Eshmuno® Q

Cat. No. 1.20079

Version 3.0

August 28th, 2013

CONTENT

1. Introduction

Eshmuno® Q is a unique ion-exchange resin especially designed for highly productive downstream purification of monoclonal antibodies, any other recombinant proteins and plasma proteins. This anion exchanger is a member of the Eshmuno® family of smart resins and is highly productive in bind-and-elute and flow-through steps.
Eshmuno® Q is a surface grafted rigid hydrophilic polyvinylether polymer bead for the purification of strong acid and neutral proteins.
Eshmuno® Q process media provides high capacity and resolution along with all the advantages of polymerbased chromatographic beads such as high throughput, easy column packing, long life-time and high mechanical stability.
The most important advantage of the tentacle chemistry, where long, linear polymer chains ("tentacles") carry the functional groups, is the large number of sterically accessible ligands for the binding of biomolecules. All tentacles are covalently attached to the polyvinylether backbone and are chemically stable under conditions applied during chromatography, regeneration and sanitization.
Due to the titration behaviour the ion exchange capacity can be used from pH 2 up to
pH 12. The separation of proteins is based on reversible electrostatic interactions between the negatively charged regions of the proteins surface and the support. Proteins are retained efficiently on Eshmuno® Q when the pH of the buffer is about 1 unit above their isoelectric points (pI).

2. General Properties of Eshmuno® Q

Table 1: General properties of Eshmuno® Q.

	Eshmuno® Q
Mean particle size (D50)	85 µm
Type of chromatography	Strong anion exchange chromatography
Functional group	Trimethylammoniumethyl (TMAE)
Ionic capacity	90 - 190 µeq/ml settled resin
Protein binding capacity (static)	120 - 190 mg BSA/ml settled resin
Polyclonal IgG dynamic binding capacity	≥ 40 mg/ml (2 min residence time, 10% breakthrough)
pK value	≥ 13
pH range (working)	pH 2 up to pH 12
pH range (CIP)	pH 0 up to pH 14
Elution conditions	high salt concentrations
Mechanical stability	8 bar
Pressure drop	≤ 1.0 bar (100x16mm, 5ml/min, 150 cm/h)
Operating temperature	4 °C to room temperature
Storage, preservative	20 % ethanol, 150 mmol/l NaCl
Regeneration	1 - 2 M NaCl
Sanitization	0.1 - 1.0 M NaOH
Linear flow rate	up to 1000 cm/h (< 2.5 bar net pressure) 20 x 10 cm i.d. column, 8% compression, 150 mM NaCl as mobile phase

3. Chromatographic Properties

3.1. Separation of Standard Proteins

A mixture of conalbumin and human serum albumin (HSA) was separated under standard conditions for an anion exchanger with Eshmuno® Q. Absorption at 280 nm and conductivity were monitored.

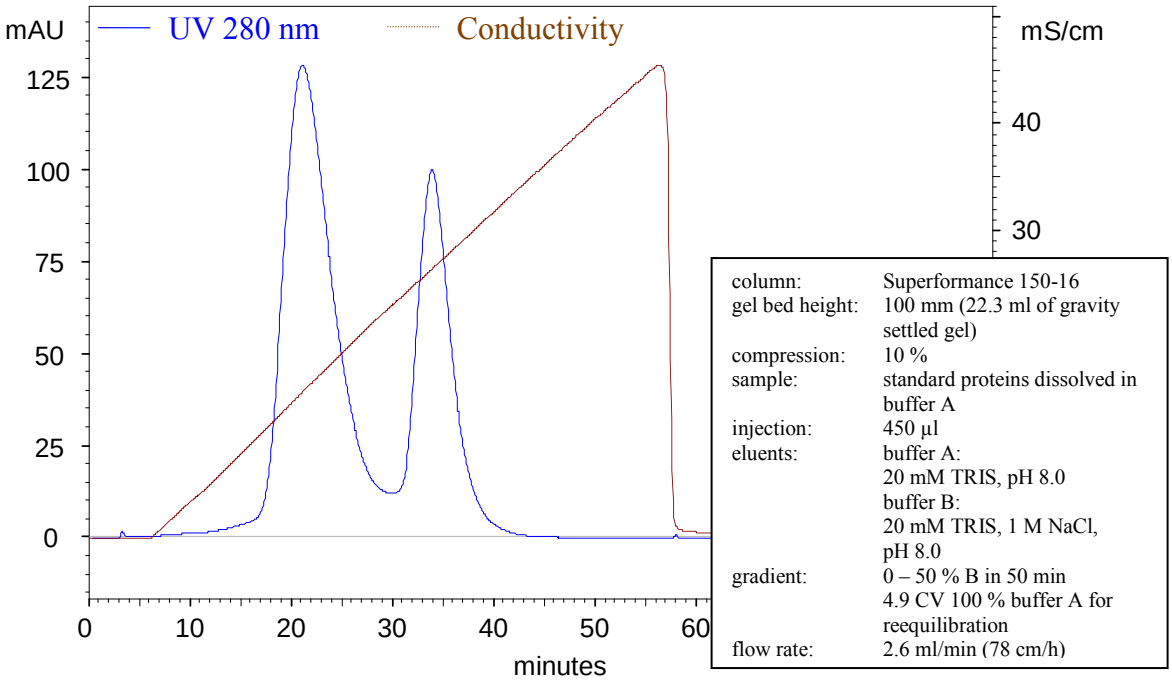

Figure 1: Separation of conalbumin (4 mg/ml) and HSA (5 mg/ml) in a linear salt gradient.

Conductivities at elution peak maxima:

conalbumin	14 mS/cm
human serum albumin (HSA)	26 mS/cm

3.2. Pressure versus Flow Curves

Eshmuno® Q was packed in a 10 cm i.d. column to 20 cm bed height and
8 % compression. Pressure versus flow curves were recorded according to the standard protocol.

Column:	Superformance®
Gel:	Eshmuno® Q, lot no. 09SAM024-10
Bed height:	20 cm
Compression:	8 % (sedimented gel volume is equivalent to 0% compression)
Eluent:	150 mM NaCl

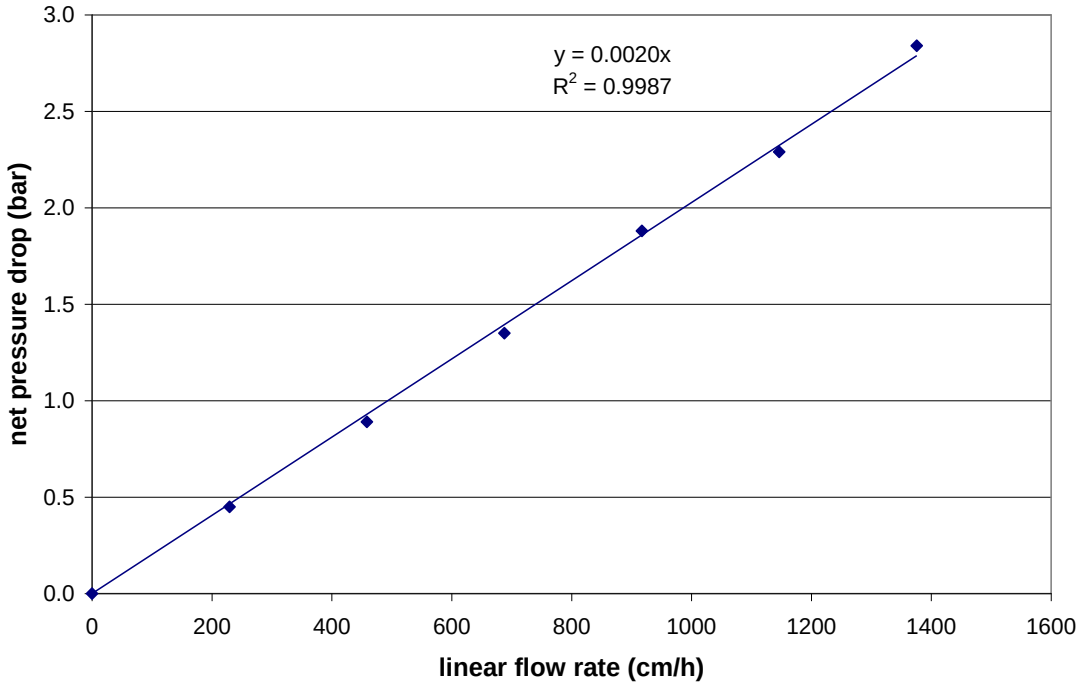

Figure 2: Pressure versus flow curve of Eshmuno® Q (lot no. 09SAM024-10).

Conclusion:
Due to the excellent pressure-flow behaviour, column pressure can be readily predicted as a function of scale. This feature allows the selection of operating conditions that maximize productivity. In contrast to many of the stationary phases used in biochromatography the new Eshmuno® Q is not compressible and, thus, exhibits a linear relationship between column pressure and flow rate independent on column diameters.

3.3. Dynamic Capacity

To demonstrate the dependence of the binding capacity of Eshmuno® Q on the flow rate, the following dynamic experiments with BSA were carried out. Binding capacities were calculated at 10 % breakthrough in the eluate, as monitored at 280 nm.

Column:	1 ml Scout column (18.95 x 8.2 mm i.d.)
Gel:	Eshmuno® Q, lot no. 09SAM024-10
Binding buffer	50 mM TRIS, pH 8.0
Sample	bovine serum albumin (BSA, 5 mg/ml) in binding buffer
Eluent:	50 mM TRIS, 1 M sodium chloride, pH 8.0

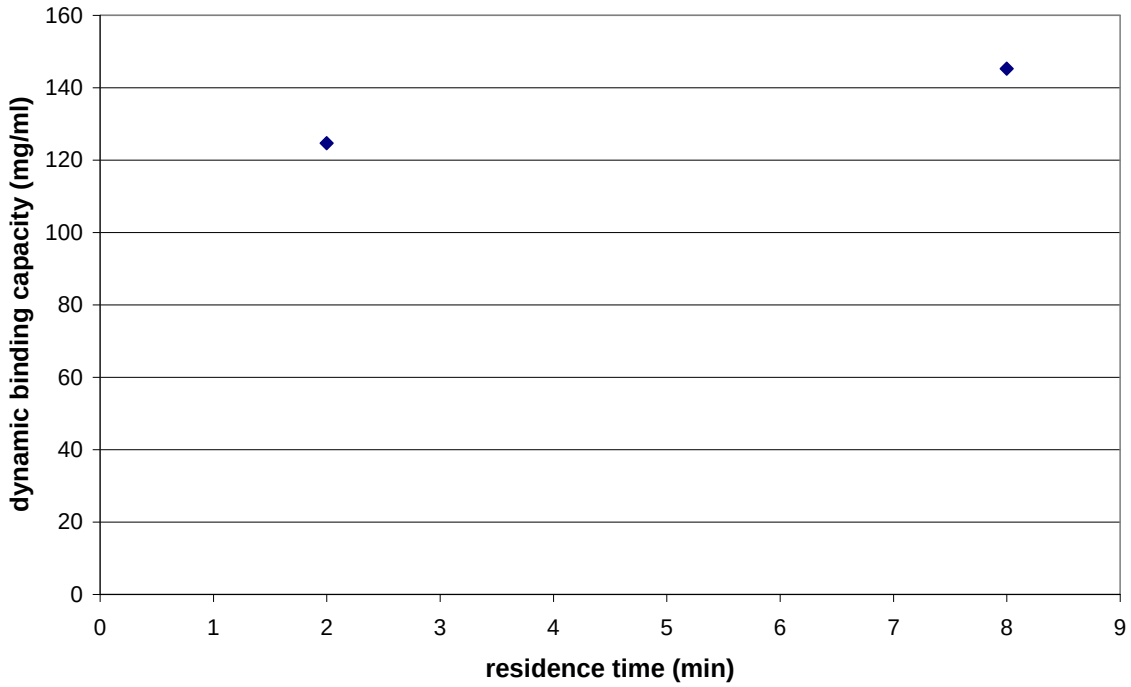

Figure 3: Influence of residence time on dynamic capacity of Eshmuno® Q for BSA at 10% break-through, 5 mg/ml BSA in 50mM TRIS buffer at pH 8.

Conclusion:
Eshmuno® Q resin has a high dynamic protein binding capacity even at low residence times. Therefore, high flow rates can be applied while binding capacity is still high enough for efficient purification steps.

3.4. Resolution versus Loading

To demonstrate the impact of loading on chromatographic resolution the following experiments with conalbumin and bovine serum albumin (BSA) were carried out.

