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High-performance protein separations with novel strong ion exchangers

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Abstract

In this paper, properties of new ion exchangers specifically designed for protein separations are reported. These sorbents are constituted of two main parts: a rigid, porous polystyrene–silica composite material which forms a rigid skeleton and a soft hydrogel bringing strong ionic groups. The later is regularly distributed inside the pores of the skeleton. Characterization of these materials was performed by measuring dynamic sorption capacity, resolving power, separation efficiency and protein recovery. These studies were done using various known proteins and protein mixtures. Some comparisons have been made with commercially available ion exchangers also designed for protein separations.

1. Introduction

High-performance ion-exchange liquid chromatography is a powerful method for separation and purification of biopolymers. Various types of stationary phases were developed based on surface modified silica, polysaccharides and synthetic hydrophilic resins. The stationary phases vary in charge, functionality and pore diameter. Besides the surface chemistry, the pore structure is one of the most important characteristics of a stationary phase, since it determines the surface area of the support as well as the intraparticle solute transport.

Micropellicular [1,2] and non-porous stationary phases [3–5] are designed to achieve very fast and very efficient separations of biopolymers. The main advantage of these stationary phases is the rapid mass transfer between the stationary phase and the solutes. By eliminating the pores, the "stagnant mobile phase mass transfer" which is the main cause of the band spreading in the case of porous supports is diminished. The limitation of these phases is the small surface area and the low column permeability due to the small, non-porous spherical particles.

At present the majority of available products are the well-known conventional porous diffusive stationary phases. The size of the pores (100–1000 Å) is large enough to let the biopolymers diffuse rapidly to access to ionic sites [6]. With this pore size these stationary phases have significantly larger surface area and thus considera-

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bly higher capacity compared to the micropellicular and non-porous phases. However, the slow mass transfer between the pores and the analytes results in peak broadening and in a significantly slower separation process.

In 1990 new, so-called perfusive chromatographic media were introduced [7]. These separation media are constituted of particles with 6000–8000-Å pores transecting the particles, the surface area being increased by the presence of 500–1500-Å interconnecting pores. As a result of the interconnected "throughpores" the particle can be operated in perfusion mode: when the rate of the solute transport due to convective flow inside the particles is greater than the rate of diffusive transport due to concentration gradients [7]. At low flow rates solutes enter the particle through a combination of convective and diffusive transport, but at high flow (Peclet number > 1) convection dominates. Due to convective transport, mass transfer rates are described as high compared to conventional diffusive stationary phases; this gives numerous application advantages to this type stationary phases [7,8]. D.D. Frey et al. [9] studied the effect of intraparticle convection on separations of biomolecules. They concluded that perfusive particles have performance advantages over particles with standard sized pores, mainly for applications that do not require high resolving power to accomplish fast separations. Besides the speed of the separation, another consequence of the very large porous, perfusive structure is the smaller surface area, which results in significant decrease of loading capacity compared to conventional diffusive stationary phases.

More recently a new chromatographic packing material was introduced based on a new hybrid concept [10–12]. A classical, soft tridimensional gel network is used as a protein sorbent; the high capacity of this kind of material was described more than 20 years ago [13]. This gel is not appropriate for high-performance liquid chromatography, due to its softness. In the so-called HyperD particles the gel is surrounded by a polystyrene–silica composite material giving it the necessary physical hardness for use in HPLC columns. The soft hydrogel can possess chemical functions or ligands appropriate for protein separations. Adsorption–desorption mechanisms are not different from classical hydrogels and necessitate the diffusion of macromolecules inside the gel where the requisite interactions occur and not in the interface between the rigid material and the hydrophilic polymer [14].

In this work main ion-exchange properties are studied with various proteins using the new ion-exchange material known under the trade name of HyperD (see below). We also compared the results to conventional diffusive and perfusive materials. Results shown deal with 10-μm spherical particles for micropreparative high-performance separations.

2. Experimental

2.1. Columns and stationary phases

The Q and S HyperD ion-exchange stationary phase (10 μm particle size) was supplied by BioSepra, France. The stationary phase was packed into Pharmacia's HR 5/5 column system (5×50 mm glass column, with adjustable column top) using the following packing procedure. First, 2 ml of slurry (50% dry material content) was homogenized and filled into an empty reservoir. Than, the slurry was pushed into the empty column, with a Beckman HPLC pump (#126) using inverse flow (against gravity) to obtain a maximum of 950 p.s.i. (1 p.s.i. = 6894.76 Pa) pressure drop on the column. The packing liquid for the Q material was 20% MeOH, 1 *M* NaCl and 50 m*M* Tris-HCl at pH 8.62. The packing buffer for the S material was 20% MeOH, 1 *M* NaCl, 50 m*M* Na Acetate at pH 4.50. The flow was maintained for 20 min.

Mono Q and Mono S columns (50×5 mm) were purchased from Pharmacia LKB, (Piscataway, NJ, USA). The column configurations and particle size (10 μm) were identical with the Q and S HyperD columns.

