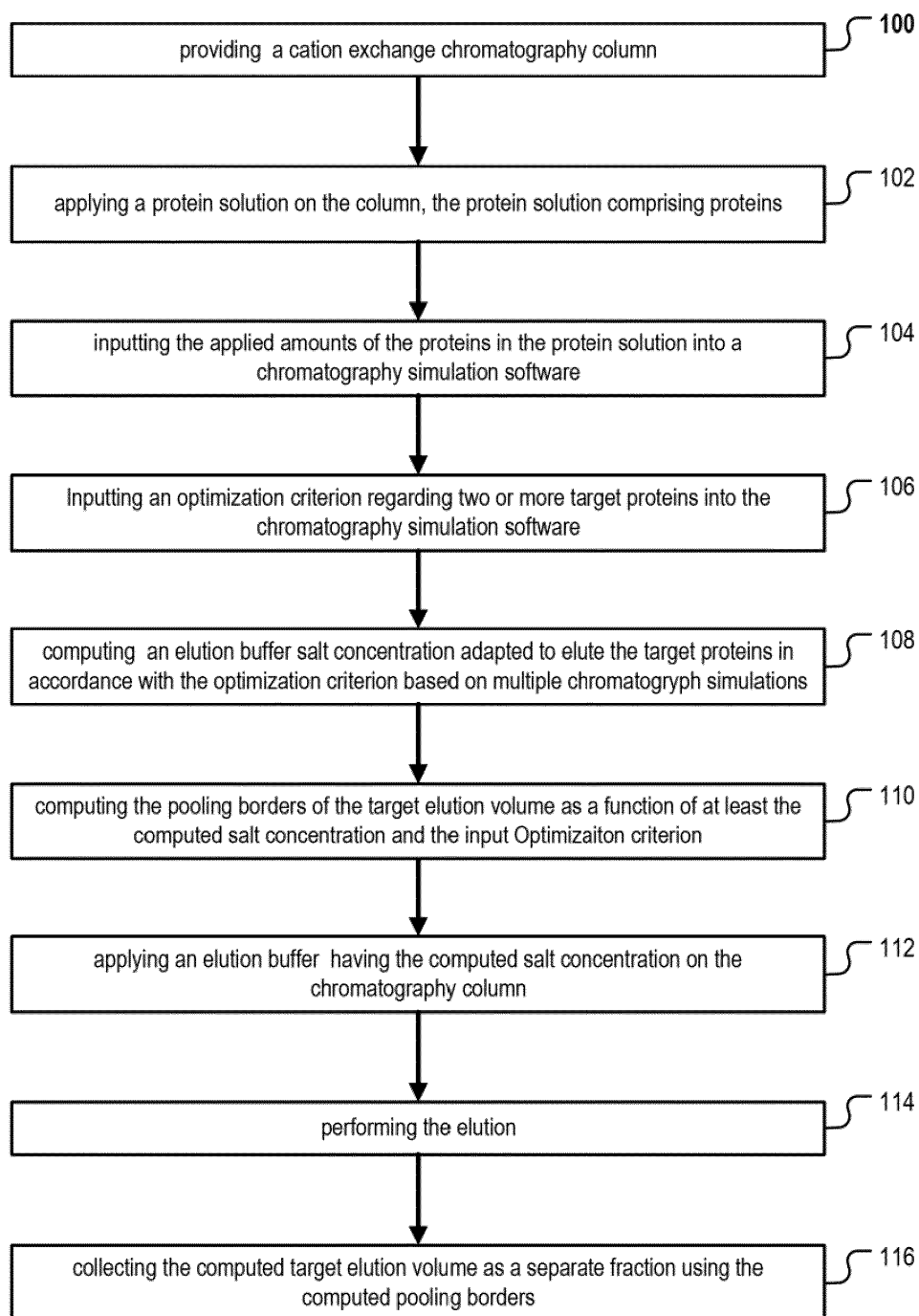
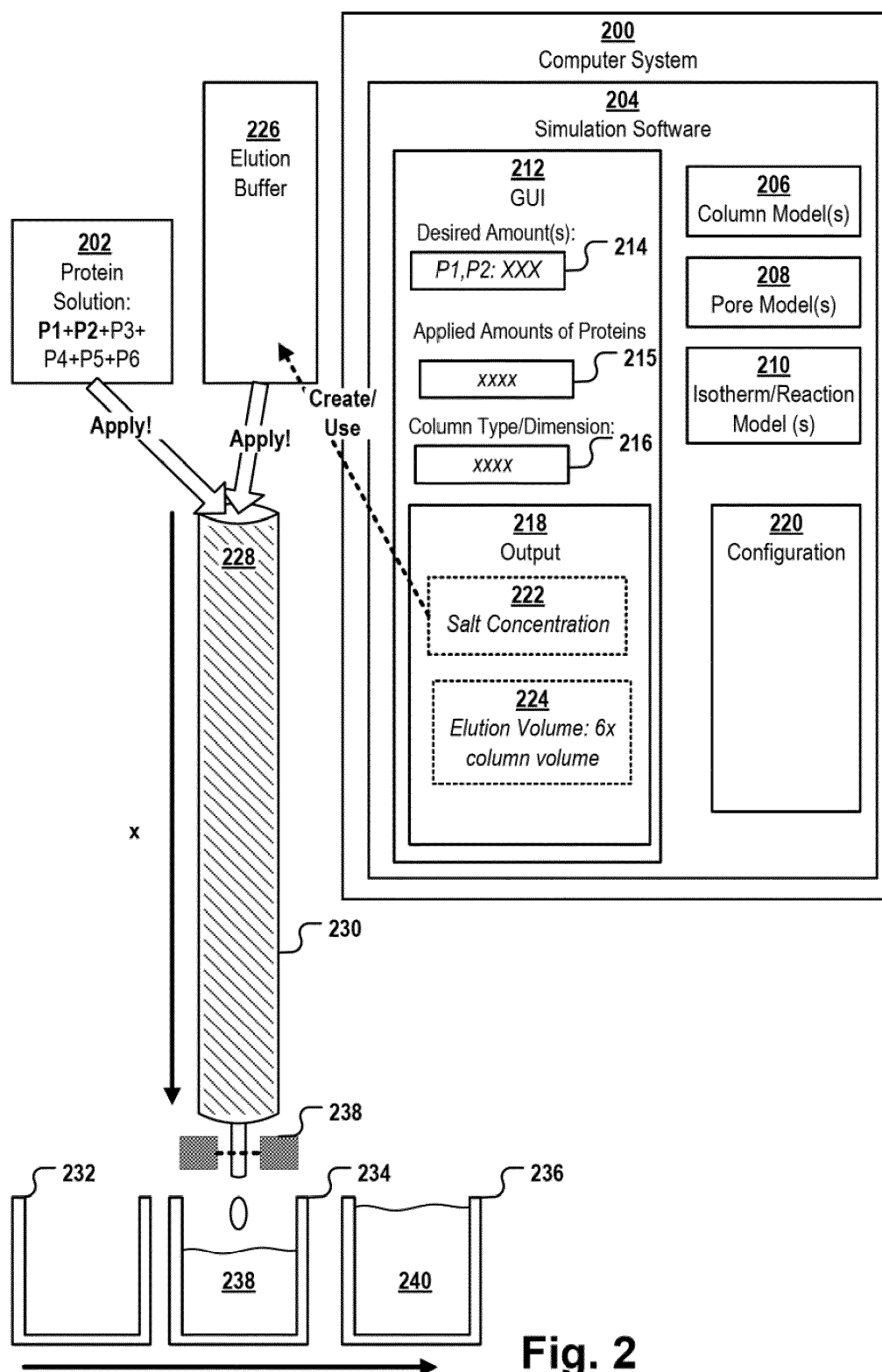
