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Retention Behavior of Reversed-Phase HPLC Columns With 100% Aqueous Mobile Phase

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Reversed-phase stationary phases such as octadecyl (C18) and octyl (C8) are the most widely used HPLC columns. However, it is well-known that C18 and C8 columns exhibit poor reproducibility of analyte retention when 98% or higher aqueous solution is used as the mobile phase. This phenomenon has been thus far explained by ligand collapse, phase collapse, or the matting effect. On the contrary, the authors propose that the decrease in retention is caused by aqueous mobile phase being expelled from the mesopores of the packing materials, which decreases the surface area on which the aqueous mobile phase can contact.[1–3] Some other parameters also influence retention stability, including pore size, the alkyl chain length of the stationary phase, temperature, pressure, endcapping, and buffer or ion-pair reagent concentration. Considering these parameters in detail, it was concluded that the decrease in retention under 100% aqueous mobile phase conditions was not caused by so-called phase collapse.[3]

This paper describes the effect of the ligand density of C18 stationary phases on the stability of analyte retention under 100% aqueous mobile phase conditions. Furthermore, it will demonstrate the effect of the pressure applied in the column on analyte retention.

Experimental

Apparatus

The LC system consisted of a PU-980 pump (Jasco, Tokyo, Japan), a UV-980 or 830-RI detector (both Jasco), a model 7725 injector (Rheodyne, Cotati, CA), and an SIC 480 integrator (System Instruments, Tokyo, Japan). The column temperature of the water bath was controlled between 20 and 40 °C using an E5C3 temperature controller (Omron, Tokyo, Japan). Particle size, pore size, and surface area were measured by a Coulter Multisizer and Coulter SA-3100 surface measurement instrument (Beckman Coulter, Fullerton, CA). The carbon content of the stationary phases was obtained with an MT-700CN-corder (Yanaco, Kyoto, Japan).

Reagents and chemicals

Distilled water was prepared in the laboratory. Other solvents and reagents, as well as test analytes, were obtained from Wako (Osaka, Japan). To evaluate the effect of the ligand density of C18 stationary phases, Develosil ODS-5, ODS-K-5, ODS-N-5, ODS-P-5, ODS-MG-5,

Table 1 Characteristics of the stationary phases examined

	Silica support				Bonded phase		
Stationary phase	Specific surface area (m^2/g)	Pore volume (mL/g)	Mean pore diam (nm)	Particle size (µm)	Carbon content (%)[b]	Ligand density ($\mu mol/m^2$)[c]	Pore diam (nm)[b]
Develosil ODS-5[a]	350	1.0	12	5	20	3.0	8.6
Develosil ODS-K-5[a]	350	1.0	12	5	19	2.8	–
Develosil ODS-N-5[a]	350	1.0	12	5	17	2.2	–
Develosil ODS-P-5[a]	350	1.0	12	5	11	1.2	–
Develosil ODS-MG-5[a]	450	1.0	10	5	15	1.6	7.6
ODS-(10)	302	1.12	13.5	5	18.4	3.2	10.3

[a] Obtained from manufacturer's literature.
[b] Measured after end-capping.
[c] Calculated from specific surface area of bare silica and carbon content before end-capping.

and ODS-(10) (Nomura Chemical, Seto, Japan) were used. These silica-based stationary phases were prepared by treating porous silica gel with dimethyloctadecylchlorosilane, followed by end-capping with trimethylchlorosilane. The physicochemical properties of the stationary phases are listed in Table 1. The stationary phases were packed into 150 × 4.6 mm i.d. stainless steel tubing (Sugiyama Shoji, Yokohama, Japan) using chloroform as the slurry solvent.

