The Role of Pore Size and Stationary Phase Composition in Preventing Aqueous-Induced Retention Time Loss in Reversed-Phase HPLC

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Abstract

The phenomenon of aqueous mobile phase induced retention time loss, which has also been referred to as phase-collapse behavior, is investigated using a variety of stationary phases with an aqueous mobile phase. The loss of retention of several water-soluble B vitamins is measured as a function of silica pore size, bonded-phase chemistry, and bonded stationary phase density. It is found that these variables influence the magnitude of the retention time loss behavior and that controlling/optimizing these variables can result in a stationary phase with sufficient retention of the analyte without the column exhibiting the so-called phase-collapse behavior.

Introduction

Reversed-phase (RP) high-performance liquid chromatography (HPLC) is a routine separation tool used for the analysis of a wide variety of organic compounds (1). The heart of the chromatographic system is typically a hydrophobic C_{18} RP column. In some applications it is necessary to retain and separate polar analytes using a nonpolar C_{18} stationary phase. This often requires a high aqueous or a buffered-water mobile phase to achieve the desired separation. However, with most procedures it is not recommended to go directly into a pure aqueous mobile phase, as it is general practice to move from an organic-aqueous mobile phase into a high-water-content mobile phase. In these situations, an interesting and undesirable problem sometimes occurs with some C_{18} columns. Use of the high-water-content mobile phase leads to a dramatic decrease in retention that occurs over time. The loss in retention time observed after moving from an organic-aqueous mobile phase into an aqueous mobile phase can be accelerated by turning off the flow for a short period of time. The loss in analyte retention over time (or instantaneously, with flow stoppage) using an RP column in a high aqueous mobile phase has commonly, but perhaps incorrectly, been referred to as "phase collapse".

Attributing the loss of analyte retention in an aqueous mobile phase to "phase collapse" is one way to rationalize the effect. The speculation has been that the hydrophobic alkyl chains are fully extended in the organic-rich mobile phase and then, in water, "lie down" onto each other and onto the silica surface. The collapse of the bonded alkyl chains on top of each other in the water-rich mobile phases will make the stationary phase surface less accessible compared with a stationary phase in which the chains are fully extended above the silica surface, as is believed to be the case in an organic-rich mobile phase. If phase collapse occurs, there would be less availability of the alkyl phase for partitioning of a polar analyte between the mobile and stationary phases. The result would be reduced retention because of the lower available surface. This is a popular explanation. However, it is not necessarily the correct one.

Much has been written on the mobility and positioning of bonded alkyl chains in various solvents (2–7). Most of these reports use spectroscopic methods to study this behavior. Unfortunately, there is no universally accepted proof that the alkyl chains of the stationary phase collapse in water compared with a typical HPLC RP mobile phase. In fact, one investigation on wetting of HPLC bonded phases using spectroscopy showed that the phase is always essentially horizontal (8). Observations focused on the probe molecule in water and in short-chain alcohols (typically used in HPLC) as solvents. Higher chain alcohols did not significantly change the orientation of the phase, and any differences that were observed were ascribed to differing amounts of interpenetration of the alcohol into the alkyl chains (8). Also, a recent investigation using Raman spectroscopy showed no evidence that the stationary phase ligands were collapsed or extended as a function of mobile phase composition even when a pure aqueous mobile phase was used (9). In another report looking at bonded phases, investigators interpreted their data to the conclusion that all alkyl-bonded phases exist in a "collapsed" state in all typical RP mobile phases (10). Thus, the mechanistic interpretation that the phase collapses is still only speculative.

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Because of this, the terms "retention time loss" or "instant retention time loss" will be used in this manuscript rather than the term "phase collapse behavior." The term "phase-collapse behavior" is an inappropriate term because it assumes the cause of the behavior, which is still very much in contention.

