

Introduction

The recent development of bio-pharmaceutical industry has been remarkable, and shortening the development time and reducing the cost become increasingly important. The development of efficient, economical and selective separation method is required for successful commercialization of bio-pharmaceutical products. To meet these demands, we have developed new polymeric resins named YMC-BioPro series, which are specially designed for ion exchange (IEX) separation and purification of proteins, peptides and nucleic acids. YMC-BioPro series includes the packed columns with 5 μm porous/non-porous polymer for analysis and laboratory scale purification, and the bulk materials of 75 μm porous polymer for capture and intermediate purification. The both materials are based on the same hydrophilic polymer beads with low nonspecific adsorption. Compared to conventional materials available in the market, the BioPro series shows higher binding capacity and higher recovery of biomolecules.

As for the analytical BioPro columns, 5 μm completely spherical and monodispersed beads, with optimal packing technology, provide high theoretical plate number and symmetrical peak shape. Excellent resolution is achieved from the high column efficiency coupled with the excellent selectivity of QA (quarternary ammonium) and SP (sulfopropyl) ion exchangers.

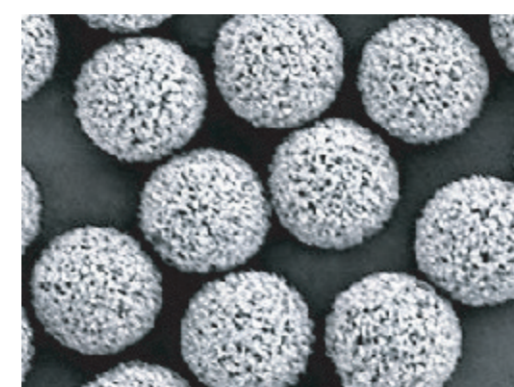
The bulk materials of 75 μm porous polymer resin, which have increased binding capacity and low pressure drop, are ideal for capture and intermediate purification steps. BioPro Q75 and S75 has similar retention selectivity to 5 μm porous type BioPro QA and SP, respectively, and it allows predictable scale-up from analytical to preparative separation in bio-processes.

In this poster, we will show benefits of YMC-BioPro series and some example cases of superior separation of important biomolecules, such as monoclonal antibody and DNA.

Features of new porous polymer resins for ion exchange chromatography 5 μm YMC-BioPro QA / SP columns and 75 μm YMC-BioPro Q75 / S75 bulk materials

- Newly developed hydrophilic porous polymer with low nonspecific adsorption
- Excellent resolution, high binding capacity and high recovery of biomolecules
- 5 μm packed column for analysis and laboratory-scale purification
- 75 μm bulk materials for capture and intermediate purification

| | YMC-BioPro QA | YMC-BioPro SP | YMC-BioPro Q75 | YMC-BioPro S75 |
|--|---|---|---|---|
| Matrix | hydrophilic porous polymer beads | | | |
| Particle size (μm) | 5 | | 75 | |
| Charged group | -CH ₂ N ⁺ (CH ₃) ₃ | -(CH ₂) ₃ SO ₃ ⁻ | -CH ₂ N ⁺ (CH ₃) ₃ | -(CH ₂) ₄ SO ₃ ⁻ |
| Dynamic binding capacity (mg/ml-resin) | > 110 (BSA) | > 70 (human-IgG) | > 160 (BSA) | > 160 (Lysozyme) |
| Available pH range | 2-12 | 2-12 | 2-12 | 2-12 |
| Column size length X i. d. (mm) | 50 X 4.6 | | Bulk materials (customized column size) | |

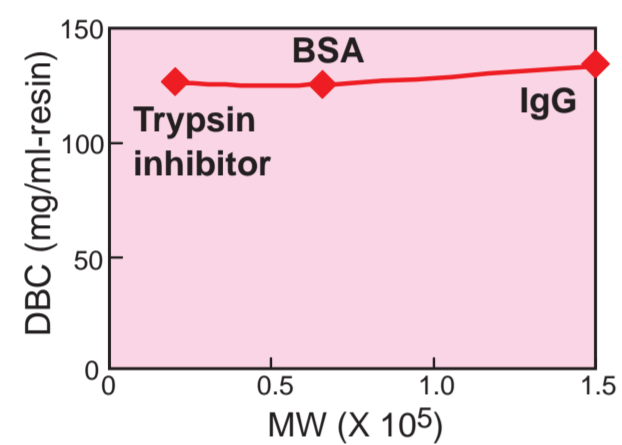


SEM images of 5 μm porous polymer beads

Dynamic binding capacity (DBC) and recovery of proteins

Comparison of dynamic binding capacity and recovery for various proteins using YMC-BioPro QA and SP

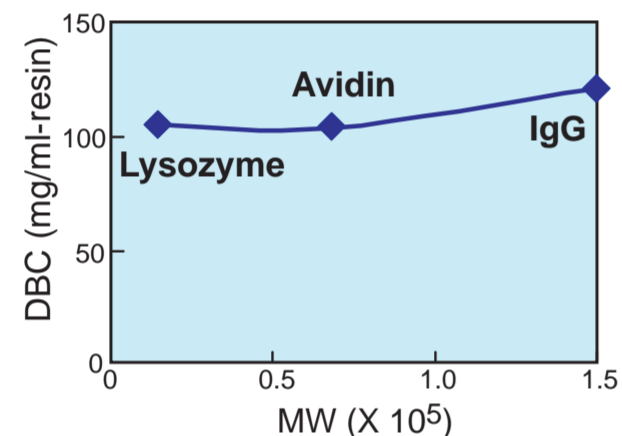
YMC-BioPro QA



| Protein | MW | DBC ^{*1} (mg/ml-resin) | Eluted amount (mg/ml-resin) | Recovery ^{*2} (%) |
|-------------------|---------|---------------------------------|-----------------------------|----------------------------|
| IgG | 150,000 | 134 | 120 | 90 |
| BSA | 66,000 | 126 | 120 | 95 |
| Trypsin inhibitor | 20,100 | 127 | 120 | 95 |

