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## SOLUTE-SOLVENT INTERACTIONS ON THE SURFACE OF REVERSED PHASES

### I. STATIONARY PHASE INTERACTIONS AND THEIR DEPENDENCE ON BONDING CHARACTERISTICS

R. P. W. SCOTT\*

*Chemical Research Department, Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)*

and

C. F. SIMPSON\*

*School of Molecular Sciences, Sussex University, Sussex (Great Britain)*

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#### SUMMARY

The retention characteristics of a number of "brush"- and "bulk"-type reversed-phase column packing materials that are commercially available for high-performance liquid chromatography are examined in the situation where an abrupt change in mobile phase from pure methanol to pure water occurs. It is shown that the brush-type reversed phases are slow to come into equilibrium with pure water, and in contact with water it would appear that the hydrocarbon chains dispersively interact with themselves. This dispersive intramolecular interaction of the hydrocarbon chains results in a reduction in effective chromatographic surface area and consequently anomalously low retentive characteristics. In contact with solvent containing 10% (w/v) or more of organic solvent such as methanol, the brush-type materials exhibit normal retention characteristics. The bulk or polymeric bonded phases, however, equilibrate rapidly with water, and due to their more rigid structure do not appear to exhibit dispersive intramolecular interactions and thus exhibit normal retention characteristics. The bulk or polymeric type reversed phases, therefore, are to be preferred for examining solute-solvent/stationary phase interactions due to their more consistent chromatographic properties.

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#### INTRODUCTION

Reversed phases are prepared in basically two forms, the so-called "brush"-type material and the "bulk" modified material<sup>1</sup>. The brush-type reversed phases are prepared by reacting a monofunctional silane such as octyl dimethylchlorosilane with the silica. Thus, to a first approximation, each molecule of the silane reagent reacts with one silanol group, producing one hydrocarbon chain per original silanol group. The bulk modified material is prepared from silica using a trifunctional silane such as trichloro-octylsilane, usually in the presence of excess water adsorbed on the

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\* Present address: Chelsea College, University of London, London, Great Britain.

silica surface. After one of the functional groups has reacted with the silanol group on the silica surface, the remaining two functional groups react with water to form more silanol groups. These new silanol groups react in turn with more reagent, forming a cross-linked polymeric structure.

The brush-type reversed phase is a material that can be pictured with the hydrocarbon chains moving freely in the mobile phase. The bulk modified material, on the other hand, would take the form of a cross-linked silicone polymer possessing a permeable, honeycomb type structure. The hydrocarbon chains of the bulk material are likely to be far more restricted in movement than the chains of the brush type as a result of the cross-linking of the polymer. Due to the significant differences between the surface structures of the two types of reversed phases, it would be expected that they would exhibit different retention characteristics and equilibrium kinetics. The different retention characteristics of the two types of phases have already been examined<sup>2</sup>, and in this paper the equilibrium kinetics associated with extreme solvent changes are examined for a number of different commercially available reversed phases.

#### EXPERIMENTAL

The apparatus used has been previously described<sup>2</sup> and consists of a Waters Assoc. (Milford, MA, U.S.A.) 6000 M pump, a thermostated column fitted with a Valco automatic injection valve having an injection volume of 2  $\mu$ l, a Waters Assoc. refractometer detector, Model 401, and an appropriate recorder. Water was circulated through the column jacket and through the detector jacket from a thermostat that was maintained at 25.75°C  $\pm$  0.05. The supply from the pump was first passed through a 3-ft. coil submerged in the thermostat prior to entering the Valco valve in order to preheat the mobile phase to the correct temperature. The outlet from the detector was connected directly to a 10-ml burette. A flow-rate of 1 ml/min was used; a burette reading was taken on injection and a reading again taken at the peak maximum. The burette was read to within 0.02 ml. A total of six different reversed phases were examined, three bulk modified materials and three brush-type materials. The three bulk modified reversed phases were ODS-2 and ODS-3, supplied by Whatman (Maidstone, Great Britain), and Bondapak as supplied by Waters Assoc. The three brush-type reversed phases were RP-2, RP-8 and RP-18, supplied by E. Merck (Darmstadt, G.F.R.), and all six reversed phases had mean particle sizes of 10  $\mu$ m. The ODS-2, ODS-3, RP-2, RP-8 and RP-18 were obtained directly from the manufacturers, packed in columns 25 cm  $\times$  0.25 in. O.D. The  $\mu$ Bondapak as supplied by Waters Assoc., however, was packed in a column 30 cm  $\times$  0.25 in. O.D. The alcohol solvent and solute employed were distilled in glass from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.), and the water was distilled-deionized and passed through an ODS-2 reversed-phase column to remove the last traces of organic matter. The concentration of the sample injected onto the column was adjusted to provide a peak 1/3 full-scale deflection at the maximum detector sensitivity. Dead volumes were measured for each mobile phase composition by determining the retention volume of sodium chloride.

