SepaFlash C18AQ Cartridge and Its Application in the Sample Desalting

Cong Zhang, Bo Xu
Application R&D Lab

Chromatography Application Note

Introduction

Reversed phase liquid chromatography (RPLC) is a widely used separation mode in liquid chromatography. RPLC has been used to separate numerous compounds, from organic acids with a small molecular weight (MW) to proteins with a MW of up to 150 kDa. However, there is a limitation for the application of RPLC, the analyte with good water solubility (i.e., hydrophilic or polar) has poor retention on the RP column. In this case, we need to increase the aqueous ratio in the mobile phase in order to enhance the interaction between the polar analyte and the hydrophobic stationary phase, thereby increasing the retention of polar analytes. The greater the polarity of the analyte, the higher the proportion of water in the mobile phase. When regular reversed phase column such as most commonly used octadecyldimethylsilane (ODS, C18) was used in pure aqueous or highly aqueous mobile phase for a long time, problems will come out including loss in retention time for the analyte as well as irreproducible separation results. This phenomenon is called as phase collapse [1]. The classic explanation for this phenomenon is that the C18 stationary phase bonded to the surface of the silica gel changed the spatial arrangement of the carbon chain in highly aqueous mobile phase, that is, changed from perpendicular to the surface of the silica gel to lying flat on the same (as shown in Figure 1). The change in the spatial arrangement of the stationary phase reduces its interaction with the analyte, thus reducing the retention time of the analyte.

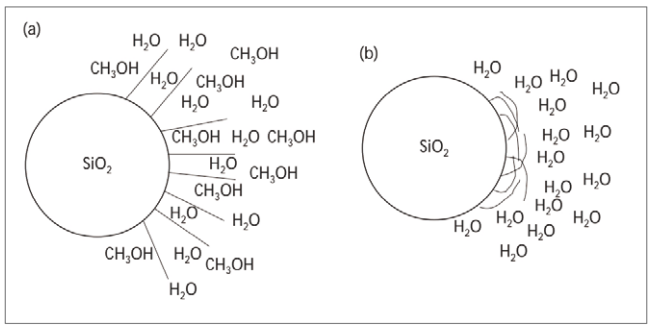

Figure 1. Illustration of the classic explanation of phase collapse in reversed phase chromatography (reproduced from Ref. 1). Shown are the configurations of long-chain bonded alkyl phases (a) in water-methanol mixtures and (b) in 100% water.

As time goes, researchers have conducted in-depth exploration of the phenomenon of phase collapse. The continuous accumulation of experimental evidences has led many researchers to accept another new theoretical explanation, that is, the loss in retention time is actually due to the "dewetting" phenomenon which occurs in the fine pores contained in the stationary phase particles [2]. In the new theoretical explanation (as shown Figure 2), a high surface tension is generated between the aqueous mobile phase and the surface of the hydrophobic stationary phase. Therefore, the mobile phase is easily expelled from the porous space of hydrophobic stationary phase. In this case, the mobile phase no longer remains in the porous space of the stationary phase particles, so the chance of the stationary phase and the analyte coming into contact with each other is also reduced, which in turn causes the reduced retention time of the analyte. The experiment confirmed that when the mobile phase in the C18 column was suddenly switched from a high proportion of organic solvent to 100% aqueous solvent, the volume of the mobile phase in the column was significantly reduced, and the magnitude of the decrease in such volume was closely related to the loss of retention time for the analyte [3, 4].

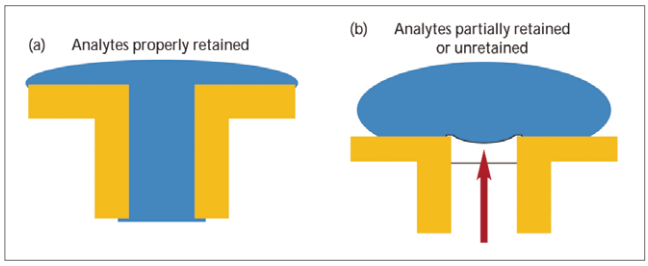

Figure 2. Illustration of a possible mechanism of pore dewetting for reversed phase chromatography (reproduced from Ref. 1). The analytes are properly retained when the alkyl chains on the stationary phase are properly solvated with pressure using a 100% aqueous mobile phase (a). When the flow has been stopped to allow expulsion of water from the pores, with flow resumed the pores are still dewetted and analytes cannot enter pores and have little or no retention (b).

