

# Hybrid Media for Biopharmaceutical Analysis and Production

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The operational effectiveness of bioseparations is measured according to four characteristics: **speed, efficiency, recovery and loading capacity**. However, existing bioseparations media for are inadequate for optimizing all four of these characteristics simultaneously. Conventional soft gel media for biomolecular separation might have good recovery and loading capacity, but they lack speed and resolution. Synthetic polymeric media might have rigidity, porosity and pore sizes that can achieve speed and resolution in the separations, but they are very hydrophobic and non-biocompatible. This can create recovery problems, especially in the protein separation and other large scale of biomolecular separation and production. The best solution is to use hybrid media of rigid synthetic polymers and biocompatible soft gels that offer both advantages.

Biopharmaceutical analysis and purification use biocompatible, natural polymers such as polysaccharides and celluloses in the production of separation media. These materials are biocompatible, friendly to biomacromolecules, such as high-molecular-weight therapeutics, recombinant proteins, antibodies, vaccines, complex biologics and biosimilars. These media are biocompatible and have large flexible pore sizes suitable for applications in biomacromolecular analysis and separation. The media can provide good recovery and loading capacity. However, most of these media can only be used under low pressure or gravitational force separation and analysis. They cannot be used for high pressure and high speed applications. They fall short on two key characteristics for modern bioseparations: speed and efficiency. This deficiency creates a bottleneck in downstream biopharmaceutical production and manufacturing. The media cannot be pressurized in large scale production. The media must be able to be pressurized in the separation to achieve speed, high efficiency, and resolution, which means that natural biocompatible polymers have limited applications in biopharmaceutical development and production.

Rigid polymer beads can be produced with high porosity and large pore size that can be operated under high pressure to provide high speed and high efficiency separation for macromolecular analysis and purification. Macroporous polymer beads can be produced with high porosity and at pore sizes greater than 1500 Å. Their densities are around 0.3 to 0.4 grams per milliliter. They can be used directly as reversed phase media to analyze and purify biomolecules from small peptides, polypeptides, insulin up to more than 290 KDa. molecular weight of proteins and enzymes (see Figure 1 and 2). This application can save significant development time and expense in early stage drug discovery and development. However, the synthetic, rigid polymeric beads are very hydrophobic and non-biocompatible. Unless the materials have adequate biocompatible coating to reduce their hydrophobicity or the materials have the specially created porosity, pore size and pore structure, the direct application of polymeric beads for biomolecular separation can result in recovery issues, especially in large-scale preparative and process application. **Hydrocell RP 5D and RP 10D** are produced from plain macroporous polymer beads.

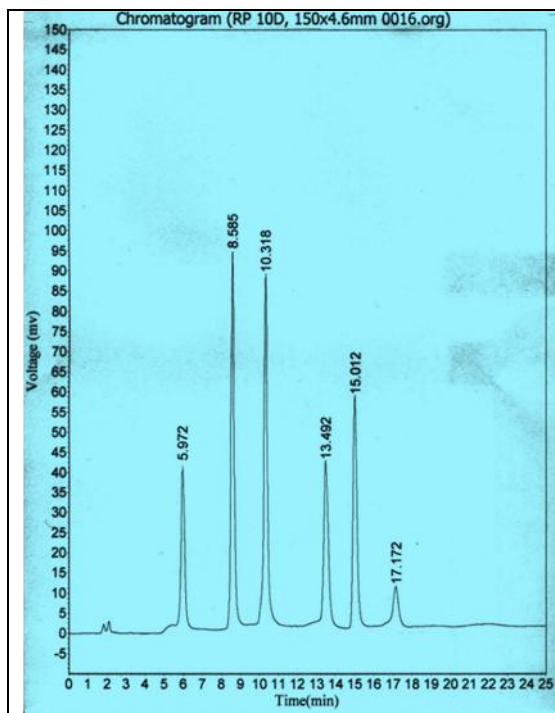


Figure 1: Standard Protein separated by **Hydrocell** RP 10D, macroporous Reversed Phase HPLC column  
**Column:** 150 x 4.6 mm;  
**Mobile Phase:** A: 5% Acetonitrile in 0.1% TFA; B: 95% Acetonitrile in 0.1% TFA  
**Gradient:** 20-70% B in 25 minutes;  
**Flow Rate:** 1.0 mL/min; **Detection:** UV 280 nm;  
**Sample:** Reversed Phase Standard Protein Mixture: 1. Ribonuclease A; 2. Cytochrome C 3. Lysozyme 4. Myoglobin 5. Ovalbumin; **Injection Volume:** 20 $\mu$ L; Catalog Number: 34-35RP-D

