Journal of Chromatography, 199 (1980) 259–273
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,964

ROLE OF ORGANIC MODIFIER SORPTION ON RETENTION PHENOMENA IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

Distribution phenomena associated with the elution of solutes of varying retention from reversed-phase chromatographic columns have been examined. For solutes slightly more retained than the organic modifier component of the mobile phase, displacement of a portion of the modifier that has been extracted into the bonded phase resulted. For longer retained solutes, a vacancy band was produced, indicating a net flux of organic solvent into the bonded phase. These effects revealed that the composition of the extracted modifier system could be varied by the addition of a second solvent to the mobile phase, such as occurs in ternary mobile phase systems. Polar group selectivities of solutes could in large part then be rationalized on the basis of specific solute–modifier interactions in the stationary phase. On the basis of these results unusual organic modifiers have been used in ternary mobile phase systems to achieve large selectivity differences.

INTRODUCTION

The popularity of reversed-phase liquid chromatography (RPLC) using *n*-alkyl chemically bonded phases is well known. The wide use of RPLC arises from the general simplicity of the method, the broad range of substances that can be chromatographed and the inherent selectivity and efficiency of the chromatographic system. The popularity remains high, despite the lack of a full understanding of the mechanism of the retention. Control and manipulation of separation requires an understanding of the retention process and a knowledge of the role that the composition of the mobile phase plays on that process. While a major factor in retention and selectivity is hydrophobic expulsion from a mixed aqueous–organic modifier mobile phase[1,2], polar and ionic forces can also influence the thermodynamics of distribution.

As in the case of polar adsorbents[3], a distribution of mobile phase constituents between the mobile and the stationary phase occurs, thereby altering the composition and properties of the latter. As equilibrium in an RPLC column must exist with respect to the distribution of mobile phase components between the aqueous and bonded phases, the organic modifier will be extracted into the stationary

0021-9673/80/0000–0000/$02.25

phase in large measure on the basis of hydrophobicity. This point was first suggested by Knox and Pryde[4] and then examined by others[5–7].

In a previous paper[8], we have studied in detail the extraction of three common organic modifiers—methanol (MeOH), acetonitrile (ACN) and tetrahydrofuran (THF), into the stationary phase. The extent of extraction, as measured by the corresponding distribution isotherms, was shown to be related to the solvent strength of the organic modifier, in direct analogy to normal-phase liquid chromatography (NPLC). The consequences of the extracted organic solvent on the elution behavior of species identical or similar (*i.e.*, deuterated solvents) to the mobile phase constituents was investigated. On the basis of finite concentration chromatographic effects, the elution behavior of each species could be understood. In particular, displacement and/or vacancy concentration pulses of the organic modifier were observed. Based on these studies, ^2H_2O was shown to be an appropriate dead volume marker in RPLC.

In this paper, we explore the role of the extracted organic solvent on retention and separation in RPLC. Specifically, the elution behavior of retained solutes including common organic modifiers is examined. Both displacement and vacancy peaks which can be attributed to concentration pulses of the organic modifier are observed. These responses to equilibrium disturbances in the column reflect changes in the extracted modifier system that occur when the solute becomes a part of the mobile phase. When ternary mobile phases (*i.e.*, water plus two organic modifiers) are used, the second organic modifier added to the binary mobile phase can significantly alter the stationary phase by virtue of its concentration in that phase. This in turn permits a powerful manipulation of chromatographic selectivity based on specific interactions of solutes with the extracted modifier system. These principles will be illustrated with unusual RPLC organic modifiers such as trifluoroethanol and chloroform.

EXPERIMENTAL

Equipment and chemicals

Chromatographic experiments were performed on an SP 8000 liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.) equipped with a built-in data handling system. All liquid chromatographic measurements were performed on either a 10- or 20-cm analytical column packed with octyl bonded Hypersil (Shandon Southern Instruments, Sewickley, PA, U.S.A.) (5 μm particle diameter) at 25.0°C in an air-thermostated oven. Preparation and characterization of the packing and columns were previously reported[8]. Liquid chromatography grade solvents were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.) and Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Other chemicals and solute samples were purchased from a variety of suppliers.

Modifier displacement/vacancy measurements

Organic modifier displacement/vacancy measurements were performed using the liquid chromatographic system with a Model R 401 refractometer (Waters Assoc., Milford, MA, U.S.A.) to monitor the column effluent. The typical sensitivity setting of the refractometer was $48 \cdot 10^{-6}$ refractive index units full scale, though

sensitivities differing by a factor of 2 from the nominal setting were also employed. The refractometer was thermostated at 25.0°C via an external circulating water bath. Samples for the displacement/vacancy studies were prepared by dissolution of the solute in an aliquot of the bulk mobile phase. Sample injection volumes were 10 μl, introduced into the column by pneumatically actuated valve injection. Corrected retention volumes and capacity factors were calculated using the elution volume of ^2H_2O as the column dead volume.

Modifier distribution measurements

Modifier distribution measurements were produced by the gas chromatographic (GC) method described previously[8]. Either isopropanol or propionitrile was used as an internal standard for GC analysis on a Model 350 gas chromatograph (Tracor Instruments, Austin, TX, U.S.A.) equipped with a flame-ionization detector. The distribution isotherm for benzene was measured by spectrophotometric analysis of the samples stripped from the column on a Cary 14 spectrophotometer (Cary Instruments, Monrovia, CA, U.S.A.).

