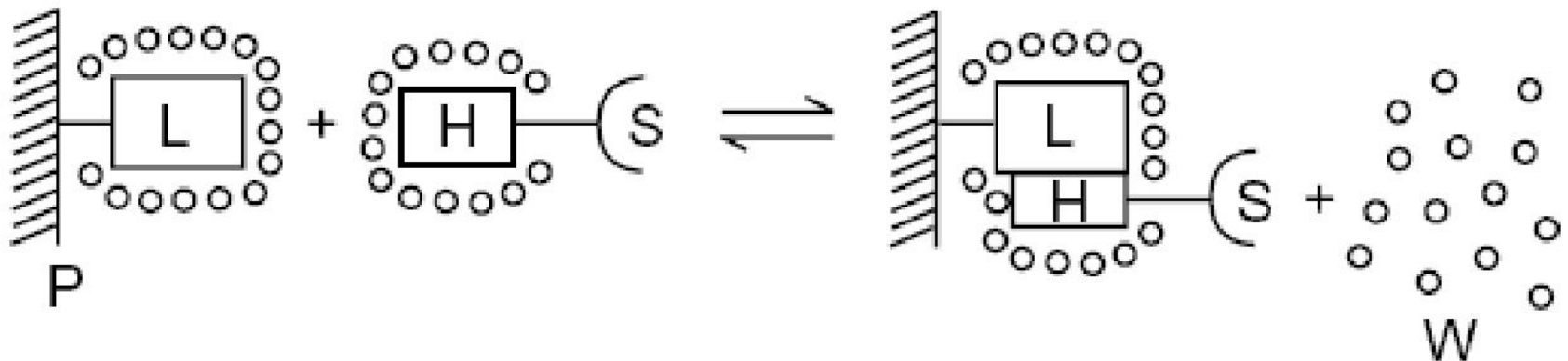


# Гидрофобная хроматография белков



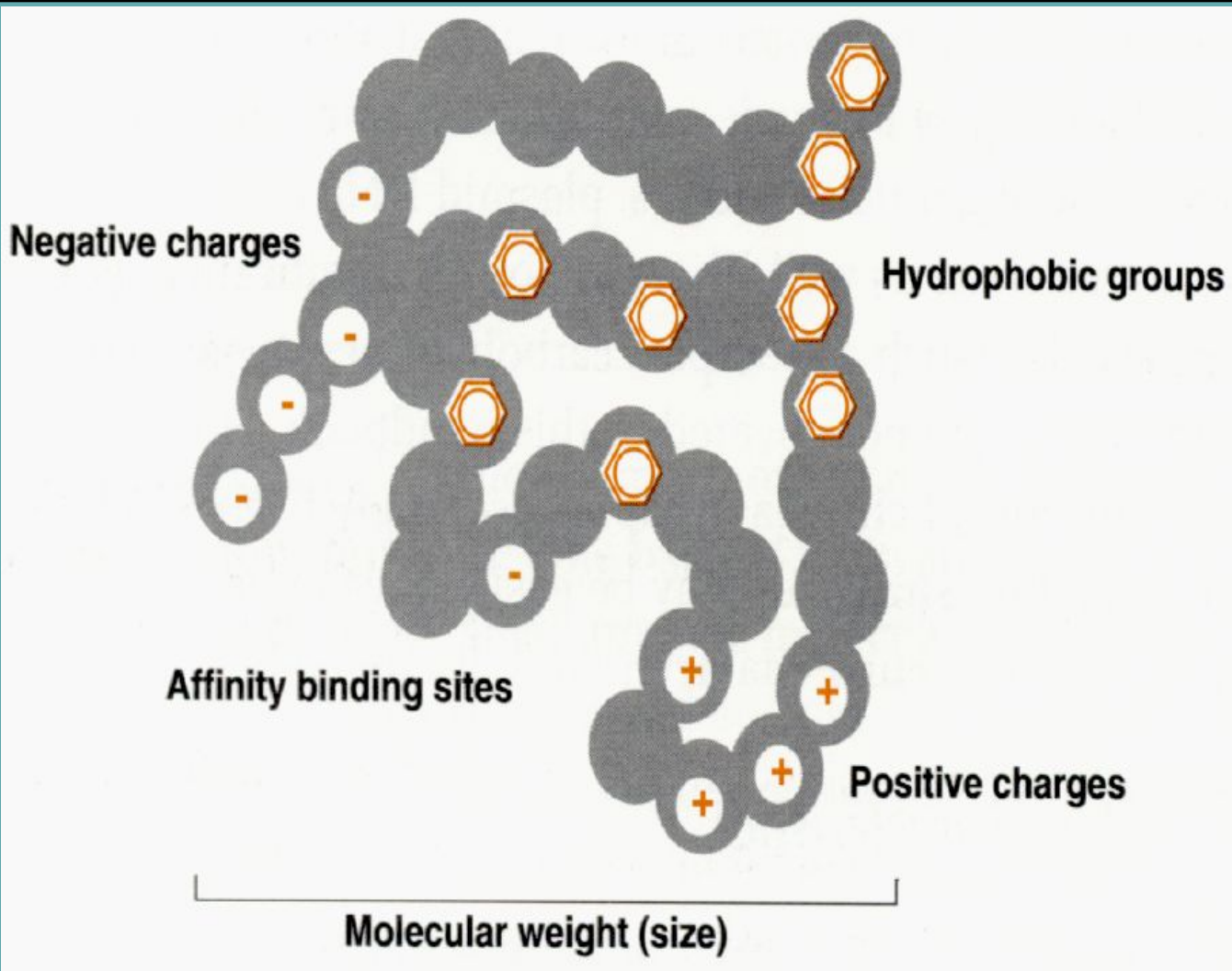
P=Polymer matrix

S=Solute molecule

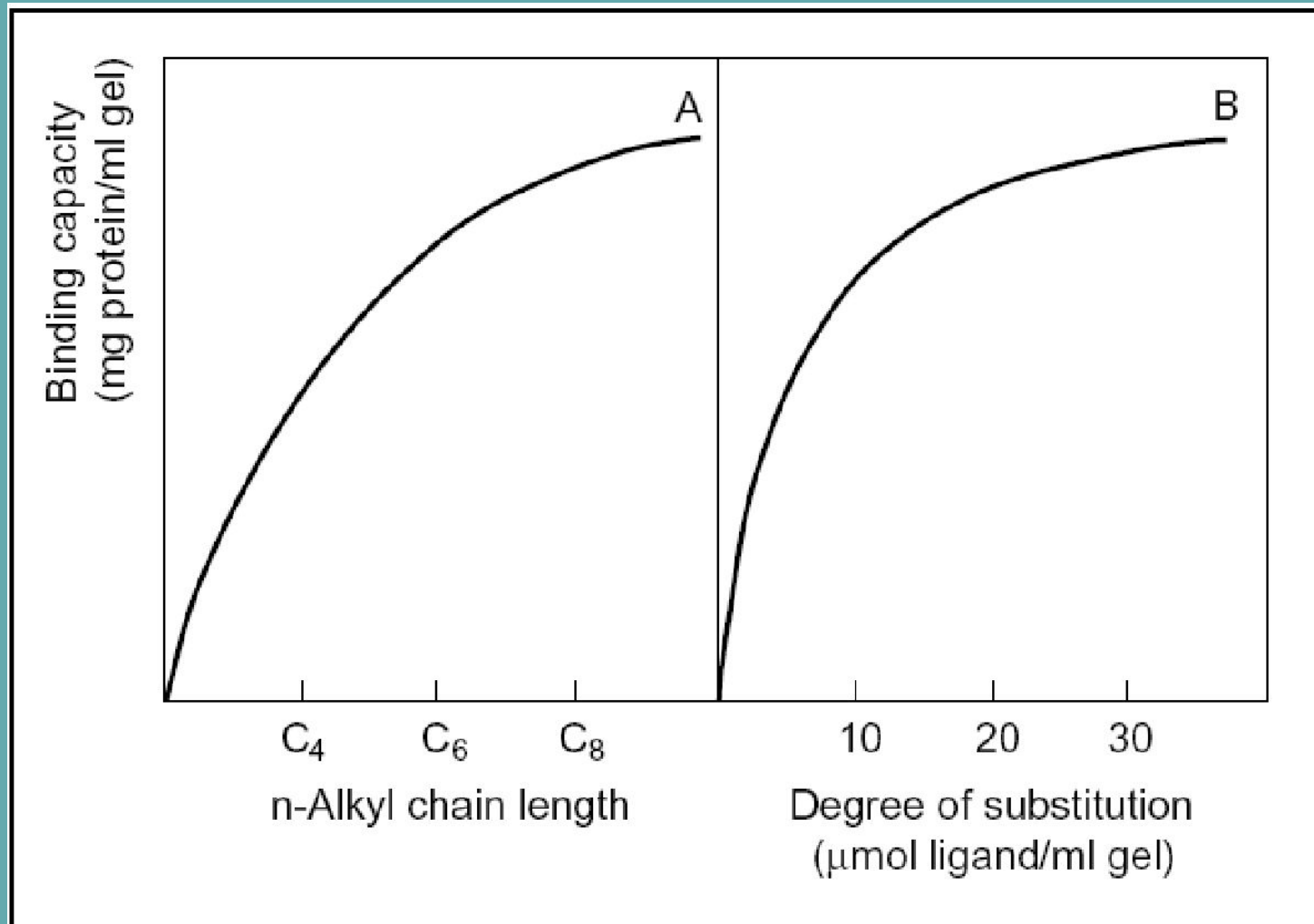
L=Ligand attached to polymer matrix

H=Hydrophobic patch on surface of solute molecule

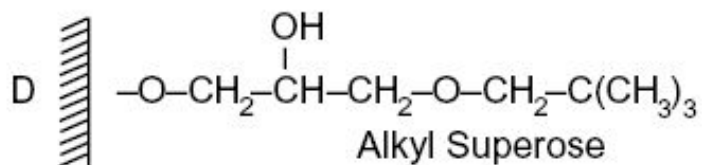
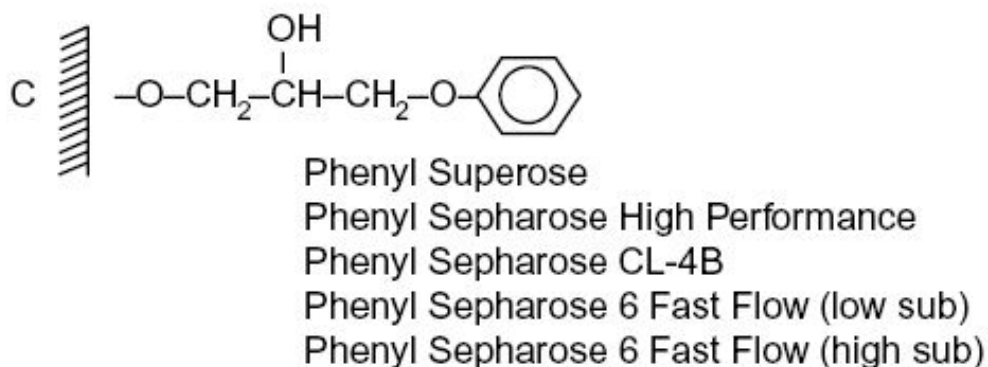
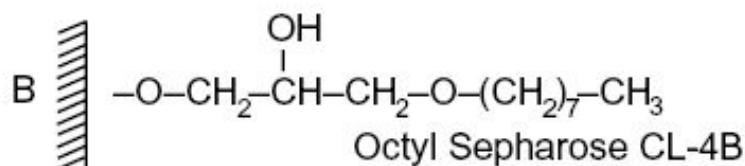
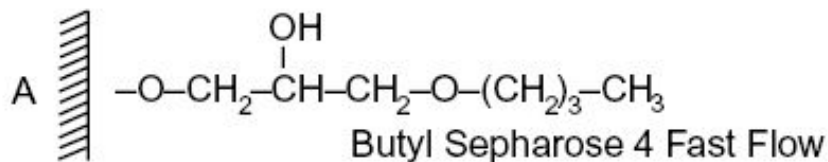
W=Water molecules in the bulk solution



# Влияние длины 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:  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{CH}_3 \cdot \text{COO}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{ClO}_4^-$ ,  $\text{I}^-$ ,  $\text{SCN}^-$   
Cations:  $\text{NH}_4^+$ ,  $\text{Rb}^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cs}^+$ ,  $\text{Li}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$   
Increasing chaotropic (“salting-in”) effect →

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

