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Characterisation of ANX Sepharose® 4 Fast Flow media

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Abstract

ANX Sepharose 4 Fast Flow (low sub) and ANX Sepharose 4 Fast Flow (high sub) are two new media developed at Amersham Pharmacia Biotech. They are weak anion exchangers with different amounts of tertiary amine groups attached via a spacer arm to Sepharose 4 Fast Flow. They have been characterised by the separation of some model proteins under different conditions and by determination of breakthrough capacities for proteins of different molecular masses. Functional performance after storage in different solutions at ambient temperature has been monitored. Carbon release after storage at different pH and temperatures has been measured using the total organic carbon technique. The selectivity results show that these new media are interesting complements to already existing Fast Flow anion exchangers. They are very stable and can be especially useful in applications involving the purification of high-molecular-mass proteins. © 1998 Elsevier Science B.V.

Keywords: Stationary phases, LC; Proteins

1. Introduction

Ion exchange is one of the most commonly used techniques in chromatographic purification of biomolecules and most industrial protein purification processes contain one or more ion-exchange steps.

The literature in the area is too vast to cover in this context but we would like to refer to reviews like [1] for a general overview. In a recently published Handbook of Process Chromatography [2] theoretical as well as practical aspects of ion exchange are discussed with a large number of references cited.

Important features of industrial ion exchangers are selectivity, dynamic binding capacity, flow properties and stability towards different chemicals used in the actual application as well as in necessary cleaning procedures [3]. Structural parameters that determine these properties are ligand structure, ligand density, coupling chemistry and matrix properties.

There are a large number of ion exchangers commercially available, based on wide variety of base matrices. For anion exchangers the most common ligands are Q (strong) and DEAE (weak) and there are products available for both capture and polishing steps [4].

In this paper we present two new weak anion-exchangers —ANX Sepharose 4 Fast Flow (low sub) and ANX Sepharose 4 Fast Flow (high sub)— developed in cooperation with leading large-scale drug manufacturers especially for use in industrial processes. They are based on Sepharose 4 Fast Flow and have the functional group, the diethylamino group, attached to the base matrix via a hydrophilic spacer arm. The coupling of the ligand is performed in a way that precludes formation of quaternary groups, resulting in a truly weak ion exchanger, cf. titration curves in Fig. 1. Some general characteristics of the products are presented in Table 1.

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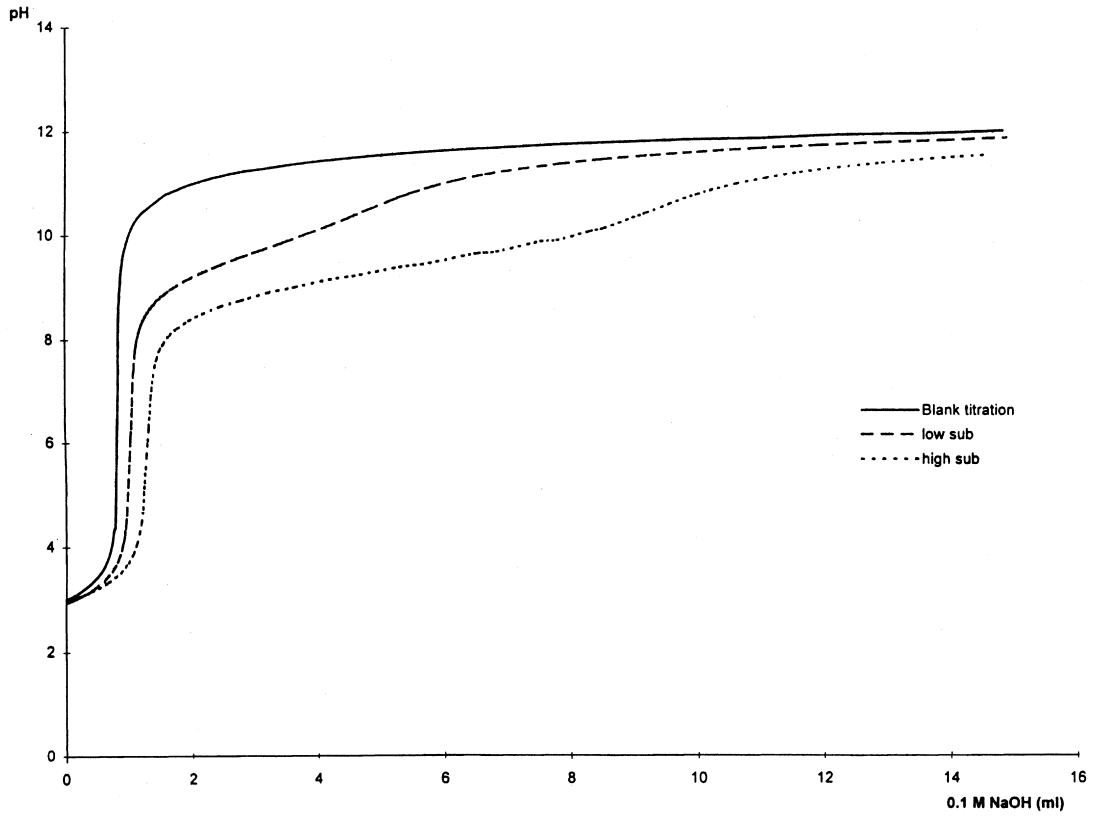

Fig. 1. ANX Sepharose 4 Fast Flow (low sub) and (high sub) in 1 *M* KCl titrated from pH 3 to 12 with 0.1 *M* NaOH.

The new media have been characterised chromatographically in a series of experiments where model proteins are separated under different conditions. Dynamic binding capacities for proteins of different molecular mass [bovine serum albumin (BSA)] and porcine thyroglobulin, have also been determined. The already established Fast Flow anion exchangers Q and DEAE Sepharose Fast Flow are used as references in these experiments.

