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Journal of Chromatography A, 1075 (2005) 177–183
JOURNAL OF CHROMATOGRAPHY A
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Mechanism of retention loss when C_8 and C_{18} HPLC columns are used with highly aqueous mobile phases

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Received 16 November 2004; received in revised form 5 April 2005; accepted 20 April 2005

Abstract

We describe investigations into the cause of retention losses encountered when C_8 and C_{18} HPLC columns are used with highly aqueous (>90% water) mobile phases. A procedure for quantifying these losses is described, involving stopping and restarting the flow. This procedure was used to study the dependence of retention loss on the pore size, surface concentration, and chemical structure of the bonded phase. Experiments were also carried out to determine how to restore the original retention of the columns by changing the composition of the mobile phase, or by increasing the pressure applied to the column. The results are shown to be consistent with a mechanism based on the theory of pore filling by non-wetting liquids, as employed in Mercury Porosimetry. The retention losses are attributed to the highly aqueous mobile phase being forced out of the pores when the flow is stopped and the pressure released. Retention is lost because the mobile phase is no longer in contact with the interior surface of the particles, where most of the surface area is located. The implications of this phenomenon for maximizing the reversed phase retention of polar analytes are discussed.

Keywords: Reversed-phase; HPLC; Highly aqueous mobile phases; Retention loss

1. Introduction

Reversed-phase high-performance liquid chromatography (RP HPLC) is often carried out using mobile phases containing high water concentrations in order to retain polar analytes that would otherwise elute in the void [1]. However, there have been many reports of anomalous behavior when C_8 and C_{18} columns are used with highly aqueous (>90% water) mobile phases [2]. These anomalies have most often been attributed to aggregation of the bonded hydrocarbon chains in the presence of highly aqueous mobile phases, rendering them inaccessible to analytes [3].

In 1997, we suggested an alternative explanation for the loss of retention following stopping and restarting the flow when using highly aqueous (>90% water) mobile phases [4]. This explanation, extrusion of the mobile phase from the pores of the particles, was based on the observation that retention losses were dependent on the pore size of the bonded phase, and that column pressure was a key variable. Partial accounts of these results have been reported [5,6]. The same theory has been applied to explain retention losses in reversed-phase solid phase extraction that occur when the sorbent bed dries out after conditioning [7]. Similar observations have since been published by other workers [8–11], although in some cases different explanations have been proposed to account for the results. Reid and Henry [8] attributed retention losses to the folding of stationary phase alkyl chains. Referring to retention losses after stopping and restarting the flow, Bidlingmeyer and Broske [11] state that "the main driving force is the formation of interfacial structure of the aqueous mobile phase onto the stationary phase." In this report, we describe a procedure to measure retention losses caused by stopping and restarting the flow when using highly aqueous mobile phases. This procedure was used to study the dependence of retention loss on pore size and the surface concentration and chemical structure of the bonded phase. We

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doi:10.1016/j.chroma.2005.04.039

also report two methods for restoring retention: by increasing the organic content of the mobile phase, and by increasing the pressure applied to the column. The results are shown to strongly support the mobile phase extrusion mechanism. We also discuss the implications of this mechanism as they relate to maximizing the reversed phase retention of polar analytes.

2. Experimental

2.1. Apparatus

Elemental analyses were performed using an Exeter Analytical (North Chelmsford, MA, USA) model 240XA CHN analyzer. Specific surface areas (A_s), specific pore volumes (V_p), and average pore diameters (D_p) were measured using the multipoint nitrogen sorption method, employing a Micromeritics (Norcross, GA, USA) ASAP 2400. The A_s values were calculated using the multipoint BET method, the V_p values were determined at a single point for $P/P_0 > 0.98$, and the D_p values were calculated from the desorption leg of the isotherm using the BJH method. Bonded phase surface concentrations were calculated using the equation of Berendsen and de Galan [12].