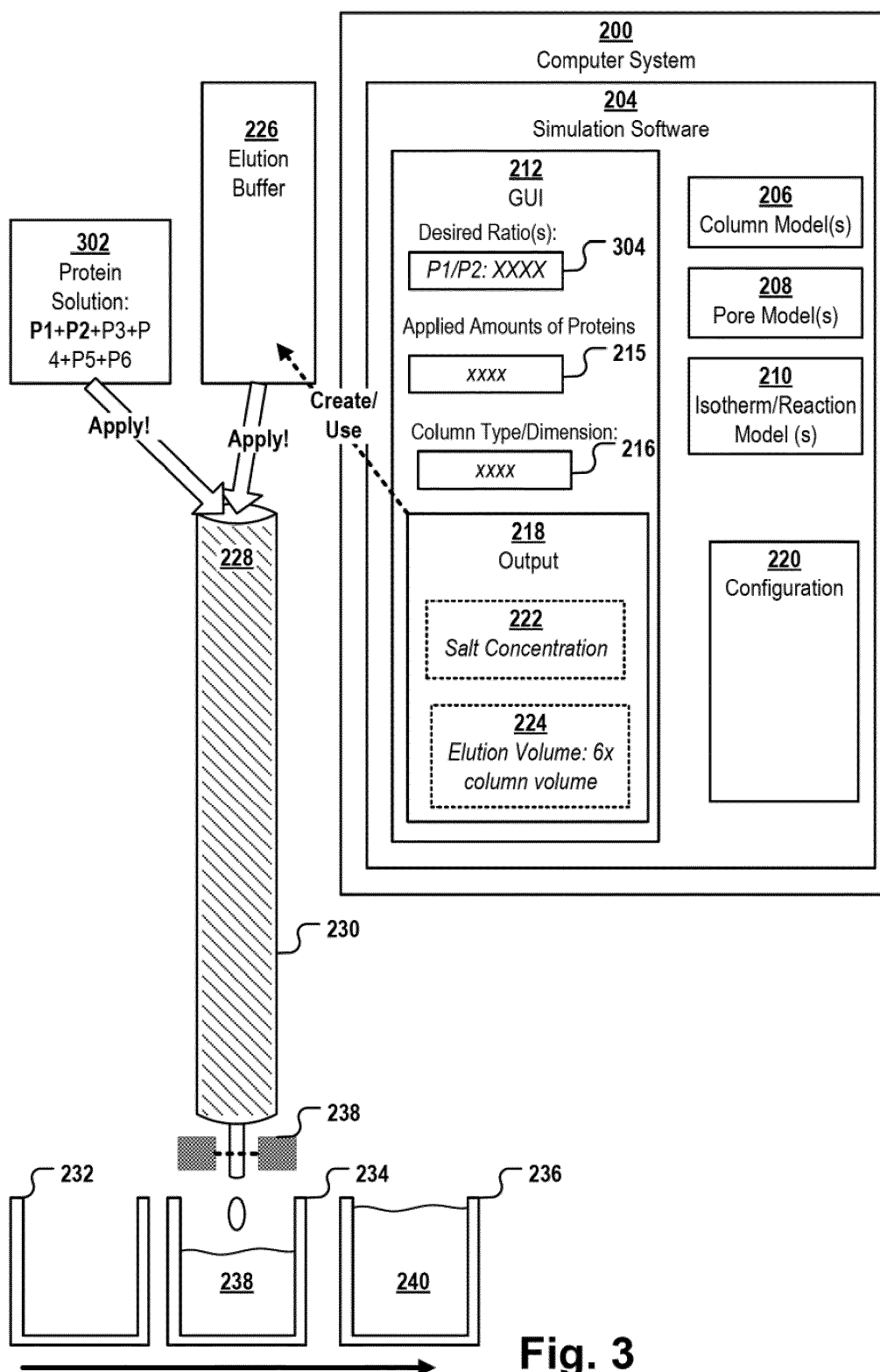