$\text{Na}_2\text{SO}_4 > \text{K}_2\text{SO}_4 > (\text{NH}_4)_2\text{SO}_4 > \text{Na}_2\text{HPO}_4 > \text{NaCl} > \text{LiCl} \dots > \text{KSCN}$

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

# Влияние pH на взаимодействие белков с гидрофобными сорбентами

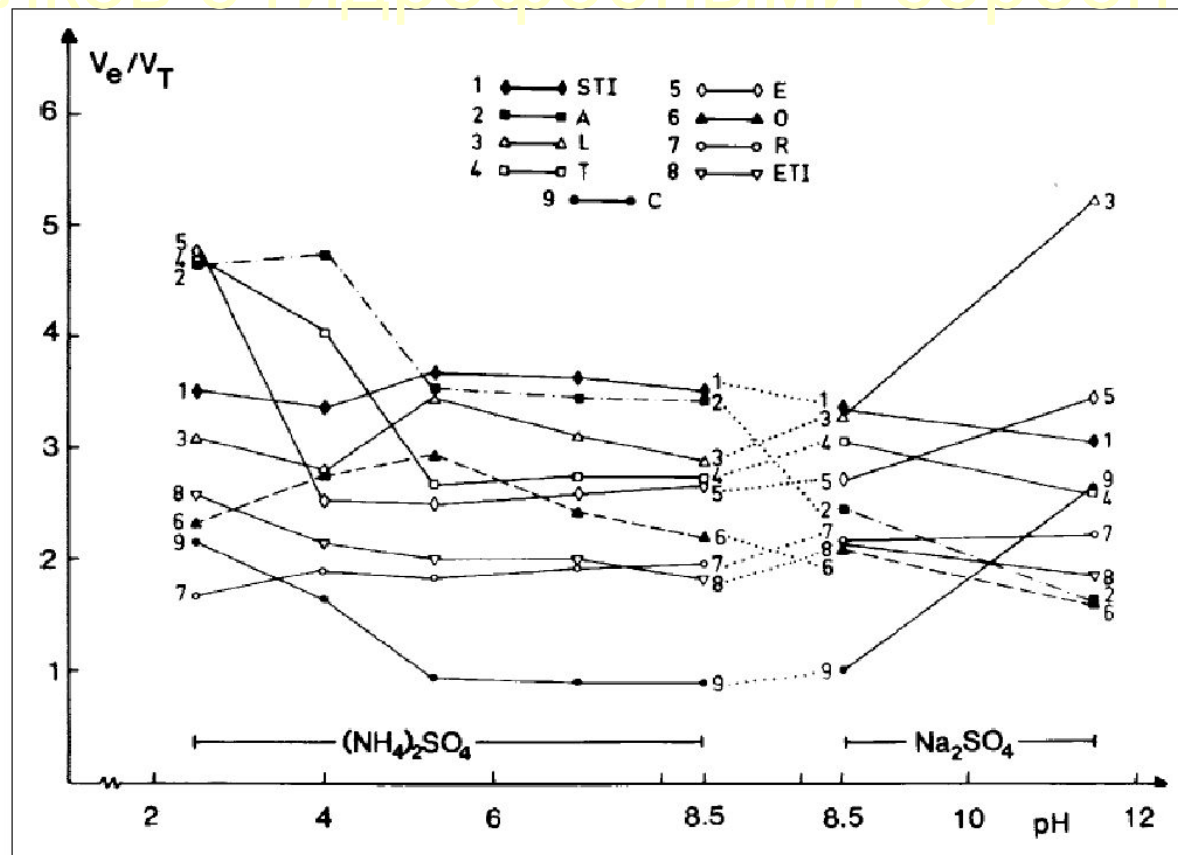


Fig. 5. The pH dependence of the interaction between proteins and an octyl agarose gel expressed as  $V_e/V_T$  ( $V_e$  is the elution volume of the different proteins and  $V_T$  is the elution volume of a non-retarded solute). Elution was by a negative linear gradient of salt. The model proteins used 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).

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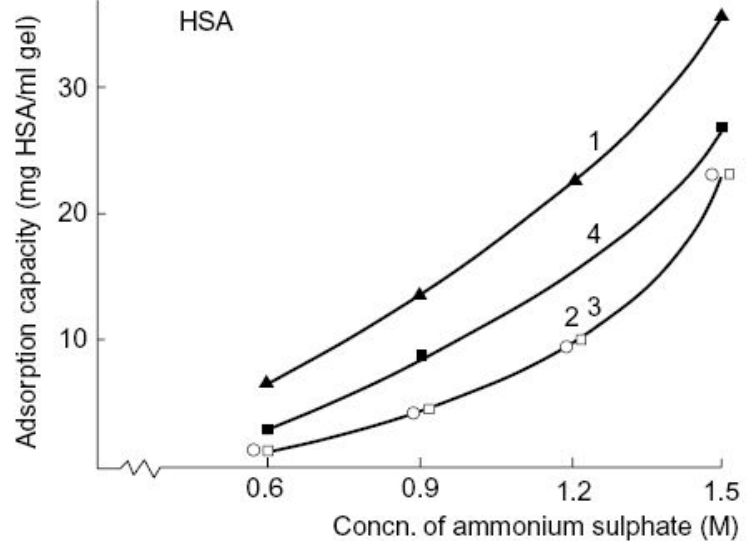
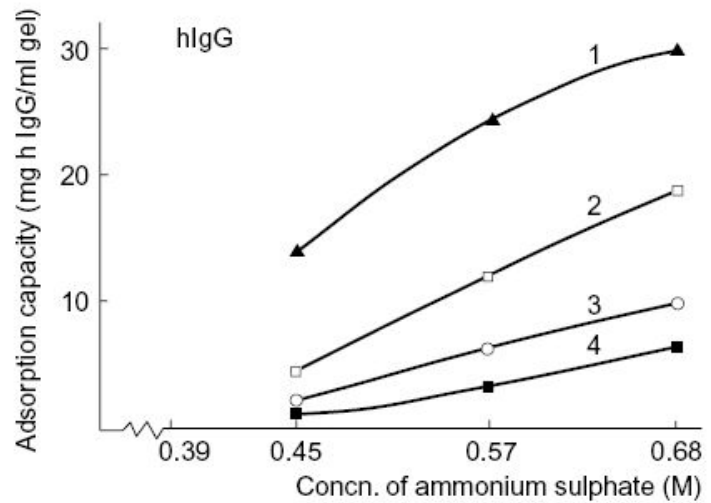
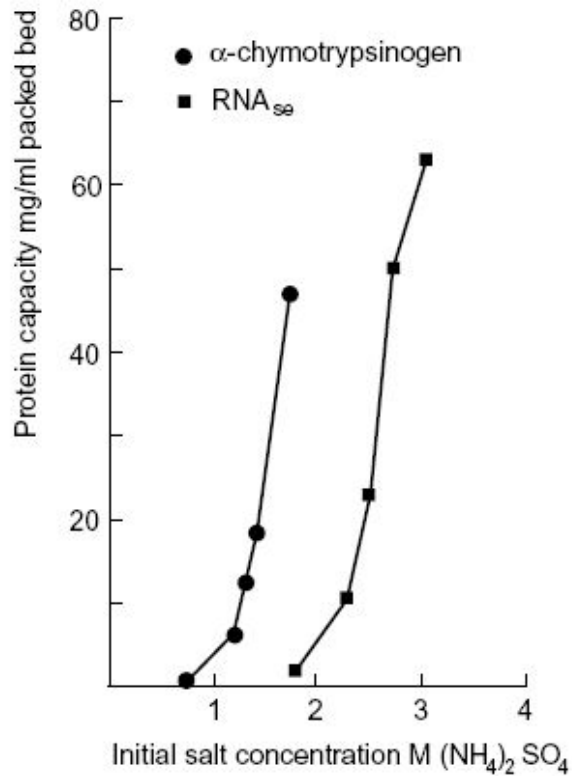
<b>Solvent</b>	<b>Viscosity (centipoise)</b>	<b>Dielectric constant</b>	<b>Surface tension (dynes/cm)</b>
Water	0.89	78.3	72.00
Ethylene glycol	16.90	40.7	46.70
Dimethyl Sulphoxide	1.96	46.7	43.54
Dimethyl Formamide	0.796	36.71	36.76
n-propanol	2.00	20.33	23.71

