INTip™ Size Exclusion Chromatography

HIGHLIGHTS: High throughput group separation in < 15 minutes

SEC

INTRODUCTION

Size exclusion chromatography (SEC), also known as gel filtration, is a method which allows molecules to pass through SEC resin media and become 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 create specified pores. Some recommended protocols suggest swelling the medium in excess buffer for 3 hours. The media slurry is made to have a ratio of 75% gel with 25% buffer and degassed under vacuum. The media slurry is then 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) cleanup.

DPX Technologies developed a patent-pending size exclusion chromatography pipette tip designed to automate group separations of complex biological mixtures. DPX's SEC Tips incorporate a novel INTip™ Swelling method for packing 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 provides efficient gel packing to prevent cross contamination of samples. INTip SEC offers a highly reproducible solution for group separation applications.

MATERIALS AND METHODS

DPX's SEC Tips comprise of a pipette tip with a bottom filter (frit) at the narrow end, dry SEC media loosely contained inside the tip, and a pierceable barrier positioned at the top to contain sorbent during transit (**Figure 1A**). Column packing for the formation of the gel is performed by aspirating solvent (such as a buffer) into the SEC Tips, holding the solvent and allowing the gel to swell and settle (**Figure 1B**).

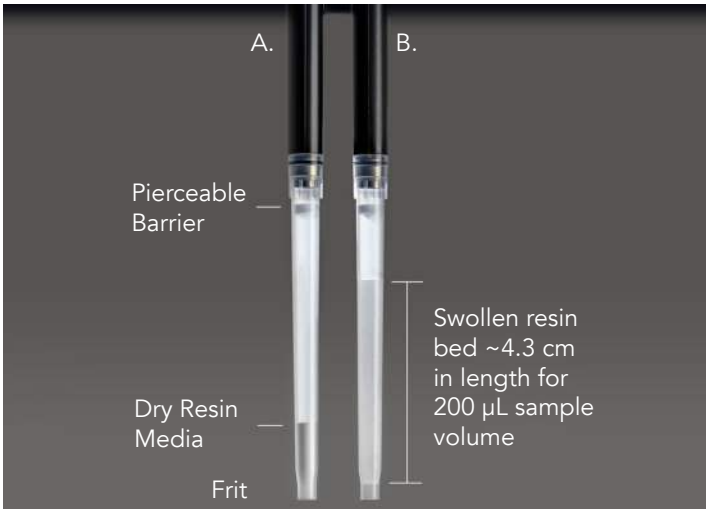

Figure 1.
A. SEC Tip anatomy. The pipette tip pictured contains 180 mg of dry resin.
B. Same resin bed after INTip Swelling.

This INTip Swelling 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. 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.

This protocol is specific for catalog # DPX170584 (SEC Tips: 250 µL Column with 5000 Da Cut Off in 1 mL Hamilton) and a sample volume of 250 µL. Sample volume is dependent on column void volume and can range from 50-250 µL depending on SEC catalog number. For this study, a Hamilton NIMBUS was

used as the automated liquid handler (ALH). After INTip swelling with PBS (phosphate buffered saline), blank transfer tips loaded the sample solution (250 µL) on top of the SEC resin bed through the pierced barrier. After the sample was loaded, the SEC Tips were picked up, and the sample was dispensed into the column. Elution buffer (300 µL PBS) was added and dispensed into the column in the same manner as the sample, with the SEC Tips positioned over a well plate to collect the large molecules of interest. (**Figure 2**)

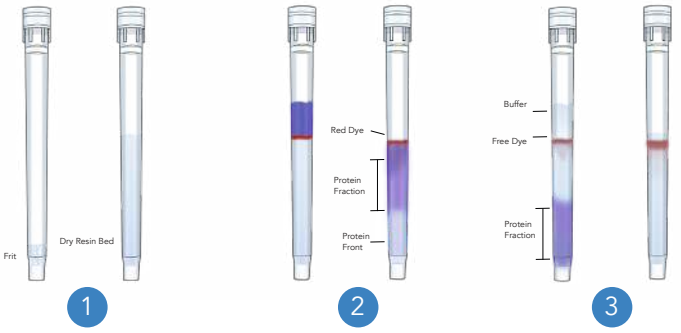

Figure 2. *Schematic for the group separation workflow using SEC Tips as laid out in **Table 1**.*

Table 1. *Schematic for the group separation workflow using SEC Tips.*

1	INTip Swelling	Buffer is aspirated into tip for reproducible column packing.
2	Load Sample	Add sample of <1000 Da red dye and 10-250 kDa purple protein. Apply positive pressure.
3	Collect	Add buffer and apply positive pressure to collect protein fraction.

