Figure 1

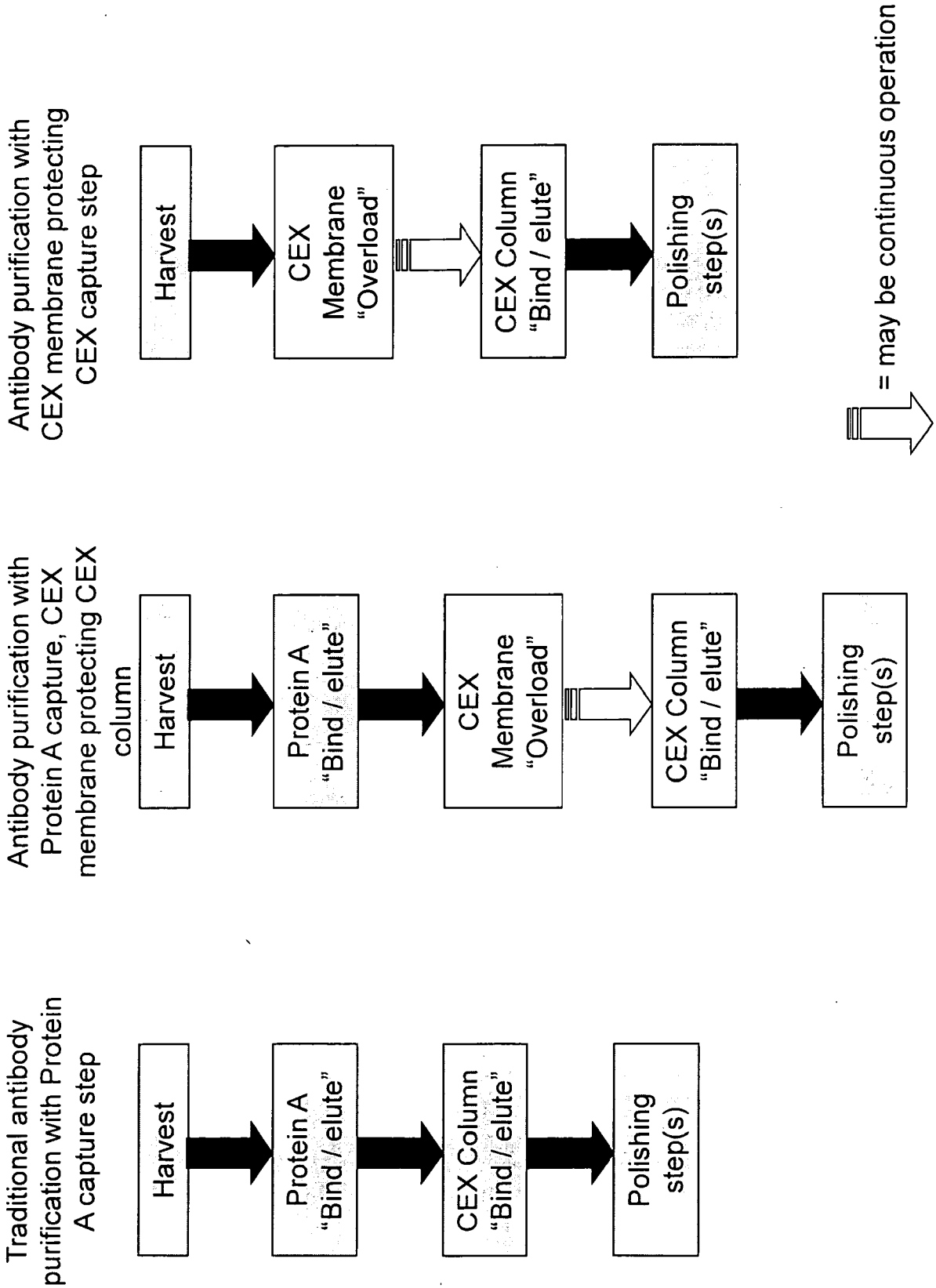
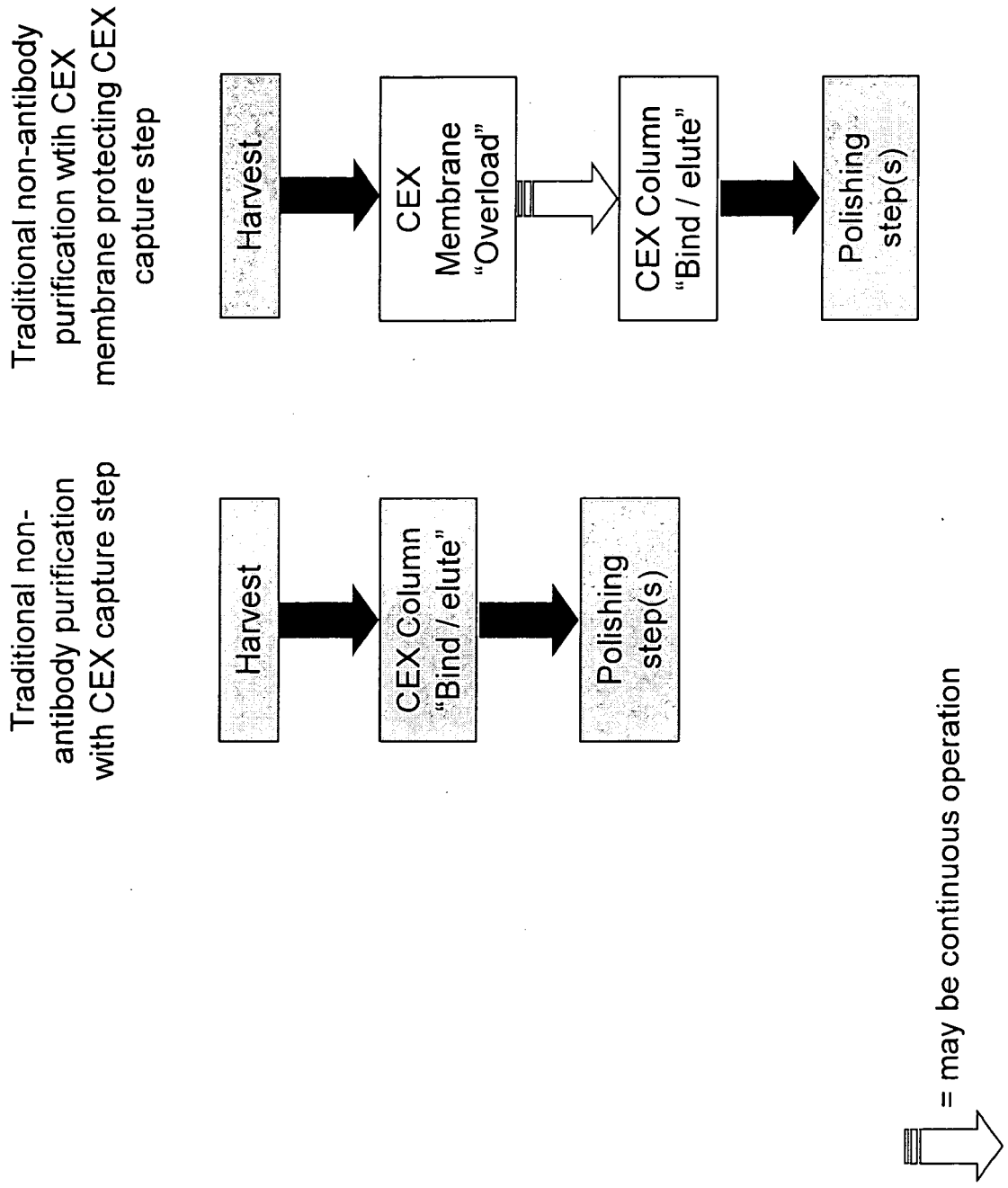

