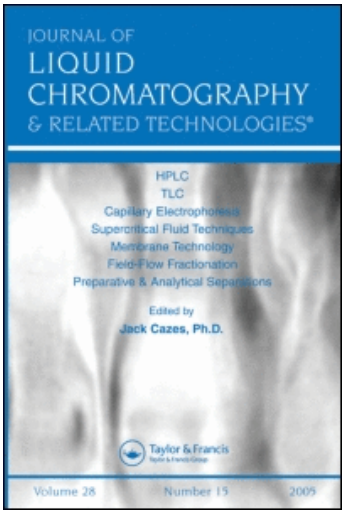

Journal of Liquid Chromatography & Related Technologies

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

On the Determination of the Hold-Up Time in Reversed Phase Liquid Chromatography

Gert E. Berendsen[a]; Peter J. Schoenmakers[a]; Leo de Galan[a]; Gyula Vigh[b]; Zita Varga-puchony[b]; János Inczédy[b]

[a] Laboratory for Analytical Chemistry, Delft University of Technology, Delft, The Netherlands [b] Institute for Analytical Chemistry, University of Chemical Engineering, Veszprém, Hungary

To cite this Article Berendsen, Gert E. , Schoenmakers, Peter J. , de Galan, Leo , Vigh, Gyula , Varga-puchony, Zita and Inczédy, János(1980) 'On the Determination of the Hold-Up Time in Reversed Phase Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 3: 11, 1669 — 1686

To link to this Article: DOI: 10.1080/01483918008064759

URL: http://dx.doi.org/10.1080/01483918008064759

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(11), 1669-1686 (1980)

ON THE DETERMINATION OF THE HOLD-UP TIME IN REVERSED PHASE LIQUID CHROMATOGRAPHY

Gert E. Berendsen, Peter J. Schoenmakers and Leo de Galan
Laboratory for Analytical Chemistry, Delft University of Technology
Jaffalaan 9, 2628 BX Delft, The Netherlands

Gyula Vigh, Zita Varga-Puchony and János Inczédy
Institute for Analytical Chemistry, University of Chemical Engineering
P.O. Box 28, H-8201 Veszprém, Hungary

ABSTRACT

The determination of the hold-up time in reversed phase liquid chromatography has been studied extensively for the mobile phase system methanol-water. Hold-up times obtained by static methods, linearization of homologous series and so-called "unretained compounds" are discussed and mutually compared. Several n-alkyldimethylsilyl bonded phases have been used for this investigation.

A rough estimate of the hold-up time can be obtained by using components of the mobile phase or highly concentrated salt solutions, but only for mobile phase compositions around 60% (v/v) methanol. Hold-up times accurate to 1% can be obtained over the complete range of mobile phase compositions from the linearization of net retention times of homologous series.

INTRODUCTION

An important problem in reversed phase liquid chromatography, RPLC, is the determination of the hold-up time. The problem is particularly serious when chemically bonded reversed phase packings are used because the boundary between the stationary phase and the mobile phase is not well defined. For comparison of retention data and for the interpretation of physical phenomena, the capacity factor, k, is the fundamental parameter. However, determination of k requires the knowledge of the hold-up time, t_o.

The concept of hold-up volume has been discussed in depth by Horvath and Lin [1]. According to their analysis the hold-up volume of a solute will vary between the interparticle volume, V_{ex}, and the sum of the intraparticle and

interparticle volume, $V_{in} + V_{ex}$, of the column. The distinction depends on the extent to which the pores are accessible to the solute in concern. If a solute is totally excluded from all the pores (e.g. by its size or by electrostatic repulsion), it will be swept through the column in the shortest possible time, the exclusion time. If exclusion effects are not operative and it is postullated that all chromatographically active solutes have free access to all pores, then we conclude that such solutes experience one common hold-up time. In other words, our considerations are not valid if molecular size or other effects play a role (as, e.g. in GPC).

In contrast to gas chromatography, GC, no preferred and generally accepted method for the determination of t_o has emerged in RPLC. Even for the widely used mobile phase system methanol-water many different procedures have been suggested that can be divided into three general categories.

1. Unretained compounds

In analogy to the use of air in GC, several possibly unretained compounds have been proposed for LC.

Frequently, one of the mobile phase components is used. Colin et al. use pure water |2|, whereas others |3,4| opt for pure methanol at all binary compositions of methanol and water. Scott and Kucera used water |5|, but recently they proposed the use of methanol for eluents rich in organic modifier |6|. They assume a constant retention for methanol for mobile phases containing more than 70% (v/v) methanol.

Instead of water, the use of deuteriumoxide has also been advanced for all mobile phase compositions |4,7|. An extension of this is the suggestion of Halász |8| to use radioactively labeled solvent components. Also water-organic modifier solutions having a slightly different composition ratio from the mobile phase are used |9|.

