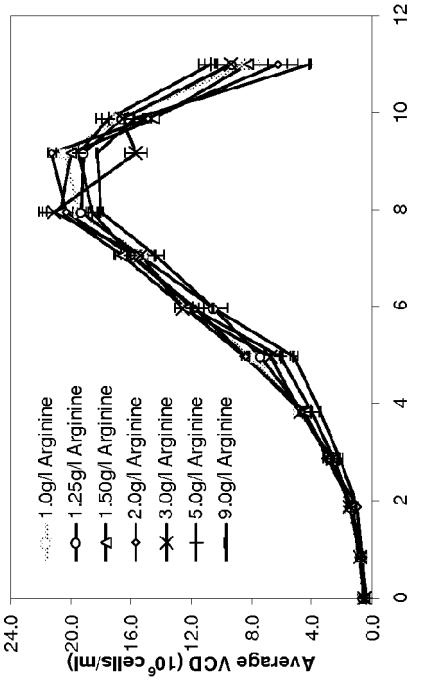


Figure 1) Effect of total arginine concentration in adalimumab producing cell line 2, media 1 on viable cell density (n=2)

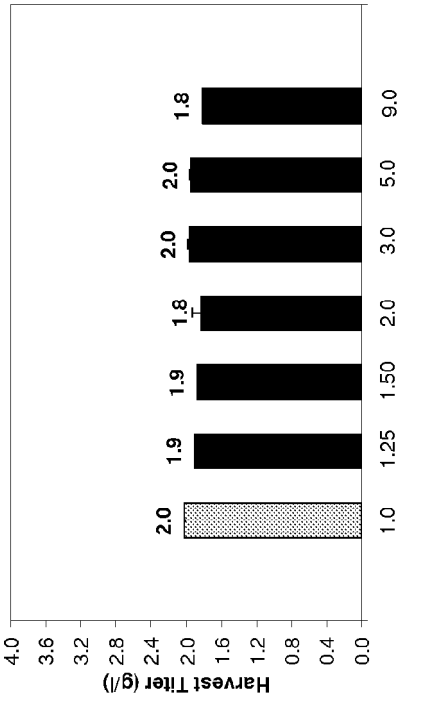


Figure 3) Effect of total arginine concentration in adalimumab producing cell line 2, media 1 on harvest titer (n=2)

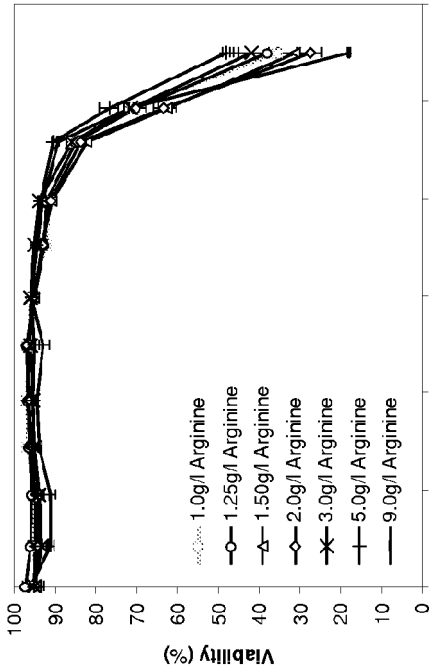


Figure 2) Effect of total arginine concentration in adalimumab producing cell line 2, media 1 on viability (n=2)

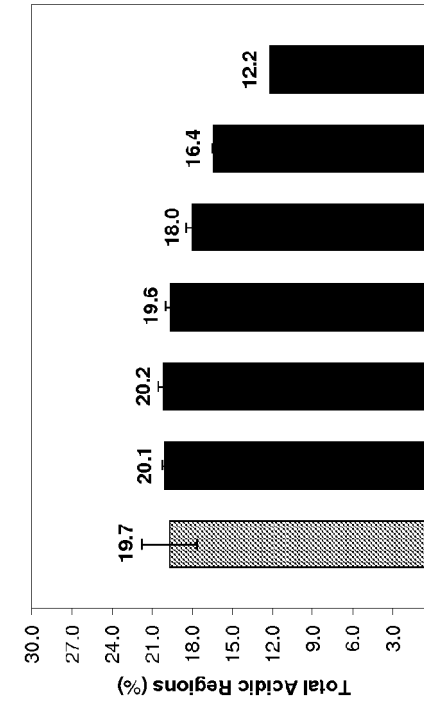


Figure 4) Effect of total arginine concentration in adalimumab producing cell line 2, media 1 on day 10 WCX 10 profile total acidic regions (n=2)

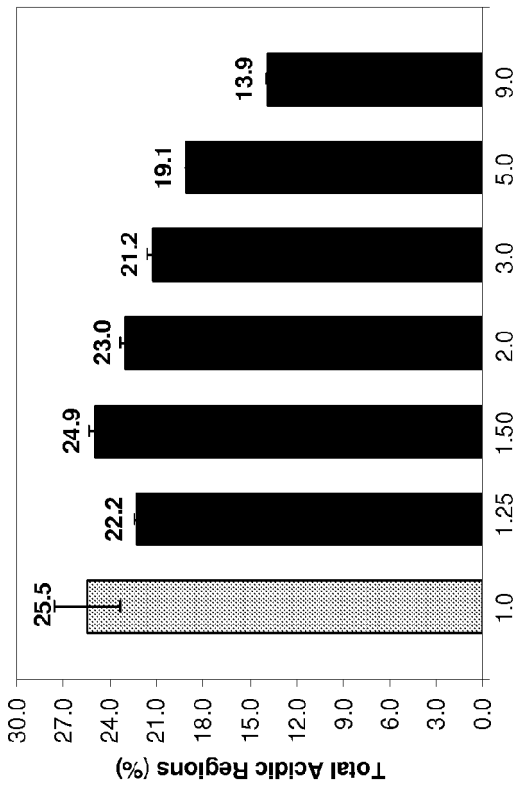


Figure 5) Effect of total arginine concentration in adalimumab producing cell line 2, media 1 on day 12 WCX 10 profile total acidic regions (n=2)

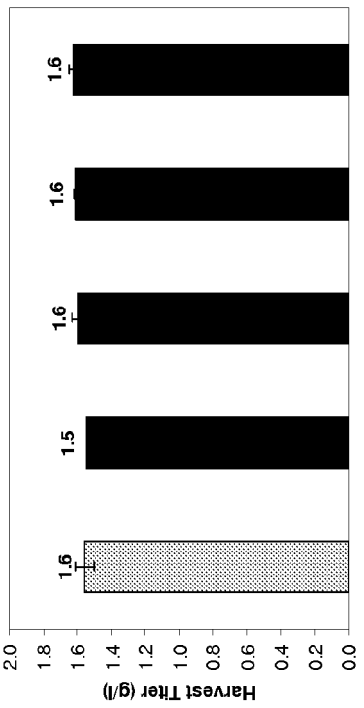


Figure 8) Effect of total arginine concentration in adalimumab producing cell line 3, media 1 on harvest titer (n=2)

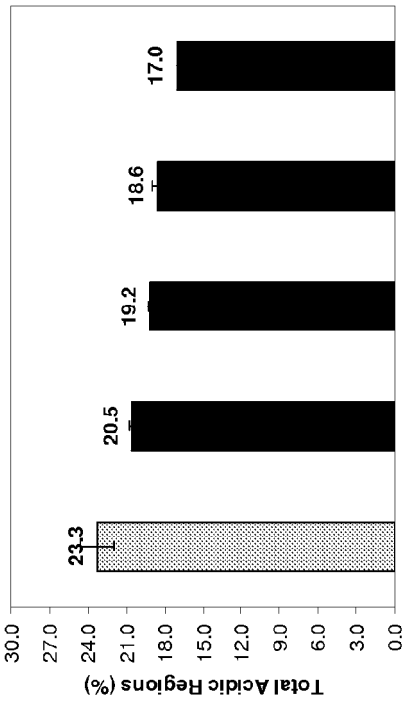


Figure 9) Effect of total arginine concentration in adalimumab producing cell line 3, media 1 on WCX 10 profile total acidic regions (n=2)

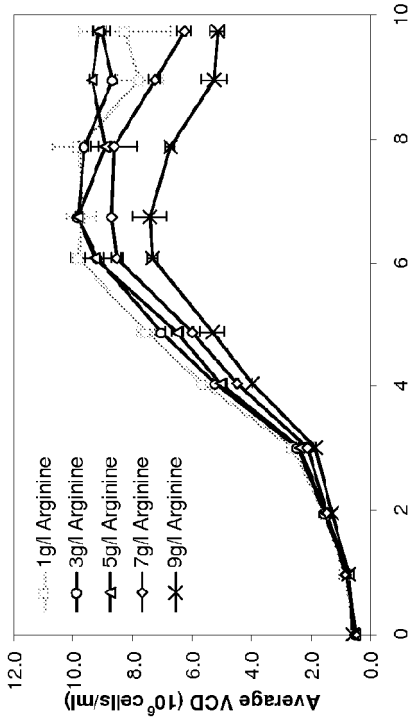


Figure 6) Effect of total arginine concentration in adalimumab producing cell line 3, media 1 on viable cell density (n=2)

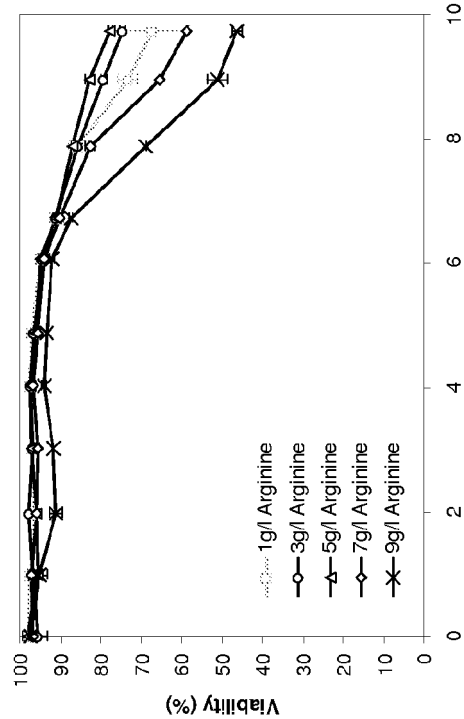


Figure 7) Effect of total arginine concentration in adalimumab producing cell line 3, media 1 on viability (n=2)

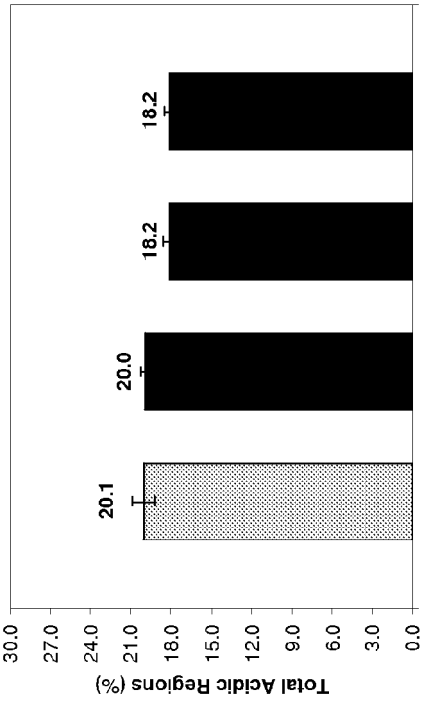


Figure 11) Effect of arginine addition to adalimumab producing cell line 1, media 2 on day 11 on WCX-10 profile total acidic regions (n=2)

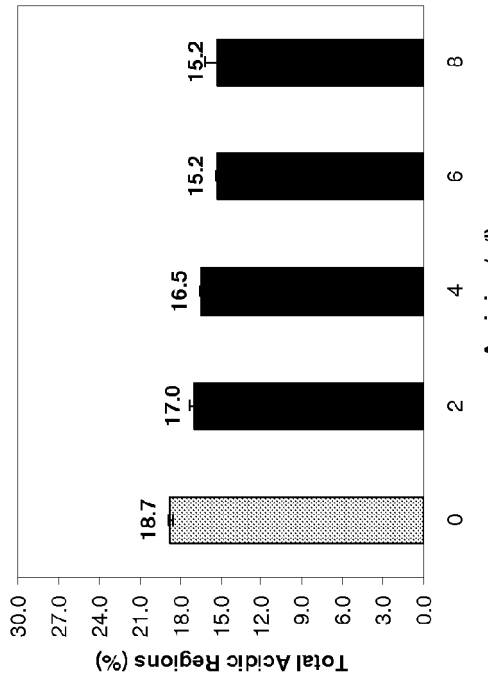


Figure 12) Effect of arginine addition to adalimumab producing cell line 2, media 3 on WCX-10 profile total acidic regions (n=2)

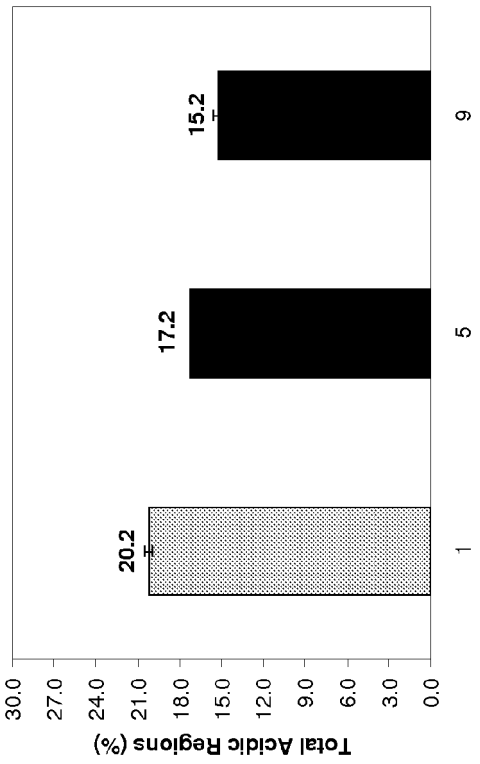


Figure 10) Effect of total arginine concentration in adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions (n=2)

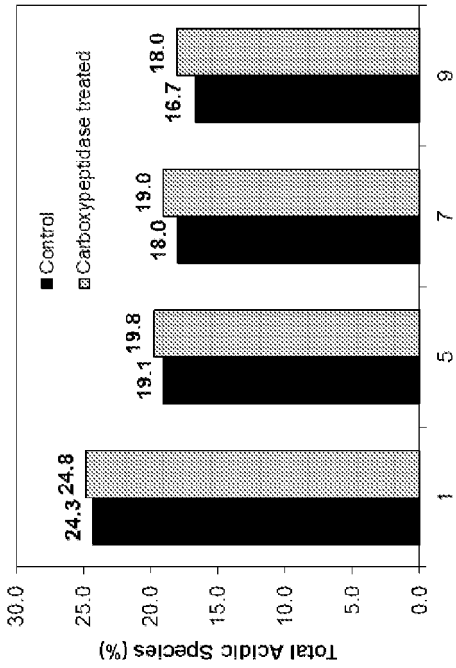


Figure 15) Effect of carboxypeptidase digestion of product from adalimumab producing cell line 3, media 1 experiment on WCX-10 profile total acidic regions (n=1)

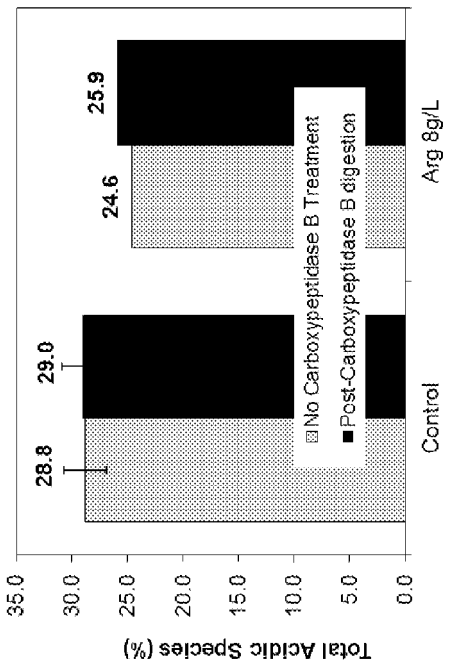


Figure 16) Effect of carboxypeptidase digestions of product from mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

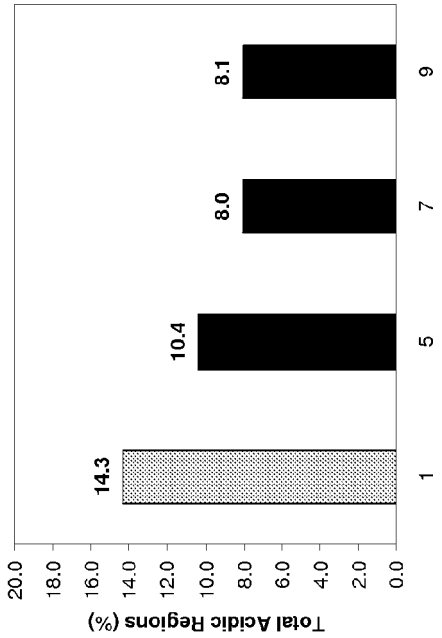


Figure 13) Effect of total arginine concentration in mAB1 producing cell line on WCX-10 profile total acidic regions (n=1)

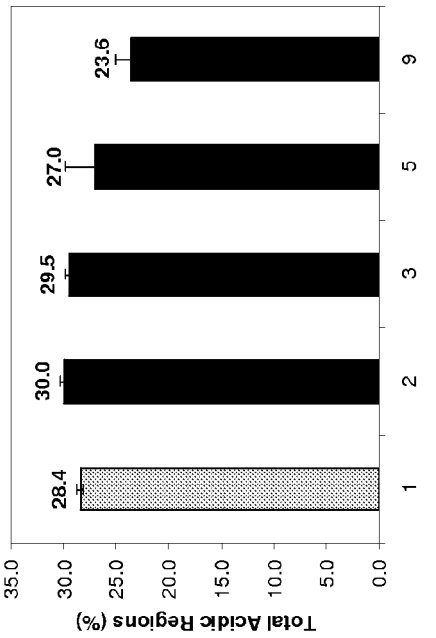


Figure 14) Effect of total arginine concentration in mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

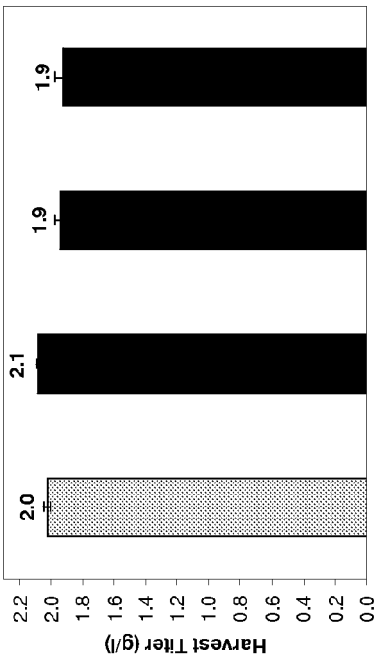


Figure 19) Effect of total lysine concentration in adalimumab producing cell line 2, media 1 on harvest titer (n=2)

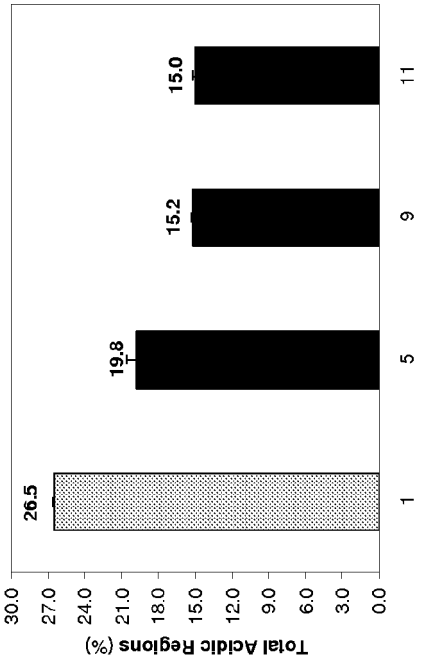


Figure 20) Effect of total lysine concentration in adalimumab producing cell line 2, media 1 on WCX 10 profile total acidic regions (n=2)

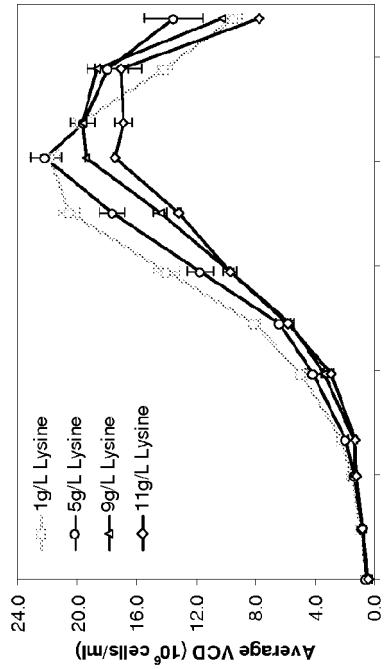


Figure 17) Effect of total lysine concentration in adalimumab producing cell line 2, media 1 on viable cell density (n=2)

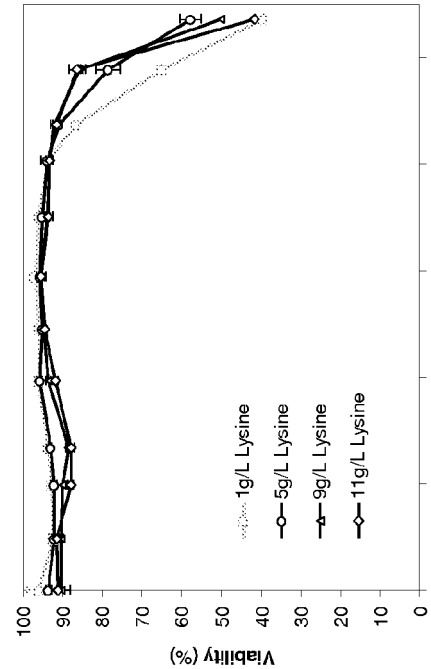


Figure 18) Effect of total lysine concentration in adalimumab producing cell line 2, media 1 on viability (n=2)

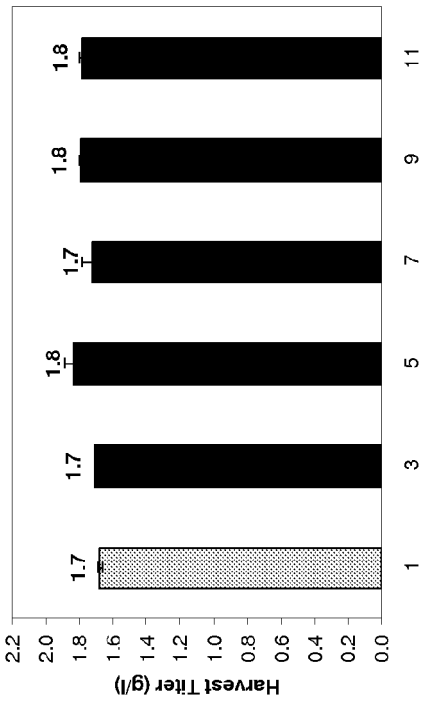


Figure 23) Effect of total lysine concentration in adalimumab producing cell line 3, media 1 on harvest titer (n=2)

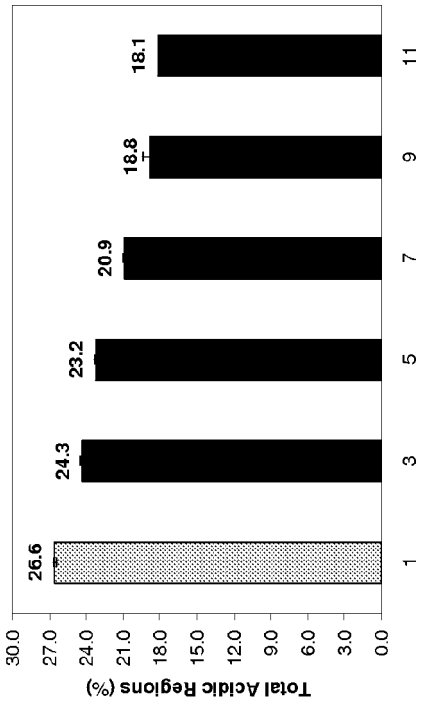


Figure 24) Effect of total lysine concentration in adalimumab producing cell line 3, media 1 on WCX 10 profile total acidic regions (n=2)

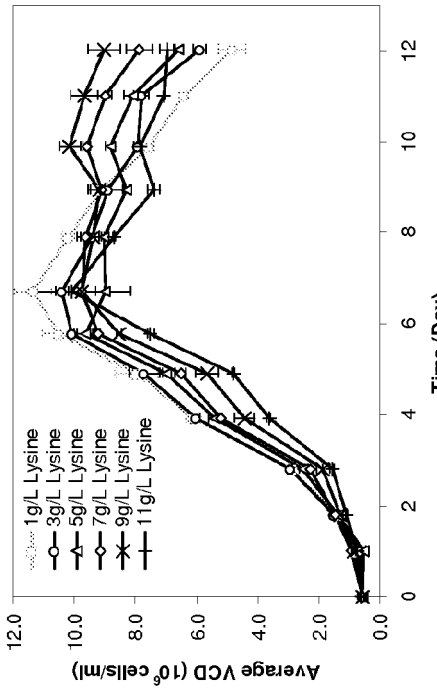


Figure 21) Effect of total lysine concentration in adalimumab producing cell line 3, media 1 on viable cell density (n=2)

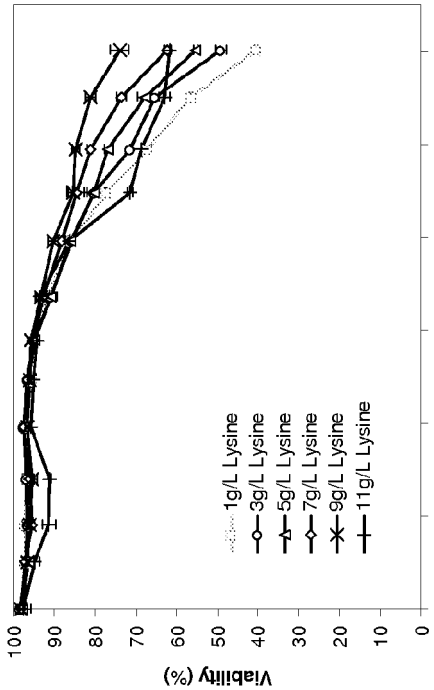


Figure 22) Effect of total lysine concentration in adalimumab producing cell line 3, media 1 on viability (n=2)

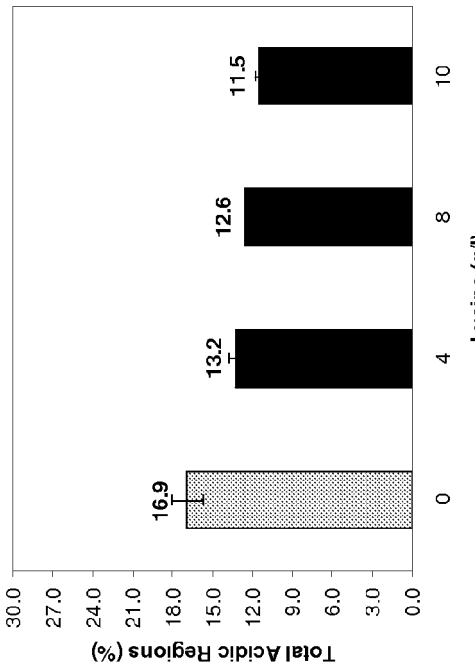


Figure 26) Effect of lysine addition to adalimumab producing cell line 1, media 2 on WCX-10 profile total acidic regions (n=2)

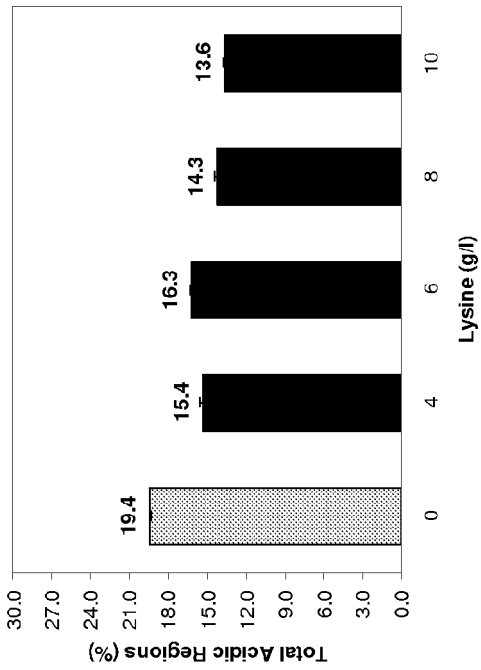


Figure 27) Effect of lysine addition to adalimumab producing cell line 2, media 3 on WCX-10 profile total acidic regions (n=2)

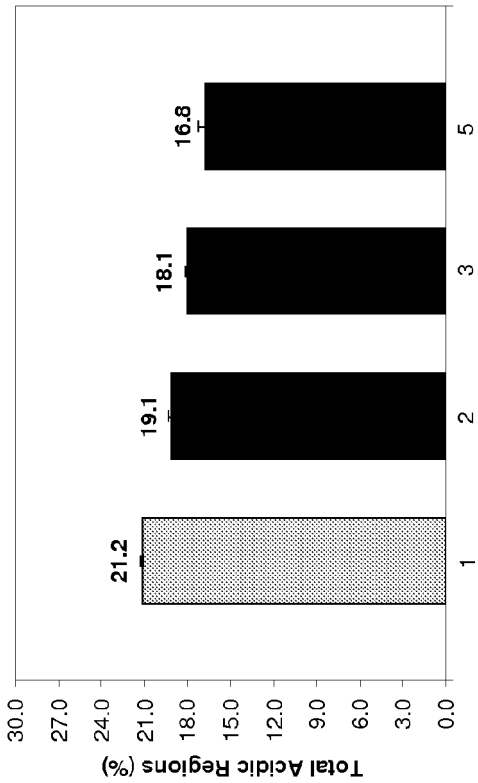


Figure 25) Effect of total lysine concentration in adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions (n=2)