Even though the recent evidence substantiates that the stationary phase does not change its orientation in highly aqueous mobile phases (8–10), there is still a pragmatic interest to manage/eliminate the retention time loss behavior. Chromatographers have been addressing this retention time loss behavior in a number of ways. One way is to rinse the column with several column volumes of a high-organic-content mobile phase after the retention drops to an unacceptable level. This rinsing with organic-rich mobile phase followed with the original aqueous mobile phase can increase retention to original levels as long as the column remains under flow and operating pressures. However, this is a reversible process, and retention will again slowly drop when operating in the original aqueous mobile phase.

A second method of preventing the retention time loss behavior on a C_{18} stationary phase is to use a stationary phase that incorporates a polar group—such as an amide, carbamate, or urea linkage—in the backbone of the alkyl chain near the silica surface (11). In concept, these stationary phases incorporating a polar-embedded alkyl group produce a highly solvated silica surface because of the strong interaction of the polar groups in the alkyl chain with the aqueous mobile phase. With the solvated surface, retention is constant over time. Unfortunately, stationary phases containing an embedded polar group are not a pure C_{18} phase and retention is generally significantly lower than on a true C_{18}-bonded surface. Therefore, although columns that contain an embedded polar group do not exhibit the retention time loss behavior, they often do not have sufficient retention to be useful for all analytes.

Another very common approach to eliminate the retention time loss behavior is to use a column that has a bonded stationary phase consisting of two types of phases that are balanced for solvation and retention (12–14). Often, the phases contain a certain amount of bonded alkyl chains and bonded polar groups attached to the surface. These "mixed-phase" columns are commercially available from a large variety of manufacturers and are generally named "Aq", "high aqueous", or some other notation that implies operation in aqueous mobile phases. Because of the balance of the two types of bonded phases, the exact nature and chemical composition of these columns are generally kept proprietary.

Although much has been discussed and presented at technical forums, only recently have reports appeared that investigate the retention time loss behavior. In one report (12), the cause of the retention time loss behavior was attributed to phase collapse induced by the bonding density of the stationary phase. They hypothesized that when the bonding density of the C_{18} chains is not sufficiently high, phase collapse will occur. The manner in which the ligand density was increased was by bonding a C_{18} phase to a wider-pore particle. This approach of using a wider-pore packing to increase bonding density is consistent with the work of Englehardt, who showed a higher coverage on the wider pore and also demonstrated better efficiencies than on a narrow pore (15). This more densely bonded phase did not exhibit the retention time loss behavior compared with the lower bonding density. Thus, the higher density will not have the ability for the phase to collapse.

Certainly the mobile phase will play a role as deposition of a liquid on a solid generates an interface between dissimilar materials. This interface involves considerations of wettability, spreading, interfacial structure, and sorption (adhesion). It is know that mobile phase adsorbs onto and perhaps interpenetrating between the bonded phase ligand (8,16). A wetted surface, therefore, involves multiple forces, not just "wettability".

It has also been proposed that the retention time loss behavior is caused by water being forced out of the pores when the pressure becomes too low (13). Although this explanation has some merit, it appears that only a part of the surface area becomes inaccessible rather than the entire pore becoming nonaccessible. Thus, the role of pore size may be under-considered, and there may be some other mechanism to explain the retention time loss behavior.

In this article, we investigate the phenomenon of retention time loss behavior in more detail and study the behavior as a function of silica pore size, surface silanol content, and bonded-phase chemistry. There are a number of contributing factors to retention time loss behavior, and the data support a simple explanation. The retention time loss behavior occurs when the analyte has limited access to the surface area of the stationary phase. Anything that promotes a wetted stationary phase will eliminate the retention time loss behavior.

Our observation is that the pore size is a significant contributor to the management or the elimination (or both) of the retention time loss behavior. An easy way of making hydrophobic phases that do not exhibit retention time loss behavior is proposed.

