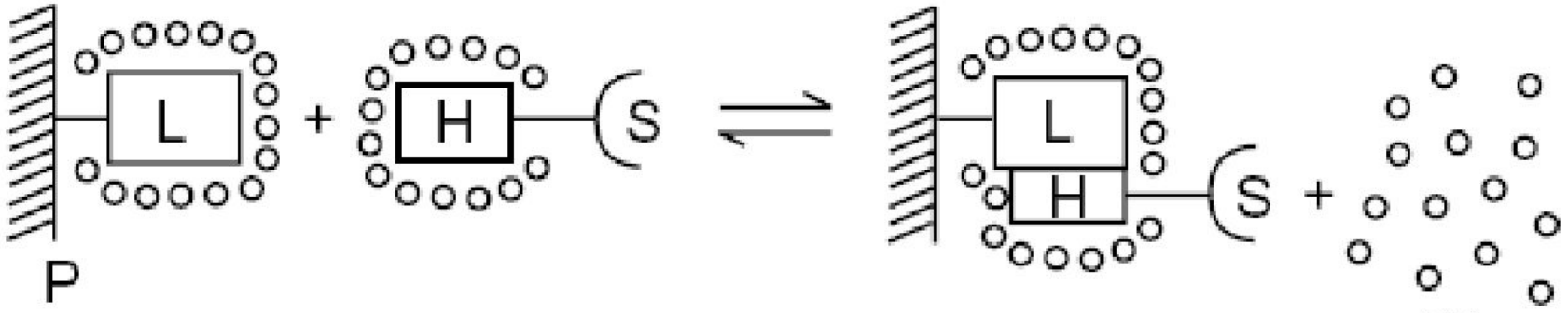
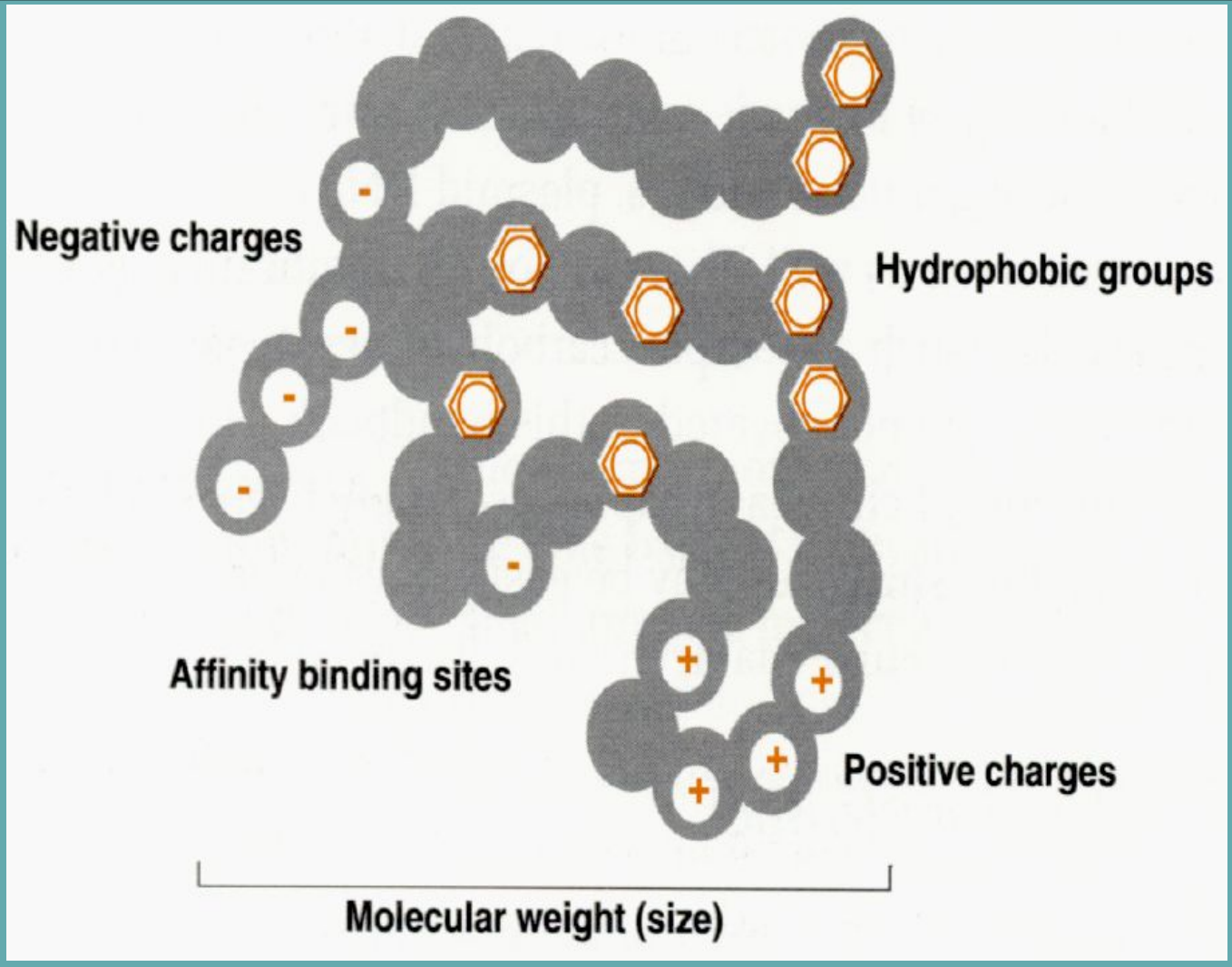
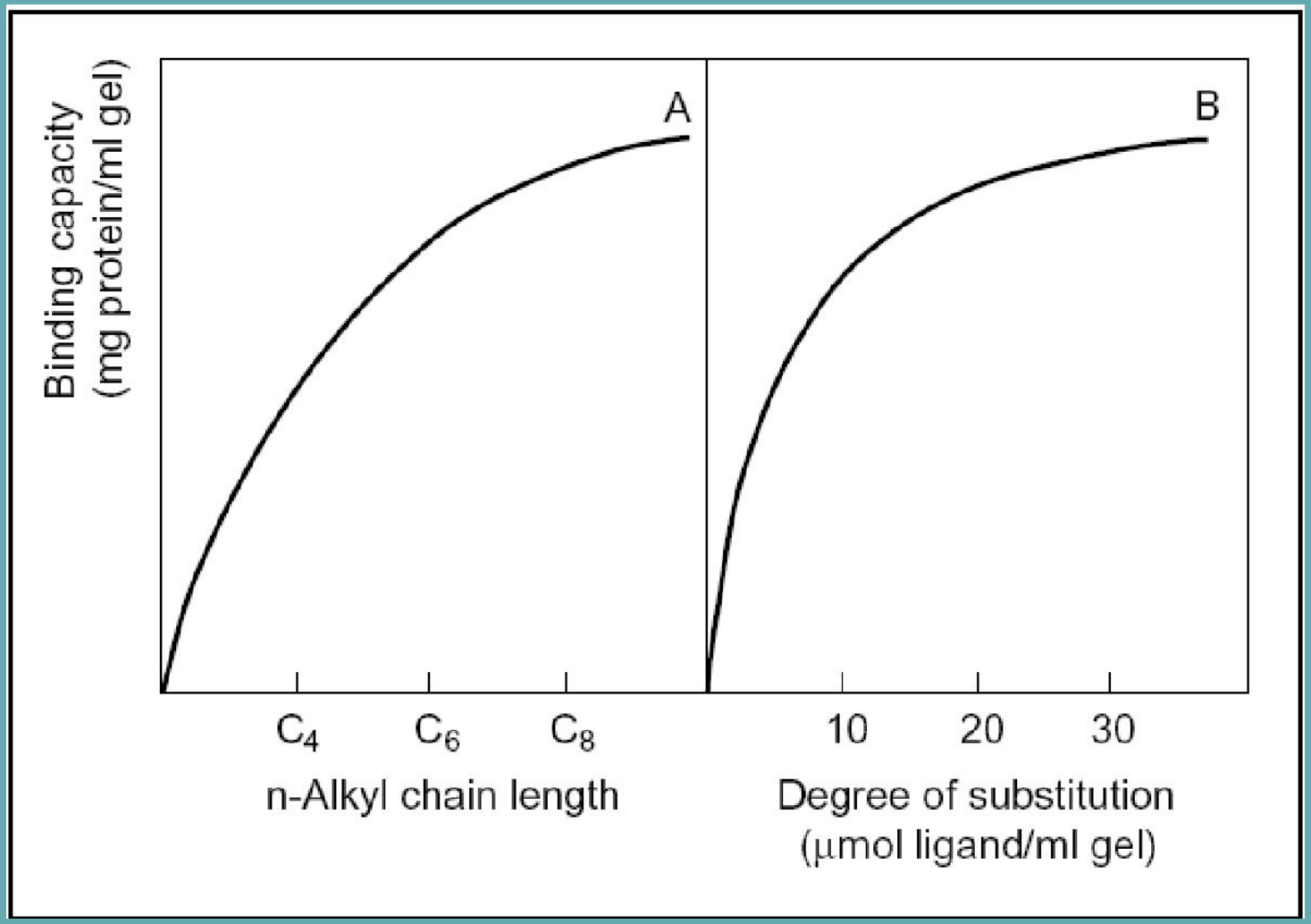
Гидрофобная хроматография белков



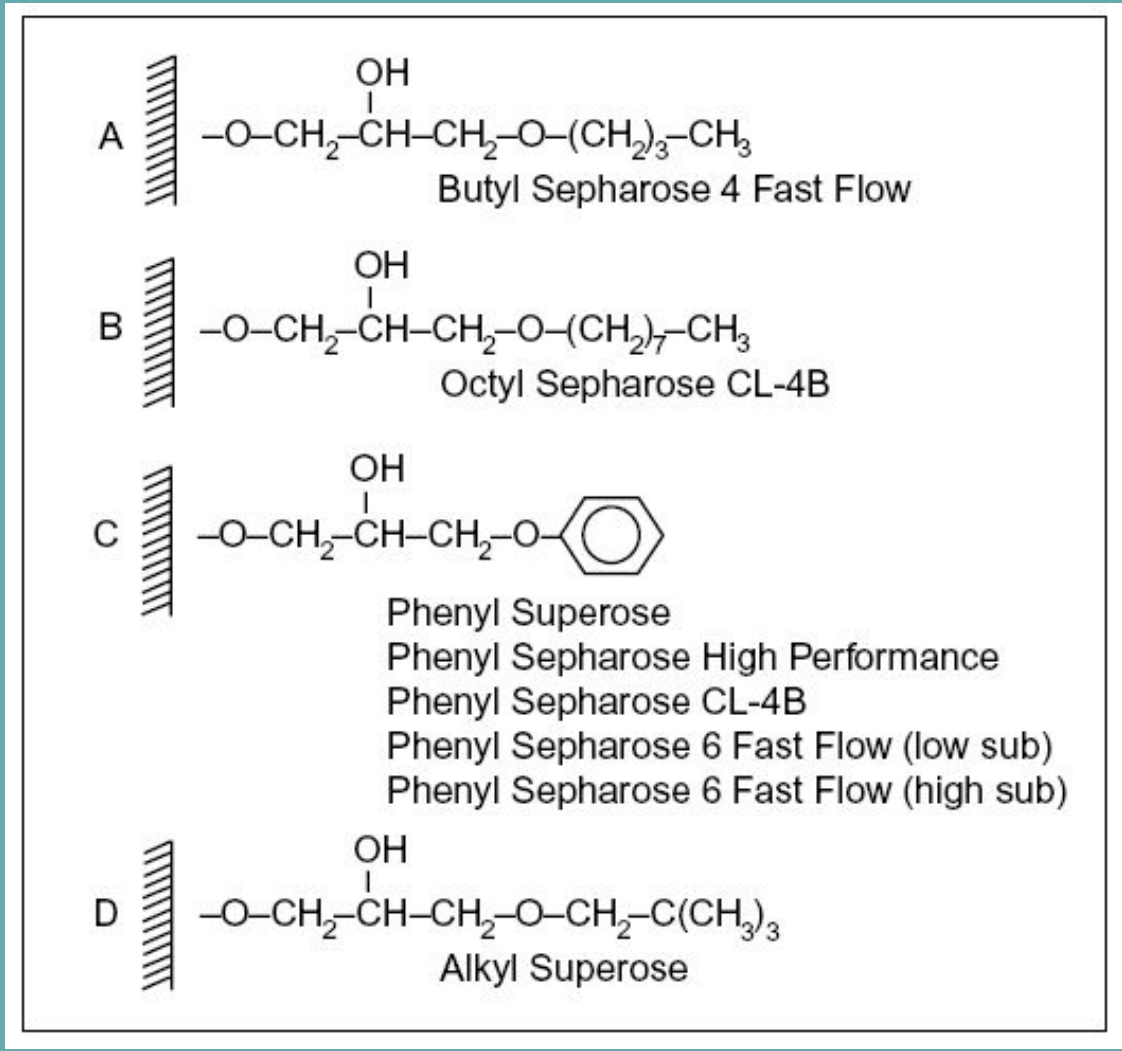
P=Polymer matrix
S=Solute molecule
L=Ligand attached to polymer matrix
H=Hydrophobic patch on surface of solute molecule
W=Water molecules in the bulk solution