Instead of eluent components other compounds have also been used occasionally, such as uracil |9|, phenol in pure methanol |10| or cytosine |11|. In all these cases, a possible retention of the injected compound is tacitly or explicitly ignored. Such problems seem to be absent if salt solutions are used to derive the hold-up time. In most cases a small amount of a UV-active salt is injected, such as sodium nitrate |1,12|, sodium benzene sulfonate |12| and potassium dichromate |13-16|, but also UV-non-active salts as sodium chloride |17| are used. As will be shown later, such low concentrations grossly underestimate the true hold-up time of the column.

2. Static methods

According to one method the packed column filled successively with two solvents of sufficiently different density (e.g. tetrachloromethane and methanol)

is weighed [18,19]. The total volume taken up by the mobile phase can then be calculated as

$$V_m = \frac{w_1 - w_2}{\rho_1 - \rho_2} \qquad (1)$$

where w and ρ are the weight of the column and the density of the solvents, respectively.

Obviously, eq. (1) ignores the possibility that the stationary phase is solvated by part of the mobile phase. Unless a correction is made for the solvation layer [19] the weighing method provides an upper limit to the hold-up volume of the column.

A similar problem arises, if the hold-up time is derived from a plot of gross retention times versus distribution coefficients determined by static methods.

This method may be applicable to liquid-liquid partition chromatography, for which the static distribution coefficients can be measured between the two corresponding bulk phases [20].

However, it is difficult to see how it can be applied to chemically bonded stationary phases, where bulk partition coefficients are invalid and the solute concentration can only be measured in the mobile phase, the volume of which is undefined once it has been brought into contact with the chemically bonded phase.

3. Linearization of the net retention time for homologous series

A method, well known and extensively discussed in gas chromatography ([21,22] and references quoted therein), has been reconsidered for LC by Al-Thamir et al. [23]. It assumes a linear relationship between the logarithm of the net retention time and the carbon number of a homologous series. The hold-up time is then derived from a series of measured gross retention times either graphically by trial and error, [24], or by calculation [25,26].

Applying the retention equation, $t_R = t_o (1 + k)$, to two consecutive homologous, n+1 and n, we obtain

$$\frac{t_{R,n+1} - t_o}{t_o} \Big/ \frac{t_{R,n} - t_o}{t_o} = \frac{t_{R,n+1} - t_o}{t_{R,n} - t_o} = \frac{k_{n+1}}{k_n} = A \qquad (2)$$

where A is constant within one homologous series. Hence

$$t_{R,n+1} = A \cdot t_{R,n} - (A - 1) \cdot t_o \qquad (3)$$

By plotting $t_{R,n+1}$ against $t_{R,n}$ we obtain A as the slope, and with this, t_o, from intercept. Note that every member of the series is used twice in

the linear regression except the first and the last one. The possible weak poin of this procedure is the assumption that linearity is valid throughout the whole series, down to the smallest members.

In this paper the above methods for the determination of the hold-up time will be compared. In order to be independent of the column dimensions the hold-up time will be converted to column porosity according to

$$t_o = \varepsilon_c V_c/F$$

where V_c is the empty column volume and F the flow rate of the mobile phase. The data obtained will vary between a minimum value (the exclusion porosity $\varepsilon_{ex} = V_{ex}/V_c$) and a maximum value ($\varepsilon_{max} = (V_{in} + V_{ex})/V_c$) as determined by weighing (eq.1). The latter value can also be calculated from the molecular properties of the chemically bonded chains [27].

EXPERIMENTAL

Apparatus and chemicals

The chromatographic apparatus used is a Waters Liquid Chromatograph equipped with a M6000 pump, a U6K injector, and a detector (401 RI or 440 UV). Injector, column and detector were thermostatted [28], whereas the connections between column and injector, and column and detector were carefully isolated. The dimensions of the columns were 300 x 4.6 mm.

A home-made conductivity detector, CD, [29], connected to a Radiometer, Model CDM 3, was used to measure non UV-active salts, such as KBr.

Chemicals, home-made bonded packings (RP-1 up to RP-22), and the packing procedure have been described elsewhere [30-32]. In addition, some experiments have been performed on commercial alkylsilyl phases, i.e. Merck RP-18 and Varian MicroPak CH-10.

Standardization

All elution and exclusion times obtained were corrected for outer column residence time and normalised to a flow rate of 1.5 ml/min. The flow rate, and in the case of the commercial columns the elution volumes were continuously measured with a calibrated buret which was carefully dried before every measurement. The buret was filled via a special inlet constructed at the bottom.