Experimental

All columns used for evaluation were 4.6-mm i.d. × 150-mm long and were based on 5-µm particles. Four main column families were used in this study: the Zorbax StableBond (SB) C_{18} family, consisting of SB-C_{18} 80A, SB-C_{18} 100A, SB-C_{18} 150A, SB-C_{18} 300A, and an endcapped SB-C_{18}; the Zorbax Eclipse eXtra-Densily Bonded (XDB) family, consisting of XDB-C_{18} 80A, XDB-C_{18} 150A, XDB-C_{18} 300A, and XDB-C_{18} 800A; the Zorbax Extend family, consisting of Extend-C_{18} 80A and Extend-C_{18} 300A; and the Zorbax StableBond-C_8 family, SB-C_8 80A and SB-C_8 300A (all columns from Agilent Technologies, Wilmington, DE). Other columns used were: Zorbax SB CN 80A, Zorbax SB Phenyl 80A, and Zorbax NH_2 80A (all columns from Agilent Technologies). Those columns that are commercially available were used as received. Those columns that are not commercially available were prepared using the same bonding procedures as used in the commercial preparation. The pore sizes of the columns were measured by using inverse gel permeation chromatography procedures as previously described (17).

All columns were tested using an Agilent Technologies 1100 HPLC equipped with solvent degasser, binary pump, heated column compartment (set to 25°C), autosampler, and diode array detector set at 254 nm. The test sample used in these evaluations was a mixture of water-soluble B vitamins. The test sample was prepared by dissolving 0.25 mg/mL of nicotinic acid, pyridoxine,

thiamin, and niacinamide (Aldrich Chemical Co., St. Louis, MO) in mobile phase B. For the analysis, 1 µL of the test mixture sample was injected.

Two mobile phases were prepared. The first was a high organic mobile phase (mobile phase A) consisting of 75% pesticide grade (Burdick and Jackson, Muskegon, MI) methanol and 25% HPLC-grade deionized water. The second mobile phase (mobile phase B) was a high aqueous phase consisting of 50mM sodium acetate (J.T. Baker, Phillipsburg, NJ) in HPLC-grade deionized water at pH 4.6. The flow rate of each mobile phase was set at 2 mL/min.

The following procedure was used for all tests. Mobile phase A was pumped through each column for 20 min to insure the column was fully equilibrated. Mobile phase B was then pumped for 20 min followed by measurement of k' for duplicate injections of the test sample. After the analysis was complete, the flow was turned off for 10 min. The flow was then turned back on for 2 min followed by measurement of k' for duplicate injections of the test sample. The capacity factors (k') calculated before and after the flow of the mobile phase had been turned off were compared. The percent difference between the two capacity factors [(k' in mobile phase B after flow was stopped/k' in mobile phase B before flow was turned off) × 100] was then a measure of the magnitude of the instant retention time loss behavior for a given column. A value of 100% indicates that no instant retention time loss was observed, though a value of a 65% indicates a 35% loss in retention time. This number may be thought of as the amount of retention remaining after exposure to an aqueous mobile phase.

Results and Discussion

Role of pore size

To investigate the effect of pore size, a series of SB-C_{18} phases were chosen with different pore sizes. These columns were prepared using diisopropyl side groups rather than dimethyl side groups. Also, the surface was not end-capped after the primary bonding of the alkyl phase.

Analysis of the B vitamin test mix on an SB-C_{18} bonded phase, 80A-pore size, using the described procedure is shown in Figure 1. As seen, there is a drop in capacity factor of approximately 30–35% (depending upon the analyte) when switching into mobile phase B (100% buffered water). This behavior illustrated the observation of instantaneous retention time loss and is typical of the chromatograms obtained in the remainder of the study when retention time loss occurred.