Negative charges
Hydrophobic groups
Affinity binding sites
Positive charges
Molecular weight (size)

Влияние длинны алифатической цепи и плотности ее посадки на гель на взаимодействие белка с носителем



Основные типы лигандов, используемые в гидрофобной хроматографии



← Increasing precipitation ("salting -out") effect

Anions: PO_4^{3-}, SO_4^{2-}, $CH_3 \cdot COO^-$, Cl^-, Br^-, NO_3^-, CLO_4^-, I^-, SCN^-

Cations: NH_4^+, Rb^+, K^+, Na^+, Cs^+, Li^+, Mg^{2+}, Ca^{2+}, Ba^{2+}

Increasing chaotropic ("salting-in") effect →

Table 1.
The Hofmeister series on the effect of some anions and cations in precipitating proteins.

$Na_2SO_4 > K_2SO_4 > (NH_4)_2SO_4 > Na_2HPO_4 > NaCl > LiCl. . . > KSCN$

Table 2.
Relative effects of some salts on the molal surface tension of water.

Влияние pH на взаимодействие белков с гидрофобными сорбентами

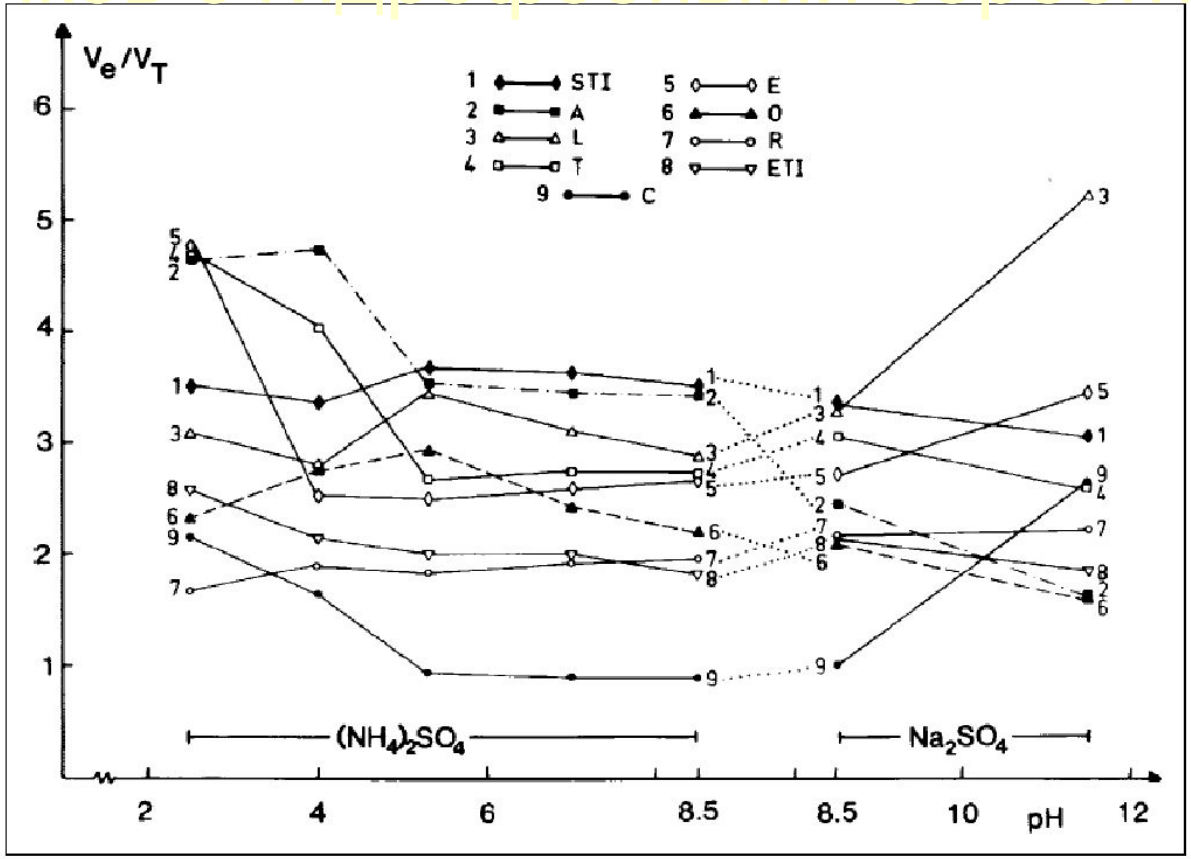


Fig. 5.
The pH dependence of the interaction between proteins and an octyl agarose gel expressed as V_e/V_T (V_e is the elution volume of the different proteins and V_T is the elution volume of a non-retarded solute). Elution was by a negative linear gradient of salt. The model proteins used were STI=soy trypsin inhibitor, A=human serum albumin, L=lysozyme, T=transferrin, E=enolase, O=ovalbumin, R=ribonuclease, ETI=egg trypsin inhibitor and C=cytochrome c. (Reproduced with permission, from ref. 42).

Solvent	Viscosity (centipoise)	Dielectric constant	Surface tension (dynes/cm)
Water	0.89	78.3	72.00
Ethylene glycol	16.90	40.7	46.70
Dimethyl Sulphoxide	1.96	46.7	43.54
Dimethyl Formamide	0.796	36.71	36.76
n-propanol	2.00	20.33	23.71

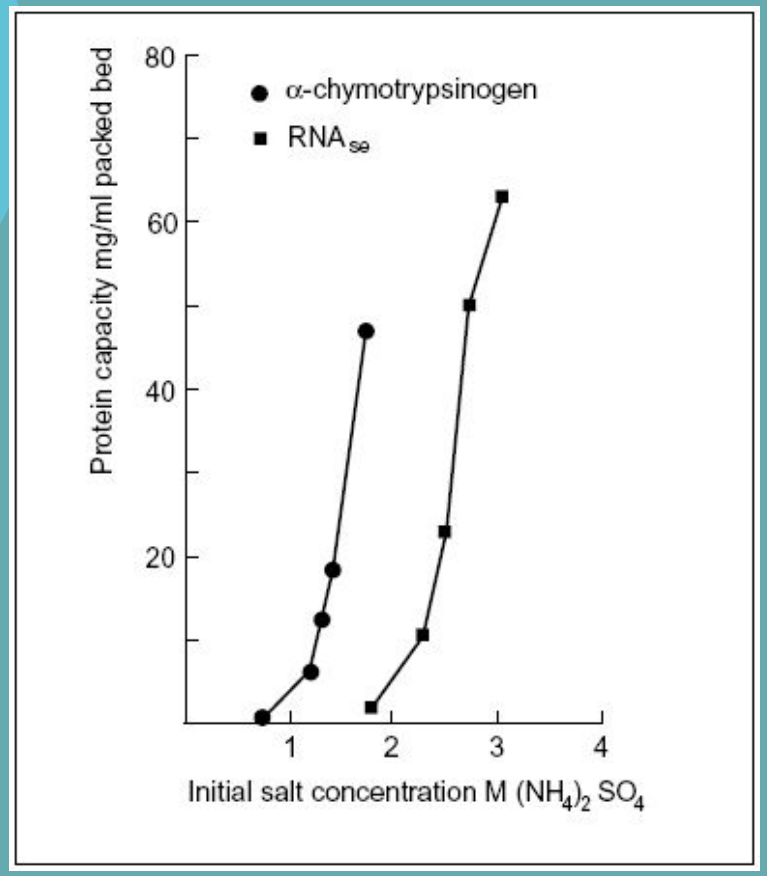
Tested media	Test solutions							
	1 M NaOH	1 M acetic acid	1 mM HCL	3 M $(NH_4)_2SO_4$	70% ethanol	30% isopropanol	6 M GuHCl	8 M Urea
Phenyl Sepharose 6 Fast Flow (low sub)	X	(n. t.)	(n. t.)	X	X	X	X	X
Phenyl Sepharose 6 Fast Flow (high sub)	X	(n. t.)	(n. t.)	X	X	X	X	X
Butyl Sepharose 4 Fast Flow	X	(n. t.)	X	(n. t.)	X	X	X	(n. t.)
Phenyl Sepharose High Performance	X	X	(n. t.)	(n. t.)	X	X	X	X