Ternary solvent selectivity

Mobile phases containing the various concentrations of ternary solvents were prepared by pipetting the stated volume percentages of the three solvents into a common vessel. The pure solvents were degassed briefly via a helium purge prior to preblending. Ultraviolet detection at 254 nm was used to monitor the column effluent. Samples were prepared by dissolving approximately 1 mg of each solute in 2 ml of methanol–water (50:50); 10 μl of the resultant solution were injected into the column.

RESULTS AND DISCUSSION

Displacement/vacancy effects occurring upon solute injection

When equilibrium is established between the mobile and stationary phases in an RPLC column, the organic modifier is preferentially extracted into the stationary phase by virtue of its hydrophobic expulsion from the mobile phase[4–8]. If this equilibrium is disturbed by either the injection of solute components or by an actual change of the mobile phase, the composition of the extracted organic modifier system will vary in order to accommodate the changes in the mobile–stationary phase equilibrium.

In the case of solute injection (*i.e.*, elution chromatography), the response of the system to the equilibrium disturbance at the top of the column will involve either a net outflow or a net inflow of organic modifier into the solute band. Net outflow will result in a displacement concentration pulse of organic modifier eluting from the column with a retention volume determined by the distribution isotherm of the modifier between the mobile and stationary phases. In contrast, a net inflow of organic modifier into the solute band will cause a vacancy concentration pulse which will elute at the same time as the displacement pulse, but the refractive index of the band will be in the direction opposite to that for the displacement pulse. The appearance of a displacement or vacancy band in the chromatogram (in addition to the solute band) provides information concerning the solute distribution process in the RPLC column.

In addition, the production of a displacement or vacancy band from injection of a solute in a binary mobile phase indicates changes that will occur in the extracted organic modifier system if that solute were made the third component of a ternary mobile phase.

We have previously examined displacement/vacancy organic modifier concentration pulses produced by the injection of constituents of the mobile phase into an RPLC column[8]. Here we study the effects of injection of small amounts of THF into a bonded phase column equilibrated with a binary aqueous–organic mobile phase, the organic solvent in the mobile phase being either MeOH or ACN. We will then turn to an examination of the effects of injection of other solutes with varying retention.

Fig. 1A shows a concentration pulse of MeOH resulting from injection of a 10-μl sample of mobile phase enriched in MeOH, the mobile phase composition being MeOH–H_2O (10:90, v/v). Detection was achieved using an RI monitor. Upon injection of 10 μl of mobile phase containing 1% (v/v) THF, the chromatogram shown in Fig. 1B results. The peak eluting at the same time as the MeOH peak in Fig. 1A is a methanol rich concentration pulse, a direct result of the displacement of MeOH from the stationary phase at the top of the column by THF molecules.

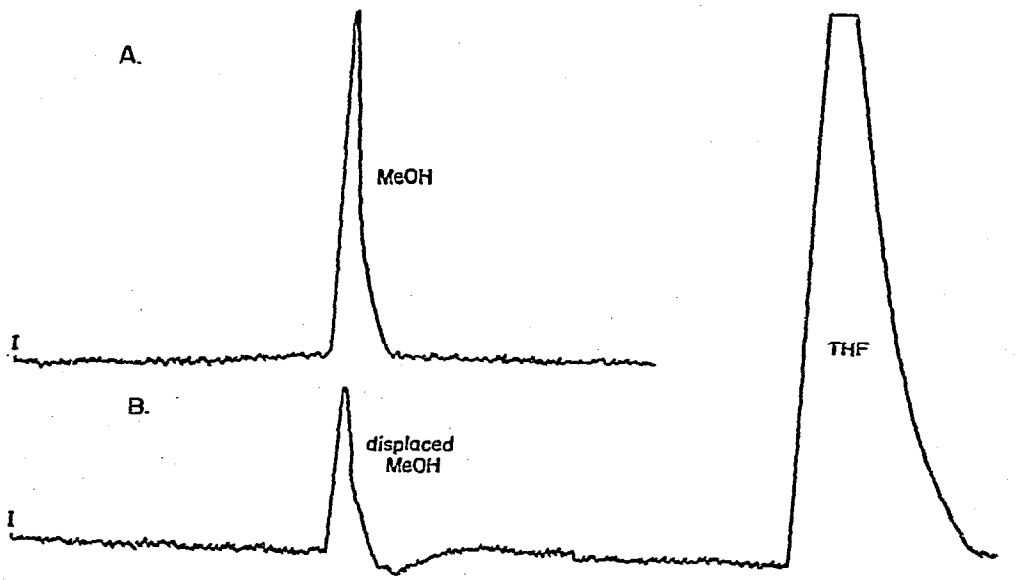

Fig. 1. Chromatograms illustrating the displacement of MeOH from the stationary phase by the injection of THF. Mobile phase: MeOH–H_2O (10:90, v/v). Bonded phase, *n*-octyl bonded Hypersil. RI detection. A, 10-μl injection of mobile phase enriched in MeOH. B, 10-μl injection of mobile phase enriched with 1% (v/v) THF.