To determine the effect of pore size on the magnitude of instantaneous retention time loss, the testing procedure was repeated using columns with differing pore sizes and included a 100A-, 150A- and 300A-pore-sized silica. Representative pore size distributions are shown in Figure 2. The "R" value is the cumulative percentage between 0% and 100% of the available pore volume. The silica with varying pore sizes was bonded with SB-$C_{18,}$ and all produced a bonding density of 2.2µM/m^2. Table I summarizes the results of testing the SB-C_{18} phases on 80A-, 100A-, 150A-, and 300A-pore sizes. The data contained in Table I demonstrate that as the pore widens with a constant bonding density, the instant retention time loss behavior is reduced. With a sufficiently wide pore, the behavior is not observed. Interestingly, the behavior of the SB-C_{18} bonded phase on the 100A-pore-size silica shows some, though minimal (2%), loss of retention, but not to the extent observed with the 80A-pore sized silica. This 2% loss of retention might be considered by some to be too small of a loss in retention to be a meaningful example. When using the bonded C_{18} phase on the 100A- and 150A-pore material, there was essentially no change in retention between the organic containing mobile phase and the buffered water mobile phase. And when using the B vitamins test mix, absolutely no instant retention time loss behavior was observed for the 150A- and the 300A-pore materials. This is also interesting because it suggests that bonding density may not be an important variable in predicting instant retention time loss behavior, as was earlier reported (12,13).

The SB-C_{18} stationary phase on the 100A- and 150A-pore size silica gave increased retention compared with the 300A silica because of the higher surface area. Thus, it appears there may be an optimum pore size for this phase chemistry. The pore size can be chosen such that there would not be any evidence of instant retention time loss behavior, but there would be sufficient analyte retention.

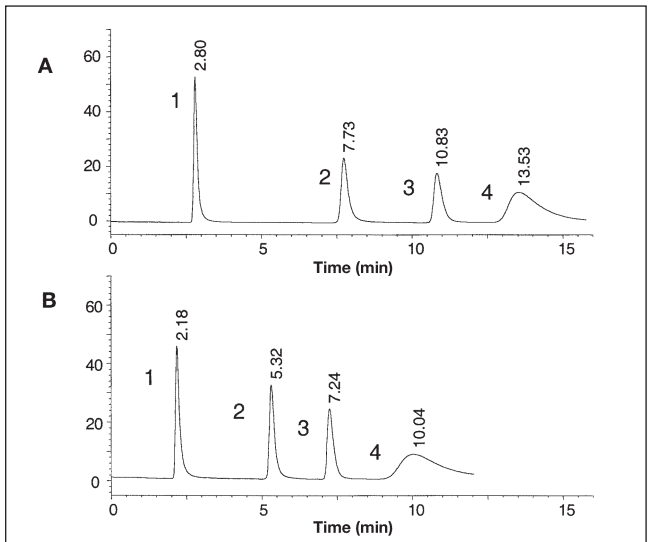

Figure 1. Chromatograms demonstrating phase collapse behavior. The column is a Zorbax C_{18} 80A. (A) Analysis immediately after change over to aqueous mobile phase and (B) run after flow is turned off. Compounds are: (1) nicotinic acid, (2) pyridoxine, (3) thiamin, and (4) niacinamide.

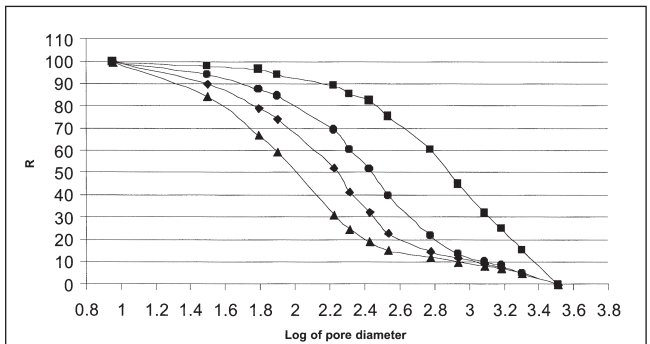

Figure 2. Pore size distributions of various packings. For the figure, (▲) 80A, (♦) 150A, (●) 300A, and (■) 800A. See text for calculation details.

Effect of bonded phase density

It is well known that C_{18} stationary phases in HPLC can be very different from each other. It was important to look at different C_{18} preparations to examine which of these preparations could give a phase that would have constant retention when operated in an aqueous mobile phase.