## RETENTION CHARACTERISTICS OF THE DIFFERENT REVERSED PHASES EMPLOYING WATER AS THE MOBILE PHASE

The respective reversed phase was brought into equilibrium with methanol by passing 250 ml of the pure solvent through the column. The mobile phase was then abruptly changed to pure water, and after 20 ml had passed through the column an injection of 2.0  $\mu$ l of a 1% aqueous solution of sodium chloride and ethanol was placed on the column and the retention volume of each solute measured. Due to the necessity for the refractive index detector to come into equilibrium with the new mobile phase, injection could not be made prior to the passage of 20 ml of water through the column. After 30 ml of water had passed through the column, another injection of the same sample was made and the respective retention volumes again measured. This process was repeated for every subsequent 10 ml of water passed through the column until a constant retention volume for each solute was obtained. The dead volume of the column was taken as the retention volume of the sodium chloride and the corrected volume of ethanol calculated as the difference between the retention volume of ethanol and the retention volume of sodium chloride. This series of experiments was repeated for every reversed phase, and the results obtained are shown as curves relating the corrected retention volume of ethanol to the volume of water passed through each column shown in Fig. 1.

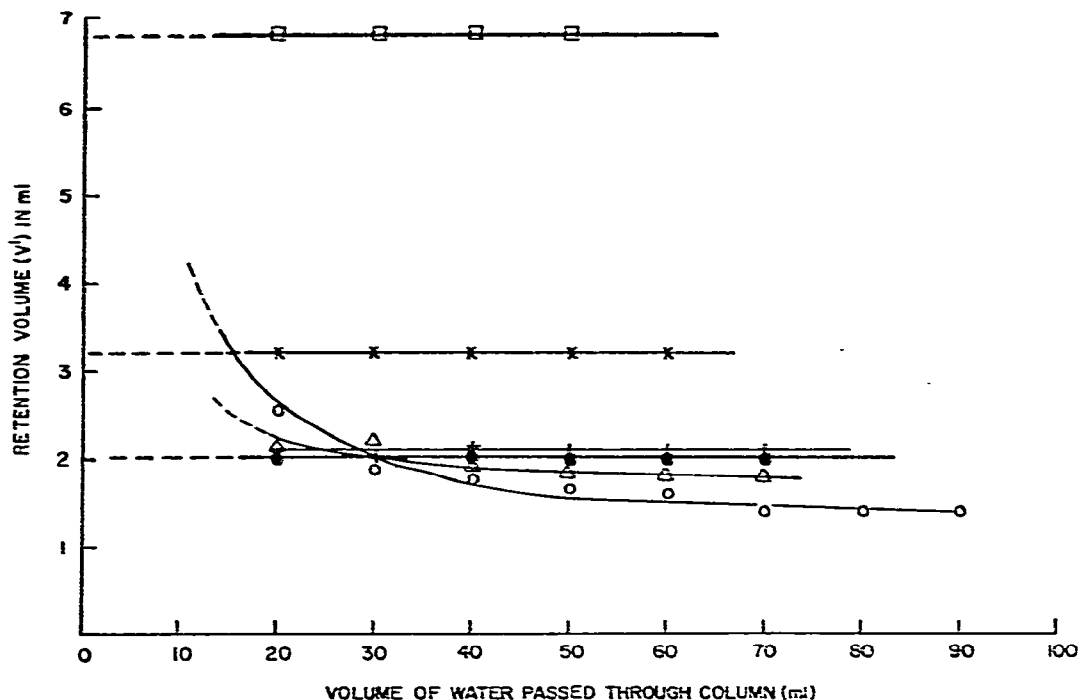


Fig. 1. Graph of retention volume ( $V'$ ) of ethanol against volume of water passed through the column for six different reversed phases.  $\square$ , ODS-2 (16.9%, w/w);  $\times$ , ODS-3 (10.5%, w/w);  $+$ , RP-2 (5.0%, w/w);  $\bullet$ ,  $\mu$ Bondapak (10.5%, w/w);  $\triangle$ , RP-18 (19.8%, w/w);  $\circ$ , RP-8 (12.2%, w/w).