The pressure/flow properties of chromatographic media are important for their behaviour when packed into various columns, especially with large columns where no substantial influence from the column walls can help stabilize the bed. Some pressure/flow

Table 1
Characteristics of ANX Sepharose 4 Fast Flow media

	Low sub	High sub
Chloride ion capacity	0.05–0.08 mmol/ml gel	0.13–0.17 mmol/ml gel
Bead structure	Highly cross-linked agarose, 4%, spherical	
Particle size	45–165 μm	
Flow at 0.1 MPa 25 cm b.h., XK 50/60	>200 cm/h	
pH stability:		
Long term	3–13	
Short term	2–14	
Temperature stability	4–40°C	

curves in different solutions were determined for ANX Sepharose 4 Fast Flow (high sub) using a BPG 100/500 column.

The chemical stability for ANX Sepharose 4 Fast Flow gels was investigated in two separate studies. In the first study samples of the gels were incubated in different storage solutions relevant for the use of the chromatography media and then tested for chloride ion capacity, chromatographic function and flow properties after different times of storage at ambient conditions.

A second study was performed in order to investigate the carbon release from the chromatography media under extreme conditions (pH 1–14, at 20 and 40°C, 1–2 weeks). The leakage levels were determined with total organic carbon (TOC) analysis of the supernatant from the storage samples.

2. Experimental

2.1. Equipment

The chromatographic studies were performed using either the fast protein liquid chromatography (FPLC®) System or ÄKTA explorer system both from Amersham Pharmacia Biotech (Uppsala, Sweden). Detectors used were either UV-1 or UV-M 280 nm (Amersham Pharmacia Biotech).

Columns used in the different experiments were HiTrap (1 ml), HR 16/10, XK 16/20, XK 50/60 and BPG 100/500, all from Amersham Pharmacia Biotech. The pH-titration of the media was performed with a Titralab 90 titration system (Radiometer Analytical, Lyon, France) and a pH electrode (Ingold, Mettler-Toledo, Greifensee, Switzerland).

Potentiometric titration of chloride ions was performed using a Mettler Memotitrator with a silver electrode (Mettler-Toledo, Greifensee, Switzerland).

For the pressure/flow test a pressure vessel (Fiberglass reservoir 11.8 l, Amicon, Stonehouse, UK) was used.

For the measurement of the carbon leakage a TOC analyzer (TOC-5000, Shimadzu, Kyoto, Japan) with an autosampler (ASI-5000, Shimadzu, Kyoto, Japan) was used. A CO_2-purifier (Peak Scientific, Renfrew, UK) was used to eliminate carbon dioxide from the air used in the TOC analyzer.

2.2. Chemicals

Model proteins used in the different tests were hemoglobin (prepared according to the procedure described in [5]), human serum albumin, bovine serum albumin, porcine thyroglobulin, β-lactoglobulin, α-lactalbumin, RNAse A (all from Sigma-Aldrich Sweden, Stockholm, Sweden), ovalbumin (Worthington, Lakewood, NJ, USA), and transferrin (Pharmacia and Upjohn, Stockholm, Sweden).

Chromatographic supports ANX Sepharose 4 Fast Flow (low sub), ANX Sepharose 4 Fast Flow (high sub), DEAE Sepharose Fast Flow and Q Sepharose Fast Flow were all obtained from Amersham Pharmacia Biotech.

All buffer chemicals used were analytical reagent grade.

2.3. Titration of ANX Sepharose 4 Fast Flow (low sub) and ANX Sepharose 4 Fast Flow (high sub)

Approx. 10 ml of the chromatographic support was washed with distilled water on a glass filter funnel. 5 ml of the washed gel was transferred to a titration vessel with approx. 70 ml 1 *M* KCl solution. The pH was adjusted to pH 3 with HCl. The sample was titrated with 0.1 *M* NaOH to pH 12. Finally a blank (containing only the potassium chloride solution) was titrated over the same interval (pH 3–12).

2.4. Selectivity studies

2.4.1. Separation of model proteins at pH 8.3

ANX Sepharose 4 Fast Flow (low sub) or ANX Sepharose 4 Fast Flow (high sub) was packed in an XK 16/20 column using 0.05 *M* Tris buffer, pH 8.3 (buffer A) at a flow-rate of 20 ml/h and the gel bed height was adjusted to approx. 10 cm. The column was attached to FPLC System with UV and conductivity monitors connected at the outlet. After equilibration with buffer A (20 ml/h) sample was applied, i.e. 1.0 ml of hemoglobin concentrate diluted 1:15 in buffer A and 1.0 ml of a 5% solution of HSA in buffer A, followed by a few ml of buffer A. The sample was desorbed using a linear salt gradient

0–41% of gradient buffer (0.05 *M* Tris containing 1 *M* sodium chloride, pH 8.3) in total 240 ml. From the recorded conductivity the salt concentration at the eluted protein peaks (two peaks from hemoglobin and one from HSA) was calculated using a calibration curve.

2.4.2. Separations of model proteins at pH 8.0 and 9.5

ANX Sepharose 4 Fast Flow (low sub), ANX Sepharose 4 Fast Flow (high sub), Q Sepharose Fast Flow and DEAE Sepharose Fast Flow were packed in small HiTrap (1 ml) columns, which were attached to ÄKTA explorer system. UV and conductivity monitors were connected at the outlet. The columns were equilibrated using a buffer (buffer A) consisting of 0.05 *M* 1-methylpiperazine, 0.05 *M* bis-Tris and 0.025 *M* Tris, pH 8.0 and 9.5, respectively, at a flow-rate of 2 ml/min. The sample was applied, i.e. 200 μl of a solution of apo-transferrin (10 mg/ml), RNase A (10 mg/ml) and α-lactalbumin (10 mg/ml) in buffer A. The sample application was followed by washing with 10 ml of buffer A. Desorption of the sample was performed using a linear salt gradient 0–0.5 *M* NaCl (20 ml) in the same buffer (see Fig. 2). From the recorded conductivity the salt concentration at the eluted protein peaks was obtained.