Traditional antibody purification with Protein A capture step
Harvest
Protein A "Bind / elute"
CEX Column "Bind / elute"
Polishing step(s)
Antibody purification with Protein A capture, CEX membrane protecting CEX column
Harvest
Protein A "Bind / elute"
CEX Membrane "Overload"
CEX Column "Bind / elute"
Polishing step(s)
Antibody purification with CEX membrane protecting CEX capture step
Harvest
CEX Membrane "Overload"
CEX Column "Bind / elute"
Polishing step(s)
= may be continuous operation

Figure 2


Traditional non-antibody purification with CEX capture step
Harvest
CEX Column "Bind / elute"
Polishing step(s)
Traditional non-antibody purification wtih CEX membrane protecting CEX capture step
Harvest
CEX Membrane "Overload"
CEX Column "Bind / elute"
Polishing step(s)
= may be continuous operation

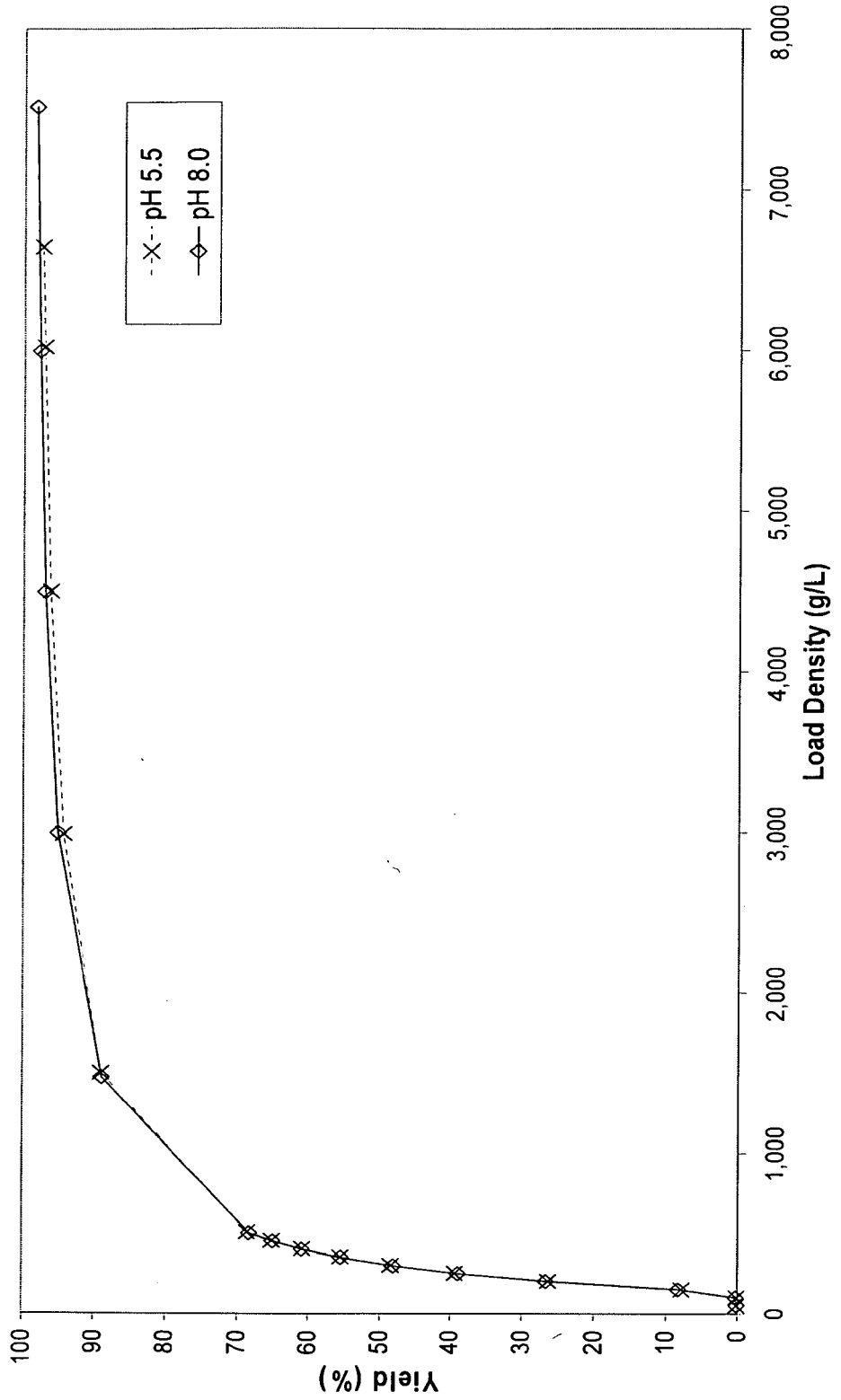

pH 5.5
pH 8.0
Yield (%)
Load Density (g/L)
0
10
20
30
40
50
60
70
80
90
100
0
1,000
2,000
3,000
4,000
5,000
6,000
7,000
8,000

