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Comparison of chromatographic ion-exchange resins II. More strong anion-exchange resins

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Abstract

A comparative study was performed on strong anion exchangers to investigate the pH dependence, titration curves, efficiency, binding strength, particle size distribution, and static and dynamic capacity of the chromatographic resins. The resins tested included Q Sepharose XL, UNO Q-1, Poros 50 HQ, Toyopearl QAE 550c, Separon HemaBio 1000Q, Q-Cellthru Bigbeads Plus, Q Sepharose HP and Toyopearl SuperQ 650s. Testing was performed with five different proteins: anti-Factor VII monoclonal antibody (immunoglobulin G), aprotinin, bovine serum albumin, lipolase and myoglobin. The dependence of pH on retention varies from generally low to very high for proteins with a low isoelectric point (p*I*). An unexpected binding at pH 7–8 of aprotinin with p*I* >11 was observed on Separon HemaBio 1000Q. No link between pH dependence on retention and titration curves of the different resins was observed. Efficiency results show the expected trend of higher dependence of the plate height with increasing flow-rate of soft resins compared to resins for medium- and high-pressure operation. No or a very small difference in particle size distribution was obtained between new and used resins. Binding to anion-exchange resins as a function of ionic strength varies to some extent depending on the specific protein. Generally, binding and elution at high salt concentration may be performed with Q Sepharose XL, Toyopearl QAE 550c, Q Sepharose HP and Poros 50 HQ, while binding and elution at low salt concentration may be performed with Q-Cellthru Bigbeads Plus. A very high binding capacity was obtained with Q Sepharose XL. Comparison of static capacity and dynamic capacity at 10% breakthrough shows approx. 50–80% utilization of the total available capacity during chromatographic operation. A general good agreement was obtained between this study and data obtained by the suppliers. The results of this study may be used for selection of resins for testing in process development.

Keywords: Anion exchangers; Stationary phases, LC; Preparative chromatography; Proteins

1. Introduction

Ion exchangers belong to the most commonly used stationary phase types for downstream processing of pharmaceutical proteins and peptides, and industrial purification processes usually comprise one or several ion-exchange steps. An advantage of ion-exchange chromatography is that elution takes place under mild conditions where the protein or peptide can maintain its native structure. Comparison of chromatographic resins is performed during process development, but due to time constraints only a limited number of resins are regularly tested for the specific application. Results of this resin comparison are hardly ever published unless very surprising or patentable discoveries are made.

This work is part of a continuing study performed at Novo Nordisk to characterize and compare old and new commercial ion exchangers for improved selec-

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tion for testing in process development. We have previously published the results of nine strong anion-exchange resins comparing dynamic capacities, titration curves, binding strength, elution dependence on pH and efficiency [1]. A number of other papers comparing anion exchangers has been published [2–21] comparing various parameters including: dynamic [2,5–7,10,14,16,18–20], static [2,3,6,16,17], and ionic [2,3,6,13,18,19] capacities, titration curves [18], binding strength [4,9,21], elution dependence on pH [8,18,21], efficiency [5,7,11,12,15,19], resolution [2,3,5–8,14,15,18,21], adsorption isotherms [11,14,17,19,20], plate height [4,7,11,12,15,19], pressure drop [18], compressibility [3,6], protein recovery [3,19], operating flow-rate [3,5,6,10,14,16,18–21], cost [5,6], chemical stability [6,13,18], retention factors [4,9,15], spacer arm [2,13,15], base matrix chemistry [2,13,15,18], and others. The number of resins compared in these papers is typically two to four, however, the papers of Boschetti [2], Dennis et al. [3], and Bai [4] include more than four anion exchangers. The test proteins used are typically serum albumins.

The scope of this paper is to compare a number of commercial strong anion exchangers by a systematic and consistent experimental set-up. The comparison include data on efficiency, binding strength, pH dependence, titration curves, particle size distribution, and dynamic and static capacity of the anion-exchange resins performed under the same, relevant conditions. Some comparison with our previous results [1] will also be made. The five test proteins used cover a broad range of isoelectric points (p*I*) and molecular masses. They include both standard test proteins [bovine serum albumin (BSA) and myoglobin] and other proteins/peptides obtained at Novo Nordisk {anti-Factor VII monoclonal antibody (anti-FVII Mab) an immunoglobulin G [22], aprotinin [23], and lipolase [24]}. The study represents approx. 1300 experimental measurements.

2. Experimental

2.1. Materials

Q Sepharose XL, DEAE Sepharose Fast Flow, and Q Sepharose High Performance beads were kindly donated by Amersham Pharmacia Biotech (Uppsala, Sweden). A prepacked Separon HemaBio 1000Q column (5 cm×0.75 cm I.D., 10 μm beads) was purchased from Tessek (Århus, Denmark). Poros 50 HQ beads were kindly donated by PE Biosystems (Cambridge, MA, USA). A prepacked UNO Q-1 monolithic column (3.5 cm×0.7 cm I.D.) was purchased from BioRad (Nazareth, Belgium). Toyopearl QAE 550c and Toyopearl SuperQ 650c beads were kindly donated by TosoHaas (Philadelphia, PA, USA). Q-Cellthru Bigbeads Plus beads were purchased from Sterogene (Carlsbad, CA, USA).

Bovine serum albumin (BSA, A6918) and horse skeletal muscle myoglobin (M0630) were purchased from Sigma (St. Louis, MO, USA). All pure industrial proteins/peptides (anti-FVII Mab, aprotinin and lipolase) were obtained from Novo Nordisk (Bagsværd, Denmark).

Tris(hydroxymethyl)aminomethane (Tris, Sigma 7-9) and Bis-Tris-propane (B6755) were purchased from Sigma. Other chemicals: sodium chloride, hydrochloric acid and acetone were analytical-reagent grade and purchased from Merck (Darmstadt, Germany).

