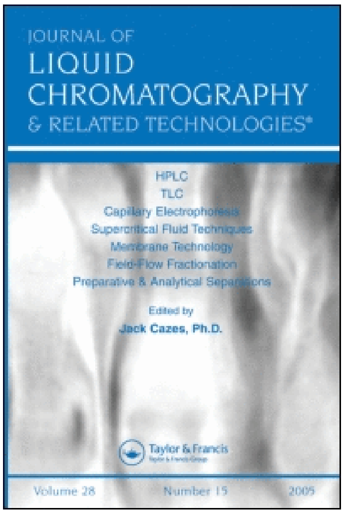

Journal of Liquid Chromatography & Related Technologies

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

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J. Gorse[a]; M. F. Burke[a]
[a] Department of Chemistry, University of Arizona, Tucson, Arizona

To cite this Article Gorse, J. and Burke, M. F.(1987) 'The Effects of an Ionic Mobile Phase Modifier and Thermal History on a Reversed-Phase Liquid Chromatographic System', Journal of Liquid Chromatography & Related Technologies, 10: 6, 1049 — 1064

To link to this Article: DOI: 10.1080/01483918708066753

URL: http://dx.doi.org/10.1080/01483918708066753

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(6), 1049-1064 (1987)

THE EFFECTS OF AN IONIC MOBILE PHASE MODIFIER AND THERMAL HISTORY ON A REVERSED-PHASE LIQUID CHROMATOGRAPHIC SYSTEM

J. Gorse* and M. F. Burke
Department of Chemistry
University of Arizona
Tucson, Arizona 85721

ABSTRACT

The addition of millimolar quantities of potassium chloride to a totally aqueous mobile phase increased retention and reduced band broadening with an RP-8 column and polar solute as compared to pure water. This effect was minimized after cycling the solvent-conditioned column through a temperature gradient. These effects are explained by a dynamic surface model in which entrapped conditioning solvent as well as imbibed ionic species all play a part in structuring the interfacial region where retention occurs.

INTRODUCTION

Liquid chromatographic stationary phases which have been bonded with organosilanes comprise a very large and important group of chemically modified surfaces. When the bonded group is a hydrocarbon, the term "reversed-phase" is used to describe this general mode of liquid chromatography. Reversed-phase systems normally

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*Present address: Box K 35, Knox College, Galesburg, IL 61401 employ a mixture of water and a miscible organic solvent such as methanol, acetonitrile or tetrahydrofuran as the mobile phase.

Reversed-phase separations have been shown to be a function of both the mobile phase and the stationary phase (1,2). In most reversed-phase studies the main focus is on organic modifiers in the mobile phase such as those mentioned above as well as the so-called "ion-pair" additives and/or soaps (3,4). In this study relatively small amounts of a neutral inorganic salt in the mobile phase are shown to have a large effect on retention and band broadening for a polar solute. These effects are compared to the chromatographic properties of a non-polar solute as well. Thermally induced changes in the chromatographic system are also used to provide further insight as to the mechanism of the observed effects.

Gilpin and co-workers have shown that the use of totally aqueous mobile phases can provide unique information in fundamental studies of reversed-phase systems as well as useful separations (5,6). It was shown that the retention of a reversed-phase system can be modified as a function of the thermal history of that system when the mobile phase is pure water. The modification of the stationary phase with ionic species such as inorganic salts is also possible. These compounds can be solvated by entrapped mobile phase which is in the solid-liquid interface. Also, there are always a significant number of underivatized silanols and an associated water layer on the surface of chemically-modified silica. These silanols can act as cation exchangers (7).

Mills and Morariu have shown, by the use of an NMR technique, that adsorbed cations can significantly affect the solvated structure of the solid-liquid interface at a non-modified silica surface depending on the cation used (8). Potassium ions were found to be particularly effective in exchanging with protons on the surface silanols and also caused the structured layer of water to have a lower microviscosity than when ions with a higher charge density were used. It is known that the residual silanols on a chemically modified surface can cause band broadening (tailing) when highly polar solutes are chromatographed (3,9). This effect is due to a dual retention mechanism involving the bonded moiety as well as the residual silanols. One should expect that cation-modified silanols will have different tailing characteristics and will possibly exhibit altered retention volumes as well. Weber and Tramposch have used a cation exchange technique on commercially-available RP-I8 to test the effectiveness of various amines to bind with residual silanols (in order to prevent tailing of highly polar compounds) (7). Potassium was found to be a highly effective exchanger for protons on the residual silanols as evidenced by its relatively higher exchange capacity (i.e. the order of exchange capacity is: $K^+ > Na^+ > Li^+ > H^+$). The effectiveness of the various amines in binding with the residual silanols was determined by the exchange capacity of K^+ after equilibration with a particular amine compound.

