

# Mobile Phase Preparation for UHPLC: Impact of Filtration Through 0.2- $\mu\text{m}$ Membranes on System Performance

UHPLC/UPLC<sup>®</sup> (Waters Corp., Milford, MA) is a revolutionary chromatography technique that is gaining wide acceptance among researchers due to improved resolution, shorter chromatographic runs, and the capability for fast method development. The presence of sub-2- $\mu\text{m}$  particles in UHPLC (ultra-high-performance liquid chromatography) columns provides these benefits but also poses challenges in sample and mobile phase preparation. Particulate impurities in the sample or mobile phase can cause backpressure buildup in the UHPLC system, causing system failure. In fact, most UHPLC instrument vendors recommend filtration of mobile phase using 0.2- $\mu\text{m}$  filters, but there is a lack of data showing the benefits of filtration. In this article, the authors show the benefits of filtering mobile phases through 0.2- $\mu\text{m}$  membrane filters and the effects on UHPLC system performance. Mobile phases prepared with three commonly used solvents (water, acetonitrile, and methanol) were filtered through four types of membrane filters, and the effect of the various filters on system backpressure was compared. The results clearly indicate that filtration of mobile phase components using the optimal membrane filter will help protect UHPLC systems from particulate impurities that may clog and shut down the system.

Sample throughput and separation efficiency in chromatography are improved by decreasing the size of the particles packed in the column, as predicted by the van Deemter equation<sup>1</sup>:

$$H = A + \frac{B}{\mu} + C \cdot \mu$$

In this equation,  $H$  is the plate height and  $\mu$  represents the velocity of the mobile phase.  $H$  is therefore inversely proportional to separation efficiency. To increase sample throughput, velocity ( $\mu$ ) should increase while minimizing  $H$ .

The constants  $A$  and  $B$  are diffusion terms, and  $C$  reflects the movement of the analyte between the mobile and stationary phases.  $A$ ,  $B$ , and  $C$  all contribute to wider analyte peaks in the HPLC profile, limiting resolution. Decreasing the particle size of the stationary phase makes both  $A$  and  $C$  smaller, increasing resolution and decreasing  $H$ . As a result,  $H$  is less sensitive to increases in velocity, allowing for high sample throughput without compromising peak resolution.<sup>2</sup> In fact, resolution improvements of up to 50% (compared to standard HPLC separations) are routine.<sup>3</sup>

UHPLC columns packed with sub-2- $\mu\text{m}$  particles create high system backpressure that far exceeds the limits of traditional HPLC systems. To reap the benefits of this new column technology, manufacturers have developed instruments capable of running at pressures much higher (up to 15,000 psi) than standard HPLC systems.<sup>3</sup>

The advantages that a UHPLC system can bring to an analytical laboratory include a general increase in productivity, reduced method development time, more data from small samples, and a 3–10 fold decrease in solvent usage and disposal costs. Most methods developed on traditional HPLC sys-

tems can be transferred to a UHPLC system without much difficulty. However, UHPLC, with its smaller particle sizes, lower interstitial void volumes, decreased column diameters, and higher flow rates, presents scientists with new challenges.<sup>4</sup> Columns filled with very small particles are more susceptible to premature plugging by particulates. UHPLC column life is generally shorter than that of traditional HPLC columns.

Clean mobile phase components (buffers and solvents) are the key to addressing some of the challenges associated with UHPLC technology. To minimize system failure and maximize system performance, instrument manufacturers recommend using ultrapure water and filtering mobile phase components through 0.2- $\mu\text{m}$  membrane filters.<sup>5</sup> Poor water quality and unfiltered buffer salts result in particulates in the mobile phase. Particles can cause increased backpressure, column clogging, and eventual system shutdown.

Simple filtration provides a fast and economical means of preparing samples and mobile phases for optimal UHPLC results. A precut disk filter that costs about \$1.50 is a far better place to collect damaging particulates than a high-priced column (~\$400) critical to the operation of sensitive and expensive UHPLC equipment.

## Membrane microfiltration

Microfiltration is the process of removing particles or biological entities in the 0.025- $\mu\text{m}$  to 10.0- $\mu\text{m}$  range from fluids by passage through a microporous medium such as a membrane filter. Because membrane filters, unlike depth filters such as glass fiber filters, have precisely defined pore sizes, contaminants can be quantitatively retained.<sup>6</sup> Membrane disk filters are therefore routinely used to remove particulate contamination from solvents prior to chromatographic analysis, and are well suited to preparing mobile phases for UHPLC.

