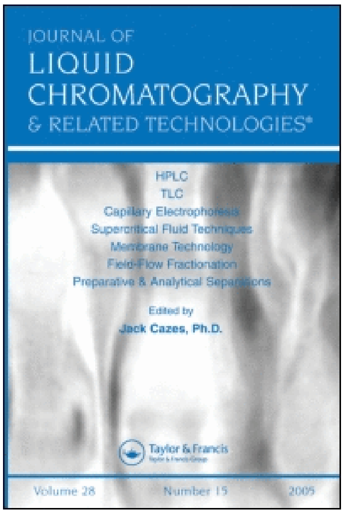

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SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. V. THE COLUMN DEAD VOLUME

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ABSTRACT

Methods of determination of the column void volume with two homologous series of solutes (alkyl-substituted aromatic hydrocarbons and phenyl-substituted aliphatic alcohols) are considered in terms of correlation coefficients r of plots of log V_R' against carbon number over the temperature range 10°-30°C. The homologous series methods provide self-consistent fits as well as temperature-invariant data for the aromatic hydrocarbons, but not for the alcohols, for which a mixed retention mechanism is said to obtain. A new graphical method of determining the best-fit value of V_A is introduced that makes use of plots of r against assumed V_A. In contrast to work reported elsewhere by others, each of the series of compounds studied in this work clearly exhibits a maximum. Those for the aromatic hydrocarbons are temperature-invariant; however, this is not so for the alcohols, which is taken as evidence of thermal and/or multiple sorption interactions of these solutes with the stationary phase.

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INTRODUCTION

Measurement of meaningful chromatographic data requires determination of the system void ("dead") time t_A or volume V_A. This is rarely a problem in gas-liquid chromatography (glc) where, typically, air or an inert gas are used that under normal working temperatures are essentially insoluble in (therefore unretained by) the stationary phase (1). In contrast, in liquid chromatography, there is at present considerable ambiguity surrounding the mechanisms of solute retention and, hence, choice of a solute that is not sorbed by the column packing (2,3). The complication may arise also of penetration of pores of the packing by small presumed non-sorbed compounds, while solutes that are retained are excluded. It is therefore assumed generally in lc that the supposed unretained solute used to measure the column void space must be of the same hydrodynamic volume as the analytes under investigation (4-6).

An approach to measurement of V_A that overcomes these difficulties makes use of the well-known relation (applicable both in gc and lc) between the adjusted retention volumes V'_R and unit numbers of members of an homologous series:

$$\log V'_R = a'n + b' \quad (1)$$

where $V'_R = V_R - V_A$; a' and b' are series constants; and n is the number representing each homologous unit. Reiterative self-consistent fitting can then be employed to deduce V_A, a', and b' from experimental V_R (7). The calculations can also be carried out exactly, as shown by Al-Thamir, Laub, Purnell, and Wellington (8) who introduced a simple variation of eqn. 1:

$$V_R = V_A + \exp(an + b) \quad (2)$$

Eqn. 2 provides for direct evaluation of a and b and, thus, of V_A, by solving the simultaneous equations provided by the retentions of three or more members of an homologous series. However, as shown in this work, both eqns. 1 and 2 can under some circumstances fail to converge on a self-consistent value of the column dead volume. We report here a study of these situations, as well as the experimental criteria that define the limits of accuracy of eqn. 2, hence, eqn. 1.

EXPERIMENTAL

Solvents. "HPLC" grade tetrahydrofuran (THF) (Fisher Scientific) was employed as received. The aqueous portion of the mobile phases comprised doubly-distilled and deionized water and reagent-grade acetic acid.

Mobile-Phase Preparation. Acetic acid at a level of 1% v/v was added to the water component of the mobile phase to diminish interactions between residual unreacted accessible silanol groups and the hydroxyl groups of solute alcohols. Solutions of the aqueous blend with the organic modifier (THF) were then prepared by volume at concentrations of 40% or 50% as noted.

