Лиганды используемые в аффинной хроматографии

1. Иммобилизованные лектины
a. ConA концевые сахара Man, NANA
b. WGA концевые сахара NAGA
c. Lentil Lectin Man, NANA

2. Иммобилизованные красители

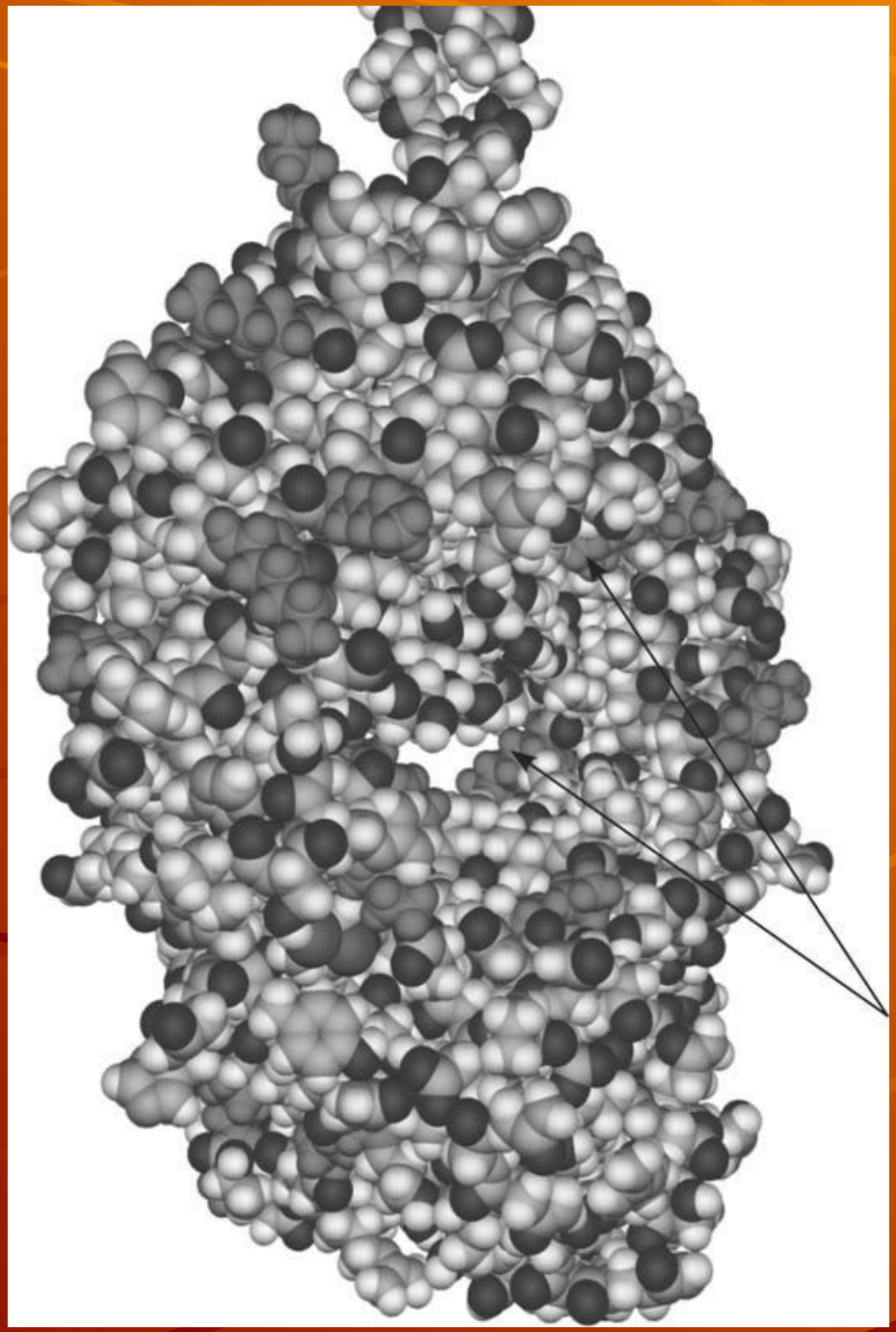
3. Иммобилизованные субстраты - кофакторы

4. Металл хелатная хроматография Zn, Co, Ca, Fe

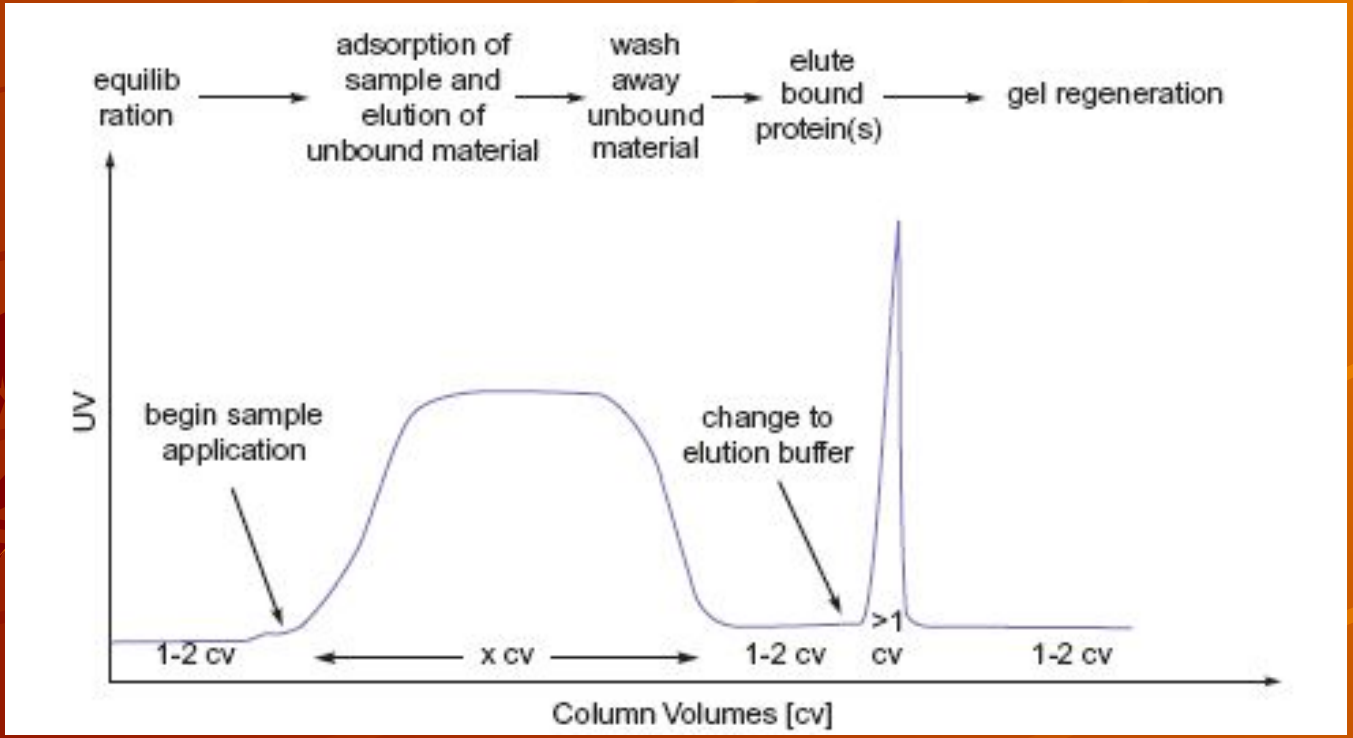
5. Иммобилизованные лиганды (гормоны)

6. Ковалентная хроматография – иммобилизация по -SH

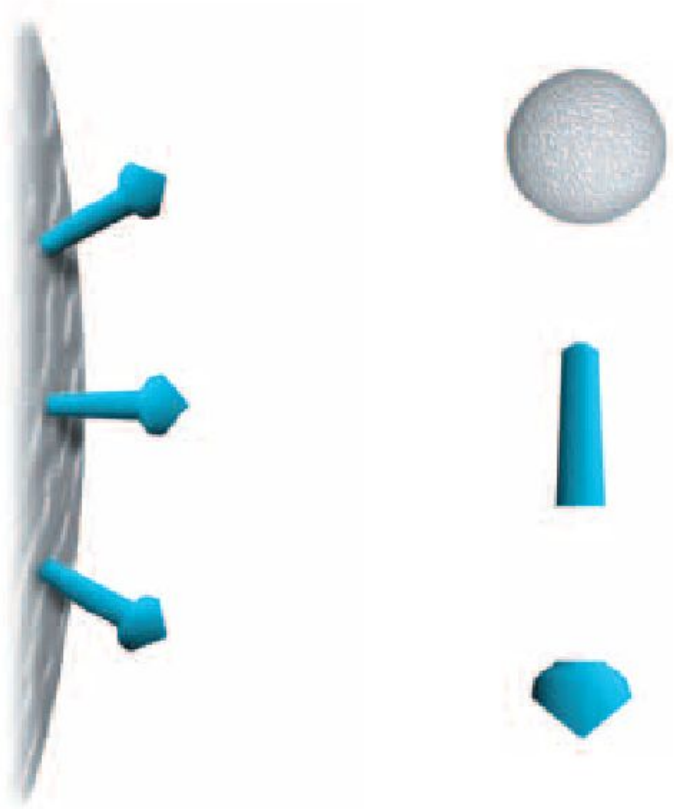
7. Хроматография на антителах - Иммуноаффинная хроматография



Аффинная хроматография



Common terms in affinity chromatography



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.

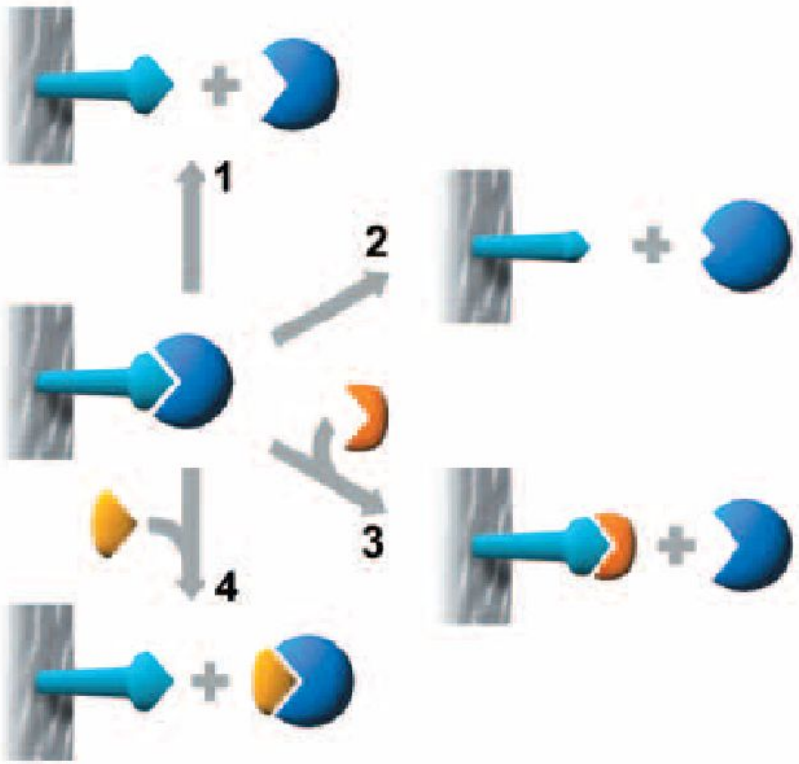
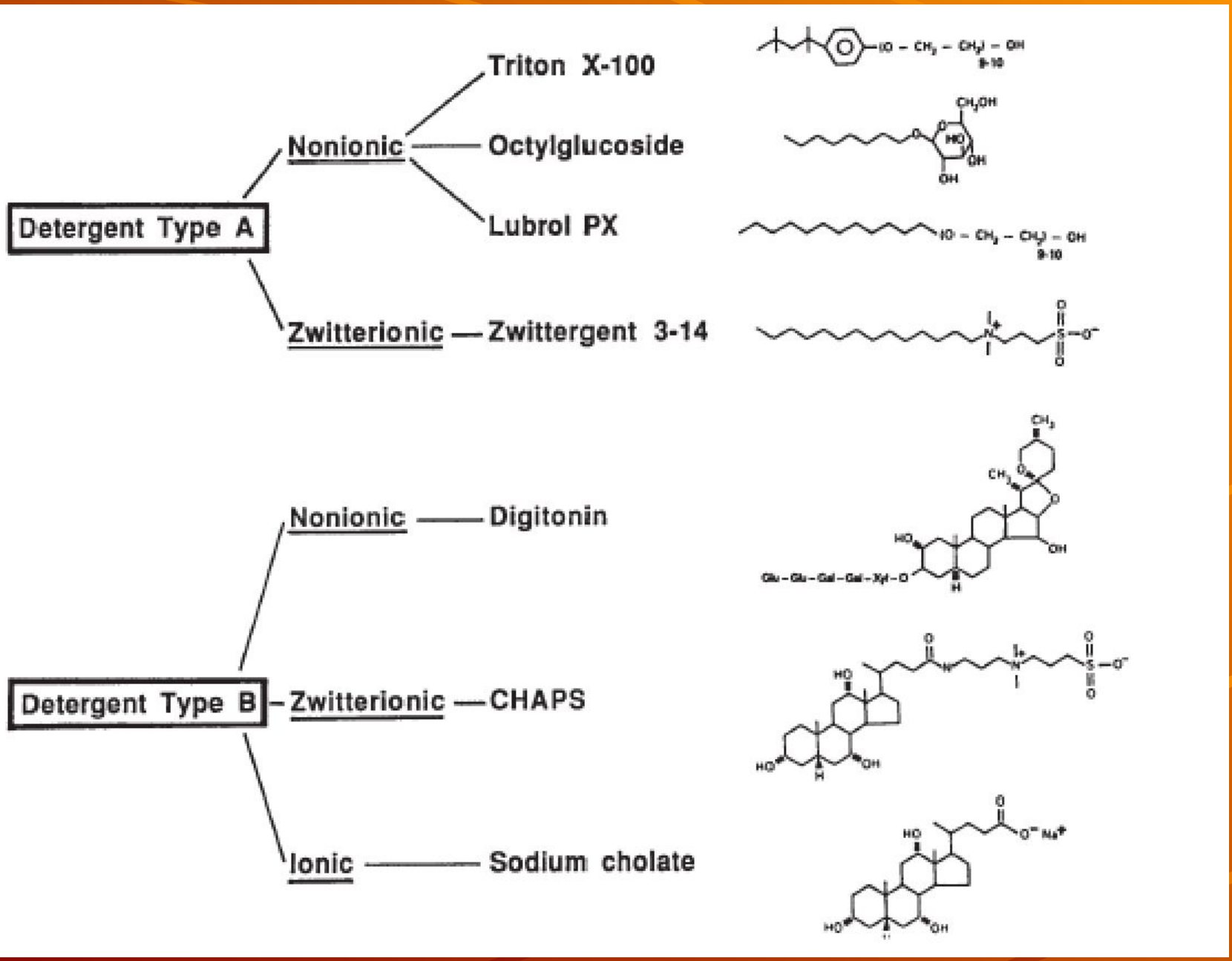


Fig. 5. Elution methods.