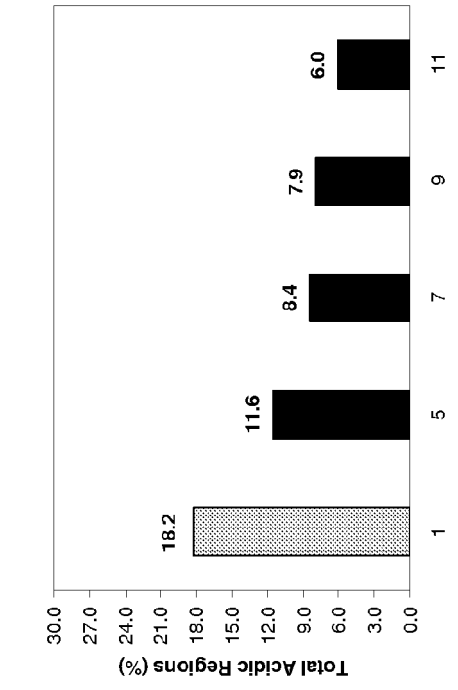


Figure 28) Effect of lysine addition to mAB1 producing cell line on WCX-10 profile total acidic regions (n=1)

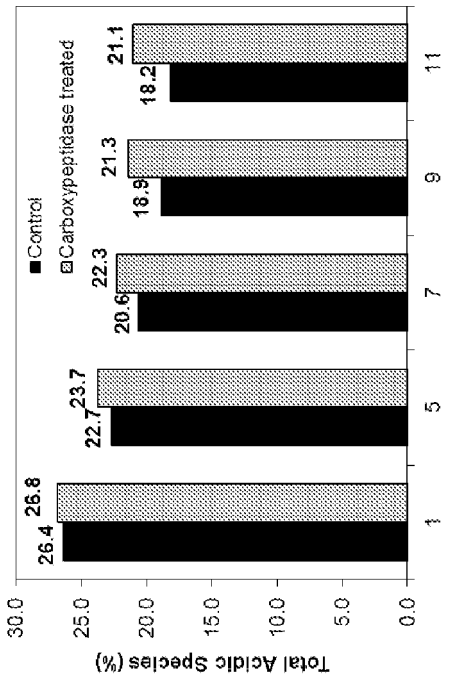


Figure 30) Effect of carboxypeptidase digestion of product from cell line 3, media 1 experiment on WCX-10 profile total acidic regions (n=1)

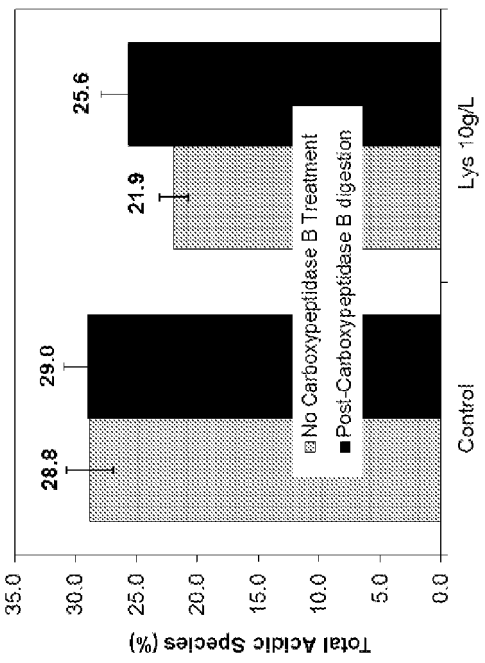


Figure 31) Effect of carboxypeptidase digestions of product from mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

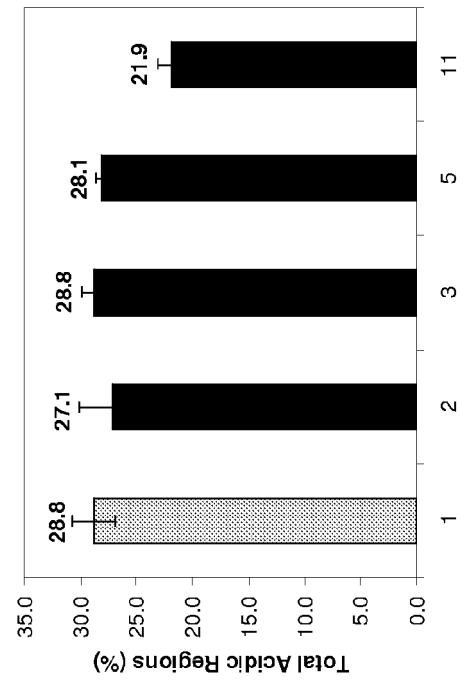


Figure 29) Effect of lysine addition to mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

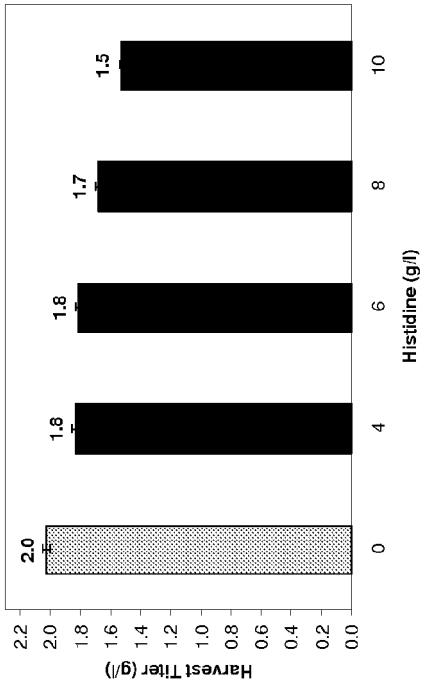


Figure 34) Effect of total histidine concentration in adalimumab producing cell line 2, media 1 on harvest titer (n=2)

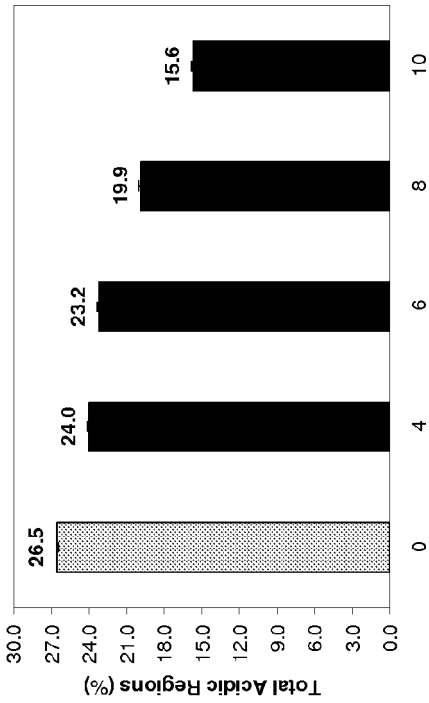


Figure 35) Effect of total histidine concentration in adalimumab producing cell line 2, media 1 on WCX 10 profile total acidic regions (n=2)

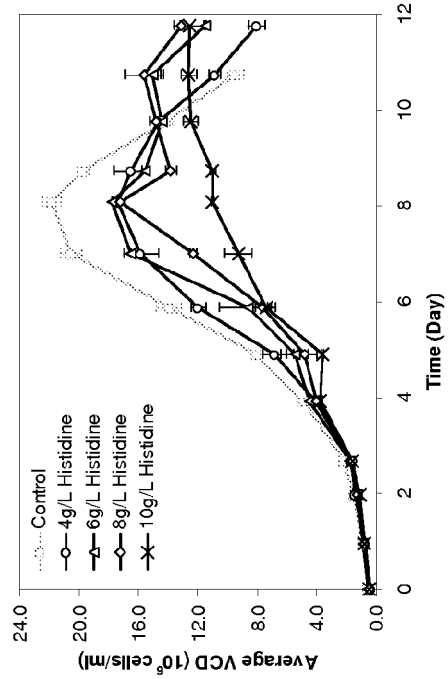


Figure 32) Effect of total histidine concentration in adalimumab producing cell line 2, media 1 on viable cell density (n=2)

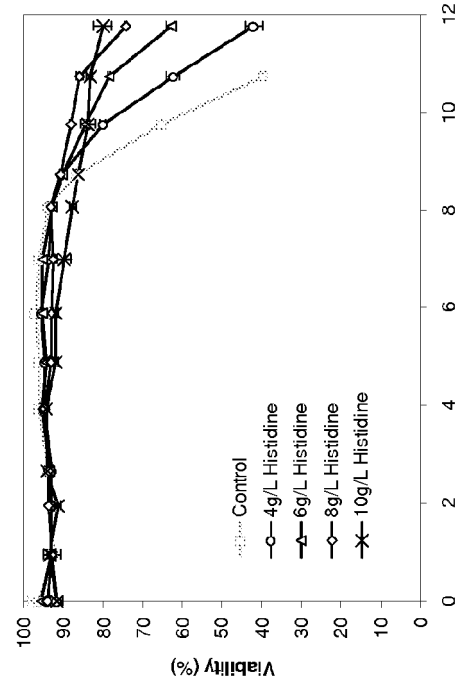


Figure 33) Effect of total histidine concentration in adalimumab producing cell line 2, media 1 on viability (n=2)

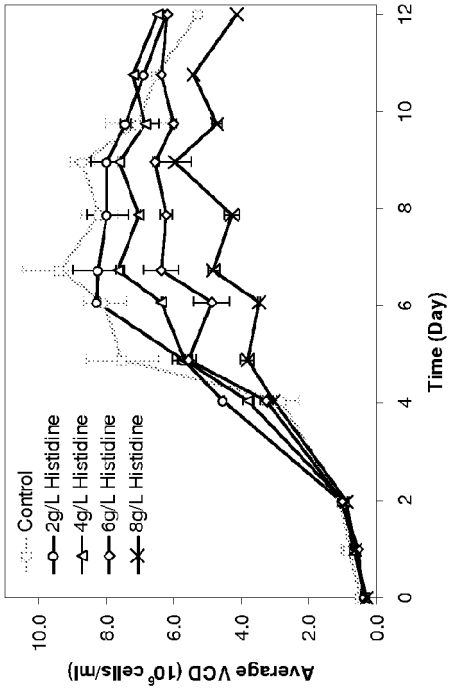


Figure 36) Effect of total histidine concentration in adalimumab producing cell line 3, media 1 on viable cell density (n=2)

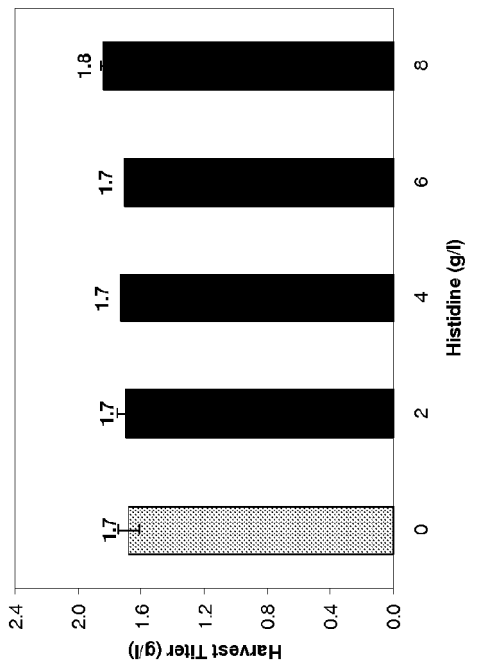


Figure 38) Effect of total histidine concentration in adalimumab producing cell line 3, media 1 on harvest titer (n=2)

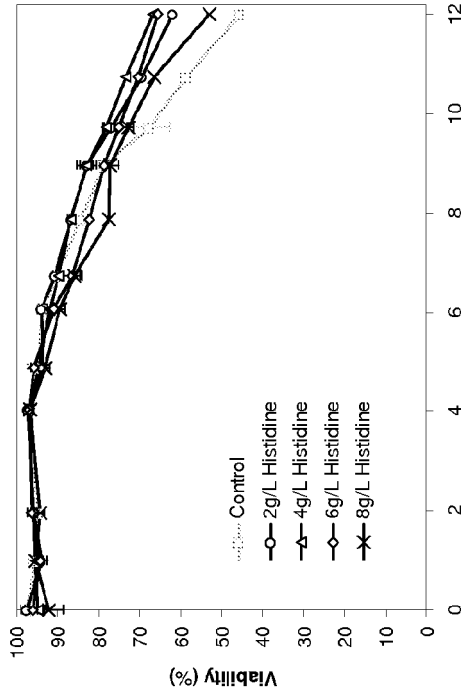


Figure 37) Effect of total histidine concentration in adalimumab producing cell line 3, media 1 on viability (n=2)

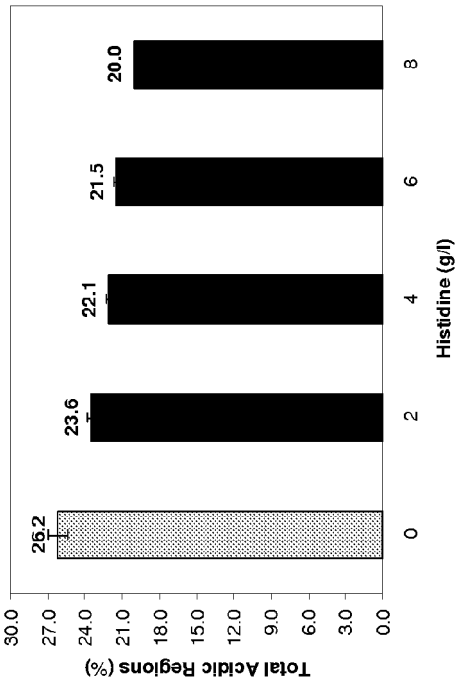


Figure 39) Effect of total histidine concentration in adalimumab producing cell line 3, media 1 on WCX 10 profile total acidic regions (n=2)

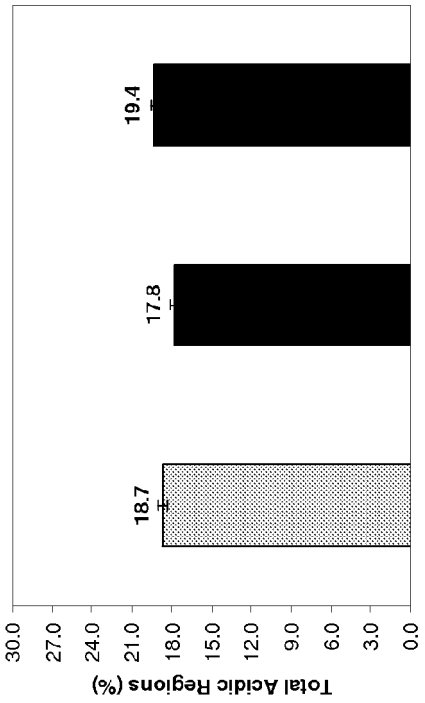


Figure 41) Effect of histidine addition to adalimumab producing cell line 1, media 2 on WCX-10 profile total acidic regions (n=2)

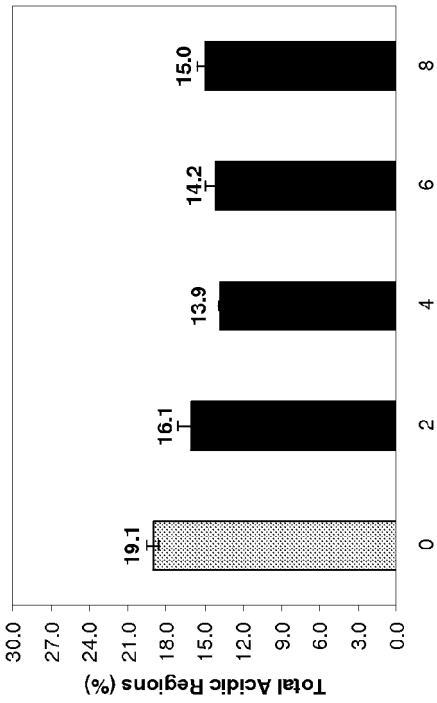


Figure 42) Effect of histidine addition to adalimumab producing cell line 2, media 3 on WCX-10 profile total acidic regions (n=2)

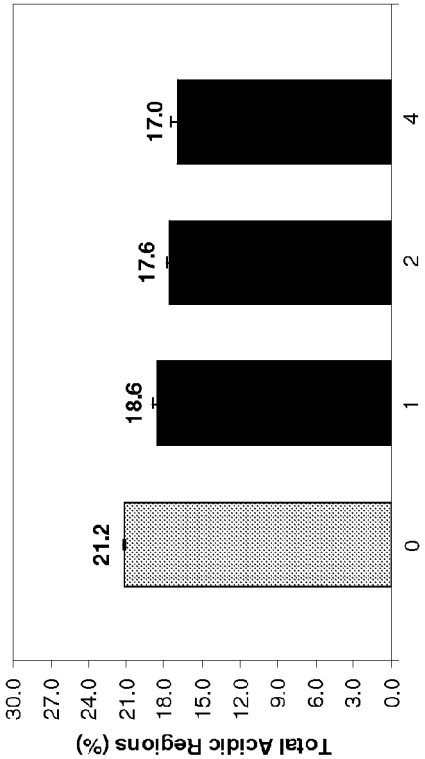


Figure 40) Effect of total histidine concentration in adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions (n=2)

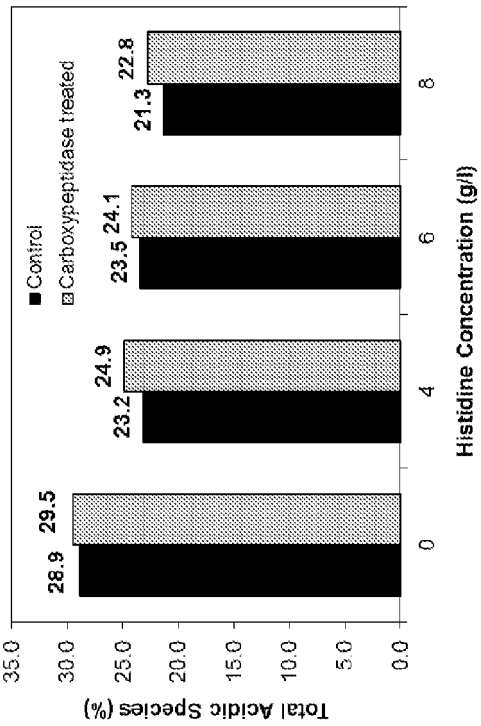


Figure 45) Effect of carboxypeptidase digestion of product from cell line 3, media 1 experiment on WCX-10 profile total acidic regions (n=1)

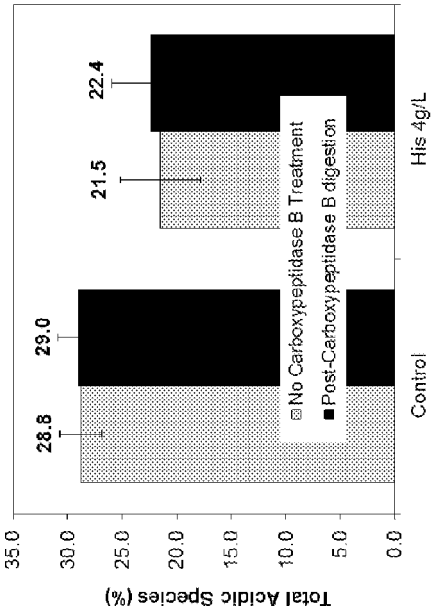


Figure 46) Effect of carboxypeptidase digestions of product from mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

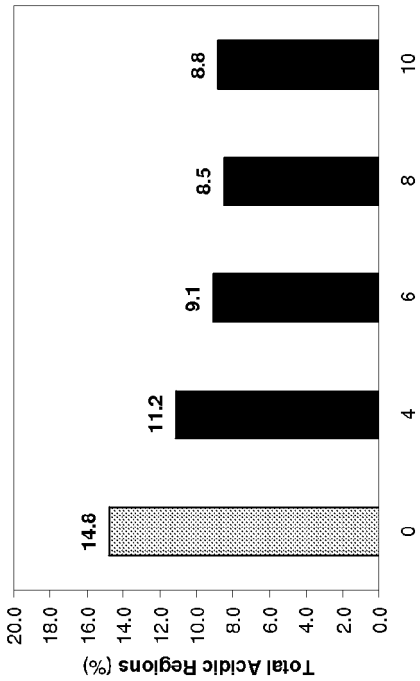


Figure 43) Effect of histidine concentration in mAB1 producing cell line on WCX-10 profile total acidic regions (n=1)

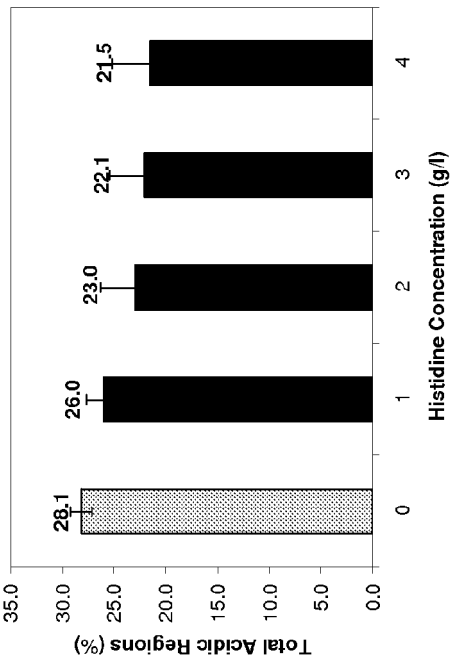


Figure 44) Effect of histidine concentration in mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

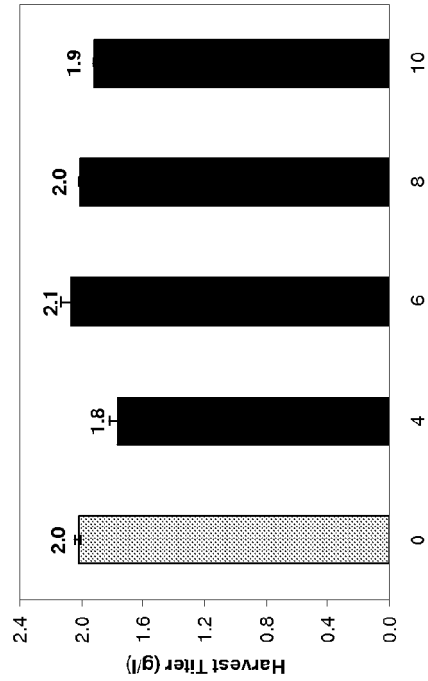


Figure 49) Effect of total ornithine concentration in adalimumab producing cell line 2, media 1 on harvest titer (n=2)

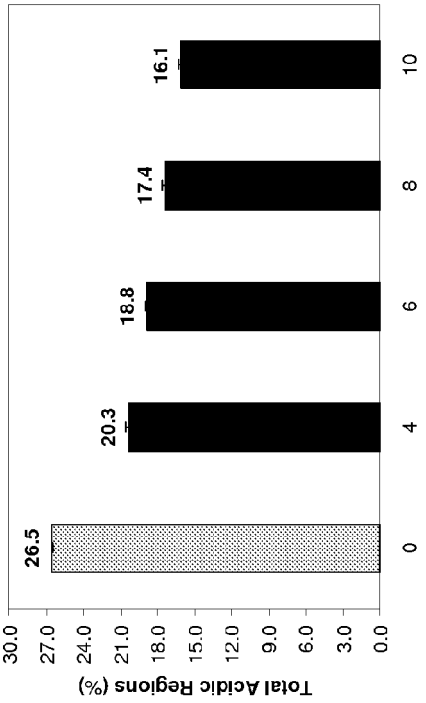


Figure 50) Effect of total ornithine concentration in adalimumab producing cell line 2, media 1 on WCX 10 profile total acidic regions (n=2)

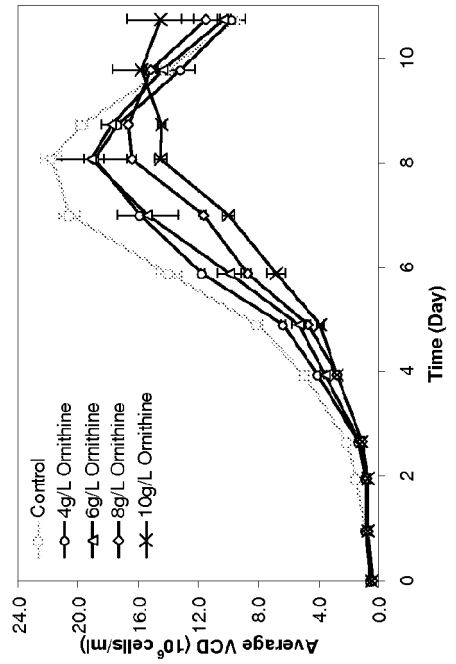


Figure 47) Effect of total ornithine concentration in adalimumab producing cell line 2, media 1 on viable cell density (n=2)

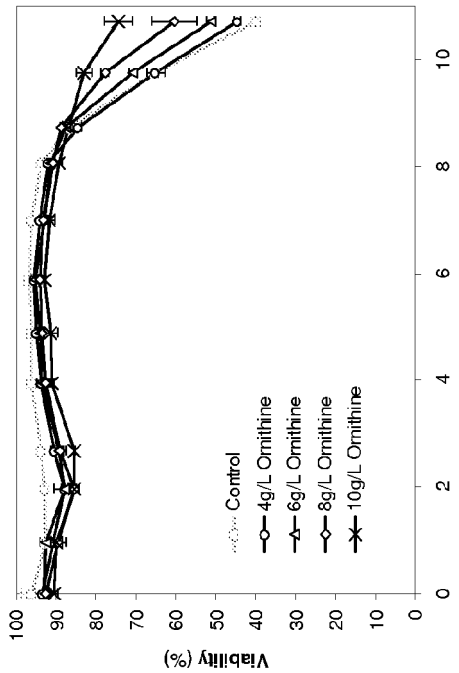


Figure 48) Effect of total ornithine concentration in adalimumab producing cell line 2, media 1 on viability (n=2)

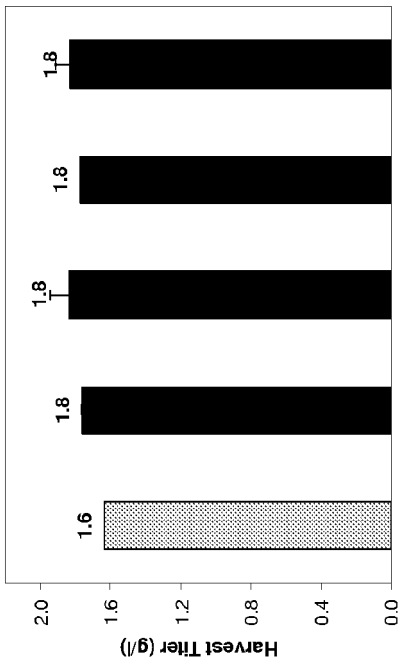


Figure 53) Effect of total ornithine concentration in adalimumab producing cell line 3, media 1 on harvest titer (n=2)

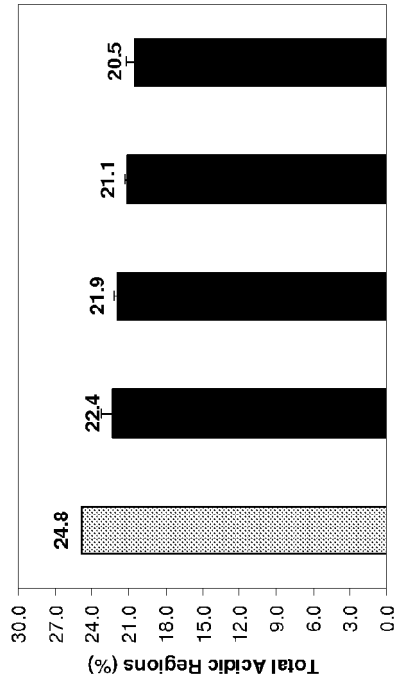


Figure 54) Effect of total ornithine concentration in adalimumab producing cell line 3, media 1 on WCX 10 profile total acidic regions (n=2)

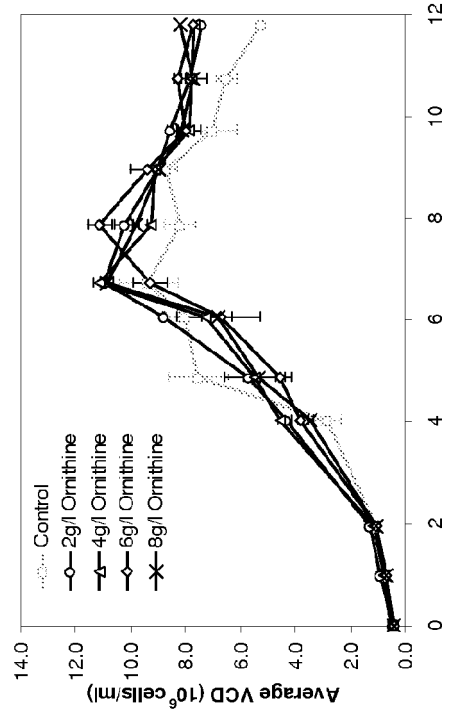


Figure 51) Effect of total ornithine concentration in adalimumab producing cell line 3, media 1 on viable cell density (n=2)

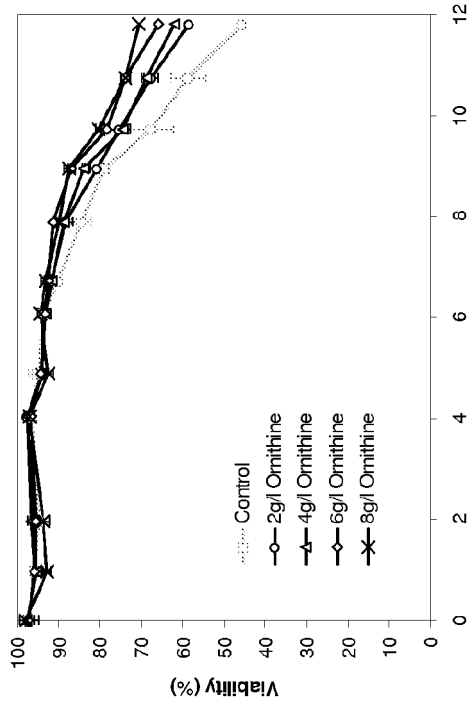


Figure 52) Effect of total ornithine concentration in adalimumab producing cell line 3, media 1 on viability (n=2)