Poros Q/H, Poros S/H and Poros HS/H columns were purchased from PerSeptive Bio-

Systems (Cambridge, MA, USA). The 10-μm particles were prepacked into 50 × 4.6 mm I.D. polyether ether ketone (PEEK) columns.

For recovery, capacity and some gradient studies Resource Q (30 × 6.4 mm) 15 μm column from Pharmacia, and the Poros Q II/P (50 × 4.6 mm PEEK) 20 μm column from PerSeptive BioSystems were also used. Due to the different particle sizes of these two last sorbents (15 and 20 μm, respectively, compared to 10 μm) results of the efficiency, resolution, and separation studies are not presented in this report.

2.2. Instruments

The Beckman (Fullerton, CA, USA) System Gold HPLC system was used throughout this study. The system consisted of a Programmable Solvent Module 126, Programmable Detector Module 166, and Autosampler 507 equipped with loops ranging in size from 25 to 1000 μl. For capacity measurements we used the 50 ml glass superloop from Pharmacia LKB.

The data was collected using an IBM PS/2 Model 56SX computer and evaluated using the System Gold Data system.

For recovery studies we used a Beckman Model DU-7 Spectrophotometer to measure UV absorbance.

2.3. Chemicals

All salts and solvents were HPLC grade. The buffers were filtered through a 0.45-μm filter and degassed before use.

All standard proteins [such as ribonuclease A (RNASE A), ovalbumin (OVA), α-chymotrypsinogen A (α-Chym A), β-lactoglobulin (β-Lact), cytochrome *c* (Cyt C), myoglobin (Myo), lysozyme (Lys), human transferrin (aTRS), human albumin (hA)] were purchased in purified form from Sigma (St. Louis, MO, USA).

βω-E. Coli extracts were prepared by Dr. Craig Adams (Beckman Instruments, Fullerton, CA, USA) and used without further treatment.

3. Results and discussion

3.1. Q ion exchangers

Isocratic elution of proteins

For preliminary testing, the efficiency was measured under isocratic conditions when flow rate and sample load were varied. Bovine serum albumin (BSA) was used as a test protein under non-retaining conditions for all columns. The lack of retention limits the practical importance of these experiments; however, they are very useful for the comparison of various stationary phases regarding their differences in intraparticle solute transport. The plate numbers were determined from three consecutive injections using the Beckman System Gold software. The data varied less than 1%. At relatively low flow rates the Q HyperD column initially showed a good efficiency. As the flow rate increases, efficiency progressively deteriorated beyond a linear velocity of 360 cm/h (Fig. 1) The Poros Q/H column demonstrated very good reduced plate height values also, which were substantially unchanged up to 550 cm/h linear velocity used in this study. The high efficiency of the Q HyperD column is attributed to the high diffusive permeability of the hydrogel [10,13] and the low level of peak broadening. However, the phenomena of non-equilibrium diffusion rate showed some degree of flow rate dependency.

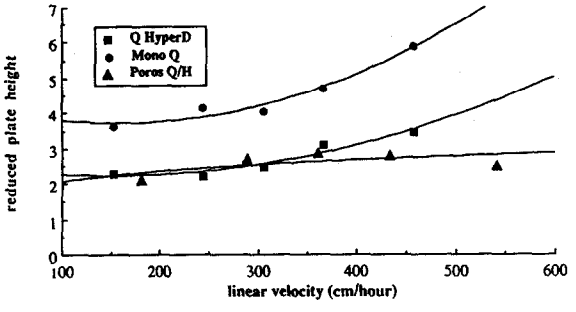

Fig. 1. Reduced plate height versus linear velocity relationship with 10-μm Q ion exchangers. 80 μg (40 μl) bovine serum albumin was injected under non-retaining conditions: 0.4 *M* NaCl in 50 m*M* Tris-HCl pH = 8.6 buffer. Detection at 280 nm.

Although some loss of efficiency can be measured at higher flow rates with the Q HyperD column, the flow range where this deterioration starts is close to the maximum operating flow rate due to back-pressure limitation. The conventional diffusive Mono Q column showed the lowest efficiency among the three columns we studied, which decreased much more with increasing flow rates. Here we need to emphasize that the diffusive Mono Q and Q HyperD do not have the speed capability of the perfusive Poros Q column. The pressure limit on the Q HyperD column is 1200 p.s.i. and on the Mono Q column 750 p.s.i. The Poros Q has superior stability: up to 2500 p.s.i. Due to pressure limitations, the maximum flow rates we could use in our comparison did not reach the perfusion limit for the Poros Q column, so all three columns were operated under diffusive conditions.

Fig. 2 shows the relationship between the column efficiency and protein load for the three columns. To determine efficiency we made three consecutive injections and used System Gold software to evaluate the chromatograms. The data varied less than 1%. Q HyperD and Poros Q possess very similar efficiency at the flow rate used (1 ml/min), Mono Q shows somewhat lower plate numbers. As expected, efficiency decreases slightly with increasing protein load for all the columns studied.