2.4.3. Separation of model proteins at pH 6.0

ANX Sepharose 4 Fast Flow (low sub), ANX Sepharose 4 Fast Flow (high sub) and Q Sepharose Fast Flow were packed in HR 16/10 columns at a flow-rate of 10 ml/min for approx. 5 min and the gel bed height was adjusted to approx. 10 cm. Each column was attached to FPLC System with UV and conductivity monitors connected at the outlet. The column was equilibrated using 0.02 *M* piperazine buffer, pH 6.0 (buffer A) at a flow-rate of 5 ml/min. The sample was applied, i.e. 2.0 ml of a solution of transferrin (10 mg/ml), ovalbumin (20 mg/ml) and β-lactoglobulin (20 mg/ml) in buffer A. The sample application was followed by a few ml of buffer A. Desorption of the sample was performed using a

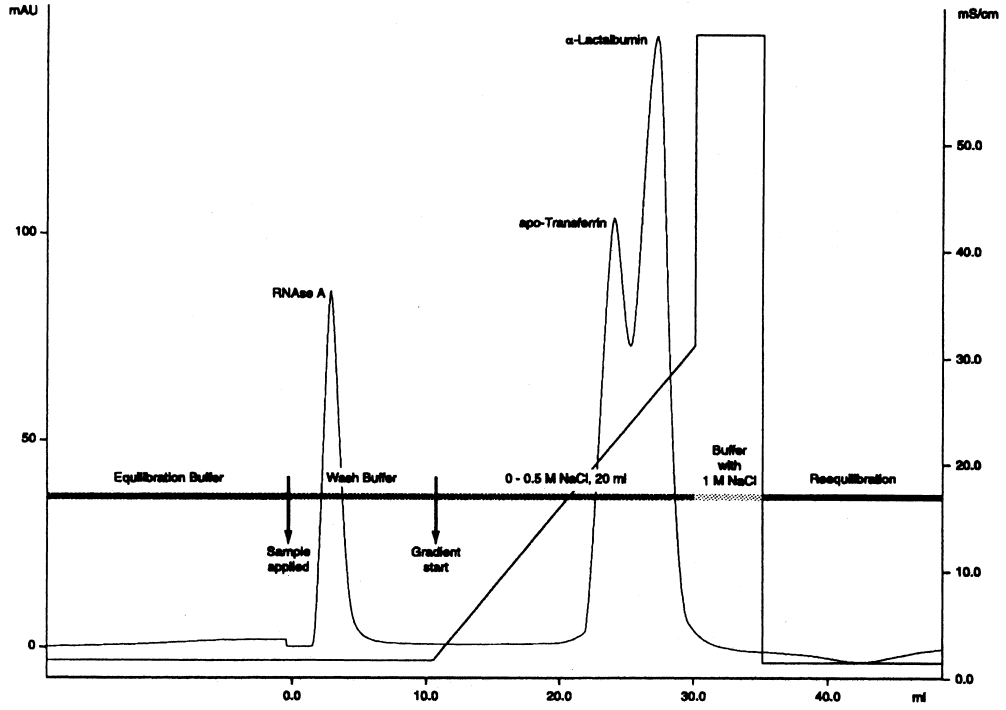

Fig. 2. Chromatographic scheme for studying the selectivity pattern of the four Fast Flow anion exchangers (ANX Sepharose 4 Fast Flow (low sub) and (high sub), Q and DEAE Sepharose Fast Flow).

linear salt gradient 0–100% of gradient buffer (0.02 *M* piperazine containing 0.3 *M* sodium chloride, pH 6.0) in total 200 ml. The elution volumes for the three main protein peaks (transferrin, ovalbumin and β-lactoglobulin) were obtained.

2.5. Determination of dynamic binding capacity of proteins

ANX Sepharose 4 Fast Flow (low sub), ANX Sepharose 4 Fast Flow (high sub) and DEAE Sepharose Fast Flow were packed in XK 16/20 columns and the bed height was adjusted to approx. 13 cm. The columns were attached to FPLC System with UV monitor connected at the outlet. The column were equilibrated using a 0.05 *M* Tris, pH 7.5 buffer (buffer A) at a linear flow of 300 cm/h. Sample solutions (BSA 2 mg/ml in buffer A and porcine thyroglobulin 0.1 mg/ml in buffer A, respectively) were applied at 300 cm/h until 10% breakthrough, i.e. when $C/C_o=0.1$. After washing with buffer A (300 cm/h) the proteins were eluted at 100 cm/h using an elution buffer consisting of buffer A containing 1 *M* NaCl. The breakthrough capacity was calculated in mg/ml drained gel for the different chromatography supports.

2.6. Study of pressure/flow properties

2.6.1. General test for flow properties

Approx. 550 ml gel was suspended in distilled water in an XK 50/60 column and the gel was allowed to settle by gravity flow. The column tube was filled with distilled water, the adaptor was mounted and connected to a manometer and pressure vessel filled with distilled water. The column was packed for 6 min at 0.1 MPa with a flow from the pressure vessel and then the bed height was adjusted to 25 cm. The pressure vessel was filled with distilled water and the pressure level adjusted to 0.100 MPa until a stable signal was obtained (at least 5 min). The flow was then measured by determining the time in seconds it took to fill a 500 ml volumetric flask. The temperature of the water was measured and a correction was made for viscosity (compared to 20°C as standard). The linear flow in cm/h can then be calculated.

