**INTRODUCTION**

Speed and resolution are two competing performance factors in chromatography using conventional porous separation media. One feature is often achieved while the other is sacrificed. This is often the case during separation of proteins and peptides, since these large molecules exhibit low diffusivity which translates into higher mass transfer resistance. The net result is significant peak broadening especially at higher flow rates.

A new generation of styrene-based reversed-phase separation media from Dionex, ProSwift™ polymer monoliths, is designed to address the above problem. They contain a network of large pores in which separation is achieved primarily by convective flow rather than diffusive flow. Highly improved mass transfer rates allow high speed analysis without band broadening or significantly sacrificing resolution thereby improving throughput and productivity. The new ProSwift phases use a patented process to produce a well defined polymer monolith. An in-situ polymerization process allows excellent batch-to-batch reproducibility and column stability.

Separations can be achieved at high flow rates without loss of resolution. These columns have an excellent longevity and are stable over a wide pH range.

We discuss the performance of three columns; two recently introduced phases, RP-2H and RP-3U, as well as our newest introduction, RP-1S. Excellent separation of a wide variety of proteins and peptides is shown here.

**OBJECTIVES**

- Characterize the morphology and pore structure of the ProSwift monoliths.
- Investigate the chromatographic properties of the ProSwift monoliths.
- Develop applications for proteomic research.

**Eluents:**

- (A) DI H₂O / CH₃CN (95:5 v/v) + 0.1% TFA
- (B) DI H₂O / CH₃CN (5:95 v/v) + 0.1% TFA

**Injection:**

10 µL (unless otherwise stated)
High Throughput Liquid Chromatography Using Polymeric ProSwift Monolithic Bioseparation Columns

Figure 4. Peptide and Protein Separation

- **Flow:** 1.5 mL/min
- **Sample:**
  1. Methionine Enkephalin acetate (Tyr-Gly-Gly-Phe-Met) (MW 573.7)
  2. Leucine Enkephalin (Tyr-Gly-Gly-Phe-Leu) (MW 555.6)
  3. Angiotensin II acetate (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) (MW 1046.2)
  4. Physalaemin (Glu-Ala-Asp-Pro-Glu-Lys-Phe-Tyr-Gly-Leu-Met) (MW 1295.6)
  5. Substance P acetate (Arg-Pro-Lys-Pro-Gln-Glu-Phe-Gly-Leu-Met) (MW 1347.6)
  6. Ribonuclease A
  7. Cytochrome C
  8. Carbonic Anhydrase
  9. Bovine Serum Albumin

- **Injection:** 5 µL
- **Gradient:** 1% B for 2 min, 1 - 75% B in 17 min

Figure 5. Protein Separation on ProSwift RP-2H

- **ProSwift RP-2H column’s faster separation enables higher productivity.**
- **Flow:** 1.5 mL/min
- **Sample:**
  1. Ribonuclease A (1.5 mg/mL)
  2. Cytochrome c (0.5 mg/mL)
  3. BSA (1.5 mg/mL)
  4. Carbonic Anhydrase (0.9 mg/mL)
  5. Ovalbumin (1.5 mg/mL)

- **Injection:** 5 µL
- **Gradient:** 1% B for 2 min, 1 - 75% B in 17 min

Figure 6. Cytochrome c Tryptic Digest Separation on ProSwift RP-1S

- **High resolution allows easy separation of digest peptides.**
- **Flow:** 1.0 mL/min
- **Sample:** Cytochrome c Tryptic Digest (2 mg Cytochrome c, 20 µg trypsin per mL)
- **Injection:** 20 µL
- **Gradient:** 1% B for 2 min, 1 - 50% B in 12 min

Figure 7. Separation of Snake Venom Proteins/Peptides

- The ProSwift RP-2H column’s uniquely engineered pore size allows separation of samples with a wide molecular weight range.

Figure 8. Separation of IgG and Albumin from Bovine Serum

- The ProSwift RP-2H column has large flow through channels that result in high resolving power for large proteins.

Figure 9. Batch-To-Batch Reproducibility of ProSwift RP-2H

- ProSwift reversed-phase media are manufactured by a patented in situ manufacturing process which has the least number of variables affecting the reproducibility among all technologies. It does not require additional sieving, coating, multiple surface modification, and packing processes.
CONCLUSION

Characteristics of Polymeric Monolithic Columns

- High resolution
- High flow velocity and low backpressure
- High capacity
- Highly reproducibility
- Much more chemically stable than silica-based media

Applications

- High-throughput, high-resolution separation of proteins, peptides, and other biomolecules

REFERENCE


ProSwift is a trademark of Dionex Corporation.