Measurements on salts

Salt solutions are commonly used for the determination of the hold-up time. Therefore, we investigated different kinds of salts and the influence of both the concentration of the injected salt solution and the ionic strength of the mobile phase on the peak-position. These measurements were carried out with various RP-18 columns and are presented in figure 1. Fig. 1a shows the actual

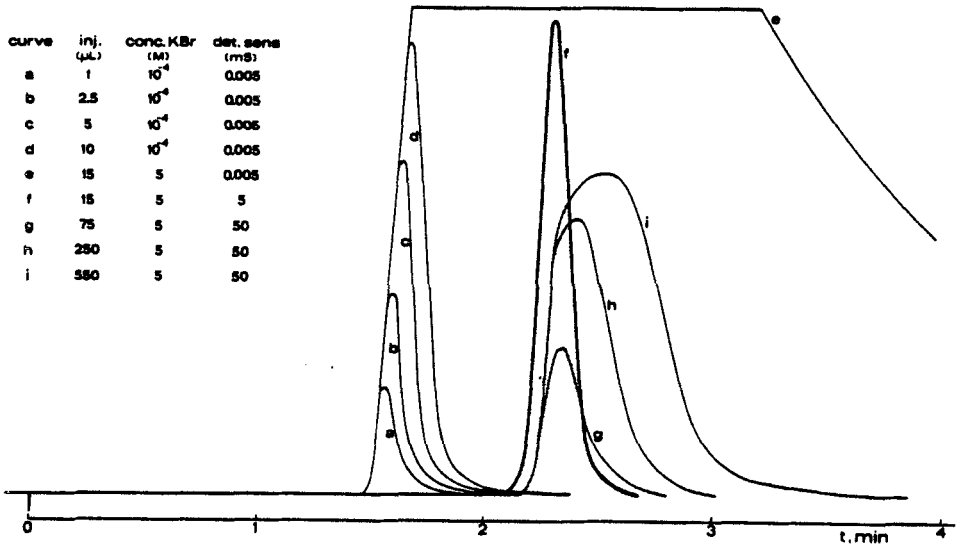

FIGURE 1a

Influence of amount of KBr injected on peak-shape measured with a conductivity detector. Conditions: 1.49 ml water per min. on a home-made RP-18 column.

chromatograms obtained for increasing amounts of KBr injected in pure water as the mobile phase.

An increase in the amount of KBr causes the peak-top to shift to longer elution times. However, the peak always starts at the same point, even for injections of the saturated solutions. This is indicated by the recorder trace for 15 µl of the saturated solution at a CD-sensitivity of 5 µS. The same injection recorded at a sensitivity of 5 mS shows the shift of the peak-top.

Although for still larger injection volumes the peak maximum keeps shifting, this effect is actually caused by the fact that the injection volume becomes too large. After correction, the true position of the peak maximum remains virtually constant from 15 µl upward. Consequently, with increasing amounts of salt injected, the apparent hold-up time in water varies from a low value of 84 seconds to a maximum value of 134 seconds.

A more extensive analysis is presented in figure 1b. Different amounts of KI in 10 µl volume have been injected using three different mobile phases. In agreement with figure 1 the elution volume of the start of the peak is independent of the injected amount and corresponds to a column porosity of 0.40.

Since the start of the peak will be influenced by the sensitivity of the detection system, it is not a meaningful parameter and its use should be avoided.

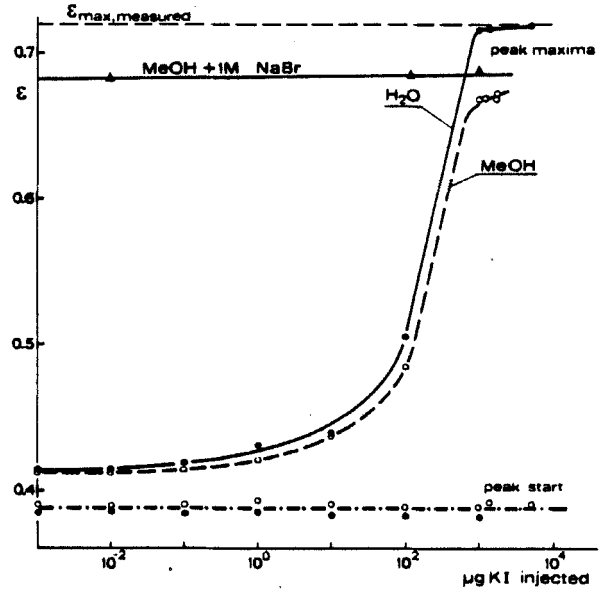

FIGURE 1b

Peak start and peak maximum with increasing amount of KI injected in water, methanol and methanol containing 1M of NaBr. The maximum column porosity has been measured by weighing, eq.(1);25 cm Knauer column packed with Merck RP-18 packing material.

If in pure water or pure methanol the amount of KI is increased, the peak-position first remains unchanged (corresponding to a porosity of 0.42), but for amounts exceeding 1 µg it starts to shift to longer residence times. Ultimately, at 1000 µg, which is close to saturation, the peak maximum levels off at a porosity of 0.68 for MeOH and 0.72 for H_2O. The latter value is virtually equal to the maximum column porosity determined by weighing (see below). Finally, if different amounts of KI are injected using a 1M NaBr solution in methanol as the mobile phase, the peak top is always close to the maximum column porosity.