**Fig. 1**





400

Model

Column Model

Transport Dispersive

Pore Model

Lumped Rate

Isotherm

IEC 2015

Model Selection Wizard

Fig. 4A

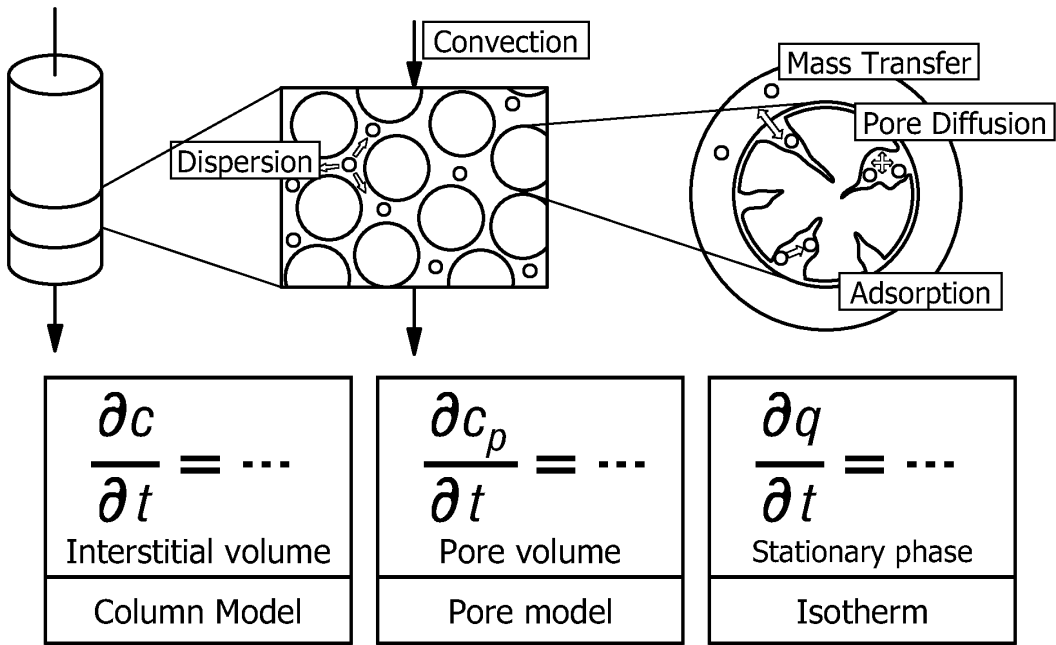


Fig. 4B

Column model (according to Schmidt Traub et al. „Preparative Chromatography“ 2012, Wiley-VCH)

Transport Dispersive

$$\frac{\partial c}{\partial t}(x,t) = -\frac{v(t)}{\varepsilon_{col}} \frac{\partial c}{\partial x}(x,t) + D_{ax} \frac{\partial^2 c}{\partial x^2}(x,t) - \frac{1-\varepsilon_{col}}{\varepsilon_{col}} \left(\varepsilon_{part} \frac{\partial c}{\partial t} + (1-\varepsilon_{part}) \frac{\partial q}{\partial t}(x,t) \right)$$

Pore model (according to Schmidt Traub et al. „Preparative Chromatography“ 2012, Wiley-VCH)

Lumped Rate Model

$$\frac{\partial c_p}{\partial t}(x,t) = \frac{3}{\tau_{part}} \frac{k_{eff}}{\varepsilon_{part}} (c(x,t) - c_p(x,t)) - \frac{1-\varepsilon_{part}}{\varepsilon_{part}} \frac{\partial q}{\partial t}(x,t)$$

Isotherm (according to Klutters et al., "Application of linear pH gradients for the modeling of ion exchange chromatography: Separation of monoclonal antibody monomer from aggregates", J Sep Sci. 2016 Feb; 39(4):663-75.):

$$k_{kin,i} \frac{\partial q_i}{\partial t} = \exp(Gp_i + v_i Gs)^{-v_i} q_{salt}^{q_i} \cdot C_{p,i}^{-q_i} \cdot C_{p,salt}^{v_i}$$
$$\text{with } v_i = \frac{-N_{Car}}{1 + 10^{pKa_{Car} - pH}} + \frac{N_{A_{mi}}}{1 + 10^{pH - pKa_{A_{mi}}}} + \frac{N_{His}}{1 + 10^{pH - pKa_{His}}} + \frac{N_{NTerm}}{1 + 10^{pH - pKa_{NTerm}}}$$

ph-dependent ION Exchange
isotherm (IEC 2015) by
Klutters et al.

Fig. 4C

Entering Parameters (Model, Column, etc.)

Geometry

Length [mm]

50

Volume [ml]

2.5

Interstibial Column Porosity

0.43

Axial Dispersion [mm²/s]

0.2

Bead Radius [mm]

0.025

Bead Porosity

0.69

Isotherm Specific Parameters

Ionic Capacity [M]

0.673

p Ka Car

4.8

p Ka His

6

p Ka Ami

10.5

p Ka Nterm

7.4

Fig. 4D

Entering Parameters (Protein 1, Protein 2,)