Solutes. Two homologous series were employed. The series of aromatic alcohols consisted of benzyl alcohol, 2-phenylethyl alcohol, 3-phenyl-1-propanol, 4-phenyl-1-butanol, and 5-phenyl-1-pentanol. The aromatic series consisted of toluene, ethylbenzene, n-propylbenzene, and n-butylbenzene. Also used were benzene, pyrene, and perylene. All solutes were supplied by Chem Service, and all mixtures were prepared as solutions in the respective mobile phase of each set of experiments.

Instrumentation. The liquid chromatograph employed in this work was a Varian Model 8500 with an LDC UV monitor set at 254 nm. Injection was carried out with a Valco valve fitted with a 10-µl external loop. The column was 25 cm x 4.5 mm i.d. containing 5µ Spherisorb ODS, and was thermostated with a water jacket. Care was taken to ensure also that the mobile phase was brought to temperature before entering the column.

Procedures. Following the establishment of temperature and column/mobile-phase equilibration, as judged by the constancy of retention times, the homologous series being investigated was injected onto the column either as individual solutes or in admixture, there being no detectable differences in observed retention times between the two methods of sample introduction. During the time of elution, an accurate determination of the flow rate was made using a calibrated and thermostated (25°C) buret.

Retention volumes were taken as the average of three repetitive measurements at 5° intervals between 10° and 30°C for both homologous series. The mobiles phases comprised a volume fraction ϕ of 0.4 (i.e., 40% v/v THF in 1% acetic acid-water) for the aromatic alcohols, and ϕ = 0.5 for the aromatic hydrocarbons. Retention volumes were also obtained for benzene, pyrene, and perylene with 100% THF at 10°-30°C.

RESULTS AND DISCUSSION

The Aromatic Hydrocarbon Homologous Series. Presented in Table 1 are the raw retention volumes V_R for the aromatic hydrocarbon homologous series. When these data were used in conjunction with eqn. 2, V_A was found to be affected rather substantially by the choice of which set of three solutes was used for the calculations, that is, toluene (n = 1), ethylbenzene (n = 2), n-propylbenzene (n = 3); or ethylbenzene (n = 2), n-propylbenzene (n = 3), n-butylbenzene (n = 4). Table 2 provides as an example the values of **a** calculated for each homologous series with eqn. 2 and the data of Table 1.

The dependence of **a** on the range of **n** was found to be due to the sensitivity of eqn. 2 to small errors in the measurement of V_R. For example, if the retention volume of n-propylbenzene at 15°C were in fact 13.43 cm^3 instead of 13.63 cm^3, the resultant value of **a** would then be 0.359, that is, approximately one-third larger for a 0.2-cm^3 change in retention volume. To circumvent this problem, the average of the **a** values

TABLE 1

Raw Retention Volumes V_R/cm^3 for the Aromatic Hydrocarbon Homologous Series (10°-30°C)

	Temperature/°C				
Solute	10.0	15.0	20.0	25.0	30.0
Toluene	9.04	8.57	8.16	7.87	7.51
Ethylbenzene	11.4	10.7	10.0	9.57	8.98
n-Propylbenzene	14.8	13.6	12.7	11.9	11.1
n-Butylbenzene	19.2	17.4	15.9	14.8	13.6

TABLE 2

Values Calculated with Eqn. 2 and Data of Table I with n = 1 to 3 and n = 2 to 4

	Temperature/°C				
	10.0	15.0	20.0	25.0	30.0
a (n = 1 to 3)	0.401	0.366	0.327	0.333	0.321
a (n = 2 to 4)	0.250	0.239	0.240	0.158	0.197
ave. **a**	0.320	0.296	0.277	0.260	0.264
ave. **b**	1.53	1.54	1.53	1.52	1.36
V_A/cm^3	2.62	2.31	2.08	1.90	2.39

was used to compute values of **b** for the two sets of homologous series, n = 1 to 3 and n = 2 to 4, and the resultant averaged **b** then used in the final calculation of the dead volume. The results for the two series at each temperature are shown in Table 2 where, if one assumes that the dead volume is temperature-independent over the range studied, the final value of V_A is found to be 2.26 cm^3. Our results therefore substantiate the point made by Al-Thamir, et al., who noted that since three data points will lead to a consistent albeit potentially inaccurate value of V_A, at least four members of an homologous series must be used (as in this work) in assessing the column void volume.

