INTip Size Exclusion Chromatography

SEC

HIGHLIGHTS: High Throughput Group Separation in < 30 minutes

BACKGROUND & INTRODUCTION

Size exclusion chromatography (SEC) also known as gel filtration is a method by which molecules pass through SEC resin media and are separated by their size. Gel filtration is a well established method used with aqueous solutions for group separation or high resolution fractionation of complex bio-molecular mixtures.

A variety of resins are commercially available with different porosities providing different molecular weight cut off values depending on the need. Group separation for desalting and buffer exchange is a technique where small molecules such as salts are separated from a group of larger molecules such as proteins.

Traditionally, resins are first wetted with solvent (like an aqueous buffer) to swell and provide specified pores, some recommended protocols suggest swelling the medium in excess buffer for 3 hours. The media slurry is then made to have a ratio of 75% gel with 25% buffer, and degas under vacuum. Then the media slurry is transferred into a column with laborious steps to avoid bubbles and air pockets. After loading the slurry, additional buffer is added to equilibrate the column.

Automation of gel filtration methods has been difficult. High resolution fractionation requires the sample volume to be approximately 2-4% of the gel bed volume. This usually requires a gel bed volume too large to be accommodated on most robotic liquid handling platforms. Group separations allow for a larger sample volume to be applied, typically 30% of the gel bed volume. Thus, the gel bed volumes can be smaller for group separations. This has allowed for the development of micro-spin columns and plates for group separation reactions; however, these protocols require a centrifugation step. Examples of group separations include desalting, buffer exchange and polymerase chain reaction (PCR) clean up.

Current commercially available pipette tip products for size exclusion contain the gel, with the size exclusion media already packed and equilibrated. Two main problems associated with these types of products are the packing gel can be readily disrupted during shipping and additional steps are often required to ensure the packing of the gel is adequate, such as centrifugation, prior to use.

DPX Technologies developed a patent-pending size exclusion chromatography (SEC) pipette tip designed to automate group separations of complex biological mixtures. The SEC pipette tip incorporates a novel INTip™ swelling and packing of the gel filtration medium to eliminate bubbles and poor packing associated with 'top-loading' a column. The formation of the gel by INTip swelling involves aspirating solvent (such as buffer) into the SEC tip. This INTip swelling provides efficient gel packing without generating air bubbles or pockets. The INTip SEC method utilizes proprietary tools to prevent concerns of potential cross-contamination of sample solutions. INTip SEC offers a highly reproducible method for group separation applications.

MATERIALS & METHODS

The device comprises a pipette tip (for the SEC column) with a bottom filter (frit) at the narrow end, and dry SEC media loosely contained inside the pipette tip (Figure 1A).

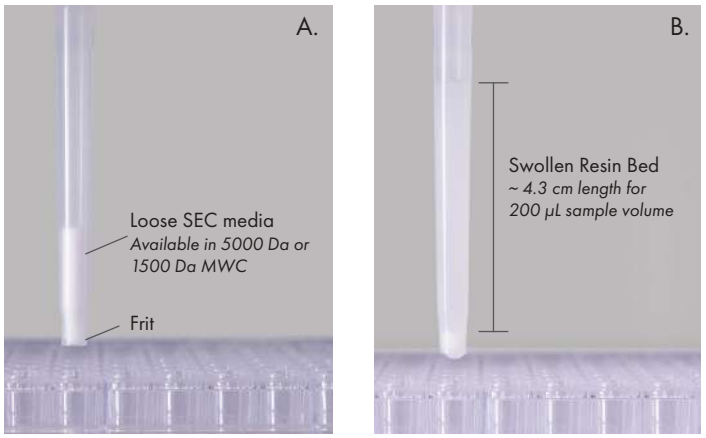


Figure 1.
A. SEC tip anatomy. The pipette tip pictured contains 180 mg of dry resin.
B. Swollen resin bed packing of ~ 4.3 cm length.

INTip Swelling:
Column packing for the formation of the gel is performed by aspirating solvent (such as a buffer) into SEC tips, holding the solvent for 5 minutes and allowing the gel to swell and settle (Figure 1B). Post swelling, for column equilibration, the excess buffer dispenses through the tip via gravity flow (~5 min). The gel is ready for sample loading in 10 minutes or less.