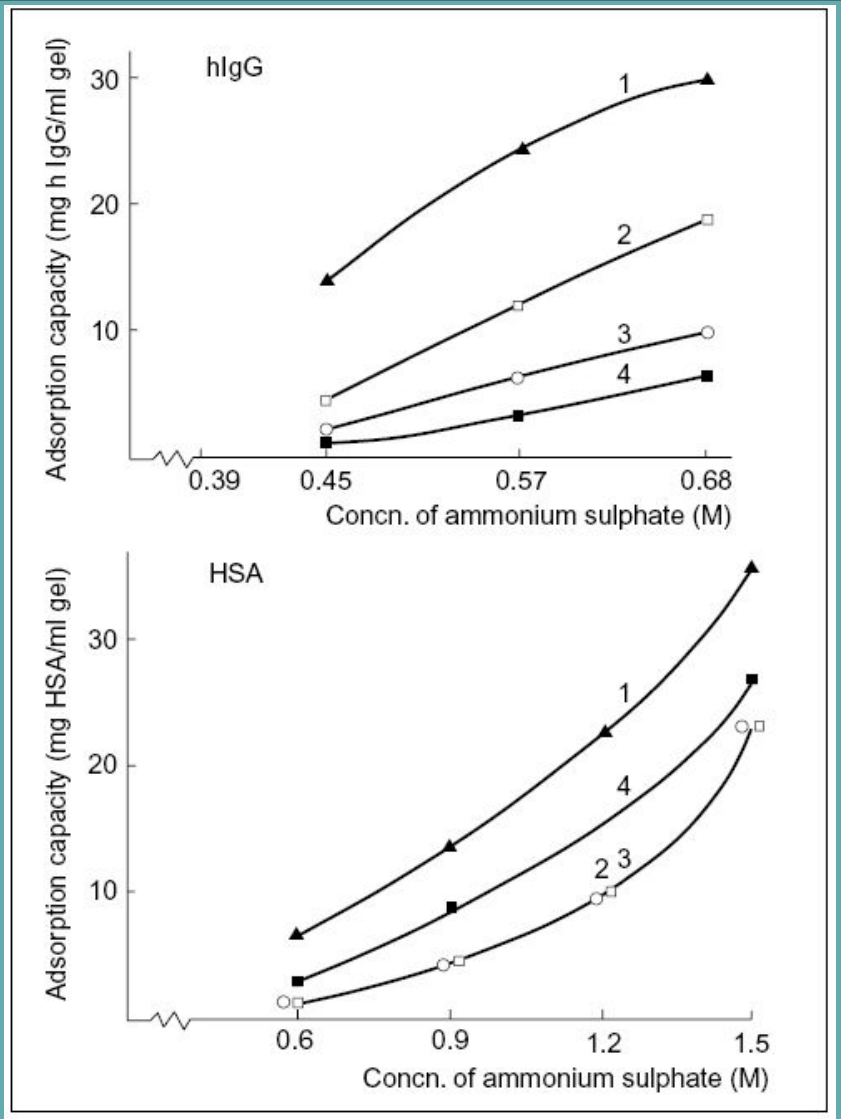
X = Functionally stable when tested for 7 days at +40°C
(n. t.) = Not tested

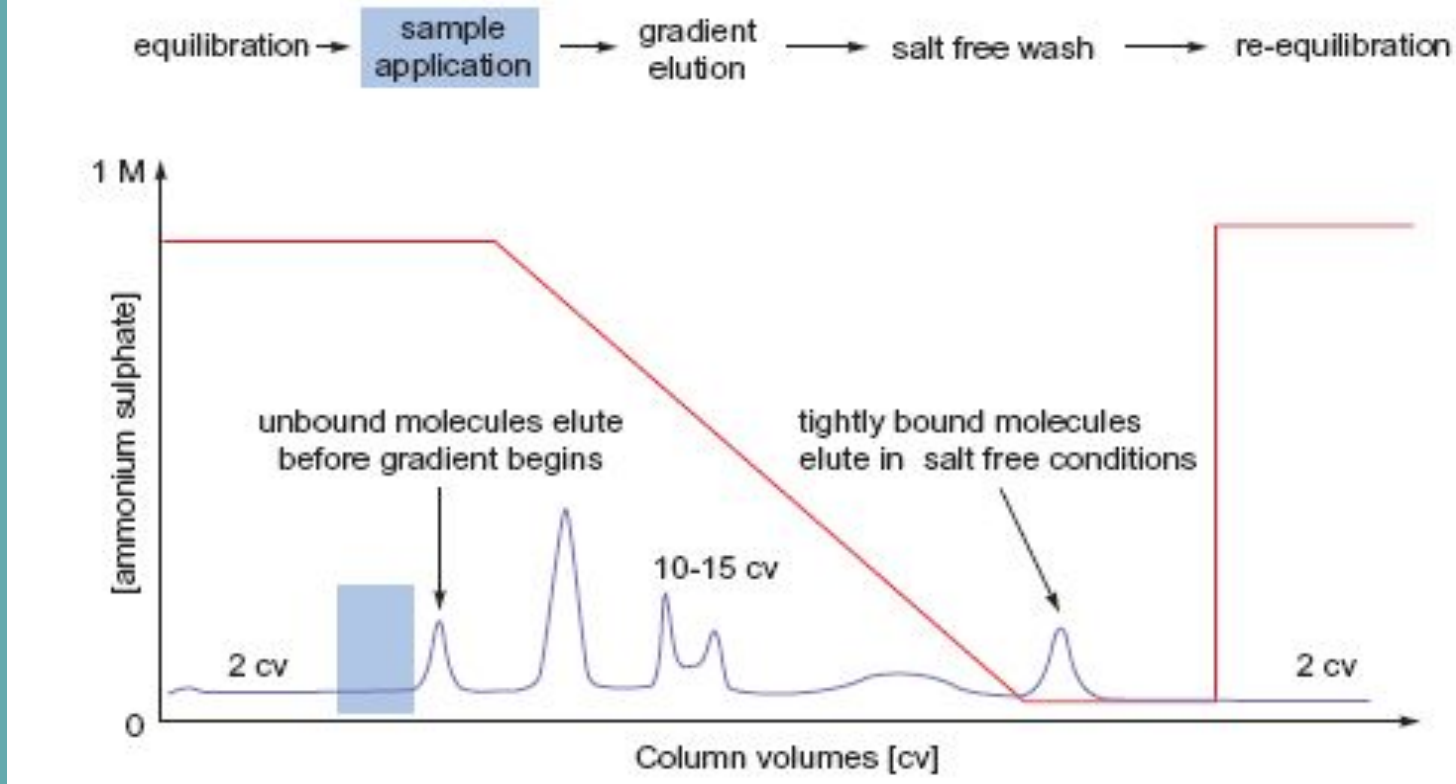
Long term stability and recommended working pH range: **3–13**

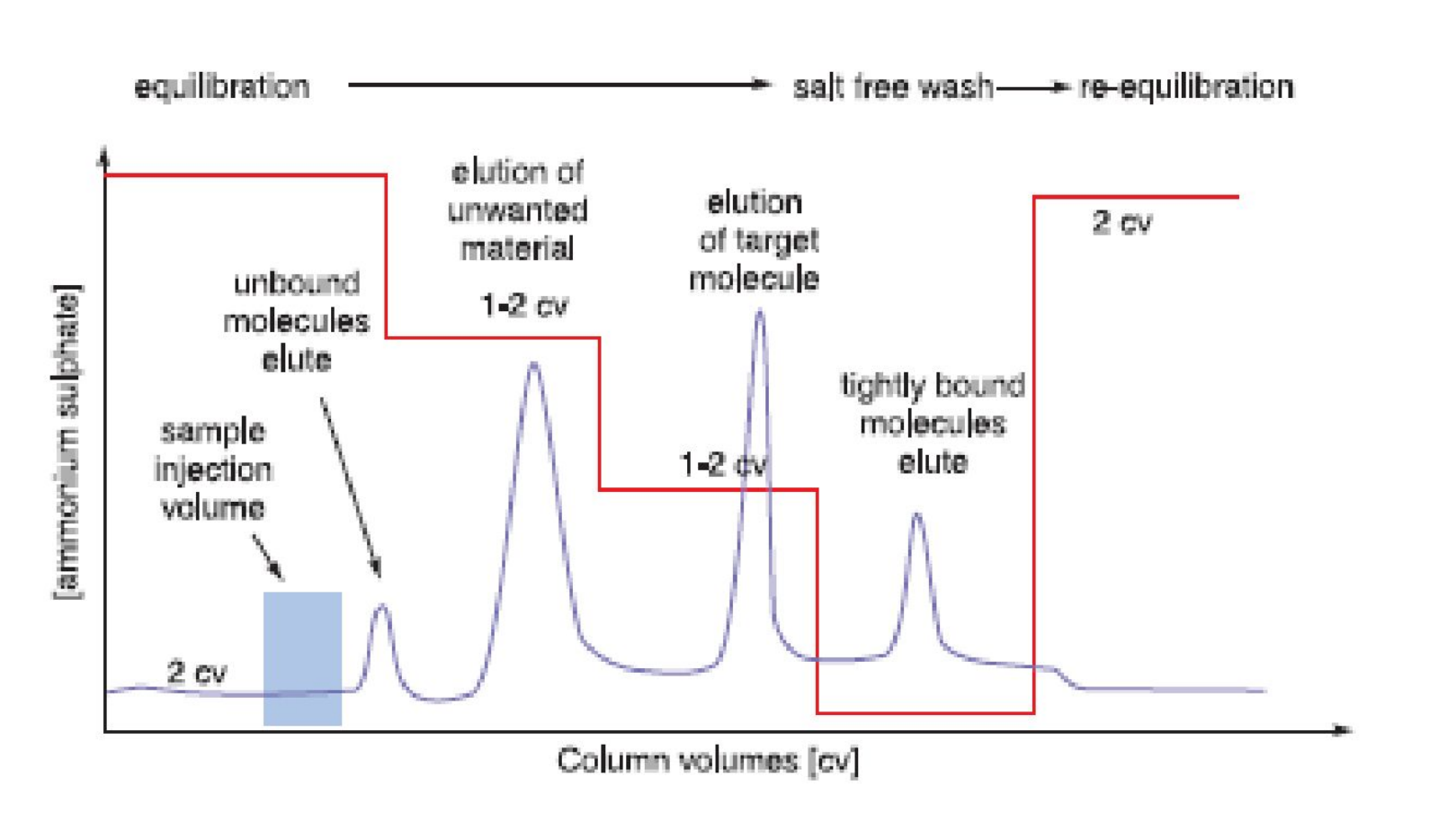
Short term stability and recommended CIP and SIP pH range: **2–14**

Recommended long term storage: **0.01 M NaOH or 20% ethanol.**

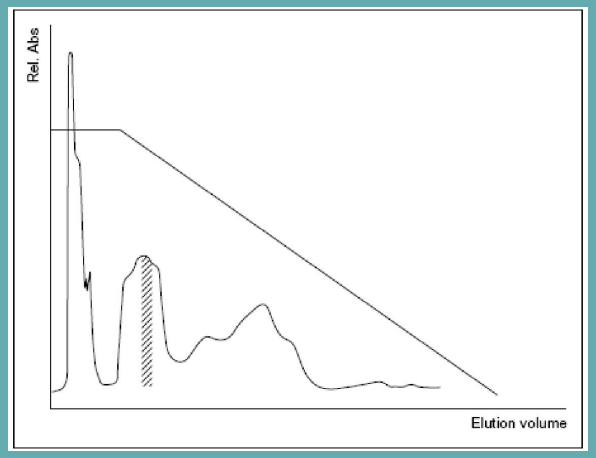

Protein capacity mg/ml packed bed
80
60
40
20
α-chymotrypsinogen
RNA$_{se}$
1
2
3
4
Initial salt concentration M $(NH_4)_2$ SO_4