We illustrate in Figure 1 plots of log V_R' against carbon number, where the value of 2.26 cm^3 was taken as V_A. The correlation coefficients at each temperature are, respectively, 0.99990 (10°), 0.99992 (15°), 0.99994 (20°), 0.99990 (25°), and 0.99990 (30°C).

The Alcohol Homologous Series. Upon applying the same analysis procedure to the raw retentions of the alcohol homologous series, Table 3, anomalous values of **a** were again found as shown in Table 4. Close inspection of the latter indicated that the values calculated with n = 1 to 3 were far too discrepant to be acceptable even when the sensitivity of the method to errors in retention-volume measurements was taken into consideration. We are therefore led to postulate that more than one retention mechanism contributes to the elution of benzyl alcohol (see later).

TABLE 3

Raw Retention Volumes V_R/cm^3 for the Alcohol Homologous Series (10°-30°C)

Solute	Temperature/°C 10.0	15.0	20.0	25.0	30.0
Benzyl alcohol	4.61	4.44	4.27	4.26	4.12
2-Phenylethyl alcohol	5.08	4.86	4.68	4.61	4.48
3-Phenyl-1-propanol	6.35	6.02	5.77	5.62	5.41
4-Phenyl-1-butanol	8.17	7.65	7.23	6.96	6.61
4-Phenyl-1-pentanol	11.0	10.1	9.36	8.85	8.29

TABLE 4

Values Calculated with Eqn. 2 and Data of Table 3 with n = 1 to 3, 2 to 4, and 3 to 5

	Temperature/°C 10.0	15.0	20.0	25.0	30.0
a (n = 1 to 3)	0.994	0.938	0.978	1.06	0.949
a (n = 2 to 4)	0.360	0.355	0.292	0.283	0.255
a (n = 3 to 5)	0.427	0.472	0.378	0.344	0.337
ave. **a**[a]	0.394	0.415	0.335	0.313	0.296
ave. **b**[a]	0.165	-0.048	0.316	0.368	0.383
V_A/cm^3 [a]	2.50	2.66	1.92	1.92	1.84

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[a] averaged values calculated from the series: n = 2 to 4 and n = 3 to 5

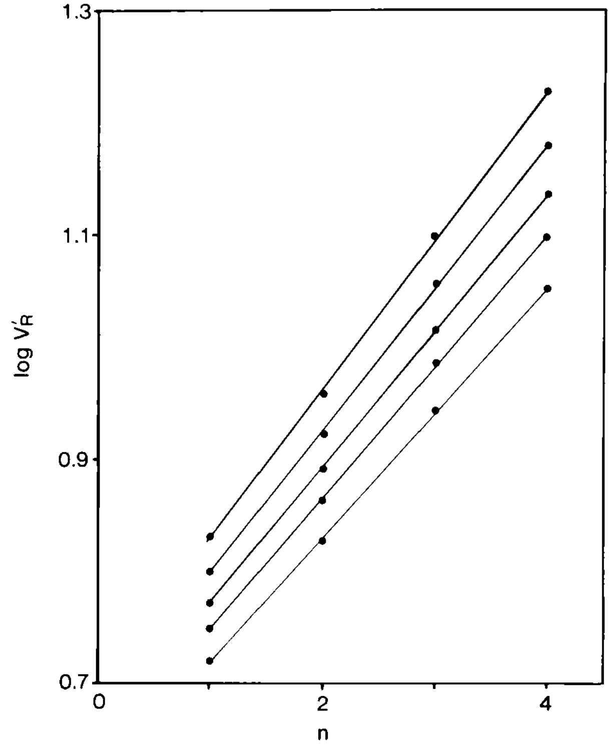

FIGURE 1. Plots of log V_R' against carbon number n for alkyl-substituted aromatic hydrocarbon solutes at 10° (upper line) to 30° (lower line) at intervals of 5°C.