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.


Detergent Type A
Nonionic
Triton X-100
Octylglucoside
Lubrol PX
Zwitterionic
Zwittergent 3-14
Detergent Type B
Nonionic
Digitonin
Zwitterionic
CHAPS
Ionic
Sodium cholate

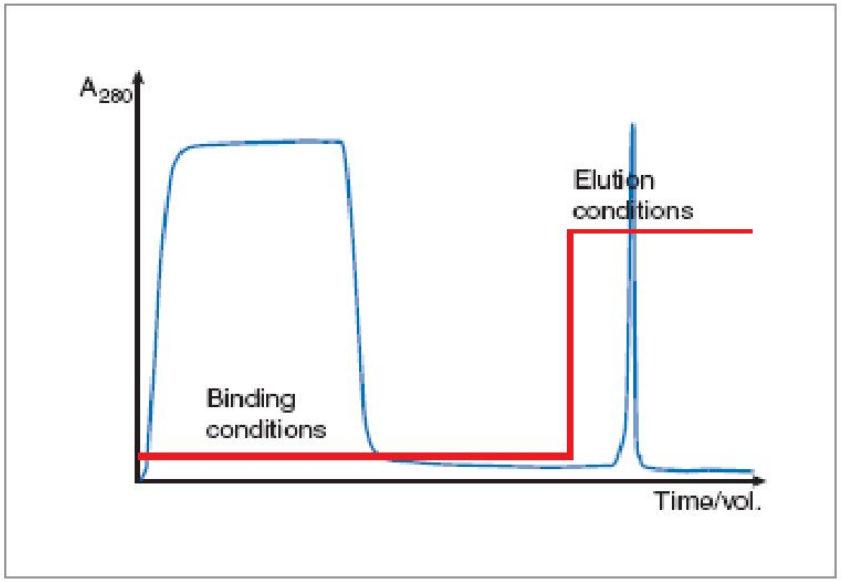


Fig. 6a. Step elution.

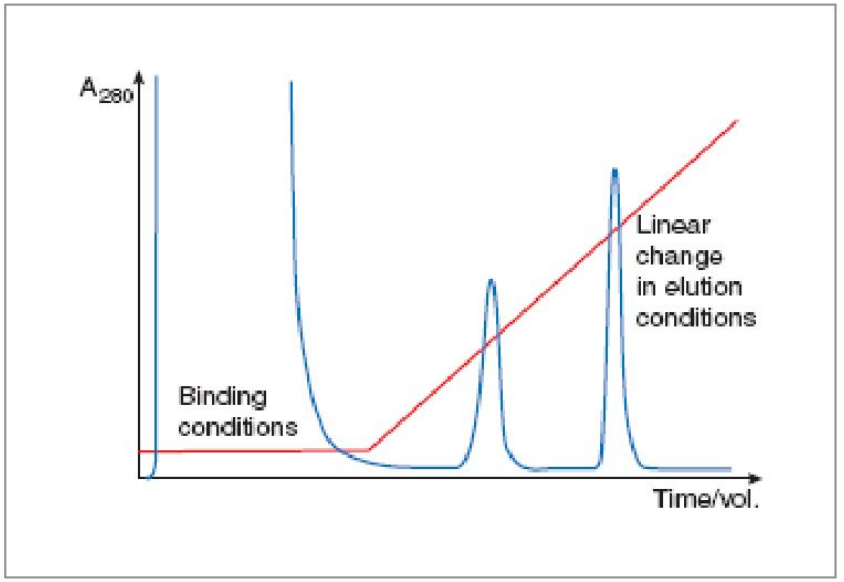


Fig. 6b. Gradient elution.

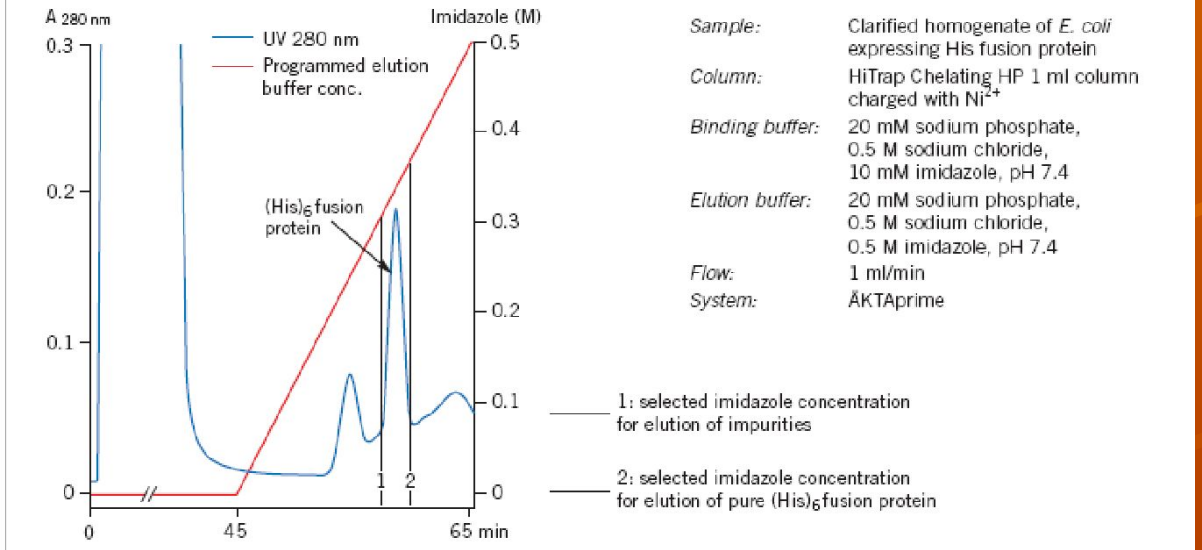
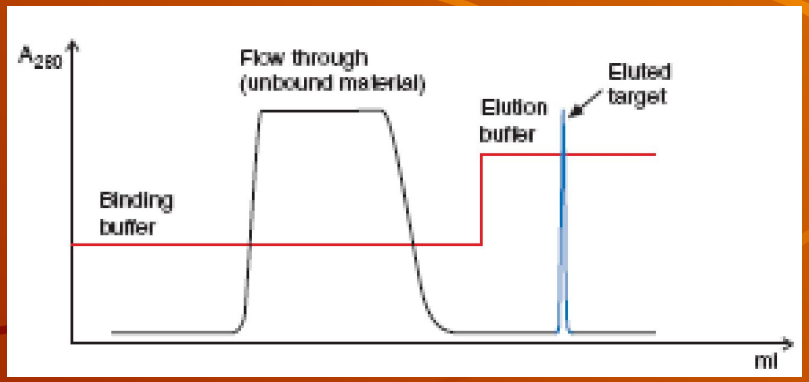
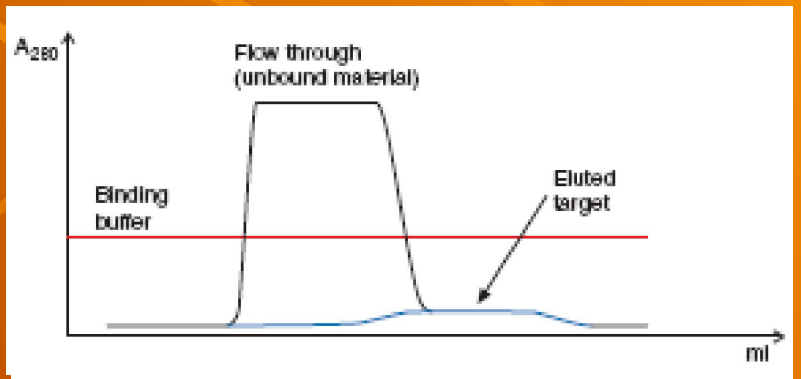
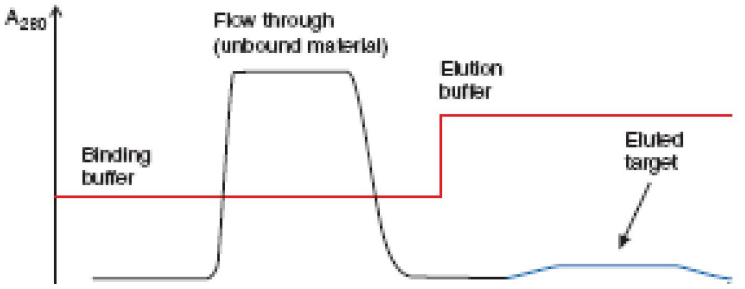
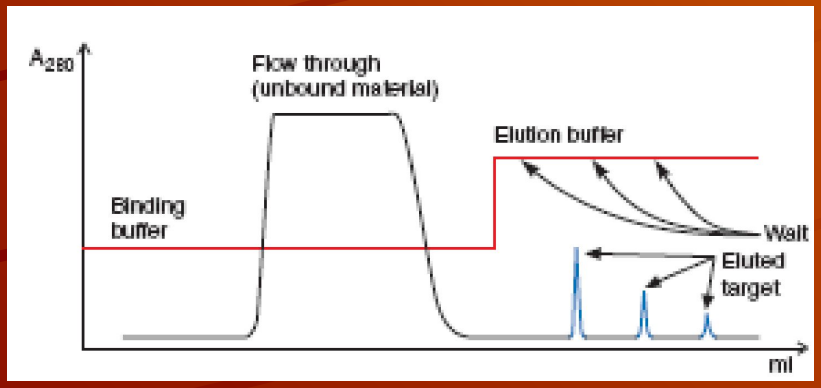


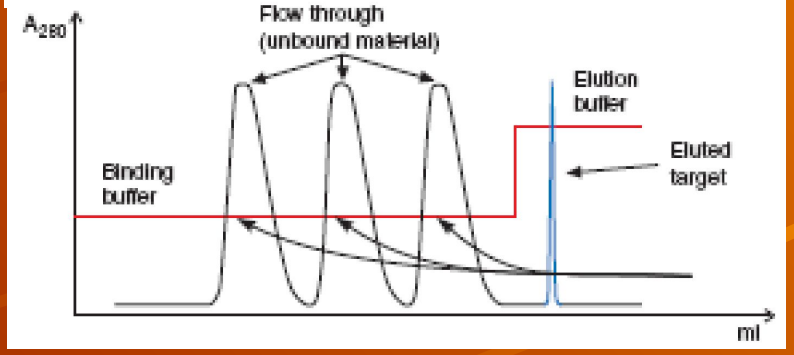
Fig. 7. Gradient elution of a $(His)_6$ fusion protein.


A280
Flow through
(unbound material)
Eluted
target
Elution
buffer
Binding
buffer
ml

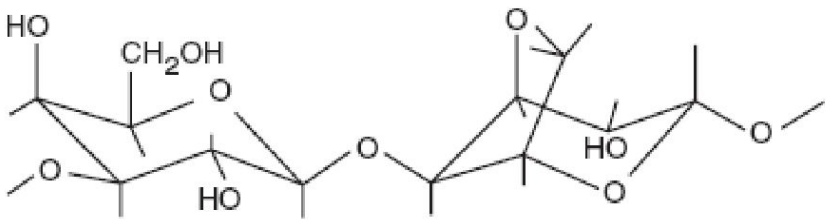

A280
Flow through
(unbound material)
Binding
buffer
Eluted
target
ml


A280
Flow through
(unbound material)
Elution
buffer
Binding
buffer
Eluted
target


A280
Flow through
(unbound material)
Elution buffer
Binding
buffer
Wait
Eluted
target
ml


A280
Flow through
(unbound material)
Elution
buffer
Eluted
target
Binding
buffer
ml

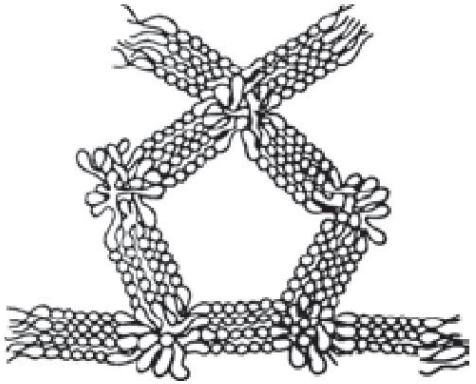
Agarose



D-galactose

3–6 anhydro
L-galactose

Structure of agarose gel



	Form	Mean particle size
Sepharose High Performance	6% highly cross-linked agarose	34 µm
Sepharose 6 Fast Flow	6% highly cross-linked agarose	90 µm
Sepharose 4 Fast Flow	4% highly cross-linked agarose	90 µm
Sepharose CL-6B	6% cross-linked agarose	90 µm
Sepharose CL-4B	4% cross-linked agarose	90 µm
Sepharose 6B	6% agarose	90 µm
Sepharose 4B	4% agarose	90 µm