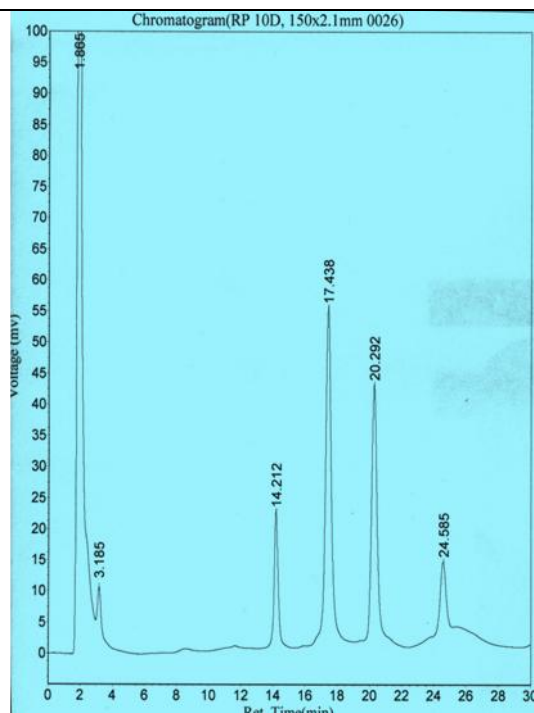


Figure 2: High Molecular Weight Proteins and Enzymes Separated by Hydrocell RP 10D, Macroporous Reversed Phase HPLC Column  
**Column:** 150 x 2.1 mm; **Mobile Phase:** A: 5% Acetonitrile in 0.1% TFA; B: 95% Acetonitrile in 0.1% TFA; **Gradient:** 20-90% B in 35 minutes  
**Flow Rate:** 0.25 mL/min; **Detection:** UV 280nm  
**Injection Volume:** 10  $\mu$ L **Sample:** Reversed Phase Standard 1. Cytochrome C (12.3 KDa); 2. Lactoferrin (80.0 KDa); 3. L-Glutamic Dehydrogenase (290 KDa); 4. Lactate Dehydrogenase (142 KDa)  
**Catalog Number:** 24-35RP-D

**BioChrom Labs, Inc.** (“BioChrom”) develops, manufactures, and markets polymeric packing materials and HPLC columns based on three major propriety technologies for the high speed purification of biopharmaceutical drugs-therapeutic agents comprised of proteins, peptides, nucleotides, nucleic acids, enzymes, DNA fragments and large macromolecules such as glycoproteins, enzymes, antibodies and vaccines.

(1). Macroporous polymer bead technology: Macroporous polymer beads are materials with high porosity and pore volume with more than 1500 Å pore size. Polymer beads can be directly used for small peptide, polypeptide, protein, high molecular weight of protein and enzyme analysis and purification with high speed, high efficiency and resolution. The processes has been developed to produce spherical, highly cross-linked polystyrene-divinylbenzene polymeric beads (PS-DVB) from 1 to 50  $\mu$ m particle sizes with macropore (1000-4000Å), mesopore (150--900 Å), micropore (20-150 Å) and non-porous beads. Polymer particle technologies not only control sizes and shape of polymer beads (spherical or irregular), but also control particle pore structure, porosity, pore volume and pore size. The pore structure, porosity,

pore volume and pore size of polymer beads are the most important factors in applying them for bioseparations, especially when they are used in the separation of macromolecules such as proteins, polypeptides, polynucleotides, and large macromolecules such as glycoproteins, antibodies and vaccines. The quality of beads affects the resolution and recoveries of macromolecules in the bioseparations.