Results and discussion

Figure 1 shows the effect of C18 ligand density on the relative retention of 2-propanol at 40 °C between the initial start of the experiment and after stopping the supply of water into the column for 1 hr, using water as the mobile phase and nitrite to measure the unretained time (t_0). It can be seen that the relative retention in the case of the 2.8- and 3.0-μmol/m^2 ligand densities is very low compared to that achieved with other stationary phases. Actually, the retention in the case of the 2.8- and 3.0-μmol/m^2 ligand densities decreased by 90% during the 1 hr that flow was stopped compared with the initial value. The relative retention for the 2.2-μmol/m^2 ligand density remained at more than 90% compared to the initial retention. For the 1.2-μmol/m^2 ligand density, the relative retention did not vary at all. When the C18 ligand density was low, it is expected that more trimethylsilyl (TMS) groups were introduced by the end-capping that followed. Since TMS is less hydrophobic than C18, the overall polarity of the stationary phase with low C18 ligand density results is higher than that with higher C18 ligand densities, leading to an improvement in the intrusion of water into the pore. This result is consistent with the authors' previous result,[3] i.e., the higher the polarity, the better the observed reproducibility of the retention time.

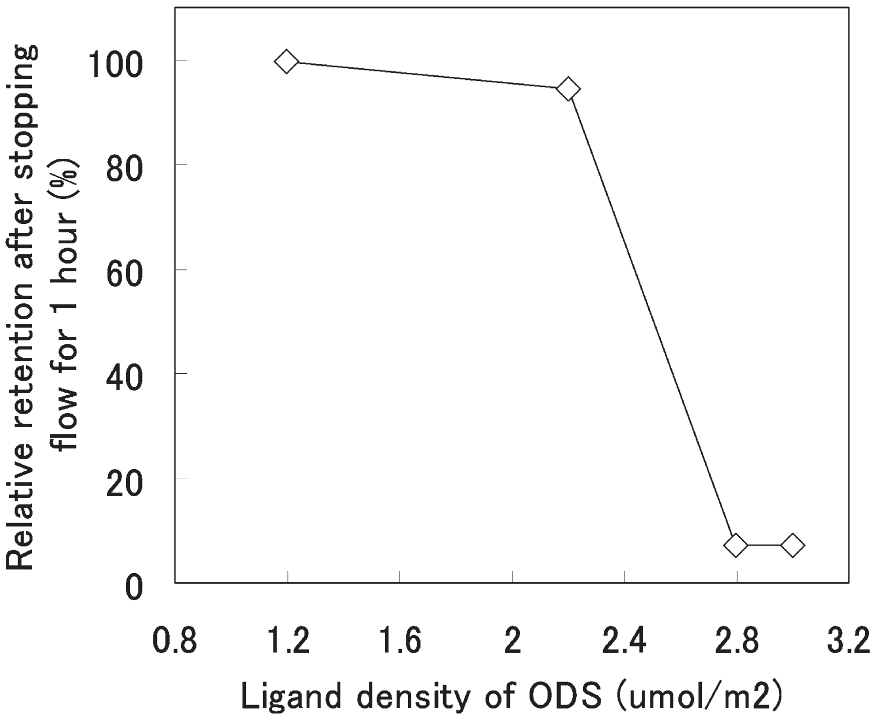

Figure 1 Relationship between C18 ligand density and relative retention of 2-propanol. Column, Develosil ODS-5, ODS-K-5, ODS-N-5, and ODS-P-5; column dimension, 150 × 4.6 mm, i.d.; mobile phase, water; flow rate, 1.0 mL/min; column temperature, 40 °C; column inlet pressure, 6 MPa; detector, RI; sample, sodium nitrite and 2-propanol.

