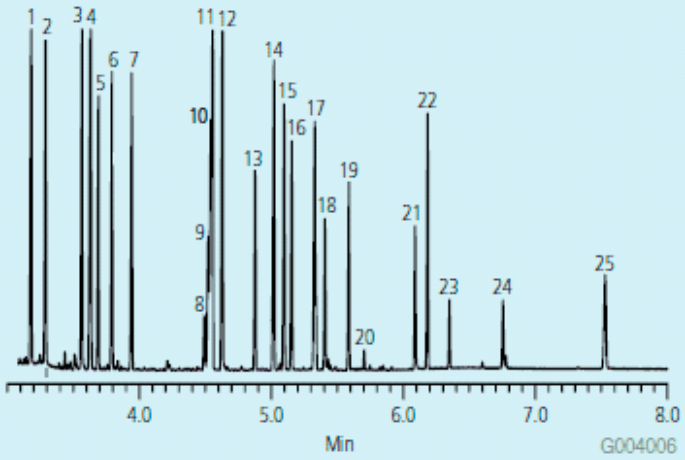
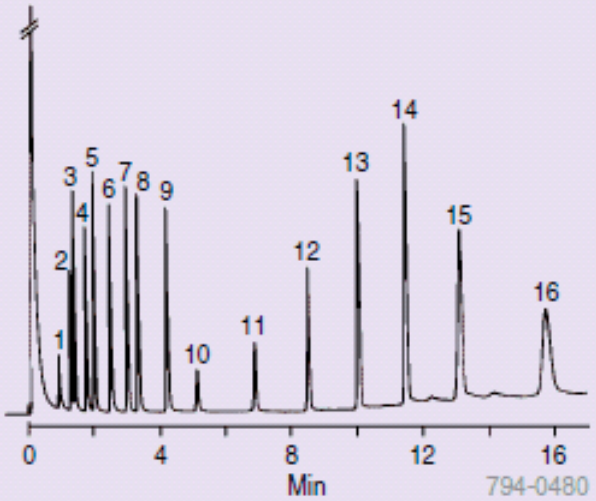
column: SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
oven: 100 °C (1 min.), 35 °C/min. to 290 °C (3 min.), 40 °C/min. to 360 °C
inj.: 250 °C
MSD interface: 325 °C
scan range: m/z = 40-450
carrier gas: helium, 1 mL/min., constant
injection: 0.5 µL, splitless (1.0 min.)
liner: 2 mm I.D., straight
sample: TBDMS derivatives of amino acids, each approximately 23 µg/mL

1. Alanine; m/z=260, 232, 158
2. Glycine; m/z=246, 218
3. Valine; m/z= 288, 260, 186
4. artifact from derivatization
5. Leucine; m/z=302, 274, 200
6. Isoleucine; m/z=302, 274, 200
7. Proline; m/z= 286, 258, 184
8. Asparagine, extra derivative; m/z= 327, 285, 243
9. Glutamine, extra derivative; m/z= 342, 300, 272
10. Methionine; m/z= 320, 292, 218
11. Serine; m/z= 390, 362
12. Threonine; m/z= 404, 376, 303
13. Phenylalanine; m/z= 336, 302, 234
14. Aspartic acid; m/z= 418, 390, 316
15. Hydroxyproline; m/z= 416, 388, 314
16. Cysteine; m/z= 406, 378
17. Glutamic acid; m/z= 432, 330, 272
18. Asparagine; m/z= 417, 302
19. Lysine; m/z= 431, 329, 300
20. Glutamine; m/z= 431, 357, 329, 299
21. Histidine; m/z= 459, 440, 338, 196
22. Tyrosine; m/z= 466, 438, 364, 302
23. Tryptophan, extra derivative m/z= 417, 375, 347, 302, 273
24. Tryptophan; m/z= 489, 302, 244
25. Cystine; m/z=639, 589, 537, 348



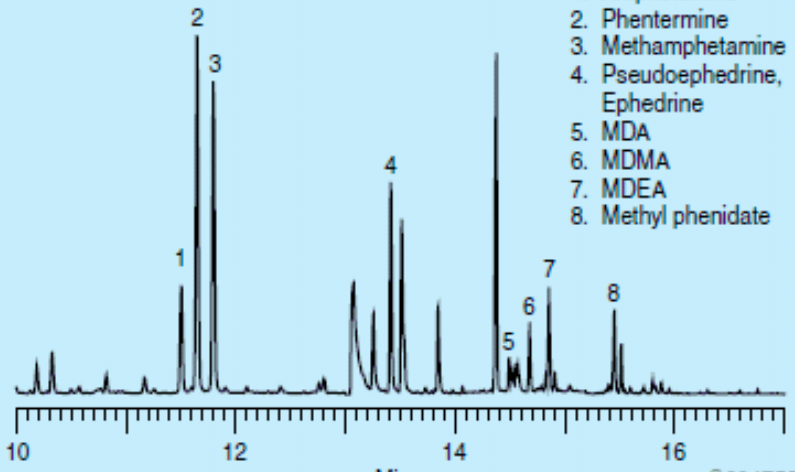
column: Nukol, 15 m x 0.53 mm I.D., 0.50 µm (25326)
oven: 100 °C, 10 °C/min. to 220 °C
det.: FID
carrier gas: helium, 30 mL/min.
injection: 0.5 µL, direct injection
sample: 16 analytes, at various concentrations from 50 to 800 µg/mL

1. Acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid
7. Isocaproic acid
8. Caproic acid
9. Heptanoic acid
10. Octanoic acid
11. Decanoic acid
12. Dodecanoic acid
13. Tetradecanoic acid
14. Hexadecanoic acid
15. Octadecanoic acid
16. Eicosanoic acid

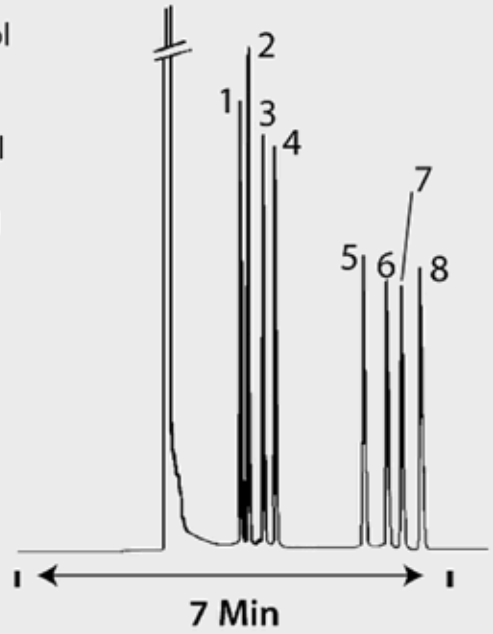


column: SLB-5ms, 20 m x 0.18 mm I.D. x 0.36 µm, 28576-U
oven: 75 °C (1 min.), 10 °C/min. to 190°C,
25 °C/min. to 300 °C (4 min.)
MSD interface: 300 °C
scan range: SIM, monitored m/z=116, 130, 144, 156 as primary ions
carrier gas: helium, 0.7 mL/min. constant flow
liner: 0.75 mm I.D. SPME

1. Amphetamine
2. Phentermine
3. Methamphetamine
4. Pseudoephedrine, Ephedrine
5. MDA
6. MDMA
7. MDEA
8. Methyl phenidate



1. Rhamnitol
2. Fucitol
3. Ribitol
4. Arabinitol
5. Mannitol
6. Galactitol
7. Glucitol
8. Inositol

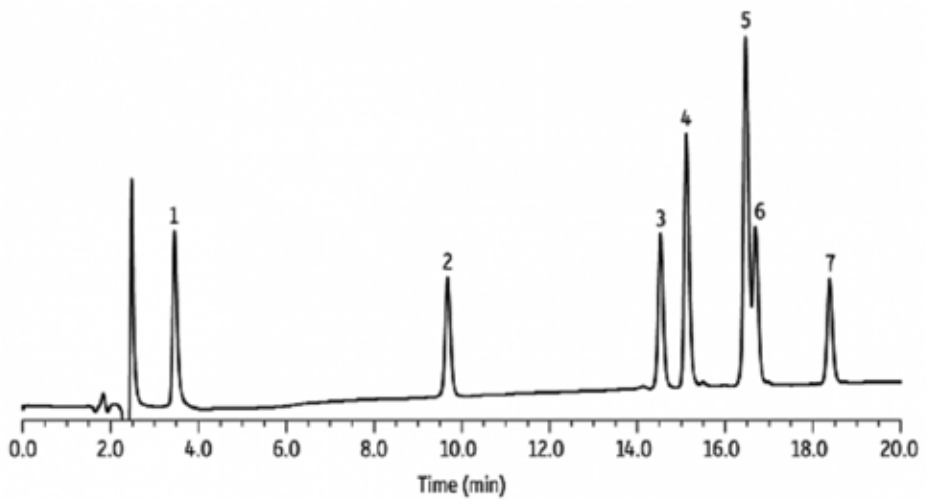


Conditions

column	SP-2380, 30 m x 0.25 mm I.D., 0.20 µm (24110-U)
oven	275 °C
carrier gas	helium, 20 cm/sec @ 275 °C
sample	alditol acetates, 6 mg/mL in chloroform
injection	1 µL100:1 split
detector	FID

Peaks
1. Val-Tyr
2. Val-Tyr-Val
3. Meth enkephalin

Peaks
4. Val-4-angiotensin III
5. Angiotensin II, III
6. Leucine enkephalin
7. Angiotensin I



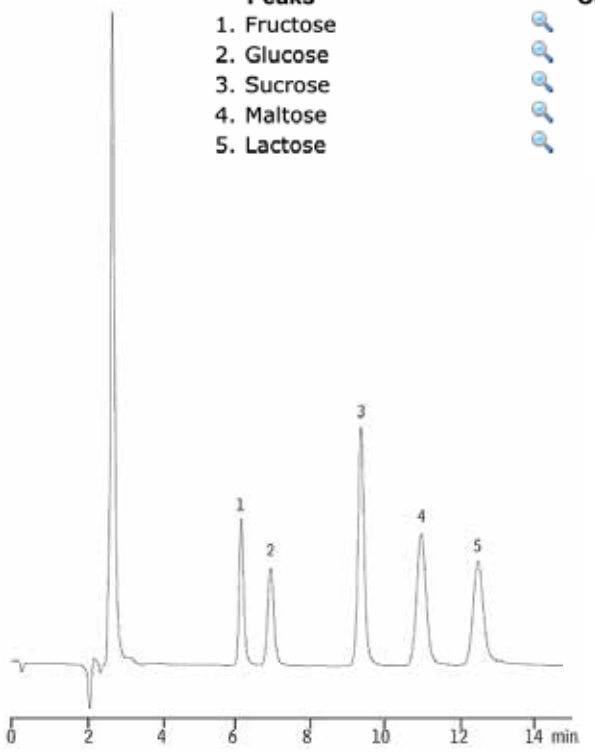
LC_BC0386

Column	Viva C18 (cat.# 9514562)
Dimensions:	150 mm x 2.1 mm ID
Particle Size:	5 µm
Pore Size:	300 Å
Temp.:	ambient
Sample	peptide test mix
Inj. Vol.:	20 µL
Mobile Phase	
A:	0.08% trifluoroacetic acid in water
B:	0.08% TFA in acetonitrile