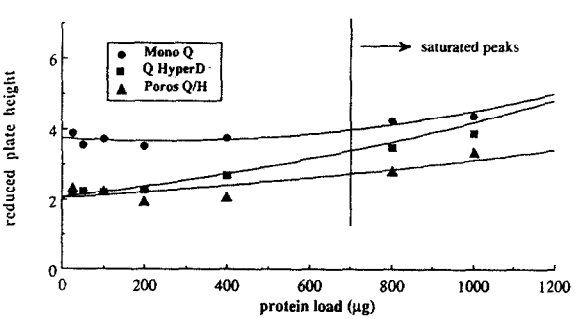

Fig. 2. Reduced plate height versus protein load relationship with 10-μm Q ion exchangers. Various amounts of bovine serum albumin were injected in 500 μl injection volume under non-retaining conditions: 1 ml/min 0.4 *M* NaCl in 50 m*M* Tris-HCl pH = 8.6 buffer. Detection at 280 nm.

Protein recovery

To measure and compare the protein recovery with various columns, the peak collection method was used. Since the capacities of the columns investigated differ greatly, the amount of BSA injected was 2% of the sorption capacity of each column. Thus, the relative sample load (injected amount compared to the capacity) on each column was similar and gave an effective comparison. The adsorbed BSA was eluted with 1 M NaCl in 50 m*M* Tris-HCl pH 8.62 buffer, and the recovered BSA was determined by measuring the BSA concentration in the collected samples using UV spectrophotometry. BSA recovery was 67% with Q HyperD and of similar order for MONO Q (73%) and Poros Q/H (70%).

After injection and elution of the protein sample the columns were washed with successive gradient runs (three times) and with 0.1 *M* sodium hydroxide injection. There was practically no protein elution during these runs. The recovery measurement was repeated three times on each column and the results varied less than 5%. On the basis of these findings, unrecovered BSA is considered here as sample impurity, worse with BSA and much better in the case of cytochrome *c* which we used for S ion exchangers (see below).

Capacity studies

One of the most important characteristics of a preparative HPLC column is its sorption capacity. It determines the highest applicable sample load and thus the productivity of the phase in protein purification. Naturally the dynamic capacity gives even more information about the column: how the capacity will be affected by increasing velocities.

To measure dynamic capacities of the three columns the frontal analysis method was used (5 mg/ml BSA solution). Breakthrough curves were measured at various linear velocities. 50% breakthrough point was taken for calculations to determine capacity; however, 10% breakthrough data for the Q HyperD column are also shown.

Under the experimental conditions studied Q HyperD showed a capacity of about 200 mg/ml

for BSA at 80 cm/h. At 900 cm/h the capacity decreased 25% to 150 mg/ml. At 10% breakthrough, the capacity difference was of about 30 mg/ml corresponding to 20%. Mono Q, Poros Q and Resource Q showed significantly lower capacities: 80, 25 and 70 mg/ml, respectively, at 80 cm/h. Increase in linear velocity resulted in capacity decrease for Mono Q and Resource Q. The sorption capacity of Poros Q remained constant over the entire range of linear velocity studied (Fig. 3).

Gradient elution of protein mixtures

To compare the chromatographic performance of the columns, standard protein samples were used under identical chromatographic conditions. Under these conditions the same reduced linear velocity was adopted for each column (linear velocity was adjusted according to particle size), the gradient length was adjusted to the column length, and the gradient volume was corrected for the dead volume of each individual column. The basis for the adjustments described above was the following equation [15]:

$$b = \frac{t_o}{t_G} \log \frac{k_a}{k_b} \quad (1)$$

where b = gradient steepness parameter, $t_o = V_m/F$, V_m = dead volume (total volume of the mobile phase inside the column), F = flow rate (ml/min), t_G = gradient duration time (min), k_a and k_b = capacity factor of the solute with A and B eluent (for given gradient solvents log k_a/k_b is constant).

Standard conditions chosen for Q HyperD were: $F = 1$ ml/min, $t_G = 20$ min, and 0–100% B concentration change during the gradient run. The value of log k_a/k_b for Q HyperD was calculated from these data and from the measured dead volume. To make the adjustments described above, as a rough estimate, log k_a/k_b was considered to be the same for each column.

Using these data as well as the measured dead volumes and the constant linear velocity, the t_G values for the other columns were calculated using Eq. 1. The calculated and applied gradient conditions for all the Q columns are summarized in Table 1.

The buffers were identical for all columns; the gradients were run from 0–0.4 M salt as described in Fig. 4 where chromatographic separations are shown.

