. The accessible volumes were determined from both Eqs. (5) and (6). Only data for which the results of the two data analysis schemes agreed within 5% were included in the analysis. Over the composition range $0.1 \leq \theta_i^M \leq 0.6$, the accessible volumes were less than either the void or mobile phase volumes but greater than the interstitial volume. Eluents rich in methanol produced no separation, and all of the oligomers eluted within the void volume. The measured accessible volumes varied (1.7–2.0 mL) with eluent composition but in a rather unsystematic manner.

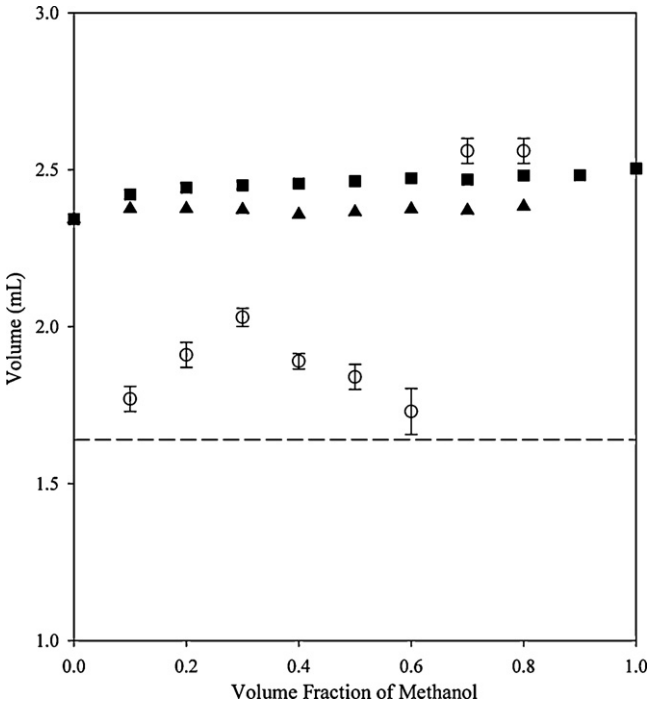

Fig. 2. Comparison of calculated volumes with methanol/water eluents. (■) Void volume from Eq. (3). (○) Accessible volume from Eqs. (5) and (6) using PEG oligomers. (▲) Volume of the mobile phase from Eq. (8). "—" interstitial volume.

4.2. Polyethylene glycols with acetonitrile/water eluents

Methanol/water was not a satisfactory eluent system for the elution of PEG oligomers from the C_{18}-bonded phase system. Thus, the eluent was changed to ACN/water. Excess isotherm data for this eluent are also given in the supplemental material. The total volume of acetonitrile taken up by the stationary phase within the composition range $0.4 \leq \theta_i^M \leq 0.8$ was determined from the excess isotherm to be 440 μL. Thus, about 4 times as much acetonitrile as methanol was taken up by the bonded phase in that composition range. This ratio is in excellent agreement with previous investigations involving both tracer pulse [24,25] and concentration pulse chromatography [27,39,40]. This significant uptake of acetonitrile by the bonded stationary phase resulted in a decreased mobile phase volume with the amount of acetonitrile in the eluent as shown in Fig. 3. The calculated accessible volumes agreed roughly with those measured for the methanol/water system at $\theta_{ACN}^M \leq 0.3$. Thus, the observed decrease in V^M did not appear to influence the calculated value of V_0^*. As the concentration of acetonitrile was increased the system collapsed the accessible volume approached the value of the void volume, so all of the solutes eventually eluted at the void volume. However, the calculated accessible volumes did not consistently agree with either the measured void, interstitial or mobile phase volumes.

Several investigators have found an inverse temperature dependence of the retention volumes of PEGs with acetonitrile [4,41,42]. The effect of this abnormal temperature dependence on the calculated accessible volumes with a fixed eluent composition of 30% ACN is shown in Fig. 4 as a plot of V_{n+1} vs. ΔV_n (Eq. (6)). The inverse temperature dependence is obvious from this graph. The inset shows that the calculated V_0^* (intercept) increased slightly as the temperature increased. The variation of V_0^* as a function of temperature is illustrated in Fig. 5 along with the experimental data of Kim et al. [5] for a system of polystyrene with 10% THF in methanol normalized to a column length of 250 mm rather than the 150 mm column used in the original publication. Although comparison of