There has been an intense interest in using reversed-phase systems for the separation of biologically active compounds which may be denatured by contact with non-aqueous solvents (e.g.10) and the expense of purchasing and handling organic solvents has prompted the development of microbore column technology as well as "soap chromatography" (e.g.11). Any liquid chromatographic system which can reduce or eliminate the need for organic modifiers, can be useful in the separation of biological compounds and economical for other applications. The understanding of reversed-phase systems with totally aqueous mobile phases will enable one to develop important separations in the future as well as to understand how the solid-liquid interface behaves in the absence of an organic modifier.

Clearly, it is seen that a reversed-phase system can undergo dynamic modification due to ion-exchange and also temperature-induced stationary phase conformation changes. In the following study, the effects of these changes are demonstrated on an RP-8 liquid chromatographic column with a totally aqueous mobile phase.

EXPERIMENTAL

The column used in this study was a monomeric RP-8, 15 cm long with a diameter of 4.6 mm and packed with 5 micron diameter silica particles (Technicon (TM), Tarrytown, New York).

The column was initially conditioned by eluting 100 column volumes (150 ml) of acetonitrile followed by 100 column volumes of mobile phase through the system. The first cycle consisted of running the column at increasing temperatures up to a minimum of 60° C. The pump was stopped after each run until thermal equilibrium was reached at the next highest temperature. After making a series of runs the pump was stopped and the temperature bath was cooled to room temperature. The same sequence of runs was again made without re-conditioning with the organic solvent and this was the second cycle.

The conditioning solvent was LC grade acetonitrile (Burdick & Jackson, Muskegon, Michigan) and was filtered before use. The mobile phases were doubly-distilled (over alkaline permanganate) water and I mM KCl. All were filtered and outgassed with helium before use. Solutes were phenol and benzene and were dissolved in mobile phase before injection. A 5% solution of $NaNO_3$ was used to determine the void volume (V_m).

The solvent delivery system was an Altex 420 HPLC with II0A pumps (Beckman Instruments, Irvine, California). Sample injections were made using a model 74I3 Rheodyne valve (Rheodyne, Cotati,California) with a I microliter internal loop. The Detector was an Altex model I53 UV detector with a custom-made 0.5 microliter flow cell to minimize instrumental band-broadening.

Temperature control was maintained by immersing the column, a coil of stainless tubing for pre-heating the mobile phase and the sample loop in a water bath which was heated with a Haake model E52 heater (Saddle Brook, New Jersey). Temperature was maintained within + or - 0.5° C.

The number of theoretical plates or N was determined by using an empirical relationship developed by Dorsey (12):

$$N = \frac{41.7(V_r/W_{.1})^2}{B/A + 1.25} \quad (I)$$

Where $W_{.l}$ is the peak width at 10% of its height and B/A is the asymmetry ratio.

RESULTS AND DISCUSSION

Retention

The retention of phenol and benzene in the different mobile phases studied is shown in figures 1 and 2 in terms of k' for the first and second cycle. The chromatographic behavior of phenol with water as the mobile phase is very similar to that described by Gilpin and Squires (5). For the first cycle, retention of phenol initially drops rapidly for each mobile phase in the low temperature range (ca 25° C) and there is little change at the higher temperatures. In this study these effects are most pronounced for the mobile phase containing I mM KCl. During the second cycle retention differences are minimal for the entire range. For benezene the same general retention behavior is seen, but there is little difference between retention values in pure water and 1 mM KCl. The change in the solvated stationary phase from the first cycle to the second was shown by Gilpin to be due to a gradual release of entrapped solvent and a concomitant change in orientation of the bonded hydrocarbon chains. At a specific temperature, depending on the hydrocarbon chainlength, all (or most) of