### Discussion

Fig. 1 shows two distinct types of curves. Ignoring the curve for RP-2 for the moment, it is seen that the bulk modified or polymeric-type bonded phase reaches equilibrium with the mobile phase almost instantaneously, producing a straight line parallel to the  $x$ -axis. The curves for the brush-type bonded phase, RP-8 and RP-18, however, are quite different, showing a continuous decrease in retention volume of alcohol until 50–60 ml of water has passed through the column. The curve then levels off to a constant value. Furthermore, the retention volume of ethanol on RP-18 is only slightly greater than the retention on RP-8 and even less than the retention on RP-2. This order of elution is entirely different to those determined using high concentrations of methanol in water as the solvent as previously reported<sup>2</sup>. It should also be noted that the curve for the short hydrocarbon chain brush-type bonded phase, RP-2, behaves similarly to the bulk-type phase, the curve for  $V'$  for ethanol against volume of water passed through the column, giving a straight line parallel to the  $x$ -axis.

### THE EFFECT OF TIME ON PHASE EQUILIBRIUM

The slow attainment of equilibrium by the brush-type stationary phase on changing the mobile phase from methanol to water could be due to two different effects. Firstly, the methanol might require to be washed from the surface by a sufficient amount of water before the stationary phase can reach its normal condition in contact with water. Alternatively, the methanol has to diffuse from the surface into the water, and thus a certain time period has to be allowed for this transfer to become complete. It is also possible that a reorientation of the brush-type phase takes place which requires a finite time to complete. To determine which of these effects was the cause of the slow equilibration, the previous experiment was again carried out for the RP-18 column, but in this case the pump was turned off after each measurement had been made and a time interval of 10 min allowed to elapse before the next injection of sample, as opposed to waiting for 10 ml of water to pass through the column. The results are shown as a curve relating  $V'$  of ethanol against equilibration time in minutes in Fig. 2. The curve for  $V'$  of ethanol on the RP-18 stationary phase against volume of water passed through the column determined in the previous set of experiments is also included in Fig. 2. In both experiments, a flow-rate of 1 ml/min was employed; thus, the volume flow and time axis are numerically equivalent. It is seen that both sets of results fall on the same straight line, indicating that time is the factor that effects the rate of equilibration and not the volume of water passed through the column.

### Discussion

The above results show that there are two significant differences in the retentive characteristics of the two types of stationary phases. Firstly, the bulk- or polymeric-type phase comes into equilibrium rapidly with the water and for a given reversed-phase type (e.g. ODS series) gives the expected retention relative to each other as expected from their carbon content<sup>2</sup>. The brush-type phase, on the other hand, comes into equilibrium much more slowly and exhibits abnormal relative retention in that RP-18, which should exhibit a retentive capacity similar to ODS-2,

