Characteristics of a Desalting Column for Biomolecular Analysis: the TSKgel BioAssist DS Column

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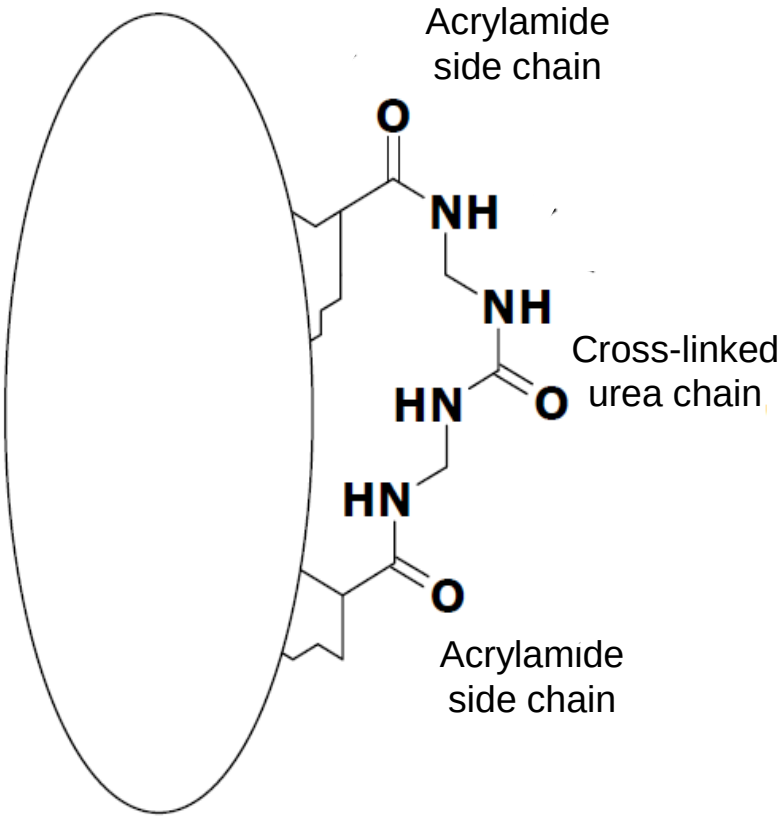
Introduction

- Desalting and buffer exchange of sample solutions containing proteins or polynucleotides are performed by various methods, such as dialysis, ultra filtration, spin-columns, or open or low-pressure size exclusion chromatography (SEC).
- In SEC, molecules differing in their physical size are separated based on their ability to fully or partially enter the pore space of a porous particle. During desalting, the concentration of low molecular weight salts in a protein or polynucleotide solution is reduced, while the protein or polynucleotide concentration usually increases.
- Dextran, cellulose and polyacrylamide are commonly used as porous gel matrices for desalting by SEC. Since these materials can withstand only minimal pressure, the use of small particle sizes to speed up the separation has not been reported in the literature.
- In this study the mechanical strength of polyacrylamide gel was improved by four fold over that of conventional gels by a continued crosslinking process. The increase in strength allowed the use of particle sizes down to 15µm. Further improvements in the manufacturing process substantially narrowed the particle size distribution.

Introduction

- The material, TSKgel BioAssist DS, was packed into PEEK columns of the following dimensions:
  - 4.6mm ID x 15cm
  - 10mm ID x 15cm
- TSKgel BioAssist DS columns are designed for desalting of proteins and polynucleotides at semi-preparative scale. The packing material shows excellent desalting results and high recovery down to ng-level protein injected.
- In this study the basic characteristics of the desalting columns are reported and the columns' properties are demonstrated in several applications.

Chemical Structure of Polyacrylamide Beads



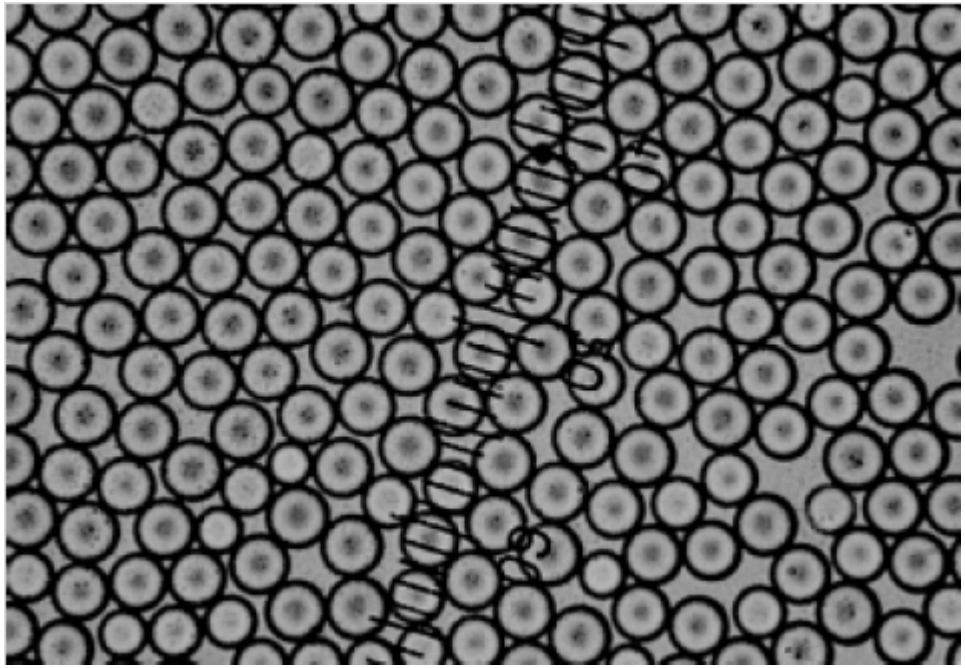
- Backbone of beads is based on polyacrylamide
- Side chains cross-linked with one another through urea
- Highly cross-linked polyacrylamide beads display high mechanical strength and low hydrophobicity

