Purification of monoclonal antibodies using Eshmuno™ S

1. Screening for optimal step conditions

Different aspects are to consider with a chromatographic purification step (e.g. dynamic binding capacity and selectivity) and several parameters influence these aspects. Efficient screening for optimal step conditions is a straighforward strategy for the development of powerful purification processes (Bensch et al., 2005).

Impact of buffer system
Denton et al. (2001) investigated the impact of buffer type (buffer system) on the dynamic binding capacity of a monoclonal antibody on a cation exchanger. In their example acetate buffer resulted in much higher capacity than phosphate buffer. Common buffer systems in antibody purification are phosphate or citrate buffer (about 20 mM), or acetate buffer (about 50 mM). With regard to comparable results, conductivity of the buffers should be similar if different buffers are tested. Buffer conductivity is usually held in the range of 3.0 to 4.5 mS/cm. Different buffer systems may behave differently with varying antibodies. We found high dynamic binding capacities of Eshmuno™ S for monoclonal antibodies using phosphate buffer or mixed acetate/phosphate buffer as running buffer.

Impact of buffer pH
The dynamic binding capacity of cation exchangers for monoclonal antibodies often depends on mobile phase pH. Screening for optimum pH can be done by using microtitre plates and/or small columns (screening/scout columns). For a given antibody (with a specific isoelectric point, pI) the pH optima for maximum binding capacity may vary for different chromatography resins (Stein and Kiesewetter, 2007). And different antibodies may have different pH optima on the same resin. For Eshmuno™ S an example for pH screening is given in figure 1 for different residence times. Very high binding capacities of 65 to 95 mg of antibody per ml of resin were found at the pH optimum of 5.5.

Buffer pH may also affect the removal of impurities. Figure 2 illustrates the influence of pH on the removal of host cell protein (HCP). In this example the efficiency of HCP removal was twice as high at pH 6 compared to pH 5.

For each new antibody the screening for optimum step conditions should be performed. If binding capacity and impurity removal show different pH optima, a trade-off for the individual step must be defined with regard to the whole purification process/sequence.

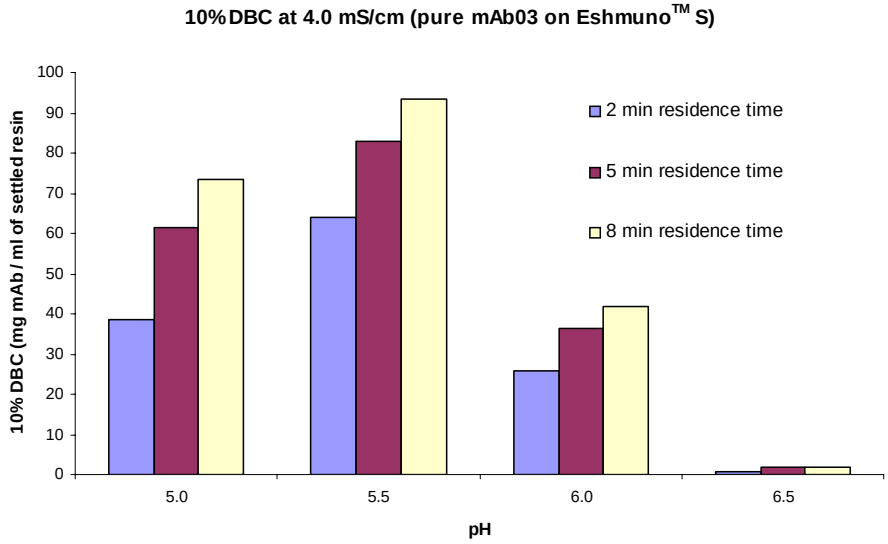

Figure 1 Impact of mobile phase pH on the dynamic binding capacity (DBC) of Eshmuno™ S for the monoclonal antibody mAb03 (pI = 8.7 - 9.2) at different residence times. Column: 50 x 10 mm i.d. Load: ≈5mg/ml mAb03 in 20 mM phosphate/ NaCl buffer (4.0 mS/cm). Loaded up to 10 % breakthrough.