According to the Young-Laplace equation, the surface of the dried hydrophobic stationary phase requires very high pressure to drive the aqueous phase solvent into the pores contained on the surface. This equation relates the intrusion pressure to the surface tension of the water and to the contact angle of the water and air in the sorbent surface:

$$\Delta P = \frac{4\gamma\cos\theta}{d}$$

where ΔP is the intrusion pressure required to drive liquid into the pores, γ is the surface tension, d is the effective pore diameter, and θ is the contact angle made between water and air on the adsorbent surface. Since the contact angle made between 100% aqueous mobile phase and hydrophobic alkyl-bonded silica-gel pore is greater than 90°, therefore, θ from the above mentioned equation is smaller than 90° and ΔP should be a positive number, which means positive pressure is required to drive the aqueous mobile phase into the silica-gel pore. In regular liquid chromatography separation, the pressure driving aqueous mobile phase into the silica-gel pore is larger than the pressure required to

drive the mobile phase flowing through the whole chromatography column. So, when the separation is suddenly stopped, the pressure inside the silica-gel pore is greater than the pressure outside the silica-gel pore, as a result the aqueous mobile phase will be squeezed out from hydrophobic alkyl-bonded silica-gel pore. When this column is reused without special treatment, due to the dewetting status of the adsorbent pore, the interaction between the adsorbent and the analyte will be greatly reduced, resulting significant loss in retention time for the analyte. However, when the stationary phase surface becomes more hydrophilic, the contact angle made between 100% aqueous mobile phase and hydrophobic alkyl-bonded silica-gel pore will be reduced. When this contact angle is smaller than 90°, which means θ is greater than 90°, in this case ΔP should be a negative number. Under this condition, the pressure outside the silica-gel pore is greater than the pressure inside the silica-gel pore and the mobile phase will spontaneously enter the silica-gel pore. This is what we call as the adsorbent pore is wetted.chromatography separation, the pressure driving aqueous mobile phase into the silica-gel pore is larger than the pressure required to drive the mobile phase flowing through the whole chromatography column. So, when the separation is suddenly stopped, the pressure inside the silica-gel pore is greater than the pressure outside the silica-gel pore, as a result the aqueous mobile phase will be squeezed out from hydrophobic alkyl-bonded silica-gel pore. When this column is reused without special treatment, due to the dewetting status of the adsorbent pore, the interaction between the adsorbent and the analyte will be greatly reduced, resulting significant loss in retention time for the analyte. However, when the stationary phase surface becomes more hydrophilic, the contact angle made between 100% aqueous mobile phase and hydrophobic alkyl-bonded silica-gel pore will be reduced. When this contact angle is smaller than 90°, which means θ is greater than 90°, in this case ΔP should be a negative number. Under this condition, the pressure outside the silica-gel pore is greater than the pressure inside the silica-gel pore and the mobile phase will spontaneously enter the silica-gel pore. This is what we call as the adsorbent pore is wetted.

The above theoretical analysis shows that for the conventional C18 column, some hydrophilic modification can be performed on the silica gel surface to improve the phase collapse in highly aqueous mobile phase [5], including

- non-endcapped, short-chain alkyl phases;
- hydrophilic, polar-endcapped, and polar-enhanced stationary phases;
- polar-embedded alkyl phases;
- long-chain alkyl phases; and
- wide-pore-diameter phases.

SepaFlash C18AQ cartridges presented by Santai Technologies are prepacked with hydrophilic C18-bonded silica gel, in which hydrophilic cyano groups are introduced on the silica gel surface (as shown in Figure 3). Thus, the alkyl chains on the silica surface could be fully extended under highly aqueous conditions and the phase collapse phenomenon could be avoided. These modified C18 columns are called aqueous C18 columns, namely C18AQ columns, which are designed for highly aqueous elution conditions and can tolerate 100% aqueous system. C18AQ columns have been widely applied in the separation and purification of highly polar compounds, including organic acids, peptides, nucleosides and water-soluble vitamins.