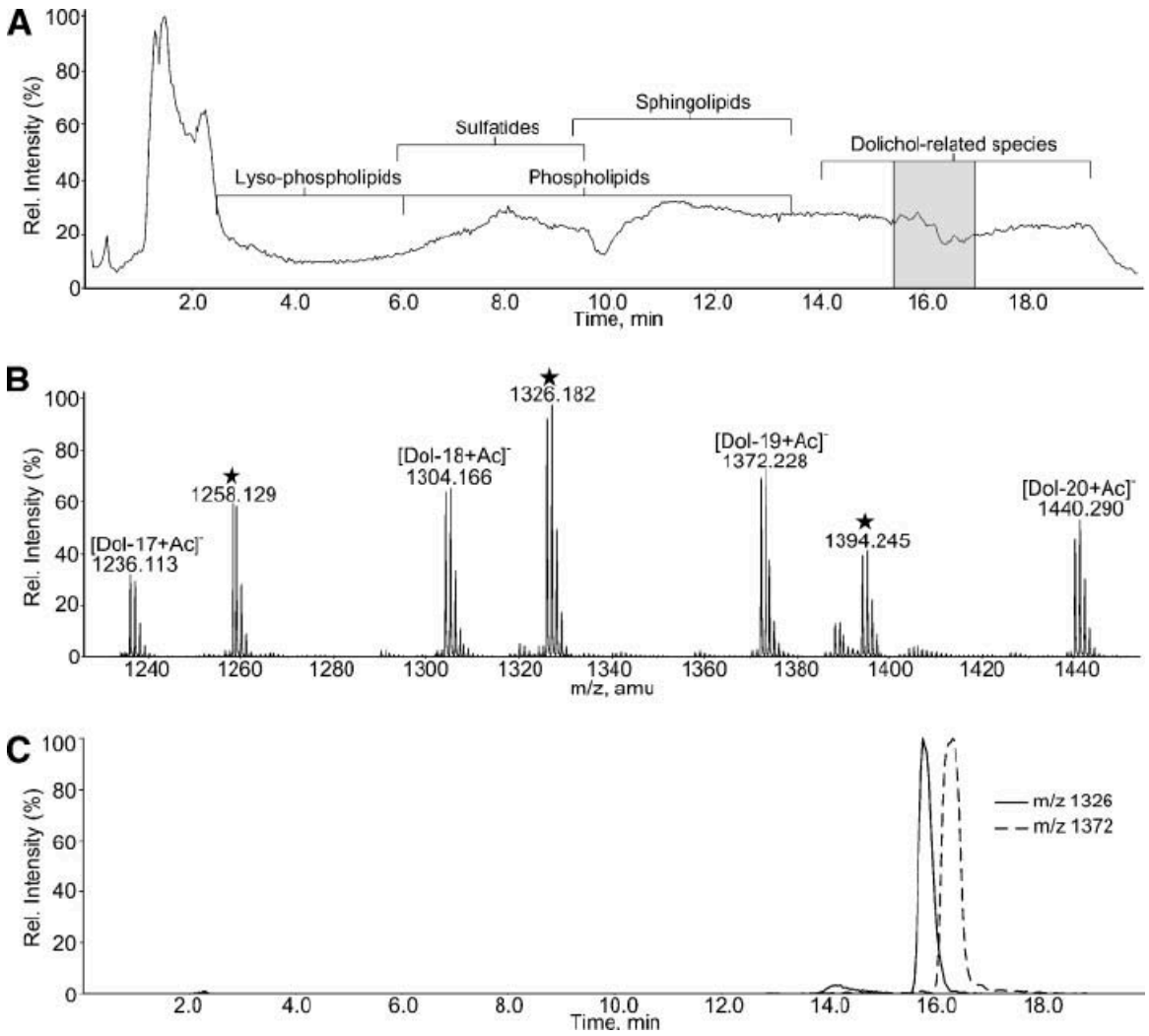
Time (min)	**%B**
0.00	10
20	40

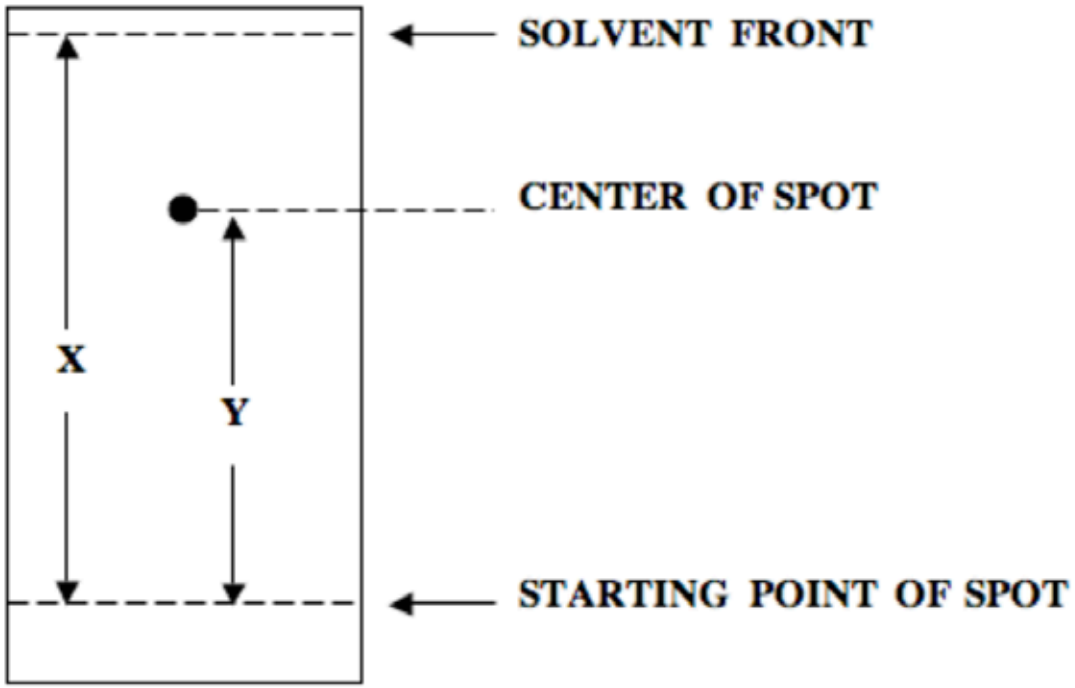
Flow:	0.2 mL/min
Detector	UV/Vis @ 214 nm

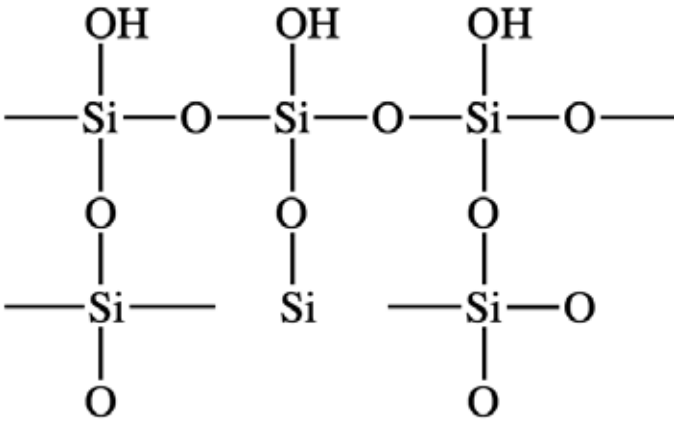
Peaks	Conc. (mg/mL)
1. Fructose	2.0
2. Glucose	2.1
3. Sucrose	4.0
4. Maltose	4.5
5. Lactose	4.4



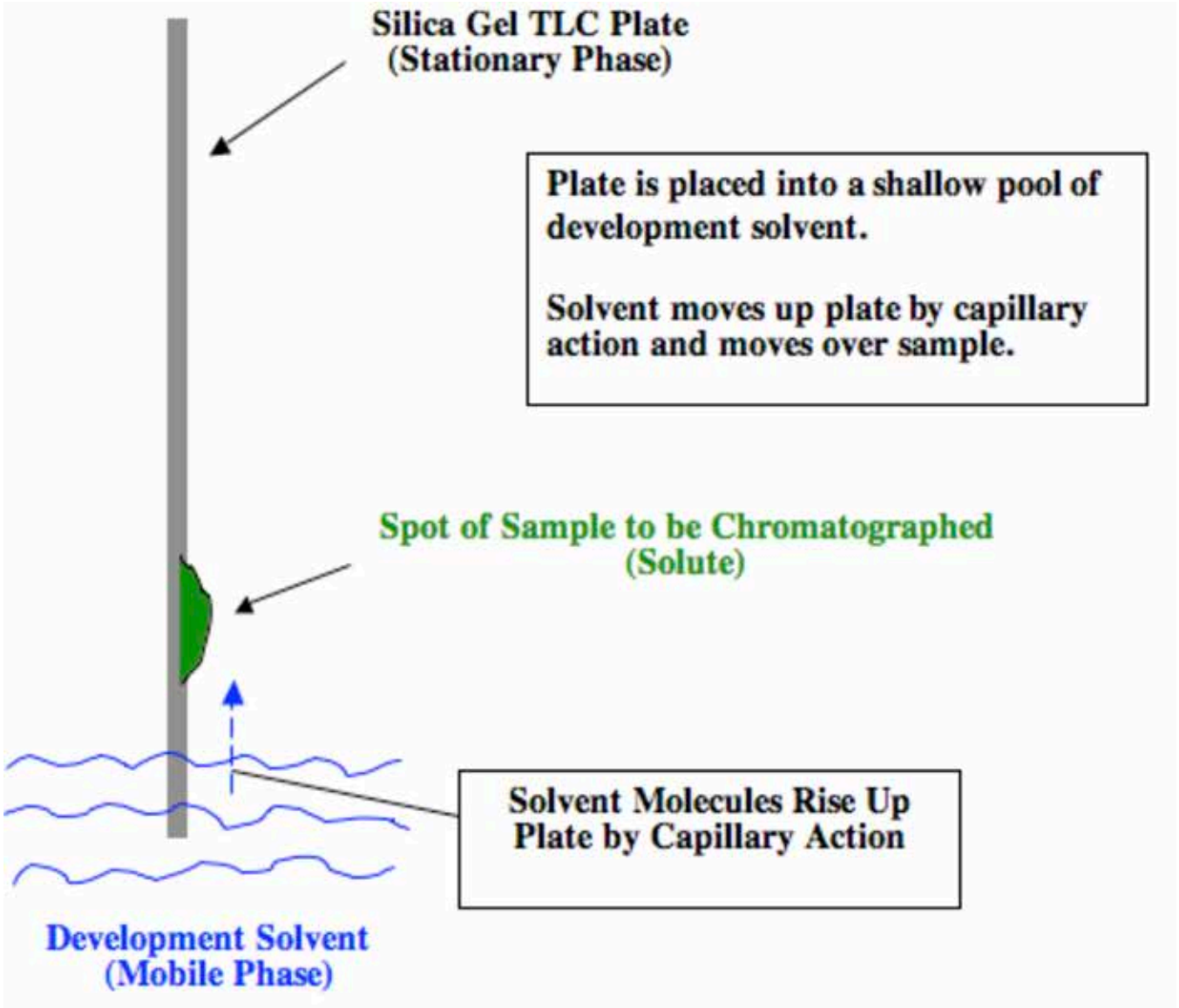
Column	Ultra Amino (cat.# 9107365)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	3 µm
Pore Size:	100 Å
Temp.:	35 °C
Sample	
Diluent:	Mobile phase
Inj. Vol.:	20 µL
Mobile Phase	Water:acetonitrile (25:75)
Flow:	0.8 mL/min
Detector	RI
Cell Temp:	35 °C