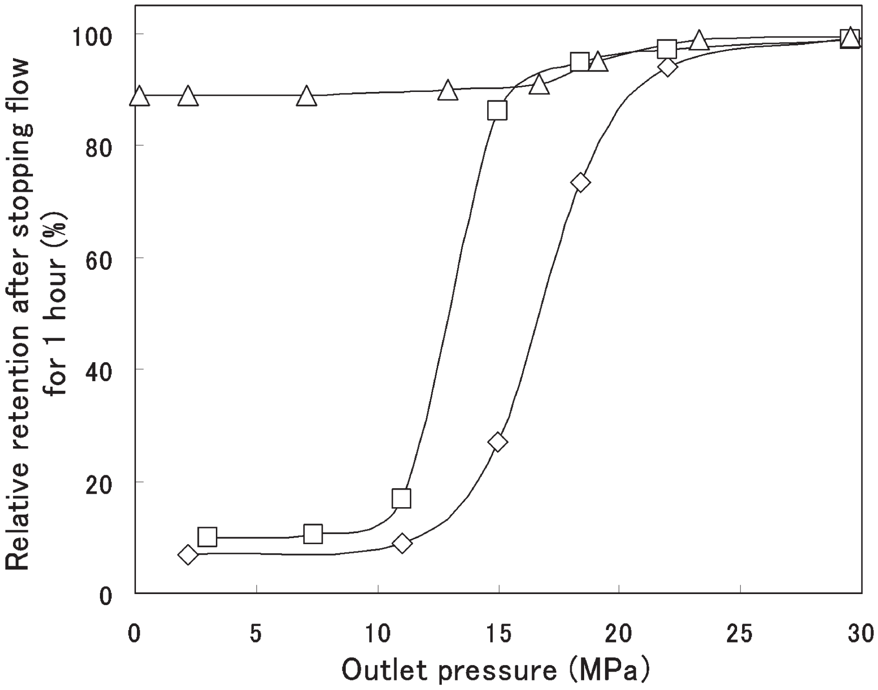

Figure 2 Effect of applied outlet pressure on relative retention of nitrite. Columns, ODS-(10) (h), Develosil ODS-5 (e), Develosil ODS-MG-5 (n); other conditions are the same as in Figure 1.

Most commercially available reversed-phase stationary phases with high reproducibility in retention time are polar-embedded alkyl phases or low ligand density phases with or without polar end-capping. They provide less hydrophobic surface than common C18 stationary phases, which provide poor retention reproducibility under 100% aqueous mobile phase conditions.

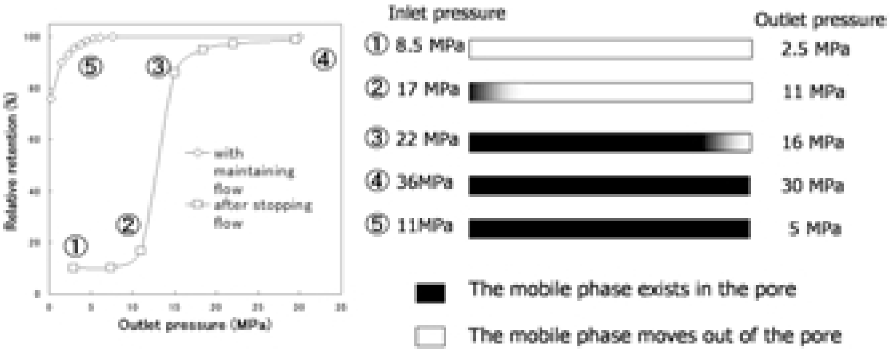

Figure 3 Effects of the outlet pressure on the relative retention and water distribution in the column. Column, ODS-(10), 150 × 4.6 mm i.d. Other operating conditions are the same as in Figure 1.

The mercury porosimetry technique is one of the most useful methods of elucidating the porous structure of solid materials such as silica gel, polycrystalline aggregates, and rocks. The theory of mercury porosimetry is based on the physical principle that a nonreactive, nonwetting liquid does not permeate into pores until sufficient pressure is applied to force its entrance. The relationship between the applied pressure (p) and the pore size, into which mercury intrudes, is given by the following Washburn equation:[4]

$$p\,r = -2\,\gamma \cos\theta$$

where r is the radius of the pore into which mercury can intrude, γ is the surface tension of mercury, and θ is the contact angle of mercury on the surface of a solid sample. In the case of an ODS (C18) surface with water as a mobile phase, the Washburn equation can be applied because water is nonwetting on an ODS surface. In other words, the pressure applied to the packing material keeps water in the pores to some degree, as expected from the above equation.