Components

Salt

pH

Protein 1

Protein 2

Protein 3

Fig. 4E

**Obtaining a Target Protein P1
(from a solution comprising P1, P2 and P3)**

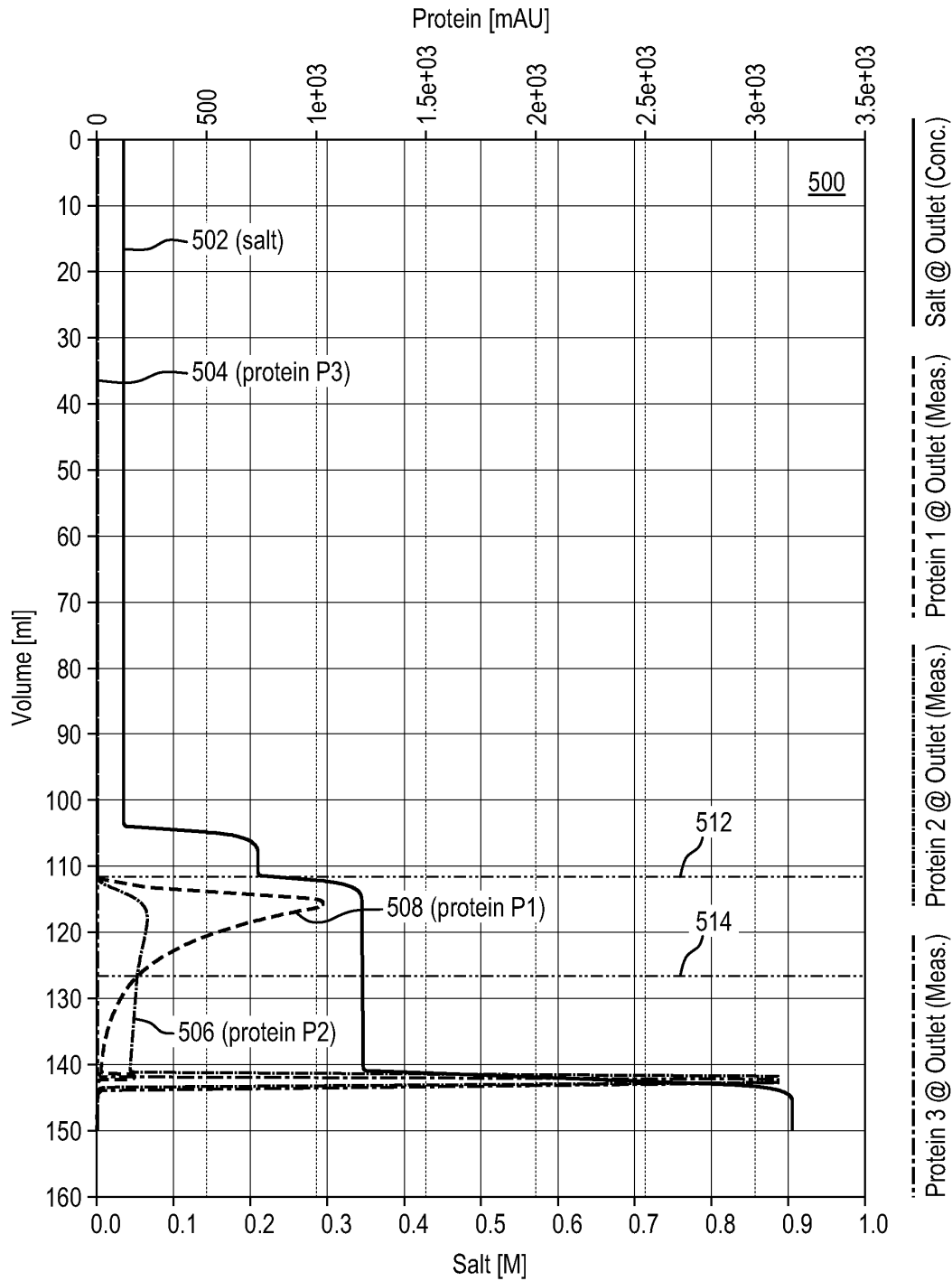


Fig. 5A

Identification of optimal salt concentration

Results					510
Iteration	Salt Stufe Concentration	Purity Protein 1	Yield Protein 1	Objective [^]	
23	0.345893	0.738685	0.862189	2.49157	
18	0.346202	0.737446	0.864401	2.49163	
1	0.34821	0.729292	0.878208	2.49298	
10	0.342544	0.751832	0.836734	2.49335	
11	0.349595	0.732567	0.887164	2.49488	
2	0.349958	0.722053	0.889435	2.4955	
22	0.338399	0.767359	0.801499	2.50167	
17	0.353815	0.705651	0.911636	2.5055	
12	0.355122	0.698624	0.918885	2.51112	

Fig. 5B

Identification of optimal salt concentration

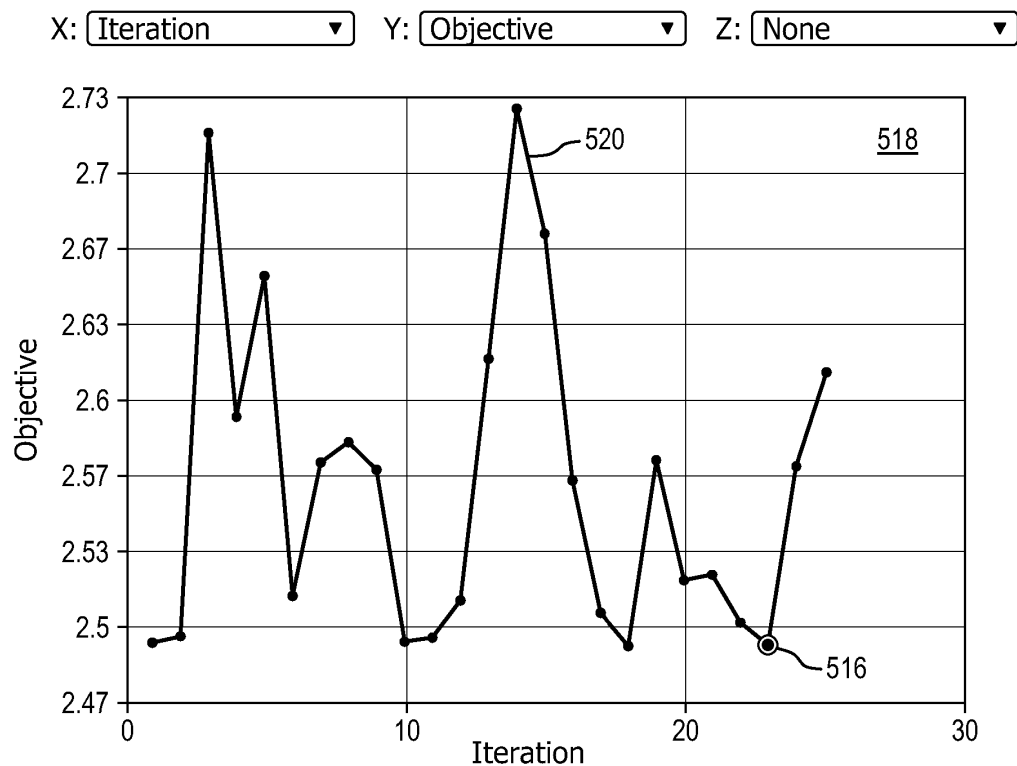


Fig. 5C

Obtaining Target Proteins P1 & P2 in a defined ratio
(from a solution comprising P1, P2 and P3)