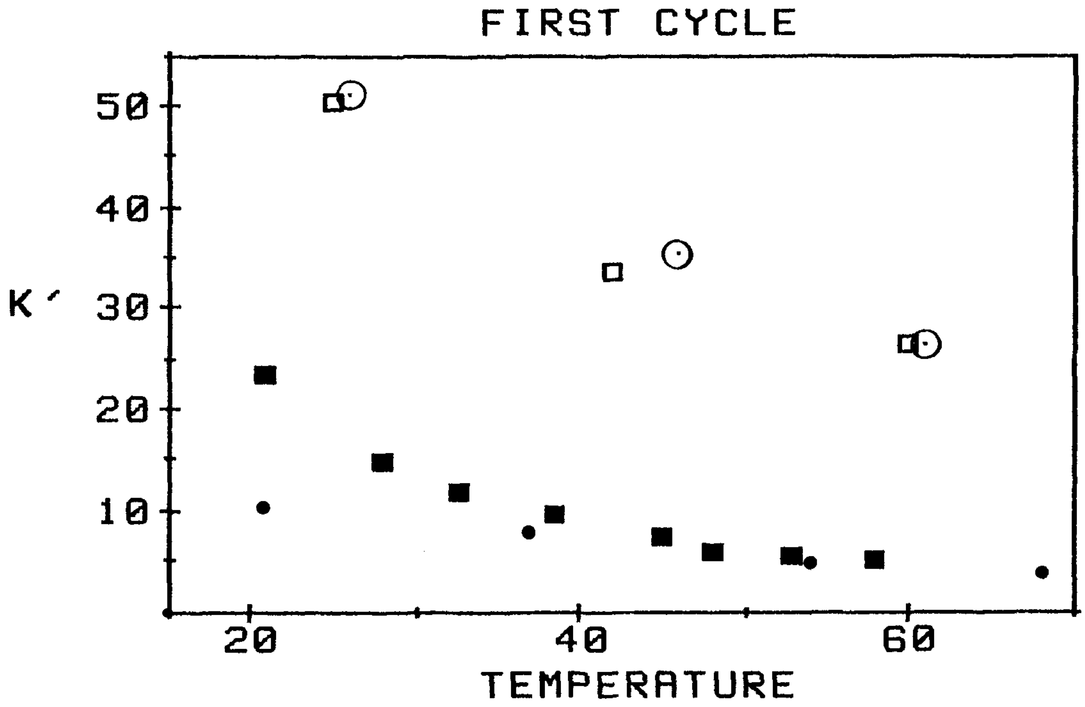

Figure 1. First cycle capacity factors vs. temperature. Circles are for pure water mobile phase and squares for 1 mM KCl. Open symbols are for benzene and closed for phenol.