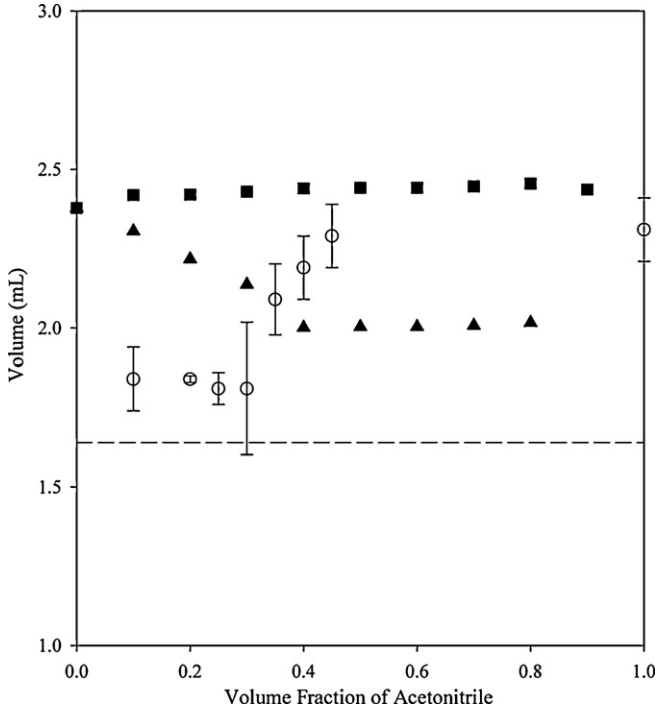

Fig. 3. Comparison of calculated volumes with acetonitrile/water eluents. (■) Void volume from Eq. (3). (○) Accessible volume from Eqs. (5) and (6) using PEG oligomers. (▲) Volume of the mobile phase from Eq. (8).“—” interstitial volume.

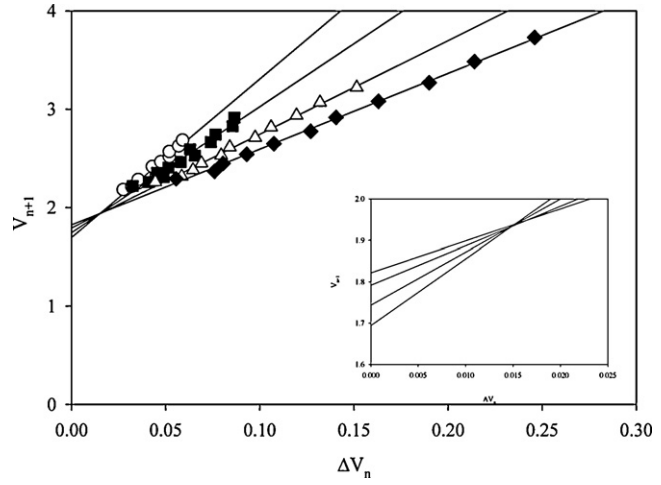

Fig. 4. Plot of V_{n+1} vs. ΔV_n for PEG oligomers as a function of temperature. (○) 30 °C, (■) 40 °C, (△) 50 °C and (♦) 60 °C.

these diverse systems is problematic, it is clear that the temperature dependence of V_0^* is not significant for these systems.

4.3. Polystyrenes with tetrahydrofuran/methanol eluents

Polystyrene oligomers were not soluble in aqueous eluents, so the eluent system was changed to THF/MeOH. The measured excess isotherms indicated that THF was preferentially taken up by the bonded phase; however, the total volume of eluent in that phase, V^s, was only 97 μL, i.e., about the same as the amount of methanol taken up from aqueous eluents. Thus, the volume of the mobile phase was approximately equal to the void volume as shown in Fig. 6. The calculated accessible volumes, however, displayed a significant decrease with increasing concentration of THF in the eluent. Surprisingly, when the system collapsed, i.e., lost resolution, the retention volume of the oligomers was much greater than the measured void volume. That is, the oligomers were retained but not resolved. The decrease in V_0^* with increasing concentration of THF has been observed previously by Kim et al. [5].