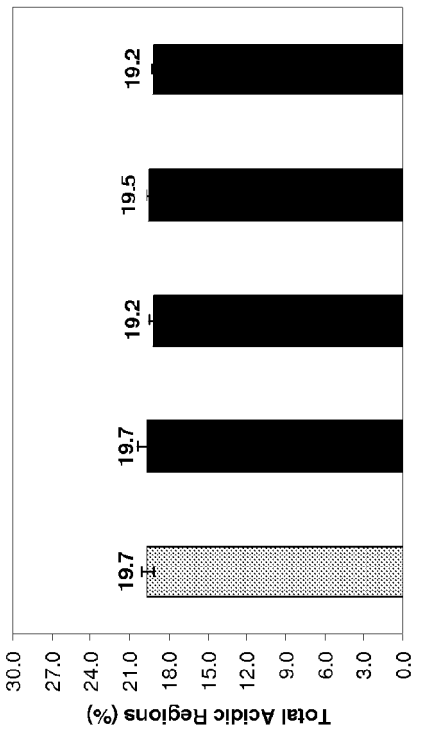


Figure 56) Effect of ornithine addition to adalimumab producing cell line 1, media 2 on WCX-10 profile total acidic regions (n=2)

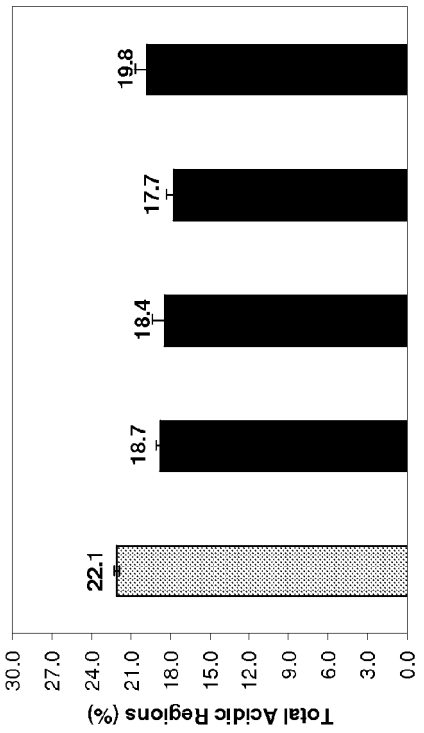


Figure 57) Effect of ornithine addition to adalimumab producing cell line 2, media 3 on WCX-10 profile total acidic regions (n=2)

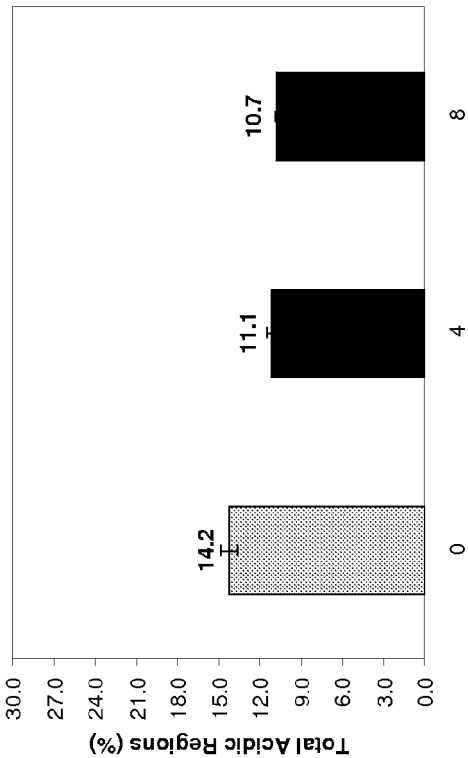


Figure 55) Effect of total ornithine concentration in adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions (n=2)

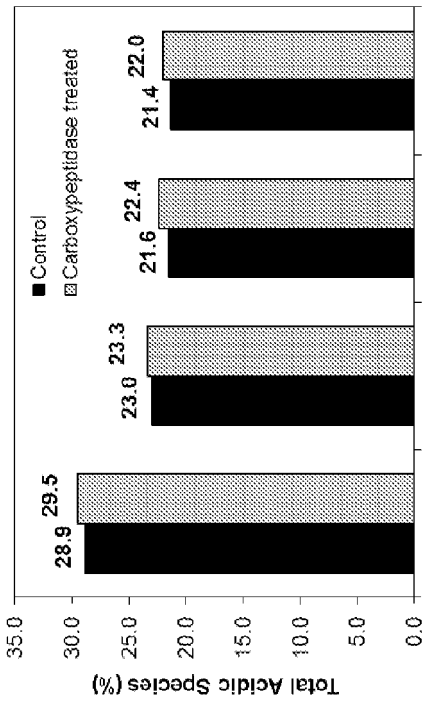


Figure 60) Effect of carboxypeptidase digestion of product from cell line 3, media 1 experiment on WCX-10 profile total acidic regions (n=1)

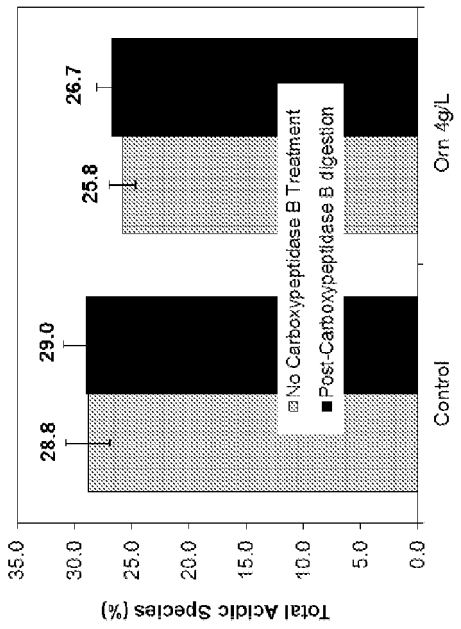


Figure 61) Effect of carboxypeptidase digestions of product from mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

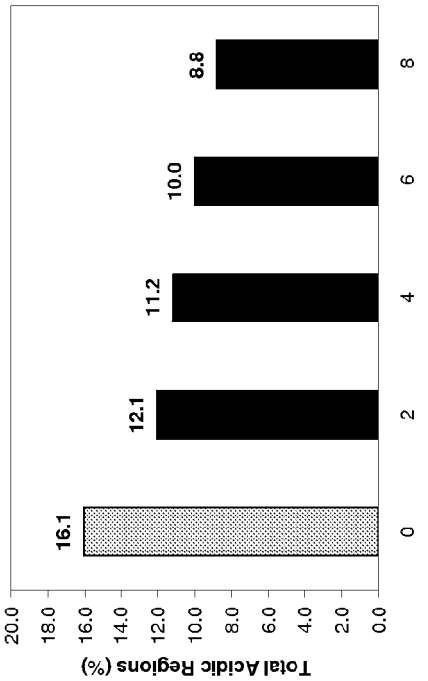


Figure 58) Effect of total ornithine concentration in mAB1 producing cell line on WCX-10 profile total acidic regions (n=1)

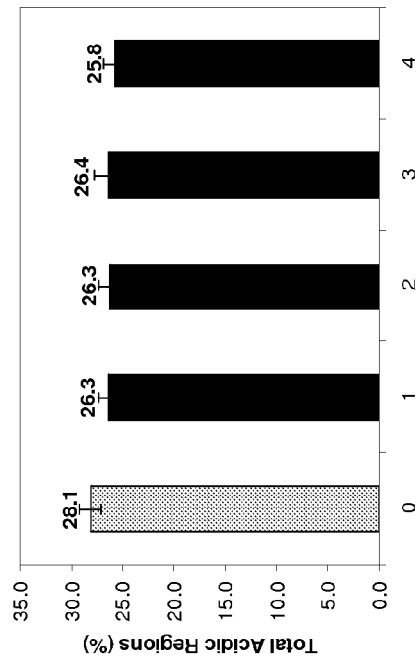


Figure 59) Effect of total ornithine concentration in mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

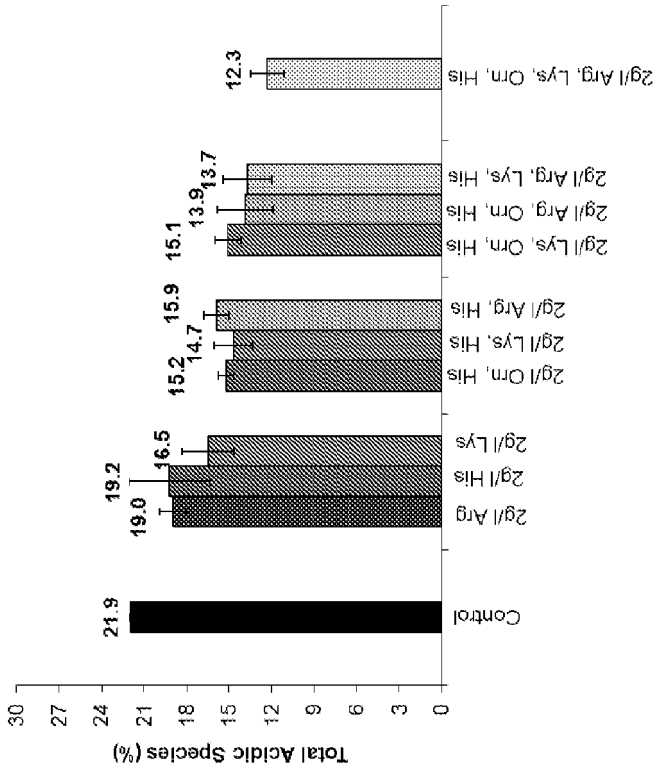


Figure 62) Effect of multiple amino acid additions to adalimumab producing cell line 2, media 1 containing 1g/l arginine and 1g/l lysine on WCX 10 profile total acidic regions (n=2)

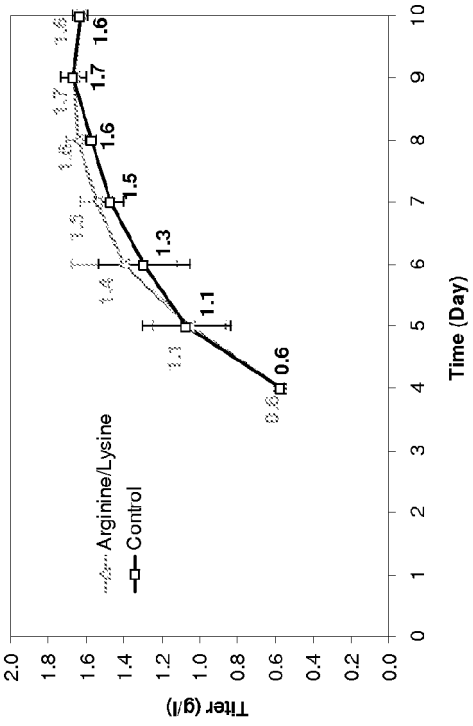


Figure 65) Effect of increased arginine and lysine concentration in adalimumab producing cell line 3, media 1 on culture titer (n=3)

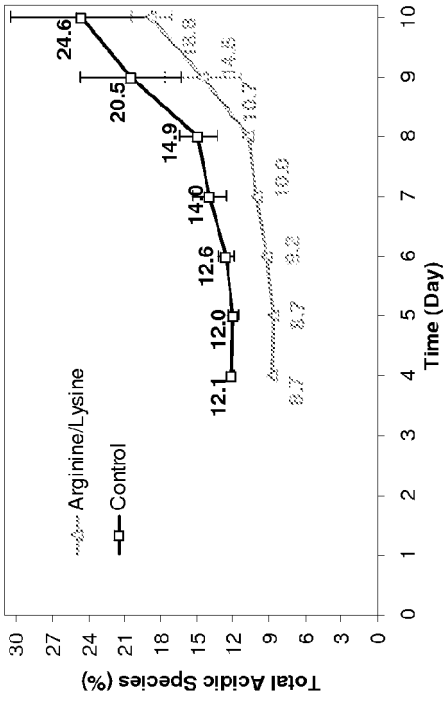


Figure 66) Effect of increased arginine and lysine concentration in adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions (n=2)

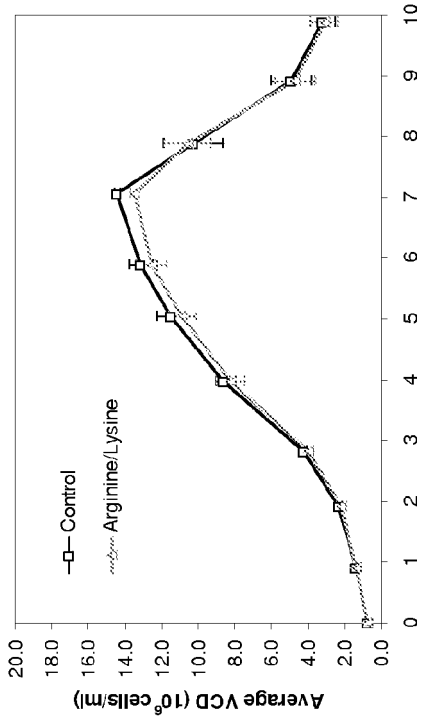


Figure 63) Effect of increased arginine and lysine concentration in adalimumab producing cell line 1, media 1 on viable cell density (n=3)

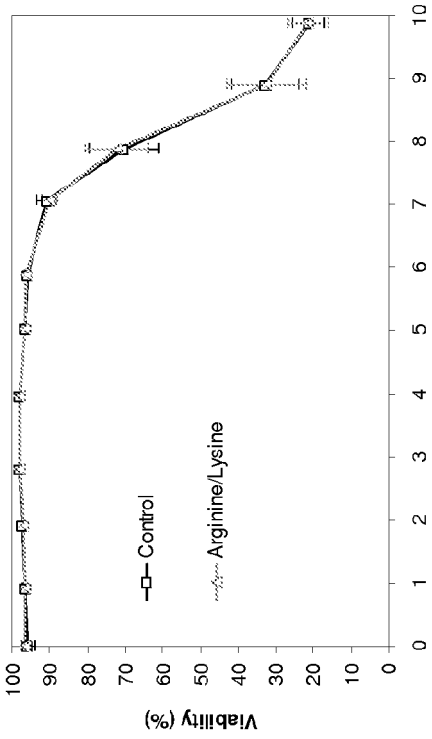


Figure 64) Effect of increased arginine and lysine concentration in adalimumab producing cell line 3, media 1 on viability (n=3)

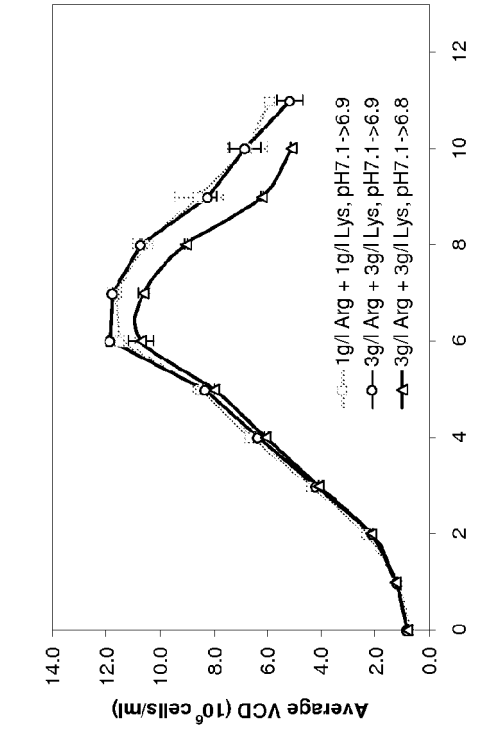


Figure 67) Effect of arginine, lysine and pH modulation to adalimumab producing cell line 1, media 1 on viable cell density (n=2)

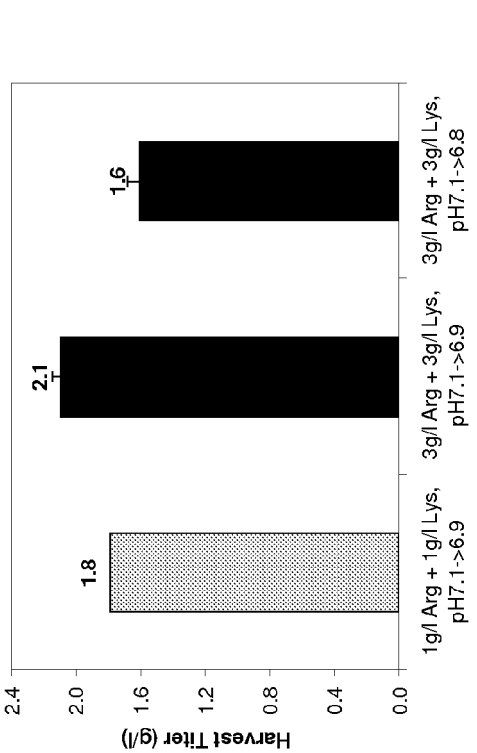


Figure 69) Effect of arginine, lysine and pH modulation to adalimumab producing cell line 3, media 1 on culture titer (n=2)

Figure 68) Effect of arginine, lysine and pH modulation to adalimumab producing cell line 3, media 1 on viability (n=2)

Figure 70) Effect of arginine, lysine and pH modulation to adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions (n=2)

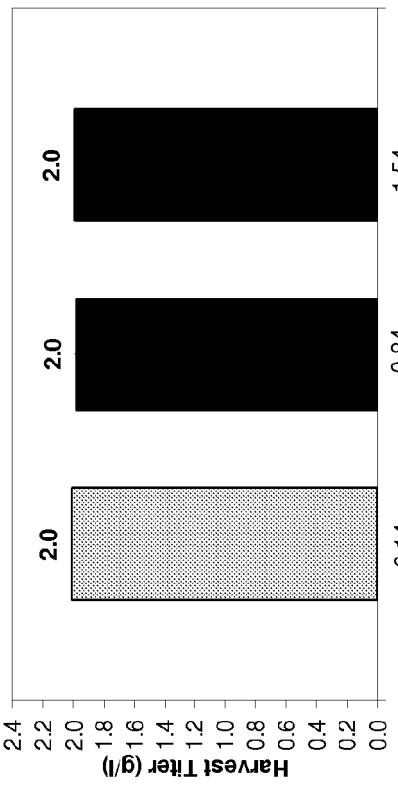


Figure 73) Effect of total calcium concentration in adalimumab producing cell line 2, media 1 on harvest titer (n=2)

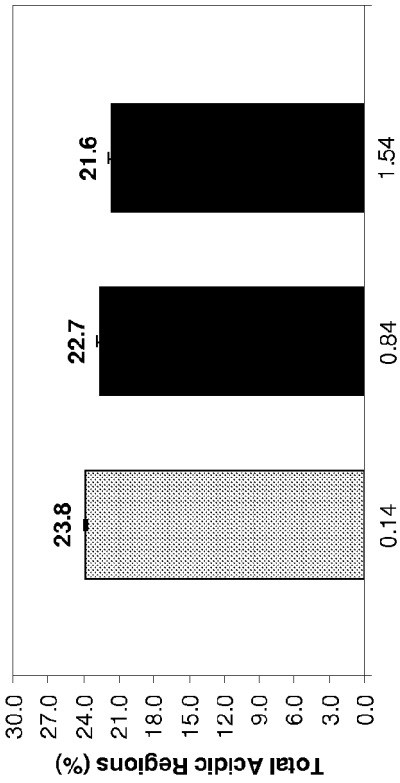


Figure 74) Effect of total calcium concentration in adalimumab producing cell line 2, media 1 on WCX 10 profile total acidic regions (n=2)

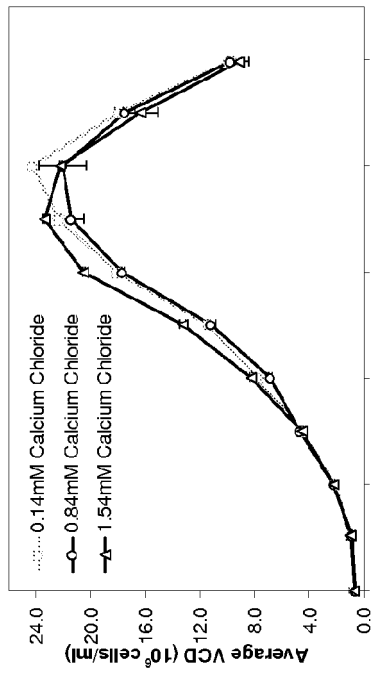


Figure 71) Effect of total calcium concentration in adalimumab producing cell line 2, media 1 on viable cell density (n=2)

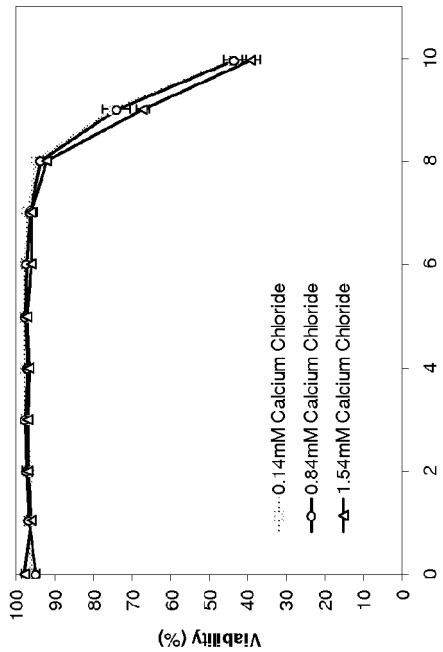


Figure 72) Effect of total calcium concentration in adalimumab producing cell line 2, media 1 on viability (n=2)

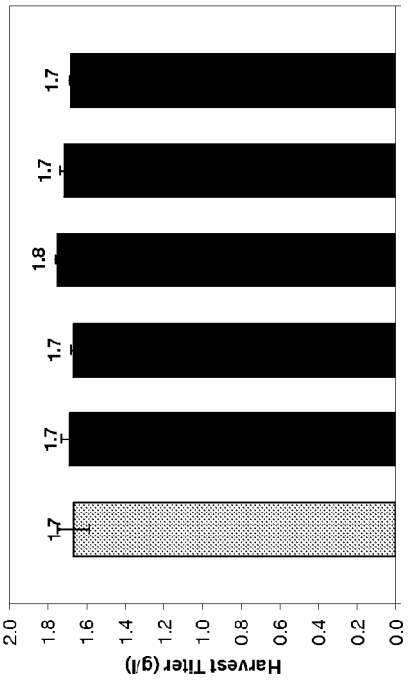


Figure 77) Effect of total calcium concentration in adalimumab producing cell line 3, media 1 on harvest titer (n=2)

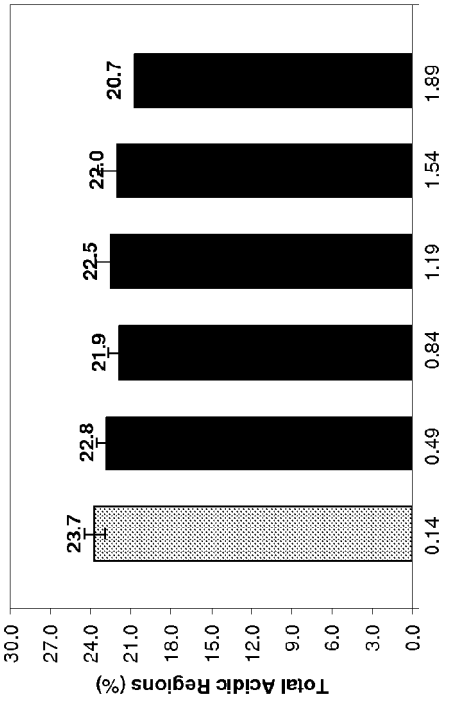


Figure 78) Effect of total calcium concentration in adalimumab producing cell line 3, media 1 on WCX 10 profile total acidic regions (n=2)

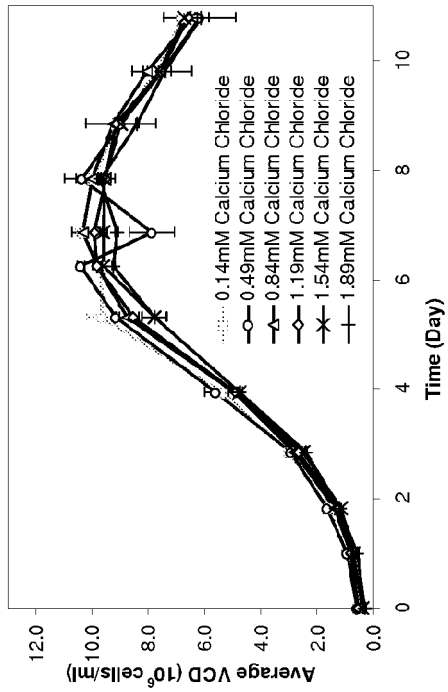


Figure 75) Effect of total calcium concentration in adalimumab producing cell line 3, media 1 on viable cell density (n=2)

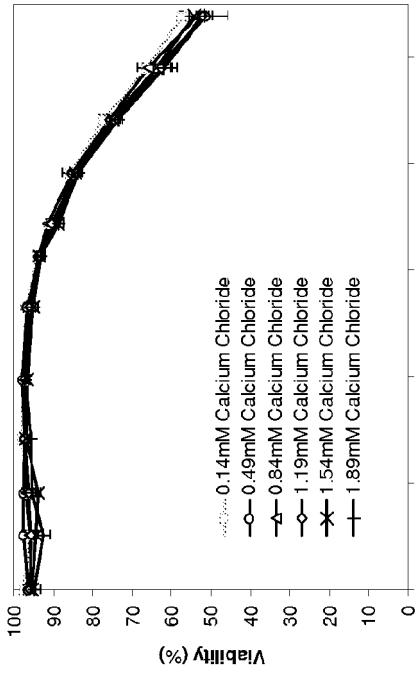


Figure 76) Effect of total calcium concentration in adalimumab producing cell line 3, media 1 on viability (n=2)

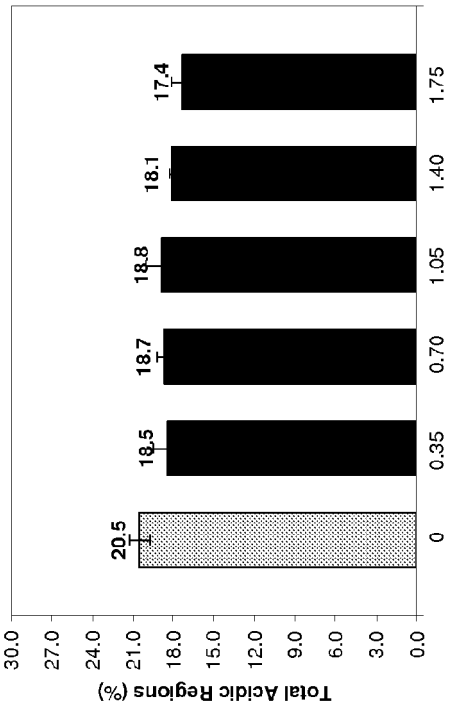


Figure 80) Effect of total calcium concentration in adalimumab producing cell line 1, media 2 on WCX-10 profile total acidic regions (n=2)

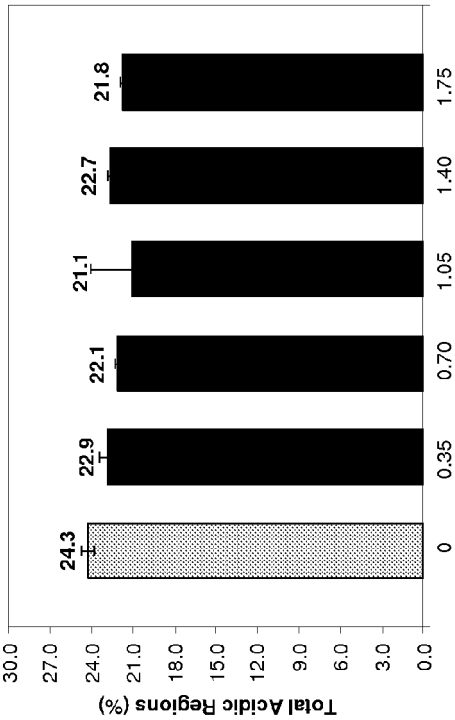


Figure 81) Effect of total calcium concentration in adalimumab producing cell line 2, media 3 on WCX-10 profile total acidic regions (n=2)

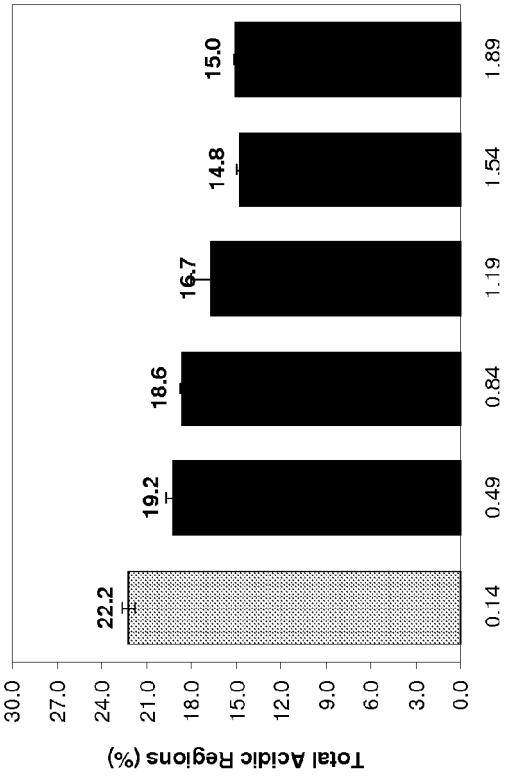


Figure 79) Effect of total calcium concentration in adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions (n=2)