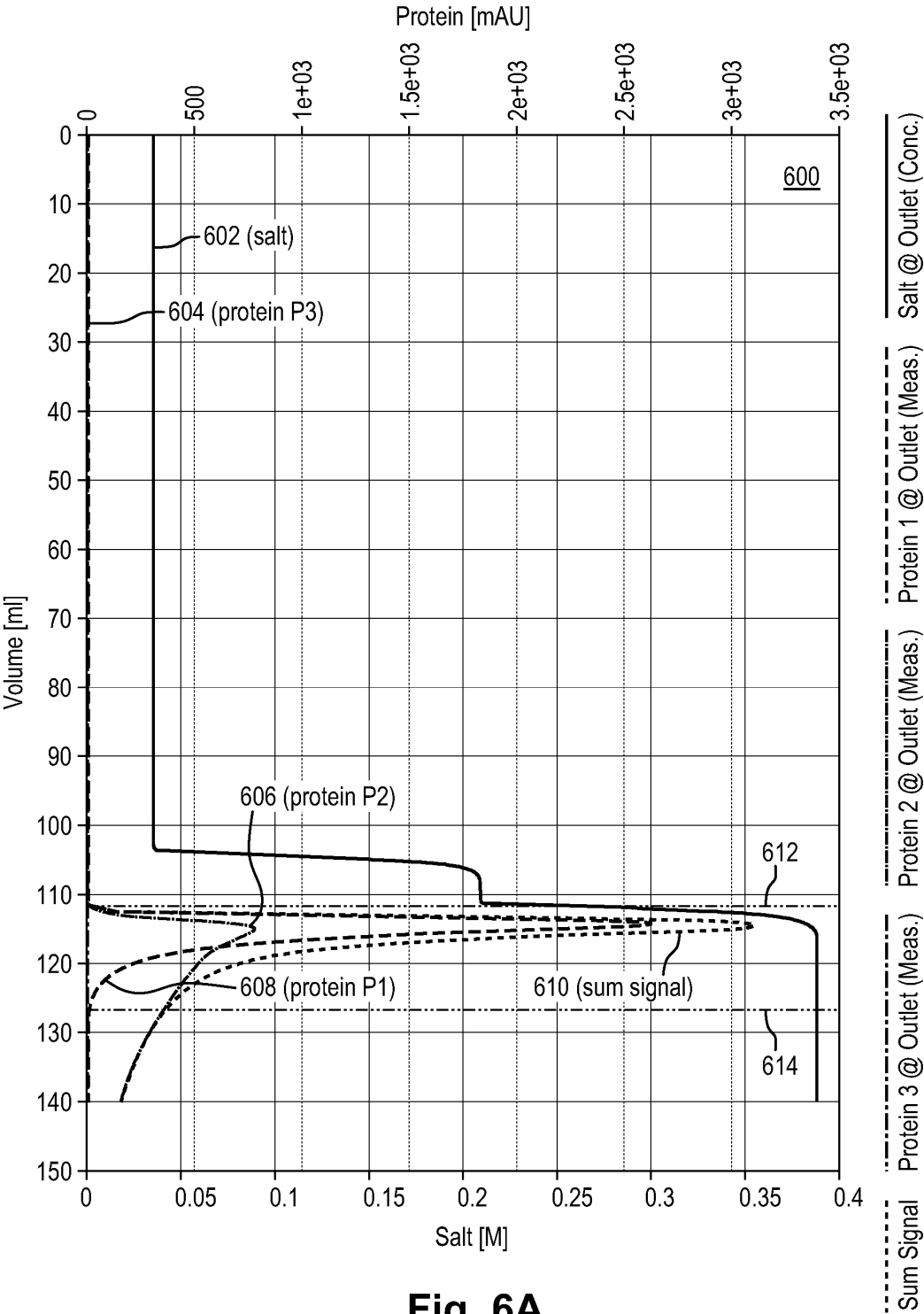
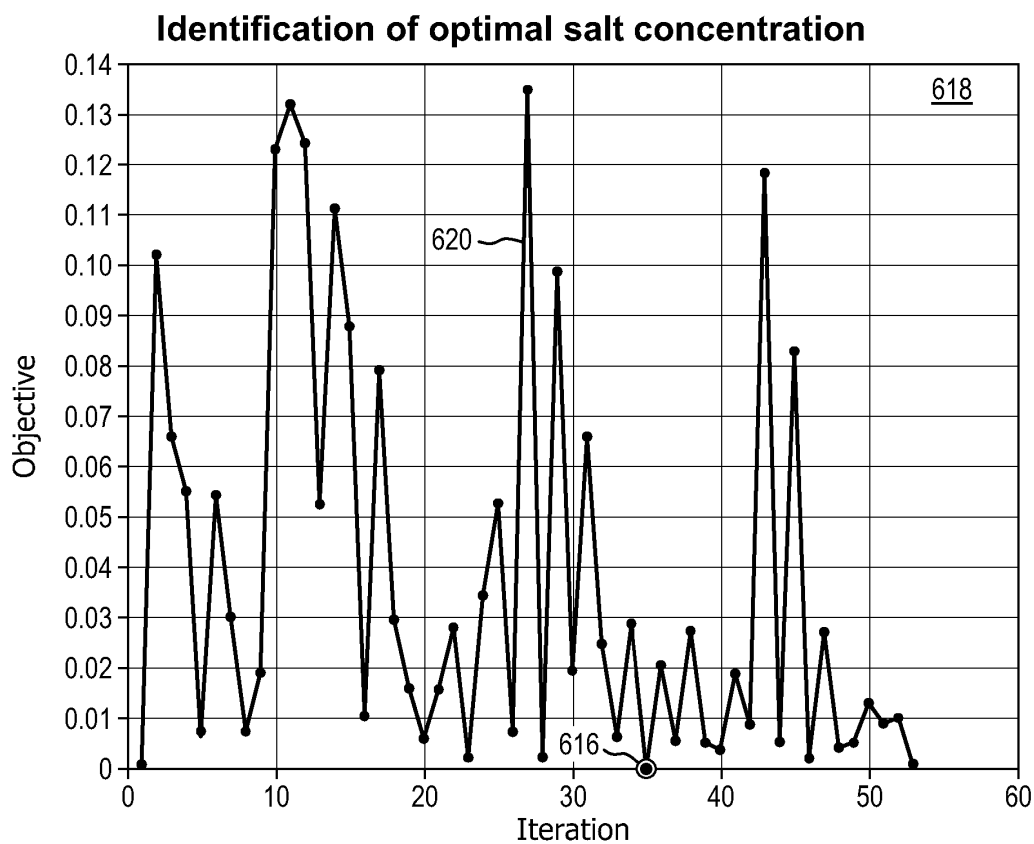


