Hydrophobic Phase Collapse, AQ Reversed Phase Chromatography Columns and Their Applications

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Introduction

According to the relative polarities of stationary phase and mobile phase, liquid chromatography can be divided into normal phase chromatography (NPC) and reversed phase chromatography (RPC). For RPC, the polarity of the mobile phase is stronger than that of the stationary phase. RPC has become the most widely used one in liquid chromatography separation modes due to its high efficiency, good resolution and clear retention mechanism. Therefore RPC is suitable for the separation and purification of various polar or non-polar compounds, including alkaloids, carbohydrates, fatty acids, steroids, nucleic acids, amino acids, peptides, proteins, etc. In RPC, the most commonly used stationary phase is the silica gel matrix which is bonded with various functional groups, including C18, C8, C4, phenyl, cyano, amino, etc. Among these bonded functional groups, the most widely used one is C18. It is estimated that more than 80%

of RPC are now using C18 bonded phase. Therefore C18 chromatography column has become a must-have universal column for every laboratory.

Although C18 column can be used in a very wide range of applications, however, for some samples which is very polar or highly hydrophilic, regular C18 columns may have problems when being used to purify such samples. In RPC, the commonly used elution solvents can be ordered according to their polarity: water < methanol < acetonitrile < ethanol < tetrahydrofuran < isopropanol. To assure good retention on the column for these samples (strong polar or highly hydrophilic), high proportion of aqueous system is necessary to be used as the mobile phase. However, when using pure water system (including pure water or pure salt solution) as the mobile phase, the long carbon chain on the stationary phase of C18 column tends to avoid the water and mix with each other, resulting in an instantaneous decrease in the retention capacity of the column or even no retention. This phenomenon is called “hydrophobic phase collapse” (as shown in the left part of Figure 1). Though this situation is reversible when the column is washed with organic solvents such as methanol or acetonitrile, it still can cause damage to the column. Therefore, it is necessary to prevent this situation from happening.

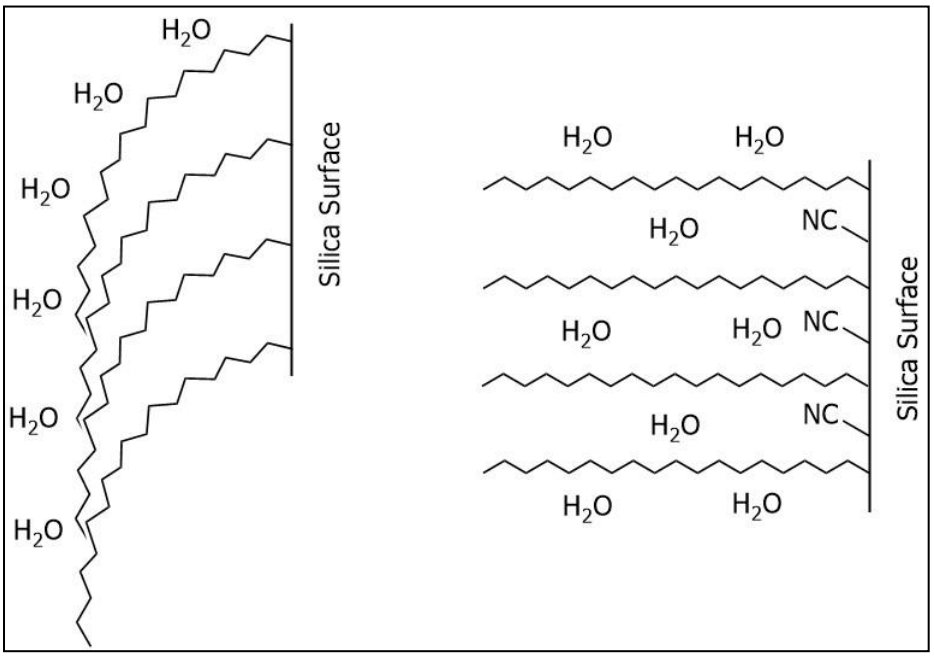

Figure 1. The schematic diagram of the bonded phases on the surface of silica gel in regular C18 column (left) and C18AQ column (right).

