ELSEVIER

Journal of Chromatography A, 897 (2000) 99–111

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Comparison of chromatographic ion-exchange resins I. Strong anion-exchange resins

Arne Staby*, Inge Holm Jensen, Inger Mollerup

Novo Nordisk A/S, Protein Purification, Hagedornsvej 1, DK-2820 Gentofte, Denmark

Received 30 November 1999; received in revised form 27 June 2000; accepted 7 July 2000

Abstract

A comparative study has been undertaken on various strong anion-exchangers to investigate the pH dependence, titration curves, efficiency, binding strength, and dynamic capacity of the chromatographic resins. The resins tested included: Macro-Prep 25Q, TSK-Gel Q-5PW-HR, Poros QE/M, Q Sepharose FF, Q HyperD 20, Q Zirconia, Source 30Q, Fractogel EMD TMAE 650s, and Express-Ion Q. Testing was performed with five different proteins: Anti-FVII Mab (IgG), aprotinin, BSA, lipolase, and myoglobin. The dependence of pH on retention varies from generally low to very high for proteins with low pI. No direct link between pH dependence on retention and titration curves of the different resins was observed. Efficiency results show the expected trend of lower dependence of the plate height with increasing flow-rate of resins for medium and high pressure operation compared to the soft resins. Binding to the anion-exchange resins as a function of ionic strength may vary depending on the specific protein. Generally, binding and elution at a high salt concentration may be performed with Poros QE/M or Macro-Prep 25Q, while binding and elution at low salt concentration may be done with TSK-Gel Q-5PW. Dynamic capacities are strongly dependent on the specific protein employed and for some resins dependent on the flow-rate. A general good agreement was obtained between this study and data obtained by suppliers for the dynamic capacity. The results of this study may be used for selection of resins for testing in process development, however, the data does not tell anything about specific selectivity differences or resolution between a target protein and a given impurity. None of the resins studied here should be regarded as good or bad, but more or less suitable for a specific purpose, and only testing for the specific application will determine which one is the optimal resin.

Keywords: Ion exchange resins; Stationary phases, LC; Anion-exchangers; Preparative chromatography; Dynamic capacity; Proteins

1. Introduction

Ion exchange chromatography is one of the most commonly used techniques for purification of pharmaceutical proteins and peptides, and practically all industrial purification processes comprise one or several ion-exchange steps. Comparison of chromatographic resins is usually performed during process development, however, often a limited number of resins is tested for a specific application, and this testing is seldom published.

Chromatographic media suppliers often publish resin comparisons in application notes and scientific papers including Refs. [1–6], which regularly fall out in favor of the publishing manufacturer under the

*Corresponding author. Tel.: +45-44-43-99-89; fax: +45-44-43-84-00.

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00780-9

specific conditions employed. More independent comparisons and comparisons between resins from the same supplier have also been published, among others: Refs. [7–17] which mainly concern cation-exchange resins, and Refs. [17–26] which compare anion-exchange resins. The parameters compared in the studies include: dynamic, static, and ionic capacities, resolution, adsorption isotherms, plate height, pressure drop, compressibility, protein recovery, operating flow-rate, cost, chemical stability, retention factors, spacer arm, base matrix chemistry, and others. Typical test proteins used for these comparisons are serum albumins for anion-exchangers and lysozyme for cation-exchangers.

This work is part of a continuing study performed at Novo Nordisk to characterise and compare old and new commercial ion-exchange resins for improved selection for testing in process development. The scope of this paper is to compare a number of commercial strong anion-exchange resins by a systematic and consistent experimental setup. The comparison include data on efficiency, binding strength, pH dependence, titration curves, and dynamic capacity of the anion-exchange resins performed at the same, relevant conditions. The five test proteins were chosen to cover a broad range of isoelectric points and molecular weights and include both standard test proteins (BSA and myoglobin) and other proteins/peptides obtained at Novo Nordisk (Anti-FVII Mab (IgG) [27], aprotinin [28], and lipolase [29]). The study represents approximately 1500 experimental chromatographic measurements.

2. Experimental

2.1. Materials

Q Sepharose Fast Flow, DEAE Sepharose Fast Flow, and Source 30Q beads were kindly donated by Amersham Pharmacia Biotech (Uppsala, Sweden). A prepacked Poros QE/M PEEK column (10 cm length×0.46 cm I.D., 20 μm beads) was purchased from Perseptive Biosystems (Cambridge, MA, USA). Macro-Prep 25Q beads were kindly donated by BioRad (Nazareth, Belgium). TSK-Gel Q-5PW-HR beads were kindly donated by TosoHaas (Philadelphia, PA, USA). Q HyperD 20 and Q Zirconia beads were kindly donated by BioSepra (Villeneuve la Garenne, France). Fractogel EMD TMAE 650s beads were purchased from Merck (Darmstadt, Germany). Express-Ion Q beads were kindly donated by Whatman (Maidstone, UK).

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 A/S (Bagsværd, Denmark).

Tris(hydroxymethyl)aminomethane (Tris, Sigma 7-9) and Bis-Tris propane (B6755) were purchased from Sigma Chemical (St. Louis, MO, USA). 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 setup) 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 (Birkerød, Denmark).

Chromatographic resins for high pressure operation (Q HyperD 20, Q Zirconia, and Source 30Q) were packed in 10 cm length×0.46 cm I.D. OmegaChrom PEEK columns from Upchurch (Oak Harbor, WA, USA). Other resins for low and medium pressure operation were packed in HR 5/10 columns (10 cm length×0.5 cm I.D.) supplied by Amersham Pharmacia Biotech (Uppsala, Sweden).