2.2. HPLC analysis conditions

Waters Corp. (Milford, MA, USA) ExpertEase V3.2 Chromatography Manager software was used for instrument control, data acquisition, and processing. The chromatographic system consisted of the following Waters components: a model 600 solvent delivery system, a 490E programmable wavelength absorbance detector set to 254 nm, and a 717plus autoinjector. Column temperature was maintained using a Euramark (Mt. Prospect, IL, USA) Mistral thermostated column oven set to 25 °C. A flow rate of 1 mL/min was used throughout the test procedure except where noted for the high-pressure rewetting experiments. The sample consisted of 100 μg/mL sulfanilamide and 100 μg/mL procainamide prepared in 80/20 (v/v) D_2O/methanol. The injection volume was 10 μL. Retention factors were calculated from the average retention times of three replicate injections using D_2O as the void marker. The mobile phases were 20 mM K_2HPO_4 (pH 6.00) neat or combined with 5 or 10% (v/v) methanol.

2.3. Chromatographic protocol for determining retention loss

The column was equilibrated for 30 min at 1 mL/min in 100% methanol, then 50/50 methanol/water, to assure complete wetting of the stationary phase and to prevent the precipitation of the pH 6 phosphate buffer, respectively. The column was then equilibrated for 30 min in the pH 6 phosphate mobile phase prior to making three injections of the sample. The run times were sufficiently long to elute all sample components and were typically 60 min, 20–25 min, and 10 min for experiments conducted using 0, 5, and 10% methanol mobile phases. Three replicate injections were made to determine the retention times, and the average values were used to calculate the retention factors. After the initial sample injections the flow rate was reduced to 0 over a 4 min period and maintained at 0 for 1 h unless specified otherwise. After this period of flow stoppage the flow was reestablished to 1 mL/min over a period of 15 s. After about 1 min, three replicate injections were made to determine the retention times, and the average values were used to calculate the retention factors. This sequence was performed first using the 100% pH 6 K_2HPO_4 mobile phase, followed by the mobile phases containing 5 and 10% methanol. The percent retention loss was calculated using the difference in the retention factor of each probe before and after flow stoppage relative to the initial retention factor.

2.4. Chromatographic protocols for restoring retention

To determine the efficacy of using pressure as a means of restoring retention after flow stoppage, the column backpressure was increased by incrementally increasing the flow rate. The retention loss protocol described above was followed using only the 100% aqueous mobile phase and a flow stoppage time of 10 min, which was determined to be a sufficient amount of time to cause complete retention loss for the bonded phase used in this study. Once the retention loss was determined after stopping the flow and restarting it at 1 mL/min, the flow rate was increased to 1.5 mL/min and held for 30 min prior to performing three replicate injections of the sample to reassess retention loss. This sequence was repeated using 2.0, 2.5, and 3.0 mL/min flow rates.

A similar approach was used to determine the minimum % methanol that was required to restore retention on the C_{18} A bonded phase after flow stoppage in the 100% aqueous mobile phase. Once the retention loss was determined after stopping the flow and restarting it at 1 mL/min, the column was equilibrated in 10/90 methanol/water (v/v) at 1 mL/min for 30 min prior to reequilibrating the column in the 100% aqueous pH 6 mobile phase. Three replicate injections were made to reassess retention loss.

This sequence was repeated using 20/80, 30/70, 40/60, and 50/50 (v/v) methanol/water mobile phases prior to reassessing the retention loss in the 100% aqueous mobile phase.

2.5. Reagents and materials

The 9.2 nm pore diameter silica used for many of the samples was 5.0 μm Symmetry silica (Waters Corp.). The larger pore size silica samples were obtained by hydrothermal pore enlargement of this silica [13]. All bonded phases were prepared using chlorodimethyl silanes, and were endcapped with trimethylsilyl groups. The high surface concentration bonded phases were prepared using an excess of silane relative to the amount that reacts with the silica. The reduced surface concentration C_{18} materials were prepared using stoichiometric amounts of n-octadecyldimethylchlorosilane (Silar

