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Note d'application

Desalting of Proteins Using MassPREP On-Line Desalting Cartridges Prior to Mass Spectrometry

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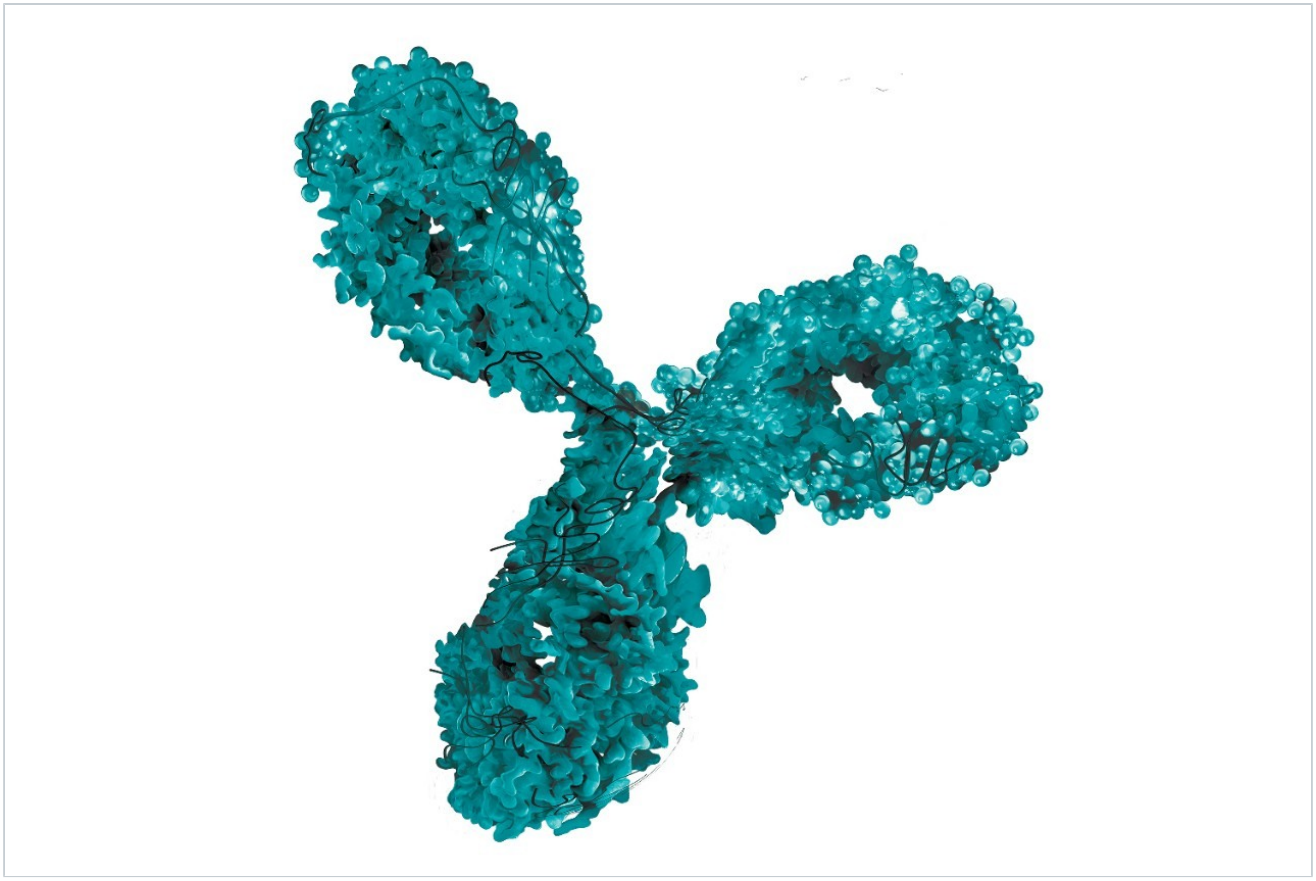


Abstract

In this application note we show a rapid, on-line desalting procedure with cycle times less than five minutes.