Fig. 6A

10/17

Identification of optimal salt concentration

Results					
617					
Iteration	Salt Step Concentration	Purity Protein 1	Purity Protein 2	Reference Compar.	Objective
35	0.388547	0.55022	0.449492	0.000553859	0.000553859
36	0.392303	0.535252	0.464414	0.0206218	0.0206218
37	0.389568	0.546074	0.453626	0.00534392	0.00534392
38	0.393545	0.53048	0.469168	0.0273574	0.0273574
39	0.389494	0.546373	0.453328	0.00492257	0.00492257
40	0.389293	0.547186	0.452518	0.00377636	0.00377636
41	0.392007	0.536403	0.463267	0.0189974	0.0189974
42	0.39018	0.543616	0.456077	0.00881355	0.00881355
43	0.412882	0.466288	0.533712	0.117882	0.117882

Fig. 6B**Fig. 6C**

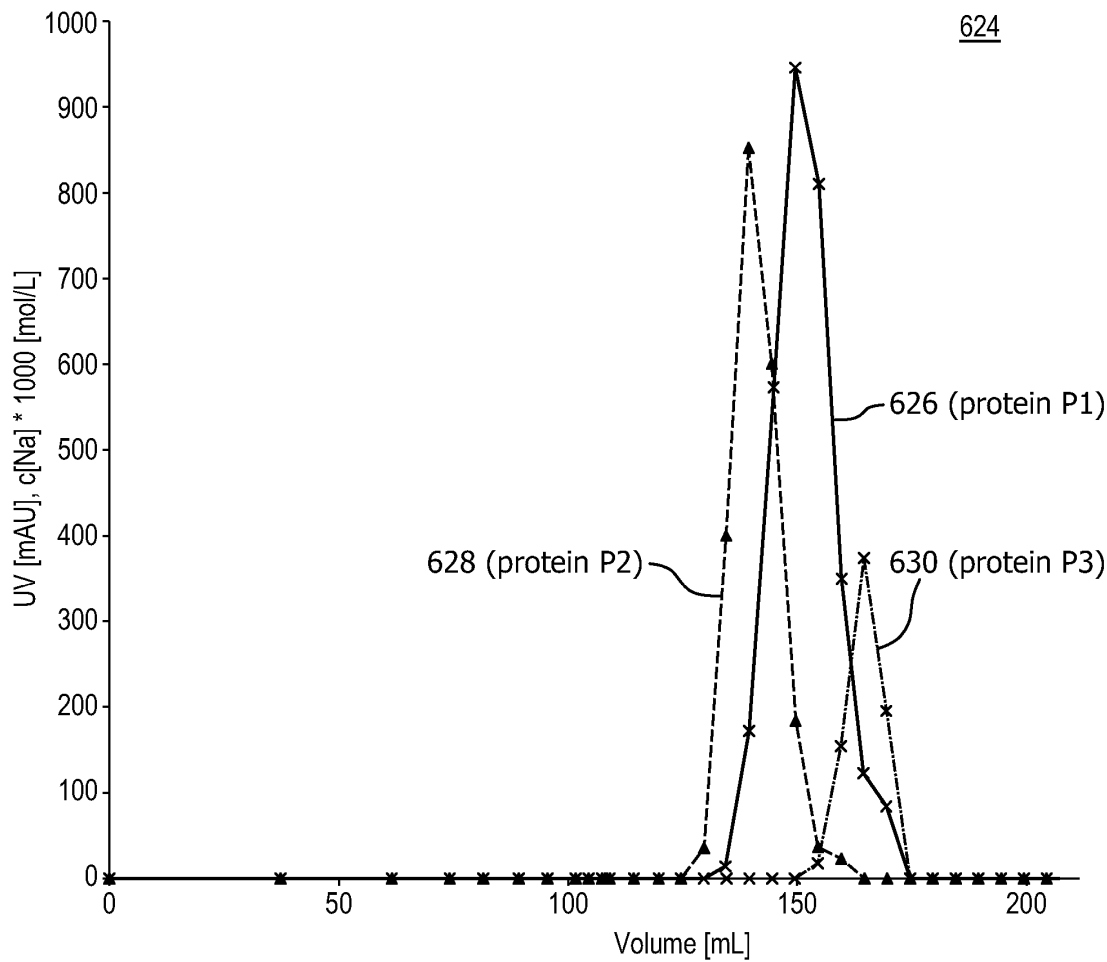
SUBSTITUTE SHEET (RULE 26)

	Elution	Load [g/L]	Column Length [cm]	Column Volume [ml]	Pooling	Pool content 2x gly. [%] Protein P1	Pool content 1x gly. [%] Protein P2
						Experiment	Model
CF200 Cycle 1	40 % B	45	22.5	11.000	0.5 OD→6 CV	56	49
→ fit ionic capacity from 670 to 690 mol/L (+3%) (PorosXS specification: ± 13%)						56	44

622

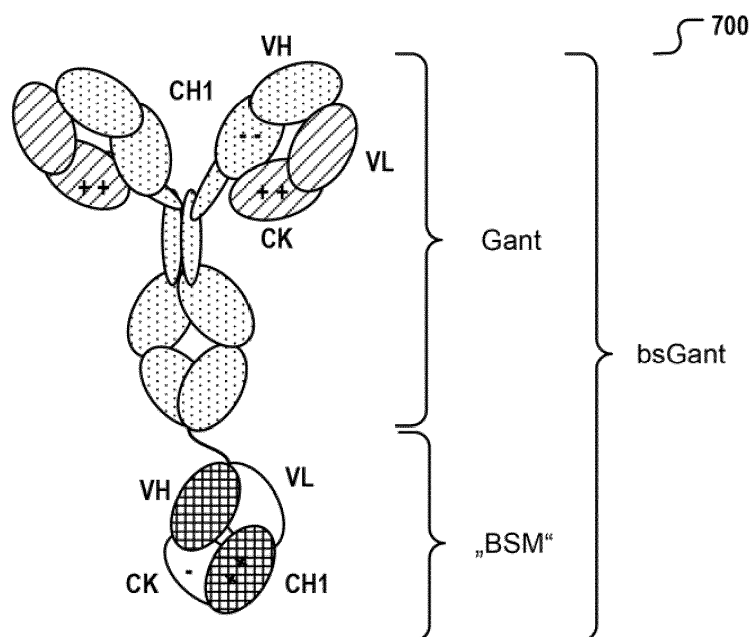
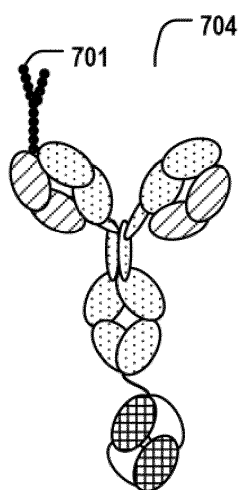
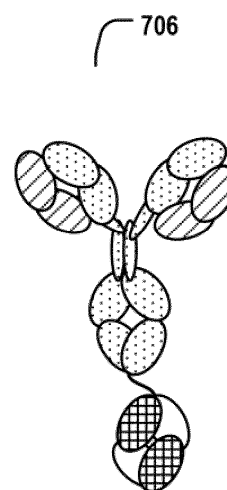
Fig. 6D

