

GE Healthcare

# Ion exchange columns and media

Selection Guide



# General information

## Principle of Ion Exchange Chromatography

Ion exchange (IEX) chromatography can separate molecules or groups of molecules that have only slight differences in charge. Separation is based on the reversible interaction between a charged molecule and an oppositely charged chromatography medium. Typically, conditions are selected to ensure that the molecules of interest bind to the medium as they are loaded onto the column. Conditions are then altered so that the bound substances are eluted differentially. Elution is most often performed by a continuous gradient or a stepwise increase in ionic strength, most commonly using NaCl. Figure 1 shows a typical high resolution gradient elution. A stepwise elution is illustrated in Figure 3.

Proteins are built up of many different amino acids containing weak acidic and basic groups (i.e. ionizable groups) that can be titrated. Hence, the net surface charge of a protein is highly pH dependent and will change gradually as the pH of the environment changes. Each protein has its own unique net charge versus pH relationship which can be visualized as a titration curve (Figure 2). This curve reflects how the overall net charge of the protein changes according to the pH of the surroundings. IEX can be repeated at different pH values to separate several proteins which have distinctly different charge properties. Figure 2 shows how selecting the correct pH is one of the most important parameters in achieving satisfactory separation.

## Choice of ion exchanger

Begin with a strong exchanger (Q, S, SP) to enable development work to be performed over a broad pH range. Use a strong anion exchanger (Q) to bind the protein(s) of interest if their isoelectric point is below pH 7.0 or unknown.

Use a strong exchanger in those cases where maximum resolution occurs at an extreme pH and the proteins of interest are stable at that pH.

Consider using a weak exchanger (DEAE, ANX, CM) if the selectivity of the strong ion exchanger is unsatisfactory, but remember that the ion exchange capacity of a weak ion exchanger varies with pH.

Multimodal ligands (MMC, adhere) provide ionic interaction, hydrogen bonding and hydrophobic interaction. MMC behaves like a weak cation exchanger, but allows binding at high conductivity. Adhere behaves as a strong anion exchanger.

## Chromatography media selection

Select the ion exchange medium according to the objective of the purification step and the condition of the starting material. Other factors such as sample stability, scale, speed, binding capacity and equipment available may also influence the final choice.

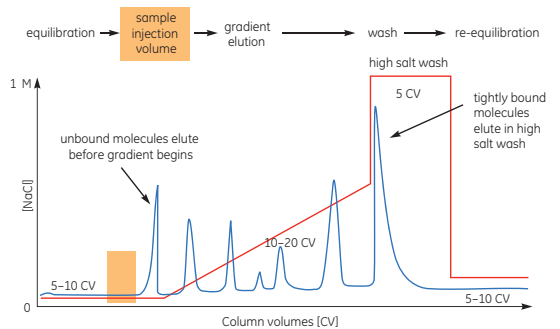


Fig 1. Typical IEX separation using gradient elution.

## Selectivity and buffer pH

Figure 2 shows the effect of pH on selectivity. The separation of three hypothetical proteins at different pH is described below, and the four scenarios are illustrated in the figure (see numbered arrows).

- 1. Most acidic pH:** all three proteins are below their isoelectric point, positively charged, and bind only to a cation exchanger. Proteins are eluted in the order of their net charge.
- 2. Less acidic pH:** the blue protein is above its isoelectric point, negatively charged, and the other proteins are still positively charged. Blue protein binds to an anion exchanger and can be separated from the other proteins which wash through. Alternatively, red and green proteins can be separated on a cation exchanger and the blue protein washes through.
- 3. Most alkaline pH:** all three proteins are above their isoelectric point, negatively charged, and bind only to the anion exchanger. Proteins are eluted in the order of their net charge.
- 4. Less alkaline pH:** the red protein is below its isoelectric point, positively charged. Red protein binds to the cation exchanger, while the other proteins wash through. Alternatively, blue and green proteins can be separated on an anion exchanger and the red protein washes through.