Polymer beads used for such applications must have macropores and high porosity to show good recoveries and efficiency in the separation. Macroporous polymer beads can also be used as ion-pair reversed phase media for DNA fragment analysis and as size exclusion media for high speed and high molecular weight synthetic polymer analysis. Macroporous polymer beads are the raw materials for producing ion exchange and hydrophobic interaction media for large biomolecular separations. The molecular weights of biomacromolecules may be close to or larger than 1,000,000 Daltons, such as glycoproteins, antibodies or vaccines that are developed by biopharmaceutical companies.

Mesoporous polymer beads have more surface area than that of macroporous polymer beads and can be used to analyze and purify single stranded recombinant DNA, DNA fragments and synthetic oligonucleotides. They are the raw materials for producing ion exchange and hydrophobic interaction media for standard protein, polynucleotide, polypeptide and carbon nanotube analysis and purification. Biomolecules such as proteins, polyoligonucleotides and polypeptides are potential therapeutic drugs or their precursors.

(2). Biocompatible coating technology: It is based on two-phase coating agents developed by BioChrom Labs, Inc. It converts hydrophobic rigid polymer surfaces into hydrophilic, biocompatible surfaces for bioseparations. Biocompatible soft gels are friendly in the separation of biomolecules, but the materials cannot be pressurized to carry out high speed and high resolution separation. It is a slow process and time consuming. Rigid polymer beads can be directly used to analyze and purify biomolecules and provide speed, efficiency and resolution in the separation. They are highly hydrophobic materials and may have recovery problems, especially in large scale of downstream development and production. Hybrid of rigid polymer beads and biocompatible soft gels can solve the problems associated with synthetic rigid polymers and natural biocompatible polymers in the bioseparation. Hybrid media carry the advantages of rigid polymer beads and biocompatible soft gels. It can be pressurized to perform high speed, high resolution separations and it is also biocompatible to large biomacromolecules with high loading capacities and recoveries. The media can be operated under a wide pH range from 1 to 14 and it is chemical resistant to most organic solvents and chemicals. The media can resolve bottleneck of large scale biopharmaceutical development and production in the high volume of downstream processes.

(3). Hybrid media of rigid polymer beads with biocompatible soft gel are developed by applying biocompatible coating reagents on the surface of rigid hydrophobic polymer beads. The modified polymer beads are then polymerized with biocompatible soft gel to increase the density of soft gel portion and immobilizing functional groups on the surface of polymers. Hybrid media can be used to produce ion exchange, hydrophobic interaction and nucleic acid HPLC columns and media for high speed biopharmaceutical analysis and separation from non-porous polymer beads. Hybrid media with macroporous polymer bead supports have the better resolution and loading capacity in comparison with hybrid media with non-porous polymer bead supports. These macroporous, Mesoporous and non-porous hybrid media can solve problems of high molecule weight of biomacromolecular separations and purification. The hybrid media can be operated under high pressure and high speed with high resolution,

loading capacity and high recoveries. The media can also be used in a wide pH range from 1 to 14. These product features of macroporous, mesoporous and nonporous hybrid media are unique and are lacking in most packing materials developed from silica gel, medium pressure polymer, or low pressure soft gels.

**Hydrocell QA 3000** is developed from hybrid media that carry three proprietary technologies: (1) macroporous polymer particle technologies, (2) biocompatible coating technologies and (3) hybrid media of rigid macroporous polymer beads and biocompatible soft gels. This product has been used for large macromolecule separations such as high molecular weight therapeutic proteins, glycoproteins, antibodies and vaccines analysis and purification (see Figure 3).

**Hydrocell C4 3000** is a Hydrophobic Interaction Column (HIC). It is developed from hybrid media with macroporous polymer supports and biocompatible coating similar to Hydrocell QA 3000. However, the packing materials have been chemically bonded with butyl (C4) functional group instead of anion exchange functional group. Hydrophobic interaction chromatography separates proteins by mechanisms similar to Reversed Phase chromatography but under gentle reverse salt gradient elution condition in aqueous buffers. Since no organic solvent is necessarily used, biological activity will be retained (see Figure 4)