Elution Profiles of P1, P2 and P3



Gradient at 40 column volumes, total protein load: 45 g/L, pH 5.5

Fig. 6E

**Fig. 7A****Protein P1****Fig. 7B****Protein P2****Fig. 7C****Protein P3****Fig. 7D**

Model prediction of the pool composition at 40 % B step elution

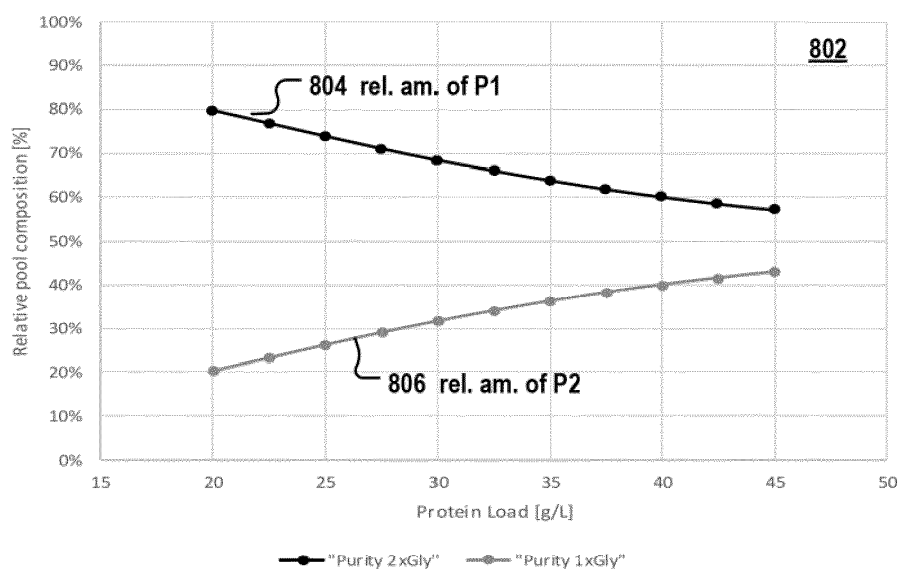


Fig. 8A

Model prediction of the pool composition at different salt step heights at 45 g/L protein load

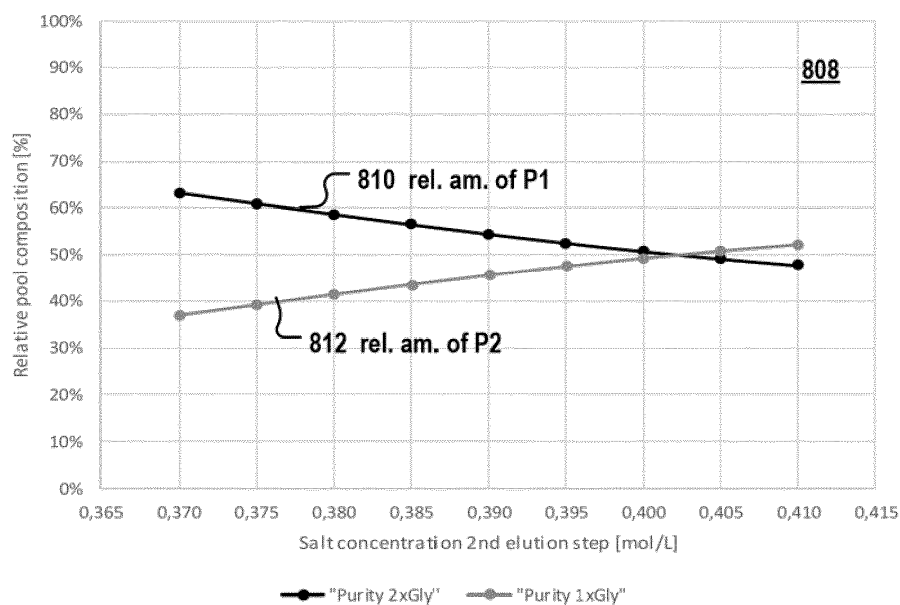


Fig. 8B

Compensating the Impact of the protein amounts loaded by adapting the elution buffer salt concentration

