Agilent Anion-Exchange Media for Proteins - Loading vs Resolution - Effect of Flow Rate and Example Protein Separations

Technical Overview

Introduction

PL-SAX is a hydrophilic strong anion-exchange chromatographic packing material from Agilent Technologies, Inc. The combination of the rigid macroporous polystyrene/divinylbenzene (PS/DVB) polymer matrix and chemically stable quaternized polyethyleneimine coating allows the analysis of biomolecules over a wide range of mobile phase conditions and pH. The physical stability of the media permits their use with high eluent flow rates and high speed gradients for very rapid separations. This excellent stability ensures both rapid equilibration between separations and the use of aggressive cleanup procedures employing high salt, NaOH, mineral and organic acids, and a wide range of organic solvents. In these examples we investigated the effects of different column and packing characteristics using common proteins as experimental analytes.

Agilent Technologies

Separation of ovalbumin and soyabean trypsin inhibitor

For globular protein analysis and purification, the PL-SAX 1000Å material has the optimum pore size for maximum loading with low band broadening. Figure 1 shows the separation of ovalbumin and soyabean trypsin inhibitor at three representative flow rates with resolution factors for their separation calculated for different flow rates, shown in Table 1.

Conditions

Column: PL-SAX 1000Å 8 µm
Buffer: 0.01 M Tris, pH 8, eluting salt NaCl
Flow rate: see Figure 6

$$R_S = \frac{2\,(t_2 - t_1)}{w_2 + w_1}$$

Table 1. Resolution factors calculated for different flow rates.

Flow Rate (mL/min)	R_s
0.5	3.79
1.0	4.27
1.5	4.46
2.0	3.68
3.0	3.37
4.0	3.09

Peak Identification

1. Ovalbumin
2. Soyabean trypsin inhibitor

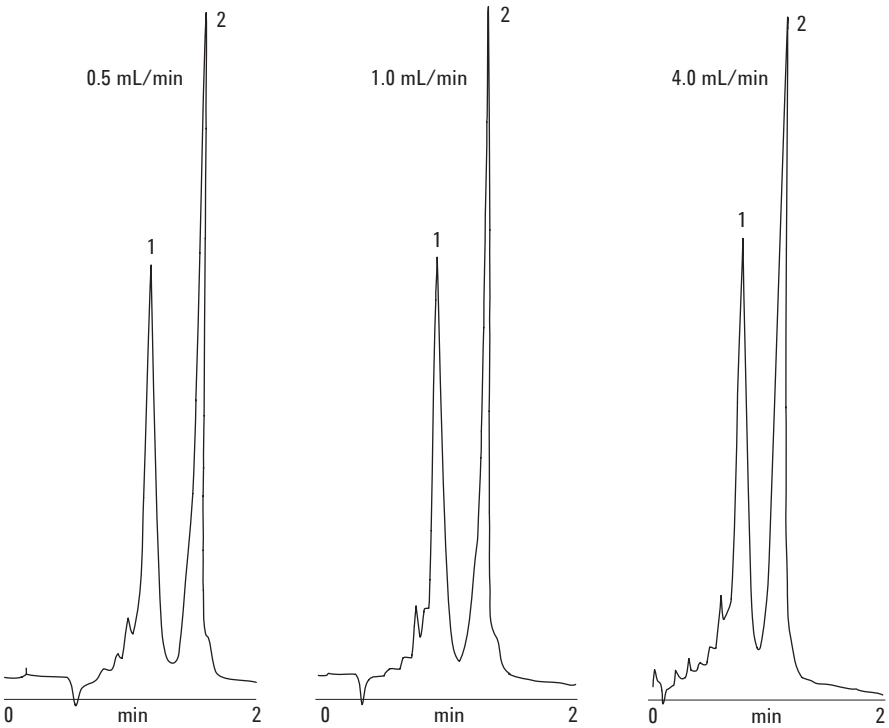

Figure 1. Separation of ovalbumin and soyabean trypsin inhibitor at three representative flow rates on Agilent PL-SAX 1000Å.