The displacement of MeOH from the bonded phase is confirmed in Fig. 2, which shows the measurement by GC analysis of the actual amounts of MeOH and THF in the stationary phase when using the ternary mobile phase of THF–MeOH–H_2O. For this ternary system, the concentration of MeOH in the mobile phase was

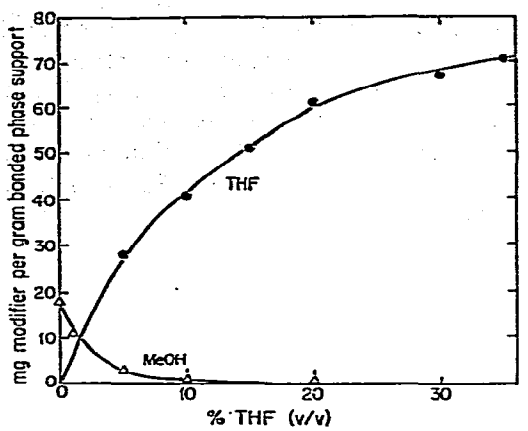

Fig. 2. Change in composition of organic modifier system extracted into the stationary phase upon addition of THF to the binary mobile phase of MeOH–H_2O (10:90, v/v). For the ternary mobile phases, the MeOH concentration is held constant at 10% (v/v), while the H_2O concentration is appropriately reduced with added THF.

held constant at 10% (v/v) and the THF concentration was varied from 0 to 35% (v/v), with a corresponding change in the concentration of H_2O. As the THF concentration in the mobile phase is increased, the concentration of methanol in the bonded phase is seen to decrease, a direct consequence of the displacement of the methanol by THF. As expected, the concentration of THF in the bonded phase increases with higher concentrations of THF in the mobile phase.

We next examined an ACN–H_2O binary mobile phase, measuring the displacement of ACN from the stationary phase by the addition of THF to the mobile phase. Fig. 3 shows the variation in the composition in the extracted organic modifier

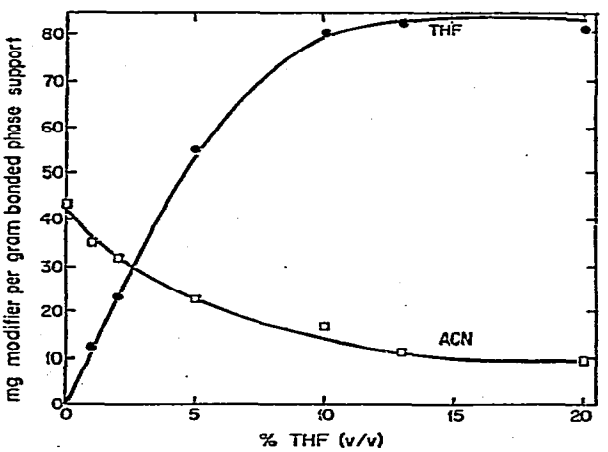

Fig. 3. Change in composition of organic modifier system extracted into the stationary phase upon addition of THF to the binary mobile phase of ACN–H_2O (13:87, v/v). For the ternary mobile phases, the ACN concentration is held constant at 13% (v/v), while the H_2O concentration is appropriately reduced with added THF.

system as the THF concentration in the mobile phase is changed. The ACN concentration is maintained at 13% (v/v) in the mobile phase, and the water concentration is appropriately varied with addition of THF. The same trends as in Fig. 2 are observed, namely that THF, the more hydrophobic solvent, selectively concentrates in the stationary phase, displacing ACN. The amount of THF in the stationary phase reaches a constant value at approximately 10% (v/v) and the concentration of ACN, contrary to MeOH, levels off to a constant finite value.