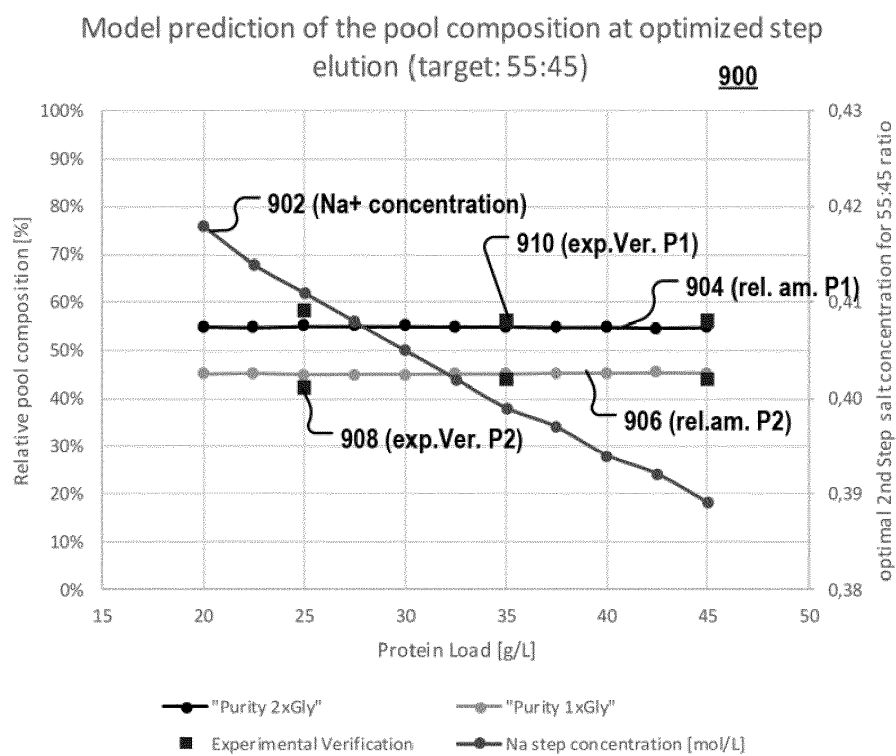


Fig. 9

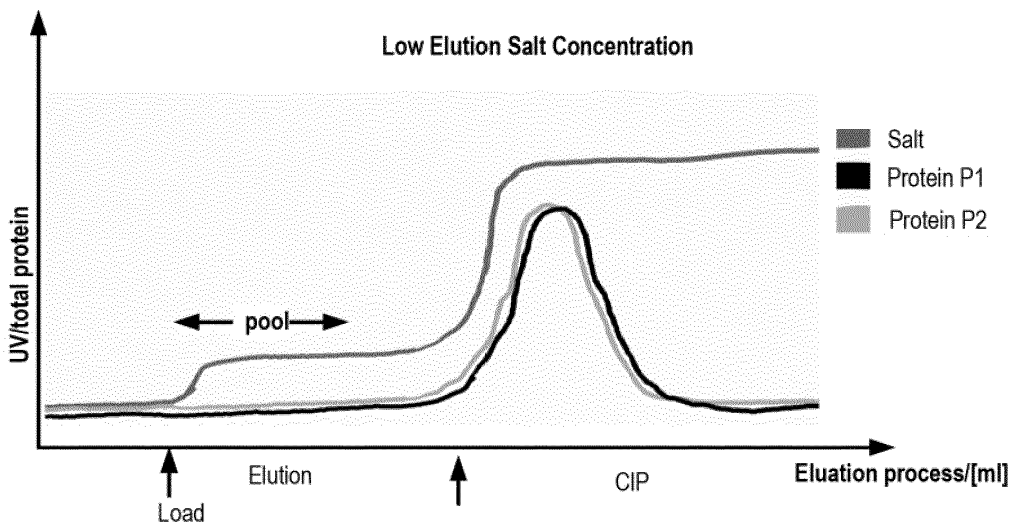


Fig. 10A

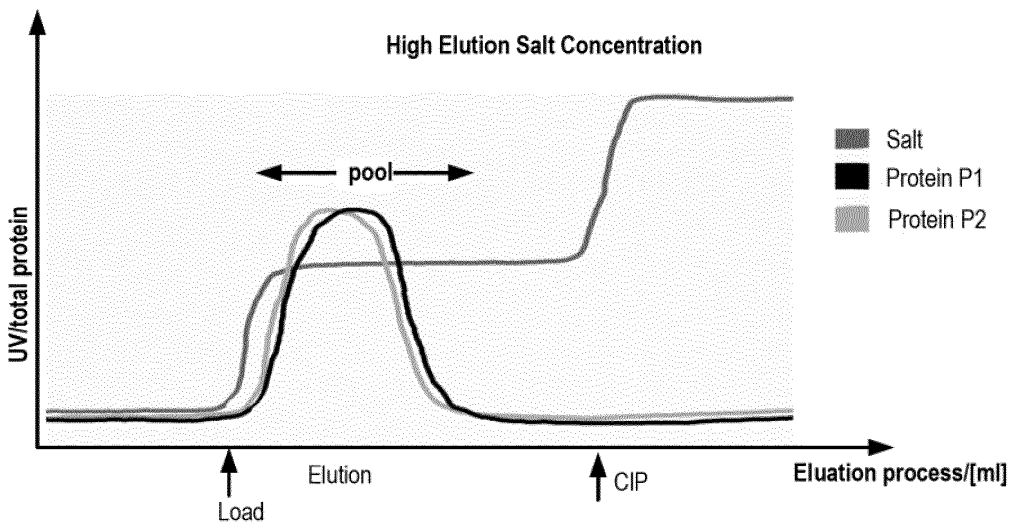
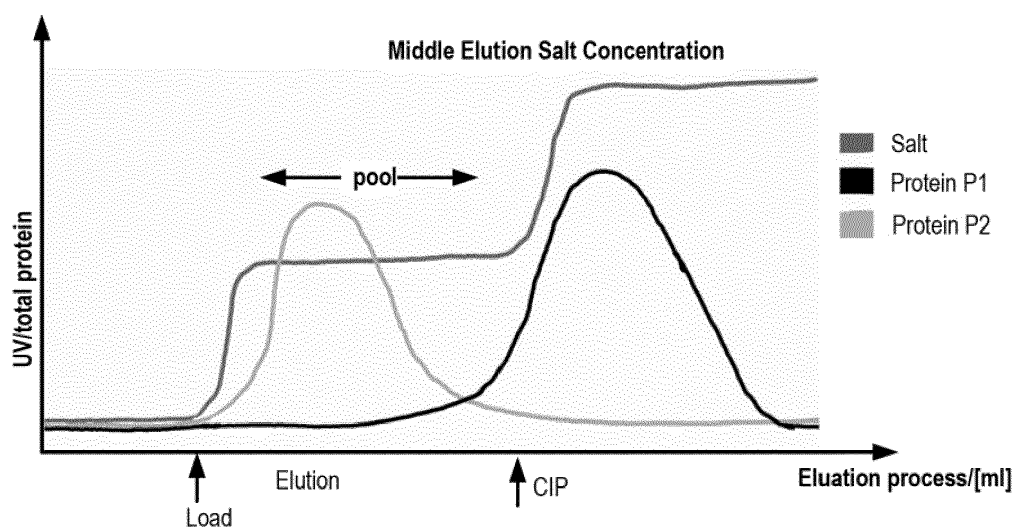


Fig. 10B

**Fig. 10C**