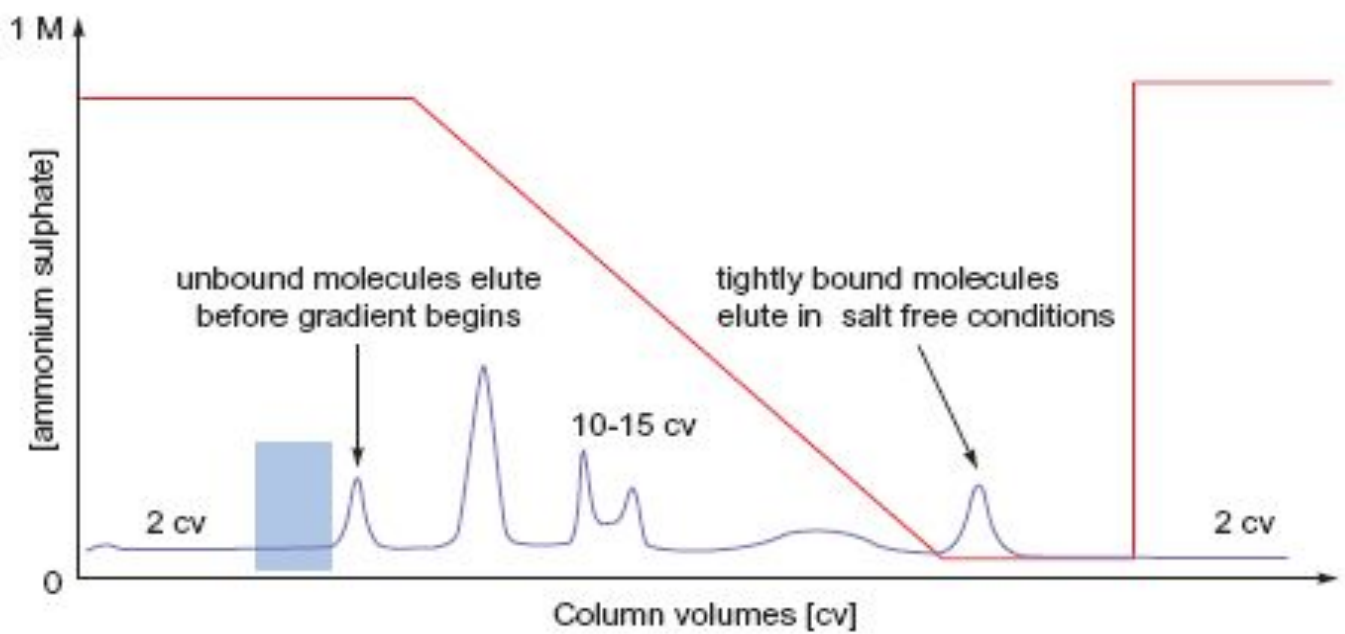
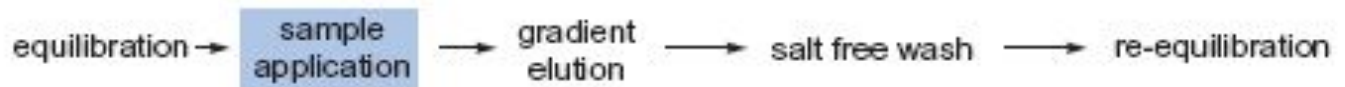
Tested media	Test solutions							
	1 M NaOH	1 M acetic acid	1 mM HCL	3 M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	70% ethanol	30% isopropanol	6 M GuHCl	8 M Urea
Phenyl Sepharose 6 Fast Flow (low sub)	X	(n. t.)	(n. t.)	X	X	X	X	X
Phenyl Sepharose 6 Fast Flow (high sub)	X	(n. t.)	(n. t.)	X	X	X	X	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

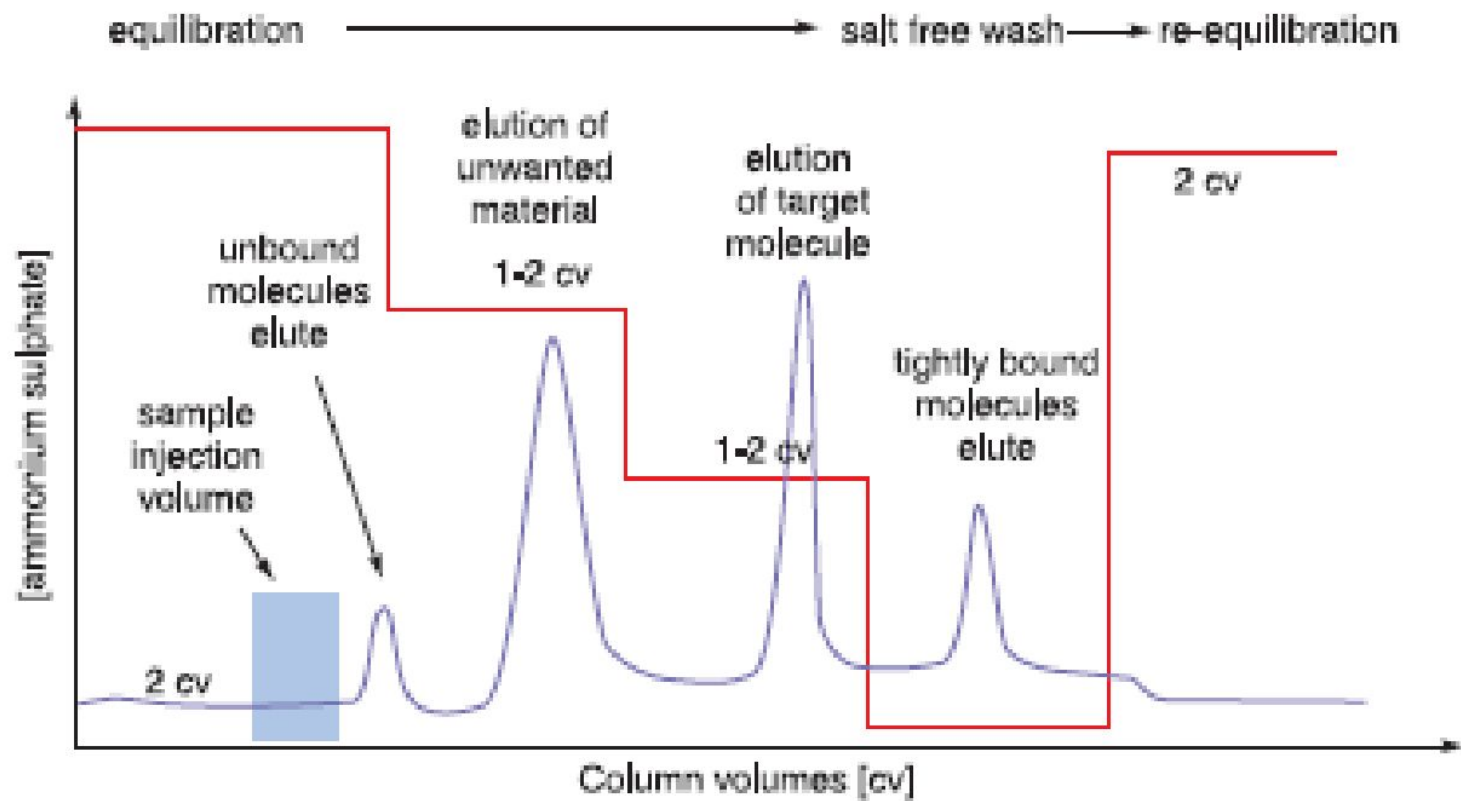
X = Functionally stable when tested for 7 days at +40°C  
(n. t.) = Not tested