2.3. Methods

All resin comparison experiments of this study

Table 1
Properties of test proteins

Protein	p*I*	Molecular weight (kDa)
Anti-FVII Mab (IgG)	~6–7	150
Aprotinin	>11	6
BSA	5.0–5.2	69
Lipolase	~4.3	35
Myoglobin	7–8	18

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. For the standard buffer and pure protein/peptide solutions, hydrochloric acid was used to adjust pH. The following general methodology was used:

The column was equilibrated with a sufficient number of column volumes (CV) of buffer (15–20 CV). 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 are given in Table 1. Standard buffer solutions were: (A) 50 m*M* Tris+50 m*M* Bis-Tris–propane, pH 6.0; (B) 50 m*M* Tris+50 m*M* Bis-Tris–propane, pH 9.0; (C) H_2O; and (D) 0.5 or 3.0 *M* NaCl. In all experiments mixing was performed to give a buffer concentration of 25 m*M* Tris+25 m*M* Bis-Tris–propane. Column regeneration was performed with 5 CV 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 2. Resin particle size and recommended maximum operating pressure were obtained from the suppliers. The general flow-rate used for pH dependence and binding strength measurements was approximately 50% of the recommended maximum operating flow-rate/pressure. The low and high flow-rates used for dynamic capacity determinations were approximately 25 and 75%, respectively, of the recommended maximum operating flow-rate/pressure.

2.3.1. Extra column volume determinations

Extra column volumes were determined by extensive measurements. For the injection system, a 15 μl pulse of 4% acetone was applied to each of four combinations of set up: two column positions and two types of column fittings for high and low

Table 2
Properties of the chromatographic anion-exchange resins and applied flow-rates[a]

Resin	Particle size (μm)	Maximum recommended pressure (bar)	Applied flow-rates (ml/min)		
			General	Capacity (low)	Capacity (high)
Macro-Prep 25Q	25	~16	2.0	1.0	3.25
TSK-Gel Q-5PW-HR	20 (15–25)	~20	2.5	1.0	4.0
Poros QE/M	20	~200	10.0	2.0	13.0
Q Sepharose FF	90 (45–165)	~3	1.2	0.6	1.8
Q HyperD 20	20	200	10.0	2.0	13.0
Q Zirconia	76	–	10.0	2.0	13.0
Source 30Q	30	~40	3.0[b]	1.0	3.0
Fractogel EMD TMAE 650s	30 (20–40)	~3	0.8	0.4	1.3
Express-Ion Q	60–130 (fibers)	~1	0.25	0.2	0.4

[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.

[b] pH dependence measurements run at 4.0 ml/min.

pressure operation. Further, three different flow-rates were employed for each of the four combinations: 0.5, 1.0, and 5.0 ml/min. To get the best determination of extra column volumes, raw data from the resulting peaks were fitted to an EMG function (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 of the system, $M_{1,S}$ and $M_{2,S}$, were calculated for each combination, and these values were used to correct data for pH dependence, efficiency, binding strength, and dynamic capacity as described below. The first and second moments showed a slight increase with the flow-rate, however, the lowest values corresponding to the lowest flow-rate were used for correction.

2.3.2. pH dependence measurements

pH dependence measurements at pH 6.0, 7.0, 8.0, and 9.0 were performed by injecting the sample through the injection system after column equilibration without NaCl, and perform a NaCl gradient from 0 to 1 *M* during 20 CV. A small isocratic segment corresponding to the dead volume from the pump mixing system to the injection system was, thus, part of the method. All proteins eluted at these conditions. 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 at non-binding conditions found from the plate height determinations below. A pH-value where sufficient binding was obtained was found from the pH dependence experiments and used in the binding strength and dynamic capacity measurements described below.

2.3.3. Plate height determinations

Non-binding conditions were aimed at for all plate height determinations at the isocratic conditions of 1 *M* NaCl, pH 6.0 as a function of flow-rate. Testing at pH 6.0 and 8.0 with 0.5 and 1.5 *M* NaCl was also performed, but the lowest retention was observed at the conditions employed. Flow rates were varied between approximately 10 and 100% of the recommended maximum flow-rate/pressure. These efficiency 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 function, and the first and second moments were used to calculate the reduced plate height, *h*. The choice of the EMG function for fitting of raw data to calculate *h* and k'-values in this study was due to the somewhat tailing peaks obtained by efficiency measurements at non-binding conditions and especially by isocratic retention measurements at binding conditions, see below. Further, the EMG function has the advantage of using all of the peak curve for the fit compared to the various graphical methods used for fitting to a Gaussian peak and optionally calculating an asymmetry factor. The function used in this study is the four parameter EMG function with an extra parameter positioning the base line, that is a five parameter model:

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

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

where t is the time; A is the scaling; μ is the Gaussian mean value; σ is the symmetrical peak width; τ is the asymmetrical peak width; and E is the peak base line 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 are [30]:

$$M_1 = \mu + \tau$$

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

Both first and second moments are additive parameters, thus for exponentially modified Gaussian

peaks the number of theoretical plates of the column, N, are:

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

where $M_{2,0}$ is the second moment of the protein peak at non-binding conditions. The theoretical plate height, H, is found from:

$$H = \frac{L}{N}$$

where L is the column length; and the reduced theoretical plate height, h, is found from the following equation:

$$h = \frac{H}{d_p}$$

where d_p is the particle diameter. h is in this study presented as a function of the linear flow-rate, v:

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

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

2.3.4. Binding strength measurements

Binding strength experiments were performed as the classical isocratic retention measurements as a function of NaCl concentration, thus equilibration and elution were performed at the same ionic strength. The NaCl concentration was varied between 0 and 350 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 performed at pH 9.0.

