DETERMINATION OF THE DEAD VOLUME OF COLUMNS IN REVERSED PHASE LIQUID CHROMATOGRAPHY

Ivan VÍT[a], Jan FÄHNRICH[b] and Milan POPL[b]

[a] *Laboratorní přístroje, 162 03 Prague 6 and*
[b] *Department of Analytical Chemistry, Prague Institute of Chemical Technology, 166 28 Prague 6*

Received May 5, 1988
Accepted August 16, 1988

The method for the determination of the dead volume of RPLC columns by measuring the retention volume of phloroglucinol is compared with the method based on the retention data of homologous series of organic solutes and that based on the use of isotopically labelled components of the mobile phase. The use of homologous series is tedious and moreover, satisfactory results are not always obtained. The phloroglucinol method is found well suited to replacing the method based on the use of isotopically labelled components of the mobile phase; poorer results with phloroglucinol are only obtained when the mobile phase contains more than 40% (v/v) water, for the Separon SE polymeric sorbent, and in alkaline medium.

Several methods are available for the determination of the dead volume V_m of columns in reversed phase liquid chromatography. The most frequently used method consists of the use of isotopically labelled molecules in the mobile phase, such as deuterated water[1-6], methanol[7], or acetonitrile[7,8]. Another widespread method is based on the measurement of the retention volumes of substances that are assumed not to be retained by the column tested; these include salts of organic or inorganic acids[9-11] or various polar organic compounds[10,12-15]. In the minor disturbance method, the detector response to a minor change in the mobile phase composition is monitored. The dead volume is also determined from retention volumes of substances constituting homologous series[6,16-18] or from retention volumes measured at different temperatures[19]. The method of weighting the dry column and column filled with liquids with different densities as the mobile phases is also employed[2,6].

This variety of methods is partly due to the fact that the definition of the dead volume is not unique. In the simplest case, the dead volume is identified with the retention volume of some compound regarded as unretained. However, that this substance is really unretained is not always certain. Moreover, retention volumes of substances with zero sorption enthalpy are not all identical; actually, they will depend on their molecular size. The different size of accessible space in the column is evident for macromolecular substances, the size exclusion mechanism, however, operates also with low molecular weight substances[20]. For example, for a sorbent

with a specific surface area of 500 $m^2 g^{-1}$, a change in the molecular size of 0·2 nm is accompanied by a change in the volume of accessible space on the order of 0·1 ml . . g^{-1}. In addition, substances ionic in nature are subjected to ion exclusion unless electrolyte is present in the mobile phase in a sufficient concentration[21]. Establishing the dead volume as the volume of the mobile phase in the column also meets with problems because the mobile phase-stationary phase interface is difficult to locate accurately. Moreover, in RPLC the components of the mobile phase permeate partly into the stationary phase, as was first pointed out by Knox and Pryde[22]; the volume of the stationary phase thus is varied in dependence on the mobile phase composition, and this is accompanied by a complementary change in the mobile phase volume.

Thus, the approach to the dead volume of a column is associated with a choice of convention. In the simplest case, this concerns the choice of the test substance. Melander and coworkers[3] suggest the assumption that that component of the mobile phase possessing the lowest affinity for the stationary phase is absent from the latter. With regard to this, D_2O is frequently employed as the test substance in RPLC. The experimental isotherms for the mobile phase components in RPLC, however, are more complex. They can serve to determine the dead volume assuming that the sorption isotherm is linear over a certain region of the mobile phase composition[5].

The simple use of D_2O is associated with a number of other inherent and technical drawbacks. Its small molecules can permeate into the tiny pores of silica gel, inaccessible to the other substances involved, as has been demonstrated by exclusion limit measurements[20]. A next problem is the possible isotope exchange at the residual silanol groups, again bringing about a higher retention of labelled hydrogen by the column. If containing exchangeable hydrogen, the organic component of the mobile phase, e.g. methanol, then intervenes into the equilibrium. Furthermore, D_2O is not amenable to detection by photometric detectors while the use of refractometric detectors is associated with many practical problems and for some modern methods, such as microcolumn liquid chromatography, it is impractical altogether.

