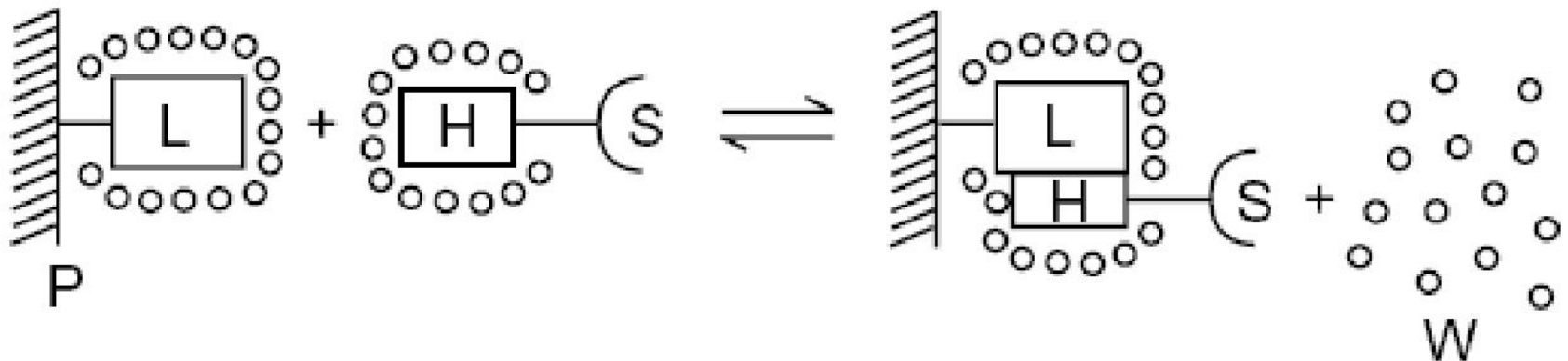


Гидрофобная хроматография белков



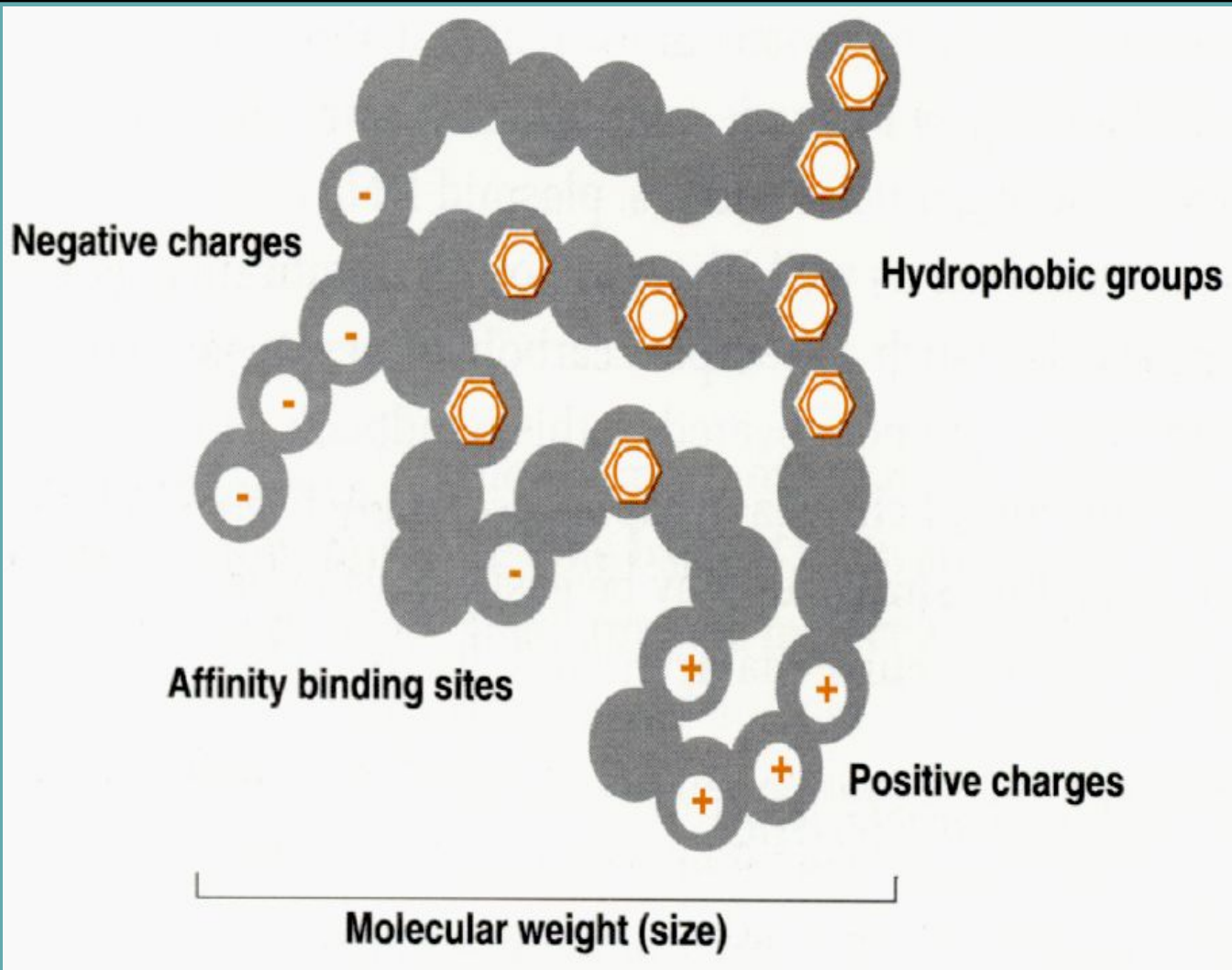
P=Polymer matrix

S=Solute molecule

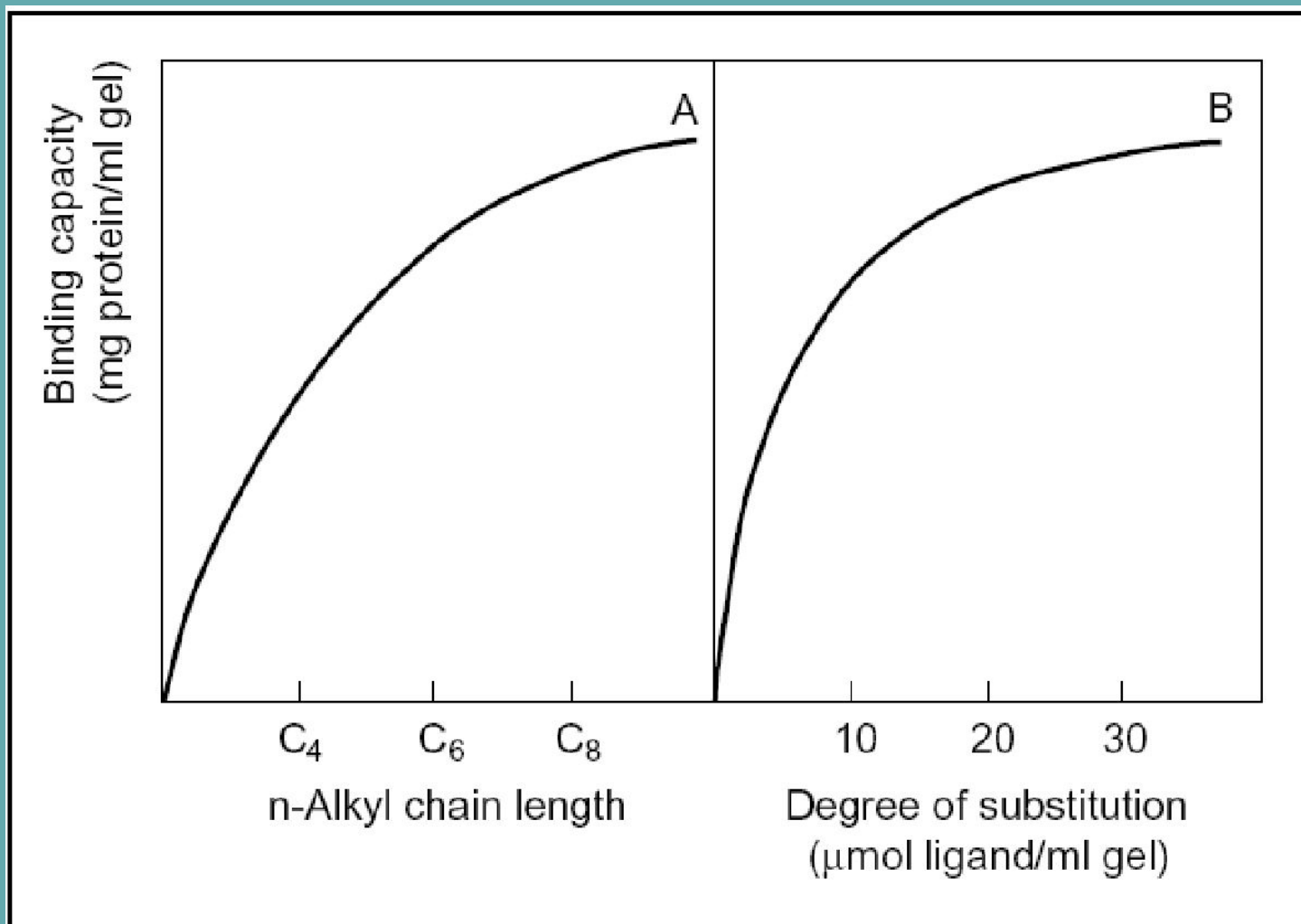
L=Ligand attached to polymer matrix

H=Hydrophobic patch on surface of solute molecule

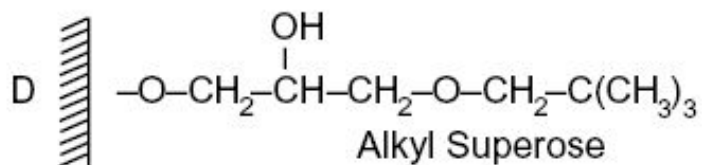
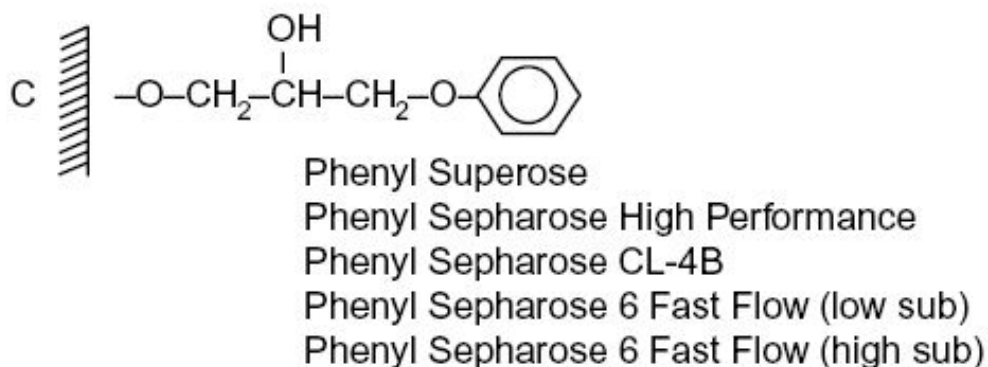
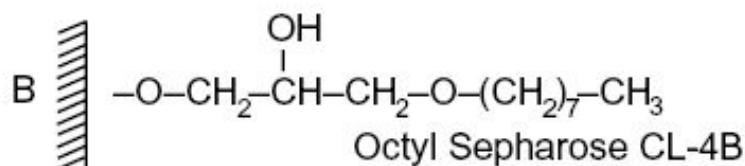
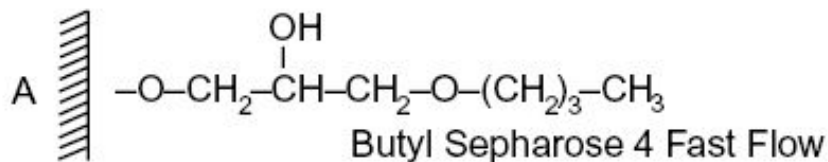
W=Water molecules in the bulk solution



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: PO_4^{3-} , SO_4^{2-} , $\text{CH}_3 \cdot \text{COO}^-$, 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.

