PALL Laboratory

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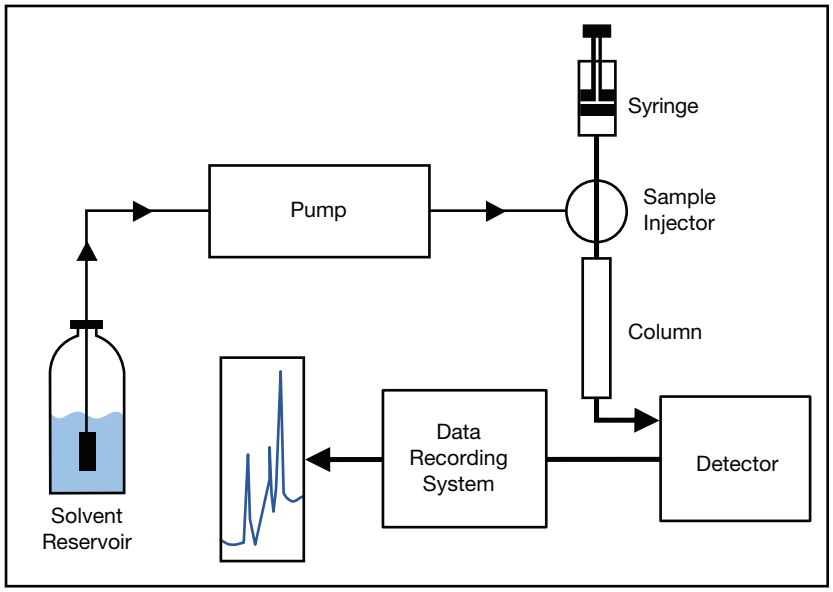
Application Note

Improve HPLC Performance with Filtration and the Increased Benefit of Using Pre-Filtration Technologies

Introduction

In many laboratories the need to consistently generate high-quality data means that laboratory managers and technicians need to ensure their instruments are performing optimally around the clock. Filtering samples before injection into a chromatography instrument is one of the primary ways that an technicians can protect their column and instrument from unnecessary wear and excess downtime. Filtration of both the sample and mobile phase prior to analysis helps increase the lifespan of chromatography columns, reducing overall instrument wear and removing any particles that may interfere with the chromatogram. Sample filtration is most often performed using syringe filters as it is time effective and an easy to implement method.

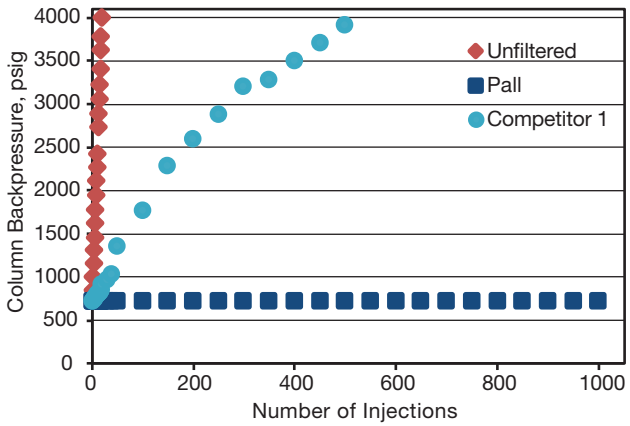
Figure 1
A Basic HPLC Configuration



Of the four common causes for HPLC column failure – plugging, voids, absorbed sample and chemical attack – plugging is the most frequently encountered by analytical chemists or analysts. Injection of samples containing particulates will eventually block the column inlet and column packing, cause high column back-pressure and shorten the normal lifetime of the column.

Figure 2

Effects of Filters on HPLC Column Life



In the Analytical Technical Guide, we have shown that particulate removal through filtration can extend column life up to 52 times (Figure 2) compared to unfiltered samples. In addition to extending the life of the column, particulate removal also protects the pump, injector, and other components from premature wear. Accurate, reproducible data depends on proper HPLC column performance. Column blockages can be prevented by filtering the mobile phase through a 0.2 µm or 0.45 µm disc filter, filtering the samples through a 0.2 µm or 0.45 µm Pall Acrodisc One™ syringe filter, and utilizing inline filtration within the instrument. Without filtration particles present in the sample can cause higher system pressures, shifted retention times, poor peak shape and separation.

Difficult to Filter Samples

For filtration to help improve column life and data quality, filters must remove extremely small particles, as small as 0.2 µm. In the course of their daily work, laboratories perform their analyses using a wide variety of starting materials, extraction methods and preparation methods. These differences in materials and methods can result in a wide variety of samples, including some that can be filtered easily, and others that can have a very high number of particles or a wide distribution of particle sizes. These samples can be challenging to filter, often clogging the filter prematurely or increasing the pressure required to pass the sample through the filter.

To keep progressing through their workflows, analysts adopt several different strategies to help remove particulates including dilution and centrifugation. While both methodologies will increase the sample throughput of filter, both are not without their own challenges and drawbacks.

If the analyst chooses to dilute their sample, they risk introducing errors. The glassware used by the analyst and the analyst's technique must be accurate so the true concentration of the starting material can be calculated. If the volumes used are inaccurate the dilution factors will be incorrect, resulting in a calculation that quantifies the starting concentration incorrectly.