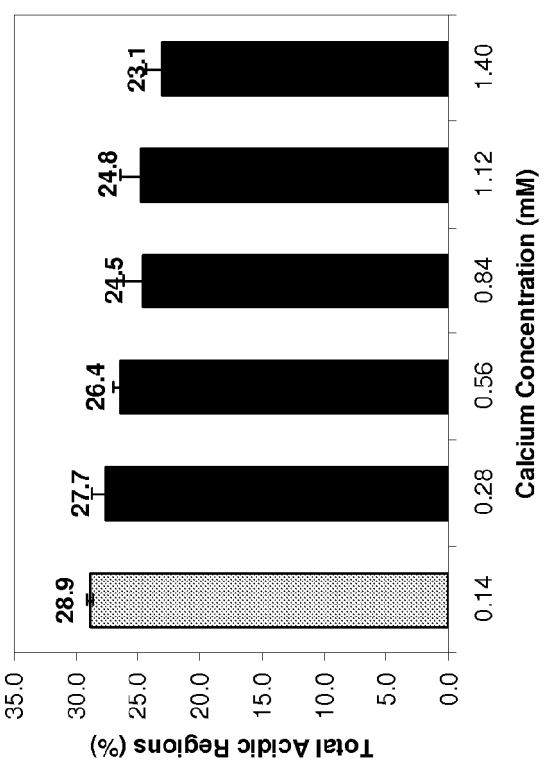


Figure 83) Effect of total calcium concentration in mAB2 producing cell line on WCX-10 profile total acidic regions (n=2)

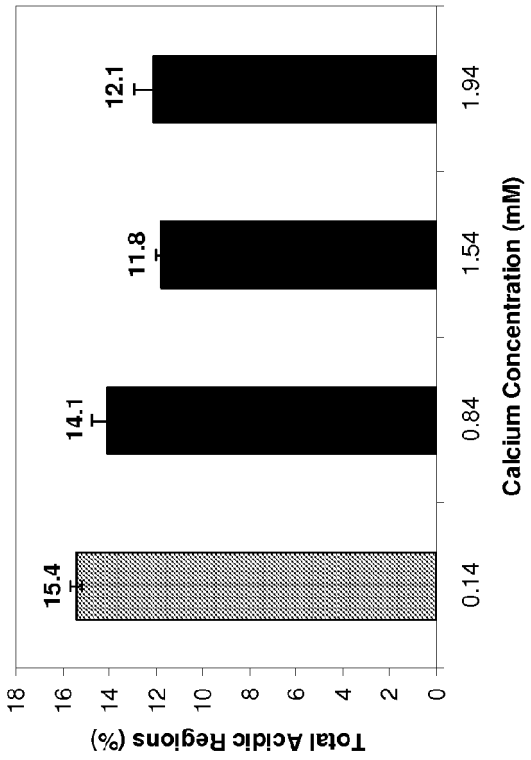


Figure 82) Effect of total calcium concentration in mAB1 producing cell line on WCX-10 profile total acidic regions (n=2)

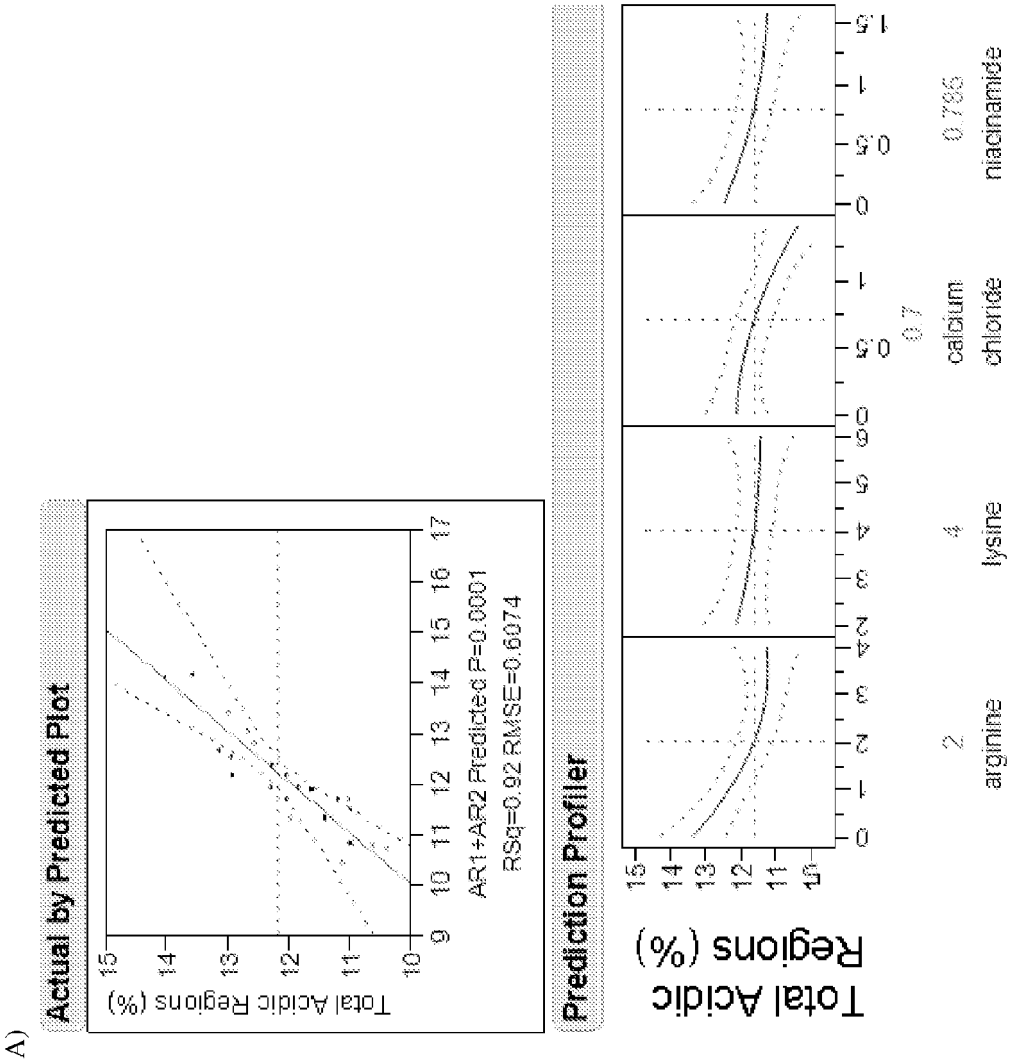


Figure 84) Effect of multiple amino acid additions to cell line 1, media 1 on WCX 10 profile total acidic regions a) overall prediction plot, b) prediction plots for each additive (n=2)

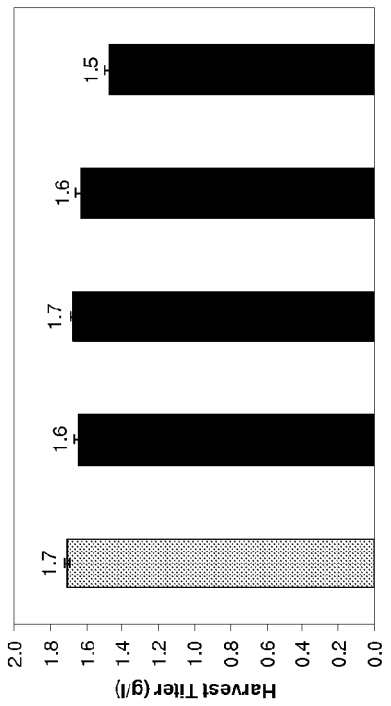


Figure 87) Effect of niacinamide addition to adalimumab producing cell line 1, media 1 on harvest titer (n=2)

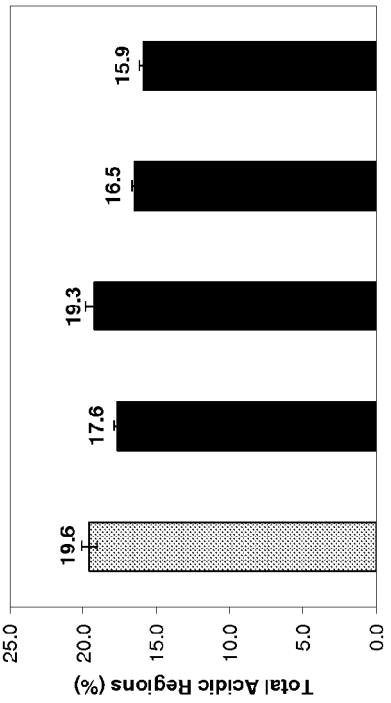


Figure 88) Effect of niacinamide addition to adalimumab producing cell line 1, media 1 on Day 11 WCX 10 profile total acidic regions (n=2)

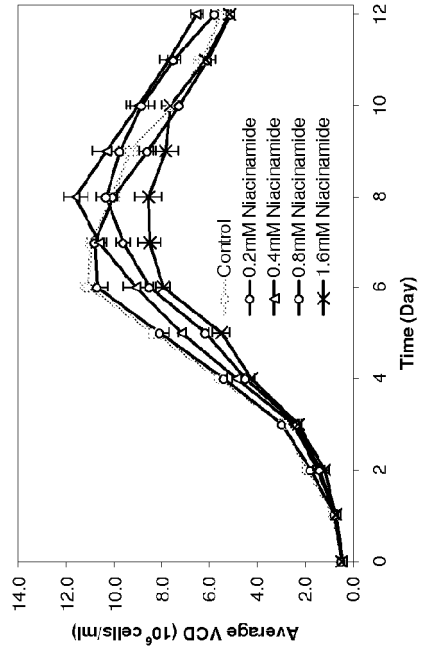


Figure 85) Effect of niacinamide addition to adalimumab producing cell line 1, media 1 on viable cell density (n=2)

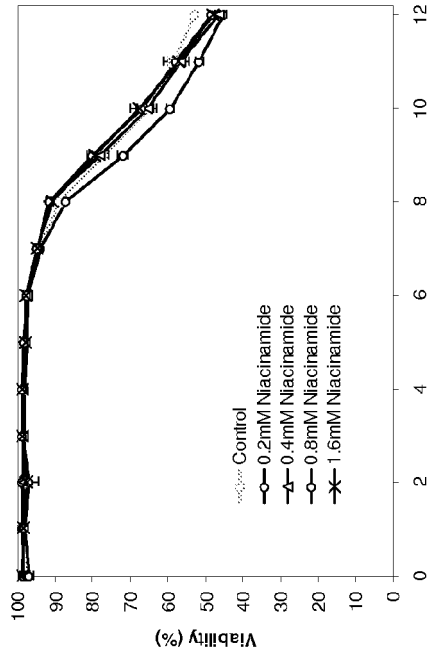


Figure 86) Effect of niacinamide addition to adalimumab producing cell line 1, media 1 on viability (n=2)

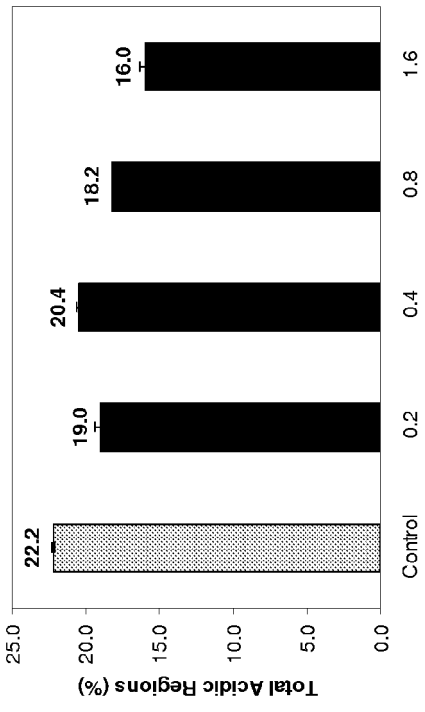


Figure 89) Effect of niacinamide addition to adalimumab producing cell line 1, media 1 on Day 12 WCX-10 profile total acidic regions (n=2)

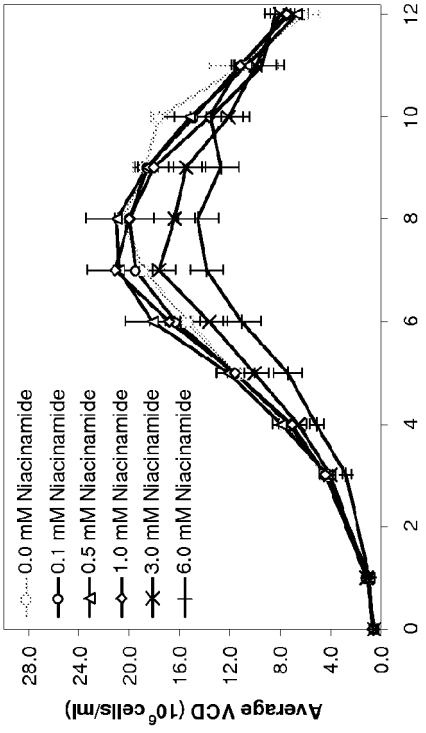


Figure 90) Effect of niacinamide addition to mAB2 producing cell line, media 1 on viable cell density (n=2)

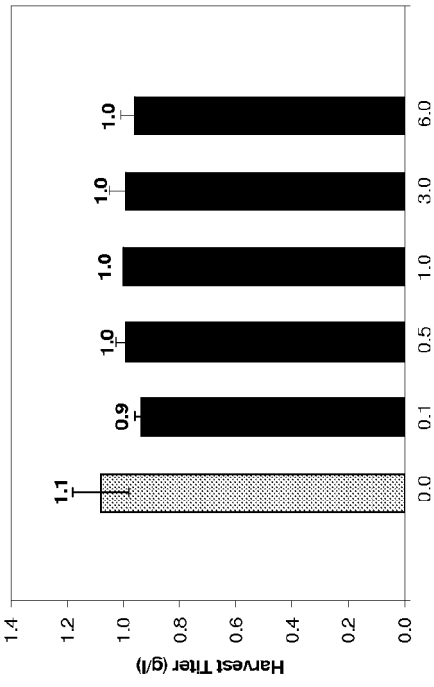


Figure 92) Effect of niacinamide addition to mAB2 producing cell line, media 1 on harvest titer (n=2)

Figure 91) Effect of niacinamide addition to mAB2 producing cell line, media 1 on viability (n=2)

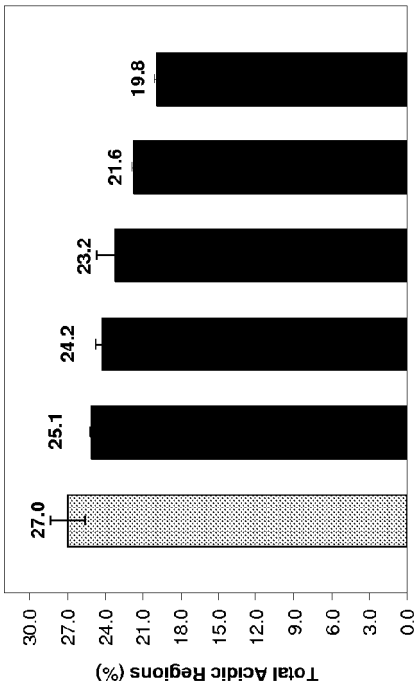


Figure 93) Effect of niacinamide addition to mAB2 producing cell line, media 1 on WCX 10 profile total acidic regions (n=2)

Figure 94A

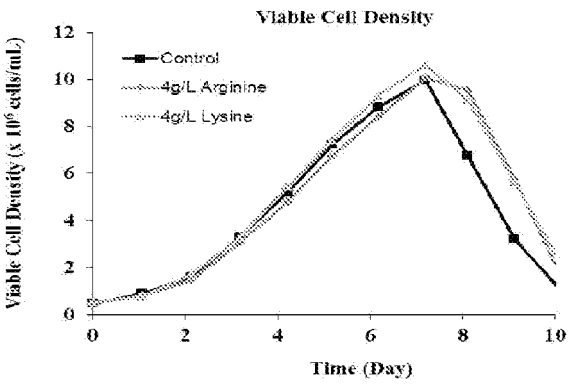


Figure 94B

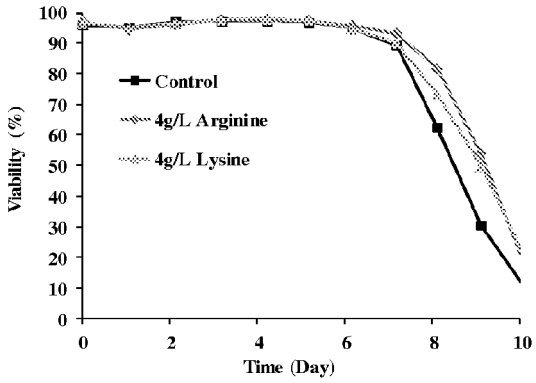


Figure 94C

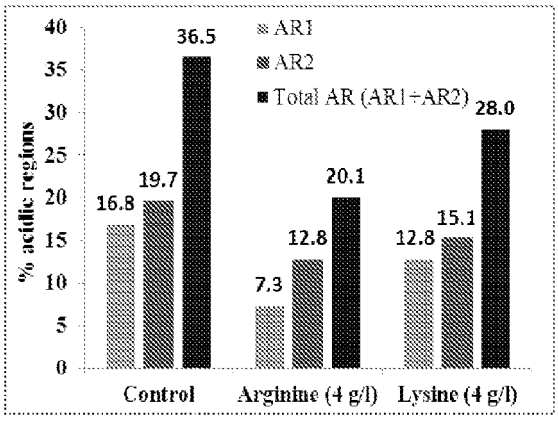
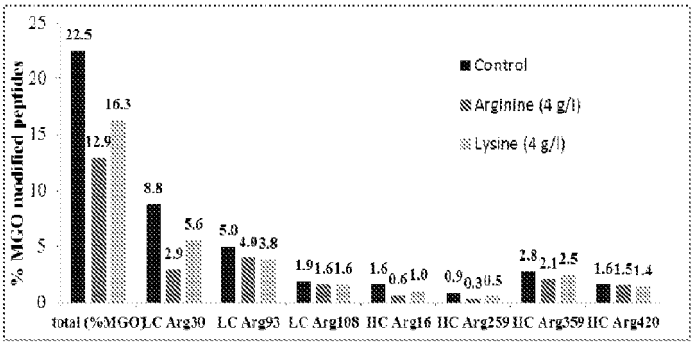


Figure 94D



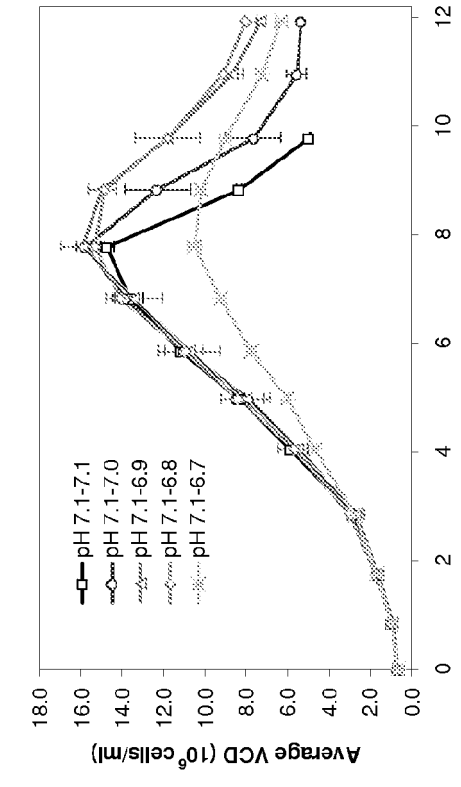


Figure 95) Effect of pH modulation of adalimumab producing cell line 1, media 1 on viable cell density (n=2, except for pH7.1-6.9 n=3)

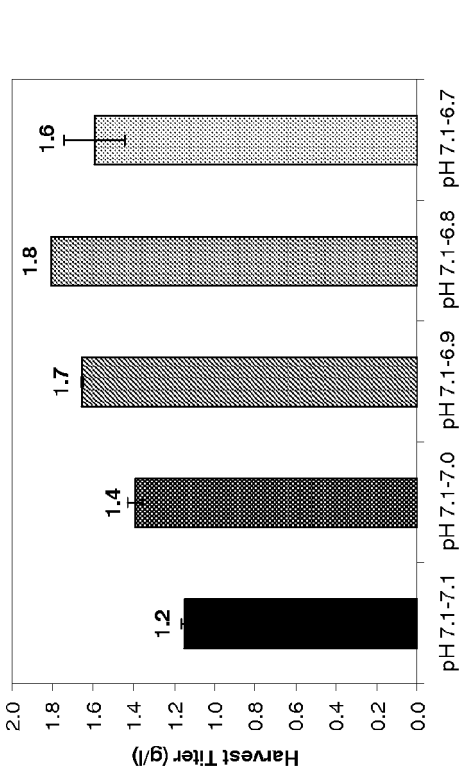


Figure 97) Effect of pH modulation of adalimumab producing cell line 1, media 1 on harvest titer

Figure 96) Effect of pH modulation adalimumab producing cell line 1, media 1 on viability (n=2, except for pH7.1-6.9 n=3)

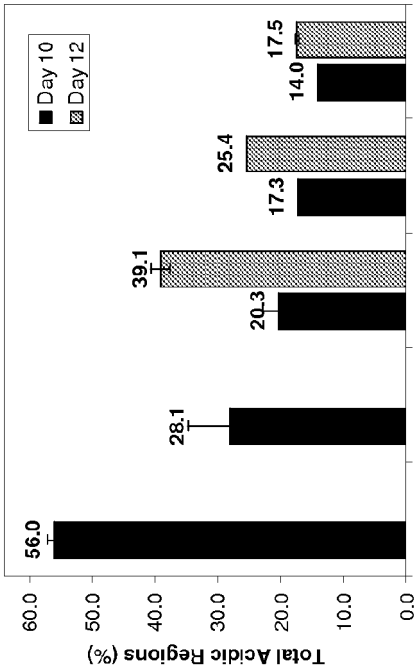


Figure 98) Effect of pH modulation of adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions

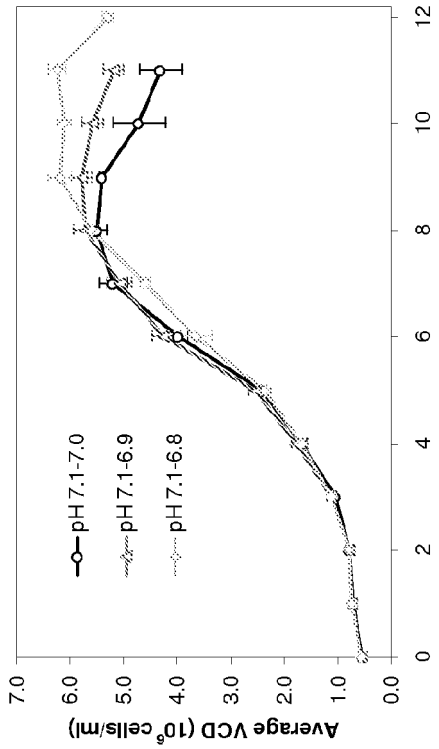


Figure 99) Effect of pH modulation of adalimumab producing cell line 1, media 2 on viable cell density (n=2, except for pH7.1-6.9 n=3)

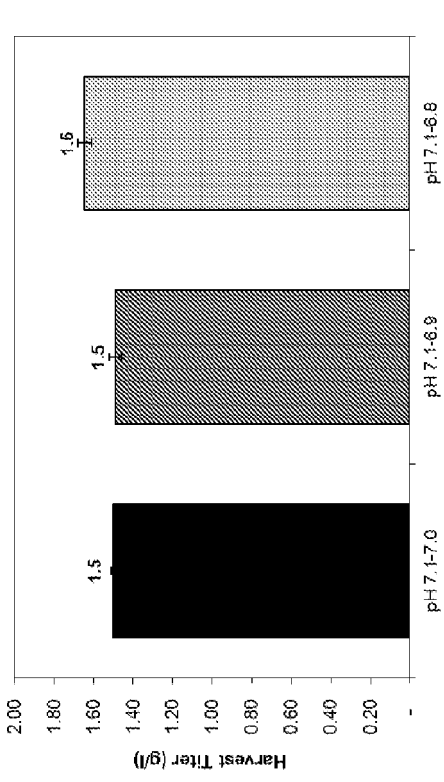


Figure 101) Effect of pH modulation of adalimumab producing cell line 1, media 2 on harvest titer

Figure 100) Effect of pH modulation of adalimumab producing cell line 1, media 2 on viability (n=2, except for pH7.1-6.9 n=3)

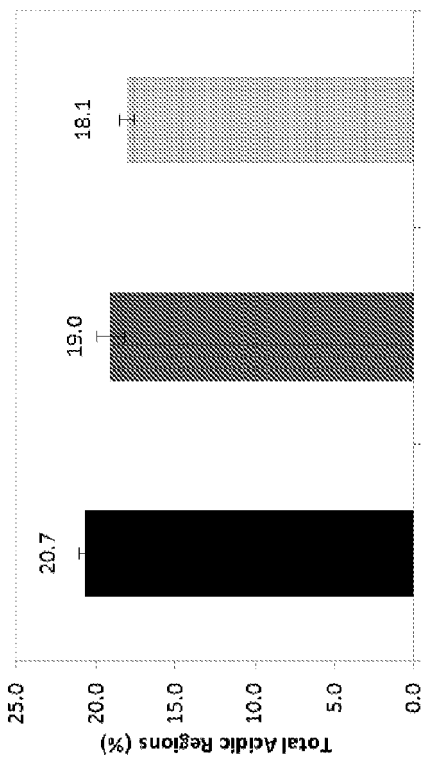


Figure 102) Effect of pH modulation of adalimumab producing cell line 1, media 2 on WCX 10 profile total acidic regions

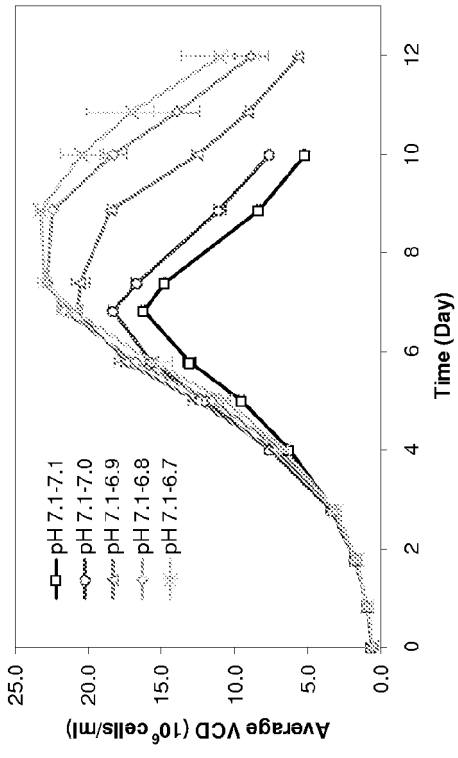


Figure 103) Effect of pH modulation of adalimumab producing cell line 3, media 1 on viable cell density (n=2, except for pH7.1-6.9 and pH7.1-6.8 n=3)

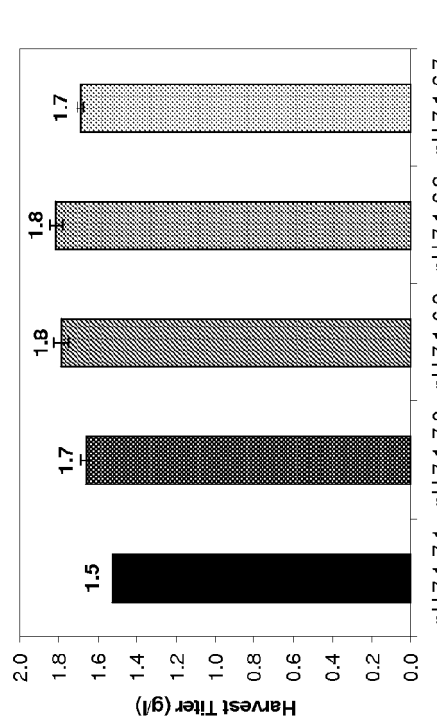


Figure 105) Effect of pH modulation of adalimumab producing cell line 3, media 1 on harvest titer

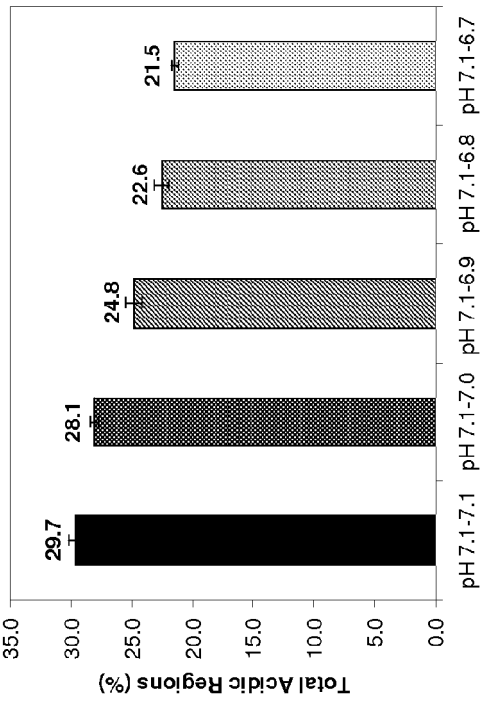


Figure 106) Effect of pH modulation of adalimumab producing cell line 3, media 1 on WCX 10 profile total acidic regions

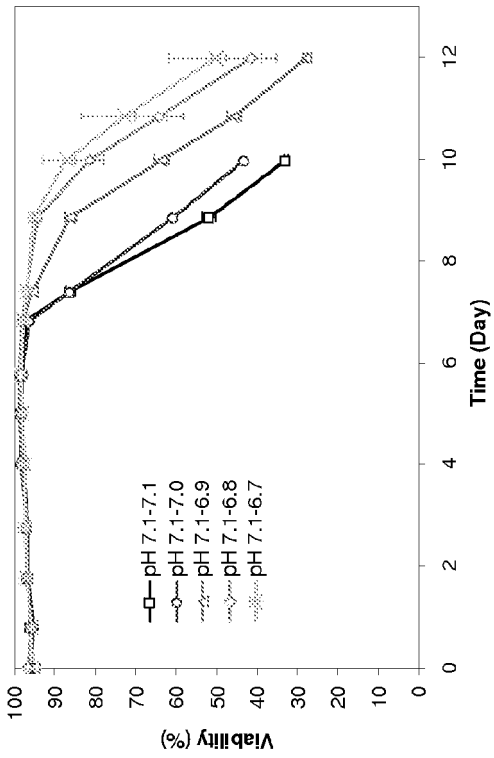


Figure 104) Effect of pH modulation of adalimumab producing cell line 3, media 1 on viability (n=2, except for pH7.1-6.9 and pH7.1-6.8 n=3)