2.6.2. Pressure/flow curves in a large-scale column

ANX Sepharose 4 Fast Flow (high sub) was suspended in distilled water and an amount of slurry corresponding to 2 l sedimented gel was poured into a BPG 100/500 column (gives approx. 20 cm bed height) connected to a pump and a pressure gauge. The pressure over the gel bed was measured at different linear flow and the data were collected into a pressure/flow curve.

Using the same procedure pressure/flow curves were registered in 1 *M* acetic acid, 1 *M* sodium hydroxide and 1 *M* sodium chloride.

2.7. Determination of chloride ion capacity

An accurately determined volume (approx. 20 ml) of ANX Sepharose 4 Fast Flow (high sub) or (low sub) was transferred to a glass filter and washed with distilled water. The gel was then saturated with chloride ions by letting 3 portions of 20 ml of 0.5 *M* sodium choride solution slowly drop through the filter (without any suction). Then the excess of chloride ions was washed away using 1 m*M* sodium chloride solution (4×20 ml) and the gel was sucked dry. It was transferred to a titration beaker and 0.1 *M* sodium nitrate solution was added to 40 ml volume. Titration with 0.1 *M* silver nitrate using the Memotitrator gave the total chloride ion capacity of the gel.

2.8. Stability studies

2.8.1. Storage of ANX Sepharose 4 Fast Flow (low sub) and ANX Sepharose 4 Fast Flow (high sub) in different solutions under ambient conditions

About 700 ml of ANX Sepharose 4 Fast Flow (low sub) and ANX Sepharose 4 Fast Flow (high sub) was rinsed with 10×1 l of distilled water on a glass filter funnel. The gel was sucked dry and portions of approx. 110 ml were transferred to plastic beakers. Storage chemicals and distilled water were added to get the right concentrations. All the resulting different slurries were transferred to plastic bottles in portions corresponding to 35 ml dry sucked gel.

The bottles were stored on the laboratory shelf, i.e. normal daylight and room temperature (18–25°C), on average 22°C.

Storage stability was tested in six different media: 20% ethanol, 70% ethanol, 1 *M* sodium hydroxide, 1 *M* acetic acid, 8 *M* urea and 8 *M* guanidine hydrochloride.

Samples were removed after different time intervals, washed thoroughly and tested for chloride ion capacity according to the procedure in Section 2.7 and for chromatographic function according to the test procedure in Section 2.4.1.

In addition, some larger (approx. 600 ml gel) samples of ANX Sepharose 4 Fast Flow (high sub) were prepared similarly and stored in 1 *M* sodium hydroxide and 1 *M* acetic acid, respectively. Flow properties of samples removed after 91 and 406 days were tested as described in Section 2.6.1.

2.8.2. Determination of carbon release from ANX Sepharose 4 Fast Flow (low sub) and ANX Sepharose 4 Fast Flow (high sub) at different pH and temperatures

Approx. 500 ml ANX Sepharose 4 Fast Flow (low sub) and ANX Sepharose 4 Fast Flow (high sub) was washed thoroughly on a glass filter with 10×200 ml of 0.5 *M* NaCl, followed by 10×200 ml high-purity low-carbon Milli-Q water (MQ water, obtained from Milli-Q Plus 185 water purifier, Millipore). The gel was sucked dry and transferred to a plastic beaker with carbon-free water where it was kept overnight. Then it was washed again with 10×200 ml of Milli-Q water and sucked dry.

Small portions of 10.0 g were weighed into 50 ml glass filters. Each sample was equilibrated with the storage solution of different pH (2×25 ml). The sample was sucked dry and the gel together with 50 ml storage solution were transferred to flasks, which were stored at 20 and 40°C for 1 or 2 weeks.

Approx. 15 ml of the supernatant was collected from the stored flasks.

The samples were acidified to approx. pH 2 with HCl and purged with nitrogen gas to eliminate any adsorbed carbonates.

They were then injected in the TOC-analysator and the non-purgeable organic carbon was detected. The carbon content was calculated from the calibrations curves that were prepared in advance.

3. Results and discussion

3.1. Selectivity studies

3.1.1. Separation of hemoglobin and human serum albumin at pH 8.3

This separation is a standard function test for DEAE media from Pharmacia Biotech and Fig. 3 shows the elution positions for the ANX Sepharose 4 Fast Flow products. As reference in this case is shown the specification intervals for two important standard products DEAE Sepharose Fast Flow and DEAE Sephadex A-50 using this test. The selectivity of the ANX adsorbents is clearly different from that of DEAE Sepharose Fast Flow, giving a better separation of the proteins. Interestingly, ANX Sepharose 4 Fast Flow (low sub) shows a similar selectivity to DEAE Sephadex A-50. It has also been found to perform analogously in certain customer applications.

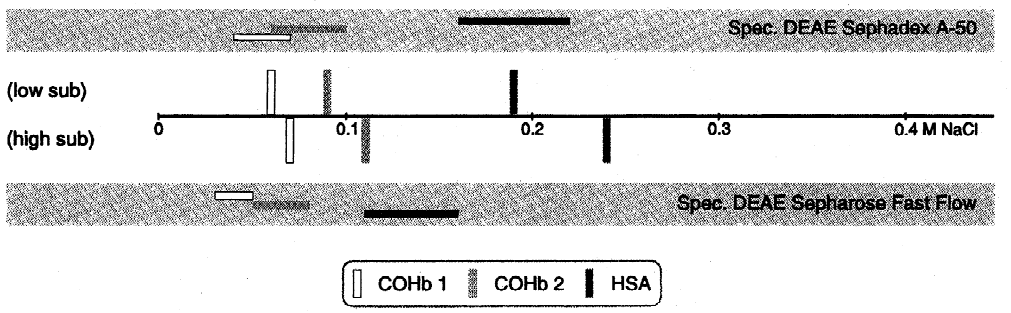

Fig. 3. Typical elution positions for the proteins hemoglobin (two peaks) and HSA on ANX Sepharose 4 Fast Flow (low sub) and (high sub) when run according to the Pharmacia test method for functional behaviour of DEAE anion exchangers.