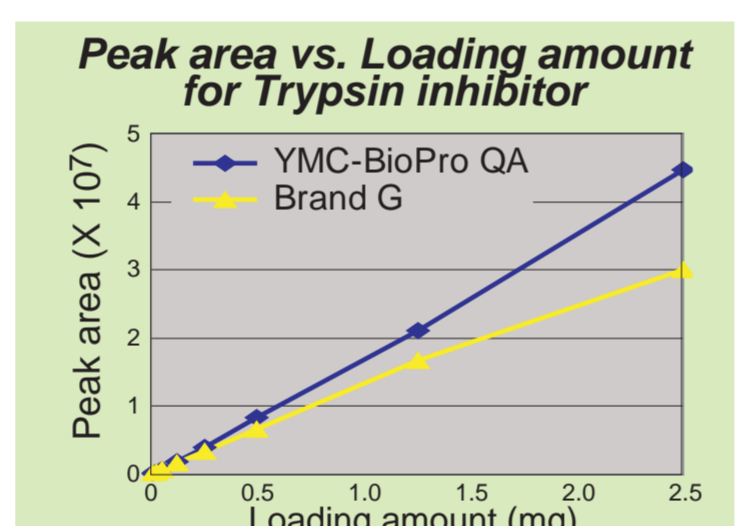
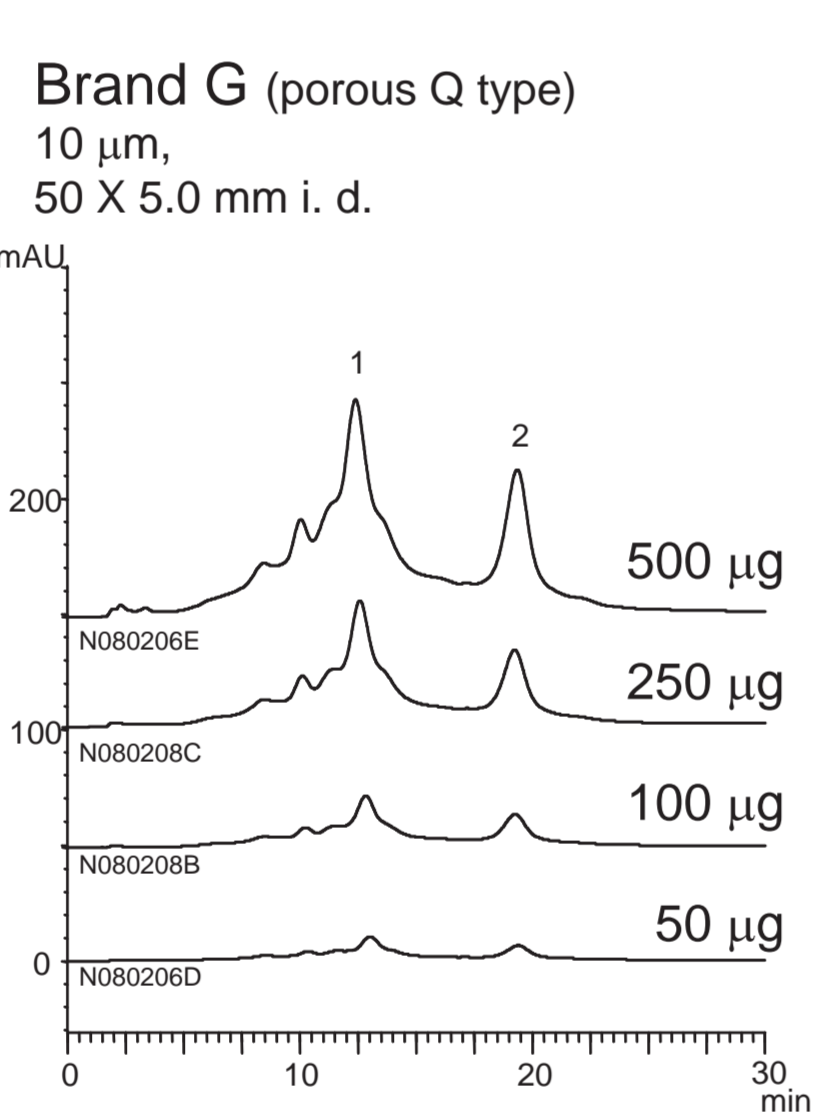
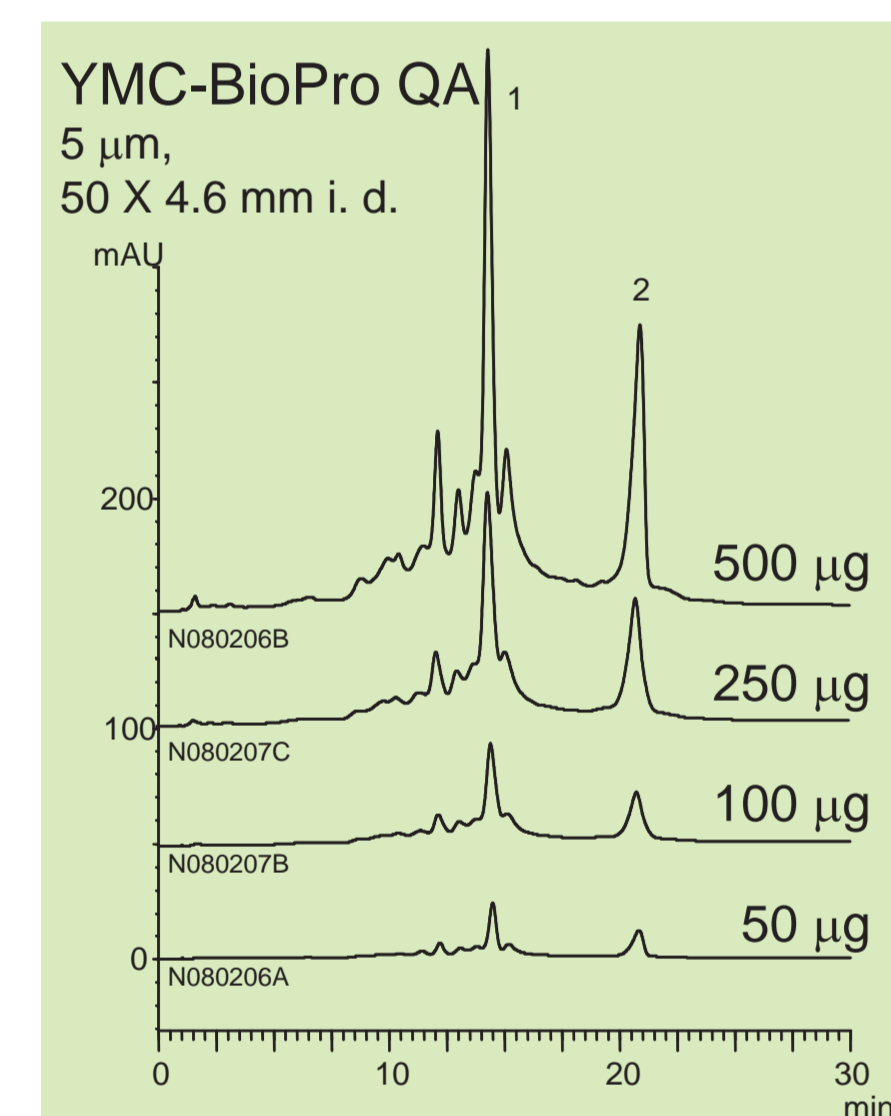
5 μm YMC-BioPro QA and SP gives the superior DBC and recovery for various proteins which have different molecular weight (MW). The hydrophilic properties of the matrix polymer remarkably reduce nonspecific adsorption of proteins on YMC-BioPro resins.