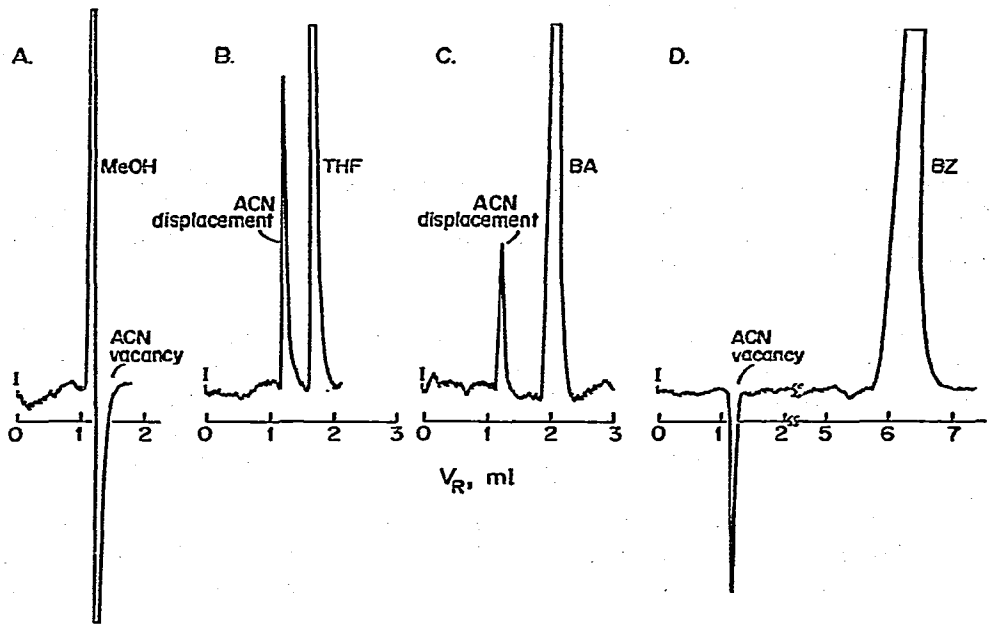

Fig. 4. Chromatograms illustrating displacement and vacancy peaks of ACN arising from the injection of solutes with increasing retention volumes. Mobile phase: ACN–H_2O (33:67, v/v). RI detection. A, ACN vacancy band from injection of 10 μl of 0.6% (v/v) MeOH dissolved in bulk mobile phase. B, ACN displacement band from injection of 10 μl of 0.6% (v/v) THF in mobile phase. C, ACN displacement band from injection of 10 μl of 0.6% (v/v) BA in mobile phase. D, ACN vacancy from injection of 0.6% (v/v) BZ in mobile phase.

The displacement accompanying the injection of THF in a column equilibrated with an ACN–H_2O binary mobile phase, as well as the phenomena occurring from the injection of other solutes of various retention volumes, are presented in Fig. 4. In agreement with Fig. 3, injection of a 10-μl sample of mobile phase containing 0.6% (v/v) THF produces a displacement peak (Fig. 4B) with a retention volume identical to that of a concentration pulse caused by the injection of a sample of ACN enriched mobile phase. This result also follows that observed for the injection of THF in a binary MeOH–H_2O mobile phase (Fig. 1).

For the injection of a sample of 0.6% (v/v) benzyl alcohol (BA) in the mobile phase, displacement of acetonitrile is again noted (Fig. 4C), though the magnitude of the displacement pulse is less than that for the injection of an equivalent quantity of THF (Fig. 4B). The retention volume of the benzyl alcohol is also larger than that

of THF (2.03 ml *vs.* 1.73 ml). For the injection of 10 μl of 0.6% (v/v) benzene (BZ) dissolved in the mobile phase, a negative peak, indicative of an ACN vacancy concentration pulse, is observed (Fig. 4D). As noted, the occurrence of this vacancy band corresponds to the elution of an acetonitrile deficient pulse relative to the bulk mobile phase. More hydrophobic solutes, such as toluene, also produce vacancy peaks, the magnitude of which appear roughly comparable to that of the vacancy band produced by injection of benzene. Finally, injection of 0.6% (v/v) of MeOH in the mobile phase also results in a vacancy peak (Fig. 4A).

The generality of the displacement/vacancy behavior seen in Fig. 4 was explored by an examination of the effect of injection of a homologous series of α,ω-alkyl diols in a mobile phase of THF–H_2O (5:95, v/v). The results are presented in Table I, with the THF concentration pulse reported in terms of μg THF per μg of solute injected. The amount of THF in the concentration pulse was determined from a calibration plot of peak area *vs.* quantity of THF in 10-μl injected samples of mobile phase. A linear relationship between the quantity of alkyl diol injected into the column and the magnitude of the resultant displacement or vacancy peak was observed over a wide solute concentration range.

TABLE I

QUANTITY OF MODIFIER DISPLACED FROM OR ADDED TO THE STATIONARY PHASE AS A FUNCTION OF RETENTION VOLUME (V_R) FOR A HOMOLOGOUS SERIES OF *n*-ALKYL DIOLS

Mobile phase: THF–H_2O (5:95, v/v).

Solutes	V_R *(ml)*	*THF (μg) displaced per solute injected (μg)* *
1,2-Ethanediol	1.23	−0.37
1,3-Propanediol	1.27	−0.51
1,4-Butanediol	1.39	−2.23
THF	1.61	.....
1,6-Hexanediol	2.39	+0.37
1,7-Heptanediol	4.63	+0.30
1,8-Octanediol	11.9	+0.20
1,9-Nonanediol	35.2	−0.39
1,10-Decanediol	large	−0.36

* Sign refers to refractive index signal; − corresponds to a vacancy; + corresponds to a displacement.

Table I reveals that solutes which elute immediately after the THF pulse (*i.e.*, hexanediol to octanediol) produce a net displacement of THF from the bonded phase. As retention of successive homologues increases, however, the magnitude of the displacement peak diminishes, leading to a THF vacancy peak for strongly retained solutes (*e.g.*, nonanediol and decanediol). Furthermore, it is seen that those solutes that elute prior to the modifier pulse also result in the production of a vacancy band, the magnitude of which increases as the retention volume of the solute approaches that of the modifier pulse.

Using different solutes in both the ACN–H_2O and THF–H_2O binaries, comparable results are obtained (Fig. 4 and Table I), supporting the idea that the observed

displacement/vacancy phenomena are common in RPLC systems. The results in Fig. 4 and Table I suggest that upon injection of solutes significantly more hydrophobic than the organic modifier, a net increase in the amount of the organic modifier in the stationary phase over that in the absence of these solutes is obtained. This behavior should also occur in the ternary mobile phase system consisting of ACN, BZ and water. The composition of the extracted modifier system as a function of BZ concentration in this ternary mobile phase is presented in Fig. 5. In the top portion of the figure it can be seen that the amount of ACN in the bonded phase increases significantly with BZ concentration in the mobile phase. For example, the quantity of extracted ACN doubles when the concentration of BZ in the mobile phase is raised from 0 to 0.2% (v/v). The distribution isotherm for BZ in the lower portion of the figure is concave in shape, unlike the convex isotherms for the distribution of modifiers such as MeOH, ACN or THF[8]. The concave shape of the isotherm implies, as expected, that BZ and other hydrophobic solutes have a higher affinity for themselves than for the aqueous mobile phase.