Q HyperD showed a complete separation of the five components of this mixture with the tendency also to separate the two forms of transferrin. Q HyperD, Mono Q and Resource

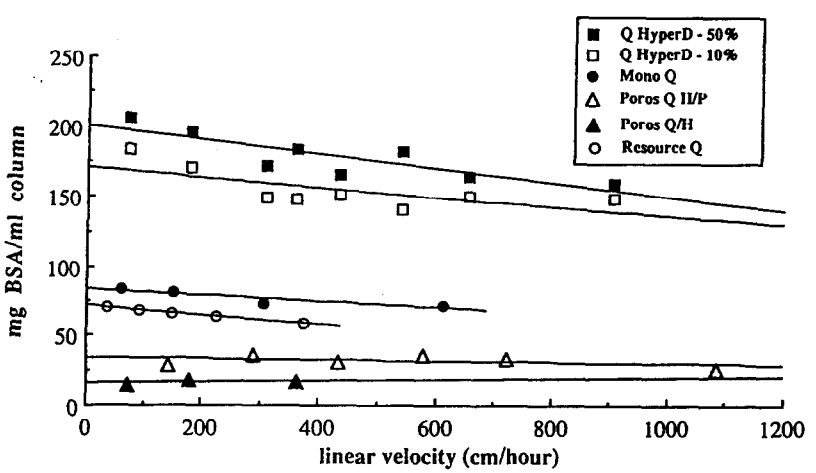

Fig. 3. Dynamic capacity of various Q columns. Method: frontal chromatography of 5 mg/ml bovine serum albumin solution, 50 mM Tris-HCl pH = 8.2 buffer; detection, 290 nm, 50% breakthrough.

Table 1
Established gradient conditions for Q ion-exchange columns

Column	Particle size (μm)	Column size (mm)	V_m (ml)	Linear velocity (cm/h)	Flow rate (ml/min)	t_G (min)
Q HyperD	10	50 × 5	0.87	306	1	20
Mono Q	10	50 × 5	0.89	306	1	20.5
Poros Q/H	10	50 × 4.6	0.66	306	0.85	17.8
ResourceQ	15	30 × 6.4	0.84	204	1.09	17.7

Q showed similar selectivity, the last peak (hA) eluted at around 75% of buffer B concentration. In Poros Q/H proteins were eluted more rapidly probably as a consequence of the weaker ion-exchange groups than the other Q columns: the last peak (hA) eluted at about 50% buffer B. However, using 0.2 *M* NaCl buffer B instead of the 0.4 *M* NaCl, the separation for this column became similar to the separation obtained with Q HyperD and Mono Q columns using 0.4 *M* NaCl. The Resource Q column resulted in broader peaks than the other Q columns, which