Characteristics of TSKgel BioAssist DS Desalting Column*

Packing Material	
Base polymer	Urea cross-linked polyacrylamide
Particle diameter	15µm (uniform)
Pore size	Excludes ca. 2500 MW PEG
Particle porosity	ca. 60%
Mechanical strength	<4MPa
Column Dimensions	4.6mm ID x 15cm PEEK 10mm ID x 15cm PEEK

* US Patent No. 7,659,348

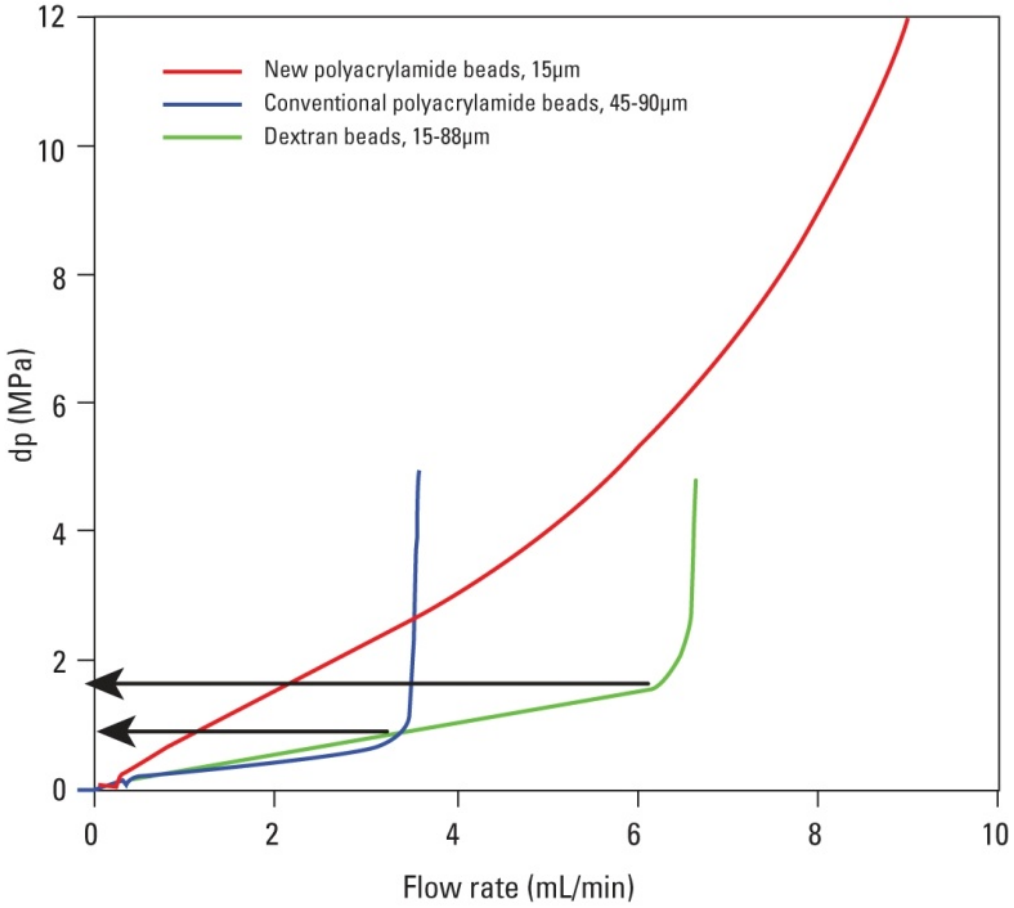
Polyacrylamide Beads



Optical microscopy image of polyacrylamide beads, x200

- **Conventionally, polyacrylamide beads have been prepared by reversed phase suspension polymerization or by using a spray dry method.**
- **The uniform and more pressure-stable polyacrylamide beads packed in TSKgel BioAssist DS columns were prepared using a normal phase suspension method.**