MATERIALS & METHODS CONTINUED

This process allows for reproducible packing of the gel without concerns of air pockets or channeling. By loading the buffer or solvent from the bottom narrow end of the pipette tip, the media particles absorb the solvent and swell, making a packed gel column. There are no air bubbles created by performing this bottom-up loading of the gel. The solvent displaces air and removes any potential for air pockets to form, thus eliminating concerns of channeling.

This method of loading and swelling the size exclusion media is much faster than conventional top loading of buffer or solvents. Instead of taking large volumes of solvent and hours for swelling, the size exclusion gel is made in minutes. Most importantly, the gel is reproducibly made to the desired column length.

Group Separation Method:

The method for separation is performed by loading the sample solution into the SEC resin after INTip swelling. Sample volume is dependent on column void volume and can range from 50-250 µL. After the sample is loaded and enters the column by gravity flow, elution buffer is added; purified sample is collected by gravity flow or positive pressure. SEC tips are positioned over collection vials or a well plate during elution to collect the large molecules of interest (Figure 2).

Automated Protocol for SEC tips: 5000 Da MWC for 250 µL sample in 1 mL Hamilton

This protocol is specific for this product and sample volume. SEC tips (DPX170236) were positioned on a Tip Isolator Pedestal (Hamilton P/N 65422-01) over a solvent reservoir containing PBS (phosphate buffered saline) solution. The liquid handler picks up the SEC tips and aspirates the buffer from below (0.8 mL) to begin the INTip Swelling. The solution was held in the SEC tips for 5 minutes allowing the resin to swell and settle. The tips were released back onto the rack to allow gravity flow for column equilibration.

A sample solution contained a mixture of FD&C red 40 and red 3 dyes (small molecules with molecular weights of 497 and 880 Da, respectively) and a blue stained protein standard (molecular weights 10-250 kDa). A DPX Funnel Plate was positioned on top of the SEC tips. The DPX Funnel Plate is designed to protect the top of the SEC tips from contamination during sample addition. The Nimbus aspirated 0.25 mL of the sample mixture and dispensed through the Funnel Plate onto the top of the column bed in the SEC tips. The sample mixture passed through the gel bed in the SEC tip via gravity flow. The sample penetrated the column, displacing PBS buffer back into the reservoir. The Funnel Plate is removed from the SEC tips and discarded.

The Nimbus picks up the SEC tips and moves them to the collection location. The final buffer addition (0.3 mL) is applied to the column and allowed to gravity flow. The protein fraction is collected in the well plate. See Figure 3 for the deck layout.

