PALL Life Sciences

USD 2443

HEA and PPA **HyperCel**™ Sorbents

Mixed-mode Chromatography For Protein Separation

- Direct hydrophobic capture of proteins at low ionic strength
- Provide new selectivities in process separations
- Environmentally-friendly, no salt waste recycling
- Orthogonal to ion exchange or other chromatography steps

Introduction

Pall HEA and PPA **HyperCel** sorbents are novel industry-scalable chromatography sorbents designed for protein capture and impurity removal in a biopharmaceutical environment.

Operating on a "mixed-mode" mechanism, their chromatographic behavior is based on a combination of electrostatic and hydrophobic properties of the protein and ligands.

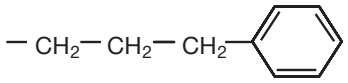
HEA and PPA **HyperCel** sorbents provide unique and different selectivities not accessible with traditional ion exchange or HIC, that can be screened to facilitate process development.

For example, the mixed-mode interaction mechanism can be exploited to achieve discrimination of proteins having similar or very close isoelectric points, a separation which cannot be performed by methods like ion exchange.

Figure 1. Structure of HEA and PPA ligands.

- **HEA HyperCel** → **n-hexyl** substituent

- **PPA HyperCel** → **phenylpropyl** substituent



- **Ligand density > 50 µmoles /mL**
- **Critical working ligand pKa = 8**

Properties

Particle size	80 – 100 µm (av.)
Bead composition	High porosity cross-linked cellulose
Dynamic binding capacity for BSA (10% breakthrough)(1)	40 – 60 mg/mL
Ligand : • Aliphatic (HEA) • Aromatic (PPA)	Hexylamine Phenylpropylamine
BSA recovery	≥ 90 %
Adsorption pH	7.0 – 9.0
Elution pH	By gradient or step-elution, e.g., pH 7.0 – 2.6
Cleaning pH	1 – 14
Pressure resistance	< 3 bar (44 psi)
Typical working pressure	< 1 bar (14 psi)

(1) Determined using 5 mg/mL BSA in PBS, flow rate: 100 cm/h.

*Filtration. Separation. Solution.*SM

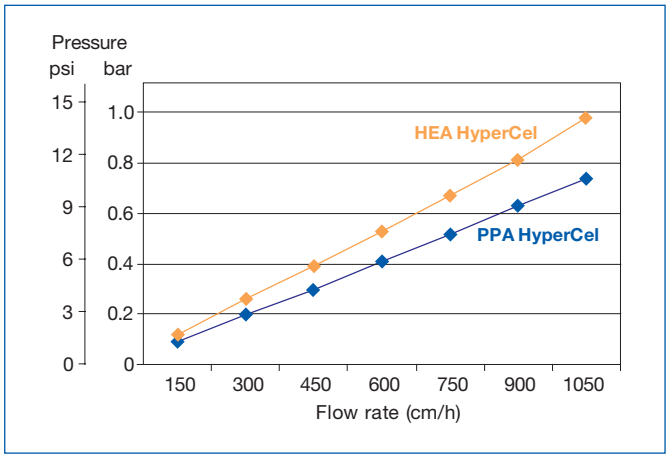
Ligand design

HEA and PPA **HyperCel** sorbents are new members of a family of Pall chromatography sorbents, complementing MEP **HyperCel** sorbent (Hydrophobic Charge Induction).

HEA and PPA **HyperCel** sorbents carry mixed-mode synthetic ligands, immobilized on **HyperCel**, a mechanically-stable base matrix currently used in the production of material for late-stage clinical trials (MEP **HyperCel**).

The ligands include aliphatic (HEA – hexylamine) and aromatic (PPA – phenylpropylamine) amines (see Figure 1), which offer different selectivity and hydrophobicity options.

Figure 2. Pressure vs. flow rate curve.



Column: 16 mm I.D. x 20 cm height.
HEA and PPA **HyperCel** sorbents provide linear pressure/flow behavior, and have characteristics well suited to process-scale operations in low pressure columns.

Principles of operating mechanism and general guidelines

(Refer to product insert for details on column packing, buffers and recommendations).

- **Protein binding** is achieved at neutral pH (i.e., PBS, pH 7.4), principally by hydrophobic interaction. Binding of very basic proteins may require increased pH (pH 9.0).

