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A Rapid Desalting Procedure for the Mass Spectrometric Analysis of Intact Proteins

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Introduction

Reversed phase chromatography of intact proteins coupled to on-line mass spectrometric analysis, has enabled detection of structural differences in proteins. Reversed phase chromatography is the most preferred method for coupling to a mass spectrometer as it utilizes a volatile mobile phase. Biotherapeutics and other proteins are usually stored in salt containing buffer solutions. Non-volatile salts such as sodium phosphate, sodium chloride, etc. can cause adducts with proteins and also suppress ionization during MALDI-MS and ESI-MS analysis. Hence mass spectrometric analysis of proteins in salt solution can be challenging. We have developed a rapid on-line desalting procedure for the analysis of proteins by ESI-MS. The method has a cycle time of 15 min and could be configured in a parallel mode for high throughput analysis of proteins. We evaluated several reversed phase chemistries for protein desalting. The LC-MS conditions such as the concentration of acid modifier were studied for intact protein analysis. We show application of online desalting to the characterization of intact IgG. We were able to detect several glycoforms in intact IgG using this method. This method should be effective in rapid analysis of biotherapeutic formulations.

System Components

Waters® BioSuite™ Intact Protein System

Waters® 2796 Separations module.
Waters® 2487 Dual Wavelength Absorbance Detector
Waters® ZQ™ Mass detector
Waters® Q-Tof-2™

Columns:

BioSuite™ Prototype desalting column 5 µM (2.1 X 20 mm)

Experimental

MS Conditions

-Source = ESI(+)
-Capillary (kV) = 3.3
-Cone (V) = 30
-Temperature (°C)
  -Source = 150
  -Desolvation = 350
-Gas Flow (L/Hr)
  -Cone = 50
  -Desolvation = 500
-Scan Mode
-Collision Energy = 10

HPLC Conditions

A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile
Flow Rate = 0.2 ml/min

Gradient Table:

Time	Flow ml/min	% A	% B
0	0.2	100	0
5	0.2	100	0
9	0.2	30	70
9.1	0.2	20	80
9.2	0.2	100	0
15	0.2	100	0

Figure 1A: MS spectrum of IgG1 obtained by infusion.
IgG and other proteins are typically stored in salt containing buffer. Salt suppress ionization of proteins during ESI-MS analysis. Salts also form adducts with proteins increasing the heterogeneity in the sample. Infusion of 20 picomoles of IgG1 in 20 mM Tris, 0.2% formic acid 50% Acetonitrile is shown in Figure 1 A.

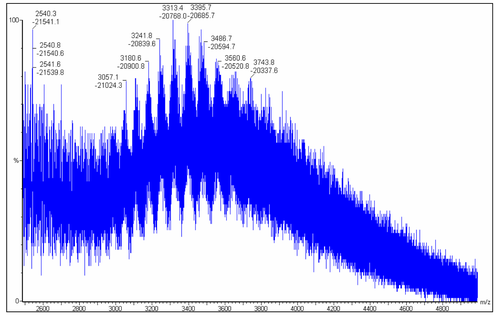


Figure 1B: Deconvoluted spectrum of IgG1 obtained by infusion.
MaxEnt1 deconvolution improves the resolution and can enhance a weak spectrum. However, heterogeneity caused by salt adducts increases the complexity of proteins and hence yields poor results after deconvolution. The deconvoluted spectrum of mass spectrum from 1 A is shown. As can be seen from the figure the presence of salts results in poor MS data

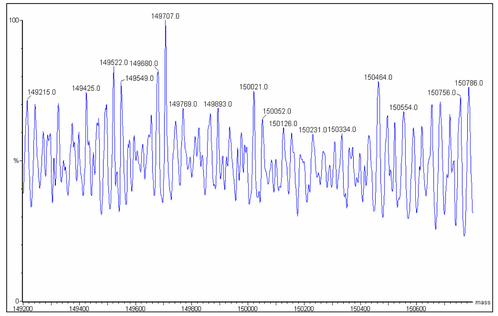


Figure 2A: On-line SPE-MS of IgG1
On-line SPE-MS is widely used for the analysis of small molecules. SPE can also be very effective for the desalting of proteins. MS spectrum of IgG1 obtained after on-line desalting is shown in Figure 2A. The amount of sample used (20 picomoles) is identical to that from Figure 1 B. A distinct enhancement in signal-to-noise ration can be observed after online desalting.

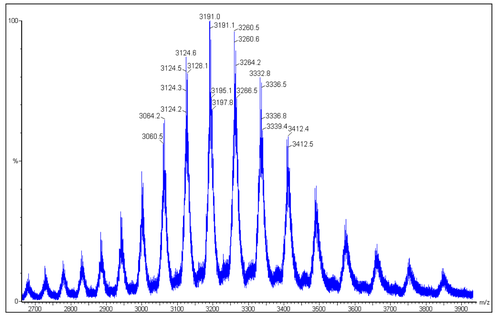


Figure 2B: Deconvoluted spectrum of IgG1 obtained on-line SPE-MS.
SPE-MS analysis eliminates salt adducts and hence reduces the hetrogeinity in protein samples. The deconvoluted spectrum of mass spectrum from Figure 1 B is shown. Several different glycoforms of IgG1 were observed. A detailed analysis of the glycoforms is presented in poster # P-71-TH

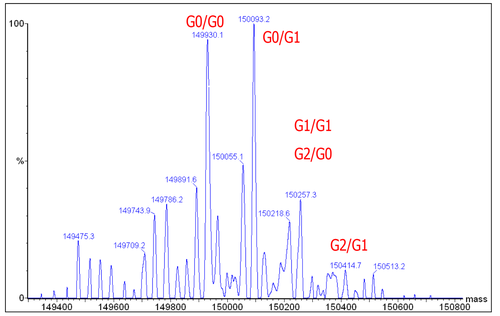
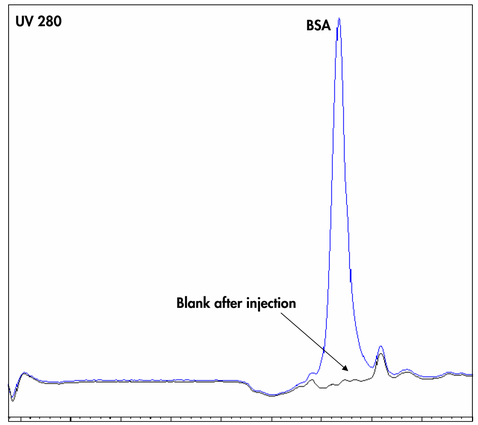


Figure 3: Oasis® HLB shows low protein carry-over.
Protein carry-over is one of the major issues with reversed phase chromatography of large proteins. Oasis® HLB is very effective for desalting and also shows very low carry over of proteins. Low carry over for 10 µg injection of BSA on Oasis® HLB is shown in Figure 3.



Conclusion

- Salt suppresses ionization in ESI.
- Salt adducts can complicate analysis.
- SPE-MS allows rapid desalting of protein samples.
- SPE-MS could be used for QC of IgG formulations.