Measurements of the detector response to a small change in the mobile phase composition give dead volume values dependent on the slope of the sorption isotherms of the mobile phase components. The calculation of the dead volume from retention volumes of substances making up homologous series is based on the assumption that the quantity log k progresses linearly in this series. In some cases this method yields good results[21] but the availability of accurate data is a prerequisite, and for some series the assumption of linearity is not satisfied[23].

In contrast to the dead volume itself, its upper possible limit V_{max}, determined by the total porosity, is well defined. This is the total volume of eluent present in the column in both the mobile and stationary phases. This quantity can be established by weighing the dry column and column wetted by the mobile phase, or by weighing the column filled with solvents of different densities. It can be also determined from the retention volumes of isotopically labelled components of the mobile phase.

It is clear that the determination of the dead volume of chromatographic columns is associated with inherent problems. It is noteworthy that this quantity actually need not be included when describing the chromatographic process[24]; it is, however, used for the calculation of conventional retention parameters of classical chromatography, such as the capacity ratio or the distribution constant, which are directly related to the sorption thermodynamic quantities. Therefore, convenient methods for the determination of the dead volume continue to be sought. Recently, phloroglucinol has been proposed[25] as a suitable test substance in RPLC. It has been demonstrated by comparison with additional hydroxybenzenes and other test substances that phloroglucinol is well suited to this purpose unless the methanol content of the mobile phase is higher than approximately 60% (v/v). Its application to alkaline mobile phases is less suitable.

In the present work, the phloroglucinol method is compared with the methods for the calculation of the dead volume based on linearization in homologous series and on the use of isotopically labelled components of the mobile phase. In addition to methanol, mixtures of acetonitrile and tetrahydrofuran with water also were used as the mobile phases.

EXPERIMENTAL

Mobile phase was delivered using an HPP 4 001 high pressure pump (Laboratorní přístroje, Prague). A UV M4 photometric detector (Development Workshop, Czechoslovak Academy of Sciences) and an RIDK 101 refractometric detector (Laboratorní přístroje, Prague) were employed. A six-way injection valve with a 10 μl sample loop (Czechoslovak Academy of Sciences) or an LCI 02 septum injector (Laboratorní přístroje, Prague) with a 50 μl Hamilton syringe were used for the on-column injection. Columns used are given in Table I.

The basic components of the mobile phases were methanol (MeOH), acetonitrile (MeCN) and tetrahydrofuran (THF) combined with redistilled water. The organic components were of reagent grade purity and were rectified prior to use. The flow rate of the mobile phase was controlled by means of a microburette inserted after the detector. Retention volumes were calculated from the retention times obtained by using a Minigrator integrator (Spectra Physics) or were directly measured via the microburette.

RESULTS AND DISCUSSION

Linearization of Retention Data of Homologous Seris of Organic Substances

In the method of retention data linearization, series of substances whose members only differ in the number of certain structure units are employed. In such series the reduced retention volumes or capacity ratios frequently exhibit a linear dependence on the number of these units. Conversely, assuming that this dependence is linear, parameter b can be so chosen that the dependence of $\log(V_R - b)$ on the number of the structure units approach linearity as close as possible, and this parameter

then is regarded as the dead volume of the column. This approach has been adopted by many researchers[6,15–18,21]; the results compare favourably with those obtained using D_2O. Some other authors, however, observed[23,26] that this linearity is not obeyed in general, deviations occurring in homologous series after the solute chain length surpasses that of the stationary phase.

In the present work, the linear dependence of $\log (V_R - b)$ on the number of structure units z is transformed into the exponential form

$$V_R = aC^z + b \qquad (1)$$

and constants a, b and C are calculated by the least squares method.

This approach was applied particularly to series of hydroxybenzenes, in combination with which methanolic mobile phases have been studied[25]. Two hydroxybenzene series were used, viz. a series of symmetrically substituted hydroxybenzenes including benzene ($z = 0$), phenol ($z = 1$), hydroquinone ($z = 2$) and phloroglucinol ($z = 3$), and a series of nonsymmetrically substituted hydroxybenzenes containing benzene ($z = 0$), phenol ($z = 1$), pyrocatechol ($z = 2$) and pyrogallol ($z = 3$). Parameters of Eq. (*1*) for these series and different columns, using methanol–water mobile phases, along with the retention volumes of phloroglucinol are given in Table II.