YMC-BioPro SP



| Protein | MW | DBC ^{*1} (mg/ml-resin) | Eluted amount (mg/ml-resin) | Recovery ^{*2} (%) |
|----------|---------|---------------------------------|-----------------------------|----------------------------|
| IgG | 150,000 | 119 | 117 | 98 |
| Avidin | 68,000 | 101 | 93 | 92 |
| Lysozyme | 14,300 | 103 | 110 | 107 |

Comparison of the effect of sample load on YMC-BioPro QA and commercial porous polymer Q type product



| Eluent | Gradient | Flow rate | Temperature | Detection | Injection | Sample |
|--|--------------------|---|-------------|--------------|-----------|--------------------------------------|
| A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl | 10-80%B (0-30 min) | 0.5 ml/min (180 cm/hr for 4.6 mm i. d., 150 cm/hr for 5.0 mm i. d.) | 25°C | UV at 280 nm | 100 μl | 1. Ovalbumin 2. Trypsin inhibitor |

YMC-BioPro QA shows the excellent peak shapes even when the loading amount increases. The column of Brand G cannot achieve acceptable peak shapes and resolution even in small amount of injection. The excellent linearity is observed between peak area and loading amount for Trypsin inhibitor on YMC-BioPro QA. These results indicate that YMC-BioPro QA would be suitable for laboratory-scale purification of proteins.

Higher DBC of 75 μm BioPro resins for capture and intermediate purification

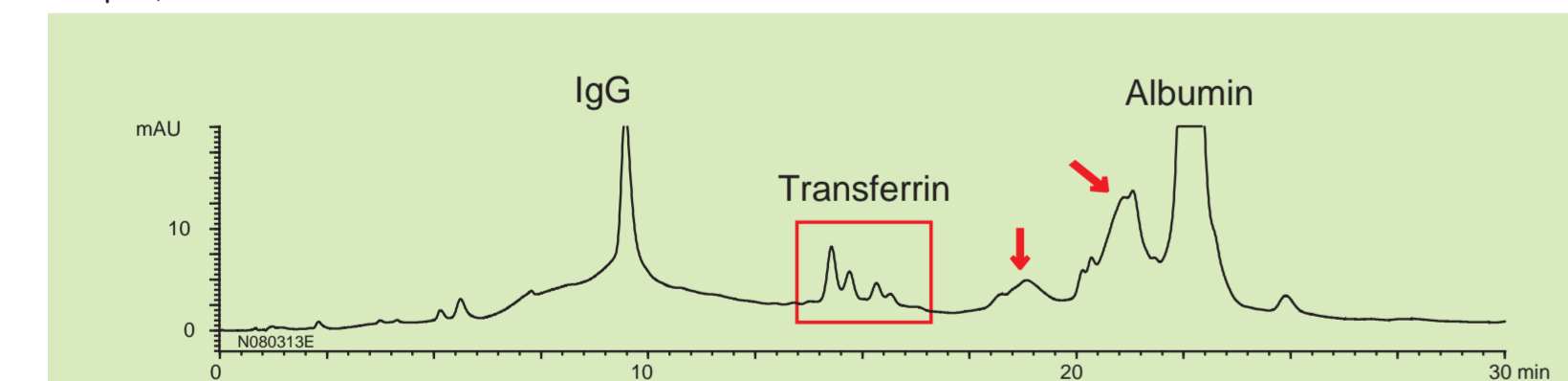
| Resin | Particle size (μm) | Ion-exchange capacity (meq/ml-resin) | DBC ^{*3} (mg/ml-resin) | Resin | Particle size (μm) | Ion-exchange capacity (meq/ml-resin) | DBC ^{*4} (mg/ml-resin) |
|-------------------------|--------------------|--------------------------------------|---------------------------------|-------------------------|--------------------|--------------------------------------|---------------------------------|
| YMC-BioPro QA | 5 | 0.08 | 118 | YMC-BioPro SP | 5 | 0.09 | 103 |
| YMC-BioPro Q75 | 75 | 0.13 | 187 | YMC-BioPro S75 | 75 | 0.12 | 187 |
| Brand G (porous Q type) | 90 | 0.19 | 102 | Brand G (porous Q type) | 90 | 0.13 | 134 |

The DBC of 75 μm BioPro resin is about 1.4-1.8 times as high as that of 5 μm BioPro and also that of a commercially available resin for capture purification (90 μm, Brand G). 75 μm BioPro would give increased productivity and reduced cost in bio-processes.