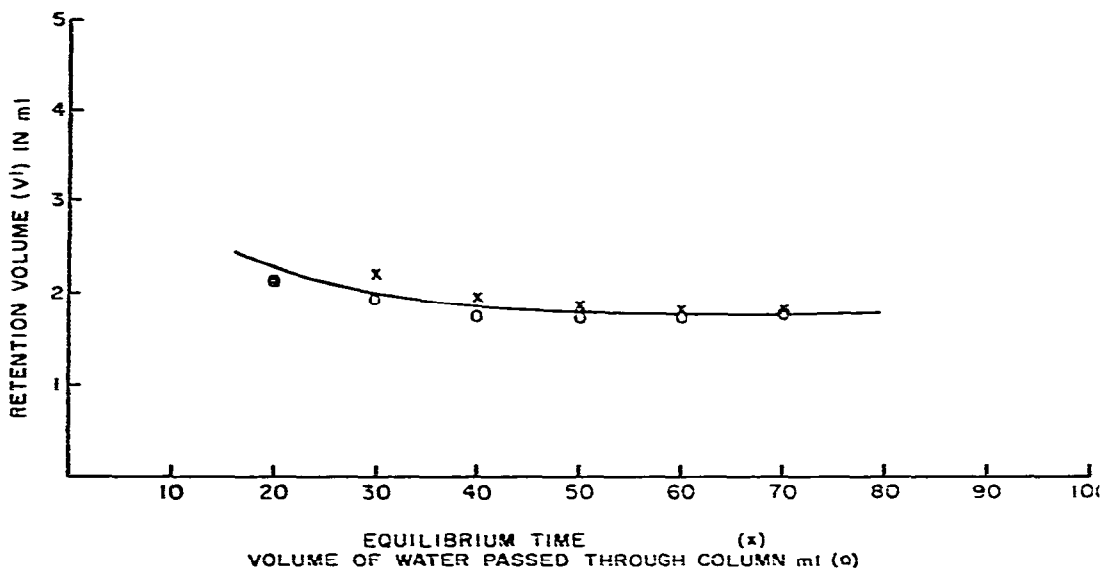


Fig. 2. Graph of retention volume ( $V'$ ) of ethanol against volume of water passed through the column and equilibrium time in minutes for the reversed phase, RP-18.

has a lower retention volume for alcohol than RP-2 and only slightly greater than RP-8. A possible explanation for this anomalous effect has been put forward by Lochmuller and Wilder<sup>3</sup> and by Gilpin and Squires<sup>4</sup>, who suggest that under certain conditions the hydrocarbon chains of a brush-type reversed phase can interact more strongly with themselves than with the surrounding mobile-phase. Hydrocarbon chains can only exhibit dispersive interactions, and thus they will compete for either water or an adjacent hydrocarbon chain to interact with. Obviously, if only dispersive interactions are considered, hydrocarbon-hydrocarbon interactions will be much stronger than hydrocarbon-water interactions. It follows that the stationary phase could agglomerate and, therefore, could have a greatly reduced effective chromatographic surface area. It is possible that it will adopt a spatial arrangement where the chains are lying almost flat upon the surface. This would, in effect, reduce the effective chromatographic surface area very significantly and could account for the extensive reduction in retention capacity of the aggregated bonded phase. This would explain why the RP-2 bonded phase retains the solute to a greater extent than RP-18 in pure water and also explains why at higher alcohol concentrations where the hydrocarbon chains could move more freely in the mobile phase the retention characteristics are reversed and exhibit normal elution behavior. Originally in contact with wet methanol, the hydrocarbon chains would be free to move in the solvent, and the methanol between the chains would permit them to remain apart as the dispersive interactions between the hydrocarbon chains and the methanol are more nearly equivalent to the interactions between the chains themselves. However, when in contact with water, the methanol would slowly diffuse from between the hydrocarbon chain matrix, permitting the chains to interact with each other and collapse onto the surface with the resulting reduction in effective chromatographic surface area. Thus, the retention volume which is a product of the distribution coefficient and

TABLE I  
DEAD VOLUME OF REVERSED-PHASE COLUMNS

Column	Dead volume (ml)
ODS-2	1.92
ODS-3	2.47
RP-18	2.25
RP-8	2.10
RP-2	2.55
$\mu$ Bondapak	2.75

the effective surface area would also be significantly reduced. The cross-linked polymeric structure of the bulk-type phase, however, will be more rigid; thus, the structure prevents collapse of the hydrocarbon chains, and its chromatographic surface area will be maintained even in the presence of water, and normal solute retention properties will be exhibited. In the collapsed state, however, it would be expected that the bonded phase, RP-18, would at least exhibit the equivalent retention to RP-2. However, in Table I the dead volumes for the respective reversed-phase columns are given as measured by the retention of sodium chloride. It is seen that the dead volume of the RP-2 column is 2.55 ml, as opposed to RP-18 column of 2.25 ml; thus, a possible explanation would be that the porosity and thus the effective surface area of the RP-2 would be significantly greater than the collapsed RP-18