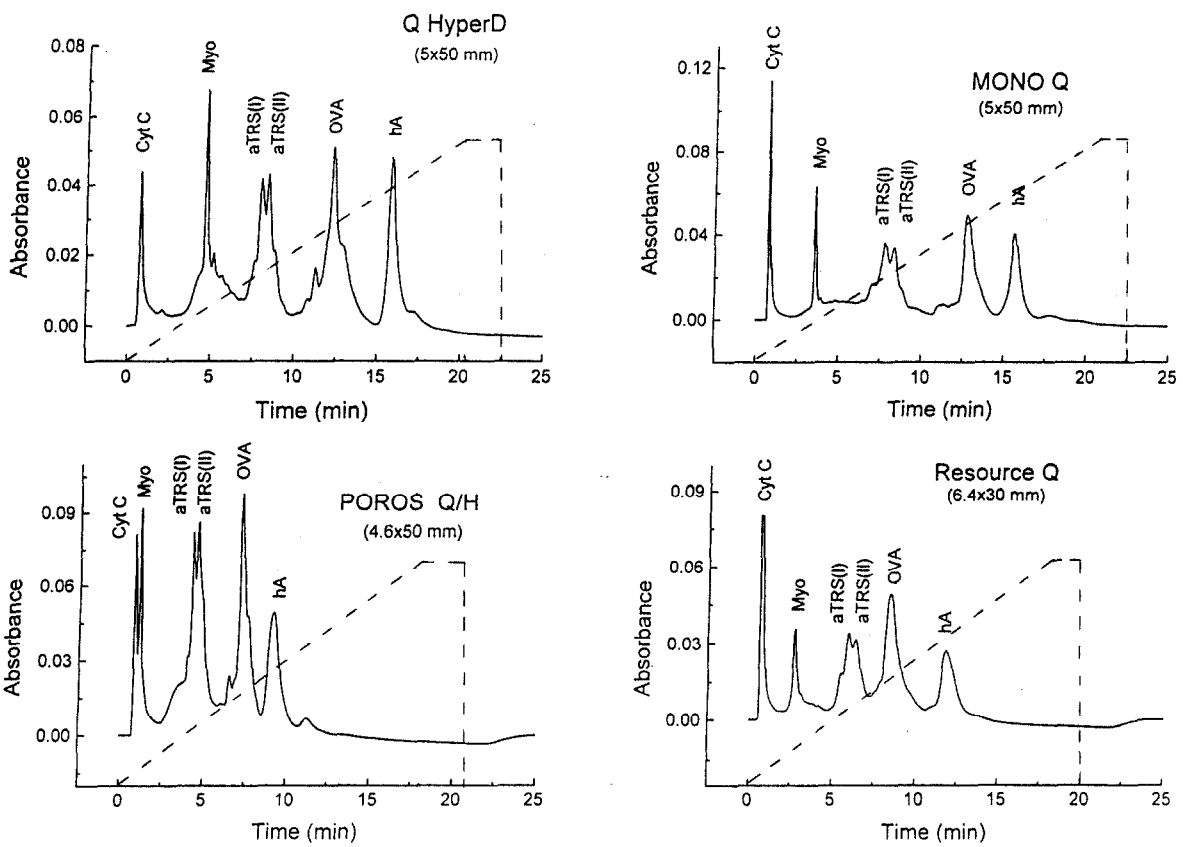

Fig. 4. Separation of 5 standard proteins with gradient elution using various Q columns. Gradient conditions (flow rate and duration time) are described in Table 1. Buffer A, 50 m*M* Tris-HCl pH = 8.6; buffer B, 0.4 *M* NaCl in 50 m*M* Tris-HCl pH = 8.6; detection at 280 nm, total injected protein amount was 297.5 μg in 50 μl.

was understandable considering the particle size differences (15 μm compared to 10 μm). As a summary of these experiments, it can be concluded that in the flow rate range we studied (0–1.5 ml/min), no dramatic difference in separation power and selectivity between the columns existed when they were used for the separation of clean, standard proteins under individually adjusted gradient conditions.

Besides testing the columns for standard protein separation, a separation of a crude extract of β-ω E. Coli was tried, with the 10-μm Q sorbents only. Fig. 5 shows the results obtained using the same, standard gradient conditions. Under standard condition the best resolution by the number of peaks and the best peak width was gotten with the Q HyperD column. Mono Q showed little resolution of the last two main peaks and a low number of peaks were obtained using the Poros Q column. Under adjusted gradient conditions Q HyperD again showed good resolution and efficiency in comparison to the other two columns. The separation performance with Q HyperD was practically independent of the sample load up to 5 mg (2% of the capacity) sample injection. Mono Q performance was strongly dependent on the injected sample amount (up to 2% of the capacity was injected); Poros Q gave very similar separations with increasing sample load and separation speed.

3.2. S ion exchangers

Protein recovery study

To measure and compare the protein recovery of S HyperD, cytochrome *c* was used as a model

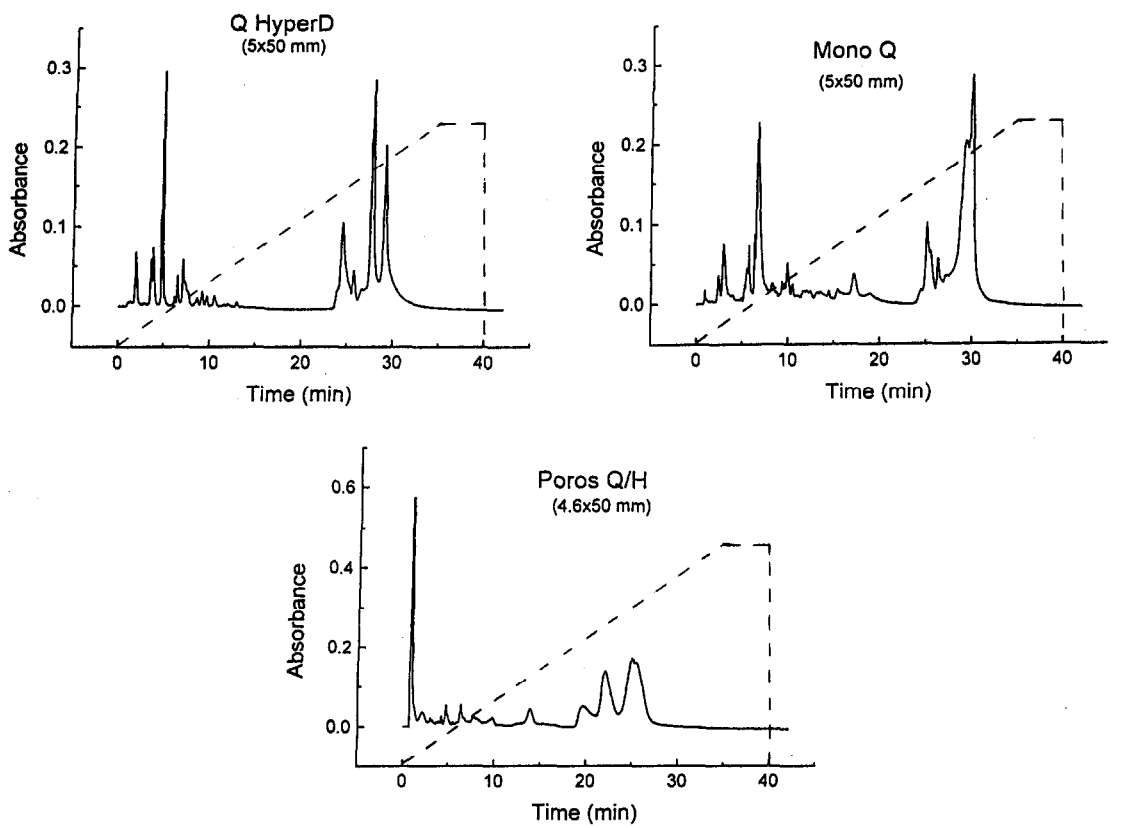

Fig. 5. Separation of $\beta\omega$-E. Coli extract on Q sorbents using the same gradient conditions for all columns. Conditions: 1 ml/min flow, 35 min 0–1 *M* NaCl in 50 m*M* Tris-HCl pH = 8.6 buffer; detection at 280 nm; injection volume, 30 μl; total injected amount 150 μg.