As for the efficiency experiments 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 at non-binding conditions for adjustment. In this study binding strength is illustrated by plotting k' vs. reciprocal total ionic strength of the solution for elution. The total ionic strength, I_{Total}, was found from:

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

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

2.3.5. Dynamic capacity determinations

Dynamic capacity was determined by frontal analysis experiments. The column was equilibrated with 15 CV of the standard buffer solution (25 m*M* Tris + 25 m*M* Bis-Tris–propane) without salt at pH 8.0. The extra column volume from the pump to the UV detector excluding the column was filled with the protein solution and the detector was set to zero when the UV signal was stabile. The column was then put in-line by switching a valve and a sudden decrease of the UV signal was experienced. The protein solution was pumped through the column until the UV signal had increased again and was stabile at or close to zero. 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 break-through was calculated by normalising the protein concentration with the initial protein concentration, C/C_0. The dynamic capacity at 10 and 50% break-through is presented in this study.

2.3.6. Titration curve measurements

Titration curves were measured by a method similar to that used by Lagerlund et al. [23]. Two milliliters of the chromatographic resin was packed in a column (10 cm length × 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.

3. Results and discussion

The general approach of this study is to compare a number of strong anion-exchange resins at similar conditions employing new test proteins/peptides, Anti-FVII Mab (IgG), aprotinin, and lipolase, as well as standard test proteins, BSA and myoglobin. The test proteins/peptides presented in Table 1 were chosen to cover a broad range of isoelectric points and molecular weights. The chromatographic resins presented in Table 2 range from typical capture resins to resins used for final purification in a purification process. Resins used for a capture step are characterised by having a fairly large particle size and 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, such as Express-Ion Q, Q Zirconia, and Q Sepharose FF. Resins for final purification steps are typically characterised by having a small particle size with high selectivity, possibly at high pressure, that will give sharper peaks and a higher resolution of the target protein to the related impurity, which cover the rest of the resins in Table 2. The resins also cover a broad range of the different base matrix chemistries available for commercial use. The column diameter employed in the experiments is not optimal for some large particle size resins, but was used anyway 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 at linear chromatography conditions.

The experimental results of the pH dependence measurements are presented in Figs. 1 and 2. The results of Anti-FVII Mab, BSA, and lipolase shown in Fig. 1 display the same 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, the retention of Anti-FVII Mab in Fig. 1a and lipolase in Fig. 1c is almost constant for Q HyperD 20, but shows some increase for BSA in Fig. 1b. In contrast, Q Zirconia is an expanded bed adsorption beta test material with a different base matrix chemistry from the same supplier, which displays a large retention dependence with pH. Poros QE/M and Q Sepharose FF also present general low variation of retention with pH.

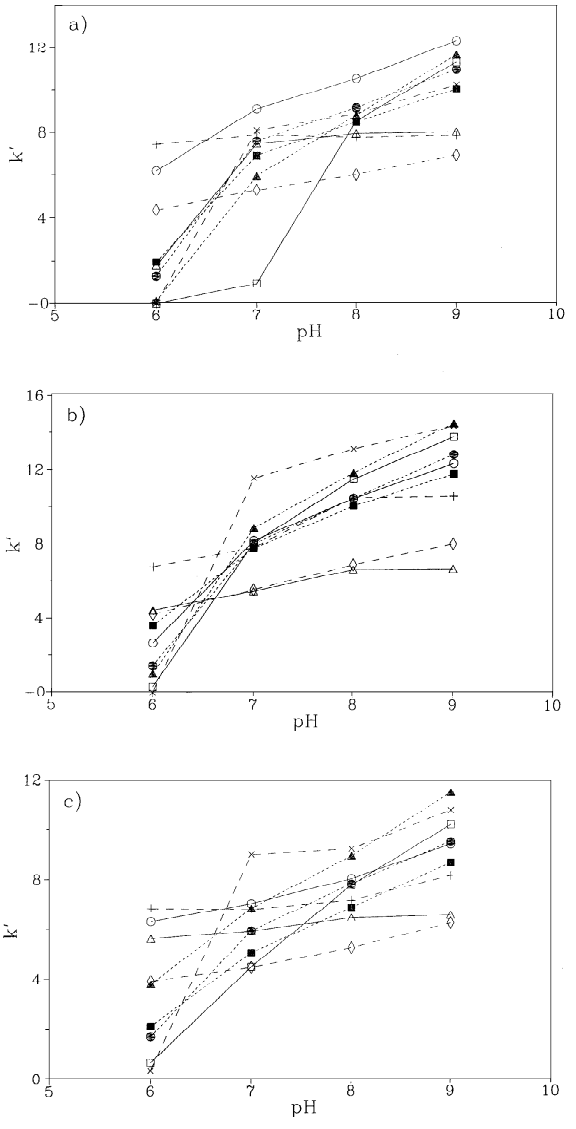

Fig. 1. pH dependence plots (k' − pH) of (a) Anti-FVII Mab; (b) BSA; and (c) Lipolase on anion-exchange resins at approximately 50% of recommended maximum operating flow-rate. The actual flow-rates are given in Table 2. pH dependence was determined by applying a 20 μl pulse of 1 mg/ml protein solution in 20 CV linear gradient from 0 to 1 *M* NaCl in 25 m*M* Tris+25 m*M* Bis-Tris–propane buffer through a 10×0.46 cm or a 10×0.5 cm column. Symbols are: ○, Macro-Prep 25Q; □, TSK-Gel Q-5PW-HR; △, Poros QE/M; ◊, Q Sepharose FF; +, Q HyperD 20; ×, Q Zirconia; ●, Source 30Q; ■, Fractogel EMD TMAE 650s; ▲, Express-Ion Q.

The 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.