These results can be explained as follows. Initially, at low electrolyte concentration the salt is excluded from the pores of the packing, presumably due to electrical charges on the phase surface [33]. With increasing electrolyte concentration in the mobile phase the ion exclusion effect is suppressed and the pores become accessible to the salt. It is immaterial whether the enhanced ionic strength of the mobile phase results from the injected salt itself or

from an electrolyte added to the mobile phase. Obviously, at low concentrations ($<10^{-3}$M) an injected salt solution yields the exclusion volume. At high concentration ($<10^{-1}$M) the maximum of the salt peak may indicate the maximum hold-up time; this will be discussed later.

Various inorganic salts (NH_4NO_3, $NaNO_3$, $FeCl_3$, and $K_2Cr_2O_7$) and HCl were also used to measure the exclusion porosity. All show exclusion porosities similar to that obtained with KBr and KI.

However, $CuSO_4$ and $FeSO_4$ showed a porosity of 0.53 and 0.63, respectively. This indicates that these salts are not completely excluded.

All exclusion data were found to be independent of the temperature (over the range of 25-60°C).

Residence time of mobile phase components

The residence time of mobile phase components was measured with a refractive index detector. Methanol, water, deuterium oxide and deuterated methanol (CD_3OD) were tested.

Linearization of homologous series

The derivation of hold-up time from the linearization of retention data for seven homologous series is illustrated in figure 2. The t_o data were obtained by lineair regressing using eq. 2.

The precision of the method was tested graphically by plotting apparent k-values using various holp-up times. If the hold-up time is severely under-estimated by using the exclusion porosity (ε=0.42, t_{ex}= 84 s) the curve is strongly convex. If, on the other hand, the residence time of water (135 s) is used, the curve is significantly convex.

Perfect linearity and mutually consistent data are obtained with an average value of 136 $\pm$ 1 s. This uncertainty agrees with the precision of each individual result, so that there is no reason to assume significant variation between the linearization time of the different homologous series. The same equality of $t_{o,lin}$ was also observed between n-alkyliodides and nitroalkanes on RP-18 in pure methanol and in 80:20 (v/v) methanol-water.

The question remains, how far the assumption of linearity between log k and n_C can be extended to the smaller members. Two arguments can be offered in favour of linearity. The first is the completely random scatter of data points around the calculated straight lines. If linearity were enforced artificially, we would observe a systematic deviation of consecutive data points around the straight line (e.g. a bow shape). This was never observed. Secondly, no significant variations in hold-up time were observed when the analysis was restricted to a smaller number of homologues.

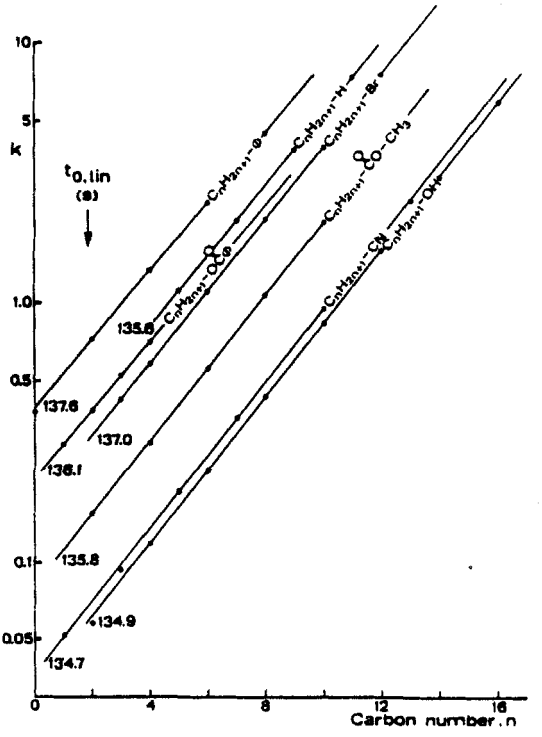

FIGURE 2

Linearization of retention for various homologous series (Ø = phenyl). The linearization times obtained from linear regression (eq. 3) are indicated next to each straight line. Conditions: 90:10 (v/v) MeOH/H_2O, 1.40 ml/min, 30 cm RP-22 column.

The choice of an appropiate series is very much dictated by practical considerations, such as solubility, availability, and detectability. For example the selection of a UV-active series, such as alkyliodides or benzoic esters has obvious advantages in terms of detectability. However, the solubility of even the shortest four alkyliodides and benzoic esters is low in mobile phases with low modifier content.

We, therefore, prefered to use n-alcohols. All members from ethanol upward were used. For pure water as mobile phase even methanol can be included in a series that runs only up to n-butanol. The disadvantage of requiring a RI-detector is far outweighed by the good solubility in mobile phase compositions ranging from water to pure methanol.

RESULTS AND DISCUSSION

$\varepsilon_{c,max}$ porosities

In order to determine the maximum total column porosity, the columns were filled with methanol and tetrachloromethane and weighed.