Column:	Superformance® 150-16
Gel:	Eshmuno® Q, lot no. 09SAM001-08
Bed height:	10 cm
Compression:	10%
Binding buffer	50 mM TRIS, pH 8.0
Sample	conalbumin (6.7 mg/ml) and BSA (13.3 mg/ml) in binding buffer
Eluent:	50 mM TRIS, 1 M sodium chloride, pH 8.0
Elution gradient	0% – 73% eluent buffer in 9.5 CV
Flow rate	150 cm/h during elution

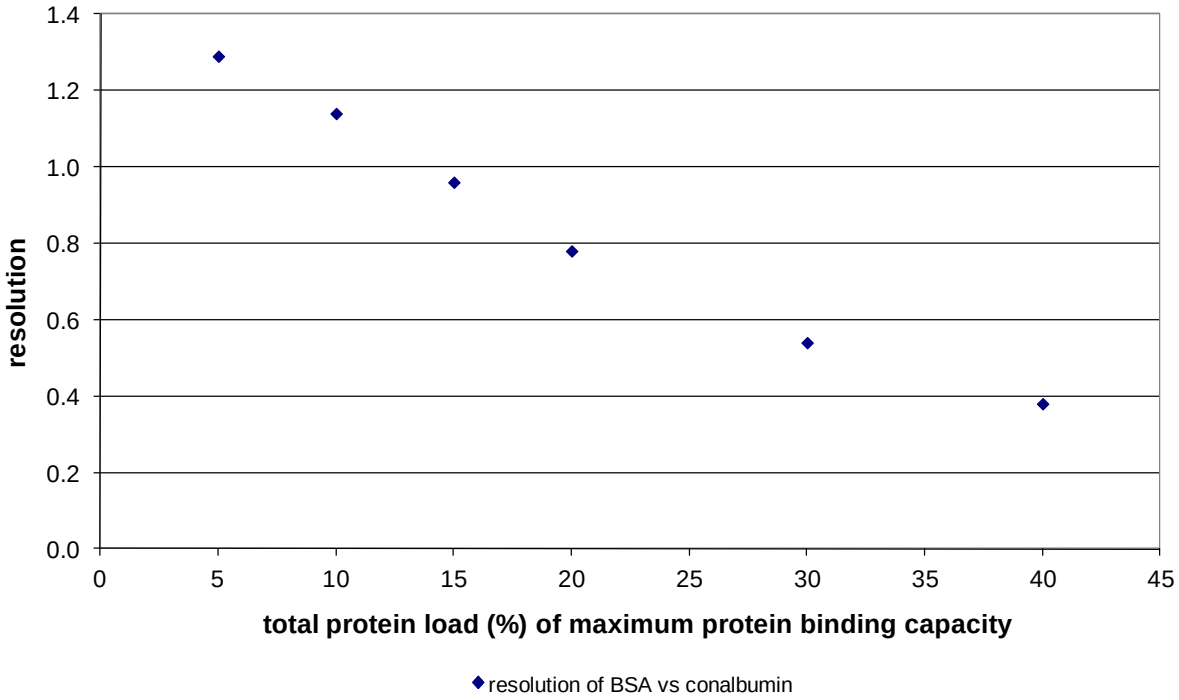

Figure 4: Conalbumin and BSA were separated on Eshmuno® Q at different loadings in a linear salt gradient.

Conclusion:

The chromatographic resolution is inversely proportional to the total protein load due to band broadening. Even though resolution is decreasing at higher protein loads according to the theory, Eshmuno® Q resin gives good separations at higher loads and enables one to develop economical process steps.

3.5. Resolution versus Flow Rate

To demonstrate the impact of flow rate on chromatographic resolution the following experiments were carried out with standard proteins at low and high loading.

Analytical load:

Column:	Superformance® 150-16
Gel:	Eshmuno® Q, lot no. 09SAM021-09
Bed height:	10 cm
Compression:	10%
Binding buffer:	50 mM TRIS, pH 8.0
Sample:	Conalbumin (4 mg/ml) and BSA (5 mg/ml) in binding buffer
Load:	500 µl
Eluent:	50 mM TRIS and 1 M sodium chloride, pH 8.0
Elution gradient	0% – 65% eluent buffer in 8.5 CV

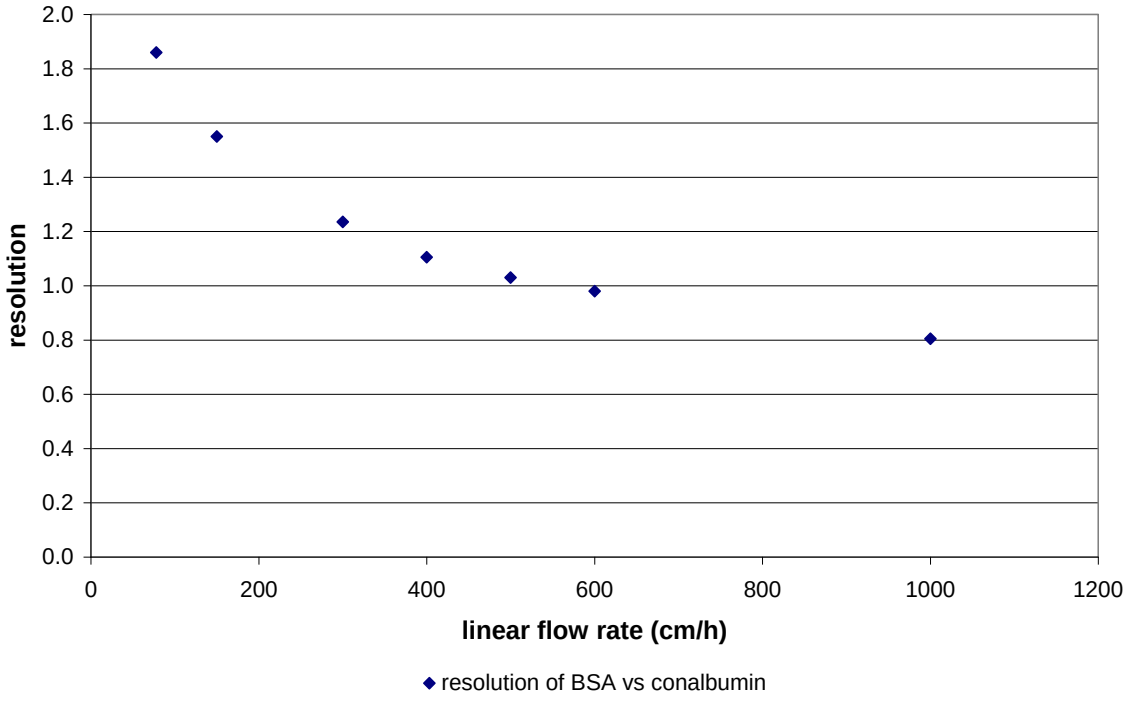

Figure 5: Conalbumin and BSA were separated on Eshmuno® Q at different flow rates in a linear salt gradient (load: 0.13% of total column binding capacity based on BSA).
As expected, the resolution of two distinct peaks is decreasing at higher flow-rates. Depending on the desired separation power, even more rapid separations are possible.

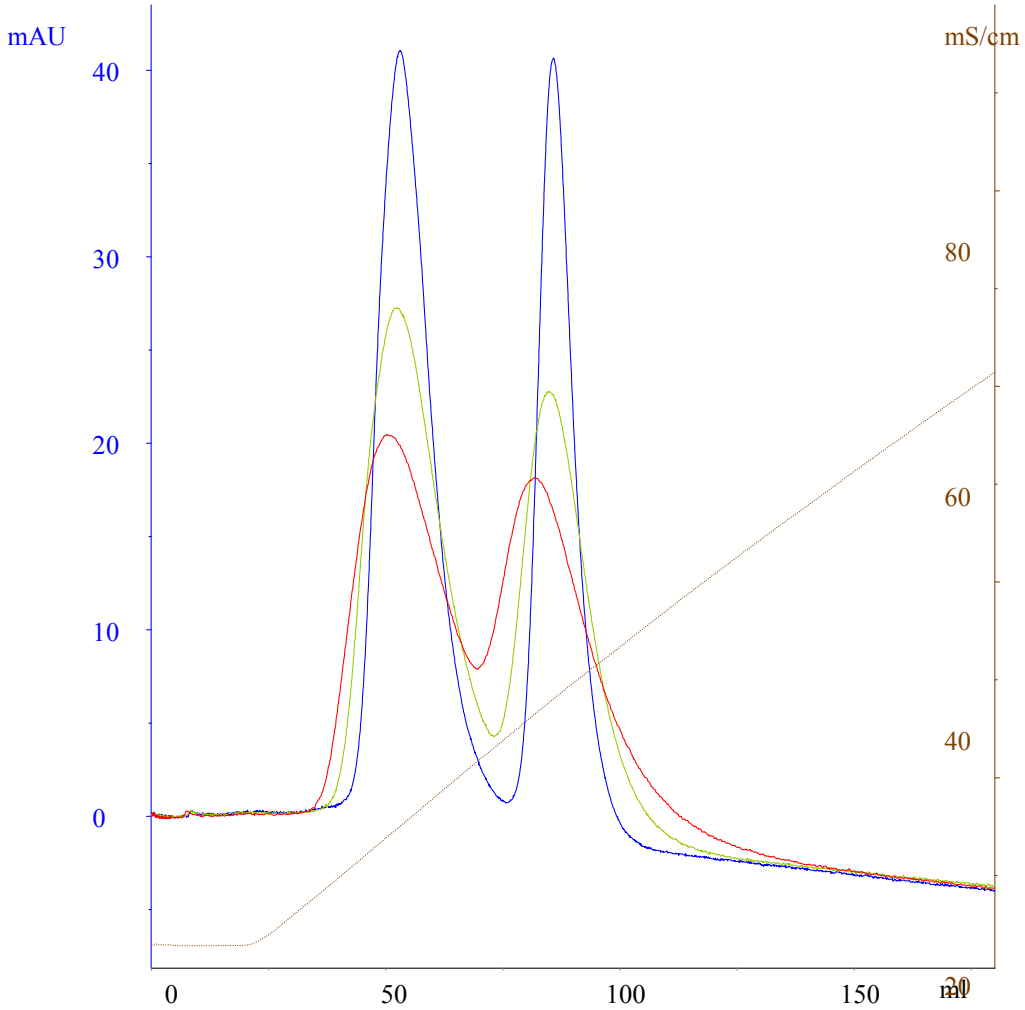

Figure 6: Separation of conalbumin and BSA on Eshmuno® Q in a linear salt gradient (conductivity brown) after loading the column to 0.13% of total column binding capacity at 78 cm/h (blue), 400 cm/h (green) and 1000 cm/h (red curve).

Process load

Column:	Superformance® 150-16
Gel:	Eshmuno® Q, lot no. 09SAM001-08
Bed height:	10 cm
Compression:	10%
Binding buffer	50 mM TRIS, pH 8.0
Sample	conalbumin (6.7 mg/ml) and BSA (13.3 mg/ml) in binding buffer
Load	26 ml
Eluent:	50 mM TRIS, 1 M sodium chloride, pH 8.0
Elution gradient	0% – 73% eluent buffer in 9.5 CV

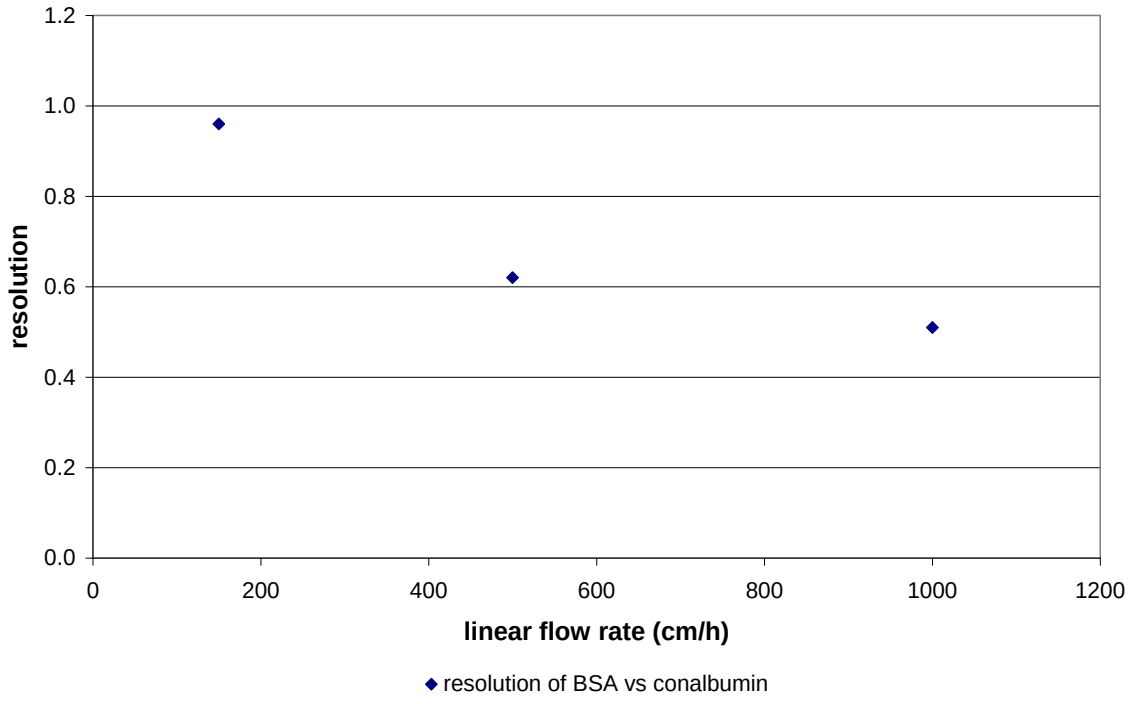

Figure 7: Conalbumin and BSA were separated on Eshmuno® Q at different flow rates in a linear salt gradient (load: 15% of total column binding capacity).