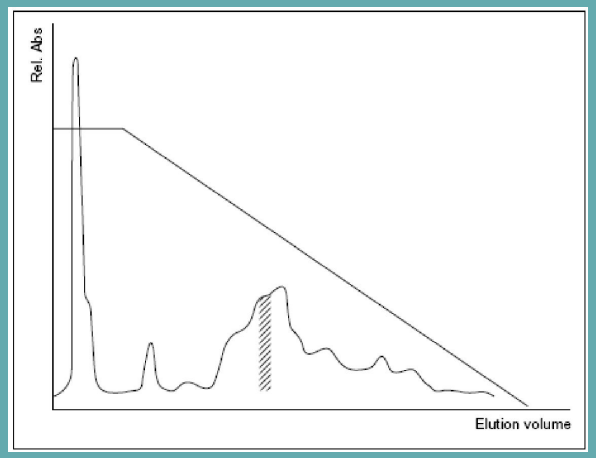

hIgG
Adsorption capacity (mg h IgG/ml gel)
30
20
10
1
2
3
4
0.39
0.45
0.57
0.68
Concn. of ammonium sulphate (M)
HSA
Adsorption capacity (mg HSA/ml gel)
30
20
10
1
4
2
3
0.6
0.9
1.2
1.5
Concn. of ammonium sulphate (M)

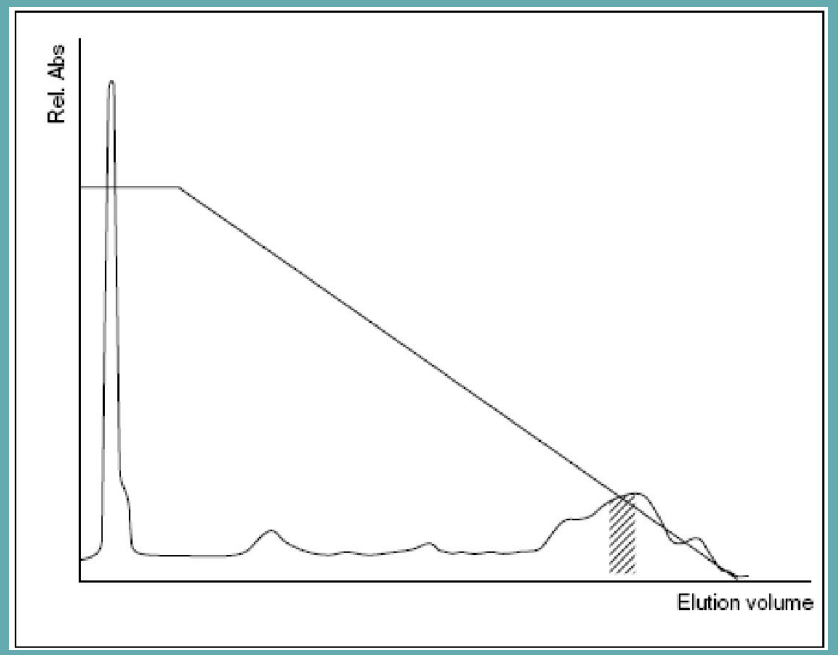

equilibration
sample application
gradient elution
salt free wash
re-equilibration
1 M
[ammonium sulphate]
0
unbound molecules elute before gradient begins
tightly bound molecules elute in salt free conditions
10-15 cv
2 cv
2 cv
Column volumes [cv]


equilibration
salt free wash
re-equilibration
elution of unwanted material
1-2 cv
elution of target molecule
2 cv
unbound molecules elute
sample injection volume
1-2 cv
tightly bound molecules elute
2 cv
[ammonium sulphate]
Column volumes [cv]

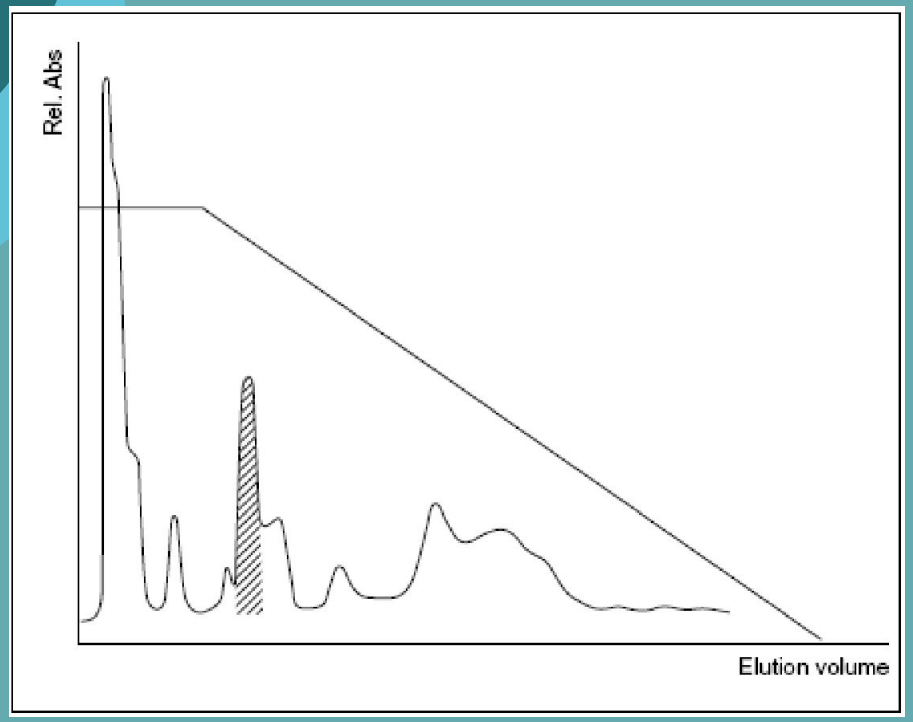
Оптимизация условий разделения гидрофобной хроматографией

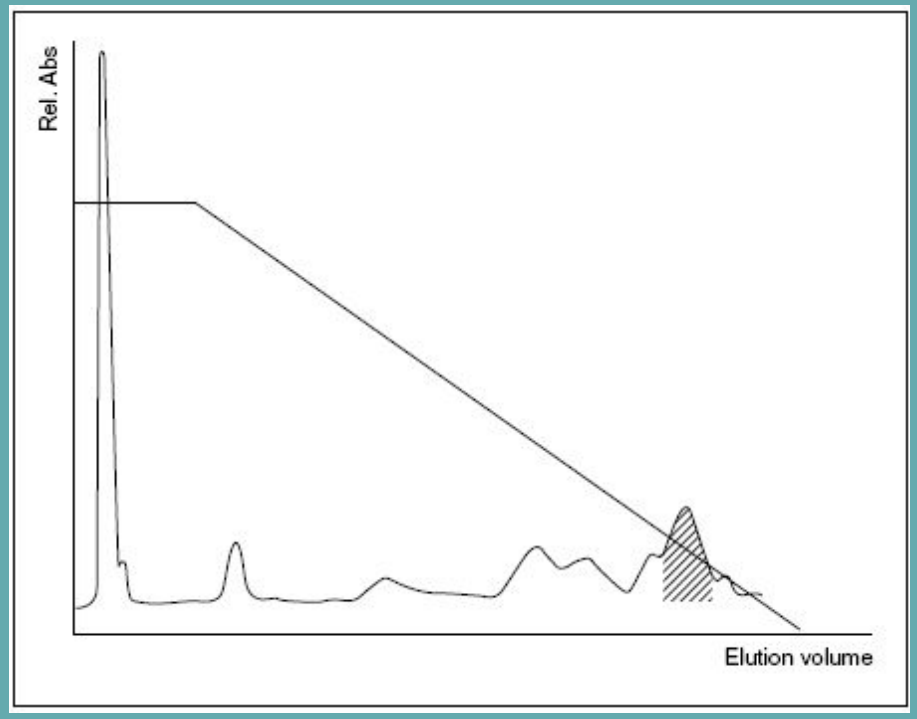






Оптимизация условий разделения гидрофобной хроматографией





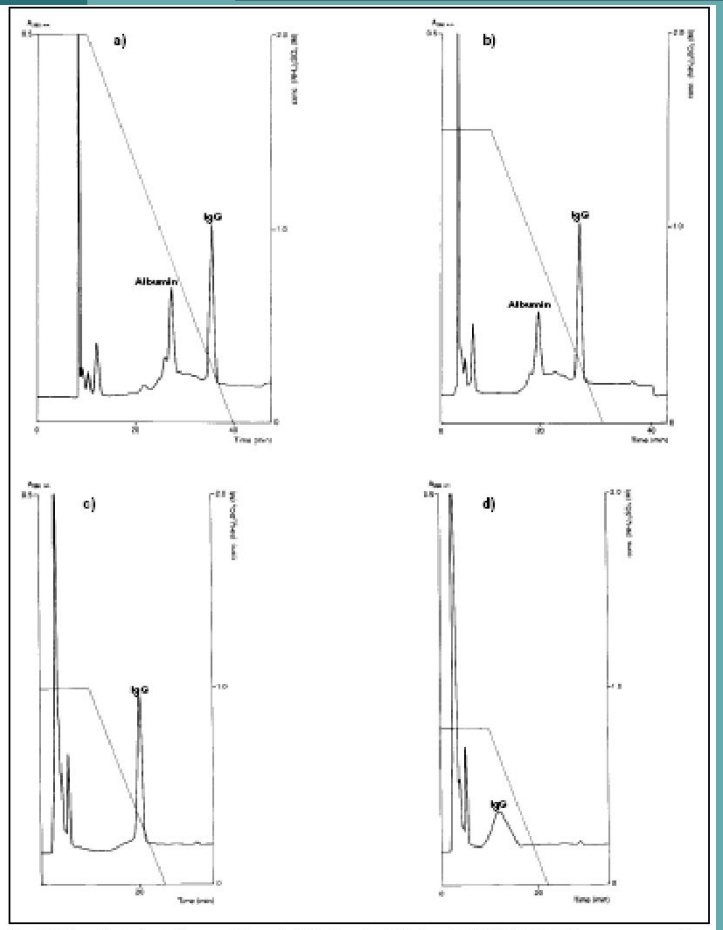


Fig. 16. The effect of starting conditions in HIC. Sample, 100 µl anti-CEA MAB (-IgG$_1$) from mouse ascites fluid in 0.8 M $(NH_4)_2SO_4$ (corresponding to 20 µl ascites); column. Alkyl Superose HR 5/5; flow rate, 0.5 ml min^{-1}; buffer A, 0.1 M sodium phosphate, pH 7.0, $(NH_4)_2SO_4$. (a) Sample applied in 2 M $(NH_4)_2SO_4$: both albumin and IgG are absorbed. (b) Sample applied in 1.5 M $(NH_4)_2SO_4$: less albumin binds and IgG elutes earlier in the gradient. (c) Sample applied in 1.0 M $(NH_4)_2SO_4$: albumin does not bind and, therefore, the column has a greater capacity for binding IgG. (d) Sample applied in 0.8 M $(NH_4)_2SO_4$: albumin does not bind; IgG is retarded, but elutes in a broad peak. (Work from Amersham Pharmacia Biotech, Uppsala, Sweden).