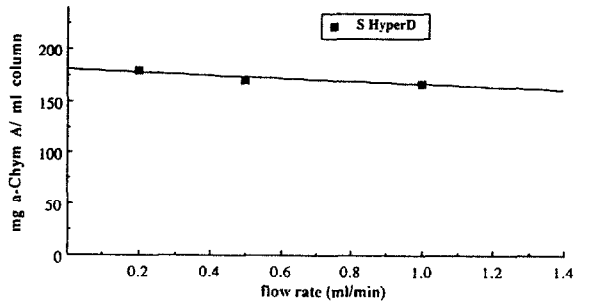

Fig. 6. Dynamic capacity study of S HyperD column. Method: frontal chromatography of 8 mg/ml α-chymotrypsinogen A solution; 50 m*M* Na acetate pH = 4.5 buffer; detection, 290 nm, 50% breakthrough.

protein. The injected amount was 2% of the capacity of the column. The elution buffer was 2 *M* NaCl in 50 m*M* Na acetate pH 4.5 buffer, and the concentration of Cyt C was determined by UV spectrophotometry. The protein recovery was found close to 86%. For Mono S, Poros S/H and Poros HS/H capacity results under the same experimental conditions were 80%, 85% and 80%, respectively. The recovery measurement was repeated three times on each column; the results were in a range of less than 6%.

Dynamic capacity

8 mg/ml α-Chymotrypsinogen A solution was used in frontal analysis to measure the capacity of the S HyperD at various flow rates. Fig. 6 shows that the capacity decreases very slowly as a function of flow rate. The measured capacity data of S HyperD compared to the other S columns' capacities as specified by the manufacturers in their operating instructions is summarized in Table 2.

Gradient elution of protein mixtures

Mixtures of purified, standard proteins were used to test the columns' performance in gradient elution. Identical gradient conditions were defined as described above; adjustments of buffers and buffer concentrations were also made to obtain the best separation for each protein mixture. It was found that, for complex mixtures (6 proteins), formate buffer permitted better separations than acetate buffer. Preliminary studies gave evidence that the selectivities of the packings studied were rather different. Since the effect of sample load and speed of the separation on resolution was to be measured, the adjustment of final salt concentration during the gradient run was necessary to get approximately the same retention time for the last protein peak with all sorbents. Nevertheless, the absolute resolution values should be interpreted very carefully because of the major differences in surface chemistry. Rather than making a quantitative evaluation based on specific values, all elements of qualitative and quantitative performance of the various columns should be taken into consideration.

Resolution–protein load study

For this study a two-protein mixture was chosen (cytochrome *c* and β-lactoglobulin). The resolutions obtained for this protein mixture were influenced not only by the ion-exchange performance of each packing material and the

Table 2
Comparison of capacity data of various S ion exchangers

Column	Capacity (mg/ml column)	Experimental conditions
S HyperD	160	α-Chymotrypsinogen A; 50 m*M* Na acetate pH 4.5
Mono S	20–50[a]	Not available[a]
Poros S/H	20[a]	Lysozyme; pH 6.2[a]
Poros HS/H	60[a]	Lysozyme; pH 6.2[a]

[a] From manufacturer's specifications (operating instructions)

Table 3
Established gradient conditions for S ion-exchange columns

Column	Column size (mm)	V_m (ml)	Linear velocity (cm/h)	Flow rate (ml/min)	t_G (min)	Final concentration (M)
S HyperD	50 × 5	0.87	382.8	1.25	18.8	1.08
Mono S	50 × 5	0.85	382.8	1.25	18.4	0.45
Poros S/H	50 × 4.6	0.65	382.8	1.06	16.6	1.23
Poros HS	50 × 4.6	0.59	382.8	1.06	15.0	0.75

particle size, but also by the indirect influence of the polymer structures. The working conditions used are summarized in Table 3. The runs were repeated three times at every load, and System Gold was used to evaluate the chromatograms. The relative standard deviation was less than 1%. Fig. 7 shows the result of the experiment: the resolution of S HyperD at low protein loading is close to the resolution of Mono S. When protein loading increased up to 600 μg, resolution diminished by 30 to 50% for Poros S/H and HS/H and by about 15% for Mono S while it remained constant for S HyperD.

Nevertheless, we need to state again that the actual resolution values are strongly dependent on the sample–column combination selected, due to the very different selectivities.