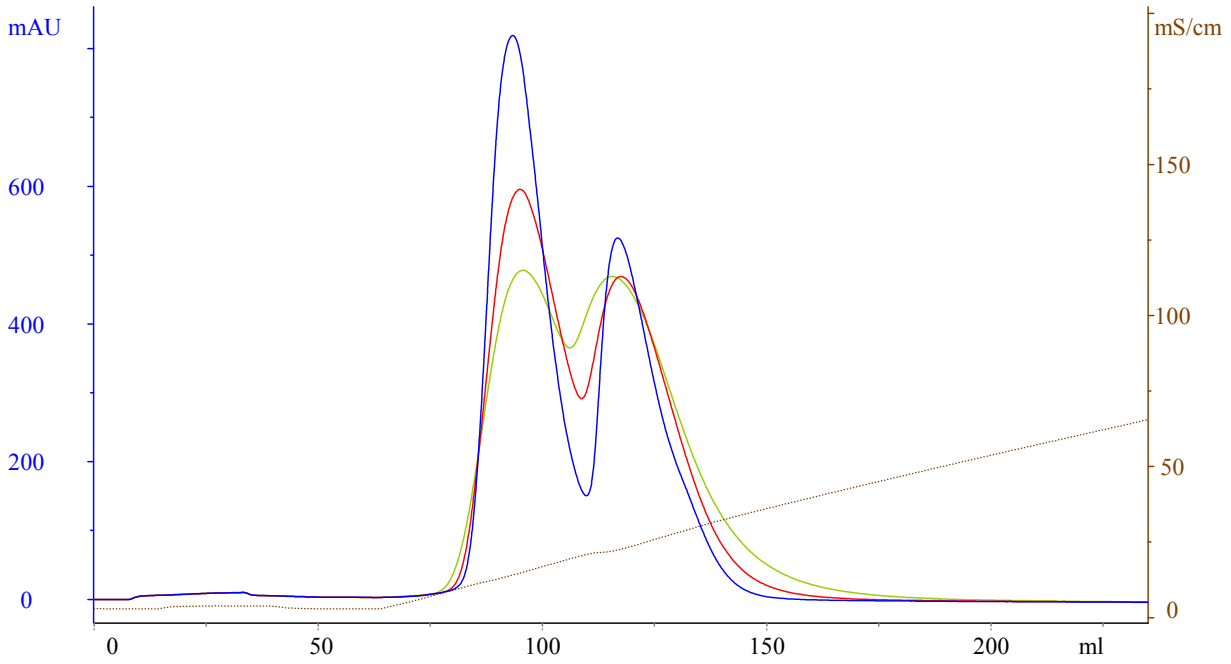

Figure 8: Separation of conalbumin and BSA on Eshmuno® Q after loading the column to 15% of total column binding capacity at 150 cm/h (blue), at 500 cm/h (red) and at 1000 cm/h (green curve).

Conclusion:
Conalbumin and BSA were separated on Eshmuno® Q at different flow rates in a linear salt gradient (load: 15% of total column binding capacity based on BSA). As expected, the resolution of two distinct peaks is decreasing at higher flow rates. Depending on the desired separation power, even more rapid separations are possible.

4. Chromatographic Stability

4.1. Reproducibility of 100 Cycles

To demonstrate the reproducibility of chromatography runs obtained with Eshmuno® Q, separation of a protein mixture was repeated 100 times.

After each run with standard proteins, the column was cleaned with two column volumes (CV) of 1 M sodium hydroxide for 60 min at a flow rate of 20 cm/h, before re-equilibration with running buffer was performed.

Therefore, the total exposure time of the column to 1 M sodium hydroxide was at least 100 h.

Chromatographic conditions:

Column:	Superformance® 150-16
Gel:	Eshmuno® Q, lot no. 09SaM021-09
Bed height:	10 cm
Compression	10%
Sample:	solution of BSA (42 mg/ml) and conalbumin (21 mg/ml) in buffer A (total protein concentration 63 mg/ml)
Sample volume:	5 ml (315 mg protein)
Buffer A:	50 mM TRIS, pH 8.0
Buffer B:	50 mM TRIS, 1 M NaCl, pH 8.0
Gradient:	linear, from 0 % B to 50 % B within 6.5 CV
Flow rate:	5 ml/min
Detection:	280 nm
Elution:	1 CV buffer B
CIP:	1 CV 1 M NaOH at 5 ml/min and 2 CV at 0.67 ml/min
Re-equilibration:	1 CV buffer B and 6 CV buffer A

The initial dynamic binding capacity at 600 cm/h and 10 % breakthrough for BSA was 140 mg/ml of packed resin, the final binding capacity after 100 cycles was determined to be 140 mg/ml.

Resolution

The figures show that the conductivities at peak maxima, retention times, and resolution for two different sample proteins are independent of the number of runs. Within a very narrow range the elution of the sample proteins remains the same for at least 100 chromatography cycles.

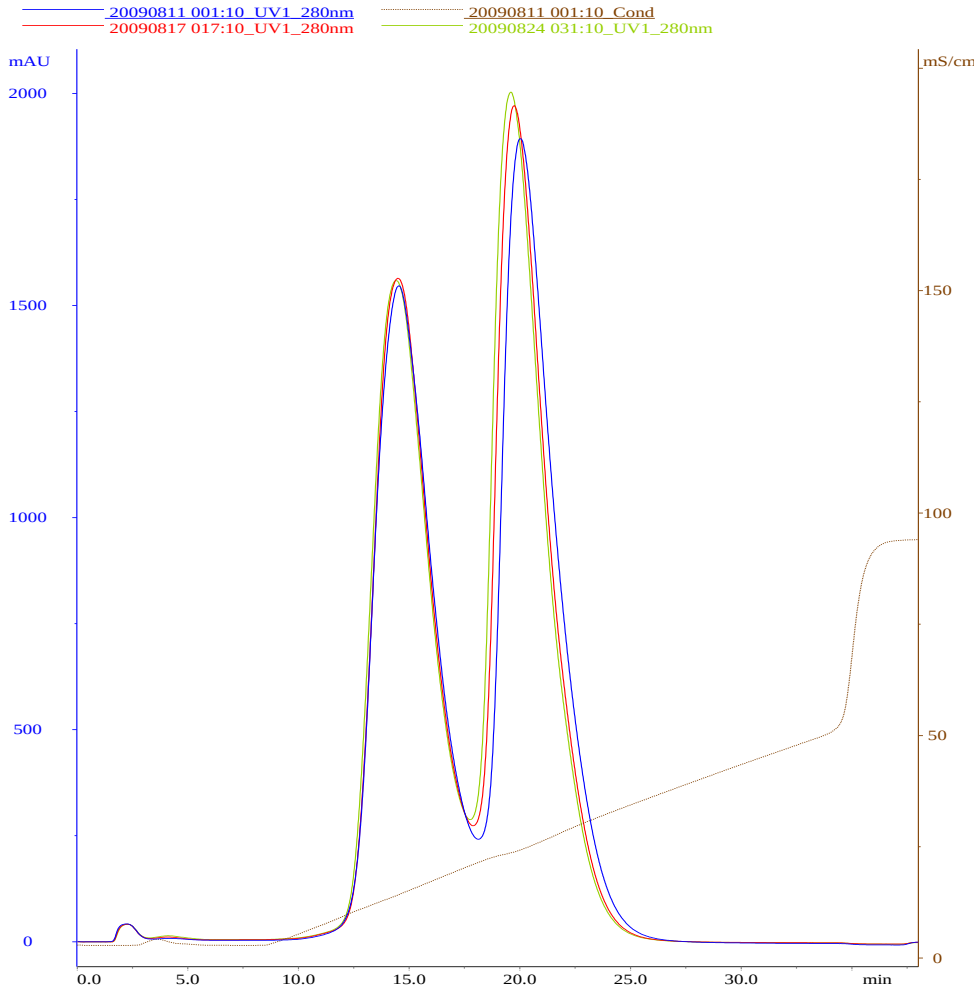

Figure 9: Separation of conalbumin and BSA on Eshmuno® Q, overlaid chromatograms of run 1 (blue), run 50 (red) and run 101 (green).

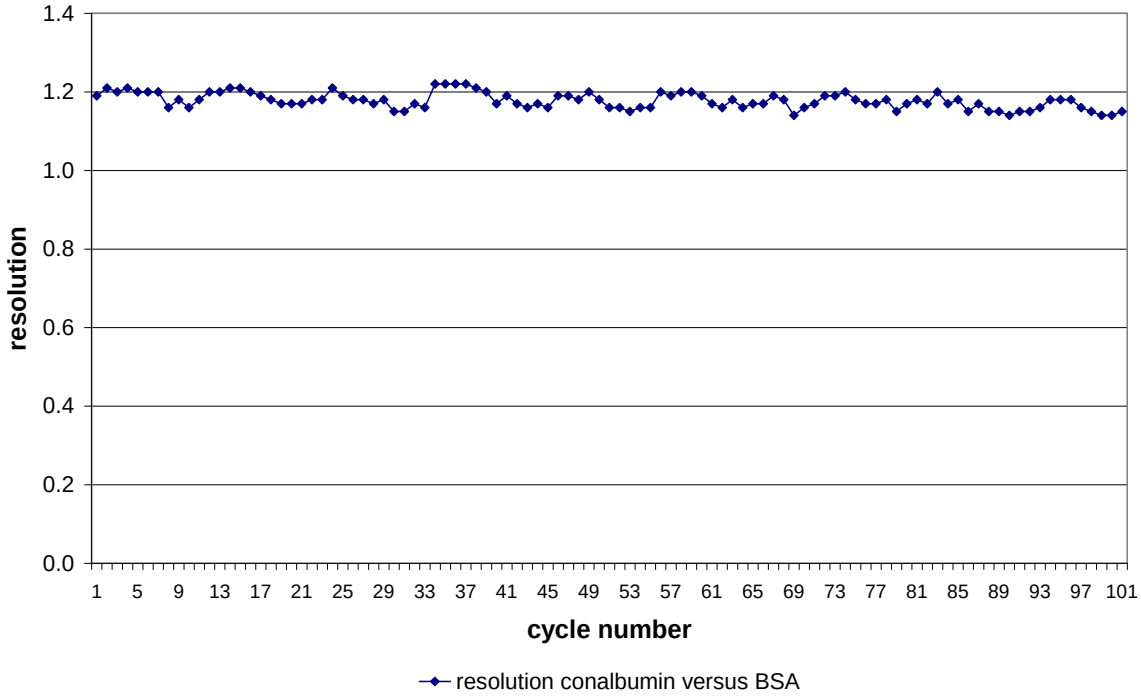

Figure 10: Resolution of conalbumin (peak 1) and BSA (peak 2) after consecutive runs.

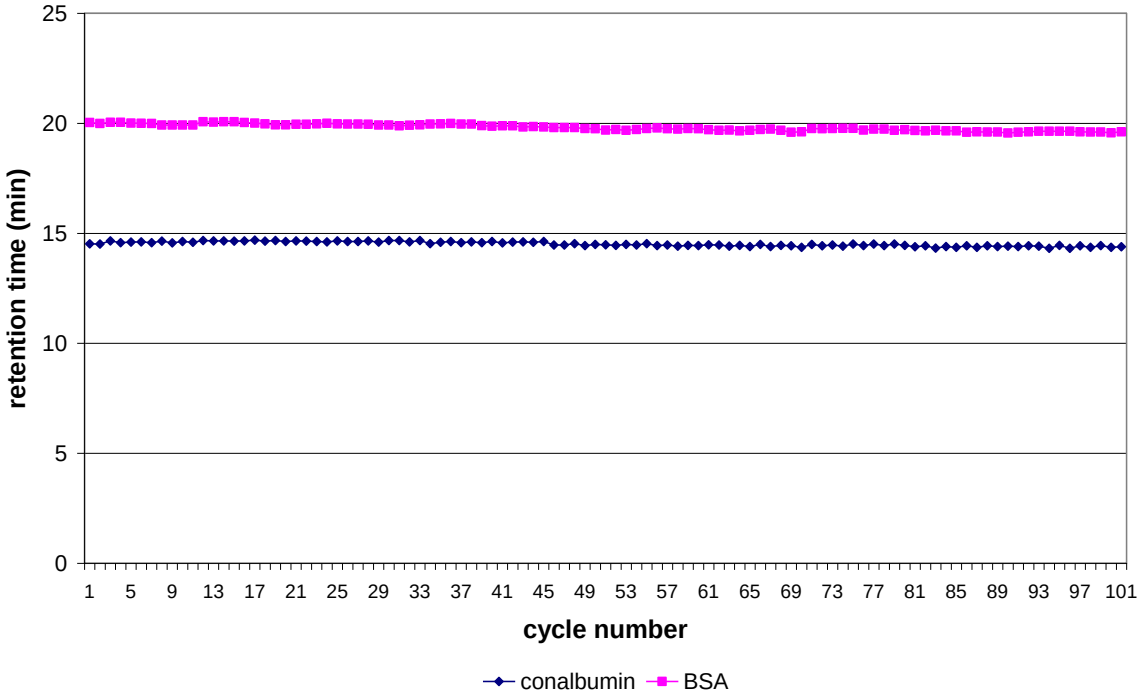

Figure 11: Retention times of conalbumin peak and BSA peak after consecutive runs.