The method was assessed both qualitatively and quantitively using two samples and means of analysis. To visually observe the efficacy of the DPX SEC Tips, a sample solution containing a mixture of FD&C red 40 and red 3 dyes (small molecules with molecular weights of 497 and 880 Da, respectively) and a purple stained protein standard (molecular weights 10-250 kDa) was used. To quantitatively assess the performance of the method, samples of 4 mM tartrazine in PBS and samples containing 2.3 mg BSA in PBS were applied to SEC Tips in triplicate. Absorbance was measured at 280 nm and 425 nm, for BSA and tartrazine, respectively.

RESULTS

Figure 3 shows a visual representation of group separation using SEC Tips. The red dyes stayed at the top of the bed and the purple stained proteins were collected in a well plate as the target compounds eluted from the gel.

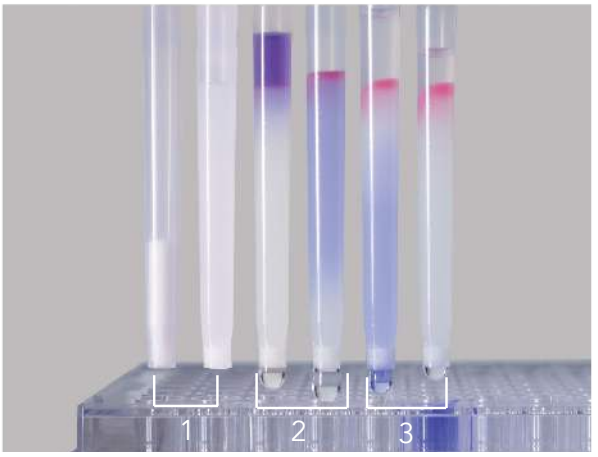

Figure 3. *A photograph illustrating the method in **Table 1** and **Figure 2**.*

Figure 4 shows NanoDrop recovery data before and after sample preparation with SEC Tips. Results showed 90% of the protein was present in the elution with no tartrazine detected.

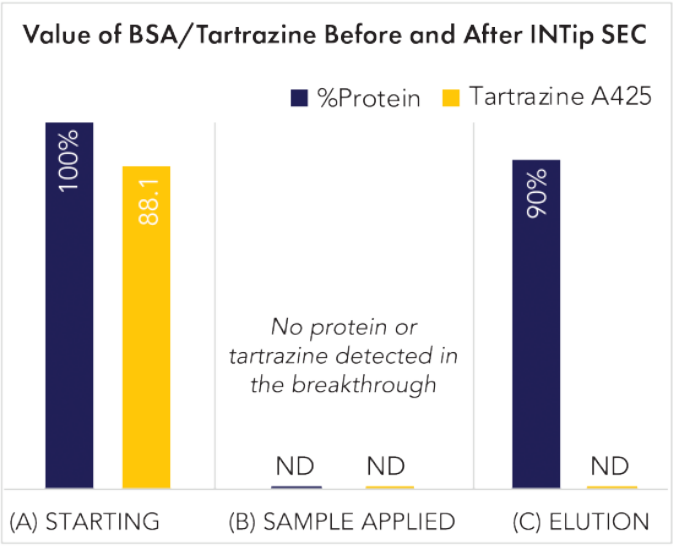

Figure 4. *Absorbance values at 280 nm for protein and 425 nm for tartrazine were collected on a NanoDrop One spectrophotometer. Measurements were taken for (A) the starting material, (B) after 250 µL sample application, and (C) 300 µL elution. Results are displayed as the average % protein concentration and average absorbance value of tartrazine at 425 nm.*

CONCLUSIONS

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