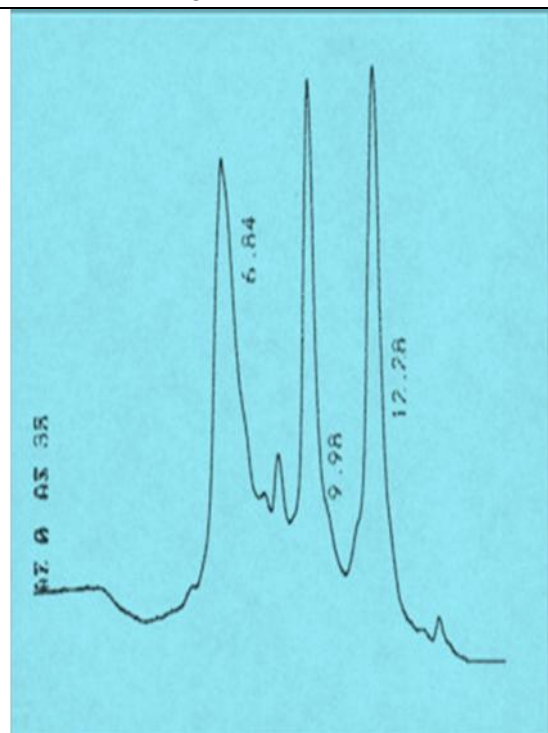


Figure 3: Protein Mixture Separated by Hydrocell QA 3000, Macroporous Anion Exchange Column  
**Column:** 150 x 4.6 mm; **Mobile Phase:** A: 10 mM Tris, pH 8.0; B: 10 mM Tris + 0.5 M NaCl, pH 8.0  
**Gradient:** Linear 0-50% B in 20 minutes  
**Flow Rate:** 1.0 mL / minutes; **Detection:** UV 280nm  
**Peak Identification:** 1. Conalbumin 2. Ovalbumin 3. Soybean Trypsin Inhibitor; **Catalog #:** 32-35QA

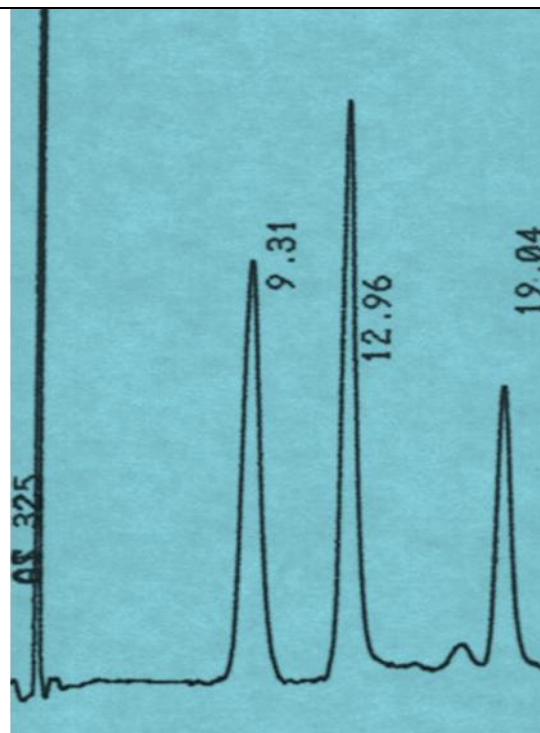


Figure 4: Protein Mixture Separated by Hydrocell C4 3000, Macroporous Hydrophobic Interaction Column  
**Column:** 150 x 4.6 mm; **Mobile Phase:** A: 2.5 M Ammonium Sulfate in Eluent B; B: 0.1 M Potassium Phosphate, pH 7.0; **Gradient:** Linear 0-100% B in 20 minutes; **Detection:** UV 280 nm;  
**Flow Rate:** 1.0 mL/min; **Peak Identification:** 1. Ribonuclease A 2. Lysozyme 3. Chymotrypsinogen A; **Catalog No.:** 34-35C4

Non-porous, high speed ion exchange and hydrophobic interaction hybrid media and columns are designed for high throughput of quality control and process monitoring, which need rapid scanning complex samples. The hybrid media and columns are produced from non-porous polymer bead supports with biocompatible coating for ultra high speed protein, enzyme, polynucleotide and polypeptide analysis and purification. The performances of these media and columns are different from the media produced from porous polymer beads. These media can be operated under extra high flow rate and steep gradient to test complex protein samples in few minutes and use for on-line process monitoring. Nonporous high speed ion exchange media and columns have two anion exchange bonded phases: **DEAE NP10**, **QA NP10** (see Figure 5) and two cation exchange bonded phased **CM NP 10** and **SP NP10**. Non-porous high speed hydrophobic interaction columns have three bonded phases **C3 NP10**, **C4 NP10** and **Phenyl NP10** (see Figure 6). All of non-porous hybrid media and columns are produced from non-porous polymer beads with biocompatible coating.