The calculated values of parameter b are nearly identical with the retention volume of phloroglucinol, particularly at higher methanol contents. This warrants the approximation of the dead volume directly by the retention volume of this substance.

TABLE I
Chromatographic columns used

Label	Length mm	Inner diameter mm	Material	Packing	
				sorbent	particle size μm
A	250	4·0	stainless steel	Separon SiC 18	10
B	150	3·3	glass	Separon SiC 18	5
C	150	3·3	glass	LiChrosorb RP-8	5
D	150	3·3	glass	LiChrosorb RP-18	5
E	125	6·0	stainless steel	Separon SE	25—30

Poorer results, where the values for phloroglucinol are lower than the total column volume only in 100% and 90% (v/v) methanol, were obtained for column E packed with Separon SE, a styrene–ethylene dimethacrylate copolymer. The calculated *b*

TABLE II

Parameters of Eq. (*1*) for symmetrically and nonsymmetrically substituted hydroxybenzenes and retention volumes of phloroglucinol

MeOH % (v/v)	Symmetrically substituted hydroxybenzenes				Nonsymmetrically substituted hydroxybenzenes				V_p ml
	a ml	*b* ml	log *C*	$\sum\Delta^2$ 10^{-4} ml^2	*a* ml	*b* ml	log *C*	$\sum\Delta^2$ 10^{-4} ml^2	
				Column A					
100	0·746	2·15	−0·425	1·6	0·656	2·23	−0·501	8	2·19
90	1·21	2·15	−0·462	15	1·08	2·28	−0·566	10	2·19
80	2·63	2·24	−0·589	0·5	2·41	2·46	−0·694	110	2·29
70	4·59	2·19	−0·544	89	4·07	2·71	−0·719	610	2·25
60	9·03	2·14	−0·662	17	8·51	2·65	−0·748	610	2·26
				Column B					
70	1·397	0·884	−0·609	1·1	1·249	1·030	−0·776	20	0·91
				Column C					
100	0·142	0·95	−0·360	4·2	0·136	0·95	−0·331	0·2	0·97
90	0·261	0·93	−0·331	3·4	0·240	0·95	−0·285	0·6	0·96
80	0·513	0·90	−0·325	3·1	0·432	0·98	−0·373	4·3	0·96
70	0·805	0·90	−0·373	7·5	0·703	0·99	−0·420	8·9	0·97
60	1·516	0·90	−0·434	16	1·371	1·04	−0·480	30	0·99
				Column D					
100	0·227	0·77	−0·356	8·1	0·185	0·82	−0·543	2·7	0·79
90	0·412	0·78	−0·438	4·1	0·356	0·83	−0·538	0·9	0·80
80	0·727	0·75	−0·462	8·1	0·629	0·85	−0·560	8·5	0·79
70	1·230	0·77	−0·558	4·6	1·119	0·88	−0·649	18	0·81
60	2·491	0·77	−0·601	4·2	2·288	0·97	−0·711	44	0·82
				Column E					
100	1·608	2·51	−0·665	30	1·755	2·36	−0·717	0·2	3·00
90	4·231	1·70	−0·657	160	3·052	2·86	−0·558	3·1	2·94
80	6·516	1·84	−0·617	2 800	5·690	2·61	−0·619	14	3·55
70	15·566	2·19	−0·423	5 200	14·221	3·44	−0·411	2 400	3·67

values also fail to correspond to the dead volume of the column, and the high sum of squares of residuals, particularly for lower methanol contents, indicates a failure of the exponential dependence (*1*).