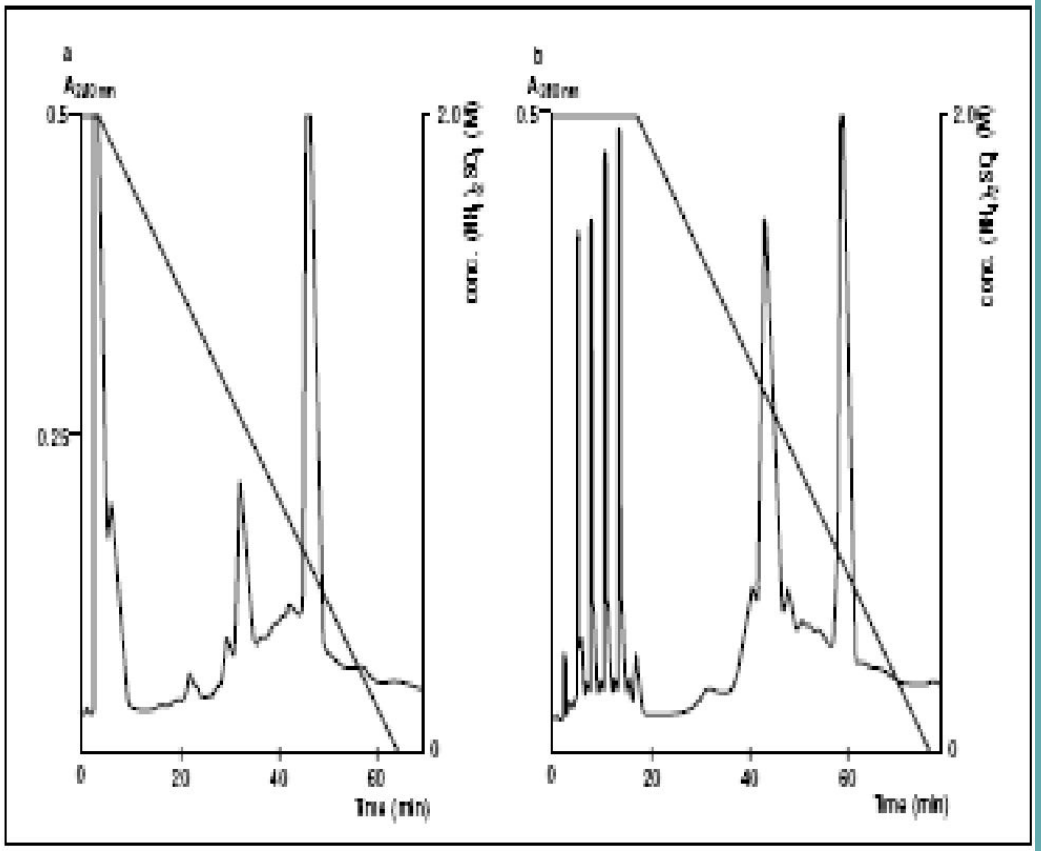


Fig. 17. The effect of loading conditions in HIC. Column, Alkyl Superose HR 5/5; flow rate, 0.5 ml min^{-1}; buffer A, 0.1 M sodium phosphate, pH 7.0, 2 M $(NH_4)_2SO_4$. (a) Sample (500 µl anti-CEA MAB (IgG$_1$) from mouse ascites fluid in 0.9 M $(NH_4)_2SO_4$ (corresponding to 115 µl ascites) applied in one injection. (b) Sample as (a) applied in five 100 µl injections with 1.3 ml 2.0 M $(NH_4)_2SO_4$ after each portion. (Work from Amersham Pharmacia Biotech, Uppsala, Sweden).

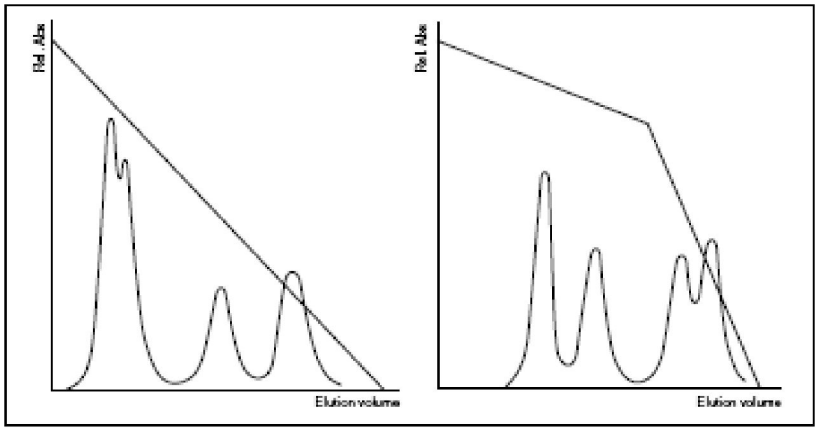


Fig. 18. Effect of a complex gradient on resolution.

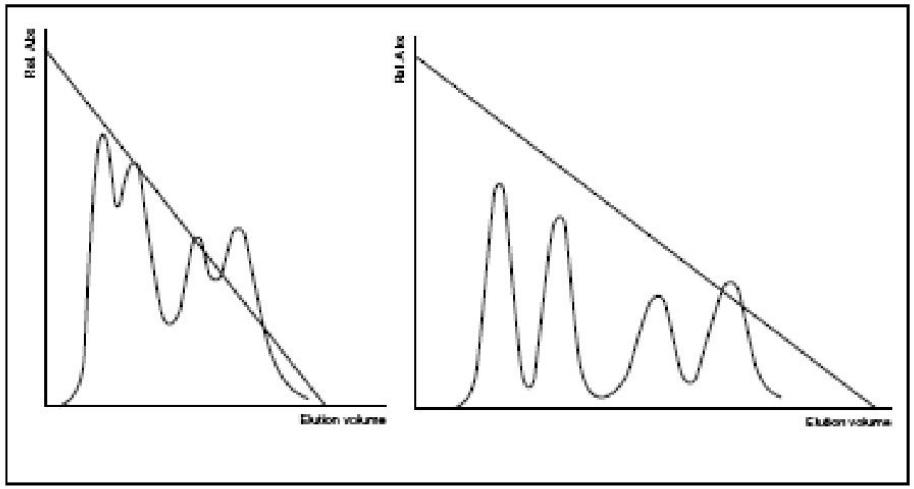


Fig. 19. Effect of gradient slope on resolution.

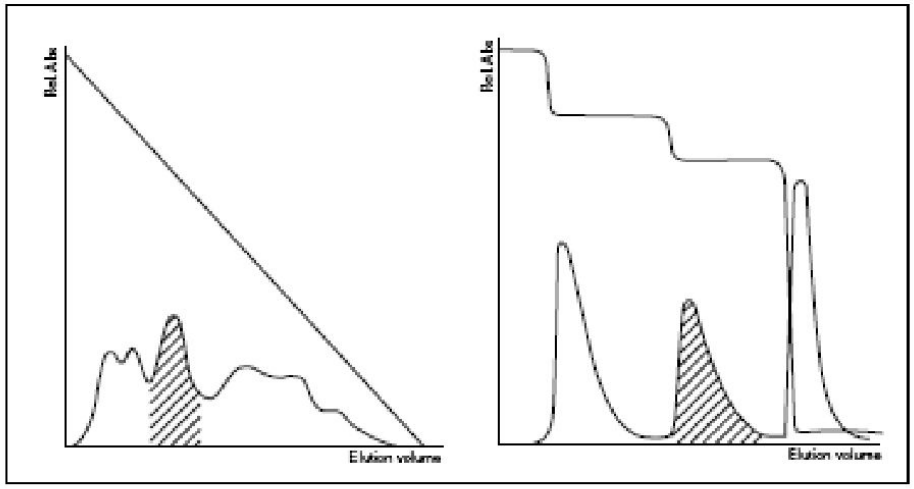
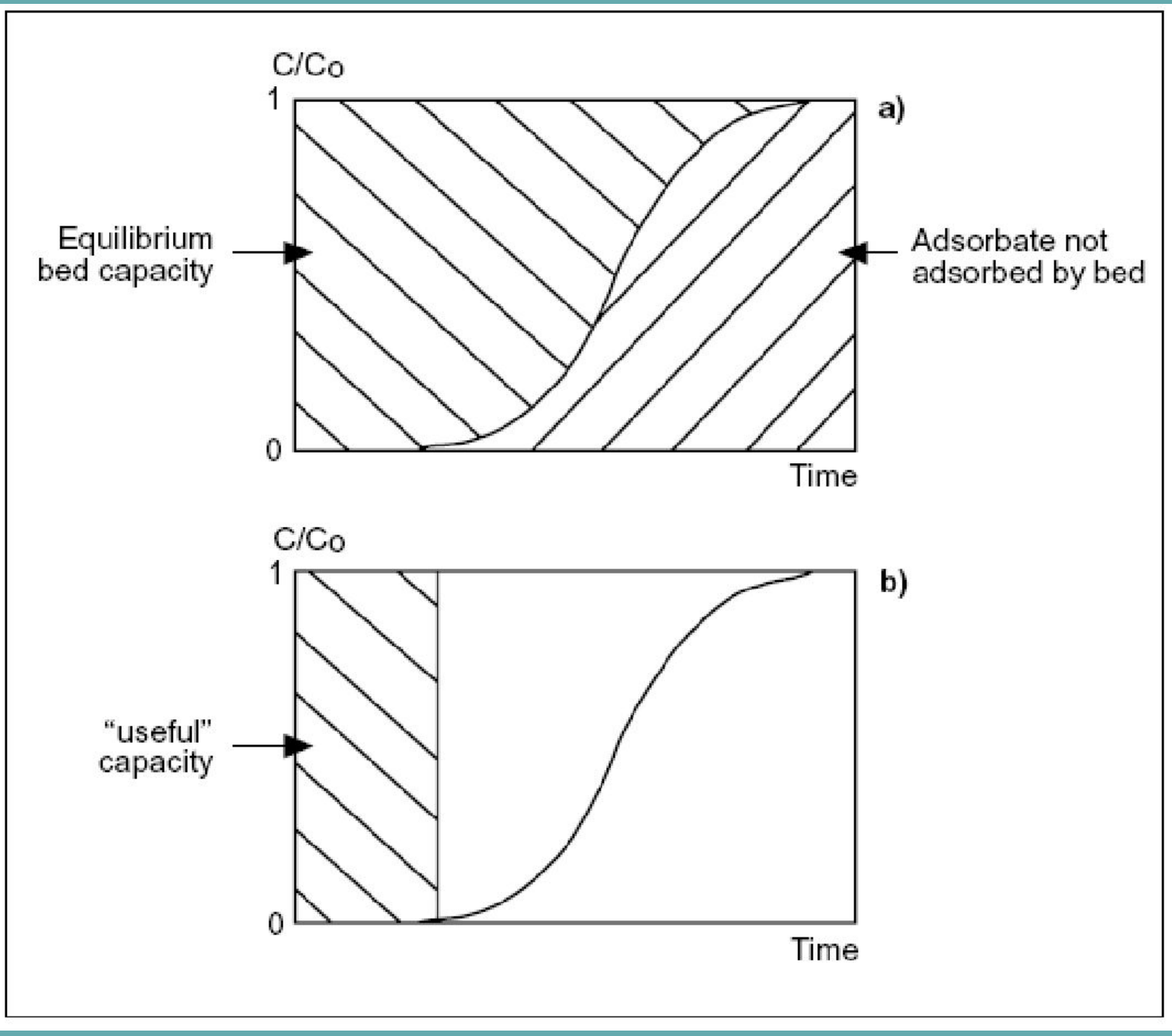
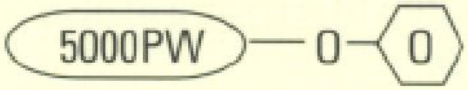
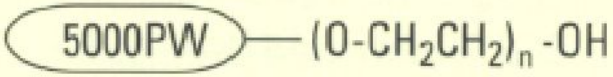


Fig. 20. Switching from a continuous gradient to step-wise elution.