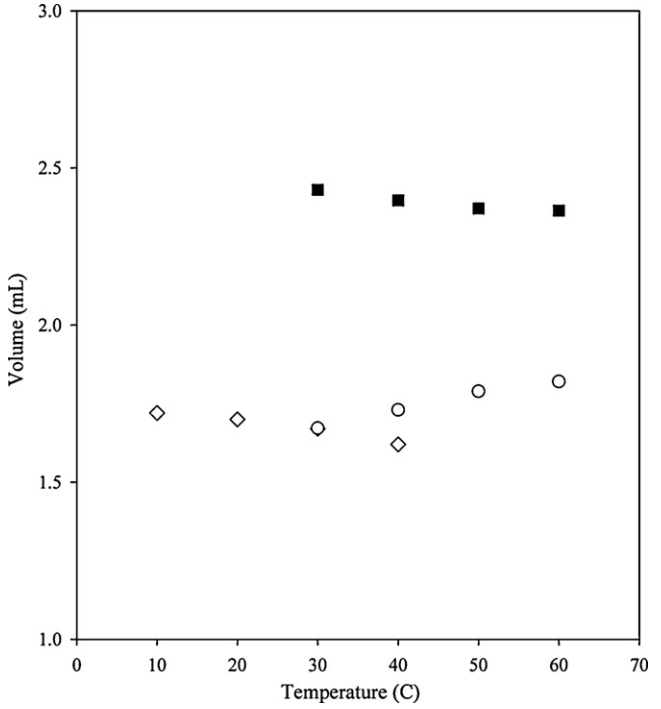

Fig. 5. Temperature dependence of void volumes and accessible volumes. (■) Void volume from Eq. (3). (○) Accessible volume from Eqs. (5) and (6) using PEG oligomers with acetonitrile/water eluents. (◊) Accessible volume from Ref. [23] using polystyrene oligomers with tetrahydrofuran/methanol eluents.

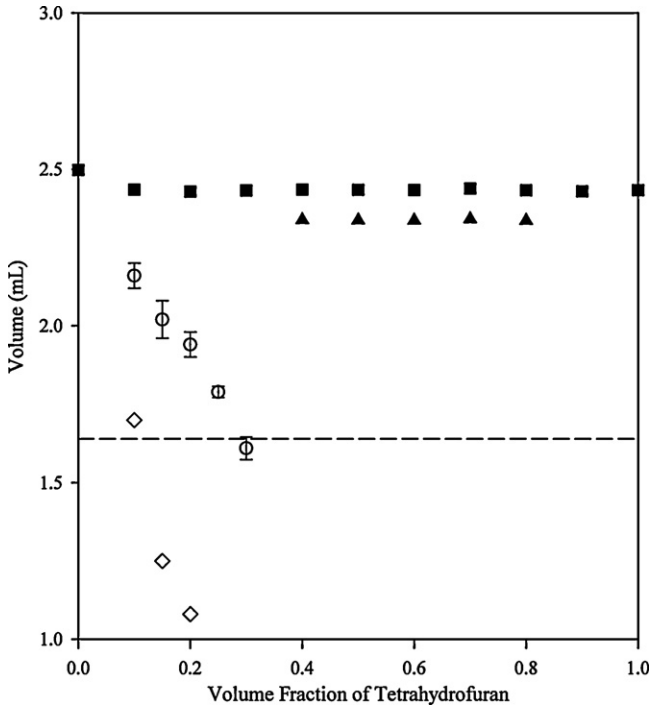

Fig. 6. Comparison of calculated volumes with tetrahydrofuran/methanol eluents. (■) Void volume from Eq. (3). (○) Accessible volume from Eqs. (5) and (6) using polystyrene oligomers. (◊) Accessible volume from Ref. [23]. (▲) Volume of the mobile phase from Eq. (8). “—” interstitial volume