Comparison of protein separation on porous polymer anion-exchange columns

Analysis of proteins in human serum

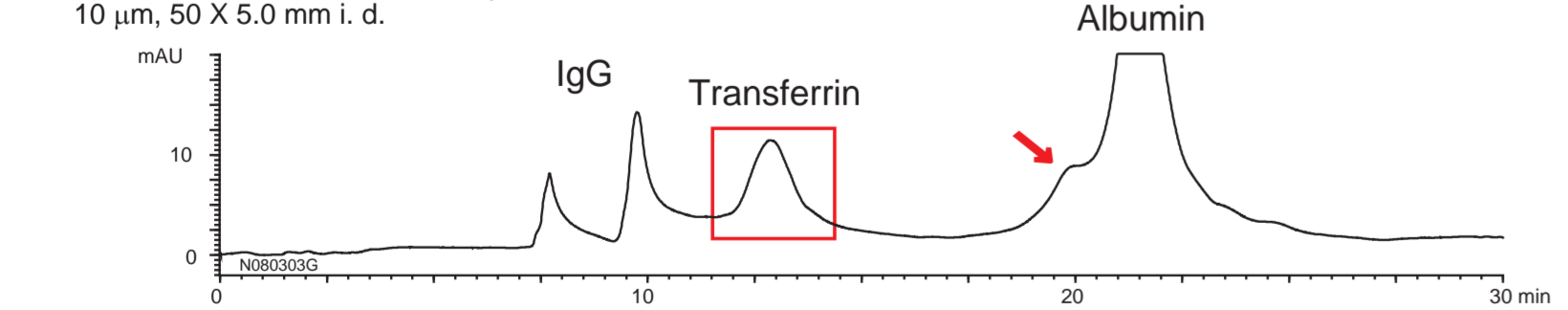
YMC-BioPro QA
5 μm, 50 X 4.6 mm i. d.



| Eluent | Gradient | Flow rate | Temperature | Detection | Injection | Sample |
|--|---|---|-------------|--------------|-----------|-------------------------|
| A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl | 0-30%B (0-15 min), 30-100%B (15-30 min) | 0.5 ml/min (180 cm/hr for 4.6 mm i. d., 150 cm/hr for 5.0 mm i. d.) | 25°C | UV at 280 nm | 20 μl | Human serum (100 μl/ml) |

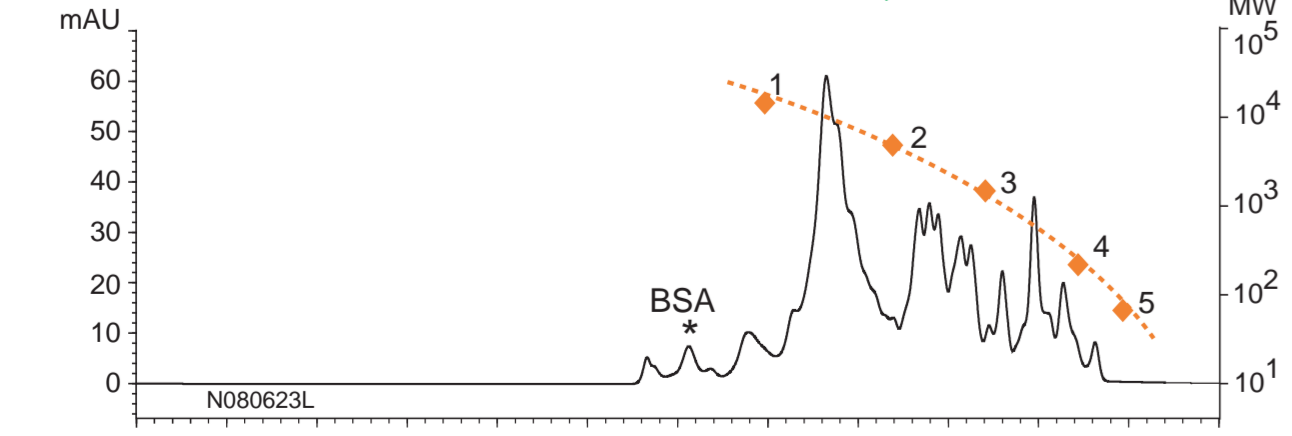
The separation of the proteins in human serum is compared among YMC-BioPro QA and a commercial porous polymer anion-exchange column. YMC-BioPro QA shows superior resolution in analysis of biological samples containing a large amount of impurities.

Brand G (porous Q type)
10 μm, 50 X 5.0 mm i. d.