Additional measurements with three conventional organic components, viz. methanol (70% (v/v)), acetonitrile (70% (v/v)) and tetrahydrofuran (50% (v/v)), were performed using three glass columns packed with chemically bonded silica gel phases. In addition to the two hydroxybenzene series, series of alcohols (C_2-C_6, even members of the C_8-C_{20} series), fused aromatics (benzene, naphthalene, phenanthrene), alkanes (C_5-C_8), *p*-alkyl phenols (C_1-C_6) and chloromethanes (di-, tri- and tetrachloromethane) were also measured. The calculated *b* values are given in Table III.

The calculated parameter *b* frequently attains unreasonable values. The experimental retention volumes are largely too far from the *b* value, whereupon a small experimental error brings about an intolerably high error of calculation. For some substances, e.g. chloromethanes, the results are seen to be incorrect at first glance. Figs 1–3 demonstrate that the curve shapes are similar for sorbents with octadecyl chains whereas LiChrosorb RP-8 retains nonpolar substances to a considerably lesser extent, as has been also observed by other authors. This difference diminishes with increasing polarity of the solutes, and polyhydroxybenzenes are even retained more by LiChrosorb RP-8 than by the octadecyl groups-containing sorbents. This trend applies to all eluents used.

It can be concluded that the calculation of the dead volume from retention volumes in homologous series is questionable and the experimental procedure is tedious. In our case, reproducible and mutually consistent values failed to be obtained although V_R was measured using a burette with a 0·02 ml precision. Using methanol–water and acetonitrile–water mobile phases, the *b* values approached the expected value (0·85 ml) for the majority of series, the *b* values, as well as the phloroglucinol retention volumes V_P, on Separon SiC 18 and LiChrosorb RP-8 sorbents being lower in acetonitrile than in methanol. On LiChrosorb RP-18, on the other hand, all *b* values are higher in acetonitrile than in methanol while the reverse is true of the phloroglucinol retention volumes. This fact on its own points to a specificity in the sorption in different mobile phases, and documents how dubious this calculation of the dead volume is. When using tetrahydrofuran, the dispersion of the *b* values for the individual series is high to the extent that approximation to the actual dead volume can hardly be treated whatsoever.

Comparison of Retention Properties of Phloroglucinol and Deuterated Mobile Phase Components

Retention volumes of deuterated water (V_D), deuterated mobile phase component (V_{deut}), phloroglucinol (V_P) and volume of eluate for which a small change in the

Collect. Czech. Chem. Commun. (Vol. 54) (1989)

TABLE III

Values of parameter *b* calculated from Eq. (*1*) and retention volumes of phloroglucinol

Column	*b* parameter							V_P ml
	Alcohols	Symmetric phenols	Asymmetric phenol[a]	Alkylphenols	Aromatics	Alkanes	Chloromethanes	
				Mobile phase: methanol–water 70 : 30 (v/v)				
B	0·95	0·88	1·03	0·95	1·07	0·79	1·47	0·91
D	0·82	0·77	0·88	0·91	0·93	0·60	1·33	0·92
C	0·98	0·90	0·99	1·01	1·09	0·98	1·30	0·97
				Mobile phase: acetonitrile–water 70 : 30 (v/v)				
B	0·87	0·77	0·85	0·88	0·96	1·03	—	0·80
D	0·95	0·87	0·98	0·93	0·98	1·02	—	0·88
C	0·93	0·92	0·97	0·88	0·71	0·88	—	0·86
				Mobile phase: tetrahydrofuran–water 50 : 50 (v/v)				
B	0·24	0·77	1·09	0·55	1·28	—	—	0·91
C	0·39	0·88	1·20	1·26	1·42	−0·51	—	1·13

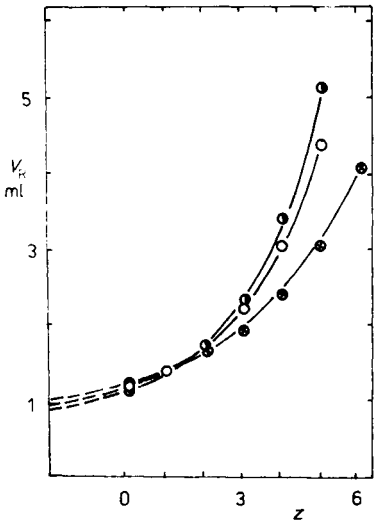

FIG. 1

Dependence of retention volume V_R of n-alkylphenols on the number of carbon atoms in the alkyl chain. Column 150 × 3·3 mm, mobile phase: methanol–water 70 : 30 (v/v). Column packing: ○ Separon SiC 18, ◑ LiChrosorb RP-18, ● LiChrosorb RP-8. Curves, constructed according to Eq. (*1*), approach the *b* value at $z \to -\infty$

