STREAMLINE SP
STREAMLINE DEAE

Data File
Expanded Bed Adsorption

STREAMLINE is a range of adsorbents and columns designed for expanded bed adsorption. In this new unit operation, proteins are recovered directly from crude feedstock in a single pass operation, without the need for prior clarification; this results in significantly reduced process times and increased product yield.

STREAMLINE SP and STREAMLINE DEAE are modified Sepharose matrices developed for expanded bed adsorption. They allow:

- Capture of biomolecules directly from unclarified feedstocks
- High binding capacities and product yields because of stable expanded beds
- Long life because of high chemical and mechanical stability

Fig. 1. STREAMLINE SP and STREAMLINE DEAE adsorbents for expanded bed adsorption.

Principle of expanded bed adsorption

The bed is expanded by the upward liquid flow. Controlled, even flow is assured by the design of the liquid distribution system in STREAMLINE columns. Adsorbent particles are suspended in equilibrium due to the balance between particle sedimentation velocity and upward flow (Fig. 2). The defined particle distribution and high density of STREAMLINE adsorbents yield a stable, uniformly expanded bed; smaller, lighter particles in equilibrium in the upper part of the column and larger, heavier particles in the lower part. There is no back-mixing and the expanded bed is ready for efficient adsorption.

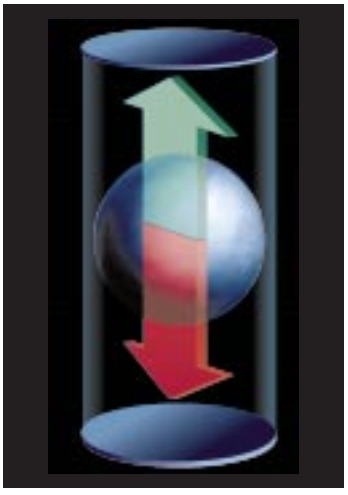

Fig 2. STREAMLINE adsorbent particles have high density and a range of sizes. When there is constant upward flow, the beads are suspended and each finds its own equilibrium position; the bed is expanded and stable.

Crude, unclarified feed is applied to the expanded bed with an upward flow. Target proteins are captured on the adsorbent while cell debris, cells, particulates and contaminants pass through unhindered. Flow is then reversed, the adsorbent particles quickly settle and target proteins are desorbed by an elution buffer as in conventional packed bed chromatography. Two examples, given under Applications, illustrate the recovery of recombinant proteins from *E. coli* homogenates using this technique.

Characteristics

The adsorbents are based on cross-linked agarose that has been modified through the inclusion of an inert, crystalline quartz, core material to provide the required high density for stable bed expansion.

STREAMLINE SP is a strong cation exchange adsorbent. The sulphonate groups maintain full protein binding capacity over the entire operating pH range. STREAMLINE DEAE is a weak anion

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exchanger, the number of ligand groups that are charged varies with pH (operating pH range 3–9). Tables 1 and 2 summarize the general properties of these adsorbents.

Table 1. Characteristics of STREAMLINE SP.

Product	Description
Type of ion exchanger	Strong cation
Functional group	–O–$CH_2CHOHCH_2O$-$CH_2CH_2CH_2SO_3^-$
Ionic capacity	0.17–0.24 mmol H^+/mL adsorbent
Porosity	4×10^6 daltons (globular proteins)
Matrix structure	Macroporous, cross-linked agarose, 6%, containing crystalline quartz core material
Particle form	Spherical, 100–300 µm
Mean particle size	200 µm
Mean particle density	Approx 1.2 g/mL
Degree of expansion (H/H_0) at 300 cm/h	2–3
pH stability	
working range	3–13
cleaning range	3–14
Chemical stability	All commonly used aqueous buffers – 1 M NaOH – 70% ethanol – Organic solvents Avoid – Oxidising agents – Long exposure (1 week, 20°C) to pH <4
Physical stability	Generation of fines during normal operation in expanded bed is negligible. Avoid handling drained adsorbent in shearing operations.
Binding capacity[1]	At least 60 mg lysozyme/mL adsorbent
Storage	0.2 M Sodium acetate in 20% ethanol

[1] Breakthrough capacity; 15 cm sedimented bed height, 300 cm/h linear flow rate, 50 mM sodium phosphate, pH 7.5.