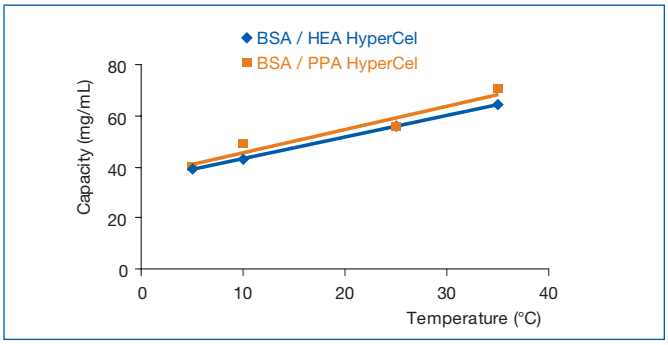
At salt concentrations recommended for binding, there is no significant ion exchange binding. Unlike traditional HIC, binding occurs at low ionic strength, in "physiological-like" conditions. No addition of lyotropic or other salt is required, limiting the risk of protein aggregation and denaturation.

PPA **HyperCel** sorbent carries an aromatic ligand, and has a stronger hydrophobicity than HEA **HyperCel** sorbent. The binding capacity is a function of the protein. For protein models like BSA, typical capacities of 40 – 60 mg/mL are obtained (PBS, pH 7.4, 0.14 M NaCl buffer, flow rate 100 cm/h). The factors which affect capacity include temperature, residence time, and column packing.

- **Protein elution** is driven by electrostatic charge repulsion, as pH is reduced to values below the pI of the protein and below the pKa of the ligand. Elution is triggered by reducing the pH (from 5 to 3), usually in a step-elution mode. This approach can also serve to resolve the target protein from impurities whose hydrophobic characteristics differ. Basic proteins will desorb earlier in the pH gradient or step-elution sequence, followed by more acidic proteins.

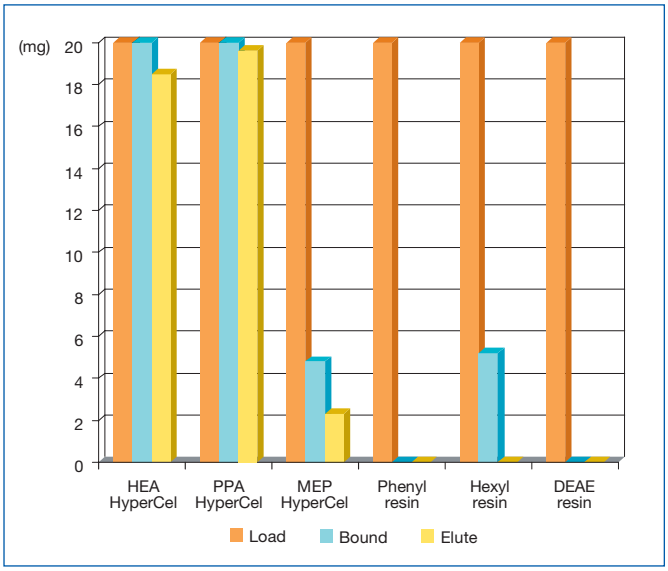
Unlike traditional HIC, the target protein is recovered in dilute buffer, reducing the need for intermediate diafiltration.

Figure 3. Effect of temperature on protein binding capacity.



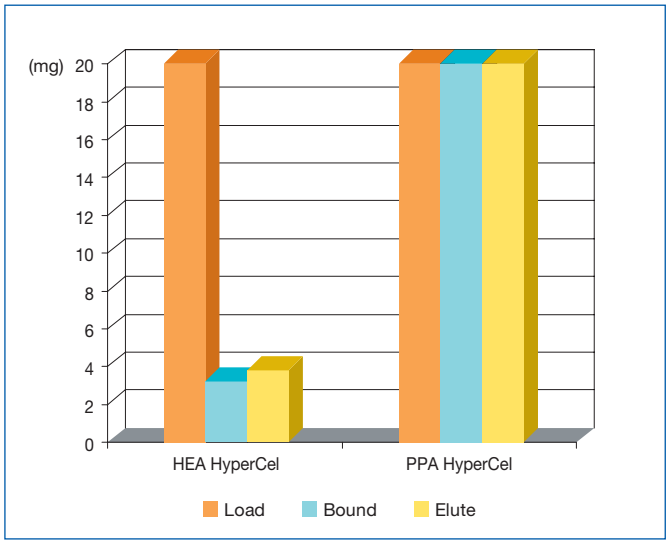
Model protein: BSA (5 mg/mL) bound to HEA and PPA **HyperCel** (7 mL column, 100 cm/h) in PBS, pH 7.4.
This experiment was carried out to demonstrate the dominant hydrophobic interaction component of protein binding to HEA and PPA **HyperCel** sorbents. The binding of proteins by HIC is entropy-driven, and the interaction increases with rise of temperature, as shown by the binding capacity increase for BSA. In practice, for robustness and capacity optimization studies, special attention should be given to keep buffer and operation room temperatures consistent.