In order to investigate the effect of increasing the silanol concentration with decreasing the bonded phase coverage, a SB-C_{18} bonded phase (80A) was prepared at a loading level of one-half of that of a fully bonded SB-C_{18} packing. This half-bonded phase had a bonding density of 1.1µM/m^2. Because the reaction was "starved", the bonded phase had approximately 50% of the available silanols unbonded. This packing exhibited longer retention times for the B vitamin sample components (18) compared with a fully bonded SB-C_{18}, indicating that the silanols played a role in retention. This observation of increased retention on a half-bonded stationary phase compared with a fully bonded stationary phase is similar to that observed in a previous published report for separation of bases at pH 7.8 (19). Both observations emphasize the role of silanols in facilitating unique separation capabilities when using very low bonded phase densities in combination with high silanol populations.

The packing with a lower bonding density, containing 50% C_{18} and 50% free silanols, showed no evidence of instant retention time loss behavior. This data is contrasted to the fully bonded SB-C_{18}, 80A listed in Table I, which exhibited instant retention time loss behavior. This data again suggested that in addition to the pore size, the bonded phase coating level is important in determining whether instant retention time loss behavior will be manifested on a particular bonded phase.

To clarify the effect of bonding density, a series of XDB-C_{18} phases were prepared. The XDB-C_{18} phase differs from SB-C_{18} in that the XDB-C_{18} is prepared using dimethyl alkyl silane rather than the diisopropyl version of the alkyl silane used for the SB-C_{18}. Also, the XDB-C_{18} is highly endcapped, resulting in higher bonding densities (4.0µM/m^2) and lower levels of surface silanols. Using the same testing protocol as mentioned previously, instant retention time loss behavior was determined using the capacity factor ratios (as previously described). The data for the amount of retention remaining is summarized in Table II for a series of Eclipse XDB bonded phases on silica with different pore sizes.

In the case of the Eclipse XDB-C_{18} series, instant retention time loss behavior was observed to a much greater degree than was observed using the SB-C_{18} family with the same pore sizes. Because the XDB-C_{18} phase has much higher bonding density than SB-C_{18}, the behavior is reversed of what was reported earlier (12). The higher bonding density exhibited more apparent instant retention time loss behavior compared with the SB-C_{18} phases shown in Table I. This suggests that the more hydrophobic the surface, the more difficult it is for water to wet the bonded phase. Thus, it is important to have a surface wetted in order to prevent instant retention time loss behavior. It is not the stationary phase bonding density that improves the ability to prevent retention time loss; quite the contrary, the data suggests that the higher the bonding density, the more the apparent instant retention time loss behavior.

Here again, there appears to be an optimum pore size for this particular phase chemistry to attain sufficient analyte retention and a minimum of instant retention time loss behavior. The optimum pore size for this phase chemistry is approximately 150A. The endcapped XDB-C_{18} has a higher surface coverage, thereby increasing the optimum pore size compared with the nonendcapped SB-C_{18} performance discussed earlier.

To confirm this hypothesis, Extend-C_{18} was bonded to different pore size silica and tested with the same methodology as used for earlier reported instant retention time loss behavior. Although this phase is highly endcapped, it should be noted that the Extend-C_{18} is a bonded phase prepared with a bidentate silane (20). One side chain of the reactive silane is a methyl group and the other side group is a propyl bridge connecting the silicon atom to another silica atom. The Extend-C_{18} phase has a bonding density of 3.8µM/m^2. This phase showed the most phase collapse of any of the C_{18} columns tested, even on the 300A-pore silica. The data is summarized in Table III.

This data for the Extend-C_{18} may be explained in the following way. Because this material has two C_{18} groups attached to an alkyl bridge forming a bidentate attachment to the surface of the silica, it forms a more hydrophobic surface for a similar bonding den-

Table I. Capacity Factor Difference for SB-C_{18} on Different Pore Size Silica*

	Nicotinic acid	Pyridoxine	Thiamin	Niacinamide
SB-C_{18} 80A	70	65	64	73
SB-C_{18} 100A	97	97	98	98
SB-C_{18} 150A	100	100	100	100
SB-C_{18} 300A	100	100	100	100

* A value of 100% indicates no loss in retention. See text for calculation details.