Protein loading study with PL-SAX 1000Å

For globular protein analysis and purification, the PL-SAX 1000Å material has the optimum pore size for maximum loading with low band broadening. The more open pore structure of the 4000Å is preferred for high resolution and high speed applications or for the separation of very large biomolecules. The resolution factors for the separation of ovalbumin and soyabean trypsin inhibitor were calculated for various protein loadings using the 1000Å and 4000Å material.

Conditions

Columns: see Figure 2
Eluent A: 0.01 M Tris HCl, pH 8
Eluent B: A + 0.35 M NaCl, pH 8
Gradient: Linear 0-100% B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

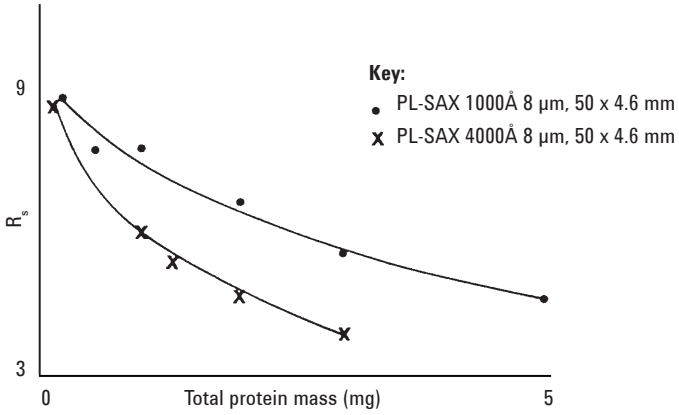

Figure 2. Calculation of resolution factors for various protein loadings using Agilent PL-SAX 1000Å and 4000Å material for the separation of ovalbumin and soyabean trypsin inhibitor.

The resolution factors for the separation of ovalbumin and soyabean trypsin inhibitor were calculated for various protein loadings on PL-SAX 1000Å columns with different internal diameters. With the 10 mm column, over 12 mg of protein can be loaded before the resolution factor falls by 40%. This compares with 1.5 mg on the 5 mm id columns (Figure 3).

Conditions

Columns: PL-SAX 1000Å 8 µm, 50 x 5 mm
PL-SAX 1000Å 8 µm, 100 x 10 mm
Eluent A: 0.01 M Tris HCl, pH 8
Eluent B: A + 0.35 M NaCl, pH 8
Gradient: Linear 0-100% B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

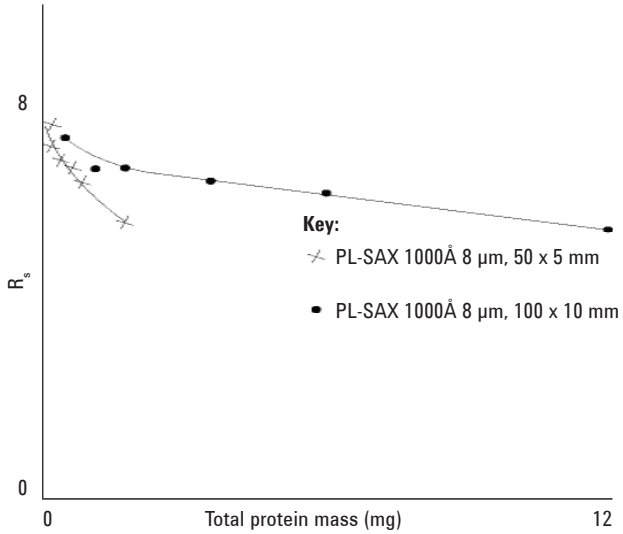

Figure 3. Calculation of resolution factors for various protein loadings on Agilent PL-SAX columns with different internal diameters for the separation of ovalbumin and soyabean trypsin inhibitor.