Figure 3

Figure 4

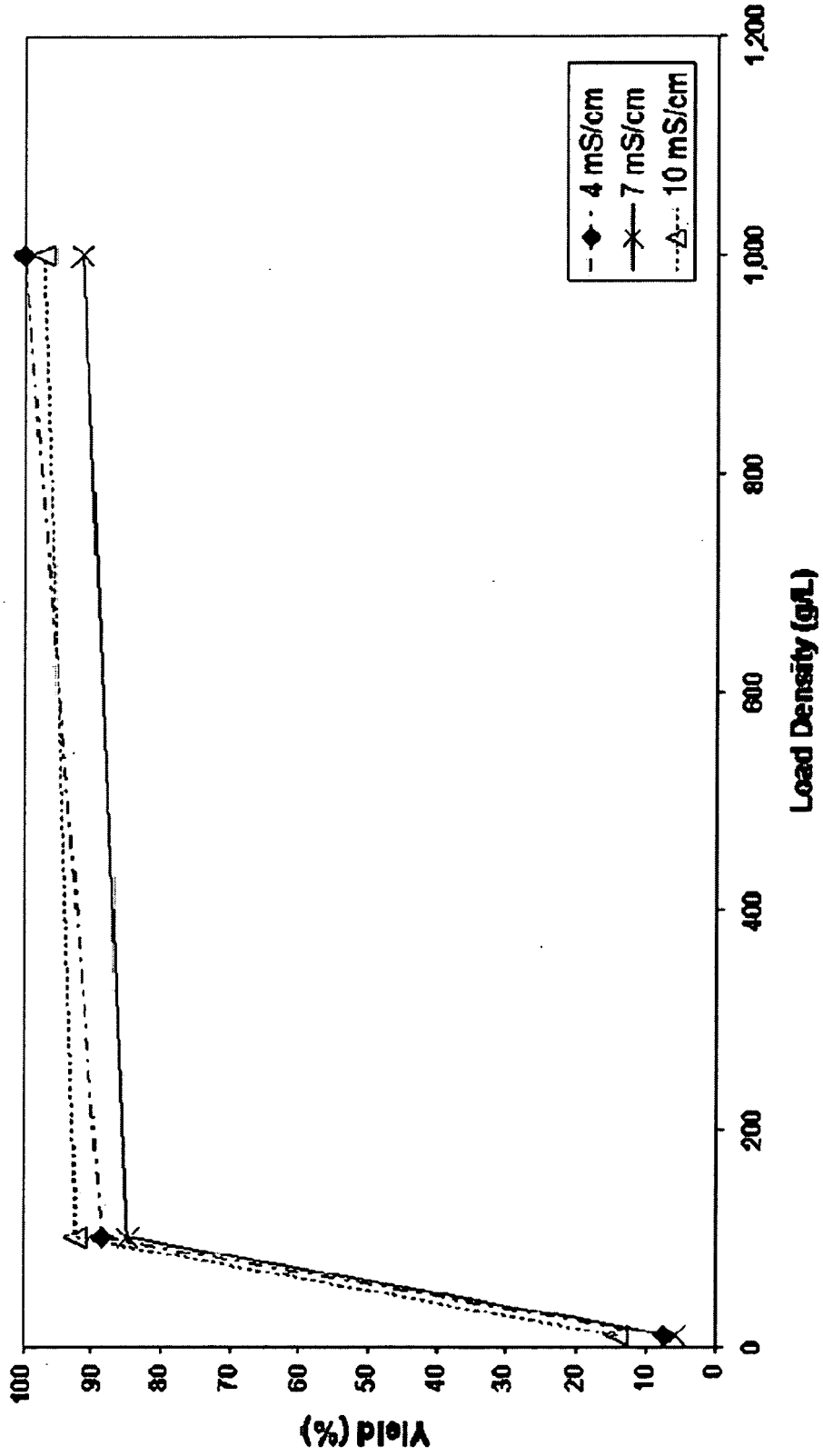

4 mS/cm
7 mS/cm
10 mS/cm
Load Density (g/L)
Yield (%)
0
200
400
600
800
1,000
1,200
100
90
80
70
60
50
40
30
20
10
0

Figure 5

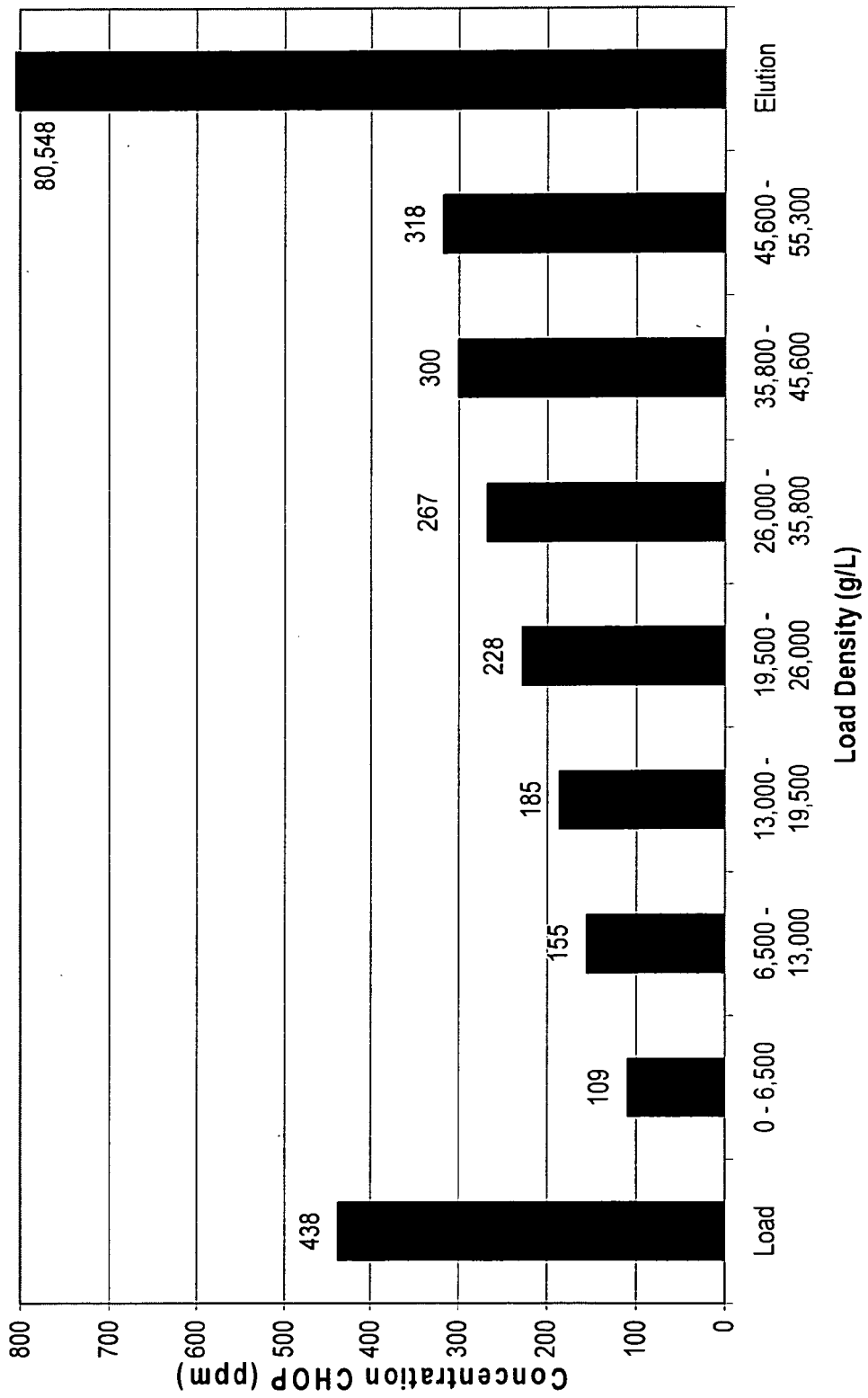
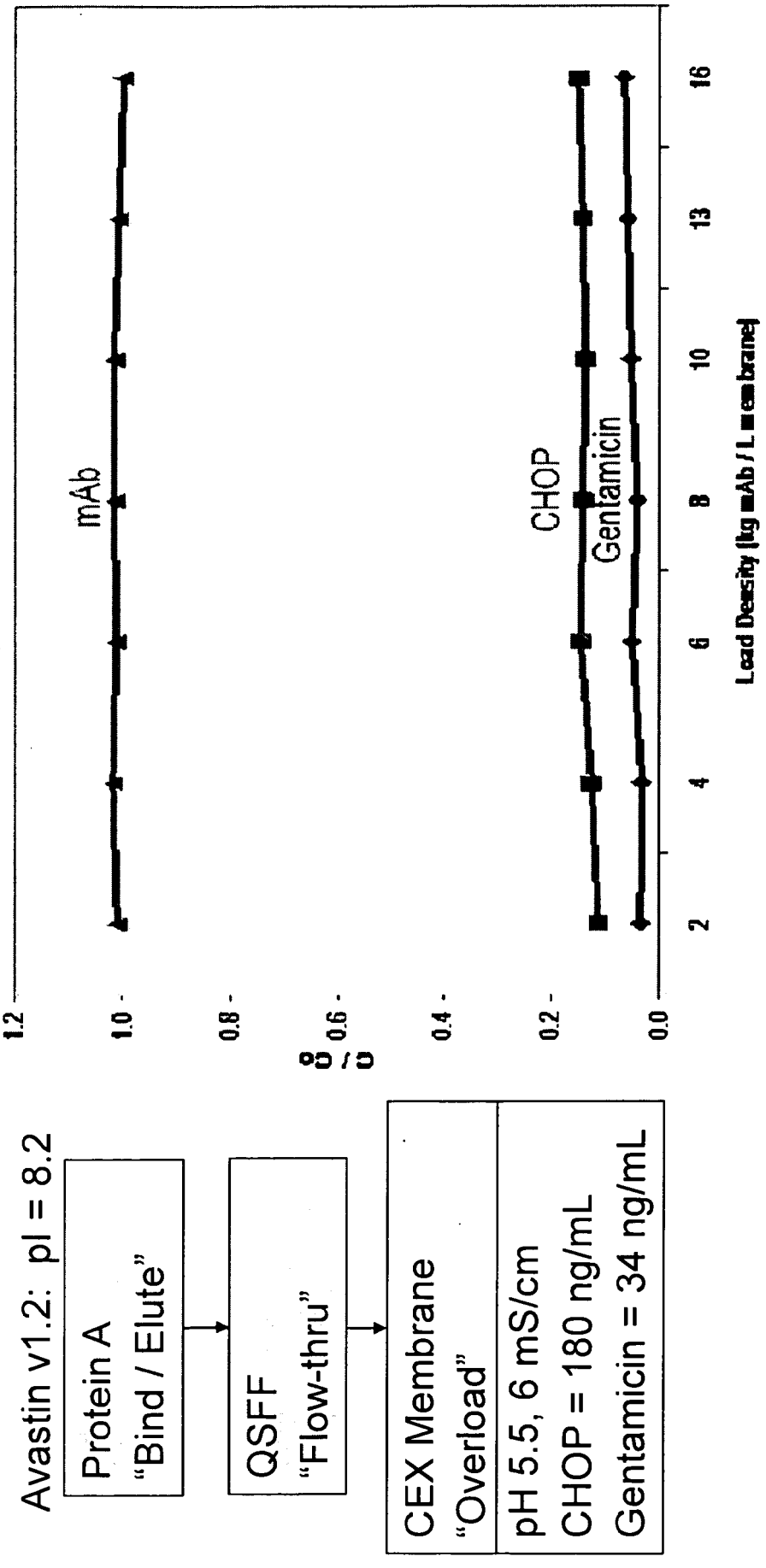