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

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

Влияние pH на взаимодействие белков с гидрофобными сорбентами

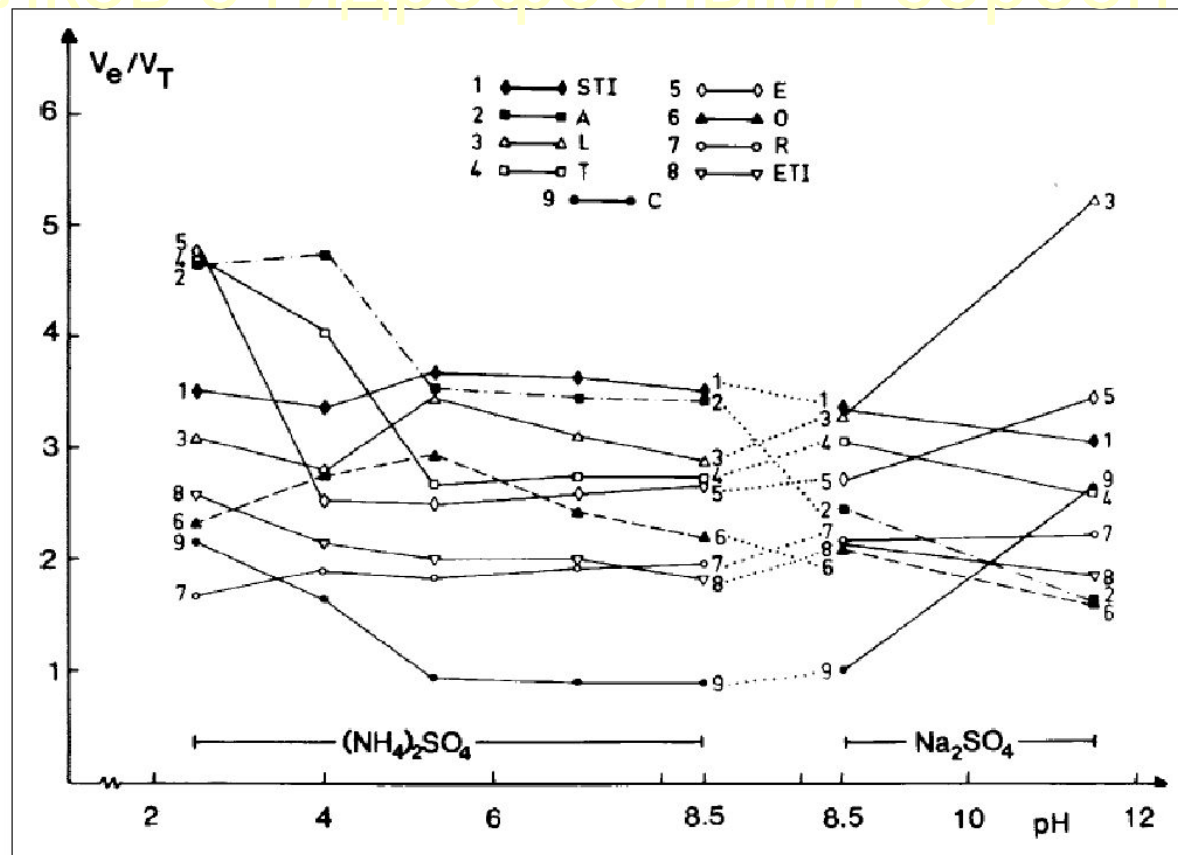


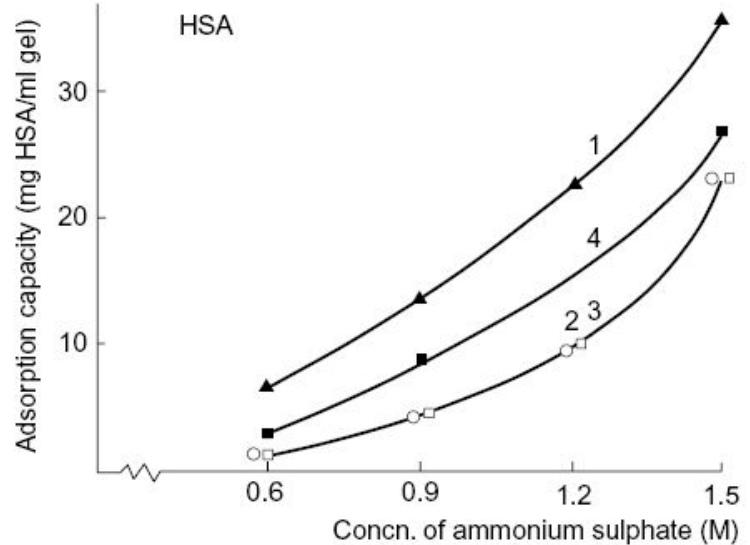
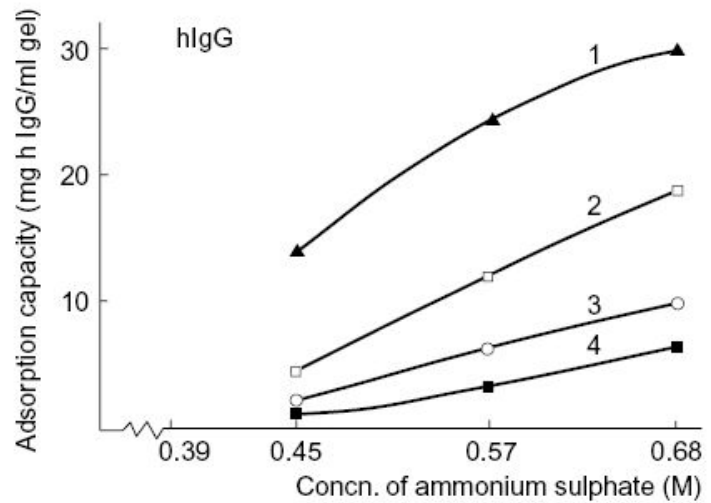
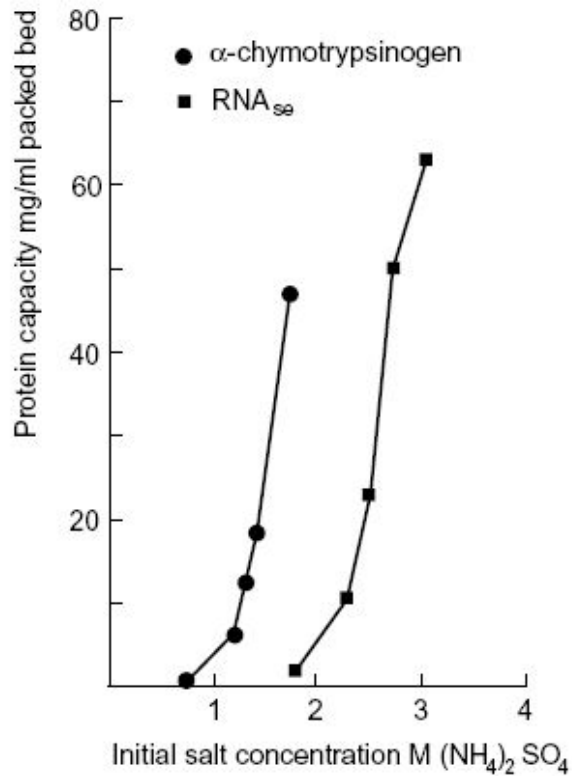
Fig. 5. The pH dependence of the interaction between proteins and an octyl agarose gel expressed as V_e/V_T (V_e is the elution volume of the different proteins and V_T is the elution volume of a non-retarded solute). Elution was by a negative linear gradient of salt. The model proteins used were STI=soy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