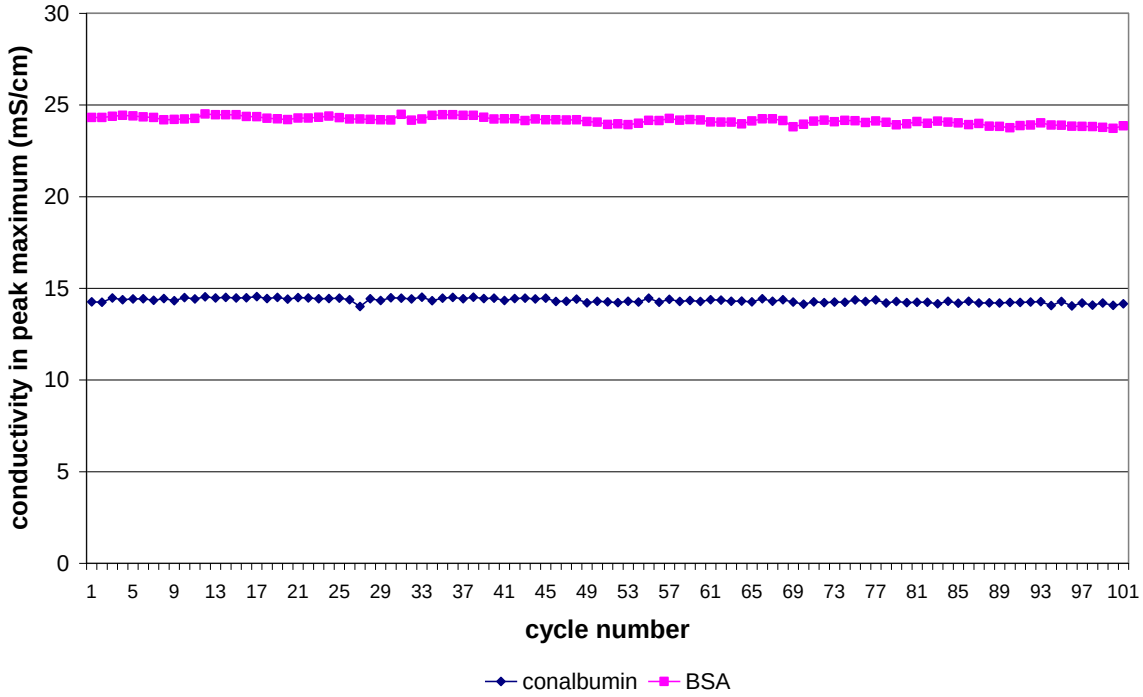

Figure 12: Conductivities at peak maxima of conalbumin peak and BSA peak after consecutive runs.

Conclusion:
During repeated 100 cleaning-in-place (CIP) cycles, no significant changes in chromatographic properties were detected. Experimental results showed no modification of the chromatographic properties of the Eshmuno Q after 100 cycles of bovine serum albumin and conalbumin separation. The retention times and elution conductivities of peak maxima of the sample proteins remain the same. Also the dynamic binding capacity for BSA remains unchanged after 100 chromatographic cycles including 100 CIP cycles.

5. Chemical Stability

5.1. Alkaline Stability

Due to the chemical structure of the Eshmuno® polymer matrix, which is a cross-linked rigid hydrophilic vinylether/urea copolymer and due to the surface modification with linear functionalized acrylamide polymers (graft polymer), the resulting Eshmuno® ion exchanger has a very high chemical stability even during prolonged exposure to usual clean-in-place conditions.

Both the polymeric matrix backbone, consisting of long alkyl chains with ether linkage to the hydroxyalkyl side groups, as well as the urea cross-linking units are very stable to caustic conditions. The same applies to the grafted functionalized polyacrylamide, which contain the ion exchanger ligands.

However, slow hydrolysis on the surface can occur by prolonged exposure of the support to strong alkaline conditions causing some release of hydrolysis products. These products could originate from the base matrix as well as in very small amounts from the graft polymer.

The immobilized chromatographically relevant functional group of the strong anion exchanger Eshmuno® Q is the quaternary ammonium group. The major drawback of this group is the relative chemical instability under strong alkaline conditions. If cleaning-in-place or sanitization of resins with quaternary ammonium groups is carried out utilizing sodium hydroxide concentrations of > 1 M, a significant loss of functional groups is observed after a number of cycles due to its slow chemical degradation. Storage of these resins in diluted sodium hydroxide (0.05 – 0.5 M) at room temperature for long time intervals cause the same effect, albeit more slowly. Even storage of these resins under neutral conditions in aqueous suspension will cause very slow degradation of the functional groups. This can easily be recognized by the amine odour present in the inner atmosphere of stored containers with the respective gel. At that point it should be emphasized that all known ion exchangers with quaternary ammonium groups ("Q-type") undergo the described degradation and normally lose binding capacity.

5.2. Stability against Sodium Hydroxide

Bulk resin (for measurement of protein binding capacity) and a packed column (for recording of chromatographic resolution and pressure versus flow curves) of
Eshmuno® Q, lot no. 09SAM021-09, were stored in 1.0 M, 0.5 M and 0.1 M sodium hydroxide solution at room temperature.
For capacity testing, aliquots of the gel suspension were taken after specified time intervals, washed with water and neutralized with 100 mM sodium phosphate buffer pH 7.0. Protein binding capacities were determined according to the standard procedures described in the monograph.

For measurement of chromatographic resolution and pressure versus flow curves the resin was packed into a Superformance® 150-16 column to 10 cm bed height at 10% compression. After testing the column according to the monograph the column was rinsed with sodium hydroxide and stored at room temperature. After specified

time intervals, the column was rinsed with 2 column volumes (CV) of equilibration buffer, 2 CV of elution buffer at a flow rate of 150 cm/h (5 ml/min), followed by 5 CV of equilibration buffer (2.6 ml/min). For determination of pressure versus flow curves the column was rinsed with 3 CV of 150 mM sodium chloride at a flow rate of 210 cm/h (7 ml/min). Then pressure versus flow curves were recorded. In addition, chromatographic resolutions were determined according to the standard procedures described in the monograph (separation of conalbumin and BSA, BSA was used instead of HSA) after rinsing the column with 10 CV of equilibration buffer. After testing the column was again rinsed with sodium hydroxide solution and stored at room temperature.

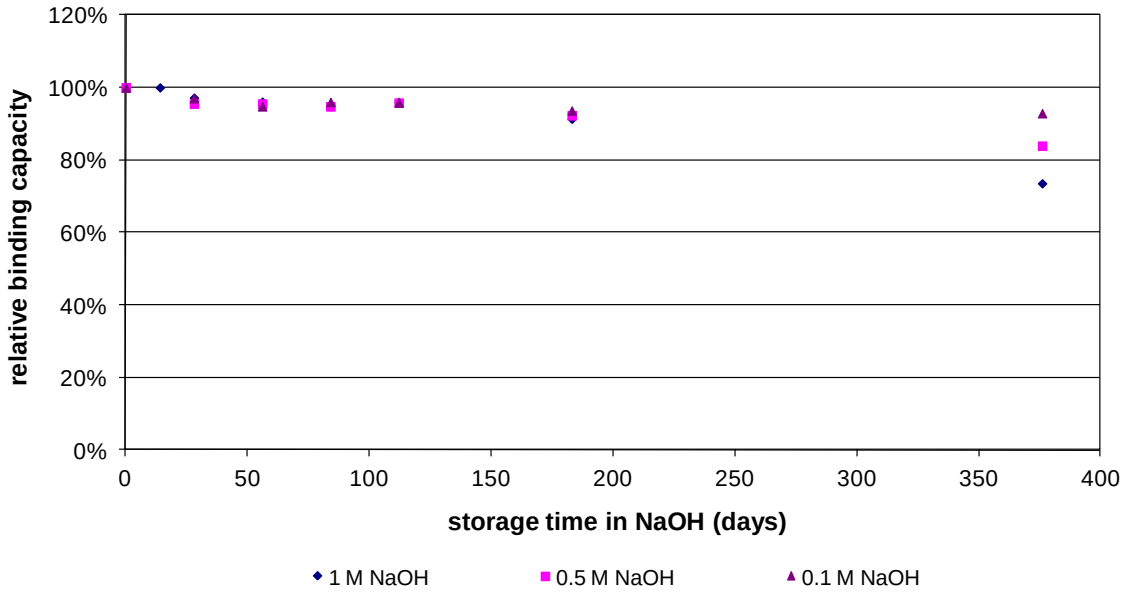

Figure 13: Relative static BSA binding capacity after prolonged treatment with 1.0 M, 0.5 M or 0.1 M sodium hydroxide.

Conclusion:

Maximizing the useful lifespan of preparative columns is a central issue during process development. Eshmuno® Q is stable in both acidic and alkaline solutions. Thus, the resin can be easily regenerated under defined conditions for repeated chromatographic use. Specific CIP protocol can be designed according to the stability data shown above for each process according to the type of contaminants.

Eshmuno® Q does not lose significantly protein binding capacity even after exposure to 1 M sodium hydroxide solution for several months at room temperature. Concurrently, the resolution of standard proteins (see below) remains nearly unchanged in this period. Pressure versus flow properties (see below) are hardly altered during long term storage in 1 M sodium hydroxide solution. Although the frequency of CIP depends on the nature and the condition of the sample, more than 100 CIP cycles are generally possible as documented here.

5.2.1. Stability against 1 M Sodium Hydroxide

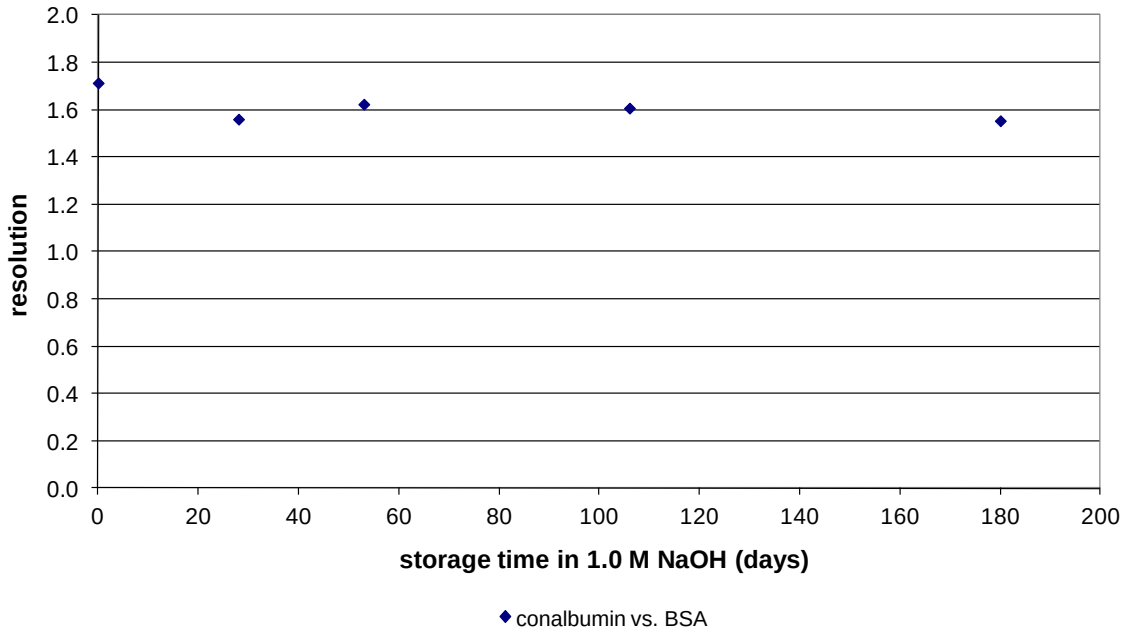

Figure 14: Resolution data for a standard separation on a packed column
(100 mm x 16 mm i.d., 10% compression) stored in 1 M sodium hydroxide for certain times after re-equilibration.