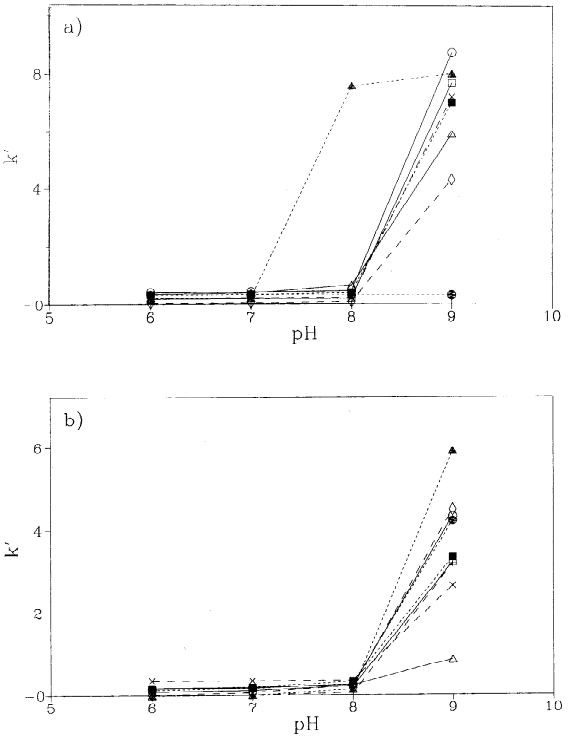

Fig. 2. pH dependence plots ($k'-$pH) of (a) Aprotinin; and (b) Myoglobin on anion-exchange resins at approximately 50% of recommended maximum operating flow-rate. The actual flow-rates are given in Table 2. pH dependence was determined by applying a 20 μl pulse of 1 mg/ml protein solution in 20 CV linear gradient from 0 to 1 *M* NaCl in 25 m*M* Tris+25 m*M* Bis-Tris–propane buffer through a 10×0.46 cm or a 10×0.5 cm column. Symbols are: ○, Macro-Prep 25Q; □, TSK-Gel Q-5PW-HR; △, Poros QE/M; ◊, Q Sepharose FF; +, Q HyperD 20; ×, Q Zirconia; ●, Source 30Q; ■, Fractogel EMD TMAE 650s; ▲, Express-Ion Q.

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. These test solutes having a p*I* generally higher than the pH used in this study should not be suitable for anion-exchange operation according to general text books [31]. Experience from a number of commercial purification processes shows, however, that removal of certain related or unrelated impurities with anion-exchange chromatography may be performed with great advantage. Even 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 the studies of Kopaciewicz et al. [32], who found retention of a number of proteins up to one pH unit below the p*I* of the proteins on Mono Q resin.

Data on retention as a function of pH could be of great importance in process development and optimisation in a situation where a change in pH is necessary to obtain, for instance, higher buffer strength of your selected buffer components. Thus, these figures could give a hint to how much or if the retention would be influenced by this change in pH. Further, such data could be useful for planning of flow-through mode operation, where the relationship between pH of operation and the selected resin is important to ensure flow-through of the target protein and binding of impurities. Finally, these data give an idea of the pH sensitivity of the resins when a pH range in a purification process step is settled or wanted. If the resin displays too much variation in retention of the protein as a function of pH in the desired pH range, it could be necessary to displace it with a less pH sensitive resin.

To what extent the pH dependence of the various resins is due to a different degree of quarternisation, i.e. the resins may contain both tertiary and quaternary amino groups, might be found from comparable titration curves of the resins. Fig. 3 shows the titration curves of all resins in this study, and the titration curves of a blank titration and DEAE Sepharose FF for comparison. A ‘true’ strong anion-

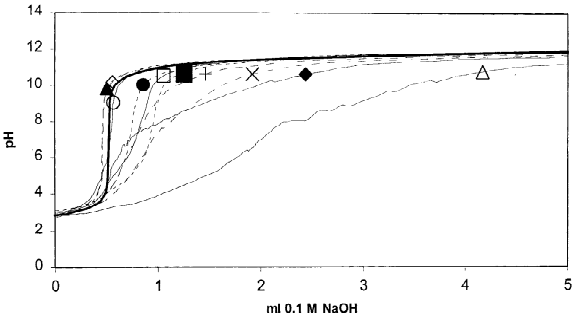

Fig. 3. Titration curves of anion-exchange resins in 1 *M* KCl from pH 3 to 12 with 0.1 *M* NaOH. The thick line (—) is the blank titration. Symbols are: ○, Macro-Prep 25Q; □, TSK-Gel Q-5PW-HR; △, Poros QE/M; ◊, Q Sepharose FF; +, Q HyperD 20; ×, Q Zirconia; ●, Source 30Q; ■, Fractogel EMD TMAE 650s; ▲, Express-Ion Q; ♦, DEAE Sephaose FF.

exchange resin would have a titration curve corresponding to the blank titration, while a weak or weaker anion-exchange resin would have a titration curve tending more to that of DEAE Sepharose FF. As shown in Fig. 3, Source 30Q, TSK-Gel Q-5PW-HR, Fractogel EMD TMAE 650s, Q HyperD 20, and Q Zirconia display an increasing trend towards the titration curve of DEAE Sepharose FF indicating some presence of tertiary amino groups, while Poros QE/M seems to contain a lot of tertiary amino groups with the titration curve placed well below that of DEAE Sepharose FF. The titration curves of Express-Ion Q, Q Sepharose FF, and Macro-Prep 25Q follow the blank titration curve indicating that they are truly strong anion-exchange resins. These titration curves show, that there is no clear link between the pH dependence of some resins in Fig. 1 and their titration curves.

