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Reverse Phase Ion-Pairing Chromatography at Pressures up to 345 MPa

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Abstract

The effects of pressure up to 345 MPa (50,000 psi) on a reverse phase ion-pairing system were studied in 50% methanol–water. Retention increased by a factor of 3.2 for methyl orange when the pressure was increased to 241 MPa (30,000 psi) and still larger (not measurable) at 345 MPa (50,000 psi). The retention of methyl red was increased by a factor of 2.2 by pressures of 241 MPa (30,000 psi) and by a factor of 2.5 by 345 MPa (50,000 psi). When the methanol concentration was increased to 75%, retention of methyl orange increased by a factor of 2.6 between 68.9 MPa (10,000 psi) and 345 MPa (50,000 psi).

Other variables studied were concentration of counterion and temperature. For methyl orange, retention decreased with pH, whereas for methyl red it increased. The effect of tetrahexylammonium ion concentration on retention was linear in the working range 0.5 to 2.0 m*M*. All samples eluted faster with increased temperature.

INTRODUCTION

Pressure has been shown to affect the retention of samples in several modes of liquid chromatography. Retention was changed by pressurizing the column in adsorption (*1, 2*), steric exclusion (*3, 4*), and in ion exchange (*5*). In the present study the effects of pressure on reverse phase partitioning

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have been examined both for regular partitioning and for ion-pairing systems using two symmetrical tetraalkylammonium ions as the counterions.

Ion-pairing chromatography has advanced primarily due to the work of Schill who extended the use of ion pairing from discrete liquid–liquid extractions to partition chromatography (*6, 7*). Wahlund and Gromingsson then introduced reverse phase ion-pairing chromatography (*8*). Since the publication by Whittmer et al. (*9*) using ion-pairing with high efficiency bonded-phase packings, reverse phase ion-pairing chromatography has been receiving increased use as an alternative to ion exchange. The main advantages of ion pairing over ion exchange are that it is very versatile regarding selectivity, and amphoteric compounds can usually be chromatographed easily.

Ion-pairing chromatography of weak acids is controlled by several equilibria. Probably the most important is ionization of the sample itself. If un-ionized, the sample can be retained by an interaction with the stationary phase. This technique has been used in the past (*10*) and is often called ionic suppression or hydrophobic chromatography. Otherwise, if the sample is ionized, it can interact with a counterion to form an ion pair. Then, the ionization equilibrium of the counterion is also important. In light of these equilibria, there are several variables which can be used to control retention in an ion-pairing system: the composition of the mobile phase, including the concentration of an organic modifier; apparent pH; concentration and type of the counterion; temperature: and pressure.

The primary purpose of the present study was to examine the effects of pressurizing the system up to 345 MPa (50,000 psi). In order to minimize the pressure drop across the column, coarse particles were used together with slow linear flow rates. Most of the pressure drop occurred across an exit valve; thus the pressure was nearly the same throughout the column. Use of a bonded-phase packing material was particularly important in these high-pressure studies because one did not have to be concerned with the effect of pressure on the solubility of a liquid stationary phase.

Systematic studies of variables other than pressure were also made. Conditions which gave a capacity factor, k', from 1 to 10 were desired. This was obtained primarily by adjusting the methanol concentration in the mobile phase. The type of counterion and its concentration were also useful variables. From extraction studies (*6*), a linear relationship between carbon number and logarithm of the distribution ratio was expected. Thus, data from the counterions we studied, tetraethylammonium (TEA) and tetrahexylammonium (THA), could, in principle, be used to predict the behaviors of other tetraalkylammonium ions.

The pH of the mobile phase had a useful upper limit of about 8 due to attack of the silica skeleton of the column packing. The lower limit of pH, especially for the high-pressure studies, was determined by the corrosion of the equipment, particularly the exit valve. Hence, at high pressures, pH 7 was used. However, low-pressure studies were made at lower pH since the compounds had interesting protonation reactions.

There were several reasons for studying the effects of temperature. Although temperature does not have as large an effect in liquid chromatography as in gas chromatography, there is indeed a significant temperature dependence (*11*). Since there was no temperature control (room temperature) in the high-pressure experiments, it was important to evaluate its importance. In addition, there was evidence to indicate that in some types of ion-pairing chromatography, temperature studies could easily distinguish between regular partitioning and ion-pair behavior (*12*). Retentions have been reported to increase with temperature in ion-pairing interactions while in regular partitioning, as in most other liquid chromatographic modes, retention decreased (*13*).

Finally, a comparative study of the temperature behaviors of liquid–liquid vs bonded phase was made. The liquid–liquid system was expected to be affected more due to a change in the solubility of the stationary phase in the mobile phase as temperature increased.