Although, theoretically, microporous membranes should retain all particles greater than the reported pore size, the true retention properties depend upon both the physicochemical characteristics of the membrane, as well as pore size uniformity. Most membrane pores have unequal sizes, which are statistically distributed. This, together, with variations in membrane constriction, tortuosity, and electrostatic interaction with particles, results in retention cutoffs that are not absolute.<sup>7</sup>

In this study, the authors wanted to determine the effects of variations in retention by membrane filters on UHPLC performance. They determined the percent retention of various microporous membranes, and then used the same membranes to filter a typi-

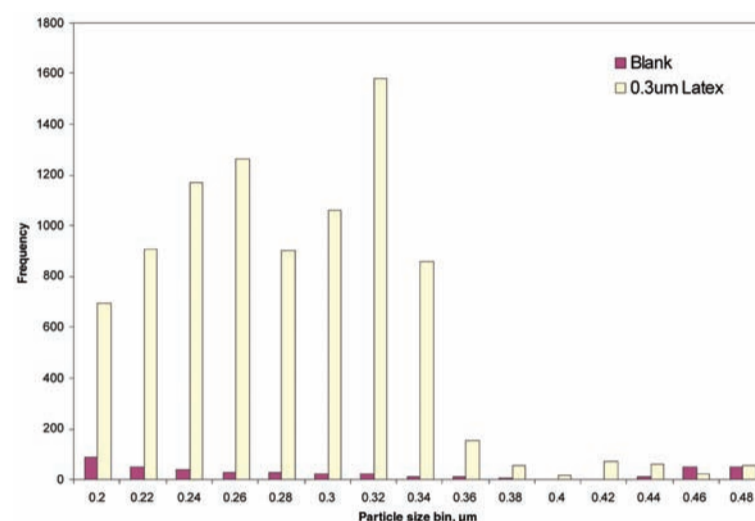


Figure 1 Particle size distribution of 0.3- $\mu\text{m}$  latex suspension, measured by laser light scattering.

cal UHPLC mobile phase (1:1 water:acetonitrile). They measured the increase in UHPLC system backpressure upon running this filtered mobile phase through a UHPLC system.

## Materials and methods

Ten percent polystyrene latex suspension (cat. #LB3) and Triton-X 100 (Rohm and Haas, Philadelphia, PA) were obtained from Sigma-Aldrich (St. Louis, MO). Acetonitrile for chromatography was obtained from Merck (Darmstadt, Germany).

## Determination of nanoparticle retention efficiency

A 0.005% suspension (v/v) of 0.3- $\mu\text{m}$  latex particles was prepared by diluting a 10% polystyrene suspension with a 0.1% solution of Triton-X 100 in water. Particle size distribution was determined by laser light diffraction using a LiQuilaz particle counting spectrometer LS 200 (Particle Measuring Systems, Boulder, CO). As can be seen in Figure 1, the latex suspension includes particles of diameters ranging from 0.2 to 0.34  $\mu\text{m}$ .

The UV absorbance at 272 nm of a dilution series of latex particles was measured to create a standard curve relating UV absorbance to particle concentration. Then, the latex suspension was filtered through various syringe filters and the UV absorbance of the filtrate was measured. This value was then compared to the absorbance of the unfiltered suspension to determine the percent retention of each membrane type. Each measurement was repeated three times, and the mean, standard deviation, and coefficient of variation were determined.