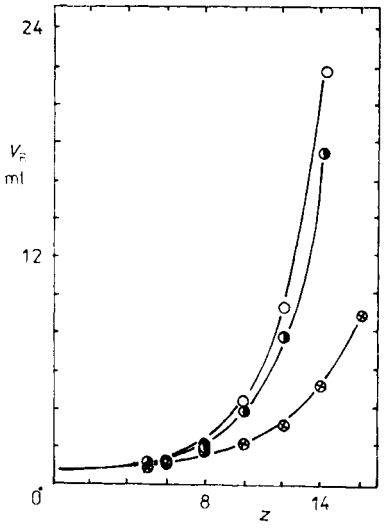

FIG. 2

Dependence of retention volume V_R of n-alcohols on the number of carbon atoms in the molecules. Column 150 × 3·3 mm, mobile phase: acetonitrile–water 70 : 30 (v/v). Column packing: ○ Separon SiC 18, ◑ LiChrosorb RP-18, ● LiChrosorb RP-8. Curves, constructed according to Eq. (*1*), approach he *b* value at $z \to -\infty$

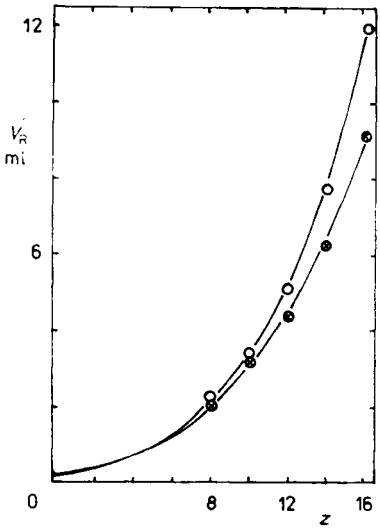

FIG. 3

Dependence of retention volume V_R of n-alcohols on the number of carbon atoms in the molecules. Column 150 × 3·3 mm, mobile phase: tetrahydrofuran–water 50 : 50 (v/v). Column packing: ○ Separon SiC 18, ● LiChrosorb RP-8. Curves, constructed according to Eq. (*1*), approach the *b* value at $z \to -\infty$

mobile phase composition mainfests itself (V_{md}) were examined on three glass columns 150 × 3·3 mm packed with Separon SiC 18, LiChrosorb RP-18 and LiChrosorb RP-8, respectively, using methanol, acetonitrile and tetrahydrofuran, either neat or in mixtures with water, as the mobile phases.

Some relations valid for the retention volumes of isotopically labelled components of binary mobile phases and for small changes in their composition are given in the Appendix. They are all derived based solely on the total equilibrium amounts of substance of the two mobile phase components in the column and on their dependence on the mobile phase composition; they do not include the dead volume,

TABLE IV

Retention volumes of phloroglucinol V_P, deuterated water V_D, deuterated organic component V_{deut}, retention volume corresponding to a small change in the mobile phase composition V_{md} and values of the left-hand side of Eq. (*10*) (acetonitrile, tetrahydrofuran) or Eq. (*13*) (methanol)

