Choosing the Right Filters for Biological Sample Filtration

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Introduction

Filter filtration is a common method for preparing and sterilizing biological samples to remove impurities and micro-organisms. However, using filters could cause loss of significant amounts of biological materials due to unwanted protein binding with the membrane or introduce unexpected interferences to the samples from the filter. Therefore, low protein binding and cleanliness are important features of a filters' performance. Polyethersulfone (PES) and polyvinylidene fluoride (PVDF) membranes are typically used for biological sample filtration and are claimed to provide very low protein binding. In this study, PES and PVDF membranes were evaluated and compared. A group of common proteins was used for the protein binding evaluations, including BSA, myoglobin, ovalbumin, cytochrome C, and thyroglobulin. After protein samples were filtered through syringe filters, the protein samples were evaluated using HPLC/UV by comparing samples with or without filtration for their monomer, dimer, or aggregates peaks. In addition, filter cleanliness was evaluated by filtering membrane-compatible solutions and monitoring the filtrate with LC/MS under positive and negative modes.

Experimental

Protein Binding Test

- Five proteins were selected for the filtration protein binding evaluation

Proteins	BSA	Myoglobin	Ovalbumin	Cytochrome C	Thyroglobulin
MW	66.5 kDa	17.2 kDa	45 kDa	12 kDa	660 kDa
Number of amino acids	583	154	386	105	~ 5000
pI	4.7	7.1	4.5	9.6	4.5

- LC Conditions
  - HPLC Agilent 1200 SL Series
  - Column Agilent Bio SEC-3, 300Å, 7.8 × 100 mm, 3 µm
  - Mobile phase 150 mM Phosphate buffer, pH 7.0
  - Flow rate 1.0 mL/min, isocratic
  - Injection volume 6 µL
  - Total run time 8 min
  - Detector DAD SL, wave length = 214 nm

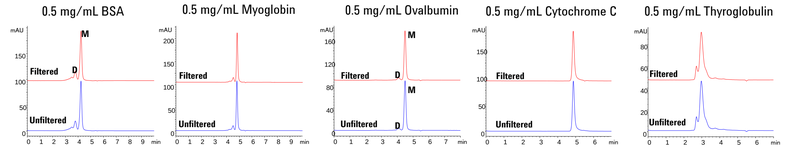
Extractables Test

- A 30:70 MeOH/water (v/v) solution spiked the internal standard was used to evaluate filter extractables.
- LC/MS Conditions
  - UHPLC: Agilent 1290 Infinity LC System
  - Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
  - Mobile phases A: H2O + 0.01% formic acid (FA)
    B: Acetonitrile + 0.01% FA
  - Flow rate: 0.5 mL/min, gradient
  - Gradient: Hold at 30% B for 1 min, then ramped to 90% B in 3 min, and hold at 90% B for 1 min
  - Injection volume: 8 µL
  - Internal standard: 50 µg/mL Naproxin
  - MS: Agilent 6150 Single Quadrupole LC/MS System
  - Source: ESI with Agilent Jet Stream Technology (AJS-ES)
  - Capillary voltage: 4,000 V
  - Nozzle voltage: 2,000 V
  - Drying gas flow: 12 L/min
  - Drying gas temp: 250 °C
  - Nebulizer pressure: 35 psig
  - Sheath gas flow: 3.0 L/min
  - Sheath gas temp: 150 °C
  - Mass range: 100 – 1350 *m/z*
  - Fragmentor: 150 V (pos), 80 V (neg)

Results and Discussion

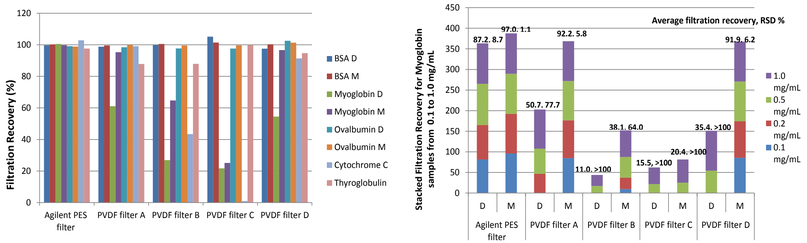
Sample Loss Caused by Protein Binding during Filtration

Chromatograms of Tested Proteins

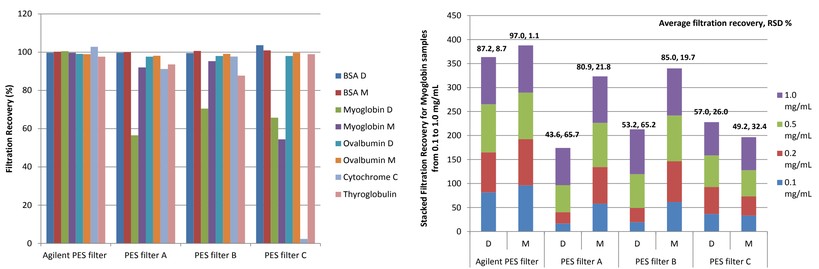


HPLC/UV chromatograms of common proteins and the comparison of unfiltered sample to filtered sample by Agilent PES filter. Protein samples were filtered with Agilent PES 0.2 µm syringe filter, 15 mm. D: Dimmer, M: Monomer.

PES Filter vs Other PVDF Filters



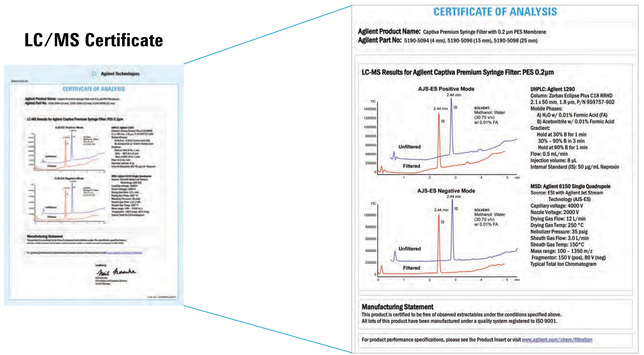
Different PES Filters Comparison



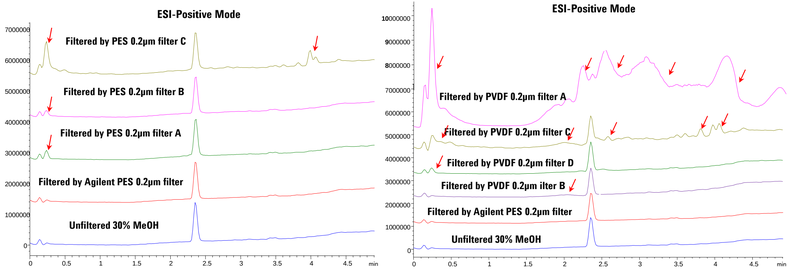
Results and Discussion

Chemical Cleanness

LC/MS Certificate



Filter cleanness comparison for Agilent PES filter vs other PES and PVDF filters using LC/MS under positive mode.



Conclusions

- Agilent PES filter provide excellent and consistent filtration recovery for different variety of proteins and low to high concentrations.
- Agilent PES filters are much cleaner than other PES and PVDF filters without introducing chemical contamination through filtration.

To learn more about Agilent Capitiva syringe filters, visit us online at www.Agilent.com/chem/SamplePrep/Filtration