## UHPLC experiments

Milli-Q<sup>™</sup> water (Millipore Corp., Billerica, MA) and acetonitrile were filtered through polypropylene,

**Table 1** Percent retention efficiency for filtration of 0.005% latex suspension (0.3  $\mu\text{m}$ ) through 0.2- $\mu\text{m}$  membrane filters

	0.2- $\mu\text{m}$ nylon	0.2- $\mu\text{m}$ PTFE	0.2- $\mu\text{m}$ polypropylene	0.2- $\mu\text{m}$ PVDF
Average retention efficiency	95.06	97.57	78.64	97.91
Std. deviation	4.53	1.70	1.88	2.63
% CV	4.76	1.74	2.39	2.38

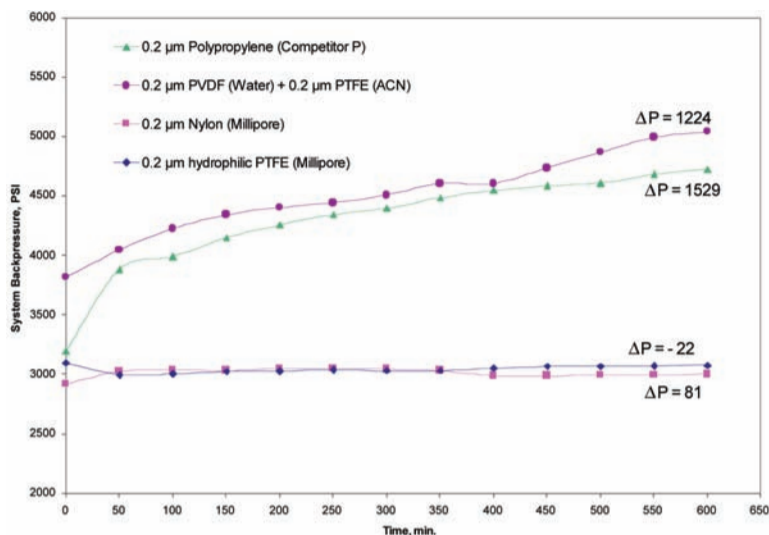


Figure 2 Change in system backpressure depends on filtration membrane used to prepare UHPLC mobile phase. Water and acetonitrile were passed through polypropylene, PVDF, PTFE, or nylon syringe filters (membranes indicated in legend), then used 1:1 (v/v) to prepare the mobile phase for UHPLC. The system was run at 0.25 mL/min for 600 min with backpressure recorded every 50 min.  $\Delta\text{P}$  represents total change in backpressure after 600 min.

nylon, polyvinylidene fluoride (PVDF), or polytetrafluoroethylene (PTFE) membrane filters. Acetonitrile could not be filtered through PVDF due to chemical incompatibility. Filtered water and acetonitrile were then mixed 1:1 (v/v) to create a mobile phase for UHPLC. Chromatography was performed using an ACQUITY UPLC® (Waters Corp.) system with an ACQUITY UPLC BEH- C18 column (2.1 mm  $\times$  100 mm, 1.7  $\mu\text{m}$ ). The system was run for 600 min at a flow rate of 0.25 mL/min, and backpressure was monitored continuously using the system.

## Results and discussion

### Retention of particles by microporous membranes

Of the four different 0.2- $\mu\text{m}$  membranes tested for retention of latex particles in suspension, nylon, PVDF, and PTFE membranes all retained more than 95% of particles in the suspension. Polypropylene membrane, however, retained only 79% of the particles (Table 1). Although the particle size distribution (Figure 1) shows that many particles in the suspension are between 0.2 and 0.3  $\mu\text{m}$  in diameter, variations in polypropylene membrane pore size, as well as variations in the particle size, may cause latex particles to pass through polypropylene membrane.

### Effect of membrane filtration of mobile phase on UHPLC system backpressure

Figure 2 presents data showing the benefits of filtration of mobile phase through 0.2- $\mu\text{m}$  membrane filters on the performance of a UHPLC system. Of the various membrane filters evaluated, hydrophilic PTFE provided the best filtration performance, as indicated by the lowest

backpressure increase in a UHPLC system—in fact, backpressure actually decreased after 600 min of run time. The hydrophilic polypropylene (PP) filter was unable to retain particulate impurities present in the solvents, as indicated by highest backpressure gain of all the filters studied. This behavior was consistent with its poor percent retention of latex particles as measured in Table 1. Nylon and hydrophilic PVDF filters showed an intermediate performance in terms of backpressure increase.

## Summary

Using membrane filtration techniques to purify mobile phases is an easy way to achieve the highest levels of UHPLC system performance. However, given the wide range of physicochemical properties of filters and UHPLC solvents, one filter type is unlikely to be optimal for all applications. Carefully choosing the correct filters will maximize retention of damaging particulates, enabling any laboratory to benefit from increased throughput and resolution provided by UHPLC.

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