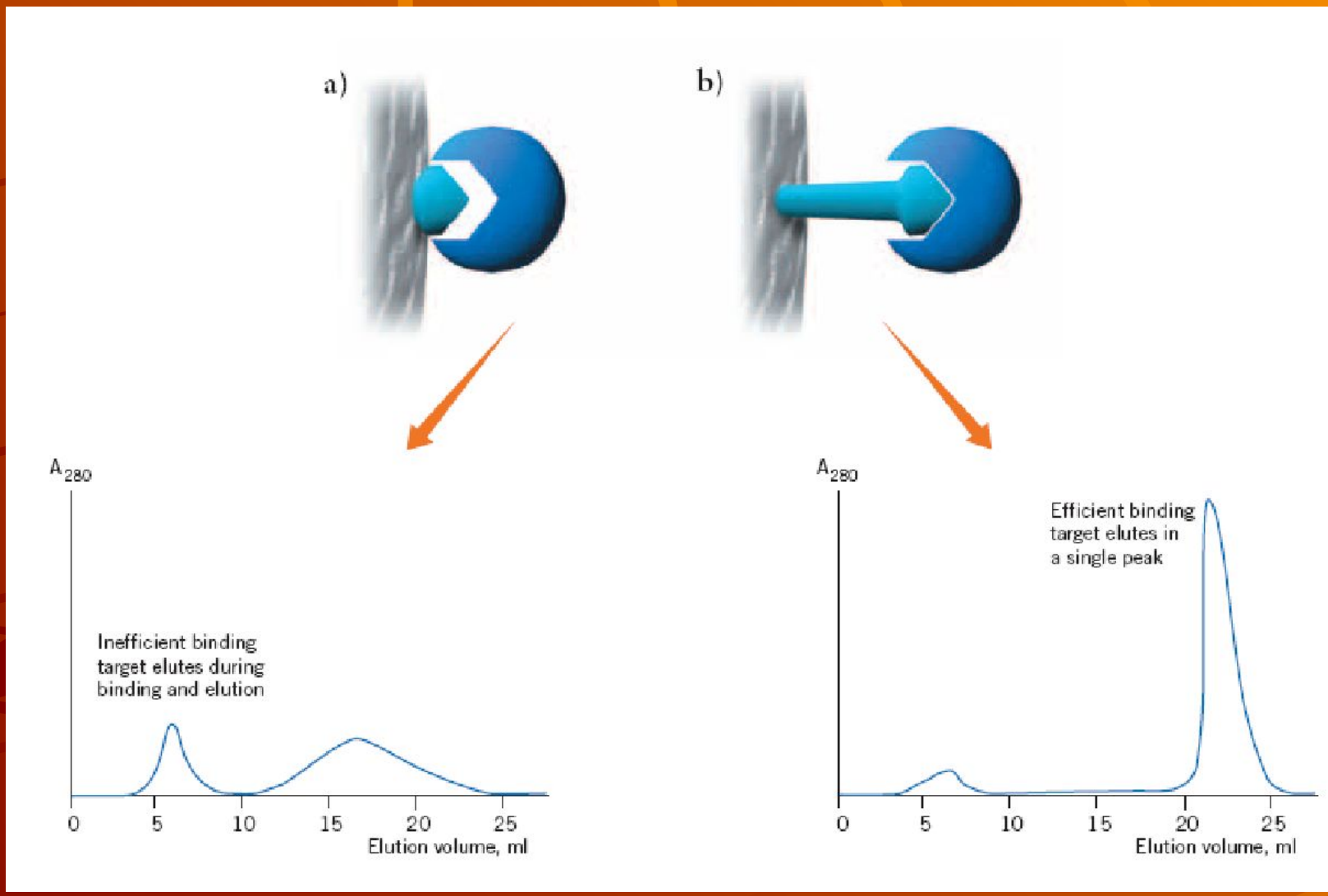
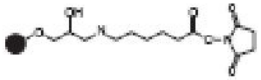
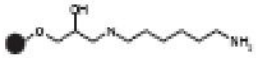
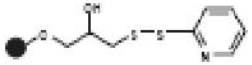
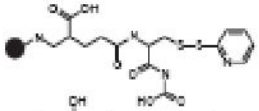
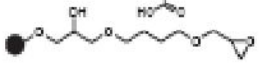
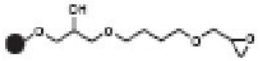
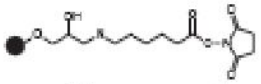
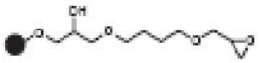
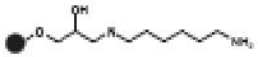
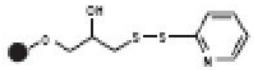
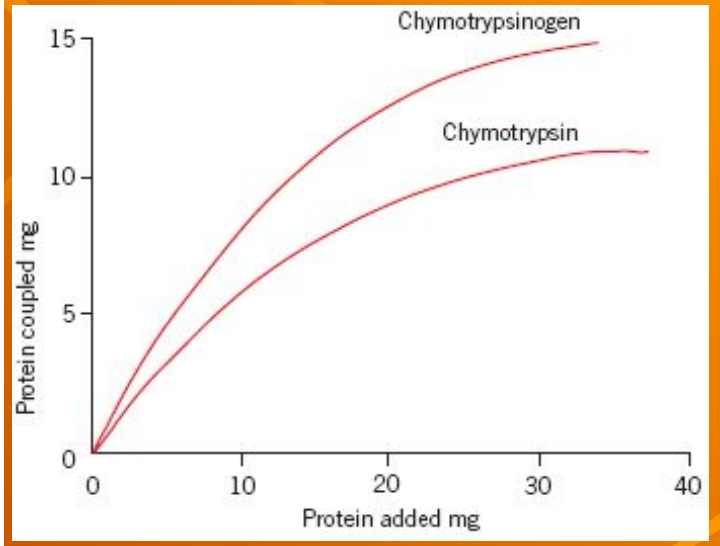

a)
b)
A_{280}
Inefficient binding target elutes during binding and elution
0
5
10
15
20
25
Elution volume, ml
A_{280}
Efficient binding target elutes in a single peak
0
5
10
15
20
25
Elution volume, ml

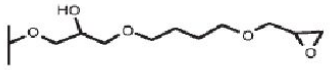
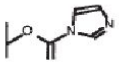
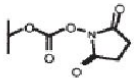
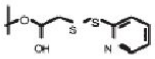
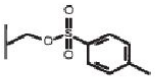
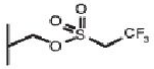
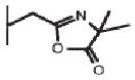
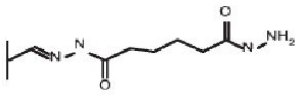
Table 7. Examples of pre-activated media.

NHS-activated Sepharose High Performance	12-atom hydrophilic spacer arm to couple via amino groups.
NHS-activated Sepharose 4 Fast Flow	As above.
CNBr-activated Sepharose 4 Fast Flow	Coupling via primary amino groups.
EAH Sepharose 4B	10-atom spacer arms to couple via amino groups.
ECH Sepharose 4B	9-atom spacer arms to couple via carboxyl groups.
Epoxy-activated Sepharose 6B	12-atom hydrophilic spacer arm to couple through hydroxyl, amino or thiol groups.
Activated Thiol Sepharose 4B	10-atom spacer arm for reversible coupling through free thiol groups.
Thiopropyl Sepharose 6B	4-atom hydrophilic spacer arm for reversible coupling of proteins and small thiolated ligands through thiol groups. Also reacts with heavy metal ions, alkyl and aryl halides and undergoes addition reactions with compounds containing C=O, C=C and N=N bonds.

Chemical group on ligand	Length of spacer arm	Structure of spacer arm	Product
Proteins, peptides, amino acids			
amino	10-atom		HiTrap NHS-activated HP NHS-activated Sepharose 4 Fast Flow
	None	–	CNBr-activated Sepharose 4B CNBr-activated Sepharose 4 Fast Flow
	10-atom		ECH Sepharose 4B
carboxyl	11-atom		EAH Sepharose 4B
thiol	4-atom		Thiopropyl Sepharose 6B
	10-atom		Activated Thiol Sepharose 4B
	12-atom		Epoxy-activated Sepharose 6B
Sugars			
hydroxyl	12-atom		Epoxy-activated Sepharose 6B
amino	10-atom		HiTrap NHS-activated HP
	10-atom		ECH Sepharose 4B
	12-atom		Epoxy-activated Sepharose 6B
carboxyl	11-atom		EAH Sepharose 4B
Polynucleotides			
amino	None		CNBr-activated Sepharose 4B CNBr-activated Sepharose 4 Fast Flow
mercurated base	4-atom		Thiopropyl Sepharose 6B
Coenzymes, cofactors, antibiotics, steroids			
amino, carboxyl, thiol or hydroxyl			use matrix with spacer arm (see above)



Reactive Groups for Derivitization

Functional group	Structure on matrix	Target moiety on ligand
Cyanogen Bromide		$-NH_2$
Epoxide / Oxarine		$-NH_2$, -OH, -SH, Sugars
Carbonyldiimidazole		$-NH_2$
N-Hydroxysuccinimide		$-NH_2$
Vinyl Sulphone		$-NH_2$, -SH, -OH
Thiol		-SH
Tosyl Chloride		$-NH_2$, -SH
Tresyl Chloride		$-NH_2$, -SH
Azlactone		$-NH_2$, -SH, -OH
Hydrazine		Sugars
Avidin	Protein[a]	Biotinylated proteins, etc.
Protein A/protein-G	Protein[b]	Immunoglobulin via Fc region

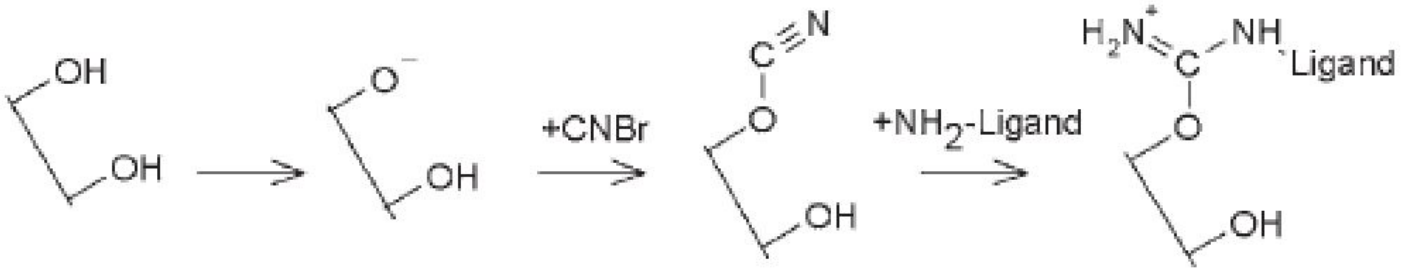


Fig. 2. Activation and immobilization via cyanogen bromide.