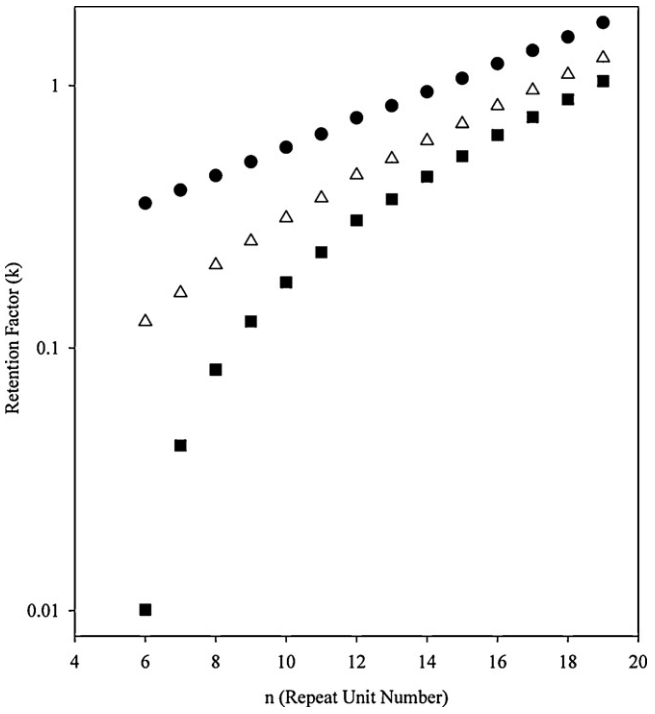

Fig. 7. Martin's rule plot for retention factors with various volumes in a 25%. acetonitrile/water eluent. (●) Calculated using the accessible volume (V_0^*). (△) Calculated using the volume of the mobile phase (V^M). (■) Calculated using the void volume (V^0).

5. Conclusions

The accessible volume calculated from the retention volumes of polyethylene glycol and polystyrene oligomers did not consistently agree with the void volume of RPLC columns measured by other techniques. Moreover, the calculated accessible volumes appear intermediate between the interstitial volume and the volume of the mobile phase. The derived volumes do not vary significantly with temperature; however, they do appear to vary with the type and composition of eluents although the variations do not show any consistent pattern.

The physical meaning of the accessible volume has been called into question by other authors [2,3,5]. Hence, the common practice of substituting the accessible volume, V_0^*, or the total volume of eluent in the column, V^0, for the volume of mobile (freely flowing) phase in the column, V^M, is open to question. This uncertainty is especially important if the volumes are used for the calculation of retention factors with subsequent derivation of thermodynamic parameters. The error caused by inaccurate estimates of the mobile phase volume for the calculation of k may be tolerable for large k values ($k \geq 20$) or for systems in which the eluent does not interact with the stationary phase. However, unless these restrictions are fulfilled for a given system, caution should be exercised in the calculation of retention factors based on the substitution of the void volume, V^0, or the accessible volume, V_0^*, for the mobile phase volume, V^M.

The question then remains of whether or not the polymeric systems used for the calculation of the accessible volume conform to Martin's rule, i.e., Eq. (4). This in turn raises the question of whether or not a volume exists that produces a linear plot of $\ln k$ vs. n. To investigate these points, plots of $\ln k$ vs. n were produced from a typical data set obtained for PEG-600 with a 25% ACN/water eluent where the retention factors were calculated with the accessible volume, void volume and mobile phase volumes were used in Eq. (4). The results are shown in Fig. 7. The only volume that produced a linear relation between $\ln k$ vs. n was the accessible volume. The plots observed for the void volume and the volume of mobile phase were nonlinear.

6. Glossary of volume expressions and symbols

Expression	Symbol	Definition
Retention volume	$V_{R,i}$	The volume of eluent required to elute any analyte, *i*, from a column.
	$V_{R,n}$	The retention volume of a member of a homologous series with carbon number *n*
	$V^*_{R,i}$	The retention volume of a detectable tracer pulse analyte
	V_R	The retention volume of a detectable concentration pulse
Void volume	V^0	The total volume of eluent in a column. The void volume represents the interstitial volume plus the pore volume
Mobile phase volume	V^M	The volume of eluent that moves freely through the column
Interstitial volume		The volume of eluent in the interparticle regions of the column
Pore volume		Volume of eluent contained within the pores of the solid support.
Stationary phase volume (HPLC)		The volume of the bonded phase plus any eluent taken up by the bonded phase
Stationary phase volume (SEC)		The pore volume
Accessible volume	V_0^*	The volume of eluent that produces a linear relation between $\ln k$ and the carbon number for a series of homologous analytes (Eq. (4)).
Volume increment	ΔV_n	The difference in retention volumes of two homologous with carbon numbers of $n+1$ and n

Acknowledgments

This research was supported by grant CHE-0715094 from the National Science Foundation. The University of Mississippi provided funds for the instrumentation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.03.037.