Concentration CHOP (ppm)
800
700
600
500
400
300
200
100
0
Load
0 - 6,500
6,500 - 13,000
13,000 - 19,500
19,500 - 26,000
26,000 - 35,800
35,800 - 45,600
45,600 - 55,300
Elution
Load Density (g/L)
438
109
155
185
228
267
300
318
80,548

Figure 6


Avastin v1.2: pI = 8.2
Protein A
"Bind / Elute"
QSFF
"Flow-thru"
CEX Membrane
"Overload"
pH 5.5, 6 mS/cm
CHOP = 180 ng/mL
Gentamicin = 34 ng/mL
mAb
CHOP
Gentamicin
C / C0
Load Density (kg mAb / L membrane)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2
4
6
8
10
13
16

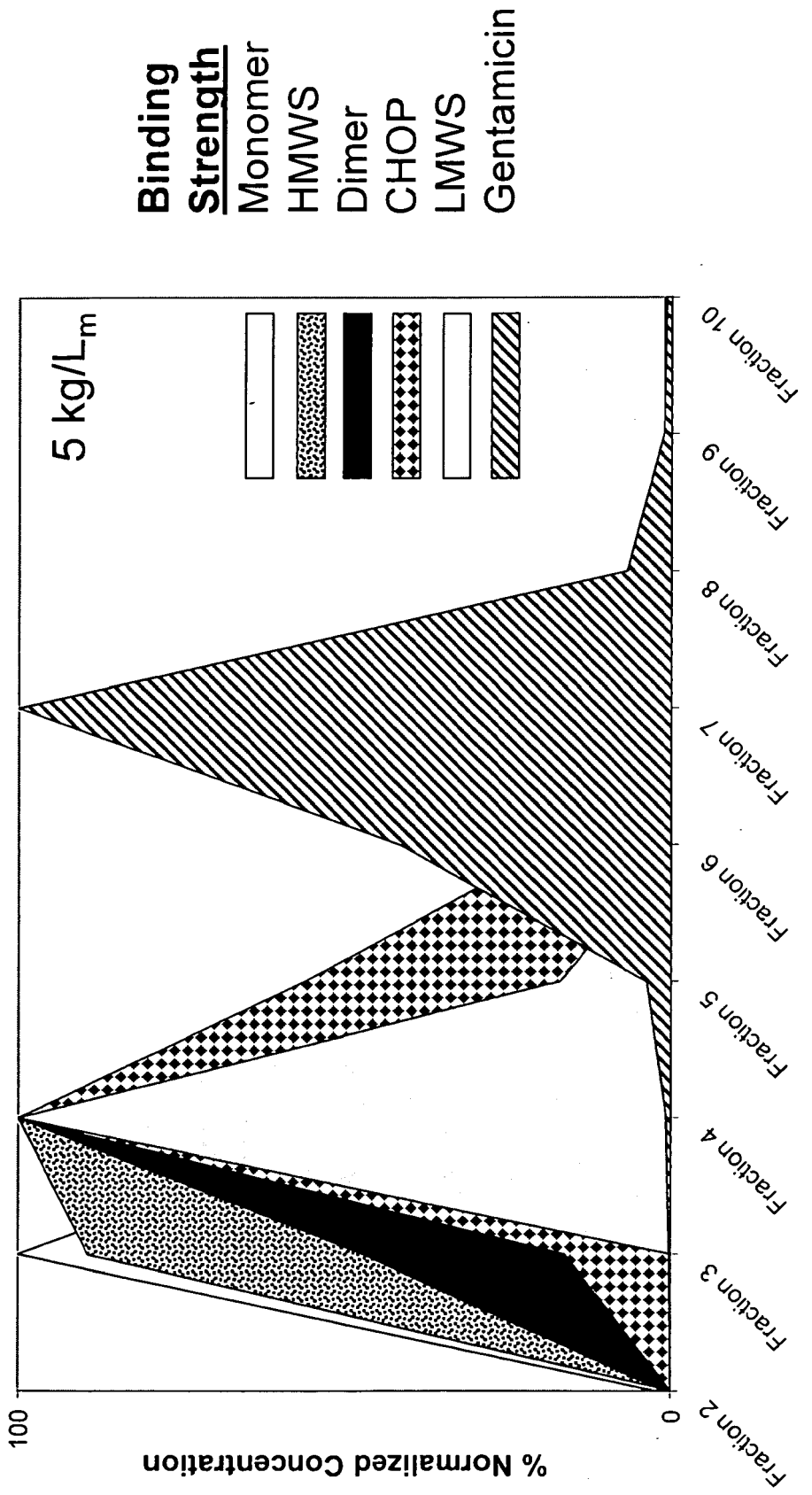

Binding Strength
Monomer
HMWS
Dimer
CHOP
LMWS
Gentamicin
5 kg/L$_m$
% Normalized Concentration
100
0
Fraction 2
Fraction 3
Fraction 4
Fraction 5
Fraction 6
Fraction 7
Fraction 8
Fraction 9
Fraction 10