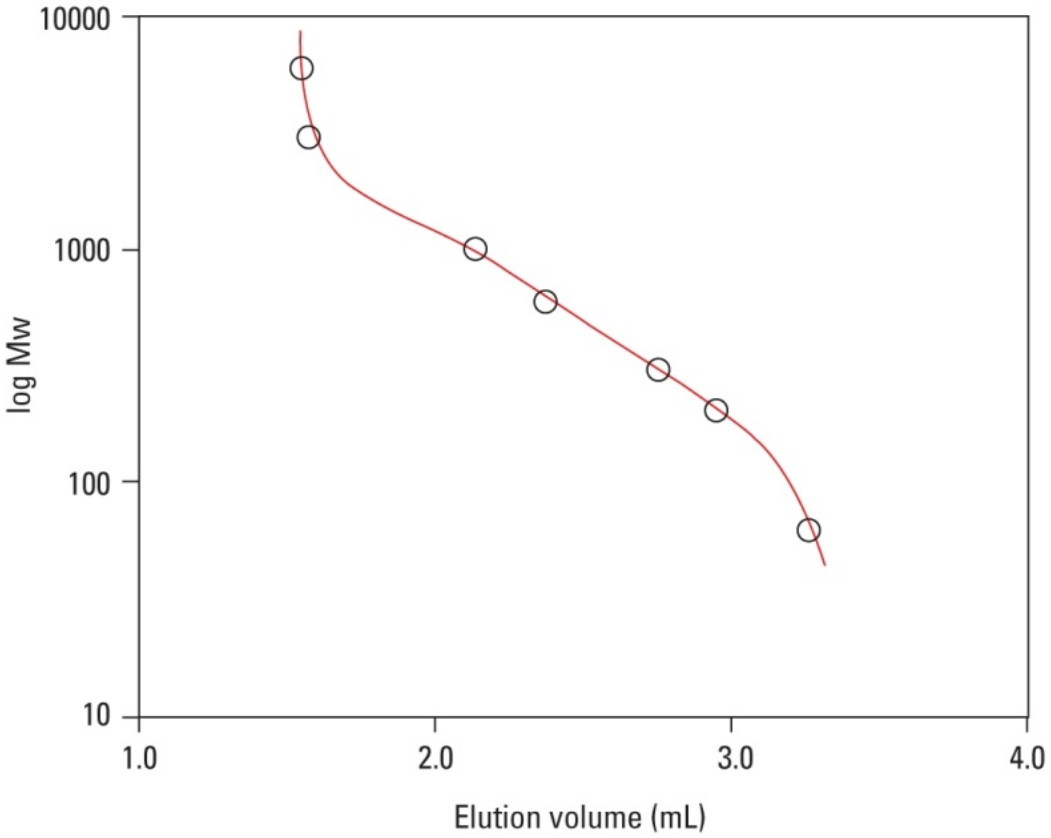
Comparison of Mechanical Strength



Column size: 4.6mm ID x 10cm
Mobile phase: H_2O

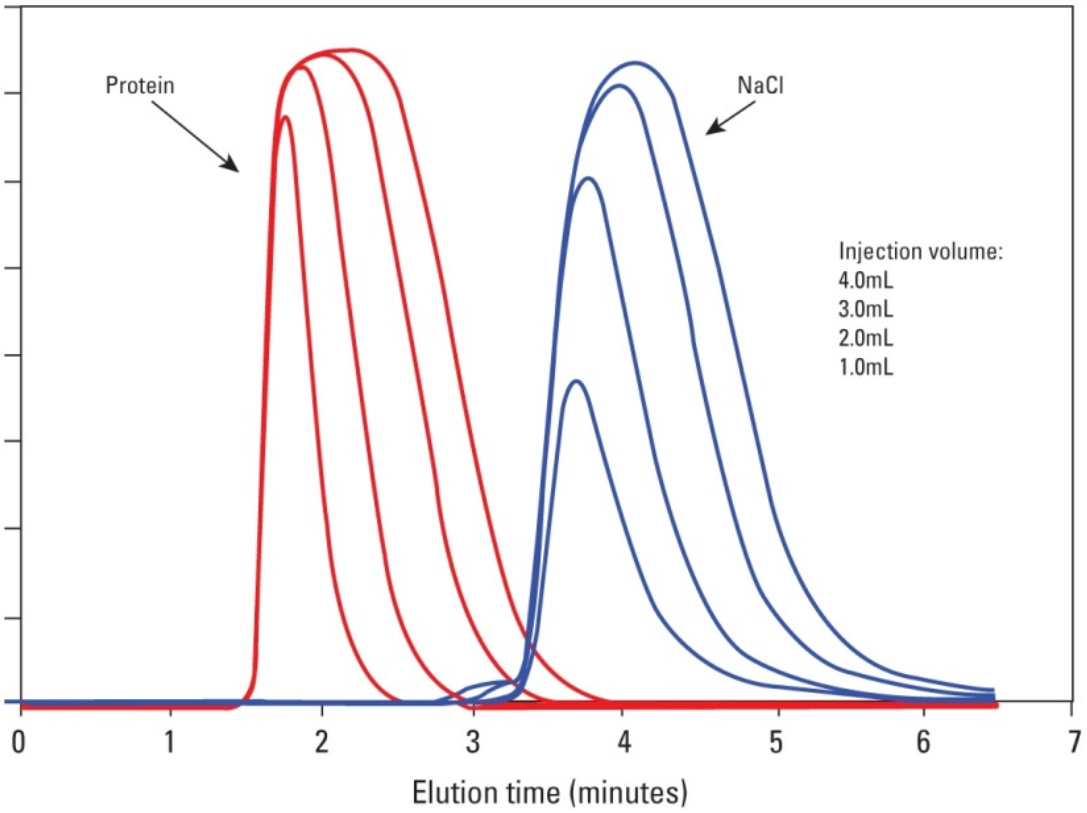
- **The mechanical strength of classical and new polyacrylamide desalting beads were compared. Each type of polyacrylamide was packed into the same size column. Operating pressure was measured at various flow rates.**
- **This figure shows that conventional beads collapse at pressures of 1 to 1.6MPa. TSKgel BioAssist DS polyacrylamide beads did not collapse even if the pressure was increased to 12MPa.**