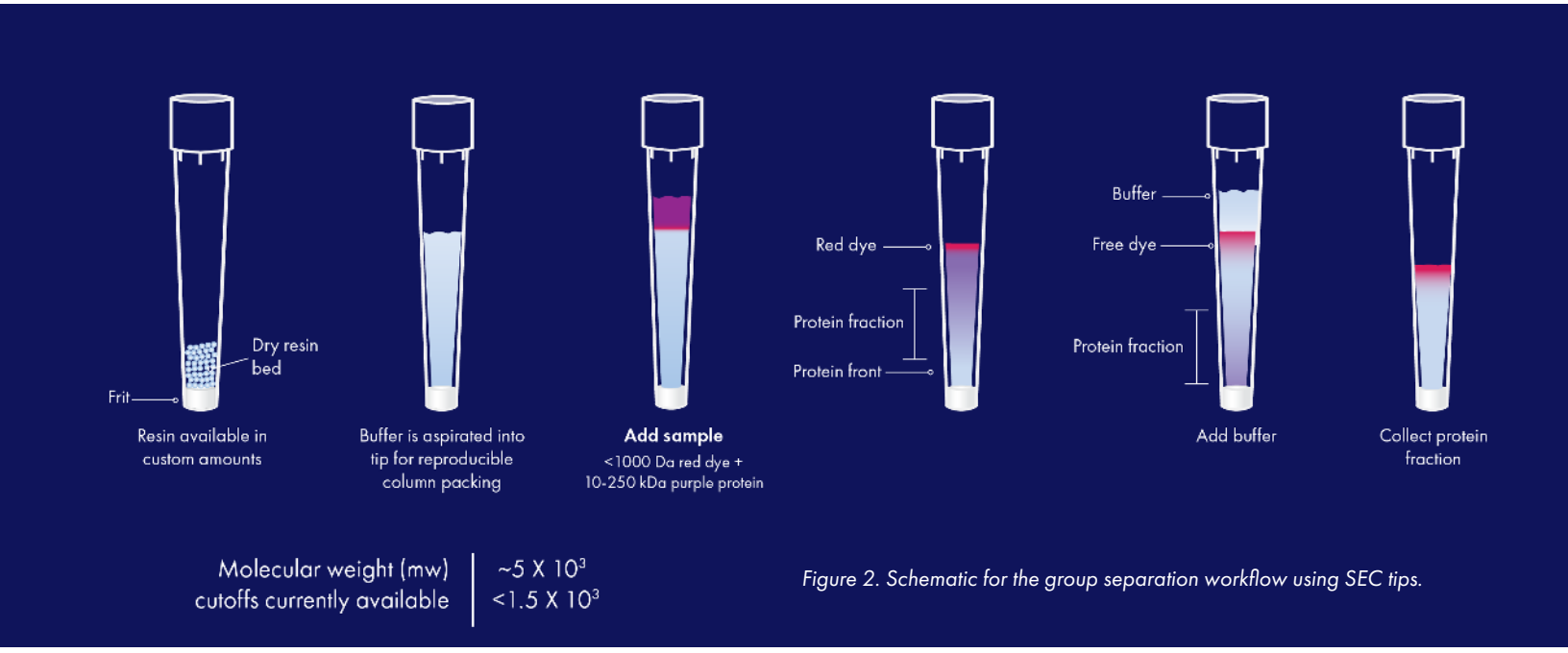
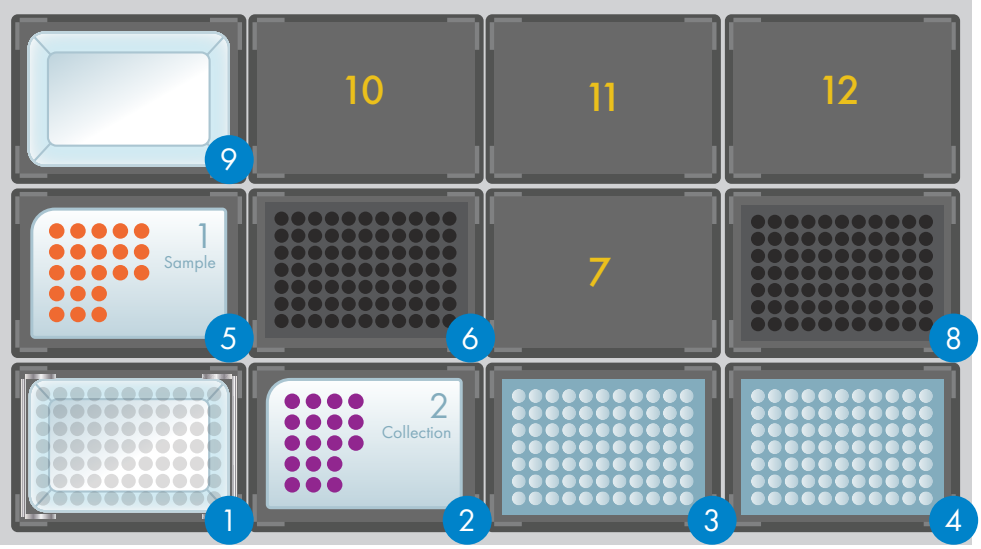


Figure 2. Schematic for the group separation workflow using SEC tips.



1 Buffer- INTip Swelling

2 Final Collection Plate

Location 1 and 2 are Tip Isolator Pedestals from Hamilton (P/N 65422-01) which allows for the SEC tips to be loaded on top of a buffer reservoir or collection plate, respectively.

