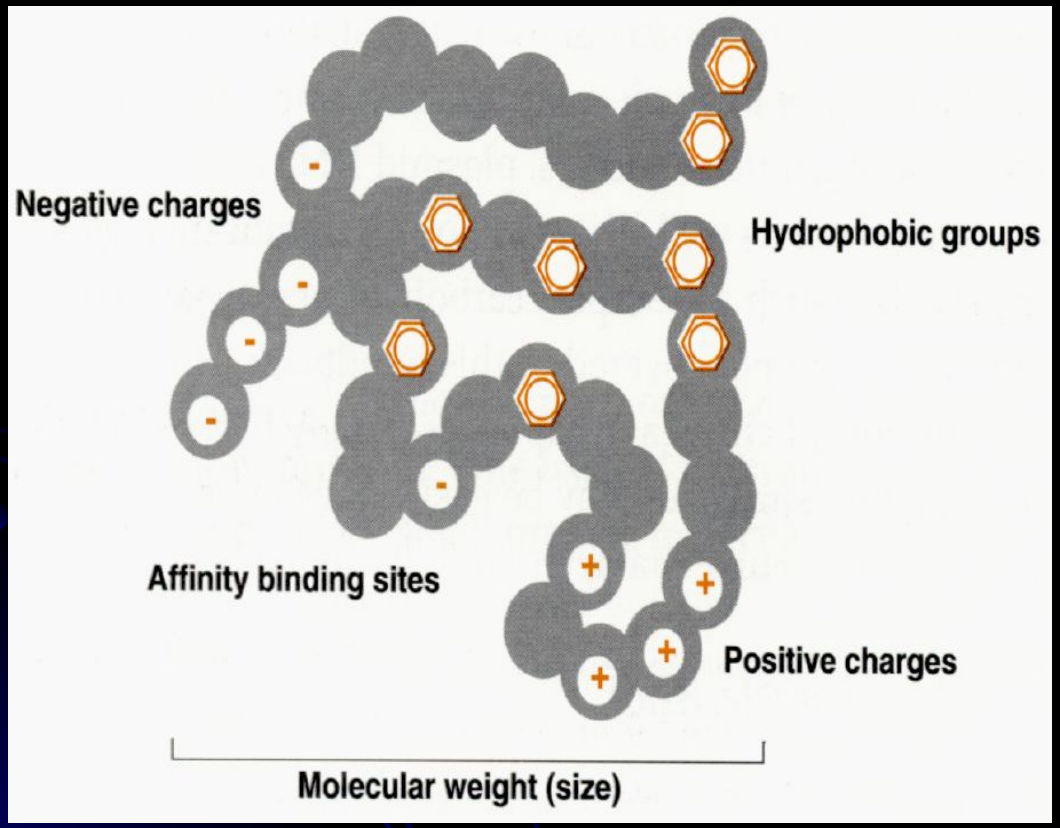
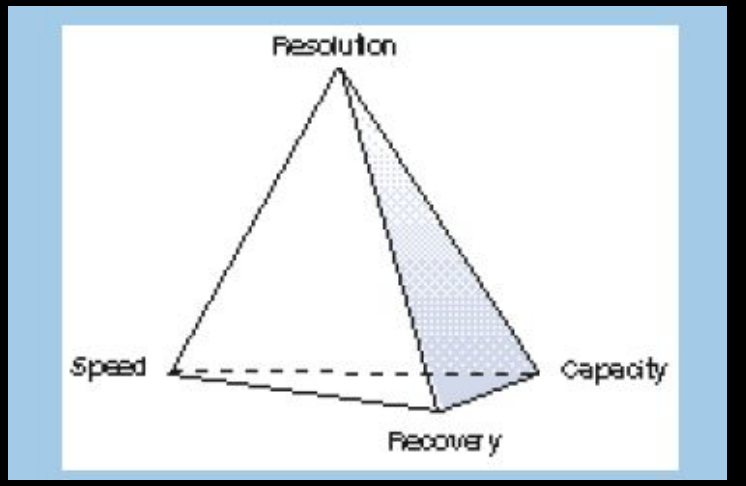
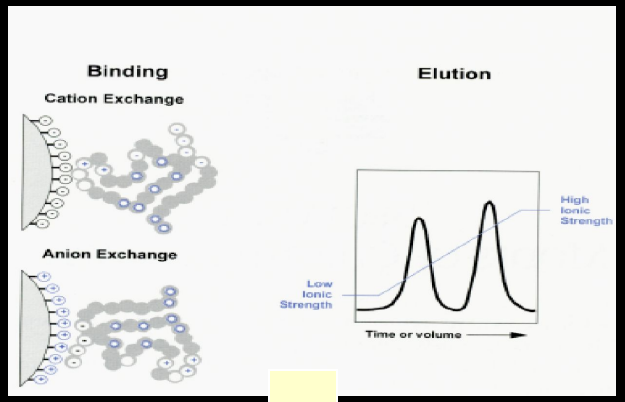
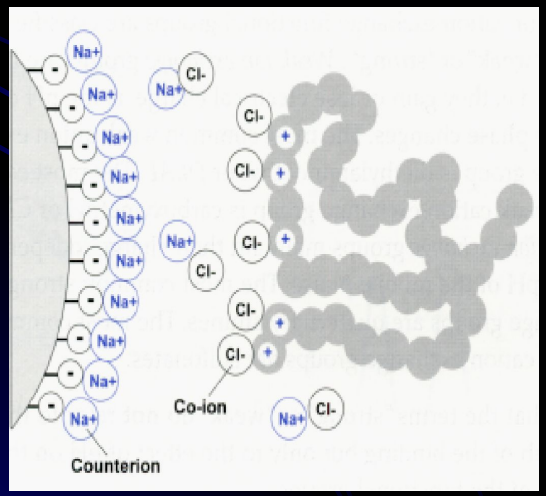
Ионообменная хроматография