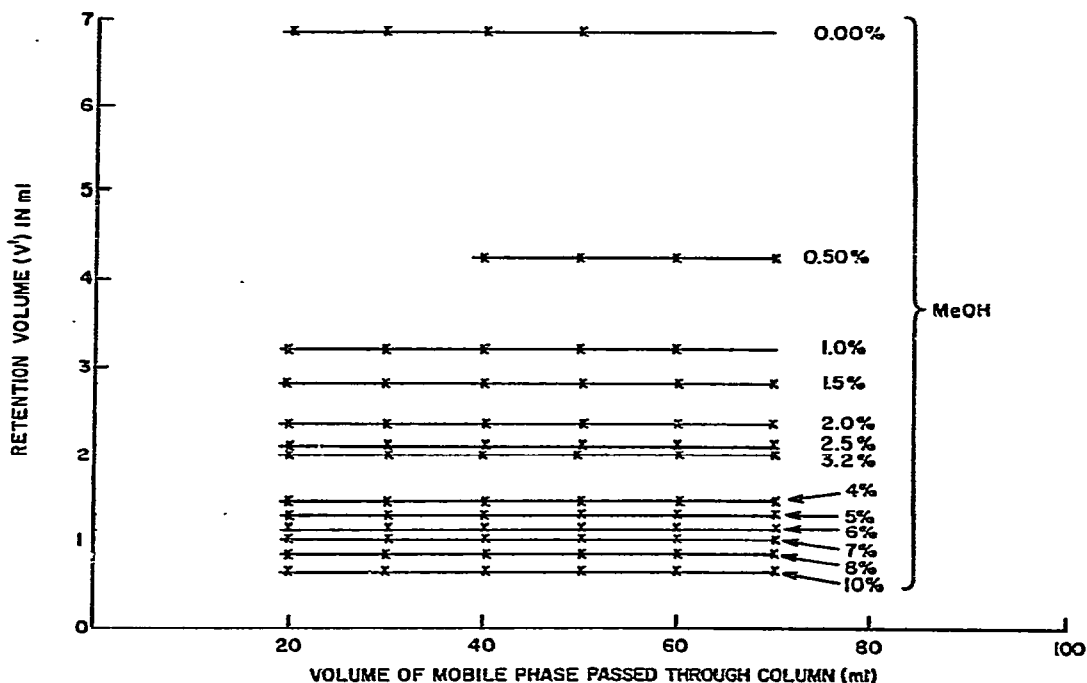


Fig. 3. Graph of retention volume ( $V'$ ) of ethanol against volume of mobile phase passed through the column for an ODS-3 reversed phase operated with different concentrations of methanol (MeOH) in the mobile phase.

brush-type phase, where apparently a large number of the pores in this material have been blocked and, therefore, not available for interaction with a solute.

#### THE EFFECT OF ALCOHOL CONTENT OF THE MOBILE PHASE ON SOLVENT RETENTION FOR TWO DIFFERENT TYPES OF REVERSED PHASES

It has been shown that in pure water the brush-type reversed phase, RP-18, behaves very differently from its characteristic performance in the presence of high concentrations of alcohol or acetonitrile<sup>2</sup> due possibly to dispersive interactions between the hydrocarbon chains themselves. At high concentrations of methanol the hydrocarbon chains are free moving in the mobile phase, whereas in pure water they associated by dispersive interactions and formed clumps on the surface. It was, therefore, of interest to determine the concentration of alcohol at which normal