2.2. Instrumentation

A BioCAD Workstation from Perseptive Biosystems (Cambridge, MA, USA) was used for chromatographic measurements and evaluation in these studies. The standard BioCAD Workstation was equipped with a 100-μl sample injection loop, a 0.6-cm flow cell, pump heads for flow-rates between 0.2 and 60 ml/min, and mixing of standard buffer solutions (standard BioCAD buffer set-up) was obtained through a mixing valve. UV detection was operated at 280 nm. The BioCAD was placed in a temperature-controlled airbath from Brønnum (Herlev, Denmark) to maintain a constant temperature of 22±1°C throughout the measurements. UV–Vis spectrophotometry for sample concentration adjustment was carried out on a HP8452A system (Birkerød, Denmark).

Chromatographic resins were packed in HR 5/10 columns (10 cm×0.5 cm I.D.) supplied by Amersham Pharmacia Biotech.

Measurement of absorbance at 280 nm (A_{280}) for static capacity determination was performed using a

diode array spectrophotometer 8452A from Hewlett-Packard (Palo Alto, CA, USA).

Coulter counting for particle size distribution measurement was performed using a Coulter Multisizer and sampling stand Model S ST II from Coulter Electronics (Luton, UK).

2.3. Methods

The methods used in this study are equal to those of our previous experiments [1]. The resin comparison experiments were performed employing similar conditions, that is, the same scale, buffers, buffer concentration, temperature, protein concentration, solution conductivity, pH, gradients and corresponding flow-rates, where appropriate. Experiments were made in duplicate. The pH of buffer and protein/peptide solutions was adjusted with hydrochloric acid. The following general methodology was used:

The column was equilibrated with a sufficient number of column volumes (CVs) of buffer (15–20 CVs). Samples of 1 mg/ml pure protein solutions were applied through the injection system or in case of frontal analysis experiments through the pump. Aprotinin, BSA and myoglobin freeze–dried products were dissolved directly in equilibration buffer for frontal analysis experiments and in water for other experiments. Wet lipolase crystals were dissolved in equilibration buffer, and the concentration was controlled and adjusted by UV spectrophotometry. Anti-FVII Mab was obtained at a concentration of 2 mg/ml in a 50 m*M* Tris+100 m*M* NaCl, pH 8.0 solution, which was diluted with one volume of water. Properties of the test proteins and standard buffer solution compositions are given elsewhere [1]. In all experiments a buffer concentration of 25 m*M* Tris+25 m*M* Bis-Tris-propane was used. Column regeneration was performed with 5 CVs of 1.0 *M* NaCl in binding strength and frontal analysis experiments.

Packing of columns was performed according to manufacturer specifications. Properties of the anion-exchange resins and flow-rates applied in these studies are presented in Table 1. Recommended maximum operating pressures were obtained from the suppliers. The general flow-rate used for pH dependence and binding strength measurements was approx. 50% of the recommended maximum operating flow-rate/pressure. The low and high flow-rates used for dynamic capacity determinations were approx. 25% and 75%, respectively, of the recommended maximum operating flow-rate/pressure.

Extra column volume measurements of the system were performed as described elsewhere [1].

2.3.1. pH dependence measurements

pH dependence measurements were performed at pH 6.0, 7.0, 8.0 and 9.0. Sample injection was done after column equilibration followed by an NaCl gradient from 0 to 1 *M* during 20 CVs. A small isocratic segment corresponding to the dead volume

Table 1
Properties of the chromatographic anion-exchange resins and applied flow-rates[a]

Resin	Particle size (μm), supplier data	Mean particle size (μm), Coulter counting	Maximum recommended pressure (bar)	Applied flow-rates (ml/min)		
				General	Capacity, low	Capacity, high
Q Sepharose XL	90 (45–165)	24	~3	1.2	0.6	1.8
UNO Q-1	–[b]	–[b]	48	2.5	1.0	2.5
Poros 50 HQ	50	37	100	4.0	2.0	10.0
Toyopearl QAE 550c	100 (50–150)	20	~5	1.0	0.5	1.7
Separon HemaBio 1000Q	10 (8–12)	11	200	10.0	2.0	10.0
Q-Cellthru Bigbeads Plus	400 (300–500)	Not measured	0.6	1.0	0.5	1.7
Q Sepharose HP	34 (24–44)	18	~3	1.2	0.3	1.2
Toyopearl SuperQ 650s	100 (50–150)	78	~5	1.0	0.5	1.7

[a] General flow-rate is used for pH dependence and binding strength measurements, while capacity low and high flow-rates are used for dynamic capacity determinations. Mean particle size is found from Coulter counting experiments.
[b] Monolithic column.

from the pump mixing system to the injection system was part of the method. The pH dependence experiments were performed with all five test proteins.

Retention factors, k', for the gradient runs were defined and calculated based on the retention time of the almost symmetrical peaks:

$$k' = \frac{t_R - M_{1,0}}{M_{1,0} - M_{1,S}}$$

where t_R is the retention time of the protein, $M_{1,S}$ is the first moment of the extra column volume, and $M_{1,0}$ is the first moment of the protein under non-binding conditions found from the plate height determinations below.

2.3.2. Efficiency determinations

Efficiency determinations were performed as plate height determinations under the non-binding, isocratic conditions of 1 *M* NaCl, pH 6.0 as a function of flow-rate. Flow-rates were varied between approx. 10 and 100% of the recommended maximum flow-rate/pressure. The experiments were performed with anti-FVII Mab, aprotinin, lipolase and myoglobin.