References

[1] J.H. Knox, R. Kaliszan, J. Chromatogr. 349 (1985) 211.
[2] B. Trathnigg, M. Veronik, A. Gorbunov, J. Chromatogr. A 1104 (2006) 238.
[3] Y. Kim, S. Ahn, T. Chang, Anal. Chem. 82 (2010) 1509.
[4] B. Trathnigg, N.V. Cuong, H. Ahmed, J. Sep. Sci. 32 (2009) 2857.
[5] Y. Kim, S. Ahn, T. Chang, Anal. Chem. 81 (2009) 5902.
[6] J. Putnam, G. Guiochon, J. Chromatogr. A 1217 (2010) 8146.
[7] A. Asnin, K. Kaczmarski, G. Guiochon, J. Chromatogr. A 1217 (2010) 7055.
[8] L. Asnin, F. Gritti, K. Kaczmarski, G. Guiochon, J. Chromatogr. A 1217 (2010) 264.
[9] J. Samuelsson, P. Sajonz, T. Fornstedt, J. Chromatogr. A 1189 (2008) 19.
[10] J. Samuelsson, T. Fornstedt, Anal. Chem. 80 (2008) 7887.
[11] G.E. Berendsen, P.J. Schoenmakers, L. de Galan, J. Liq. Chromatogr. 3 (1980) 1669.
[12] H. Engelhardt, H. Muller, B. Dreyer, Chromatographia 19 (1984) 240.
[13] B.A. Bidlingmeyer, F.V. Warren, A. Weston, C. Nugent, J. Chromatogr. Sci. 29 (1991) 275.
[14] H. Poppe, J. Chromatogr. A 656 (1993) 19.
[15] R.A. Djerki, R.J. Laub, J. Liq. Chromatogr. 10 (1987) 1749.
[16] Y.V. Kazakevich, H.M. McNair, J. Chromatogr. Sci. 31 (1993) 317.
[17] A.M. Krstulovic, H. Colin, G. Guiochon, Anal. Chem. 54 (1982) 2438.
[18] C.A. Rimmer, C.R. Simmons, J.G. Dorsey, J. Chromatogr. A 965 (2002) 219.
[19] Y.V. Kazakevich, Y.A. Eltekov, Chromatographia 25 (1988) 965.
[20] F. Helfferich, J. Chem. Educ. 41 (1964) 410.
[21] F. Helfferich, D.L. Peterson, Science 142 (1963) 661.
[22] R. Arnell, T. Fornstedt, Anal. Chem. 78 (2006) 4615.

[23] D. Samuelsson, R. Arnell, J.S. Diesen, J. Tibbelin, A. Paptchikhine, T. Fornstedt, P.J.R. Sjoberg, Anal. Chem. 80 (2008) 2105.
[24] M. Wang, J. Mallette, J.F. Parcher, Anal. Chem. 80 (2008) 6708.
[25] M. Wang, J. Mallette, J.F. Parcher, Anal. Chem. 81 (2009) 984.
[26] A. Alvarez-Zepeda, D.E. Martire, J. Chromatogr. 550 (1991) 285.
[27] Y.V. Kazakevich, R. LoBrutto, F. Chan, T. Patel, J. Chromatogr. A 913 (2001) 75.
[28] R.M. McCormick, B.L. Karger, Anal. Chem. 52 (1980) 2249.
[29] J. Samuelsson, P. Forssen, M. Stefansson, T. Fornstedt, Anal. Chem. 76 (2004) 953.
[30] P.L. Zhu, Chromatographia 20 (1985) 425.
[31] A.J.P. Martin, Biochem. Soc. Symp. 3 (1949) 4.
[32] B. Trathnigg, A. Skvortsov, J. Chromatogr. A 1127 (2006) 117.
[33] H.J. Möckel, J. Chromatogr. A 675 (1994) 13.
[34] B. Trathnigg, O. Jamelnik, J. Chromatogr. A 1146 (2007) 78.
[35] B. Trathnigg, M.I. Malik, N.V. Cuong, A. Skvortsov, J. Chromatogr. A 1207 (2008) 122.
[36] M. Wang, J. Mallette, J.F. Parcher, J. Chromatogr. A 1190 (2008) 1.
[37] F. Riedo, E. Kovats, J. Chromatogr. 239 (1982) 1.
[38] M. Wang, J. Mallette, J.F. Parcher, J. Chromatogr. A 1213 (2008) 105.
[39] F. Gritti, Y.V. Kazakevich, G. Guiochon, J. Chromatogr. A 1169 (2007) 111.
[40] F. Gritti, G. Guiochon, J. Chromatogr. A 1155 (2007) 85.
[41] D. Cho, S. Park, J. Hong, T. Chang, J. Chromatogr. A 986 (2003) 191.
[42] B. Trathnigg, M. Veronik, J. Chromatogr. A 1091 (2005) 110.