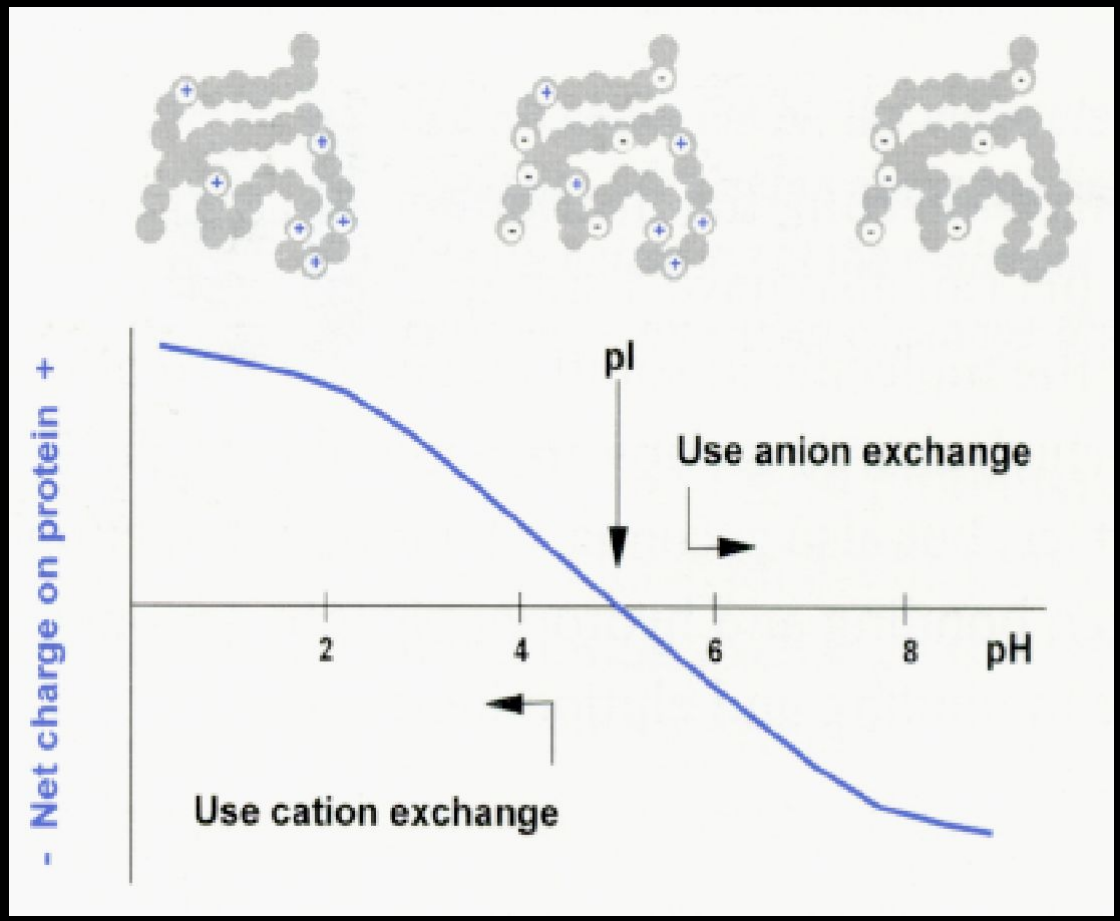




Принципы ионообменной хроматографии







Функциональные группы

Table 1. Functional groups used on ion exchangers.

Anion exchangers	**Functional group**
Diethylaminoethyl (DEAE)	$-O-CH_2-CH_2-N^+H(CH_2CH_3)_2$
Quaternary aminoethyl (QAE)	$-O-CH_2-CH_2-N^+(C_2H_5)_2-CH_2-CHOH-CH_3$
Quaternary ammonium (Q)	$-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-N^+(CH_3)_3$
Cation exchangers	**Functional group**
Carboxymethyl (CM)	$-O-CH_2-COO^-$
Sulphopropyl (SP)	$-O-CH_2-CHOH-CH_2-O-CH_2-CH_2-CH_2SO_3^-$
Methyl sulphonate (S)	$-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2SO_3^-$

Sulphonic and quaternary amino groups are used to form strong ion exchangers; the other groups form weak ion exchangers. The terms strong and weak refer to the extent of variation of ionization with pH and not the strength of binding. Strong ion exchangers are completely ionized over a wide pH range (see titration curves on page 49) whereas with weak ion exchangers, the degree of dissociation and thus exchange capacity varies much more markedly with pH.

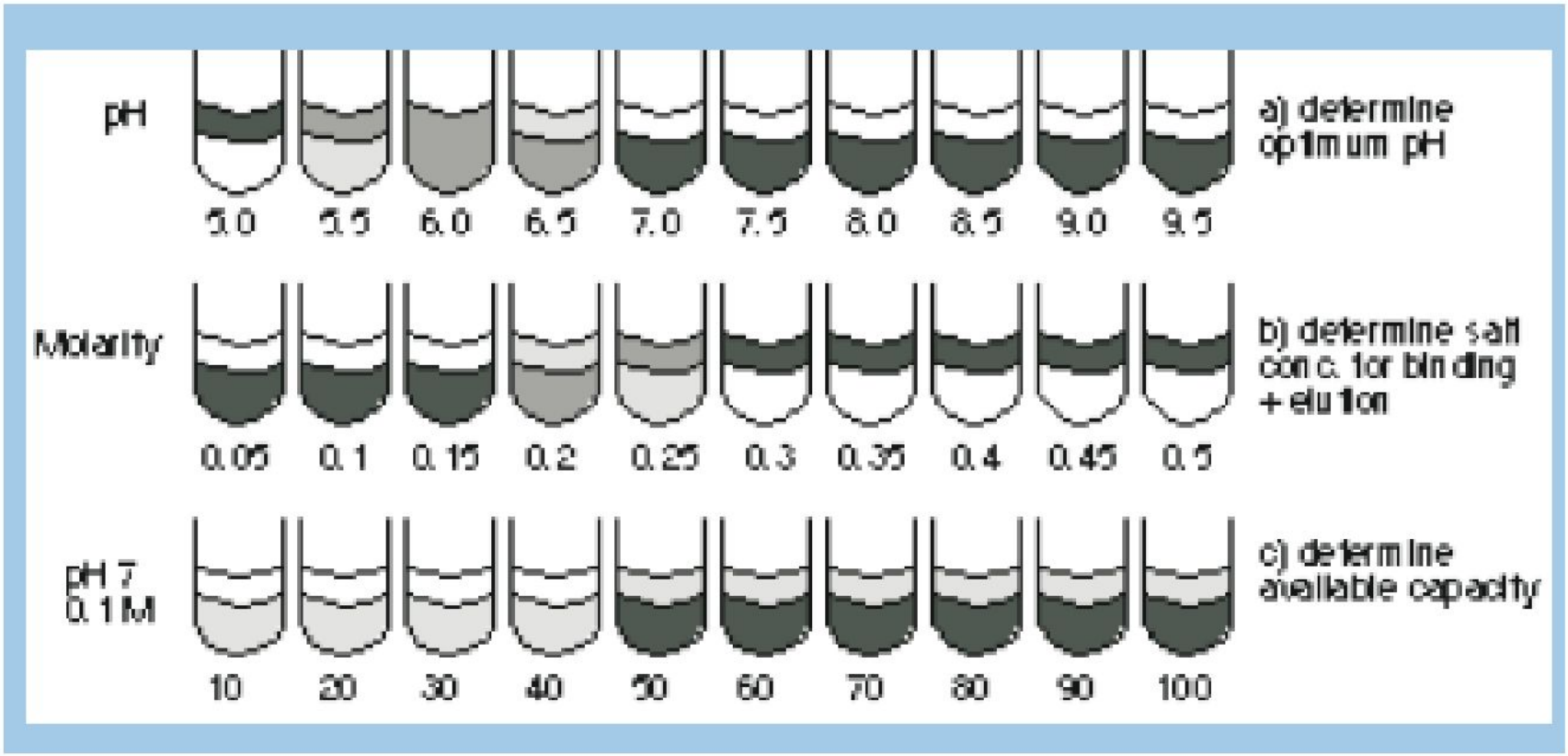
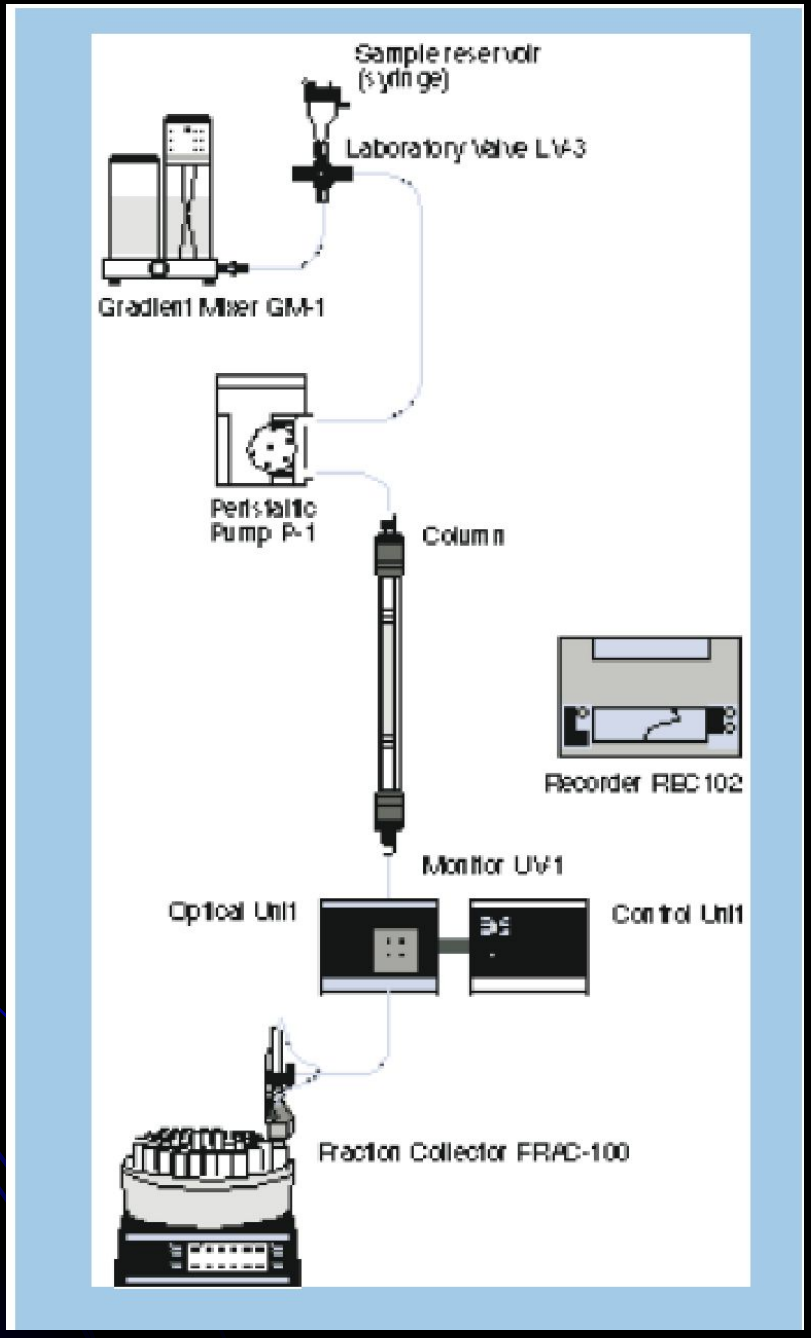


