Ion Exchange Chromatography Using Fractogel® EMD Tentacle Supports

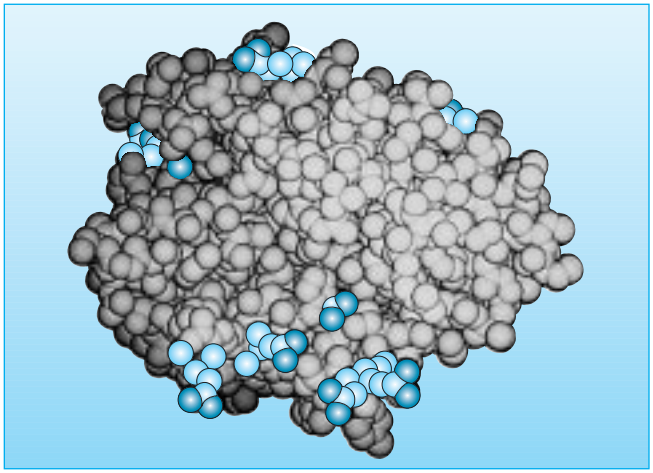

Fig.1 X-ray structure of a protein. All charged amino acids involved in binding to the ion exchanger are indicated by blue colour.

Ion Exchange Chromatography

Ion exchange chromatography is commonly used for nearly all purifications of proteins. This widely distributed method is very easy to perform in analytical applications as well as in production scale processes.

The technique is based on the fact that charged substances can be separated on column materials carrying an opposite charge. The ionic groups of exchanger columns are covalently bound to the gel matrix. The retention principle is based on competition for the ionized site between the buffer ions (counter ions) and the charged area on the protein's surface. Since proteins are multivalant anions or cations, anion exchangers can be used as well as cation exchangers. With a strongly acidic pH value all amino acids are present as cations as a result of suppression of the dissociation of the COOH group and protonation of the NH_2 group. At pH values above 12, proteins are present as anions because the amino groups are uncharged and the COOH group is dissociated. Due to the net charge of the proteins it is easy to bind them to a correspondingly charged stationary phase at low salt concentrations. The strenght of binding depends on their total charge. The bound biomolecules can be displaced according to their charge using an increasing salt gradient. The substances that have a higher charge density are stronger bound to the column while the others elute more rapidly. Alternatively the desorption of proteins from weak ion exchange columns can be performed with pH changes.

High salt concentrations, however, cause shielding of the charged groups on the protein surface and effective binding to an ion exchanger can no longer take place. Therefore buffers with a conductivity of no more than 5 mS should be used for loading the protein.

During practical application of ion exchange chromatography it is important to operate with pH values in which the exchangers are mostly ionized and the biopolymers contain positive or negative charges, e.g. they are not near their pl value (isoelectric point).

The size of the sample volume in ion exchange chromatography is not restricted. On the other hand, ion exchange chromatography can be used as a concentrating step when highly diluted samples were injected. Under such conditions, the sample components are collected at the top of the column and can be eluted in a small volume of elution buffer. Sometimes it is helpful to elute the protein at high concentrations by inversing the flow direction.

If the proteins do not bind to the column under the selected conditions, the pH value and/or the concentration of the starting buffer should be changed. Additional changes in selectivity can be found by different buffer salts since each buffer solvates the ion exchanger and the sample components uniquely. The separation can be improved by changing the gradient slope.

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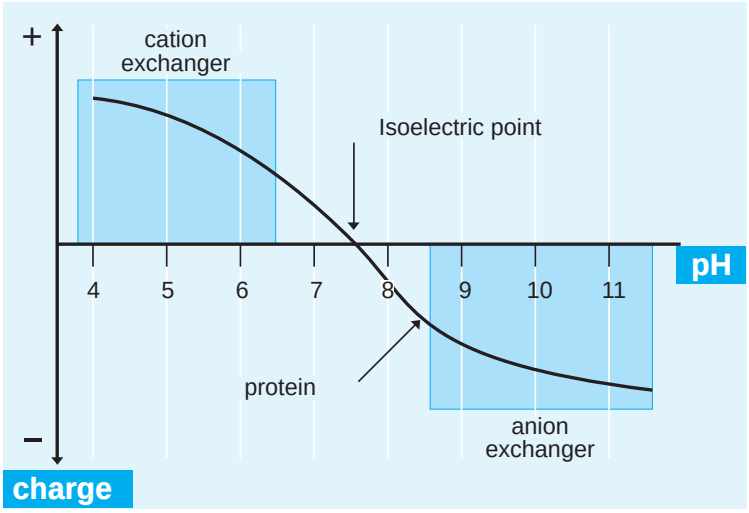

Fig. 2. Working range of anion and cation exchangers. The curve shows the titration behaviour of a protein. The isoelectric point of the protein is 7.5. Below a value of 6.5 the protein is positively charged and can be purified by a cation exchanger. Above pH 8.5 the same protein has a negativ net charge and can be bound to an anion exchanger.