In the present study, methyl red (MR) and methyl orange (MO) were shosen as model ion-pairing compounds. Both molecules are azobenzene derivatives and have functional groups capable of ion pairing. In addition, they are weak acids, so pH is a useful variable. Furthermore, there is a different type of protonation for each compound in the sense that the carboxylate of methyl red loses its ion-pairing ability at low pH but can then itself partition as an uncharged species, whereas the sulfonate of methyl orange retains its charge when a nitrogen is protonated, so it loses its ability to partition without a counterion. Two other compounds, azobenzene and 4-methoxy-2-nitroaniline (4M2NA), were used as controls because they were uncharged and did not ion pair. All solutes were easily detectable in both the UV (254 nm) and visible (450 nm) regions.

EXPERIMENTAL

Reagents

Methyl red, methyl orange, azobenzene, 4-methoxy-2-nitroaniline, tetraethylammonium bromide (TEA), and tetrahexylammonium bromide (THA) were obtained from Eastman Organic Chemicals (Rochester,

New York). Methyl orange (MO) and methyl red (MR) were recrystallized twice from hot distilled water. Standard solutions were 40 mg/ml. The dried alkylammonium salts were stored in a desiccator prior to use. Standard solutions were 0.100 *M* for tetrahexylammonium ion and 0.250 *M* for tetraethylammonium ion. All other concentrations were prepared from these stock solutions by dilution immediately prior to use.

The sodium citrate, sodium dihydrogen phosphate, and potassium acid phthalate salts used as buffers were obtained from J. T. Baker Chemical Co. (Phillipsburg, New Jersey). Solutions (0.100 *M*) of these salts in doubly distilled water were adjusted to the desired pH using either 0.1 *M* hydrochloric acid or 0.1 *M* sodium hydroxide in conjunction with a Corning Model 110 pH meter. Absolute methanol (J. T. Baker) was then added to the desired volume percent. The pH values shown in the diagrams were those before addition of the alcohol. All solutions were stored in glass-stoppered Pyrex bottles.

Column Packings

For most of the studies, a bonded octadecyl column was used. The pellicular packing, Bondapak C_{18} Corasil, was purchased from Waters Associates (Milford, Massachusetts). The packing for the liquid–liquid studies was *n*-octadecane coated on deactivated silica. The Corasil I (Waters Associates) was the same substrate as that for the bonded-phase packing. The Corasil was silanized by refluxing for 4 hr with chlorotrimethylsilane (Pierce Chemical Co., Rockfield, Illinois) in benzene from a freshly opened bottle. Then the support was washed with benzene, dried at 60°C for 2 hr, and finally coated in a rotoevaporator to 0.5% (w/w) loading of *n*-octadecane (99% pure lot 95 A Humphrey Chemical Co., New Haven, Connecticut) using purified *n*-pentane as the solvent. This *n*-octadecane was also used to saturate the mobil phase in a separatory funnel overnight prior to use.

A precolumn is usually required in liquid–liquid partition chromatography because of the slight solubility of the stationary phase in the mobile phase. This is especially true if the temperature is varied since solubility is a function of temperature. The precolumn support material was acid-washed Chromasorb W, 30/60 mesh (Johns Manville, Denver, Colorado) which had been treated with chlorodimethylsilane. Its *n*-octadecane loading of 33% w/w was obtained by rotoevaporation using purified pentane as the solvent. The precolumn was 40 cm × 6 mm i.d. stainless steel. All columns were dry packed.

Five meters of 3.17 mm o.d. stainless steel tubing before the precolumn insured that the mobile phase was at the oven temperature before entering the analytical column. This heat exchanger was also used, without the precolumn, during the temperature studies with the bonded packing.

Equipment

The high-pressure chromatographic system has been described elsewhere (*1*). Pressures were measured by an 80,000 psi Bourdon gauge (American Instrument Co., Silver Spring, Maryland). Detection of the samples was performed by a Heath Model 703 UV-VIS spectrophotometer holding a Beckman flow-through cell. Connecting tubing between exit valve and detector was 0.3 mm i.d. Teflon.

Chromatograms were recorded using a Heath/Schlumberger Model 205–11 recorder (GCA McPherson, Chicago). Retention volumes were monitored by putting event marks on the chromatogram after reading a buret which collected the eluent.

Studies that did not involve high pressures used 19.0 cm × 5.0 mm i.d. 316 stainless steel columns with zero-dead-volume fittings. The bed was held in place by 0.5 μm stainless steel frits at each end of the column. A 6.9 MPa (1000 psi) Milton Roy minipump (Laboratory Data Control, Rivera Beach, Florida) provided flow. The mobile phase was filtered through a 7-μm stainless steel Nupro filter (Georgia Valve and Fitting, Atlanta) before being pumped through Teflon and polyethylene tubing to the air-actuated 32 μl loop injector valve (Chromatronix, Laboratory Data Control). Then, 2 cm of 0.3 mm i.d. stainless steel tubing connected the injector and the column.