Fig. 38. Test-tube methods for selecting ion exchange conditions.

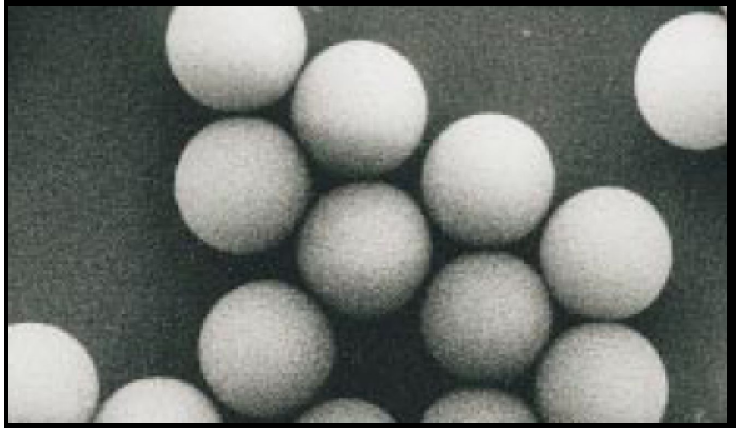

Sample reservoir
(syringe)
Laboratory Valve LV-3
Gradient Mixer GM-1
Peristaltic
Pump P-1
Column
Recorder REC 102
Monitor UV-1
Optical Unit
Control Unit
Fraction Collector FRAC-100

Характеристика носителя MonoBeads

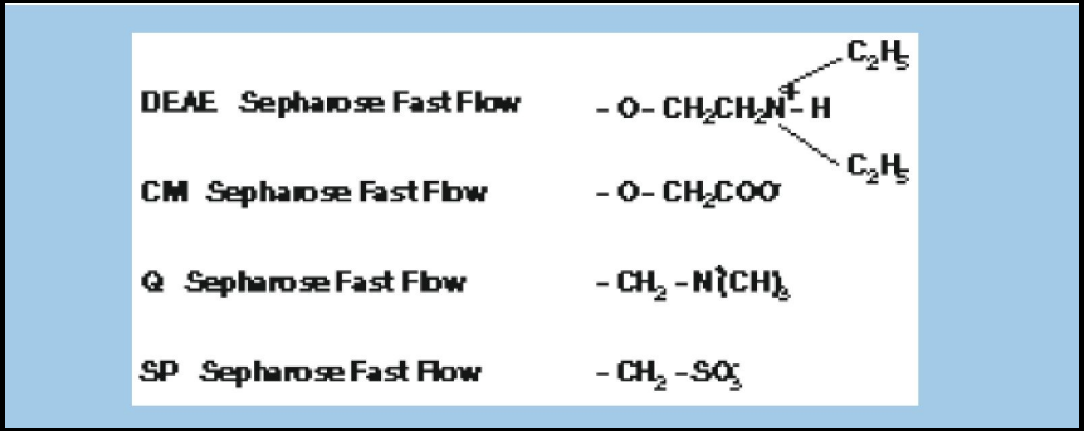
Table 2. Characteristics of MonoBeads.

Properties	Mono Q	Mono S
Type of gel	strong anion exchanger	strong cation exchanger
Charged group	$-O-CH_2-CHOH-CH_2-O-$ $-CH_2-CHOH-CH_2-N^+(CH_3)_3$	$-O-CH_2-CHOH-CH_2-O-$ $-CH_2-CHOH-CH_2SO_3^-$
Total ionic capacity (µmoles/ml gel)	270-370	140-180
Total protein binding capacity (mg/ml gel)		
Thyroglobulin (MW 669 000)	25	N.D.
HSA (MW 68 000)	65	N.D.
α-lactalbumin (MW 14 300)	80	N.D.
IgG (MW 150 000)	N.D.	75
Ribonuclease (MW 13 700)	N.D.	75
Typical protein recoveries (%)	90-100	90-100
Typical enzyme activity recoveries (%)	>80	>80
Average particle size (µm)	10 ±0.5	10 ±0.5
MW range (proteins)	up to 10^7	up to 10^7
working pH range*	3-11	3-11
pH stability**		
long term	2-12	2-12
short term	2-14	2-14

N.D. = Not determined
Solvent restrictions: The ion exchangers are stable in alcohol/water solutions (C1-C4). 100% dimethyl sulphoxide, dimethylformamide, and formic acid can change the separation properties of the gel. Avoid oxidizing and reactive reagents. Detergents can be used if they are non-ionic or have the same charge as the gel.
* working pH range refers to the pH range over which the ion exchange groups remain charged and maintain consistently high capacity.
** pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration and cleaning procedures.



Ионообменные носители на основе Sepharose



Ion Exchanger	Q Sepharose Fast Flow	SP Sepharose Fast Flow	DEAE Sepharose Fast Flow	CM Sepharose Fast Flow
Total ionic capacity (μmol/ml gel)	180-250	180-250	110-160	90-130
Dynamic binding capacity* (mg/ml gel)				
Thyroglobulin (MW 669 000)	3	N.D.	3.1	N.D.
HSA (MW 68 000)	120	N.D.	110	N.D.
α-lactalbumin (MW 14 300)	110	N.D.	100	N.D.
IgG (MW 160 000)	N.D.	50	N.D.	15
Bovine COHb (MW 69 000)	N.D.	50	N.D.	30
Ribonuclease (MW 13 700)	N.D.	70	N.D.	50

N.D. = Not determined
*Capacities were determined using the method described in Chapter 10 at a flow rate of 75 cm/h. For anion exchangers (DEAE and Q) the starting buffer was 0.05 M Tris, pH 8.3 and for cation exchangers (CM and S) 0.1 M acetate buffer, pH 5.0. Limit buffers were the respective start buffers containing 2.0 M NaCl.