Results of the efficiency experiments are presented in Fig. 4 as scaled standard van Deemter plots at unretained conditions. Use of 1 *M* NaCl at pH 6 gave the lowest retention in initial test runs with Q Sepharose FF and Poros QE/M because higher or lower salt concentration increased retention due to salting out effects and higher ionic interaction, respectively, and these conditions were employed in all subsequent efficiency experiments. Further, it was assumed that no resin swelling occurred as a function of pH in the range 6–9.

Fig. 4 shows the expected general trend of lower dependence of the plate height with increasing flow-rate of resins for medium and high pressure operation compared to the soft resins. The scatter in the data for some resins is due to difficult and inadequate fitting to the EMG function. The trend of increasing *h* values followed by a decrease with increasing flow-rate especially for Macro-Prep 25Q and Express-Ion Q, and to some extent for Q Sepharose FF and Fractogel EMD TMAE 650s may be due to emerging compression of the resins. Another explanation could

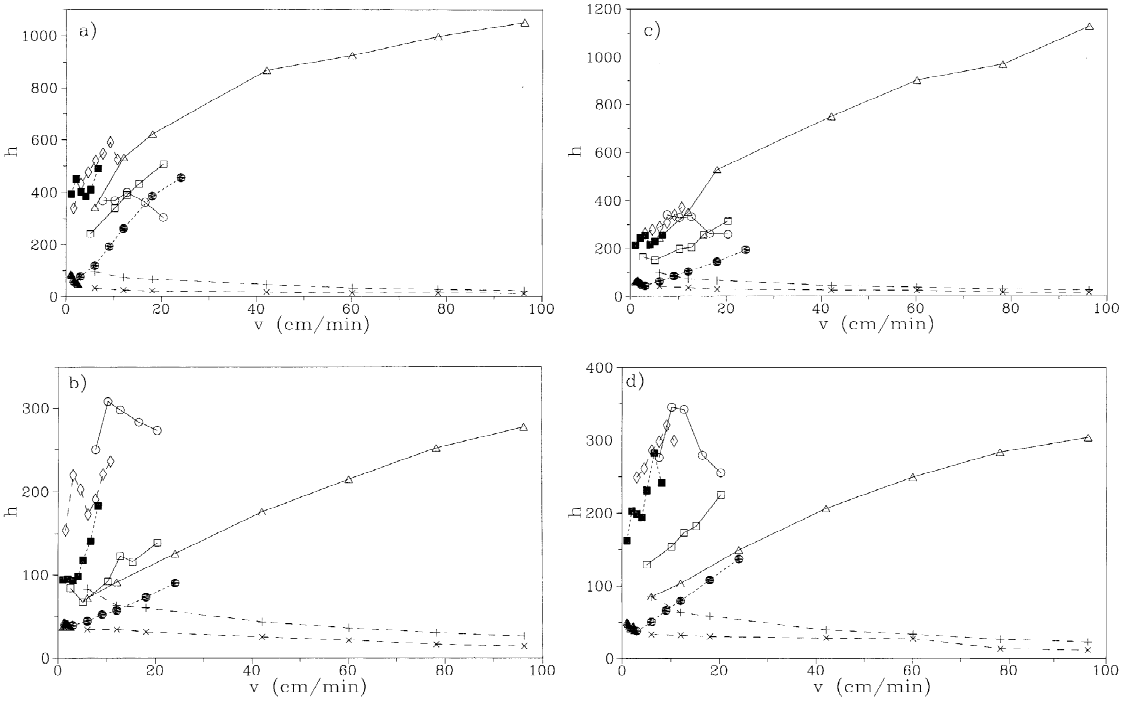

Fig. 4. Efficiency plots ($h - v$) of (a) Anti-FVII Mab; (b) Aprotinin; (c) Lipolase; and (d) Myoglobin at non-binding conditions. van Deemter curves were determined by applying a 20 μl pulse of 1 mg/ml protein solution in 1 *M* NaCl in 25 m*M* Tris+25 m*M* Bis-Tris–propane buffer at pH 6 through a 10×0.46 cm or a 10×0.5 cm column. Symbols are: ○, Macro-Prep 25Q; □, TSK-Gel Q-5PW-HR; △, Poros QE/M; ◊, Q Sepharose FF; +, Q HyperD 20; ×, Q Zirconia; ●, Source 30Q; ■, Fractogel EMD TMAE 650s; ♦, Express-Ion Q.

be that determination of HETP involves calculation with very small numbers which may be associated with some degree of uncertainty. For Q HyperD 20 and Q Zirconia, the efficiency seems to increase with increasing flow-rate. An explanation could be that the proteins simply are increasingly excluded from the soft gel part of the resins at the non-binding conditions at the increasing flow-rates resulting in a slightly smaller accessible volume for the protein. This phenomenon is also known from size exclusion chromatography. Further, it seems like Source 30Q and TSK-Gel Q-5-PW-HR follow the same trend and flow dependence for all proteins, with Source 30Q at a lower level. The high efficiency of Source 30Q at very low flow-rates is probably due to the highly mono-dispersed particle size character of this resin.

Plate height data may be used in process development as an indication of the purification efficiency that can be expected for a separate resin, that is the number of plates at a given column length and flow-rate. For process optimisation, these data will indicate the influence of an increase in the flow-rate for a given purification step on the separation efficiency.

The binding strength is in this study characterised by the standard k' vs. I_{Total}^{-1} plot obtained by isocratic runs as presented in Fig. 5, thus a stronger binding will need more salt for elution to occur and thus a smaller I_{Total}^{-1}. For the two test proteins with low isoelectric points, Anti-FVII Mab and lipolase in Fig. 5a and c, the resins with the strongest binding are Poros QE/M and Macro-Prep 25Q, while the weakest binding resin is TSK-Gel Q-5PW-HR. For the two test proteins with high isoelectric points, aprotinin and myoglobin in Fig. 5b and d, no clear trend is observed.