Detection was performed at 450 nm using a Perkin-Elmer LC55 UV-VIS spectrophotometer. Chromatograms were recorded using an Omniscribe 5000 recorder (Houston Instruments, Houston).

The circulating air bath used in the temperature study has been described (*14*). Temperature measurements were performed by an iron-constantan thermocouple with double reference junctions. A Thermotrol temperature controller Model 1053 (Hallikainen Instruments, Richmond, California) controlled the temperature to about ± 0.05°C. The temperature range, ambient to 50°C, was limited by the maximum operating temperature of the injection valve, even though the bonded packing was stable to approximately 80°C. During the temperature studies, the air bath was stabilized for at least 30 min before injection. The precolumn, 5 m of heat

exchanger, and the column were centered over the oven fan, and the thermocouple was placed in the middle of the heat exchanger coils.

Procedures

During the high-pressure studies, sample injections were performed at atmospheric pressure with no flow. Then the exit valve was closed off and the pump started so as to attain the desired pressure before opening the exit valve and adjusting the flow to approximately 1 ml/min. During a run, the injector was kept in the sample-inject position.

Pressure pulses of about 3500 kPa were observed during pump strokes. Since the flow rate was not constant, the eluent was directed into a buret in order to monitor the flow rate and volume.

The low-pressure system was used for all studies which did not require high pressures. The flow rate was 1 ml/min. Injections were made during flow. Retention volumes were recorded by collecting the eluent in a buret.

When changing to a different mobile phase, at least seven total column volumes were passed through the system before injecting a sample. Most of the work was performed at room temperature.

Calculations

The capacity factor, k', was calculated using

$$k' = (V - V_i)/V_i \tag{1}$$

where V is the retention volume of the solute and V_i is the interstitial or void volume of the column. The capacity factor is related to the distribution coefficient, K, by

$$k' = K(V_s/V_m) \tag{2}$$

where V_s and V_m represent the volumes of the stationary and the mobile phases.

The distribution coefficient is related to the partial molar volume by the partial differential equation (*15*)

$$\frac{\partial(\ln K)}{\partial P} = \frac{-\Delta V}{RT} \tag{3}$$

If ΔV is constant, the relation of K to k' can be used to obtain ΔV by plotting ln k' vs pressure.

$$\Delta V = -RT(S_p) \tag{4}$$

where S_p is the slope. ΔV is often not constant with pressure. However, Eq. (4) is still useful in those cases to define an average volume change (*16*).

The enthalpy of solute transfer ΔH can be obtained from the slope, S_T, of a plot of log K vs $1/T$ (*17*):

$$\Delta H = -2.30RS_T \tag{5}$$

RESULTS

Preliminary Experiments

The expected (*11*) linear relationship between log k' and the concentration of methanol was obtained for MO (Fig. 1), MR, and 4M2NA in the absence of a suitable counterion. Furthermore, in the presence of such an ion, a linear relationship was observed for MO and MR. Thus, one could adjust k' in a predictable manner.

THA had a much larger effect on retention than did TEA. By adding 0.012 *M* TEA to a 33% methanol mobile phase at pH 7, retention for MO was increased by about 25%. However, when 0.0010 *M* THA was added, MO was retained so long it could not be detected. The methanol concentration had to be increased to 50% in order to elute MO in a reasonable volume. Compared to regular partitioning, retention was increased by a factor of 10 for MO by adding 0.001 *M* THA to a mobile phase of 50%

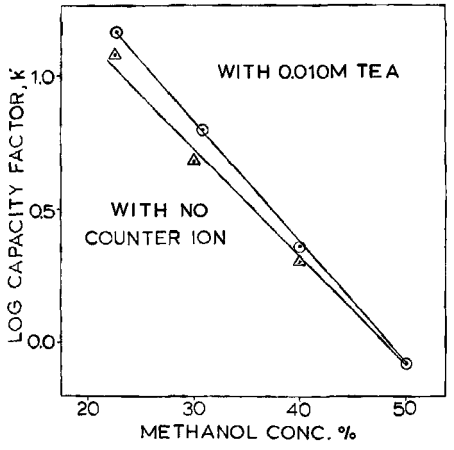

FIG. 1. Log capacity factor vs methanol concentration for methyl orange with (upper line) and without (lower line) ion-pairing on Corasil C-18.

methanol. The addition of 0.015 *M* sodium nitrate to the mobile phase gave no change in retention, indicating that the increase in retention was not due to a change in ionic strength. Using the linear relation of log k' vs length of the alkyl chain, intermediate retentions could presumably have been predicted for counterions having intermediate chain lengths.