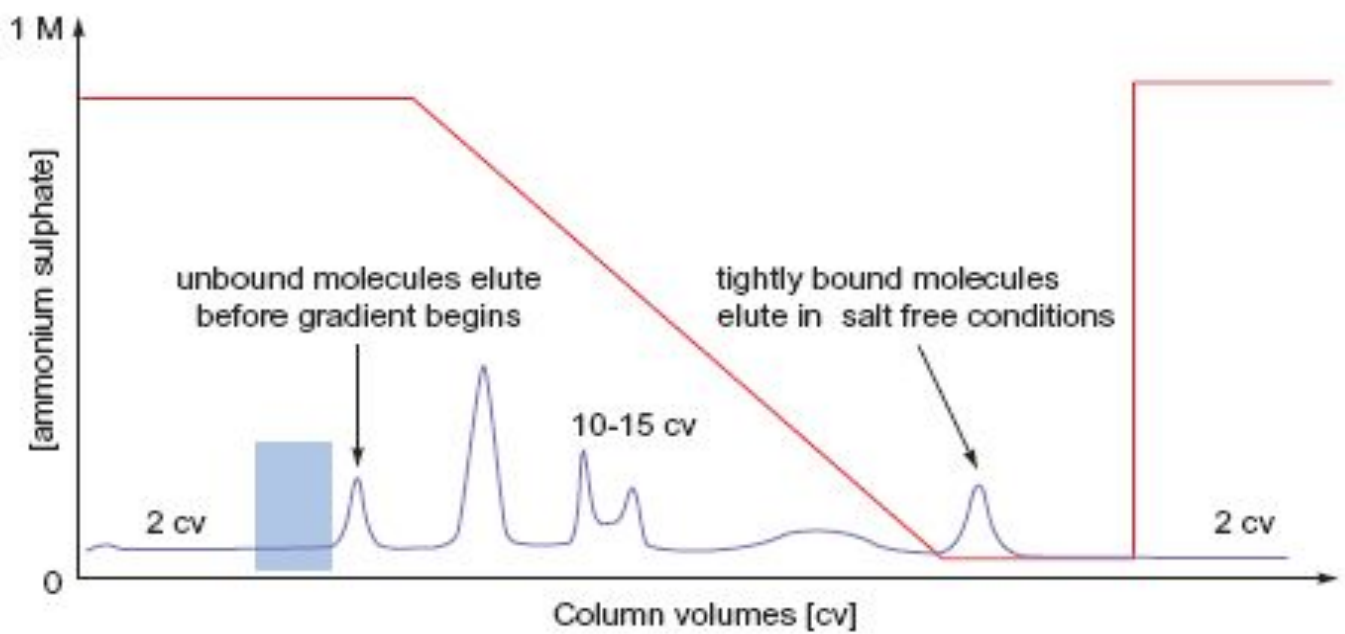
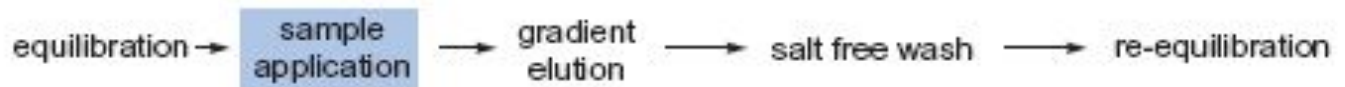
Solvent	Viscosity (centipoise)	Dielectric constant	Surface tension (dynes/cm)
Water	0.89	78.3	72.00
Ethylene glycol	16.90	40.7	46.70
Dimethyl Sulphoxide	1.96	46.7	43.54
Dimethyl Formamide	0.796	36.71	36.76
n-propanol	2.00	20.33	23.71

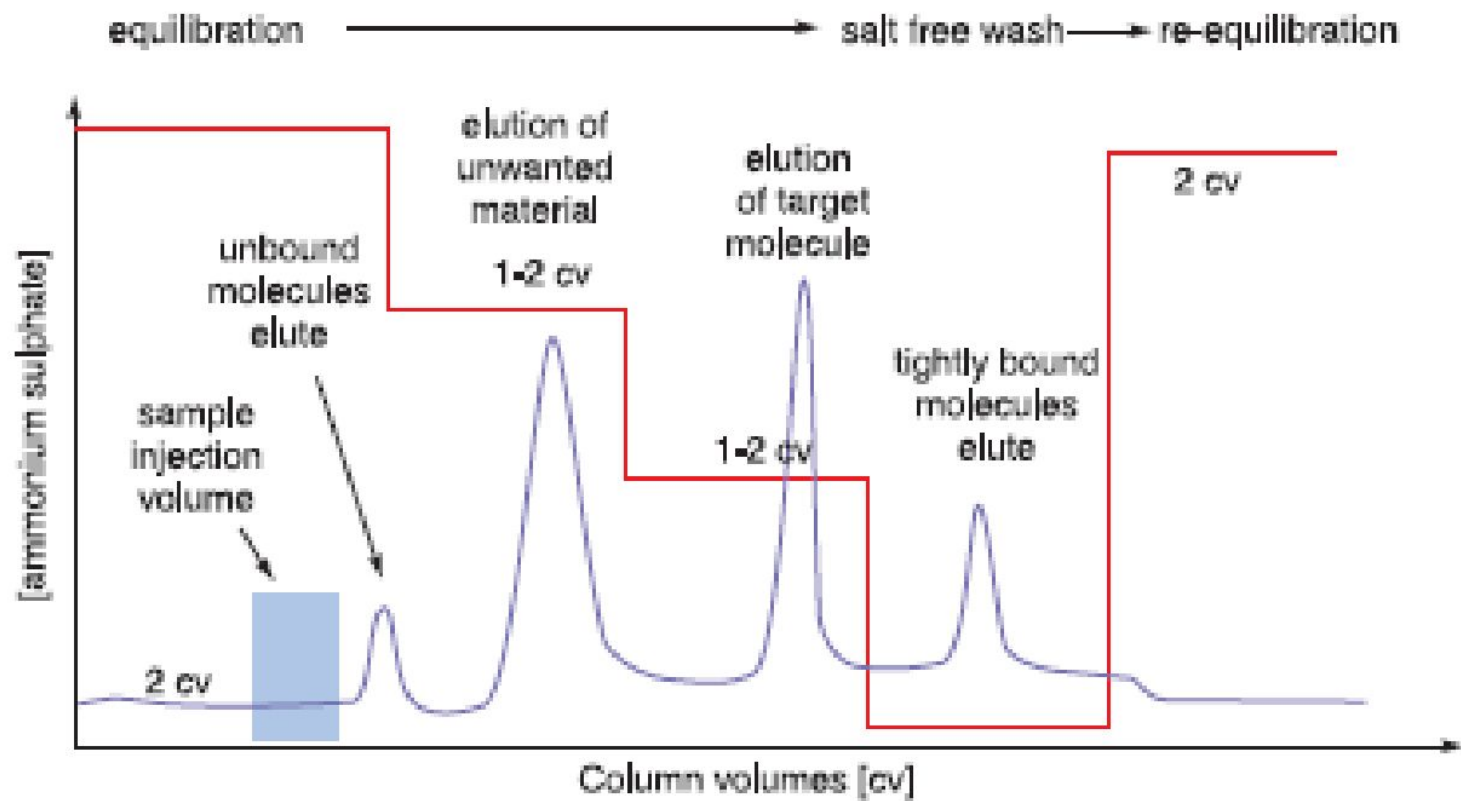
Tested media	Test solutions							
	1 M NaOH	1 M acetic acid	1 mM HCL	3 M (NH ₄) ₂ SO ₄	70% ethanol	30% isopropanol	6 M GuHCl	8 M Urea
Phenyl Sepharose 6 Fast Flow (low sub)	X	(n. t.)	(n. t.)	X	X	X	X	X
Phenyl Sepharose 6 Fast Flow (high sub)	X	(n. t.)	(n. t.)	X	X	X	X	X
Butyl Sepharose 4 Fast Flow	X	(n.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