Introduction

LC-MS is a powerful tool used for the characterization of proteins. Reversed-phase HPLC is the most common mode of chromatography coupled with mass spectrometry. This separation technique can also be an effective method for the desalting of proteins. Pharmaceutical biotherapeutics and other proteins are often prepared or stored in salt containing buffer solutions such as PBS (phosphate buffered saline). These buffer solutions contain salts that form adducts with proteins, complicating data interpretation. These salts also suppress ionization during ESI-MS analysis. Therefore, separation of proteins from non-volatile salts is an important step prior to introduction into the mass spectrometer. Here we show a rapid, on-line desalting procedure with cycle times less than five minutes. The MassPREP On-Line Desalting Cartridge is a 2.1 x 10 mm device packed with polymer sorbent and was evaluated using acidic, (bovine serum albumin), basic, (cytochrome c), and large globular, (monoclonal antibody) protein samples. Parameters such as column carry over, loading, and lifetime were investigated. The results of this study show no sample carryover and excellent lifetime for repeated sample analysis under the conditions tested.

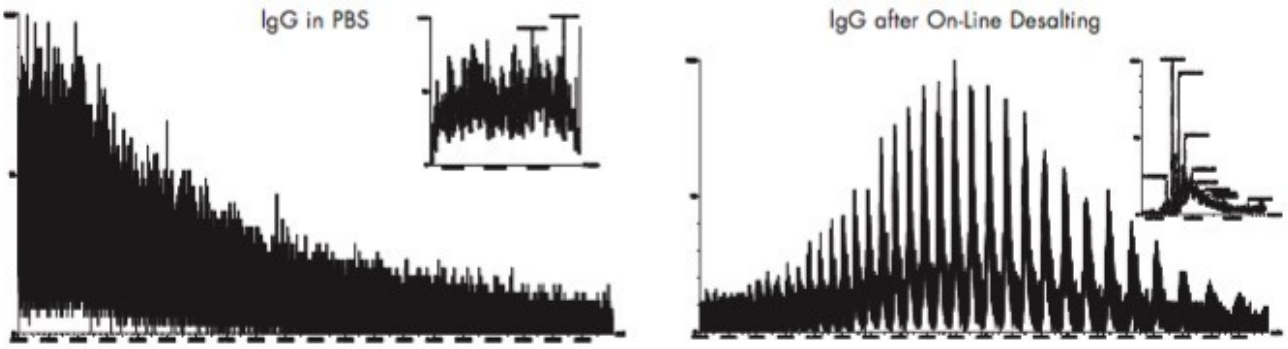


Figure 1A and 1B. Desalting of monoclonal IgG. The sample in PBS gives no recognizable protein mass spectrum. After desalting, the sample gives a typical protein spectrum that can be deconvoluted using the Waters Micromass MaxENT 1 algorithim to reveal isoforms of IgG.

Experimental

Sample Preparation

Bovine serum albumin (BSA), cytochrome c, and monoclonal IgG, were obtained from Sigma Aldrich Inc. St. Louis Mo. All proteins were dissolved in phosphate buffered saline (PBS) at a concentration on 1µg/µL.

LC Conditions

Diveter valve:	Waters Selection Valve
HPLC system:	Alliance 2796 Separations Module
Needle wash solution:	H_2O (35%) / Isopropanol (5%) / Acetonitrile (60%)
Mass spectrometer:	Waters Micromass Q-tof micro

Ionization mode:	ES+

Diveter valve, HPLC System, and Mass Spectrometer were all controlled by Waters MicroMass MassLynx Software.