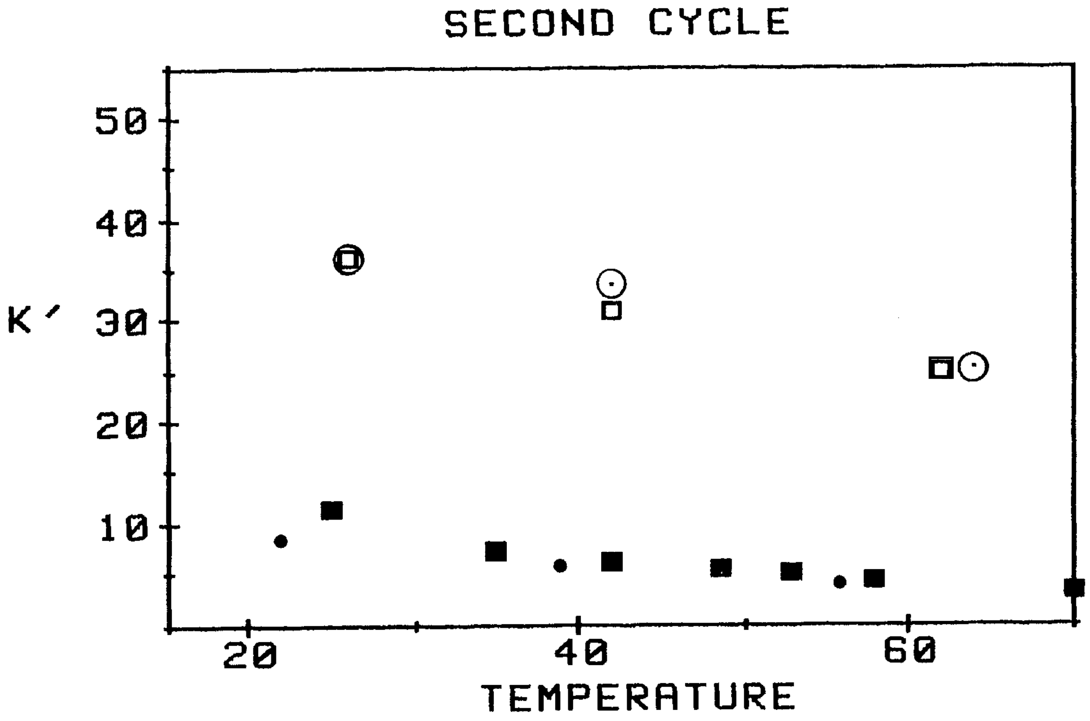

Figure 2. Second cycle capacity factors vs. temperature. See Figure 1 for symbol designation.

the solvent is released and replaced with water (5,6). In the first cycle the stationary phase interface is believed to be associated and in a folded orientation with some imbibed conditioning solvent still present. This orientation is termed the "down" state. At higher temperatures the orientation of the hydrocarbon chains is changed to what is termed the "up" state . This "up" conformation is expected to be more rigid and more ordered due to the the replacement of the organic solvent with water. This highly-ordered interfacial region has been pictured by Kovats and Riedo as being similar to a monolayer of a long-chain fatty acid or alcohol on a water surface which is known to be very rigid and somewhat compressed compared to the molar volumes of the neat fatty acids or alcohols (13). Retention in the "down" state has been shown to be a function of the conditioning solvent, while the "up" state is independent of the conditioning solvent.

Other workers have noted changes in bonded stationary phase orientation as a function of solvation, surface coverage and bonded chainlength. Scott and Simpson reported unexpedtedly low retention for 100% aqueous mobile phase when using a monomerically-bonded RP-18 stationary phase (14). Retention increased to expected values when small amounts of methanol were added. This effect was attributed to the fact that the hydrocarbon chains are self-associated under totally aqueous conditions and thus had a reduced "effective area". This condition corresponds to the "up" state in Gilpin's model described above. Likewise, Lochmuller and Wilder have invoked a surface model of bonded hydrocarbon chains which consisted of bunches of hydrocarbon chains surrounded by bare silica. These hydrocarbon chains undergo varying degrees of self-association which depend upon the chain length, the amount of surface coverage and solute size (15). Lochmuller also introduced the concept of a "variable phase ratio" which is similar to Scott's idea of "effective surface area".

For this study, the first-cycle stationary phase consists of the hydrated silica substrate, the bonded hydrocarbon chains, entrapped conditioning solvent and ionic modifier. As the temperature is increased, the imbibed conditioning solvent and ionic modifiers are gradually released due to the thermal motion until finally the bonded chains open up forming a bristle-like conformation in which water replaces the organic solvent. Solutes are retained more strongly in the first cycle. Possible reasons for increased retention include: l) interaction of the solute with previously adsorbed cations and solvent, 2) higher entropy of sorption in the well-solvated, less rigid "down" state and 3) the effective stationary phase volume (or area) may be greater in the "down" state (this would increase the phase ratio and thus increase k'). One might argue that the entropy of sorption should increase if the effective stationary phase volume is increased, so that reason 3 includes 2 as well. Since phenol is a very polar compound, an increase in the polar nature of the interface via sorption of water and/or ionic modifiers can possibly increase retention. The "down" orientation, in a more disordered state (more fluid) is able to interact with phenol in a less orientation-specific manner (higher entropy of sorption). The chemically modified silica surface consists of the bonded moiety as well as residual silanols which were not derivatized. These silanols are acidic and can exchange protons for metallic cations (7,16). This property has been used recently to provide a separation of various metal ions on bare silica (17). Both chemically modified and bare silica surfaces have been shown to have high exchange capacities for potassium. This has been explained by Mills and others as a function of the sphere of hydration which is relatively small for potassium cations because of their lower charge density compared to smaller cations (8). An interesting possibility is that the phenol may be retained by a ligand exchange mechanism the same as has been reported by Goulding, Walton and Jupille for sugars, polyhydric alcohols and substituted phenols (18,19,20). Further, potassium cations have been shown to reduce the viscosity of electrolyte solutions when highly polar solvents are used (21). These properties of potassium allow an increased penentration into the porous structure of silica and also provide a surface which is less structured in terms of tightly bound water molecules and would be expected to add to the disorder of the "down" or folded orientation.

