The Effect of NaCl Concentration on Protein Size Exclusion Chromatography

Application Note

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Introduction

Size exclusion chromatography (SEC) is a non-invasive technique that provides information on the molecular size of proteins and polymers. SEC requires a column with a uniform porous material with a defined pore size and a mobile phase appropriate to the solute. Mobile phase components, such as salts, can affect SEC separations. The purpose of adding salt to the eluent is to minimize secondary interactions of the sample components with the resin. The presence or absence of sodium chloride (NaCl) in aqueous eluents influences the elution volume of proteins by altering their hydrodynamic radius as a function of salt strength.

It is important to note that relatively minor changes in protein structure may affect protein solubility and encourage secondary hydrophobic interactions causing similarly sized proteins to elute at different times. In such cases, it is important to modify the mobile phase composition to regain a separation based on molecular size alone.

The retention behavior of three globular proteins (γ-globulins, bovine serum albumin and ovalbumin) was investigated using a Agilent ProSEC 300S column in low ionic strength mobile phases of varying NaCl concentration. The dependence of 'non-ideal' protein-protein and protein-matrix interactions on NaCl was determined by comparison of UV responses at each salt concentration.

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Methods and Materials

Conditions

Sample:	Proteins
Column:	ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Flow Rate:	1.0 mL/min
Injection Volume:	100 µL
Temperature:	5 °C
Eluent:	0.1 M KH_2PO_4 comprising various concentrations of NaCl @ pH 8.0
Detector:	UV at 310 nm

Results and Discussion

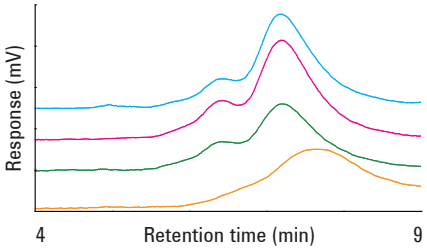

Figure 1. Overlay of UV response for a sample of γ-globulins at varying salt concentrations. Orange - no NaCl, green - 0.1 M NaCl, pink - 0.3 M NaCl, blue - 0.5 M NaCl

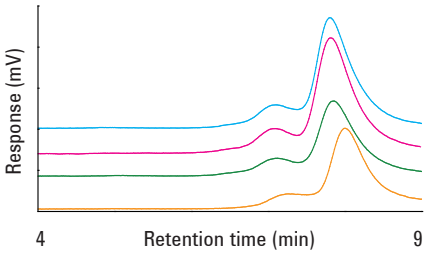

Figure 2. Overlay of UV response for a sample of bovine serum albumin at varying salt concentrations. Orange - no NaCl, green - 0.1 M NaCl, pink - 0.3 M NaCl, blue - 0.5 M NaCl

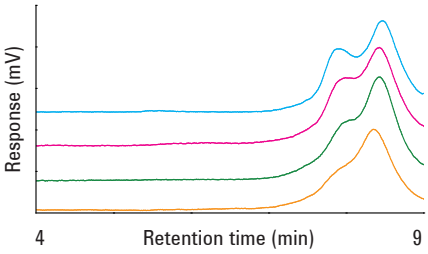

Figure 3. Overlay of UV response for a sample of ovalbumin at varying salt concentrations. Orange - no NaCl, green - 0.1 M NaCl, pink - 0.3 M NaCl, blue - 0.5 M NaCl

The figures illustrate that the absence of salt affects the chromatography of globular proteins, slightly delaying peak elution and broadening the peaks giving less definition between monomers and higher oligomers. This is consistent for all three proteins. The higher molecular weight species seen in the presence of salt are not visible in the traces in which the mobile phase did not contain NaCl, presumably due to retention and there is a shift in the protein elution time in the absence of salt indicating that the protein is being partially retained on the column. This suggests that the presence of salt reduces the interaction of protein with the matrix.

Conclusion

The presence or absence of sodium chloride influenced the elution volume of proteins. Presence of salt in the mobile phase led to more defined protein peaks and prevented any protein-matrix interactions, generating more accurate molecular size determination.

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