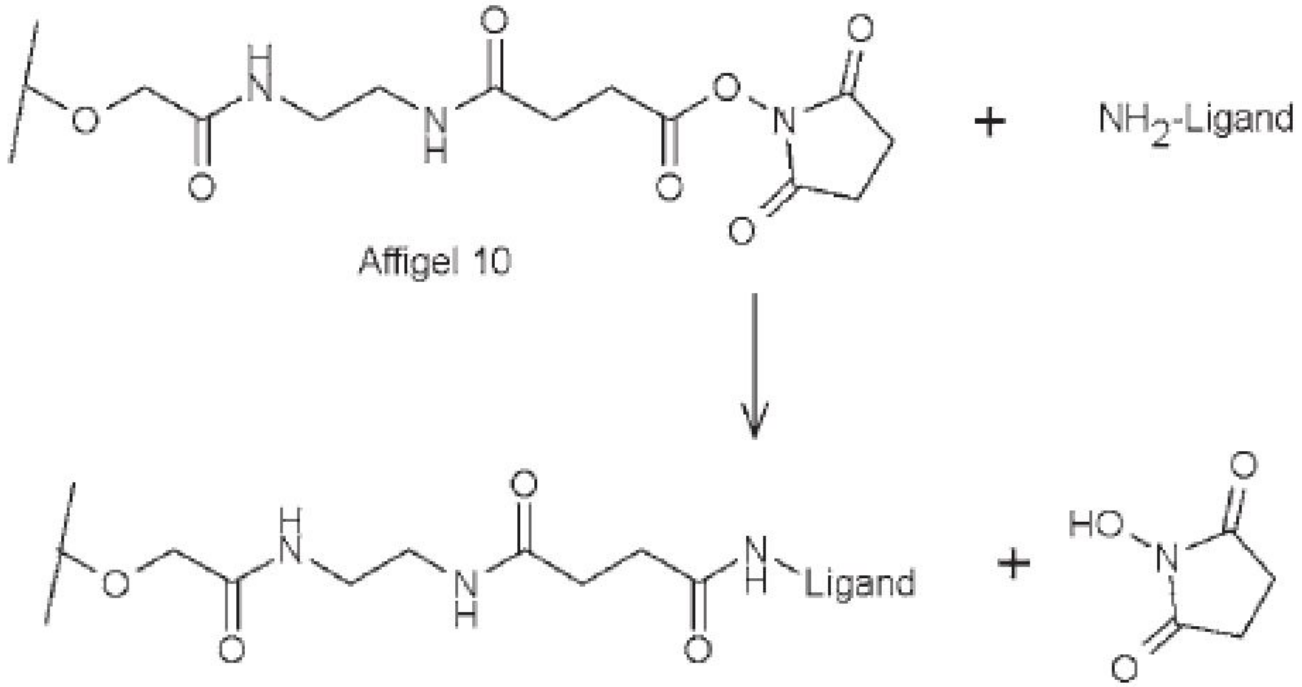
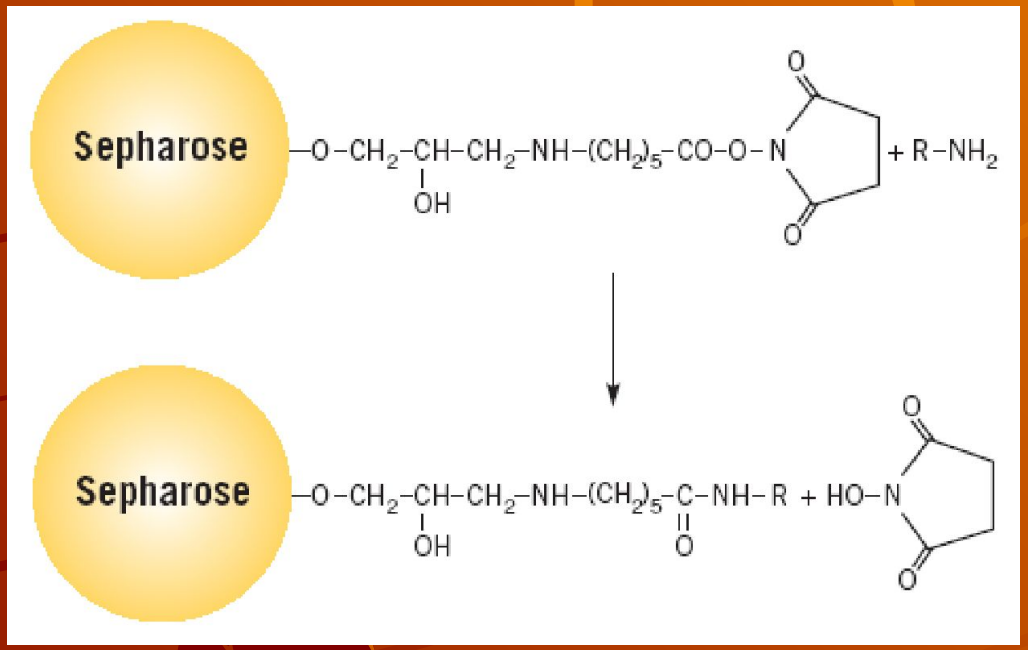
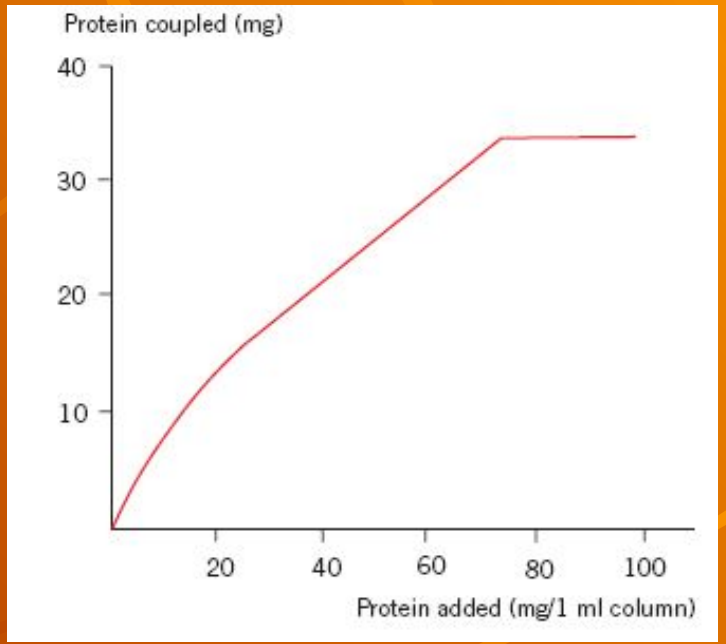
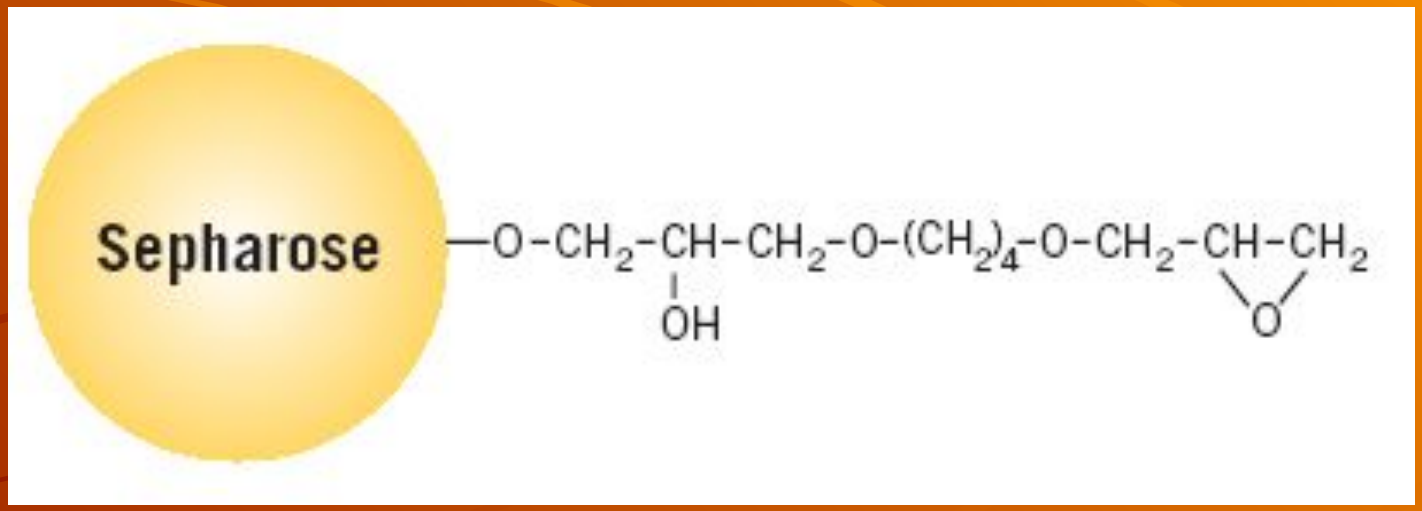


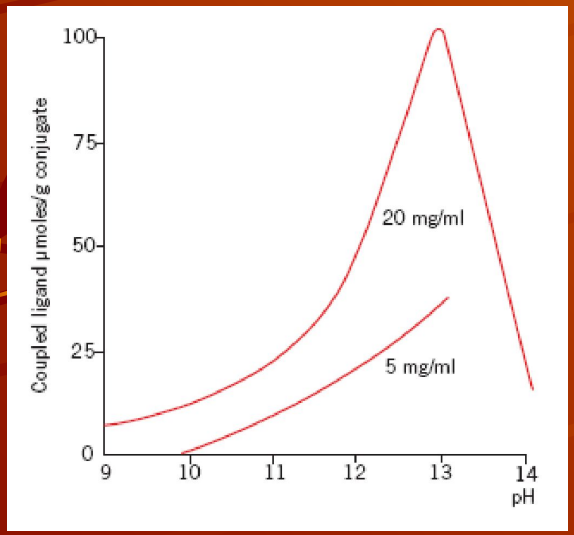
Fig. 3. Immobilization of a ligand via an *N*-hydroxysuccinimide linkage.

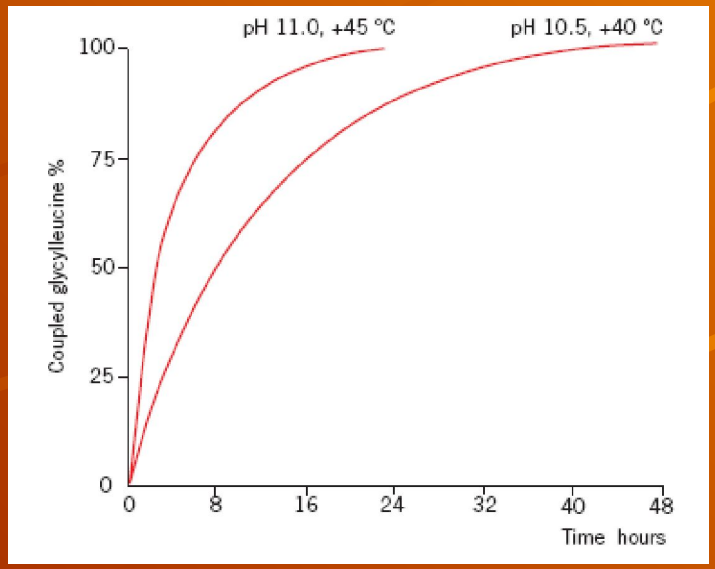
Experimental condition	Recommended concentration for coupling
Readily available ligands	10–100 fold molar excess of ligand over available groups
Small ligands	1–20 µmoles/ml medium (typically 2 µmoles/ml medium)
Protein ligands	5–10 mg protein/ml medium
Antibodies	5 mg protein/ml medium
Very low affinity systems	Maximum possible ligand concentration to increase the binding

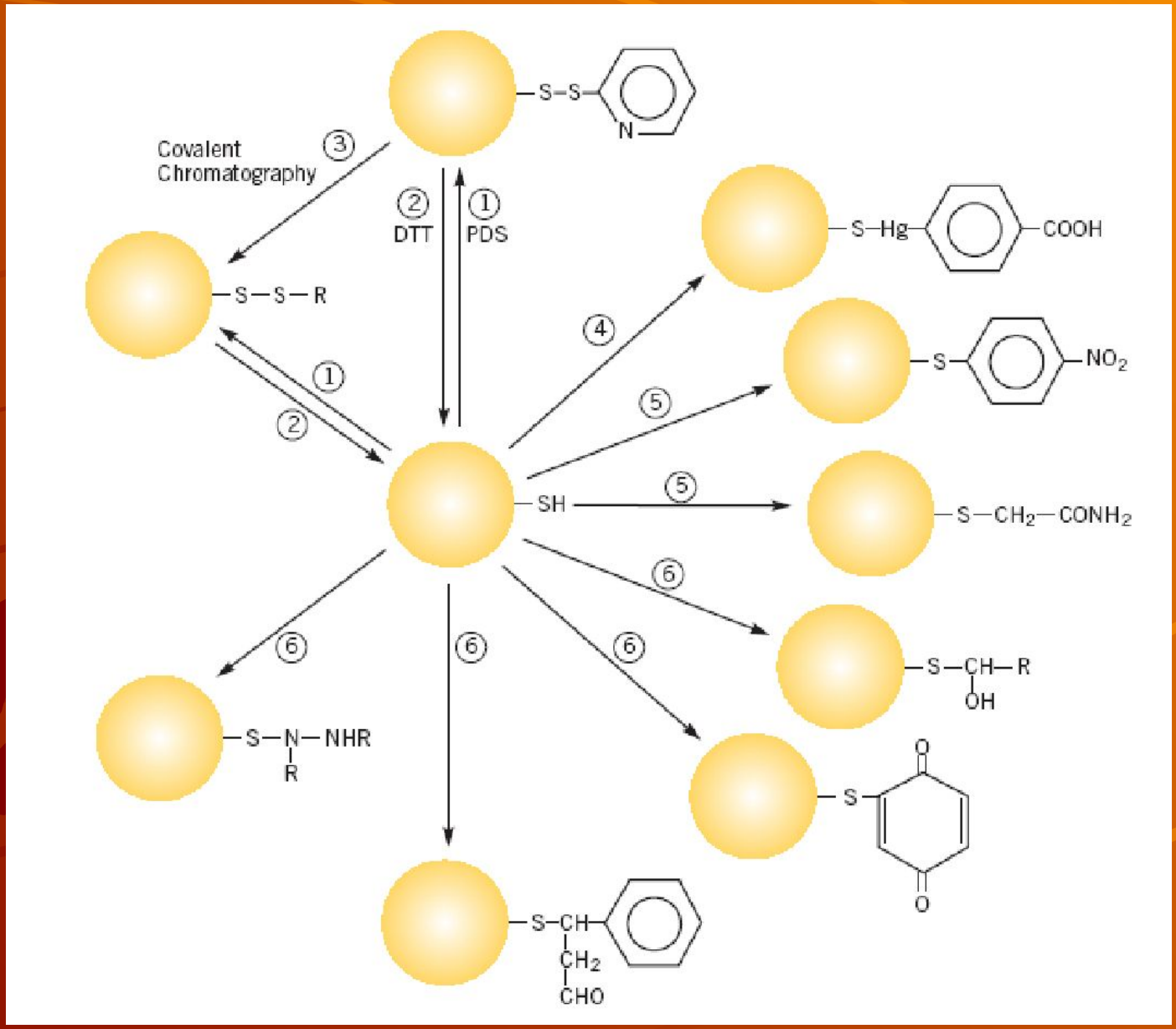

Sepharose
—O—CH2—CH—CH2—NH—(CH2)5—CO—O—N
OH
+ R—NH2
Sepharose
—O—CH2—CH—CH2—NH—(CH2)5—C—NH—R + HO—N
OH
O

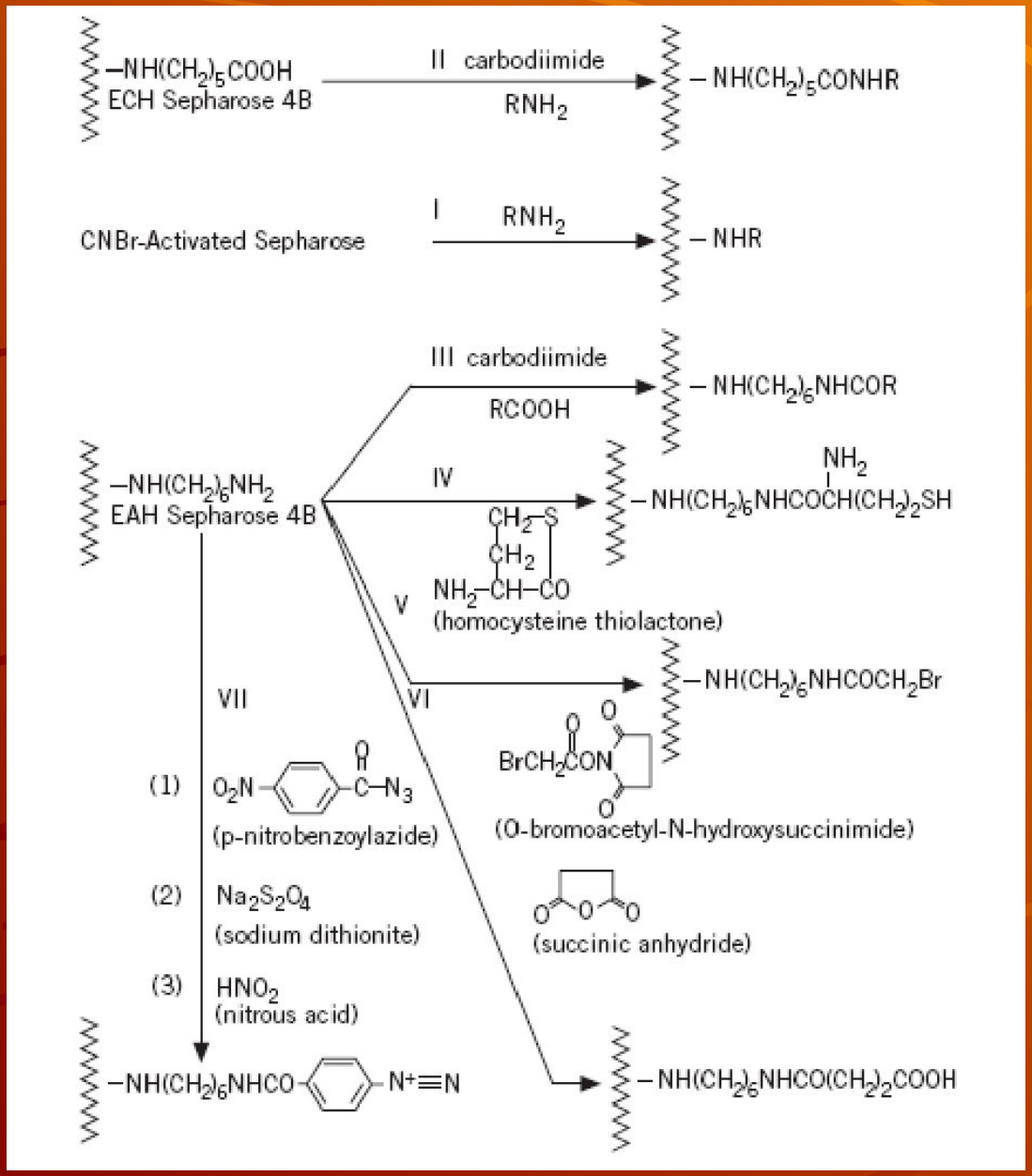

Protein coupled (mg)
40
30
20
10
20
40
60
80
100
Protein added (mg/1 ml column)


