Fast track mass spec sample prep

Introduction

Mass spectroscopy (MS) is a ubiquitous characterization tool that is vital to biologics development. Unfortunately many MS experiments require a compatible sample solution that differs from the supplied formulation to acquire high quality mass spectra. For example, electrospray ionization (ESI) experiments require a solution of water, organic solvent and organic acid as well as the removal of salts, detergents and buffering molecules. To remove these unwanted buffer components and to hit the right solution conditions, buffer exchange and/or dilution are commonly used. Buffer exchange is a time consuming hands-on process that can really put a cramp on the throughput of MS experiments, but allows for the removal of interfering contaminants to >99%. Conversely, dilution is a much quicker process, but contaminants that interfere with MS experiments will still be present. Automating buffer exchange for sample preparation provides the best of both worlds by increasing your throughput while removing spectrum degrading contaminants.

Automated buffer exchange on Junior achieves high-throughput buffer exchange, freeing up your time, while removing unwanted buffer components to >99%. Junior combines automated liquid handling with Unchained Labs' pressure-based ultrafiltration/diafiltration (UF/DF) buffer exchange technology. Junior's automation removes the need for hands-on buffer exchange, while increasing throughput (Figure 1). Simply setup the experimental parameters in the LEA software suite, load Junior with supplies, click execute, and come back to recover your MS-ready samples. In this technical note we demonstrate how Junior with buffer exchange can be used to prepare up to 96 MS-ready samples in <3 hours.

Figure 1: Junior with buffer exchange.

Methods

A stock of 1.17 µg/µL Adalimumab (Aragen Bioscience) was prepared in 105 mM sodium chloride, 5.5 mM monobasic sodium phosphate anhydrous, 8.6 mM dibasic sodium phosphate dehydrate, 1.2 mM sodium citrate dehydrate, 6.2 mM citric acid monohydrate, and 66 mM manitol at pH 5.2. The mass spectrometry compatible buffer was prepared by combining 90% 18 megOhm H_2O with 10% HPLC grade methanol and 0.1% formic acid followed by filtering with a 0.22 µm bottle top filter.

Buffer exchange was performed with Junior's pressure-based UF/DF technology using Unchained Labs' 96-well Unfilter consumable (Unchained Labs 601-2007). The automated UF/DF method applied uniform pressure at 60 psi with nitrogen on top of Unfilter and was paired with gentle orbital mixing to prevent membrane polarization (Figure 2). Well volumes were measured ultrasonically with a BioMicroLab VolumeCheck instrument to determine the volume removed after pressurization and to calculate how much new buffer needed to be added for the next buffer exchange cycle. The cycle of pressurization,