High capacity and high yield

The stability of the expanded bed during feed application provides high binding capacities (breakthrough capacity) and minimal loss of product. This is in contrast to a fluidized bed where back-mixing results in much lower binding efficiency. Characteristically, the stable expanded bed behaves much closer to a packed bed in chromatography. This is illustrated in Fig. 3 which compares the breakthrough curves for a model protein with STREAMLINE DEAE in packed bed mode, and expanded mode at two scales of operation.

Product yields from expanded bed adsorption with STREAMLINE are normally high, in the range of 80–100%. Two examples are shown under Applications.

Table 2. Characteristics of STREAMLINE DEAE.

Product	Description
Type of ion exchanger	Weak anion
Functional group	–O–CH_2CH_2–$N^+(C_2H_5)_2H$
Ionic capacity	0.13–0.21 mmol Cl^-/mL adsorbent
Porosity	4×10^6 daltons (globular proteins)
Matrix	Macroporous, cross-linked agarose, 6%, containing crystalline quartz core material
Particle form	Spherical, 100–300 µm
Mean particle size	200 µm
Mean particle density	Approx 1.2 g/mL
Degree of expansion (H/H_0) at 300 cm/h	2–3
pH stability	
working range	2–13
cleaning range	2–14
Chemical stability	All commonly used aqueous buffers – 1 M NaOH – 70% ethanol – Organic solvents Avoid – Oxidising agents
Physical stability	Generation of fines during normal operation in expanded bed is negligible. Avoid handling drained adsorbent in shearing operations.
Binding capacity[2]	At least 40 mg BSA/mL adsorbent
Storage	20% ethanol

[2] Breakthrough capacity; 15 cm sedimented bed height, 300 cm/h linear flow rate, 50 mM Tris-HCl, pH 7.5.

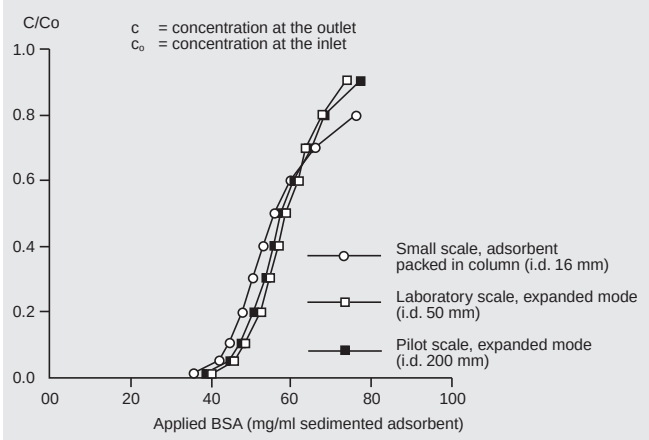

Fig. 3. Breakthrough capacity curve comparisons. Running conditions: BSA in 50 mM Tris-HCl, pH 7.5, linear flow rate 300 cm/h.

High chemical and mechanical stabilities give long life

STREAMLINE DEAE and STREAMLINE SP have high chemical stability and can be used over wide pH ranges (see Tables 1 and 2). Such high stability allows considerable flexibility when choosing conditions for adsorption and elution, as well as for efficient cleaning and sanitization.

In a test of stability, STREAMLINE SP was subjected to a cleaning-in-place (CIP) procedure, described in Fig. 4, for 40 repetitive cycles. As shown by the data in Table 3, the harsh conditions had very little effect on the performance and chemical content of the adsorbent.