Table 1
Properties of the bonded phases used in this work

Material	% C1[a]	% C2[b]	Surface concentration (μmol/m^2)	Before bonding			After bonding		
				A_s (m^2/g)	V_p (cm^3/g)	D_p (nm)	A_s (m^2/g)	V_p (cm^3/g)	D_p (nm)
C_{18} A	19.18	19.37	3.21	332	0.878	9.3	136	0.310	6.7
C_{18} B	13.64	13.68	3.50	203	0.868	15.5	114	0.517	12.1
C_{18} C	10.50	10.67	3.41	145	0.838	21.5	91.2	0.578	17.5
C_{18} D	9.55	9.61	3.53	128	0.831	24.5	85.1	0.609	19.9
C_{18} E	12.91	14.22	2.00	319	0.839	9.1	184	0.467	7.0
C_{18} F	14.34	15.42	2.30	319	0.839	9.1	170	0.444	6.9
C_{18} G	16.64	17.36	2.62	337	0.880	9.3	155	0.425	7.1
C_{18} H	17.50	18.07	2.80	337	0.880	9.3	143	0.394	7.1
C_{18}-carbamate	21.09	21.36	3.07	344	0.861	9.1	106	0.274	6.5
C_8	11.24	11.61	3.41	329	0.860	9.3	201	0.513	6.7

[a] Carbon content (w/w) after C_{18} or C_8 bonding.
[b] Carbon content (w/w) after endcapping.

Labs, Scotia, NY, USA). The structure and preparation of the *N*-octadecylcarbamate (C_{18}-carbamate) bonded phase has previously been reported [14]. The carbon contents, surface concentrations, surface areas, pore volumes, and average pore diameters of the materials used in this work are summarized in Table 1. All materials were packed into 150 mm × 3.9 mm stainless steel columns using proprietary high-pressure slurry packing procedures. All reagents and solvents were used as received.

3. Results

3.1. Quantifying retention losses

It has been reported that retention times decrease gradually when some RP columns are used with highly aqueous mobile phases [8]. However, we observed no change in retention when using a column containing a high surface concentration C_{18} bonded phase (material C_{18} A in Table 1) with a completely aqueous mobile phase over 20 h of continuous operation. But when the flow was stopped, then restarted, we observed almost no retention. When mobile phases containing more than 10% (v/v) methanol or acetonitrile were used, this retention loss was not observed. Based on this observation, we devised a procedure to quantify retention loss caused by stopping and restarting the flow using highly aqueous mobile phases. The column (150 mm × 3.9 mm) was first conditioned with 100% methanol followed by 50/50 (v/v) methanol/water for 30 min at 1 mL/min, then equilibrated with the highly aqueous mobile phase for 30 min at 1 mL/min. A test mixture was injected, containing sulfanilamide, procainamide, and D_2O as the void marker. After all analytes had eluted, the flow was stopped for a predetermined time, then restarted at 1 mL/min. The retention loss was measured as the difference in the retention factor of procainamide before and after stopping and restarting the flow, as a percentage of the original retention factor. The relative retention losses measured for procainamide and sulfanilamide were the same, within experimental error.

We investigated the kinetics of retention loss by varying the time during which the flow was stopped. For high surface concentration bonded phases (C_{18} A and C_8 in Table 1), we found that retention loss was constant after 10 min. For a C_{18} bonded phase with a surface concentration of 2.00 μmol/m^2 (C_{18} E), however, the retention factor continued to decrease after 9 h. In the experiments described below, we used a 1 h flow stoppage, unless noted otherwise.

In addition to a decrease in retention for the initially retained analytes, we also observed a decrease in the elution time for the void marker, D_2O. For a 150 mm × 3.9 mm column containing bonded phase C_{18} A, the void volume decreased from 1.24 to 0.95 mL. This 0.29 mL volume change is similar to the volume of mobile phase contained within the pores of the particles in the column (1.0 g/column × 0.31 mL/g = 0.31 mL/column). For a column containing bonded phase C_{18} E, with a C_{18} surface concentration of 2.00 μmol/m^2, a smaller change in void volume (0.08 mL) was found. This material also shows a smaller retention loss after flow stoppage (18% after 1 h; see below). McCormick and Karger [15] have reported similar changes in the elution time for D_2O when a C_8 column was used with a 100% water mobile phase.