3.1.2. Separation of apo-transferrin, RNAseA and α-lactalbumin at pH 8.0 and 9.5

When running the test at pH 8.0 all the anion exchangers give rather similar separation of the proteins (see Fig. 4), the largest difference in selectivity being between the two ANX Sepharose 4 Fast Flow media. At pH 9.5 the pattern changes in as much that RNAseA begins to adsorb to the strong anion exchanger Q Sepharose Fast Flow, while it does not bind to the three weak anion exchangers.

3.1.3. Separation of transferrin, ovalbumin and β-lactoglobulin at pH 6.0

This test is a standard function test for Q media from Pharmacia Biotech and Fig. 5 shows the elution pattern for the ANX Sepharose 4 Fast Flow products together with the standard Q Sepharose Fast Flow. In this case ANX Sepharose 4 Fast Flow (low sub) gives a separation very similar to that of the Q gel, while the (high sub) variant shows an even better selectivity.

3.2. Dynamic binding capacity

From the results in Table 2 it can clearly be seen how the base matrix influences the ability to bind very large proteins. The ANX Sepharose 4 Fast Flow media have a bead structure with larger pores than DEAE Sepharose Fast Flow, and this is probably the reason for the substantial increase in capacity for the very large thyroglobulin (M_r=650 000) obtained using the Sepharose 4 Fast Flow based ANX gels.

3.3. Pressure/flow curves for ANX Sepharose 4 Fast Flow (high sub) in BPG 100/500 column

In Fig. 6 is shown that ANX Sepharose 4 Fast Flow (high sub) can be packed in a large-scale column giving good flow-rates even with a rather high bed height. However, it is important to note that the higher viscosity of the sodium hydroxide or acetic acid solution has an influence on the flow properties. This fact has to be taken into account, for

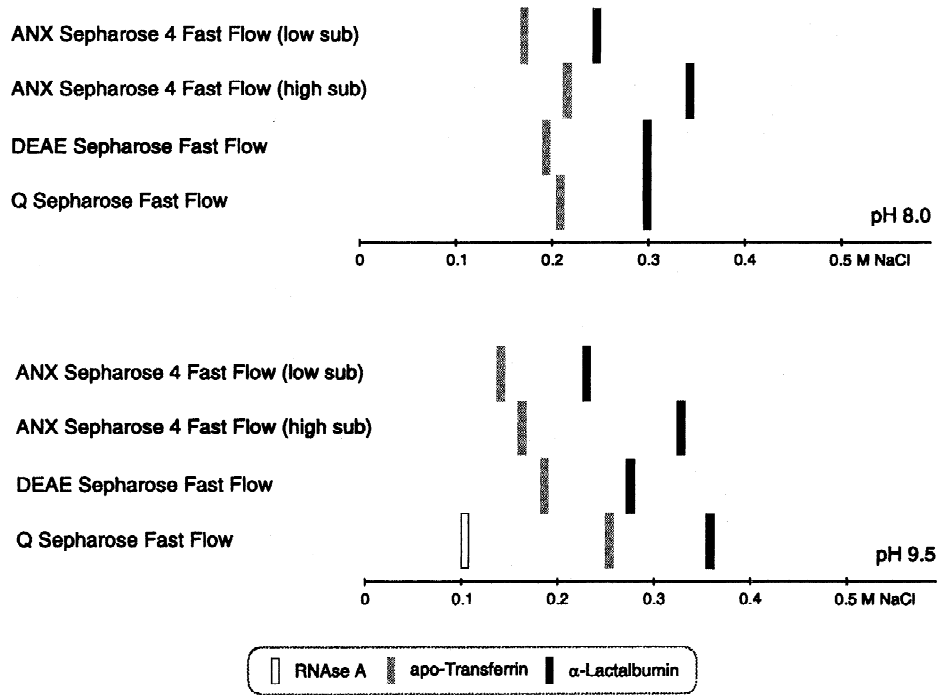

Fig. 4. Elution positions for RNAse A, apo-transferrin and α-lactalbumin on the four Fast Flow anion exchangers (ANX Sepharose 4 Fast Flow (low sub) and (high sub), Q and DEAE Sepharose Fast Flow) when run according to the scheme in Fig. 2 at two different pH (8.0 and 9.5).

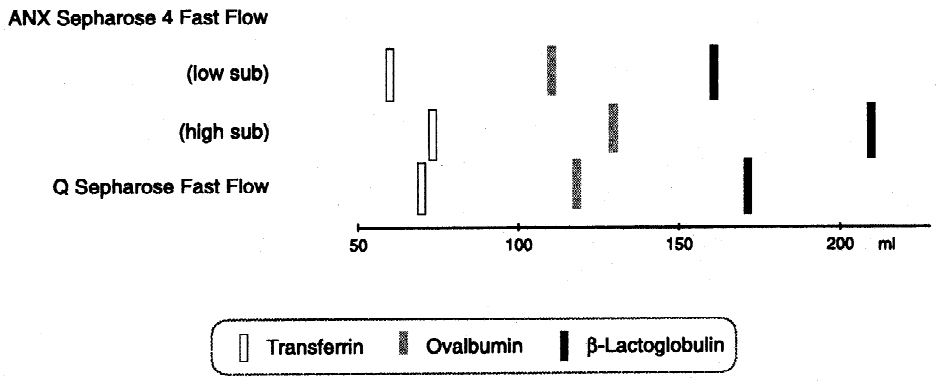

Fig. 5. Typical elution volumes for the proteins Transferrin, Ovalbumin and β-lactoglobulin on ANX Sepharose 4 Fast Flow (low sub) and (high sub) and Q Sepharose Fast Flow when run according to the Pharmacia test method for functional behaviour of Q anion exchangers.

example when creating cleaning or sanitisation procedures.