Column	Mobile phase		V_p	V_{md}	V_D	V_{deut}	Eq. (*10*), Eq. (*13*)
	organic component	concentration % (v/v)	ml	ml	ml	ml	
B	MeOH	100	0·87	0·95	0·95	0·97	—
		70	0·91	0·92	0·97	0·99	0·98
	MeCN[a]	100	0·85	0·99	—	0·91	—
		70	0·80	0·69	0·81	0·96	0·92
	THF[a]	100	0·80	0·86	—	0·88	—
		50	0·91	0·76	0·76	1·09	0·93
D	MeOH	100	0·78	0·88	0·88	0·84	—
		70	0·81	0·81	0·86	0·85	0·85
	MeCN[a]	100	0·92	1·02	—	0·96	—
		70	0·88	0·78	0·88	1·02	0·98
	THF[a]	100	0·83	0·92	—	0·96	—
C	MeOH	100	0·94	1·07	1·07	1·02	—
		70	0·97	1·00	1·05	1·05	1·05
	MeCN[a]	100	0·89	0·98	—	0·93	—
		70	0·86	0·75	0·86	0·98	0·94
	THF	100	0·85	0·98	—	0·93	—
		50	1·13	0·86	0·85	—	—

[a] Column different from, although of the same type as, that used for methanol was employed.

wherefrom it is clear that the magnitude of the dead volume can be only indirectly inferred from these data.

The results given in Table IV are consistent with the preferential sorption of one or the other component into the stationary phase, reported in refs[2,5]. If this preferential sorption did not take place, then according to Eq. (*6*) (Appendix) the V_{md} and V_{deut} values would be identical and equal to V_{max}, and in the case of negligible amounts of residual silanol groups in the column, the V_D value would be the same. According to Eq. (*5*), the V_{deut} values given in Table IV for the nonaqueous organic mobile phases correspond to V_{max} of the columns used. The V_{deut} values for acetonitrile and tetrahydrofuran approach each other closely whereas for methanol, for which, however, a different column of the same type was used, the data are different.

With nonaqueous acetonitrile and tetrahydrofuran, deuterium is not washed out of the column after injection of D_2O, because it substitutes hydrogen atoms in the silanol groups of the support, and only a peak of water with the retention volume V_{md} is obtained (Eq. (*9*)). For deuterium to be eluted from the column, the mobile phase must contain water or some other substance capable of exchanging hydrogen atoms with the silanol groups, an alcohol for instance. If methanol is used, deuterium after the injection of D_2O is eluted in the form of CH_3OD, which appears in addition to the H_2O (md) peak. Actually, for all columns a peak was only observed at the same volume as after the injection of ordinary water, hence, at V_{md}. Thus, either V_D is identical with V_{md} or the deuterium signal was too low to be detected. The latter alternative is borne out by the fact that for column B, the observed V_{md} is lower than V_{deut} which is equal to V_{max}, while for V_D the reverse should be true according to Eq. (*12*).

The extent to which the mesurement is affected by the presence of silanol groups can be assessed by enumerating the left-hand side of Eq. (*10*) or Eq. (*13*). For the cases where this was possible, the data are given in the last column in Table IV. They are only slightly higher than V_{max}, and the effect of silanol groups in the experimental conditions used is hardly discernible from the experimental error. At low water concentrations in acetonitrile or tetrahydrofuran, however, the effect of silanol groups manifests itself appreciably in the V_D value according to Eq. (*9*).

In the majority of cases, the retention volume of phloroglucinol is lower than V_{md}, V_D and V_{deut}, so that its retention is difficult to prove. The dead volume of the column then can be well approximated by the retention volume of this substance. Only in 70% (v/v) acetonitrile and 50% (v/v) tetrahydrofuran, V_P is higher than V_{md}. In this case it is V_{md} that presumably approaches the dead volume better; actually, if the dead volume V_m were higher than V_{md}, we would have to admit that at least for one component, increase in its concentration in the mobile phase brings about decrease in its amount in the stationary phase.

APPENDIX

Retention Volumes of Isotopically Labelled Components of a Binary Mobile Phase

Relations given in this paragraph can be derived readily, similarly as some equations in the paper by Slaats and coworkers[5], without having to specify the retention mechanism. The retention volumes of the labelled components of the mobile phase and small changes in the composition of the mobile phase will be expressed for a binary mobile phase with components A and B from the total equilibrium amounts of substance n_A and n_B in the column in dependence on the mobile phase composition. Riedo and Kováts[24] refer to this general function as the column capacity or the molar component capacity. Its course for a binary mobile phase is shown in Fig. 4, for the ideal case where no preferential sorption of one component takes place, and schematically, for the case encountered with actual RPLC sorbents and mobile phases[2,5]. The quantities n_A and n_B are not independent; instead, they are interrelated through