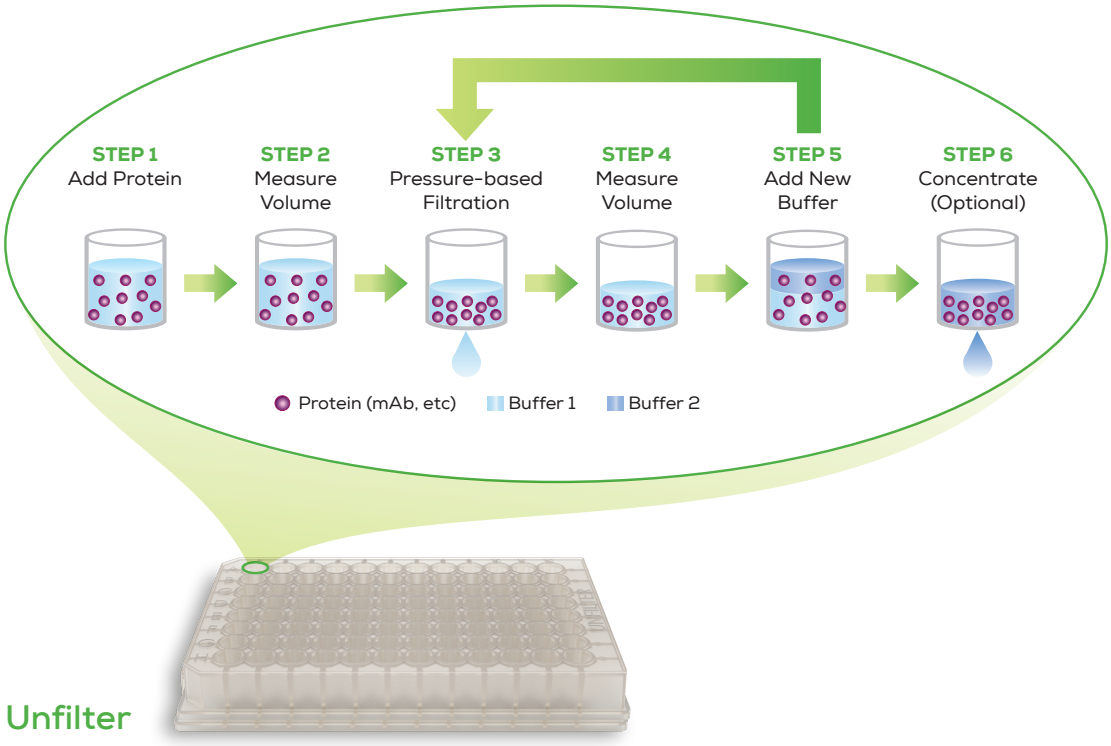

Figure 2: Junior automates the process of removing unwanted buffer, measuring solution volumes, and refilling each well with new buffer. Junior's UF/DF technology relies on applying pressure with gentle orbital mixing to remove buffer from Unfilter. After buffer exchange is complete, Junior can concentrate up to 4.5 times the starting protein concentration.

volume measurement, and new buffer addition was repeated until 99% exchange was achieved.

Key experimental details from the buffer exchange experiment, such as the final volume, buffer exchange cycle durations, volume removed per cycle and percent buffer exchanged were recorded by Automation Studio. Mass recovery was calculated using the initial and final volumes measured by Junior and absorbance measurements (A_{280}) from a Lunatic. After the buffer exchange run was complete the total time including system setup and protein mass recovery were analyzed.

Results

The major drawbacks of traditional MS sample preparation techniques are the required hands-on time and inability to adequately remove contaminants. Setting up buffer exchange with Junior took less than 30 minutes. The buffer exchange experimental design was created in Unchained Labs' Library Studio software by quickly modifying a supplied template (Figure 3). Experimental information, such as concentration, initial volume, final volume and buffers used were input to create a Library Studio design. Once the design was created, 450 µL of the Adalimumab stock was added to each well of an Unfilter with a multichannel pipette. The filled Unfilter and a buffer tray with the 90/10 H_2O/Methanol solution were loaded onto Junior. With the design created and Junior loaded, Unchained Labs Automation Studio was used to launch Junior's buffer exchange workflow. The buffer exchange workflow walked through the setup of key user controlled parameters, such as target percent exchange (99%) and volume removed per cycle (66%). Once the workflow parameters were input, Junior took over and automated buffer exchange began.

Automating buffer exchange leads to an increase in sample preparation throughput. Buffer exchange to 99% with the 96-well Unfilter was completely unattended and took 2 hours and 24