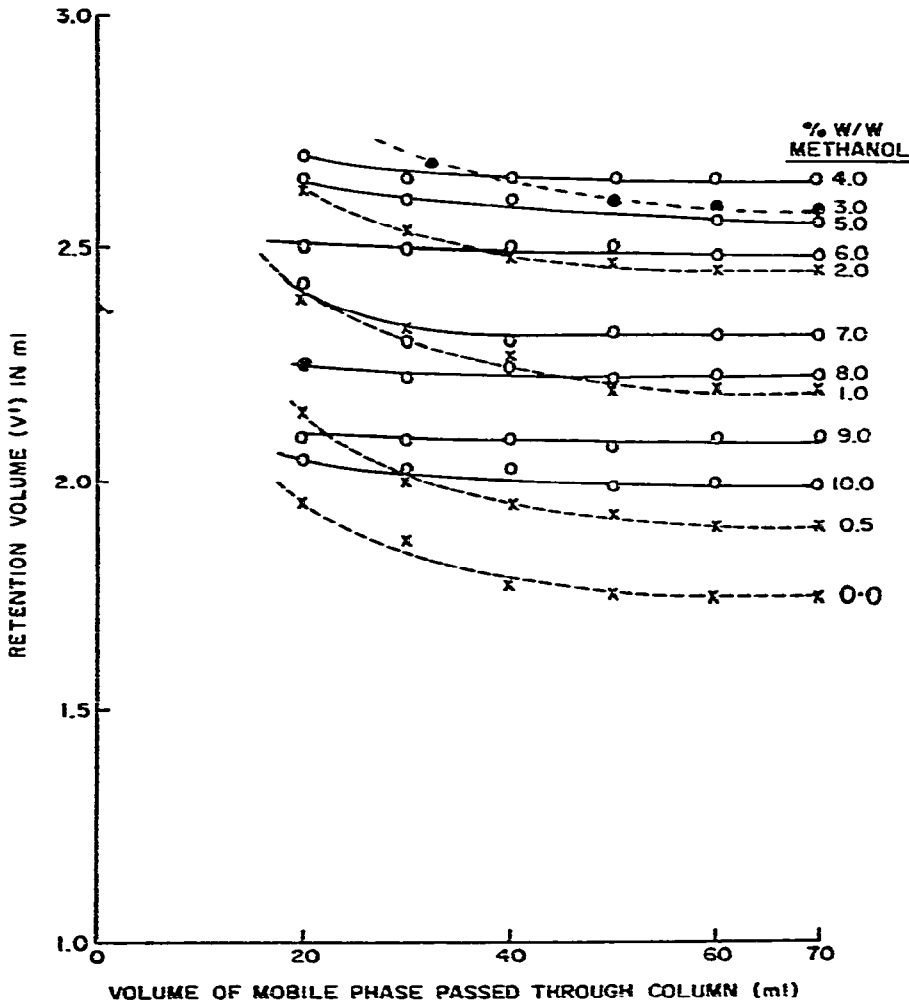


Fig. 4. Graph of retention volume ( $V'$ ) of ethanol against volume of mobile phase passed through the column for an RP-18 reversed phase with different concentrations of methanol in the mobile phase.

retention behavior was achieved by the brush-type reversed-phase and to compare it with the performance of a bulk modified or polymeric bonded phase. The previous experiments were, therefore, repeated employing both ODS-3 bulk- or polymeric-type reversed phase and RP-18, the brush-type bonded phase. Equilibrium curves were determined relating corrected retention volume of ethanol against volume of mobile phase passed through the column, and the final equilibrium value was noted. The curves were obtained for both reversed phases over a range of methanol-water mixtures from 0 to 10% (v/v) methanol in water. The results obtained are shown in Fig. 3 for the bonded phase ODS-3 and in Fig. 4 for the bonded phase RP-18. In Fig. 4 in order to avoid confusion, the curves for mixtures from 0 to 3% methanol are shown in broken lines, where those mixtures between 4 and 10% methanol are shown in solid lines. When the curves are close together, different symbols are used for the points on each curve to again avoid confusion. The equilibrium values for the corrected retention volume of ethanol for each reversed-phase were then plotted against the methanol concentration of the mobile phase, and the curves for the two phases are shown in Fig. 5.