To get the best representation of the plate height, the peaks were fitted to an EMG (exponentially modified Gaussian) function using the software program TableCurve 2D ver. 2.03 from Jandel Scientific (San Rafael, CA, USA). The first and second moments were used to calculate the reduced plate height, *h*. The EMG function for fitting of raw data was chosen because of the somewhat tailing peaks obtained by efficiency measurements under non-binding conditions and especially by isocratic retention measurements under binding conditions, see below. The EMG function has the advantage of using the entire peak curve for the fit compared to the various graphical methods used for fitting to a Gaussian peak. The EMG function used is a five-parameter model:

$$f(t) = \frac{A\sigma}{\tau\sqrt{2}} \exp\left[\left(\frac{\sigma}{\tau}\right)^2 \cdot \frac{1}{2} - \frac{t-\mu}{\tau}\right] \int_{-\infty}^{Z} e^{-x^2} dx + E,$$

$$\text{with } Z = \frac{1}{\sqrt{2}} \cdot \left(\frac{t-\mu}{\sigma} - \frac{\sigma}{\tau}\right)$$

where t is the time, A is the scaling, μ is the Gaussian mean value, σ is symmetrical peak width, τ is the asymmetrical peak width, and E is the peak baseline level. Fits to the EMG function were generally performed with a correlation factor higher than 0.99, and in most cases higher than 0.999. In a few difficult cases, fits to the EMG function were performed with a correlation factor down to 0.90. The first and second moments, M_1 and M_2, of the peak curve [25]:

$$M_1 = \mu + \tau$$

$$M_2 = \sigma^2 + \tau^2$$

are additive parameters, thus for an exponentially modified Gaussian peak the reduced theoretical plate height of the column, *h*, is found from:

$$h = \frac{H}{d_p} = \frac{L}{d_p N} = \frac{L(M_{2,0} - M_{2,S})}{d_p(M_{1,0} - M_{1,S})^2}$$

where H is the theoretical plate height, d_p is the particle diameter, L is the column length, N is the number of theoretical plates of the column, $M_{2,0}$ is the second moment of the protein peak under non-binding conditions, and $M_{2,S}$ is the second moment of the extra column volume. *h* is in this study presented as a function of the linear flow-rate, v:

$$v = \frac{v_{vol}}{\pi r^2}$$

where v_{vol} is the volumetric flow-rate and r is the column radius.

2.3.3. Binding strength measurements

Binding strength experiments were performed as the classical isocratic retention measurements as a function of NaCl concentration, which was varied between 0 and 250 m*M* depending on the resin and the protein. For anti-FVII Mab and lipolase, the binding strength experiments were performed at pH 8.0, while experiments with aprotinin and myoglobin were done at pH 9.0.

The best representation of data was achieved by fitting the peaks to an EMG function. The first moment of the fit was used to calculate the retention factor, k':

$$k' = \frac{M_1 - M_{1,0}}{M_{1,0} - M_{1,S}}$$

using the first moments of the extra column volume and the efficiency data under non-binding conditions for adjustment. The binding strength is illustrated by plotting k' versus reciprocal total ionic strength of the solution for elution. The total ionic strength, I_{Total}, was found from:

$$I_{\text{Total}} = 0.5\sum_i c_i z_i^2$$

where c_i is the molar concentration and z_i is the ionic charge of the ionic species i in the solution for elution.

2.3.4. Dynamic capacity determinations

Dynamic capacity was determined by frontal analysis experiments according to the method described elsewhere [1]. Frontal analysis experiments were performed at pH 8.0 with anti-FVII Mab, BSA and lipolase. Based on the UV signals obtained, the level of breakthrough was calculated by normalizing the protein concentration with the initial protein concentration, C/C_0. The dynamic capacity at 10% and 50% breakthrough is presented in this study.

2.3.5. Titration curve measurements

Titration curves were measured by a method similar to that used by Lagerlund et al. [18]. A 2-ml volume of the chromatographic resin was packed in a column (10 cm×0.5 cm I.D.) according to manufacturer specifications and washed with 50 ml of 1 *M* KCl, pH 3. The resin was transferred to a beaker containing 30 ml of 1 *M* KCl, pH 3, and titrated with 0.1 *M* NaOH to pH 12. For comparison, the DEAE Sepharose FF resin in 30 ml of 1 *M* KCl, pH 3 and a blank solution containing only 30 ml of 1 *M* KCl, pH 3 were titrated over the same pH interval.

2.3.6. Static capacity determinations

Static capacity was determined by batch adsorption experiments with BSA. Resins were packed and equilibrated as stated previously with buffer solution at pH 8. A_{280} of a 1 mg/ml BSA solution was measured. Resins were poured out of the column into a beaker containing the BSA solution. Based on results from dynamic capacity experiments, the total amount of BSA in solution was two times that found per ml of resin. Solutions were left standing overnight (16–20 h) with slow agitation. A_{280} of the supernatant was measured. Assigning the difference in A_{280} to the amount of BSA bound, static capacity was determined as this amount divided by the amount of resin present in the solution.

2.3.7. Particle size distribution measurements

Particle size distribution was measured by Coulter counting. A few drops of resin in suspension were added to 100 ml of 0.9% NaCl solution. A preliminary counting of the solution was performed to ensure that the coincidence factor was below 10%. The final measurement was thus performed, counting more than 60 000 particles. A 280 μm I.D. orifice tube was employed for the measurements.

3. Results and discussion

The general approach of this study is to compare a number of strong anion-exchange resins under similar conditions. The chromatographic resins presented in Table 1 cover a variety of functions and uses including capture, DNA removal, and purification in a purification process. Resins used for a capture step are characterized by having a fairly large particle size and a high binding capacity that may concentrate the target protein, remove water, and avoid clogging of the column by fermentation products at a high flow-rate, e.g., Q Sepharose XL, Toyopearl QAE 550c and Toyopearl SuperQ 650c. The intended use of Q Cellthru Bigbeads Plus is DNA removal, and the resin has a very large particle size. This resin could be operated anywhere in the process even before the capture step in flow-through mode letting the target protein pass the column and binding DNA and related impurities. Resins for purification steps are typically characterized by having a small particle size with high selectivity, possibly at high pressure. This will result in sharper peaks and a higher resolution of the target protein to the related impurity, e.g., Poros 50 HQ, Separon HemaBio 1000Q and Q Sepharose HP. UNO Q-1 is a monolithic column which due to its perfusive character should be the optimal solution to obtain high resolution. This technology is still at the experimental stage regarding preparative use. The ion exchangers also cover a broad range of commercially available base matrix chemistries including agarose, acrylic

and vinylic polymers, and polystyrene. The column diameter employed in the experiments is not optimal for large particle size resins, but was used to minimize the protein consumption. The protein load employed in pH dependence, efficiency, and binding strength experiments was low and it is assumed that the experiments were performed under linear chromatography conditions.