Table 4 also provides the values of **a** and **b** for the series n = 2 to 4 and n = 3 to 5, averaged in a similar fashion as before. The resultant dead volumes lead to an overall average V_A of 2.17 cm^3, which compares favorably with the value of 2.26 cm^3 calculated with the aromatic hydrocarbon series. The validity of the result is illustrated in Figure 2, which shows plots of log V_R' against carbon number for the solutes of n = 1 to n = 5, and where the correlation coefficients (excluding benzyl alcohol, n = 1) are 0.99991 (10°), 0.99996 (15°), 0.99994 (20°), 0.99994 (25°), and 0.99990 (30°).

Anomalous Retention of Benzyl Alcohol. The plots in Figure 2 show clearly that benzyl alcohol (n = 1) is indeed anomalous, as was deduced also

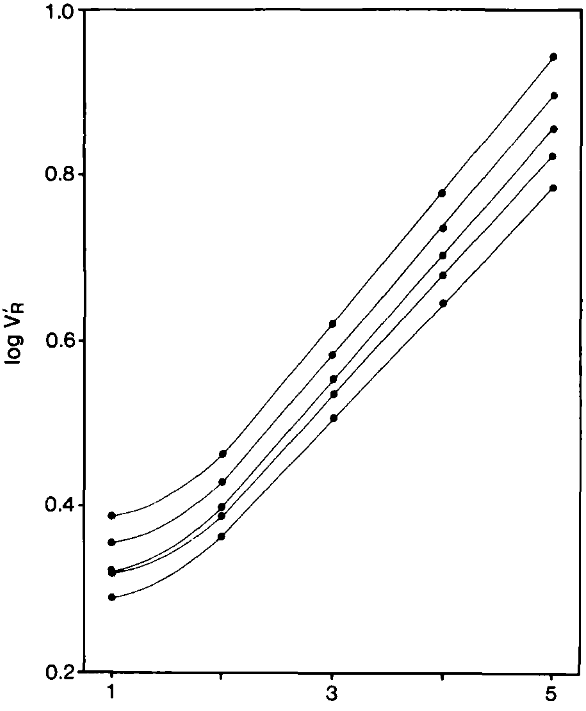

FIGURE 2. As in Figure 1; phenyl-substituted aliphatic alcohol solutes.

from the data of Table 4. It might be supposed that the larger than expected retention volume could be due to increased pore penetration by the relatively small benzyl alcohol molecule, thus increasing the surface area available for sorption of it by the stationary phase. However, we would then expect the first member of the aromatic hydrocarbon series to exhibit analogous behavior also, there being little size difference between toluene and benzyl alcohol. This was not observed.

Corroborative evidence that a size exclusion mechanism is not involved in the retention of benzyl alcohol is provided in Table 5, which gives the raw retentions of a variety of aromatic compounds ranging in size from benzene to perylene (100% THF mobile phase).

TABLE 5

Raw Retention Volumes V_R/cm^3 for Indicated Aromatic Compounds; Mobile Phase: 100% THF

Solute	Temperature/°C 10.0	15.0	20.0	25.0	30.0
Benzene	2.30	2.29	2.32	2.19	2.26
n-Butylbenzene	2.30	2.29	2.27	2.21	2.26
5-Phenyl-1-propanol	2.32	2.28	2.24	2.28	2.27
Pyrene	2.30	2.26	2.24	2.26	2.25
Perylene	2.30	2.26	2.24	2.24	2.24

In view of these data, there is little question that there is only negligible retention of the solutes by the stationary phase with pure THF carrier. Indeed, to within an experimental error of ± 2%, there is in fact no difference in the retentions of any of these compounds at any temperature.

