ARTICLES

Reduction of Reequilibration Time following Gradient Elution Reversed-Phase Liquid Chromatography

Lynn A. Cole[1] and John G. Dorsey*,[1]

Department of Chemistry, University of Florida, Gainesville, Florida 32611

A simple and convenient method for the reduction of column reequilibration time following gradient elution reversed-phase liquid chromatography is described. This method utilizes the addition of a constant volume of 3% 1-propanol to the mobile phase throughout the solvent gradient to provide consistent solvation of the reversed-phase stationary phase. Reductions in reequilibration time of up to 78% have been observed. The effect of alkyl chain bonding density on reequilibration volume is also examined. A maximum in the mobile phase volume necessary to reequilibrate the column is found at a bonding density of about 2.9 μmol/m². The relationship of reequilibration volume to bonding density supports the partitioning model of retention for reversed-phase liquid chromatography.

INTRODUCTION

Gradient elution is the most common solution to the general elution problem in liquid chromatography (*1-4*). In reversed-phase liquid chromatography (RPLC) this technique involves increasing the percentage of an organic modifier with respect to an aqueous phase as the chromatographic run progresses. This time-dependent increase in mobile phase strength provides greater retention of early eluting compounds and decreases retention of late eluting compounds with respect to isocratic conditions, thus improving the limits of detection and peak shapes for later eluting compounds and providing more evenly spaced bands in the chromatogram. Practical and theoretical aspects of gradient elution have been thoroughly reviewed (*1, 2, 4-7*).

One problem commonly associated with gradient elution is the time required for reequilibration of the column to the starting gradient conditions following elution of the last compound in the chromatogram (*1, 2, 5*). As the mobile phase is varied during the course of the gradient, the stationary phase composition changes due to varying solvation of the bonded alkyl chains, thus making it necessary to flush the column with many volumes of the initial mobile phase to return the stationary phase to its original conditions. Generally it is believed that 15-20 column volumes of the starting mobile phase must be passed through the column to achieve column reequilibration (*2, 5*). Often this reequilibration time is as long as the sample elution time, thus doubling the time of analysis and the volume of solvent used and making gradient elution impractical for routine analysis (*2*). Several attempts have been made to understand and reduce column reequilibration time. Frenz and Horvath (*8*) found that column regeneration time may be reduced by passing a carefully chosen series of regenerating solvents through the column in order of decreasing affinity for the stationary phase. In a recent study of column reequilibration, Payne et al. (*9*) suggest that the rate-limiting steps in column regeneration are mass transfer into and diffusion of mobile phase components within the pores of the column and that column reequilibration time may be reduced by promoting these processes. Finally, a reverse-gradient may be employed to achieve column regeneration, although there does not appear to be a significant advantage to running a reverse-gradient over stepping from the final to the initial solvent conditions to achieve column reequilibration in reversed-phase systems (*5*).

The solvation structure of the stationary phase is an important factor in gradient elution, since changes in stationary phase composition make necessary excessive column reequilibration times. The solvation of RP stationary phases has been studied extensively. McCormick and Karger evaluated the extraction of the most hydrophobic component of the mobile phase into the stationary phase by using distribution isotherms (*10, 11*). Although this work predicts preferential expulsion of the least polar mobile phase constituent into the stationary phase, the stationary phase is considered to be inert with respect to the overall retention process. Yonker et al. showed the importance of various characteristics of the stationary phase to its solvation and developed models for the configuration of the stationary phase at different concentrations of organic modifier (*12, 13*). Gilpin and Gangoda (*14, 15*) and Marshall and McKenna (*16*) used solution NMR experiments to investigate solvent effects on the configuration of the bonded alkyl chains of the stationary phase and found that the configuration of the stationary phase is a function of the mobile phase composition. All of these studies (*10-16*) as well as others (*17-19*) demonstrate that the stationary phase is preferentially solvated by the organic component of the mobile phase. The changing solvation of the stationary phase during a gradient not only makes long

[1] Present address: Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172.

0003-2700/90/0362-0016$02.50/0

reequilibration times necessary but also introduces two nonideal processes for theoretical considerations. These nonideal processes are solvent demixing due to the preferential uptake of the organic modifier by the stationary phase and changes in column dead time due to changes in the stationary-phase composition (*20*).