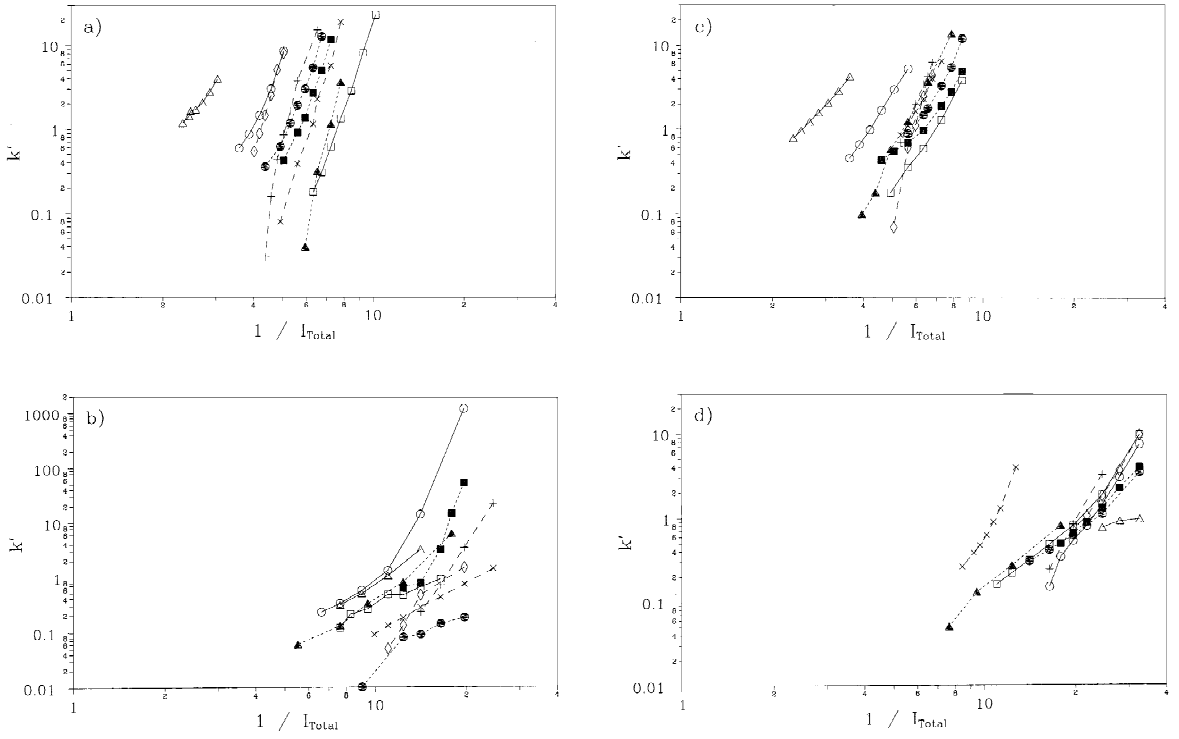

Fig. 5. Binding strength plots ($k'-I_{\text{Total}}^{-1}$) of (a) Anti-FVII Mab at pH 8; (b) Aprotinin at pH 9; (c) Lipolase at pH 8; and (d) Myoglobin at pH 9 on anion-exchange resins at approximately 50% of recommended maximum operating flow-rate. The actual flow-rates are given in Table 2. Binding 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 10×0.46 cm or a 10×0.5 cm column. Symbols are: ○, Macro-Prep 25Q; □, TSK-Gel Q-5PW-HR; △, Poros QE/M; ◊, Q Sepharose FF; +, Q HyperD 20; ×, Q Zirconia; ●, Source 30Q; ■, Fractogel EMD TMAE 650s; ▲, Express-Ion Q.

Binding strength measurements are of great importance for process development and optimisation. In the case where the target protein binds weakly perhaps due to a high conductivity solution before loading to the column, a resin giving a curve to the left in the figures would probably be selected for further testing. In the case of a strongly binding protein to all resins, a resin giving one of the rightmost curves would probably be chosen to avoid the use of too much salt for elution. Further at flow-through mode chromatography operation for removal of impurities, the best suited resin will just let the target protein run through the column at the conditions of the sample and retain the impurities.

Dynamic capacities are determined by frontal analysis experiments of pure proteins and presented as 10 and 50% break-through data for Anti-FVII Mab, BSA, and lipolase in Table 3. Dynamic capacity data for BSA as obtained by the various suppliers is also presented in Table 3. 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 2. The purpose of this study was not to obtain a complete adsorption isotherm, but merely to experience the influence of a rather high and low flow-rate on the resin performance.

Table 3 presents the expected general trend of higher dynamic capacity for both 10 and 50% break-through at lower flow-rate compared to higher flow-rate for all proteins. In some cases like Q Sepharose FF with BSA and Fractogel EMD TMAE 650s and Express-Ion Q with Anti-FVII Mab, the difference in capacity was much higher between 10 and 50% at high flow-rate compared to low flow-rate due to poor mass transfer into the resins particles. This was also an expected observation for these fairly soft resins. Good agreement was obtained in most cases between this study and supplier data for BSA, however, for Q Sepharose FF and Fractogel EMD TMAE 650s there are some differences in favor of the suppliers. The large difference may be due to a different experimental setup. Results for BSA with Q Sepharose FF are in very good agreement with results obtained by Tsai et al. [25]. Dynamic capacity data for IgG are usually low, but the extraordinary low capacities obtained with Anti-FVII Mab may be due to a rather high conductivity of the stock solution (25 m*M* Tris + 50 m*M* NaCl), which is higher than for other proteins of this study.