A more plausible explanation for the extra-long retention of benzyl alcohol lies in the strength of hydrogen bonding exhibited by this compound relative to the latter members of the series, as exemplified in the plots of glc partition coefficients of alcohols against the volume fraction of oxygen-containing additives in admixture with hydrocarbon stationary phases reported by Laub, Purnell, and Vargas de Andrade (9,10). Particularly noteworthy are those for the aliphatic alchohols methanol to n-butanol ($n = 1$ to $n = 4$) with di-n-octyl ether/n-heptadecane stationary phase. Plots of the apparent complex formation constant K_1 against carbon number of these species are illustrated in Figure 3, where a sharp drop is seen on passing from the first member of the series to the last. Thus, the anomalously-high liquid-chromatographic retention exhibited by benzyl alcohol can be ascribed at least in part to the increased strength of hydrogen bonding of this solute with the stationary phase.

Graphical Determination of V_A. Finally, we consider a graphical method of establishing the maximal correlation coefficient r of plots of log V_R' against carbon number as an alternative technique of determining a

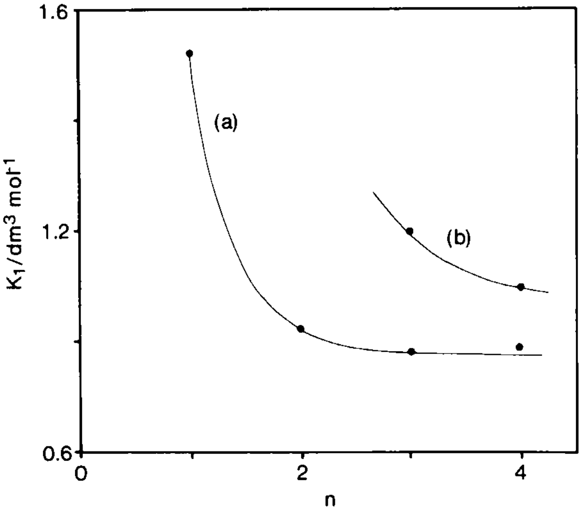

FIGURE 3. Plots of notional K_1 for the aliphatic alcohols methanol (n = 1) to n-butanol (n = 4) with (a) di-n-octyl ether/n-heptadecane and (b) di-n-octyl ether/n-octadecane. Data calculated from partition coefficients provided in Refs. 9 and 10.

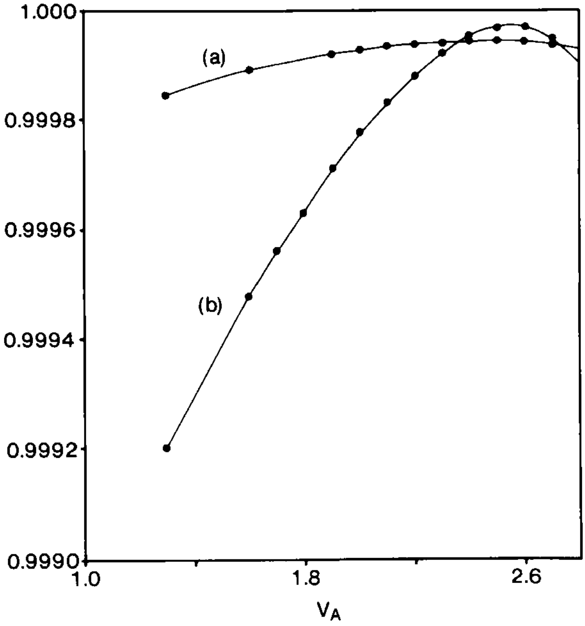

FIGURE 4. Plots of correlation coefficient r against assumed column void volume V_A for (a) aromatic hydrocarbons and (b) alcohols homologous series of solutes at 10°C.

"best-fit" dead volume. In doing so, we test the claim of Krstulovic, Colin, and Guiochon (11) that very high values of **r** can be obtained even if V_A is varied by upwards of ± 20% (cf. Table I of Ref. 11) since, although insufficient data were reported with which to test their claim, it nevertheless casts some doubt upon the method indicated by eqns. 1 and 2.