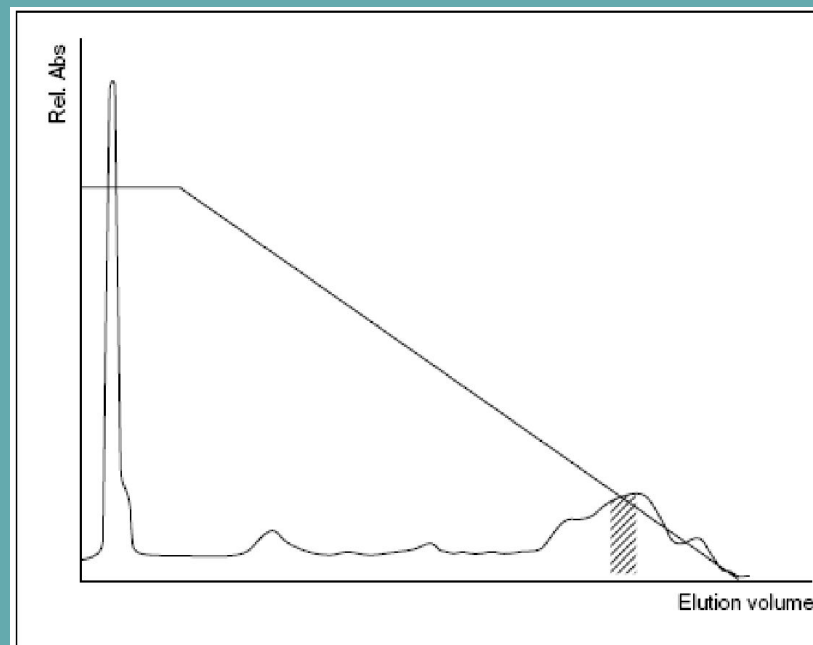
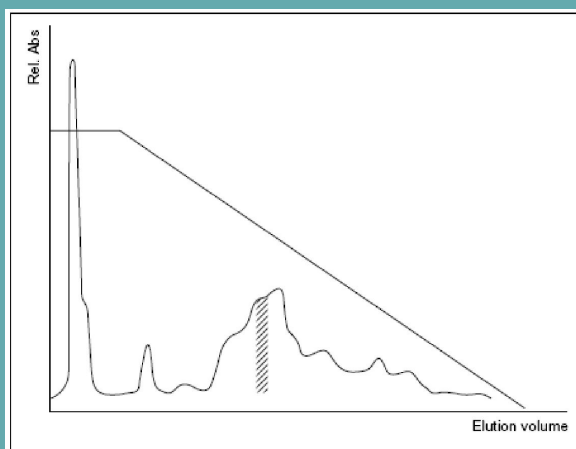
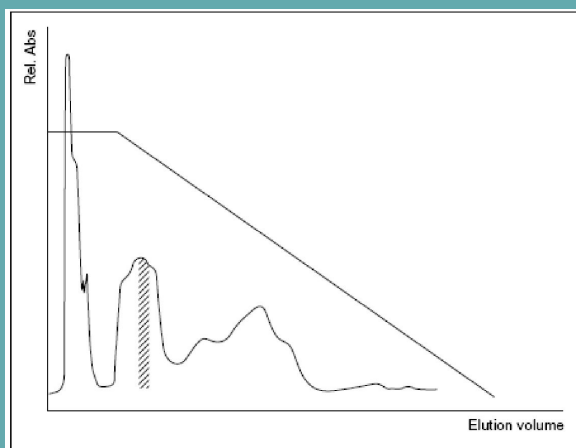
Long term stability and recommended working pH range: **3–13**
Short term stability and recommended CIP and SIP pH range: **2–14**
Recommended long term storage: **0.01 M NaOH or 20% ethanol.**



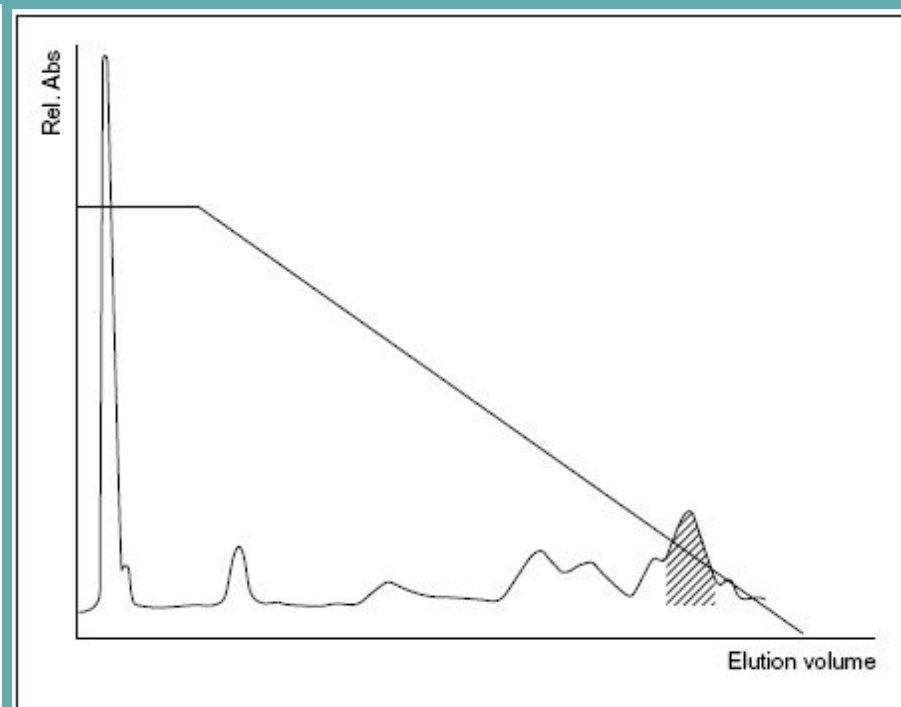
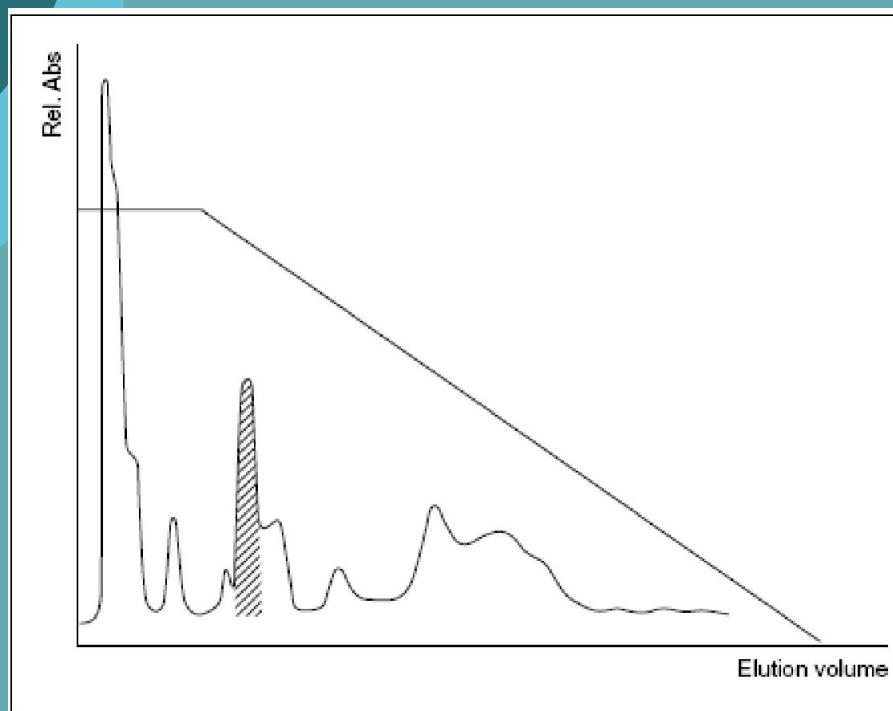




Оптимизация условий разделения гидрофобной хроматографией



Оптимизация условий разделения гидрофобной хроматографией



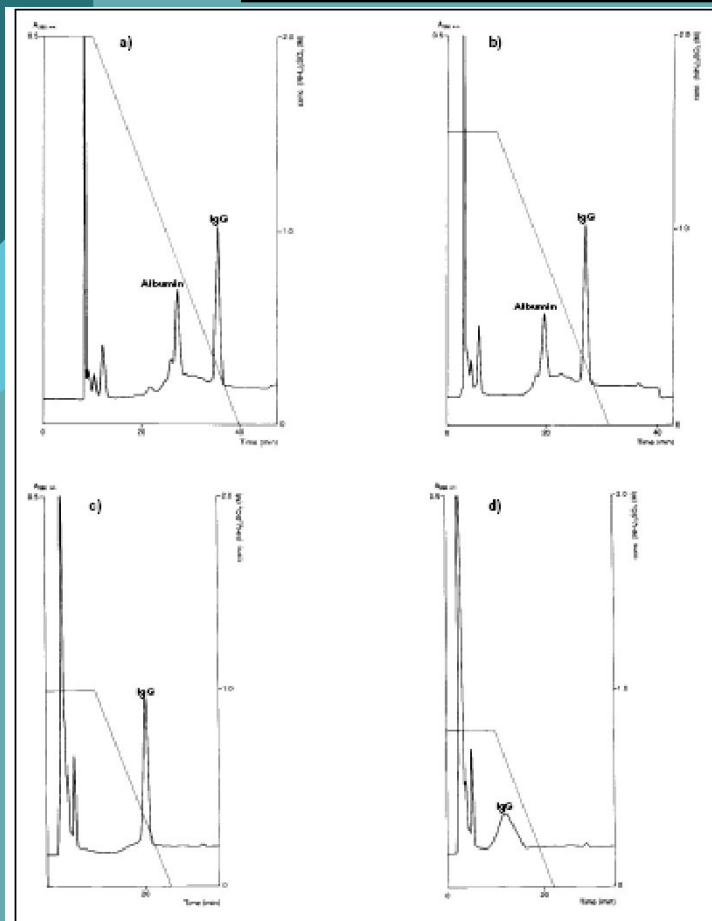


Fig. 16. The effect of starting conditions in HIC. Sample, 100 μ l anti-CEA MAB (IgG₁) from mouse ascites fluid in 0.8 M $(\text{NH}_4)_2\text{SO}_4$ (corresponding to 20 μ l ascites); column, Alkyl Superose HR 5/5; flow rate, 0.5 ml min^{-1} ; buffer A, 0.1 M sodium phosphate, pH 7.0, $(\text{NH}_4)_2\text{SO}_4$. (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).