3.4. Stability studies

3.4.1. Storage stability tests

The results from this investigation are presented in Tables 3–6. They show that there is no significant change of chloride ion capacity after storage of either ANX Sepharose 4 Fast Flow adsorbent for more than 1 year in the different solutions.

Further, there is no reduction of the flow properties of ANX Sepharose 4 Fast Flow (high sub) when stored in 1 *M* sodium hydroxide or 1 *M* acetic acid for 406 days.

Looking at the chromatographic function there is no change in elution positions for the three protein peaks in the test after more than 70 days of storage in any of the solutions.

For the samples stored at approx. 1 year or more there is a slight shift towards higher elution concentrations for the proteins in all samples. This shift (it is hardly significant) could be a result of a slow degradation, however, it could also be an influence of the day-to-day variation of the test method.

Anyhow, the ANX media are very stable even towards rough conditions, which makes it easy to find suitable cleaning procedures. Important in this respect is the stability towards sodium hydroxide, as alkaline treatment decharges the weak ion-exchangers and thus is especially suitable for cleaning in place (CIP) of the ANX Sepharose adsorbents.

3.4.2. TOC investigation

The TOC technique is a very suitable test method

Table 2
Breakthrough capacity (10%) of BSA and thyroglobulin for ANX Sepharose 4 Fast Flow (high sub), ANX Sepharose 4 Fast Flow (low sub) and DEAE Sepharose Fast Flow at 300 cm/h

Anion exchange medium	Q_B (10%)	
	BSA (mg/ml drained gel)	Thyroglobulin (mg/ml drained gel)
ANX Sepharose 4 Fast Flow (low sub)	42	6
ANX Sepharose 4 Fast Flow (high sub)	43	5
DEAE Sepharose Fast Flow	47	1

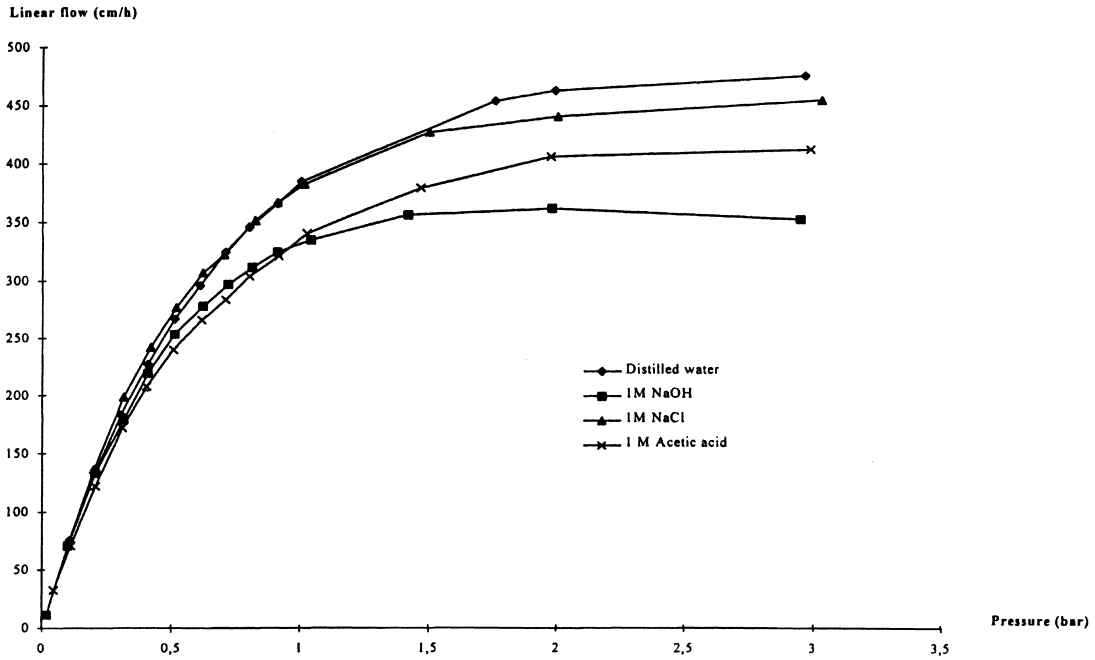

Fig. 6. Pressure/flow curves for ANX Sepharose 4 Fast Flow (high sub) in BPG 100/500 column (20 cm bed height) using different eluents.

for leakage of the ANX Sepharose media, since the main component of the products is carbon. However, it has the limitation that it is impossible to study leakage in any carbon-containing media, (e.g. ethanol, urea or guanidine–HCl).

The carbon leakage from ANX Sepharose 4 Fast Flow media is low all over the tested pH-range; see Fig. 7 Fig. 8. It is expected that ANX Sepharose 4 Fast Flow media should be more stable at high pH compared to e.g. DEAE Sepharose Fast Flow [6].

The reason is that the ligand in the ANX gels is coupled in a way that creates no tandem groups that could be eliminated by Hoffman elimination.