Product	Q Sepharose Fast Flow	SP Sepharose Fast Flow	DEAE Sepharose Fast Flow	CM Sepharose Fast Flow
Type of gel	strong anion	strong cation	weak anion	weak cation
Total ionic capacity (μmol/ml gel)	180-250	180-250	110-160	90-130
Recommended working flow rate range (cm/h)	100-300	100-300	100-300	100-300
Approx. mean particle size (μm)	90	90	90	90
Particle size range (μm)	45-165	45-165	45-165	45-165
working pH range*	2-12	4-13	2-9	6-10
pH stability**				
short term	1-14	3-14	1-14	2-14
long term	2-12	4-13	2-13	4-13

Table 9. Characteristics of Q, SP, DEAE and CM Sepharose Fast Flow.

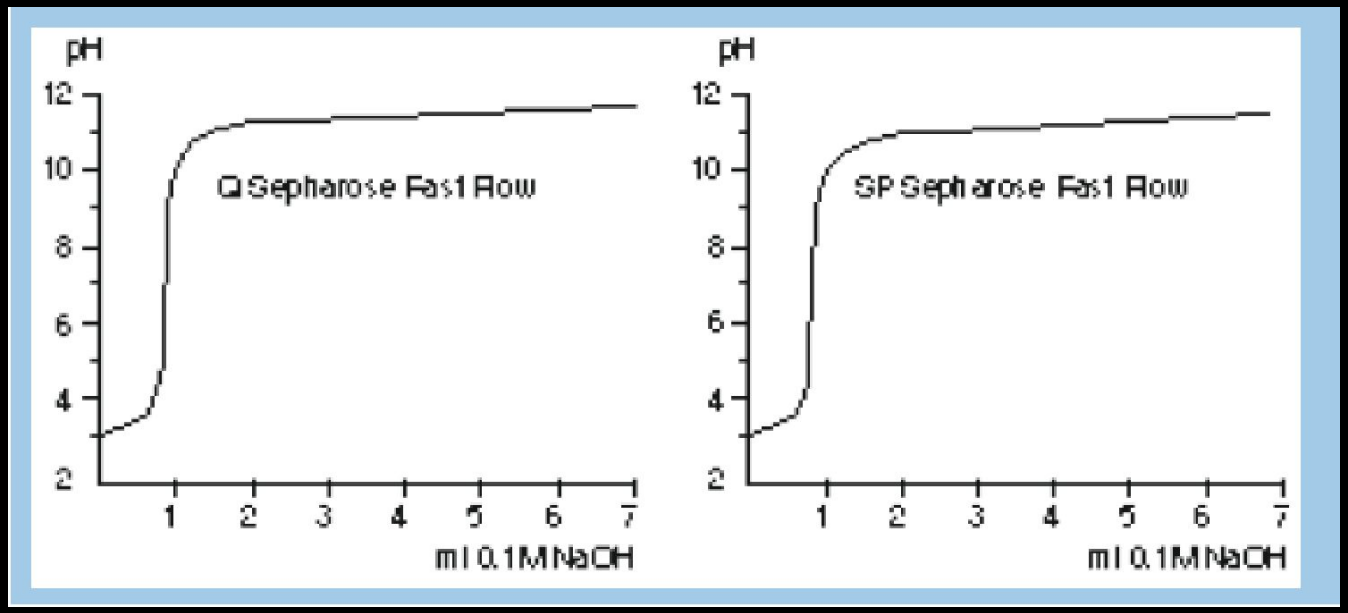
Product	Q Sepharose Fast Flow	SP Sepharose Fast Flow	DEAE Sepharose Fast Flow	CM Sepharose Fast Flow
Type of gel	strong anion	strong cation	weak anion	weak cation
Total ionic capacity (µmol/ml gel)	180-250	180-250	110-160	90-130
Recommended working flow rate range (cm/h)	100-300	100-300	100-300	100-300
Approx. mean particle size (µm)	90	90	90	90
Particle size range (µm)	45-165	45-165	45-165	45-165
working pH range*	2-12	4-13	2-9	6-10
pH stability**				
short term	1-14	3-14	1-14	2-14
long term	2-12	4-13	2-13	4-13

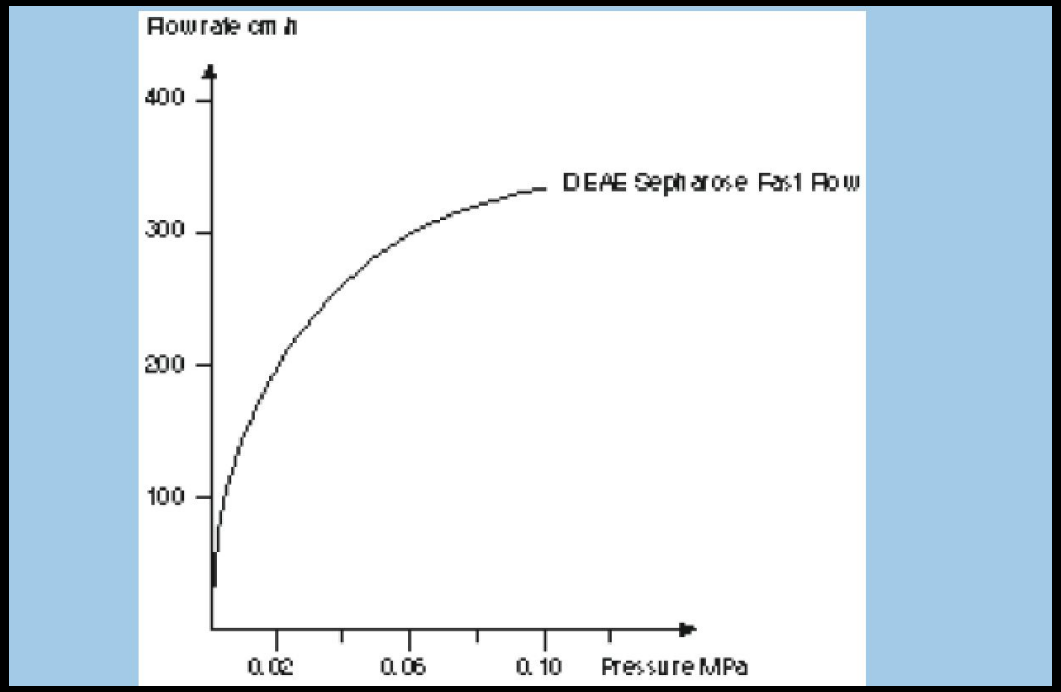
* working pH range refers to the pH range over which the ion exchange groups remain charged and maintain consistently high capacity.
** pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration and cleaning procedures.

Table 10. Capacity data for Sepharose Fast Flow ion exchangers.

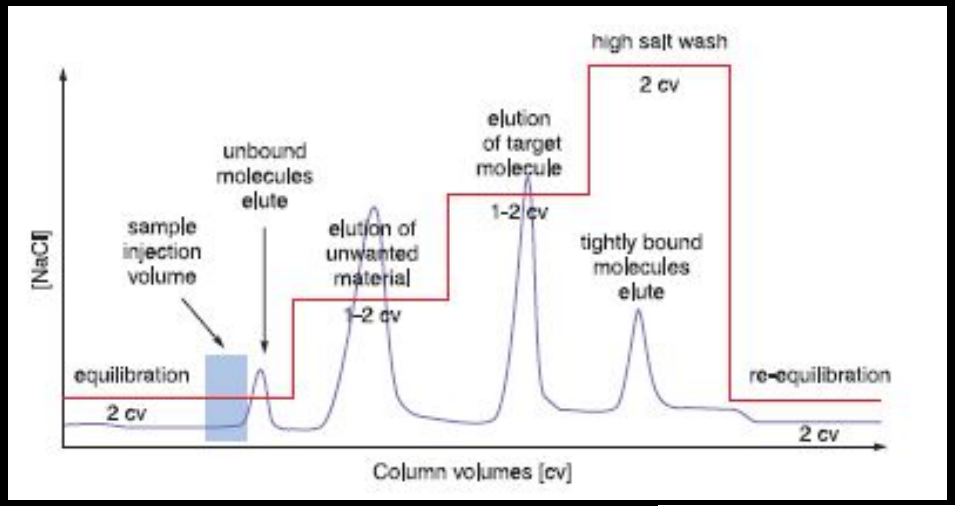
Ion Exchanger	Q Sepharose Fast Flow	SP Sepharose Fast Flow	DEAE Sepharose Fast Flow	CM Sepharose Fast Flow
Total ionic capacity (µmol/ml gel)	180-250	180-250	110-160	90-130
Dynamic binding capacity* (mg/ml gel)				
Thyroglobulin (MW 669 000)	3	N.D.	3.1	N.D.
HSA (MW 68 000)	120	N.D.	110	N.D.
α-lactalbumin (MW 14 300)	110	N.D.	100	N.D.
IgG (MW 160 000)	N.D.	50	N.D.	15
Bovine COHb (MW 69 000)	N.D.	50	N.D.	30
Ribonuclease (MW 13 700)	N.D.	70	N.D.	50