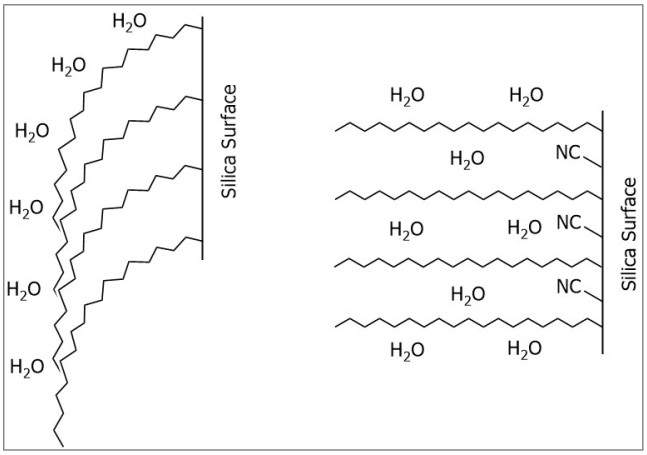

Figure 3. The schematic diagram of the bonded phases on the surface of silica gel in regular C18 column (left) and C18AQ column (right).

Desalting is one of the typical applications of C18AQ columns in the Flash purification for samples, which removes the salt or buffer components in the sample solvent to facilitate the application of the sample in subsequent studies. In this post, a highly polar compound was used as the sample and purified on a SepaFlash C18AQ cartridge. The salty components contained in the raw sample was removed and the sample solvent was replaced by organic solvent from aqueous solvent, thus facilitating the following rotary evaporation as well as saving solvents and operating time.

Experimental Section

1.Sample information

The sample was the final product of a synthesis reaction which was shown in Figure 4.

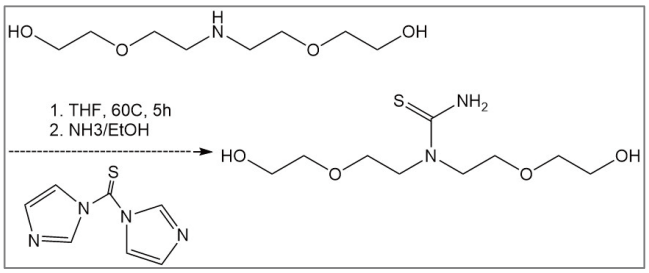

Figure 4. The synthesis reaction formula of the sample in this post.

As shown in Figure 4, in addition to the target product in the raw sample, there is also excess ammonium salt (tetrabutylammonium fluoride, MW 261) used in the synthesis reaction. The sample should be desalted in order to obtain the target product meeting the purity requirement. In chromatographic separation technology, gel filtration based on the principle of molecular sieving is generally used to desalt the macromolecular sample, including proteins, peptides, nuclei acids, etc. However, for the target product with MW of 252 in this post, it is a nearly impossible task for gel filtration to distinguish the target molecule from salt impurities with very similar MW. Other separation mode must be considered.

2.Sample purification by Flash chromatography

The sample was purified by a Flash chromatography system SepaBean machine 2 according to the parameters as shown in Table 1.

Instrument	SepaBean machine 2	
Flash cartridge	12 g SepaFlash C18AQ cartridge (spherical silica, 20 – 45 μm, 100 Å, Order number: SW-5222-012-SP(AQ))	
Wavelength	254 nm; 220 nm	
Mobile phase	Solvent A: water Solvent B: methanol	
Flow rate	15 mL/min	
Sample load	0.5 mL (100 mg)	
Gradient	Time (CV)	Solvent B (%)
	0	0
	20	0
	21	100
	30	100

Table 1. The experimental setup for Flash purification.

Results and Discussion

The sample used in this post was highly polar and soluble in water. Due to its high polarity and strong retention on normal phase silica cartridge, thus normal phase separation mode was excluded. In reversed phase separation mode, if a conventional C18 RP Flash cartridge was used, the sample will be eluted from the stationary phase quickly since the organic phase ratio was more than 10% in the initial mobile phase. Furthermore, there are still certain amount of salt impurities in the collected fractions, leading to a bad desalting result. Therefore, aqueous C18AQ cartridge was utilized to purify the sample.

A step gradient was set for the Flash chromatography. To ensure that the salt impurities with a MW very close to the target product are fully removed, pure water was used as the mobile phase to flush the cartridge for about 20 column volumes (CV). As shown in Figure 5, the sample was fully retained on the C18AQ cartridge when pure water was used as the mobile phase. Next, methanol in the mobile phase was directly increased to 100% and the gradient was maintained for 10 CVs. The target product was eluted out from 22.5 to 24 CVs. In the collected fractions, the sample solution was replaced from water to methanol. Comparing with highly aqueous solution, methanol was much easier to be removed by rotary evaporation in the subsequent step, which facilitates the following research.