It is apparent from these studies of stationary-phase solvation that in order to reduce column reequilibration time following gradient elution it is necessary to control the solvation of the bonded alkyl chains. The addition of a good wetting agent to the mobile phase has been shown to be valuable for altering the chromatographic properties of the stationary phase. Scott and Simpson (*21*) have shown that the addition of a small percentage of a short-chain alkanol (such as 1-propanol) to the mobile phase provides near monolayer coverage of the RP stationary phase. Their work showed that over 90% of the surface of the stationary phase is solvated by the alcohol when the mobile phase contains 3% (w/v) 1-propanol, but only about 50% of the surface is solvated with the same concentration of methanol. The addition of small percentages of short-chain alkanols to hydroorganic mobile phases has been used previously to improve the selectivity of separations. MacCrehan and Schönberger showed that the addition of 10% 1-butanol to a methanol/water mobile phase significantly improved the selectivity in separations of retinol isomers (*22*), and retinol, α-tocopherol, and β-carotene isomers in serum (*23*). MacCrehan and Brown-Thomas added 3% 1-propanol to an acetonitrile/water mobile phase to improve selectivity in the determination of phenols and naphthols in shale oil (*24*). The addition of small percentages of short-chain alkanols has also been shown to improve the efficiency in separations involving micellar mobile phases. Dorsey et al. (*25*) demonstrated that the addition of 3% 1-propanol to micellar mobile phases dramatically improves the efficiency of the separation without adversely affecting the viscosity or retention characteristics of the mobile phase. The improved efficiency was attributed to better wetting of the stationary phase surface by the 1-propanol, providing better mass transfer of the solutes. In a thorough study of optimization of secondary chemical equilibria in RPLC, Foley and May also found that 4% 1-propanol greatly improved the efficiency of columns used with highly aqueous mobile phases (*26*, *27*).

In addition to mobile phase and solvation structure considerations, the nature of the bonded phase, particularly the bonding density of the alkyl chains, must be considered in order to completely understand column reequilibration following gradient elution. Dill has developed a retention model (*28–30*) that predicts a critical alkyl chain bonding density of 2.7 μmol of bonded alkyl chains per square meter of silica surface. At surface densities below this critical bonding density, Dill's theory predicts a linear increase in solute partitioning with increasing surface density. Above the critical bonding density alkyl chain configurational constraints increase the amount of free energy necessary to create a solute-sized cavity in the stationary phase, and partitioning of solutes should decrease. Sentell and Dorsey (*31*) have recently investigated the relationship between partitioning and stationary phase bonding density by determining partition coefficients for several compounds on well-characterized stationary phases of known high and low bonding densities, and their findings closely correlate with Dill's predictions. From this work it is clear that bonding density plays a significant role in the partitioning process. It follows that stationary phase bonding density should play a role in column regeneration following gradient elution, since the solvation of the alkyl chains at any mobile phase composition (i.e., starting and final gradient conditions) is directly influenced by the partitioning of solvent molecules into the stationary phase. Thus columns of different bonding densities should require different volumes of starting mobile phase to achieve column reequilibration.

On the basis of the work with micellar mobile phases in our laboratory (*25*) and the work of Scott and Simpson (*21*), we predicted that the addition of 3% 1-propanol to the mobile phase would significantly reduce column reequilibration time following gradient elution. In this paper we present evidence which shows that the addition of a constant volume of 3% 1-propanol to the mobile phase provides consistent solvation of the stationary phase throughout the mobile phase solvent gradient due to preferential wetting of the stationary phase by the 1-propanol. This consistent solvation provides a robust stationary phase structure, which in turn leads to a dramatic decrease in the volume of mobile phase which must be passed through the column following a solvent gradient to achieve stationary phase reequilibration. We also show the relationship of the bonding density of the stationary phase to the volume of starting mobile phase required to achieve column reequilibration when 1-propanol is not added and to the reduction in reequilibration time achieved by adding 3% 1-propanol to the mobile phase.

EXPERIMENTAL SECTION

Apparatus. All retention measurements were made with a Spectra-Physics SP8700 ternary pump (Spectra-Physics, San Jose, CA) and UV detection at 254 nm with a Beckman Model 153 fixed-wavelength detector (Beckman Instruments, San Ramon, CA). Sample injection was performed with a Rheodyne Model 7125 manual injector (Rheodyne, Inc., Cotati, CA) fitted with a 20-μL sample loop, and detector output was recorded on a Scientific Products Quantigraph chart recorder (Houston Instruments, Austin, TX). Each column was thermostated at 30 °C with a water jacket and a Fisher Scientific Model 73 immersion circulator (Fisher Scientific, Fair Lawn, NJ). The mobile phase flow rate for all experiments was 1.0 mL/min.