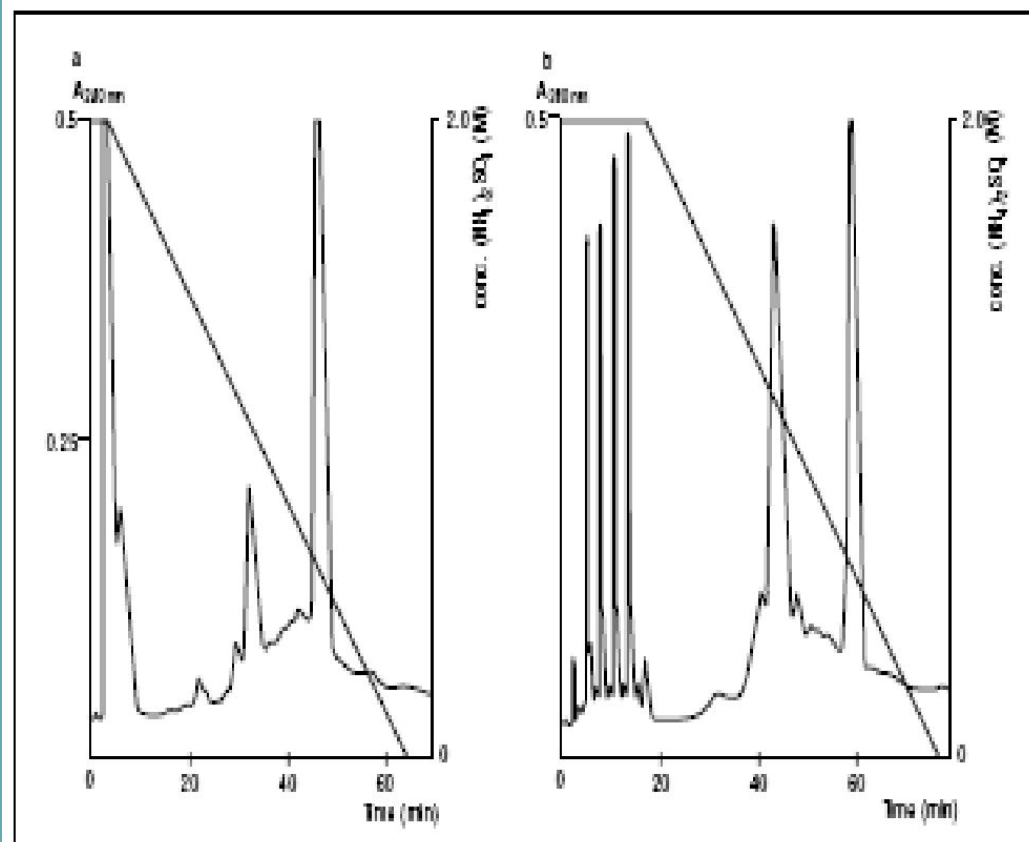


Fig. 17. The effect of loading conditions in HIC. Column, Alkyl Superose HR 5/5; flow rate, 0.5 ml min^{-1} ; buffer A, 0.1 M sodium phosphate, pH 7.0, 2 M $(\text{NH}_4)_2\text{SO}_4$. (a) Sample (500 μ l anti-CEA MAB (IgG₁) from mouse ascites fluid in 0.9 M $(\text{NH}_4)_2\text{SO}_4$ (corresponding to 115 μ l ascites) applied in one injection. (b) Sample as (a) applied in five 100 μ 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).

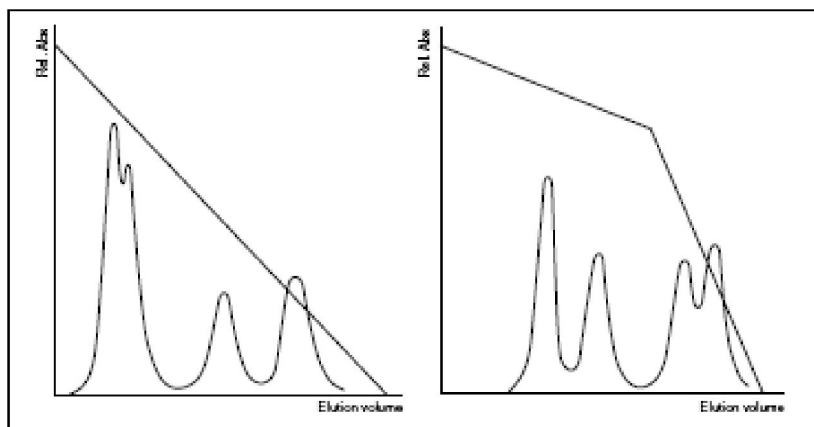


Fig. 18. Effect of a complex gradient on resolution.

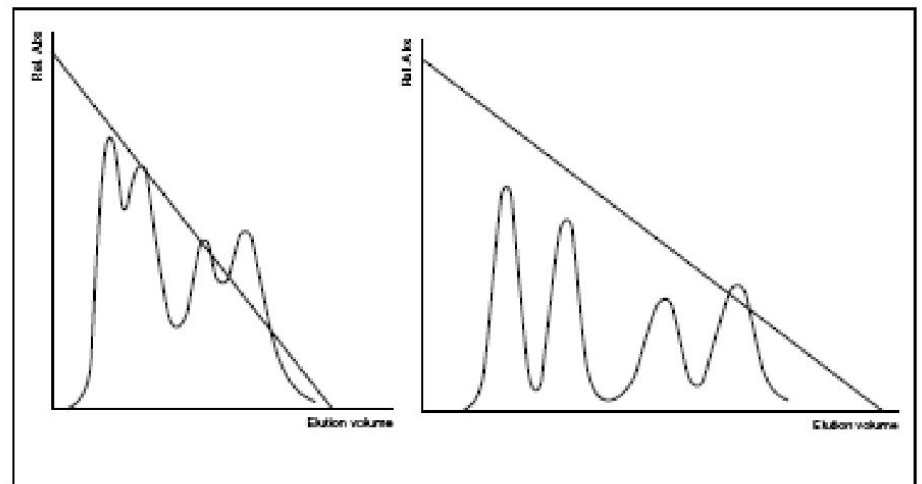


Fig. 19. Effect of gradient slope on resolution.

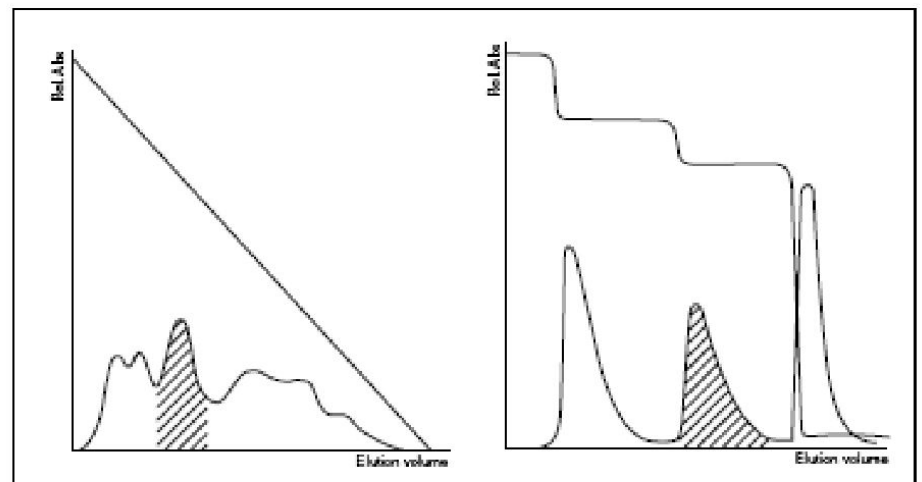
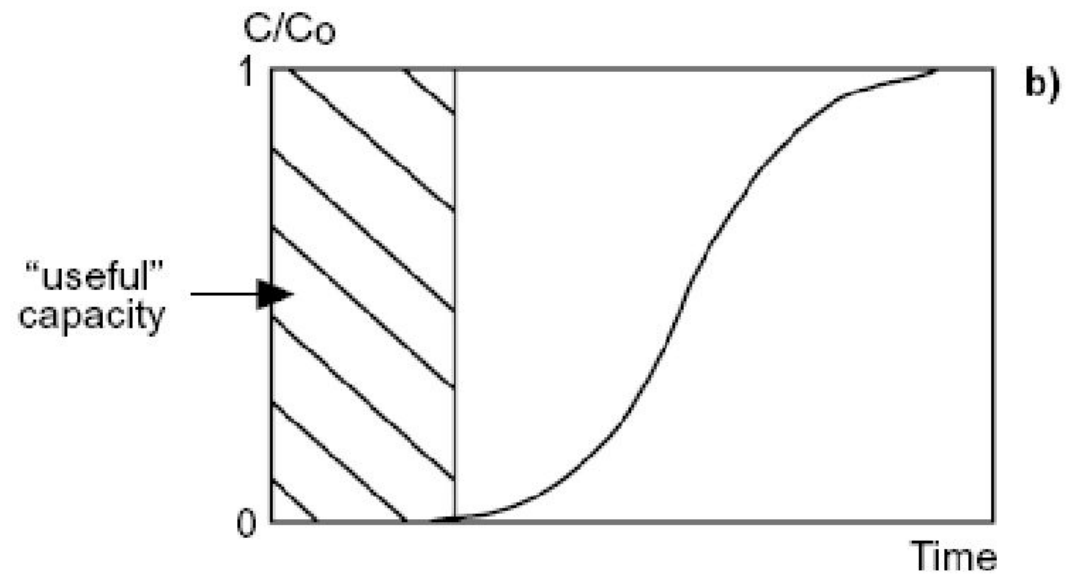
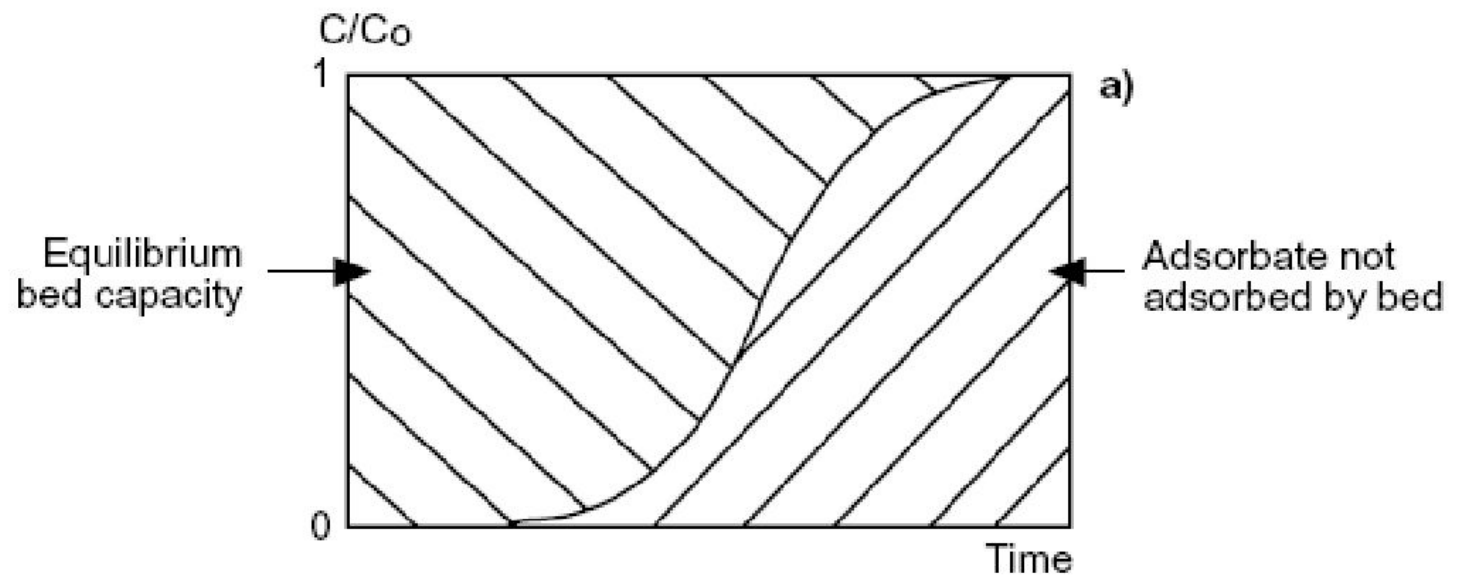
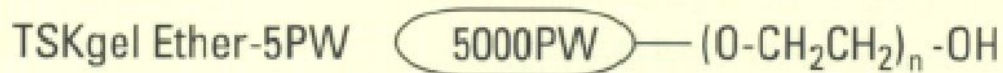
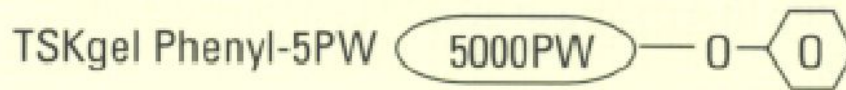