Figure 4. Comparison between HEA and PPA HyperCel, MEP HyperCel, conventional HIC and anion exchange sorbents.



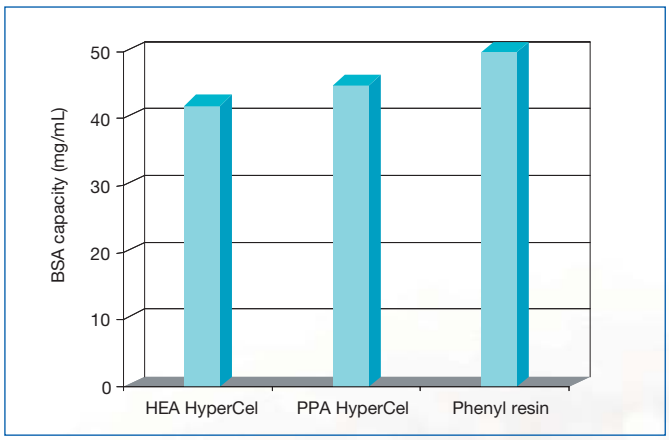
A series of experiments with various standard proteins were carried out to show the selectivity differences of HEA and PPA **HyperCel** sorbents compared to MEP **HyperCel** sorbent (HCIC – Hydrophobic Charge Induction Chromatography), conventional HIC sorbents (Phenyl and Hexyl ligands), as well as anion exchange resins (DEAE). Figure 4 illustrates the adsorption/desorption of Bovine Serum Albumin (BSA), in PBS buffer, pH 7.4. Data shows that BSA is efficiently retained on both HEA and PPA **HyperCel** sorbents, but is poorly retained on MEP **HyperCel** sorbent (as this ligand is antibody-selective). In PBS, without lyotropic salt addition, low binding of BSA to both Phenyl and Hexyl HIC sorbents is observed. The anion exchange (DEAE) resin did not bind BSA at these non-optimal conditions either (pH 7.4 and too high salt concentration).

Figure 5. Selectivity difference between HEA and PPA HyperCel sorbents: Adsorption/desorption of α-chymotrypsinogen A.



HEA and PPA ligands are of different nature (respectively aliphatic and aromatic). This translates in differences in selectivity for various proteins. Figure 5 illustrates the binding and elution properties for α-chymotrypsinogen A (2 mg/mL, binding in PBS, pH 7.4, 0.14 M NaCl), elution in 0.02 M sodium acetate at pH 5.0, 4.0 or 3.0. Data suggests a stronger binding of the model protein to PPA **HyperCel**, as well as a good recovery (elution at pH 4.0 is the most efficient).

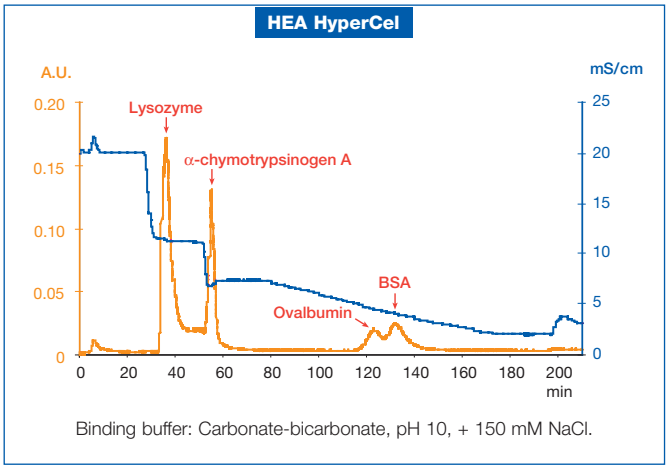
Figure 6. BSA binding capacity of HEA and PPA HyperCel sorbents in the presence of 1.7 M ammonium sulphate.



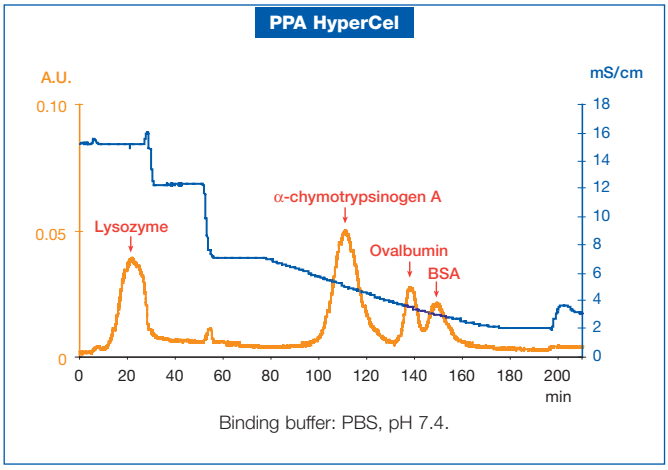
When needed, HEA and PPA **HyperCel** sorbents can be used in conventional hydrophobic interaction conditions, in the presence of high concentrations (1 – 2 M) of lyotropic salt like ammonium sulphate. Data shows that BSA binding capacity in these conditions is close to the capacity of a conventional HIC sorbent (phenyl resin).