Figure 7

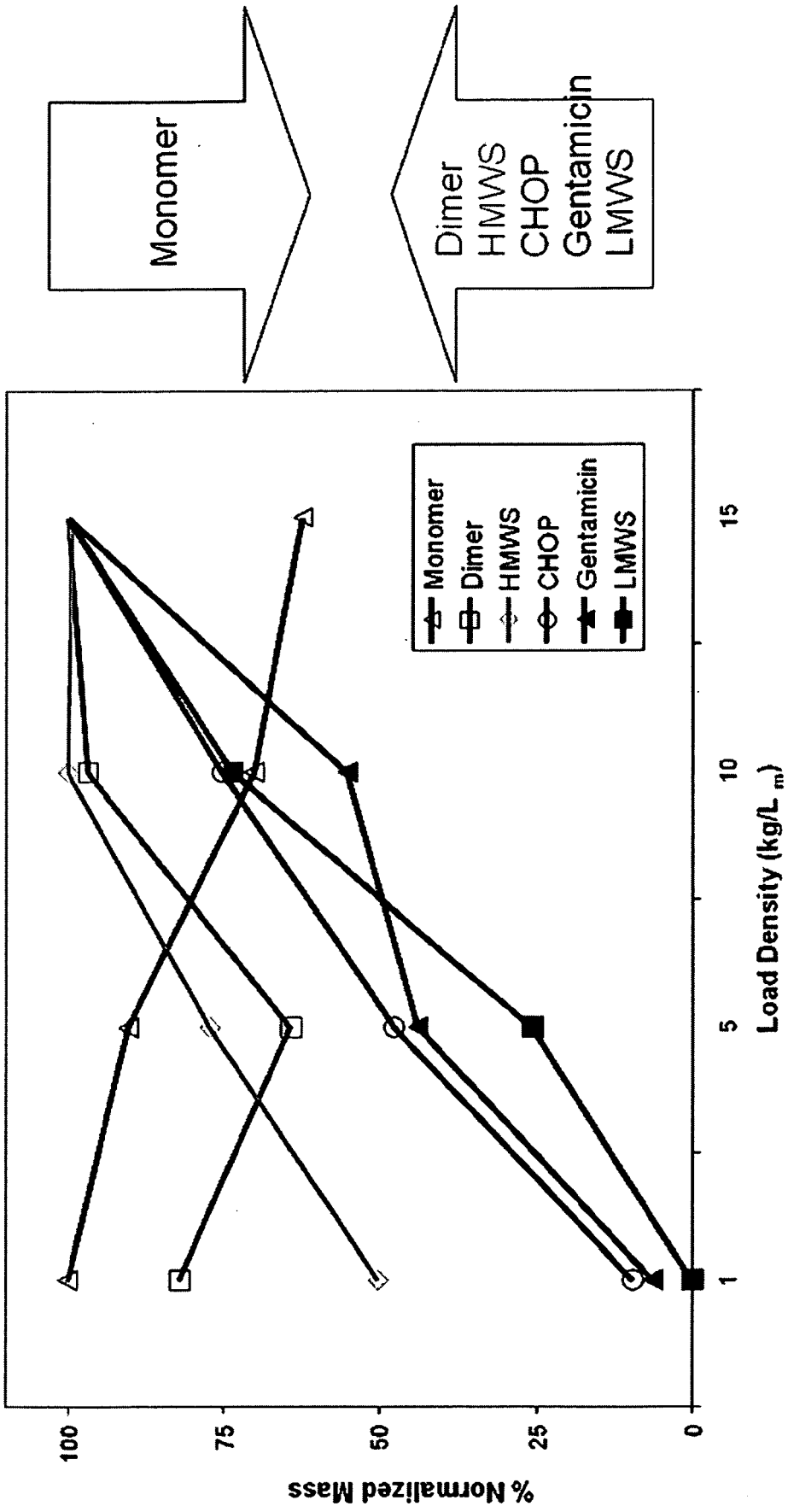

Monomer
Dimer
HMWS
CHOP
Gentamicin
LMWS
Monomer
Dimer
HMWS
CHOP
Gentamicin
LMWS
% Normalized Mass
0
25
50
75
100
1
5
10
15
Load Density (kg/L m)

Figure 8

Figure 9

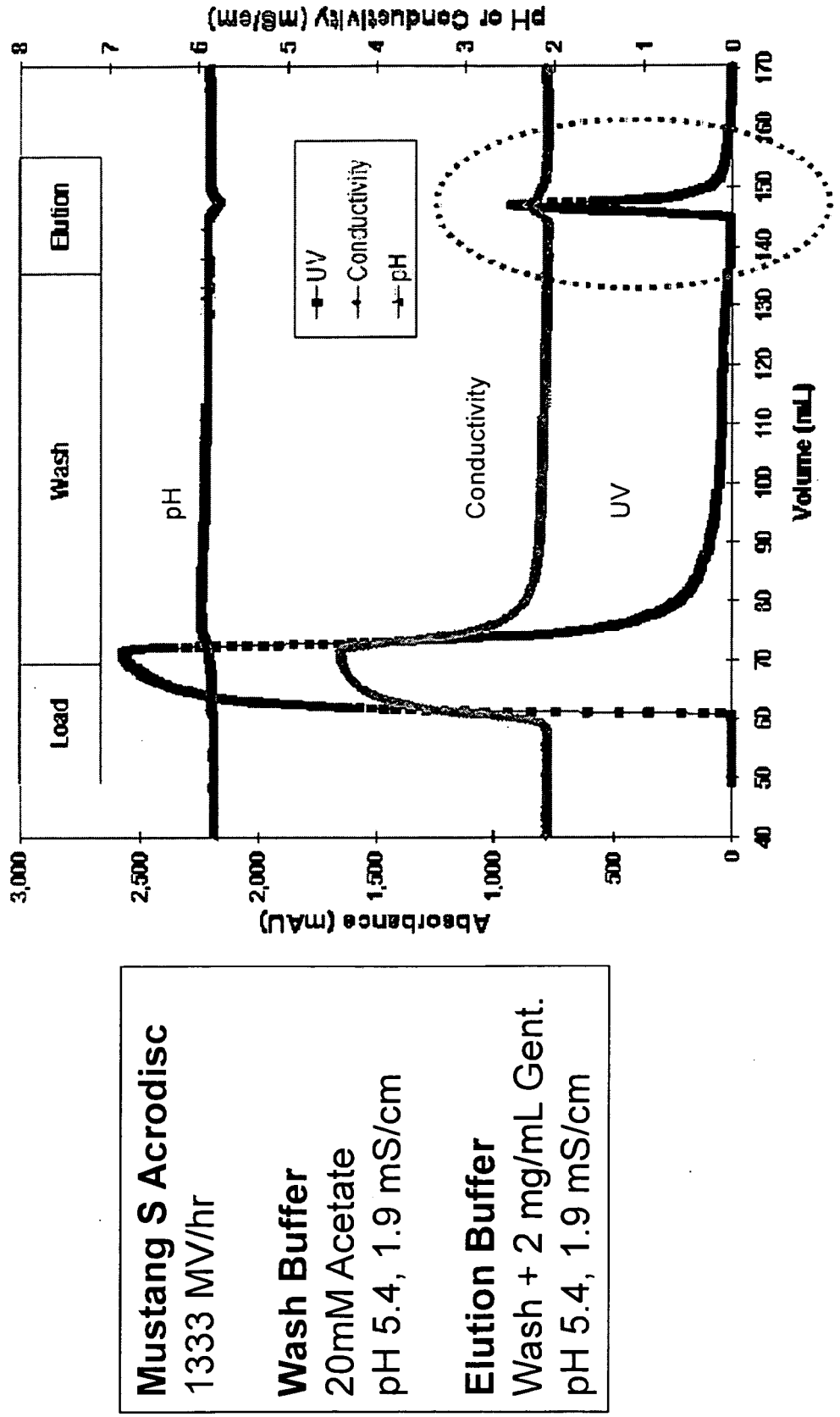

Load
Wash
Elution
pH
Conductivity
UV
UV
Conductivity
pH
Absorbance (mAU)
pH or Conductivity (mS/cm)
Volume (mL)
Mustang S Acrodisc
1333 MV/hr
Wash Buffer
20mM Acetate
pH 5.4, 1.9 mS/cm
Elution Buffer
Wash + 2 mg/mL Gent.
pH 5.4, 1.9 mS/cm