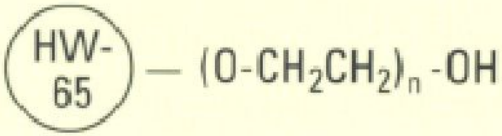

C/Co
1
0
a)
Equilibrium
bed capacity
Adsorbate not
adsorbed by bed
Time
C/Co
1
0
b)
"useful"
capacity
Time

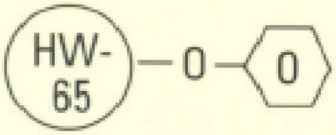
Structure of TSK-GEL HIC resins

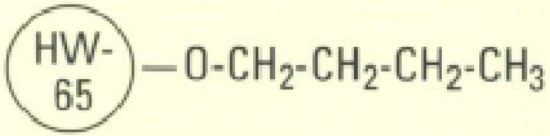
TSKgel Phenyl-5PW 

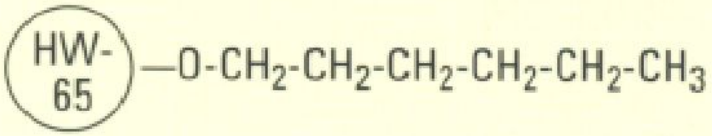
TSKgel Ether-5PW 

Structure of Toyopearl HIC resins

Toyopearl Ether-650 

Toyopearl Phenyl-650 

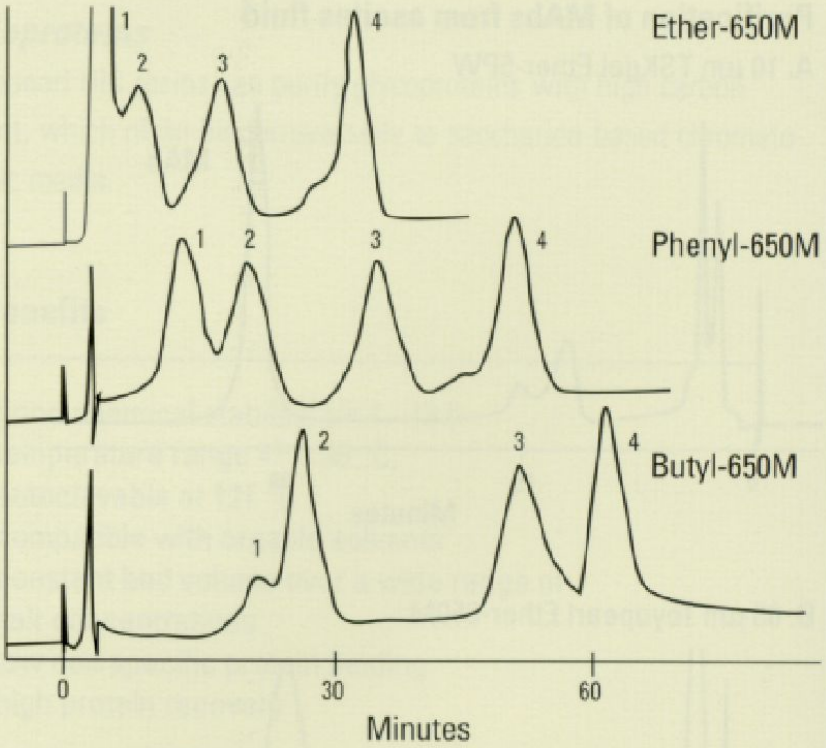
Toyopearl Butyl-650 

Toyopearl Hexyl-650 

Resolution values (R_s) of Toyopearl resins for lysozyme and α-chymotrypsinogen

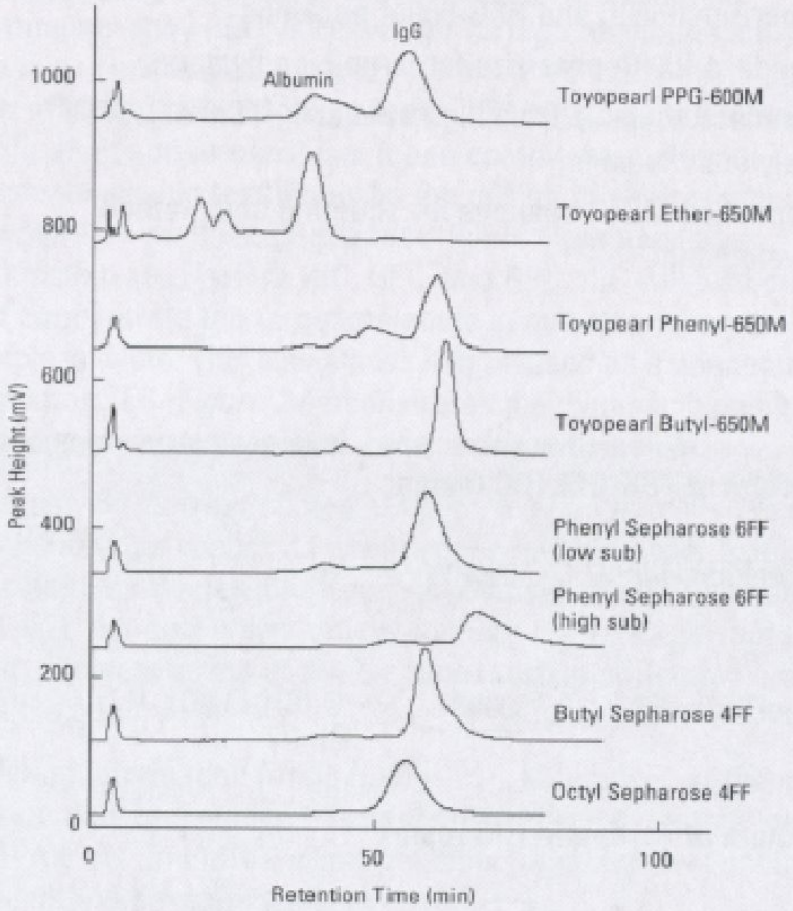
	Particle size grade		
Resins	***C***	***M***	***S***
Phenyl-650	1.12	1.52	2.19
Butyl-650	0.91	1.37	2.20

Selectivity of Toyopearl HIC resins



Column:	7.5 mm I.D. x 7.5 cm L
Sample:	1. myoglobin, 2. ribonuclease A, 3. lysozyme, 4. α-chymotrypsinogen
Elution:	60 min linear gradient from 1.8 M to 0 M $(NH_4)_2SO_4$ in 0.1 M phosphate buffer (pH 7.0)
Flow rate:	136 cm/h
Detection:	UV @ 280 nm

Separation of mouse ascites fluid by HIC



Column size:	7.5 mm ID x 7.5 cm L
Elution:	A. 0.1 mol/L phosphate buffer containing 1.8 mol/L ammonium sulfate (pH 7.0) B. 0.1 mol/L phosphate buffer (pH 7.0) linear gradient from A to B for 60 min.
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Injection:	100 µL
Sample:	mouse ascites fluid (x 4 diluted) (Antibody: Anti-IgE)

Proposed Two Step Adsorption Model for Mab to Toyopearl Resins

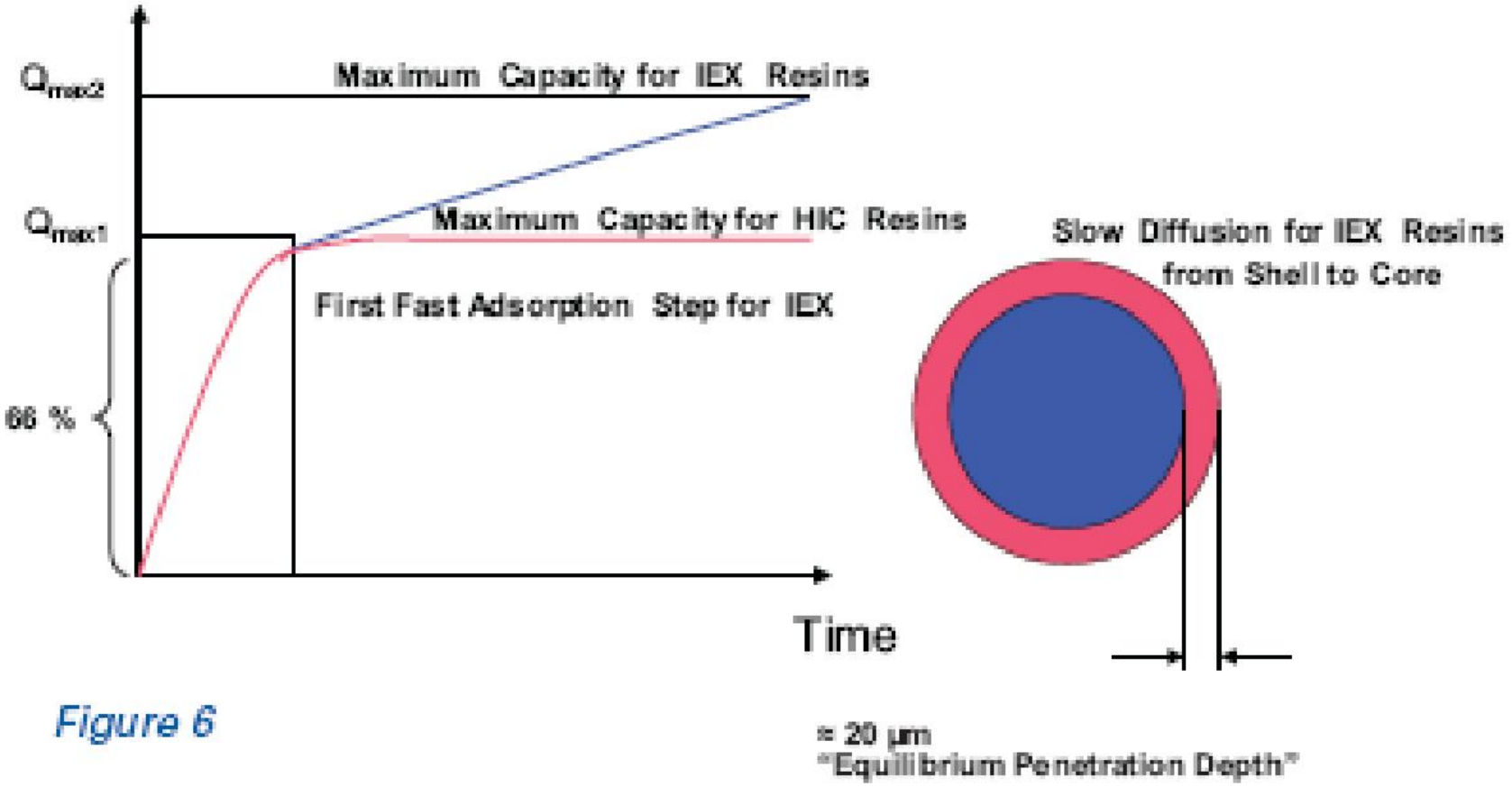
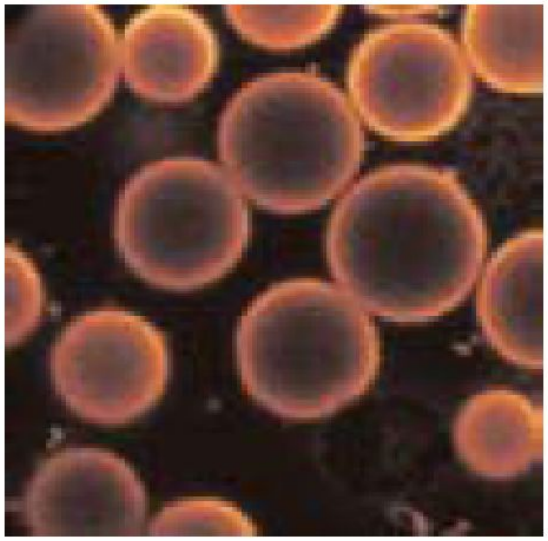