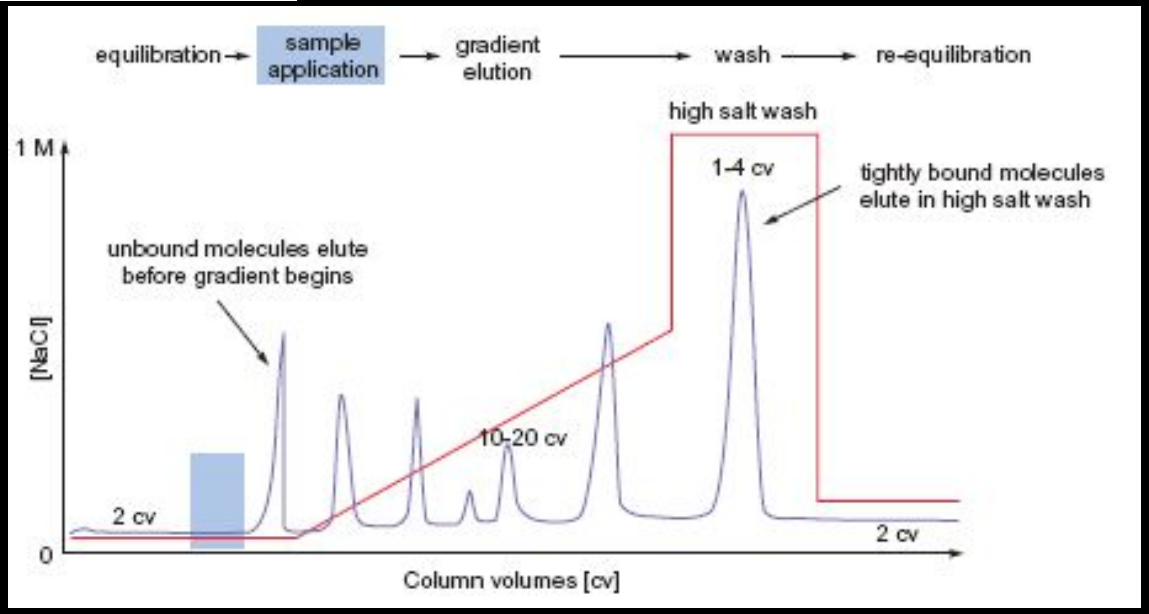
N.D. = Not determined
*Capacities were determined using the method described in Chapter 10 at a flow rate of 75 cm/h. For anion exchangers (DEAE and Q) the starting buffer was 0.05 M Tris, pH 8.3 and for cation exchangers (CM and S) 0.1 M acetate buffer, pH 5.0. Limit buffers were the respective start buffers containing 2.0 M NaCl.


pH
12
10
8
6
4
2
Q Sepharose Fast Flow
1 2 3 4 5 6 7
ml 0.1M NaOH
pH
12
10
8
6
4
2
SP Sepharose Fast Flow
1 2 3 4 5 6 7
ml 0.1M NaOH


Flow rate cm/h
400
300
200
100
DEAE Sepharose Fast Flow
0.02
0.06
0.10
Pressure MPa

Градиентная элюция





Sample: Chymotrypsinogen A, Ribonuclease A, Cytochrome C, Lysozyme (6:10:6:5), 25 µg/ml gel (6 µg Mini S, 2.5 µg Mono S)
Buffer A: 20 mM acetic acid, pH 5.0
Buffer B: Buffer A with 0.5 M lithium chloride
Gradient: 0–100% B in 20 col. vols. (6 min Mini S, 10 min Mono S).
Flow rate: 10 cm/min (800 µl/min Mini S, 200 µl/min Mono S)
Instrument: SMART System with µPeak Monitor

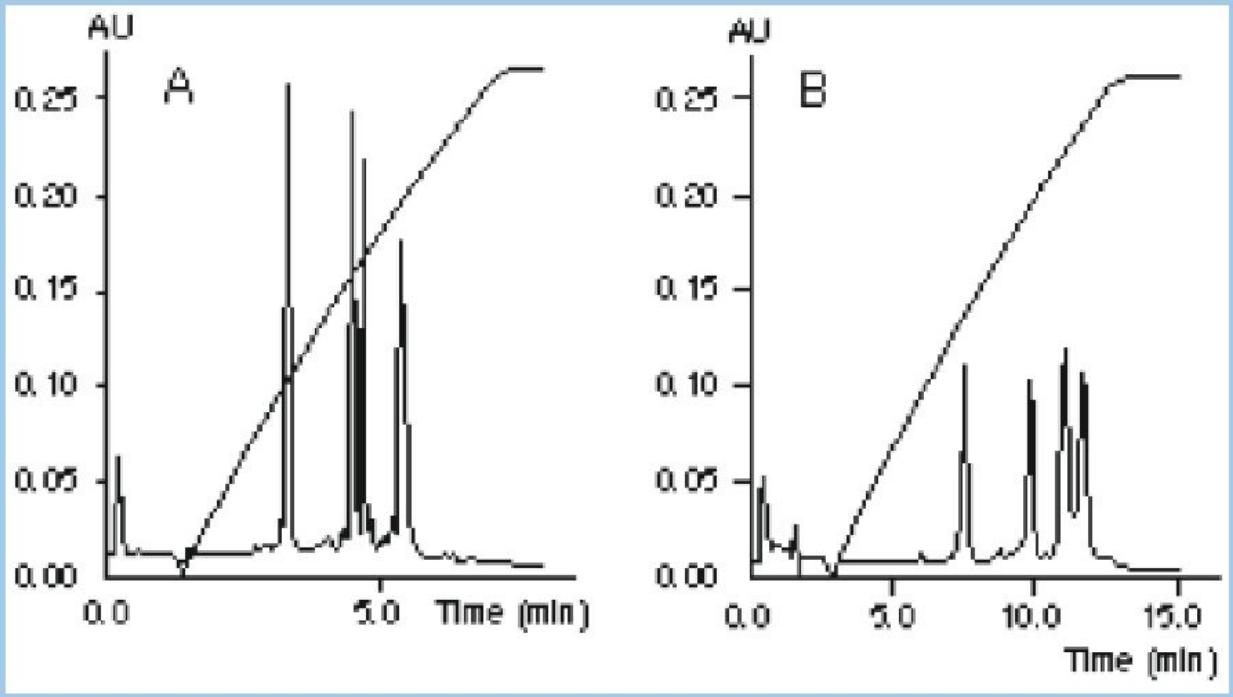


Fig. 12. Comparison of (A) Mini S PC 3.2/3 and (B) Mono S PC 1.6/5. Mini S PC 3.2/3 gives a faster separation and a better resolution of the peaks. Similar results have been found with Mini Q PC 3.2/3 and Mono Q PC 1.6/5. (Work by Pharmacia Biotech, Uppsala, Sweden.)