3.2. Dependence of retention loss on pore size

To study the dependence of retention loss on the pore size of the packing material, we tested a series of high surface concentration endcapped C_{18} bonded phases prepared using silicas with average pore diameters ranging from 9.3 to 24.5 nm (materials C_{18} A–D in Table 1). The pore diameters after bonding were found to range from 6.7 to 19.9 nm. This reduction in average pore size is a well-known effect caused by partial filling of the pore network with the bonded groups [16]. The dependence of retention loss after stopping and restarting the flow on the pore size of the bonded material is shown in Fig. 1 for three different mobile phase compositions. The largest retention losses were seen when using a 100% aqueous mobile phase. While the retention loss was nearly 100% for the smallest pore size material with this

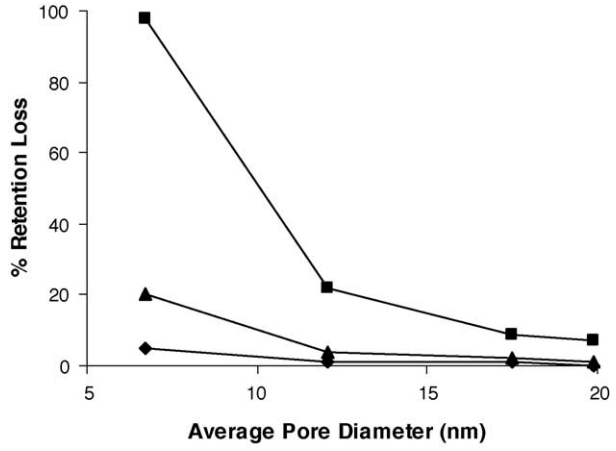

Fig. 1. Dependence of retention loss for procainamide after stopping and restarting the flow on the average pore diameter of high surface concentration C_{18} bonded phases (square symbols: 100% 20 mM K_2HPO_4 pH 6 mobile phase, triangles: 95/5 buffer/methanol, diamonds: 90/10 buffer/methanol).

mobile phase, it dropped to only 8% for the largest pore size material. This dependence on pore size has been reported by several other workers [8,10,11].

3.3. Dependence of retention loss on C_{18} surface concentration

To study the dependence of retention loss on surface concentration, we tested a series of C_{18} bonded phases prepared on 9.1–9.3 nm pore diameter silicas. The C_{18} surface concentrations of the five materials ranged from 2.00 to 3.21 μmol/m^2 (materials C_{18} A and E–H in Table 1). All were completely endcapped in a subsequent step. The dependence of retention loss on C_{18} surface concentration is shown in Fig. 2 for three different mobile phase compositions. Again, the largest retention losses were seen when using a 100% aqueous mobile phase. For this mobile phase, a strong relationship is observed, with retention loss decreasing with decreasing C_{18} surface concentration. A similar trend has been reported by Bidlingmeyer and Broske [11].

3.4. Dependence of retention loss on chemical structure of the bonded phase

To examine how retention loss varies with the chemical structure of the bonded phase, we tested three different high surface concentration endcapped materials: C_{18} A, C_8, and *N*-octadecylcarbamate (C_{18}-carbamate). The characteristics of these materials are given in Table 1. Again, the largest retention losses were seen when using a 100% aqueous mobile phase. For this mobile phase, the C_{18} A and C_8 bonded phases exhibited 98% retention losses, while the C_{18}-carbamate bonded phase showed less than a 3% loss. The C_{18} A and C_8 bonded phases also showed significant retention losses when using a 95/5 buffer/methanol mobile phase, with