Solvents and Columns. HPLC grade acetonitrile and methanol (Fisher Scientific, Fair Lawn, NJ) were used without further purification. Water was obtained from a Barnstead Nanopure II water purification system (Barnstead Co., Boston, MA) fitted with a 0.45-μm filter. Reagent grade 1-propanol (Fisher Scientific, Fair Lawn, NJ) was filtered through a 0.45-μm Nylon-66 membrane filter (Rainin Instruments, Woburn, MA) before being used. Mobile phases containing 3% (v/v) 1-propanol were degassed by sonicating for 30 min prior to use; unmixed mobile phases were degassed by sparging with helium. The acetone sample was prepared by diluting spectral grade acetone (Mallinckrodt, Paris, KY) 1 part to 1000 in water.

Three commercial C_{18} chromatographic columns were used for the gradient elution reequilibration studies: Phenomenex Nucleosil, 5 μm, 25 cm × 4.6 mm i.d. (Phenomenex, Rancho Palos Verdes, CA); Burdick and Jackson OD5, 5 μm, 25 cm × 4.6 mm i.d. (Baxter Healthcare Corp., Muskegon, MI); and Zorbax ODS, 5 μm, 15 cm × 4.6 mm i.d. (Du Pont Instruments, Wilmington, DE). In addition, five columns containing well-characterized stationary phases prepared, packed, and previously used (*31*) in our laboratory were used for the gradient elution studies. The C_{18} bonding densities of these columns were calculated as previously described (*32*) and ranged from 1.60 to 4.07 μmol/m^2. Detailed synthesis procedures for the preparation of these stationary phases may be found elsewhere (*33*). All "homemade" columns were 15 cm × 4.6 mm i.d.

Reequilibration Studies. Column reequilibration following gradient elution was evaluated by measuring the variation in the retention time of acetone after a change in mobile phase conditions. Acetone was chosen as the solute for these studies since it is an early eluting compound and thus will show the greatest variation in retention time with a change in stationary phase solvation (*2*, *5*). For each column a gradient was run from 0% to 100% organic modifier (acetonitrile or methanol) and then held at the 100% organic composition for 15 min to ensure complete equilibration of the stationary phase with the organic modifier, as indicated by a steady base line. Following the 15-min equil-

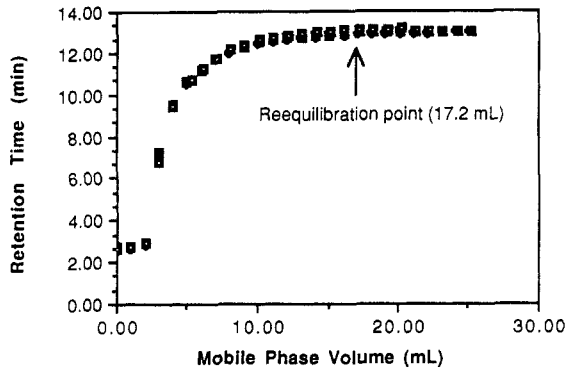

Figure 1. Acetone retention time as a function of the volume of starting mobile phase (100% water) for the Burdick and Jackson column with acetonitrile as the organic modifier.

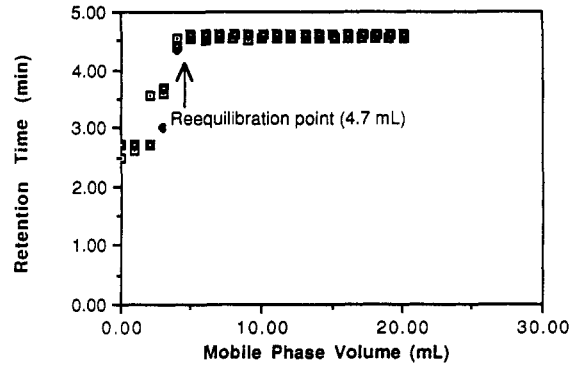

Figure 2. Acetone retention time as a function of the volume of starting mobile phase (97/3 water/1-propanol) for the Burdick and Jackson column with acetonitrile as the organic modifier and a constant volume of 3% 1-propanol added to the mobile phase.