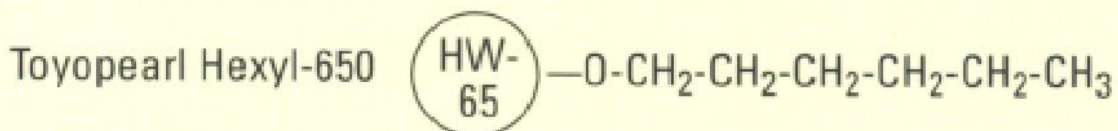
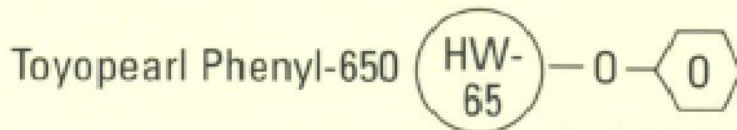
Fig. 20. Switching from a continuous gradient to step-wise elution.



Structure of TSK-GEL HIC resins



Structure of Toyopearl HIC resins

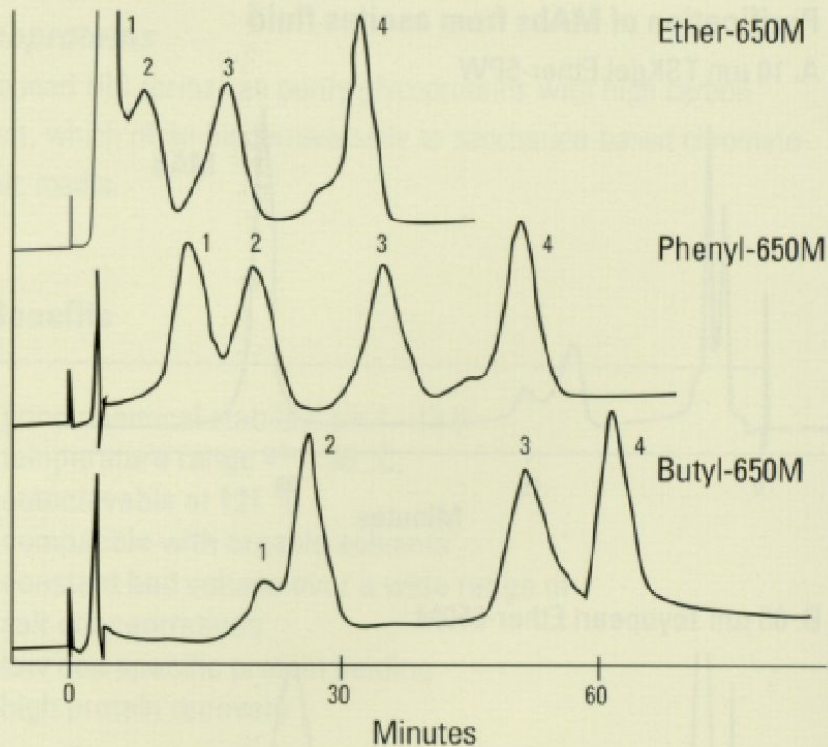


Resolution values (R_s) of Toyopearl resins for lysozyme and α -chymotrypsinogen

Particle size grade

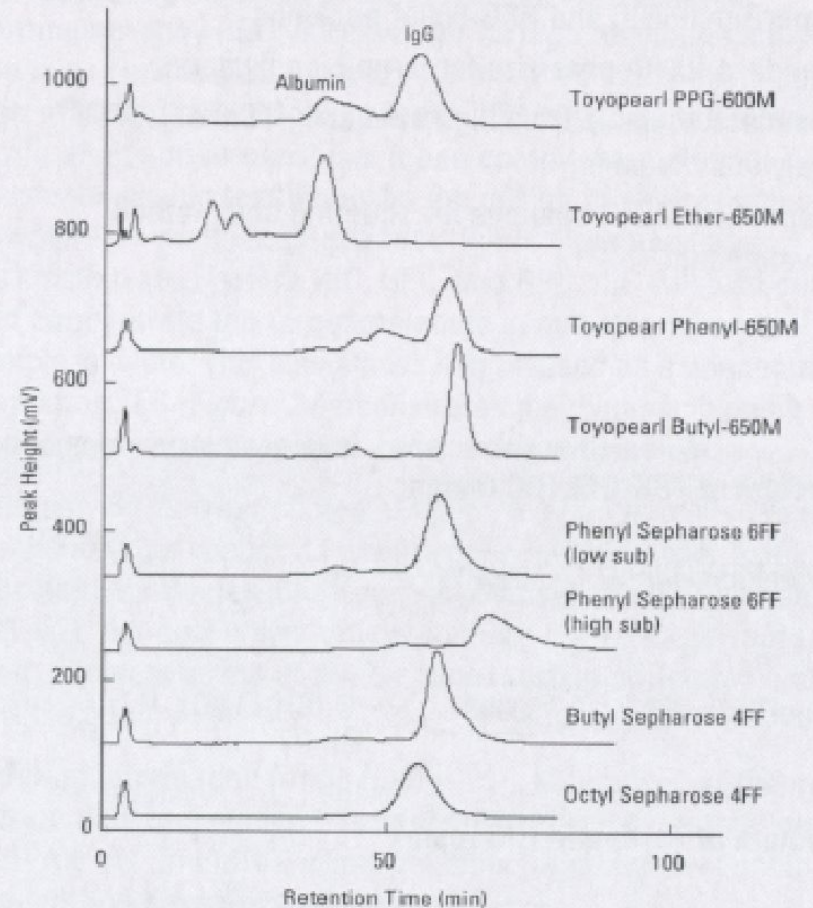
<i>Resins</i>	<i>C</i>	<i>M</i>	<i>S</i>
Phenyl-650	1.12	1.52	2.19
Butyl-650	0.91	1.37	2.20

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 $(\text{NH}_4)_2\text{SO}_4$ 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 μL
 Sample: mouse ascites fluid (x 4 diluted) (Antibody: Anti-IgE)