Table II. Capacity Factor Difference for XDB-C_{18} on Different Pore Size Silica*

	Nicotinic acid	Pyridoxine	Thiamin	Niacinamide
XDB-C_{18} 80A	17	21	21	22
XDB-C_{18} 150A	90	89	90	89
XDB-C_{18} 300A	100	100	100	100
XDB-C_{18} 800A	100	100	100	100

*A value of 100% indicates no loss in retention. See text for calculation details.

Table III. Capacity Factor Difference for Extend-C_{18} on Different Pore Size Silica*

	Nicotinic acid	Pyridoxine	Thiamin	Niacinamide
Extend-C_{18} 80A	ND†	7	8	8
Extend-C_{18} 300A	99	98	95	98

* A value of 100% indicates no loss in retention. See text for calculation details.
† ND indicates an accurate retention time could not be determined because retention was too close to the void volume.

sity. This data is consistent with the observation that an endcapped C_{18} exhibits more instant retention time loss behavior than the nonendcapped material even though the bonding density is higher.

With Extend C_{18}, the optimum pore size needed to minimize retention time loss behavior is approximately 300A. Unfortunately, retention of the analytes is too short for accurate quantitation of the test compounds. The Extend type of phase chemistry would not be suitable for small molecule separations in high aqueous environments.

As a result of this investigation, it can be concluded that the "key" parameter influencing instant retention time loss behavior is pore size. However, there are several parameters that are important in predicting the likelihood of instant retention time loss behavior. All of these parameters are related to the ability of the mobile phase to coat the surface. It is not important whether the stationary phase is fully extended or collapsed. The important attribute is that the surface is freely accessible. Nonendcapped phases have more hydration because of the residual silanols on surface of the silica and exhibit less phase collapse. The higher the silanol content, as in the case of lower density bonded StableBond-C_{18}, the less instant retention time loss behavior compared with the fully bonded XDB-C_{18}.

Pore size plays a very large role in limiting instant retention time loss behavior, not because of increased bonding density, but because as the pore size widens, water can more easily form an appropriate interface on the surface. There is an optimum pore size that is somewhat unique for each type of bonding chemistry. At this optimum, instant retention time loss is eliminated. Bonding density, itself, has relatively little effect on retention time loss behavior.

Narrow pores inhibit the transport of the analyte to the stationary phase. One explanation is that the nonpolar surface is not wetted, perhaps because of the van der Waals forces involved. Because of the interface formed on the stationary phase from the bulk water to the surface, there is a barrier for the analyte to access the entire pore. Widening the pore reduces the barrier to complete entry. The behavior is similar to a Donnan membrane effect with ionic compounds in which the transport from the bulk mobile phase into or through a pore is restricted. It is as if a double layer exists on the surface, which retards the analytes access to the entire surface (21), as it was demonstrated in ion-pair chromatography. However, double layers are most often thought of when ionic interactions are involved.

Another possibility is that a surface layer of structured water exists on the hydrophobic surface in high aqueous mobile phases. This concept is supported by evidence in the literature that supports the existence of a hydration shell formed around a hydrophobic entity. This data was reviewed extensively and was used to corroborate the role of water as an active participant in an explanation of pressure-induced retention changes for azo dyes in liquid chromatography occurring at ultrahigh pressure (22). Investigations of the properties of water at the surface of oxide materials have shown that water associated with these surfaces exhibits properties that differ from those of the bulk water (23). In fact, the structure of water occurs at many interfaces and has been reported to range from 50A on clay surfaces up to 0.1 µm on mica (24,25).

It is known that mobile phase solvents like methanol will be adsorbed into the stationary phase (4,5). Therefore, it is conceivable that the surface, which was initially wetted in the organic aqueous mobile phase, remains wetted when switched to the 100% aqueous mobile phase. When the flow is stopped, the pressure is removed and the surface transitions to a nonwetted (water-rich) surface. This lends additional credibility to the hypothesis that reduced retention observed for the instant retention time loss behavior is attributable to a lack of access to the nonpolar alkyl surface induced by the structured water established on the very hydrophobic surfaces.