ibration period, the mobile phase was returned to its original conditions (100% water) and injections of acetone were immediately begun at a rate of one injection per minute for 20–30 min. The retention time of acetone was measured and plotted versus the volume of starting mobile phase (100% water) that had passed from the ternary proportioning valve (TPV) of the pump at the time of injection. The time at which the mobile phase was returned to starting conditions and acetone was first injected was considered to be the starting time for the reequilibration experiments. Thus, the retention time of the first acetone injection was plotted versus 0.00 mL of mobile phase, as no volume of starting mobile phase had passed from the TPV at the time of this injection. The column was determined to be completely reequilibrated when the retention time of acetone reached a constant value. This experiment was repeated with mobile phases containing 3% 1-propanol, and the volume of mobile phase necessary to reequilibrate the column without 3% 1-propanol present was compared to the volume of mobile phase necessary to reequilibrate the column with 3% 1-propanol added. Each experiment was run in triplicate.

RESULTS AND DISCUSSION

A plot of the retention time of acetone as a function of the volume of starting mobile phase (100% water) that had passed from the TPV of the pump for the Burdick and Jackson commercial column is shown in Figure 1. A gradient was run from 0% to 100% acetonitrile, held at 100% acetonitrile for 15 min, and then returned to the starting mobile phase conditions, at which time acetone injections were begun. All three reequilibration trials are plotted on the same axes, and the average reequilibration volume, as determined from a constant retention time of acetone, was 17.2 mL of the starting mobile phase. This same experiment was repeated with a constant volume of 3% 1-propanol added to the mobile phase, and the results are shown in Figure 2. Again, all three trials are plotted, but for this experiment the average volume of starting mobile phase (97/3, water/1-propanol) needed to achieve column reequilibration was only 4.7 mL. At a flow rate of 1.0 mL/min these reequilibration volumes correspond to reequilibration times of 17.2 and 4.7 min. The reduction in reequilibration time achieved by adding 3% 1-propanol to the mobile phase was calculated as

$$\text{RT(red)}\ (\%) = \frac{\text{RT(w/o)} - \text{RT(w)}}{\text{RT(w/o)}} \times 100$$

where RT(red) is the reduction in reequilibration time (or improvement in column regeneration) expressed as a percentage, RT(w/o) is the reequilibration time of the system without 3% 1-propanol added to the mobile phase, and RT(w) is the reequilibration time of the system with 3% 1-propanol added to the mobile phase. Thus, for the Burdick and Jackson column with acetonitrile as the organic modifier the time necessary for column reequilibration is reduced by 73% when 3% 1-propanol is added to the mobile phase. This represents a significant savings in time and solvents necessary for a complete gradient run.

Although chromatographic data are most frequently expressed in terms of capacity factors, the data here are expressed in terms of retention times. In order to express these data in terms of capacity factors, it would be necessary to know the dead time (t_0) of the column at the time of each acetone injection. Since t_0 may change as the solvation of the stationary phase changes (*20*), and, in the process of reequilibration, the column solvation is changing, it is impossible to know accurately the value of t_0 at each injection time. Additionally, the value of most interest in these studies is the mobile phase volume at which the retention time becomes constant and not the actual value of the capacity factor. Therefore, retention time is the most convenient and useful way of expressing these data.

Obviously, the process of column reequilibration cannot begin until the starting mobile phase reaches the column. The dead volume of the chromatographic system from the TPV of the pump to the head of the column was determined to be 1.22 mL. Therefore, the starting mobile phase does not reach the column until after the second injection of acetone. Subtraction of this dead volume from the reequilibration volume would give a true measure of the volume of mobile phase needed to reequilibrate the column. However, in practice, the volume (or time) necessary to reequilibrate the column includes the dead volume of the system, so this value was included in all of our data. Taking this dead volume into account for the Burdick and Jackson column, the actual volume of mobile phase needed to reequilibrate the column when 1-propanol is added is 3.5 mL, which is approximately one and one-half column volumes, and considerably less than the 15–20 column volumes generally thought necessary for column regeneration (*2, 5*). This provides evidence that 1-propanol creates consistent stationary phase solvation, leading to a more robust stationary phase structure.