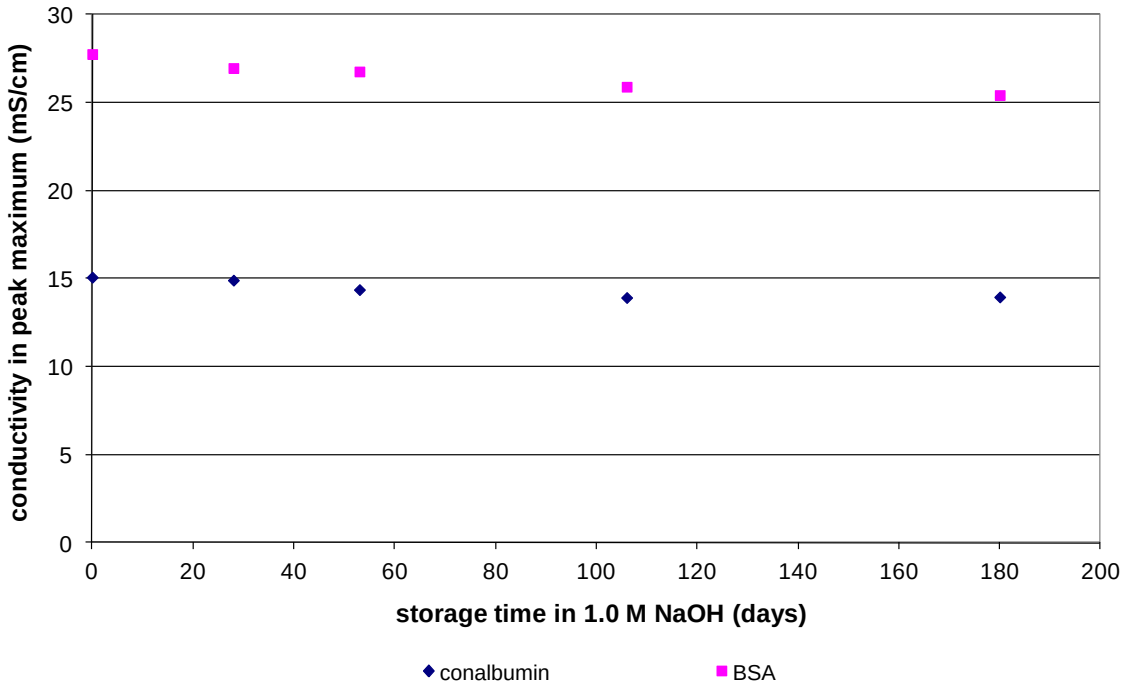

Figure 15: Elution conductivities at peak maxima for a standard separation on a packed column (100 mm x 16 mm i.d., 10% compression) stored in 1 M sodium hydroxide for certain times after re-equilibration.

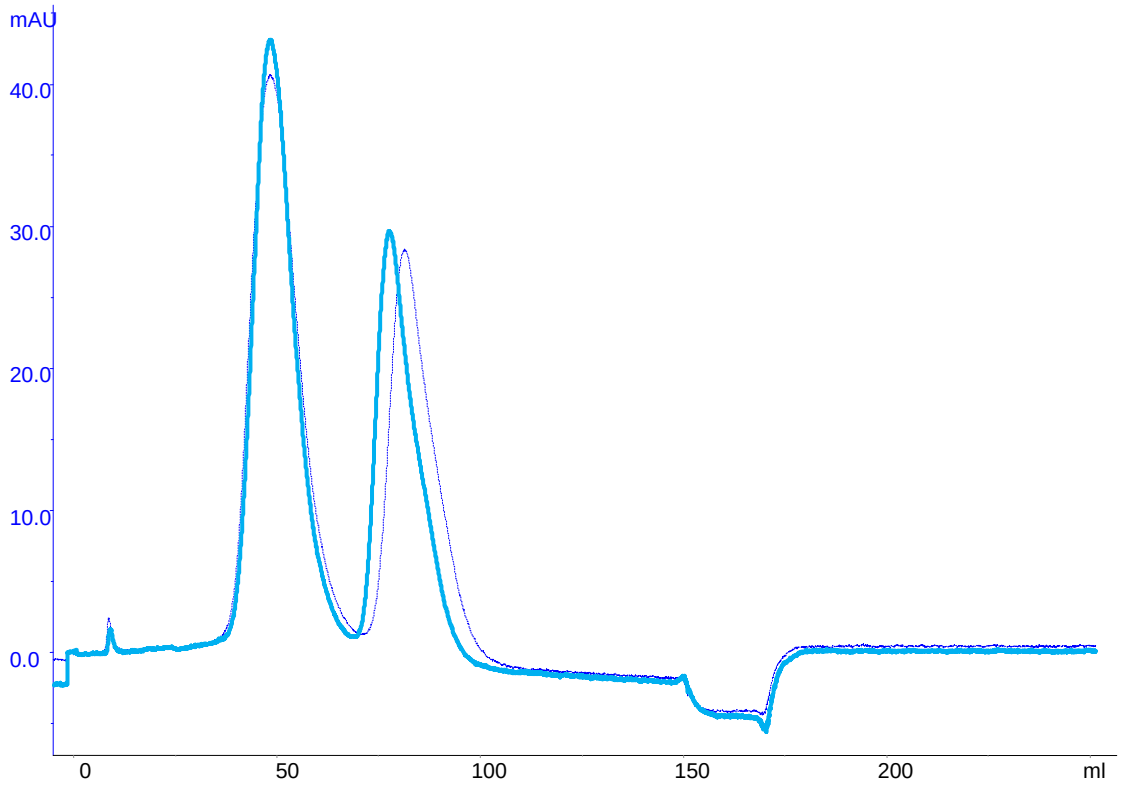

Figure 16: Chromatograms of conalbumin and BSA eluted from a packed column (100 mm x 16 mm i.d., 10% compression) with Eshmuno® Q after storage in 1 M NaOH (solid cyan) for 6 months compared to storage in 20 vol% ethanol / 150 mM NaCl (blue, dashed line).

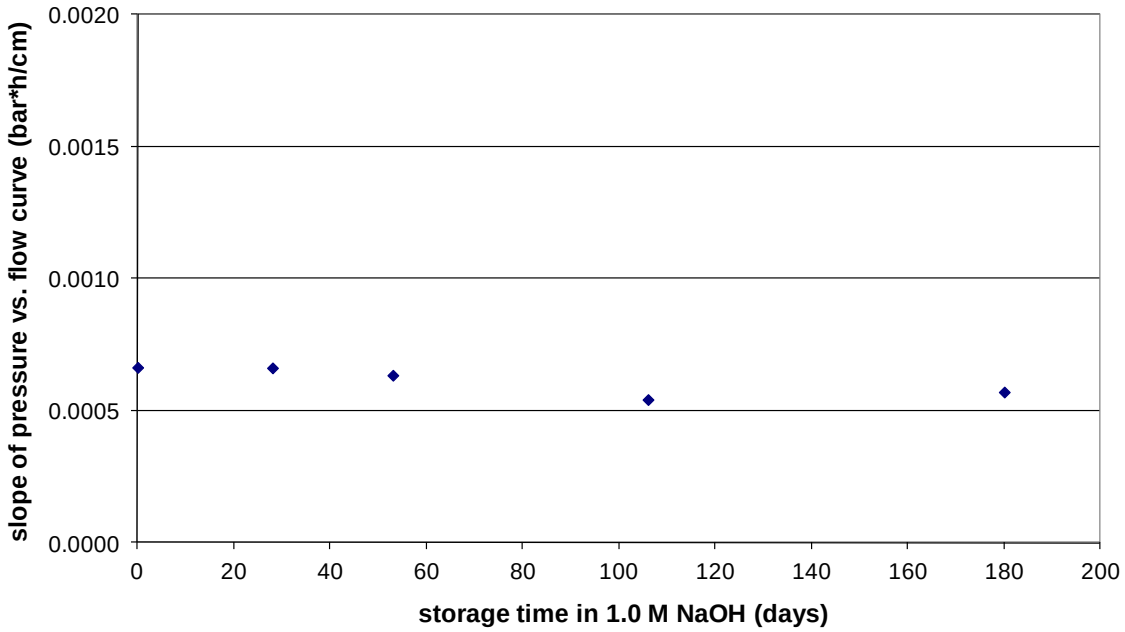

Figure 17: Slope of the net pressure drop versus flow curve after treatment with 1 M sodium hydroxide for certain times. Pressure versus flow curves were recorded in 100 x 16 mm i.d. columns packed to 10% compression at 150 – 1500 cm/h with 150 mM sodium chloride as mobile phase. The slope of the net pressure drop versus flow curve after treatment with 1.0 M sodium hydroxide for certain times was determined by linear regression. The coefficient of determination (R^2) was always greater than 0.989.

5.2.2. Stability against 0.5 M Sodium Hydroxide

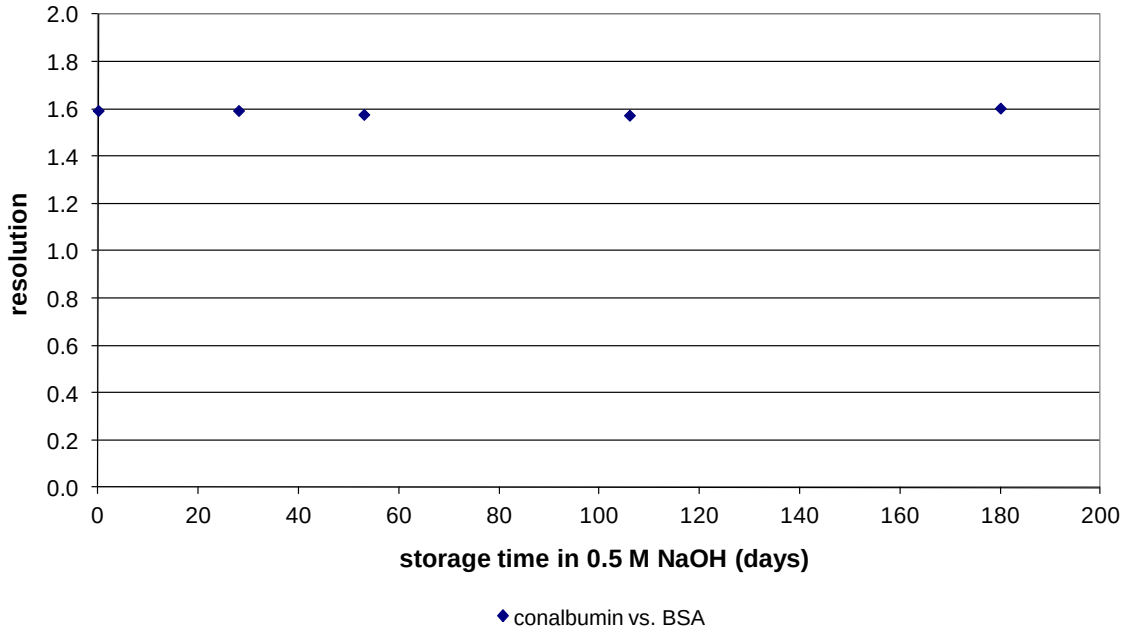

Figure 18: Resolution data for a standard separation on a packed column
(100 mm x 16 mm i.d., 10% compression) stored in 0.5 M sodium hydroxide for certain times after re-equilibration.

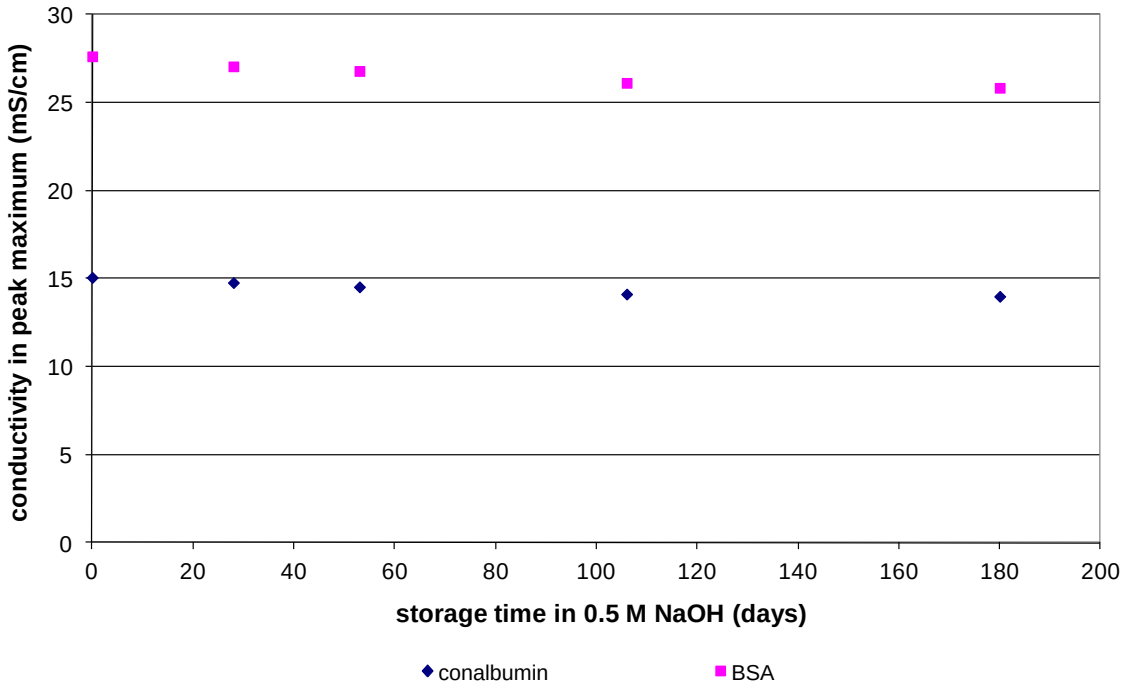

Figure 19: Elution conductivities at peak maxima for a standard separation on a packed column (100 mm x 16 mm i.d., 10% compression) stored in 0.5 M sodium hydroxide for certain times after re-equilibration.