Sepharose
—O-CH2-CH-CH2-O-(CH2)4-O-CH2-CH-CH2
OH
O


Coupled ligand µmoles/g conjugate
100
75
50
25
0
9
10
11
12
13
14
pH
20 mg/ml
5 mg/ml


pH 11.0, +45 °C
pH 10.5, +40 °C
Coupled glycylleucine %
100
75
50
25
0
0
8
16
24
32
40
48
Time hours


Covalent
Chromatography
③
S–S
N
② DTT
① PDS
S–S–R
①
②
④
S–Hg–COOH
S–NO2
⑤
⑤
SH
S–CH2–CONH2
⑥
⑥
⑥
⑥
S–CH–R
OH
S–N–NHR
R
O
S
O
S–CH
CH2
CHO


–NH(CH2)5COOH
ECH Sepharose 4B
II carbodiimide
RNH2
– NH(CH2)5CONHR
CNBr-Activated Sepharose
I
RNH2
– NHR
III carbodiimide
RCOOH
– NH(CH2)6NHCOR
–NH(CH2)6NH2
EAH Sepharose 4B
IV
NH2
– NH(CH2)6NHCOCH(CH2)2SH
CH2–S
CH2
NH2–CH–CO
V
(homocysteine thiolactone)
VI
– NH(CH2)6NHCOCH2Br
BrCH2CON
(O-bromoacetyl-N-hydroxysuccinimide)
VII
(1)
O2N
C–N3
(p-nitrobenzoylazide)
(2)
Na2S2O4
(sodium dithionite)
(3)
HNO2
(nitrous acid)
(succinic anhydride)
–NH(CH2)6NHCO
N+≡N
– NH(CH2)6NHCO(CH2)2COOH

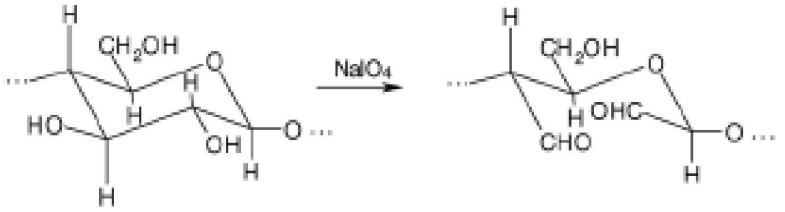
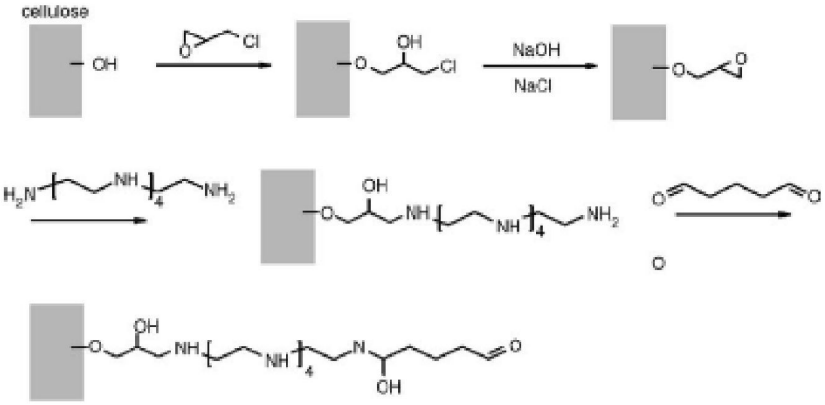


Fig. 2. Oxidation of cellulose with sodium periodate.

Activation of Granocel matrix with sodium periodate

Concentration of $NaIO_4$ (M)	Concentration of aldehyde groups (meq/g)			
	1 h	2 h	3 h	
0.1	1.12	1.12	1.24	1.55
0.2	1.43	1.86	1.92	3.35
0.3	1.92	2.23	2.23	3.84



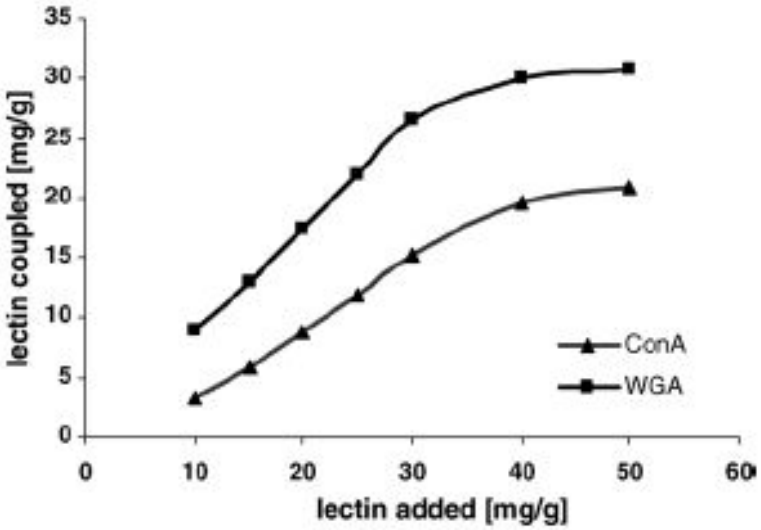


Fig. 4. Immobilization of the lectins from solutions of rising concentration on PEHA(1.1)-Cel. 0.05 M phosphate buffer containing 5×10^{-3} M of Mg^{2+} (pH 7.5) were used for the immobilization at 4 °C for 15–17 h.

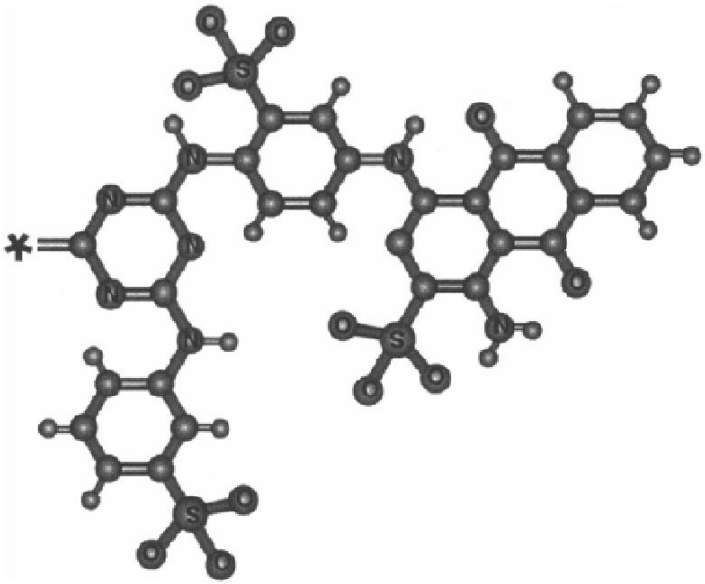
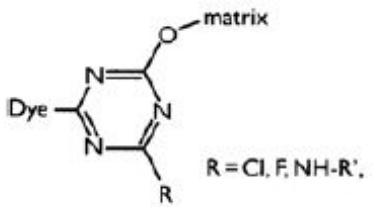
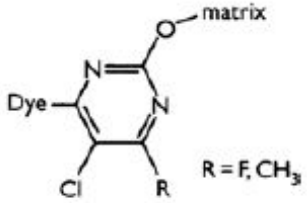


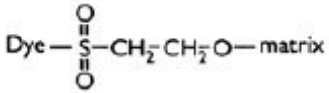
Figure 7.1 Two-dimensional structural diagram of Cibacron Blue F3G-A. The attachment to the matrix occurs at the starred linkage, by replacement of a chlorine at this position in the dye with the oxygen of a primary hydroxyl group in the matrix, or a nitrogen if coupling through an amine on the matrix.



Triazinyl: Procion, Cibacron

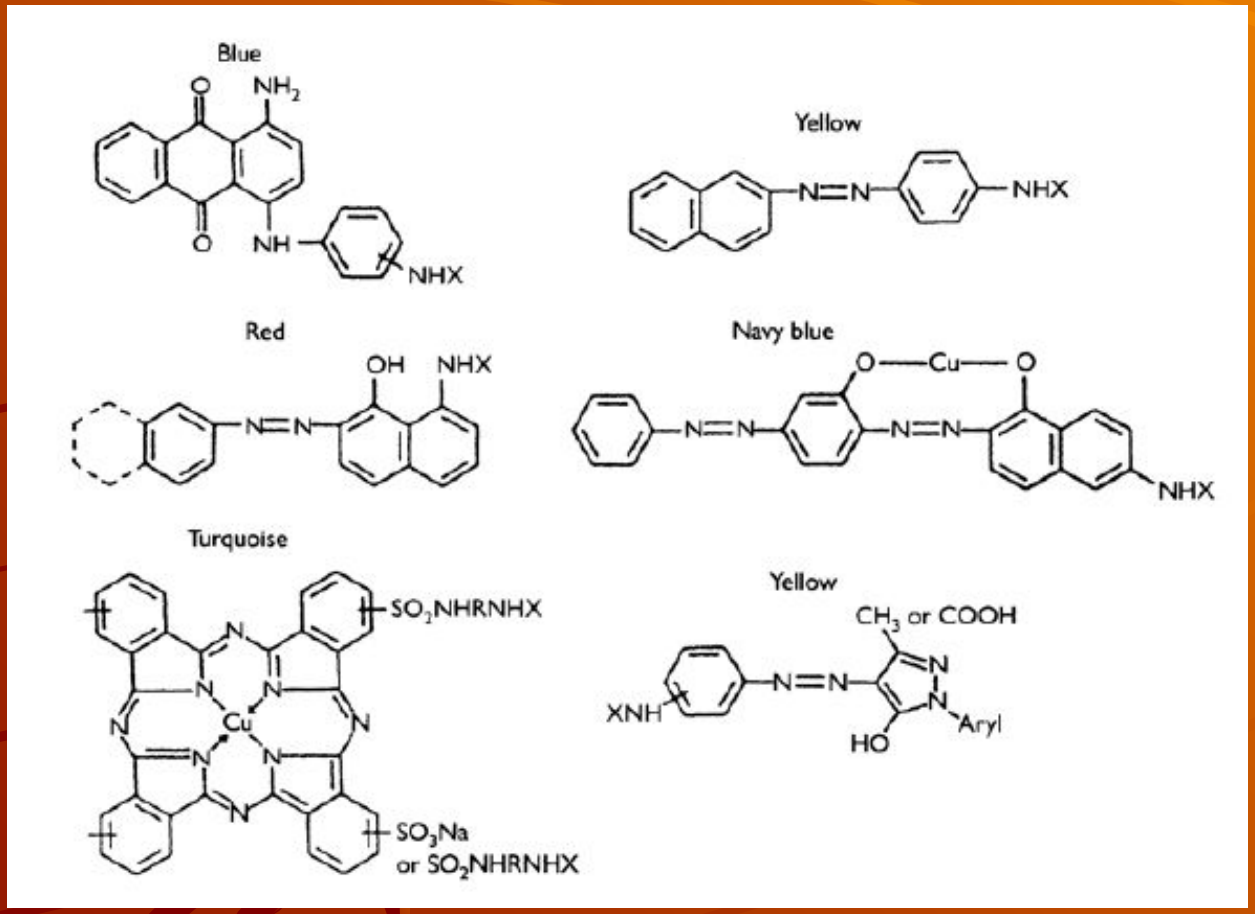


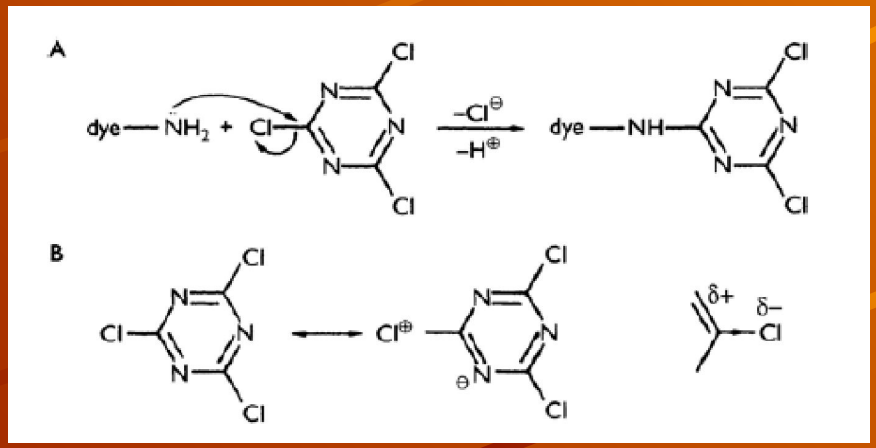
Pyrimidine: Levafix



Ethyl sulphonyl: Remazol

Figure 7.2 Three examples of reactive groups on dyes used in dye-ligand chromatography. Illustrated after reaction has occurred with a hydroxyl matrix.