Experimental results of pH dependence measurements are presented in Figs. 1 and 2. Results of anti-FVII Mab, BSA and lipolase with isoelectric points of approx. 6.5, 5.1 and 4.3, respectively [1], are shown in Fig. 1. They display the same expected trend of increasing retention with increasing pH for all resins, however, the degree of change in retention varies for the various resins and depends on the test protein. As an example, retention of anti-FVII Mab in Fig. 1a and BSA in Fig. 1b is smoothly increasing for Toyopearl QAE 550c, but shows a sudden large increase for lipolase at pH 9 in Fig. 1c. In contrast, Toyopearl SuperQ 650c with the same base matrix chemistry from the same supplier displays large retention dependence with pH ranging from almost no retention at pH 6 to a rather high retention at pH 9 for all three proteins. Poros 50 HQ and Q Sepharose HP also present general low variation of retention with pH. Q Sepharose XL shows a much higher retention trend in this pH study and the previous study [1] compared to other resins for anti-FVII Mab and BSA, while an average retention trend is obtained for lipolase.

Results of aprotinin and myoglobin are presented in Fig. 2 and show the same general trend of no binding at pH 6–8, but some retention at pH 9. This trend is expected for myoglobin with a p*I* of 7–8, but is also noticed for aprotinin with a p*I* above 11.

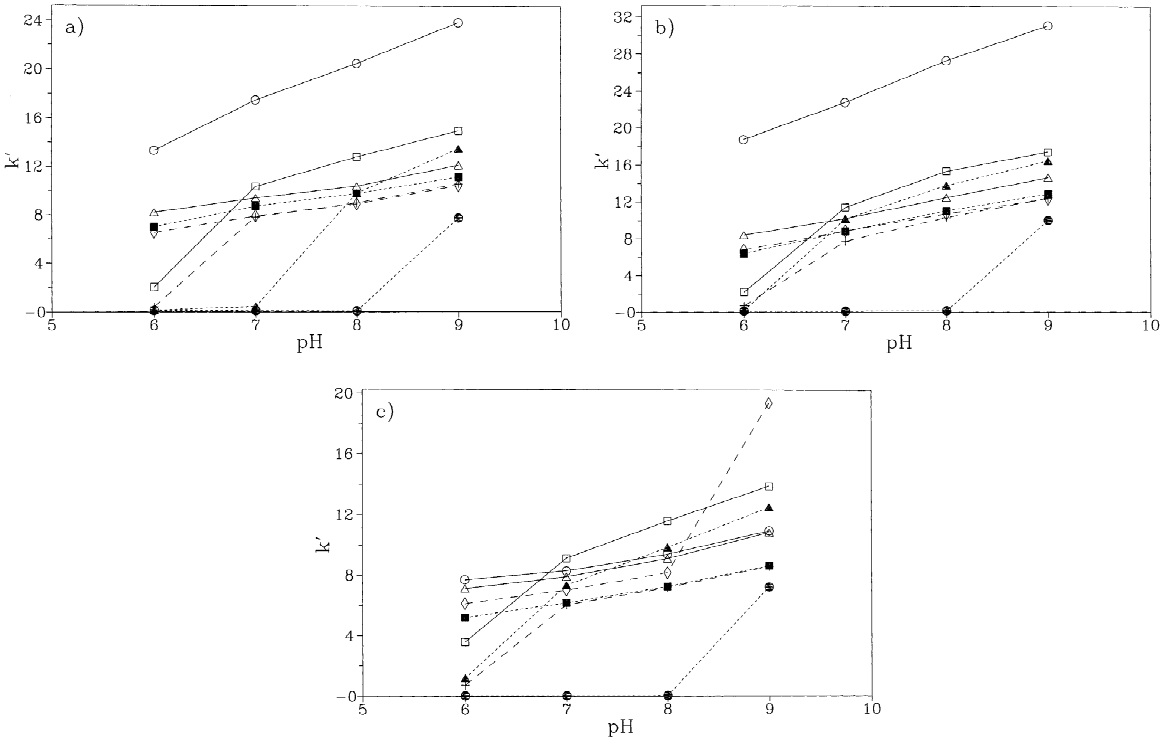

Fig. 1. pH dependence plots (k'–pH) of (a) anti-FVII Mab, (b) BSA, and (c) Lipolase on anion-exchange resins. Flow-rates are given in Table 1. pH dependence was determined by applying a 20-μl pulse of 1 mg/ml protein solution in a 20 CV linear gradient from 0 to 1 *M* NaCl in 25 m*M* Tris+25 m*M* Bis-Tris-propane buffer through a 3.5×0.7 cm, a 5×0.75 cm, or a 10×0.5 cm column. Symbols: ○=Q Sepharose XL; □=UNO Q-1; △=Poros 50 HQ; ◊=Toyopearl QAE 550c; +=Separon HemaBio 1000Q; ●=Q-Cellthru Bigbeads Plus; ■=Q Sepharose HP; ▲=Toyopearl SuperQ 650c.