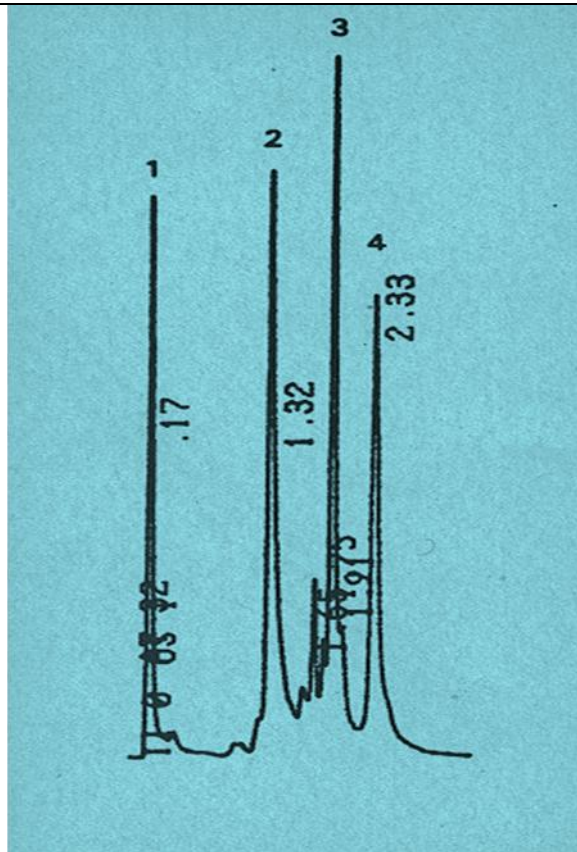


Figure 5: Protein Mixture Separated by Hydrocell DEAE NP10, Nonporous High Speed Anion Exchange HPLC Column  
 Column: 35x4.6 mm; Mobile Phase: A: 10 mM Tris-HCl, pH 8.0; B: Eluent A + 0.5 M NaCl, pH 8.0  
 Gradient: Linear 0-50% B in 5 min; Detection: UV 280 nm  
 Flow Rate: 2.0 mL / minutes; Injection: 10  $\mu$ L  
 Protein Mixture: 1. Myoglobin 2. Conalbumin  
 3. Ovalbumin 4. Soybean Trypsin Inhibitor;  
 Catalog #: 31-30DE