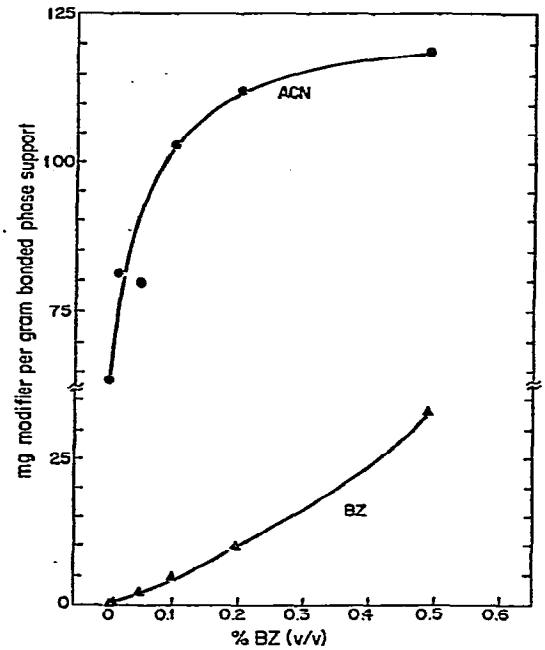

Fig. 5. Change in composition of organic modifier system extracted into the stationary phase upon addition of BZ to the binary mobile phase of ACN–H_2O (35:65, v/v).

The trends in Fig. 5 relate to the chromatographic behavior of BZ and ACN as a function of mobile phase composition. The elution volumes of concentration pulses of BZ and ACN, as well as ^2H_2O, as a function of concentration of BZ in the mobile phase are given in Fig. 6. An increase in the elution volume of the BZ pulse is observed as the concentration of BZ in the mobile phase is increased; this behavior is a direct consequence of the concave nature of the BZ isotherm. The retention volume of the ACN pulse also increases with increasing concentration of BZ, supporting the

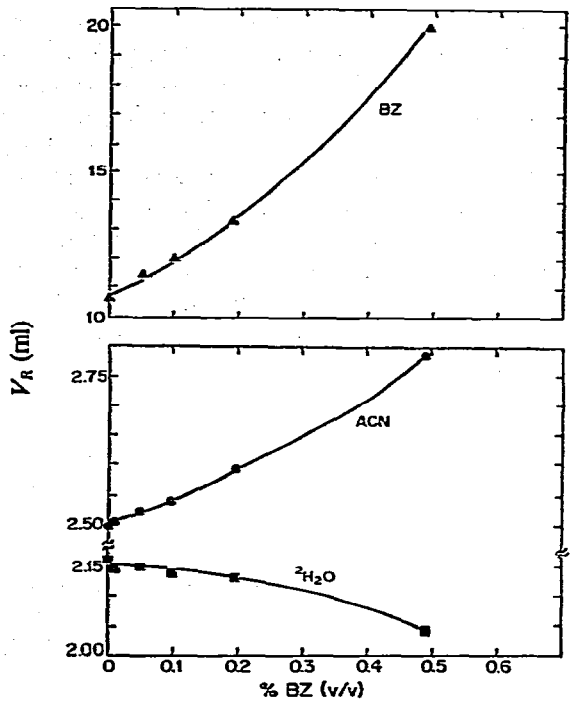

Fig. 6. Plot of elution volume of ACN, BZ and ^2H_2O as a function of concentration (%, v/v) of BZ in the ternary mobile phase BZ–ACN–H_2O. The concentration of ACN in the mobile phase was held constant at 35% (v/v).

observation in Fig. 5 that the amount of ACN in the stationary phase increases with increasing concentration of BZ. The retention volume of the ^2H_2O band, which is a good measure of the column dead volume, decreases with increasing BZ concentration. This decrease in the dead volume is again indicative of the increased extraction of ACN and BZ in the bonded phase. Note that the dead volume change is not large, varying by only 3% over the BZ concentration range of 0 to 0.5%; however, the dead volume of the column without the extracted modifer is roughly 15% higher than that obtained with the BZ–ACN–H_2O ternary mobile phase.

The production of organic modifier displacement/vacancy pulses appears to be at variance with the results of Scott and Kucera[5], who observed no organic modifier displacement band upon injection of a solute species. It is difficult to compare the results of those authors with the results presented in this paper since numerous differences in experimental parameters, *e.g.*, different mobile phase compositions, bonded phase materials, etc., exist between the two works. However, based on our present study and our previous paper[8], several possible reasons can be given to explain the different observations.

First, an implicit assumption in ref. 5 was that an amount of modifier would be removed from the stationary phase approximately equivalent to the amount of solute injected. However, as seen in Fig. 4 and Table I, either displacement or vacancy peaks can be produced, and there will be a range of retention volumes for which little or no effect will be observed.