Blue
NH2
O
O
NH
NHX
Yellow
N=N
NHX
Red
OH
NHX
N=N
Navy blue
O—Cu—O
N=N
N=N
NHX
Turquoise
SO2NHRNHX
N
Cu
SO3Na
or SO2NHRNHX
Yellow
CH3 or COOH
XNH
N=N
N
N
Aryl
HO


A
dye—NH2 + Cl
Cl
N
N
Cl
−Cl⊖
−H⊕
dye—NH
B
Cl
N
Cl
N
Cl
Cl⊕
δ+
δ−
Cl

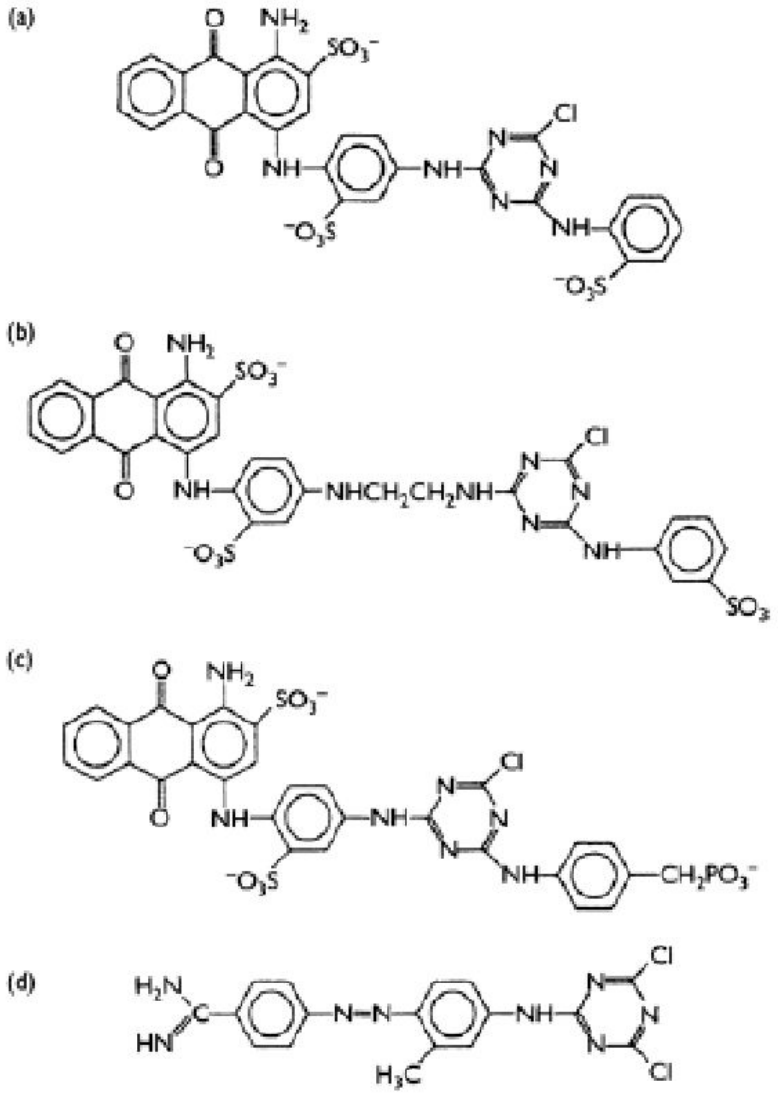


Figure 8.8 Structures of parent dye Cibacron Blue 3GA (a) followed in sequence by three biomimetic dyes: two blue-analogues (b and c) and a benzamidino-cationic yellow (d) (Labrou and Clonis, 1994).

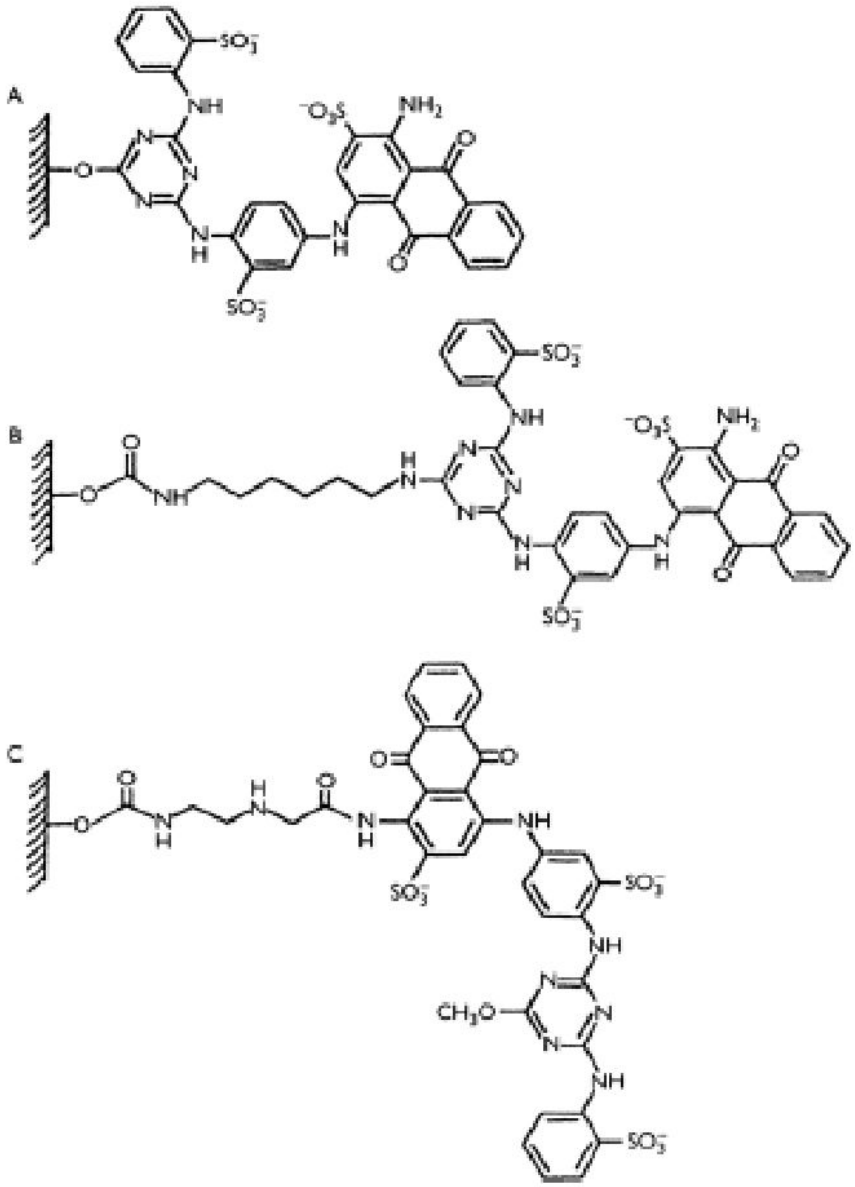


Figure 8.6 Immobilization of chlorotriazinyl anthraquinone dyes. A: coupled directly to agarose; B: coupled to 1, 1′-carbonyldiimidazole-activated agarose by a triazine ring-coupled 6-aminohexyl spacer arm; C: coupled to 1,1′-carbonyldiimidazole-activated agarose by an anthraquinone 1-amino group.

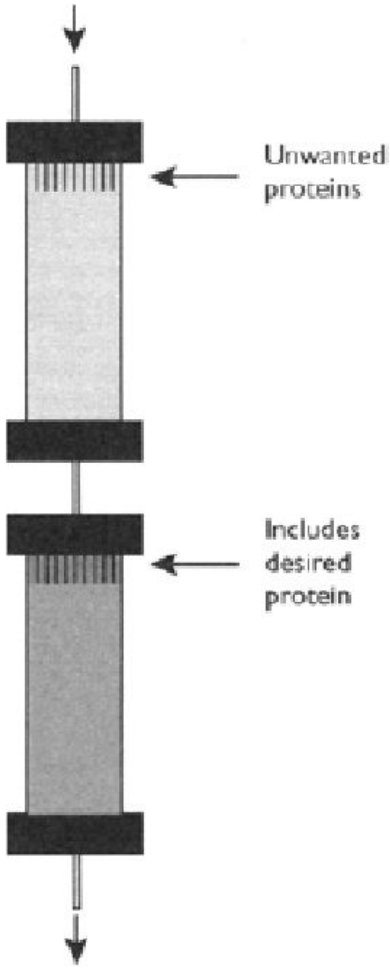


Figure 7.4 Principle of tandem column operation. The sample is passed through both columns and washed through with buffer. The desired protein is bound only on the second (positive) column, while most unwanted proteins are removed on the first (negative) column.

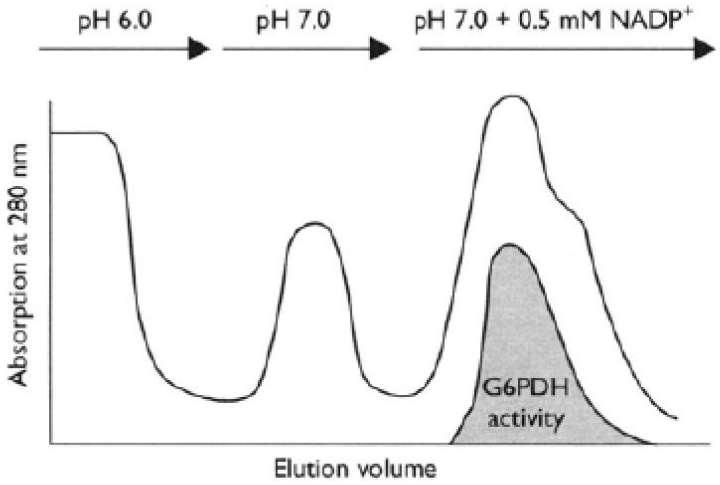


Figure 7.7 Elution of *Zymomonas mobilis* glucose 6-phosphate dehydrogenase from a Procion Yellow HE-3G column using $NADP^+$. Efficient elution at pH 7 (0.1M phosphate) can be achieved with as little as 0.5mM nucleotide, which is approximately 10 times its K_m value.

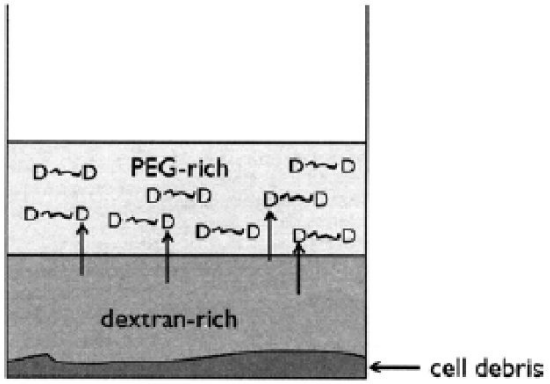
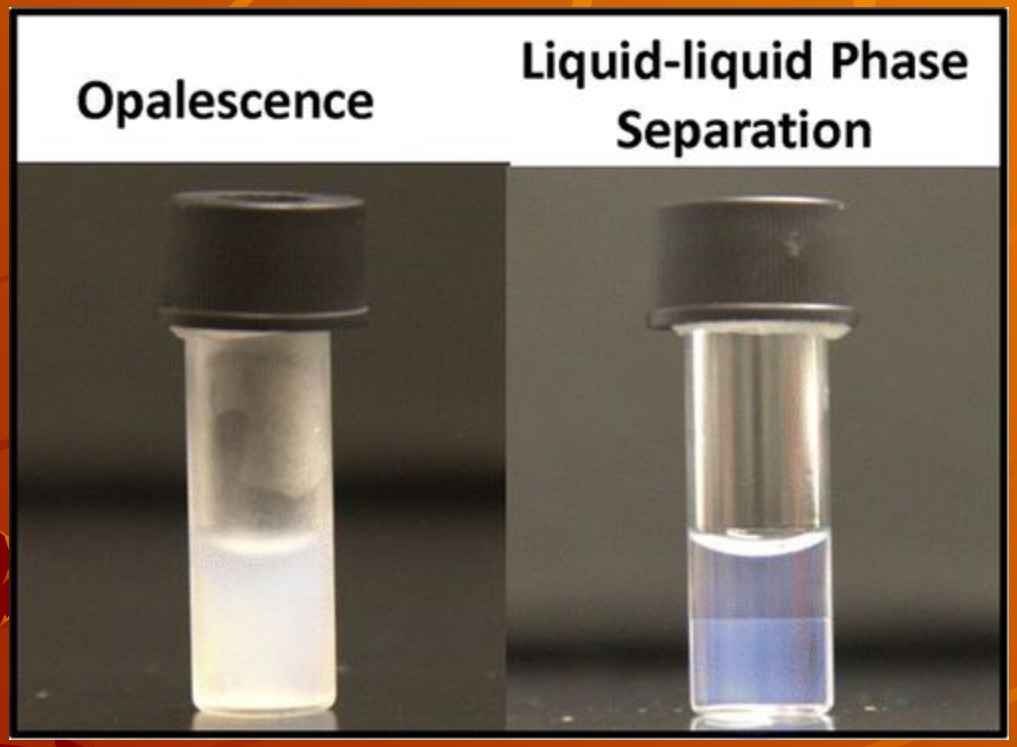


Figure 7.8 Principle of affinity phase partition. The dye molecules (D) are covalently attached to polyethylene glycol (PEG) molecules, which partition in the upper layer.


Opalescence
Liquid-liquid Phase Separation

Молекулярные основы металхелатной хроматографии

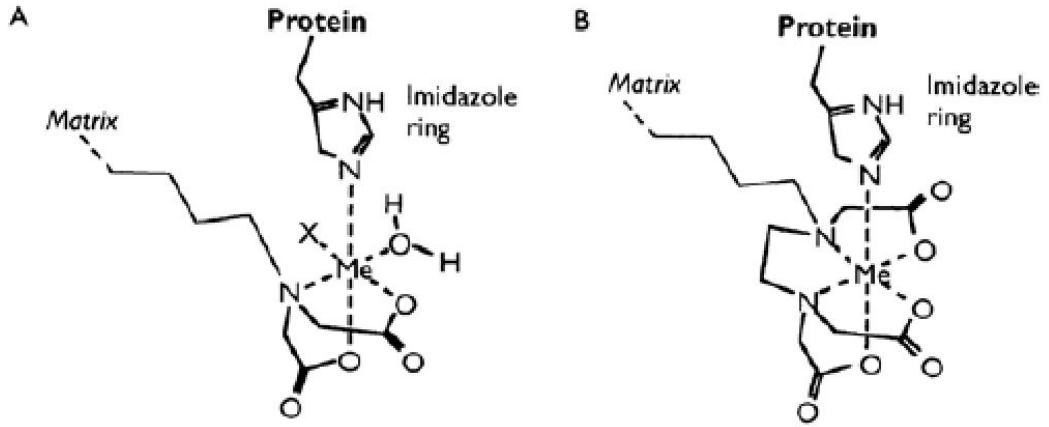


Figure 10.1 Interaction scheme between metal chelate and imidazole ring (Histidine) of protein. A with iminodiacetic acid (IDA) as chelating agent. B with tris(carboxymethyl)ethylenediamine (TED) as chelating agent.