Proposed Two Step Adsorption Model for Mab to Toyopearl Resins

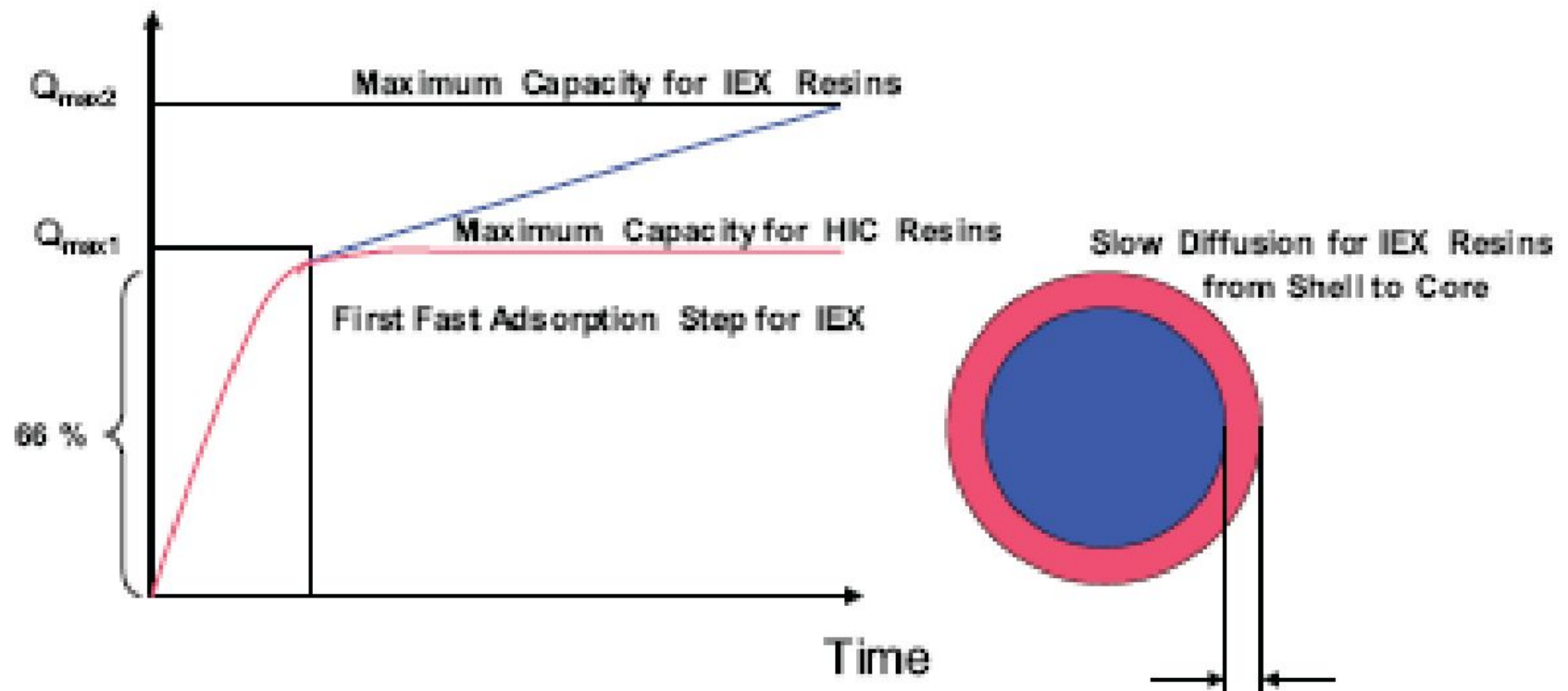
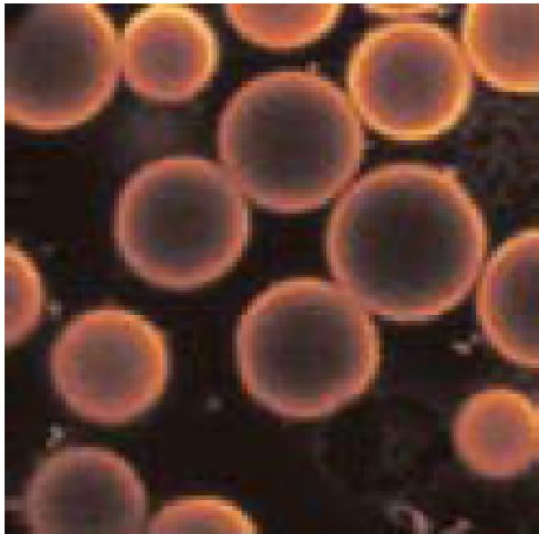


Figure 6

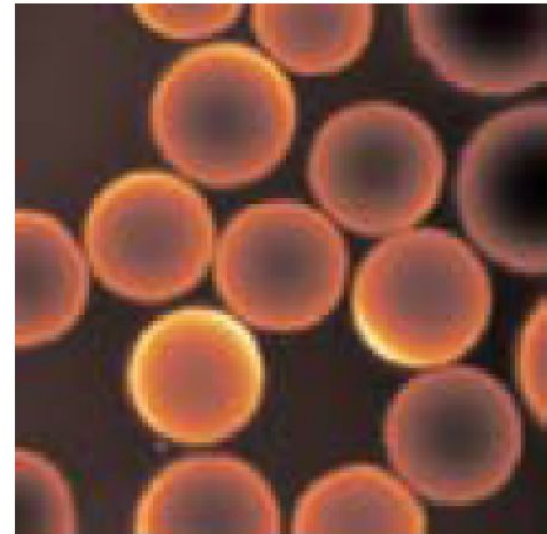
$\approx 20 \mu\text{m}$
"Equilibrium Penetration Depth"