Secondly, the investigations in ref. 5 were limited to high ACN concentrations in the mobile phase. In order to be certain that the phenomena we observed were not limited to the low mobile phase modifier concentrations, we examined whether organic modifier concentration pulses were produced upon solute injection at a mobile phase composition of ACN–H_2O (75:25, v/v). Results identical to those shown in Fig. 4 were observed, *i.e.*, the appearance of an ACN displacement peak for the injection of a sample of THF in the mobile phase, and an ACN vacancy peak for the injection of benzene. However, a significant decrease in the detector sensitivity for ACN was observed, because the RI difference between a pulse of ACN and the mobile phase is lower at higher concentrations of ACN in the mobile phase. Thus, the displacement/vacancy pulses produce less sensitive signals at the higher modifier concentrations; however, they would appear exitso t at these mobile phase compositions as well.

Consider next the meaning of the preceding results with respect to the distribution (or retention) process in RPLC. As noted, upon injection of a solute into the column, the mobile–stationary phase system is forced out of equilibrium at the top of the column. The equilibrium will be rapidly reestablished; however, the solute band will contain a different organic modifier composition than other parts of the column. In order to achieve equilibrium within the solute band, organic modifier must either be added to or removed from the solute zone. Both processes will undoubtedly occur, but only the net effect will be observed at the detector. This movement of modifier creates a separate zone in which the total modifier concentration in the mobile and stationary phase differs from that in the rest of the column. This band travels down the column as a concentration pulse with an elution time corresponding to the injection of a sample of mobile phase enriched in modifier or water.

Injection of solutes more hydrophobic than the organic modifier causes a displacement of that modifier from the solute band. One can envision a competitive effect in which organic solvent molecules which solvate the *n*-alkyl bonded chains in the stationary phase are displaced by solutes of greater hydrophobicity.

As the retention volume of the solute relative to that of the modifier increases, the height of the displacement band decreases until a vacancy band appears. As revealed in Fig. 4 for the ternary phase system BZ–ACN–H_2O, the net inflow of organic modifier into the solute band results in an increase in the amount of extracted modifier in the stationary phase. As the retention of the injected solute increases, the distribution at the head of the column will favor higher concentrations of solute in the stationary phase. This solute distribution will modify the hydrophobic character of the stationary phase, favoring a net flux of organic solvent into the bonded phase, possibly solvating the extracted solute and the solute–*n*-alkyl chain complex. Organic modifier displacement from the *n*-alkyl chains may also occur when the solute is sorbed into the stationary phase, but this effect is overshadowed by the enrichment of organic solvent in the bonded phase, and a vacancy band is observed at the detector.

For solutes that elute before the concentration pulse of the organic modifier, a vacancy band results, in accordance with the vacancy produced when samples of mobile phase enriched in H_2O are injected[8]. In this case the solute will remain predominantly in the mobile phase upon injection. A net flux of organic modifier from the stationary to the mobile phase will result in part due to the increased mobile phase

strength in the solute band. As in the case of solutes with long retention, a net flow of modifier into the solute zone occurs, creating a modifier deficient or vacancy band.

The increase in the magnitude of the vacancy band as one proceeds from ethanediol to butanediol may be a result of the increase in solvent strength of the higher homologues relative to ethanediol. Note that the fraction of butanediol in the stationary phase is still quite small (only about 0.1 % of the amount injected), and thus the presence of the butanediol will have a significant effect upon the hydrophobic strength of the mobile phase in the band.

As we have noted, the changes in the extracted organic modifier observed for solute injection have direct relevance to separations using ternary mobile phases in which the solute becomes the third component of the mobile phase. In the next section, we will examine some of the characteristics of ternary mobile phases from this point of view.

Selectivity with ternary mobile phases

The manipulation of the mobile phase for control of selectivity in RPLC is now well established[9,10]. Fine tuning of separations is possible using ternary mobile phases which consist of water plus two organic modifiers. Such powerful controls on separation through the use of ternary mobile phases are also known in NPLC using silica gel[11,12]. We will now show that the composition of the extracted modifier system can play an important role in the polar group selectivities found for ternary mobile phases. This result leads directly to the introduction of new organic solvents that may be used as the third solvent in a ternary system for control of separation.

In a previous paper, we have studied the changes in retention of mono- and disubstituted polar aromatic solutes relative to benzene as a function of the mole fraction ratio of THF to THF plus MeOH in ternary aqueous mobile phases[9]. The concentration of the water in the ternary mobile phase was such that the α value of toluene to benzene (an approximate measure of hydrophobic selectivity) was maintained constant over the whole composition range. In Fig. 6 of ref. 9, the α value of *p*-chlorophenol to benzene increased rapidly upon addition of THF to a binary MeOH–H_2O mobile phase, and then leveled to a constant α value at approximately 0.25 mole fraction THF. In contrast, the α value of methyl benzoate relative to benzene was observed to decrease with increasing concentration of THF, again leveling off at about 0.25 mole fraction THF. It has previously been shown (Fig. 2) that THF concentrates in the stationary phase at the expense of MeOH. Since THF is a stronger base than methanol, the acidic phenol is retarded upon addition of THF to the mobile phase by virtue of hydrogen bonding between the solute and extracted THF in the stationary phase. On the other hand, the basic methyl benzoate is accelerated through the column as THF is added to the mobile phase.