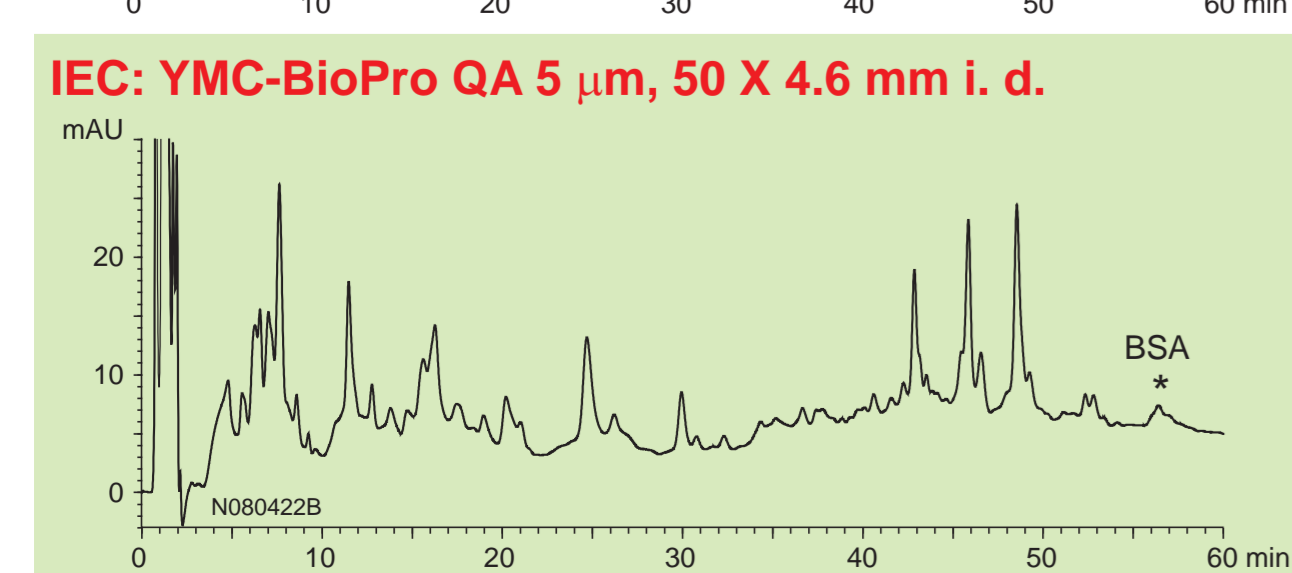
Comparison of separation of BSA tryptic digests on between IEC and SEC

SEC: YMC-Pack Diol-120 + Diol-60 5 μm, 500 X 8.0 mm i. d. X 2



| Eluent | Flow rate | Temperature | Detection | Injection |
|---|-----------------------|----------------|--------------|-----------|
| A) 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl/acetonitrile (70/30) | 0.7 ml/min (85 cm/hr) | ambient (25°C) | UV at 220 nm | 5 μl |

These chromatograms show separation of tryptic digests of BSA (MW:66,000) on ion-exchange chromatography (IEC)-column and size-exclusion chromatography (SEC)-column. The molecular weight (MW) of the digests is estimated to be approximately from 100 to 20,000 by SEC chromatogram. IEC chromatogram shows YMC-BioPro column is also applicable for separation of low-MW peptides.

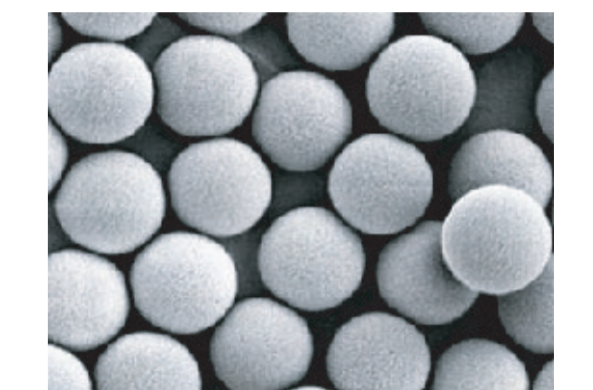


| Eluent | Flow rate | Temperature | Detection | Injection |
|--|--|-------------|--------------|-----------|
| A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl | 0-15%B (0-30 min), 15-60%B (30-60 min) | 25°C | UV at 220 nm | 20 μl |

Features of new non-porous polymer resins for ion exchange chromatography YMC-BioPro QA-F / SP-F

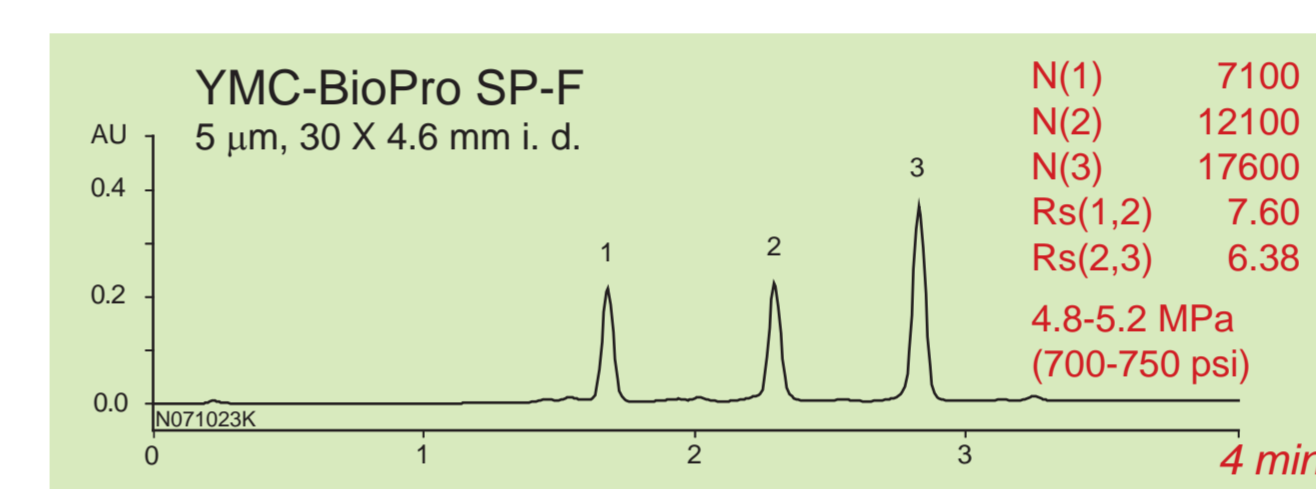
- Non-porous polymer beads with high chemical and mechanical stabilities
- 30 mm-length column for high-throughput analysis with low operating pressure
- 100 mm-length column for high-resolution analysis

| | YMC-BioPro QA-F | YMC-BioPro SP-F |
|--|---|---|
| Matrix | hydrophilic non-porous polymer beads | |
| Particle size (μm) | 5 | |
| Charged group | -CH ₂ N ⁺ (CH ₃) ₃ | -(CH ₂) ₃ SO ₃ ⁻ |
| Dynamic binding capacity (mg/ml-resin) | > 12 (BSA) | > 10 (human-IgG) |
| Available pH range | 2-12 | 2-12 |
| Column size length X i. d. (mm) | 30 X 4.6, 50 X 4.6, 100 X 4.6 | |