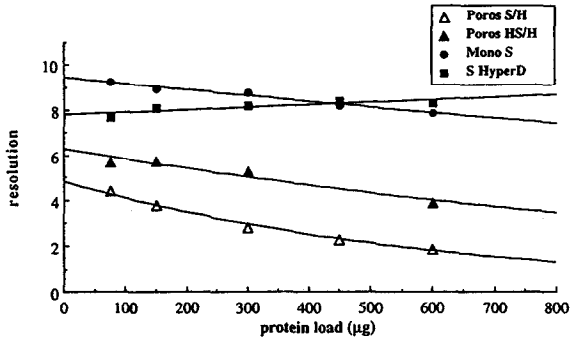

Fig. 7. Effect of the protein load on resolution in gradient elution. Various amounts of cytochrome c and β-lactoglobulin were separated using optimized gradient conditions to get similar retention time for the second peak. The exact conditions are summarized in Table 3. Detection, 280 nm; buffer A, 50 mM Na acetate pH = 4.5; buffer B, 1.5 M NaCl in A buffer.

Resolution–speed study

The effect of flow rate on resolution was also investigated while keeping the applied gradient volume constant with steeper gradient profile. The flow rates were chosen to be the original, standard flow (see Table 3) multiplied by 0.33, 0.5, 0.67, 1 and 1.2, termed "speed factor". The applied flow rates and gradient duration times for each of the columns are summarized in Table 4. The maximum flow rate we could use was 1.5 ml/min, determined by the pressure limit of Mono S and S HyperD columns. Resolution was determined using System Gold software from three consecutive injections. The result varied less than 1%. The measured resolution was plotted as a function of the speed factor (Fig. 8), which is the same for each individual column. S HyperD shows decreasing resolution with increasing speed as well as Mono S. Nevertheless, its resolution for this particular protein mixture remains higher than the Poros S/H and Poros HS/H columns' resolution at the maximum speed tested. The two Poros columns showed no resolution decrease versus flow rate as theory on perfusion predicts [7].

Separation of a mixture of 6 proteins

To study and compare the resolving power of the columns, the same mixture of 6 proteins was used for all columns with the normalized gradient conditions described above (same linear velocity and dead volume correction). The final salt concentration was chosen in such a way that the elution time of the last protein would be similar. Fig. 9 shows chromatographic separations obtained. S HyperD gave better resolution

Table 4
Gradient conditions used in speed–resolution study of S ion exchangers

Column	Speed factor									
	0.33		0.50		0.67		1.00		1.20	
	Flow	t_G	Flow	t_G	Flow	t_G	Flow	t_G	Flow	t_G
S HyperD	0.41	57.3	0.625	37.6	0.84	27.9	1.25	18.8	1.5	15.7
Mono S	0.41	56.1	0.625	36.8	0.84	27.4	1.25	18.4	1.5	15.3
Poros S/H	0.35	50.3	0.53	33.2	0.71	24.8	1.06	16.6	1.27	13.9
Poros HS	0.35	45.4	0.53	30	0.71	22.4	1.06	15	1.27	12.5

Flow measured in ml/min; t_G in min.

for this sample compared to the other packing materials; with the Poros columns 4 or 5 peaks were separated against 6 for S HyperD. It was also interesting that the elution order of Cyt C and Lys was inverse with Mono S compared to S HyperD; lysozyme possesses a higher isoelectric point than cytochrome *c* (about 11 versus 9.5) and should normally be more retained by strong cation exchangers.

Once again, the influence of the sorbent polymer modified the selectivity to a certain extent which increased the difficulties of interpretation.

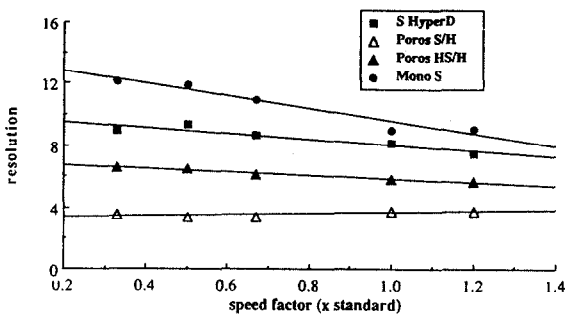

Fig. 8. Effect of the separation speed on the resolution in gradient elution. 150 μg of cytochrome *c* and β-lactoglobulin were separated using optimized gradient conditions to get similar retention time for the second peak. The speed factor is the number the original flow rate (Table 3.) was multiplied with. The gradient conditions used (flow rate, gradient duration) are summarized in Table 4. The final salt concentrations are summarized in Table 3. Buffer A, 50 m*M* Na acetate pH = 4.5; buffer B, 1.5 *M* NaCl in A buffer; detection, 280 nm.

So, regardless of the adjustments we made to establish comparable gradient conditions, results could easily be different for another sample mixture.

4. Conclusions

A performance study of a new ion-exchange packing material (commercially available under the trade name of HyperD) was performed. The results were compared to the most well-known existing packings such as Mono, Resource and Poros columns.