To address the above mentioned problems, the chromatographic packing materials manufacturers have made technical improvements. One of these improvements is making some modifications on the surface of the silica matrix, such as the introduction of

hydrophilic cyano groups (as shown in the right part of Figure 1), to make the surface of the silica gel more hydrophilic. Thus the C18 chains on the silica surface could be fully extended under highly aqueous conditions and the hydrophobic phase collapse 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 strong polar compounds, including organic acids, peptides, nucleosides and water-soluble vitamins.

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, the Brilliant Blue FCF with strong polarity was used as the sample and purified on the C18AQ column. The sample solvent was replaced by organic solvent from buffer solution, thus facilitating the following rotary evaporation as well as saving solvents and operating time. Furthermore, the purity of the sample was improved by removing some impurities in the sample.

Experimental Section

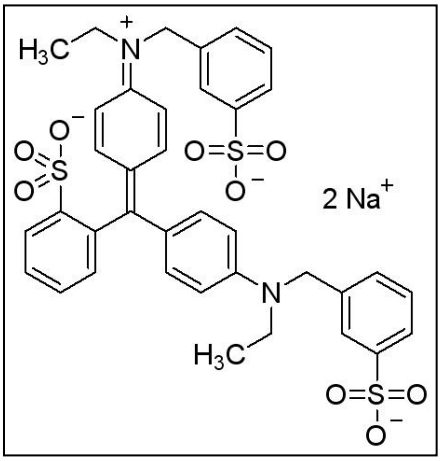

Figure 2. The chemical structure of the sample.

The Brilliant Blue FCF was used as the sample in this post. The purity of the raw sample was 86% and the chemical structure of the sample was shown in Figure 2. To prepare the sample solution, 300 mg powdery crude solid of Brilliant Blue FCF was dissolved in 1 M NaH_2PO_4 buffer solution and shook well to become a completely clear solution. The sample solution was then injected into the flash column by an injector. The experimental setup of the flash purification is listed in the Table 1.

Instrument	SepaBean™ machine 2			
Cartridges	12 g SepaFlash® C18 RP flash cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number: SW-5222-012-SP)		12 g SepaFlash® C18AQ RP flash cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number：SW-5222-012-SP(AQ))	
Wavelength	254 nm			
Mobile phase	Solvent A：Water Solvent B：Methanol			
Flow rate	30 mL/min			
Sample loading	300 mg (Brilliant Blue FCF with the purity of 86%)			
Gradient	Time (CV)	Solvent B (%)	Time (CV)	Solvent B (%)
	0	10	0	0
	10	10	10	0
	10.1	100	10.1	100
	17.5	100	17.5	100
	17.6	10	17.6	0
	22.6	10	22.6	0

Results and Discussion

A SepaFlash® C18AQ RP flash cartridge was used for sample desalting and purification. Step gradient was utilized in which pure water was used as the mobile phase at the start of elution and run for 10 column volumes (CV). As shown in Figure 3, when using pure water as the mobile phase, the sample was completely retained on the flash cartridge. Next, the methanol in the mobile phase was directly increased to 100% and the gradient was maintained for 7.5 CV. The sample was eluted out from 11.5 to 13.5 CV. In the collected fractions, the sample solution was replaced from NaH_2PO_4 buffer solution 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.