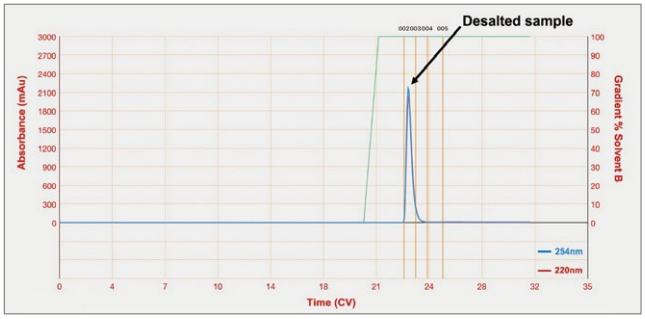

Figure 5. The Flash chromatogram of the sample on a C18AQ cartridge.

Comparing with linear gradient, the use of step gradient has the following advantages:

- Solvent usage and run time for sample purification is reduced.
- The target product elutes in a sharp peak, which reduces the volume of collected fractions and thus facilitates the following rotary evaporation as well as saving time.
- The collected product is in methanol which is easy to be evaporated, thus drying time is reduced.

In conclusion, for the purification of the sample which is strongly polar or highly hydrophilic, SepaFlash C18AQ cartridges combining with the Flash chromatography system SepaBean machine is undoubtedly a fast and efficient solution.

About the SepaFlash C18AQ RP Flash cartridges

There are a series of the SepaFlash C18AQ RP Flash cartridges with different specifications from Santai Technology (as shown in Table 2).

Item Number	Column Size	Sample Size	Flow Rate (mL/min)	Max Pressure (psi/bar)
SW-5222-012-SP(AQ)	20 g	20 mg–0.40 g	10-25	400/27.5
SW-5222-025-SP(AQ)	33 g	33 mg–0.66 g	10-25	400/27.5
SW-5222-040-SP(AQ)	48 g	48 mg–0.96 g	15-30	400/27.5
SW-5222-080-SP(AQ)	105 g	105 mg–2.1 g	25-50	350/24.0
SW-5222-120-SP(AQ)	155 g	155 mg–3.1 g	30-60	300/20.7
SW-5222-220-SP(AQ)	300 g	300 mg–6.0 g	40-80	300/20.7
SW-5222-330-SP(AQ)	420 g	420 mg–8.4 g	40-80	250/17.2

Table 2. SepaFlash C18AQ RP Flash cartridges. Packing materials: High-efficiency spherical C18(AQ)-bonded silica, 20 - 45 μm, 100 Å.

For further information on detailed specifications of SepaBean machine, or the ordering information on SepaFlash series flash cartridges, please visit our website: http://www.santaitech.com/index/.

References

1. Przybyciel M, Majors RE (2002). Phase collapse in reversed-phase liquid chromatography. LCGC North Am. 20 (6): 516–523.

2. Majors RE (2013). The top 10 HPLC and UHPLC column myths. LCGC North Am. 31 (7): 522-537.

3. Bidlingmeyer BA, Broske AD (2004). The role of pore size and stationary phase composition in preventing aqueous-induced retention time loss in reversed-phase HPLC. J Chromatogr Sci. 42: 100-106.

4. Nagae N, Enami T and Doshi S (2002). The retention behavior of reversed-phase HPLC columns with 100% aqueous mobile phase. LCGC North Am. 20 (10): 964-972.

5. Majors RE, Przybyciel M (2002). Columns for reversed-phase LC separations in highly aqueous mobile phases. LCGC North Am. 20 (7): 584-593.

Santai Technologies Inc.
Website: www.santaitech.com

a santai science company
SANTAI TECHNOLOGIES quality since 2004

CHINA
Tel.: +86 (519) 8515 0175
Fax: +86 (519) 8515 3561
Email: info@santaitech.com
Address: No.78 Qingyang Road, Xinbei District, Changzhou, Jiangsu Province, China

CANADA
Tel: +1 418-580-0437
Order mail: ca_order@santaitech.com
Support mail: ca_support@santaitech.com
Office: Thetford Mines. 375, Labbe Street, Thetford Mines, Québec, Canada

INDIA
Tel: +91 937-181-2696
Order mail: in_order@santaitech.com
Support mail: in_support@santaitech.com
Office: T5-204, Highlands Forest Trails Township Bhugaon, Pune-412115 Maharashtra, India