$$n_A\bar{V}_A + n_B\bar{V}_B = V_{max} . \qquad (2)$$

For the retention volumes of isotopically labelled components of the mobile phase we have

$$V_R^A = n_A/c_A \qquad (3)$$

$$V_R^B = n_B/c_B . \qquad (4)$$

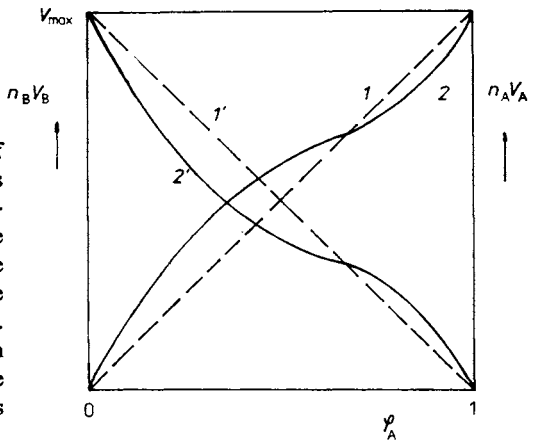

FIG. 4

Dependence of the equilibrium amounts of substance of the mobile phase components A and B in the column on the volume fraction of the component in the mobile phase φ_A. In the plot, the amounts of substance n_A and n_B are multiplied by the molar volume of the respective component in the mixture. Curves *1*, *1'* refer to the ideal case free from preferential sorption, curves *2*, *2'* are schematic drawings for real RPLC systems

Inserting in Eq. (2) we obtain an expression interrelating the two volumes, viz.

$$V_R^A \varphi_A + V_R^B \varphi_B = V_{max} . \tag{5}$$

In the ideal case where no preference of one component exists, we have

$$V_R^A = V_R^B = V_{max} . \tag{6}$$

For the retention volume of a small change in the mobile phase composition,

$$V_{md} = dn_A/dc_A = dn_B/dc_B , \tag{7}$$

the latter equality following from Eq. (2). For the ideal case, free from preferential sorption,

$$V_{md} = V_{max} . \tag{8}$$

If component B is water, deuterium-labelled water is injected, and if component A does not participate in the exchange of deuterium (assuming that the deuterium exchange obeys the same patterns as regarded by Slaats and coworkers[5]), the retention volume of deuterium is

$$V_D = (2n_{H_2O} + n_{SiOH})/2c_{H_2O} , \tag{9}$$

where n_{SiOH} is the amount-of-substance of silanol groups (or other groups capable of isotope exchange with D_2O) in the column. For a nonzero silanol group content and c_{H_2O} approaching zero, V_D approaches infinity, i.e. deuterium from the water injected is exchanged for hydrogen atoms from the silanol groups and remains bonded on the column. Eq. (5) transforms into

$$V_R^A \varphi_A + V_D \varphi_{H_2O} = V_{max} + (1/2)\, n_{SiOH} \overline{V}_{H_2O} \tag{10}$$

for $\varphi_{H_2O} \neq 0$.

If A is methanol or some other solvent capable of isotope exchange of deuterium with water and silanol groups, the retention volume of exchangeable deuterium is

$$V_D = (n_{CH_3OH} + 2n_{H_2O} + n_{SiOH})/(c_{CH_3OH} + 2c_{H_2O}) \tag{11}$$

which for a zero concentration (or amount) of water transforms into

$$V_D = (n_{CH_3OH} + n_{SiOH})/c_{CH_3OH} = V_{max} + n_{SiOH} \overline{V}_{CH_3OH} . \tag{12}$$

The equation analogous to Eqs (5) and (10) is more complex in this case and can be rearranged, e.g., to the form

$$V_D\varphi_{H_2O} + V_R^{CD_3OH}\varphi_{CH_3OH} + (1/2)\,\varphi_{CH_3OH}(\bar{V}_{H_2O}/\bar{V}_{CH_3OH})\,(V_D - V_R^{CD_3OH}) = \\ = V_{max} + (1/2)\,n_{SiOH}\bar{V}_{H_2O} \qquad (13)$$

which for $\varphi_{H_2O} = 0$ transforms into Eq. (12).