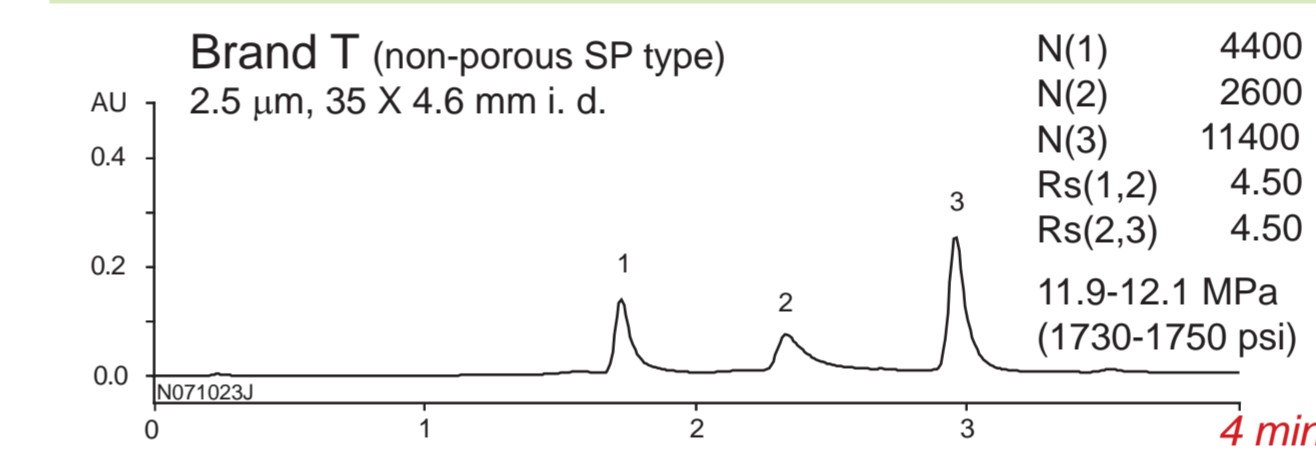


SEM images of 5 μm non-porous polymer beads

High-throughput analysis of proteins on non-porous polymer cation-exchange columns



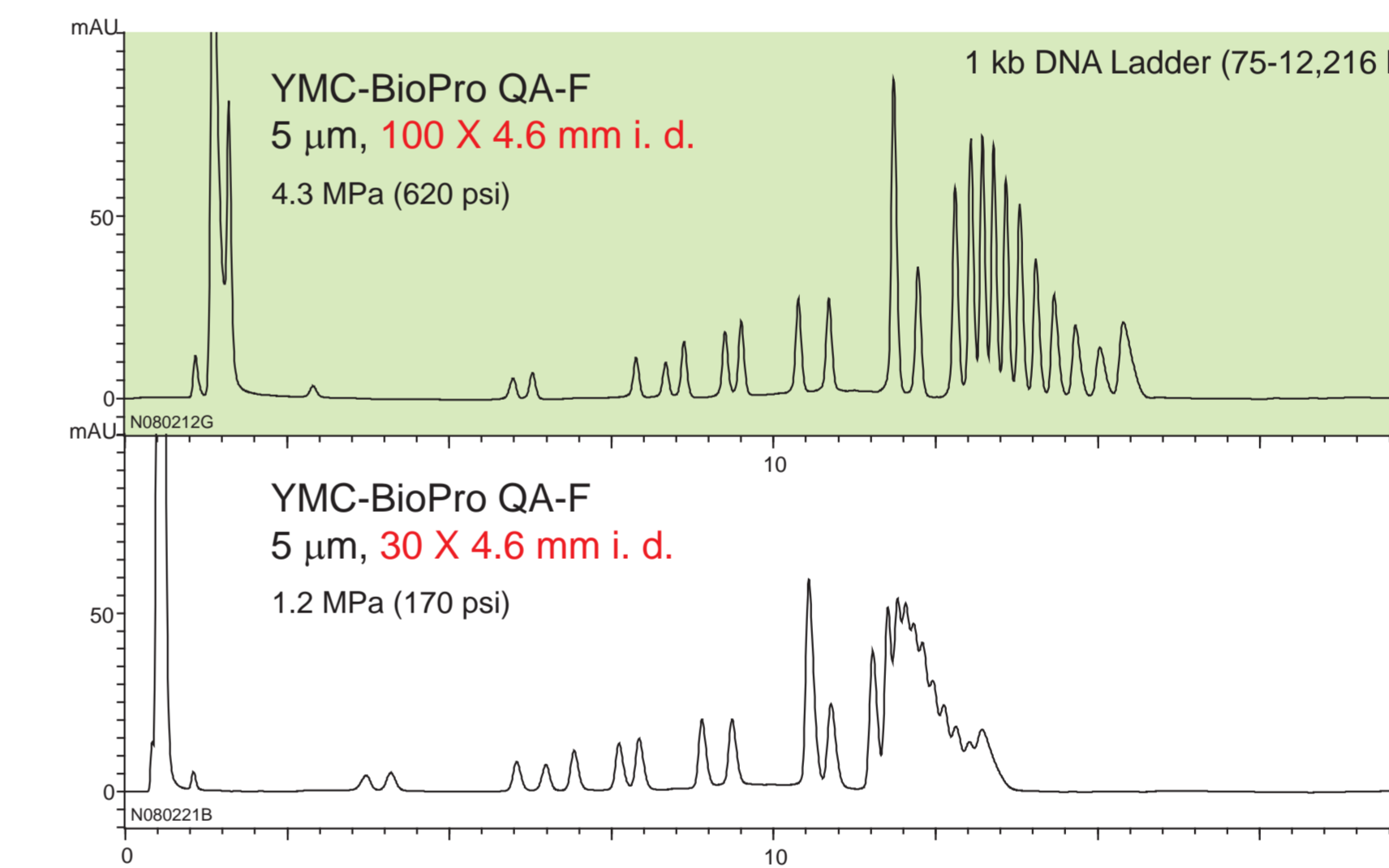
| Eluent | Gradient | Flow rate | Temperature | Detection | Injection | Sample |
|--|-------------------|------------------------|-------------|--------------|-----------|---|
| A) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.8) B) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.8) containing 0.5 M NaCl | 0-100%B (0-4 min) | 1.5 ml/min (540 cm/hr) | 25°C | UV at 220 nm | 20 μl | 1. Ribonuclease A (0.1 mg/ml) 2. Cytochrome c (0.1 mg/ml) 3. Lysozyme (0.1 mg/ml) |