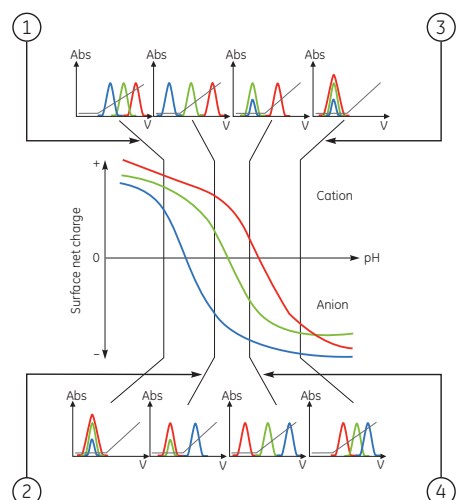


Fig 2. Effect of pH on selectivity (elution patterns).

## Sample preparation

Correct sample preparation is essential in order to achieve optimal separation and avoid deterioration in column performance. Samples must be clear and free from particulate matter.

To remove particulate matter, filter (see Buffer Preparation for filter sizes) or centrifuge (10 000 g for 15 min).

Desalt samples and transfer into the chosen start buffer using HiTrap™ Desalting 5 ml (volumes up to 1.5 ml) or HiPrep™ 26/10 Desalting (volumes up to 15 ml).

Very small sample volumes in high salt concentration, with no major contaminants, can be diluted with start buffer to lower the salt concentration to a level that does not interfere with binding to the medium.

## Column preparation

Wash away storage solutions and preservatives before using any IEX medium.

Use prepacked columns to ensure the best performance and reproducible results.

The volume required for the packed bed is determined by the amount of sample to be purified and the binding capacity of the medium. Pack a column that will have approximately 5-fold excess of the binding capacity required with a bed height up to 20 cm.

## Buffer preparation

See Table 1 for recommendations on volatile and non-volatile buffer systems.

Buffering ions should have the same charge as the functional groups on the IEX medium and a pKa within 0.6 pH units of the working pH.

Use a buffer concentration sufficient to maintain buffering capacity and constant pH, typically 20–50 mM.

Filter buffers after all salts and additives have been included, and always use high quality water and chemicals. Use 1 µm filters for particle sizes > 90 µm, 0.45 µm filters for 34 µm particles, or 0.22 µm filters for particles sizes < 15 µm or when sterile or extra clean samples are required.

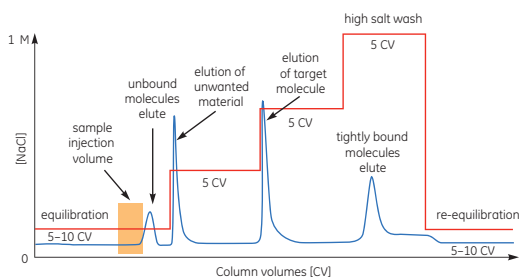
To avoid formation of air bubbles, ensure that the column and buffers are at the same temperature.

For samples with unknown charge properties, try the following:

- anion exchange (Q)  
start buffer: pH 8.0  
elution buffer: start buffer including 1 M NaCl, pH 8.0
- cation exchange (S or SP)  
start buffer: pH 6.0  
elution buffer: start buffer including 1 M NaCl, pH 6.0

## Method development and optimization (in priority order)

1. Scout for optimum pH by testing a range of pH values within which the proteins of interest are known to be stable. If the isoelectric point of the target protein is known, then begin with a narrower pH range, for example, 0.5–1 pH unit away from the isoelectric point.
2. If required, scout for optimum selectivity (testing strong or weak exchangers) using automatic media scouting.
3. Scout for the steepest gradient that gives acceptable resolution at the selected pH.
4. Scout for the highest flow rate that maintains resolution and minimizes separation time. Check recommended flow rates for the specific medium.
5. Scout for the maximum sample load that can be applied while maintaining satisfactory resolution. In general, loading 20–30% of the total binding capacity of the column gives optimal resolution with gradient elution.
6. For large-scale purification, separation times and buffer consumption can be reduced by transfer to a stepwise elution as shown in Figure 3.