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.**

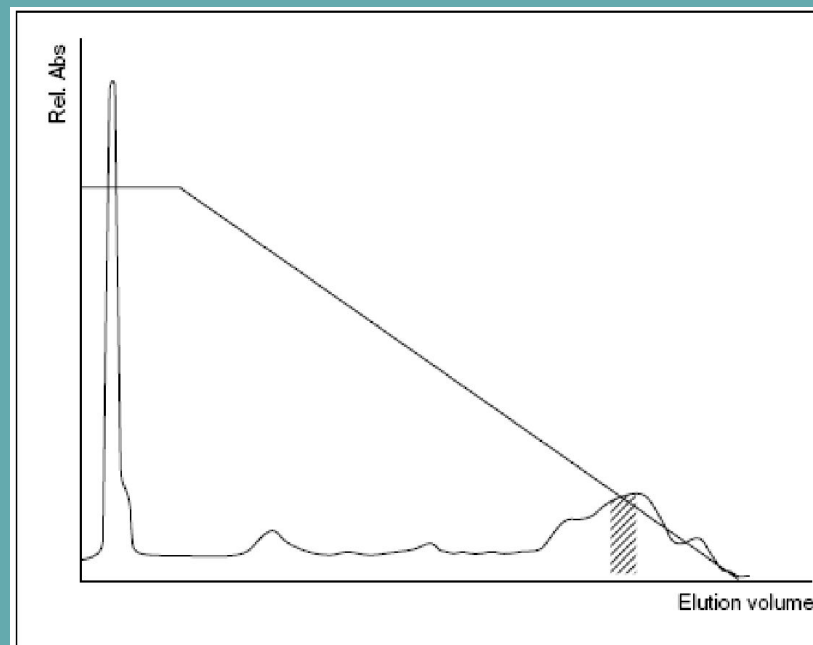
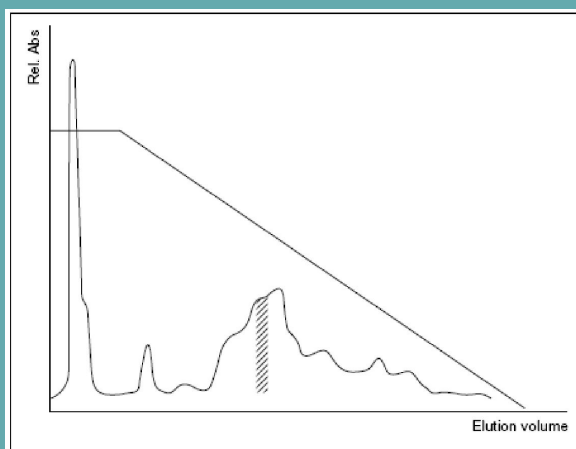
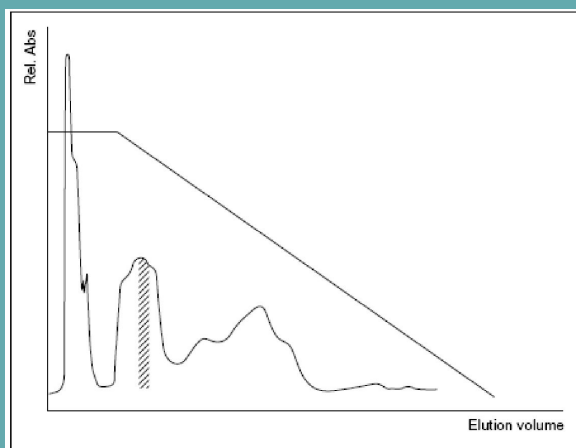






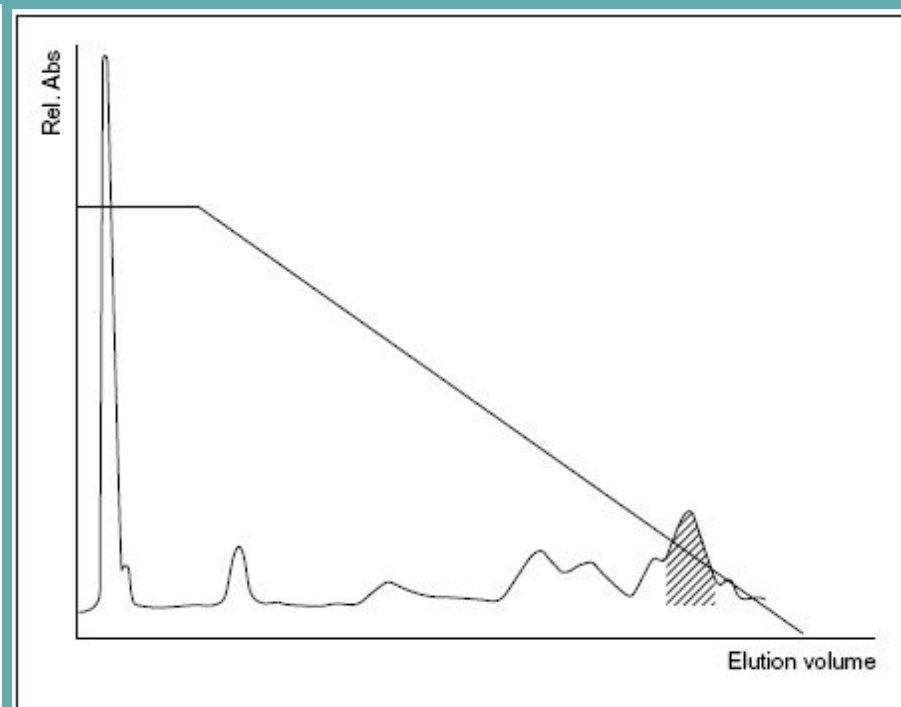
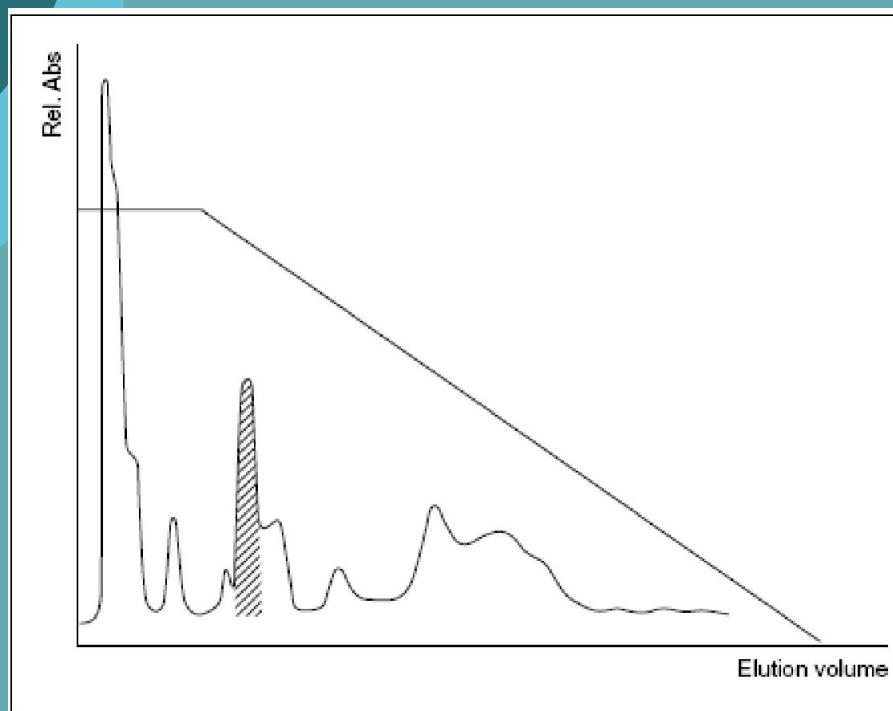


# Оптимизация условий разделения гидрофобной хроматографией



# Оптимизация условий разделения гидрофобной хроматографией

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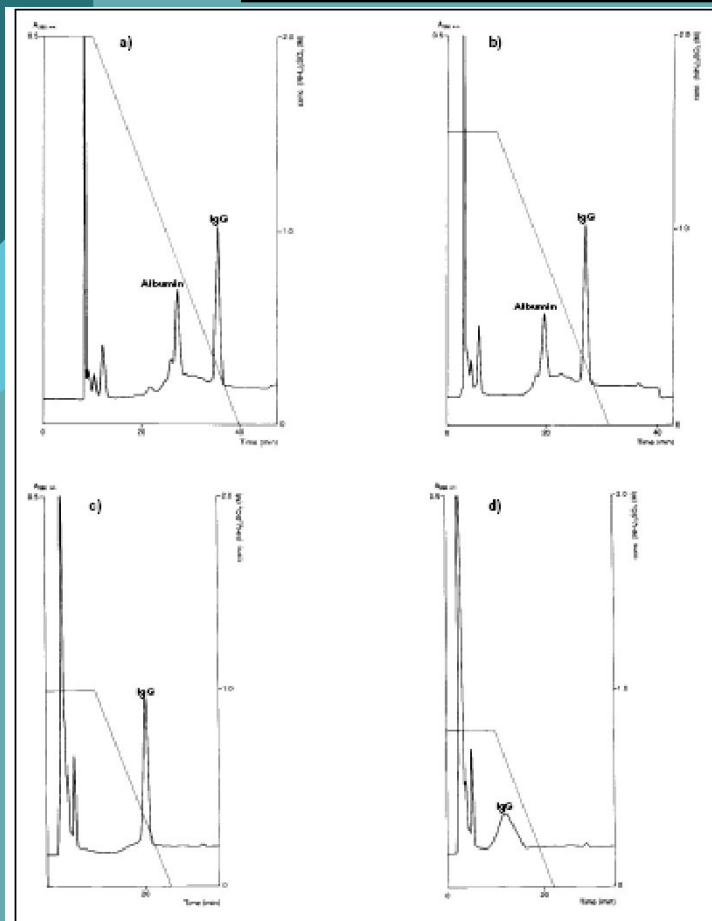


Fig. 16. The effect of starting conditions in HIC. Sample, 100  $\mu$ l anti-CEA MAB (IgG<sub>1</sub>) from mouse ascites fluid in 0.8 M  $(\text{NH}_4)_2\text{SO}_4$  (corresponding to 20  $\mu$ l ascites); column, Alkyl Superose HR 5/5; flow rate, 0.5 ml  $\text{min}^{-1}$ ; buffer A, 0.1 M sodium phosphate, pH 7.0,  $(\text{NH}_4)_2\text{SO}_4$ . (a) Sample applied in 2 M  $(\text{NH}_4)_2\text{SO}_4$ ; both albumin and IgG are absorbed. (b) Sample applied in 1.5 M  $(\text{NH}_4)_2\text{SO}_4$ ; less albumin binds and IgG elutes earlier in the gradient. (c) Sample applied in 1.0 M  $(\text{NH}_4)_2\text{SO}_4$ ; albumin does not bind and, therefore, the column has a greater capacity for binding IgG. (d) Sample applied in 0.8 M  $(\text{NH}_4)_2\text{SO}_4$ ; albumin does not bind; IgG is retarded, but elutes in a broad peak. (Work from Amersham Pharmacia Biotech, Uppsala, Sweden).