Properties	Mini Q PC 3.2/3	Mini S PC 3.2/3
Type of gel	strong anion exchanger	strong cation exchanger
Charged group	$-O-CH_2-CHOH-CH_2-O-$ $-CH_2-CHOH-CH_2-N^+(CH_3)_3$	$-O-CH_2-CHOH-CH_2-O-$ $-CH_2-CHOH-CH_2SO_3^-$
Total ionic capacity (µmoles/ml gel)	60-90	16-30
Column dimensions i.d. x bed height (mm)	3.2 x 30	3.2 x 30
Column volume (ml)	0.24	0.24
Average particle size (µm)	3 µm	3 µm
Binding capacity (mg/column)		
α-amylas (MW 49 000)	≈ 1.4	N.D.
Trypsin inhibitor (MW 20 100)	≈ 1.4	N.D.
Ribonuclease (MW 13 700)	N.D.	≈ 1.3
Lysozyme (MW 14 300)	N.D.	≈ 1.3
Max loading capacity (mg/column)	1-1.5	1-1.5
Practical loading capacity (µg/column)	≤200	≤200
Typical protein recoveries (%)	70-90	70-90
working pH range*	3-11	3-11
pH stability**		
long term	3-11	3-11
short term	1-14	1-14
Maximum flow rate (ml/min)	1.0	1.0
Operational pressure limit (MPa)	10	10
Normal separation times (min)	5-20	5-20

N.D. = Not determined
* working pH range refers to the pH range over which the ion exchange groups remain charged and maintain consistently high capacity.
** pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration and cleaning procedures.

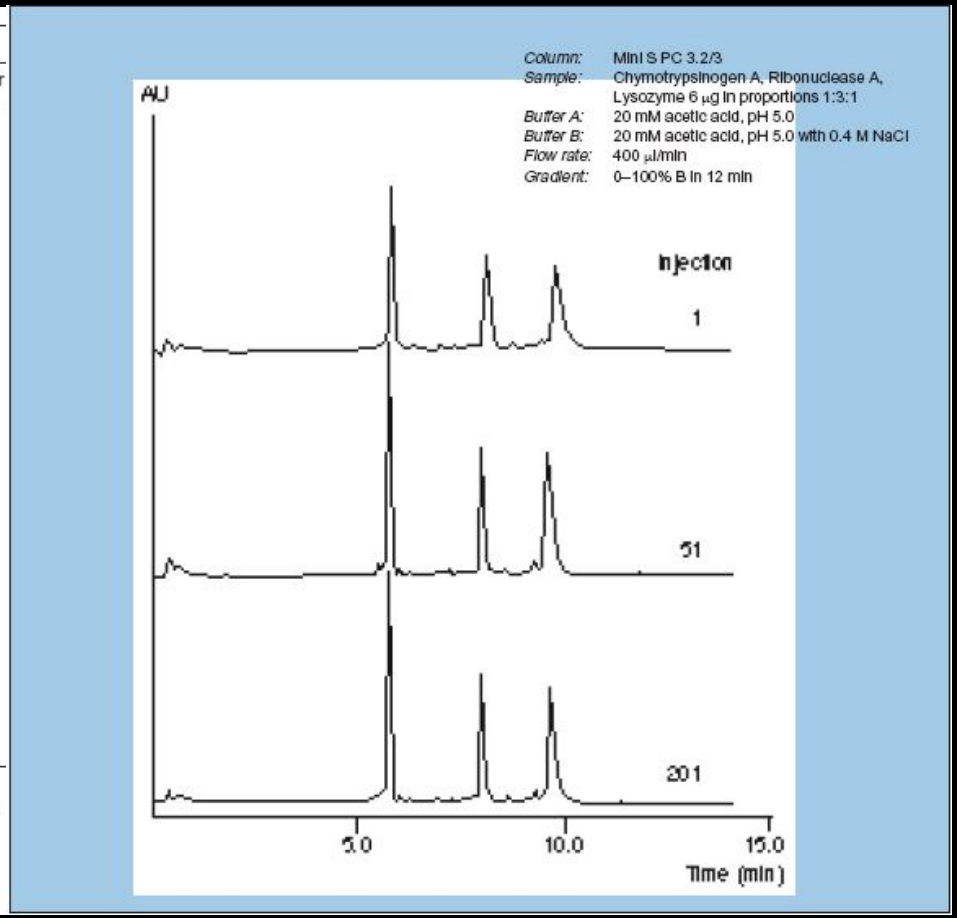


Table 6. Characteristics of SOURCE 15Q and 15S, and SOURCE 30Q and 30S.

Properties	SOURCE 15Q	SOURCE 30Q	SOURCE 15S	SOURCE 30S
Type of gel	strong anion exchangers		strong cation exchangers	
Charged group	-O-CH_2-CHOH-CH_2-O-		-O-CH_2-CHOH-CH_2-O-	
	-CH_2-CHOH-CH_2-$N^+(CH_3)_3$		-CH_2-CHOH-$CH_2SO_3^-$	
Matrix	Polystyrene/divinyl benzene			
Bead form	Rigid, spherical, porous monodisperse			
Mean particle size (µm)	15	30	15	30
Dynamic binding capacity* (mg/ml gel)				
Lysozyme (MW 14 500)	N.D.	N.D.	80	80
BSA (MW 67 000)	45	45	N.D.	N.D.
MW range (proteins)	up to 10^7	up to 10^7	up to 10^7	up to 10^7
working pH range**	2-12	2-12	2-12	2-12
pH stability***				
long term	2-12	2-12	2-12	2-12
short term	1-14	1-14	1-14	1-14
Maximum flow rate (cm/h)	1800[1]	2000[2]	1800[1]	2000[2]
Recommended working flow rate range (cm/h)	30-600	300-1000	30-600	300-1000
Operating temperature (°C)	4-40	4-40	4-40	4-40

N.D. = Not determined

Solvent restrictions: SOURCE is stable in alcohol/water solutions (C1-C4). 100% dimethyl sulphoxide, dimethylformamide, and formic acid can change the separation properties of the gel. Avoid oxidizing and reactive reagents. Detergents can be used if they are non-ionic or have the same charge as the gel.

* Determined by frontal analysis at a flow rate of 300 cm/h, using a 5.0 mg/ml solution of protein in 20 mM sodium phosphate buffer, pH 6.8 (lysozyme) and 20 mM BIS TRIS PROPANE buffer, pH 7.0 (BSA).

** working pH range refers to the pH range over which the ion exchange groups remain charged and maintain consistently high capacity.

*** pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

pH stability, short term refers to the pH interval for regeneration and cleaning procedures.

1) Maximum flow rate to be applied, will depend on the pressure specification of the chromatographic system used. A linear flow rate of 1800 cm/h will give a pressure drop of approximately 10 MPa at a bed height of 3 cm.

2) Maximum flow rate to be applied, will depend on the pressure specification of the chromatographic system used. A linear flow rate of 2000 cm/h will give a pressure drop of approximately 10 MPa at a bed height of 10 cm.