Calibration Curve for New Polyacrylamide Beads



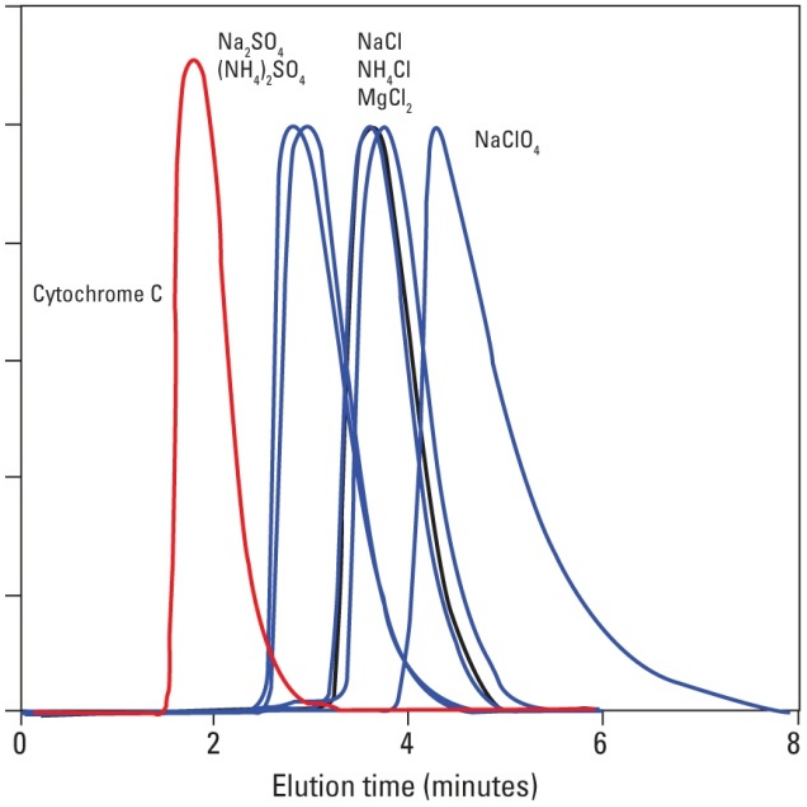
Column:	**TSKgel BioAssist DS, 6.0mm ID x 15cm PEEK**
Mobile phase:	**H_2O**
Flow rate:	**0.5mL/min**
Sample:	**polyethylene glycols and ethylene glycol**

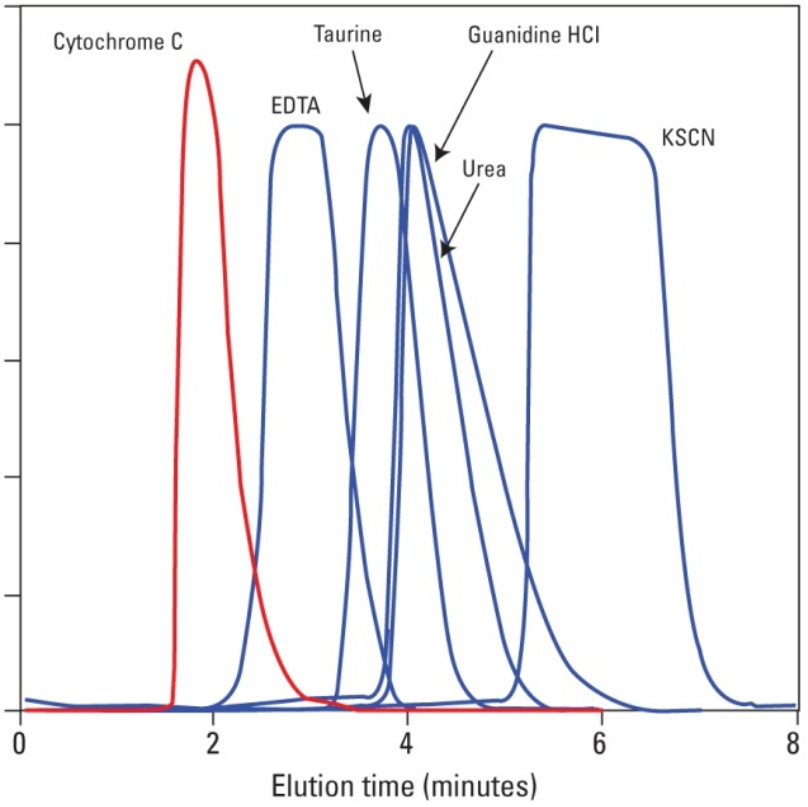
Typical Desalting Chromatograms on TSKgel BioAssist DS Column