Table 10.1 Classification of metal ions and their ligands. From Chottard *et al.* (1984) and Glusker (1991)

Classification	*Metal ions (Lewis acids)*	*Principal ligands (Lewis bases)*
Hard	H^+, Li^+, Na^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cr^{3+}, Co^{3+}, Al^{3+}, Ga^{3+}, La^{3+}, Nd^{3+}, Eu^{3+}	H_2O, ROH, OH^-, RO^-, NH_3, RNH_2, CO_3^-, $RCOO^-$ (essentially oxygen ligands)
Soft	Cu^+, Ag^+, Au^+, Ti^+, Pd^{2+}, Pt^{2+}, Cd^{2+}, Hg^+, Hg^{2+}	RSH, RS, R_2S, CN^-, H^-, I^- (essentially sulfur ligands)
Borderline	Zn^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, Co^{3+}, Sn^{2+}, Pb^{2+}, Rh^{3+}, Ir^{3+}, In^{3+}, Ru^{3+}	Imidazole, Pyridine, N_2, NO_2^-, N_3^-, Br^- (essentially nitrogen ligands)

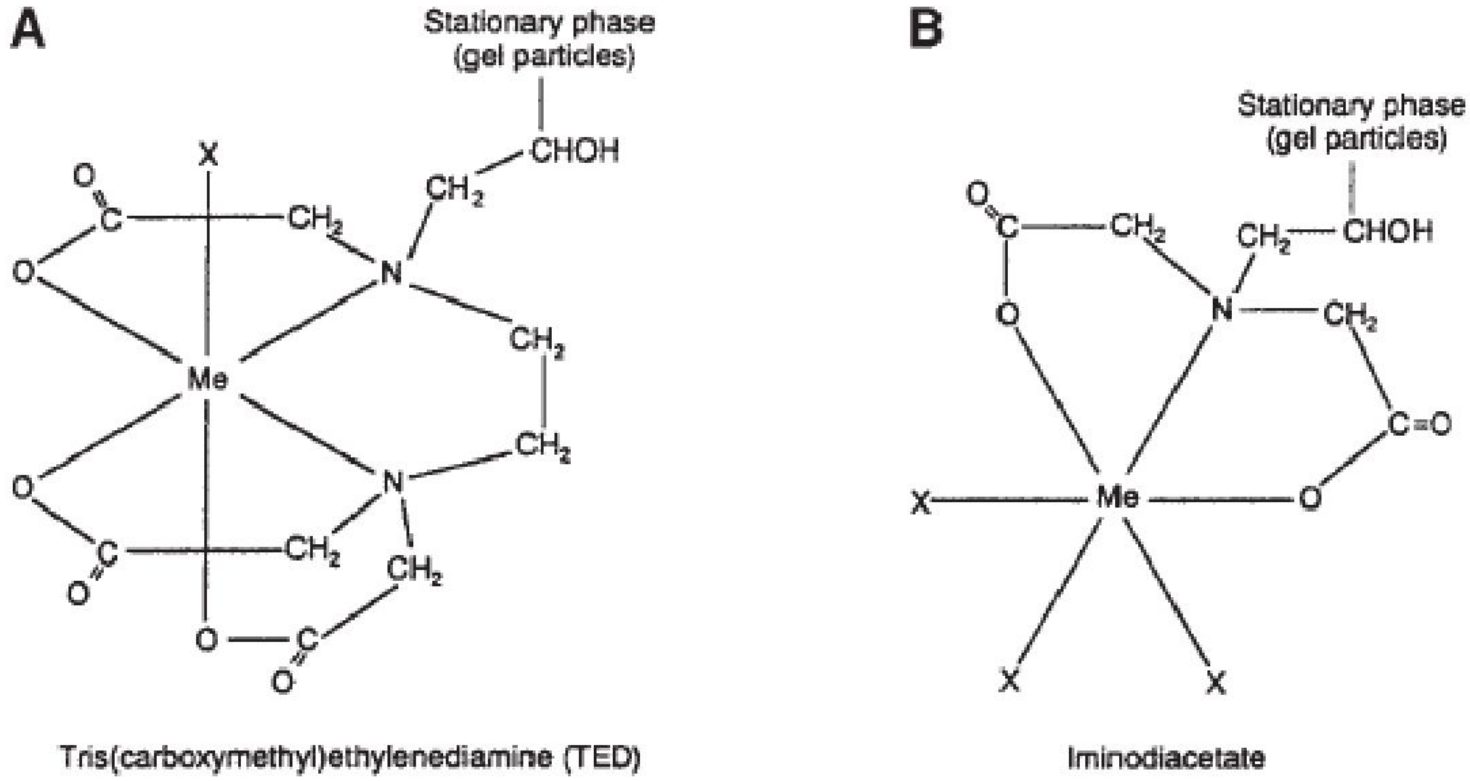
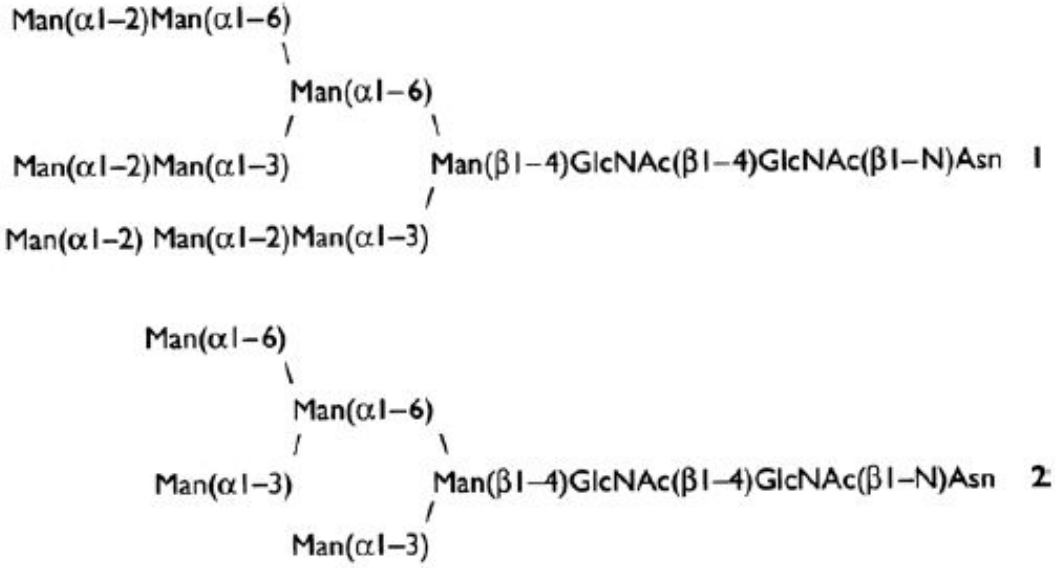


Fig. 1. Schematic illustration of several types of immobilized metal-chelating group, including iminodiacetate (IDA), tris(carboxymethyl) ethylenediamine (TED), and the metal-binding peptides $(GHHPH)_nG$ (where $n = 1, 2, 3$, and 5. (From **refs.** *17* and *18*.)



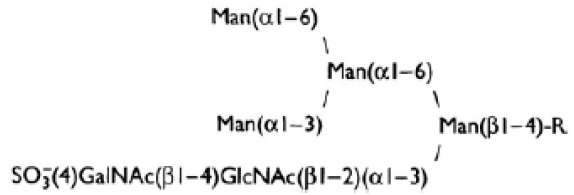


Figure 13.5 Glycan of the hybrid type of a pituitary hormone. R: GlcNAc(β1−4) GlcNAc (β1− N)Asn.

Figure 13.6 Structure of glycans of the *N*-acetyllactosaminic type (complex type). 1: biantennary glycan of human serum transferrin; 2: difucosylated biantennary glycan of human lactotransferrin; 3: triantennary glycan of human serum transferrin; 4: tetraantennary glycan of human α_1-acid glycoprotein

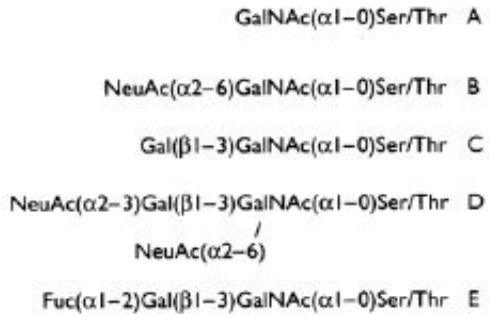


Figure 13.7 Structure of some O-glycans of the "mucin type". A: epitope of Tn group; B: human and ovine submaxillary mucin; C: antifreeze glycoprotein from antarctic fish, porcine submaxillary mucin, human chorionic gonadotropin, human IgA, T-reactive erythrocytes, rat brain glycoproteins, intestinal mucin; D: human glycophorin, fetuin, bovine kappa casein, human gonadotropin, lymphocyte membranes; E: human erythrocyte of O group, IgA: immunoglobulin A.

Лектины

используемые в аффинной хроматографии гликопротеинов

α-D-mannose, α-D-glucose	
Canavalia ensiformis	Con A
Lens culinaris	LCA
β-D-galactose, *N*-acetyl-β-D-galactosamine	
Ricinus communis	RCA_I
	RCA_{II}
Glycine max (Soybean)	SBA
Arachis hypogaea (Peanut)	PNA
α-D-Galactose, *N*-acetyl-α-D-galactosamine	
Griffonia simplicifolia I	GSA_I
Dolichos biflorus	DBA
N-acetyl-β-D-glucosamine	
Triticum vulgare (Wheat germ)	WGA
α-L-fucose	
Aleuria aurantia	AAA
Lotus tetragonolobus	LTA
Ulex europeus I	UEA_I
α-*N*-acetylneuraminic acid	
Limulus polyphemus (Limulin)	

Специфичность и способы элюции гликопротеинов с лектинсодержащих сорбентов

Common Lectins Used in Affinity Chromatography

Lectin	Specificity	Useful eluants	Uses
Concanavalin A	α-D-Mannopyranosyl with free hydroxyl groups at C3, C4, and C6	0.01–0.5 *M* Methyl α-D-mannoside	Separation of glycoproteins
		D-Mannose	Purification of glycoprotein ecozymes
		D-Glucose	Partial purification of IgM
			Separation of lipoproteins
Lens culinaris	α-D-Glucopyranosyl residues	Methyl-α-D-glucoside	Purification of gonadotrophins
	α-D-Mannopyranosyl	0.1 *M* Na borate, pH 6.5	Purification of HeLa cells
		0.15 *M* Methyl α-D-mannoside	Isolation of mouse H antigens
	Binds less strongly than Con A		Purification of detergent-solubized glycoproteins
			Schistosoma mansoni: surface membrane isolation
Triticum vulgaris	*N*-Acetyl-D-glucosamine	0.1 *M N*-Acetyl-glucosamine	Biochemical characterization of H4G4 antigen from HOON pre-B leukemic cell line
			Purification and analysis of RNA polymerase transcription factors
Ricinus communis	α-D-Galactopyranosyl residues	0.15 *M* D-Galactose	Fractionation of glycopeptide-binding proteins
Jacalin	D-Galactopyranosyl residues	0.1 *M* Melibiose in PBS	Separating IgA1 and IgA2
			Purification of C1 inhibitor
Bandeira simplicifolia	α-D-Galactopyranosyl and *N*-acetyl-D-galactosamyl	PBS	Resolving mixtures of nucleotide sugars

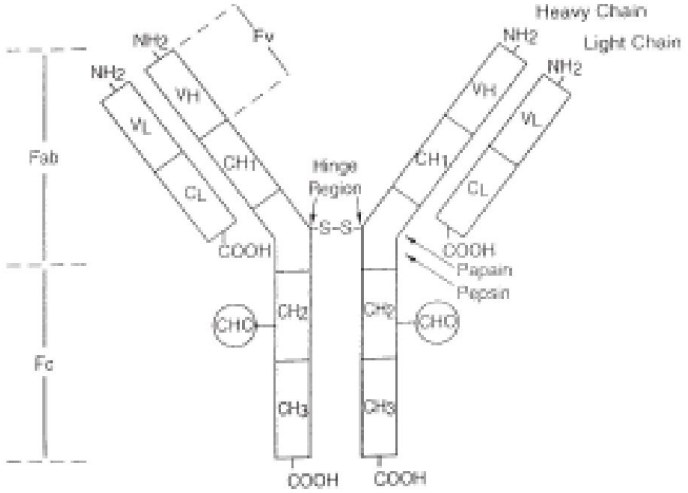
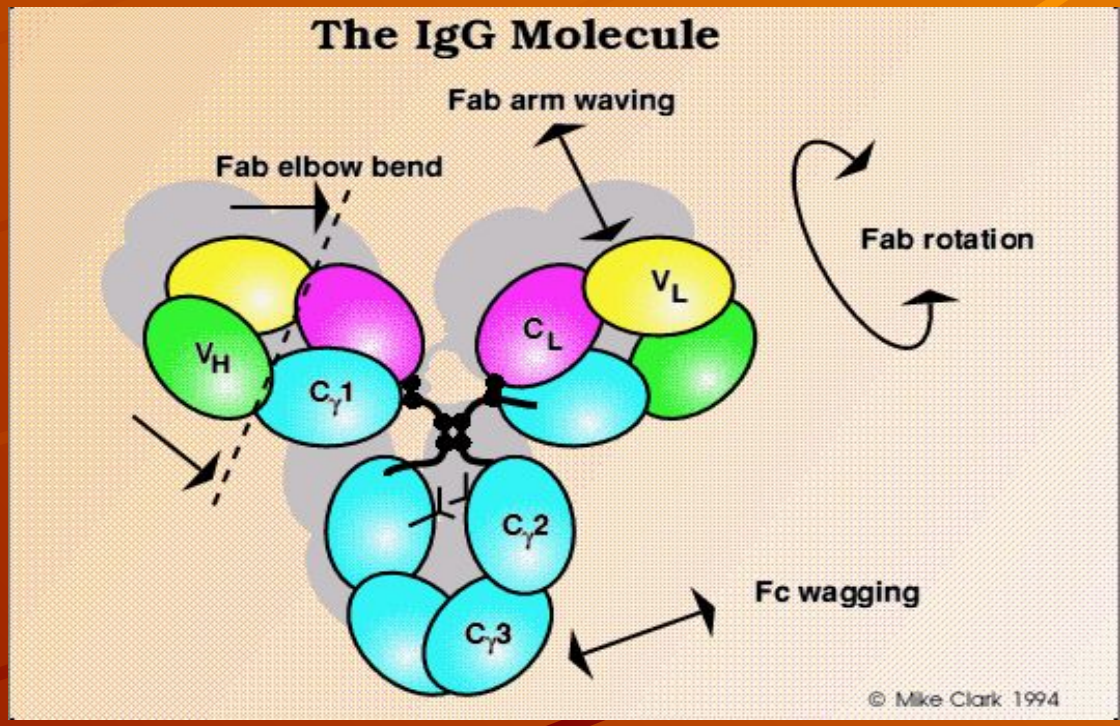
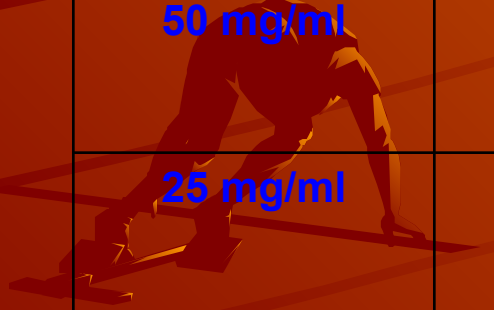