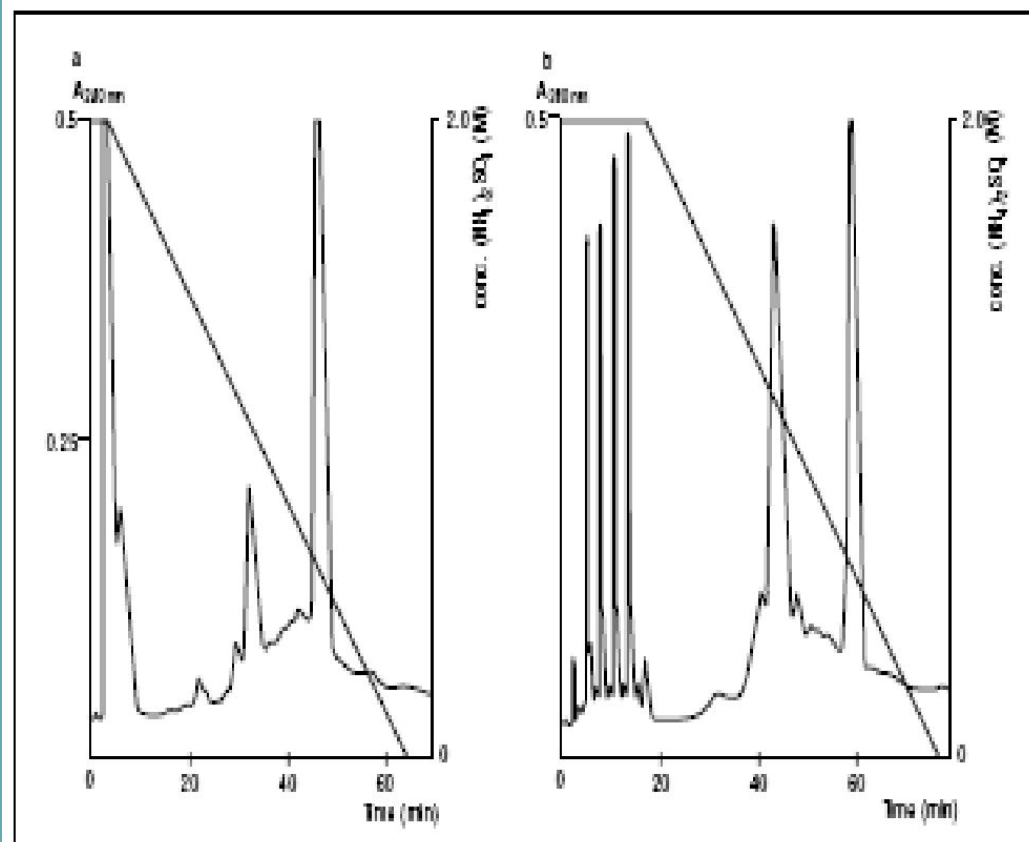


Fig. 17. The effect of loading conditions in HIC. Column, Alkyl Superose HR 5/5; flow rate, 0.5 ml  $\text{min}^{-1}$ ; buffer A, 0.1 M sodium phosphate, pH 7.0, 2 M  $(\text{NH}_4)_2\text{SO}_4$ . (a) Sample (500  $\mu$ l anti-CEA MAB (IgG<sub>1</sub>) from mouse ascites fluid in 0.9 M  $(\text{NH}_4)_2\text{SO}_4$  (corresponding to 115  $\mu$ l ascites) applied in one injection. (b) Sample as (a) applied in five 100  $\mu$ l injections with 1.3 ml 2.0 M  $(\text{NH}_4)_2\text{SO}_4$  after each portion. (Work from Amersham Pharmacia Biotech, Uppsala, Sweden).

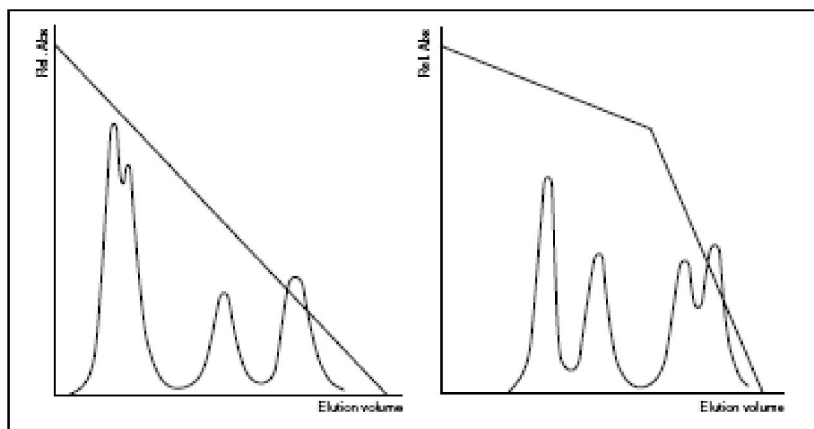


Fig. 18. Effect of a complex gradient on resolution.

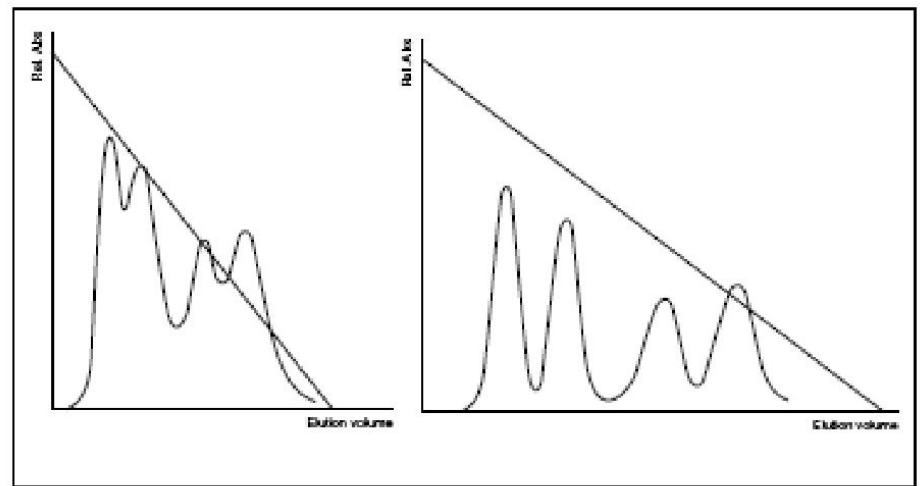


Fig. 19. Effect of gradient slope on resolution.