The results show an expected decrease with increasing length of the chemically bonded alkyl chain. A low value of 0.70 is observed for RP-22, whereas RP-1 and RP-3 show high porosities of 0.80. These data agree well with the porosity of 0.84 measured for pure silica and also observed by others |7,8,34|. If there would be a sharp boundary layer between the bonded phase and the mobile phase, these $\varepsilon_{c,max}$ values would present the actual column porosity. However, if the bonded chains are solvated, the actual value will be smaller, and consequently, the hold-up time will be smaller.

Residence time of the solvent components

The possibility to use the components of the eluent or their deuterated forms as unretained compounds has been extensively investigated for all n-alkyl-silyl bonded packings.

Data for the RP-3 and RP-18 packing are presented in figure 3. Data of the RP-6, RP-10 and RP-14 packings are situated in-between. In this figure the column porosity and the absolute retention of MeOH, H_2O and D_2O are presented as a function of the mobile phase composition, ϕ.

The curve for the RP-18 column is situated lower than for the RP-3 column. This is due to the larger volume occupied by the octadecyl chains. The longer the chains, the smaller the pore volume and hence the quicker the elution.

Within the experimental error all four compounds, MeOH, H_2O, D_2O and CD_3OD, elute from the column in the same time, at all mobile phase compositions, except in the region of $\phi = 0$. In this region D_2O elutes faster than methanol and CD_3OD.

For all packings the residence time varies with the modifier content in exactly the same way, and shows a minimum at about $\phi = 0.7$. Scott and Kucera |6| who carried out measurements only between $\phi = 0$ and 0.7, found the same dependency for methanol. However, our data are in contradiction with those of Elgass |7|, who did not find a dependency of the elution of D_2O on ϕ.

The shape of these curves is rather difficult to explain. However, if the $\varepsilon_{c,max}$ values for both columns are inserted in figure 5 it is clear that for methanol contents up to $\phi = 0.3$ and over $\phi = 0.9$ the solvent components are retained. This is confirmed by measurements at 60°C. At $\phi = 0.6$ the absolute retention decreases 3%, which can be completely attributed to the expansion of the mobile phase |2|. In other words the residence time is independent of

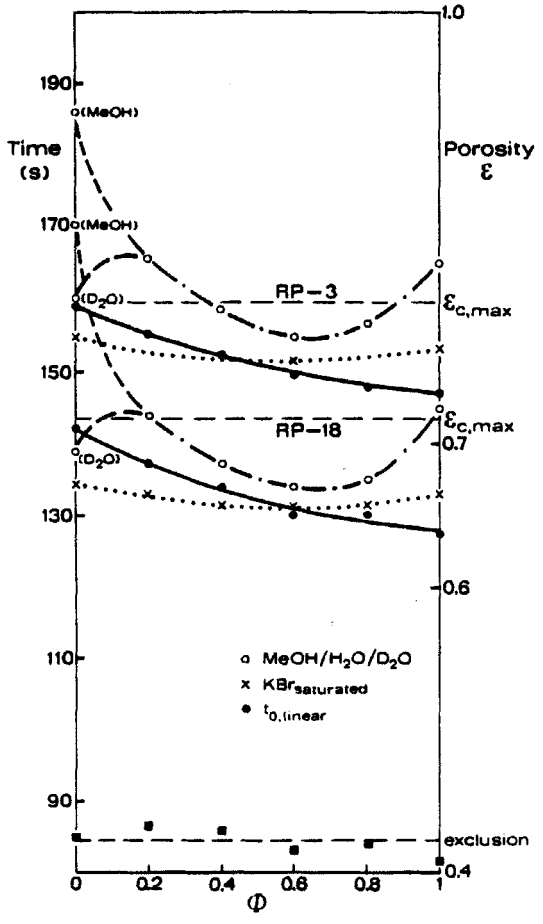

FIGURE 3

Possible estimate of hold-up time and column porosity as a function of the mobile phase composition Ø (MeOH/H_2O, v/v), for two different columns (RP-3 and RP-18). Different curves refer to:

—.—. *mobile phase components CH_3OH, CD_3OD, H_2O, D_2O*
..... *peak maximum of 15 µl saturated KBr*
——— *linearization of n-alcohols*
– – – *peak start of 10 µl 10^{-4} M KBr*

Maximum column porosities determined by weighing (eq. 1).

temperature at $\phi = 0.6$, which must be true for the real hold-up time. However, at $\phi = 0.2$ the mobile phase expansion from room-temperature to 60°C is 2%, whereas the absolute retention decreases over 4%. Hence, the net result shows a slight decrease in the residence time as would be expected for true retention.

It can be concluded from these results that at mobile phase compositions between $\phi = 0.6$ and 0.7 the elution times of methanol and water might approach the hold-up time, because of the temperature-independency and the position with respect to $\varepsilon_{c,max}$. The fact that the hold-up time is somewhat smaller than the one corresponding to the maximum column porosity may be attributed to the presence of a solvation layer on the stationary phase.

However, it remains questionable whether the hold-up time derived from solvent components at $\phi \approx 0.6$ can be used over the complete range of mobile phase composition.