The effect of partitioning or adsorption of the counterion itself into the stationary phase was observed indirectly by mixing excess THA with a sample containing MO, MR, and 4M2NA, then injecting the mixture onto a column and using a mobile phase of low (33%) methanol concentration without counterion. After elution of the first series of peaks, another injection was made. This injection procedure was repeated several times, and the chromatograms are shown in Fig. 2. In each run the retention of the first peak, 4M2NA, was unchanged. However, MR and MO, which

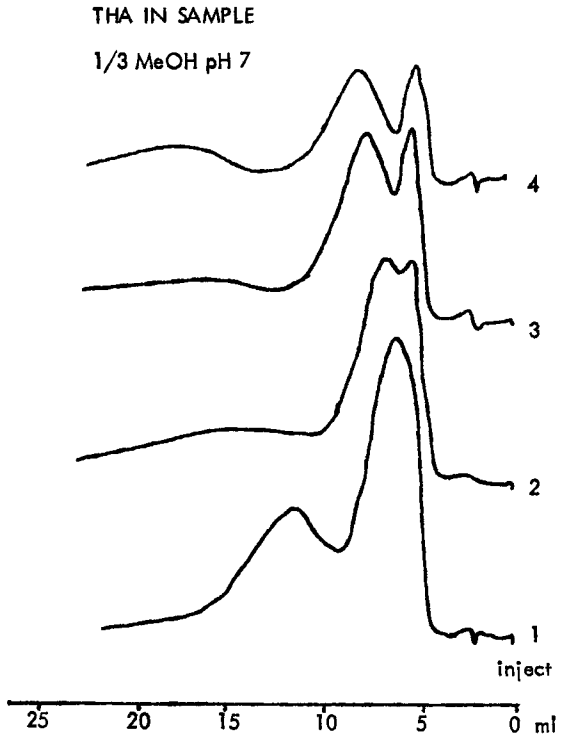

FIG. 2. Effect of repetitive injections of sample containing counterion onto column containing no counterion. Sample methyl orange, methyl red, and 4-methoxy-2-nitroaniline (left to right).

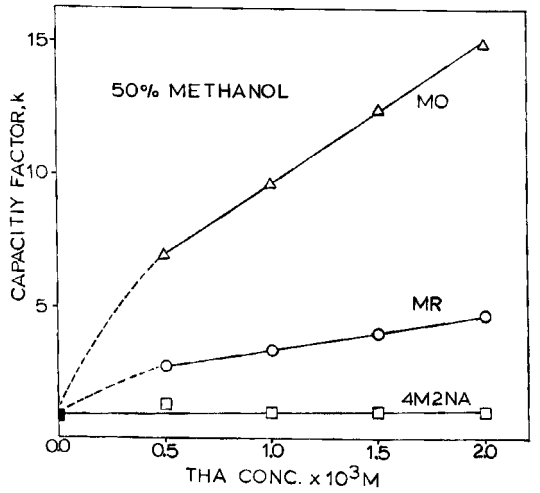

FIG. 3. Capacity factor vs concentration of tetrahexylammonium counterion using 50% methanol at pH 7.

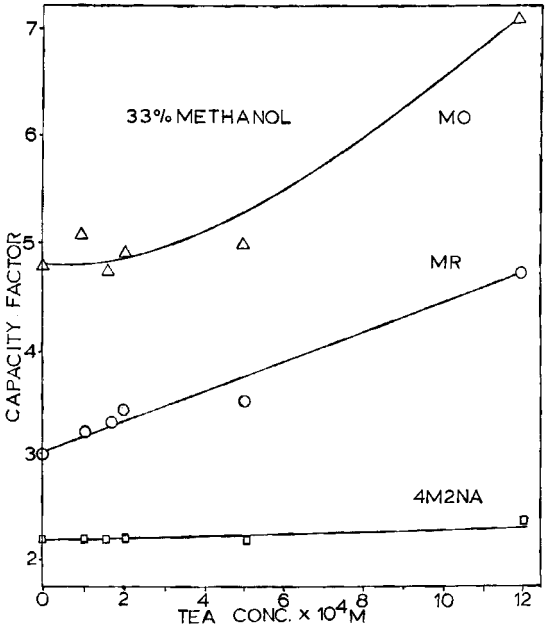

FIG. 4. Capacity factor vs concentration of tetraethylammonium counterion in 33% methanol at pH 7.

can form ion pairs, took longer and longer to elute after each successive injection, presumably due to the build-up of the concentration of counterions on the column. At this methanol concentration the counterion apparently partitioned more strongly than did the sample components. Hence, to obtain a reasonable k' and rapid equilibration of the column, the methanol concentration should be sufficient to minimize the partitioning of the counterion.

The effect of counterion concentration on retention is shown for THA in Fig. 3. A linear relationship was obtained from 0.5 to 2.0 mM. Similar results have been obtained using tetrabutylammonium ion in liquid–liquid chromatography where the slope was related to the conditional extraction constant (*18*). In contrast, the data for TEA in Fig. 4 were not as close to linear, and a much higher concentration was required before ion pairing significantly contributed to retention.