Figure 10

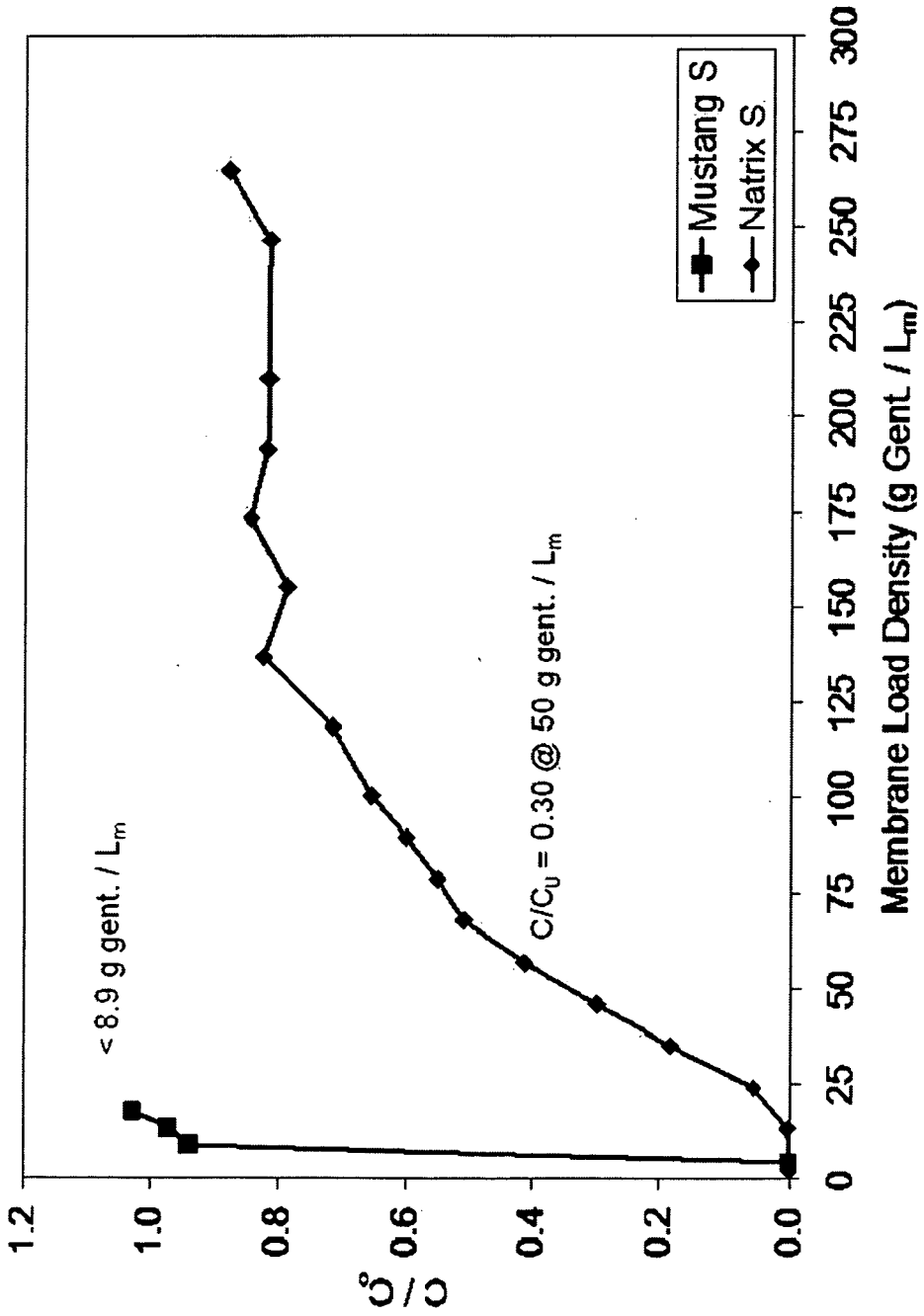

Mustang S
Natrix S
< 8.9 g gent. / L_m
C/C_U = 0.30 @ 50 g gent. / L_m
C / C°
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
25
50
75
100
125
150
175
200
225
250
275
300
Membrane Load Density (g Gent / L_m)

Figure 11

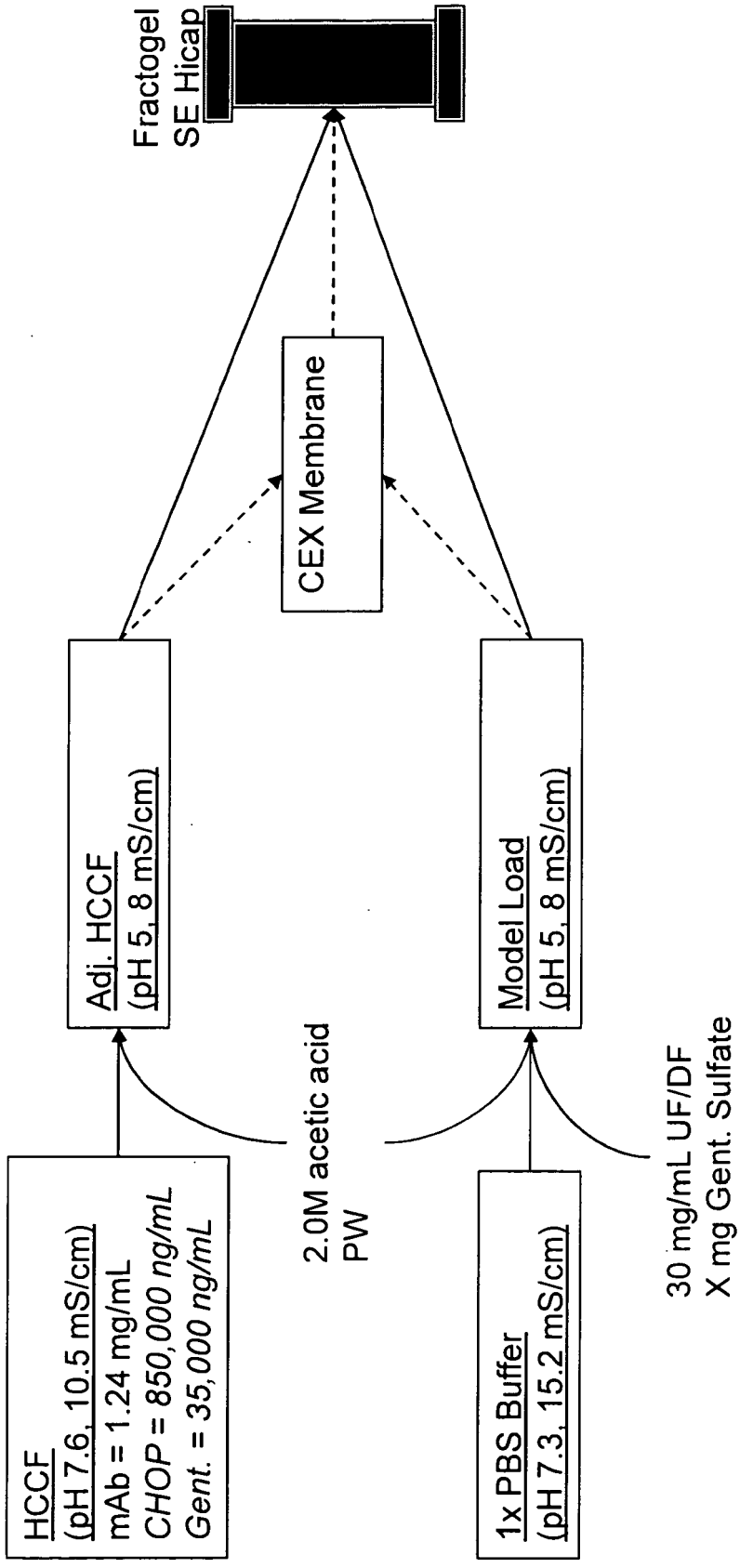

Fractogel SE Hicap
CEX Membrane
Adj. HCCF (pH 5, 8 mS/cm)
Model Load (pH 5, 8 mS/cm)
HCCF (pH 7.6, 10.5 mS/cm) mAb = 1.24 mg/mL CHOP = 850,000 ng/mL Gent. = 35,000 ng/mL
2.0M acetic acid PW
1x PBS Buffer (pH 7.3, 15.2 mS/cm)
30 mg/mL UF/DF X mg Gent. Sulfate

Figure 12

	Load	mAb (mg/mL)	Gentamicin (ng/mL)	CHOP (ng/mL)	DBC (g/L)	Exp. Order
■	Adj. HCCF 1	0.89	24,100	408,000	68	4th
◆	Adj. HCCF 2	0.87	30,000		71	1st
●	Pure mAb	1.62	30,500	0	88	3rd
▲	Pure mAb	1.64	24,100		89	5th
X	Pure mAb	1.64	0		108	2nd