**Fig 3.** Typical IEX separation using stepwise elution.

## Column cleaning

Correct preparation of samples and buffers and application of a high salt wash (1 M NaCl) at the end of each separation should keep most columns in good condition. However, reduced performance, a slow flow rate, increasing back pressure or complete blockage are all indications that the medium needs to be cleaned using more stringent procedures in order to remove contaminants.

It is recommended to reverse the direction of flow during column cleaning so that contaminants do not need to pass through the entire length of the column. The number of column volumes and time required for each cleaning step may vary according to the degree of contamination. If the cleaning procedure to remove common contaminants does not restore column performance, change the top filter (when possible) before trying alternative cleaning methods. Care should be taken when changing a filter as this may affect the column packing and interfere with performance.

**Note:** For recommended flow rates, refer to the table "Products for Ion Exchange."

### Removal of common contaminants

The following procedure should be satisfactory to remove common contaminants:

1. Wash with at least 2 column volumes (CV) of 2 M NaCl.
2. Wash with at least 4 CV of 1 M NaOH (same flow as step 1).
3. Wash with at least 2 CV of 2 M NaCl (same flow as step 1).
4. Rinse with at least 2 CV of distilled water (same flow as step 1) until the UV-baseline and eluent pH are stable.
5. Wash with at least 4 CV of start buffer or storage buffer (same flow as step 1) until pH and conductivity values have reached the required values.

### Removal of precipitated proteins

1. Inject 1 CV of pepsin (1 mg/ml in 0.5 M NaCl, 0.1 M acetic acid). Leave overnight at room temperature or for 1 h at 37°C.
2. Rinse with at least 2 CV of distilled water until the UV-baseline and the eluent pH are stable.
3. Wash with at least 4 CV of start buffer or storage buffer, same flow as step 2, until eluent pH and conductivity have reached the required values.

*Alternative procedure:*

1. Wash with 2 CV of 6 M guanidine hydrochloride.
2. Wash immediately with at least 5 CV of buffer at pH 7–8.
3. Rinse with at least 2 CV of distilled water (same flow as step 2) until the UV-baseline and eluent pH are stable.
4. Wash with at least 4 CV of start buffer or storage buffer (same flow as step 2) until pH and conductivity values have reached the required values.

### Removal of lipids, hydrophobically bound proteins or lipoproteins

Organic solvents or detergents may be required to completely remove contaminants of this type.

Before using organic solvents, wash the medium with at least 4 CV of distilled water to avoid any salts precipitating on the column.

When applying organic solvents or solutions it may be necessary to reduce the flow rate to avoid over-pressuring the column.

Use cleaning solutions such as up to 100% isopropanol, up to 100% methanol, up to 100% acetonitrile, up to 2 M NaOH, up to 75% acetic acid, up to 100% ethanol, ionic or non-ionic detergents. Always check for solvent compatibility in the instructions supplied with the medium or column.

Avoid anionic detergents with Q, DEAE and ANX charged groups. Avoid cationic detergents with S, SP and CM charged groups.

*Cleaning procedure: alternative 1*

1. Wash with 4 CV of up to 70% ethanol or 30% isopropanol.
2. Rinse with at least 2 CV of distilled water until the UV-baseline and eluent pH are stable.
3. Wash immediately with 3 CV of start buffer (same flow as step 2).

*Cleaning procedure: alternative 2*

1. Wash with 2 CV of detergent in a basic or acidic solution (e.g. 0.1–0.5% non-ionic detergent in 0.1 M acetic acid).
2. Rinse with 5 CV 70% ethanol to remove residual detergent.
3. Rinse with at least 2 CV of distilled water (same flow as step 1) until the UV-baseline and the eluent pH are stable.
4. Wash with 3 CV of start buffer (same flow as step 1).