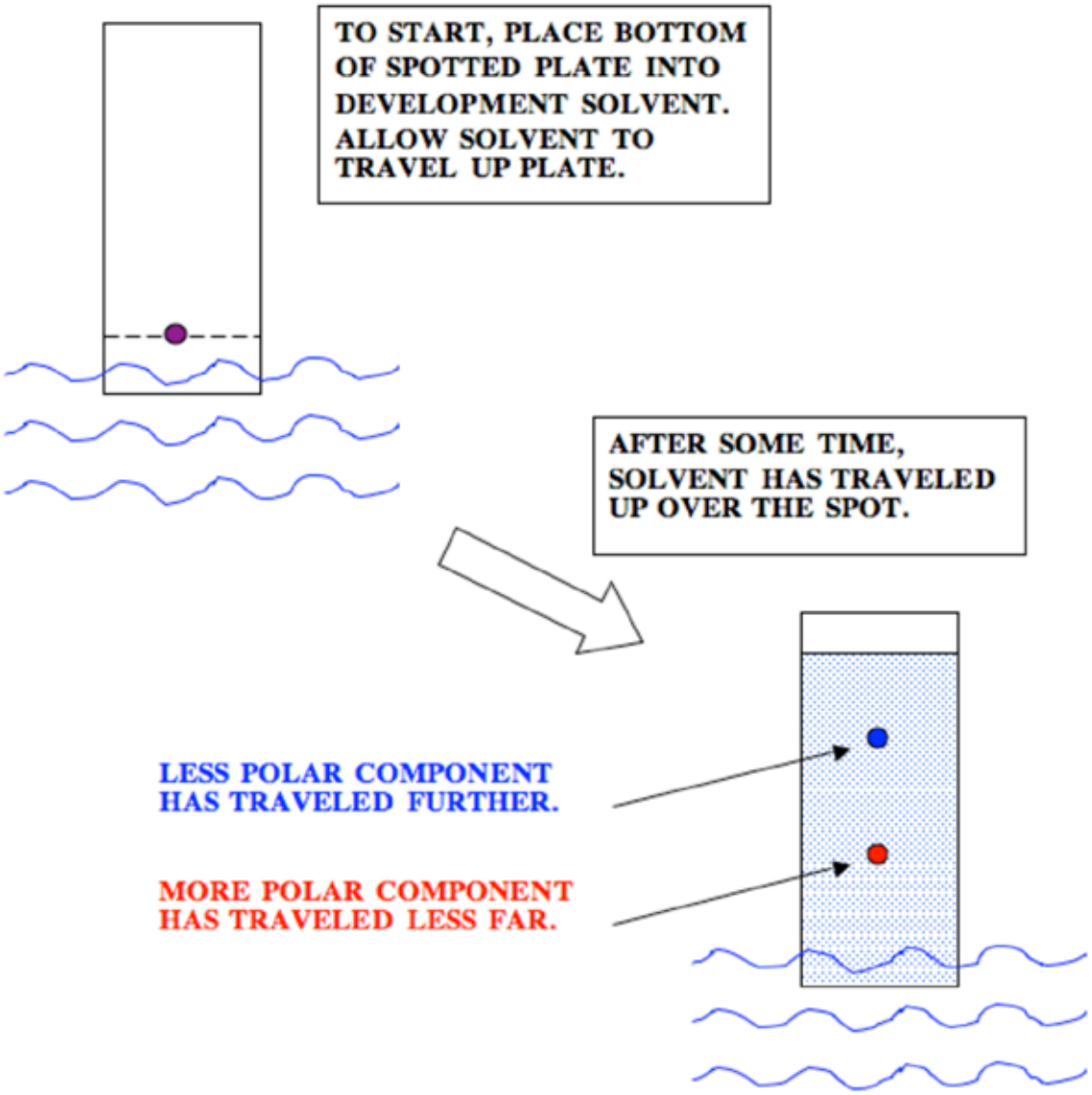

A
Rel. Intensity (%)
Time, min
Lyso-phospholipids
Phospholipids
Sulfatides
Sphingolipids
Dolichol-related species
B
Rel. Intensity (%)
m/z, amu
[Dol-17+Ac]⁻ 1236.113
1258.129
[Dol-18+Ac]⁻ 1304.166
1326.182
[Dol-19+Ac]⁻ 1372.228
1394.245
[Dol-20+Ac]⁻ 1440.290
C
Rel. Intensity (%)
Time, min
m/z 1326
m/z 1372

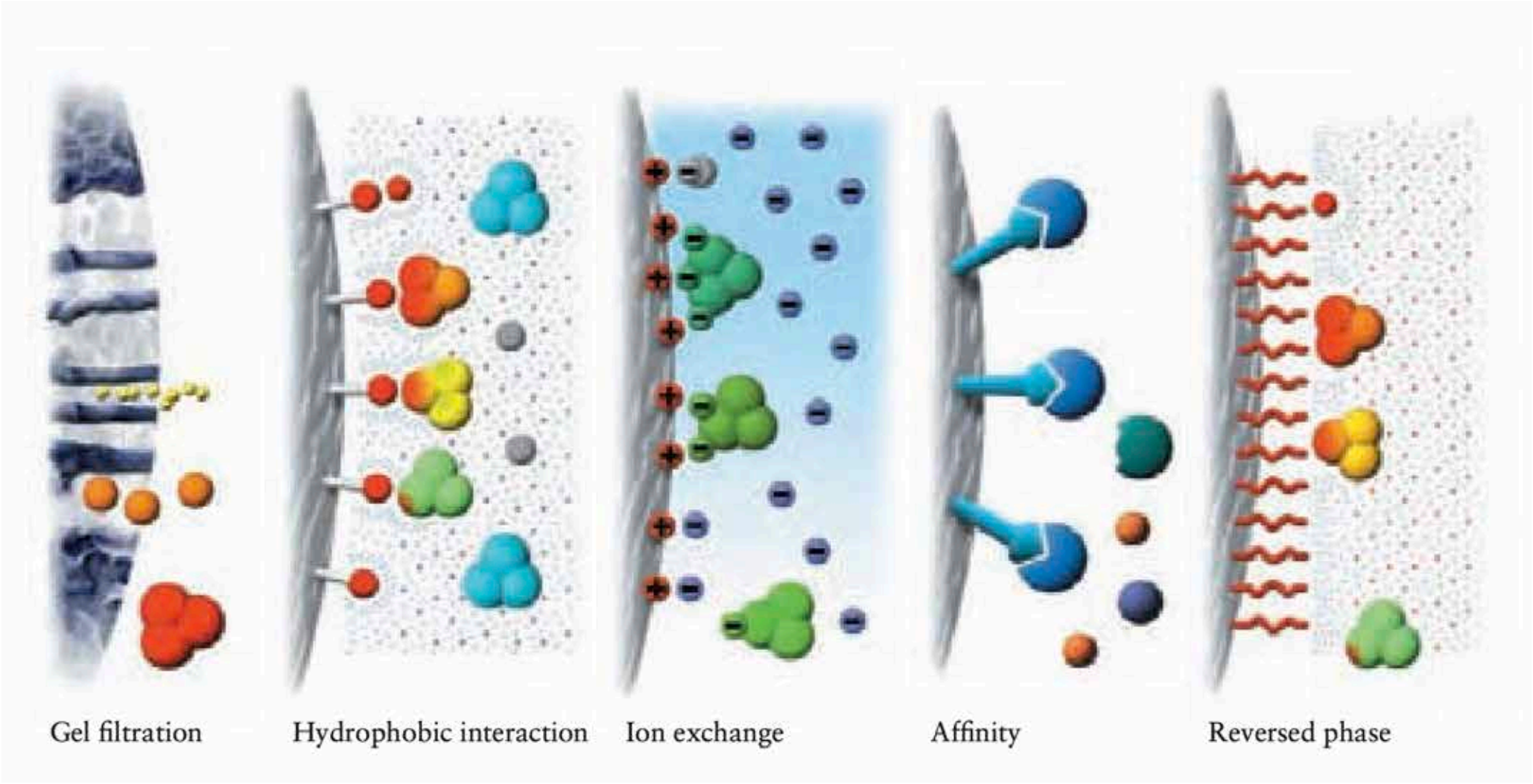




$R_f = Y/X$ (always ≤ 1)


Silica Gel TLC Plate
(Stationary Phase)
Plate is placed into a shallow pool of development solvent.
Solvent moves up plate by capillary action and moves over sample.
Spot of Sample to be Chromatographed
(Solute)
Solvent Molecules Rise Up Plate by Capillary Action
Development Solvent
(Mobile Phase)

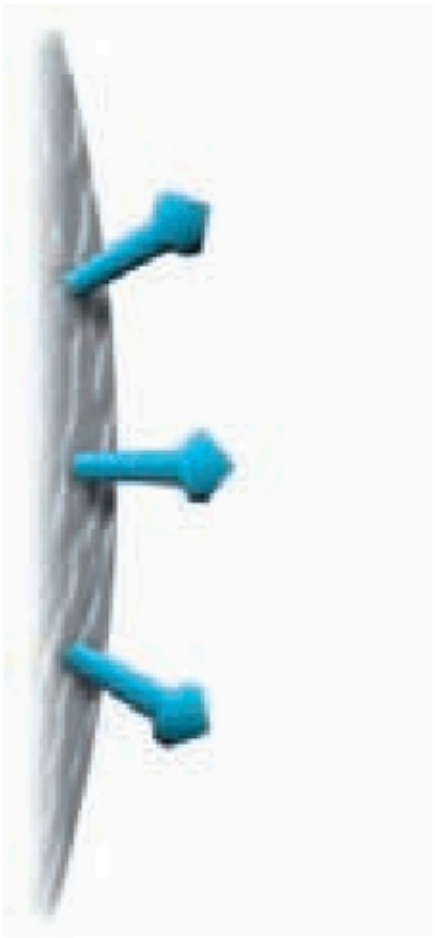

TO START, PLACE BOTTOM OF SPOTTED PLATE INTO DEVELOPMENT SOLVENT. ALLOW SOLVENT TO TRAVEL UP PLATE.
AFTER SOME TIME, SOLVENT HAS TRAVELED UP OVER THE SPOT.
LESS POLAR COMPONENT HAS TRAVELED FURTHER.
MORE POLAR COMPONENT HAS TRAVELED LESS FAR.



Affinity Chromatography Principles and Methods, GE Healthcare

Affinity chromatography separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand coupled to a chromatography matrix. The technique is ideal for a capture or intermediate step in a purification protocol and can be used whenever a suitable ligand is available for the protein(s) of interest. With high selectivity, hence high resolution, and high capacity for the protein(s) of interest, purification levels in the order of several thousand-fold with high recovery of active material are achievable. Target protein(s) is collected in a purified, concentrated form.

Affinity Chromatography Principles and Methods, GE Healthcare



Matrix: for ligand attachment. Matrix should be chemically and physically inert.

Spacer arm: used to improve binding between ligand and target molecule by overcoming any effects of steric hindrance.

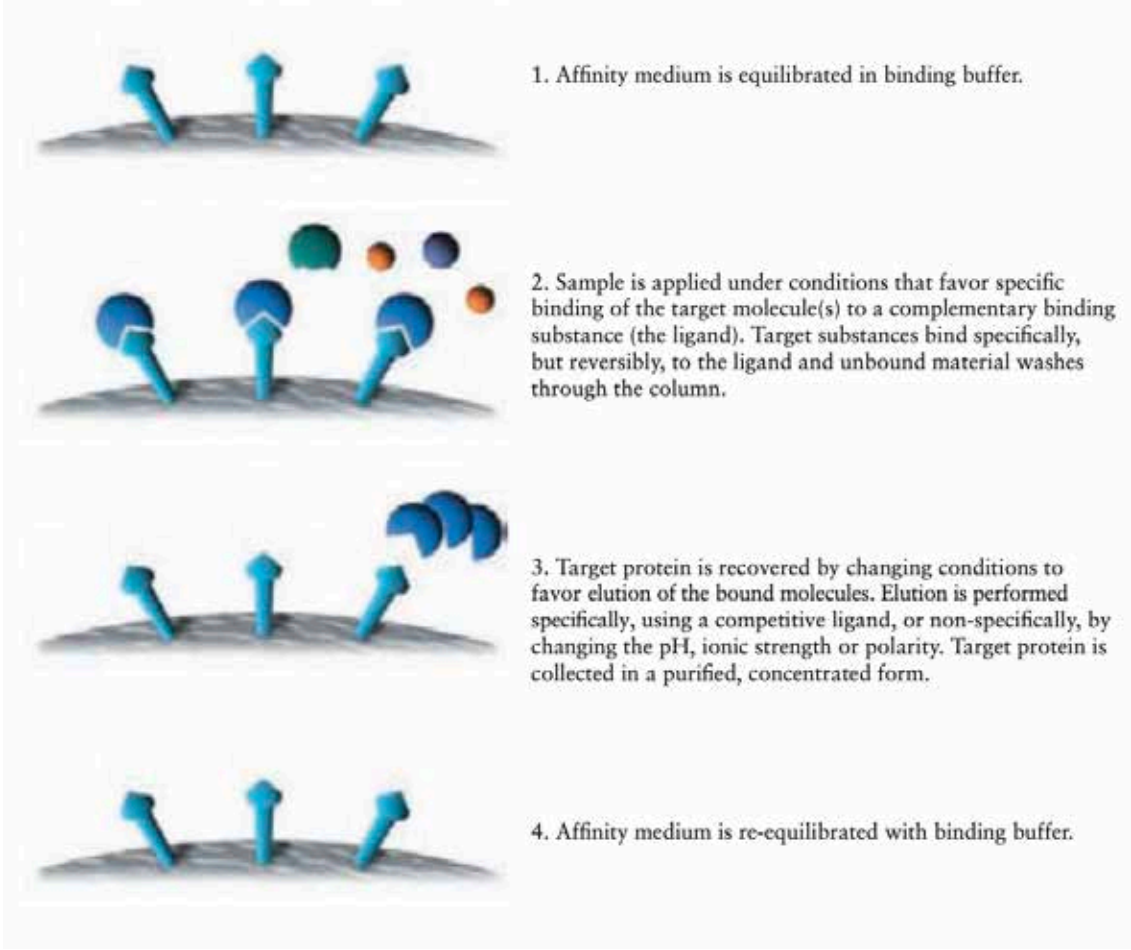
Ligand: molecule that binds reversibly to a specific target molecule or group of target molecules.