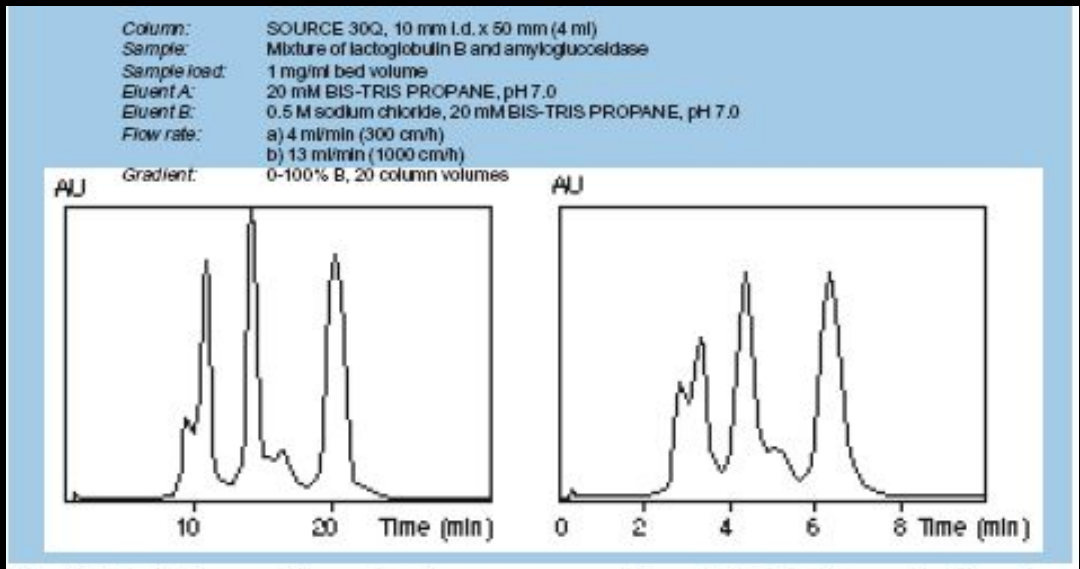
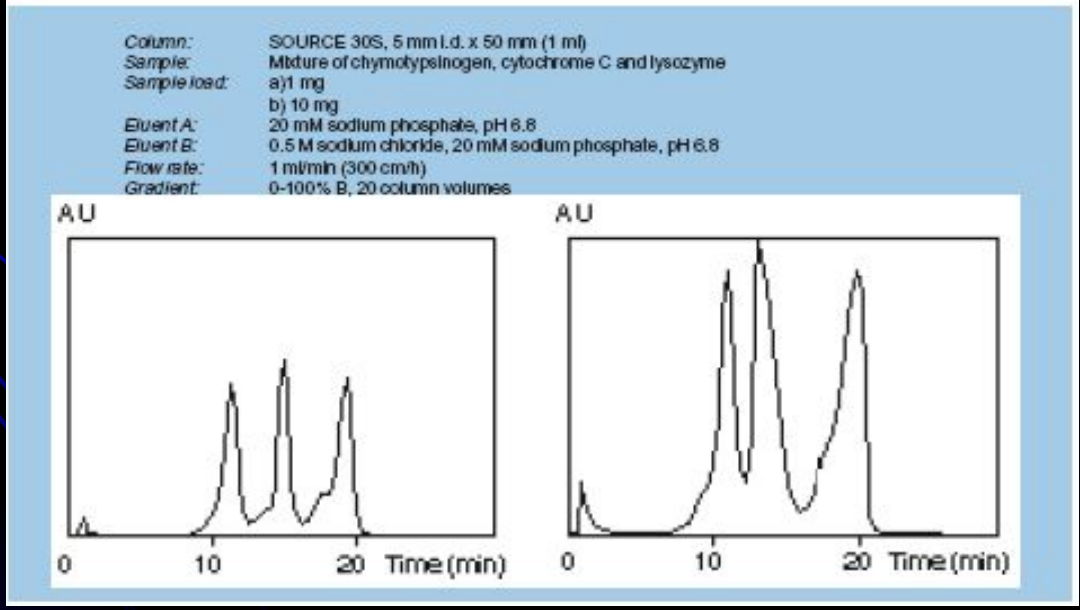
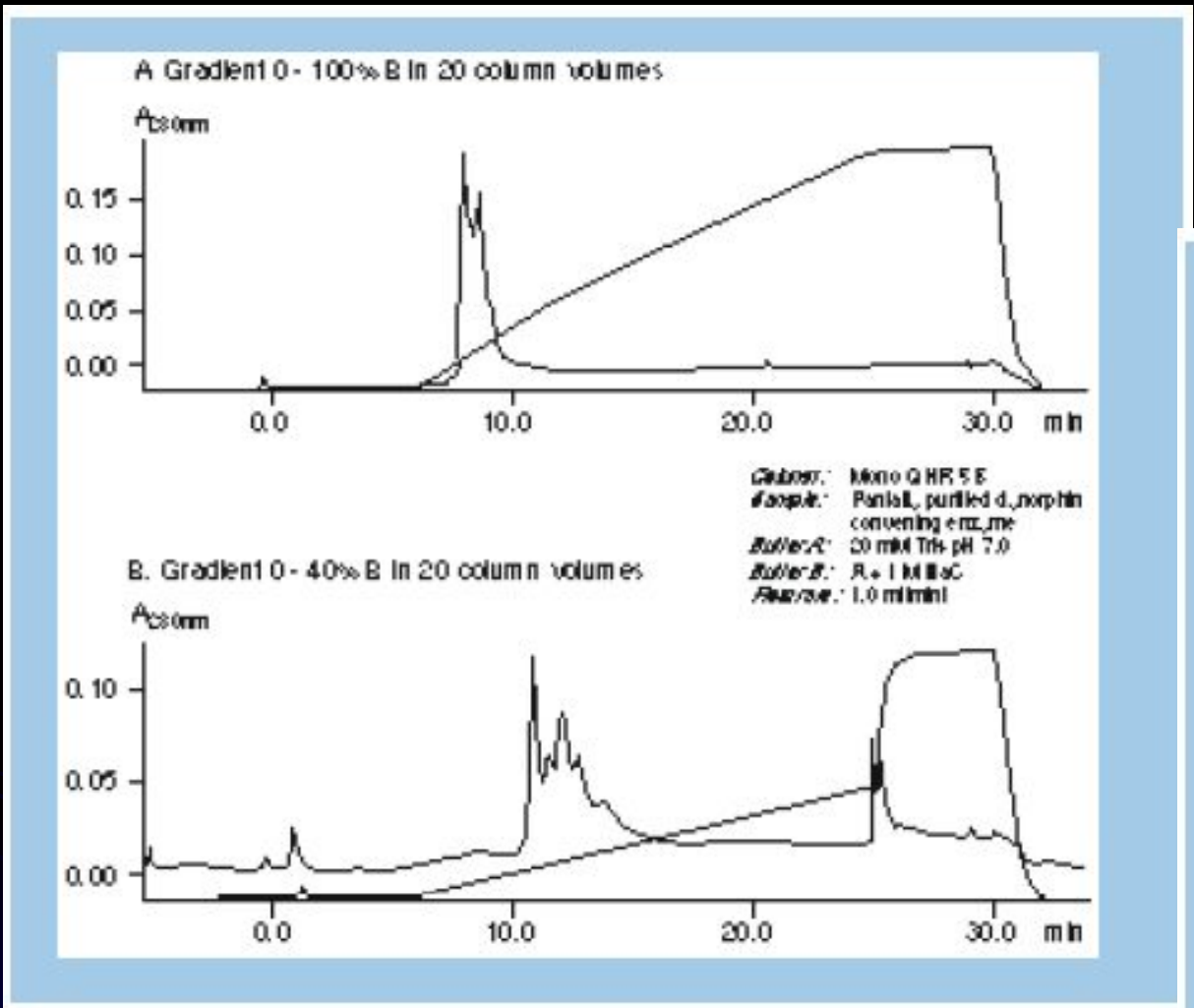


Fig. 16. The influence of increasing flow rate on resolution. (Work by Pharmacia Biotech, Uppsala, Sweden.)