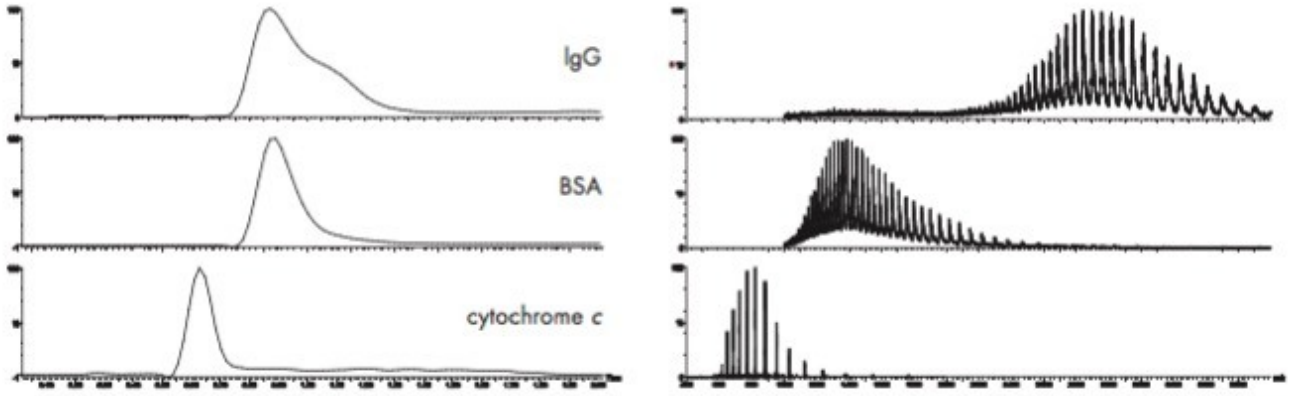
Separation Method

Eluent A:	H_2O with 0.1% Formic Acid
Eluent B:	Acetonitrile with 0.1% Formic Acid
Flow:	0.4 mL/minute

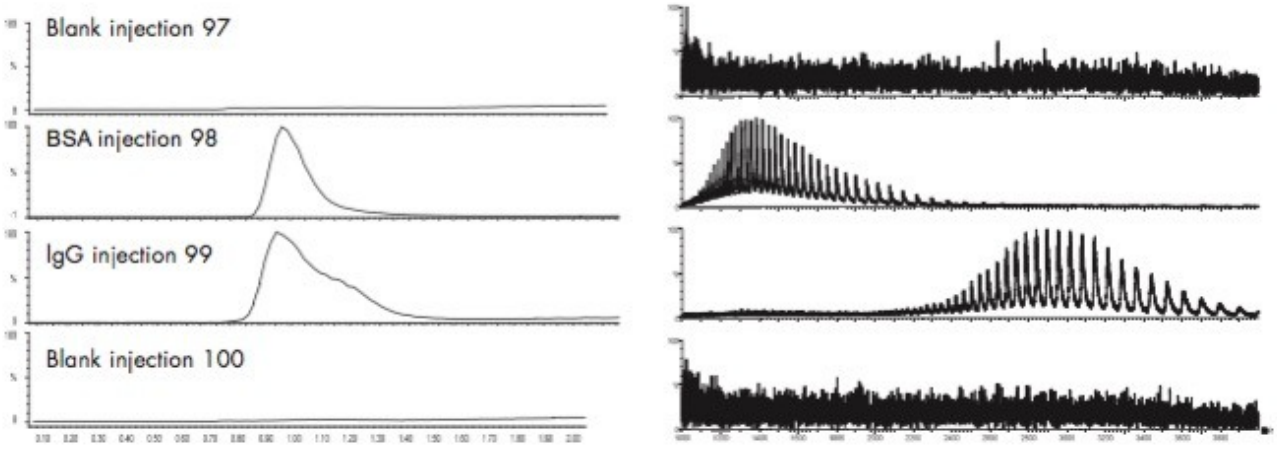
Gradient

Time	Eluent A	Eluent B	Valve position
0.0	95	5	waste
0.5	95	5	waste
2.0	20	80	Mass spectromete
3.0	95	5	waste