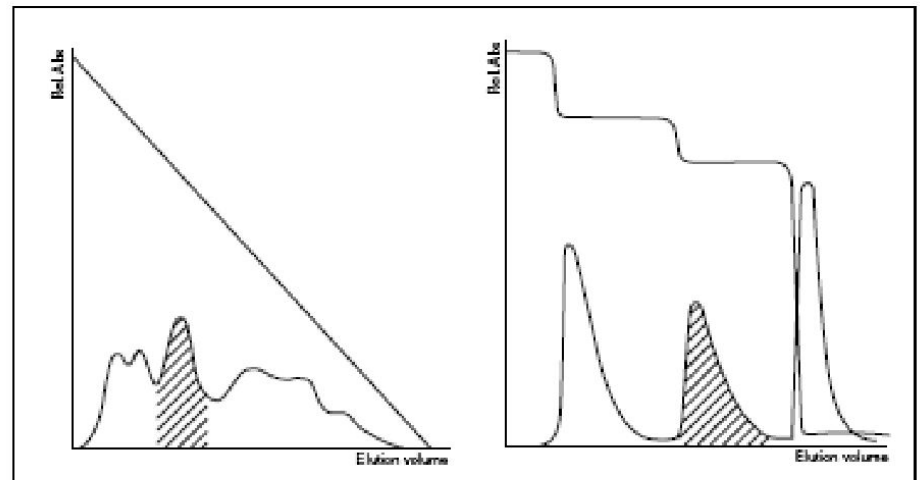
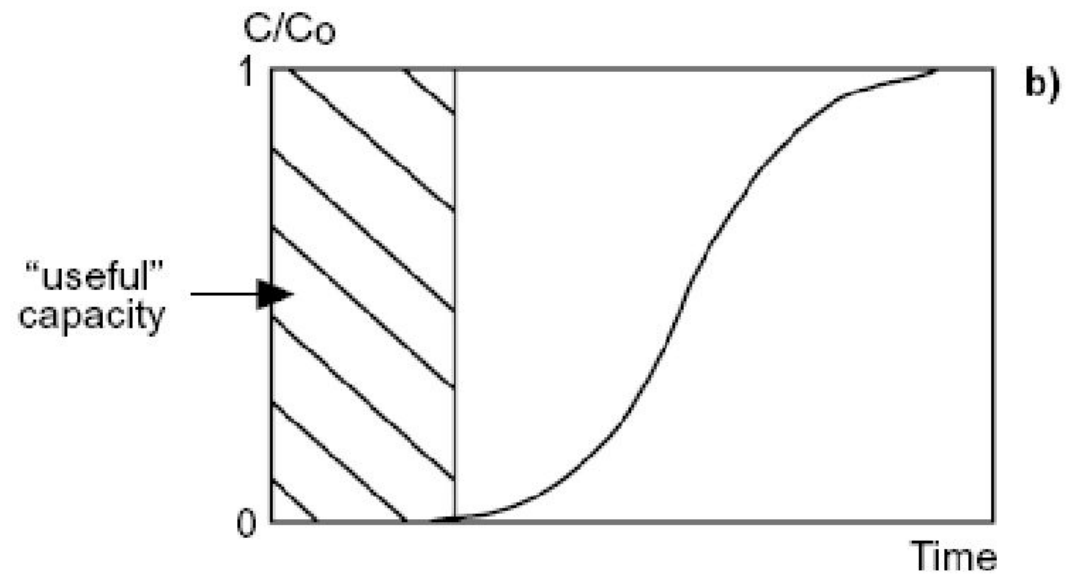
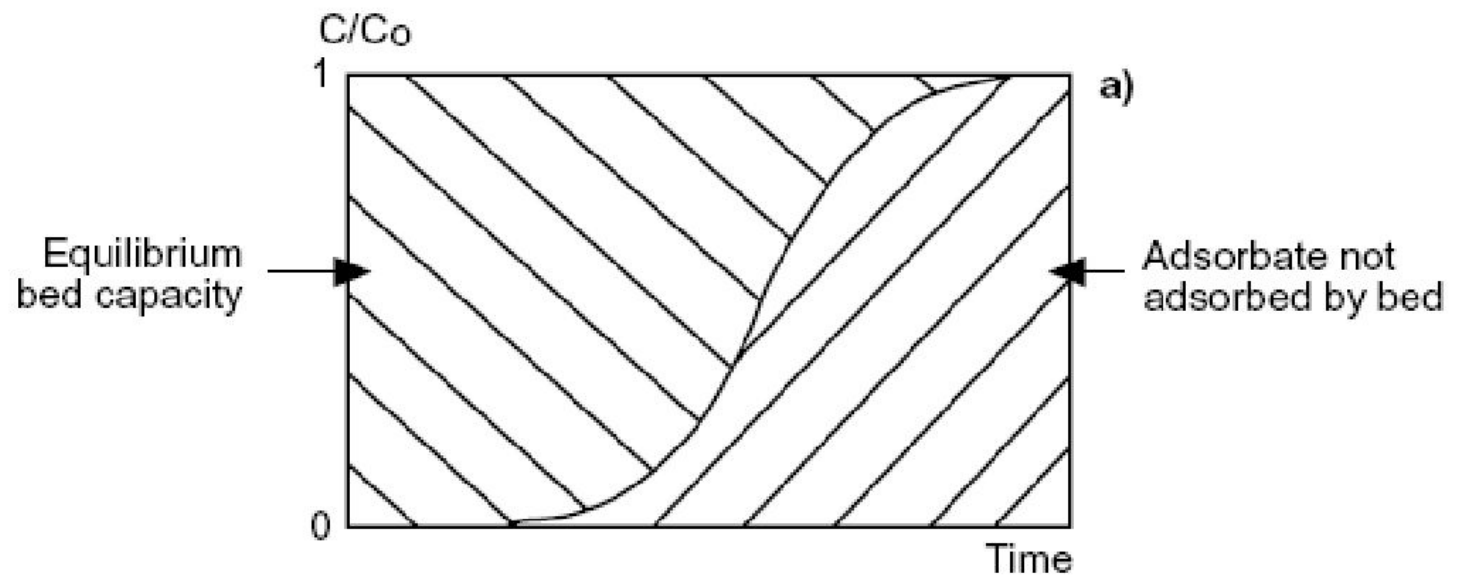
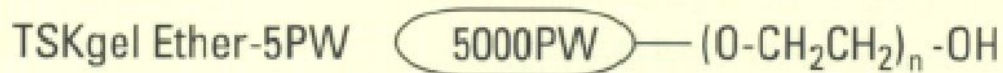
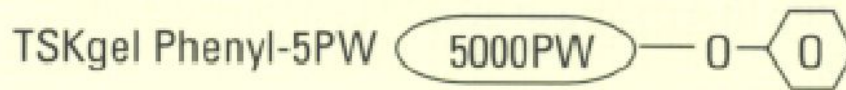


Fig. 20. Switching from a continuous gradient to step-wise elution.

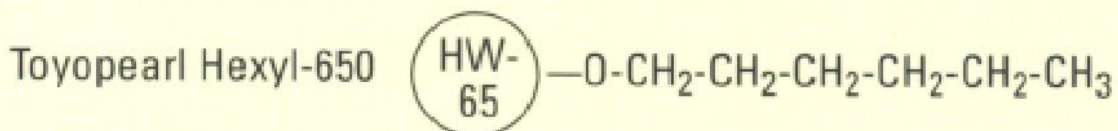
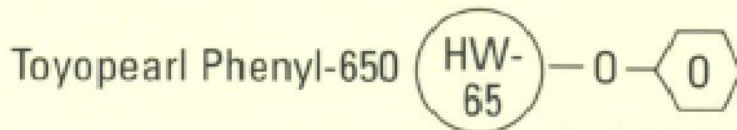




## Structure of TSK-GEL HIC resins



## Structure of Toyopearl HIC resins

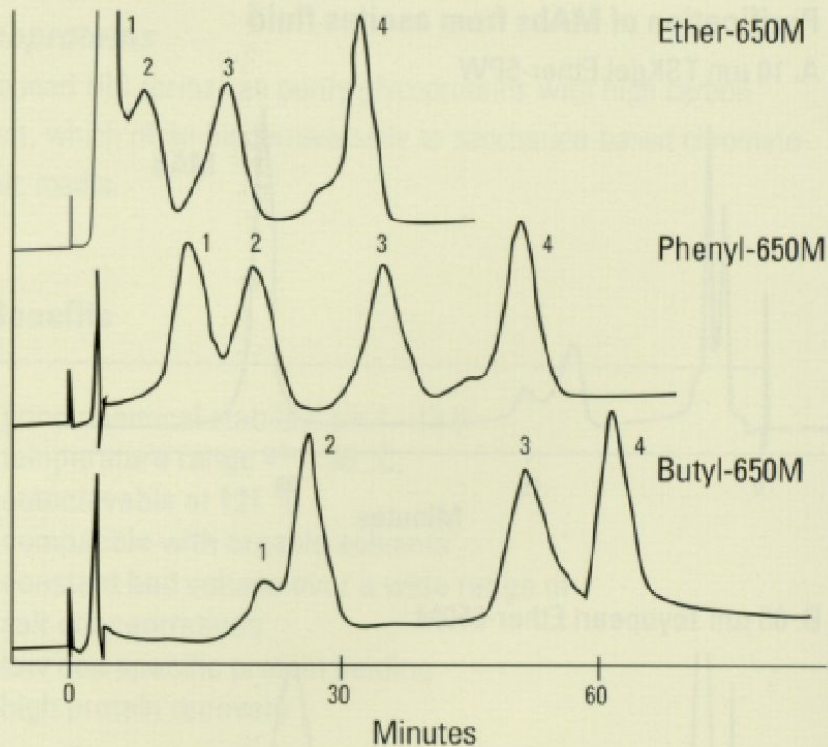


## Resolution values ( $R_s$ ) of Toyopearl resins for lysozyme and $\alpha$ -chymotrypsinogen

*Particle size grade*

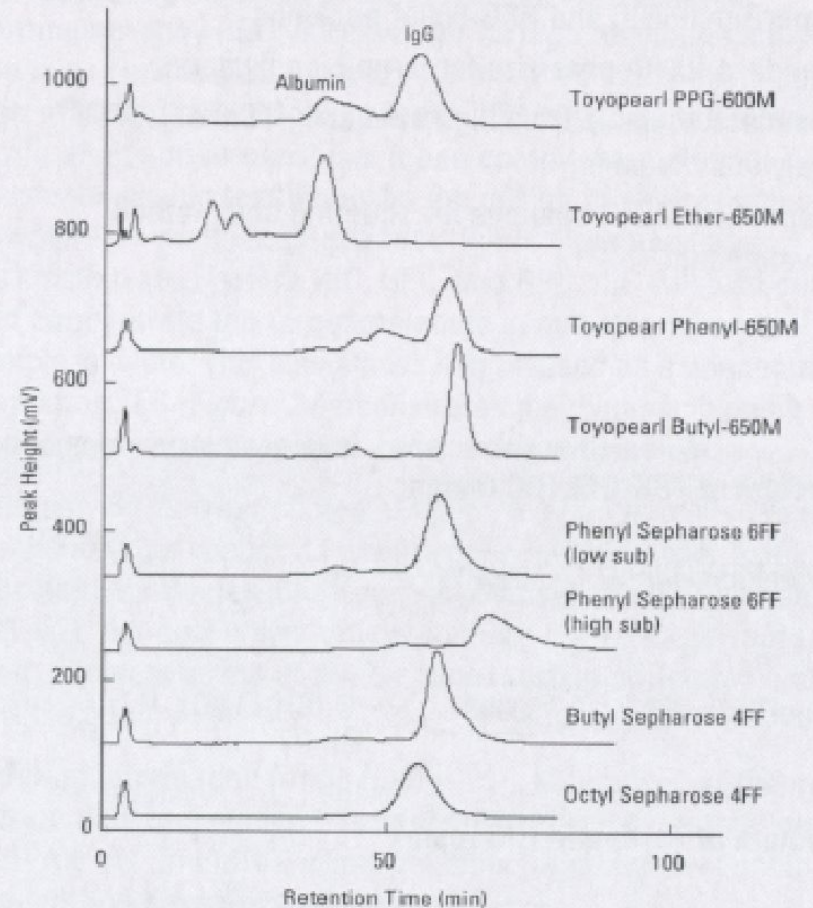
<b><i>Resins</i></b>	<b><i>C</i></b>	<b><i>M</i></b>	<b><i>S</i></b>
Phenyl-650	1.12	1.52	2.19
Butyl-650	0.91	1.37	2.20

### 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.  $\alpha$ -chymotrypsinogen  
 Elution: 60 min linear gradient from 1.8 M to 0 M  $(\text{NH}_4)_2\text{SO}_4$  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  $\mu$ L  
 Sample: mouse ascites fluid (x 4 diluted) (Antibody: Anti-IgE)