We show in Figures 4-8 plots of **r** against assumed V_A for both homologous series at 10^o-30^oC. Those for the aromatic hydrocarbons are indeed quite shallow although do in fact provide a distinct maximum at each temperature. Further, the values of these remain very nearly constant at 2.2-2.3 cm^3. In addition, the midpoint of the range, 2.25 cm^3, is in good agreement with the average of 2.26 cm^3 deduced from the data of Table 2. In contrast, there is a well-defined maximum exhibited in each of the plots of the alcohols data. However, there is clearly an appreciable shift also in the "best-fit" void volume on passing from 10^o (V_A = 2.6 cm^3) to 30^oC (V_A = 1.8 cm^3). This result can be explained only on the basis either of a mixed retention mechanism of the alcohols with the stationary phase, and/or as arising from thermal interaction(s) as described above and as illustrated in Figure 3. Moreover, the situation is obviously potentially quite complex since, if hydrogen bonding is presumed to occur, the magnitude of the interaction will be diminished as the column temperature is increased. However, there is no evidence to support the notion that the value of apparent K_1 (i.e., the various thermodynamic properties of sorption) for each member of the homologous series will be decreased by precisely the amount required overall in order that the "best-fit" V_A remain constant when deduced from plots of log V_R' against carbon number. Indeed, quite the opposite would be expected to be the case. We find, then, that the graphical procedure, as well as the calculational methods of eqns. 1 and 2, provide an accurate and self-consistent value of the column void volume (including invariance as a function of temperature) provided that the test solutes are not subject to specific thermal interactions with the stationary phase, the latter accounting for the anomalies reported by Krstulovic, et al. (11).

CONCLUSIONS

It is evident that precise measurement of the column dead volume in lc is not a trivial matter. Analysis of the data reported in this work makes

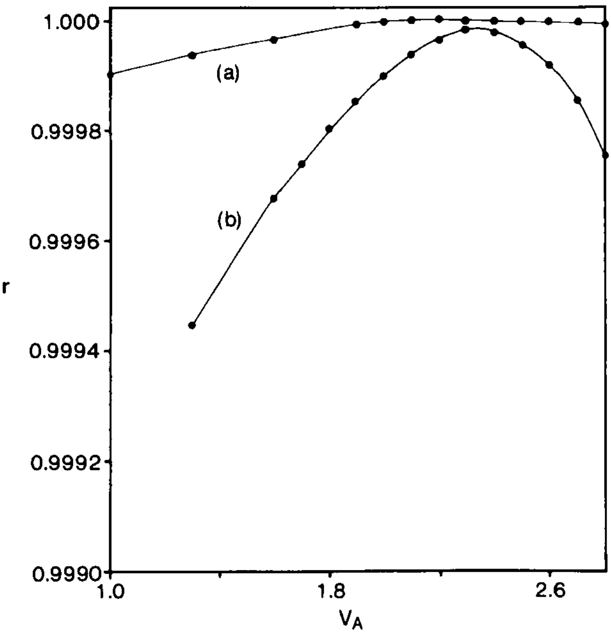

FIGURE 5. As in Figure 4; 15°C.

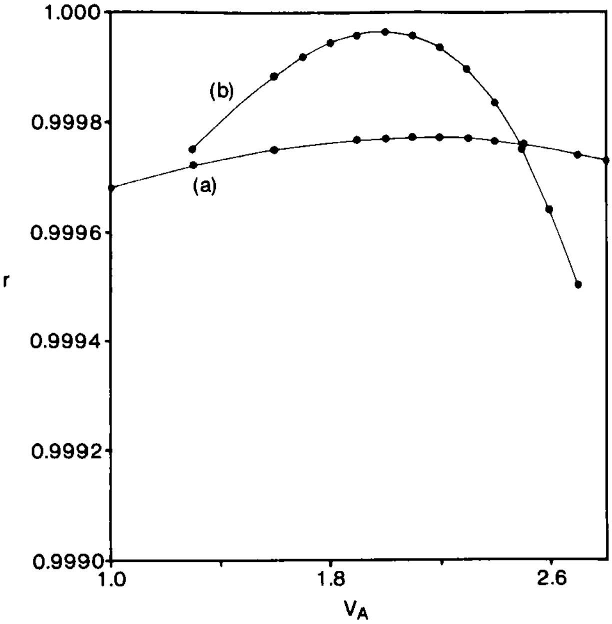

FIGURE 6. As in Figure 4; 20°C.