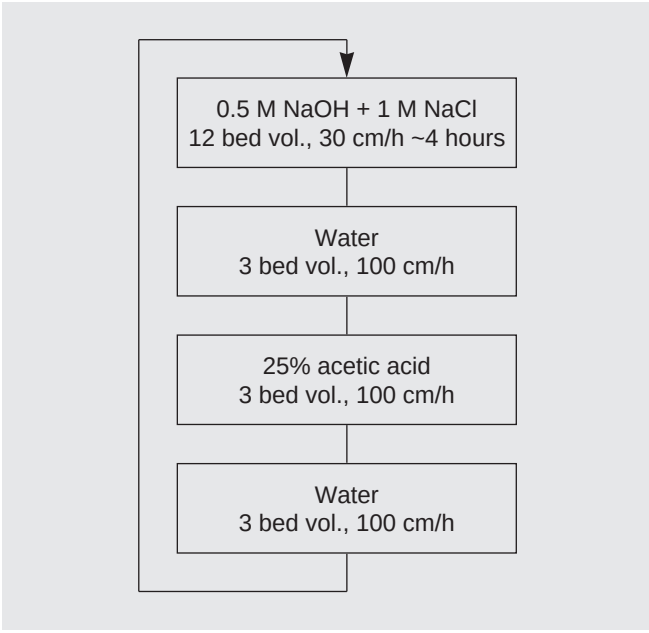

Fig. 4. Cleaning-in-place procedure used for STREAMLINE SP, 200 mL adsorbent in STREAMLINE 50 column.

Table 3. The effect of 40 CIP cycles on STREAMLINE SP.

Analysis	Before CIP	After 40 CIP cycles
Ionic capacity (mmol H^+/mL adsorbent)	0.20	0.19
Degree of expansion (H/H_0)	2.54	2.56
Organic substance (%)	100	96
Quartz (%)	100	98

STREAMLINE adsorbents have high mechanical stability and can be expanded repeatedly with little generation of fines. In one study, STREAMLINE DEAE was expanded using different flow rates up to 320 cm/h. Flow rate was increased abruptly to promote turbulence in the bed. The procedure was repeated 140 times during which a total of 800 L eluent were pumped through the column (STREAMLINE 50) and expanded bed; the volume of fines generated amounted to less than 0.2% of the sedimented bed volume (elutriated from the column) and the degree of expansion was unaffected (22).

Operation

Method design and optimisation

Determination of optimum pH and conductivity for expanded bed adsorption is often conveniently determined through breakthrough analysis of clarified material using the adsorbent in packed bed mode. This is followed by optimization of the method with unclarified material in expanded bed mode. The following are some general considerations:

- Flow rates are typically 200–300 cm/h.
- Low conductivity is important for efficient binding of the target protein in some applications. In extracellular systems, dilution, or other methods may be needed to lower conductivity. In intracellular systems, homogenization in water, or low ionic strength buffer will suffice.
- Biomass content and viscosity of the feed can affect the degree of expansion and may sometimes limit operational flow rates. Typically biomass content is around 5% (dry weight), but optimum values vary with feedstock (18).
- Washing of cells, cell debris and unbound material from the expanded bed is done efficiently using 15–20 (sedimented) bed volumes of equilibration buffer (25,26).
- Elution is normally carried out in packed bed mode with the adaptor lowered and downward flow. A typical elution buffer contains 0.5–1.0 M sodium chloride; elution flow rates are in the range 50–150 cm/h (25,26).

Cleaning-in-place (CIP)

Efficient cleaning methods should be developed as an integral part of the complete process to maximize the life of the adsorbents and to minimize problems such as clogging. Each cleaning protocol is dependent on the nature of the feed material and must be optimized on a case-to-case basis. The following protocols have been developed for STREAMLINE adsorbents used in different applications. They have enabled the repetitive use of the adsorbents, applied to different types of *E. coli* homogenates, without affecting bed expansion and/or adsorption properties.

CIP procedure 1
Flow direction: upward

– 0.5 M NaOH containing 1 M NaCl, flow rate 30 cm/h, contact time 4 hours
– distilled water, flow rate 100 cm/h, 3 sedimented bed volumes
– 30% isopropanol, flow rate 100 cm/h, 3 sedimented bed volumes
– 25% acetic acid, flow rate 100 cm/h, 3 sedimented bed volumes
– equilibration buffer, flow rate 100 cm/h, 5–10 sedimented bed volumes

CIP procedure 2
Same as above except that 30% isopropanol is exchanged for:

– distilled water, 85–90 °C, flow rate 100 cm/h, 10 sedimented bed volumes

CIP procedure 3
Flow direction: upward

– 1% DARACLEAN* 8471, flow rate 30 cm/h, contact time 4 hours
– equilibration buffer, flow rate 100 cm/h, 5–10 sedimented bed volumes

* DARACLEAN (Grace Dearborn Ltd) is a commercially available cleaning agent containing caustic soda, alkaline salts and the non-ionic detergent Triton CF 10.