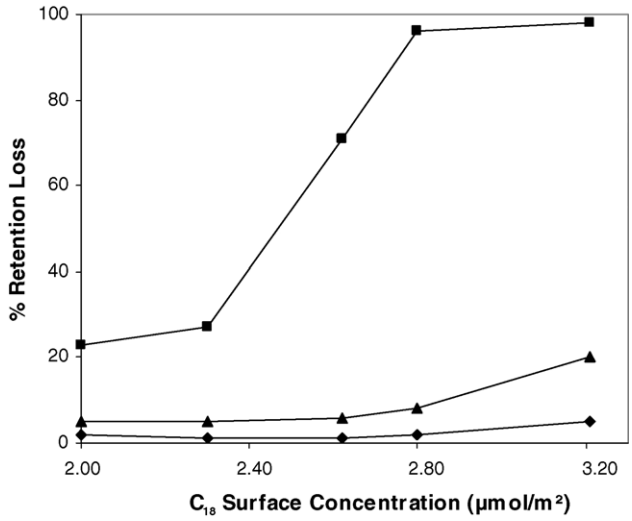

Fig. 2. Dependence of retention loss for procainamide after stopping and restarting the flow on C_{18} surface concentration for bonded phases based on 9.1–9.3 nm average pore diameter silicas (square symbols: 100% 20 mM K_2HPO_4 pH 6 mobile phase, triangles: 95/5 buffer/methanol, diamonds: 90/10 buffer/methanol).

the C_8 material showing a larger loss (84%) than the C_{18} A material (20%).

3.5. Restoring retention

When retention has been lost following stoppage of flow, the original retention may be restored by conditioning the column with a mobile phase containing a higher concentration of methanol. To determine the methanol concentration required to restore the original retention, we carried out an experiment using a column containing high surface concentration 9.3 nm pore diameter bonded phase C_{18} A. After carrying out the retention loss procedure described above using a 100% aqueous mobile phase and a 10 min stoppage time, the column showed nearly a 100% retention loss. Different methanol/water mobile phases were then passed through the column, starting at 10% methanol and increasing to 50%. At each composition, the mobile phase was passed through the column for 30 min at 1 mL/min, then the column was equilibrated in the 100% aqueous mobile phase for 30 min at 1 mL/min, and the test mixture was injected to determine the retention factors of the retained analytes. The results are shown in Fig. 3. As the methanol content of the reconditioning mobile phase was increased, the retention began to return to the original value. For this 9.3 nm pore diameter high surface concentration C_{18} bonded phase, 40% methanol was required to restore the original retention. Others have reported that retention may be restored by reconditioning with mobile phases containing about 50% methanol or acetonitrile [8–10].

Another way we found to restore retention was to increase the pressure by increasing the flow rate, using a 100% aqueous mobile phase. To study the effect of pressure on retention,

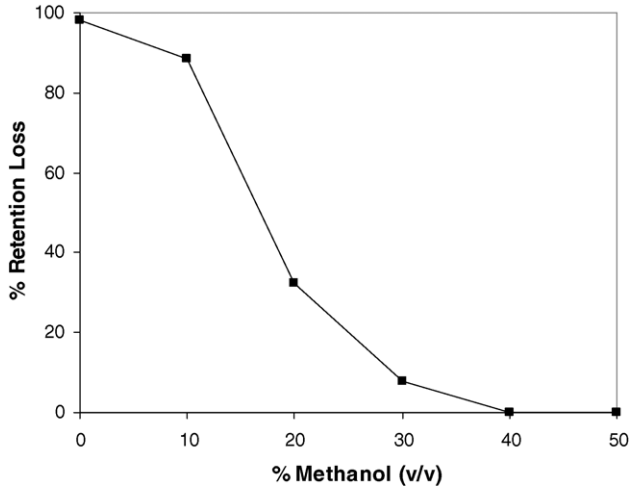

Fig. 3. Dependence of retention loss for procainamide after stopping and restarting the flow on methanol content of reconditioning mobile phase (for bonded phase C_{18} A).