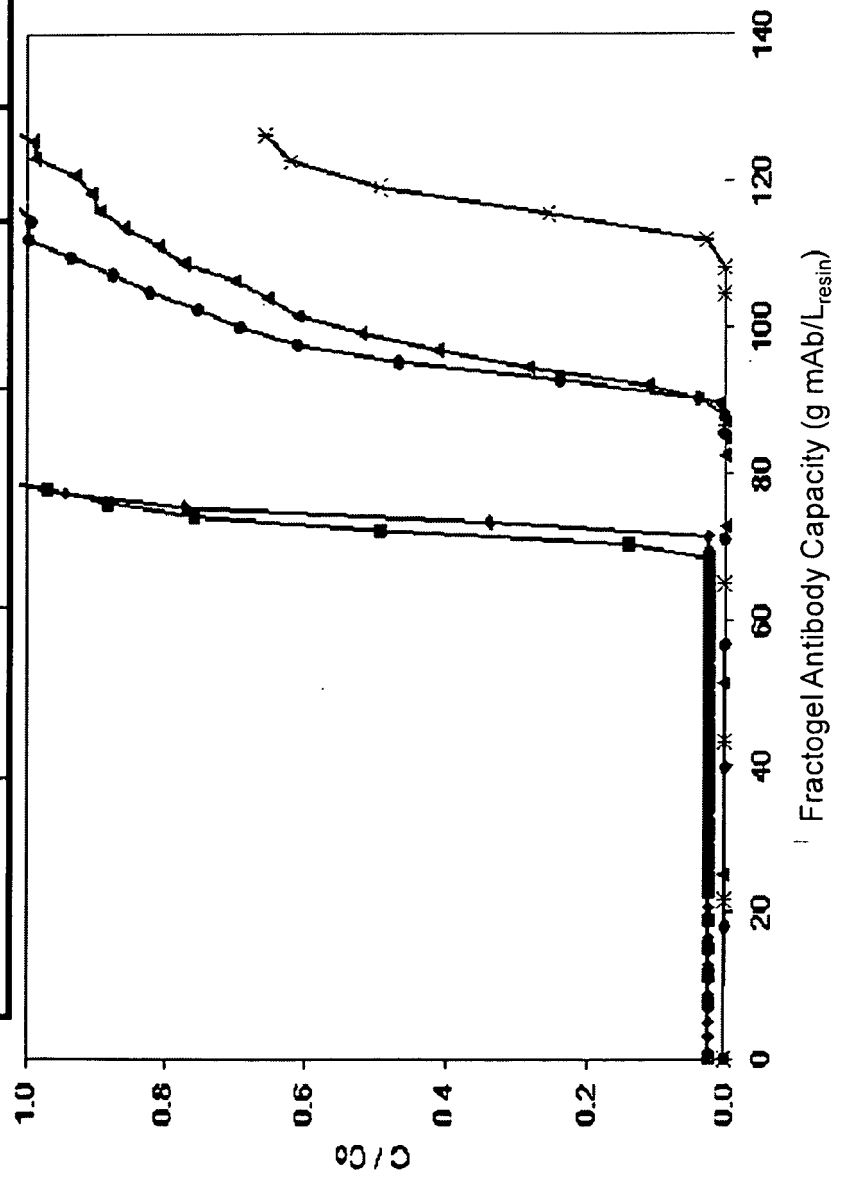
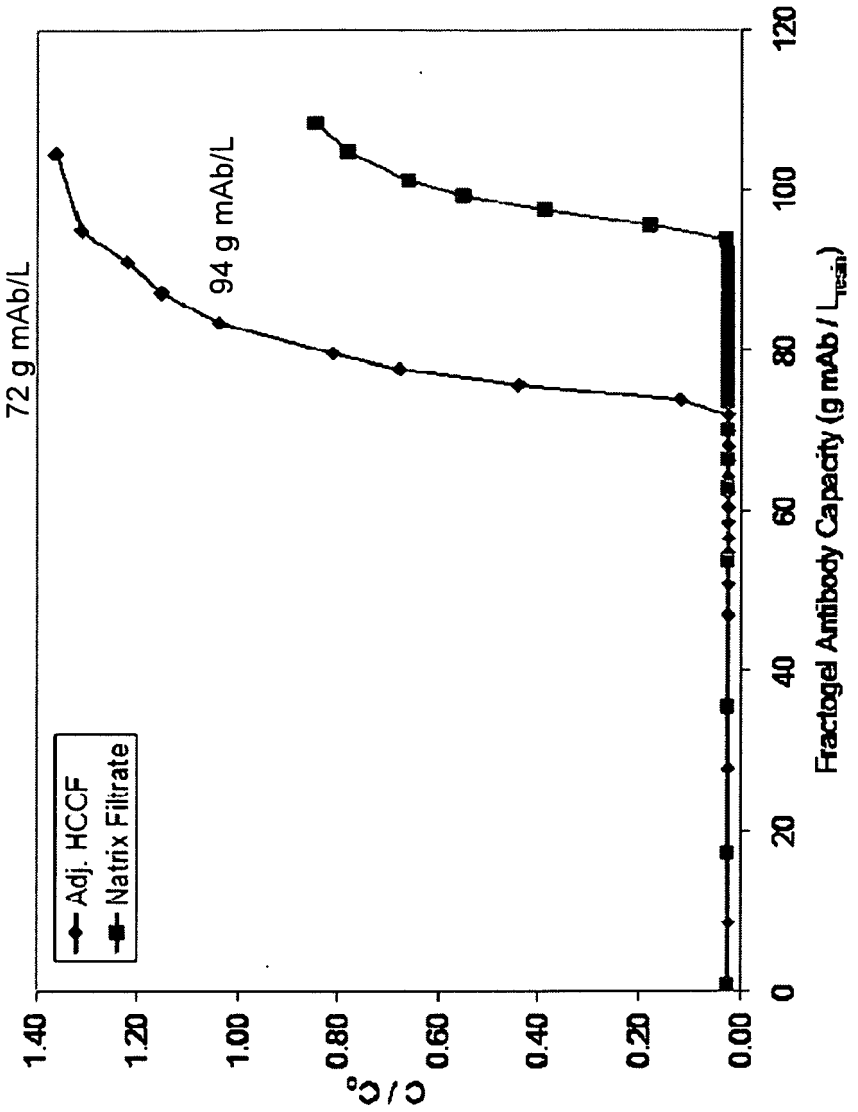
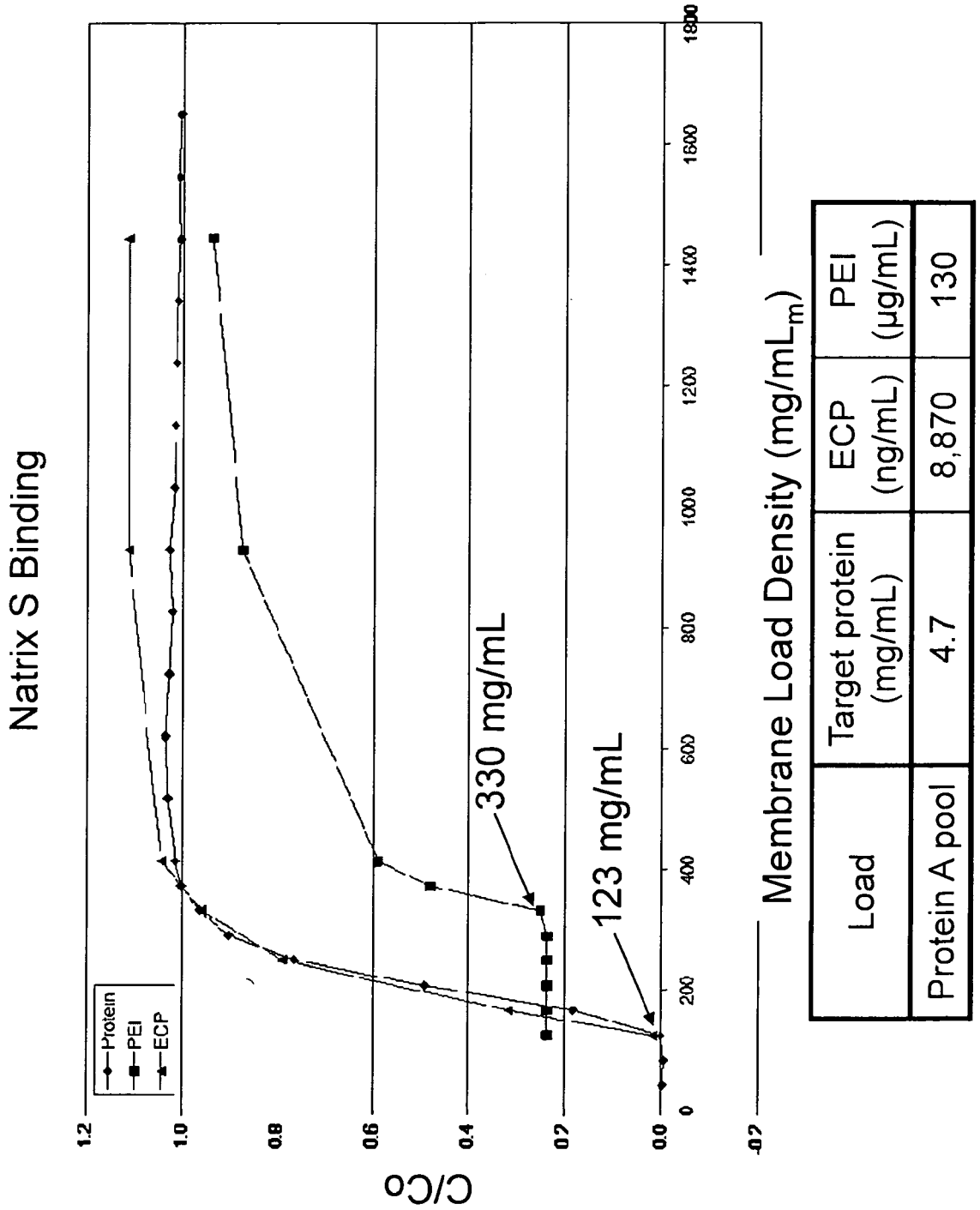