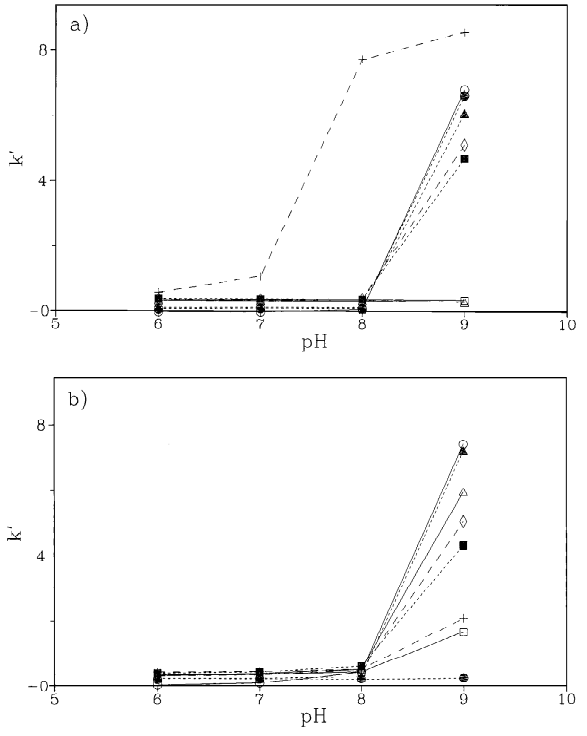

Fig. 2. pH dependence plots (k'–pH) of (a) aprotinin and (b) myoglobin on anion-exchange resins. Flow-rates are given in Table 1. pH dependence was determined by applying a 20-μl pulse of 1 mg/ml protein solution in a 20 CV linear gradient from 0 to 1 *M* NaCl in 25 m*M* Tris+25 m*M* Bis-Tris-propane buffer through a 3.5×0.7 cm, a 5×0.75 cm, or a 10×0.5 cm column. Symbols: ○=Q Sepharose XL; □=UNO Q-1; △=Poros 50 HQ; ◊=Toyopearl QAE 550c; +=Separon HemaBio 1000Q; ●=Q-Cellthru Bigbeads Plus; ■=Q Sepharose HP; ▲=Toyopearl SuperQ 650c.

Though the overall charge of the protein is positive the retention experienced could be due to a local area with many negative charges. This is supported by studies of Kopaciewicz et al. [26], who found retention of a number of proteins up to one pH unit below the p*I* of the proteins on Mono Q resin. Fig. 2 also shows that binding of aprotinin is obtained on Separon HemaBio 1000Q at pH 8 and even to a slight extend at pH 7, which is highly surprising. Though solutes with p*I* higher than the pH used should not be suitable for anion-exchange operation, experience from a number of commercial purification processes shows that removal of certain related or unrelated impurities may be performed to great advantage.

Data on retention as function of pH is of great importance in process development and optimization where a change in pH is necessary to obtain higher buffer strength of the selected buffer system. Figs. 1 and 2 could give a hint to how much or if retention would be influenced by such change in pH. These data also give an idea of pH sensitivity of resins when the pH range in a purification process step is settled. If a selected resin displays too much variation in protein retention as a function of pH, it may be necessary to displace it with a less pH sensitive resin. Finally, data on retention as function of pH is useful for planning of flow-through mode operation. A suitable resin for that purpose based on these results is Q-Cellthru Bigbeads Plus as expected.

Fig. 3 presents resin titration curves of this study and titration curves of a blank titration and DEAE Sepharose FF for comparison. A strong anion-exchange resin containing solely quaternary amino groups would have a titration curve corresponding to the blank titration, while an anion-exchange resin containing some tertiary amino groups would have a titration curve tending towards that of DEAE Sepharose FF. In Fig. 3, titration curves of Separon HemaBio 1000Q, Poros 50 HQ and Toyopearl QAE 550c display a trend towards the titration curve of DEAE Sepharose FF indicating some presence of tertiary amino groups. Titration curves of the other resins follow the blank titration curve indicating that they are truly strong anion-exchange resins. The titration curve of UNO Q-1 was not measured. The

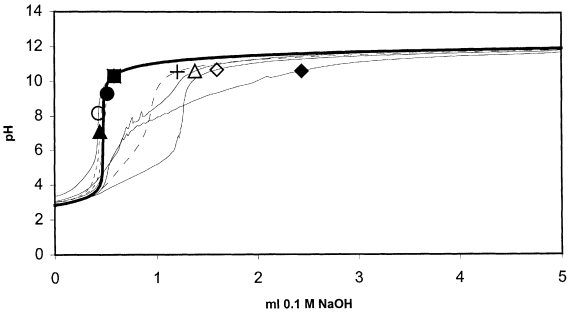

Fig. 3. Titration curves of anion-exchange resins in 1 *M* KCl from pH 3 to 12 with 0.1 *M* NaOH. The thick line (—) is the blank titration. Symbols: ○=Q Sepharose XL; △=Poros 50 HQ; ◊=Toyopearl QAE 550c; +=Separon HemaBio 1000Q; ●=Q-Cellthru Bigbeads Plus; ■=Q Sepharose HP; ▲=Toyopearl SuperQ 650c; ♦=DEAE Sepharose FF.

pH dependence of resins experienced in Fig. 1 might be due to presence of tertiary amino groups, however, these results show that there is no clear link between pH dependence of some resins and their titration curves.

Results of the efficiency experiments are presented in Fig. 4 as scaled standard Van Deemter plots under unretained conditions. It was assumed that no resin swelling occurred as a function of pH in the range 6–9. Fig. 4 shows the general trend of higher dependence of the plate height with increasing flow-rate of soft resins compared to resins for medium and high-pressure operation. Scatter in the data for some resins is due to difficult and inadequate fitting to the EMG function, and because determination of *h* involves calculation with very small numbers which may be associated with some degree of uncertainty. For UNO Q-1, efficiency seems very high and unaffected by flow-rate, however, this is caused by the large particle size of a monolithic column (apparently one particle), thus all values of *h* were found by division with a very large number for d_p. The vertical position of some resins in Fig. 4 would change if the mean particle size found in this study was used instead of supplier data (see below), however, this was not done due to lack of data for all resins. The trend of increasing *h* values followed by a decrease with increasing flow-rate obtained for Q-Cellthru Bigbeads Plus and to some extent for Toyopearl SuperQ 650c and Q Sepharose XL may be due to emerging compression of the resins. These resins are probably the softest resins of this study. Q Sepharose HP, Poros 50 HQ and Separon HemaBio 1000Q generally follow the same trend and flow dependence, with Q Sepharose HP at the lowest level for aprotinin, lipolase and myoglobin.