Figure 6

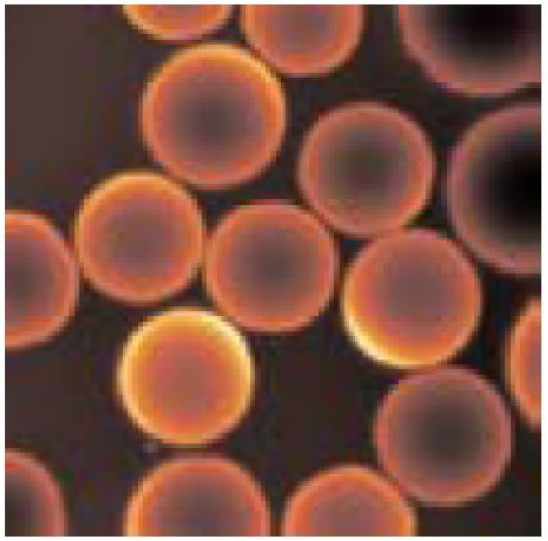
Toyopearl Phenly-650M (24 hours)



20mM sodium dihydrogenphosphate pH7 and 1 M ammonium sulfate, 1mg/ml labeled IgG

Figure 7

Toyopearl SP (100-300µm) (24 hours)



20mM sodium dihydrogenphosphate pH7, 1mg/ml labeled IgG

MAb Adsorption on Toyopearl Phenyl Resins

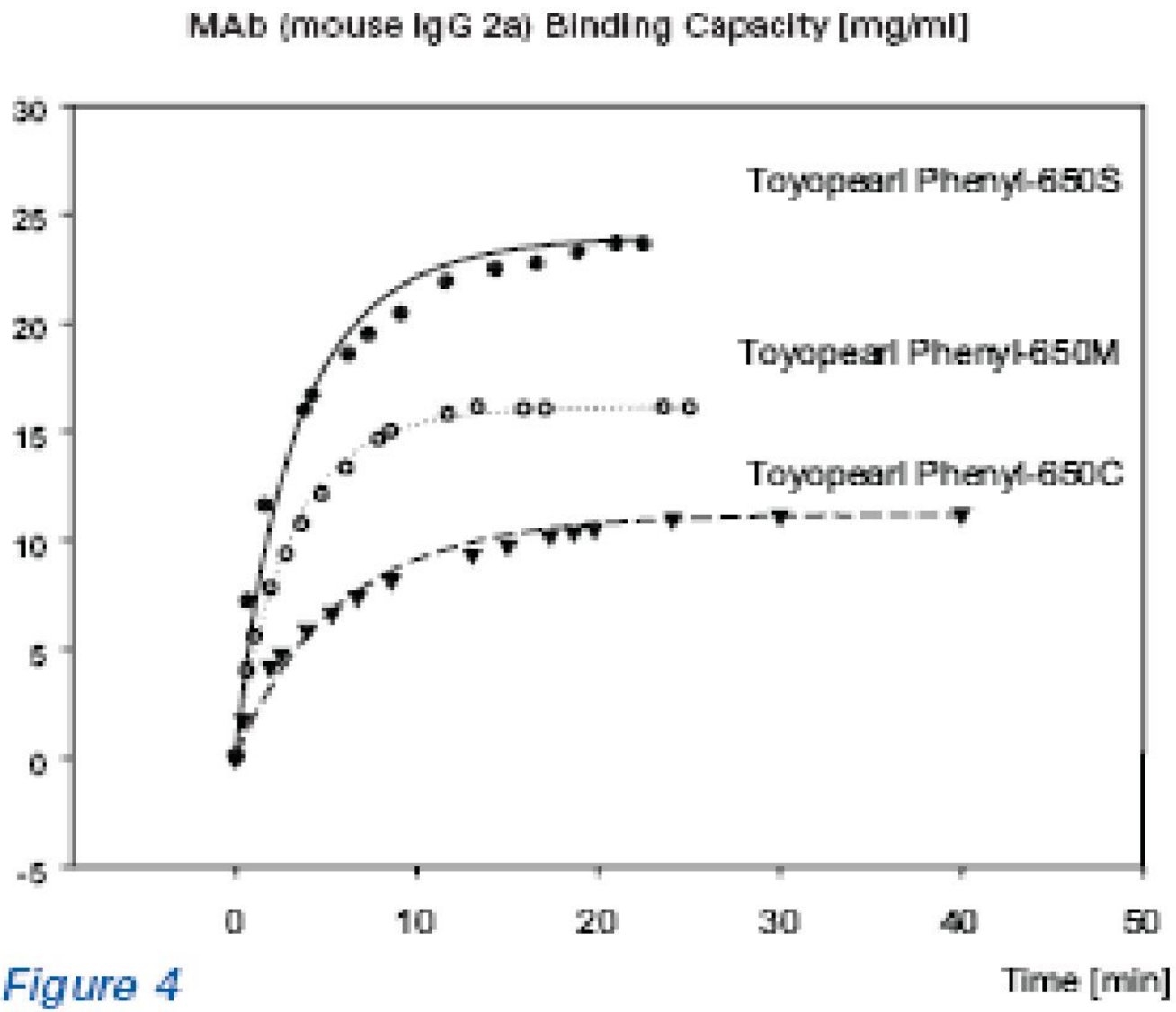


Figure 4

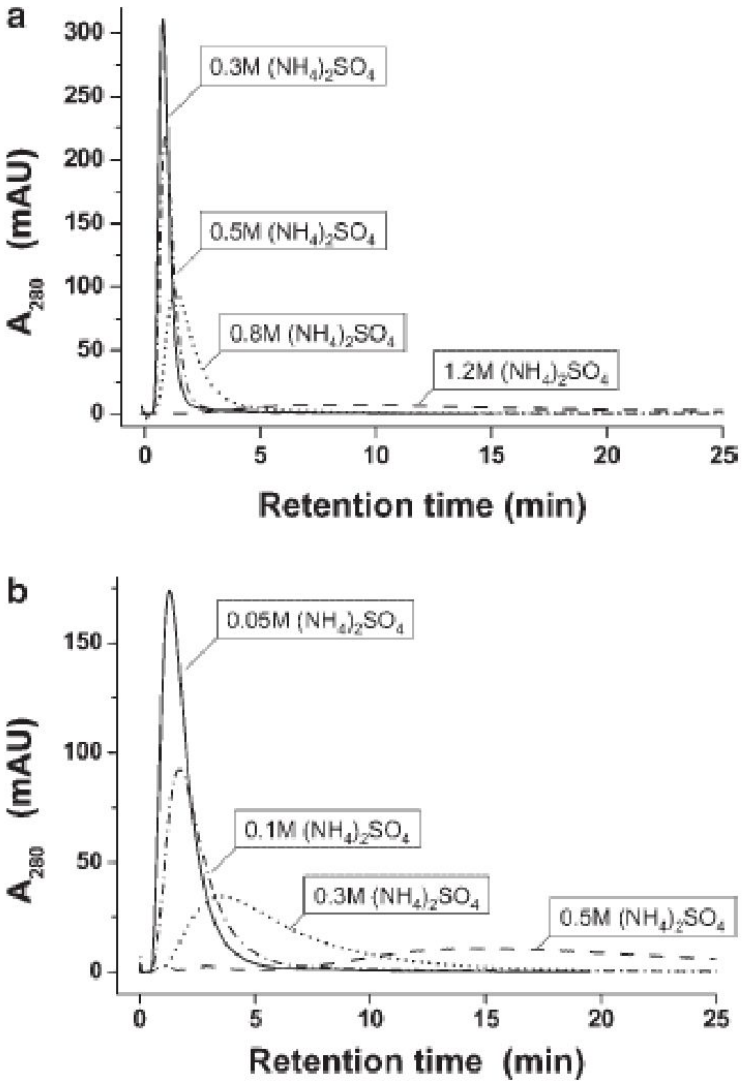


Figure 1. Effect of $(NH_4)_2SO_4$ concentration on α-lactalbumin retention for α-lactalbumin. Isocratic elution was performed on Phenyl Sepharose™ 6 Fast Flow (low sub) at a flowrate of 1.0 mL/min and 55°C. a: Calcium included in the samples and buffers at 12 mM for all conditions, with $(NH_4)_2SO_4$ concentrations varying as shown. b: 0 mM $CaCl_2$. EDTA (2 mM) was included to chelate any trace calcium.

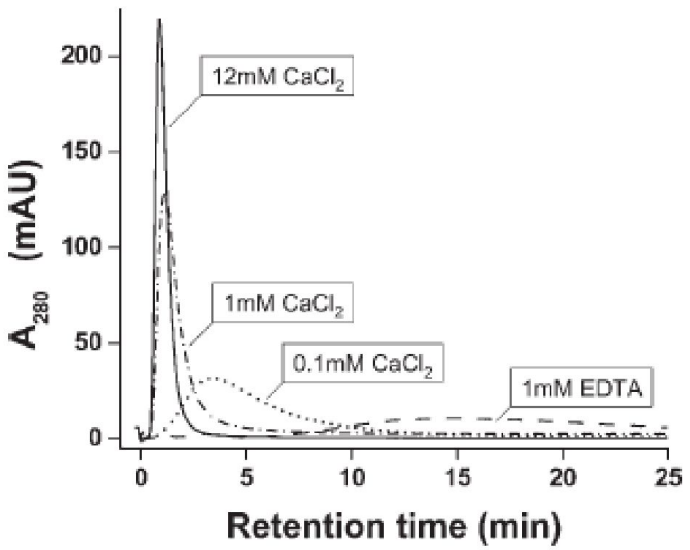


Figure 3. Effect of calcium concentration on α-lactalbumin isocratic elution. The $(NH_4)_2SO_4$ concentration was 0.5 M, and calcium concentrations varied as shown. Other conditions identical to Figure 1.