Figure 2 shows the effect of applied outlet pressure on the relative retention of 2-propanol. The retention of 2-propanol on each column was measured after the supply of the eluent (water) into the column was stopped for 1 hr. During the hour, the column temperature was kept at 40 °C. The pressure was then applied to the outlet of the column using a restrictor tubing with 0.13 mm i.d., and the pressure drop over the restrictor was adjusted by changing its length. It should be noted that once the supply of the water eluent was stopped for 1 hr, water was expelled from the pores, leading to decreased retention when the outlet pressure was atmospheric or low. The retention sharply increased at a certain outlet pressure, depending on the physicochemical property of the packing materials. This phenomenon is consistent with the Washburn equation because the pores of the packing materials for HPLC show comparatively narrow distribution. The relative retention was calculated in Figure 2 by assuming that the relative retention was 100% when the applied outlet pressure was 30 MPa. It can be seen that the smaller the average pore diameter, the higher the backpressure observed. Since the C18 ligand density of Develosil ODS-MG-5 phase is low, it showed only a 10% reduction in retention, meaning little water was expelled from the pores.

A certain level of outlet pressure was needed to repermeate the water into the pores, and it was found that the outlet pressure required for this purpose depended on the pore size. This result indicates that the present method can estimate the pore size of C18 stationary phases using water as the mobile phase.

Figure 3 shows the effect of outlet pressure on the relative retention and water distribution in the column. After stopping the supply of water into the column for 1 hr, the retention time was measured with increasing outlet pressure up to 30 MPa. Then, in the same way, after stopping the supply of water into the column for 1 hr, the outlet pressure was kept at 30 MPa for only 2 sec using the long restrictor tubing. Without stopping flow, the outlet pressure was immediately reduced by shortening the restrictor tubing; subsequently, retention time was measured. It took approx. 2 min for the outlet pressure increased up to 30 MPa because the pumping system had a bumper in order to flow steadily. Hysteresis-like curves were observed for measurement of retention between increasing and decreasing the outlet pressure, as shown in Figure 3. Such a hysteretic phenomenon is often observed when the porous structure of solid materials is measured by mercury porosimetry. It can be seen that water as mobile phase cannot enter into the pores of the packing materials at 40 °C even if the outlet pressure is kept at 10 MPa. Water began to permeate into the pores at pressure higher than 10 MPa and completely permeated the pores at 30 MPa, as shown in Figure 3. Once water filled the pores, it remained there unless the outlet pressure was lowered to below 5 MPa. It is concluded that highly reproducible separations can be achieved using a conventional C18 column under 100% aqueous conditions when the outlet pressure is maintained at 30 MPa. In a practical sense, a much more important finding is that if the outlet pressure is applied at 30