Affinity Chromatography Principles and Methods, GE Healthcare

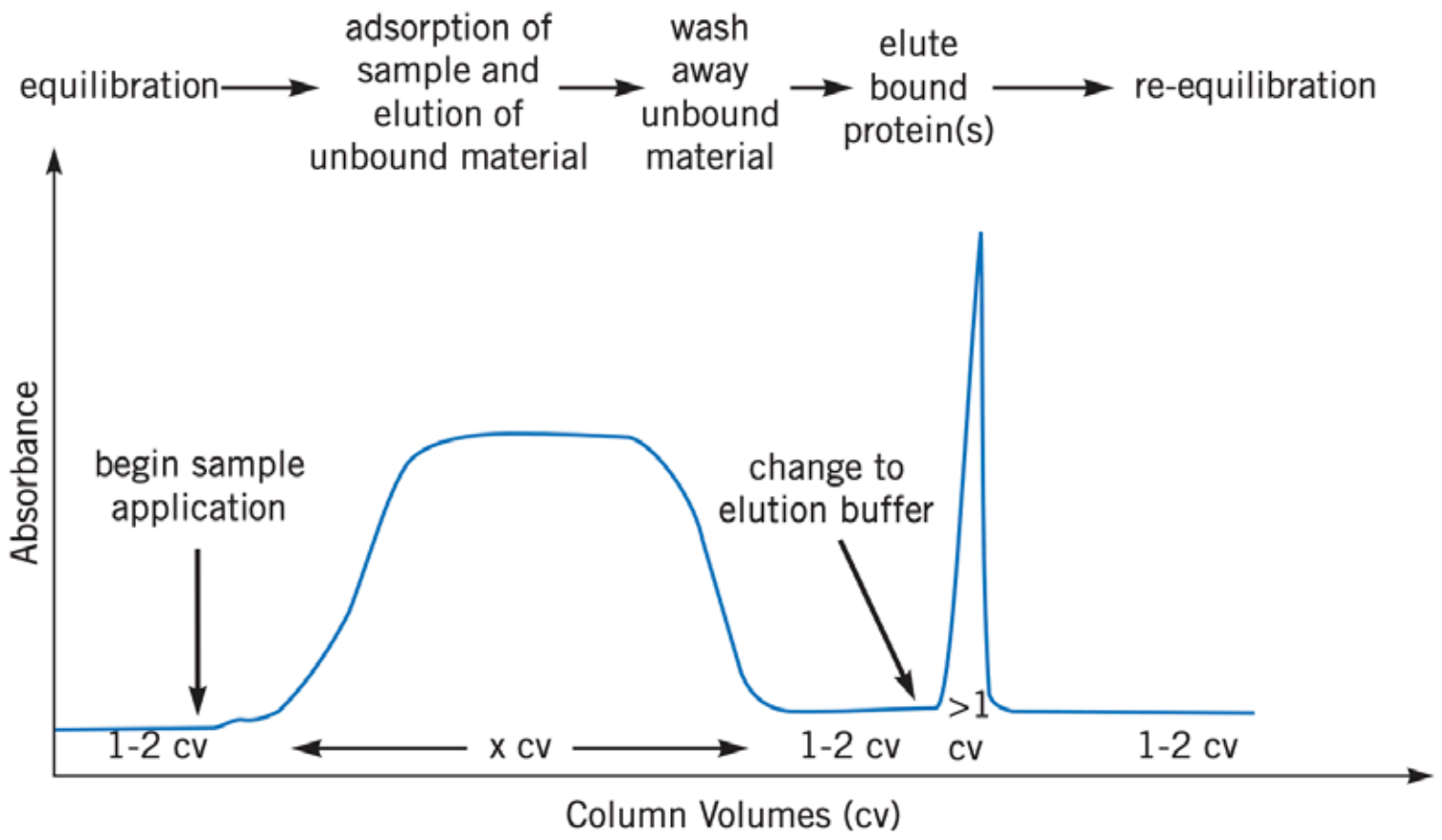
Any component can be used as a ligand to purify its respective binding partner. Some typical biological interactions, frequently used in affinity chromatography, are listed below:

- Enzyme ☐ substrate analogue, inhibitor, cofactor.
- Antibody ☐ antigen, virus, cell.
- Lectin ☐ polysaccharide, glycoprotein, cell surface receptor, cell.
- Nucleic acid ☐ complementary base sequence, histones, nucleic acid polymerase, nucleic acid binding protein.
- Hormone, vitamin ☐ receptor, carrier protein.
- Glutathione ☐ glutathione-S-transferase or GST fusion proteins.
- Metal ions ☐ Poly (His) fusion proteins, native proteins with histidine, cysteine and/or tryptophan residues on their surfaces.

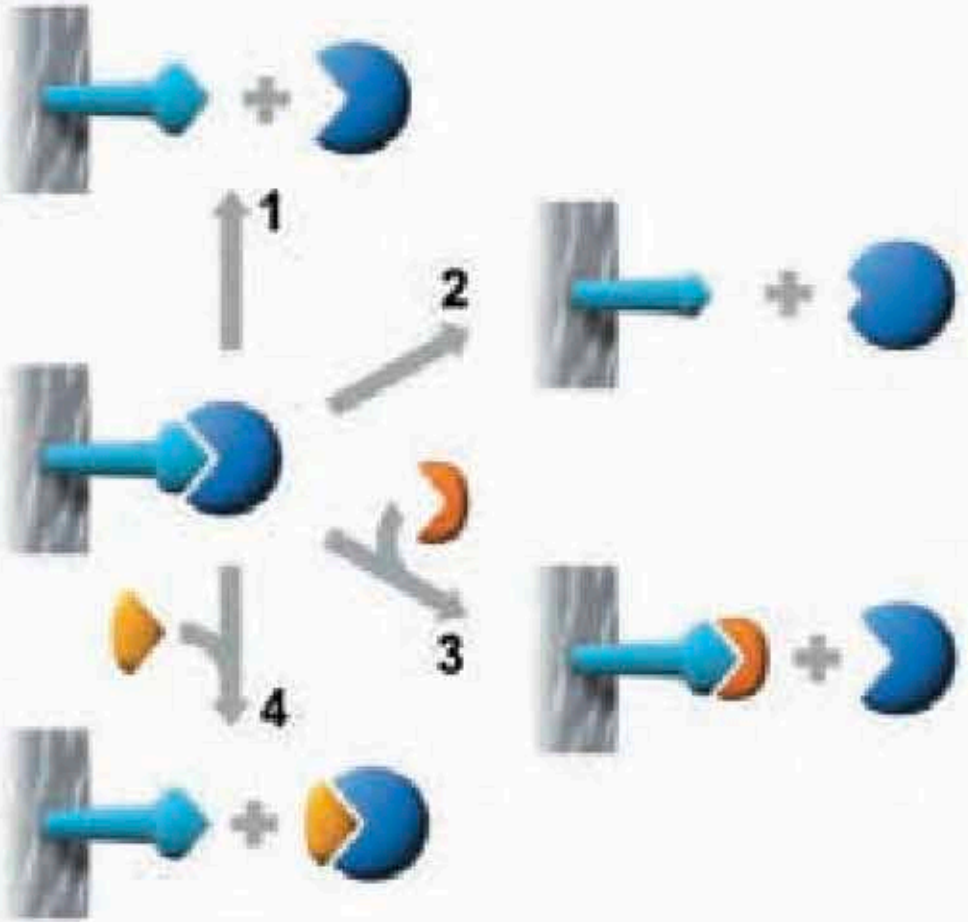
Affinity Chromatography Principles and Methods, GE Healthcare



Affinity Chromatography Principles and Methods, GE Healthcare



Affinity Chromatography Principles and Methods, GE Healthcare

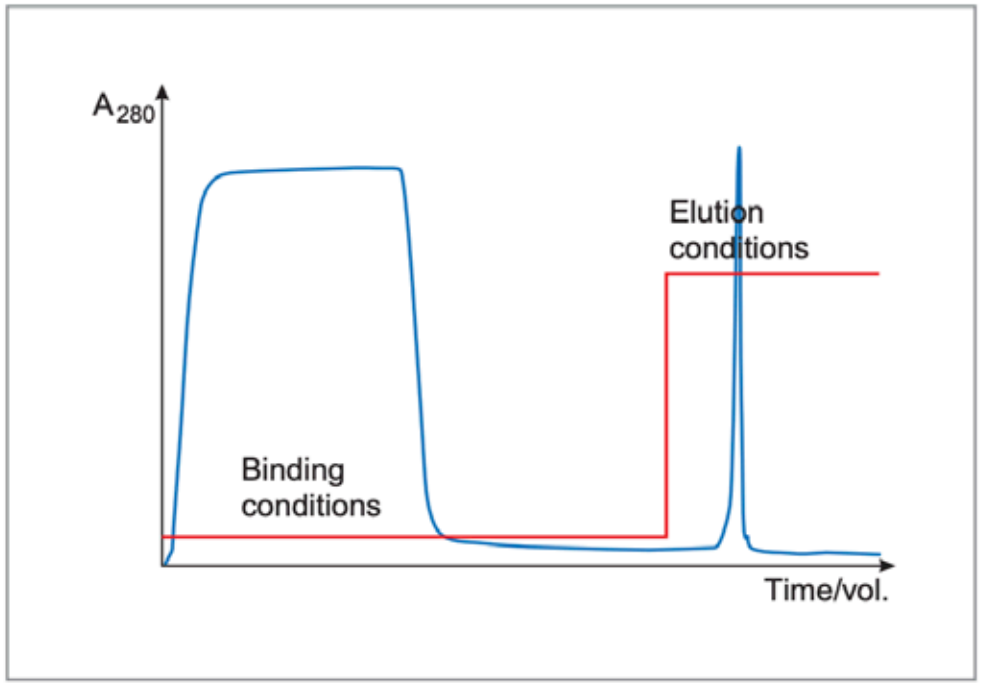


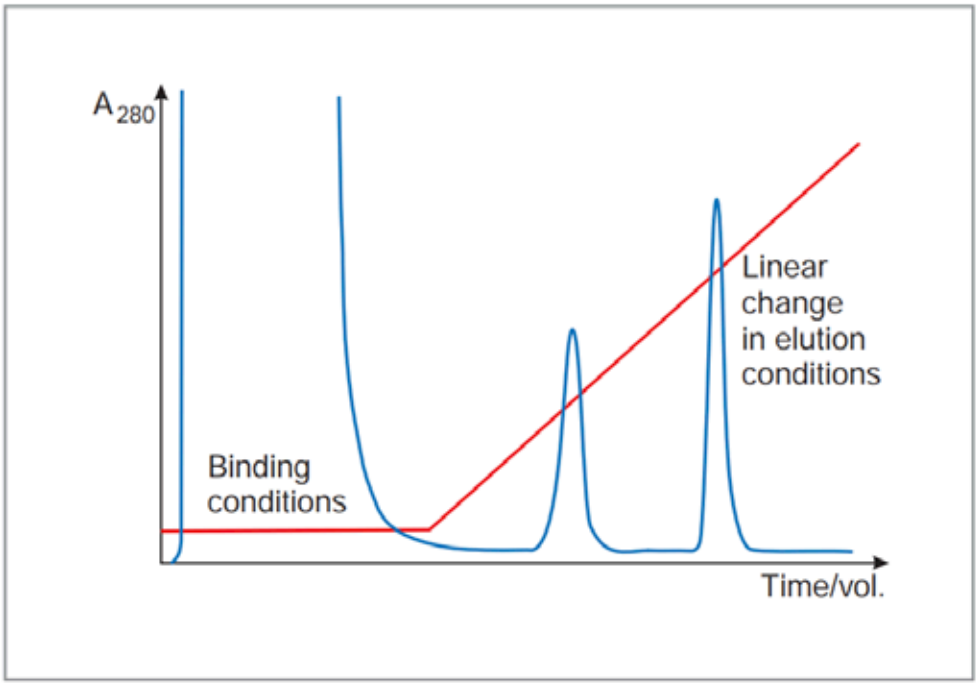
Method 1
The simplest case. A change of buffer composition elutes the bound substance without harming either it or the ligand.

Method 2
Extremes of pH or high concentrations of chaotropic agents are required for elution, but these may cause permanent or temporary damage.