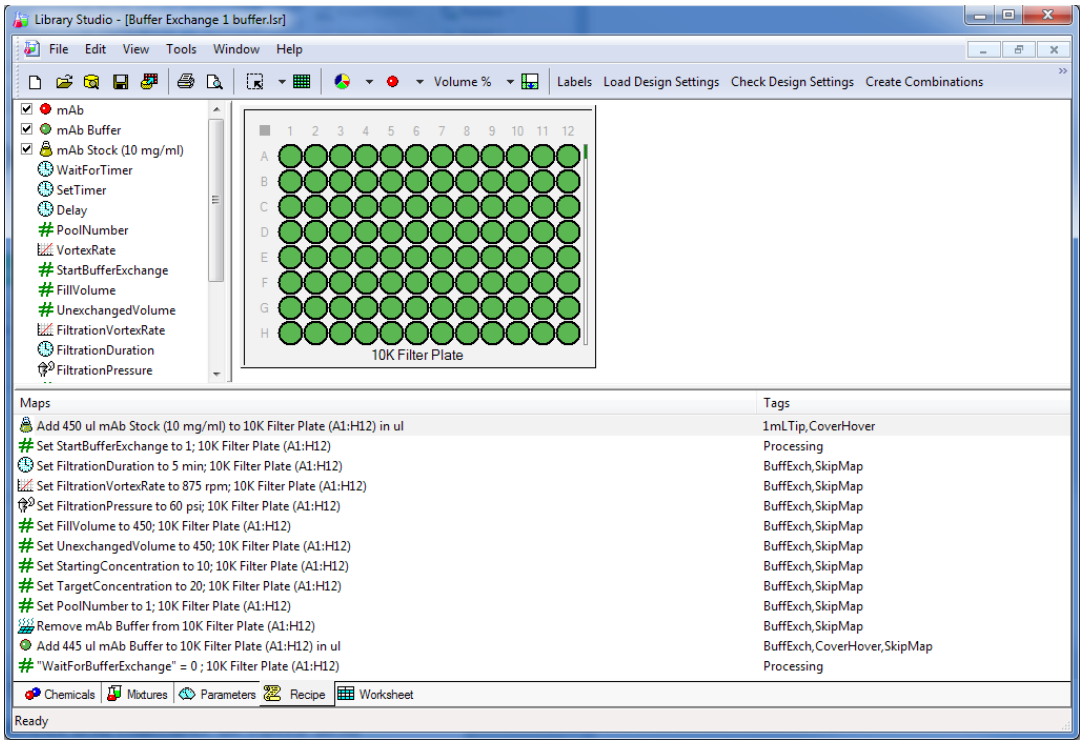

Figure 3: Junior buffer exchange experiments are designed with the LEA Library Studio software. Templates can quickly be altered to fit your desired experimental conditions. Users control the initial volumes, final volumes, initial concentrations, final concentrations and orbital mixing rate in the Library Studio design. Library Studio designs are used by Automation Studio to run the buffer exchange workflow. Additional parameters, such as target percent exchange (99%) and volume removed per cycle (66%), are set after the buffer exchange workflow is started.

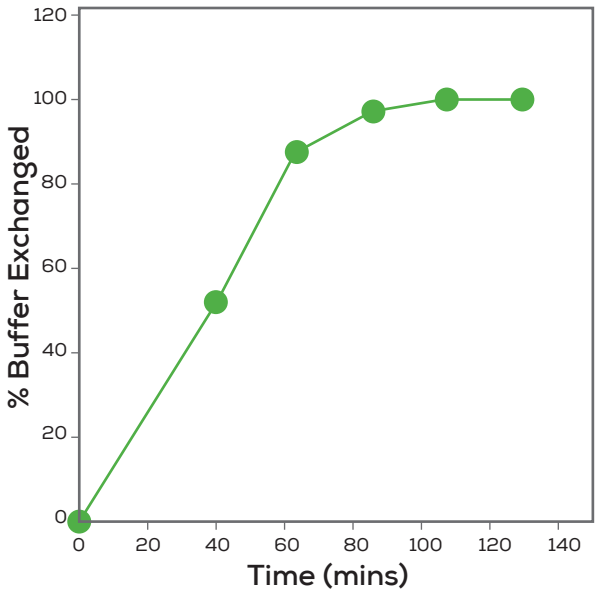

Figure 4: Junior reports the percent buffer exchanged after each buffer exchange cycle. Percent buffer exchange progress was highly similar between wells. All wells completed buffer exchange, to 99%, by the fifth buffer exchange cycle. Lowering the percent buffer exchange (ex. 97%) target during the workflow setup will decrease total experiment time.

minutes to complete (Figure 4). In that time, the Adalimumab samples were buffer exchanged from a complex formulation into a H_2O/methanol/formic acid solution ready MS characterization (Table 1). To further increase throughput, the experiment can be setup with a lower percent buffer exchange target. For the Adalimumab samples, 97% exchange and 98% exchange were achieved in 1 hour 26 minutes and 1 hour 48 minutes respectively. Additionally, Junior gave back what was put in with an average mass recovery yield of 97.3%. Junior buffer exchange was able to produce 96 samples at ~1 µg/µL in a MS compatible solution in less than 3 hours, including setup time. Automating buffer exchange with Junior allows for the rapid preparation of up to 96 MS samples per run.

	Result
Setup Time	<30 minutes
Buffer Exchange Time	2 hours 24 minutes
Total Time (Setup + Exchange)	**2 hours 54 minutes**
Sample Input	96 wells @ 450 µL each of Adalimumab
	105 mM NaCl, 14 mM sodium phosphate, 1.1 mM sodium citrate, 6.2 mM citric acid, 66 mM mannitol, pH 5.2
	1.17 mg/mL starting concentration
Sample Output	96 wells @ 456 ± 14 µL each of Adalimumab
	90% H_2O, 10% methanol, 0.1% formic acid
	1.15 ± 0.03 mg/mL average final concentration
	97.3% average mass recovery

Table 1: Summary of experimental results.

Conclusion

Common approaches to mass spectroscopy sample preparation come with serious draw backs. Manual buffer exchange takes time and dilution does not remove contaminants. Automating MS sample preparation with Junior clears these hurdles and will allow you to:

- Increase sample preparation throughput
- Remove up to 99% of unwanted buffer components
- Walk away with up to 96 unique samples ready for analysis

Automated buffer exchange removes the sample preparation bottleneck from your lab, allowing you to get more critical characterization done.

Unchained Labs
6870 Koll Center Parkway
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

Rev C