Measurements on salts

The data obtained for KBr solutions are also included in figure 5. The bottom trace represents the exclusion porosity determined from the start of the peak measured with an RI-detector for a 10^{-4}M KBr solution. *This exclusion porosity is equal for all RP-phases, and independent of the mobile phase composition.*

As formulated earlier the elution time of the peak maximum from an injection of 15 µl of a saturated solution of KBr in the mobile phase gives an estimate of the true hold-up time of the column. As expected from the decreased internal porosity the derived hold-up time decreases drastically when going from an RP-3 to an RP-18 phase. Also, all column porosities fall below the maximum possible column porosity for both RP-phases. This may be attributed to the presence of a solvation layer.

In figure 5 we observe hardly any variation of the hold-up time of KBr-saturated with mobile phase composition. Naturally, we would expect the solvation layer to be smaller for pure water than for pure methanol. In another series of measurements the hold-up time of 1000 µg of KI on a Merck RP-18 column varied much more strongly with the methanol content of the mobile phase. It approached the maximum column porosity of 0.72 very closely at $\phi = 0$ and $\phi = 1$, whereas a minimum porosity of 0.65 was measured at $\phi = 0.5$. In neither series of measurements did the temperature dependence of the hold-up time exceed the variation to be expected on the basis of mobile phase expansion. Consequently, all data appear to be independent of temperature.

These observations cast some doubt about the general use of concentrated salt solutions for the determination of hold-up times. At present, they seem

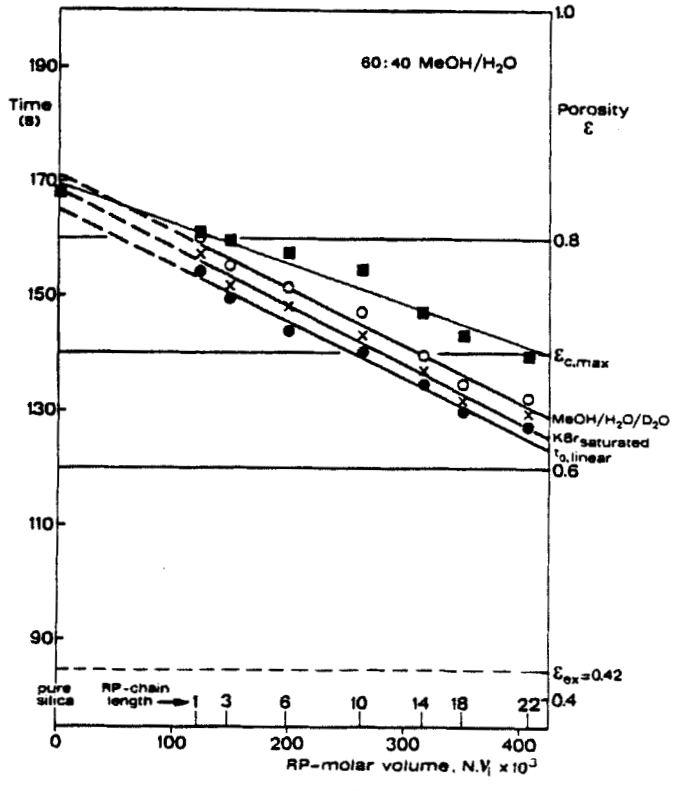

FIGURE 4

Possible estimates of hold-up time and column porosity in 60:40 MeOH/H_2O as a funtion of the volume of different RP-phases. RP-volume can be calculated as the product of the molar volume of the chain and the surface coverage [30]. *Symbols according to fig. 3.*

to give good results only for mobile phases composed of nearly equal volumes of water and methanol ($0.4 < \phi < 0.6$).

Linearization of homologous series

At every mobile phase composition we determined the linearization times, $t_{o,lin}$ of a homologous series of n-alcohols.

The $t_{o,lin}$ values obtained by linear regression show a steady decrease in going from pure water, $\phi = 0$, to pure methanol, $\phi = 1$, This can be best ex-

plained by the phenomenon of solvation. At $\phi = 0$, the total column porosity calculated from $t_{o,lin}$, closely resembles the $\varepsilon_{c,max}$ value. This agrees with the notion that solvation of a reversed phase packing will be minimal for pure water, because the interaction between the hydrocarbon chains and water will be minimal. Minimal solvation means a maximal internal porosity and hence a maximal hold-up time.

If methanol is added to the aqueous mobile phase a solvation layer may be built up gradually, reaching its maximum thickness in pure methanol. This results in a minimum internal porosity and hence in a minimum hold-up time.

For the RP-3 and RP-18 bonded packings the maximum solvation volumes at $\phi = 1$ are 310 and 375 µl, respectively, which corresponds to solvation layers of 0.56 and 0.69 nm, respectively.

Both values exceed the average monolayer thickness of 0.35 nm proposed by Snyder [35]. However, from the average methanol molecule (0.45 nm) we conclude that the solvation is between one and two molecules thick.