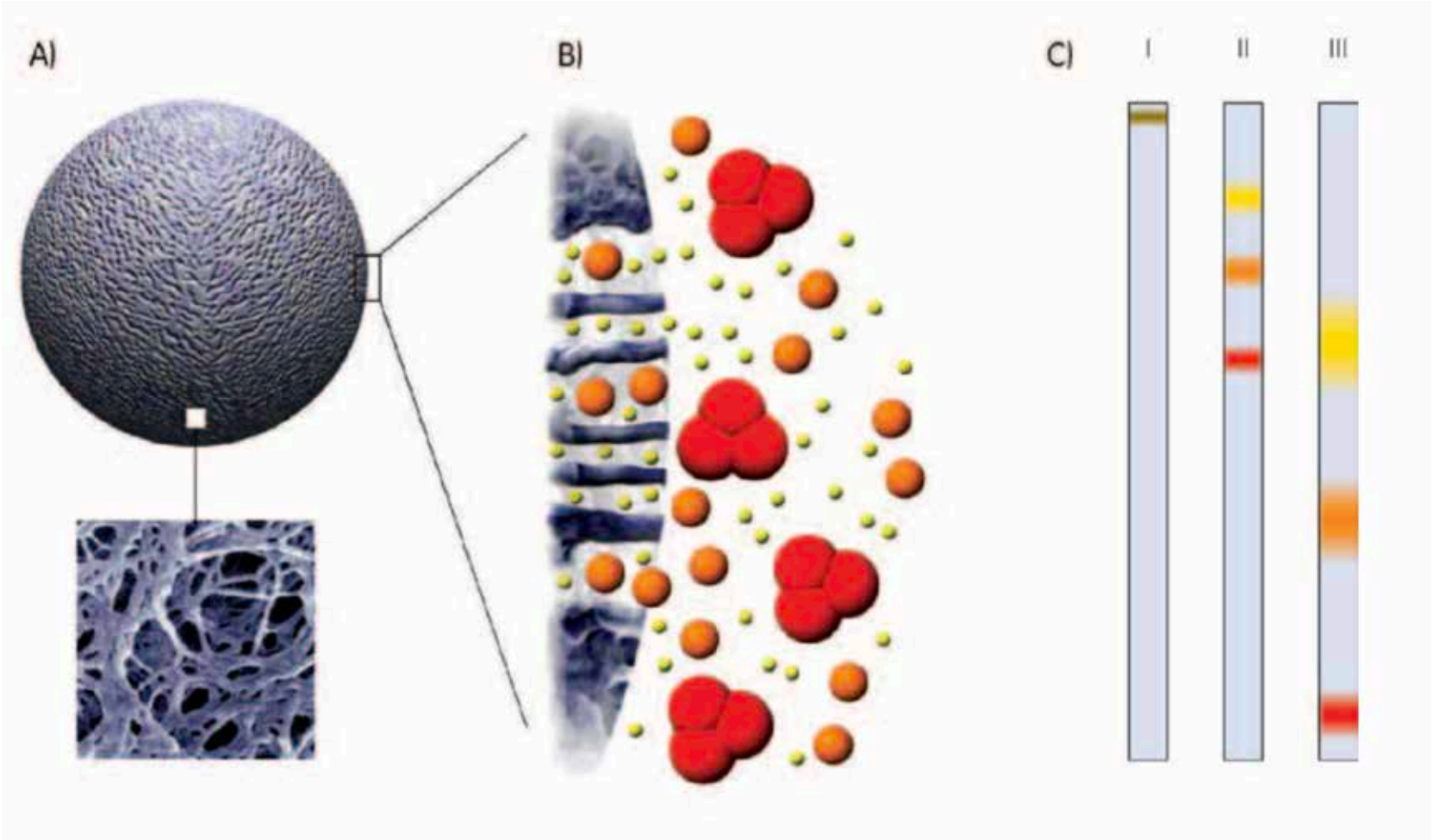
Methods 3 and 4
Specific elution by addition of a substance that competes for binding. These methods can enhance the specificity of media that use group-specific ligands.

Affinity Chromatography Principles and Methods, GE Healthcare

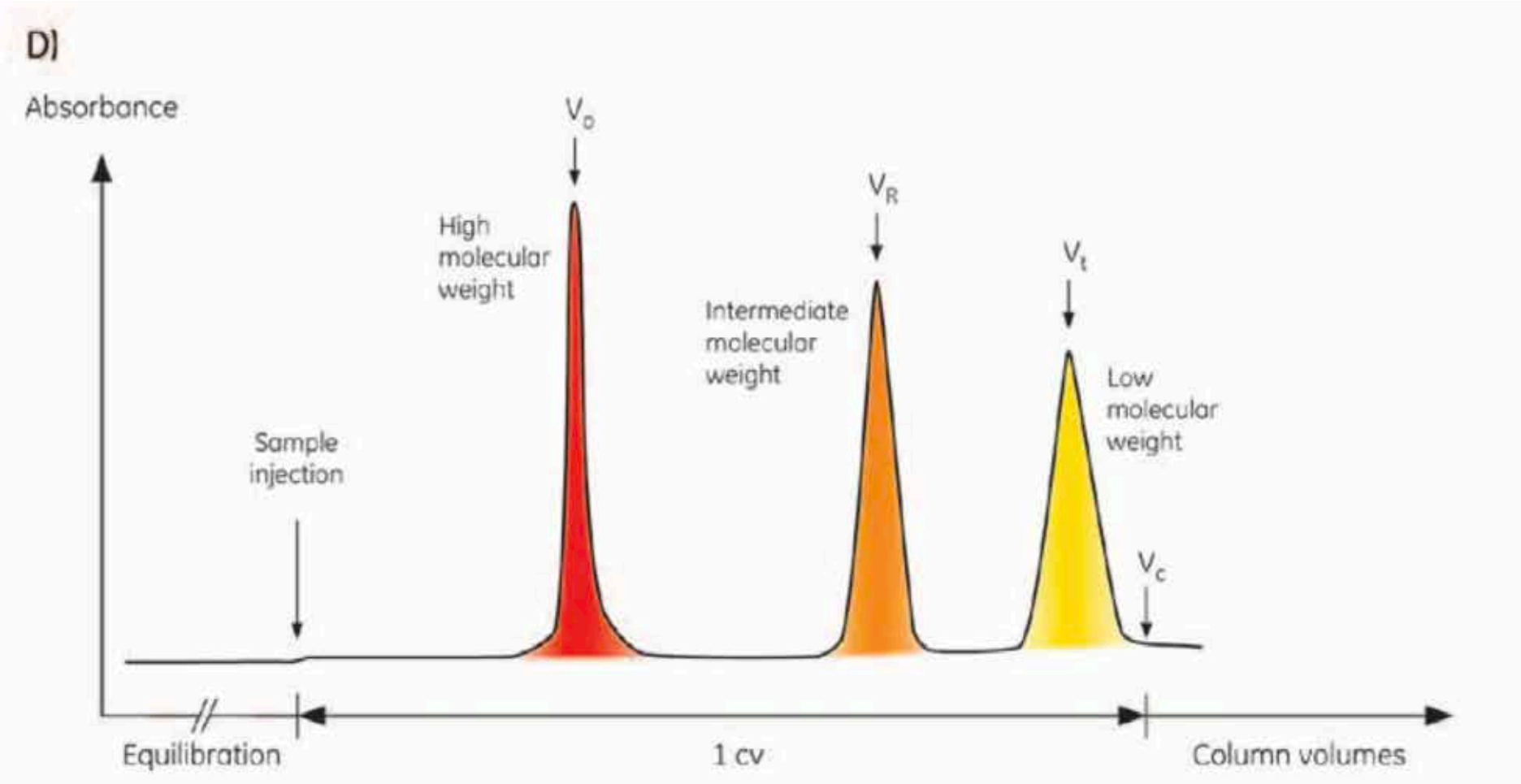




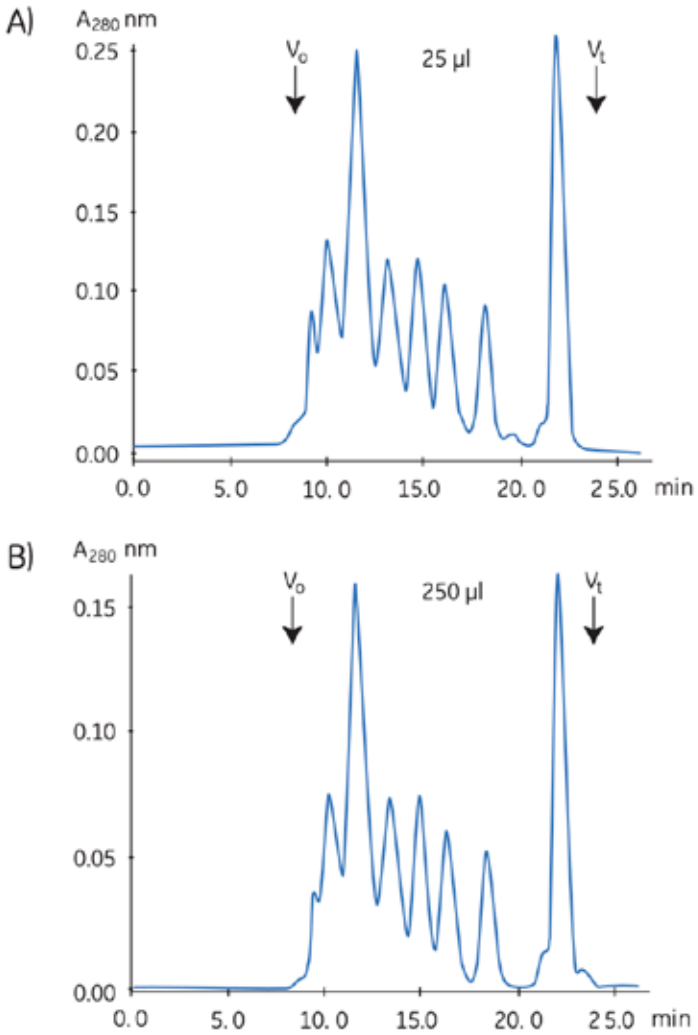
Affinity Chromatography Principles and Methods, GE Healthcare