Column:	**TSKgel BioAssist DS, 10mm ID x 15cm PEEK**
Mobile phase:	**50mmol/L sodium phosphate buffer, pH 6.5**
Flow rate:	**3.0mL/min**
Temperature:	**4°C**
Samples:	**10mg/mL ovalbumin in 0.5mol/L NaCl and 25mmol/L sodium phosphate buffer, pH 6.5**

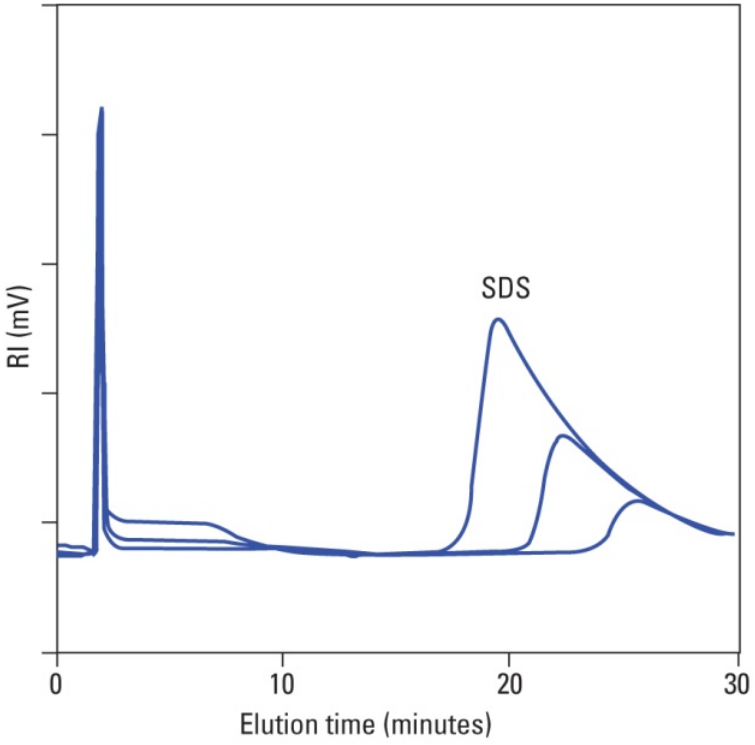
Elution Profile of Various Salts and Additives



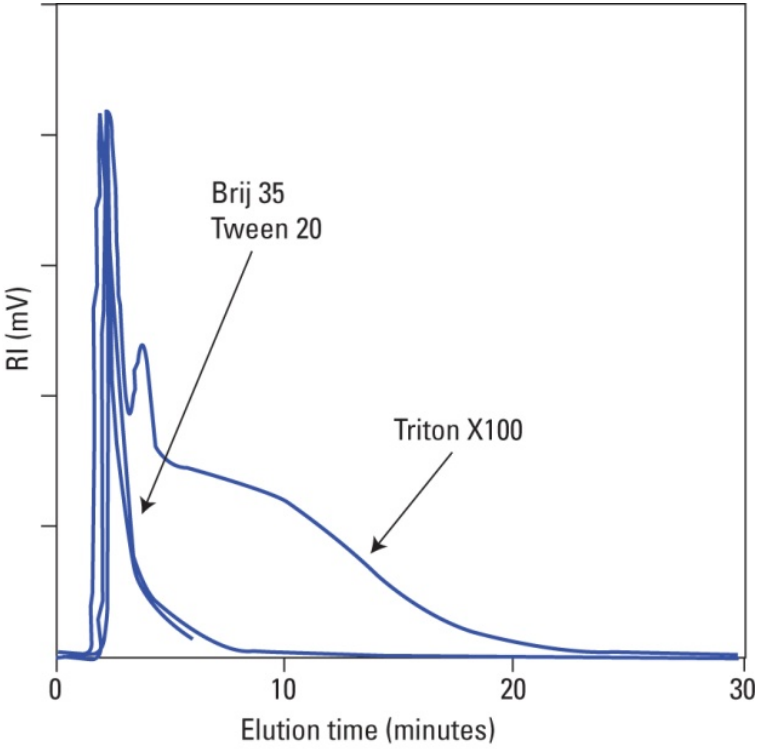


Column:	**TSKgel BioAssist DS, 10mm ID X 15cm PEEK**
Mobile phase:	**H_2O**
Flow rate:	**3.0mL/min**
Temperature:	**4°C**
Injection vol.:	**2mL**
Salt conc.:	**0.5mol/L each**