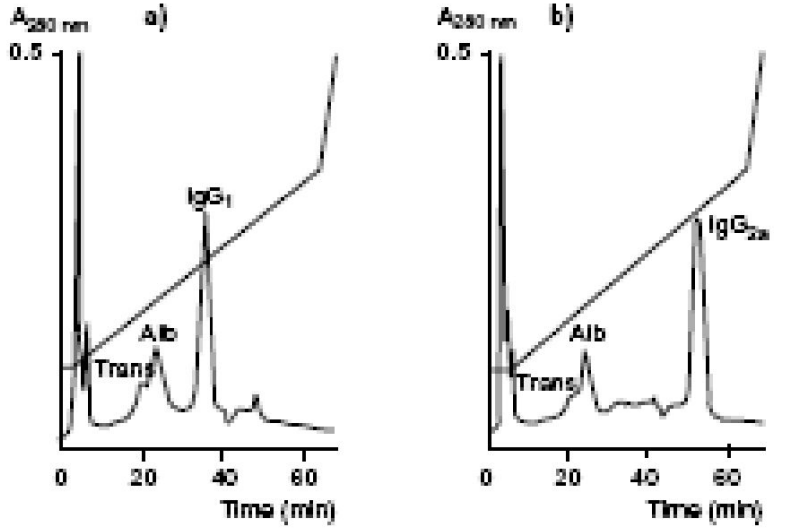
Column: Alkyl Superose HR 5/5

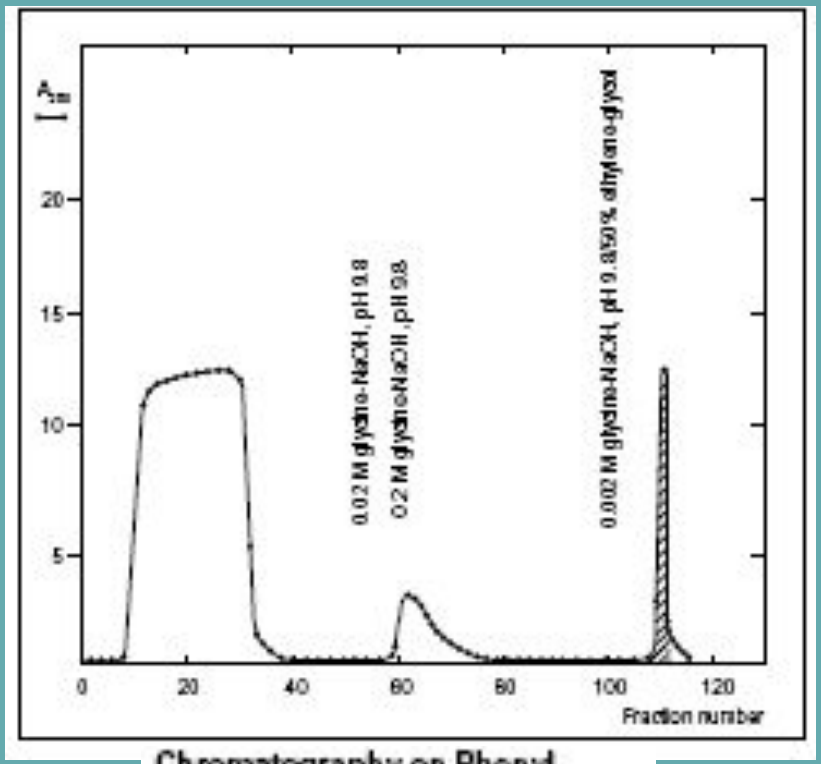
Sample: 100 µl mouse ascites containing monoclonal IgG_1 (a) or IgG_{2a} (b) + 100 µl buffer A, centrifuged and filtered

Buffer A: 0.1 M phosphate, pH 7.0, 2.0 M amminium sulphate

Buffer B: 0.1 M phosphate, pH 7.0

Detection: A_{280}. Proteins were identified by SDS-PAGE with PhastSystem. The programmed gradient (FPLC System) is also shown.





Chromatography on Phenyl Sepharose CL-4B of a prolactin preparation. The hatched area represents the prolactin-containing fractions. (reproduced with permission, from ref. 53.)

Column:	HiLoad 16/10 Phenyl Sepharose High Performance, 10 cm bed height
Sample:	Hybridoma cell culture supernatant; mouse IgG_1 anti-IgE. Ammonium sulphate added to 0.5 M.
Sample volume:	130 ml
Sample load:	4.5 mg Mab/ml gel
Flow rate:	100 cm/h (3.3 ml/min)
Buffer A:	20 mM potassium phosphate, pH 7.0 + 0.5 M ammonium sulphate
Buffer B:	20 mM potassium phosphate, pH 7.0
Gradient:	0-100% B; 10 column volumes

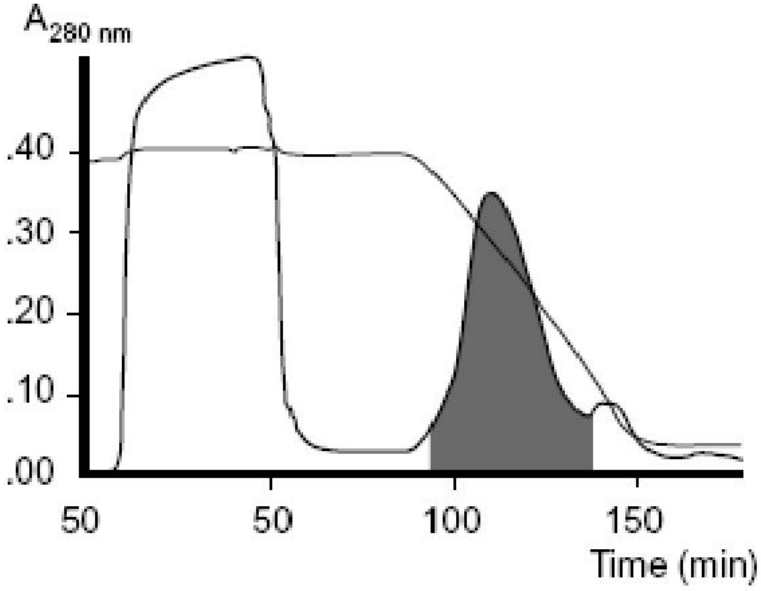
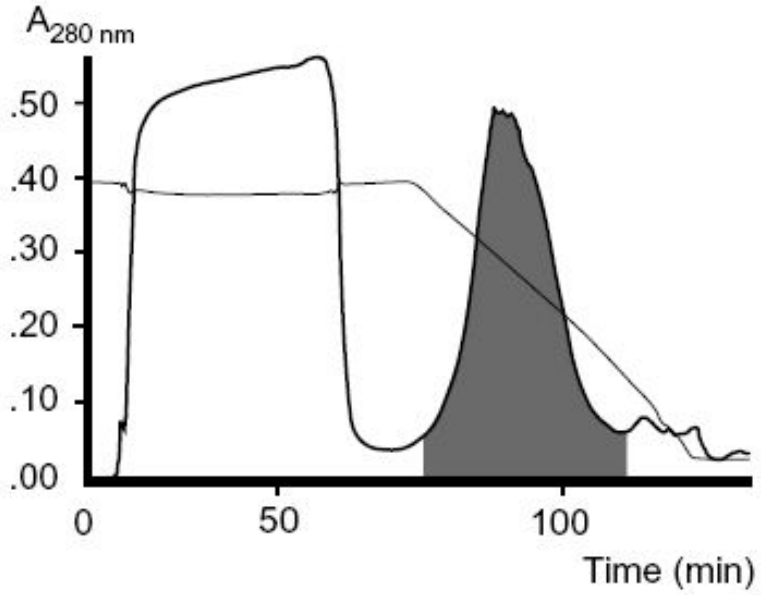
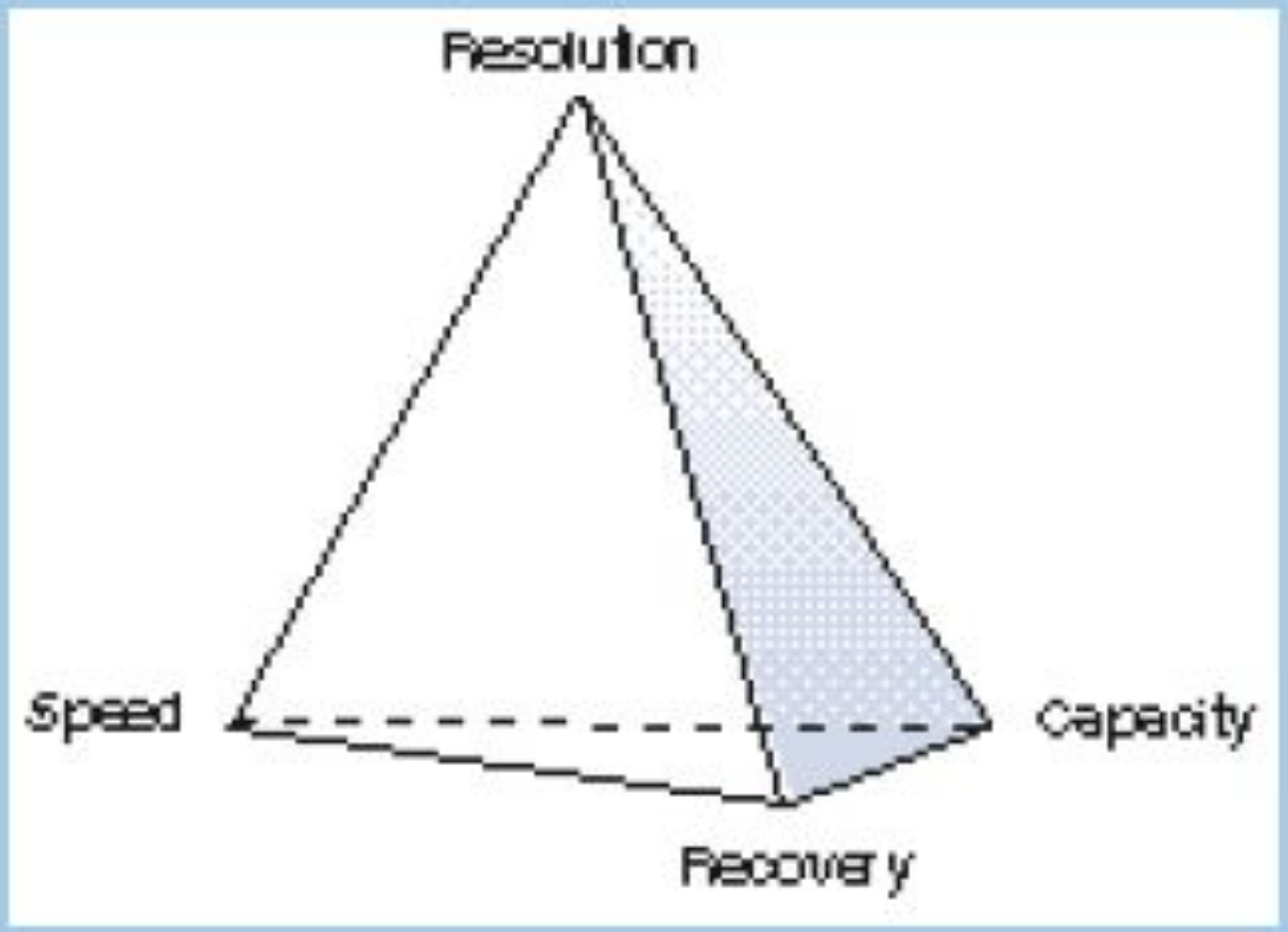


Fig. 48. Laboratory scale purification of mouse IgG_1, anti-IgE, on Phenyl Sepharose High Performance. (Work from Amersham Pharmacia Biotech, Uppsala, Sweden).

Column:	BioPilot Column 35/100
Gel:	Phenyl Sepharose High Performance, 10 cm bed height
Sample:	Hybridoma cell culture supernatant; mouse IgG_1, anti-IgE. Ammonium sulphate added to 0.5 M.
Sample volume:	735 ml
Sample load:	4.5 mg Mab/ml gel
Flow rate:	100 cm/h (16.7 ml/min)
Buffer A:	20 mM potassium phosphate, pH 7.0 + 0.5 M ammonium sulphate
Buffer B:	20 mM potassium phosphate pH 7.0
Gradient:	0–100 % B; 10 column volumes




Resolution
Speed
Capacity
Recovery