YMC-BioPro SP-F can elute the proteins sharply without peak-tailing rather than commercial non-porous SP column, Brand T. Furthermore, despite larger particle size, the theoretical plate number of SP-F is higher than that of Brand T.

High-resolution analysis with 100 mm-length column packed with non-porous polymer

Analysis of DNA fragments

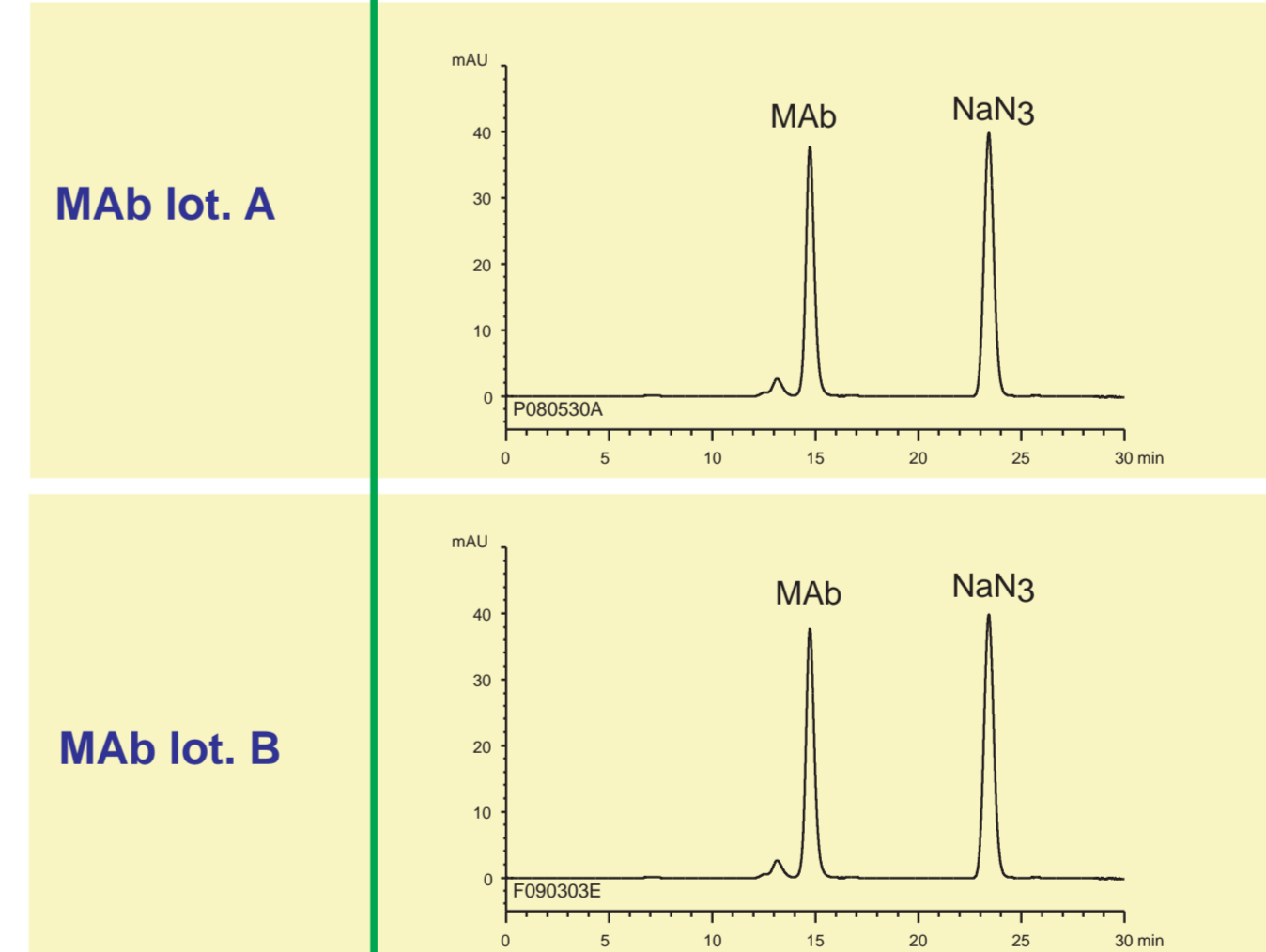


| Eluent | Gradient | Flow rate | Temperature | Detection | Injection |
|--|---------------------|------------------------|-------------|--------------|--------------------|
| A) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl | 40-100%B (0-30 min) | 0.5 ml/min (180 cm/hr) | 25°C | UV at 260 nm | 20 μl (0.25 mg/ml) |