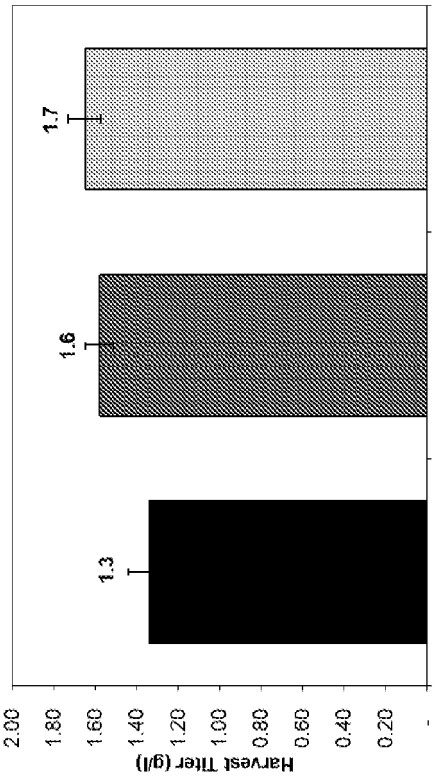


Figure 109) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 35°C on harvest titer

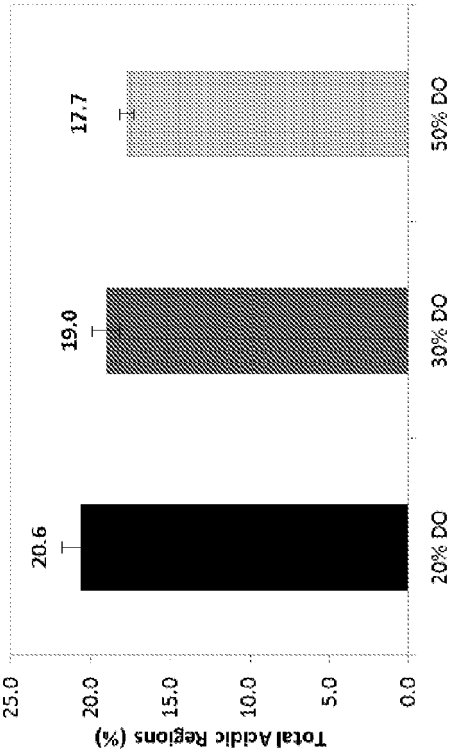


Figure 110) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 35°C on WCX 10 profile total acidic regions

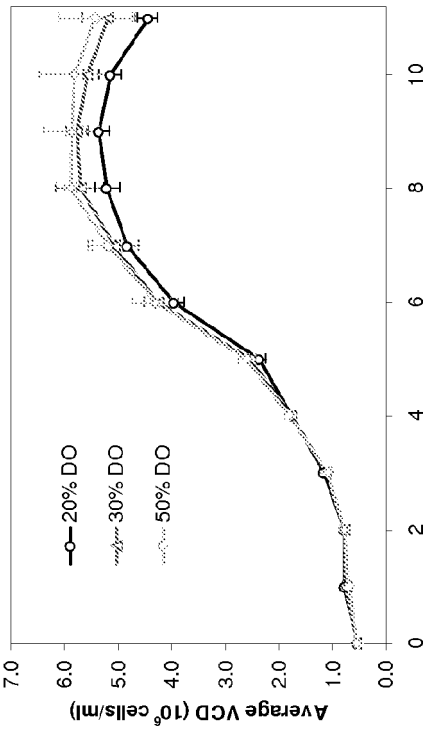


Figure 107) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 35°C on viable cell density (n=2, except 30% DO n=3)

Figure 108) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 35°C on viability (n=2, except 30% DO n=3)

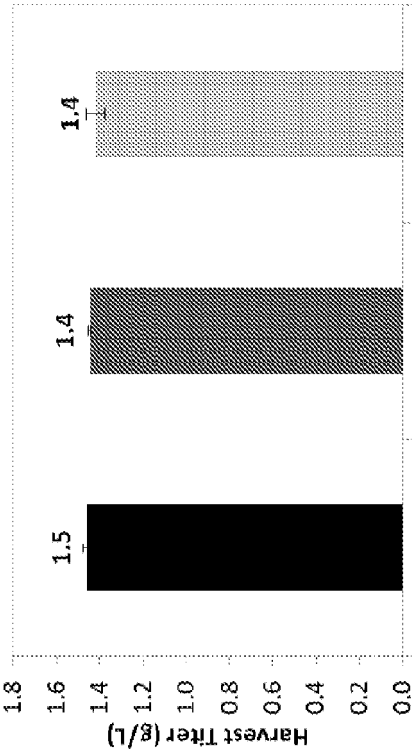


Figure 113) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 33°C on harvest titer

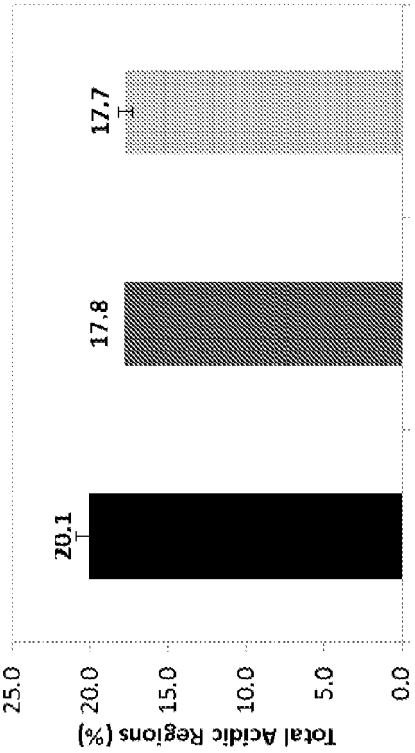


Figure 114) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 33°C on WCX 10 profile total acidic regions

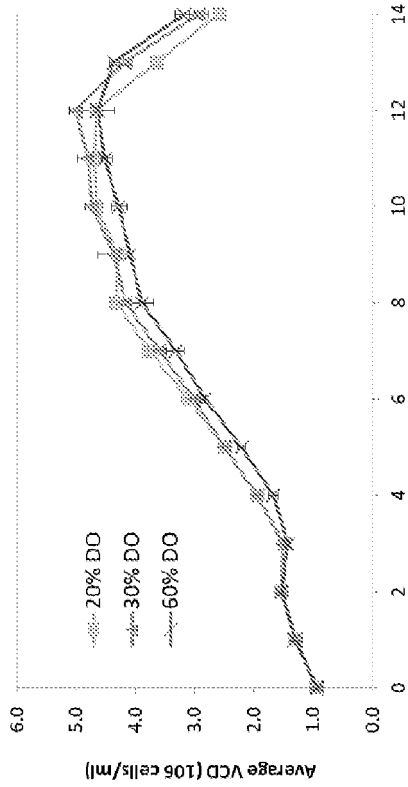


Figure 111) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 33°C on viable cell density (n=2)

Figure 112) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 2 at 33°C on viability (n=2)

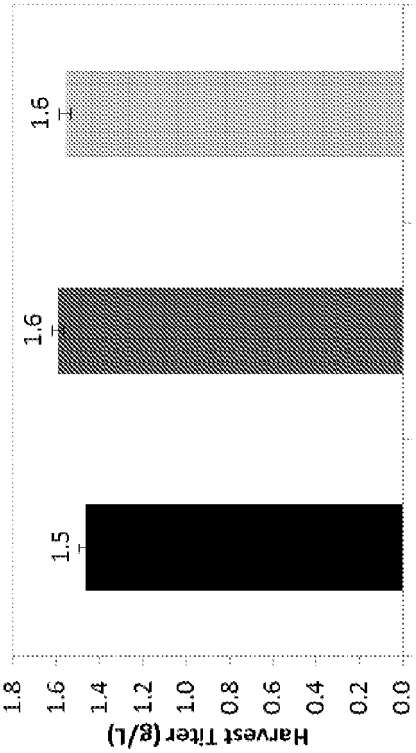


Figure 117) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 1 at 35°C on harvest titer

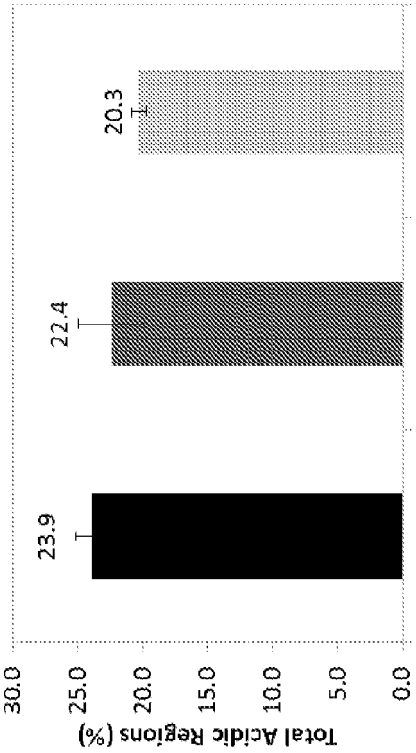


Figure 118) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 1 on WCX 10 profile total acidic regions

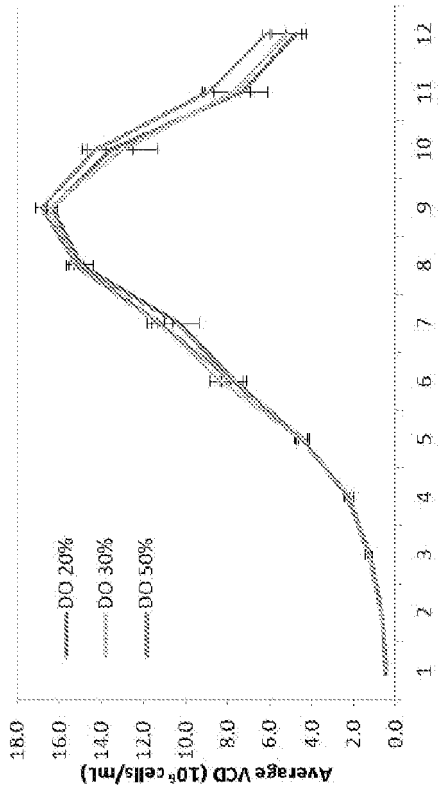


Figure 115) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 1 at 35°C on viable cell density (n=3, except for 30% DO n=2)

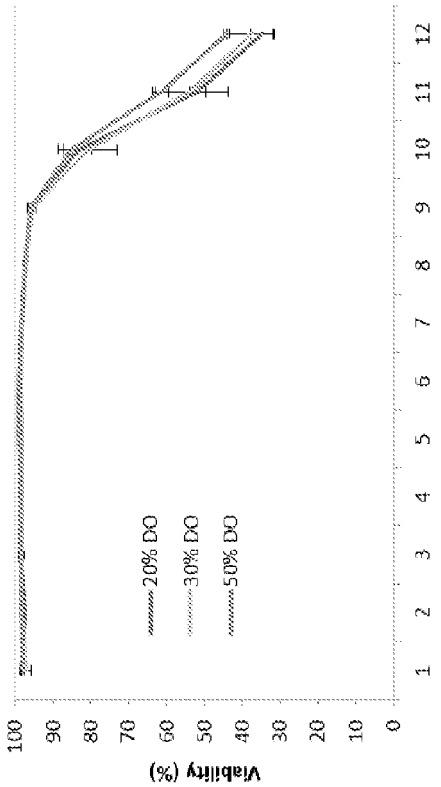


Figure 116) Effect of dissolved oxygen modulation of adalimumab producing cell line 1, media 1 at 35°C on viability (n=3, except for 30% DO n=2)

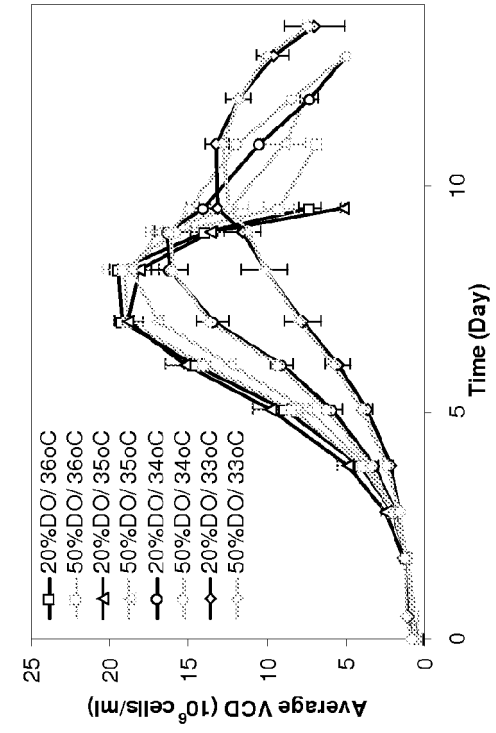


Figure 119) Effect of dissolved oxygen modulation of adalimumab producing cell line 3, media 1 on viable cell density (n=2)

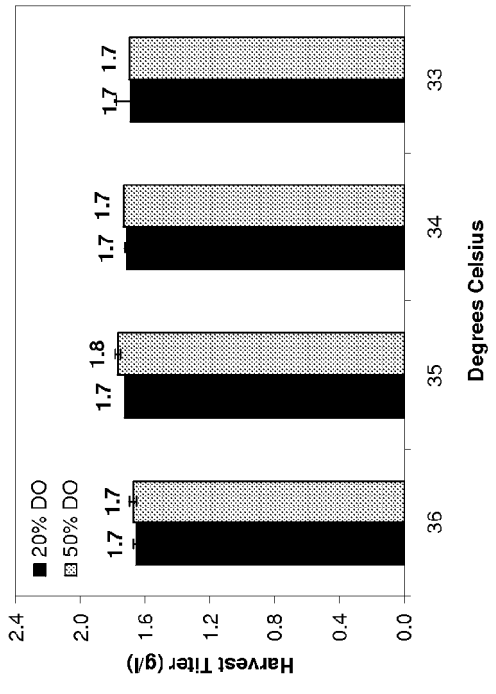


Figure 121) Effect of dissolved oxygen modulation of adalimumab producing cell line 3, media 1 on harvest titer

Figure 120) Effect of dissolved oxygen modulation of adalimumab producing cell line 3, media 1 on viability (n=2)

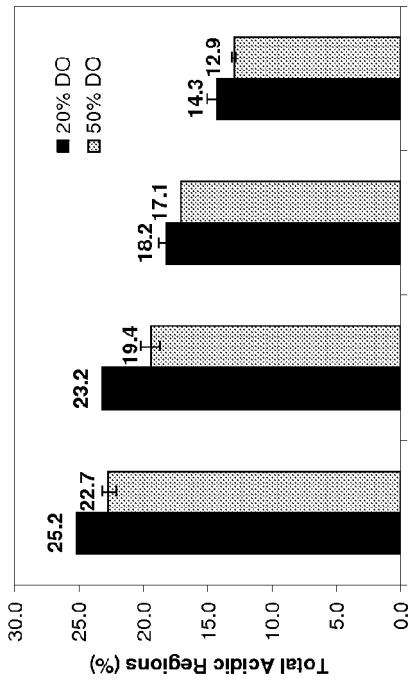


Figure 122) Effect of dissolved oxygen modulation of adalimumab producing cell line 3, media 1 on WCX 10 profile total acidic regions

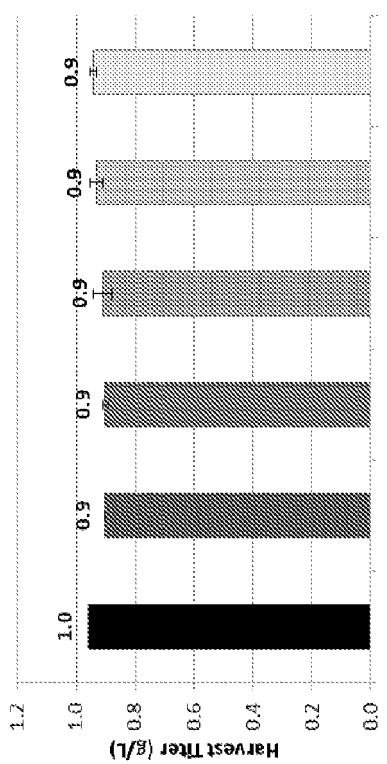


Figure 125) Effect of dissolved oxygen modulation of mAB2 producing cell line, media 1 on harvest titer

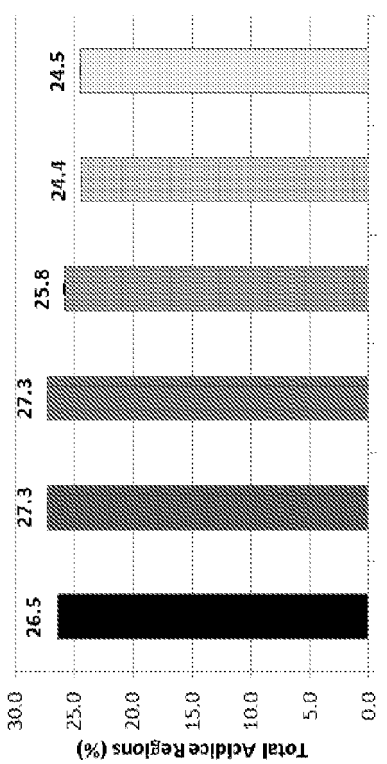


Figure 126) Effect of dissolved oxygen modulation of mAB2 producing cell line, media 1 on WCX 10 profile total acidic regions

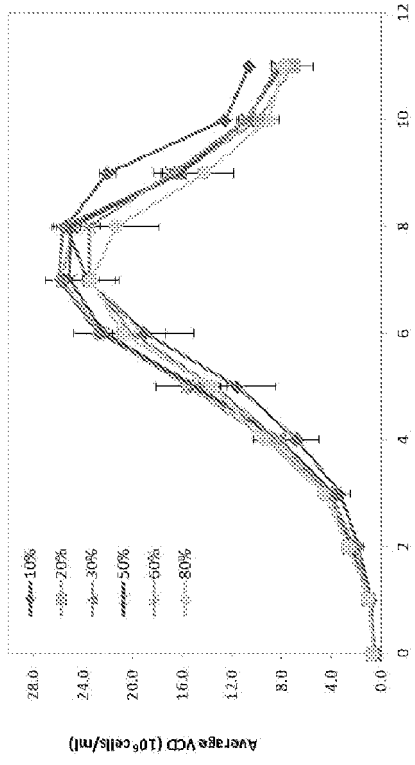


Figure 123) Effect of dissolved oxygen modulation of mAB2 producing cell line, media 1 on viable cell density (n=2)

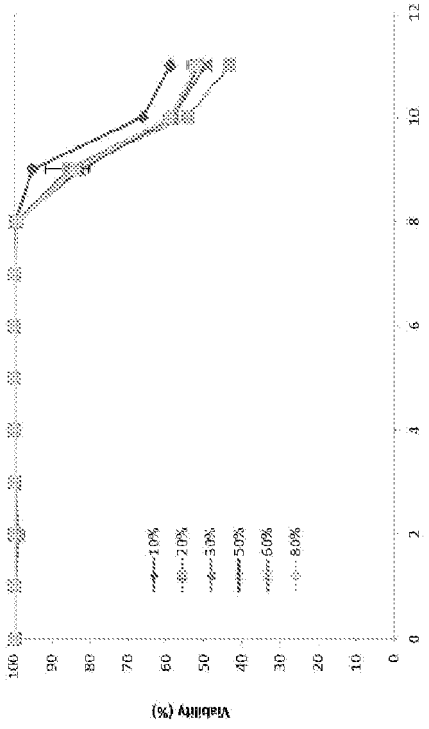


Figure 124) Effect of dissolved oxygen modulation addition of mAB2 producing cell line, media 1 on viability (n=2)

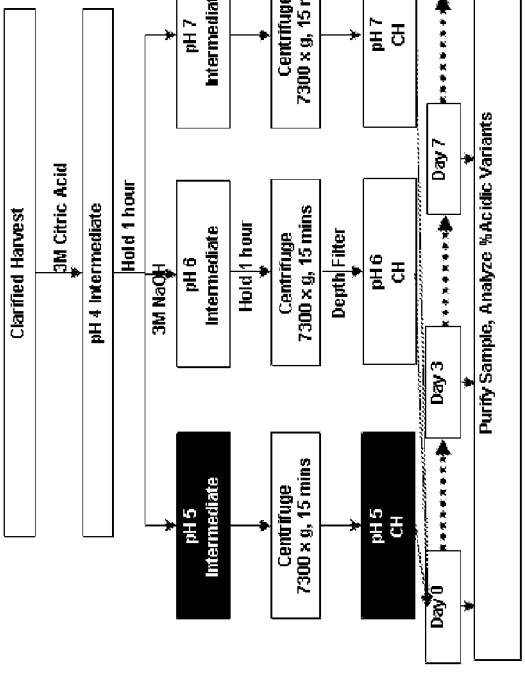


Figure 127) Acidification sample preparation scheme

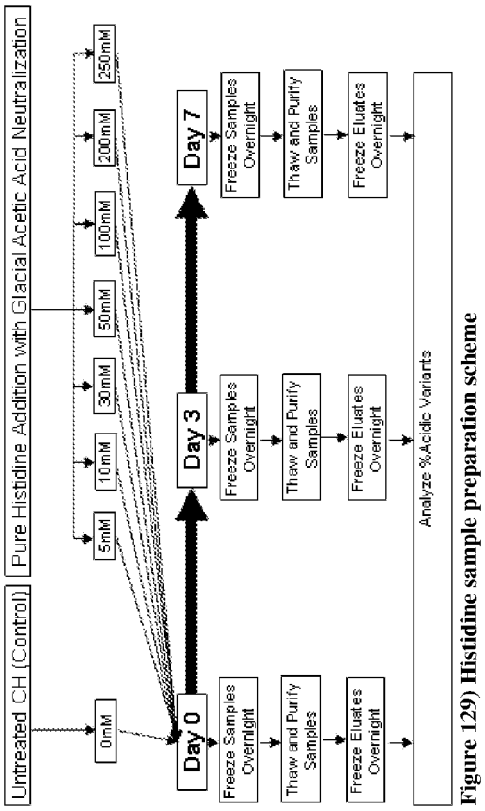


Figure 129) Histidine sample preparation scheme

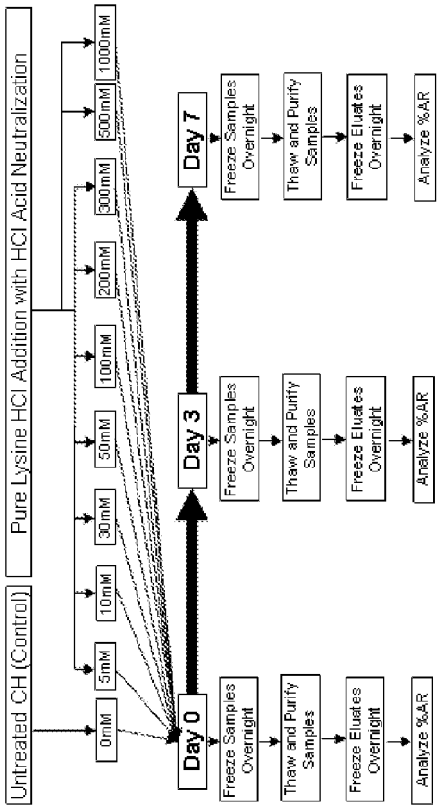


Figure 130) Lysine sample preparation scheme

Figure 128) Arginine sample preparation scheme

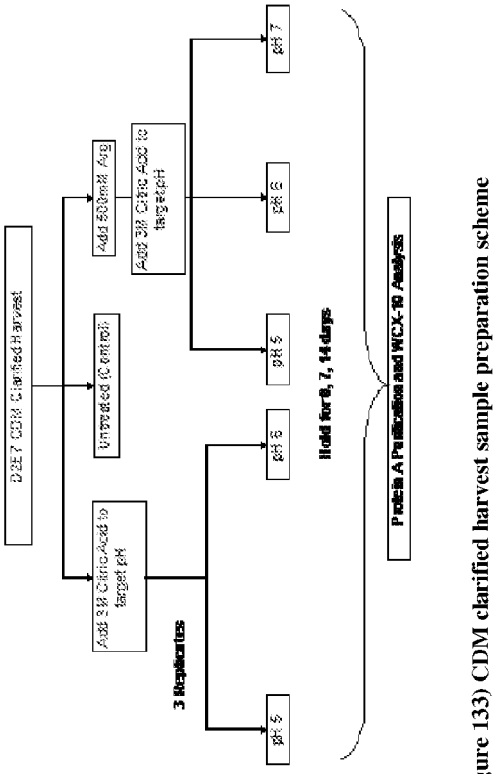


Figure 133) CDM clarified harvest sample preparation scheme

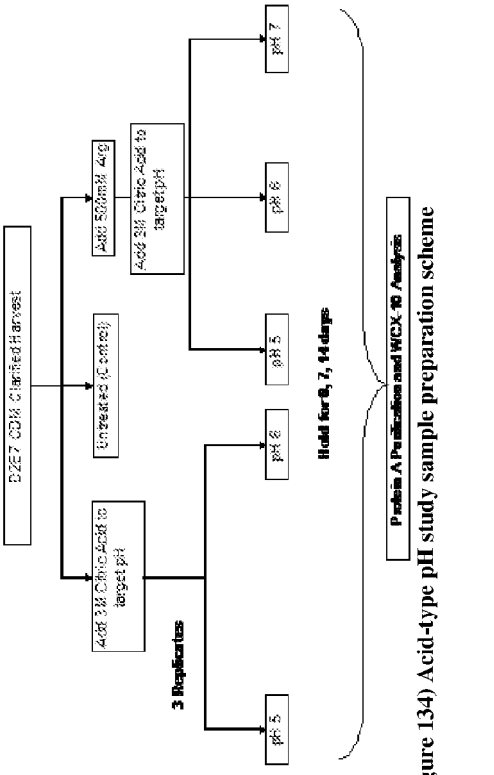


Figure 134) Acid-type pH study sample preparation scheme

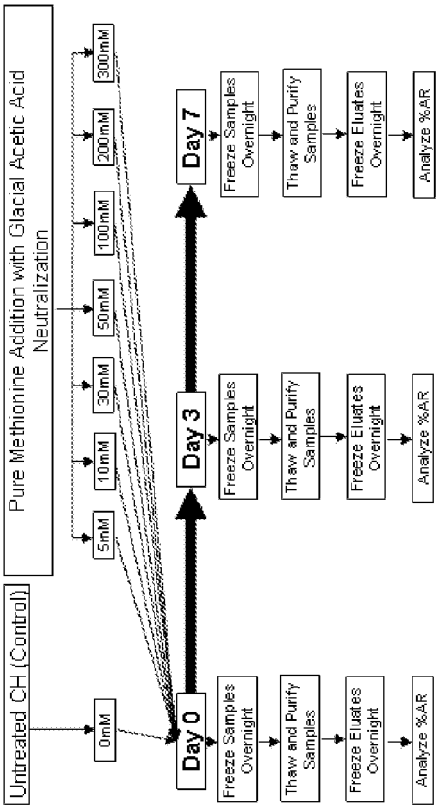


Figure 131) Methionine sample preparation scheme

Figure 132) Amino acid sample preparation scheme

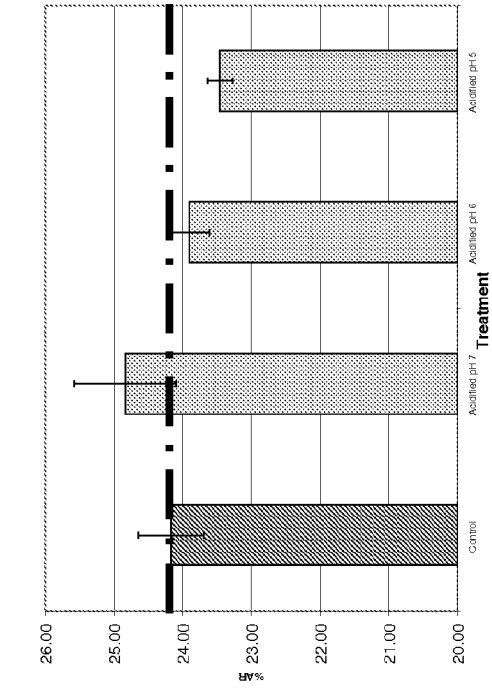


Figure 135) Effect of low pH treatment with subsequent neutralization on initial acidic variant content

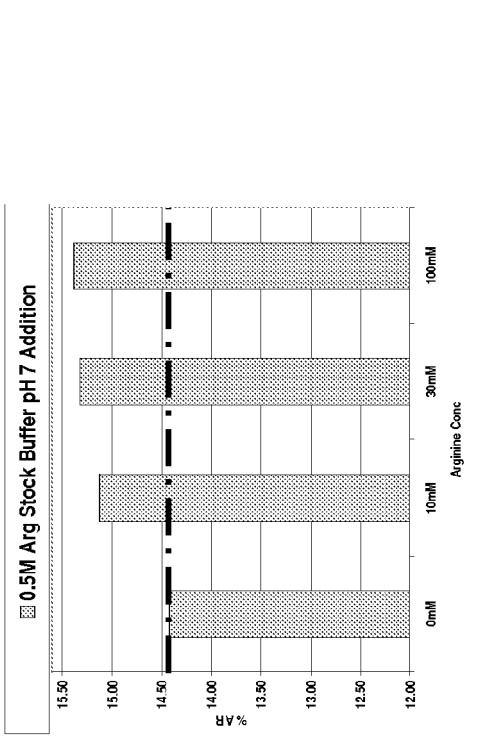


Figure 137) Effect of sample preparation method on initial acidic variant content

Figure 136) Effect of low pH treatment with subsequent neutralization on acidic variant formation rate