The highest carbon leakage after 1 week in 20°C was obtained in the pH 14 samples, where approx. 5 ppm carbon in the supernatant were detected. This leakage level indicates a loss of approximately 0.1% of the carbon content of the gel.

Table 3
Chloride ion capacity (mmol/ml drained gel) of ANX Sepharose 4 Fast Flow (high sub) and ANX Sepharose 4 Fast Flow (low sub) after storage under ambient conditions in different solutions

	ANX Sepharose					
	4 Fast Flow (low sub)			4 Fast Flow (high sub)		
Days	0	83	385	0	84	370
Storage solution						
20% Ethanol	0.07	0.07	0.07	0.16	0.16	0.16
70% Ethanol	0.07	0.07	0.07	0.16	0.16	0.16
1 *M* NaOH	0.07	0.07	0.07	0.16	0.16	0.16
1 *M* Acetic acid	0.07	0.07	0.07	0.16	0.16	0.16
8 *M* Urea	0.07	0.07	0.07	0.16	0.16	0.16
8 *M* Guanidine chloride	0.07	0.07	0.07	0.16	0.16	0.15

Table 4
Chromatographic function test results for ANX Sepharose 4 Fast Flow (low sub) after storage in different solutions for 71 and 517 days, respectively

Storage solution	ANX Sepharose 4 Fast Flow (low sub)								
Days	0			71			517		
Elution peak nr I–III (*M* NaCl)	I	II	III	I	II	III	I	II	III
20% Ethanol	0.05	0.08	0.19	0.05	0.08	0.19	0.06	0.09	0.20
70% Ethanol	0.05	0.08	0.19	0.05	0.08	0.19	0.06	0.09	0.20
1 *M* NaOH	0.05	0.08	0.19	0.05	0.08	0.19	0.06	0.09	0.20
1 *M* Acetic acid	0.05	0.08	0.19	0.05	0.08	0.19	0.06	0.09	0.20
8 *M* Urea	0.05	0.08	0.19	0.05	0.08	0.19	0.06	0.09	0.20
8 *M* Guanidine chloride	0.05	0.08	0.19	0.05	0.08	0.19	0.06	0.09	0.20

Table 5
Chromatographic function test results for ANX Sepharose 4 Fast Flow (high sub) after storage in different solutions for 84 and 342 days, respectively

Storage solution	ANX Sepharose 4 Fast Flow (high sub)								
Days	0			84			342		
Elution peak nr I–III (*M* NaCl)	I	II	III	I	II	III	I	II	III
20% Ethanol	0.06	0.10	0.23	0.06	0.10	0.23	0.07	0.11	0.24
70% Ethanol	0.06	0.10	0.23	0.06	0.10	0.23	0.07	0.11	0.24
1 *M* NaOH	0.06	0.10	0.23	0.06	0.10	0.22	0.07	0.10	0.24
1 *M* Acetic acid	0.06	0.10	0.23	0.06	0.10	0.22	0.09	0.12	0.24
8 *M* Urea	0.06	0.10	0.23	0.06	0.09	0.23	0.06	0.09	0.20
8 *M* Guanidine chloride	0.06	0.10	0.23	0.06	0.10	0.23	0.08	0.11	0.25

This very limited decomposition fits well with the fact that no significant decrease of the chloride ion-capacity is obtained during long-term storage of the gels (cf. Table 3).

The low leakage from the static experiments indicates that the leakage levels at chromatographic conditions, (Cleaning In Place, CIP) would be even lower. An estimation based on earlier experience is that the leakage with a conventional CIP-procedure, (e.g. 1 *M* NaOH for 1 h) could be expected to be approximately 100 times lower.

At low pH and temperature the leakage is also

Table 6
Flow-rate of ANX Sepharose 4 Fast Flow (high sub) after storage for 406 days in 1 *M* sodium hydroxide and 1 *M* acetic acid under ambient conditions

Days of storage	Storage	
	1 *M* NaOH flow (cm/h)	1 *M* Acetic acid flow (cm/h)
0	343	343
91	330	340
406	346	338