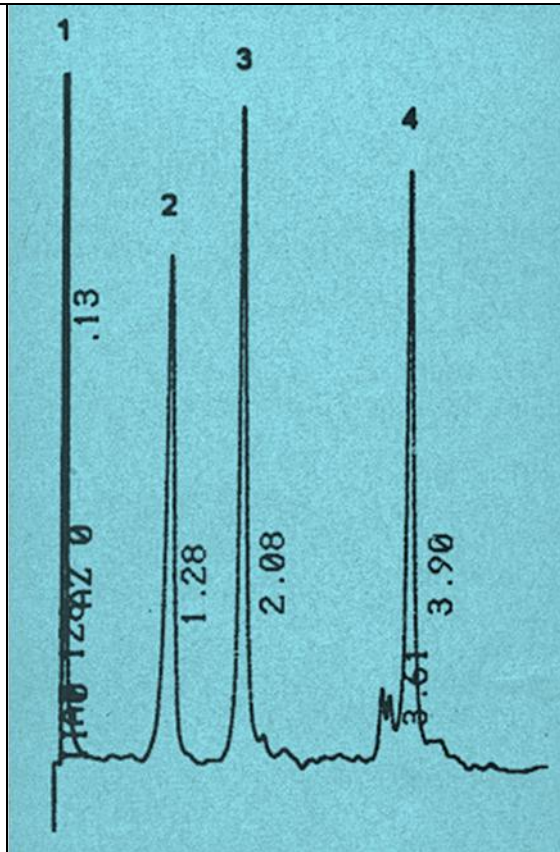


Figure 6: Protein Mixture Separated by Hydrocell C4 NP10, Nonporous High Speed Hydrophobic Interaction HPLC Column  
**Column:** 35 x 4.6 mm; **Mobile Phase:** A: 2.5 M Ammonium Sulfate in Eluent B; B: 0.1 M Potassium Phosphate, pH 7.0; **Gradient:** Linear 0-100% B in 5 minutes; **Detection:** UV 280 nm; **Flow Rate:** 2.0 mL/min; **Injection:** 10  $\mu$ L; **Peak Identification:** 1.Cytochrome C 2. Ribonuclease A 3. Lysozyme 4. Chymotrysinogen A; **Catalog No.:** 31-30C4

Anion exchange hybrid media produced from mesopore PS-DVB polymer beads are used for the analysis and purification of DNA fragments and synthetic oligonucleic acid. They can also be used for carbon nanotube (CNT) sorting and purification. Wrapping of carbon nanotubes by synthetic single-stranded DNA was found that electrostatics of DNA-CNT hybrid depends on tube diameter and electronic properties, enabling carbon nanotube separation by anion exchange column CNT-NS 1500. After hybrid with the single-stranded synthetic oligonucleic acid, semiconductive carbon nanotubes can be analyzed and separated from metallic CNT by **Hydrocell CNT-NS 1500 column** with up to 99.0% purity. The column exhibits decent resolution and recoveries when carbon nanotube sorting is operated under high pressure conditions. CNT-NS 1500 column can be further improved, if CNT raw materials can be sorted by reducing internal diameter, length and shape distribution (see Figure 7).

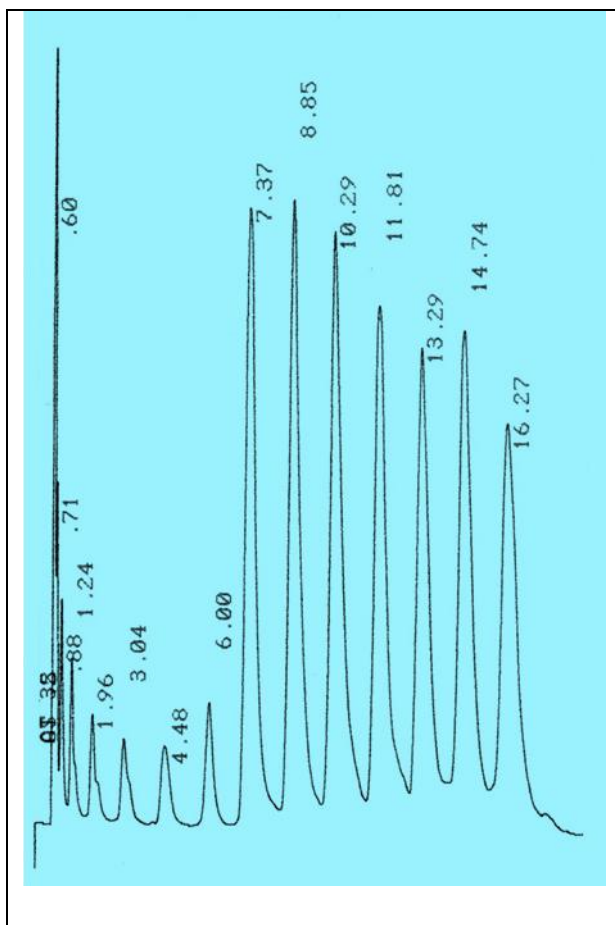


Figure 7: Synthetic Oligothymidylic Acid d(pT) 12-18 mer Separated by Hydrocell CNT-NS 1500, Anion Exchange HPLC Column.

**Column:** 75 x 7.8 mm; **Mobile Phase:** Eluent A: 25 mM CHES, pH 8.0; Eluent B: Eluent A + 1.2 M Ammonium Sulfate, pH 8.0; **Gradient:** Linear 10-50% B in 40 minutes; **Detection:** UV 260 nm.; **Flow Rate:** 2.5 mL/min.

**Sample:** Oligothymidylic Acid d(pT) 12-18 mer, 5 units in 1.0 mL of eluent A; **Injection:** 30  $\mu$ L  
**Catalog No.:** 43-34NS-CNT