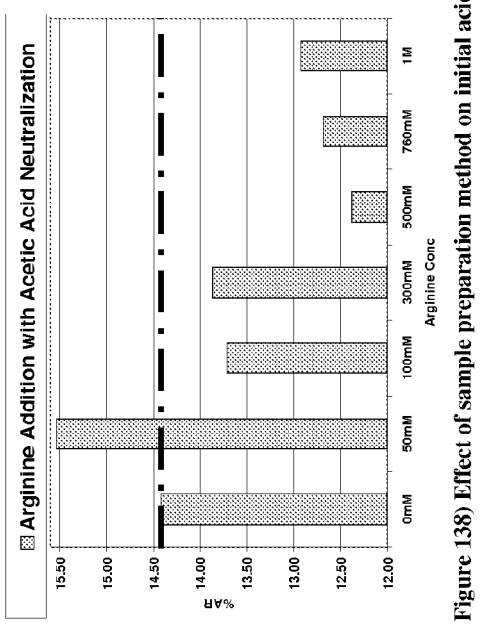


Figure 138) Effect of sample preparation method on initial acidic variant content

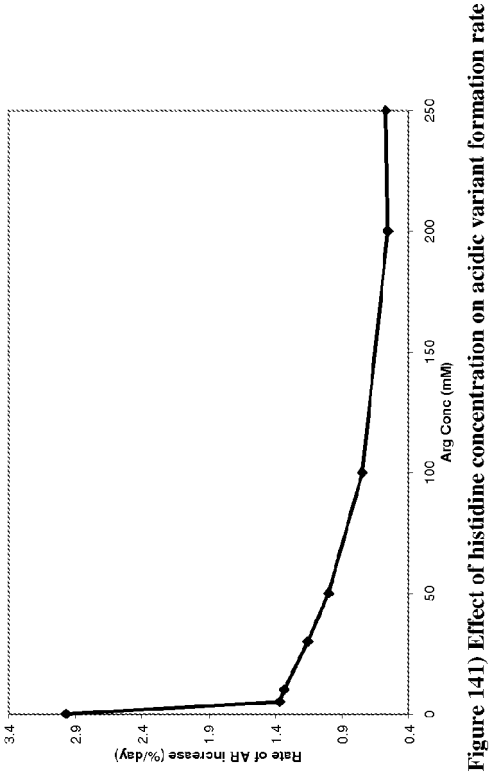


Figure 139) Dose dependent effect of arginine on reduction of acidic variant formation rate

Figure 141) Effect of histidine concentration on acidic variant formation rate

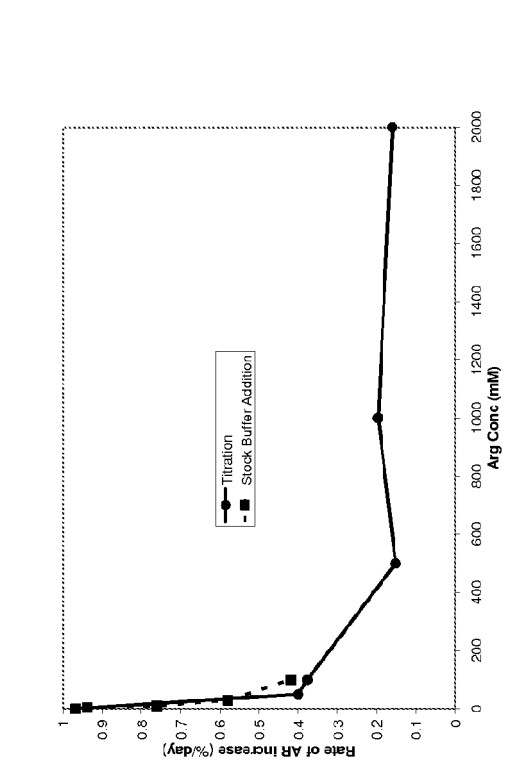


Figure 140) Effect of histidine concentration on initial acidic variant content

Figure 142) Effect of lysine on initial acid variant content

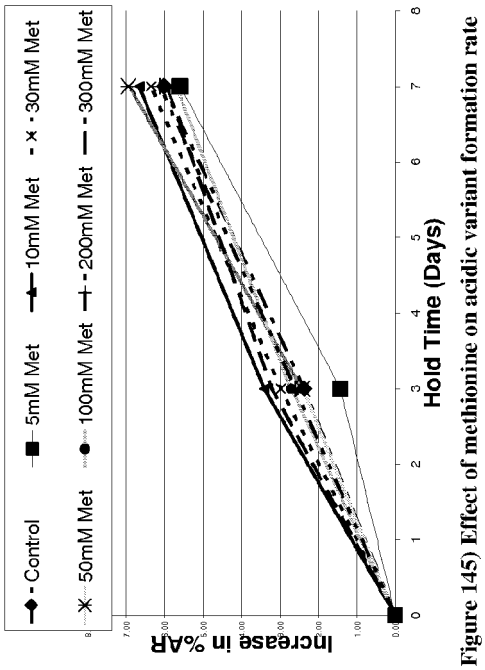


Figure 145) Effect of methionine on acidic variant formation rate

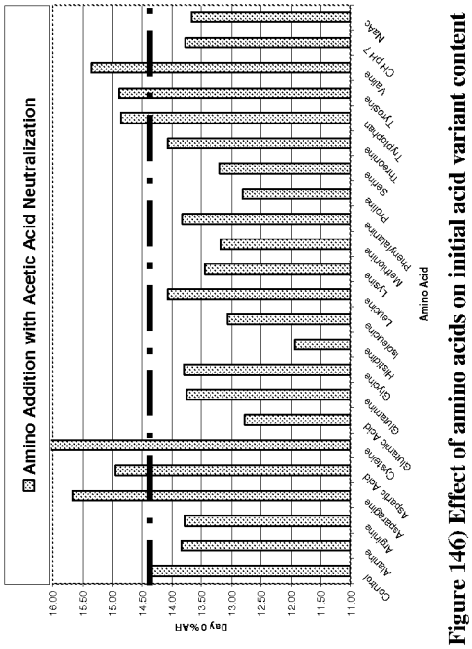


Figure 146) Effect of amino acids on initial acid variant content

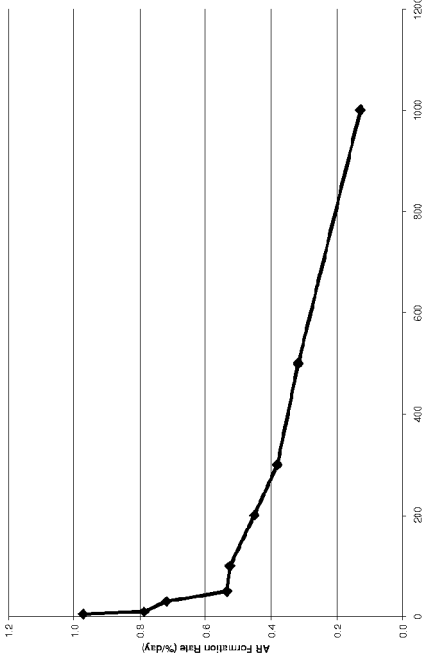


Figure 143) Effect of lysine on acidic variant formation rate

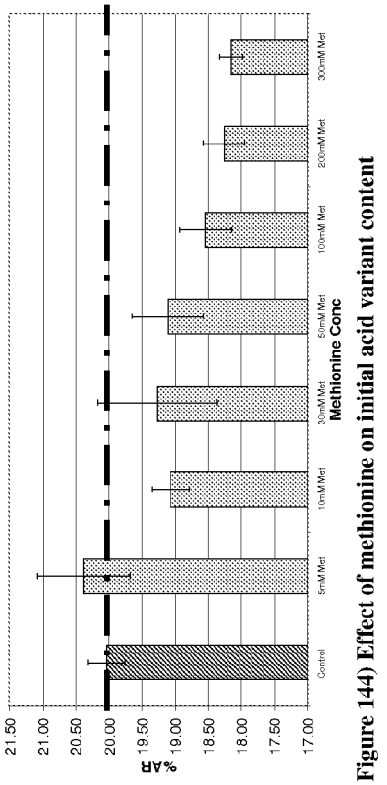


Figure 144) Effect of methionine on initial acid variant content

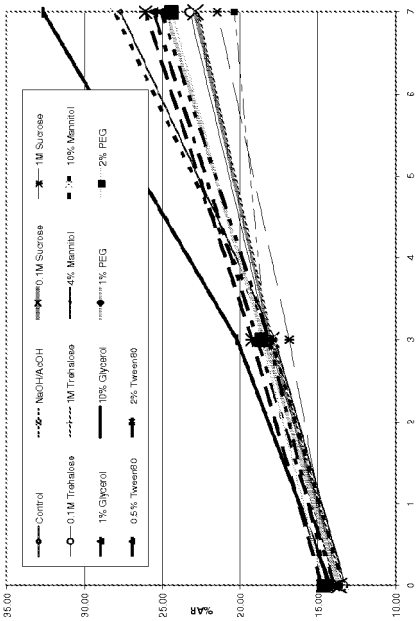


Figure 149) Effect of alternative additives on acidic variant formation rate

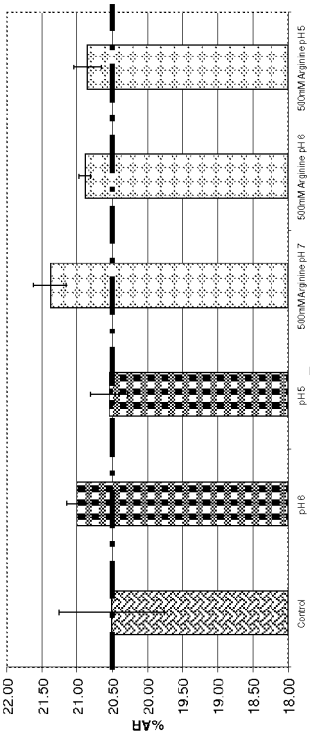


Figure 150) Effect of low pH/arginine treatment on adalimumab CDM initial acid variant content

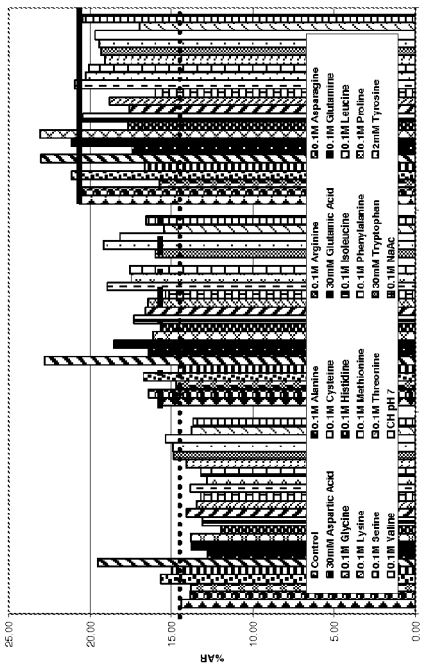


Figure 147) Effect of amino acids on acidic variant formation rate

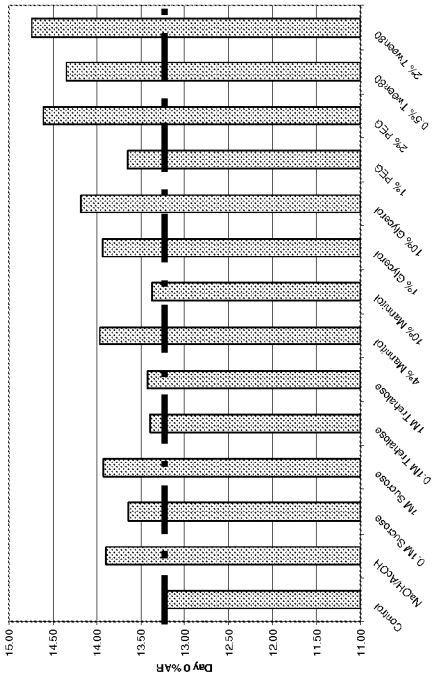


Figure 148) Effect of alternative additives on initial acid variant content

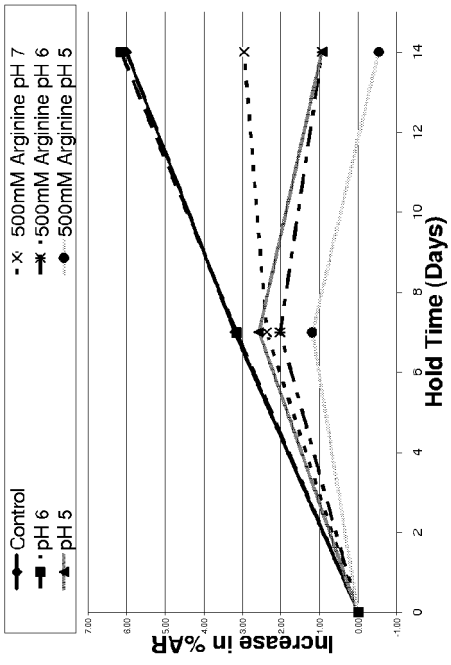


Figure 151) Effect of low pH/arginine treatment on adalimumab CDM acidic variant formation rate

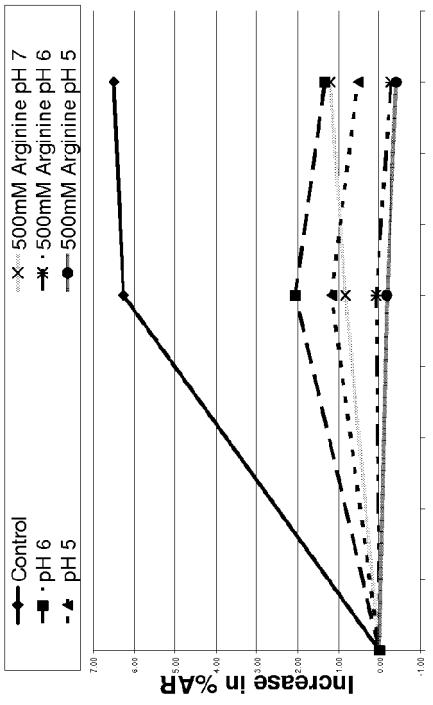


Figure 153) Effect of low pH/arginine treatment on mAb B hydrolysate acidic variant formation rate

Figure 152) Effect of low pH/arginine treatment on mAb B hydrolysate initial acid variant content

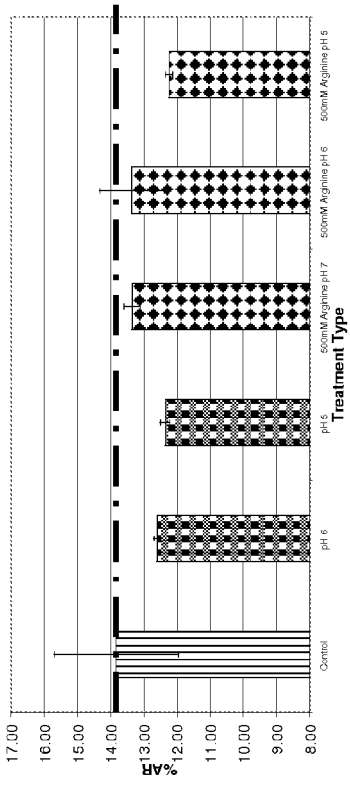


Figure 154) Effect of low pH/arginine treatment on mAb C hydrolysate initial acid variant content

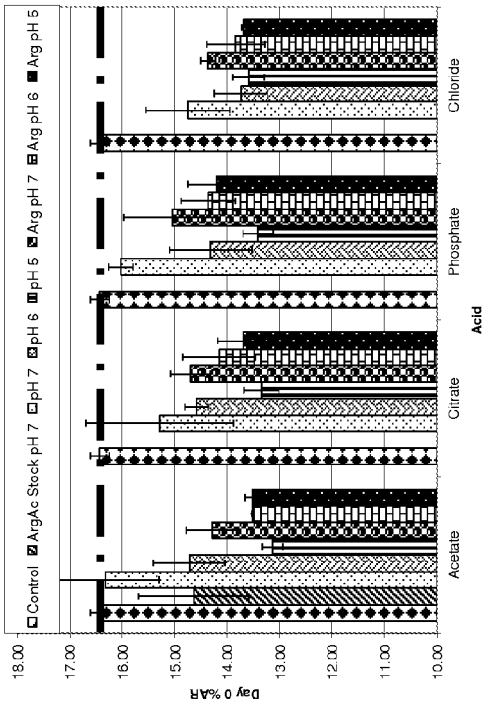


Figure 156) Effect of acid type/pH on acid variant content

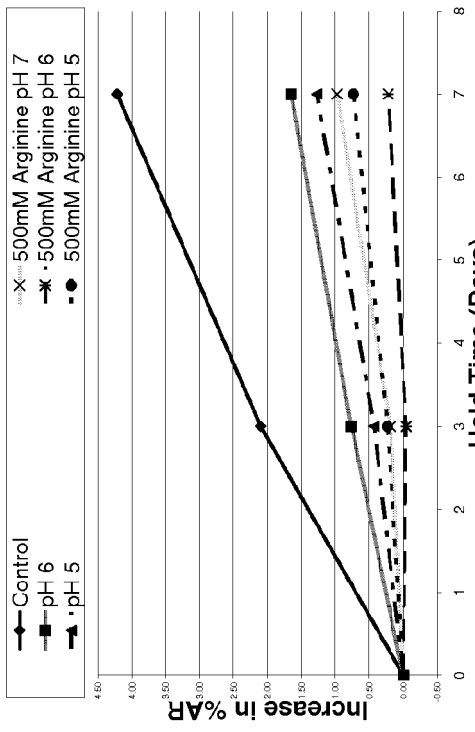


Figure 155) Effect of low pH/arginine treatment on mAb C hydrolysate acidic variant formation rate

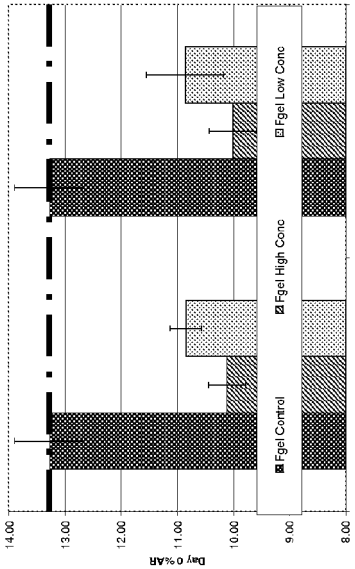


Figure 158) Effect of acid concentration on acid variant content

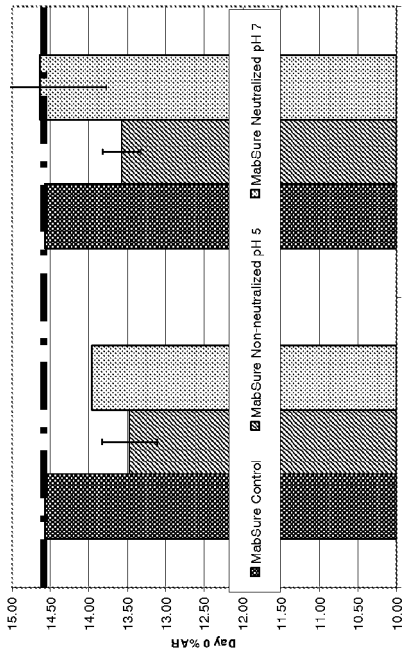


Figure 159) Effect of neutralization on acid variant content

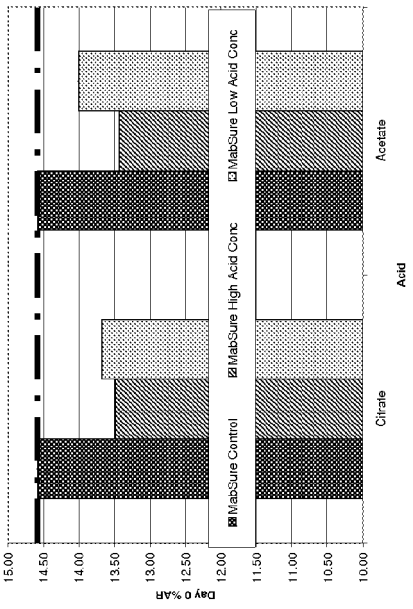


Figure 157) Effect of acid concentration on acid variant content

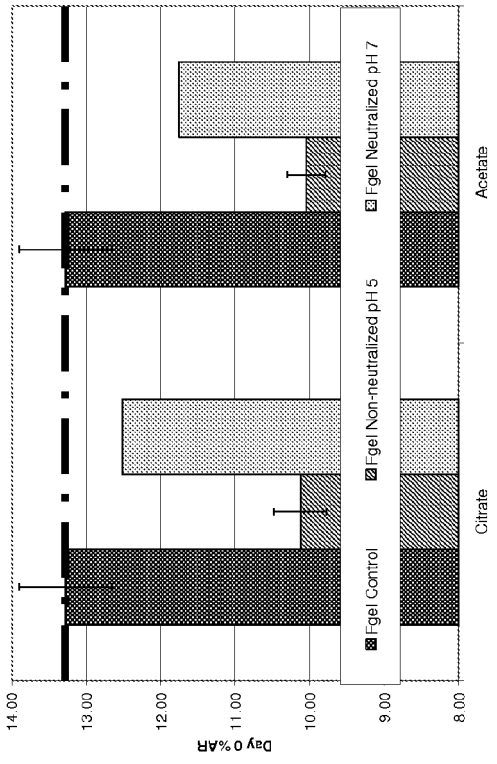


Figure 160) Effect of neutralization on acid variant content

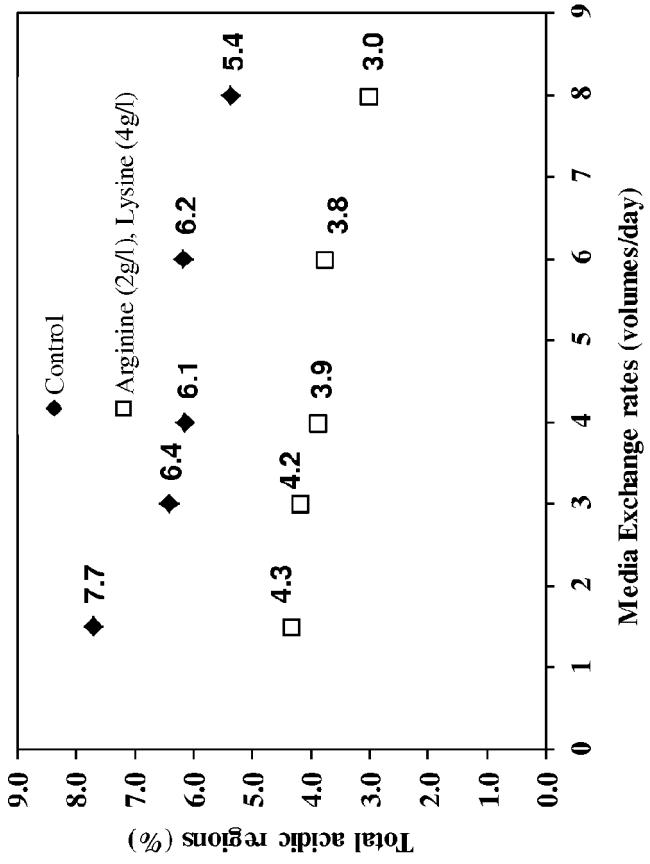
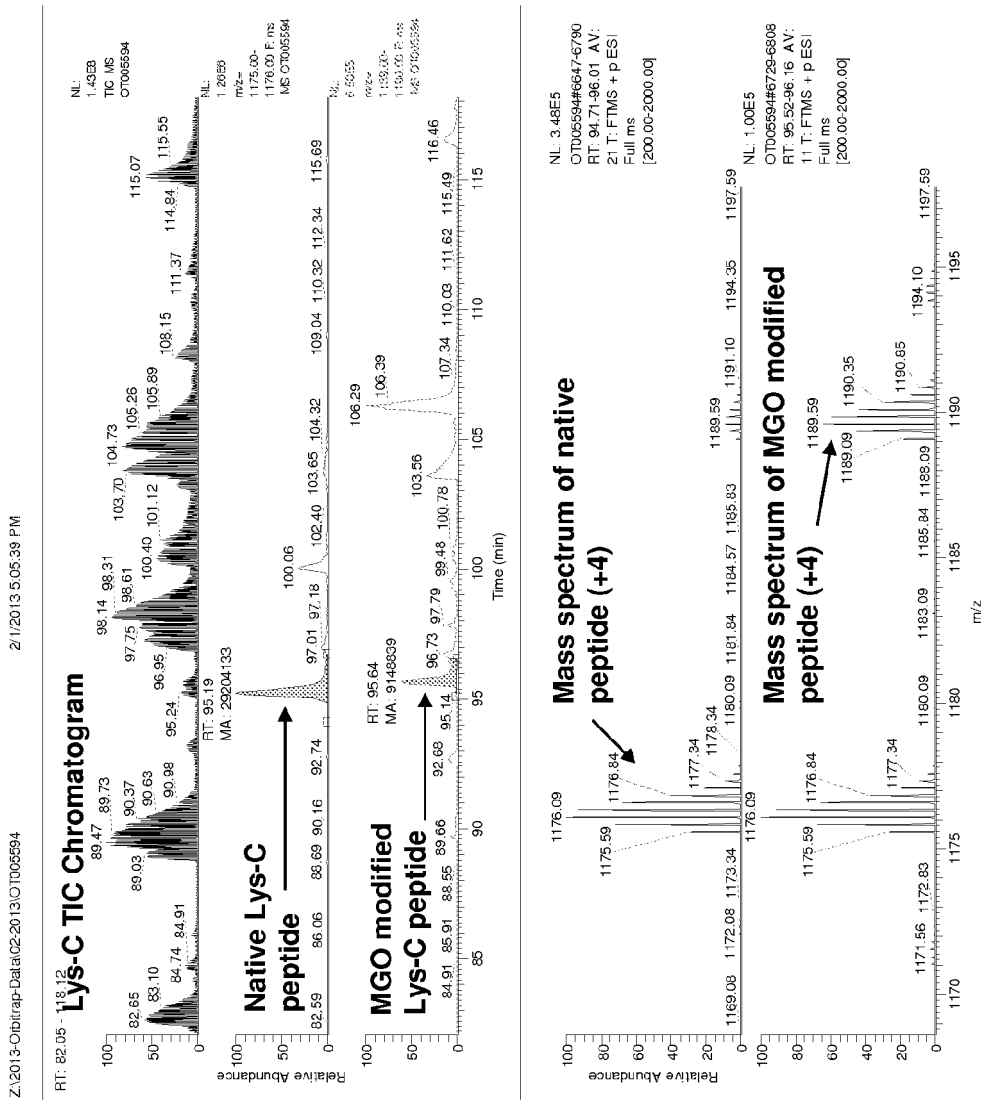

Total acidic regions (%)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
Media Exchange rates (volumes/day)
Control
Arginine (2g/l), Lysine (4g/l)
7.7
6.4
6.1
6.2
5.4
4.3
4.2
3.9
3.8
3.0

Figure 161

Figure 162: Total ion current of the Lys-C peptide map and mass filter traces of a modified and non-modified peptides used for quantification. Spectra below confirm identity.