We also investigated the influence of temperature on $t_{o,lin}$ between 27.5° and 60.0° C, for some mobile phase compositions. Naturally, there is a strong influence of the temperature on the retention of the individual members of the homologous series. For example, the retention of the C_2, C_4, C_6 and C_8 members of the n-alcohol series, progressively decrease with 4, 15, 34 and 53%, respectively, at $\phi = 0.6$ on an RP-14 column. However, after correction for mobile phase expansion the values derived for $t_{o,lin}$ appeared to be completely independent of the temperature.

The fact that the saturated KBr-curve, the $t_{o,lin}$-curve and the solvent data between $\phi = 0.6$ and 0.7 show no influence of temperature indicates that the real hold-up time for the RP-18 column corresponds to a total column porosity between 0.64 and 0.72; and for the RP-3 column, between 0.74 and 0.80.

Comparison of n-alkylsilyl bonded phases

We have also compared the n-alkylsilyl bonded phases, RP-1 up to RP-22, mutually and with the original silica support. Fig. 4 presents data for MeOH, H_2O, D_2O, saturated KBr and the linearization times at a mobile phase composition of $\phi = 0.6$. This composition was chosen because of our conclusion that the elution times of MeOH, H_2O and D_2O might approach the hold-up time only between $\phi = 0.6$ and 0.7. Remarkably, all three curves run closely parallel. Obviously, the data obtained for the hold-up time decrease linearly with increasing volume occupied by the bonded phase. Only if all phases had equal surface coverage, then this volume and hence the

t_o-values, would also be proportional to the bonded phase chain length.

The maximum column porosities of the different bonded packings, have also been included in figure 4. Note that the $\varepsilon_{c,max}$ value calculated for silica is not placed at the chain length corresponding to RP-0, but rather at zero RP-volume. All three extrapolated straight lines fall close to the porosity of pure silica, so that on this basis no further discrimination can be made between the methods applied for the approximation of the hold-up value.

CONCLUSIONS AND RECOMMENDATIONS

The hold-up time of a chromatographic column is a valid concept for solutes that have free access to all the pores of the (modified) support material. It breaks down for solutes that are excluded from some or all the pores. In fact, completely excluded solutes can be used to determine the intraparticle or exclusion volume of the column. As such we recommend the use of very dilute monovalent salt solutions at zero ionic strength. The peak position of up to 1 nmol salt as detected with a high sensitivity detector provides an estimate of the exclusion porosity. In agreement with with other authors [8, 34], an external porosity of about 0.42 was found for well-packed columns, independent of the type of the chemically bonded stationary phase and the composition of the mobile phase.

For the determination of the true chromatographic hold-up time a variety of methods has been tested. The following conclusions apply only to methanol-water as the mobile phase.

Static measurements, such as weighing of the column or correlation with static distribution coefficient can only provide an upper limit for the total column porosity. As expected, the measured values decrease with increasing chain length of the bonded alkylsilyl phase. However, neglecting solvation effects may result in overestimating the true hold-up time of the column by as much as 15% in pure methanol. Consequently, accurate hold-up times can only be determined by dynamic methods conforming to chromatographic practice.

The use of potentially unretained compounds [4, 11] is clearly dangerous. Somewhat surprisingly, we found strong indications that even the mobile phase components may be subject to chromatographic retention. The variation with mobile phase composition, figure 3,

and the slight temperature dependence of the elution times of solvent components all warn us to be careful. It is only for methanol-water compositions around 65:35 (v/v) that the true hold-up time is approached.

A usually better estimate of the hold-up time in methanol-water can be derived from the maximum elution time of salts in highly concentrated salt solutions. The choice of an appropriate monovalent salt is determined by the detector. With a sensitive UV-detector a minute amount of KI or $NaNO_3$ may be added to a large excess of NaBr. With a conductivity or RI-detector saturated solutions of NaBr or KBr can be used. Here too, however, our results warn us to be cautious. We have noticed that different conditions (stationary phase, the kind of salt, the injected quantity) can lead to different results. This raises some doubt about the validity of hold-up times derived from concentrated salt solutions.

In our opinion the most accurate hold-up time in methanol-water is derived from the linearization of the logarithmic net retention times of a homologous series. Despite the disadvantage of a lengthy procedure, it remains the method of choice for fundamental work. The results are precise to within 1%, independent of the column temperature and equal for different homologous series. The results suggest the presence of a solvation layer, the thickness of which increases with the chain length of the bonded alkylsilyl phase and with the increasing modifier content of the mobile phase.

In fact, the results presented in figures 3 and 4 may be described by the following expression for the column porosity.

$$\varepsilon_c = 0.79 - 0.06 \cdot \phi - 0.005 \cdot n_{RP} \qquad (4)$$

where ϕ is the volume fraction of methanol and n_{RP} is the number of carbon atoms in the alkylsilyl bonded chain. This expression, which is obviously valid only for the system presently investigated, describes the porosity to about 0.01 units.