In order to demonstrate that it is the presence of 1-propanol in the mobile phase that provides consistent solvation of the alkyl chains, and not just the presence of any organic modifier, an experiment was performed in which the starting mobile phase contained 10% acetonitrile and the final mobile phase contained 90% acetonitrile. This experiment was performed on one of the stationary phases prepared in our laboratory with a bonding density of 3.06 μmol/m^2. Three trials were performed in which no 1-propanol was added to the mobile phase, and the average reequilibration time was 7.1 min. The experiment was repeated with 3% 1-propanol added to the mobile phase, and the average reequilibration time was 3.7 min. This represented a 48% reduction in reequilibration time, and shows that 1-propanol is preferentially solvating the bonded alkyl chains, creating a consistent solvation structure

Table I. Reequilibration Data for Commercial Columns

column	organic modifier	reequilibration volume, mL: without 1-propanol	reequilibration volume, mL: with 1-propanol	reduction in reequilibration time, %
Phenomenex	acetonitrile	14.0	6.0	57
	methanol	9.0	5.0	44
Zorbax	acetonitrile	15.9	4.4	72
	methanol	11.5	4.1	64
Burdick and Jackson	acetonitrile	17.2	4.7	73
	methanol	11.5	4.0	65

of the stationary phase. This experiment also demonstrates that the addition of 3% 1-propanol to the mobile phase improves column reequilibration in gradient separations where the starting mobile phase is a hydroorganic mixture rather than 100% water. Another experiment was performed in which 3% 1-butanol was added to the mobile phase, but this study proved to be experimentally difficult due to bubble formation within the pump when the mobile phase was switched from the final mobile phase (97/3 acetonitrile/1-butanol) to the starting mobile phase (97/3 water/1-butanol). The choice of 1-propanol as the most useful and practical solvating agent and the volume of 1-propanol needed to achieve consistent solvation (3%) were previously optimized in our laboratory (*25*) and are in agreement with the results of Foley and May (*26*, *27*).

Careful attention to Figures 1 and 2 reveals that the addition of 3% 1-propanol to the mobile phase reduces the retention time of the test solute acetone. In Figure 1, the average retention time of acetone when the Burdick and Jackson column is equilibrated with 100% water is 13.0 min, whereas in Figure 2, the average retention time of acetone when the column is equilibrated with 97/3 water/1-propanol is only 4.6 min. In this particular experiment the reduction in retention time of acetone by the addition of 3% 1-propanol is substantial, primarily because the retention time of any solute in 100% water is extraordinarily long compared with the retention time of that solute in a hydroorganic mobile phase. In the experiment on the 3.06 μmol/m^2 bonding density column in which the starting mobile phase contained 10% acetonitrile and the final mobile phase contained 90% acetonitrile, the reduction in retention time of acetone with the addition of 3% 1-propanol to the mobile phase was less severe. In this experiment the average retention time of acetone when the column was equilibrated with 10/90 acetonitrile/water was 3.2 min, whereas the average retention time of acetone when the column was equilibrated with 87.3/9.7/3 acetonitrile/water/1-propanol was 2.7 min, which is only a 16% decrease in retention time. The effect of adding 3% 1-propanol to the mobile phase on the retention of solutes will vary according to the gradient conditions of the experiment, and in practice, this effect may be diminished by appropriate adjustment of the gradient parameters.

Two other commercial columns were studied in addition to the Burdick and Jackson column. A summary of results for all the commercial columns studied is given in Table I. In every case, the addition of 3% 1-propanol to the mobile phase decreased the column reequilibration time significantly. The results for the Zorbax column were very similar to those for the Burdick and Jackson column, but the results for the Phenomenex column were somewhat different. Since the experimental conditions were identical for each of the columns, the differences in the column reequilibration times were attributed to differences in the stationary phases. Based on knowledge of the effect of alkyl chain bonding density on partitioning, and therefore on column regeneration, the variation in the results is most likely due both to differences in the alkyl chain bonding density and to the surface area of the silica of the three commercial columns.