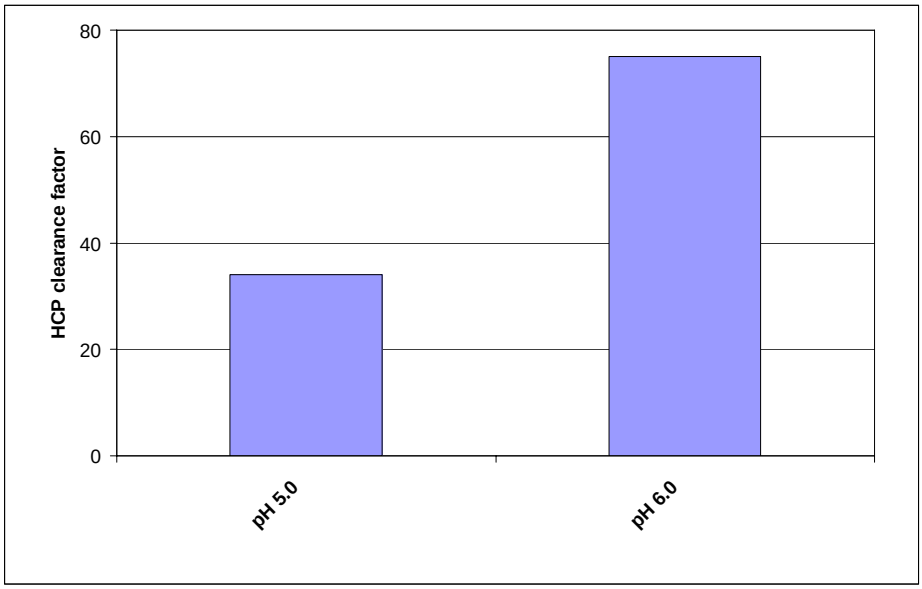

Figure 2 Impact of mobile phase pH on removal of host cell proteins using Eshmuno™ S in capture step. Sample: Monoclonal antibody mAb01 (pI = 7.9 - 8.5) in conditioned NS0 cell culture supernatant (4.4 mS/cm, pH 6.0 or 5.0), mAb01 conc. = 0.7 mg/ml. Column load: 10 mg of mAb01 per ml of column volume (CV). Column: 100 x 10 mm i.d., resin packed to 7 % compression. Buffer A (column equilibration): 25 mM Na-phosphate + 25 mM Na-acetate, pH=6.0 or 5.0. Buffer B (elution): 25 mM Na-Pi + 25 mM Na-Acetate + 1 M NaCl, pH=6.0 or 5.0. Gradient elution: 0 - 50 % buffer B in 20 CV. Flow rate: 150 cm/h.

2. Preparative chromatography

- Column dimensions: 100 mm (L) x 10 mm (I.D.)
  Packed with Eshmuno™ S, resin compression = 8 %
- Buffers: Buffer A: running buffer (column equilibration), conductivity ≈ 3.0 to 4.5 mS/cm
  Buffer B: elution buffer, = Buffer A plus 1 M NaCl
  Adequate buffer should be established in screening studies.
  20 mM phosphate buffer is a good buffer to start with for Eshmuno™ S.
  pH: Optimum pH should be determined in screening studies
- Sample: Conditioned cell culture supernatant (CCS), conductivity ≈ 3.0 to 4.5 mS/cm
  pH: Optimum pH should be determined in screening studies
  Depending on the scale conditioned CCS was obtained by either
  i) dilution with water and then pH adjusting with 1 M hydrochloric acid or
  ii) Ultra-/diafiltration using buffer A
- Column equilibration: 5 CV (column volumes) buffer A at 153 cm/h (2 ml/min)
- Sample load: To 5 % breakthrough at 300 cm/h (3.9 ml/min) = 2 min residence time
- Washing step: 7 CV buffer A at 153 cm/h
- Elution: Linear gradient of 0 - 50 % buffer B in 20 CV at 153 cm/h
  Fractionation: 3 ml fractions
  Pooling criterion for the antibody pool: A_{280} = 80 mAU
  The flow rate during wash and elution may be increased if appropriate
- Regeneration: 2 CV buffer B at 153 cm/h
- Cleaning-in-place: 2 CV 1 M NaOH at 153 cm/h in upflow mode
- Re-equilibration: 2 CV buffer B plus 5 CV buffer A at 153 cm/h

3. Analytics

- Analysed fractions: Sample, flow-through, wash, antibody pool, regeneration pool, cleaning-in-place pool
- Antibody: Quantification by analytical size-exclusion chromatography
- Host cell protein: Quantification by commercial host cell protein ELISA
- Host cell DNA: Quantification by OliGreen kit (ssDNA), PicoGreen kit (dsDNA)

4. References

Bensch, M., Schulze Wierling, P., von Lieres, E., and Hubbuch, J. (2005)
High throughput screening of chromatographic phases for rapid process development.
Chem. Eng. Technol. **28**(11), 1-11

Denton, G., Murray, A., Price, M.R., and Levison, P.R. (2001)
Direct isolation of monoclonal antibodies from tissue culture supernatant using the cation-exchange cellulose Express-Ion S.
J. Chromatogr. A **908**, 223-234

Stein, A. and Kiesewetter, A. (2007)
Cation exchange chromatography in antibody purification: pH screening for optimised binding and HCP removal.
J. Chromatogr. B **848**, 151-158