Protein loading study with PL-SAX 4000Å

Resolution factors for the separation of ovalbumin and soyabean trypsin inhibitor were also calculated for protein loadings using PL-SAX columns of different sizes containing 4000Å, 8 µm material. At low protein loadings (0.1 mg), the resolution of the standard protein mixture is comparable. However, the resolution, as would be expected, falls off more rapidly with the shorter column length. At increased protein loads, less dependency is observed with increasing column id (Figure 4).

Conditions

Columns: PL-SAX 4000Å, 50 x 4.6 mm (p/n PL1551-1803)
PL-SAX 4000Å, 150 x 4.6 mm (p/n PL1551-3803)
Eluent A: 0.01 M Tris HCl, pH 8
Eluent B: A + 0.35 M NaCl, pH 8
Gradient: Linear 0-100% B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

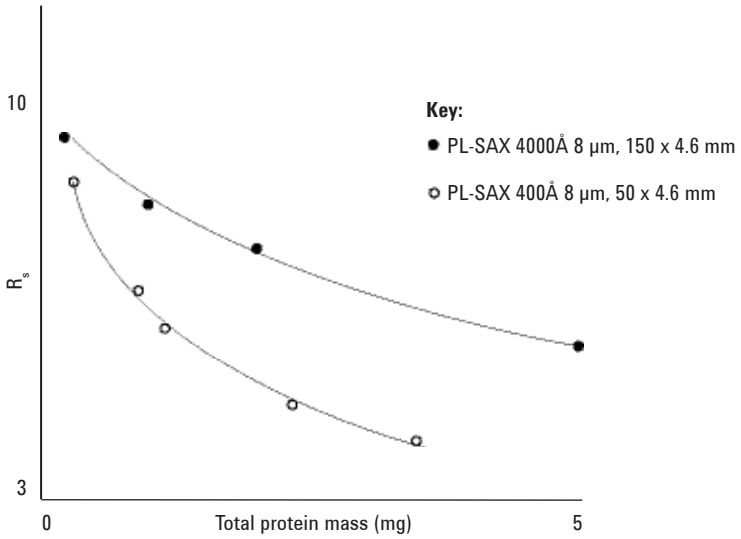

Figure 4. Calculation of resolution factors for various protein loadings using different sized columns containing Agilent PL-SAX 4000Å 8 µm material for the separation of ovalbumin and soyabean trypsin inhibitor.

Protein standards

The physical stability of PL-SAX media permits their use with high eluent flow rates and high speed gradients for rapid separations (Figure 5).

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm
Eluent A: 0.01 M Tris HCl, pH 8
Eluent B: A + 0.35 M NaCl, pH 8
Gradient: Linear 0-100% B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

Peak Identification

1. Myoglobin
2. Conalbumin
3. Ovalbumin
4. Soyabean trypsin inhibitor

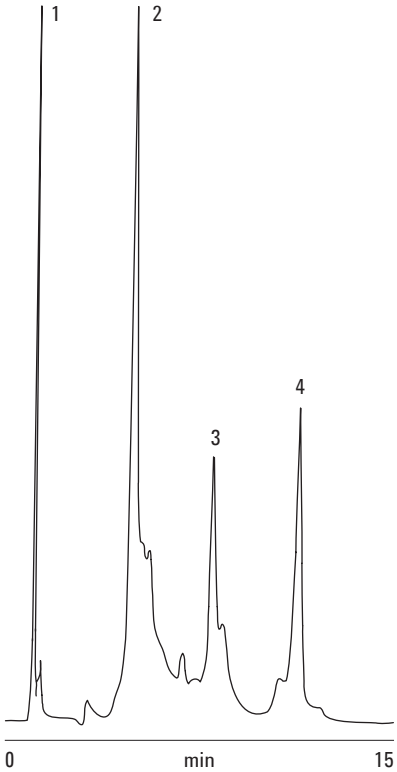

Figure 5. Raw data chromatogram for a mixture of protein standards on Agilent PL-SAX 1000Å.