Elution Profiles for Common Surfactants

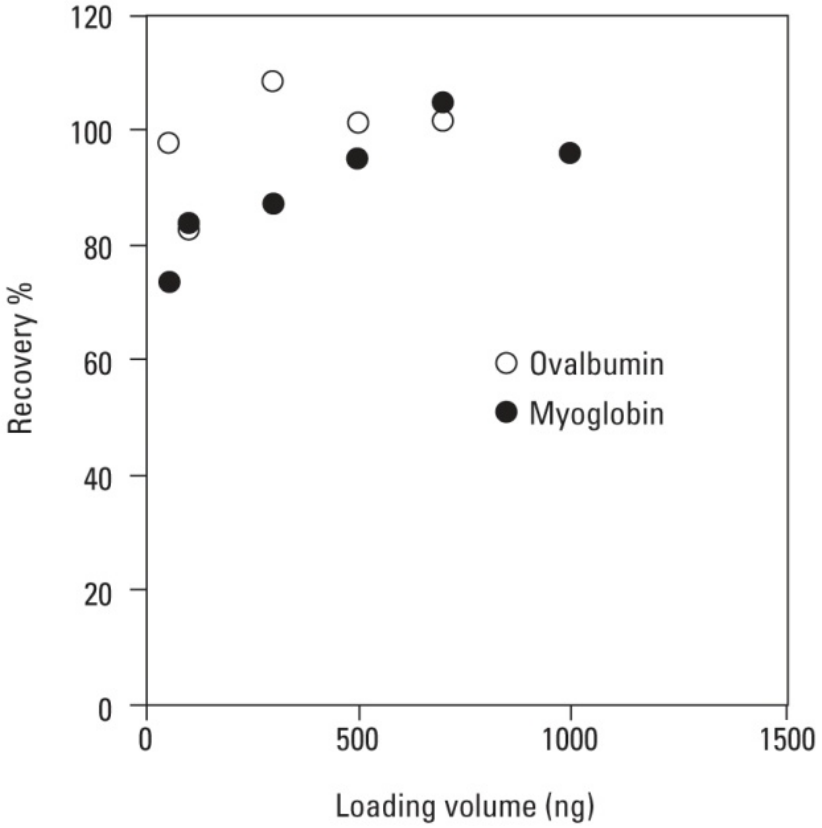


Column:	TSKgel BioAssist DS, 4.6mm ID x 15cm PEEK
Mobile phase:	20mmol/L phosphate buffer, pH 6.9
Flow rate:	1.0mL/min
Temperature:	25°C
Sample:	0.1-0.5wt% SDS in eluent

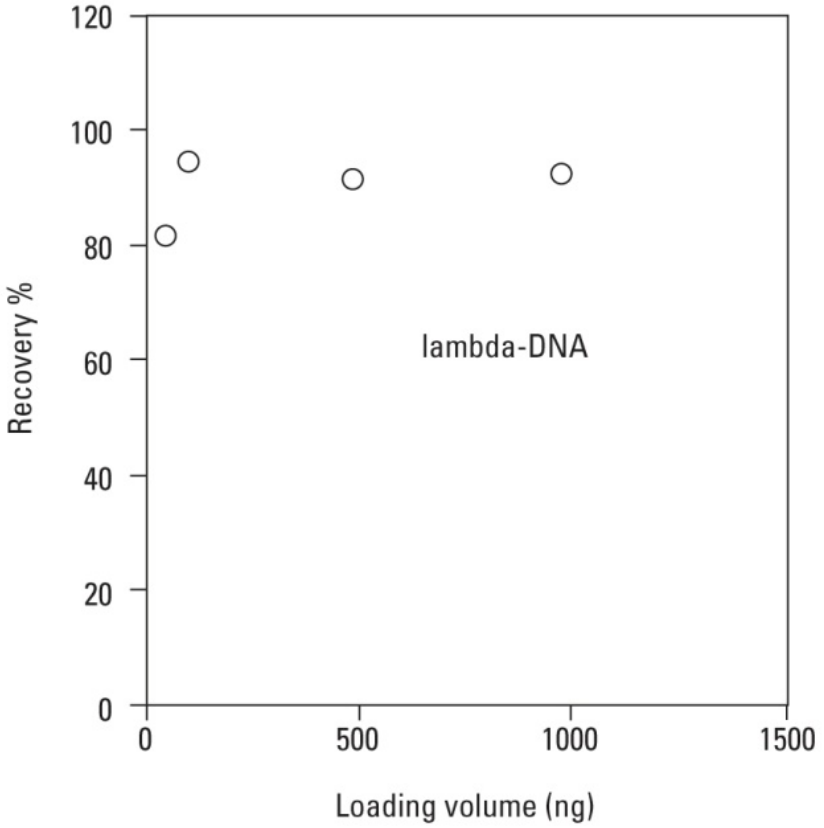


Column:	TSKgel BioAssist DS, 10mm ID x 15cm PEEK
Mobile phase:	D-H_2O
Flow rate:	3.0mL/min
Temperature:	25°C