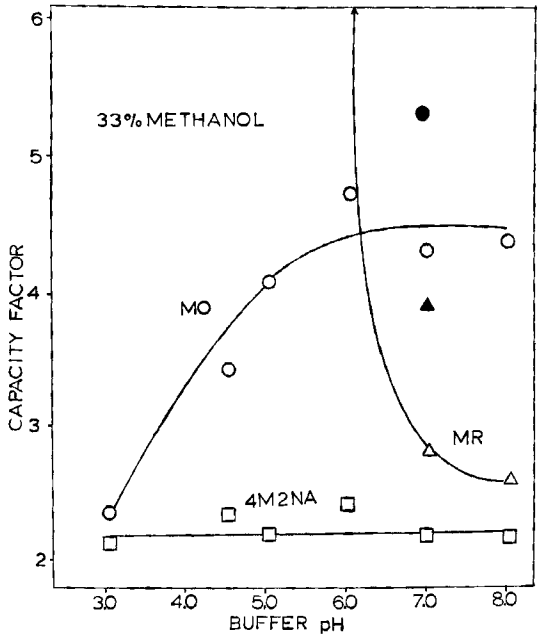

FIG. 5. Capacity factor vs pH for methyl red (triangles), methyl orange (circles), and 4-methoxy-2-nitroaniline (squares) for regular partitioning (open symbols) and for ion pairing with 0.010 M tetraethylammonium ion (solid symbols) in 33% methanol.

Effects of pH

Because the pK_a values of MR and MO are about 5.1 and 3.8, respectively, pH was expected to have a large effect on their retention behaviors. Figure 5 shows the behavior for regular partitioning when the mobile phase was 33% methanol. As expected, retention of the uncharged sample, 4M2NA, was independent of pH. On decreasing the pH, MR showed a rapid increase in retention due to its increased partition as an uncharged species. Not shown on the graph is a k' of 12 that was observed for MR at pH 6. When 0.012 *M* TEA was added to the mobile phase at this pH, there was no change in retention, indicating that the pairing site was blocked.

The effect of pH on MO was exactly the opposite. As the pH decreased, so did retention. That was because the pK_a refers not to the strong sulfonic acid but to protonation of an azo nitrogen (*19*). Thus protonation formed a zwitterion which was a weak ion-pair former. In addition, the pairs that

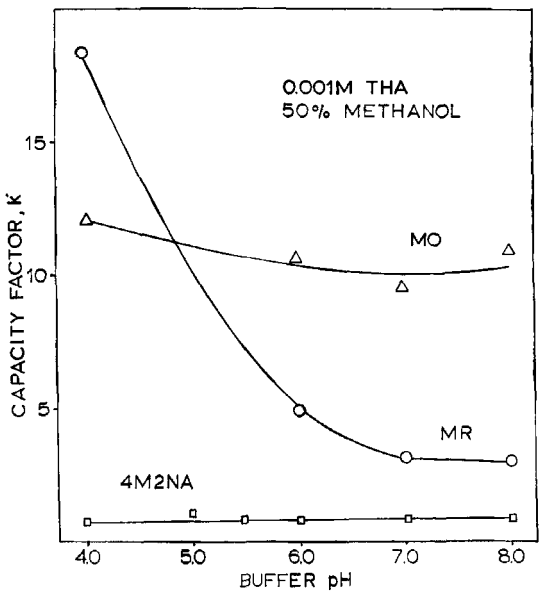

FIG. 6. Capacity factor vs pH in ion-pairing chromatography for methyl red, methyl orange, and 4-methoxy-2-nitroaniline in 50% methanol and 0.001 *M* THA.

formed did not extract well due to the more hydrophilic character of that pair (*20*).

Phthalate buffers were used for pH values below 4.5. Phosphate buffers were used for the higher pH values. In order to see if the buffer salts had any effect, citrate was used at pH 5 for MO and gave excellent agreement with the phthalate results. At pH 6, MR had a retention volume of 24.0 ml in a phosphate buffer and 25.0 ml in citrate, values that were within the range of experimental error. MO had a retention volume of 12.4 and 12.5 ml with the respective buffers. Hence, partitions were not significantly affected by the anions of the buffer.

With 0.001 *M* THA present, the effect of pH could not be studied using 33% methanol since the retentions of MO and MR were prohibitively long. Therefore, pH changes were studied in 50% methanol as shown in Fig. 6. As the apparent pH decreased, there was again a reversal in the elution order around pH 5 with MR going to longer retentions and MO remaining essentially unchanged throughout the range of pH 4 to 8.