Fig. 6 presents the frontal analysis plots of Anti-FVII Mab on Q HyperD 20 and Poros QE/M at the high flow-rate. The overshoot phenomenon shown with Anti-FVII Mab and Q HyperD 20 where the break-through concentration gives a concentration higher than the feed concentration and ends up at the

Table 3
Dynamic capacity results of strong anion-exchange resins at 10 and 50% break-through for Anti-FVII Mab, BSA, and lipolase at flow-rates of approximately 25 and 75% of recommended maximum operating flow-rate/pressure[a,b]

Resin	Dynamic capacity, % breakthrough, Anti-FVII Mab (mg/ml)				Dynamic capacity, % breakthrough, BSA (mg/ml)				Dynamic capacity, % breakthrough, Lipolase (mg/ml)				Dynamic capacity obtained by the suppliers, BSA (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%	
Macro-Prep 25Q	8	9	7	8	21	22	19	21	26	26	24	27	>30
TSK-Gel Q-5PW-HR	4	4	1	2	42	49	39	47	62	75	46	58	70±18
Poros QE/M	8	9	5	6	38	39	38	40	16	17	17	18	40
Q Sepharose FF	5	6	4	5	54	60	42	53	66	75	61	73	120*
Q HyperD 20	5	5	5	5	85	87	79	81	63	65	65	69	80**
Q Zirconia	3	3	2	3	76	80	67	71	56	61	40	46	68***, 78****
Source 30Q	3	3	3	3	38	41	39	42	41	44	41	44	≥40****
Fractogel EMD TMAE 650s	0.9	1.3	0.6	1.2	28	30	27	30	29	35	29	36	100
Express-Ion Q	0.4	1.4	0.2	1.4	50	51	50	52	60	63	57	60	55

[a] Flow rates are presented in Table 2.
[b] * HSA, ** 90% break-through, *** 10% break-through, **** 50% break-through.

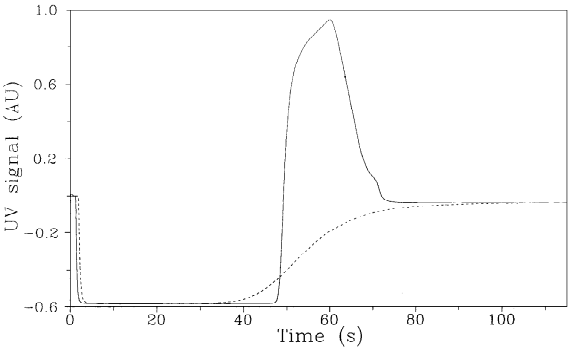

Fig. 6. Frontal analysis plots of Anti-FVII Mab at pH 8 on Q HyperD 20 (———) and Poros QE/M (— — —) at 13 ml/min. Frontal analysis curves were measured by loading of a 1 mg/ml Anti-FVII Mab solution in 25 m*M* Tris buffer and 50 m*M* NaCl through a 10×0.46 cm column.

feed concentration was experienced for several resins (Q HyperD 20,TSK-Gel Q-5PW-HR, Express-Ion Q, Source 30Q, and Q Zirconia) with Anti-FVII Mab at both the high and low flow-rates applied in this study. Whether this is due to at least two different populations of Anti-FVII Mab for example in glycosylation and that the solution should be regarded as a binary solution where one population is displacing the other in a competitive system [33] is not known. It is, however, not likely when the phenomenon only appears for certain resins.

Dynamic capacities for pure proteins were measured in this study, though dynamic capacities of true mixtures such as fermentation broth give a more realistic picture of what to expect in a process development situation [34]. However, supply of uniform fermentation broth is difficult to obtain over a long period of time, and the resins tested in this study are intended for different purposes, from capture to final purification. The need for dynamic capacity data for the industry is obvious: In a situation where two or more resins perform equally well with respect to resolution, flow-rate, price, etc. the resin with the highest capacity will be chosen for a process to reduce the number of purification cycles necessary per gram of protein.

Comparison of the results obtained in this study with literature data [17–26] was difficult because most literature data are of a different kind performed with different resins and/or at different conditions. A lot of experiments in this study were performed below the p*I* of the proteins. It is not the text book advise for anion-exchange chromatography, however, it is often a powerful purification tool in many industrial processes to run at such conditions.

The data generated here may be used for selection of resins for testing in process development, and the selection can be done according to dynamic capacity, binding strength, pH dependence, and flow dependence. The data does, however, not tell anything about specific selectivity differences between a target protein and a given related or unrelated impurity and thus nothing about the resolution one should expect to obtain. There are no good or bad resins, but more or less suitable resins for a specific purpose.

4. Conclusion

A comparative study has been undertaken on various strong anion-exchangers to investigate the pH dependence, titration curves, efficiency, binding strength, and dynamic capacity of the resins. There are clear differences among the results on the nine different resins studied with five different test proteins. The dependence of pH on retention varies from generally low, i.e. Q HyperD 20, Poros QE/M, and Q Sepharose FF to very high, i.e. TSK-Gel Q-5PW and Express-Ion Q for proteins with low p*I*. All resins will generally bind proteins of high p*I* at pH 9. No direct link between pH dependence on retention and titration curves of the different resins was observed. Efficiency results show the expected trend of lower dependence of the plate height with increasing flow-rate of resins for medium and high pressure operation compared to the soft resins. Binding to the anion-exchange resins as a function of ionic strength may vary depending on the specific protein. Generally, binding and elution at a high salt concentration may be performed with Poros QE/M or Macro-Prep 25Q, while binding and elution at low salt concentration may be done with TSK-Gel Q-5PW. Dynamic capacities are strongly dependent on the specific protein employed and for some resins dependent on the flow-rate. A general good agreement was

obtained between this study and data obtained by suppliers for the dynamic capacity.