## Proposed Two Step Adsorption Model for Mab to Toyopearl Resins

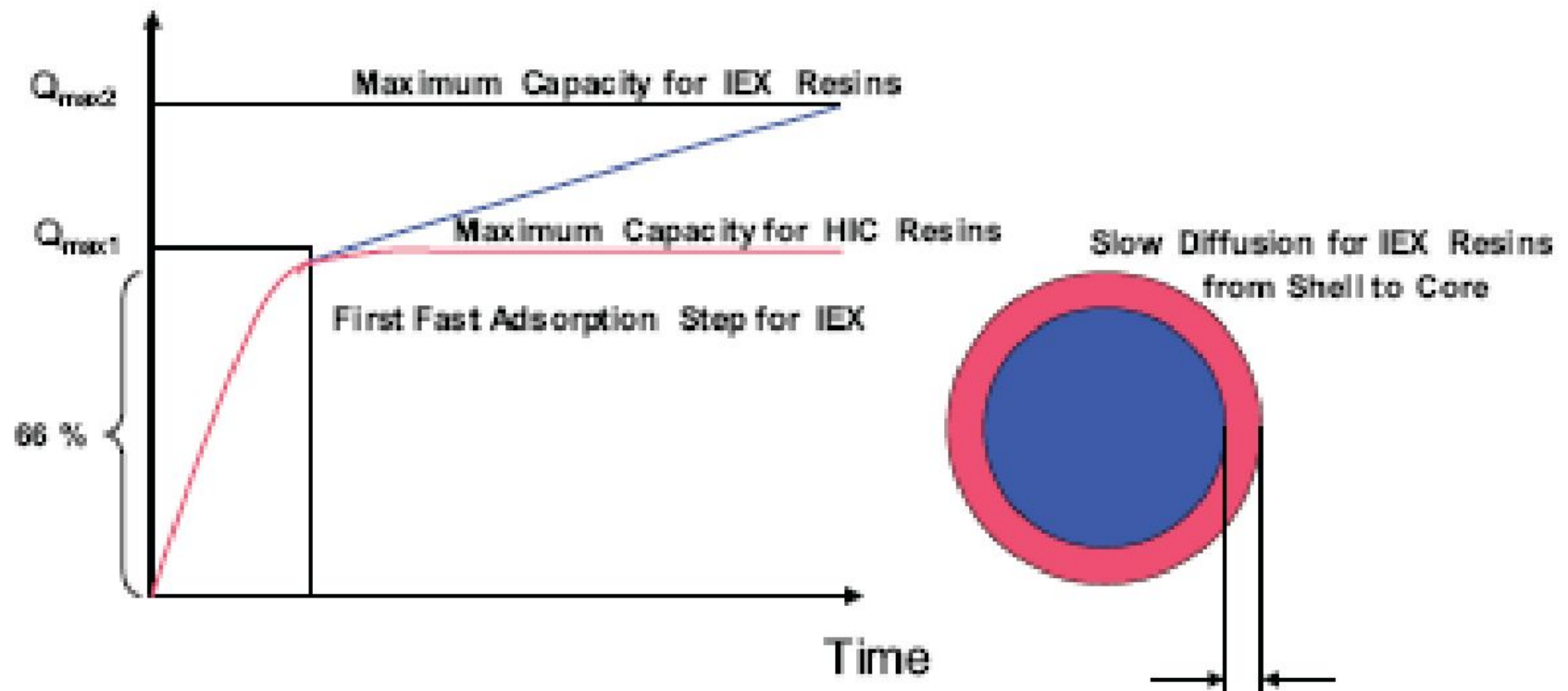
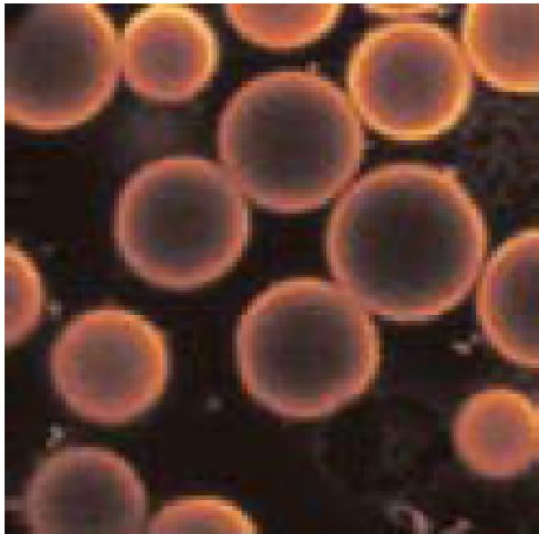


Figure 6

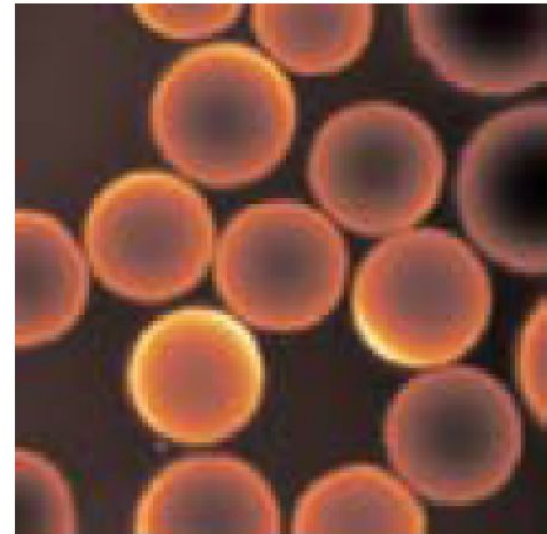
$\approx 20 \mu\text{m}$   
"Equilibrium Penetration Depth"

**Toyopearl Phenly-650M  
(24 hours)**



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

**Toyopearl SP (100-300 $\mu$ m)  
(24 hours)**



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

*Figure 7*

# MAb Adsorption on Toyopearl Phenyl Resins

MAb (mouse IgG 2a) Binding Capacity [mg/ml]

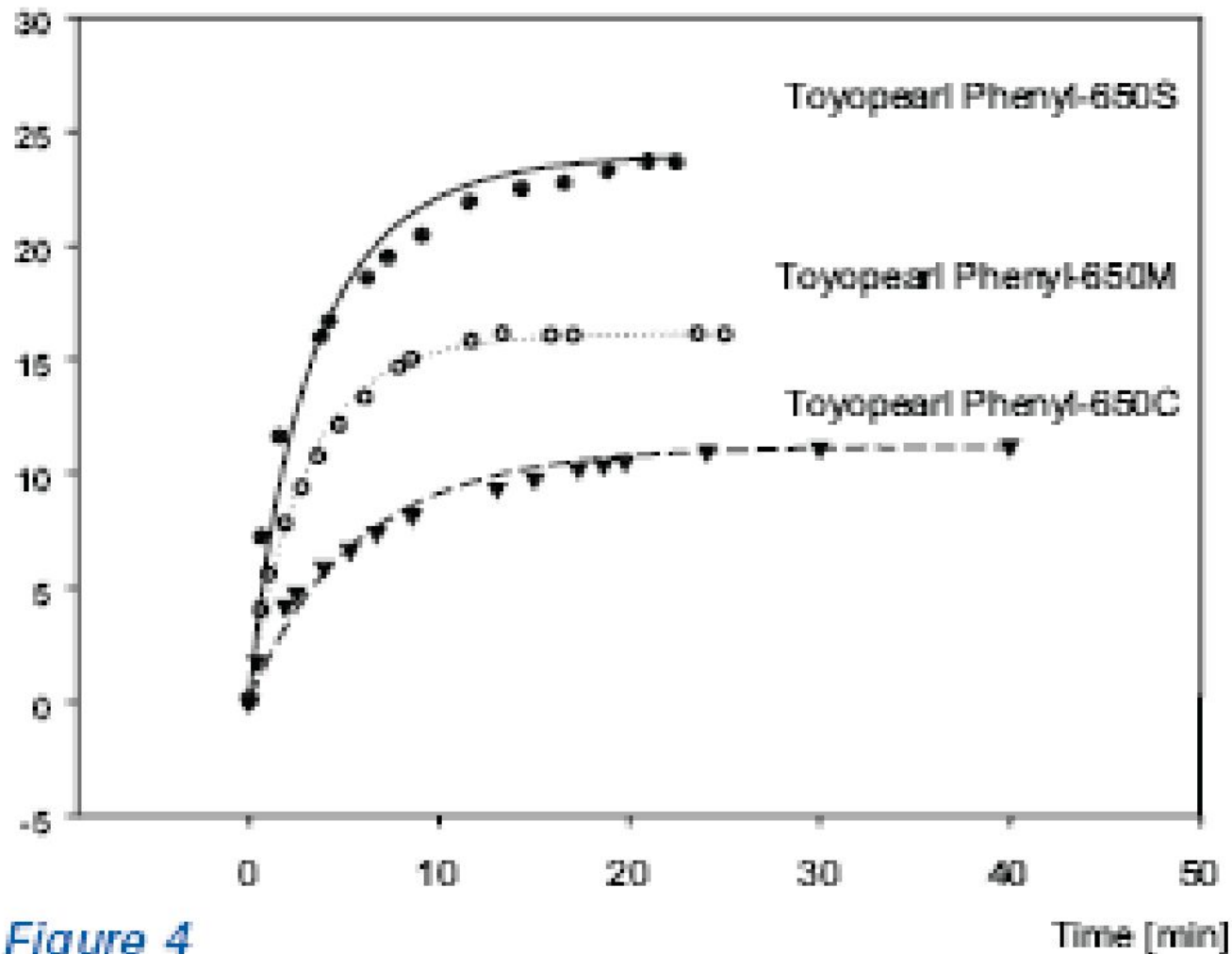
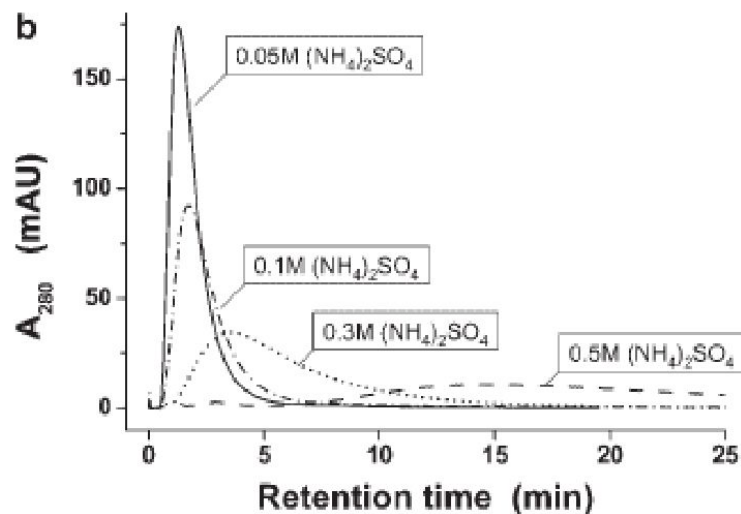
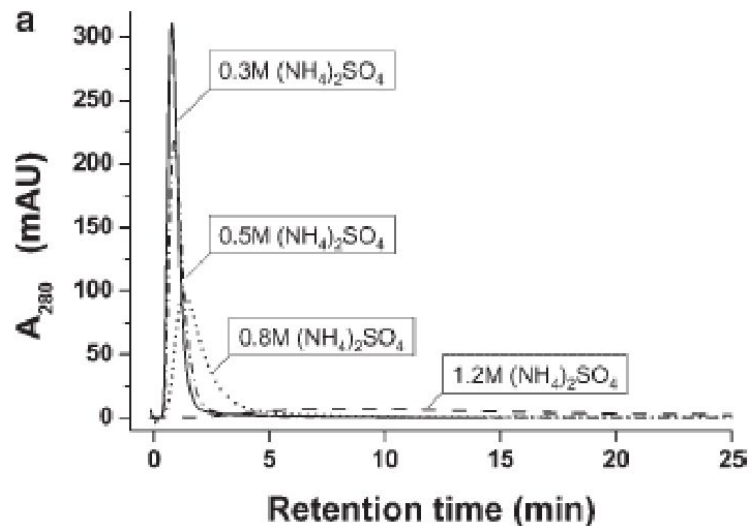
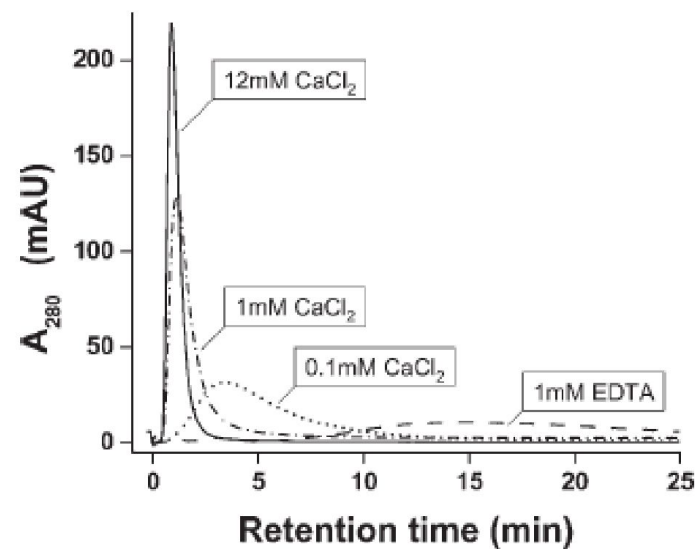