Commonly Used Functional Groups

Two ion exchanger types exist: basic (positively charged) and acidic (negatively charged). They can be divided into those with weak basic or acidic character or strong basic or acidic character. With strong basic or acidic materials all functional groups are always present in ionized form independent from the pH value in the specified operating range.

For example, the quaternary ammonium groups (R_3N^+) are positively charged, while the sulfonic acid groups (SO_3^-) are negatively loaded. The pK values of the quaternary ammonium groups are around 13, those of the sulfonate residues below 1. In addition, weak basic types (pK values between 8 and 11) and weak acidic types (between 4 and 6) exist.

The weak basic types consist of secondary and tertiary amino functional groups; the weak acidic types have carboxyl functional groups. Thus weak basic exchanger should only be used at pH values under 8.5, weak acidic exchangers only at pH values above 6. Outside these ranges strong basic, or strong acidic exchangers should be used or one has to consider that the weak ion exchangers display a lower capacity. Many proteins can be separated as polyanions (pH > pI) or as polycations (pH < pI), as long as the pH stability of the protein of interest allows this selection. The most common groups are summarized in Table 1 below with their abbreviations and pK values:

Tab. 1

functional group	pK-value	characteristic	working range	description
TMAE-Group	pK > 13	strongly basic	pH 2-12	**tri**methyl**a**mmonium**e**thyl group
DEAE-Group	pK > 11	weakly basic	pH 2-9.5	**die**thyl**a**mino**e**thyl group
DMAE-Group	pK ~ 10	weakly basic	pH 2-8.5	**di**methyl**a**mino**e**thyl group
COO-Group	pK 4.5	weakly acidic	pH 6-12	**c**arb**o**xy group
SE-Group	pK < 1	strongly acidic	pH 2-12	**s**ulfo**e**thyl group
SO_3-Group	pK < 1	strongly acidic	pH 2-12	**s**ulfoisobutyl

Advantages of the Tentacle Ion Exchangers

The synthetic polymer Fractogel® EMD, which is pressure stable and can easily be regenerated with sodium hydroxide, is the basic matrix for the tentacle exchangers. This proven biochromatographic support material has the following characteristics:

- Biocompatibility (non-toxic)
- Mechanical stability (pressure stable)
- Particle size of 20-40µm for analytical applications
- Particle size of 40-90 µm for preparative columns
- High selectivity
- Chemical stability (acids, alkali)
- Large pore diameter (> 800 Å)
- Resistance to microorganisms
- Reduced non-specific interactions
- High capacity

With most ion exchange gels the ionic groups (exchangers) are fixed directly on the surface of the matrix. The ion exchange capacity of these types of packings depends on the surface of the gel matrix. On any given gel matrix, only a certain number of ionic groups can be attached because of the chemistries used and the porosity of the matrix. Once attached, these ionic groups have to be accessible to the biopolymers being separated. Any ionic group which cannot be approached by the biopolymer because of steric hinderence are essentially of no use in the separation process. Thus the capacity of directly attached ion exchangers is often limited with regard to the binding of biopolymers.

With tentacle ion exchangers, however, the exchanger groups are anchored to the surface of the matrix via linear polymer chains. The medium length of the polymer chain is 15 to 20 monomer units resulting in an increased capacity. The reason is a better accessibility of the functional groups with respect to the binding of biopolymers. Especially for large proteins tentacle media provide higher capacities compared to conventional supports. Due to the good selectivities and high efficiencies, columns packed with tentacle media give good chromatographic results and highly concentrated fractions can be obtained.

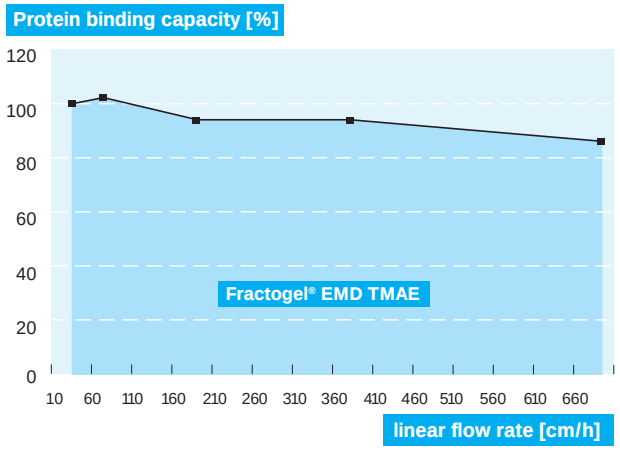

Fig. 3: BSA binding capacity of Fractogel® EMD TMAE (S) at linear flow rates up to 600 cm/h.

Recently, more applications are published showing the superior binding of very large compounds like plasmids and viruses.

The special pressure stability of the Fractogel® tentacle material also allows the use of high flow rates (for a 1 cm column up to 5 ml/min) without loss of resolution and capacity (Fig. 3).

Practical Tips

Buffer:

Any of the conventional buffers can be used for ion exchange chromatography. However, positively charged buffering ions should be used on anion exchangers to avoid an interaction or binding to the functional group. Therefore, Tris (pKa 8.2) is preferred with Cl- as counterion. For cation exchangers, the buffering ion should be negatively charged, for example carbonate, acetate or MES, and the counterion K+ or Na+. Phosphate buffers are generally used on both exchanger types. The buffer concentration is in the range of 5 mM up to 50 mM, usually.