Table II. Relevant Characteristics of Columns Used for Bonding Density Study

C_{18} bonding density, μmol/m^2	particle size, μm	endcapped?
1.60	20	yes
2.84	20	no
3.06	20	no
3.56	20	no
4.07	10	no

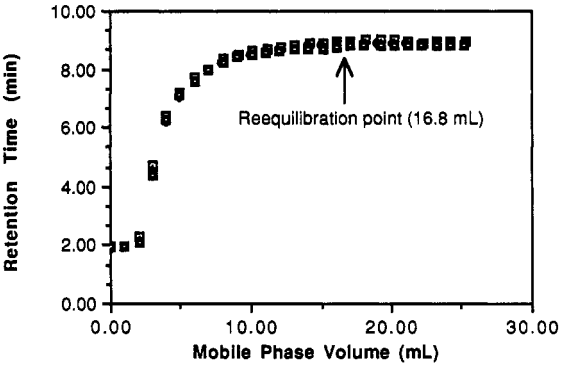

Figure 3. Acetone retention time as a function of the volume of starting mobile phase (100% water) for the column with a C_{18} bonding density of 3.06 μmol/m^2 with acetonitrile as the organic modifier.

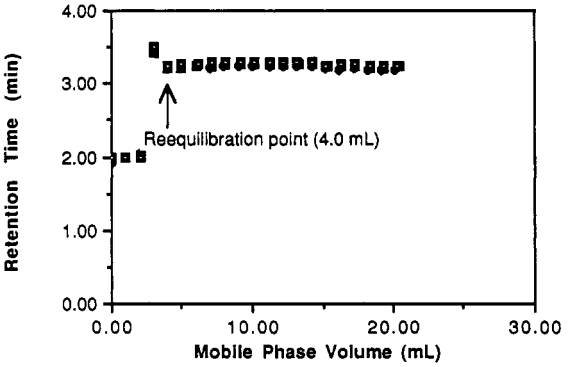

Figure 4. Acetone retention time as a function of the volume of starting mobile phase (97/3 water/1-propanol) for the column with a C_{18} bonding density of 3.06 μmol/m^2 with acetonitrile as the organic modifier and a constant volume of 3% 1-propanol added to the mobile phase.

Relationship to Bonding Density. Five columns of known C_{18} bonding density prepared and packed in our laboratory were used to evaluate the effect of alkyl chain bonding density on column regeneration time. Relevant characteristics of these columns, ranging in bonding density from 1.60 to 4.07 μmol/m^2, are given in Table II. Reequilibration studies were performed in triplicate for each of the columns using methanol and acetonitrile as the organic modifier. Figure 3 shows a plot of the retention time of acetone as a function of the volume of starting mobile phase (100% water) for the column with a C_{18} bonding density of 3.06 μmol/m^2 with acetonitrile as the organic modifier. The average reequilibration time for this column without 1-propanol added to the mobile phase is 16.8 mL. A plot of acetone retention time versus the volume of starting mobile phase with 3% 1-propanol added for this column is shown in Figure 4. With 3% 1-propanol added to the mobile phase the reequilibration volume is only 4.0 mL, which represents a 76% reduction in reequilibration time.

A summary of reequilibration results for all of the columns of known bonding density is given in Table III. Again, in all

Table III. Reequilibration Data for Columns of Known Bonding Density

bonding density, $\mu mol/m^2$	organic modifier	reequilibration volume, mL: without 1-propanol	reequilibration volume, mL: with 1-propanol	reduction in reequilibration time, %
1.60	acetonitrile	12.1	6.0	50
	methanol	9.1	5.3	42
2.84	acetonitrile	16.9	3.7	78
	methanol	13.8	3.7	73
3.06	acetonitrile	16.8	4.0	76
	methanol	13.6	4.0	71
3.56	acetonitrile	15.2	4.0	74
	methanol	9.8	5.7	42
4.07	acetonitrile	8.5	5.0	41
	methanol	5.0	3.7	26

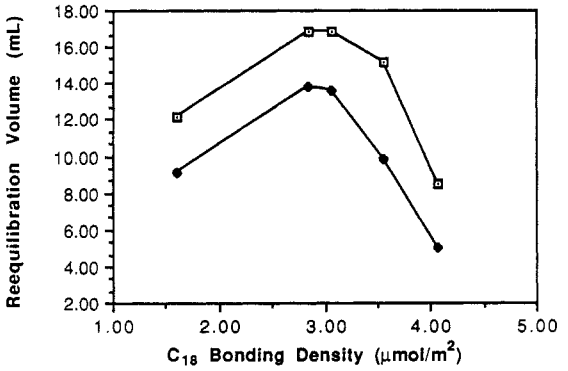

Figure 5. Reequilibration volume as a function of C_{18} bonding density with no 1-propanol added to the mobile phase. Open boxes represent experiments with acetonitrile as the organic modifier, and closed boxes represent experiments with methanol as the organic modifier.