It was found that the HyperD ion exchangers have very high capacity and excellent resolution. The presence of the hydrophilic and permeable ionic gel in the HyperD particles yielded capacities which were improved compared to the conventional porous stationary phases [10,12]. Slightly decreasing resolution and capacity with increasing flow rates are limitations linked to mass transfer issues in the presence of high linear velocities with macromolecules when compared to convection based separations. However, capacity and resolution are high enough, that even when using the column at its maximum, pressure-limited flow rate, both resolution and capacity are the best among the columns studied. Even though the column does not have the flow rate independent performance and the speed capability of the perfusive columns (Poros), the high capacity of HyperD phases enables high

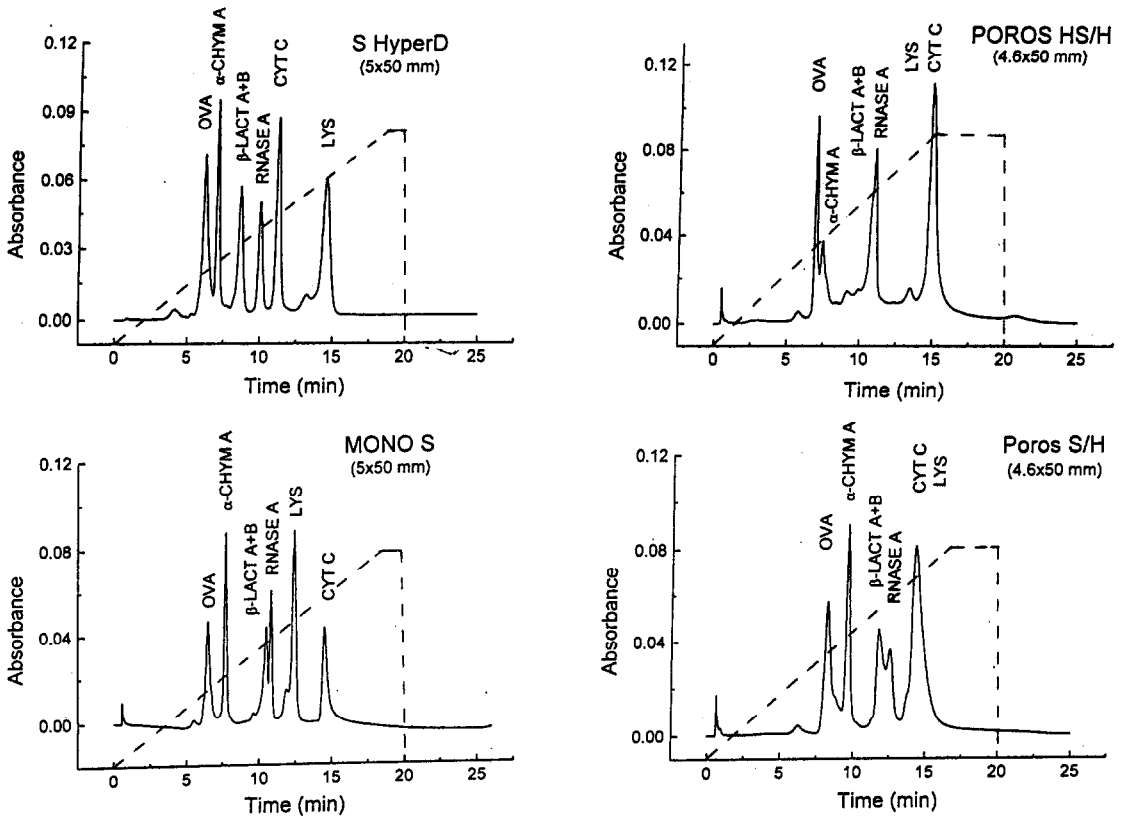

Fig. 9. Gradient separation of 6 standard proteins with S ion exchangers. Normalized gradient conditions were used. Starting buffer, 20 m*M* Na formate pH = 4.00; injection, 285 μg total; detection, 280 nm; linear velocity, 382.8 cm/h for all columns. The individual gradients were: S HyperD, 1.25 ml/min 18.8 min (0–0.75M LiCl); Mono S, 1.25 ml/min 18.4 min (0–1.35 *M* LiCl); Poros S/H, 1.06 ml/min 16.6 min (0–0.34 *M* LiCl); Poros HS/H, 1.06 ml/min 15 min (0–1.2 *M* LiCl).

sample load onto the column. High capacity combined with high resolution and efficiency makes HyperD media very attractive for most separation, purification and isolation problems.

It should also be mentioned that HyperD stationary phases designed primarily for preparative purposes could be appropriate for scaling up the separations. With a larger particle diameter and the same chemistry as the 10-μm support a particular separation could be transferred easily from analytical and semipreparative to preparative scale.

To summarize the situation of chromatographic packing materials existing today, it can be stated that for the most efficient, fast separation of small amount of samples, non-porous and micropellicular stationary phases are the most suitable [1–4]. When efficiency and resolution can be sacrificed to gain speed for the separation of relatively small sample amounts convective stationary phases can be recommended [9]. When resolution is more important than the speed of the separation, and the sample amount is relatively small, conventional diffusive stationary phases are suitable [9]. For separations where the sample amount varies from analytical to semi-preparative scale, and especially when future large scale separation is planned, hyperdiffusive stationary phases (HyperD) seem to be the best choice for the chromatographer because it preserves good efficiency and resolution power.

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