Accurate hold-up times must be determined experimentally for each particular phase combination. Unfortunately, potentially attractive homologous series (e.g. alkyliodides) fail in solubility for mobile phases of low methanol content. Therefore, we recommend

the use of n-alcohols despite the fact they require the use of an RI-detector.

Finally, one should be very careful in applying the methods presently discussed for methanol-water to other mobile phase systems. For acetonitrile and tetrahydrofuran-water systems the addition of large amounts of inorganic salts leads to demixing. We are presently investigating the possibility to use the linearization method in other mobile phase systems and we shall report on this in a future publication.

ACKNOWLEDGEMENTS

The authors gratefully thank Dr. H. Poppe for helpful comments and Ing. H.A.H. Billiet and Mr. K.A. Pikaart for useful discussions.

REFERENCES

1. Horvath, H. and Lin, H.J. *J. Chromatogr.*, 149, 43, 1978
2. Colin, H., Ward, N. and Guiochon, G., *J. Chromatogr.*, 149, 169, 1978
3. Roumeliotis, P. and Unger, K.K., *J. Chromatogr.*, 149, 211, 1978
4. Karch, K., Sebastian, I., Halász, I. and Engelhardt, H., *J. Chromatogr.*, 122, 171, 1976
5. Scott, R.P. and Kucera, P., *J. Chromatogr.*, 142, 213, 1977
6. Scott, R.P. and Kucera, P., *J. Chromatogr.*, 175, 51, 1979
7. Elgass, H., *Thesis.*, University of Saarbrücken, Saarbrücken, 1978
8. Halász, I., in *Modern Practice of Liquid Chromatography*, ed. J.J. Kirkland, Wiley, New York, 1971
9. Karger, B.L., Gant, J.R., Hartkopf, A. and Weiner, P.H., *J. Chromatogr.*, 128, 65, 1976
10. Hennion, M.C., Picard, C. and Caude, C., *J. Chromatogr.*, 166, 21, 1978
11. Wal, Sj. van der, and Huber, J.F.K., *J. Chromatogr.*, 149, 431, 1978

12. Melander, W., Campbell, D.E. and Horváth, C., *J. Chromatogr.*, 158, 215, 1978

13. Schoenmakers, P.J., Billiet, H.A.H., Tijssen, R. and Galan, L. de, *J. Chromatogr.*, 149, 519, 1978

14. Berendsen, G.E. and Galan, L. de, *J. Liquid Chromatogr.*, 1, 561, 1978

15. Hermansson, J., *J. Chromatogr.*, 152, 437, 1978

16. Tjaden, U.R., *Thesis*, University of Amsterdam, Amsterdam, 1976

17. Hoffman, N.E. and Liao, J.C., *Anal. Lett.*, A11, 307, 1978

18. Venne, J.L.M. van de, *Thesis*, University of Eindhoven, Eindhoven, 1979

19. Slaats, E.H., Kraak, J.C., Brugman, W.J.T. and Poppe, H., *J. Chromatogr.*, 149, 255, 1978

20. Huber, J.F.K., Alderlieste, E.T., Harren, H. and Poppe, H., *Anal. Chem.*, 45, 1337, 1973

21. Haken, J.K., Wainwright, M.S. and Smith, R.J., *J. Chromatogr.*, 133, 1, 1977 and ibid 147, 65, 1978

22. Gassiot, M., Guardino, X., Albaigés, J., Firpo, G. and Rodrígnez-Viñals, R., *J. Chromatogr.*, 147, 540, 1978

23. Al-Thamir, W.K., Purnell, J.H., Wellington, C.A. and Laub, R.J., *J. Chromatogr.*, 173, 388, 1979

24. Sevcik, J., *J. Chromatogr.*, 135, 183, 1977

25. Grobler, E. and Balizs, G., *J. Chromatogr., Sci.*, 12, 57, 1974

26. Wainwright, M.S., Haken, J.K. and Srisukh, D., *J. Chromatogr.*, 179, 160, 1979 and previous papers

27. G.E. Berendsen, *Preparation and Characterizatiob of well-defined chemically bonded stationary phases for HPLC*, Ph.D. Thesis, Delft, 1980

28. Vigh, Gy., *J. Chromatogr.*, 117, 424, 1976

29. Billiet, H.A.H. Keehnen, P.D.M. and Galan, L. de, *J. Chromatogr.*, 185, 515, 1979

30. Berendsen, G.E., Pikaart, K.A. and Galan, L. de, *J. Liquid Chromatogr.*, in press

31. Berendsen, G.E. and Galan, L. de, *J. Chromatogr.*, in press

32. Berendsen, G.E., Regouw, R. and Galan, L. de, *Anal. Chem.*, 51, 1091, 1979

33. Stenlund, B., in *Advances in Chromatography*, Vol. 14, Marcel Dekker, New York, 1976, Chapt. 2

34. Engelhardt, H., *Hochdruck Flüssigkeits Chromatographie*, Springer-Verlag, Berlin. 1975

35. Snyder, L.R., *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968