cases, the addition of 3% 1-propanol to the mobile phase produces a marked reduction in reequilibration time. Examination of these data reveals that for every bonding density with either acetonitrile or methanol as the organic modifier, the average reequilibration volume when 3% 1-propanol is added to the mobile phase is roughly the same, but without 1-propanol added the reequilibration volumes are quite different. The fact that the reequilibration volumes for all columns are similar when 3% 1-propanol is added to the mobile phase demonstrates that 1-propanol provides consistent wetting of the stationary phase. A plot of the reequilibration volume as a function of C_{18} bonding density when no 1-propanol is added to the mobile phase is shown in Figure 5. This plot shows that a maximum in the amount of mobile phase required for reequilibration occurs at bonding densities of about 2.9 $\mu mol/m^2$. This matches closely the critical alkyl chain bonding density of 2.7 $\mu mol/m^2$ predicted by Dill (*29*) and of 3.1 $\mu mol/m^2$ observed by Sentell and Dorsey (*31*) and indicates that alkyl chain bonding density plays a prominent role in the partitioning process and therefore in the reequilibration process. At bonding densities greater than the critical bonding density, the mobile phase volume necessary to achieve column reequilibration decreases. At these higher bonding densities chain ordering increases due to configurational constraints imposed by the increased chain density, which in turn decreases partitioning (*28–30*). Since chain ordering increases and partitioning decreases at higher bonding densities, the stationary phase will undergo fewer changes with a change in mobile phase composition. Therefore, as our experimental evidence indicates, the reequilibration volume for columns of high bonding densities should decrease due to the additional rigidity of the stationary phase structure imposed by the higher density of the alkyl chains.

It is interesting to note that for every column examined, both commercial and "homemade", the volume of mobile phase necessary for stationary phase reequilibration when no 1-propanol is added to the mobile phase is greater when acetonitrile is used as the organic modifier than when methanol is used as the organic modifier. Since acetonitrile is less polar than methanol, it will have more affinity for the stationary phase with respect to water than will methanol. Therefore, when the column is equilibrated with acetonitrile (as at the end of a gradient), it is more difficult for water (the starting mobile phase) to replace the acetonitrile in the stationary phase than it is for water to replace methanol. This is in agreement with the results of McCormick and Karger (*10*, *11*), who found that acetonitrile is extracted into the stationary phase to a greater extent than methanol, and with Yonker et al. (*13*), who found that acetonitrile is a better C_{18} solvating agent than methanol. This trend is also observed when the mobile phase contains some acetonitrile or methanol at the beginning of the gradient. However, when 1-propanol is added to the mobile phase, this trend is not observed, since the 1-propanol is present at a constant volume percentage throughout the gradient and preferentially wets the alkyl chains.

By maintaining consistent solvation of the stationary phase through the addition of a constant volume of 3% 1-propanol to the mobile phase, it is possible to markedly reduce the column reequilibration time needed following gradient elution. The use of this simple method to reduce column reequilibration time should lead to a significant savings in time, solvents, and money when used for routine gradient separations. Additionally, identification of a critical alkyl chain bonding density of about 2.9 $\mu mol/m^2$ provides support for the partitioning mechanism of retention as proposed by Dill (*28–30*) and demonstrates the significance of examining and controlling stationary phase characteristics when performing chromatographic studies.

ACKNOWLEDGMENT

The authors are grateful to Carol Fung-Kee-Fung for preliminary data and to Joe P. Foley for helpful discussions.

Registry No. 1-Propanol, 71-23-8.

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RECEIVED for review July 17, 1989. Accepted October 6, 1989. The authors are grateful for support of this work by NIH GM33382 and by Burdick and Jackson BJ 8803.

Immobilized 8-Oxine Units on Different Solid Sorbents for the Uptake of Metal Traces

O. Abollino, E. Mentasti,* V. Porta, and C. Sarzanini

Department of Analytical Chemistry, University of Torino, Via Giuria 5, 10125 Torino, Italy

The sorption of 8-hydroxyquinoline and 8-hydroxyquinoline-5-sulfonic acid on a polystyrene–divinylbenzene resin (Amberlite XAD-2) and on an anion exchange resin (Bio-Rad AG MP-1) has been used for the uptake and enrichment of trace metal ions. The investigated metal ions were Ca(II), Cd(II), Cu(II), Mg(II), Mn(II), Ni(II), Pb(II), and Zn(II). The uptake and recovery yields were determined by use of inductively coupled plasma atomic emission spectroscopy. The behavior of the sorbed ligands was determined in different conditions, and the results have been discussed and compared with those computed according to a model described. The chelating solid substrates have been used for the enrichment of metal traces from environmental samples. Enrichment factors of up to 100, together with low blank levels of the optimized procedures, allow the simple determination of the above elements at concentrations down to a few nanograms per milliliter.