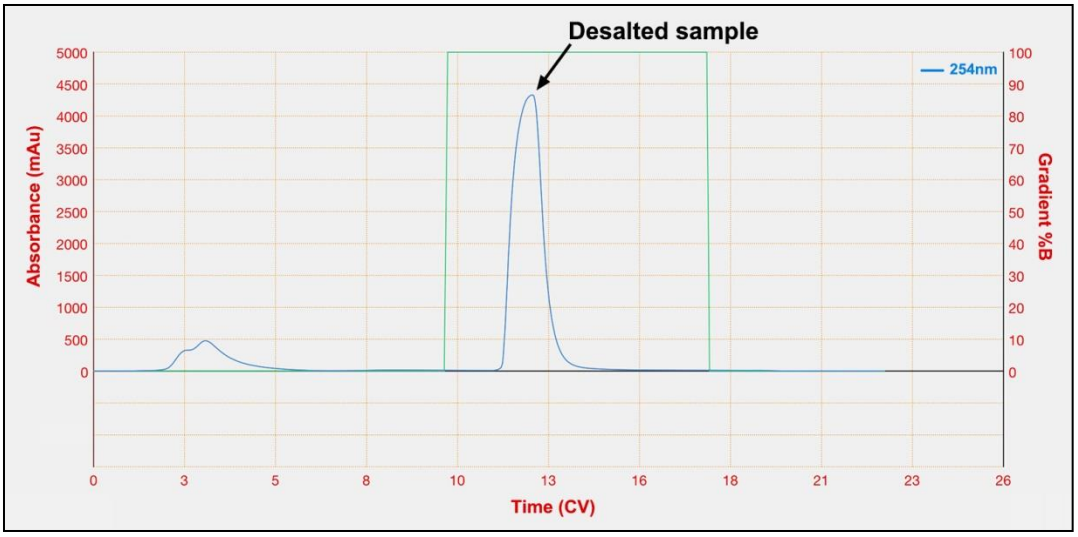

Figure 3. The flash chromatogram of the sample on a C18AQ cartridge.

To compare the retention behavior of C18AQ cartridge and regular C18 cartridge for samples of strong polarity, parallel comparison test was performed. A SepaFlash® C18 RP flash cartridge was used and the flash chromatogram for the sample was shown in Figure 4. For regular C18 cartridges, the highest tolerated aqueous phase ratio is about 90%. Therefore the start gradient was set at 10% methanol in 90% water. As shown in Figure 4, due to the hydrophobic phase collapse of the C18 chains caused by high aqueous ratio, the sample was barely retained on the regular C18 cartridge and was directly eluted out by the mobile phase. As a result, the operation of sample desalting or purification cannot be completed.

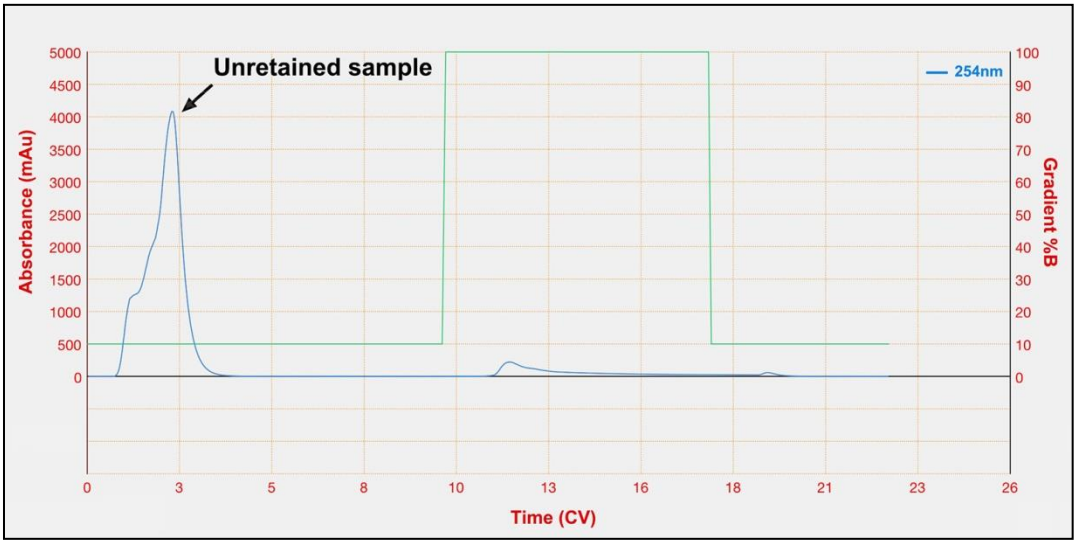

Figure 4. The flash chromatogram of the sample on a regular C18 cartridge.

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 RP flash cartridges combining with the preparative flash chromatography system SepaBean™ machine could offer a fast and efficient solution.

About the SepaFlash® Bonded Series C18 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	Flow Rate (mL/min)	Max.Pressure (psi/bar)
SW-5222-004-SP(AQ)	5.4 g	5-15	400/27.5
SW-5222-012-SP(AQ)	20 g	10-25	400/27.5
SW-5222-025-SP(AQ)	33 g	10-25	400/27.5
SW-5222-040-SP(AQ)	48 g	15-30	400/27.5
SW-5222-080-SP(AQ)	105 g	25-50	350/24.0
SW-5222-120-SP(AQ)	155 g	30-60	300/20.7
SW-5222-220-SP(AQ)	300 g	40-80	300/20.7
SW-5222-330-SP(AQ)	420 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:

www.velocityscientific.com.au