we again used a column containing high coverage 9.3 nm pore diameter bonded phase C_{18} A. After carrying out the standard retention loss procedure using a 100% aqueous mobile phase and a 10 min stoppage time, the column showed nearly a 100% retention loss. The flow rate was then increased, using the same 100% aqueous mobile phase. At each flow rate, the pressure was noted, and the test mixture was injected to determine the retention factors for the retained analytes. The results are shown in Fig. 4. As the pressure increased to 22.8 MPa (228 bar, 3300 psi), the retention was found to increase. However, even at a pressure of 34.5 MPa, only 62% of the original retention was obtained. When the flow rate was reduced back to 1 mL/min, giving a column pressure of 11.0 MPa, the retention remained unchanged. The reason that the retention couldn't be completely restored by increasing pressure in this manner is because the outlet end of the column was at atmospheric pressure. To completely restore the

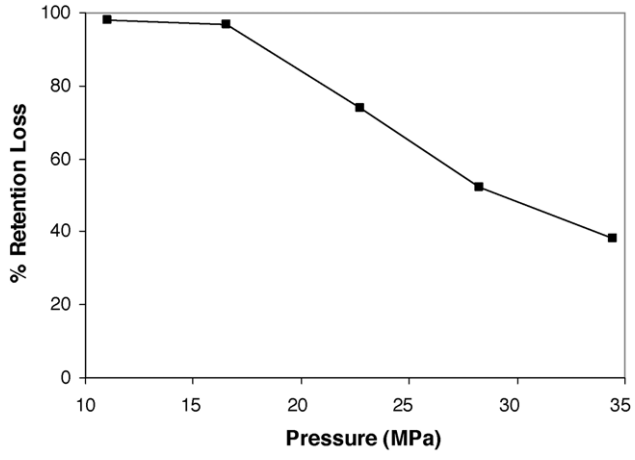

Fig. 4. Dependence of retention loss for procainamide after stopping and restarting the flow on pressure using a 100% 20 mM K_2HPO_4 pH 6 mobile phase (for bonded phase C_{18} A).

original retention, restriction must be added after the column so that the outlet end of the column is maintained above atmospheric pressure [9,10]. Other workers have reported that retention may be at least partially restored by applying pressure [8]. However, Reid and Henry [8] note that for the column they studied, this approach was only successful if some organic solvent (e.g. 10% acetonitrile) was in the column during pressurization.

4. Discussion

The mechanism that we believe best accounts for these observations is based on the theory of pore filling by non-wetting liquids. This is the basis for a common method of determining the pore size distribution of porous solids, known as Mercury Porosimetry [17]. In the Mercury Porosimetry experiment, a sample of a porous solid is evacuated, then mercury is forced into the pores under pressure. Mercury is non-wetting for many materials. The volume of mercury intruded into the sample is measured as a function of applied pressure. The pressure (ΔP) required to force mercury into a cylindrical pore of radius r is given by the Washburn equation [18]:

$$\Delta P = \frac{-2\gamma}{r} \cos\theta$$

where γ is the surface tension of mercury and θ is the contact angle between mercury and the sample. Using this equation, the intruded volume versus pressure data may be converted to plots of pore volume versus pore size.

The results presented above may be interpreted in a similar way. Most C_{18} bonded phases are known to not be wetted by water [19,20]. By definition, this means that $\theta > 90^\circ$. Although the established methods of measuring contact angles for non-wetting liquids [21] do not work for typical chromatographic particles, a simple test for wetting of a bonded phase was described by Engelhardt and Mathes [22]. A sample of the bonded phase is shaken with water, and an observation made as to whether the sample floats on top or is wetted and sinks. By this test, none of the materials used in this work are wetted by pure water. For a quantitative estimate of the contact angle for water on C_{18}-silica when this value is greater than 90°, we must look at values measured for flat surfaces. Montgomery et al. [23] reported a contact angle of 93° for pure water on a silica plate bonded with octadecyldimethylchlorosilane and end-capped with trimethylchlorosilane. Maoz and Sagiv [24] reported an advancing contact angle of 112° for pure water on a self-assembled monolayer of octadecyltrichlorosilane on glass slides. Wasserman et al. [25] reported advancing contact angles of 110° for pure water on films formed from alkyltrichlorosilanes (butyl and higher) on the silica surface of silicon wafers. The latter report also noted that receding contact angles for these films were 10° lower than the advancing contact angles. Another useful reference value is the