3 50 µL CO-RE tips

4 300 µL Slim CO-RE tips

5 Sample

6 SEC tips

8 DPX Funnel Plate

9 PBS

Figure 3. Deck layout for SEC workflow described above on a Hamilton Microlab Nimbus.

RESULTS

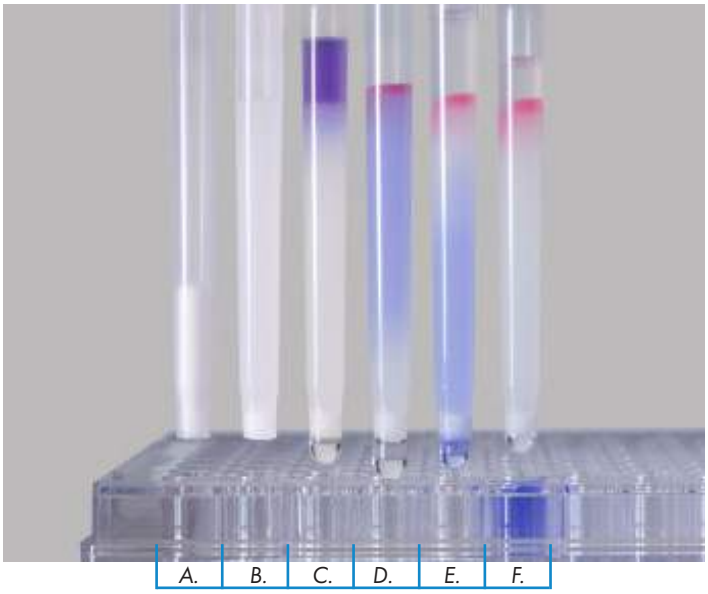


Figure 4.
A. Dry resin bed *B. Swollen resin bed*
C. Sample application *D. Sample fills void volume*
E. & F. Elution of proteins separated from low MW dye.

Figure 4 shows a visual representation of group separation using SEC tips. The red dyes stayed at the top of the bed and the blue stained proteins were collected in a well plate as these target compounds eluted from the gel. Figure 5 shows recovery data before and after sample preparation with SEC tips. Samples of 4mM tartrazine in PBS and samples containing 2.3 mg BSA in PBS were applied to SEC tips in triplicate. 90% of the protein was present in the elution solution with no detected tartrazine.

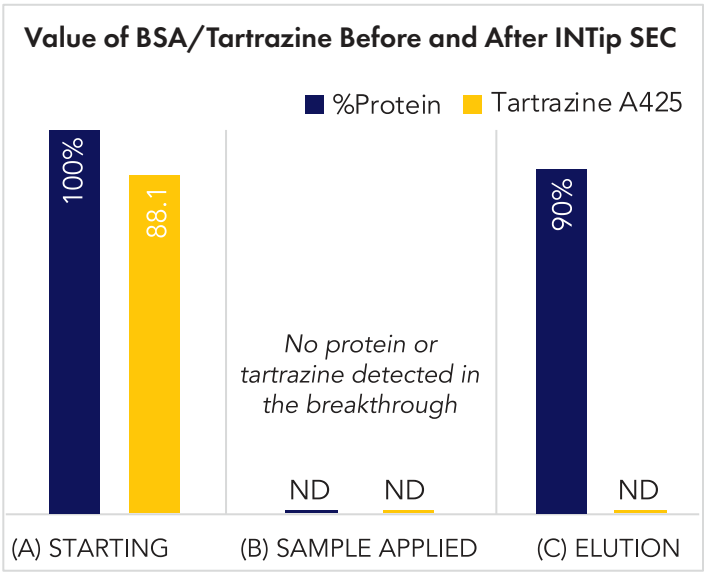


Figure 5. Absorbance values at 280 nm for protein and 425 nm for tartrazine were performed on a nanodrop one spectrophotometer. Measurements were taken for the starting material (A); after 250 µL sample application (B); and 300 µL elution (C). Results are displayed as the average % protein concentration and average absorbance value of tartrazine at 425 nm.

CONCLUSIONS

DPX SEC tips provide a robust method for automating group separations without the need for centrifugation or positive pressure manifolds. The products and methods were developed to overcome the complexities and time commitment of manual column preparation and centrifugation steps.