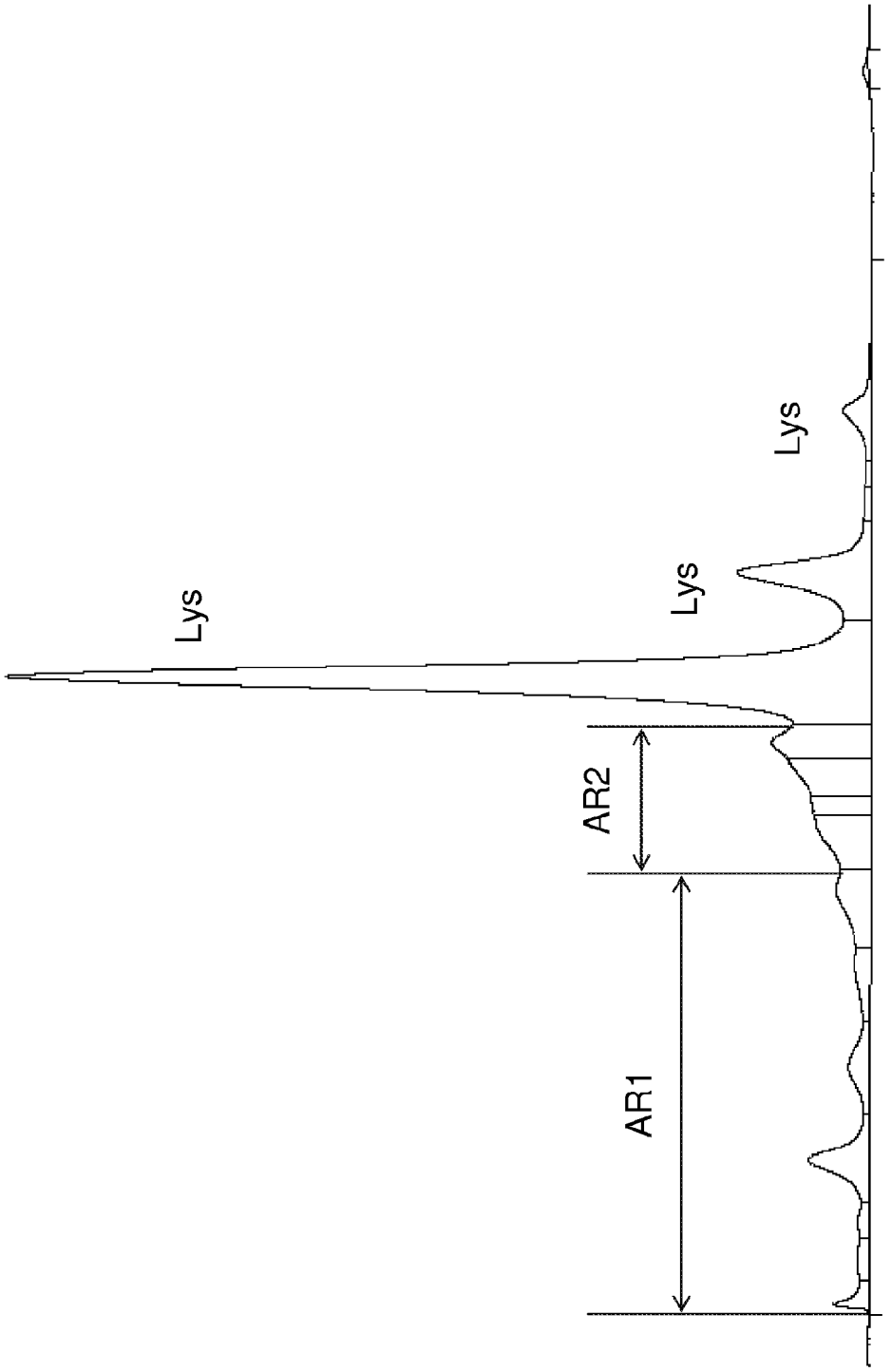


Figure 163

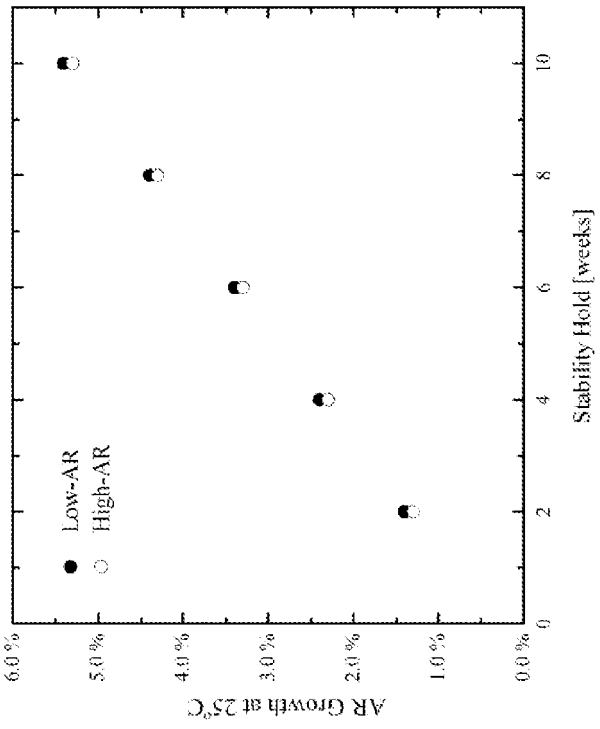


Figure 164: AR Growth at 25°C

Figure 165: Process Chromatogram of pH Gradient Elution

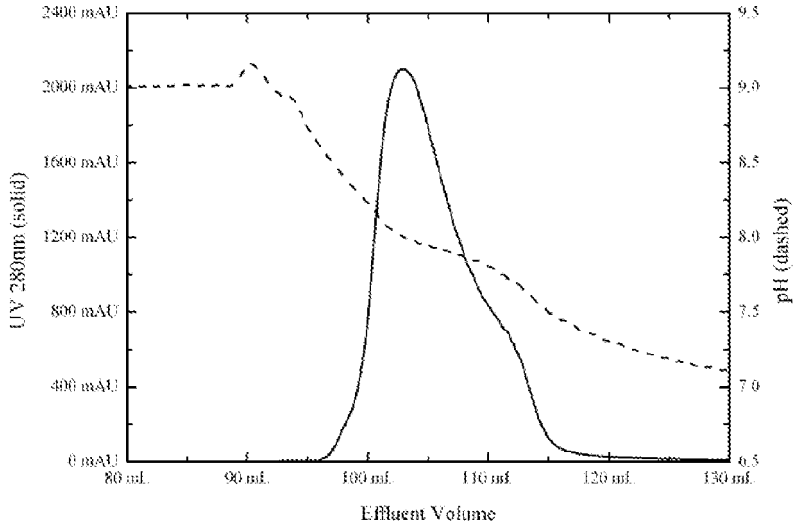


Figure 166: Process Chromatogram of Linear Gradient Elution by increasing Anion Concentration

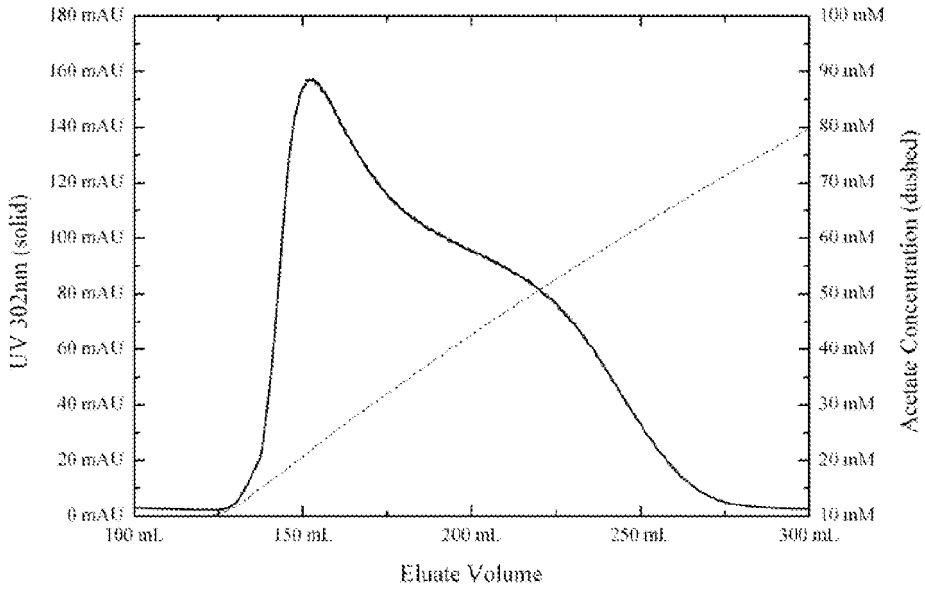


Figure 167: Process Chromatogram of Fractionation of 300 g/L Load and Wash

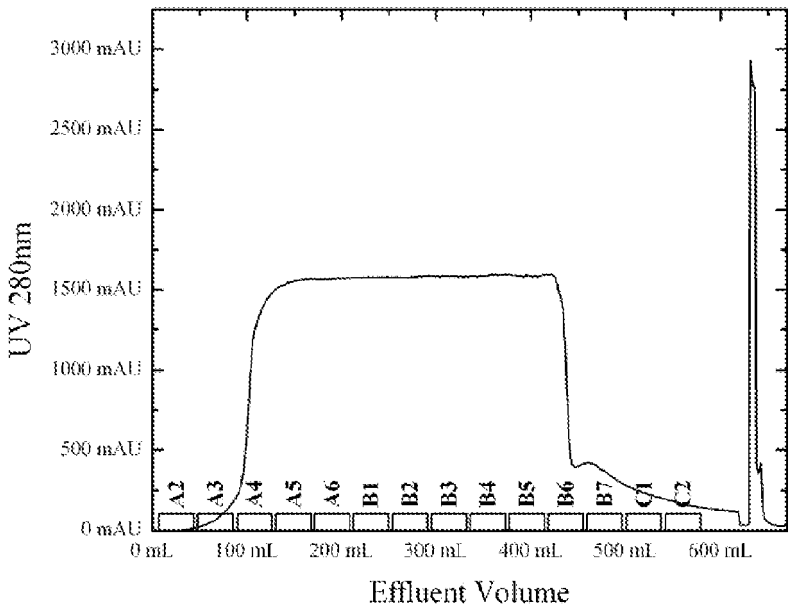


Figure 168: Effect of pH on AR reduction

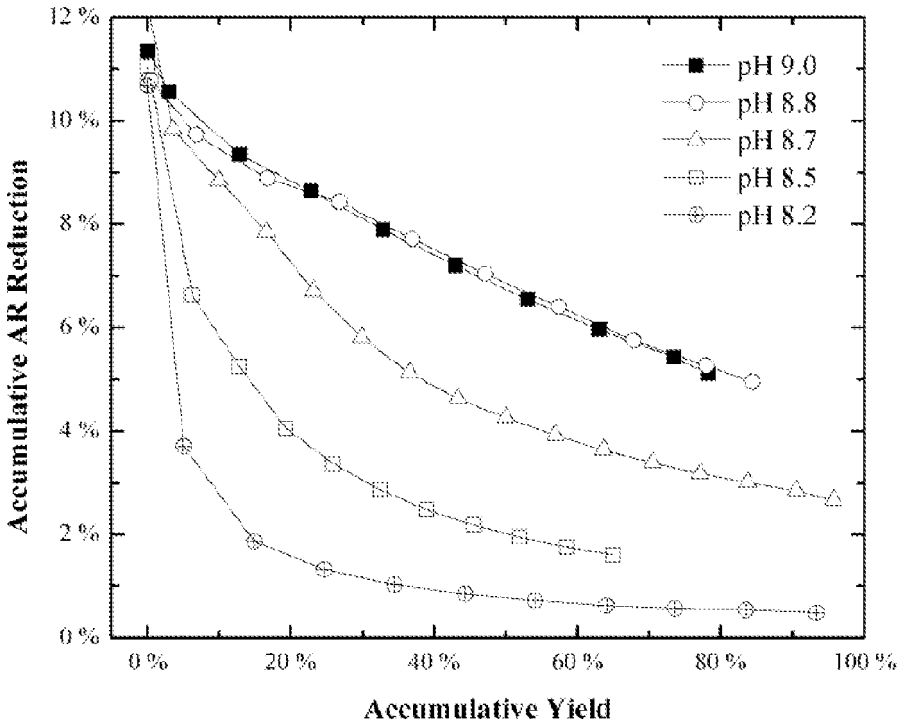


Figure 169: Process Chromotogram at Different Salt (Cation) Concentrations

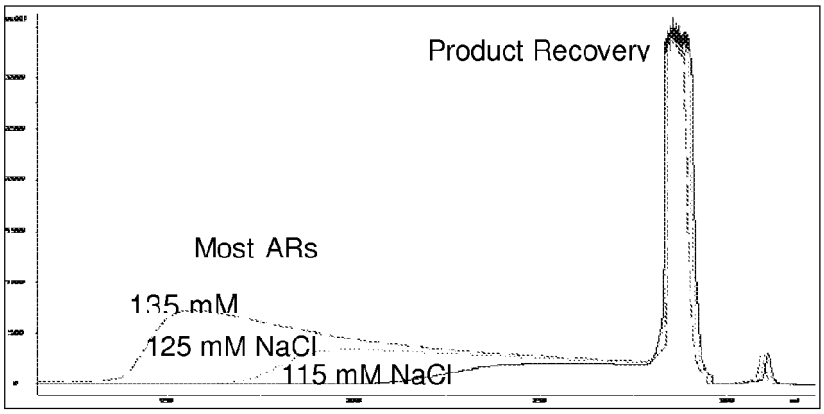


Figure 170: Recovery versus AR reduction

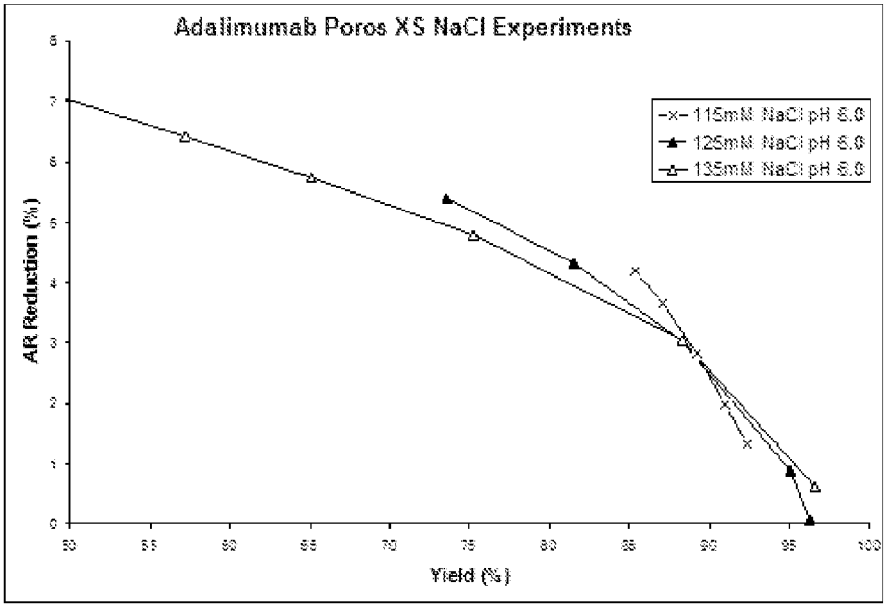


Figure 171: WCX-10 Profile of Glycated Load Material and CEX Eluate

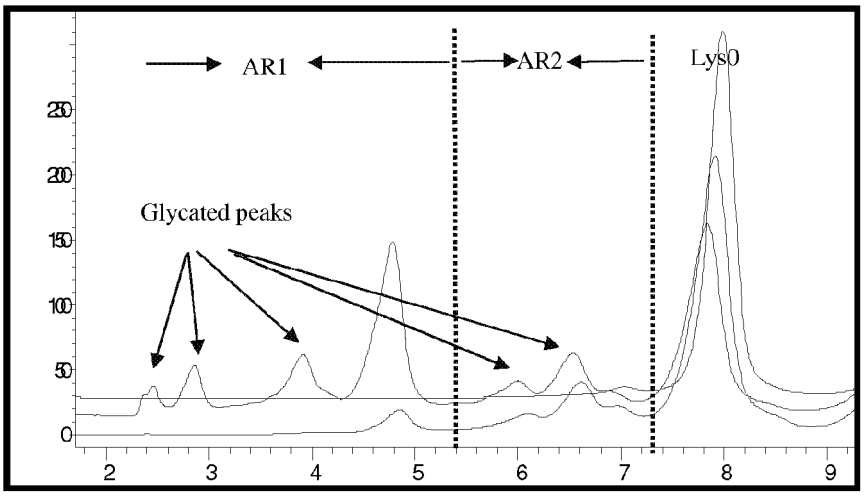


Figure 172: WCX 10 Profile of MGO Modified Load Material and Eluate from CEX Column Employing Toyo Pearl MX TRP 650M Resin

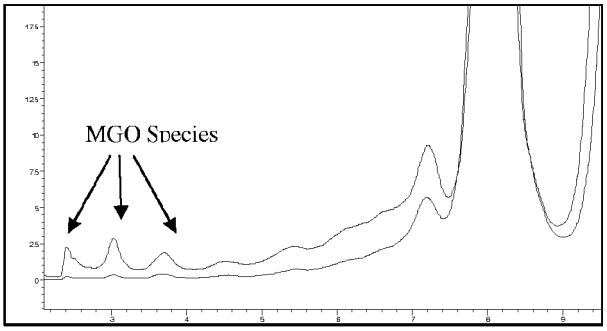


Figure 173: Change in Lysine Distribution during CEX chromatography – effect of Tris concentration

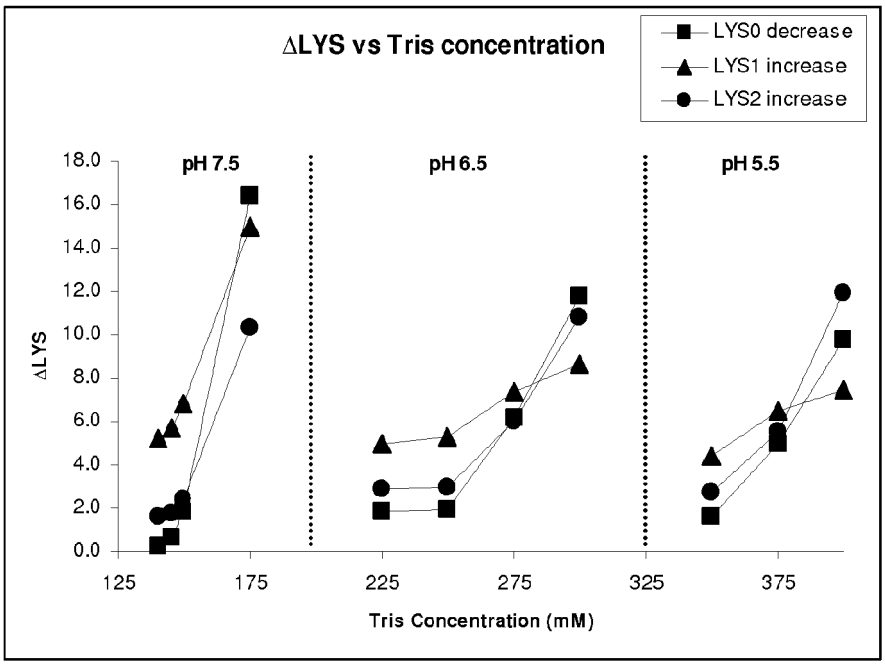
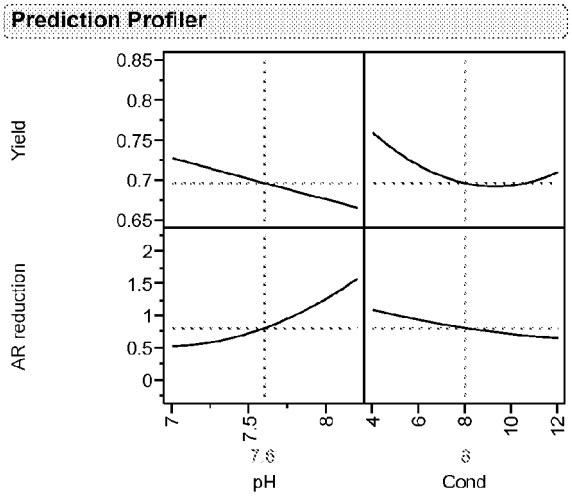


Figure 174: Effect of pH and conductivity on adalimumab AR reduction and recovery yield



Note: AR reductions and protein recovery yields were calculated based on the Flow Through fractions at about loading 200 g protein per L of resin

Figure 175: AR Reduction Achieved with the Corresponding Protein Recovery

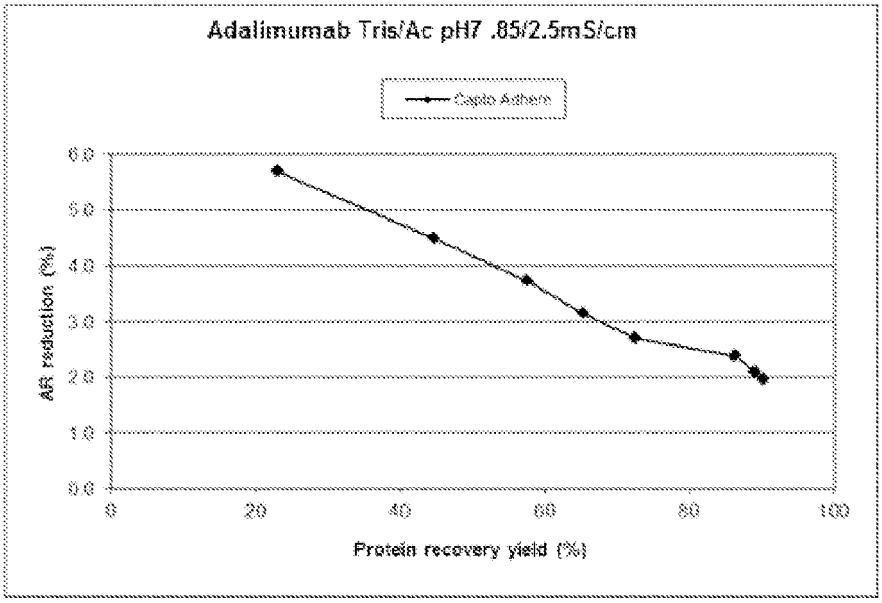


Figure 176: Total adalimumab Protein concentration levels and AR levels during Flow Through and Wash

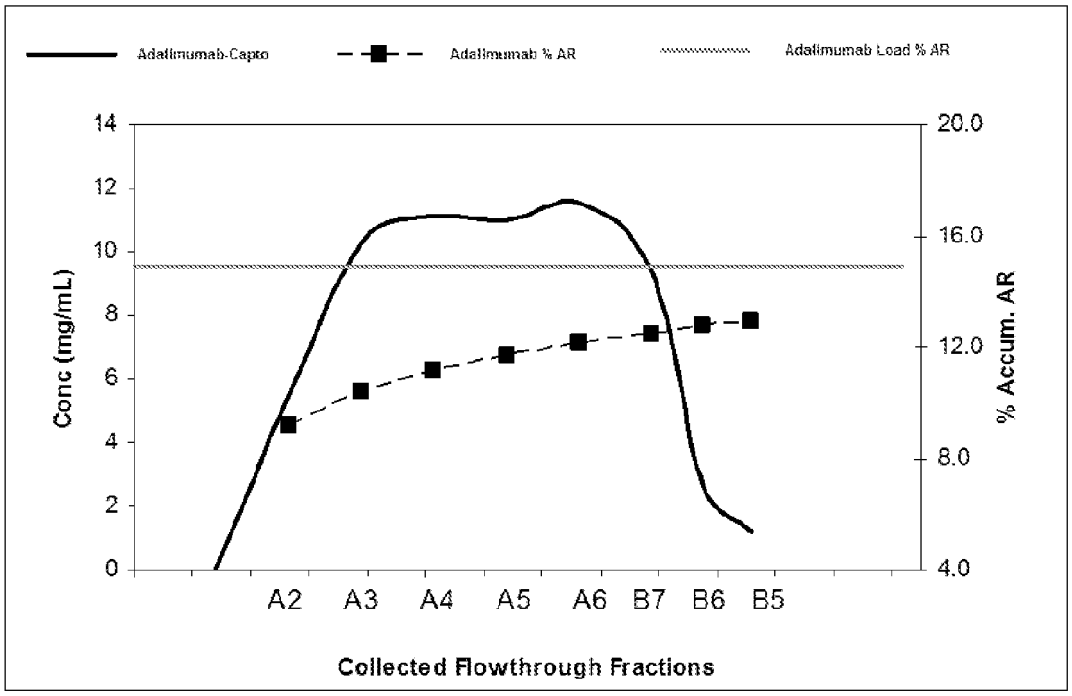


Figure 177: Total mAb B Protein concentration levels and AR levels during Flow Through and Wash

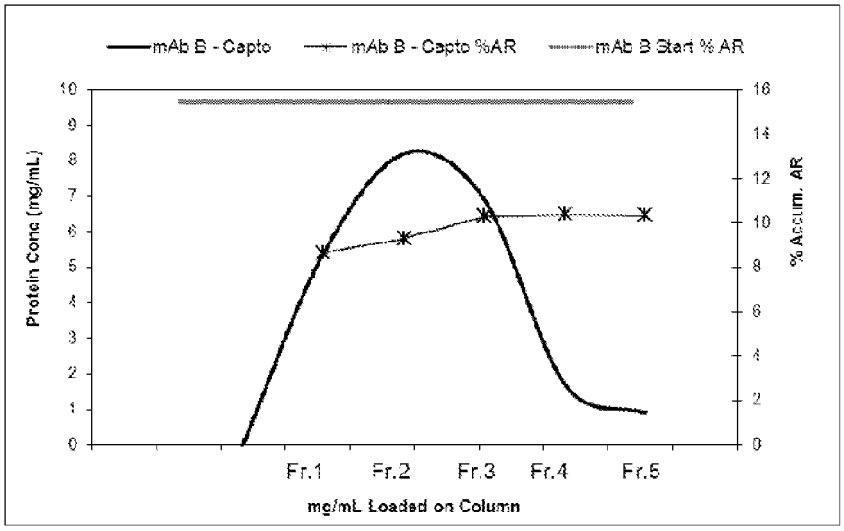


Figure 178: Total mAb C Protein concentration levels and AR levels during Flow Through and Wash

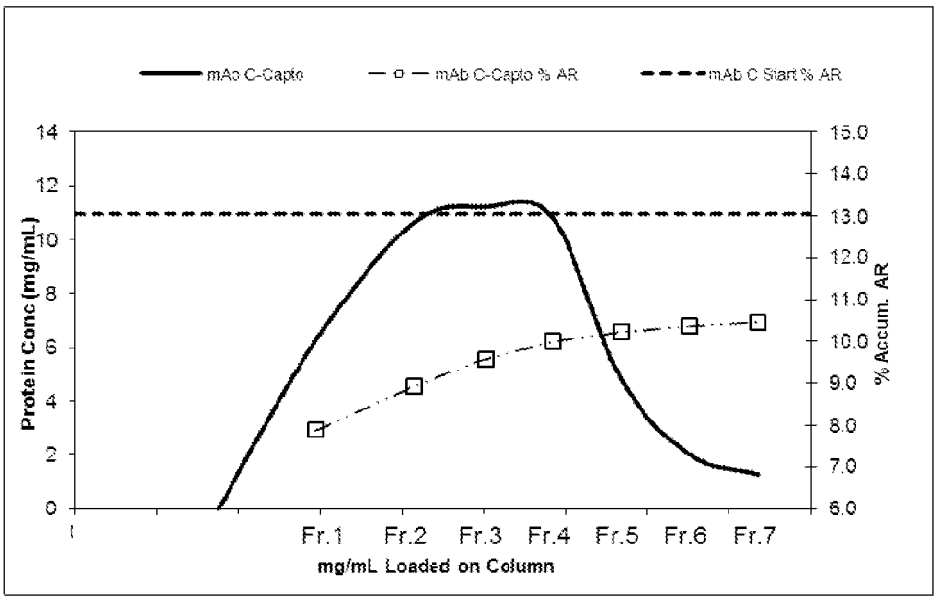


Figure 179: Cumulative % AR Breakthrough of mAb C on Different MM Resins

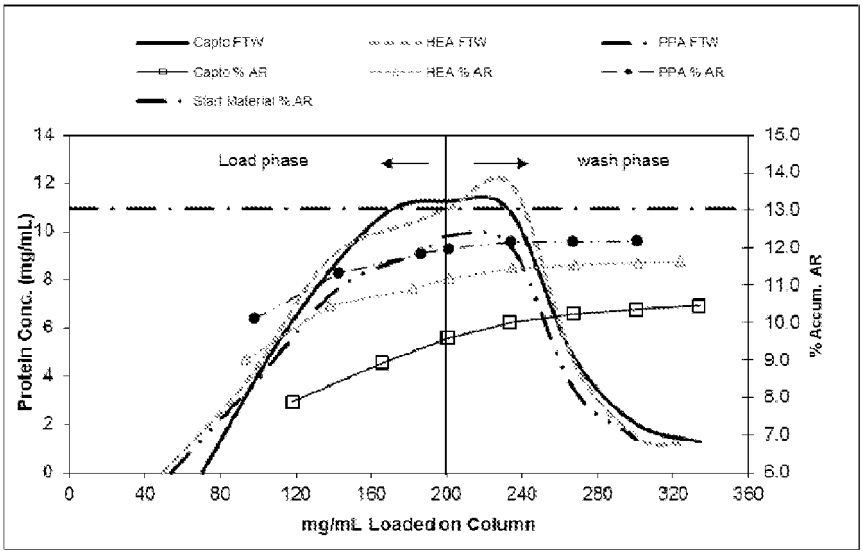


Figure 180: Impact of pH-pI and Conductivity on adalimumab AR Reduction

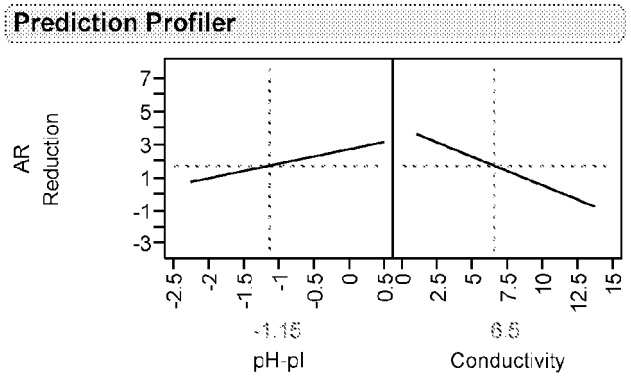


Figure 181: Impact of pH-pI and Conductivity on mAb B AR Reduction

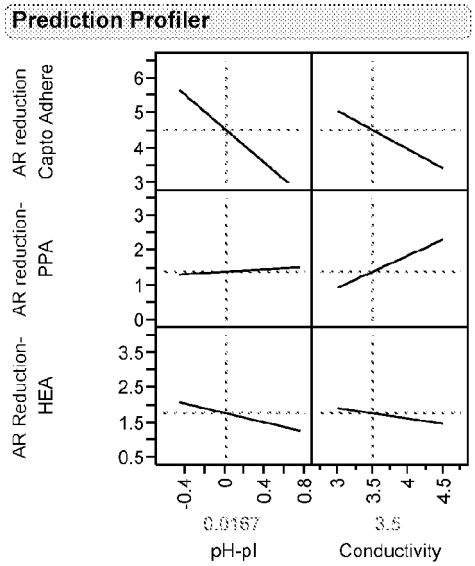


Figure 182: Impact and trend of pH-pI on mAb C AR reduction with multiple resins

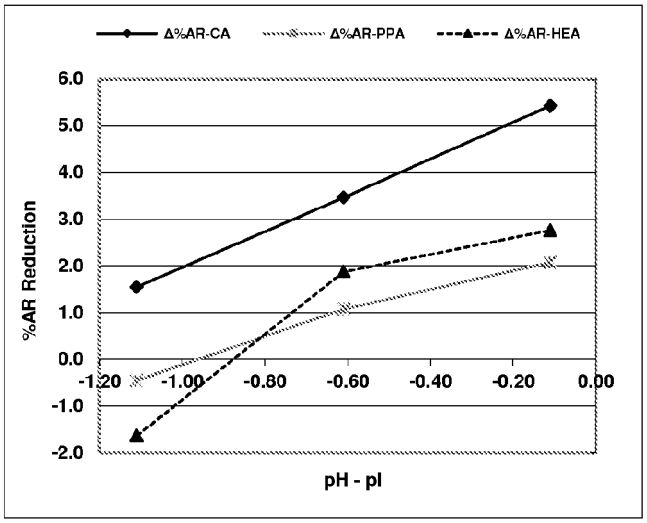
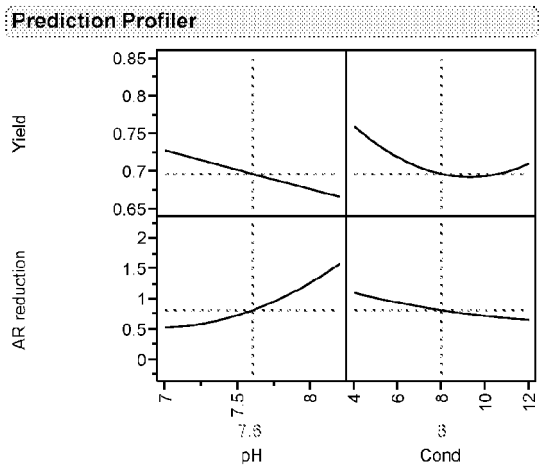
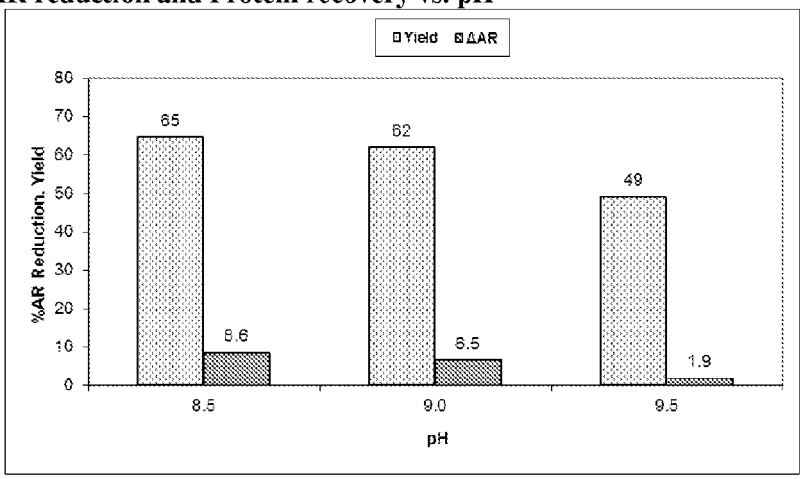


Figure 183: Effect of pH and Conductivity on AR reduction and Yield



Note: AR reductions and protein recovery yields were calculated based on the Flow Through fractions at about loading 200 g protein per L of resin

Figure 184: AR reduction and Protein recovery vs. pH



Note: adalimumab in Protein A eluate containing 20 mM acetate was adjusted to target pH with 400 mM Arginine solution. The final conductivity was 1 mS/cm.