Effect of Temperature

A temperature change of 30°C can double the capacity factor in both bonded-phase (*11*) and liquid–liquid chromatography (*21*). Figures 7 and

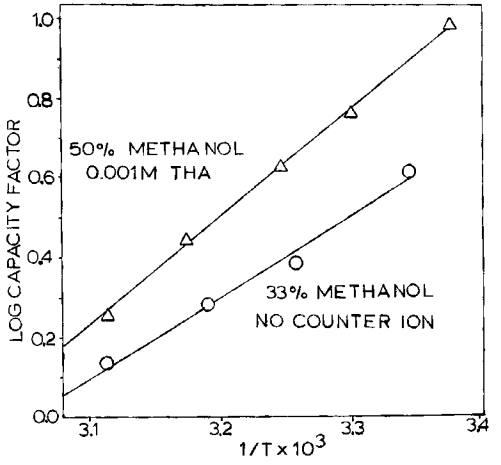

FIG. 7. Van't Hoff plots for ion pairing of methyl orange in 50% methanol (upper line) and for regular partitioning in 33% methanol on bonded octadecane (lower line).

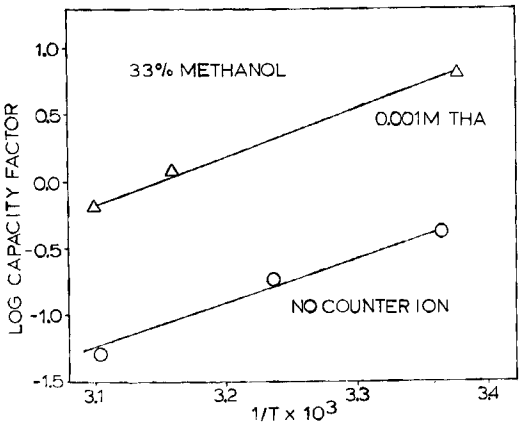

FIG. 8. Van't Hoff plots for ion pairing (upper line) and regular partitioning (lower line) of methyl orange using 33% methanol on a 0.5% octadecane column.

TABLE 1

Relative Enthalpies of Sorption for Methyl Orange in Bonded-Phase and Liquid–Liquid Systems

No counterion	-15.2 ± 1.2 kcal/mole	-9.2 ± 0.7 kcal/mole
0.001 *M* THA	-16.5 ± 0.4	-12.3 ± 0.2[a]

[a]In 50% methanol; all others were 33% methanol.

8 show that straight lines were obtained when $\ln k'$ was plotted against $1/T$ for both cases. From the slopes of the lines, relative values for enthalpies of transfer shown in Table 1 were calculated using Eq. (5). Azobenzene, which does not ion pair, had a ΔH of -6.3 ± 0.2 kcal/mole in the liquid–liquid system using 33% MeOH as the mobile phase. MR, on the other hand, had a very large ΔH of -23.9 ± 2.1 kcal/mole in the same system with 0.001 *M* THA. MR was essentially unretained without the counterion. It is interesting to note that the melting point of octadecane (28°C) fell in the temperature range studied, but a change in ΔH was not observed on going through the melting point. All samples eluted faster as temperature increased, i.e., ΔH was always negative. Dissociations are usually endothermic (*22*). Thus higher temperatures favored shorter retentions.

As a result, in our system, one could not use the sign of the slopes to

distinguish between mechanisms as Huber did in his studies (*12*). The difference in behavior may stem from the difference in the nature of the ion-pairing system. Huber's ion-pairing system resembles liquid ion-exchange more than ours does.

In the liquid–liquid case studied here, ΔH was significantly more negative than in the bonded-phase case. This difference was attributed to the increased solubility of the nonpolar stationary phase in the polar mobile phase at elevated temperatures.

Effect of Pressure

Increases in retention volume with pressure were observed as shown in Fig. 9. An apparent linear relationship was obtained using THA as the counterion up to 345 MPa. The retention of MO was so great that its peak was too flat to be detected. However, one could calculate from the change in retention as a linear function of pressure that its retention would change by a factor of 4 on going from ambient pressure to 345 MPa. Extrapolation using a semilog function increased the factor to about 9. A smaller effect, a change of 2 to 3, was found for MR under the same conditions.

Under a mobile phase of 0.001 *M* TEA and no counterion, retention changed faster with pressure. Thus log k' vs pressure was linear as shown

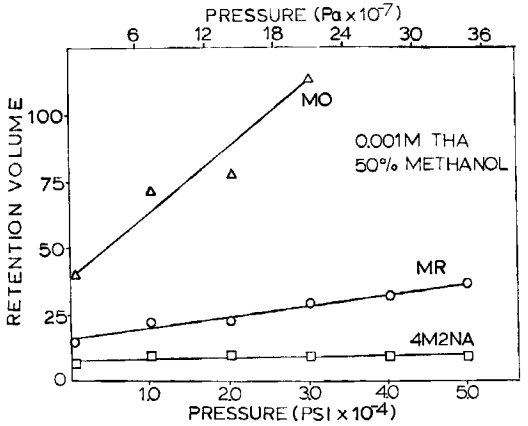

FIG. 9. Retention volume vs pressure for ion pairing of methyl orange, methyl red, and 4-methoxy-2-nitroaniline using 0.001 *M* tetrahexylammonium ion.

in Figs. 10 and 11. Accordingly, these partial molar volumes could be determined using Eqs. (3) and (4) and are shown in Table 2. Most of the data gave fairly linear relationships over the entire pressure range, indicating that ΔV values were constant. In the case of 0.001 M THA, MR was an obvious exception. Forcing those data to a straight line gave a value of -6.9 ± 0.9 whereas the average value for ΔV below 200 MPa was -10.1 ± 1.3 ml/mole, a value comparable to those found under the other solvent conditions.