# Technical information

**Table 1.** Non-volatile and volatile buffer systems

## Buffers for anion exchange chromatography

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25 °C) <sup>1</sup>
4.3–5.3	N-Methylpiperazine	20	Cl <sup>-</sup>	4.75
4.8–5.8	Piperazine	20	Cl <sup>-</sup> or HCOO <sup>-</sup>	5.33
5.5–6.5	L-Histidine	20	Cl <sup>-</sup>	6.04
6.0–7.0	Bis-Tris	20	Cl <sup>-</sup>	6.48
6.2–7.2	Bis-Tris propane	20	Cl <sup>-</sup>	6.65
8.6–9.6	Bis-Tris propane	20	Cl <sup>-</sup>	9.10
7.3–8.3	Triethanolamine	20	Cl <sup>-</sup> or CH <sub>3</sub> COO <sup>-</sup>	7.76
7.6–8.6	Tris	20	Cl <sup>-</sup>	8.07
8.0–9.0	N-Methyldiethanolamine	20	SO <sub>4</sub> <sup>2-</sup>	8.52
8.0–9.0	N-Methyldiethanolamine	50	Cl <sup>-</sup> or CH <sub>3</sub> COO <sup>-</sup>	8.52
8.4–9.4	Diethanolamine	20 at pH 8.4 50 at pH 8.8	Cl <sup>-</sup>	8.88
8.4–9.4	Propane 1,3-diamino	20	Cl <sup>-</sup>	8.88
9.0–10.0	Ethanolamine	20	Cl <sup>-</sup>	9.50
9.2–10.2	Piperazine	20	Cl <sup>-</sup>	9.73
10.0–11.0	Propane 1,3-diamino	20	Cl <sup>-</sup>	10.55
10.6–11.6	Piperidine	20	Cl <sup>-</sup>	11.12

<sup>1</sup> Ref: Handbook of chemistry and physics, 83<sup>rd</sup> edition, CRC, 2002–2003.

## Buffers for cation exchange chromatography

pH interval	Substance	Conc. (mM)	Counter-ion	pKa (25 °C) <sup>1</sup>
1.4–2.4	Maleic acid	20	Na <sup>+</sup>	1.92
2.6–3.6	Methyl malonic acid	20	Na <sup>+</sup> or Li <sup>+</sup>	3.07
2.6–3.6	Citric acid	20	Na <sup>+</sup>	3.13
3.3–4.3	Lactic acid	50	Na <sup>+</sup>	3.86
3.3–4.3	Formic acid	50	Na <sup>+</sup> or Li <sup>+</sup>	3.75
3.7–4.7	Succinic acid	50	Na <sup>+</sup>	4.21
5.1–6.1	Succinic acid	50	Na <sup>+</sup>	5.64
4.3–5.3	Acetic acid	50	Na <sup>+</sup> or Li <sup>+</sup>	4.75
5.2–6.2	Methyl malonic acid	50	Na <sup>+</sup> or Li <sup>+</sup>	5.76
5.6–6.6	MES	50	Na <sup>+</sup> or Li <sup>+</sup>	6.27
6.7–7.7	Phosphate	50	Na <sup>+</sup>	7.20
7.0–8.0	HEPES	50	Na <sup>+</sup> or Li <sup>+</sup>	7.56
7.8–8.8	BICINE	50	Na <sup>+</sup>	8.33

<sup>1</sup> Ref: Handbook of chemistry and physics, 83<sup>rd</sup> edition, CRC, 2002–2003.