Plate height data may be used in process development as an indication of the purification efficiency to

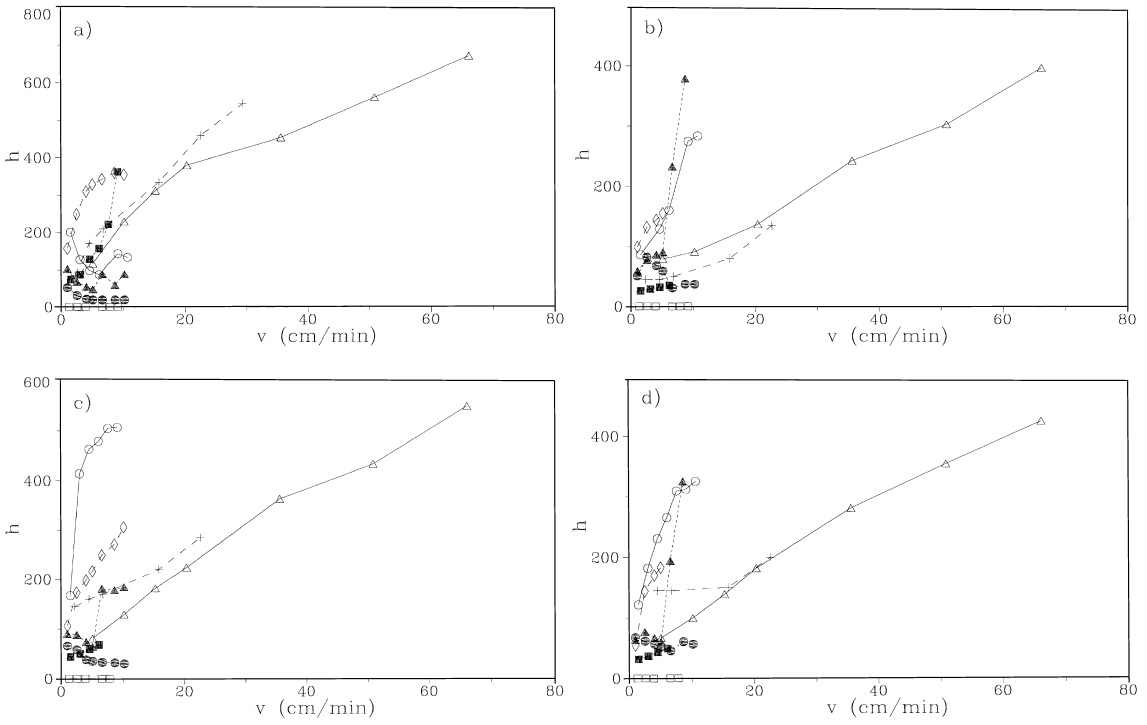

Fig. 4. Efficiency plots (*h*–*v*) of (a) anti-FVII Mab, (b) aprotinin, (c) lipolase, and (d) myoglobin under non-binding conditions. Van Deemter curves were determined by applying a 20-μl pulse of 1 mg/ml protein solution in 1 *M* NaCl in 25 m*M* Tris+25 m*M* Bis-Tris-propane buffer at pH 6 through a 3.5×0.7 cm, a 5×0.75 cm, or a 10×0.5 cm column. Symbols: ○=Q Sepharose XL; □=UNO Q-1; △=Poros 50 HQ; ◊=Toyopearl QAE 550c; +=Separon HemaBio 1000Q; ●=Q-Cellthru Bigbeads Plus; ■=Q Sepharose HP; ▲=Toyopearl SuperQ 650c.

be expected for the individual resins at a specified column length and flow-rate. For process optimization, these data will indicate the influence of an increase in flow-rate for a specific purification step on separation efficiency.

Particle size distribution was measured by Coulter counting for both new resins and resins after use for the other chromatographic experiments of this study corresponding to approx. 200 runs. Fig. 5 shows results of new resins, and the mean particle size (50%) is presented in Table 1. Particle size distributions of Q-Cellthru Bigbeads Plus and obviously UNO Q-1 were not measured. Distributions in Fig. 5 increase with the mean particle size. The results are in agreement with supplier data for Separon HemaBio 1000Q and Toyopearl SuperQ 650c, however, much smaller particles were found for Q Sepharose XL and Toyopearl QAE 550c. All results were verified by scanning electron microscopy (data not shown). Fig. 6 shows results of particle size distribution of Q Sepharose HP and Toyopearl SuperQ 650c. A slight decrease in particle size is observed after use indicating a small degree of deterioration of these resins. No change in particle size distribution was observed for the other resins.

Data on particle size distribution is important when the column filter of industrial columns is chosen. If resin particles are smaller than stated, they may clog up the filter leading to increased column backpressure and the risk of damaging column and

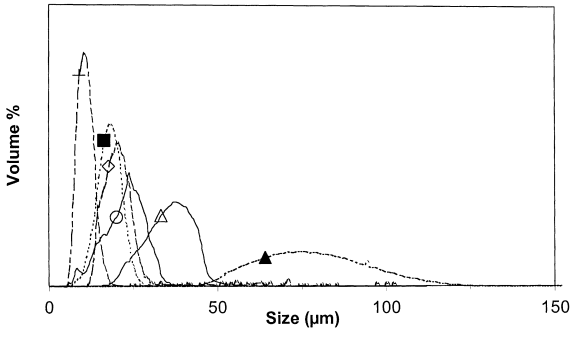

Fig. 5. Particle size distribution (%, v/v) of new anion-exchange resins in 0.9% NaCl measured by Coulter counting (particles of Separon HemaBio 1000Q were, however, used for approx. 200 runs). Symbols: ○=Q Sepharose XL; △=Poros 50 HQ; ◊=Toyopearl QAE 550c; +=Separon HemaBio 1000Q; ■=Q Sepharose HP; ▲=Toyopearl SuperQ 650c.

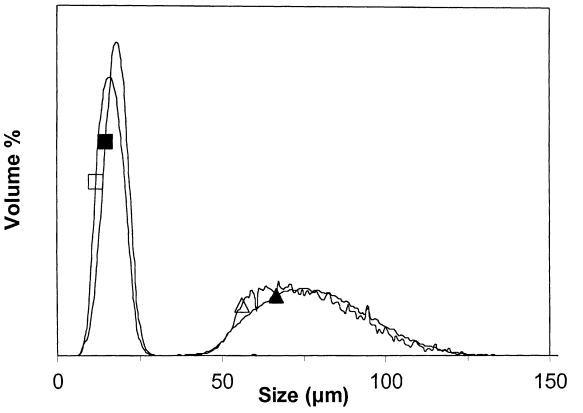

Fig. 6. Comparison of particle size distributions (%, v/v) of new and used anion-exchange resins in 0.9% NaCl measured by Coulter counting. Squares: Q Sepharose HP; triangles: Toyopearl SuperQ 650c. Filled symbols are new resins; open symbols are resins used for approx. 200 runs.

chromatographic resin. Further, data on both new and used resin tell something about the mechanical stability of the resin, and the risk of generation of fines which again may clog up column filters.