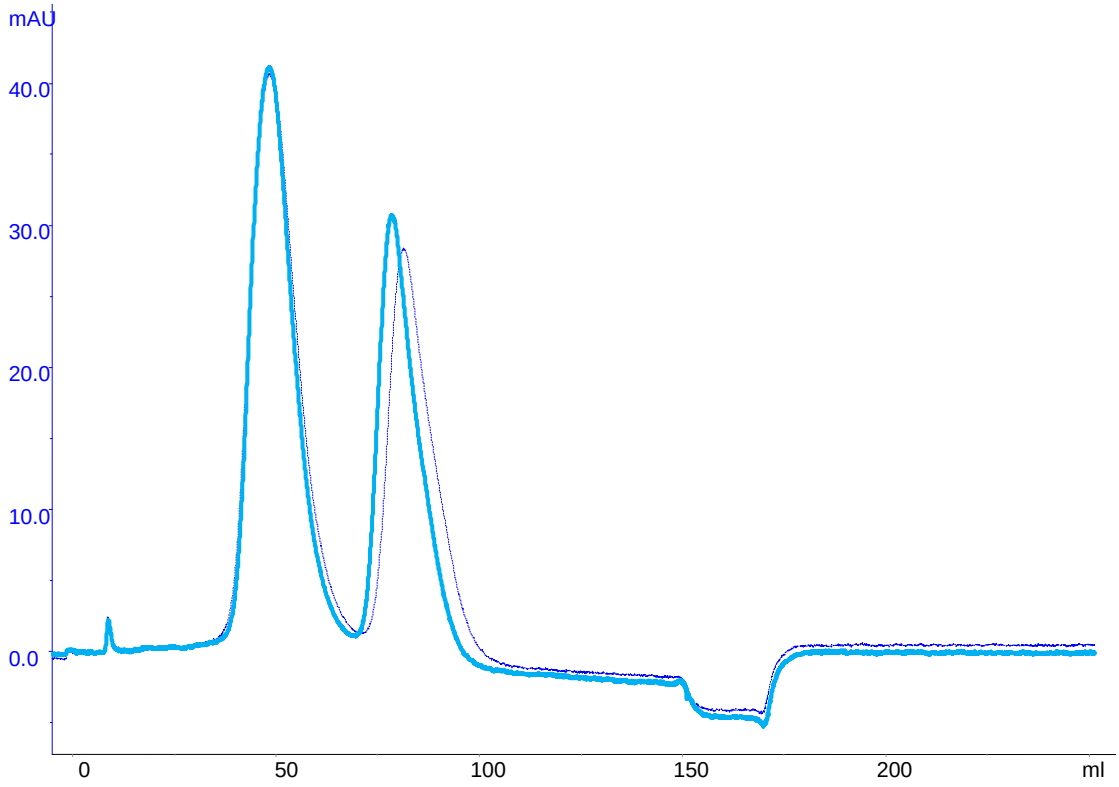

Figure 20: Chromatograms of conalbumin and BSA eluted from a packed column (100mm x 16 mm i.d., 10% compression) with Eshmuno® Q after storage in 0.5 M NaOH (solid cyan) for 6 months compared to storage in 20 vol% ethanol / 150 mM sodium chloride (dashed blue line).

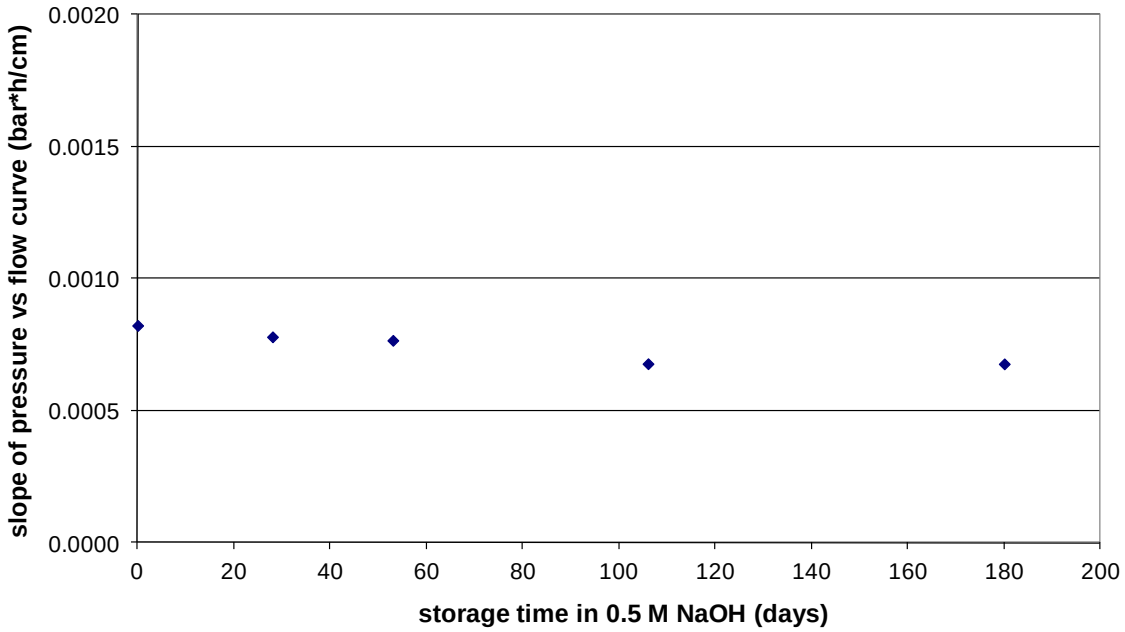

Figure 21: Pressure versus flow curves were recorded in 100 x 16 mm i.d. columns packed to 10% compression (150 – 1500 cm/h). The slope of the net pressure drop versus flow curve after treatment with 0.5 M sodium hydroxide for certain times was determined by linear regression. The coefficient of determination (R^2) was always greater than 0.992.

5.2.3. Stability against 0.1 M Sodium Hydroxide

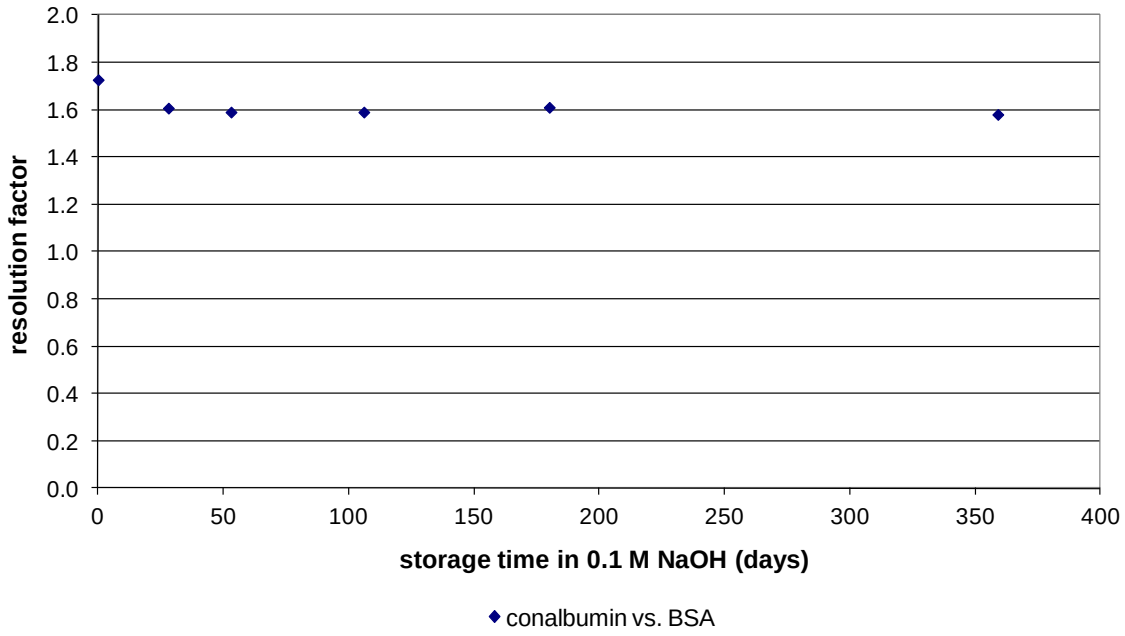

Figure 22: Resolution data for a standard separation on a packed column
(100 mm x 16 mm i.d., 10% compression) stored in 0.1 M sodium hydroxide for certain times after re-equilibration.

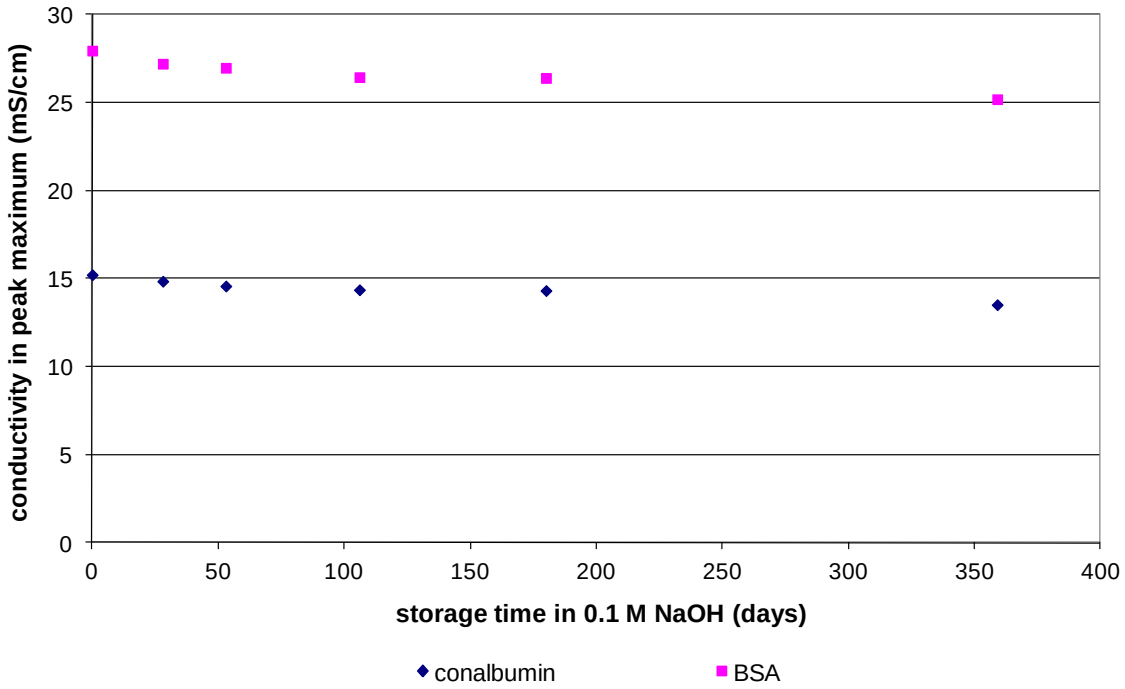

Figure 23: Elution conductivities at peak maxima for a standard separation on a packed column (100 mm x 16 mm i.d., 10% compression) stored in 0.1 M sodium hydroxide for certain times after re-equilibration.

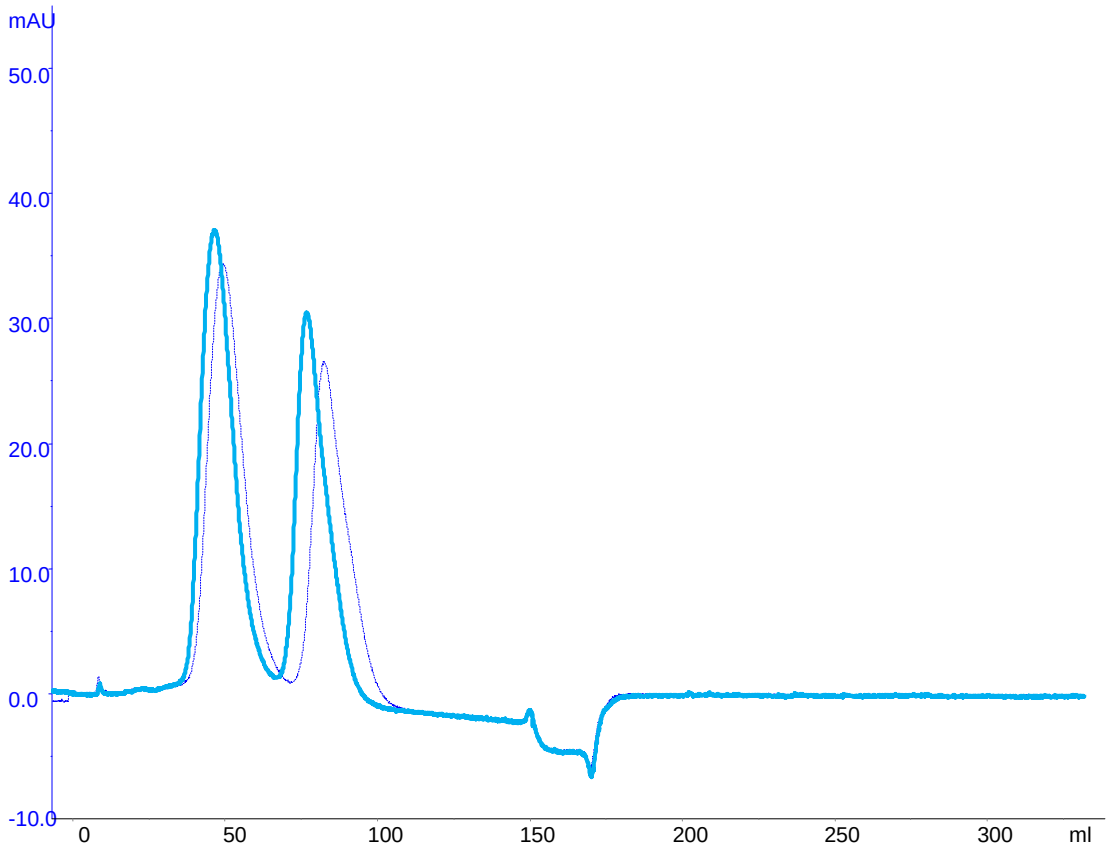

Figure 24: Chromatograms of conalbumin and BSA eluted from a packed column (100mm x 16 mm i.d., 10% compression) with Eshmuno® Q after storage in 0.1 M NaOH for 12 months (solid cyan) compared to storage in 20 vol% ethanol / 150 mM sodium chloride (dashed blue line).