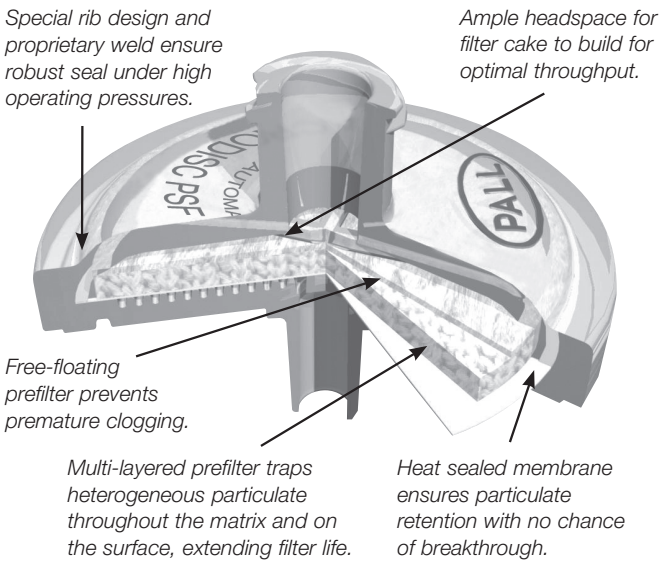
Another option that the analyst has is to centrifuge the sample. During the centrifugation process, the larger particles are forced to the bottom of the sample container, resulting in a cleaner supernatant. The main issue with centrifugation, not considering the time it takes, is it will not necessarily result in a particle free supernatant. Fine particles that can be present in a sample require longer times at higher speeds to be pelletized and are often still suspended after centrifugation. While centrifugation can help clear a sample of larger particles, there can still be a significant amount of particulate matter suspended in the sample which can clog or slow filtration.

There is a third option the analyst can use to help process their samples and that is using a filter that has an integrated prefilter. By using a filter with a prefilter, the analyst can increase their filter throughput and processing speed without having to resort to additional steps to move through their workflow. The Pall Premium Syringe Filter (PSF) family utilizes a fibrous depth filter over the final membrane filter. The fibrous depth filter media's high particulate holding capacity means that many of the contaminating particles are trapped and removed before challenging the final membrane filter. With less particles having to be removed by the final membrane filter, the filter can process more volume at higher flow rates including difficult to filter samples.

In order to maximize throughput, Pall's Premium Syring Filters utlilze a GxF glass fiber prefilter. GxF is an asymmetric glass fiber filter that is 40 µm on the upstream side, and tapers to 1 µm on the downstream side. This increases throughput over a symmetrical prefilter by trapping larger particles sooner and allowing the smaller particles to be captured further in the structure of the filter, increasing total filter capaciy.

Figure 3

The Acrodisc® PSF GxF syringe filter has a serial glass fiber (GxF) prefilter to allow for maximum throughput and faster flow rates than standard glass fiber prefilter devices. The multi-layered prefilter, rated from > 40 to 1 µm, traps particulates, thereby extending filter life.



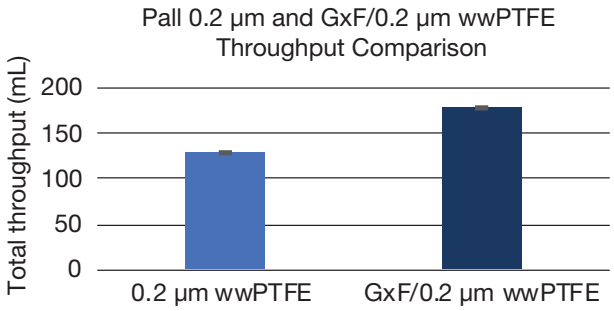
Importantance of Prefiltration

To see how much prefiltration improves throughput and filter capacity, a solution was made to challenge the filtration capacity of the Pall Acrodisc One with wwPTFE membrane and the Pall Acrodisc One with a GxF prefilter and wwPTFE membrane. The test solution was prepared by adding 80 mL of 0.02% latex bead solution to 5 L of water. Five filters were tested by allowing the test solution to flow through the filter for four minutes at a pressure of 30 psi. At the end of four minutes the volume of filtrate was measured in a graduated cylinder.

Figure 4

Throughput comparison of latex bead solution collected after 4 minutes.

Replicate	0.2 µm wwPTFE	GxF/0.2 µm wwPTFE
1	130 mL	170 mL
2	135 mL	182 mL
3	142 mL	175 mL
4	144 mL	186 mL
5	138 mL	178 mL
Mean	138 mL	178 mL
SD	1.4	1.6



By comparing the amount of sample filtered in four minutes through each filter type, it was shown that the filter with GxF prefilter had a higher filtration capacity and faster flow rates. The GxF prefilter increased throughput by 29% compared to the filter with only the final membrane.

Conclusion

Sample conditions can vary drastically based upon the starting material and steps performed to prepare them for analysis. Some of these samples can have high particle counts, be difficult to filter and reduce HPLC instrument performance and results. There are several methods an analyst can use to process difficult to filter samples. By using a syringe filter with an integrated prefilter, throughput can be increased by 29% without the added time and risk of using dilution or centrifugation techniques.

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