The binding strength is characterized by standard k' versus I_{Total}^{-1} plots obtained by isocratic runs as presented in Fig. 7, thus a stronger binding will need more salt for elution to occur and thus a smaller I_{Total}^{-1}. Strong binding of test proteins with low isoelectric points, anti-FVII Mab and lipolase in Fig. 7a and c, is obtained for Q Sepharose XL, Toyopearl QAE 550c, Q Sepharose HP and Poros 50 HQ, while the resin with the weakest binding is Q-Cellthru Bigbeads Plus. Q Sepharose XL has the same base matrix chemistry as Q Sepharose HP and Q Sepharose FF from the previous study [1], however, the dextran coating of Q Sepharose XL seems to increase the binding strength in some cases and not in others. For lipolase, binding is stronger while it is the same for anti-FVII Mab, which is in agreement with what was found in pH dependence measurements. This reveals a complex retention mechanism most probably with the interplay of hydrophobic interactions. For the two test proteins with high isoelectric points, aprotinin and myoglobin in Fig. 7b and d, no clear trend is observed. As for pH dependence measurements, a significant and surprising binding of aprotinin to Separon HemaBio 1000Q is obtained,

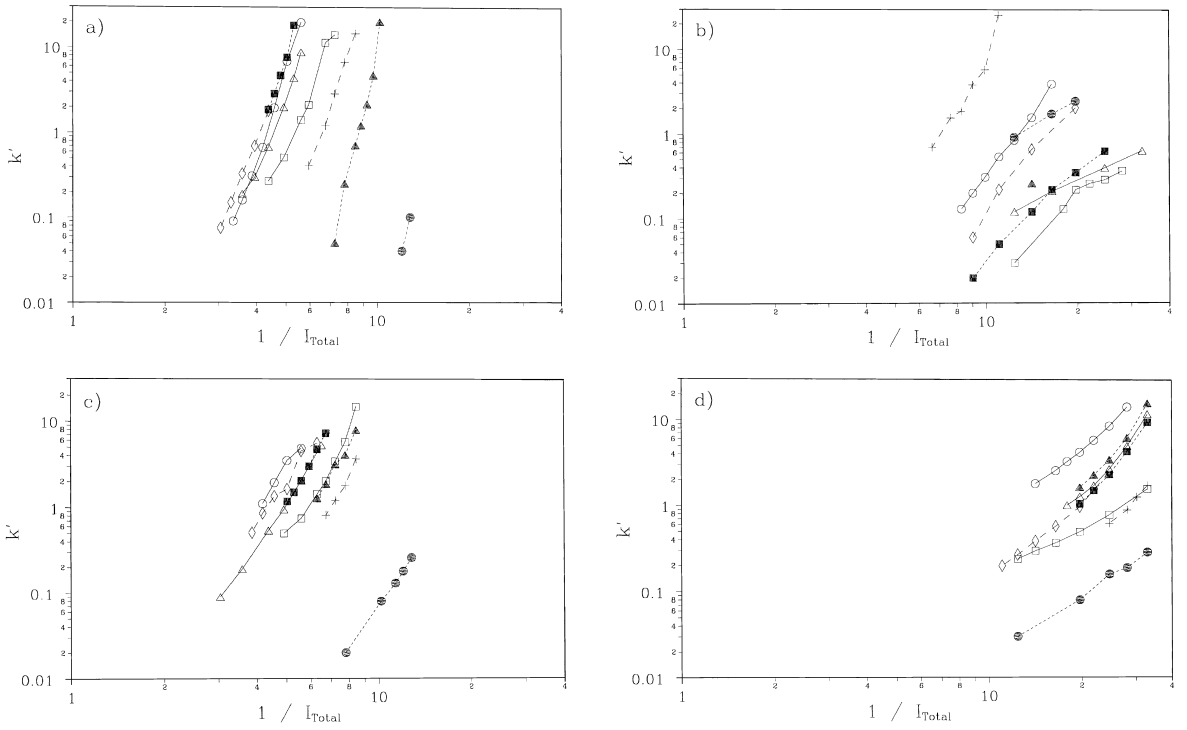

Fig. 7. Binding strength plots (k'–I_{Total}^{-1}) of (a) anti-FVII Mab at pH 8, (b) aprotinin at pH 9, (c) lipolase at pH 8, and (d) myoglobin at pH 9 on anion-exchange resins at approx. 50% of recommended maximum operating flow-rate. Actual flow-rates are given in Table 1. Binding strength was determined by applying a 20-μl pulse of 1 mg/ml protein solution in 25 m*M* Tris+25 m*M* Bis-Tris-propane buffer at various isocratic NaCl concentrations through a 3.5×0.7 cm, a 5×0.75 cm, or a 10×0.5 cm column. Symbols: ○=Q Sepharose XL; □=UNO Q-1; △=Poros 50 HQ; ◊=Toyopearl QAE 550c; +=Separon HemaBio 1000Q; ●=Q-Cellthru Bigbeads Plus; ■=Q Sepharose HP; ▲=Toyopearl SuperQ 650c.

which to some extent also may be due to hydrophobic interactions.

Binding strength measurements are of great importance for resin selection and optimization of ion-exchange processes. If the target protein binds weakly due to its presence in a high conductivity loading solution, selection of resins for further testing will be among those to the left in the figures. In case of a strongly binding protein to all resins, a resin with one of the rightmost curves would probably be selected for further testing to minimize salt consumption for elution. In flow-through mode chromatography operation for removal of impurities the target protein passes the resin, which retains the impurities preferably under the conditions of the loading sample. Again, this is the main purpose of Q-Cellthru Bigbeads Plus, but generally other resins to the right in the figures may also apply.