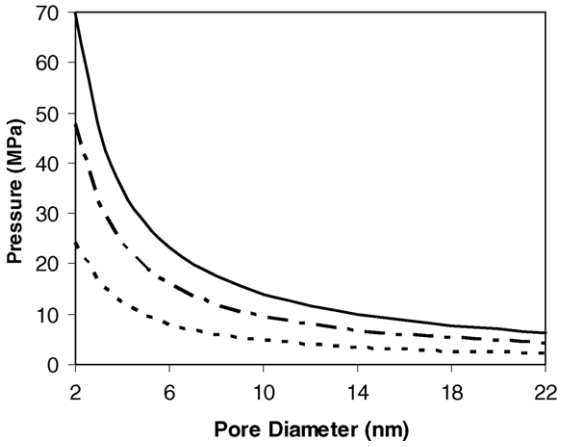

Fig. 5. Calculated pressures required to force pure water into pores of different diameters: (—) $\theta = 120^\circ$, (— - —) $\theta = 110^\circ$, (- - -) $\theta = 100^\circ$.

contact angle of 110.6° measured by Janczuk et al. [26] for pure water on a film of paraffin. Janczuk et al. also reported contact angles for mixtures of water with methanol, ethanol, and propanol on a paraffin film. The methanol concentration required to wet paraffin was found to be between 20 and 40%.

Because pure water is non-wetting for the materials we studied, it will not fill the pores unless pressure is applied. The pressure required to force water into the pores may be calculated for different pore diameters and contact angles using the Washburn equation, with γ set to the surface tension of water (72 dyn/cm). (We have neglected the small increase in surface tension caused by the 20 mM phosphate buffer. Note, however, that organic additives like acetic acid significantly lower the surface tension.) The results of this calculation for $\theta = 120^\circ$, 110°, and 100° are shown in Fig. 5. Below these pressures, water will be forced out of the pores. For $\theta = 110^\circ$, the pressures required to force water into 6.7, 12.1, and 17.5 nm pores (the average pore diameters for C_{18} A–C) are 14.2, 7.9, and 5.5 MPa, respectively.

Our interpretation of the results presented above is that when the flow is stopped, and the pressure released, highly aqueous mobile phases for which $\theta > 90^\circ$ extrude from the pores of the particles. In the case that there is contact angle hysteresis, it is the receding contact angle that should be relevant [27]. Retention is lost because the mobile phase can no longer access the interior surface of the particles, where most of the surface area is located. The only reason that retention is obtained using the highly aqueous mobile phase before the flow is stopped is because the column is under pressure. Since the outlet of the column is close to atmospheric pressure, the mobile phase may be forced out of the pores of the particles located near the outlet end. This could account for observations of partial retention losses in experiments not involving flow stoppage. This will vary with the amount of restriction in the system after the column, which depends on the diameter and length of the tubing leading to the detector, as well as the design of the detector flow cell. This same mechanism was proposed by McCormick and Karger [15] to account for changes in the elution time of D_2O for a C_8 column when a pure water mobile phase was used. In addition, Fadeev and Eroshenko [27,28] have reported water porosimetry measurements on alkyl bonded porous silicas that demonstrate behavior in agreement with the Washburn equation.