## Volatile buffer systems

pH range	Buffer system	Counter-ion	pKa-values for buffering ions <sup>1</sup>
3.3–4.3	Formic acid	H <sup>+</sup>	3.75
3.3–4.3; 4.8–5.8	Pyridine/formic acid	HCOO <sup>-</sup>	3.75; 5.25
3.3–4.3; 9.3–10.3	Trimethylamine/formic acid	HCOO <sup>-</sup>	4.75; 9.81
4.3–5.8	Pyridine/acetic acid	CH <sub>3</sub> COO <sup>-</sup>	4.75; 5.25
4.3–5.3; 9.3–10.3	Trimethylamine/acetic acid	CH <sub>3</sub> COO <sup>-</sup>	4.75; 9.81
3.3–4.3; 8.8–9.8	Ammonia/formic acid	HCOO <sup>-</sup>	3.75; 9.25
4.3–5.3; 8.8–9.8	Ammonia/acetic acid	CH <sub>3</sub> COO <sup>-</sup>	4.75; 9.25
5.9–6.9; 9.3–10.3	Trimethylamine/carbonate	CO <sub>3</sub> <sup>2-</sup>	6.35; 9.81
5.9–6.9; 8.8–9.8	Ammonium bicarbonate	HCO <sub>3</sub> <sup>-</sup>	6.35; 9.25
5.9–6.9; 8.8–9.8	Ammonium carbonate/ammonia	CO <sub>3</sub> <sup>2-</sup>	6.35; 9.25
5.9–6.9; 8.8–9.8	Ammonium carbonate	CO <sub>3</sub> <sup>2-</sup>	6.35; 9.25
4.3–5.3; 7.2–8.2	N-ethylmorpholine/acetate	HCOO <sup>-</sup>	4.75; 7.72

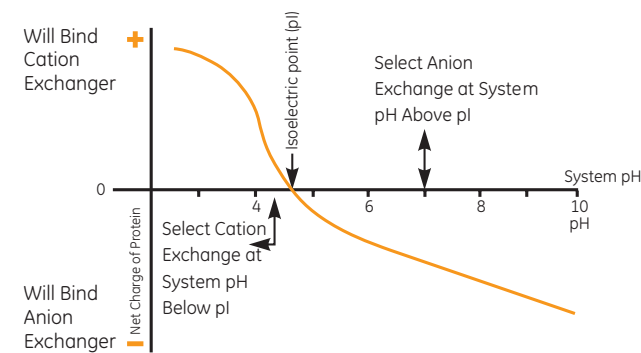
<sup>1</sup> Ref: Handbook of chemistry and physics, 83<sup>rd</sup> edition, CRC, 2002–2003.

ÄKTAdesign™ chromatography systems provide advice on buffer recipes for each pH and medium. The BufferPrep function, available in selected systems, compensates automatically for changes in pH caused by changes in salt concentration and temperature, ensuring constant pH throughout the separation.

# Selection guide - Ion Exchange Media

## Selecting an anion or cation exchanger

Ion exchange separates proteins on the basis of differences in their net surface charge in relation to the pH of the surroundings. The figure below illustrates how the net charge of a protein can vary with pH. Every protein has its own charge/pH relationship.



If isoelectric point (pI) of the target protein is known:

- select an anion exchanger (Q, DEAE, ANX) with a buffer pH above the pI.
- select a cation exchanger (S, SP, CM) with a buffer pH below the pI.

If pI is unknown:

- test for selectivity using a strong ion exchanger, Q, S or SP. Strong ion exchangers maintain their charge over a wider pH range than weak ion exchangers and are suitable for most applications.

A typical purification strategy has three phases: Capture, Intermediate Purification and Polishing (CIP). Each phase has a specific objective, dependent largely on the properties of the starting material. Select the appropriate ion exchange medium according to the objective of your purification step and the condition of your starting material.



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GE Healthcare Bio-Sciences AB  
Björkgatan 30  
751 84 Uppsala  
Sweden

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GE Healthcare Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp., 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany  
GE Healthcare Bio-Sciences KK, Sanken Bldg, 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo, 169-0073 Japan



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