Gel Filtration Principles and Methods, GE Healthcare

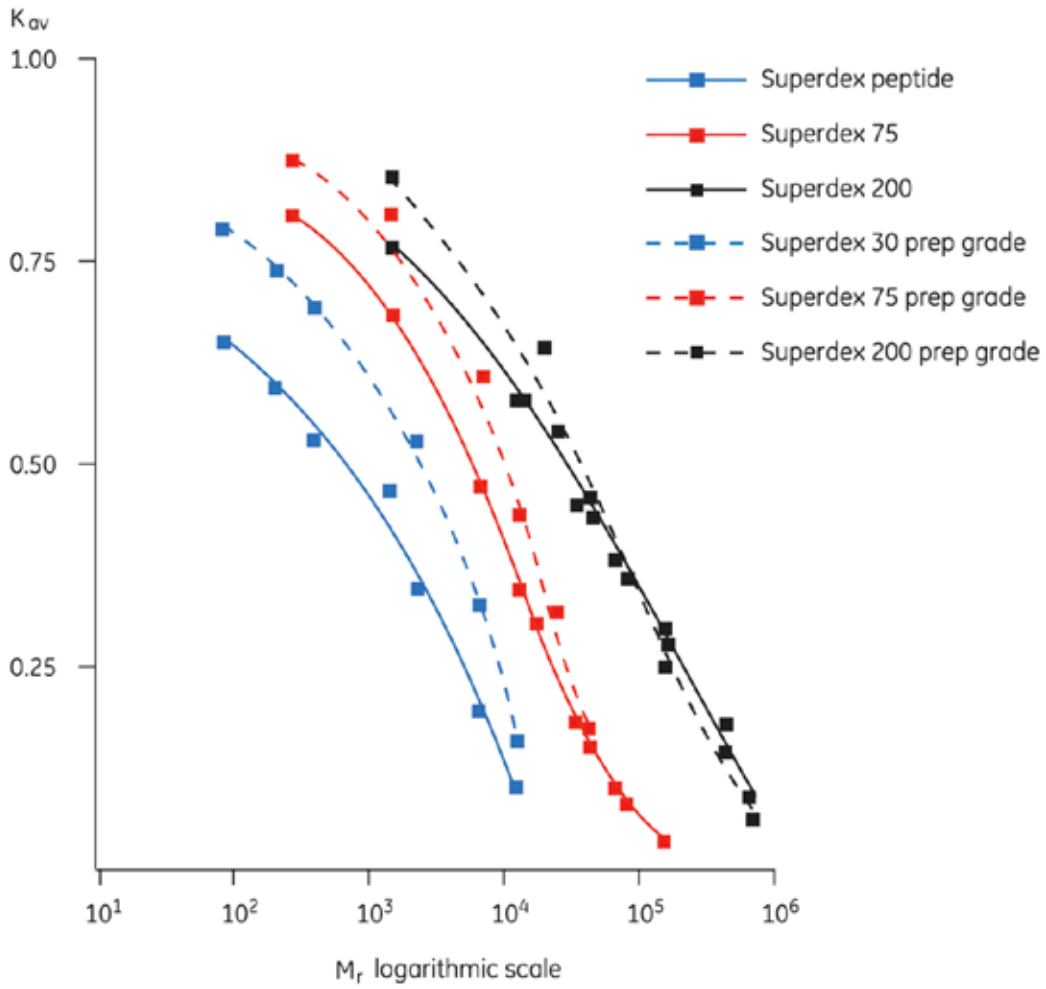


Gel Filtration Principles and Methods, GE Healthcare



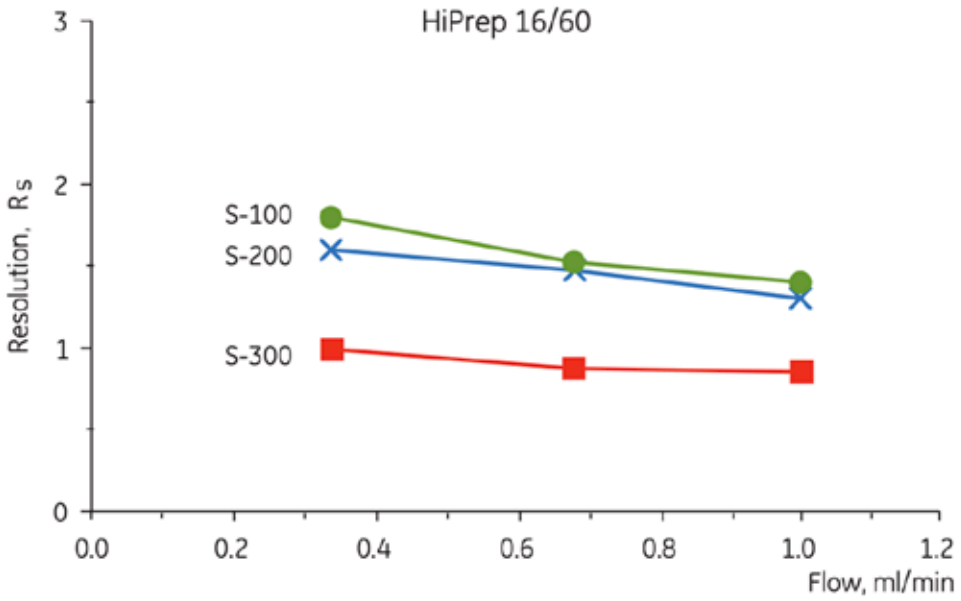
Column:	Superdex 200 HR 10/30 (V_t: 24 ml)	
Sample:	*M_r*	*Conc. (mg/ml)*
Thyroglobulin	669 000	3
Ferritin	440 000	0.7
IgG	150 000	3
Transferrin	81 000	3
Ovalbumin	43 000	3
Myoglobin	17 600	2
Vitamin B12	1 355	0.5
Total		15.2
Sample load:	A) 25 µl (0.1% × V_t) B) 250 µl (1% × V_t) C) 1000 µl (4.2% × V_t)	
Buffer:	0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0	
Flow rate:	1.0 ml/min (76.4 cm/h)	

Gel Filtration Principles and Methods, GE Healthcare

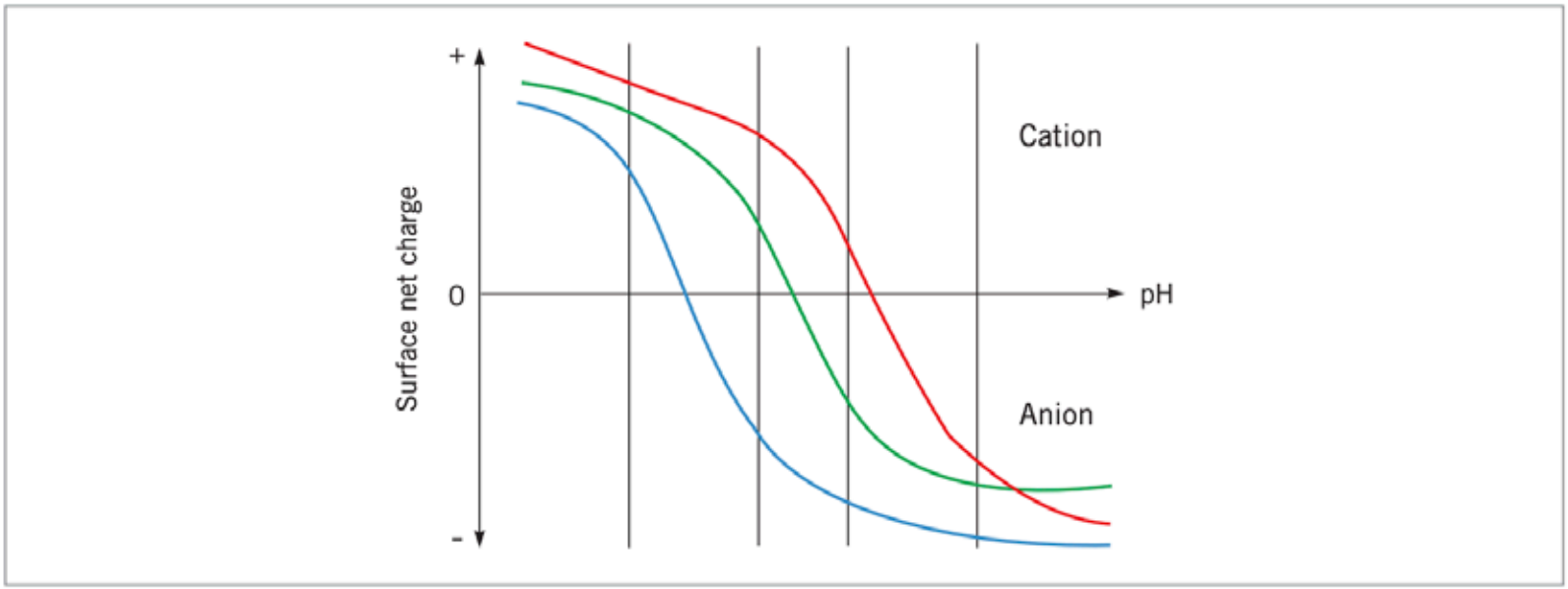


Gel Filtration Principles and Methods, GE Healthcare

Columns:	HiPrep™ 16/10 Sephacryl S-100 HR HiPrep 16/10 Sephacryl S-200 HR HiPrep 16/10 Sephacryl S-300 HR
Sample:	IgG, ovalbumin, cytochrome C, 1:2:1
Sample load:	2.4 ml (2% × V_t)
Total sample load:	8 mg
Buffer:	50 mM $NaPO_4$, 0.15 M NaCl, 0.02% NaN_3, pH 7.0

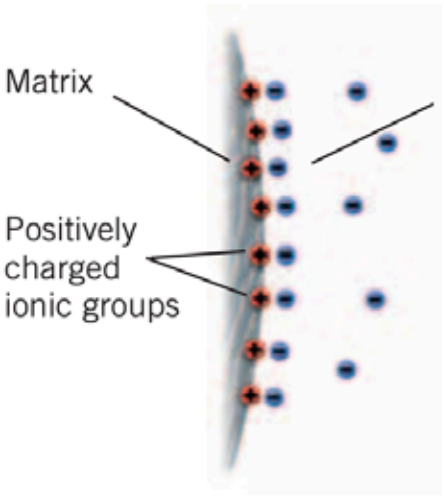


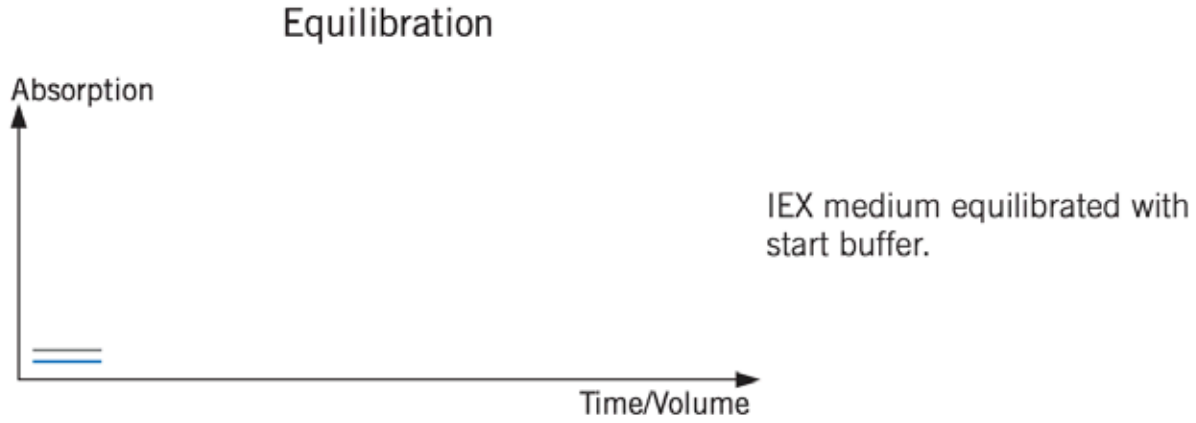
Gel Filtration Principles and Methods, GE Healthcare

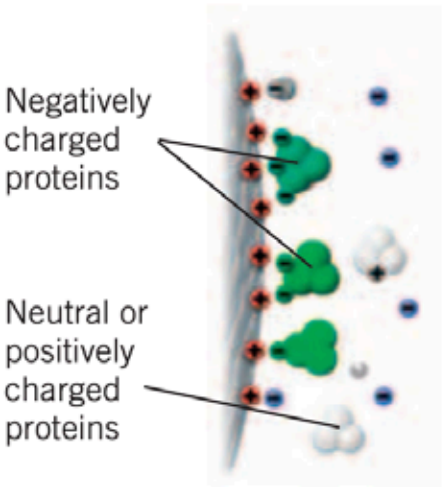


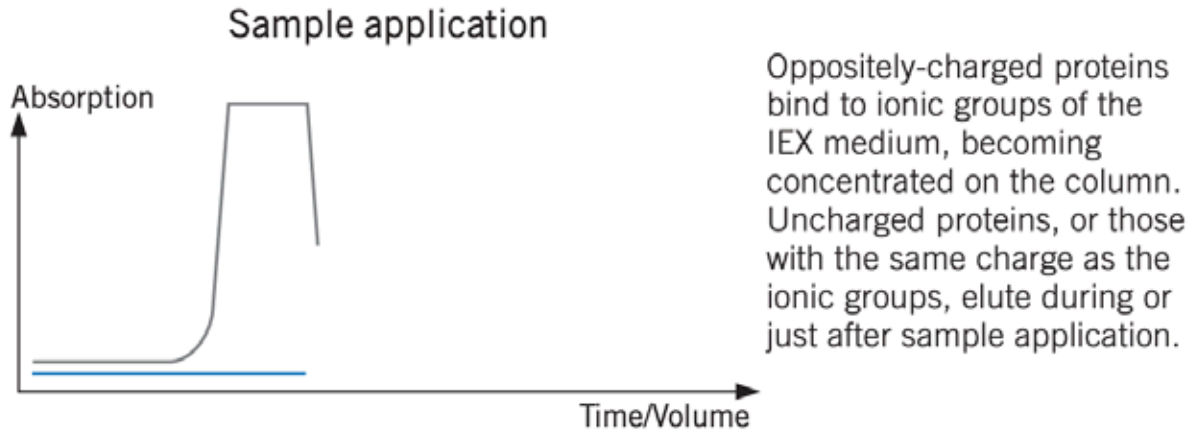
"IEX chromatography takes advantage of the fact that the relationship between net surface charge and pH is unique for a specific protein. A protein that has no net charge at a pH equivalent to its isoelectric point (pI) will not interact with a charged medium."

Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare

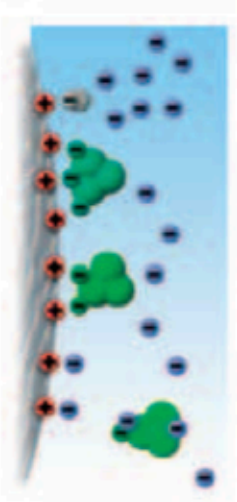


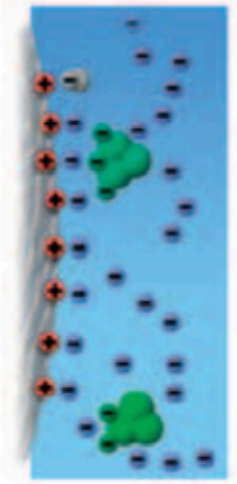


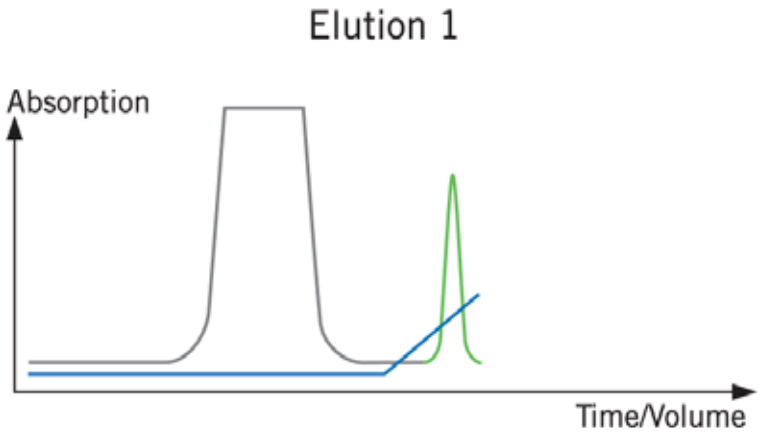




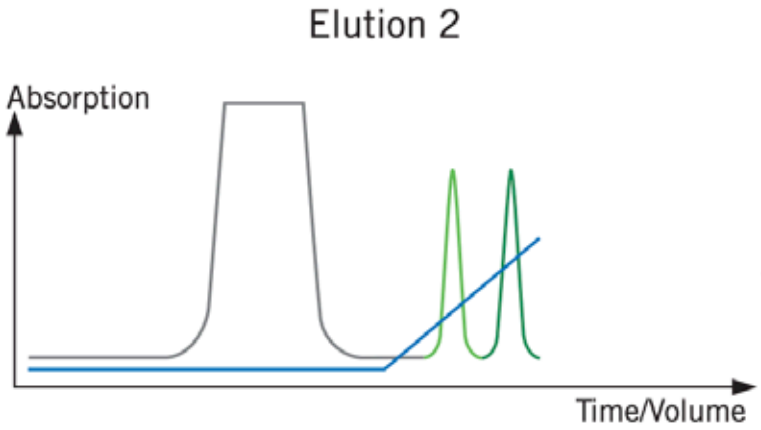
Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare





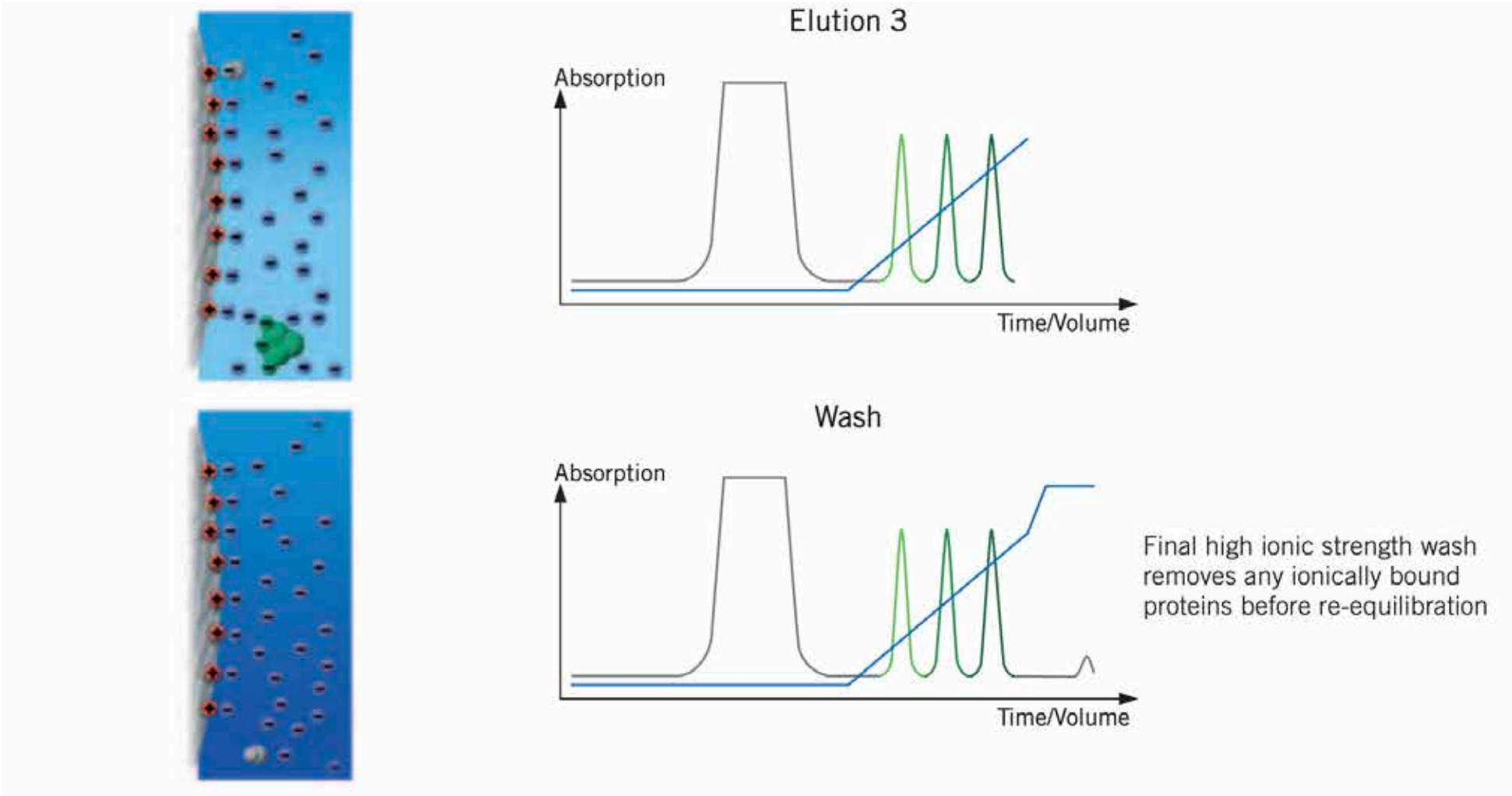


Increasing ionic strength (using a gradient) displaces bound proteins as ions in the buffer compete for binding sites.

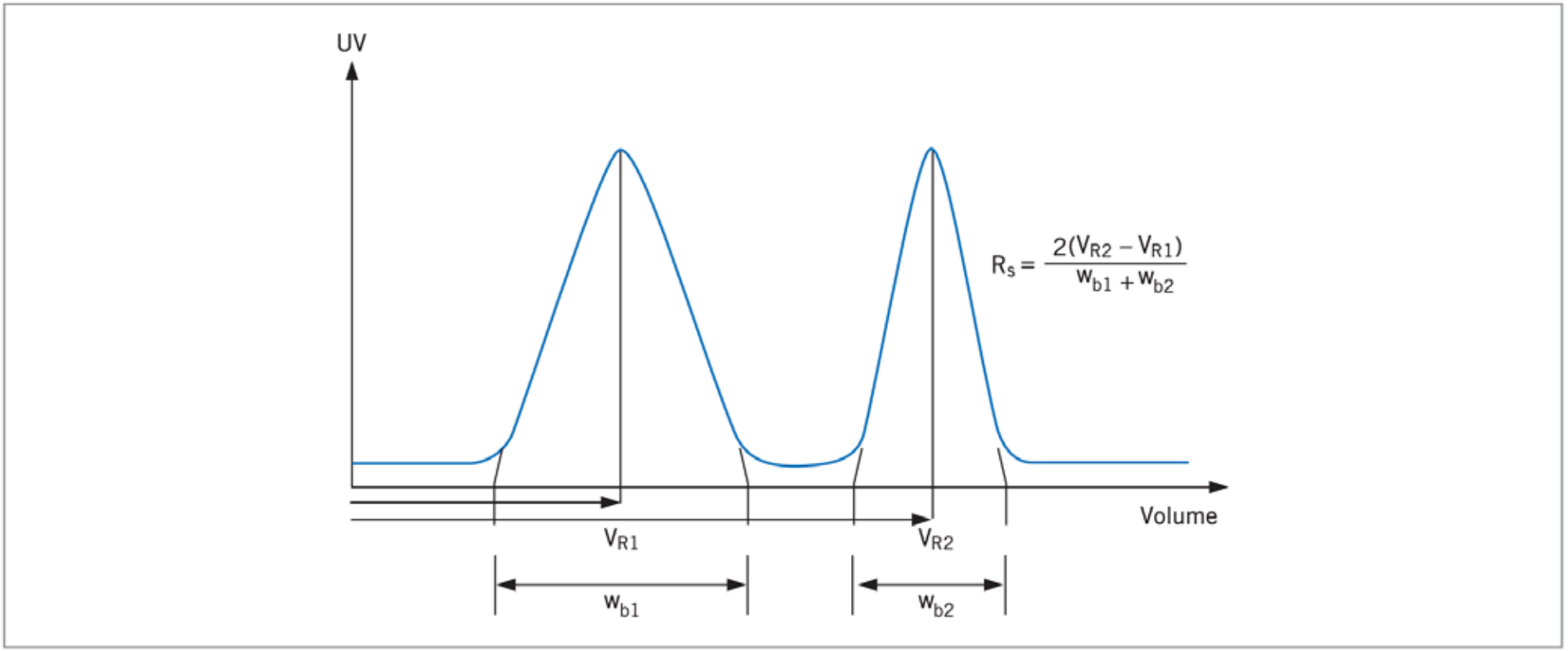


Further increases in ionic strength displace proteins that are more highly charged (more tightly bound)

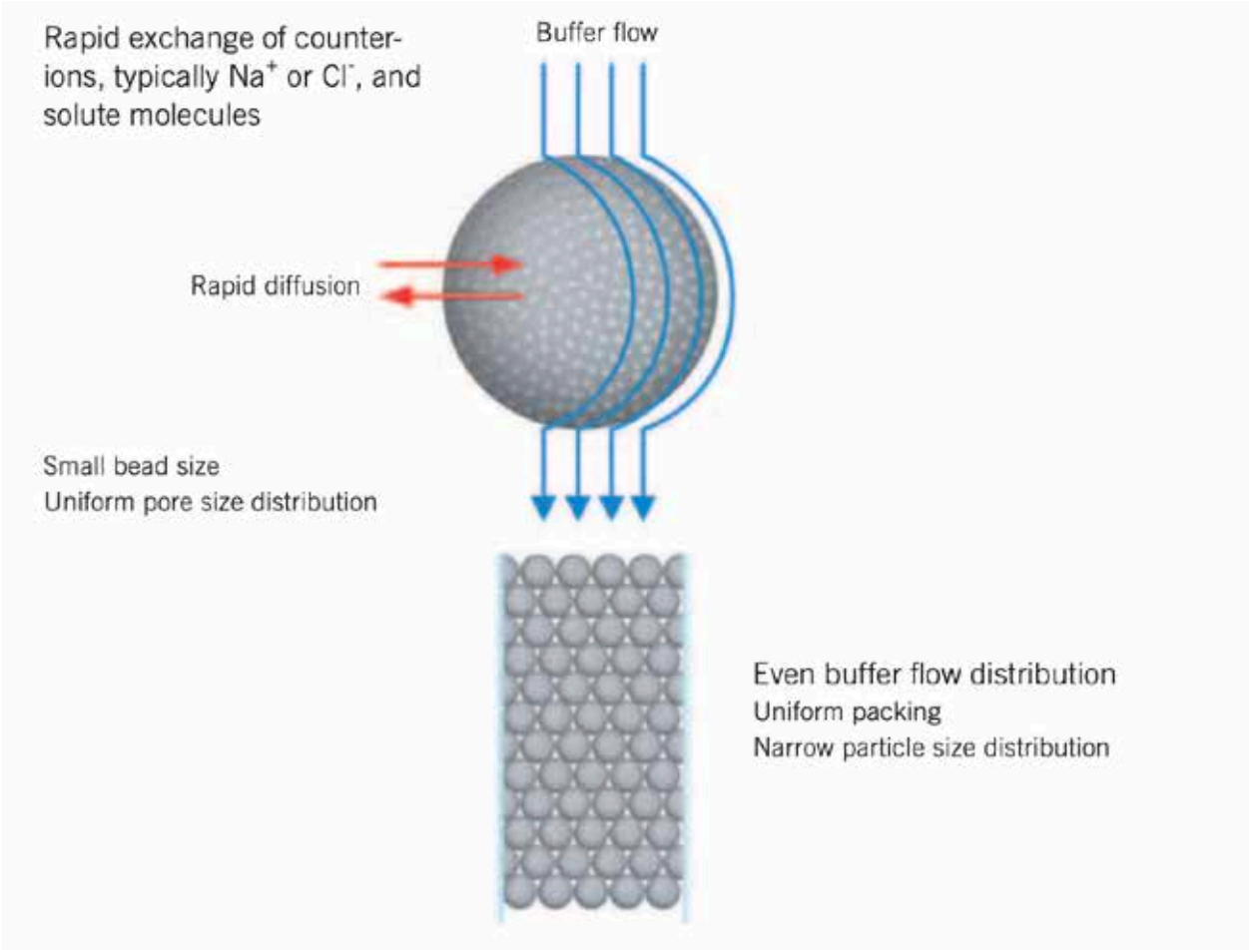
Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare



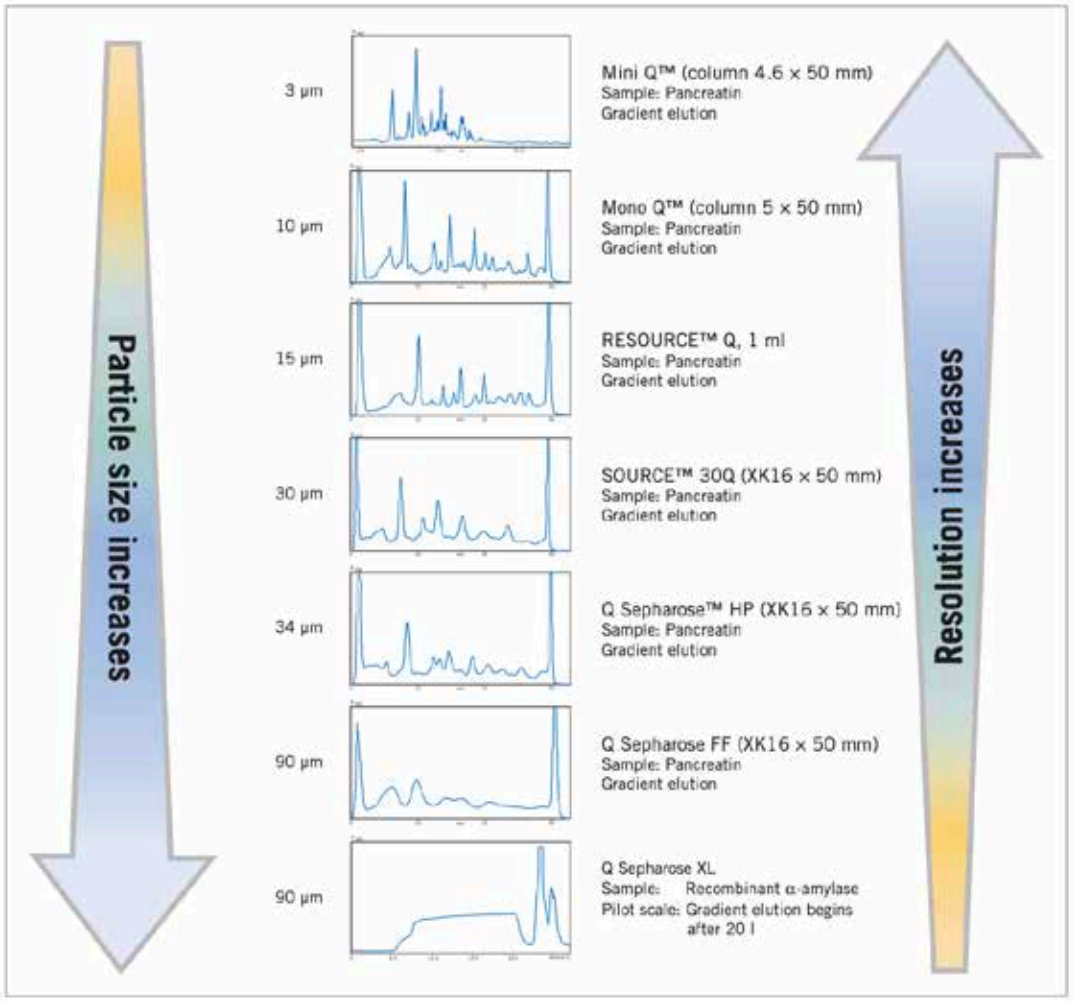
Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare



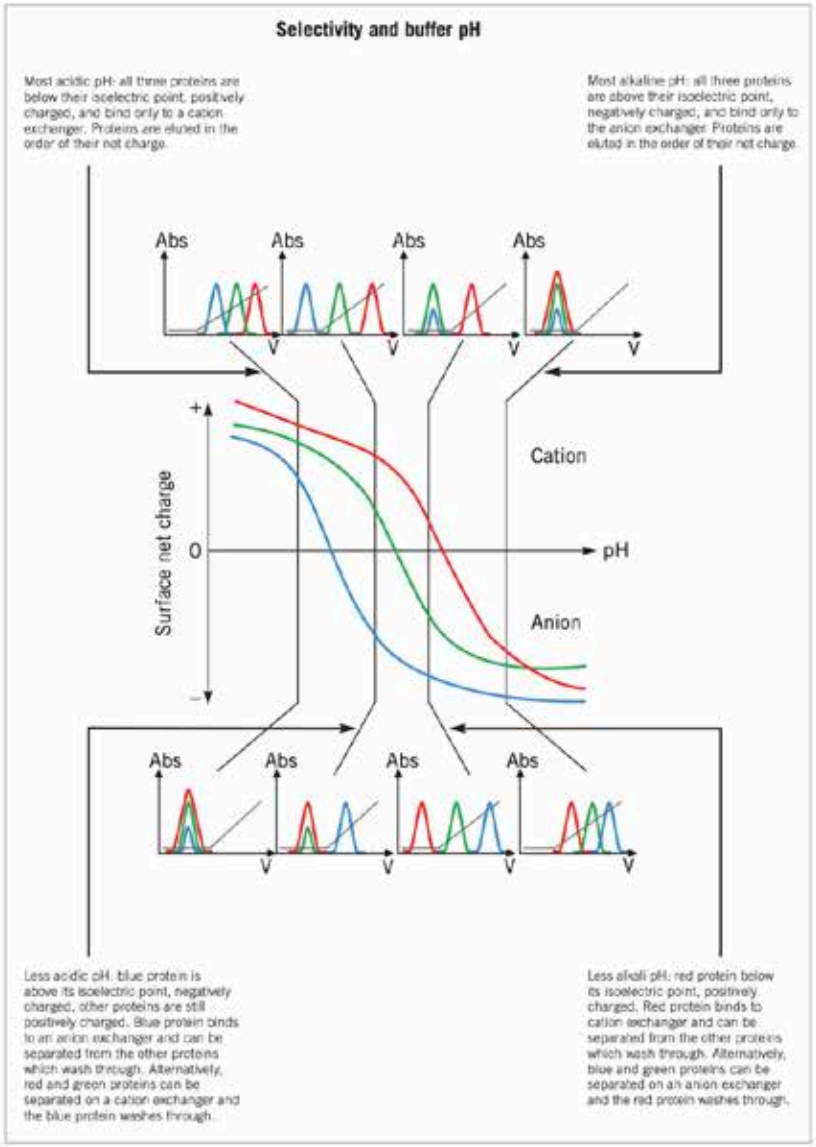
Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare



Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare



Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare



Ion Exchange Chromatography & Chromatofocusing Principles and Methods, GE Healthcare

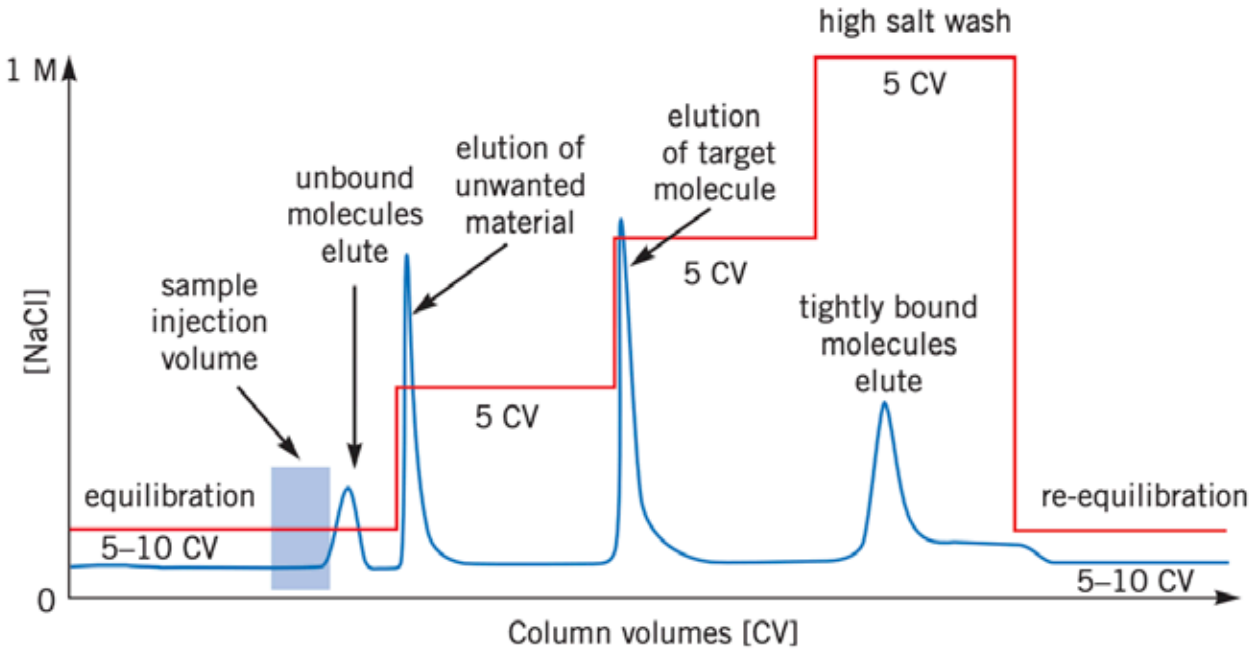


Fig. 10b. Typical IEX separation using step elution (25–30 column volumes).

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Table 1. Ion exchange matrices.

	Form	Mean particle size
MiniBeads™	Polystyrene/divinyl benzene	3 µm
MonoBeads™	Polystyrene/divinyl benzene	10 µm
SOURCE 15	Polystyrene/divinyl benzene	15 µm
SOURCE 30	Polystyrene/divinyl benzene	30 µm
Sepharose High Performance	Agarose 6%	34 µm
Sepharose Fast Flow	Agarose 6%	90 µm
Sepharose 4 Fast Flow	Agarose 4%	90 µm
Sepharose XL	Agarose 6%, dextran chains coupled to agarose	90 µm
Sepharose Big Beads	Agarose 6%	200 µm

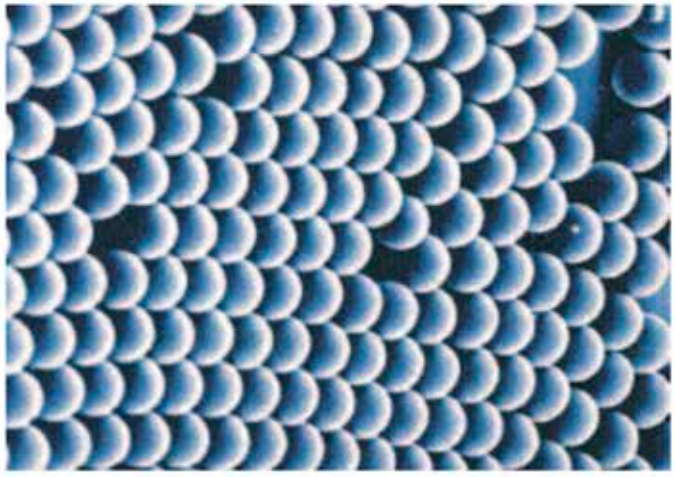

Table 2. Functional groups used on ion exchangers.

Anion exchangers		**Functional group**
Quaternary ammonium (Q)	strong	$-O-CH_2N^+(CH_3)_3$
Diethylaminoethyl (DEAE)*	weak	$-O-CH_2CH_2N^+H(CH_2CH_3)_2$
Diethylaminopropyl (ANX)*	weak	$-O-CH_2CHOHCH_2N^+H(CH_2CH_3)_2$
Cation exchangers		**Functional group**
Sulfopropyl (SP)	strong	$-O-CH_2CHOHCH_2OCH_2CH_2CH_2SO_3^-$
Methyl sulfonate (S)	strong	$-O-CH_2CHOHCH_2OCH_2CHOHCH_2SO_3^-$
Carboxymethyl (CM)	weak	$-O-CH_2COO^-$

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