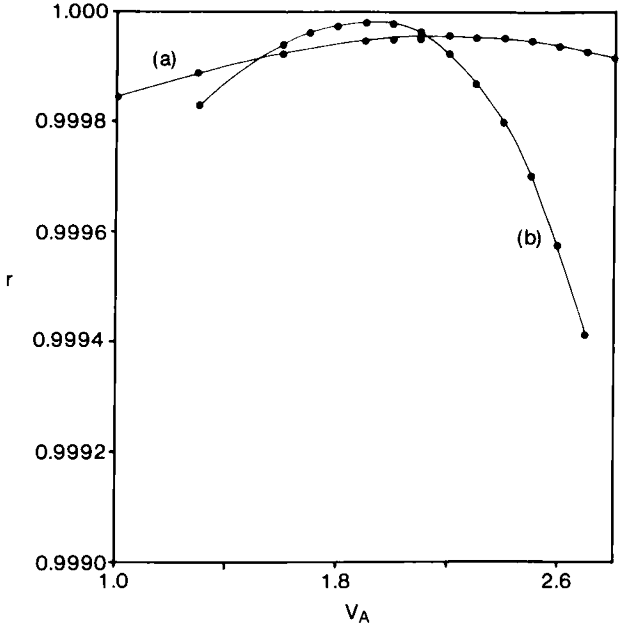

FIGURE 7. As in Figure 4; 25°C.

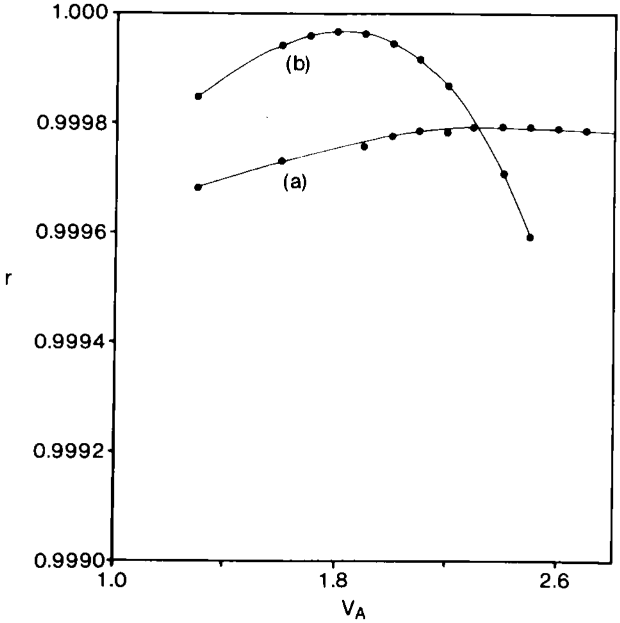

FIGURE 8. As in Figure 4; 30°C.

it clear also that when either the homologous-series calculational or graphical methods of assessment of V_A are employed, only a single sorption mechanism should ideally be operative for all members of it. Variation of the column temperature moreover provides the means of establishing if in fact the interaction accounting for the retentions is athermal. In contrast, mixed sorption mechanisms will lead to erroneous results unless each is independent of the others and all are temperature-insensitive. By way of corollary, anomalies in plots of log V_R against **n** (e.g., Figure 2) as well as those of r against assumed V_A (Figures 4-8) appear to be useful tools for detecting thermal and/or multiple retention mechanisms, and may in fact provide also means of quantitating the magnitude of each.

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