Recovery from TSKgel BioAssist DS Column

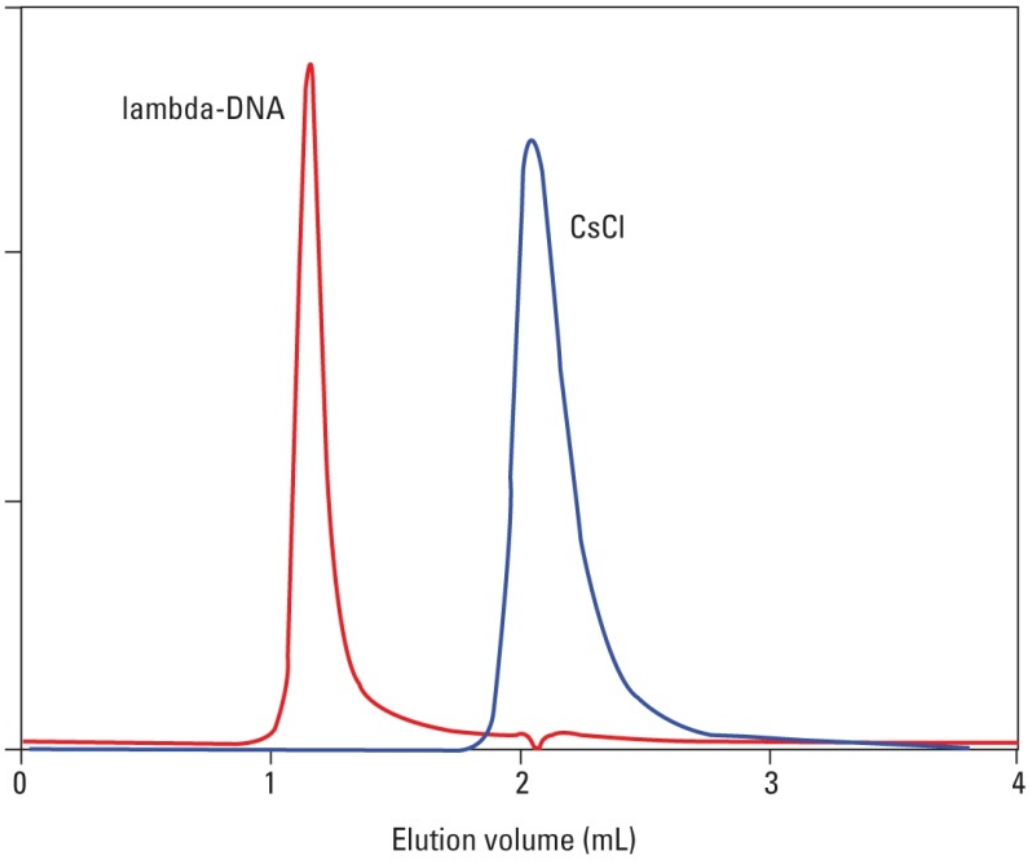


Column: TSKgel BioAssist DS, 4.6mm ID x 10cm PEEK
Mobile phase: 20mmol/L Tris-HCl buffer, pH 8.0 for ovalbumin
20mmol/L phosphate buffer, pH 6.5 for myoglobin



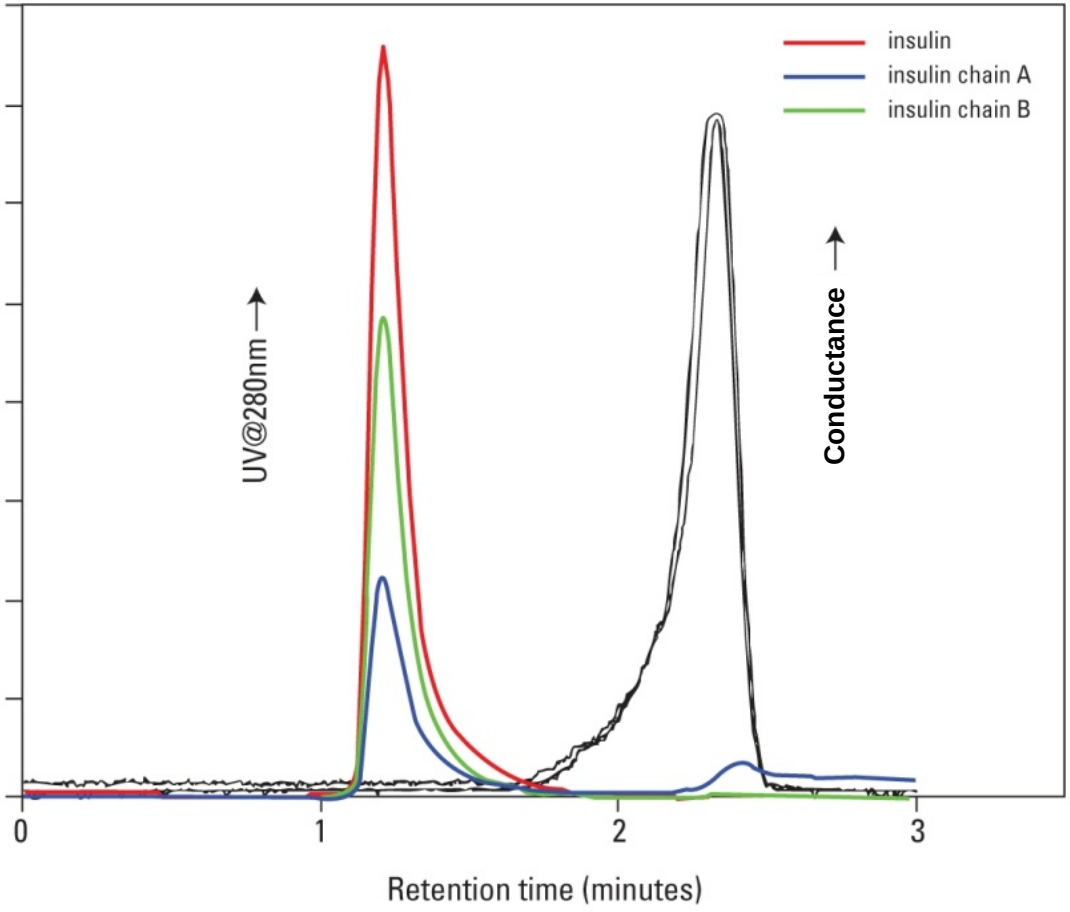
Column: TSKgel BioAssist DS, 4.6mm ID x 10cm PEEK
Mobile phase: 20mmol/L Tris-HCl buffer, pH 7.8