Fig. 1. Immunoglobulin G (IgG) structure. IgG molecules are tetramers of heavy and light chains. The Fab (antigen-binding fragment) region mediates selective target isolation. The Fc (crystallizable fragment) region controls receptor binding and complement activation. The Fc region also plays a role in binding to protein A and protein-*G*. *N*-Linked oligosaccharide moieties are found on Asn-297 of the heavy chain in the CH2 region but may be found at additional sites.

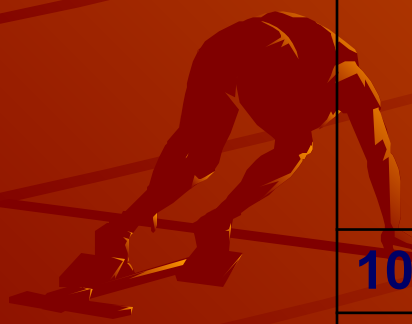


Эффективность связывания антител, Влияние концентрации BrCN и pH при связывании

BrCN	Иммобилизация Антител на Sepharose pH8,7(mgAB/2ml)(%)	pH7,2(mgAB/2ml)(%)	pH6,4(mgAB/2ml)(%)
100 mg/ml	19,3 (96,5%)	18,6 (93,0%)	18,0 (90(%)
50 mg/ml	18,6 (92,7%)	17,8 (89%)	15,3 (76,5%)
25 mg/ml	17,1 (85,5%)	15,5 (73,6%)	14,7 (73,5%)



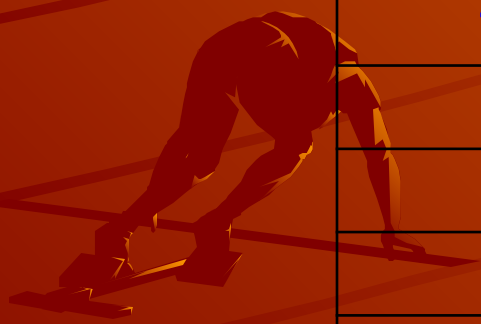
Связывающая способность BrCN активированной Сефарозы МАТ иммуноаффинного сорбента. Эффект концентрации BrCN, и pH реакции иммобилизации.



BrCN	Антиген-связывающая эффективность			Общее количество связавшегося на антителах фермента		
	pH 8,7	pH 7,2	pH 6,4	pH 8,7	pH 7,2	pH 6,4
100	0,42	0,47	0,49	8,13	8,73	8,82
50	038	0,42	0,49	6,95	7,51	7,52
25	0,36	0,47	0,51	6,07	7,20	7,42

Эффект концентрации антител на антигенсвязывающую способность AffiGel10 сорбента

Кол-во АТ связанных с сорбентом мг/мл	Выход фермента в мг.	Антиген-связывающая эффективность мг/мл
13,5	6,78	0,21
7,6	3,40	020
4,9	3,84	0,26
2,4	1,8	0,25
0,61	0,48	0,26

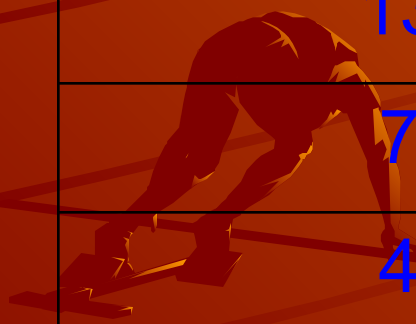


Антиген-связывающая емкость иммуносорбентов, приготовленных из различных МАТ

МАТ	Антитела иммобилизованные на сорбент		Общее количество связавшегося фермента (мг)	Антиген-связывающая эффективность Мг/мл
	мг	%		
3D11	18,7	93,5	19,4	1,00
3F3	17,2	86,0	16,0	0,91
3E1	16,5	82,5	14,2	0,83
2B12	17,5	87,5	9,8	0,56
1C4	18,7	93,5	8,7	0,48
2D3	15,3	76,5	8,0	0,53
4D4	18,6	93,0	8,0	0,043
4D7	17,7	87,0	1,6	0,09
3H11	17,7	88,5	1,0	0,06
5A6	17,4	87,0	0,4	0,02

Эффект концентрации антител на антиген-связывающую емкость AffiGel10 сорбента

Количество иммобилизованных антител мг/мл	Выход антигена мг	Эффективность связывания мг/мг
13,5	6,78	0,21
7,6	3,40	0,20
4,9	3,84	0,26
2,4	1,8	0,25
0,61	0,48	0,26



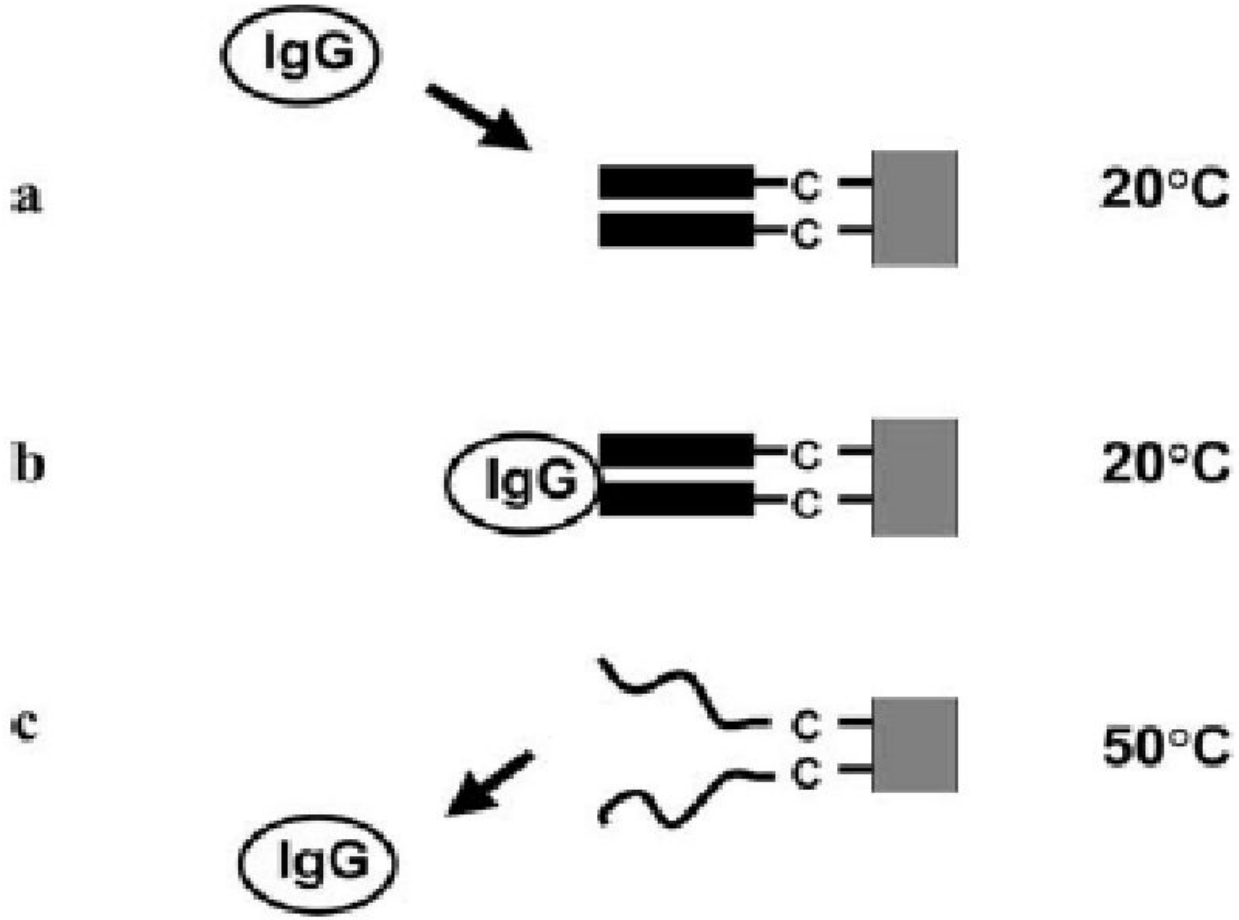
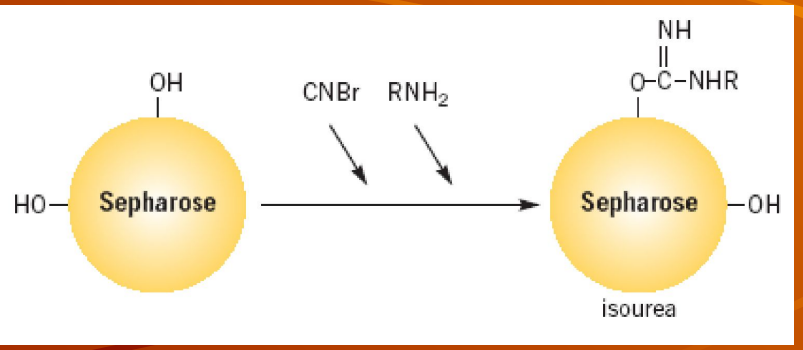
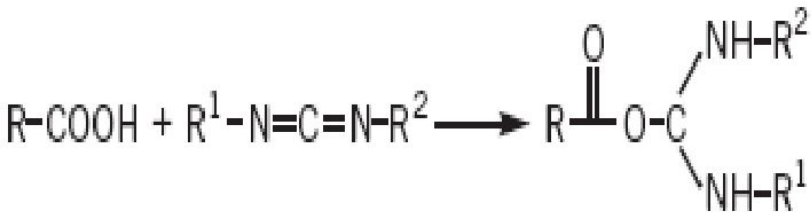


Fig. 2. Heat elution chromatography. (a) At low temperature, the immobilized prH is folded in a coiled-coil conformation (shown as black bars) with an intact binding site for IgG. IgG will therefore interact with prH and other molecules can be washed out (b). When the temperature is raised above the melting point of the dimer, the structure and the binding site are disrupted and IgG elutes from the column (c).

Mobile Phases for Elution From Immunoaffinity Supports

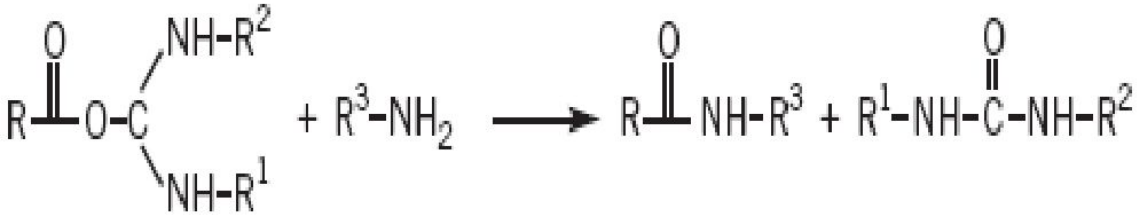
Class of eluent	Principle
0.1 *M* Glycine–NaOH, pH 10.0 50 m*M* Diethylamine pH 11.5 1 *M* NH_4OH	High pH
0.1 *M* Glycine–HCl, pH 2.0 20 m*M* HCl 0.1 *M* Sodium citrate, pH 2.5 1 *M* Propionic acid	Low pH
50% (v/v) Ethylene glycol Dimethyl sulfoxide (DMSO) Acetonitrile 10% (v/v) Dioxane	Organic solvent
0.1 *M* Tris-HCl, pH 8.0, + 2 *M* NaCl	High ionic strength
Deionized water	Low ionic strength
1 *M* Ammonium thiocyanate 3 *M* Potassium chloride 5 *M* Potassium iodide 4 *M* Magnesium chloride	Chaotropes
6 *M* Guanidine HCl, pH 3.0 6–8 *M* Urea	Denaturant
1% (w/v) SDS	Surfactant
1–10 m*M* EDTA or EGTA Sodium citrate	Metal-ion chelator





Carbodiimide Active ester

R and R^3 = matrix or ligand



Active ester Peptide bond Urea derivate (side product)

1. Иммобилизованные лектины

2. Иммобилизованные красители

3. Иммобилизованные субстраты - кофакторы

4. Металл хелатная хроматография Zn, Co, Ca, Fe

5. Иммобилизованные лиганды (гормоны)

6. Ковалентная хроматография – иммобилизация по -SH

7. Хроматография на антителах - Иммуноаффинная хроматография