Effect of temperature

Temperature is often used in RP-HPLC to attain improved separations (26). Perhaps the role of temperature would shed some light on the instant retention time loss behavior. For this set of experiments, it was desired to use a column that would have reasonable retention and exhibit moderate instant retention time loss at room temperature. This was achieved by using a pore size of 100A and bonding the SB-C_{18} and then endcapping the surface.

As expected, when temperature was increased up to 70°C, retention of the B vitamins decreased on this column. However, over the temperature range investigated, there was sufficient retention to measure the instant retention time loss as the percentage retention remaining before and after the flow was stopped. Those results are shown in Figure 3. As was mentioned earlier, a high value of percent retention remaining implies that there is a small loss in instant retention time loss behavior. From Figure 3 it can be seen that the relative retention time loss appears to vary as a function of temperature. When temperature is low there is a less instant retention time loss behavior compared with the higher temperatures; and, conversely, at high temperature there is a large instant retention time loss behavior. An explanation is that when temperature is low, there is less driving force to transition from the organic solvated surface to the water-rich surface. When temperature is high, this transition occurs quickly, and there is considerable instant retention time loss behavior observed.

This hypothesis is consistent with the observation that the stationary phase will preferentially adsorb the organic solvent component into the phase and onto the surface (27,28). Furthermore, once an abrupt mobile phase change is made from an organic solvent to a pure water mobile phase, there is an initial rapid release of the organic solvent from the surface followed by an extensively

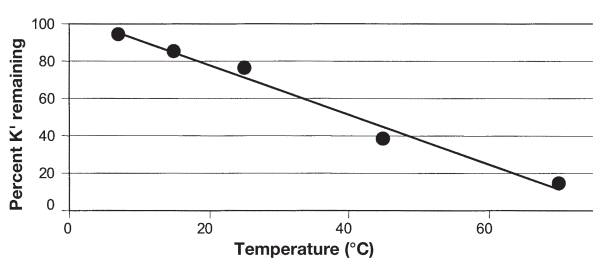

Figure 3. Percent of retention remaining as a function of temperature. A value of 100% indicates no loss in retention. See text for calculation details. Each data point represents the average of the values for the pyridoxine, thiamin, and niacinamide peaks.

prolonged release of the entrapped organic solvent (many hundreds of column volumes) (29). In observing this slow release of organic solvent into the water mobile phase, the authors speculated that one possibility is that the alkyl chains changed orientation during this slow release. However, other than the chromatographic data, there was no supporting data to substantiate this claim.

If the cause of the retention time loss were, indeed, the collapse of the phase, it is less apparent how the data in Figure 3 could be rationalized. If there were a change in orientation of the phase, operation at increased temperature should enable more free mobility of the chains, and, as such, there should be less retention time loss at the higher temperatures. The data, however, shows the opposite effect.

Effect of alkyl chain length

In order to investigate the effect of stationary phase chemistry on instant retention time loss behavior, SB-C_8 was bonded to silica with pore sizes of 80A and 300A and tested for instant retention time loss (Table IV). The SB-C_8 has a bonding density of 2.0µM/m^2 for each pore size. The SB-C_8 showed limited evidence of instant retention time loss behavior on the 80A-pore-size material and showed no instant retention time loss behavior when bonded to the 300A-pore-size silica. Comparing this data with that in Table I confirms that reducing the chain length of the bonded phase reduces the magnitude of instant retention time loss. This data also supports the speculation that instant retention time loss behavior would be rare when short-chained alkyl bonded phases such as C_3 are used (13). In fact, these results suggest that for some types of bonded phases up to a C_8 short-chain phase may indeed not exhibit instant retention time loss behavior in a small pore format.

Use of more polar phases

We then examined the effect of added polar functional groups on the potential for phase-collapse behavior. Three different functional groups were examined that included phenyl (SB-Phenyl) (2.0µM/m^2), cyanopropyl (SB-CN) (2.0µM/m^2), and aminopropyl (Zorbax NH_2) (3.0µM/m^2). Table V shows the results for all three phases.