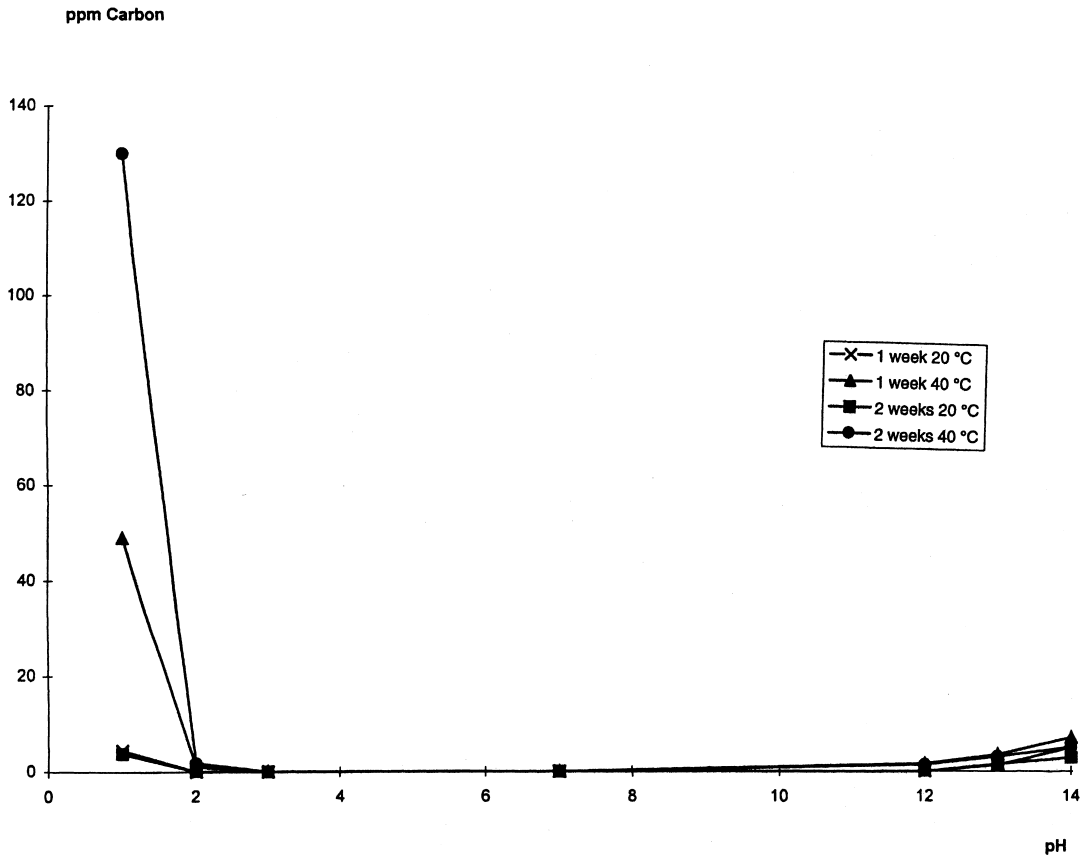

Fig. 7. The influence of pH, temperature and time on the release of carbon from ANX Sepharose 4 Fast Flow (low sub).

extremely low, in the same range for pH 1 as for pH 14. However, at pH 1 it can be seen that when increasing the temperature from 20 to 40°C the carbon leakage increases substantially especially for ANX Sepharose 4 Fast Flow (low sub). The increased decomposition of the media at higher temperature could be explained by a higher degree of hydrolysis of the matrix. The difference between the high and low substituted products in this respect probably is a result of that some additional cross-linking takes place in the derivatisation steps (thus being more efficient for the high substituted derivative).

This is supported by the fact that at pH 14, where the Sepharose matrices are generally highly stable, the leakage level was only slightly increased at the higher temperature.

4. Conclusion

The results presented above show that the new anion exchangers ANX Sepharose 4 Fast Flow (low sub) and (high sub) constitute valuable complements to the already established Fast Flow ion-exchangers. Thus, they show a different selectivity—often giving a better separation—and they have a comparatively high dynamic capacity for large size proteins like, for example, thyroglobulin. They can be packed in industrial columns giving reasonable flow-rates and

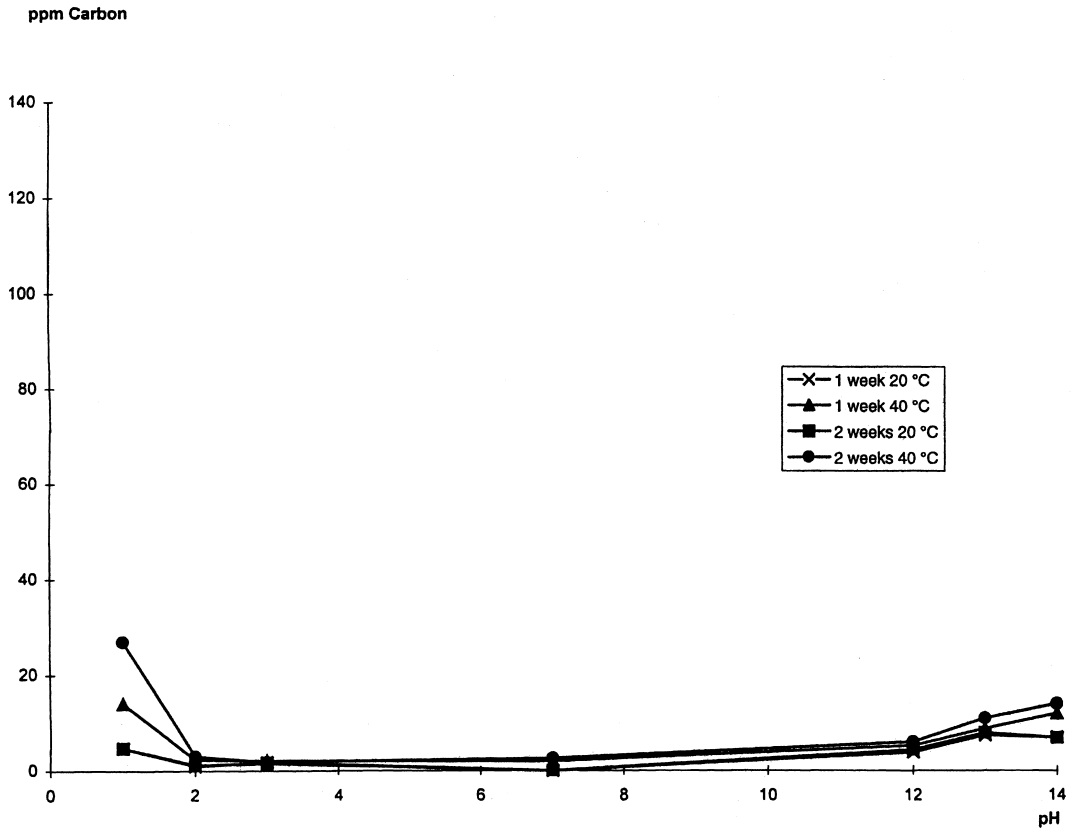

Fig. 8. The influence of pH, temperature and time on the release of carbon from ANX Sepharose 4 Fast Flow (high sub).

they are extremely stable within the whole pH-range, which gives many possibilities for the choice of CIP and sanitisation protocols.

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