Desalting DNA in CsCl Solution on TSKgel BioAssist DS column



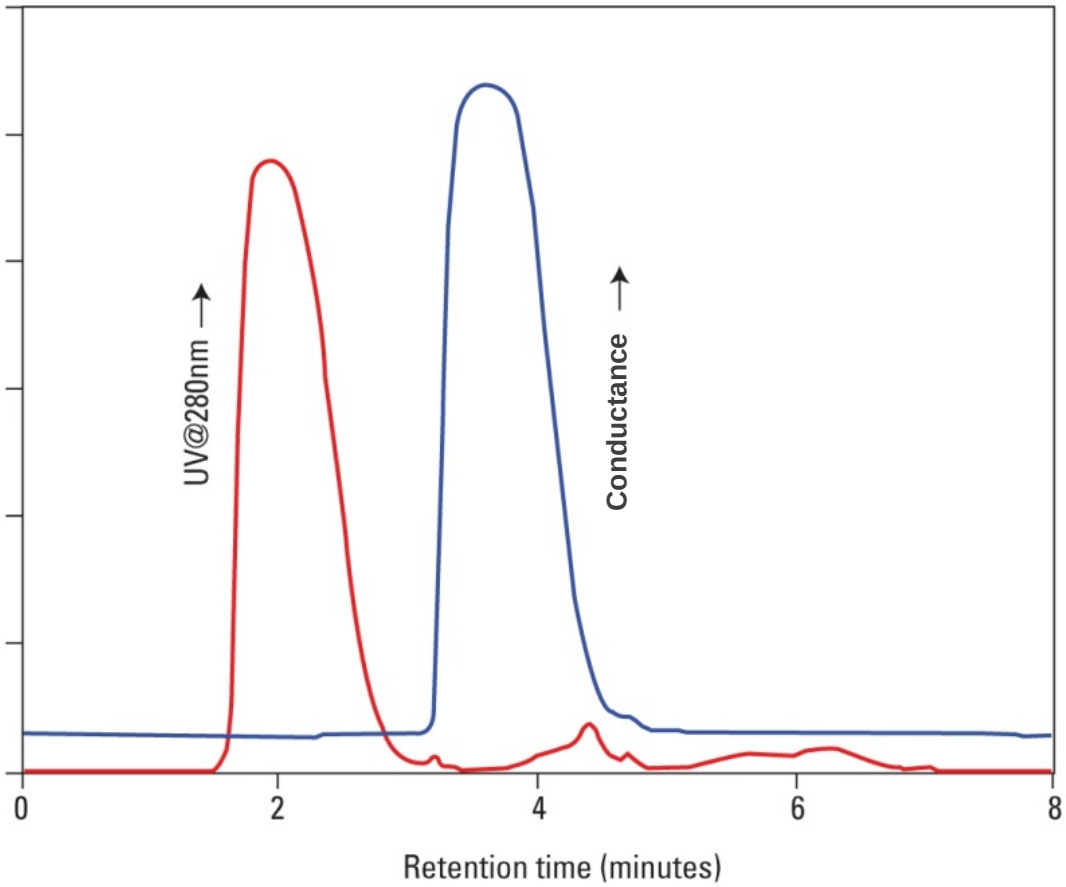
Column:	**TSKgel BioAssist DS, 4.6mm ID x 10cm PEEK**
Mobile phase:	**20mmol/L Tris-HCl buffer, pH 7.8**
Flow rate:	**1.0mL/min**
Samples:	**25µg/mL lambda DNA in 1mol/L CsCl and 20mmol/L Tris-HCl buffer, pH 7.8**

Desalting Insulin on TSKgel BioAssist DS Column



Column:	**TSKgel BioAssist DS, 4.6mm ID x 10cm PEEK**
Mobile phase:	**20mmol/L Tris-HCl buffer, pH 8.5**
Flow rate:	**1.0mL/min**
Temperature:	**4°C**
Injection vol.:	**100µL**
Samples:	**1mg/mL insulin in 0.5mol/L NaCl and 20mmol/L Tris-HCl buffer, pH 8.5**

Desalting mAb in Mouse Ascites by TSKgel BioAssist DS Column



Column:	**TSKgel BioAssist DS, 10mm ID x 15cm PEEK**
Mobile phase:	**20mmol/L Tris-HCl , pH 8.0**
Flow rate:	**3.0mL/min**
Sample:	**mouse ascites fluid diluted with 0.2mol/L NaCl (0.45µm filter)**

Conclusions

- Novel hydrophilic highly cross-linked polyacrylamide beads showed very high mechanical strength compared with conventional hydrophilic polyacrylamide beads and cross-linked dextran beads.
- Uniform particle size polyacrylamide beads were obtained using a normal phase suspension polymerization method.
- Polyacrylamide beads, packed in PEEK columns (TSKgel BioAssist DS), provided excellent desalting performance.
- Recovery of sample from the column was 80% or better when injecting as little as 50ng protein or polynucleotide on the column.