None of the three phases showed any significant evidence of instant retention time loss behavior even with the 80A-pore-size silica. However, each phase showed a different selectivity than the pure alkyl-functionalized phases, as would be expected. These phases could provide added flexibility to the method developer who wants a different selectivity than the standard alkyl phases. From all of these results presented herein, it may be assumed that retention time loss behavior is a unique situation for each type of phase chemistry.

Table V. Capacity Factor Difference for Various Phases*

	Nicotinic acid	Pyridoxine	Thiamin	Niacinamide
SB-Phenyl 80A	ND†	99	99	ND
SB-CN 80A	100	100	100	100
Zorbax NH_2 80A	100	100	100	99

* A value of 100% indicates no loss in retention. See text for calculation details.
† ND indicates an accurate retention time could not be determined (e.g., too low of retention or coelution).

Table IV. Capacity Factor Difference for SB-C_8 on Different Pore Size Silica*

	Nicotinic acid	Pyridoxine	Thiamin	Niacinamide
SB-C_8 80A	96	95	93	97
SB-C_8 300A	100	100	100	100

*A value of 100% indicates no loss in retention. See text for calculation details.

Conclusion

The degree to which a stationary phase will exhibit instant retention time loss behavior (so-called phase-collapse behavior) depends upon how wetted the stationary phase/silica surface is. When there is an abrupt change from an organic rich mobile phase to a totally aqueous mobile phase, this wettability depends upon how easily the stationary phase will release the adsorbed organic solvent and transition to a water rich surface. If the water-rich mobile phase does not easily adsorb or permeate (or both) into the stationary phase (wetting), a surface layer of structured water forms and limits access of the analyte to the surface. The two factors, diffusion of the organic out of surface and diffusion of the water into or onto the surface, contribute to the degree of instant retention time loss. It does not matter whether the phase is extended or collapsed in this model.

In addition to the physiochemical factors, the establishment of a wetted surface is governed by a number of chromatographic factors. The most important chromatographic factor is the pore size. Too narrow of a pore size of the base silica particle can result in significant instant retention time loss behavior because of the inhibition of the diffusional factors. Choosing a slightly larger pore size can minimize this effect while allowing reasonable retention of the analyte. A nonendcapped phase has more surface silanols and permits an appropriate solvent interface that results in constant retention behavior and elimination of the instant retention time loss behavior. For a relatively hydrophobic phase, opening the pore size is an effective way to attain good retention and have constant retention times in high aqueous mobile phases. Lastly, increasing phase density increases the magnitude of instant retention time loss behavior because it is more difficult to wet the phase with a highly aqueous mobile phase.

Overall, we believe the main driving force is the formation of interfacial structure of the aqueous mobile phase onto the stationary phase. One component of the interfacial structure is the "wettability" of the surface. In all cases, whether the chains are fully extended or collapsed is not important if the key factor is how well the analyte passes from the bulk mobile phase through the interfacial solvent structure to the hydrophobic surface. Clearly, having a column with adequate retention that does not exhibit the retention time loss behavior is the proper choice of

pore size, bonded-phase ligand type, and ligand density. Calling the instant retention time loss behavior "phase collapse" is incorrect at this time, as it assumes that the cause is the stationary phase collapsing onto the surface, and this assumption is far from proven and not substantiated by the data presented.

Interestingly, pore size has been considered important mainly for attaining separations with large molecules. Pore size is not considered important in influencing small molecule separations, and, as such, is an under-investigated area. For instance, pore size was recently observed to influence chromatographic behavior in micellar chromatography (MC) (30). The work contained in this paper may shed some light on that report because both techniques utilize highly aqueous mobile phases, one containing a surfactant and the other containing only a buffer. Perhaps the improved behavior when using wide pores in MC may be attributable to the same mechanism as is in operation in the work reported here. The observed improvement in chromatographic behavior when using a wider pore size in MC may not be caused by better accessibility of the pore by the micelle. It may be that the improved retention has little to do with micelles but more to do with the wettability—the improved interfacial mobile phase structure on the surface—as a result of the wider pore sizes.

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