Scale-up

Expanded bed adsorption is scaled up by increasing column diameter, while keeping the sedimented bed height, linear flow-rate and load at constant values. This procedure is similar to that used in packed bed chromatography.

Recommended equipment

Initial method development work with clarified feed in packed bed mode:

- XK 16/20 column, 20–30 mL sedimented bed volume.

Laboratory scale, method optimization in expanded bed mode with unclarified feedstocks with volumes 1–20L:

- STREAMLINE 50 column, 200–600 mL sedimented bed volume. Peristaltic pump for operation, flow capacity 6 L/h; optional pump for hydraulic drive. Manual valves and tubing, i.d. 3–4 mm, pH and conductivity meters, UV-monitor, recorder, filter funnel (1L, porosity #3), stirrer (for the sample during application), water suction (to remove air during start up).

Pilot scale, feedstock volumes 50–300 L:

- STREAMLINE 200 column, 3–9 L sedimented bed volume. Peristaltic pump for operation, flow capacity 125 L/h; optional pump for hydraulic drive. Manual valves and tubing, i.d. 8–10 mm, pH and conductivity meters, etc.

Production scale:

- Large scale stainless steel columns and systems, controlled by UNICORN, are manufactured to customers' requirements.

Applications

Capture of recombinant P. aeruginosa exotoxin A from E. coli homogenate

A complete downstream process was developed for the purification of a recombinant modified *Pseudomonas aeruginosa* exotoxin A starting from unclarified *E. coli* cell homogenate. The four step procedure included STREAMLINE DEAE for Capture, Phenyl Sepharose 6 Fast Flow (high sub) and SOURCE 30Q for the two Intermediate Purification steps and SOURCE 15PHE for Polishing. The process resulted in a pure protein, according to PAGE and RPC analysis.

Cells (4.7 kg) were subjected to osmotic shock and suspended in the final volume of 180 L. The unclarified cell suspension, obtained after lysis and addition of DNase (Benzonase, Merck) to reduce viscosity, was applied directly to the expanded bed of STREAMLINE DEAE in STREAMLINE 200 column. The expanded bed was washed and then allowed to settle, the adaptor was lowered to the surface of the packed bed and the adsorbed fraction was eluted. The chromatogram is shown in Fig. 5 and Table 4 summarizes the data. The total cycle time for this Capture step was 3 hours. The complete process is described in Application Note 218, Code No. 18-1109-16.

Column: STREAMLINE 200 (i.d. 200 mm)
Adsorbent: STREAMLINE DEAE, 4.7 L
Sample: 4.7 kg of cells were subjected to osmotic shock and suspended in a final volume of 180 litres 50 mM Tris buffer, pH 7.4, before application onto the expanded bed.
Buffer A: 50 mM Tris buffer, pH 7.4
Buffer B: 50 mM Tris, 0.5 M sodium chloride, pH 7.4
Flow rate: 400 cm/h during sample application and wash
100 cm/h during elution
System: BioProcess Modular

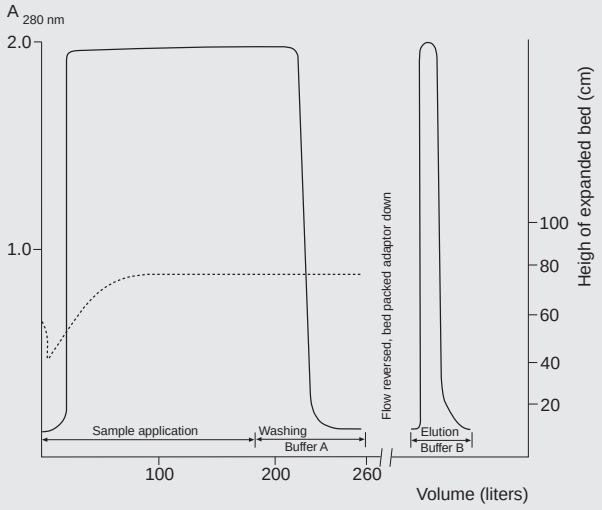

Fig. 5. Capture by expanded bed adsorption on STREAMLINE DEAE.