The evidence supporting this mechanism is: (i) the observation that releasing the pressure causes the retention decrease, and that retention may be recovered by applying pressure to the column, (ii) the observation of a reduction in void volume after flow stoppage that matches the intraparticle pore volume, and (iii) the dependence of retention loss on pore size, with larger pore materials showing reduced losses. In the experiment where retention was partially restored for a column containing bonded phase C_{18} A by applying pressure, it was found that 22.7 MPa or higher was needed to recover retention. This is roughly consistent with the pressures calculated for a pore diameter of 6.7 nm if the contact angle is in the vicinity of 110°. Further evidence that the highly aqueous mobile phase is forced out of the pore network comes from measurements of column weight before and after flow stoppage [10]. The observation that smaller retention losses are seen for reduced coverage C_{18} bonded phases, and bonded phases containing carbamate groups, would be consistent with this theory if these materials have reduced contact angles with the highly aqueous mobile phases. While these materials would be expected to have lower contact angles, we have not found a reliable method of determining these values. This model can also explain why increasing the methanol concentration to 40% restores the original retention. Increasing the methanol concentration reduces the contact angle, and when $\theta < 90^\circ$, the mobile phase is able to access the pore network. That a methanol concentration of 40% is required to wet this C_{18} material is consistent with the results of Janczuk et al. [26], if we assume that the contact angles measured for paraffin are a reasonable model for a high surface concentration C_{18} bonded phase.

This mechanism may also explain the optical transmittance behavior reported by Li et al. [2], since extrusion of the mobile phase from the pores creates solid/gas interfaces that strongly scatter light.

Although this simple description accounts for most of the results presented above, the true situation is more complex. First, the pore structure of the particles is not made up of non-intersecting cylindrical pores, as is assumed for strict interpretation using the Washburn equation. In the calculation used for Fig. 5, we further simplified our analysis by assuming that the pores all have the same diameter. The real pore structure of these particles is made up of a network of interconnected pores of different sizes and shapes. The behavior of such networks in Mercury Porosimetry experiments has been described by Conner and coworkers [29,30]. One point that is relevant to this work is that intrusion of the non-wetting liquid into the pore network is controlled by constrictions in

the pore structure, while extrusion is controlled by the larger openings in the network. This means that the pressure required to force the non-wetting liquid into the pore network will be higher than the pressure at which the liquid is extruded from the pores. This has been observed in water porosimetry studies [27,28].

Another source of complexity arises from the mobility of the hydrocarbon chains. Nagae et al. [10] have shown that retention loss after stopping and restarting the flow varies with chain length, with retention loss increasing from C_{30} to C_{18} to C_8. In addition, for C_{18} and C_{30} bonded phases they have shown that retention losses increase with increasing temperature. Bidlingmeyer and Broske [11] reported similar results for a C_{18} bonded phase. These phenomena are not readily explained by the mechanism described above, since the contact angle is not expected to vary with chain length [25]. Their interpretation of this result is that the mobile phase is more easily expelled from the pores when the temperature is above the melting point of the alkyl chain. The temperature dependence may be quite complex, since the surface tension, contact angle, and surface structure all vary with temperature.

5. Conclusions

The mechanism presented above is useful for predicting the behavior of reversed phase columns with highly aqueous mobile phases. All reports of retention losses after flow stoppage with such mobile phases indicate that high surface concentration endcapped alkyl bonded phases based on relatively small pore size silicas (<20 nm average pore diameter) show large losses [4,8–11]. High surface concentration endcapped alkyl bonded phases based on larger pore size silicas show much smaller losses [4,8,10,11]. However, because of the lower surface areas of these materials, they are not a good choice for maximizing the retention of polar analytes. All reports of the lack of retention losses after stopping and restarting the flow for bonded phases containing polar functionalities indicate that the incorporation of a polar group in the bonded phase is an effective way to prevent such losses [4,5,8,9]. However, some of these bonded phases give reduced retention compared to conventional alkyl bonded phases, particularly for basic compounds [5,31]. An alternative solution for maximizing the reversed phase retention of polar analytes is to use a reduced surface concentration endcapped C_{18} bonded phase on a small (ca. 9–10 nm) pore size silica, such as material C_{18} E. Such bonded phases not only show minimal losses after stopping and restarting the flow, but also exhibit excellent retention and peak shapes for polar analytes [32].

Acknowledgements

We thank John O'Gara, Ray Fisk, Ken Glose and Dan Walsh for preparing the bonded phases used in this work. We also thank Ed Bouvier and Randy Meirowitz for helpful discussions on the physical chemistry of surfaces.

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