LIST OF SYMBOLS

a, b, C	constants in Eq. (1)
c	concentration of component in the mobile phase
n	total equilibrium amount of substance of component in the column at a given mobile phase composition
n_{SiOH}	amount of substance of silanol groups in the column
$\bar{V}$	partial molar volume of component (the same value over the whole column is assumed for simplicity)
V_D	retention volume of deuterated water (exchangeable deuterium)
V_{deut}	retention volume of deuterated (organic) component of the mobile phase
V_m	dead volume of the column
V_{max}	upper limit of the dead volume, determined by total porosity
V_{md}	retention volume of a small change in the mobile phase composition
V_P	retention volume of phloroglucinol
V_R	retention volume
V_R^A, V_R^B	retention volumes of isotopically labelled components A and B, respectively
z	number of structure units
$\varphi = c\bar{V}$	volume fraction of component in the mobile phase

REFERENCES

1. Karch K., Sebastian I., Halász I., Engelhart H.: J. Chromatogr. *122*, 171 (1976).
2. McCormick R. M., Karger B. L.: Anal. Chem. *52*, 2249 (1980).
3. Melander W. R., Erard J. F., Horváth C.: J. Chromatogr. *282*, 211 (1983).
4. Jandera P., Churáček J.: J. Chromatogr. *197*, 181 (1980).
5. Slaats E. H., Markowski W., Fekete J., Poppe H.: J. Chromatogr. *207*, 299 (1981).
6. Krstulovic A.-M., Colin H., Guiochon G.: Anal. Chem. *54*, 2438 (1982).
7. Engelhadt H., Müller H., Dreyer B.: Chromatographia *19*, 240 (1984).
8. Engelhardt H. in: *High Performance Liquid Chromatography*, p. 127. Springer, Berlin, Heidelberg, New York 1979.
9. Jinno K.: Chromatographia *17*, 367 (1983).
10. Wells M. J. M., Clark C. R.: Anal. Chem. *53*, 1341 (1981).
11. Jinno K., Kawasaki K.: Chromatographia *17*, 337 (1983).
12. Karger B. L., Gant J. R., Hartkopf A., Weiner P. H.: J. Chromatogr. *128*, 65 (1976).
13. Unger S. H., Feuerman T. F.: J. Chromatogr. *176*, 426 (1979).
14. Gutnikov G., Hung L. B.: Chromatographia *19*, 260 (1984).
15. Johnson H. J. jr, Cernosek S. F. jr, Gutierrez-Cernosek R. M.: J. Chromatogr. *177*, 297 (1979).

16. Vigh G. Y., Varga-Puchony Z.: J. Chromatogr. *196*, 1 (1980).
17. Haken J. K., Wainwirght M. S., Smith R. J.: J. Chromatogr. *133*, 1 (1977).
18. Möckel H. J., Freyholdt T.: Chromatographia *17*, 215 (1983).
19. Neidhart B., Kringe K. P., Brockmann W.: J. Liq. Chromatogr. *4*, 1875 (1981).
20. Scott R. P. W., Kucera P.: J. Chromatogr. *125*,251 (1976).
21. Berendsen G. E., Schoenmakers P. J., de Galan L., Vigh G., Varga-Puchony Z., Inczedy J.: J. Liq. Chromatogr. *3*, 1669 (1980).
22. Knox J. H., Pryde A.: J. Chromatogr. *112*, 171 (1975).
23. Tchapla A., Colin H., Guiochon G.: Anal. Chem. *56*, 621 (1984).
24. Riedo F., sz. Kováts E.: J. Chromatogr. *239*, 1 (1982).
25. Vít I., Popl M., Fähnrich J.: J. Chromatogr. *281*, 293 (1983).
26. Möckel H. J., Freyholdt T.: Chromatographia *17*, 215 (1983).

Translated by P. Adámek.