Polar solute–organic modifier interactions in the stationary phase (in the above case in the form of hydrogen bonding) would thus appear to play an important role in the selective elution of polar solutes in a chemically bonded RPLC column. Hence, the separating power of ternary mobile phases would seem to be related in part to the ability of such phases to alter the composition of the extracted organic modifier system via the concentration of relatively hydrophobic solvents in the stationary phase. Obviously, selective solute–modifier interactions in the mobile phase cannot be ruled out. Addition of a third species to the mobile phase will also

change the hydrophobic (or non-polar) selectivity. However, the concentration of hydrophobic modifiers in the stationary phase is believed to be a significant factor in polar selectivity.

We decided to explore these concepts further by an examination of retention changes that occur for various simple polar aromatic solutes as small amounts of polar, acidic and basic organic solvents are added to a mobile phase of MeOH–H_2O (50:50, v/v). For the third solvent, some relatively uncommon organic modifiers were selected in order to enhance potential polar selectivity effects and to assess the relative usefulness of such solvents in RPLC. The retention changes resulting from the use of ternary mobile phases consisting of a low concentration of the second modifier [usually $\approx 2\%$ (v/v)] in aqueous methanol are summarized in Fig. 7. Solutes selected for study included proton donors (*e.g.*, 2-chloro-5-methyl phenol, *m*-chlorophenol), proton acceptors (*e.g.*, methyl benzoate, benzaldehyde), dipolar species (*e.g.*, nitrobenzene, benzonitrile) and non-polar species (*e.g.*, benzene).

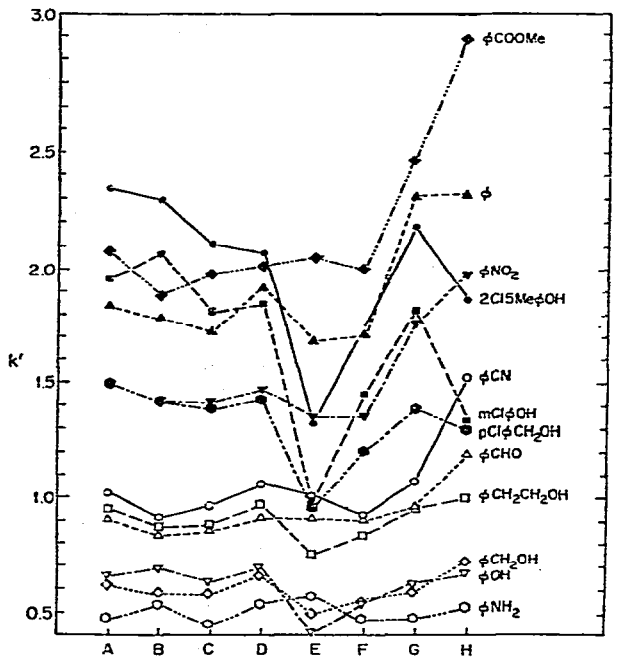

Fig. 7. Diagram illustrating the selectivity changes possible in various ternary mobile phases containing small quantities of unusual organic solvents in MeOH–H_2O (50:50, v/v). Solute capacity factors are plotted for each ternary system. Mobile phases: A, MeOH–H_2O (50:50, v/v); B, THF–MeOH–H_2O (2:49:49); C, ACN–MeOH–H_2O (2:49:49); D, propionitrile–MeOH–H_2O (2:49:49); E, hexafluoroisopropanol–MeOH–H_2O (1.8:49.1:49.1); F, trifluoroethanol–MeOH–H_2O (2:49:49); G, trichlorotrifluoroethane–MeOH–H_2O (0.9:49.55:49.55); H, chloroform–MeOH–H_2O (2:49:49).

Significant changes in retention and elution order are observed for the various ternary phases. Many of the trends can be rationalized on the basis of solute interaction with the extracted modifier in the stationary phase. Solvents which possess greater proton donating ability than MeOH such as hexafluoroisopropanol, tri-

fluoroethanol and chloroform (solvents E, F and H, respectively in Fig. 7) accelerate the migration of proton donating solutes through the column while retarding the migration of proton accepting solutes. To illustrate, *m*-chlorophenol elutes more rapidly and methyl benzoate elutes more slowly in the chloroform ternary phase (solvent H) relative to the MeOH–H_2O binary phase (solvent A), as shown in Fig. 7. In contrast, opposite behavior is observed for these two solutes when the THF ternary phase (solvent B) is compared to the MeOH–H_2O binary phase.

Another general observation is that the retention changes are largest for ternary solvents E–H. The added organic modifier in these cases is, in general, more hydrophobic than the other organic modifiers (*i.e.*, B–D), as evidenced by the retention volumes of these modifiers in the binary MeOH–H_2O mobile phase. For a given ternary phase composition, a greater extraction of the organic solvent additive will occur for the more hydrophobic species. Larger quantities of organic additives in solvents B–D in the mobile phase will be necessary to obtain the large selectivity changes observed for sovents E–H.