Dynamic capacities are determined by frontal analysis of pure proteins, and 10% and 50% breakthrough data for anti-FVII Mab, BSA and lipolase are presented in Table 2 with dynamic capacity data for BSA obtained by suppliers. A protein concentration of 1 mg/ml was employed as a realistic concentration for many commercial purification processes. Applied flow-rates are given in Table 1. The purpose of this study was to experience the influence of a rather high and low flow-rate on resin performance. The expected trend of higher dynamic capacity for both 10% and 50% breakthrough at lower flow-rate compared to higher flow-rate is generally presented in Table 2 for all proteins. In some cases such as Toyopearl SuperQ 650c with BSA and lipolase, capacity was much higher at 10% and 50% breakthrough at low flow-rate compared to high flow-rate due to poor mass transfer into resins

Table 2
Binding capacity of strong anion-exchange resins for 1 mg/ml solutions of anti-FVII Mab, BSA and lipolase[a]

Resin	Dynamic capacity (% breakthrough)												Dynamic capacity obtained by the suppliers, BSA (mg/ml)	Static capacity, BSA (mg/ml)
	Anti-FVII Mab (mg/ml)				BSA (mg/ml)				Lipolase (mg/ml)					
	Low flow-rate		High flow-rate		Low flow-rate		High flow-rate		Low flow-rate		High flow-rate			
	10%	50%	10%	50%	10%	50%	10%	50%	10%	50%	10%	50%		
Q Sepharose XL	5	5	5	6	176	196	188	215	169	209	160	200	>130[b]	244
UNO Q-1	7	8	7	8	24	26	26	27	29	32	28	33	20[c]	–
Poros 50 HQ	3	4	2	2	45	58	47	58	54	72	46	67	60–70	73
Toyopearl QAE 550c	7	9	6	9	33	53	21	47	39	61	28	61	70±10	68
Separon HemaBio 1000Q	0.5	0.6	0.4	0.5	18	19	18	19	16	18	16	18	N.A.	25
Q-Cellthru Bigbeads Plus	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	61	<1
Q Sepharose HP	7	10	6	10	64	73	63	74	74	88	76	92	70	78
Toyopearl SuperQ 650s	<0.1	0.2	<0.1	0.1	66	83	48	66	66	91	42	80	130±25	98

[a] Dynamic capacity results are determined at 10% and 50% breakthrough at flow-rates of approx. 25% and 75% of recommended maximum operating flow-rate/pressure. Flow-rates are presented in Table 1.
[b] 10% breakthrough.
[c] Maximum recommended protein load.

particles. Dynamic capacity flow-rate at the high level employed for Separon HemaBio 1000Q, UNO Q-1 and Sepharose HP was equal to the general flow-rate used to study other parameters due to pressure problems after extensive use. Good agreement was obtained between this study and supplier data for BSA, however, for Q-Cellthru Bigbeads Plus and Toyopearl SuperQ 650c there is some difference in favor of suppliers. The large difference may be due to a different experimental set-up, especially for Q-Cellthru Bigbeads Plus where the fairly high buffer solution conductivity (50 m*M*) may be the reason for the lack of protein binding. A significantly higher capacity of Q Sepharose XL with BSA and lipolase is obtained which support supplier data. Results for BSA with Q Sepharose HP are in very good agreement with results obtained by Tsai et al. [20]. Dynamic capacity of anti-FVII Mab on all anion exchangers is low as previously experienced [1].

Static capacity results obtained with 1 mg/ml BSA solution are shown in Table 2. No measurement was performed with UNO Q-1. Static capacity is regarded to be the maximum possible capacity that can be obtained for a specific resin. By comparison with dynamic capacity data at 10% breakthrough an indication of the fraction of the column utilized during preparative chromatographic operation is obtained. Excluding the result of Q-Cellthru Bigbeads Plus, the fraction utilized at 10% breakthrough range from approx. 50–80%; lowest for Toyopearl QAE 550c and highest for Q Sepharose HP. This indicates that different degree of improved productivity of the resins may be obtained in process development by decreasing flow-rate compared to this study.

Binding capacities of true mixtures such as fermentation broth give a more realistic picture of what to expect in a process development situation [27]. However, binding capacity was measured for pure proteins in this study because supply of uniform fermentation broth is difficult to obtain through a long period. Further, the resins tested are intended for different purposes as previously stated. Binding capacity data is very useful for the industry. If two or more resins during process development perform equally well with respect to resolution, flow-rate, price, etc., the resin with the highest capacity will be chosen to increase productivity.

4. Conclusion

A comparative study was performed on various strong anion exchangers to investigate the pH dependence, titration curves, efficiency, binding strength, particle size distribution, and static and dynamic capacity of the chromatographic resins. A general good agreement was obtained between this study and data obtained by suppliers. Comparison with literature data was difficult because literature data are generally performed with different resins and/or under different conditions.

The data generated in this study may be used for selection of resins for testing in process development. However, the data cannot be used to estimate selectivity differences between a target protein and a specific impurity, nor tell anything about the resolution that may be obtained between a target protein and related or unrelated components. None of the resins should be regarded as good or poor for chromatographic operation but more or less suitable for a specific purpose, and only testing for the specific application will determine which is the optimal resin.

5. Nomenclature

A	Scaling parameter
A_{280}	Absorbance at 280 nm
c_i	Molar concentration of component i
C/C_0	Normalized protein concentration
CV	Column volume
E	Baseline level
EMG	Exponentially modified Gaussian
I_{Total}	Total ionic strength
k'	Retention factor
M_1	First moment of the peak curve
M_2	Second moment of the peak curve
$M_{1,\text{S}}$	First moment of the extra column volume
$M_{2,\text{S}}$	Second moment of the extra column volume
$M_{1,0}$	First moment of the unretained protein
$M_{2,0}$	Second moment of the unretained protein
v	Linear flow-rate
v_{vol}	Volumetric flow-rate
Z	Dummy parameter

z_i	Ionic charge of component i
μ	Gaussian mean retention time
σ	Symmetrical peak width
τ	Asymmetrical peak width

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