The results of this study may be used for selection of resins for testing in process development, however, the data does not tell anything about specific selectivity differences between a target protein and a given impurity. Further, information with a view to resolution between a target protein and related or unrelated components can not be obtained from the results. None of the resins studied here should be regarded as good or bad, but more or less suitable for a specific purpose, and only testing for the specific application will determine which one is the optimal resin.

5. Nomenclature

A	Scaling parameter
c_i	Molar concentration of component i
C/C_0	Normalised protein concentration
CV	column volumes
E	Base line 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
$M_{1,S}$	First moment of the extra column volume
$M_{2,S}$	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

Acknowledgements

The supply of pure proteins from Peter Rahbek Østergaard, Birgitte Silau, and Ole Elvang Jensen, the review of the paper by Ernst Hansen, and the technical assistance and set up of dynamic capacity measurements and EMG data fitting procedures from Ulrik Borgbjerg are gratefully acknowledged.

References

[1] P.R. Levison, R.M.H. Jones, D.W. Toome, S.E. Badger, M. Streater, N.D. Pathirana, J. Chromatogr. A 734 (1996) 137.
[2] J. Horvath, E. Boschetti, L. Guerrier, N. Cooke, J. Chromatogr. A 679 (1994) 11.
[3] Y.-B. Yang, K. Harrison, J. Kindsvater, J. Chromatogr. A 723 (1996) 1.
[4] N.B. Afeyan, N.F. Gordon, I. Mazsaroff, L. Varady, S.P. Fulton, Y.B. Yang, F.E. Regnier, J. Chromatogr. 519 (1990) 1.
[5] E. Boschetti, J. Chromatogr. A 658 (1994) 207.
[6] J. Dennis, P. Levison, C. Mumford, BioPharm. 11 (1998) 44.
[7] C. Chang, A.M. Lenhoff, J. Chromatogr. A 827 (1998) 281.
[8] M. Weitzhandler, D. Farnan, J. Horvath, J.S. Rohrer, R.W. Slingsby, N. Avdalovic, C. Pohl, J. Chromatogr. A 828 (1998) 365.
[9] F. Fang, M.-I. Aguilar, M.T.W. Hearn, J. Chromatogr. A 729 (1996) 67.
[10] L.E. Weaver, G. Carta, Biotechnol. Prog. 12 (1996) 342.
[11] Y. Hu, P.W. Carr, Anal. Chem. 70 (1998) 1934.
[12] M.A. Hashim, K.-H. Chu, P.-S. Tsan, J. Chem. Tech. Biotechnol. 62 (1995) 253.
[13] R. Hahn, P.M. Schulz, C. Schaupp, A. Jungbauer, J. Chromatogr. A 795 (1998) 277.
[14] C.M. Roth, K.K. Unger, A.M. Lenhoff, J. Chromatogr. A 726 (1996) 45.
[15] D.C. Nash, H.A. Chase, J. Chromatogr. A 807 (1998) 185.
[16] P. DePhillips, A.M. Lenhoff, Determinants of Protein Retention Characteristics on Cation-Exchange Resins, Poster presentation at HPLC'96, San Francisco, CA, June 1996.
[17] M. McCoy, K. Kalghatgi, F.E. Regnier, N. Afeyan, J. Chromatogr. A 743 (1996) 221.
[18] J. Ericsson, E. Berggren, C. Lindqvist, K.-A. Hansson, K. Qvarnström, L. Lundh, G. Moen, React. Functional Polymers 30 (1996) 327.
[19] A.E. Ivanov, V.P. Zubov, J. Chromatogr. A 673 (1994) 159.
[20] C. McNeff, Q. Zhao, P.W. Carr, J. Chromatogr. A 684 (1994) 201.
[21] M.A. Fernandez, W.S. Laughinghouse, G. Carta, J. Chromatogr. A 746 (1996) 185.
[22] M.A. Fernandez, G. Carta, J. Chromatogr. A 746 (1996) 169.
[23] I. Lagerlund, E. Larsson, J. Gustavsson, J. Färenmark, A. Heijbel, J. Chromatogr. A 796 (1998) 129.
[24] D. Bentrop, H. Engelhardt, J. Chromatogr. 556 (1991) 363.
[25] A.M. Tsai, D. Englert, E.E. Graham, J. Chromatogr. 504 (1990) 89.
[26] M.B. Jensen, Ionbytningskromatografi af Valleproteiner-Isokratisk og lineær gradient eluering, Preliminary Master Thesis, Technical University of Denmark, June 1999.

[27] L. Thim, S. Bjørn, M. Christensen, E.M. Nicolaisen, T. Lund-Hansen, A.H. Pedersen, U. Hedner, Biochemistry 27 (1988) 7785.
[28] H. Fritz, G. Wunderer, Arzneim.-Forsch./Drug Res. 33 (I,4) (1983) 479.
[29] E. Boel, T. Christensen, E. Gormsen, B. Huge-Jensen, B.S. Olesen, in L. Alberghina, R.D. Schmid, R. Verger (Eds.), Lipases: Structure, Mechanism and Genetic Engineering, GBF Monographs, Vol. 16, 1990, p. 207.
[30] E. Gruska, Anal. Chem. 44 (1972) 1733.
[31] S. Yamamoto, K. Nakanishi, R. Matsuno, Ion-Exchange Chromatography of Proteins, Chromatographic Science Series, Vol. 43, Marcel Dekker, New York, 1988.
[32] W. Kopaciewicz, M.A. Rounds, J. Fausnaugh, F.E. Regnier, J. Chromatogr. 266 (1983) 3.
[33] R.M. Nicoud, A. Seidel-Morgenstern, Isolation Purif. 2 (1996) 165.
[34] A. Staby, N. Johansen, H. Wahlstrøm, I. Mollerup, J. Chromatogr. A 827 (1998) 311.