The selectivity changes that can be achieved by the addition of small amounts of hydrophobic acids to the binary mobile phases in RPLC are illustrated in Figs. 8 and 9. In Fig. 8A, a mobile phase of MeOH–H_2O (50:50, v/v) produced a separation in which phenol and aniline (compounds 1 and 2) and methyl benzoate and 2-chloro-5-methyl phenol (compounds 5 and 6) completely overlapped. Addition of 2% hexafluoroisopropanol to this mobile phase caused the acceleration of acidic phenols (compounds 1, 6 and 7) and a small retardation of aniline, benzaldehyde and methyl benzoate, as shown in Fig. 8B.

As a further example, Fig. 9A shows the coelution of nitrobenzene, indole and

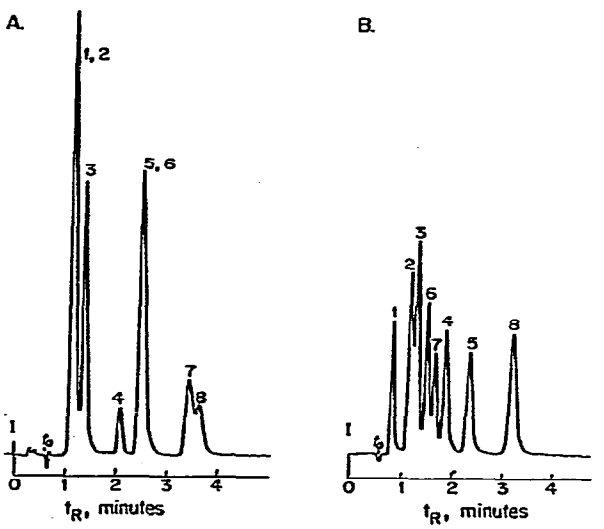

Fig. 8. Chromatograms illustrating selectivity changes when using ternary mobile phases. A, MeOH–H_2O (50:50, v/v); B, hexafluoroisopropanol–MeOH–H_2O (2:49:49, v/v). UV detection. A 10-μl volume of sample mixture in mobile phase was injected. Components: 1 = phenol; 2 = aniline; 3 = benzaldehyde; 4 = benzene; 5 = methyl benzoate; 6 = 2-chloro-5-methyl phenol; 7 = 4-chloro-2-methyl phenol; 8 = toluene.

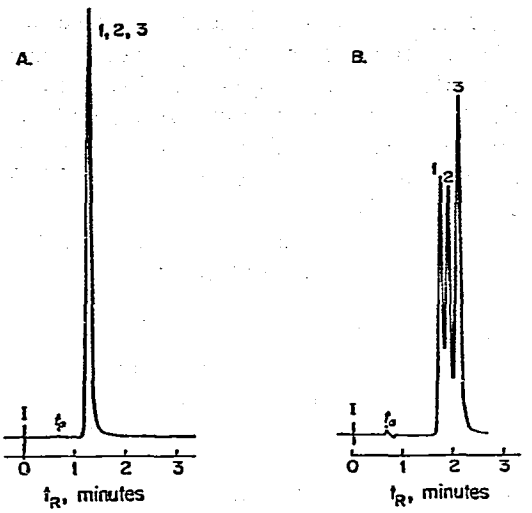

Fig. 9. Chromatograms illustrating selectivity changes when using ternary mobile phases. A, MeOH–H_2O (35:65, v/v); B, trifluoroethanol–MeOH–H_2O (2:34.5:63.5). A 10-μl volume of sample mixture in mobile phase was injected. Components: 1 = nitrobenzene; 2 = indole; 3 = 2,4-dinitroaniline.

2,4-dinitroaniline with a mobile phase of MeOH–H_2O (35:65, v/v). Addition of 2% (v/v) trifluoroethanol results in a separation in which the basic solutes (indole and 2,4-dinitroaniline) are retarded relative to nitrobenzene. Here again, hydrogen bonding in the stationary phase can explain the changes in retention.

The practical usefulness of such mobile phase solvents is obviously dependent on their availability and cost. In this regard, trifluoroethanol and chloroform are relatively inexpensive and can be considered valuable additions to the standard organic modifiers, *i.e.*, MeOH, ACN and THF. For more exotic modifiers, *e.g.*, hexafluoroisopropanol, it is noted that such solvents are typically employed in small percentages, and thus the cost factor is reduced. The use of microbore columns[13], noted for their low solvent consumption, will reduce the cost factor even further and stimulate the practical utility of these modifiers.

CONCLUSIONS

The phenomena observed in this paper have also been seen in normal-phase liquid chromatography using polar adsorbents[14]. As we have implied, other similarities exist between RPLC and NPLC. Significant amounts of strong solvents are extracted into the stationary phase in both cases (*e.g.*, THF for RPLC and MeOH for NPLC). Ternary solvents can be used for manipulations of separations for both processes as well. The comparison should obviously not be taken too far as the driving mechanism for sorption in RPLC is usually expulsion from the mobile phase and this typically does not exist in NPLC.

Further studies in the characterization and the role of the extracted modifier system are needed. In this regard, it would be interesting to explore the displacement/vacancy phenomena that arise from solute injection in ternary systems. Moreover,

spectroscopic studies (*e.g.*, nuclear magnetic resonance) of the bonded-phase system, in the presence of the organic solvent may prove to be informative. These efforts as well as others will provide much insight into the distribution phenomena occurring in RPLC systems.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the National Science Foundation for support of this work. Paper number 73 from the Institute of Chemical Analysis.

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