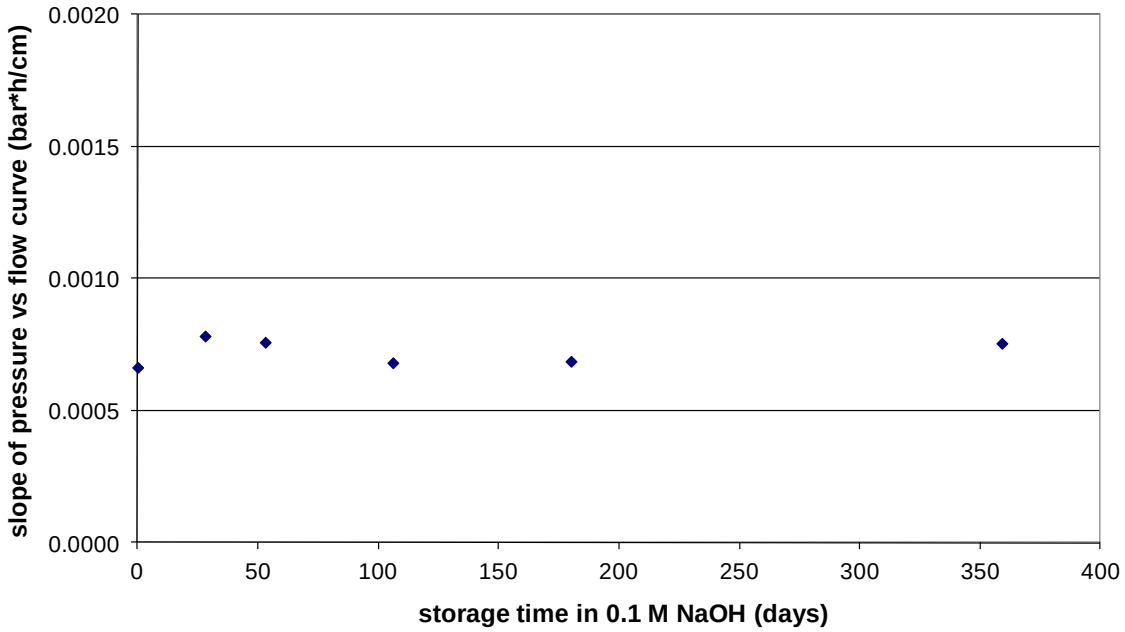

Figure 25: Pressure versus flow curves were recorded in 100 x 16 mm i.d. columns packed to 10% compression (150 – 1500 cm/h) with 150 mM sodium chloride as mobile phase. The slope of the net pressure drop versus flow curve after treatment with 0.1 M sodium hydroxide for certain times was determined by linear regression. The coefficient of determination (R^2) was always greater than 0.993.

5.3. Stability against Acids

According to the chemical structure of Eshmuno® Q the amide groups present in the matrix can be expected to be prone to hydrolysis by strong mineral acids. As the functional ligands (quaternary ammonium groups) are coupled to the polyacrylamide (tentacles), this would lead to release of soluble amine and, concomitantly, to the loss of ion exchanger functionality of the gel. However, acidic hydrolysis of the amide bond is an extremely slow reaction under normal chromatographic conditions. Of course, the functional ligands and the matrix can be hydrolyzed using harsh conditions, e.g. several hours treatment of the gel with 6 M hydrochloric acid at 120°C under elevated pressure.

5.3.1. Stability against 1 M Hydrochloric Acid

Bulk resin (for measurement of protein binding capacity) and a packed column (for recording of chromatographic resolution and pressure versus flow curves) of
Eshmuno® Q, lot no. 09SAM021-09, were stored in 1.0 M hydrochloric acid solution at room temperature.
For capacity testing, aliquots of the gel suspension were taken after specified time intervals, washed with water and neutralized with 100 mM sodium phosphate buffer
pH 7.0. Protein binding capacities were determined according to the standard procedures described in the monograph.
For measurement of chromatographic resolution and pressure versus flow curves the resin was packed into Superformance® 150-16 column to 10 cm bed height at 15% compression. After testing the column according to the monograph the column was rinsed with hydrochloric acid and stored at room temperature. After specified time intervals, the column was rinsed with 2 column volumes (CV) of equilibration buffer, 2 CV of elution buffer at a flow rate of 150 cm/h (5 ml/min), followed by 5 CV of equilibration buffer (2.6 ml/min).For determination of pressure versus flow curves the column was rinsed with 3 CV of 150 mM sodium chloride at a flow rate of 210 cm/h (7 ml/min). Then pressure versus flow curves were recorded. In addition, chromatographic resolutions were determined according to the standard procedures described in the monograph (separation of conalbumin and BSA, BSA was used instead of HSA) after rinsing the column with 10 CV of equilibration buffer. After testing the column was again rinsed with hydrochloric acid and stored at room temperature.

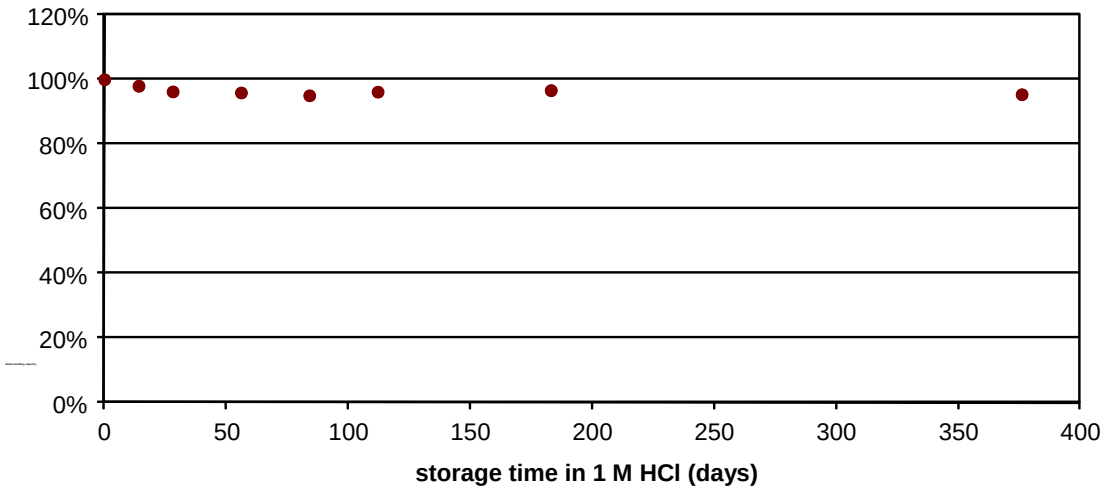

Figure 26: Relative static BSA binding capacity after prolonged treatment with 1.0 M hydrochloric acid.

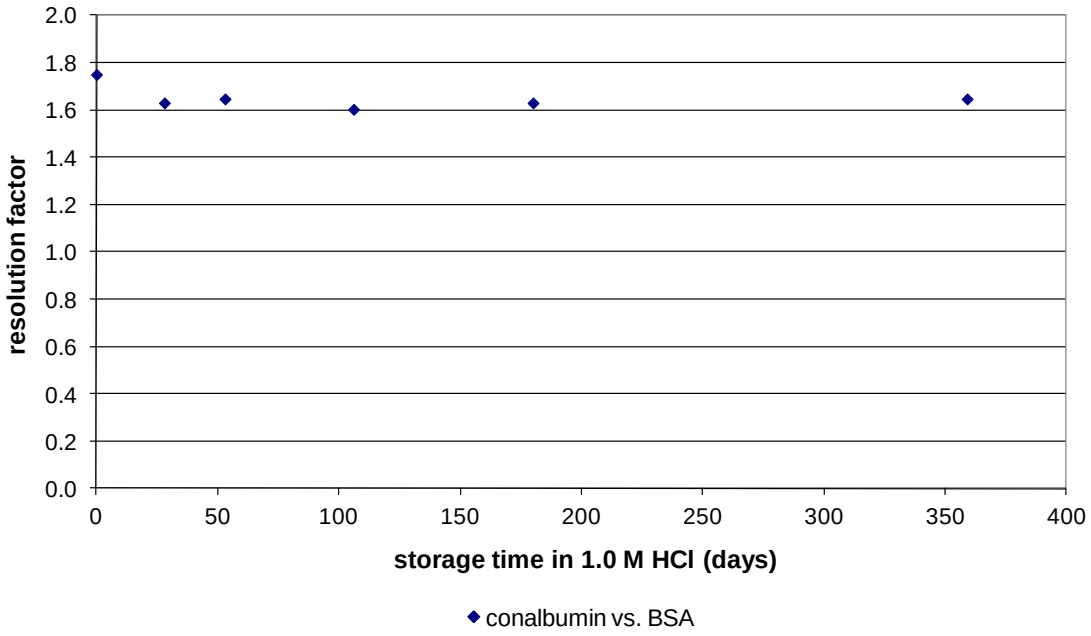

Figure 27: Resolution data for a standard separation on a packed column (100 mm x 16 mm i.d., 10% compression) stored in 1.0 M hydrochloric acid for certain times after re-equilibration.

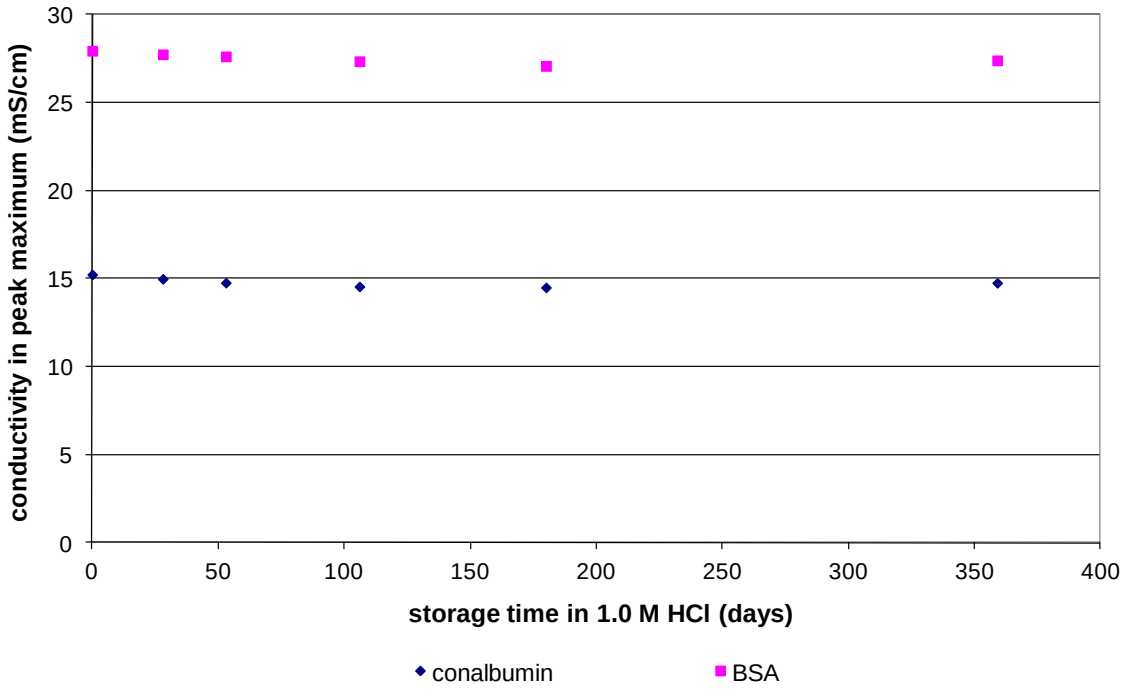

Figure 28: Elution conductivities at peak maxima for a standard separation on a packed column (100 mm x 16 mm i.d., 10% compression) stored in 1.0 M hydrochloric acid for certain times after re-equilibration.