Figure 13



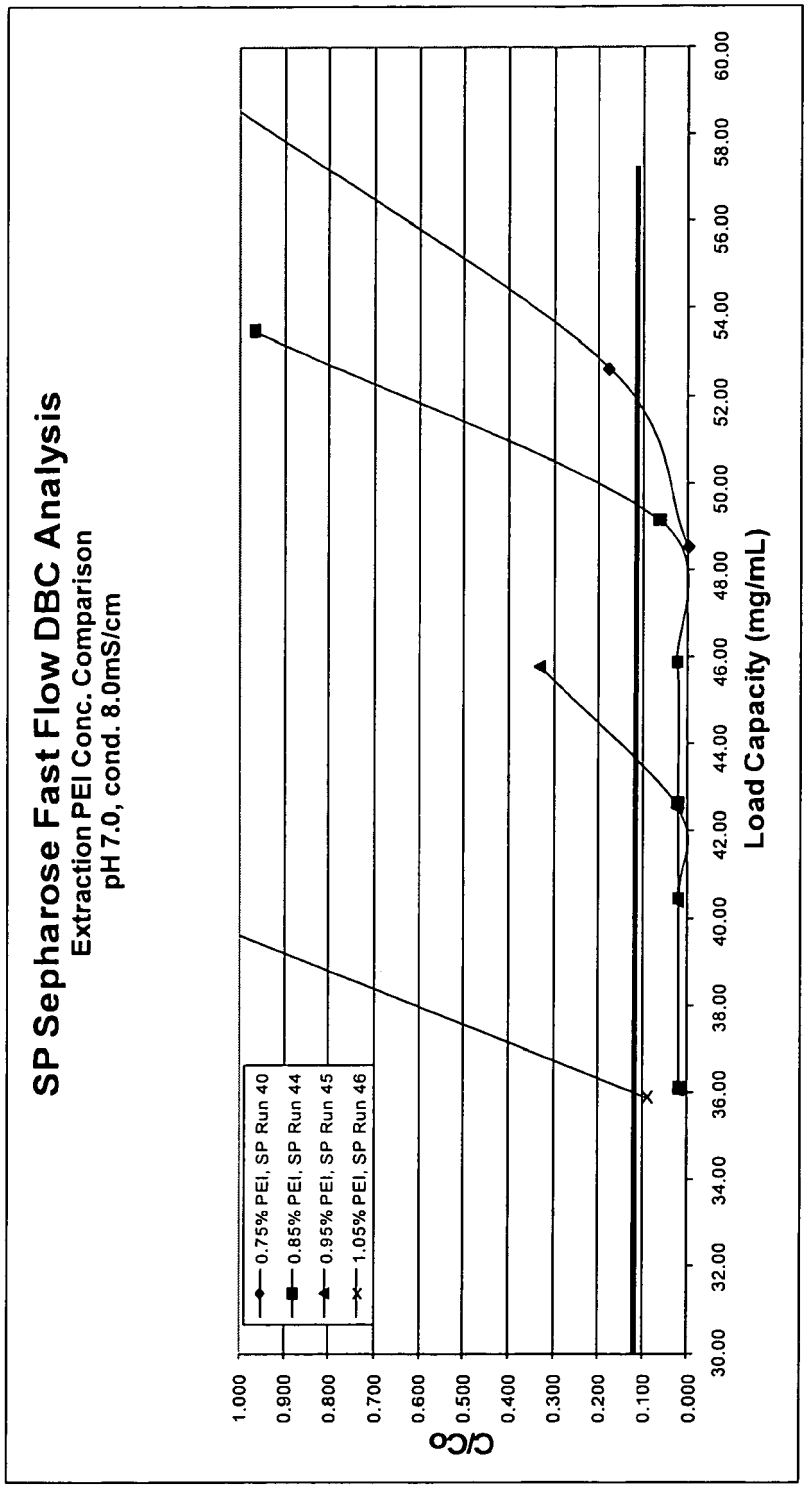
	Adj. HCCF	Natrix flow-through
mAb (mg/mL)	0.88	0.83
CHOP (ng/mL)	408,000	328,000
Gent. (ng/mL)	24,100	870

Figure 14



Load	Target protein (mg/mL)	ECP (ng/mL)	PEI (µg/mL)
Protein A pool	4.7	8,870	130

Figure 15



Run	40	44	45	46
Pre-Harvest PEI added (%)	0.75	0.85%	0.95%	1.05%
Dynamic Binding Capacity (g/L) at 10% break-through	51	49	43	36

Figure 16

Run	Pre-Harvest Condition	Load Protein (mg/mL)	SP Sepharose Fast Flow			
			Step Yield (%)	Pool ECP (ng/mg)	Pool Aggregate (%)	Pool Dimer (%)
	Effect of PEI concentration					
29	0.60% PEI	6.9	88	155	2.5	3.7
	0.70% PEI	6.6	96	145	7.5	3.8
	0.80% PEI	7.3	93	247	5.4	3.4
	0.90% PEI	7.5	91	274	3.5	3.3
	1.00% PEI	7.3	81	621	6.8	5.0
	1.10% PEI	7.4	70	904	17.3	5.9