Figure 4

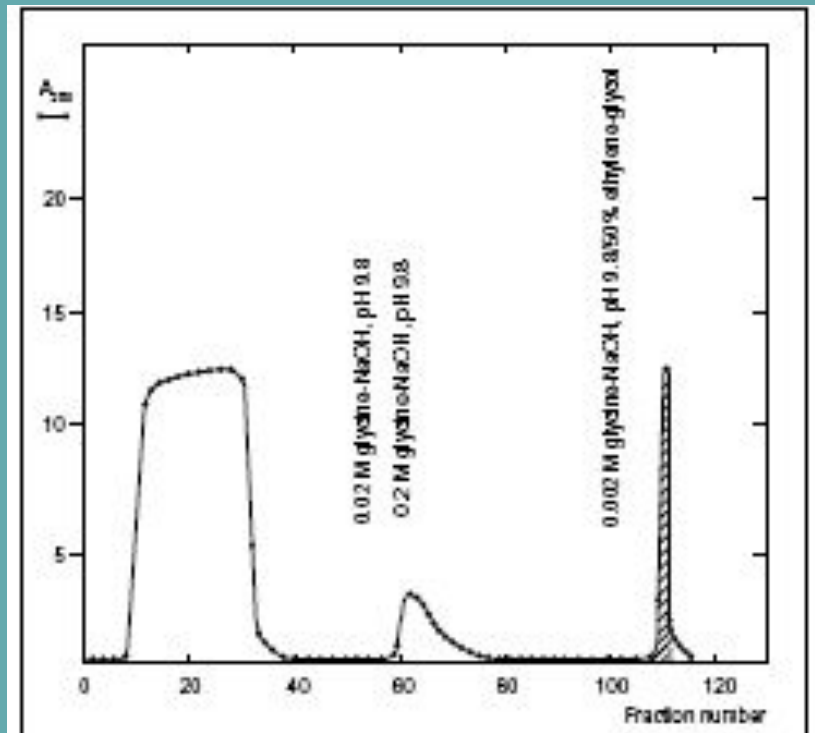
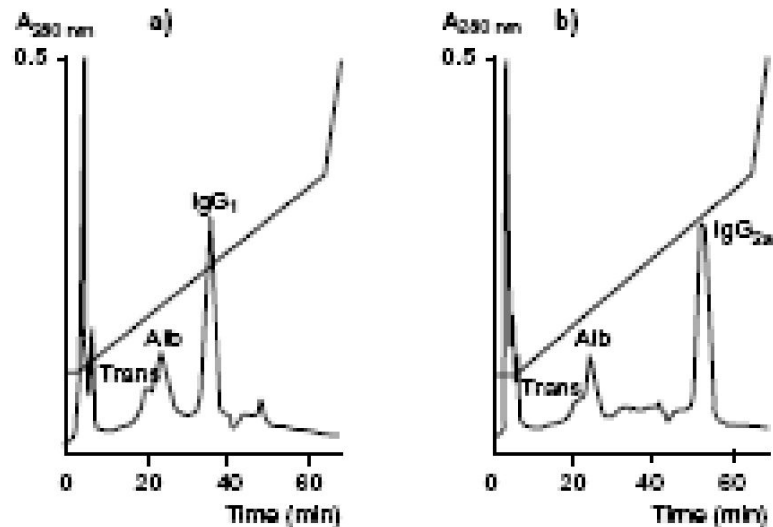


**Figure 1.** Effect of  $(\text{NH}_4)_2\text{SO}_4$  concentration on  $\alpha$ -lactalbumin retention for  $\alpha$ -lactalbumin. Isocratic elution was performed on Phenyl Sepharose™ 6 Fast Flow (low sub) at a flowrate of 1.0 mL/min and 55°C. a: Calcium included in the samples and buffers at 12 mM for all conditions, with  $(\text{NH}_4)_2\text{SO}_4$  concentrations varying as shown. b: 0 mM  $\text{CaCl}_2$ . EDTA (2 mM) was included to chelate any trace calcium.



**Figure 3.** Effect of calcium concentration on  $\alpha$ -lactalbumin isocratic elution. The  $(\text{NH}_4)_2\text{SO}_4$  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  $\mu$ l mouse ascites containing monoclonal IgG<sub>1</sub> (a) or IgG<sub>2a</sub> (b) + 100  $\mu$ l buffer A, centrifuged and filtered  
**Buffer A:** 0.1 M phosphate, pH 7.0, 2.0 M ammonium sulphate  
**Buffer B:** 0.1 M phosphate, pH 7.0  
**Detection:** A<sub>280</sub>. 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<sub>1</sub> 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

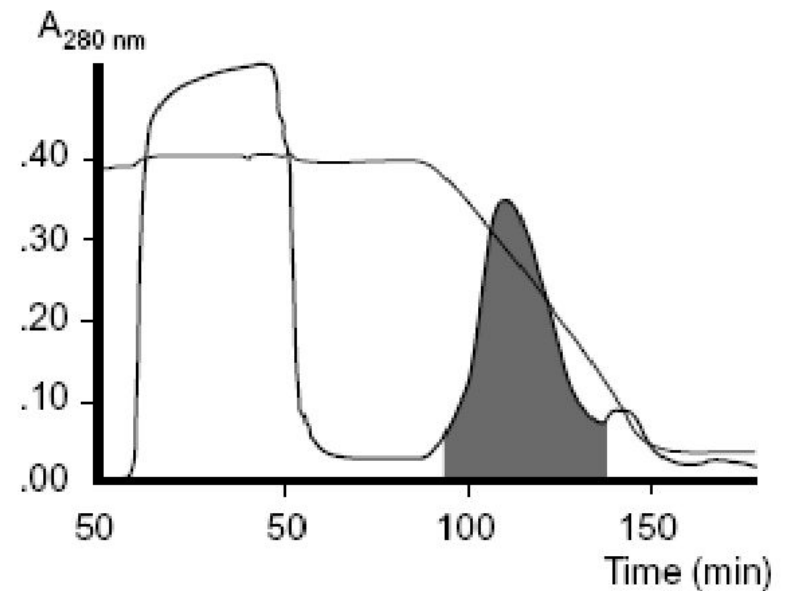


Fig. 48. Laboratory scale purification of mouse IgG<sub>1</sub> anti-IgE, on Phenyl Sepharose High Performance. (Work from Amersham Pharmacia Biotech, Uppsala, Sweden).

**Column:** BioPilot Column 35/100  
**Gel:** Phenyl Sepharose High Performance, 10 cm bed height  
**Sample:** Hybridoma cell culture supernatant; mouse IgG<sub>1</sub>, 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