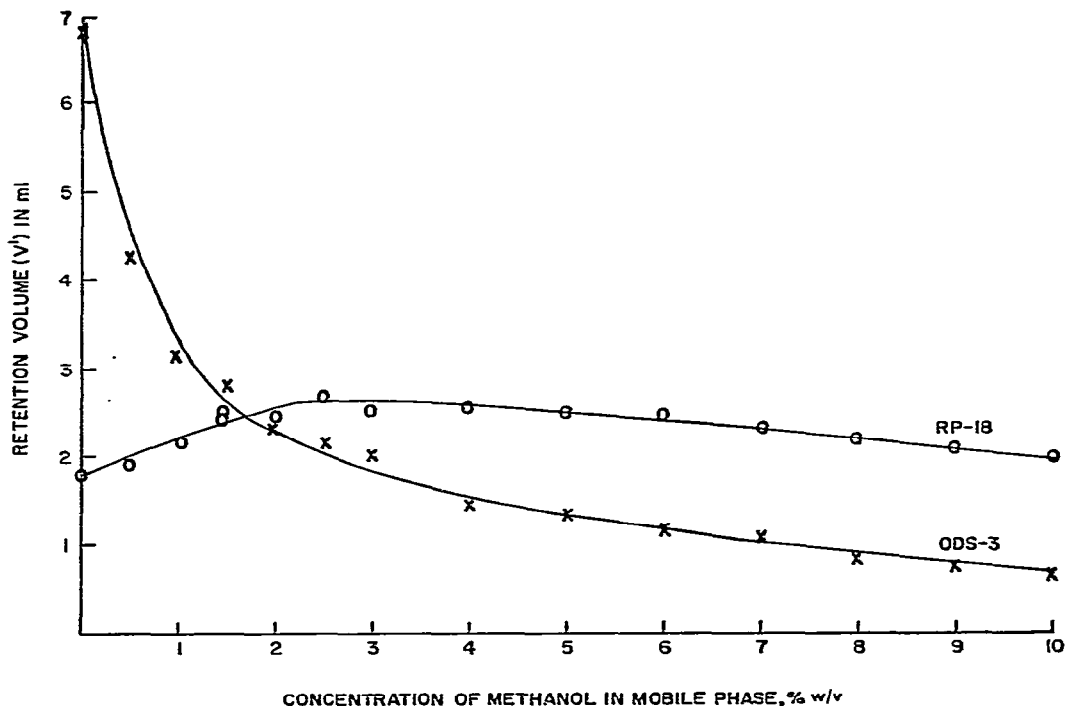


Fig. 5. Graph of retention volume ( $V'$ ) of ethanol against concentration of methanol in the mobile phase after stationary phase-mobile phase equilibrium was achieved for two reversed phases, ODS-3 and RP-18.

### Discussion

It is seen from Fig. 5 that the two reversed phases show very different types of curves. The ODS-3 curve gives the typical reciprocal relationship between the



corrected retention volume and solvent concentration. The curve for RP-18, however, first shows an increase in corrected retention volume with solvent concentration as the hydrocarbon chains become more associated with the mobile phase, free moving and less associated with each other in the form of a flat layer on the surface. This dissolution increases the effective surface area of the stationary phase, and thus the retention volume increases. However, when the concentration of methanol reaches about 5%, the solute-solvent interactions become significant and counteract the effect of the increase in surface area which results from the freeing of the hydrocarbon chains. Thus, at about 5% methanol in water the curve relating  $V'$  for ethanol against solvent concentration reaches a maximum and subsequently falls. It is also interesting to note that the normal retention behavior as predicted by the carbon content of the RP-18 bonded phase relative to that of the ODS-3 is only achieved when the methanol concentration reaches between 7 and 10% (v/v). At this stage the retention volume of ethanol on the RP-18 column is greater than the retention volume of ethanol on the ODS-3 column.

## CONCLUSIONS

The results obtained indicate that a brush-type reversed phase containing hydrocarbon moieties having significant chain length can associate with themselves at low methanol or high water concentrations and thus reduces the effective surface area of the reversed phase which will result in retention volumes of solutes being lower than would be expected from the carbon content of the reversed phase. Furthermore, the brush-type reversed phases in the presence of low concentrations of methanol take a significant time to come into equilibrium with the mobile phase. Thus, in chromatographic systems such as ion-pair chromatography where low concentrations of organic solvent are often employed, a significant period of time must be allowed for solvent-stationary phase equilibrium to be achieved after changing the solvent composition. The bulk-type or polymeric bonded phase, however, does not exhibit dispersive interactions between the hydrocarbon chains themselves as they are fairly rigidly held in the polymeric structure of the phase. It follows that equilibrium is far more rapid, and the bulk phases exhibit the expected retentive characteristics according to their carbon content at low concentrations of solvent in the mobile phase and even in the presence of pure water. It follows that, if the nature of the solute-stationary phase interactions on the surface of a bonded phase is to be examined, as a result of the uncertain surface area of a brush-type bonded phase due to dispersive intraphase interactions, this type of phase should be avoided. The bulk-type or polymeric reversed phase would, therefore, be the preferred choice for such investigations.

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