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



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

**Toyopearl SP (100-300µm)
(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]

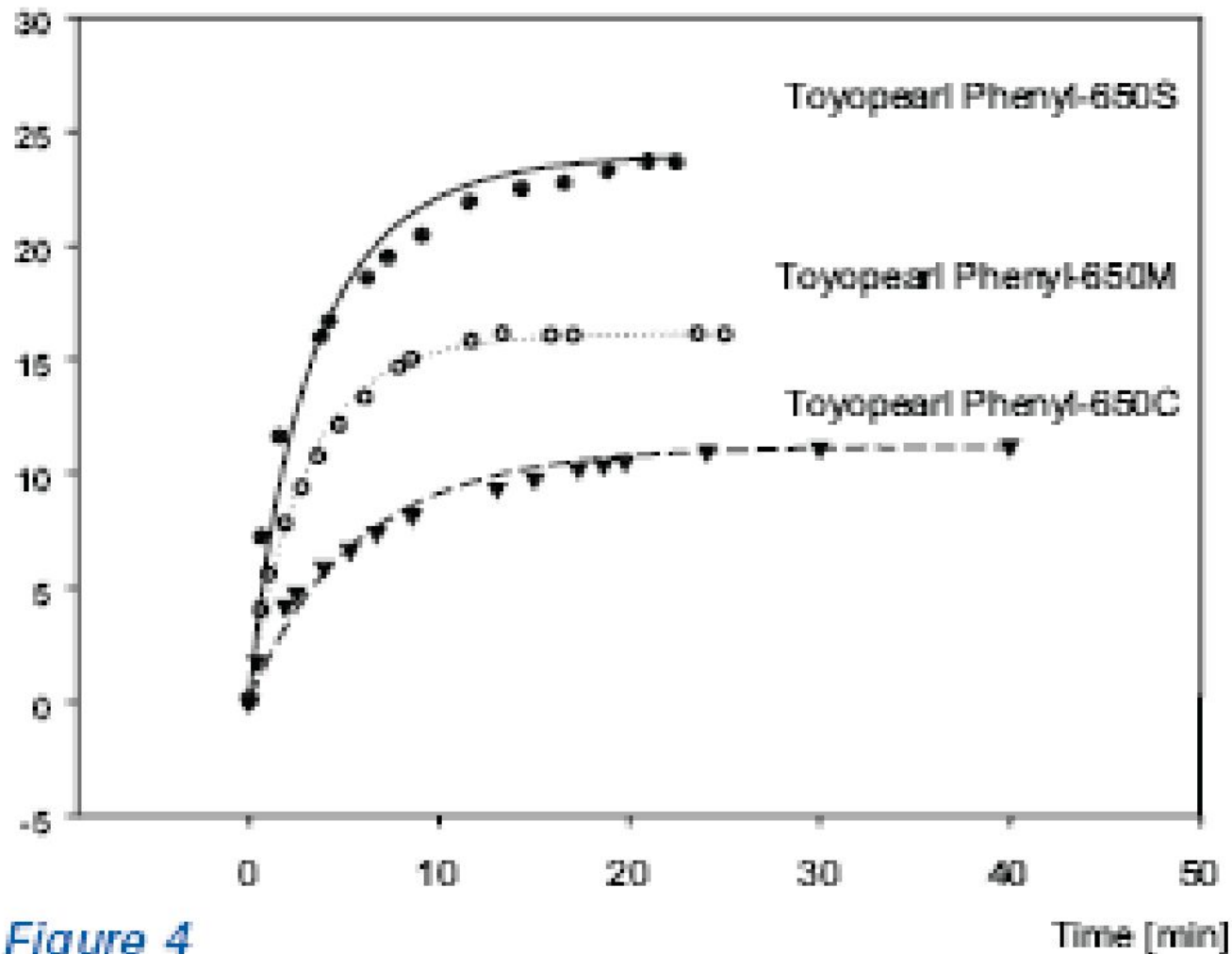


Figure 4

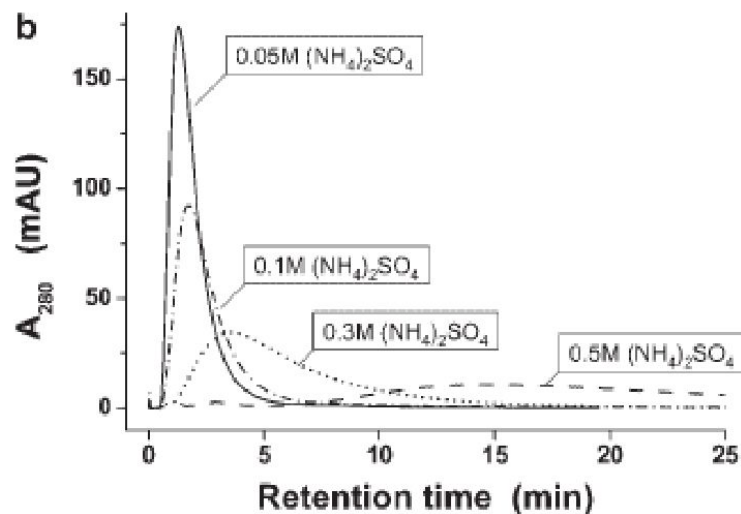
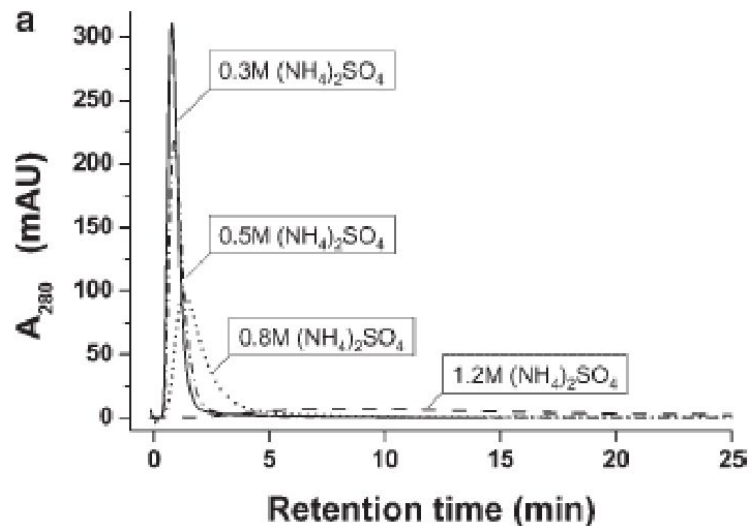


Figure 1. Effect of $(\text{NH}_4)_2\text{SO}_4$ concentration on α -lactalbumin retention for α -lactalbumin. Isocratic elution was performed on Phenyl Sepharose™ 6 Fast Flow (low sub) at a flowrate of 1.0 mL/min and 55°C. a: Calcium included in the samples and buffers at 12 mM for all conditions, with $(\text{NH}_4)_2\text{SO}_4$ concentrations varying as shown. b: 0 mM CaCl_2 . EDTA (2 mM) was included to chelate any trace calcium.

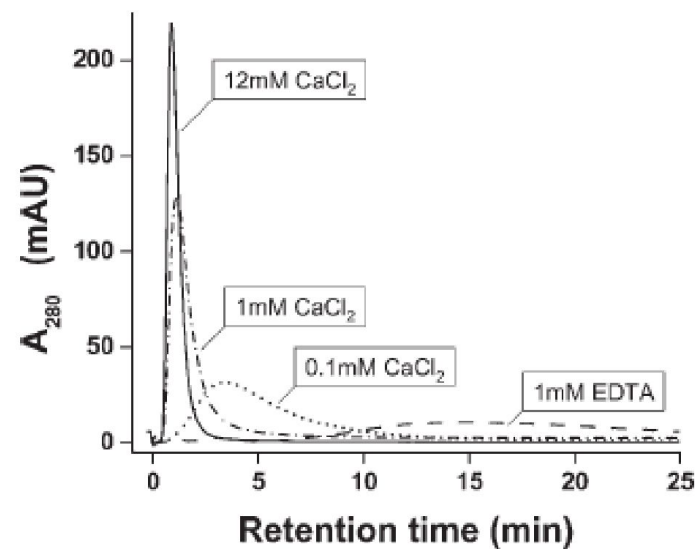
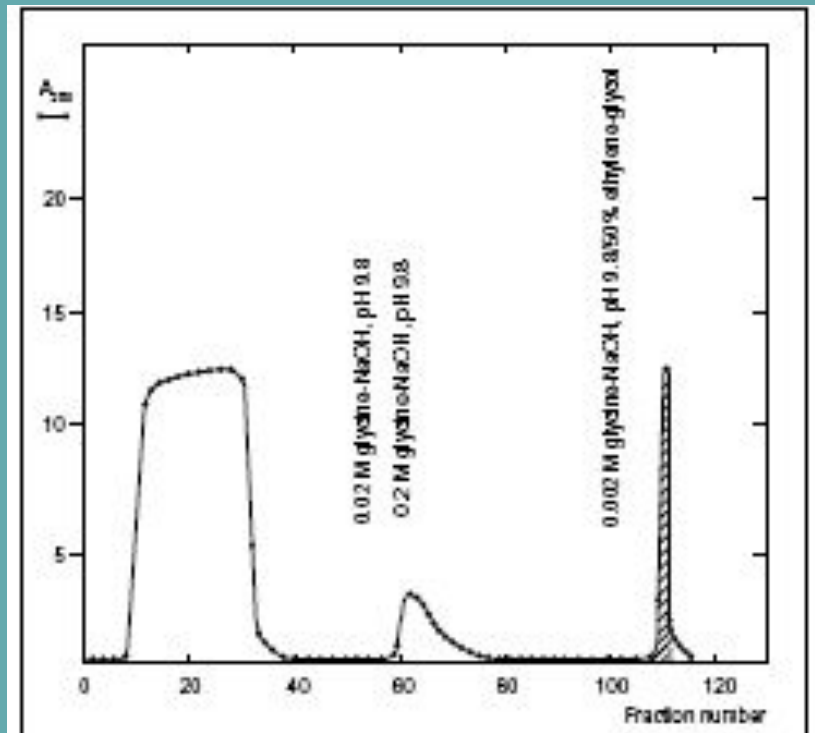
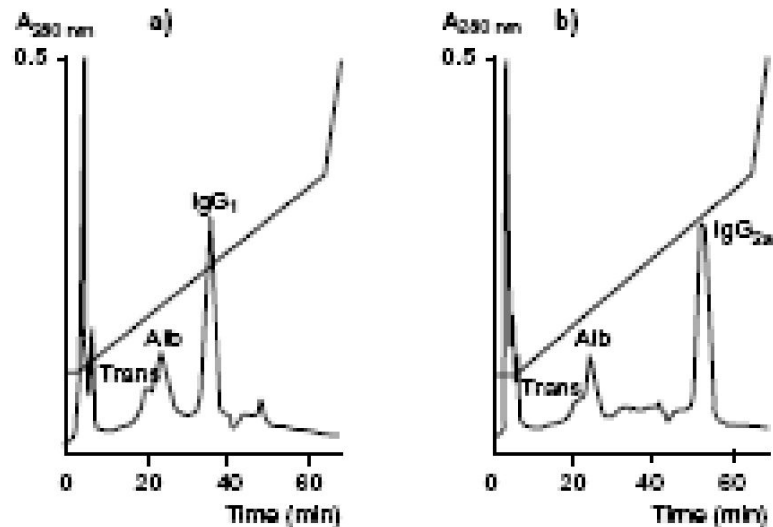


Figure 3. Effect of calcium concentration on α -lactalbumin isocratic elution. The $(\text{NH}_4)_2\text{SO}_4$ concentration was 0.5 M, and calcium concentrations varied as shown. Other conditions identical to Figure 1.

Column: Alkyl Superose HR 5/5
Sample: 100 μ l mouse ascites containing monoclonal IgG₁ (a) or IgG_{2a} (b) + 100 μ 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: A₂₈₀. 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 IgG₁ 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

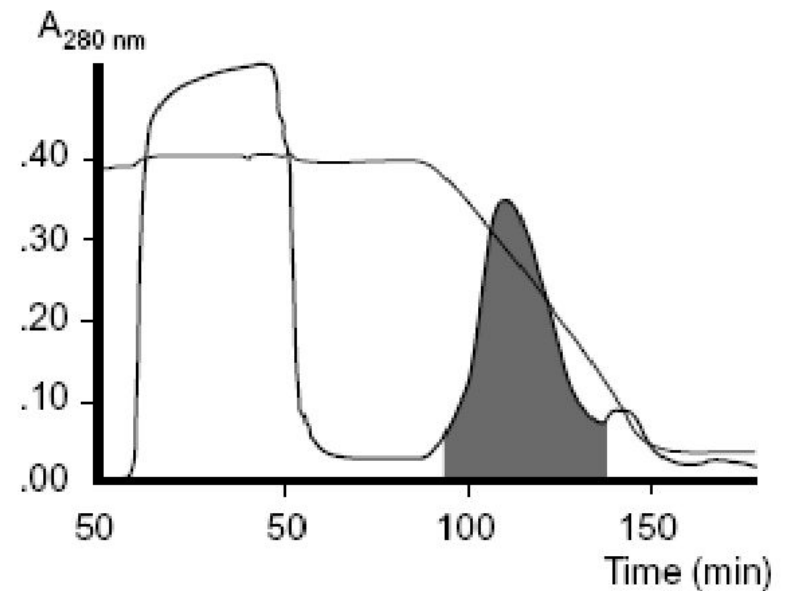


Fig. 48. Laboratory scale purification of mouse IgG₁ anti-IgE, on Phenyl Sepharose High Performance. (Work from Amersham Pharmacia Biotech, Uppsala, Sweden).

Column: BioPilot Column 35/100
Gel: Phenyl Sepharose High Performance, 10 cm bed height
Sample: Hybridoma cell culture supernatant; mouse IgG₁, 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