Analysis of egg white proteins

The hydrophilic, strong anion-exchange chromatographic packing of PL-SAX analyzes many biomolecules over a wide range of mobile phase conditions and pH. The separation of egg white proteins is shown as an example (Figure 6). The frozen egg white was thawed, passed through a 45 µm syringe filter and diluted (1:8) with Eluent A.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.01 M Tris HCl, pH 8
Eluent B: A + 0.5M NaCl, pH 8
Gradient: Linear 0-100% B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

Peak Identification

1. Conalbumin
2. Ovalbumin
3. Unknown

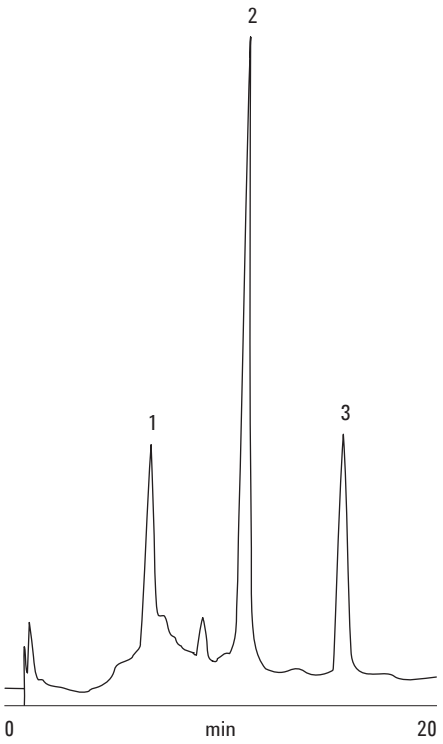

Figure 6. Raw data chromatogram from an Agilent PL-SAX 1000Å column for a mixture of egg white proteins (separation courtesy of Mary Ann Rounds, Purdue University, USA).

Analysis of representative whey proteins

The analysis of representative whey proteins is straightforward on PL-SAX 1000Å columns.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.02 M Tris HCl, pH 7
Eluent B: A + 0.5 M CH_3COONa, pH 7
Gradient: Linear 0-50% B in 10 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

Peak Identification

1. Carbonic anhydrase
2. α-lactalbumin
3. β-lactoglobulin B
4. β-lactoglobulin A

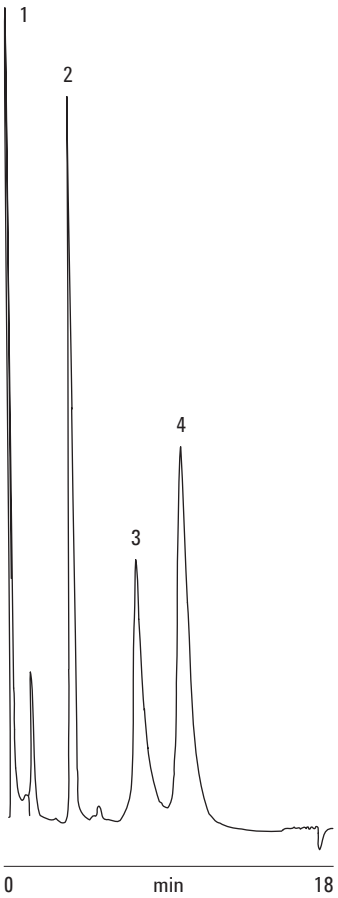

Figure 7. Raw data chromatogram for a mixture of whey proteins on Agilent PL-SAX 1000Å.

Agilent PL-SAX Strong Anion-Exchange Columns

PL-SAX is designed for anion-exchange HPLC separations of proteins and deprotected synthetic oligonucleotides under denaturing conditions. The strong anion-exchange functionality, covalently linked to a chemically stable polymer, extends the operating pH range. What's more, anion-exchange capacity is independent of pH.

These data represent typical results. For further information, contact your local Agilent Sales Office.

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