Table 4. Capture of recombinant *P. aeruginosa* exotoxin A from *E. coli* on STREAMLINE DEAE.

	Volume (L)	Total protein (g)	Exotoxin A (g)*	Recovery (%)
In	180	351	10.8	
Out	13.5	140	8.54	79

* Activity was determined with a radial immunodiffusion assay.

Capture of recombinant Fab fragment from E. coli homogenate

A process for the purification of a recombinant anti-HIV Fab fragment starting from unclarified *E. coli* homogenate was developed using STREAMLINE SP for Capture, Phenyl Sepharose 6 Fast Flow (high sub) for Intermediate Purification and SOURCE 15S for Polishing (17). High pressure homogenized *E. coli* suspension was applied to the expanded bed of STREAMLINE SP. Unbound material was washed out of the column, the bed was allowed to settle, the adaptor was lowered and the Fab-fragments were eluted. The method was optimized using the STREAMLINE 50 column and then scaled up to STREAMLINE 200, see Fig. 6. Data are summarized in Table 5. The complete process resulted in a pure recombinant Fab-fragment according to SDS-PAGE, with retained biological activity.

Column: STREAMLINE 200 (i.d. 200 mm)
Adsorbent: STREAMLINE SP, 4.6 L
Sample: 60 L high pressure homogenized *E. coli* suspension
Buffer A: 50 mM sodium acetate, pH 5.0
Buffer B: 50 mM sodium acetate, pH 5.0, 1 M NaCl
Flow rate: 300 cm/h during sample application and wash
100 cm/h during elution

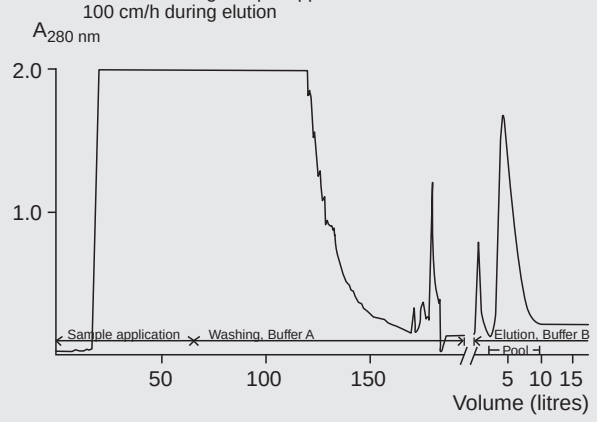

Fig. 6. Capture of recombinant anti-HIV Fab-fragment on STREAMLINE SP.

Table 5. Capture of recombinant anti-HIV Fab-fragment from *E. coli* on STREAMLINE SP at two scales.

Separation media, column		Volume (L)	Fab-fragment* (g)	Recovery (%)
STREAMLINE 50 column	In	4.8	0.033	
	Out	0.5	0.031	95
STREAMLINE 200 column	In	60	7.5	
	Out	6.0	7.5	100

* Activity was determined with an ELISA assay.

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Ordering information

Product		Code No.
STREAMLINE SP	0.3 L	17-0993-01
STREAMLINE SP	7.5 L	17-0993-02
STREAMLINE DEAE	0.3 L	17-0994-01
STREAMLINE DEAE	7.5 L	17-0994-02
XK 16/20 (16 mm i.d.)		18-8773-01
STREAMLINE 50 column (50 mm i.d.)		18-1038-01
STREAMLINE 200 column* (200 mm i.d.)		18-1100-22
*Stand (order separately)		18-1031-20

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