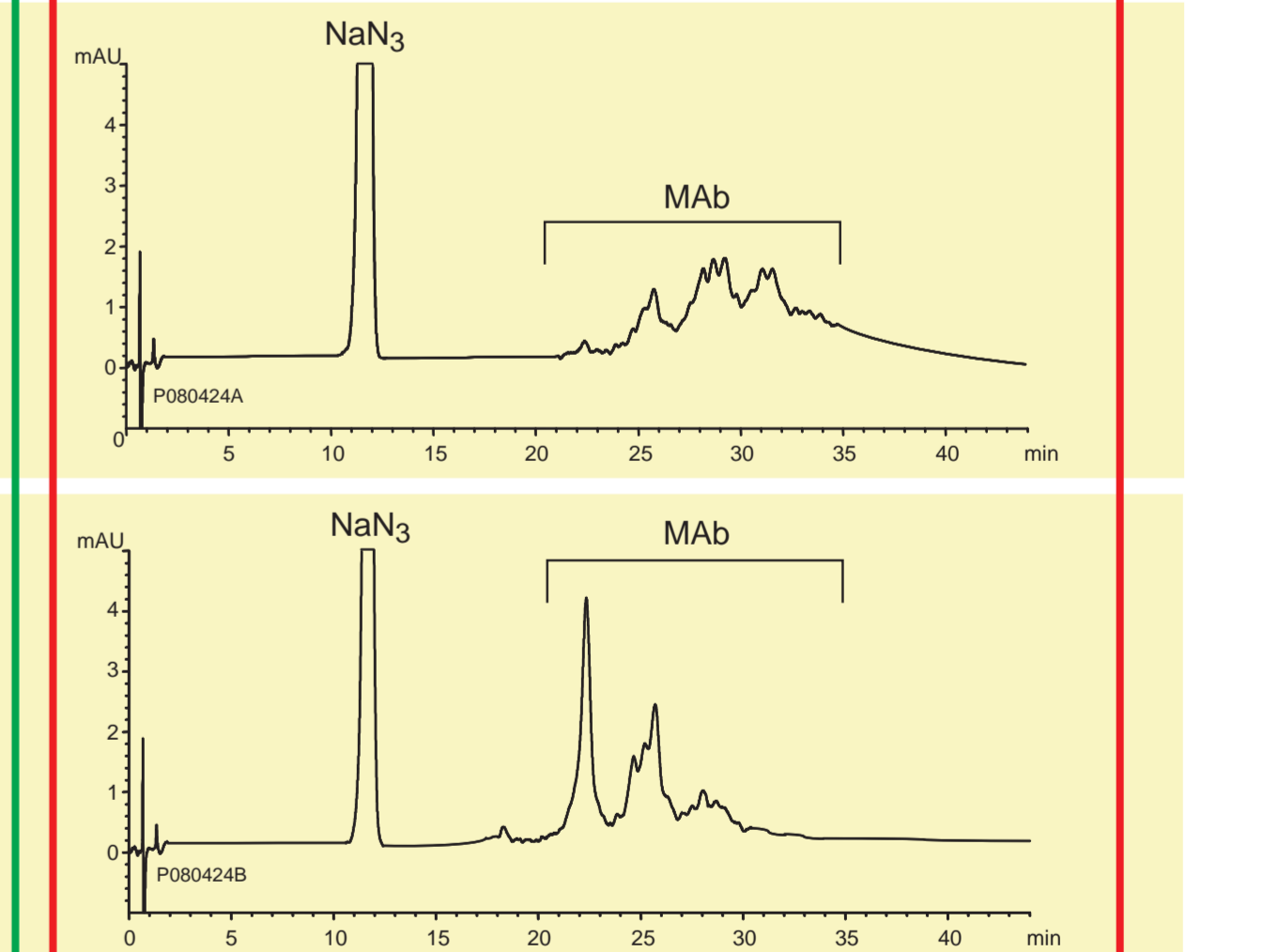
The separation of DNA fragments is compared between 100 mm-length and 30 mm-length of YMC-BioPro QA-F columns. The resolution of DNA fragments is dramatically improved by 100 mm column. The combination of non-porous polymer beads and long column provides extremely high column efficiency.

Analysis of monoclonal antibody (MAb) against human IgG4

SEC: YMC-Pack Diol-200
5 μm, 300 X 4.6 mm i. d.



IEC: YMC-BioPro QA-F
5 μm, 100 X 4.6 mm i. d.



| Eluent | Flow rate | Temperature | Detection | Injection | Sample |
|--|------------------------|----------------|--------------|-----------|---|
| A) 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl | 0.17 ml/min (80 cm/hr) | ambient (25°C) | UV at 220 nm | 10 μl | Monoclonal mouse IgG1 (0.05 mg/ml) (purified by DEAE chromatography, containing Na ₂ S ₂ O ₃) |

| Eluent | Flow rate | Temperature | Detection | Injection | Sample |
|--|--------------------|-------------|--------------|-----------|--|
| A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl | 10-25%B (0-60 min) | 25°C | UV at 220 nm | 14 μl | Monoclonal mouse IgG1 (0.1 mg/ml) (Purified by DEAE chromatography, containing Na ₂ S ₂ O ₃) |

Two different lots of commercially available MAb, purified by DEAE chromatography, are separated on SEC-column and IEC-column.

The MAb is resolved into several peaks with 100 mm-length IEC-column and the lot-to-lot variability is observed.

The 100 mm-length column of YMC-BioPro QA-F and SP-F has high efficiency and it is ideal for characterization or QC assessment of closely related proteins.

Conclusion

- The newly developed ion exchange chromatography resins based on highly hydrophilic polymer beads show significantly low non-specific adsorption of proteins.
- Porous polymer-based BioPro QA/SP columns, show superior resolution, high binding capacity and high recovery for various biomolecules. They are useful for analysis and laboratory-scale purification of biological samples containing a large amount of impurities.
- 75 μm bulk materials of porous polymer, BioPro Q75/S75, are useful for high capacity capture and high efficiency intermediate purification steps.
- Non-porous polymer-based BioPro QA-F/SP-F columns are useful for high-throughput analysis. Furthermore, 100 mm-length column is effective for high resolution analysis of complex mixtures, such as MAbs, DNA fragments and synthetic oligonucleotides.