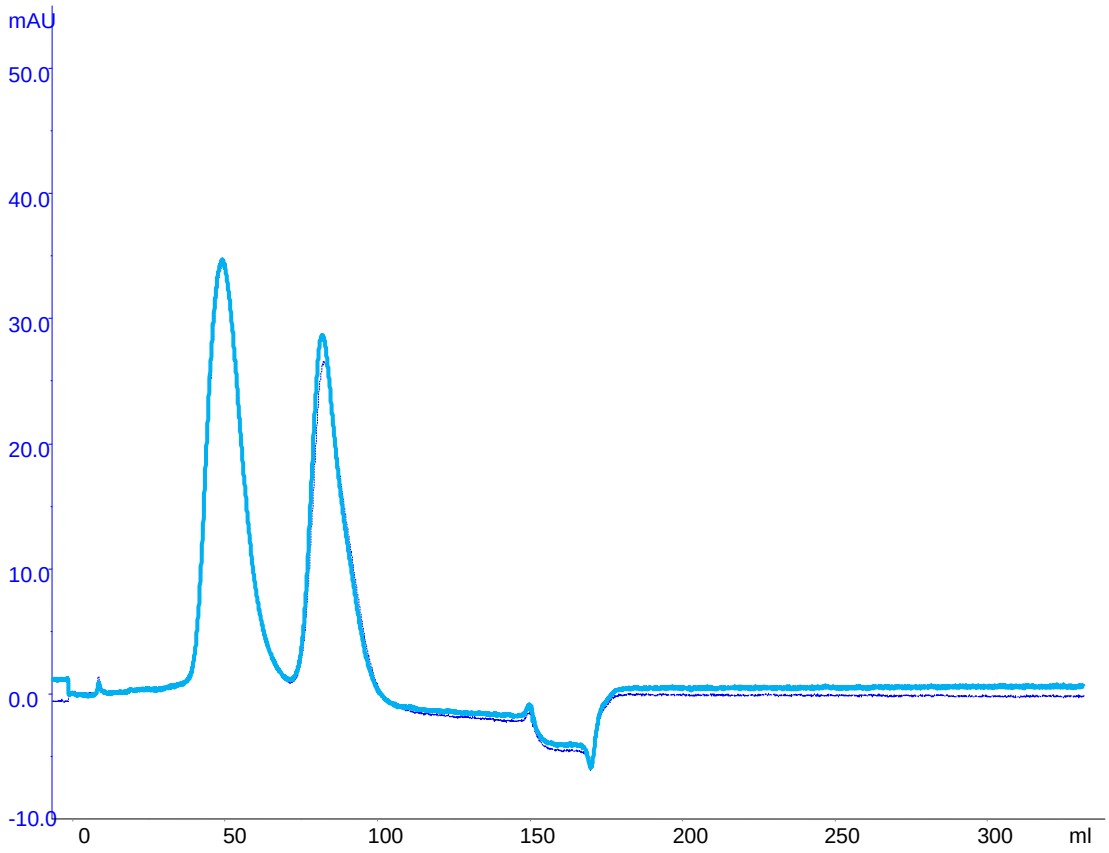

Figure 29: Chromatograms of conalbumin and BSA eluted from a packed column (100mm x 16 mm i.d., 10% compression) with Eshmuno® Q after storage in 1.0 M hydrochloric acid for 12 months (solid cyan) compared to storage in 20 vol% ethanol / 150 mM sodium chloride (dashed blue line).

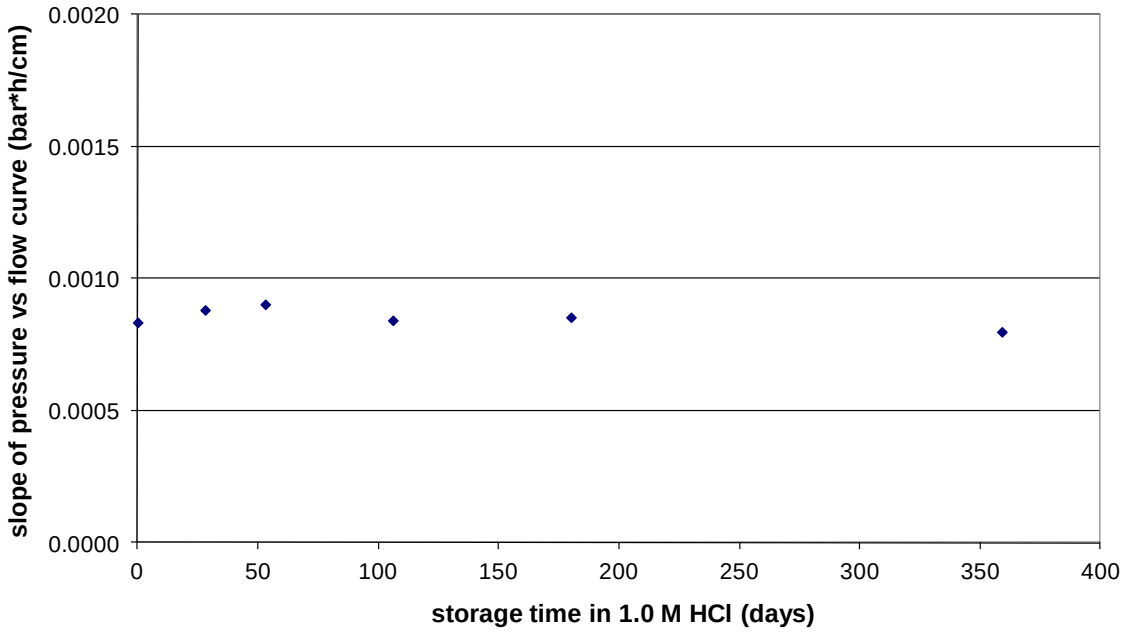

Figure 30: Pressure versus flow curves were recorded in 100 x 16 mm i.d. columns packed to 10% compression (150 – 1500 cm/h) with 150 mM sodium chloride as mobile phase. The slope of the net pressure drop versus flow curve after treatment with 1.0 M hydrochloric acid for certain times was determined by linear regression. The coefficient of determination (R^2) was always greater than 0.992.

Conclusion:
Eshmuno® Q does not lose protein binding capacity after exposure to 1 M hydrochloric acid solution for one year at room temperature. Concurrently, the resolution of standard proteins remains nearly unchanged in this period. Pressure versus flow properties are hardly altered during long term storage in 1 M hydrochloric acid.

5.4. Stability against Various Chemicals

To demonstrate the compatibility of Eshmuno® Q, columns packed with the resin were exposed to various chemicals commonly used in biochromatography. The chemicals are 5 M urea, 6 M guanidinium chloride and 30% 2-propanol (see tables below).

Experimental procedure:

Three 100 mm x 16 mm i.d. columns were packed with Eshmuno® Q to 10% compression according to the standard procedure. The columns were equilibrated to the respective chemical at 5 ml/min using ten column volumes. Afterwards the chemical was circulated through the column for about 16 hours at 6 ml/min.
After circulation with each chemical a pressure versus flow curve of the column was recorded with this chemical according to the packing protocol.
After this treatment the column was equilibrated with TRIS buffer and characterized by the separation of standard proteins as described in the monograph. Subsequently a pressure versus flow curve was performed using 150 mM sodium chloride eluent.

Results and Discussion:

After regeneration with buffer the chromatographic behaviour of Eshmuno® Q was unchanged.

Table 2: Resolution data and elution conductivities at peak maxima for the separation of standard proteins before and after treamtment with particular chemicals.

untreated and regenerated column: 50 mM TRIS, pH 8.0	elution conductivity (mS/cm)		resolution (EUP)
	conalbumin	BSA	conalbumin vs. BSA
Untreated column 1	15.2	28.1	1.7
Column 1 after 5 M urea	15.1	28.0	1.7
Untreated column 2	15.5	27.6	1.6
Column 2 after 6 M guanidinium chloride	15.0	27.6	1.6
Untreated column 3	15.1	27.6	1.5
Column 3 after 30% 2.propanol	15.4	28.2	1.7

The three chemicals listed in the table affect the slope of the pressure versus flow curves. After regeneration with aqueous buffer, the slope of the curves came back to the slope of the untreated column with some unavoidable deviations.

Table 3: Relative slope of the net pressure drop versus flow curve before, during and after treatment with particular chemicals. The slope was determined by linear regression and the coefficient of determination (R^2) was always greater than 0.97.

eluent A	Relative slope of pressure versus flow curve		
	before treatment (eluent 150 mM NaCl)	with eluent A	after regeneration (eluent 150 mM NaCl)
5M urea	100%	134%	84%
6M guanidinium chloride	100%	196%	99%
30% 2-propanol	100%	338%	91%

Conclusion:

Treatment of a column packed with Eshmuno® Q with various chemicals results in specific pressure versus flow properties. After regeneration of the column with buffer the pressure versus flow curves remain mostly unchanged when compared with the curve of the untreated column. There was no change in the chromatographic properties of the gel upon exposure to these chemicals after regeneration with buffer.

6. Removal of Ethanol

Complete removal of ethanol from bulk material is achieved by rinsing the gel with 7 column volumes of buffer or water.

The removal of ethanol is shown for a 16 mm i.d. column packed with Eshmuno® Q equilibrated in 20% ethanol. After washing with 3 column volumes of deionized water, the ethanol concentration had dropped to less than 50 ppm (figure 31). This method can also be scaled up to bigger columns.

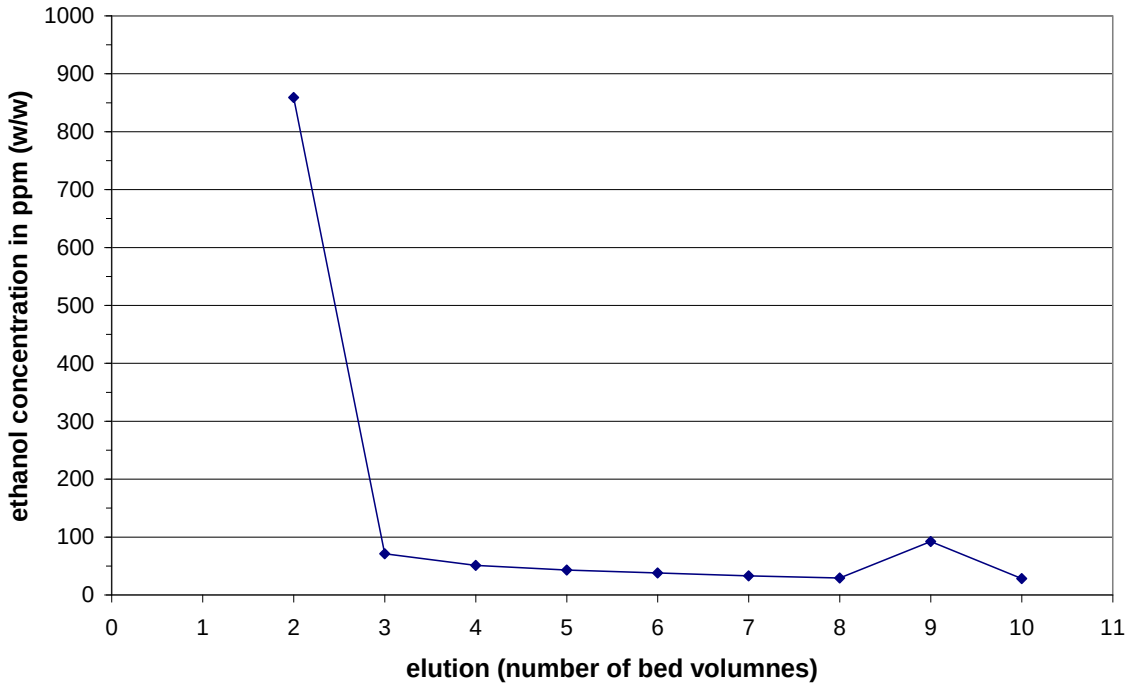

Figure 31: Ethanol concentration in the effluent.

Method:
A Superformance® 150-16 column was packed with Eshmuno® Q to 10% compression. After testing the packed column according to the standard packing procedure the gel bed was equilibrated with 4 bed volumes of storage solution (20% ethanol/150 mM sodium chloride) at 100 cm/h. The gel was allowed to stand over night (20 h) in this solution.
Subsequently, the column was again washed with deionized water, collecting the effluent in fractions of one bed volume. The flow rate was 100 cm/h.

The individual fractions were analyzed for ethanol content by gas chromatography (DB1 quartz capillary column, 30 m × 0.32 mm, equilibrated at 80°C, temperature program: 5 min at 50°C, heating up to 240°C at a rate of 8°/min, hold at 240°C for 5 min; detector: FID; carrier gas: nitrogen. The detection limit was 0.01 mg ethanol per g effluent.).

To Place an Order or Receive Technical Assistance
In Europe, please call Customer Service:
France: 0825 045 645
Germany: 01805 045 645
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
United Kingdom: 0870 900 4645
For other countries across Europe, please call: +44 (0) 115 943 0840

Or visit: www.merckmillipore.com/offices

For Technical Service visit: www.merckmillipore.com/techservice