The retention of benzene is not significantly affected by a change in mobile phase (figures 1 and 2). This indicates that the increased retention between the different mobile phases may be due to polar interactions in the stationary phase. Another possiblity is that benzene is not affected as much by the surface orientation, since it does not possess a highly polar "tail" like phenol.

EFFICIENCY

The efficiency of a chromatographic separation as measured by band broadening or number of theoretical plates, N, is dependent upon such factors as the packing efficiency of the column, the particle size of the adsorbent, the kinetics of mass transfer in the stationary phase, extra-column volume, sample size, etc. (e.g. see 3). Efficiency can also be reduced by a mixed retention mechanism as shown by Phillips, Wright and Burke (9). This mixed retention mechanism is often caused by polar solutes which "tail". Since the column and associated extra-column apparatus were the same throughout this study, band-broadening changes occurred only due to changes in mass transfer in the stationary phase and possibly multiple retention mechanisms. The amount of tailing is best measured by using the peak width at l0% of its height and also by using the asymmetry ratio B/A. This is taken into account by the use of the Dorsey-Foley relationship which was used in this study (12). The limiting number of plates for this system based upon the particle size is l5,000. In this work, N was signficantly lower due to the use of totally aqueous mobile phases. This has been attributed to the inability of water to "wet" the hydrocarbon chains (3).

In figures 3 and 4 it is seen that N follows retention behavior for the polar solute, phenol, by decreasing as temperature increases. This result would seem to exclude the multiple retention argument, since one would expect an increase in retention to accompany an increase tailing when the highly polar silanols participate in the retention process. Thus efficiency is also a function of the imbibed ionic species. It should be noted here that for an idealized static surface, efficiency is expected to increase with temperature due to the increase in the rate of mass transfer in the stationary phase (4). The expulsion of organic solvent and ion modifiers and change in orientation of the bonded hydrocarbons due to thermal motion of molecules at higher temperatures leads to lower plate count in the "up" state. The possible reasons for this are two: First, an increase in potassium ions in the "down" state may lead to an increase in the homogeneity of the surface due to the fact that not all residual silanols are energetically equivalent (e.g. 16). This would have the effect of reducing tailing, but not necessarily eliminating it. Second, the rate of mass transfer in the stationary phase may be faster due to lower microviscosity at the interface. Potassium has a small sphere of hydration and is known to be able to break up solvent structure. Therefore, one would expect the potassium-modified system to be less structured and mass transfer in the stationary phase would be correspondingly faster leading to higher efficiency (less band-broadening).

The plate counts for benzene versus temperature are shown in figures 3 and 4. Benzene is much less affected by differences in mobile phase, but nevertheless shows the same decrease in efficiency in the first cycle as temperature is increased. Higher efficiency is exhibited for the KCl-modified system. In the second cycle the differences are slight and