Applications

Example 1. Separation of a protein mix on HEA and PPA HyperCel sorbents.



Binding buffer: Carbonate-bicarbonate, pH 10, + 150 mM NaCl.



Binding buffer: PBS, pH 7.4.

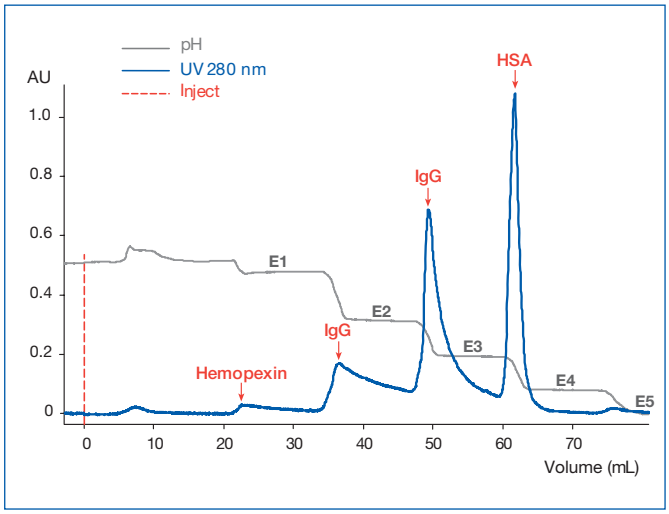
Sample volume: 1 mL; Column volume: 7 mL; Proteins: BSA, ovalbumin, lysozyme, α-chymotrypsinogen A, at a concentration of 2 mg/mL each.

Due to the difference in their ligands, HEA and PPA **HyperCel** sorbents have different retentivities and selectivities for proteins and should be screened during process development.

The example illustrates the chromatographic profiles obtained with a reference protein mixture applied to columns of 1.1 cm I.D. x 7 cm length. A step-elution sequence was performed, followed by gradient elution from pH 5.4 to 2.6, all conducted using sodium phosphate / citrate buffers. Data shows that a very basic protein – lysozyme – does not bind to PPA **HyperCel** sorbent at pH 7.4, and is found in the flowthrough; in contrast, when raising the binding pH to 10.0 by reducing the ionic repulsion, lysozyme can be retained on HEA **HyperCel** sorbent.

In practice, for a protein of unknown pI and hydrophobicity, screening of the two ligands at different pH and salt concentrations is recommended.

Example 2. Chromatography on HEA HyperCel sorbent: Separation of partially-purified polyclonal IgG from major plasma impurities.



Column: 0.66 cm I.D. x 7 cm height; Sorbent volume: 2.4 mL. Run at 100 cm/h; Equilibration in PBS, pH 7.4; Loading 5 mL of a partially-purified human polyclonal IgG (60% purity) at 3.8 mg/mL, pH 8.4, and 8.3 mS/cm; Wash with 5 CV in PBS; Elution in 0.2 M sodium phosphate / 100 mM citric acid, pH 7.0 (Elution **1**), pH 5.4 (Elution **2**), pH 4.4 (Elution **3**), pH 3.4 (Elution **4**), pH 2.6 (Elution **5**); Regeneration in 1 M NaOH.

The objective of this experiment was to separate a target IgG from contaminants present in the feedstream. Three different mixed-mode sorbents – HEA, PPA, and MEP **HyperCel** sorbents – were tested. The figure shows only the chromatogram obtained with HEA **HyperCel** sorbent, which gave the best results in this case: the IgG (main elution, E2) was well separated from HSA (elution E3). Additionally, molecular-mass based analysis using SELDI (ProteinChip* System, Ciphergen Biosystems, Inc.) – data not shown – demonstrated resolution from lower molecular weight contaminants (elution E1), as well as a partial separation of IgA, co-eluting with the HSA peak.

Ordering Information

Size	HEA HyperCel Cat. No.	PPA HyperCel Cat. No.
5 mL	20250-012	20260-015
25 mL	20250-026	20260-025
100 mL	20250-033	20260-030
1 L	20250-041	20260-040
5 L	20250-042	20260-045
10 L	20250-056	20260-052

HEA and PPA HyperCel sorbents are manufactured to ISO 9001:2000 standards.

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10/2006