Effect of pH on Protein Size Exclusion Chromatography

Application Note

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Introduction

Determination of protein structure is key to understanding the function of many proteins. Molecule size is an indicator of structure and provides information on the quaternary structure of the protein. Many proteins undergo profound conformational changes in different solution conditions, with the most extreme being denaturation. Similar structural modifications may occur as a function of ionic strength, pH, temperature and changes in various other solution conditions.

Proteins comprise amino acids, some of which have polar side chains or contain acidic or basic groups. At the isoelectric point, the protein is uncharged and charge repulsions of similar functional groups will be at a minimum, allowing aggregation to take place. Many proteins precipitate under these conditions, and even for proteins that remain in solution at their isoelectric point this is the pH of minimum solubility.

At physiological pH most proteins are above their isoelectric point and have a net negative charge. A pH lower than the isoelectric point causes protonation of acidic groups and a net positive charge on the protein. In each case, the like-charges formed repel each other, reducing the likelihood of protein aggregation. However, in areas of larger charge density the intramolecular repulsion can lead to destabilization of the protein tertiary structure and subsequent unfolding. In some cases, this unfolding may lead to exposure of hydrophobic groups and cause irreversible aggregation.

This application note demonstrates the effect of pH on the elution profiles of a range of globular proteins (γ-globulin, bovine serum albumin (BSA), ovalbumin and myoglobin). The retention behavior was determined using the Agilent ProSEC 300S column in constant ionic strength (0.3 M) mobile phases of high, intermediate and low pH (10.0, 7.0 and 4.0, respectively). Assessments on the importance of pH to protein structure were made by comparison of UV response for each protein at these pH values[1].

[1] ProSEC 300S columns are not designed for continual use above pH 7.5.

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Methods and Materials

Conditions

Samples:	Proteins
Column:	ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Eluent:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 4.0, 7.0 and 10.0
Flow Rate:	1.0 mL/min
Injection Volume:	100 mL
Temperature:	5 °C
Detection:	UV at 310 nm

Results and Discussion

The results in Figures 1-4 indicate that varying the pH affected the chromatography of globular proteins by altering retention time and broadening elution peaks, giving less definition between low and high molecular weight species.

In the case of γ-globulins (Figure 1) and BSA (Figure 2) that have isoelectric points of ~6.5 and ~4.8, respectively, a mobile phase at pH 4.0 caused a shift in the elution time of the protein, suggesting that it was being partially retained on the column or was adopting a smaller conformation in solution. Peaks due to higher oligomers of γ-globulins and BSA were broader and less defined at this pH than at higher values, indicative of increased protein-surface interactions. Figures 3 and 4 demonstrate a similar effect on ovalbumin and myoglobin, whereby eluent at pH 4.0 markedly affected elution volume. The effect of pH on the higher molecular species peaks was even more pronounced with these proteins as at pH 4.0 the monomer and dimer were not distinct individual peaks but a single broad peak.

Myoglobin showed a markedly different elution profile as a function of pH, indicating that the protein may have undergone a large conformational change as a result of protonation. At higher pH the peak shape improved and the presence of a dimer could be clearly seen.

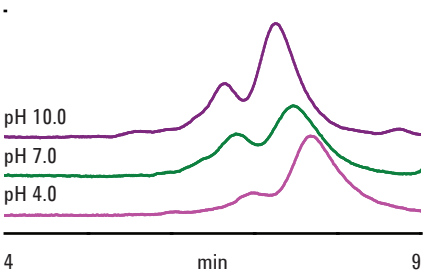

Figure 1. Overlay of UV response for a sample of γ-globulins at varying pH reveals a shift in elution time from partial retention or smaller conformation. The broader peak at pH 4.0 suggests protein-surface interactions

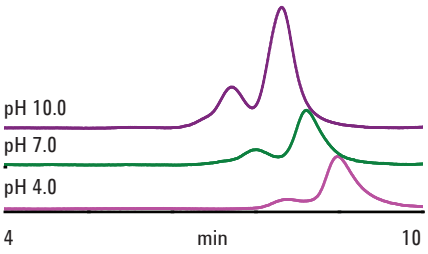

Figure 2. Overlay of UV response for a sample of BSA at varying pH also reveals a shift in elution time from partial retention or smaller conformation. Again, the broader peak at pH 4.0 suggests protein-surface interactions

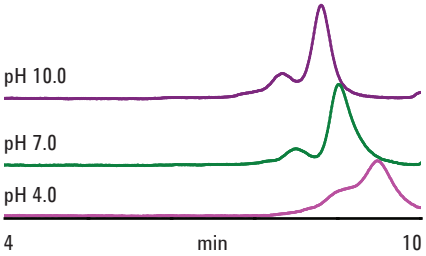

Figure 3. Overlay of UV response for a sample of ovalbumin at varying pH with elution volume shift and indistinct monomer and dimer peak at pH 4.0

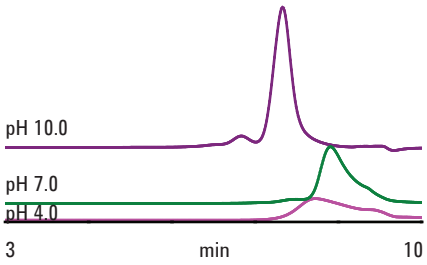

Figure 4. Overlay of UV response for a sample of myoglobin at varying pH, with markedly different elution profiles suggesting protonation-induced conformational change

Conclusion

The pH of the mobile phase influenced the elution profile of globular proteins. Reducing the pH to 4.0 resulted in peaks being less defined and an increase in protein-matrix interactions, partially retaining protein on the column. An increase of eluent pH to 10.0 did not have such an adverse effect on protein elution, with peaks being of similar definition.

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