In an earlier chromatographic study (*2*) using methyl orange and ethyl orange on silica with pure water as the mobile phase, ΔV was $+5.1$ and $+4.9$ ml/mole below 147 MPa (21.3 kpsi) for the respective azo dyes. In the pressure range 147 to 294 MPa (21.3 to 40 kpsi), the values

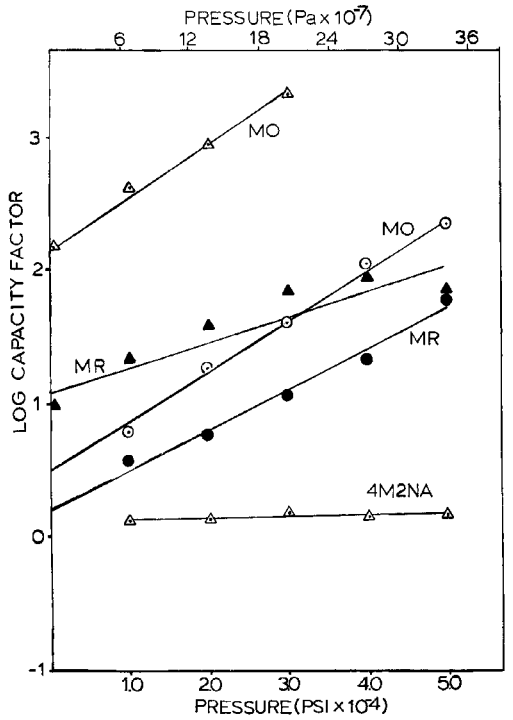

FIG. 10. Log capacity factor vs pressure using 0.001 M tetrahexylammonium counterion (triangles) and 0.001 M tetraethylammonium ion (circles) in 50% methanol, pH 7.

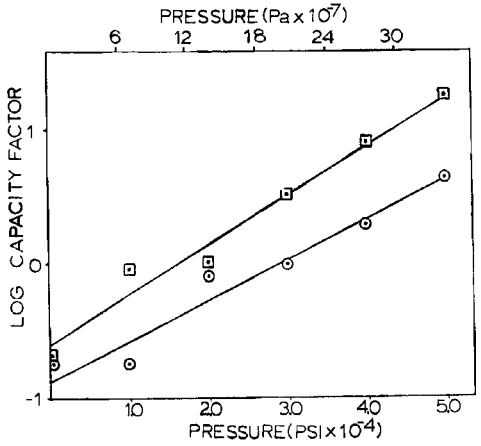

FIG. 11. Log capacity factor vs pressure with no counterion added for methyl red (circles) and methyl orange (squares) in 50% methanol, pH 7.

TABLE 2

Change in Partial Molar Volumes Determined from Slope of ln k' vs Pressure

	ml/mole		
	4M2NA	MR	MO
No counterion	—	-10.5 ± 0.6	-13.0 ± 0.7
0.001 *M* TEA	-4.0 ± 1.2	-10.8 ± 0.8	-13.4 ± 0.8
0.001 *M* THA	-3.2 ± 0.8	-6.9 ± 0.9[a]	-14.5 ± 1.5

[a] -10.1 ± 1.3 ml/mole when fitted for data up to 207 MPa (30,000 psi).

were -8.6 and -13.2 ml/mole. The change in sign was attributed to characteristics of the solvent, pure water. The anomalies of the pressure behaviors of density, viscosity, and other properties of pure water are removed when another liquid or a salt is added (*23*). Since the addition of solute has the effect of an additional external pressure, our buffered methanol/water system should be compared with the pressure range above 147 MPa. Under those conditions, the signs agree with the earlier data obtained and the magnitudes of the changes are reasonably close. The retention volume of THA ion itself was found to be 4.6 ml at 68.9 MPa and 4.7 ml at 345 MPa. Thus enhanced partitioning of the counterion was not greatly contributing to the pressure-increased retention of MO and MR under the solvent conditions used.

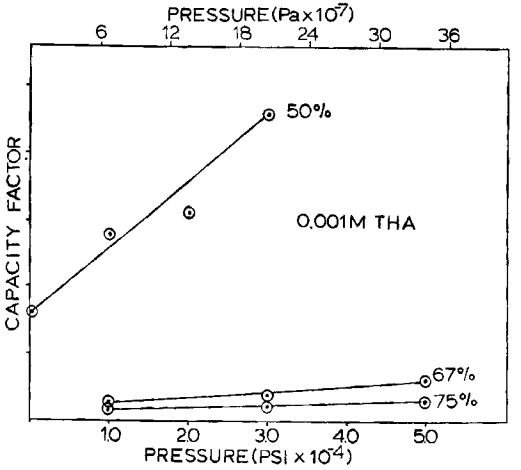

FIG. 12. Capacity factor of methyl orange vs pressure for various methanol concentrations, pH 7.