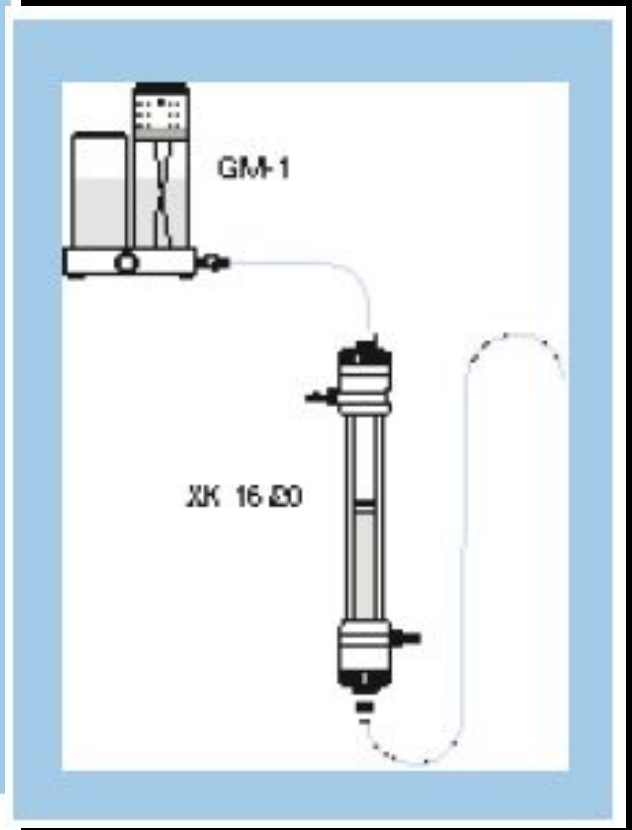
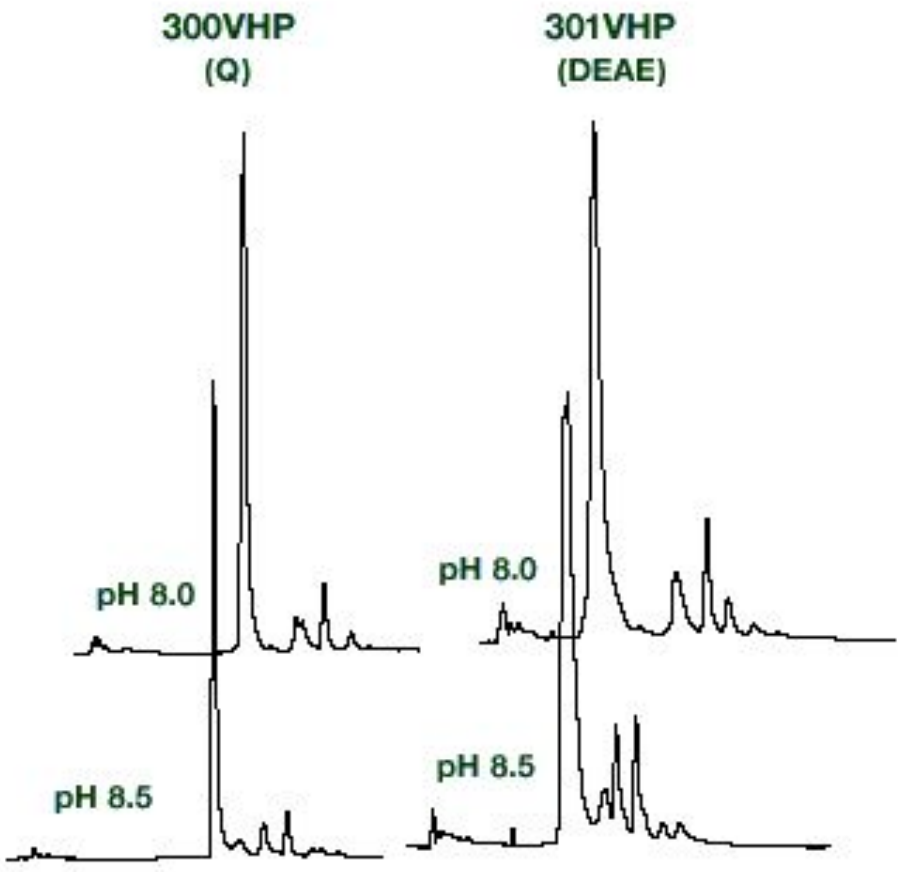


Fig. 53. Effect of gradient slope on resolution. (Work by Pharmacia Biotech, Uppsala, Sweden).



Conditions

Vydac 300VHP575 (Q anion exchange, 5 µm, 7.5 mm i.d. x 50 mm)
Vydac 301VHP575 (DEAE anion exchange, 5 µ, 7.5 mm i.d. x 50 mm)
Eluent: 10 mM Tris-HCL, pH 8.0 or 8.5, gradient 0-150 mM NaCl in 60 min.
Sample: carbonic anhydrase.

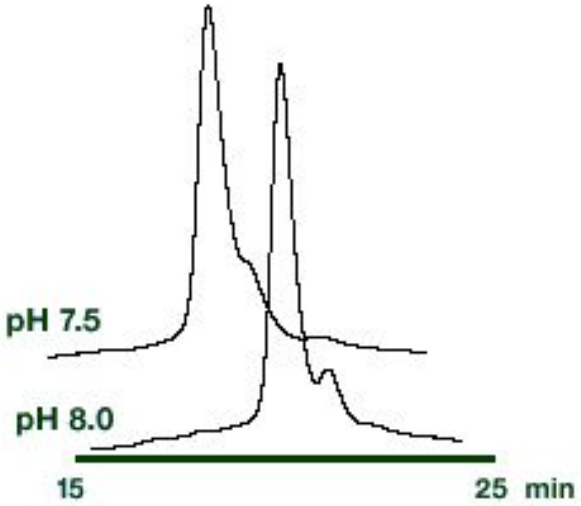
Effect of pH on protein separations

High resolution ion exchange separations can be optimized by careful adjustment of the eluent pH. For instance, by changing the eluent pH from 7.5 to 8.5, histidine converts from a plus one charge to uncharged, affecting the resolution between similar proteins.

The effect of pH on resolution is illustrated by the separation of a small peak trailing conalbumin. The trailing peak is only a shoulder at pH 7.5 but is nearly completely separated at pH 8.0.

Conditions

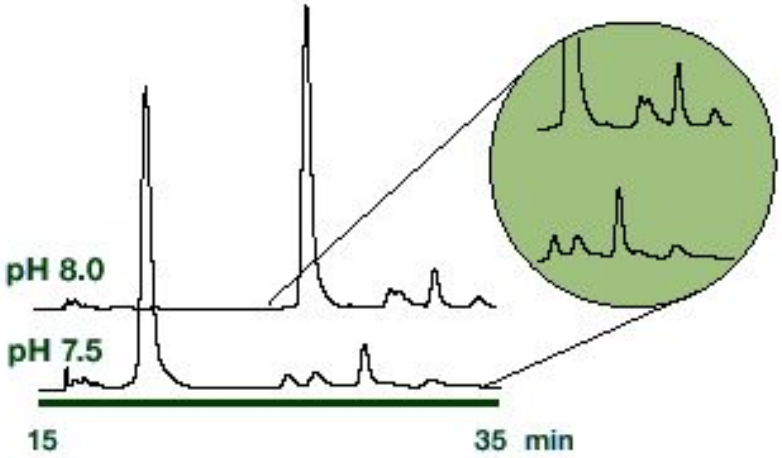
Vydac 301VHP575 (DEAE anion exchange, 5 µm, 7.5 mm i.d. x 50 mm).
Eluent: 10 mM Tris-HCL, pH 7.5 and 8.0,
gradient 0-0.5 M NaCl in 50 min.
Sample: conalbumin.



In the separation of several small peaks following carbonic anhydrase, changing the pH from 8.0 to 7.5 resulted in significantly better resolution as well as reduced retention.

Conditions

Column: Vydac 300VHP575 (DEAE anion exchange, 5 µm,
7.5 mm i.d. x 50 mm).
Eluent: 10 mM Tris-HCL, pH 7.5 and 8.0,
gradient 0-0.15 M NaCl in 60 min.
Sample: carbonic anhydrase.



Streamline Techniques