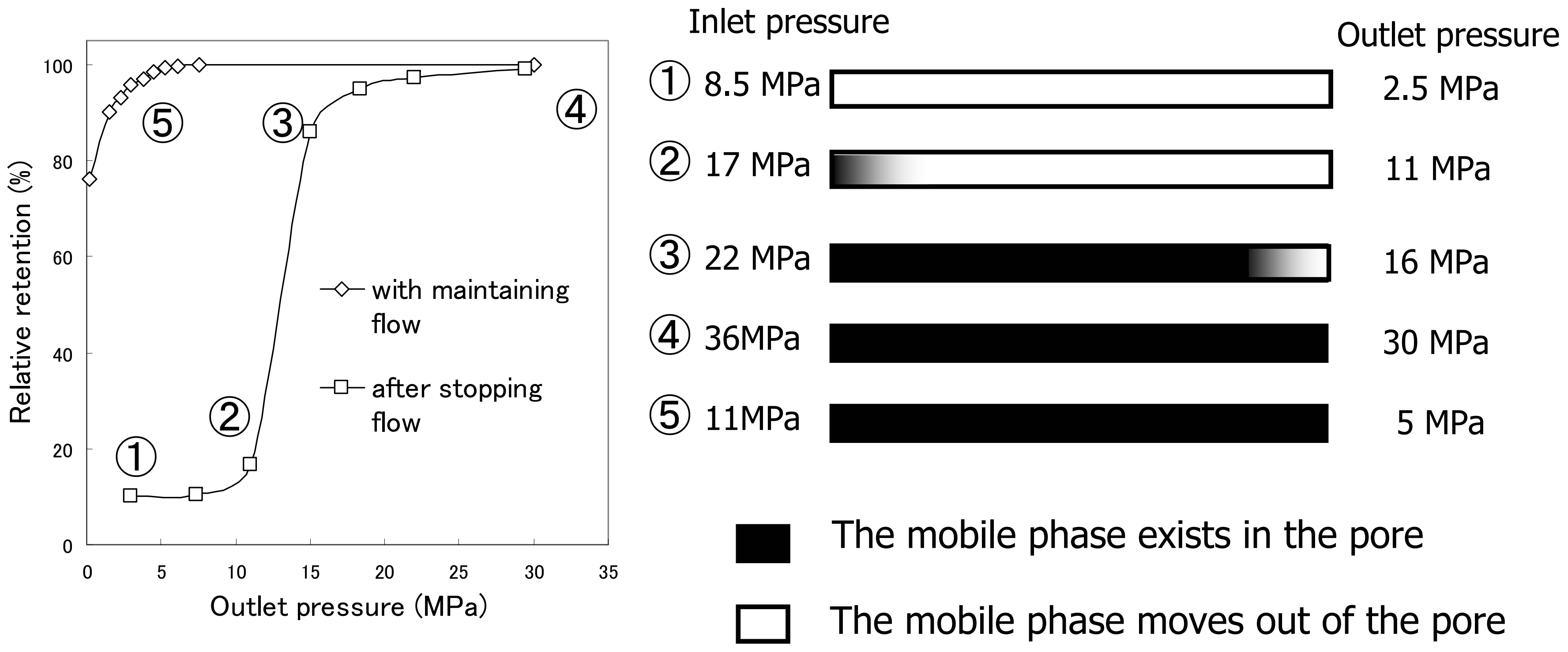

Figure 3. Effects of the outlet pressure on the relative retention and the water distribution in the column. Column, ODS (10), 150 × 4.6 mm I.D. Other operating conditions are the same as in Figure 1.

MPa for only a few seconds, reproducible retention time can be obtained as long as the outlet pressure is kept higher than 5 MPa. Outlet pressure of 5 MPa barely damages HPLC systems, while at 30 MPa, the pressure is too high to ensure system stability. It should be noted that the present technique[5] can be applied to all reversed-phase stationary phases, although the outlet pressure required depends on the materials.

As proved above, control of outlet pressure allowed the authors to recover analyte retention even under 100% aqueous conditions. There is some thought that phase collapse occurs on C18 stationary phases under 100% aqueous conditions, leading to a decrease in retention, but the results revealed here show that phase collapse has nothing to do with decreased retention.

Conclusion

Less hydrophobic C18 stationary phases with low ligand density gave reproducible retention even under 100% aqueous mobile phase conditions. Increased outlet pressure allowed water to enter the hydrophobic pores in accordance with the Washburn equation. Reproducible separation on all reversed-phase stationary phases can be achieved under 100% aqueous mobile phase conditions by controlling the outlet pressure. Once outlet pressure as high as 30 MPa is applied for a few seconds, reproducible retention is achieved unless the outlet pressure falls below 5 MPa. This is because water in the pores of the packing materials remains in the hydrophobic pores.

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