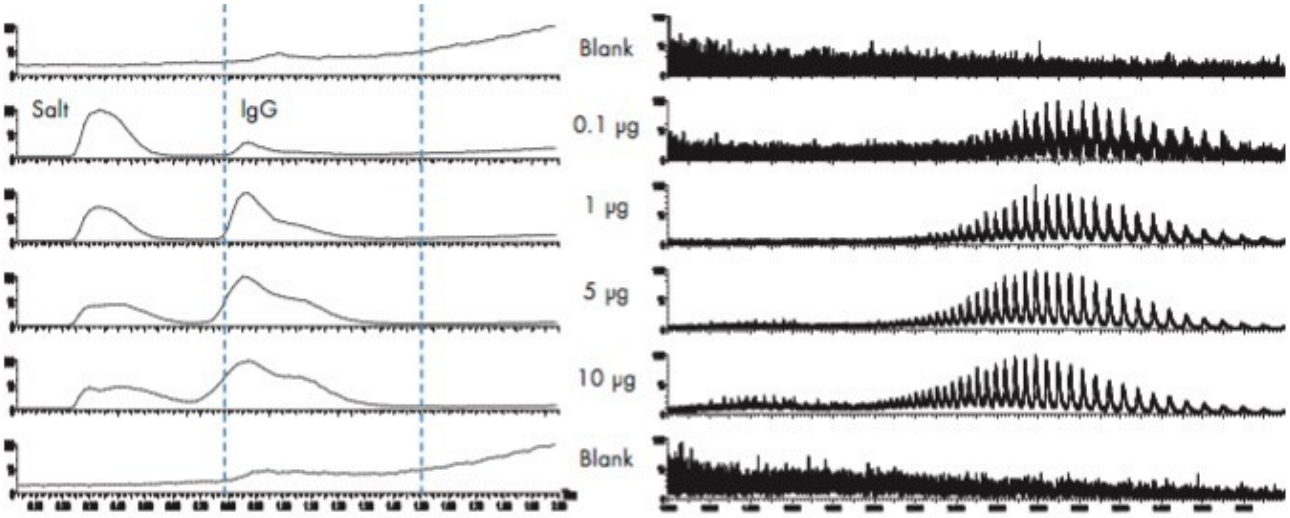
Results and Discussion



Figures 2A and 2B. The chromatographic elution profile is shown for three test proteins in figure 2A. Mass specta summed across the chromatographic peaks are shown in figure 2B. These experiments show that the cartridge can be used over a wide range of protein types.



Figures 3A and 3B. A series of injections consisting of a blank, 1 µg BSA, 1µg monoclonal IgG, and blank was repeated 25 times for a total of 100 injections. The performance of the cartridge is unaltered over this series. There is no evidence of carr yover or background inter ference in the blank injection representing the 100th injection.



Figures 4A and 4B. Total ion chromatograms and summed spectra for indicated region demonstrating loading capacity and carryover of monoclonal IgG. Blanks were injected between increasing loads of IgG1 to measure carryover (data not shown). The amount of sample can be increased to 10 µg in 10 µL of PBS without breakthrough and with no detectable carryover in the blank following a 10 µg injection.

Conclusion

Waters MassPREP On-Line Desalting Cartridges are an effective tool for the successful desalting of acidic, basic, and large globular proteins. The desalted proteins yielded abundant ESI-MS signal suitable for deconvolution and protein characterization. Studies determining loading capacity and carryover showed that up to 10 µg of monoclonal IgG was succesfully loaded with no breakthrough and no detected carryover in a subsequent analysis. The same results were obtained for BSA and cytochrome c for injected masses of up to 5 µg. Further, no loss of performance was observed after 100 consecutive injections were performed. The overall MassPREP desalting strategy provides an easy, fast (<5 minutes), and reproducible approach for the effective desalting of proteins stored in physiological salt buffers prior to high resolution structural analysis.

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