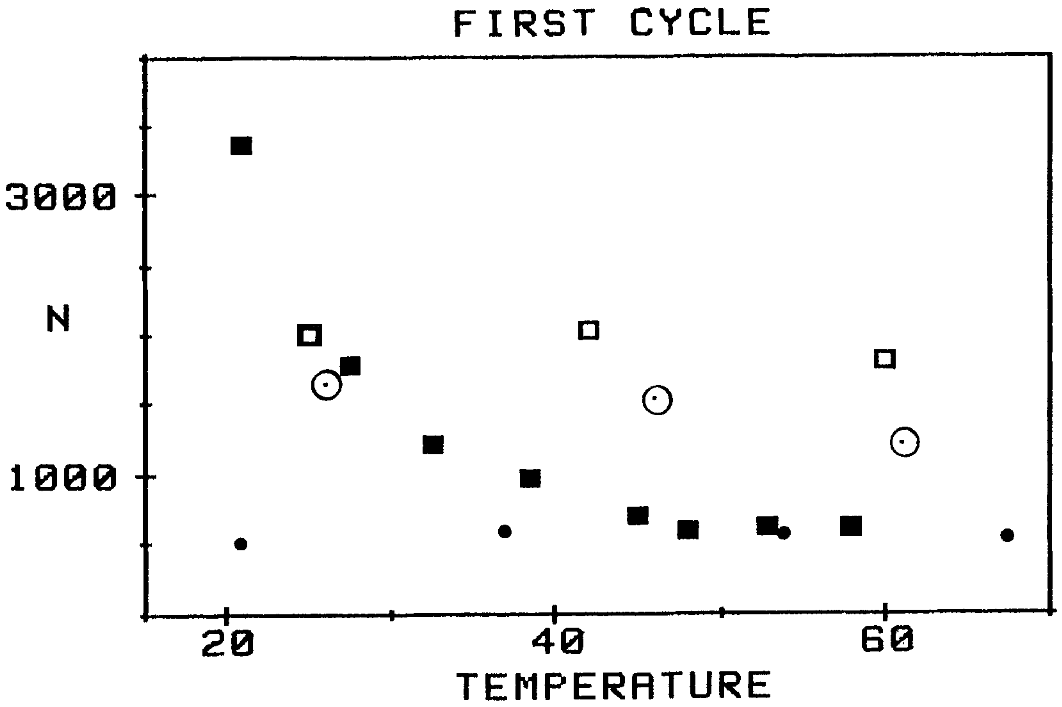

Figure 3. First cycle. Theoretical plates vs. temperature. See Figure 1 for symbol designation.

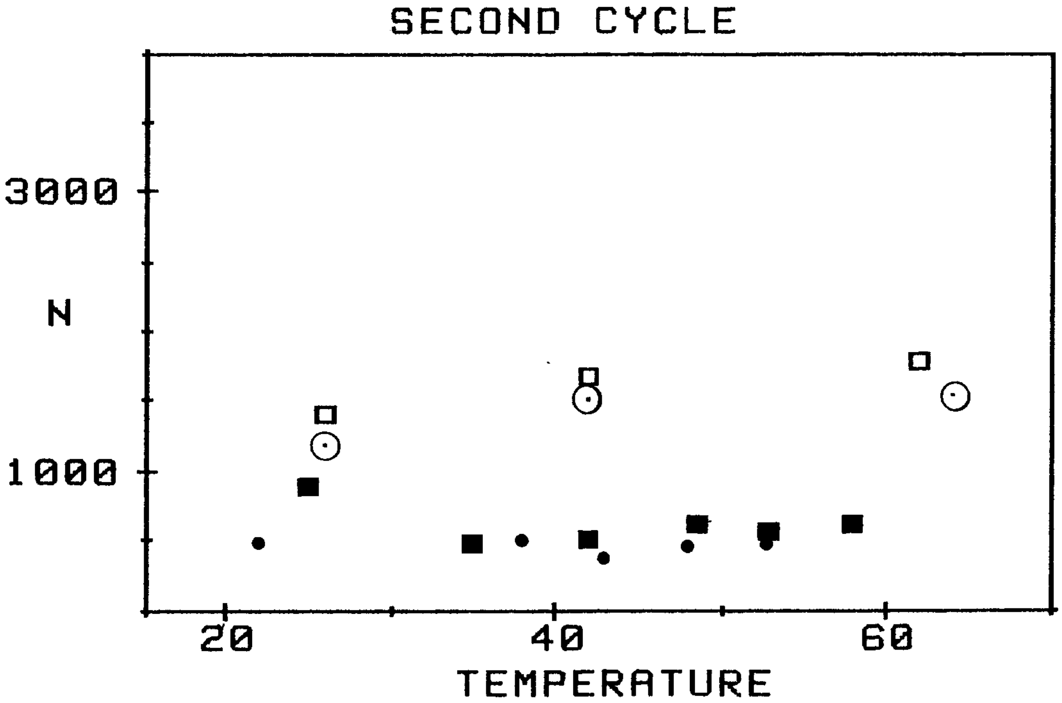

Figure 4. Second cycle. Theoretical plates vs. temperature. See Figure 1 for symbol designation.

efficiency increases with temperature as would be expected for a static surface. This behavior is indicative of adsorption on a rigid surface with little or no partitioning. Benzene is unable to penetrate the more highly structured "up" state in which the conditioning solvent has been replaced by water.

CONCLUSIONS

It is shown that reversed-phase systems can be used under totally aqueous conditions and that retention and efficiency can be modified by ionic species and temperature-induced conformation changes. The results of this study point to the need to take into account changes which may be brought about in a reversed-phase system due to the presence of ionic species as well as the temperature and mobile phase history of the column. With further development, this type of system, which employs an aqueous mobile phase, can compete with mixed solvent systems which are more expensive to use. Also, totally aqueous mobile phases are less likely to denature certain proteins as well as provide a separation for them which has better selectivity.

Further work is being carried out involving the use of mixed mobile phases which contain different ionic species and/or buffer systems.

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