As the starting condition the highest salt concentration should be used that permits binding of the target protein. The final chromatographic condition should be the lowest ionic strengths which allows the elution of the protein of interest. A third and higher concentration of salts in the buffer can be recommended as a washing step after the re-use of the column.

All buffers should be as pure as possible and should be made up from double distilled water (Milli Q® quality) and filtered if possible (0.45 or 0.22 µm filter).

Flow rate:

Depending on the desired resolution, linear flow rates up to 380 cm/h (= 5 ml/min for a 1 cm column) can be used. The protein binding capacity is not significantly affected up to these flow rates (Fig. 3).
A separation at 2 ml/min using a Superformance glass column with an inner diameter of 1 cm is shown in Fig. 4.

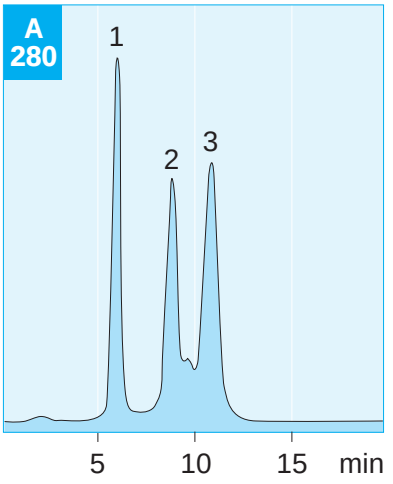

Fig. 4: Fast separation of α-lactalbumin (1), β-lactoglobulin (2) and amyloglucosidase (3) on Fractogel® EMD TMAE (S) (buffer A: 50 mM Tris/HCl, pH 8.5; buffer B: A + 1 M NaCl; flow rate: 150 cm/h).

Gradient:

In order to achieve optimum results, the gradients used for elution should not be too steep. With a column volume of 4-5 ml (= the content of a 50-10 cartridge), a gradient volume of 20 to 25 ml is sufficient. Depending on the individual separation problem step gradients can also be used. As a rule the

gradients are generated by a buffer A without salt and a buffer B, containing 1 M NaCl (or another salt).

Here are some additional rules that should be considered during optimization of a separation in bio-chromatography:

A. With anion exchangers it is better to keep the pH value of the buffer by 1.5-2 higher than the pI value of the protein; with cation exchangers operation has to be performed at 1.5 - 2 units below the protein's pI.

B. Increasing the pH value shortens the retention times with cation exchangers

C. Lowering the pH value shortens the retention time with anion exchangers

D. Increasing the ionic strength of the counter ion shortens the retention time (with anion exchangers and cation exchangers.)

Column Dimensions

For ion exchange chromatography processes short columns with a large diameter should be used. Especially recommended, for example, are Superformance columns with a length of 5 to 15 cm and an internal diameter of 1, 1.6 or 2.6 cm. Optimum results can be achieved with the pre-packed cartridges, without time consuming training in column packing.

Regeneration

Treatment with NaOH solution using concentrations of 0.1 to 0.5 (1 M NaOH for short periods of time) is best suited for the regeneration of ion exchange columns. Regeneration can also be performed with 2 % sodium lauroyl sarcosinate solution. Another successful method to regenerate a contaminated column is 20% ethanol or 6 M guanidine-hydrochloride. For rinsing Fractogel® media with organic solvents a linear flow rate of 1cm/min is recommended.

Ordering informationen

Catalog No.	Description	Particle-size	Content	Type of chromatography
1.16887	Fractogel® EMD TMAE (S)	20-40µm	100 ml, 500 ml	strong anion exchanger
1.16888	Fractogel® EMD DEAE (S)	20-40µm	100 ml, 500 ml	weak anion exchanger
1.16889	Fractogel® EMD DMAE (S)	20-40µm	100 ml, 500 ml	weak anion exchanger
1.16890	Fractogel® EMD SO_3^- (S)	20-40µm	100 ml, 500 ml	strongly acidic cation exchanger
1.16891	Fractogel® EMD COO^- (S)	20-40µm	100 ml, 500 ml	weakly acidic cation exchanger
1.16881	Fractogel® EMD TMAE (M)	40-90µm	500 ml, 5 l	strong anion exchanger
1.10316	Fractogel® EMD TMAE Hicap (M)	40-90µm	500 ml, 5 l	strong anion exchanger
1.16883	Fractogel® EMD DEAE (M)	40-90µm	500 ml, 5 l	weak anion exchanger
1.16884	Fractogel® EMD DMAE (M)	40-90µm	500 ml, 5 l	weak anion exchanger
1.16882	Fractogel® EMD SO_3^- (M)	40-90µm	500 ml, 5 l	strongly acidic cation exchanger
1.14894	Fractogel® EMD SE Hicap (M)	40-90µm	500 ml, 5 l	strongly acidic cation exchanger
1.16886	Fractogel® EMD COO^- (M)	40-90µm	500 ml, 5 l	weakly acidic cation exchanger

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