In the present study the pressure dependence of the capacity factor of MO decreased as the methanol concentration increased as shown in Fig. 12. Formation of the hydrophobic ion pairs and partitioning was favored by low methanol concentrations.

DISCUSSION

In a liquid–liquid chromatography study (*12*) using tri-*n*-octylamine as both the stationary phase and the source of the counterion, it was shown that retention of samples due to ion-pair formation (like that with sulfonic acids) increased with temperature, i.e., ΔH was positive. This was found to be true for a wide selection of sulfonic acids. A class separation of sulfonic acids, which form ion pairs easily, and carboxylic acids, that were retained mainly by partitioning of the acids themselves, was performed by temperature adjustment. In the present study, where the counterion was distributed between the bound stationary phase and the mobile phase, retentions decreased with temperature both for the sulfonic acid and the carboxylic acid even though ion pairing was known to be the main contributor to retention. As noted earlier, our results are in line with the expectation that higher temperatures would favor dissociation of an ion pair since association is usually exothermic (*24*).

In the preliminary experiments it was shown that ion-pair retentions could be adjusted in a predictable manner just as partitioning of uncharged molecular species. However, the percentage of organic modifier, in our case methanol, was also important because of the need to equilibrate the stationary phase with the counterion. Otherwise, retention times were not reproducible. If the percentage was not high enough for a particular counterion, either higher concentrations of counterion were required in the mobile phase or larger volumes of mobile phase had to be passed through the column before equilibrium was obtained. The latter was particularly undesirable since the counterion had to be removed from the column when not in use to avoid deterioration of the silica.

Since both the solute and the solvent contributed to the observed ΔV, both the destruction of the normal solvent structure and ion-pair formation could have contributed to ΔV. Pressure is known to have a strong influence on the structure of solvents (*8*, *24*). The tetrahedral structure of water is destroyed by bending and perhaps even breaking the H-bonds. However, the effect of pressure on a solvent made up of 50% methanol and 50% water is not known.

Pressure-induced ion pairing has been seen for other systems (*25*). The ion pairing of hydrophobic ions described by Diamond (*26*) became more important the higher the dielectric constant of the solvent. Since the dielectric constant increases with pressure for both pure water and pure methanol, this type of ion pairing would be favored by pressure. Furthermore, the effect of pressure on the dielectric constant is greater for methanol than for water (*24*). The effect on a mixture of two solvents should be in the same direction and is presumably intermediate between the two pure solvents. The change in dielectric constant with pressure has been used as an explanation for other pressure effects (*16*).

Change in dielectric constant may also have been a contributor in the temperature study, since the dielectric constant is decreased by increased temperature (*22*). Thus higher temperatures would favor faster elutions during ion-pairing chromatography. However, this contribution is believed to be small compared to other thermal effects.

From the present study, one can predict that pressure should be useful as a variable with conventional high performance liquid chromatography equipment (HPLC) by adding an exit valve. In addition, one can predict that pressure can have a significant effect in conventional HPLC if the operating conditions are changed so that there is a different pressure drop across the column.

In HPLC, pumps having pressure capabilities of 34.5 MPa (5000 psi)

and higher are often used. From the present study, one can calculate that by using 0.001 *M* THA ion in the mobile phase and assuming a column pressure drop of 3.4 MPa (590 psi) or less, one could change the separation factor, α, of MR and MO (2.94) by 11% by placing a valve on the exit of the column. This represents an unfavorable case since the capacity factors of MR and MO are changing in the same direction. If a sample which was not affected by pressure, like 4M2NA, was completely unresolved from MO at low pressure, α could be increased from 1.000 to 1.223 by pressurizing to 34.5 MPa (5000 psi). Although there would be loss in resolution due to the exit valve, these examples show that pressure effects could be used with equipment presently available.

For conventional HPLC (no exit valve), if one had a large pressure drop across the column of about 2000 psi, the average pressure on the column would be less than half the head pressure. But for simplicity, assuming an average column pressure of 1000 psi, the capacity factor would change by 4.2% in the most pressure-sensitive case. Thus there would be a change in α with column length, flow rate, or particle size due solely to a change in the pressure drop. Hence there may be lab-to-lab or column-to-column differences in values for distribution ratios and α values due to changes in pressure drop. In addition, since the pressure is due to the flow resistance of the column itself, the gradient of pressure across the column would contribute to band spreading due to the corresponding gradient of k' values. Hence pressure can affect resolution in two different types of ways in conventional HPLC.

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