Different solid sorbents (namely polymers, anion exchangers, reversed-phase octadecylsilica, controlled-pore glass) have extensively been used for the immobilization of organic ligands (*1*, *2*). Such substrates have been used for the uptake of metal ion traces from aqueous samples and for their enrichment, under different operating mechanisms of ligand and of complex retention, i.e. adsorption (*3–5*), ion exchange (*6*), partition (*7*), ion pair interaction (*8*), chelation (*9–11*).

Among the grafted ligands, 8-hydroxyquinoline (oxine) has been extensively used in different forms, especially grafted to controlled-pore glass (CPG) (*12–18*), or adsorbed on octadecyl reversed-phase silica (silica C-18) (*5*, *19*, *20*). In this last form standard procedures for the enrichment and subsequent determination of a series of metal traces have been investigated in detail (*2*, *21*).

In the present paper we have investigated the grafting procedures of the oxine moiety to organic polymeric supports in order to develop preconcentration procedures. In particular the systems used here are a polystyrene–divinylbenzene (ST–DVB) copolymer and an anion exchange (AE) resin, whose properties allowed metal complexes of the parent ligand oxine (OX) and of its 5-sulfonic acid derivative (SOX), respectively, to retain.

For both systems very low contaminations from the solid supports were experienced during the enrichment procedures, and this favorable condition was given by the low content of metal impurities on both investigated polymeric supports. On the contrary, this is not the case for the other silica-based solid supports, like CPG or silica C-18; with these last, higher blank values are usually found that make the developed procedures not appropriate for applications directed to the analysis of environmental samples (*5*, *22*).

The high stability of the complexes formed by the 8-hydroxyquinoline moiety with several metals prompted us to investigate in detail the behavior of Ca(II), Cd(II), Cu(II), Mg(II), Mn(II), Ni(II), Pb(II), and Zn(II). The aim of the present paper is therefore the characterization of the above chelating systems for the uptake of such trace metal ions.

The experimental results have been compared with reference to the theoretical uptake yields which can be computed on the basis of each metal speciation, obtained with the aid of the corresponding stability constants, according to a described model.

EXPERIMENTAL SECTION

Instruments. A Plasma 300 Allied Analytical Systems (Waltham, MA) inductively coupled plasma emission spectrometer (ICP-AES) was used throughout for the determination of the metals in all solutions. The operating conditions of the ICP-AES were as follows: rf power, 1.2 kW; coolant flow rate, 13 L/min; aspiration rate, 1.5 mL/min; nebulizer pressure 26 psi; observation height above the load coil, 12 mm; integration time, 5 s; window slit, 0.067 nm; readings, 2. An Orion EA 920 pHmeter equipped with a combined glass–calomel electrode was used for all pH measurements. All solutions were prepared with high-purity water HPW (Millipore Milli-Q). High-purity acids and ammonia were obtained with a sub-boiling quartz still (K. Kurner, Rosenheim, West Germany). Standard labware and glassware were used throughout and repeatedly cleaned with HNO_3 and rinsed with HPW, according to a published procedure (*23*).

Chemicals. The ligands employed were 8-hydroxyquinoline (OX) and 8-hydroxyquinoline-5-sulfonic acid (SOX). OX ligand is less polar and its dissolution may be easily achieved in a water/methanol (75/25 (v/v)) mixture, while SOX was dissolved in water.

Also the solid supports for these ligands show different properties. For SOX, a macroporous anion exchange resin (AG MP-1, 100–200 mesh, chloride form, Bio-Rad) enabled the immobilization of the ligand through its sulfonato group. OX was supported on Amberlite XAD-2 polymer beads (polystyrene–divinylbenzene copolymer, 20–50 mesh, Fluka). For some experiments (see below), AG MP-1 resin was converted in nitrate form according to the following procedure: 1 g of resin, slurry packed in a polypropylene

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