Figure 185: Effect of pH, conductivity and protein load amount on AR reduction and Yield

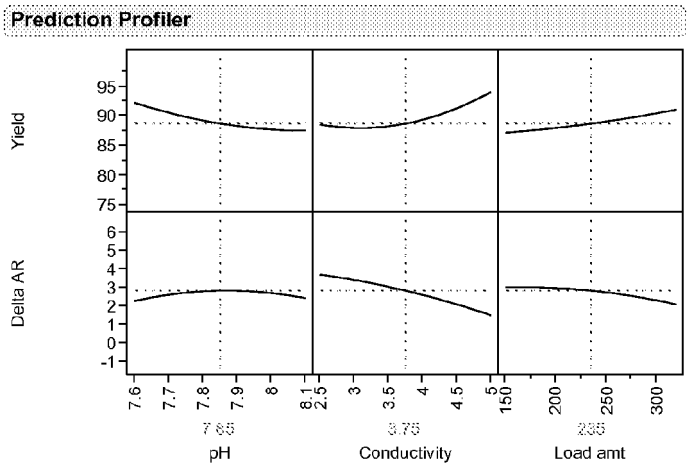


Figure 186: Effect of pH, conductivity and protein load amount on AR reduction and Yield

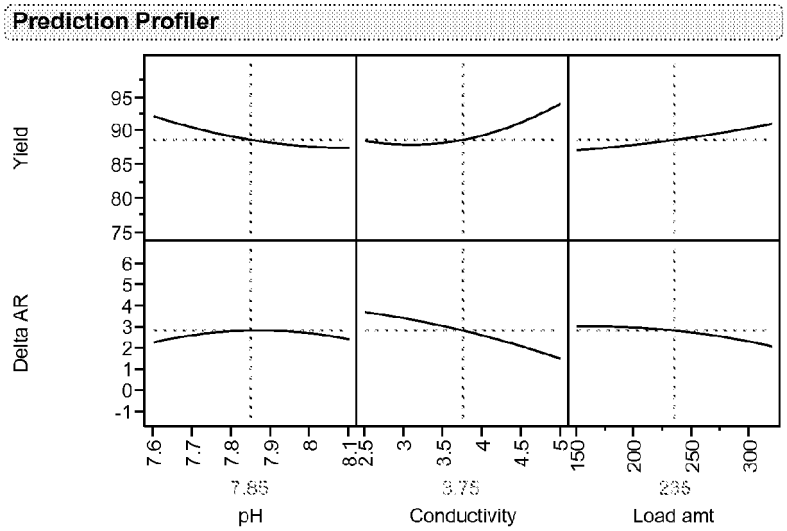


Figure 187: Effect of AEX adsorbent pKa

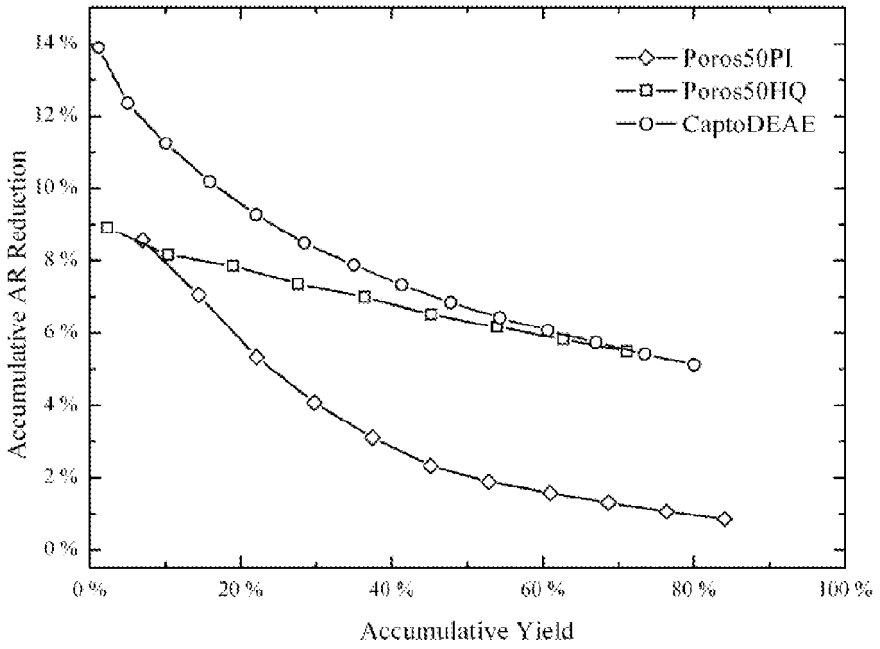


Figure 188
Acidic Variants Summary
Charge
afucosylation
deamidation
citric acid
MGO
glycation
Structure
glycosylation
acetonation
Fragmentation
HC VD missing
C-terminal truncation
Fab fragment
Acidic Region 1
glycation
deamidation
Acidic Region 2
Formed during cell culture
Formed during storage
Formed during both

Figure 189

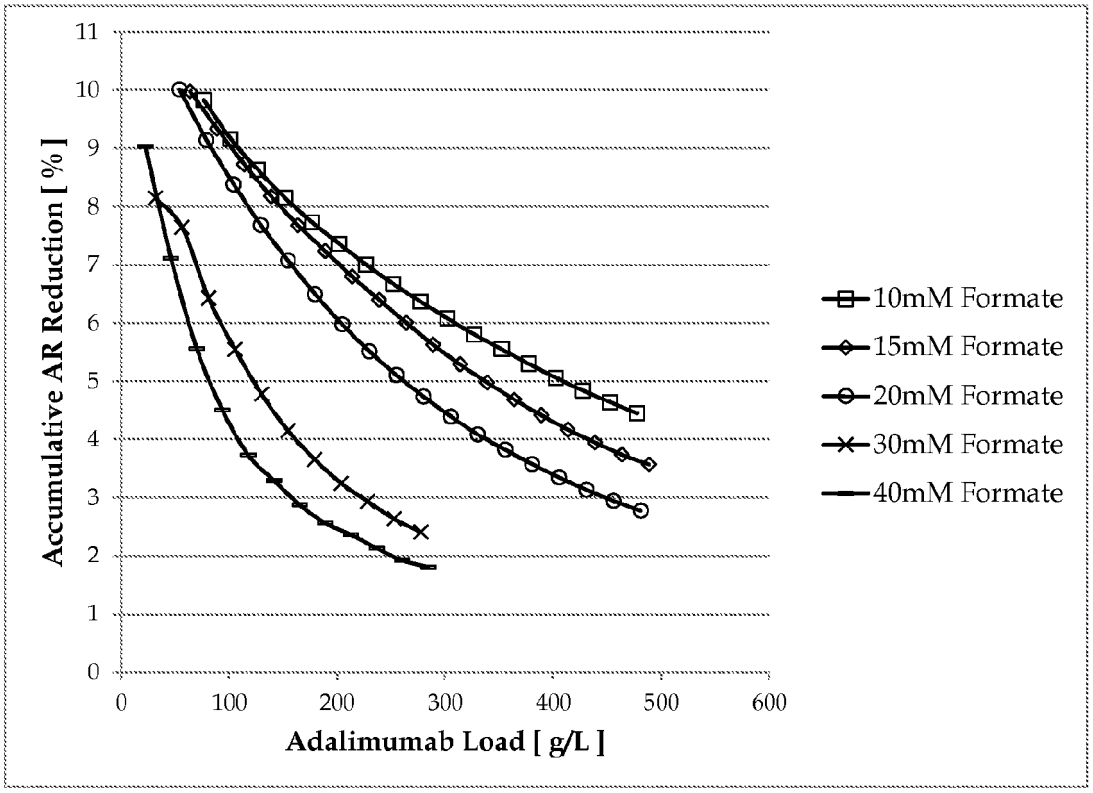

Accumulative AR Reduction [%]
Adalimumab Load [g/L]
0
1
2
3
4
5
6
7
8
9
10
11
0
100
200
300
400
500
600
10mM Formate
15mM Formate
20mM Formate
30mM Formate
40mM Formate

Figure 190

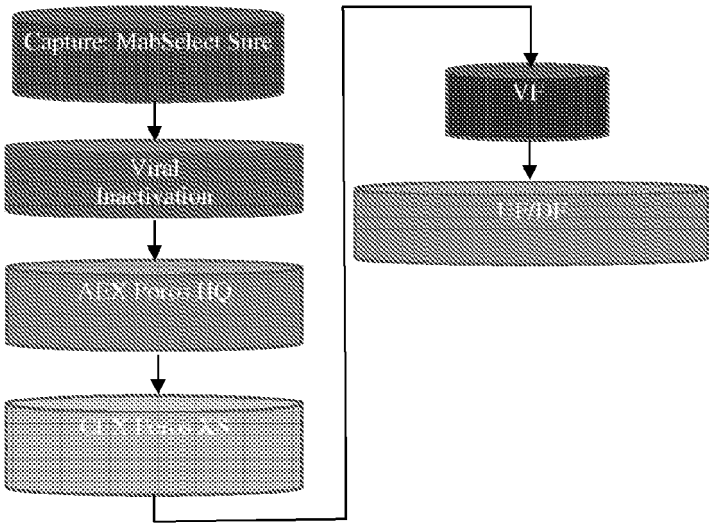

Capture: MabSelect Sure
Viral Inactivation
AEX Force HQ
VI
TFF/DF

Figure 191

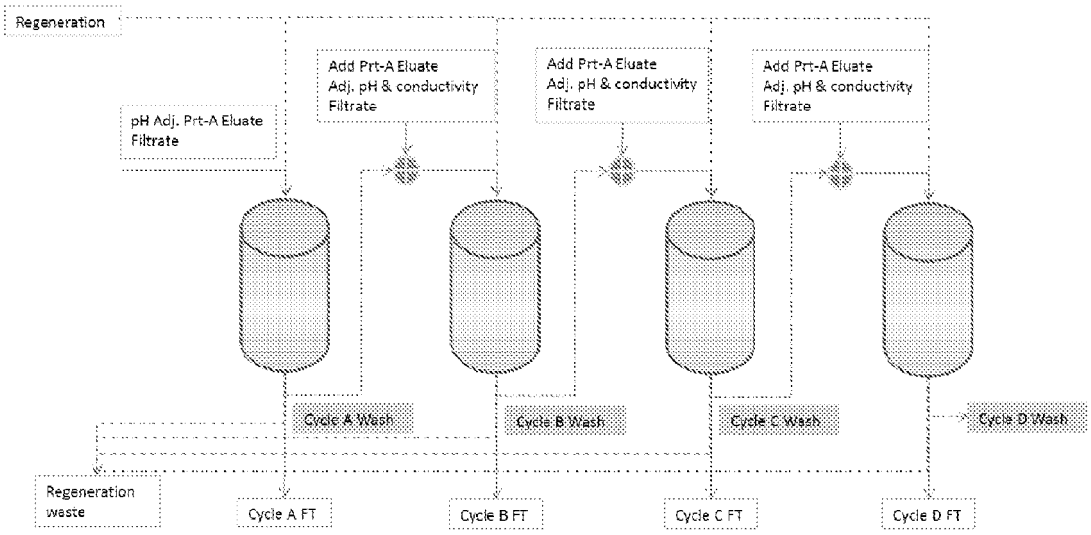

Regeneration
pH Adj. Prt-A Eluate Filtrate
Add Prt-A Eluate Adj. pH & conductivity Filtrate
Add Prt-A Eluate Adj. pH & conductivity Filtrate
Add Prt-A Eluate Adj. pH & conductivity Filtrate
Cycle A Wash
Cycle B Wash
Cycle C Wash
Cycle D Wash
Regeneration waste
Cycle A FT
Cycle B FT
Cycle C FT
Cycle D FT

Figure 192

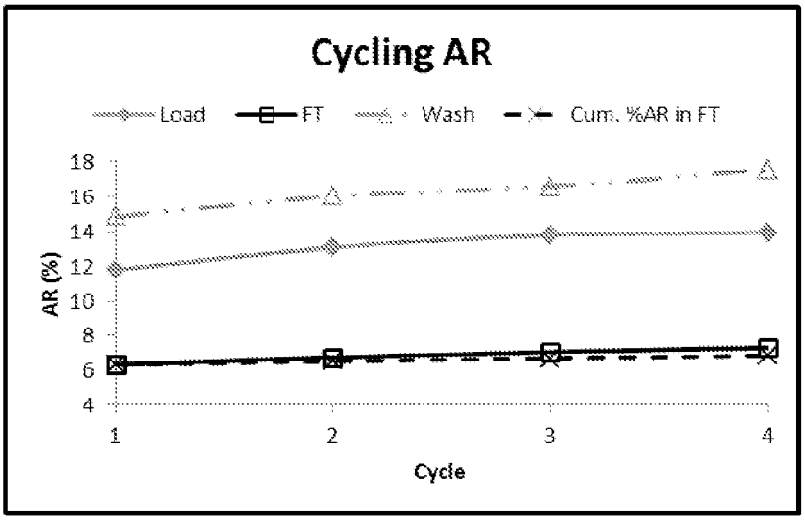

Cycling AR
Load
FT
Wash
Cum. %AR in FT
AR (%)
18
16
14
12
10
8
6
4
1
2
3
4
Cycle

Figure 193

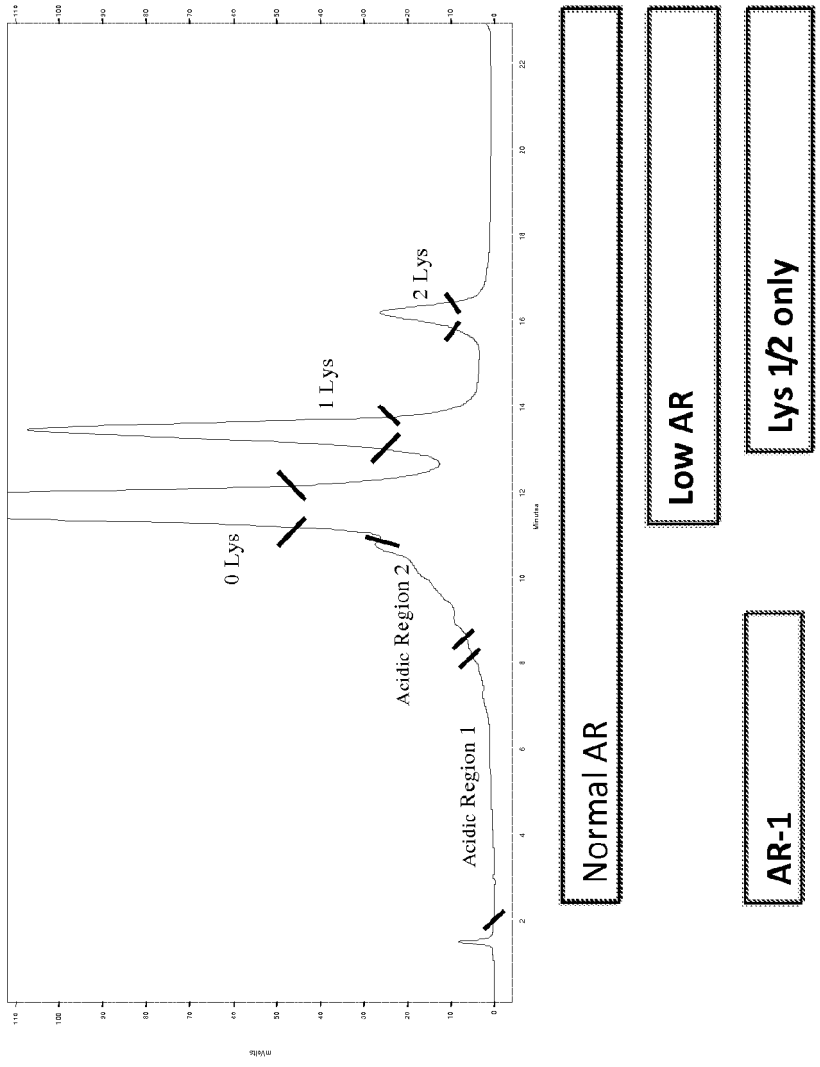

0 Lys
1 Lys
2 Lys
Acidic Region 1
Acidic Region 2
mVolts
Minutes
Normal AR
Low AR
Lys 1/2 only
AR-1

Figure 194A

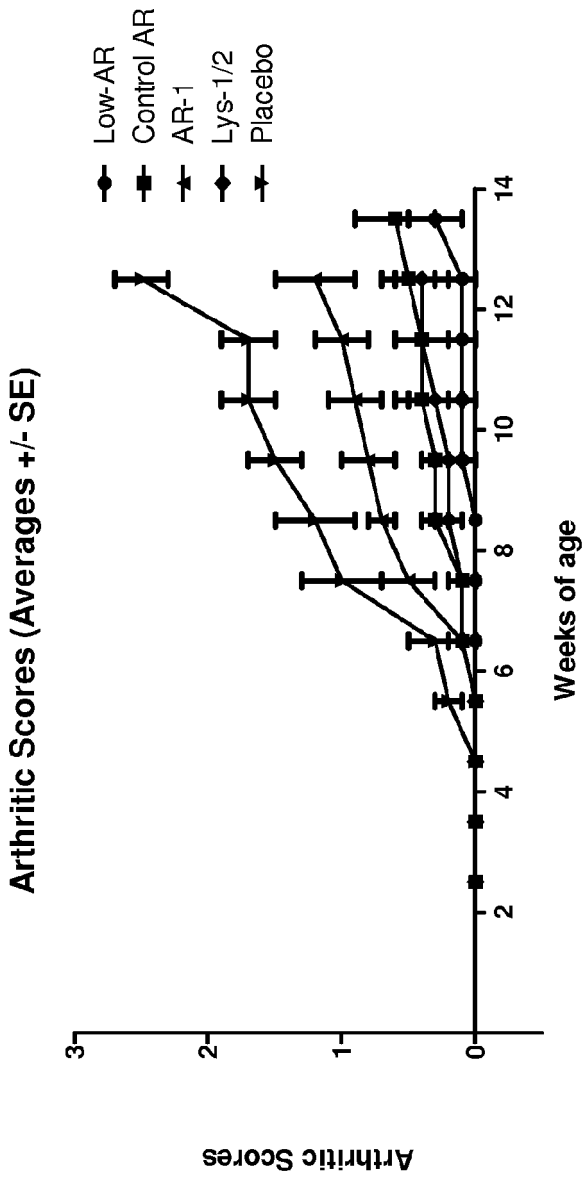
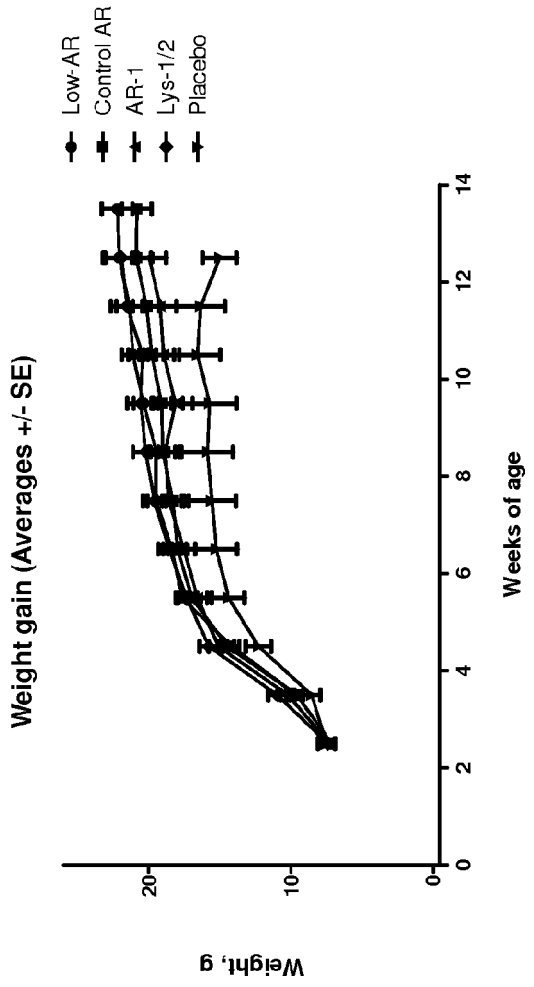

Arthritic Scores (Averages +/- SE)
Low-AR
Control AR
AR-1
Lys-1/2
Placebo
Arthritic Scores
0
1
2
3
2
4
6
8
10
12
14
Weeks of age

Figure 194B


Weight gain (Averages +/- SE)
Low-AR
Control AR
AR-1
Lys-1/2
Placebo
Weight, g
0
10
20
Weeks of age
0
2
4
6
8
10
12
14

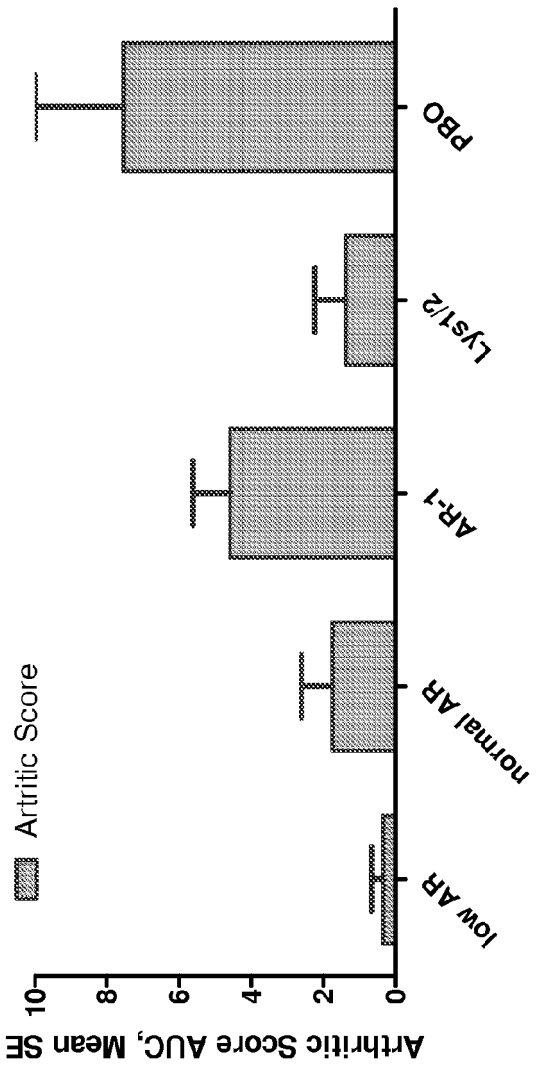

Arthritic Score AUC, Mean SE
0
2
4
6
8
10
Artritic Score
low AR
normal AR
AR-1
Lys1/2
PBO

Figure 195

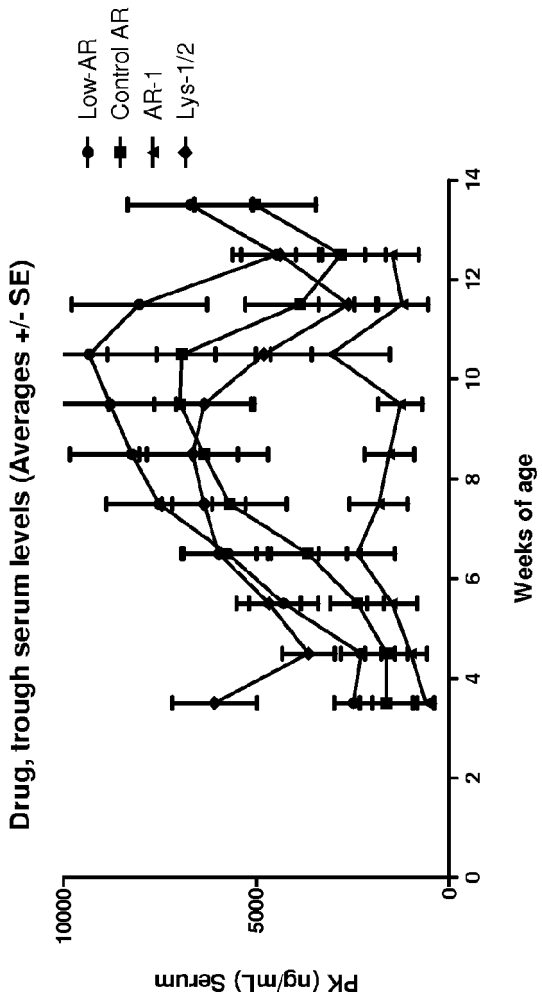

Low-AR
Control AR
AR-1
Lys-1/2
Drug, trough serum levels (Averages +/- SE)
PK (ng/mL) Serum
10000
5000
0
0
2
4
6
8
10
12
14
Weeks of age

Figure 196A

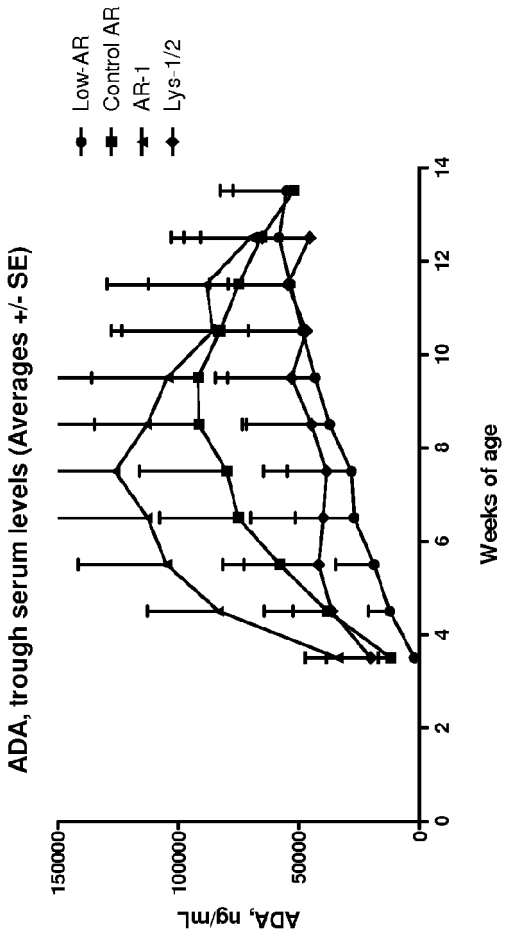

ADA, trough serum levels (Averages +/- SE)
Low-AR
Control AR
AR-1
Lys-1/2
ADA, ng/mL
150000
100000
50000
0
0
2
4
6
8
10
12
14
Weeks of age

Figure 196B

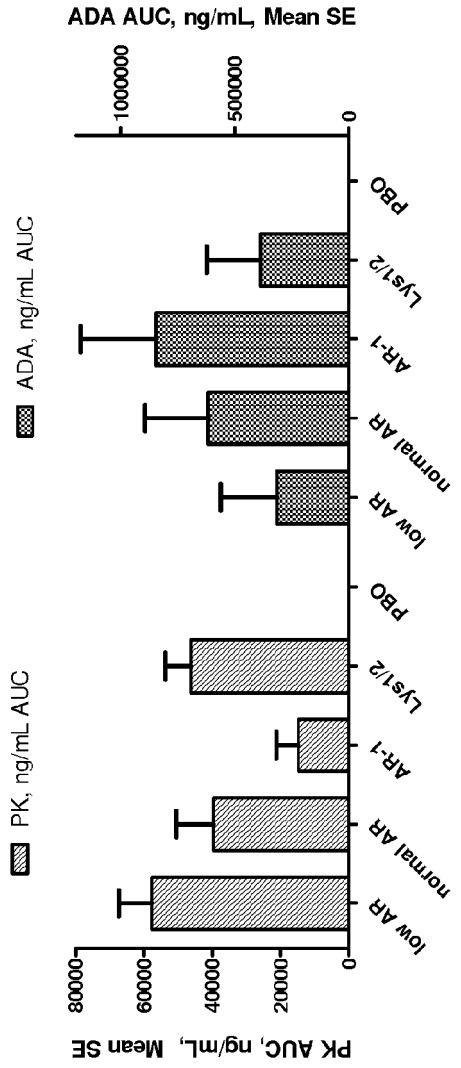

ADA AUC, ng/mL, Mean SE
1000000
500000
0
PBO
Lys1/2
AR-1
normal AR
low AR
PBO
Lys1/2
AR-1
normal AR
low AR
ADA, ng/mL AUC
PK, ng/mL AUC
80000
60000
40000
20000
0
PK AUC, ng/mL, Mean SE

Figure 197

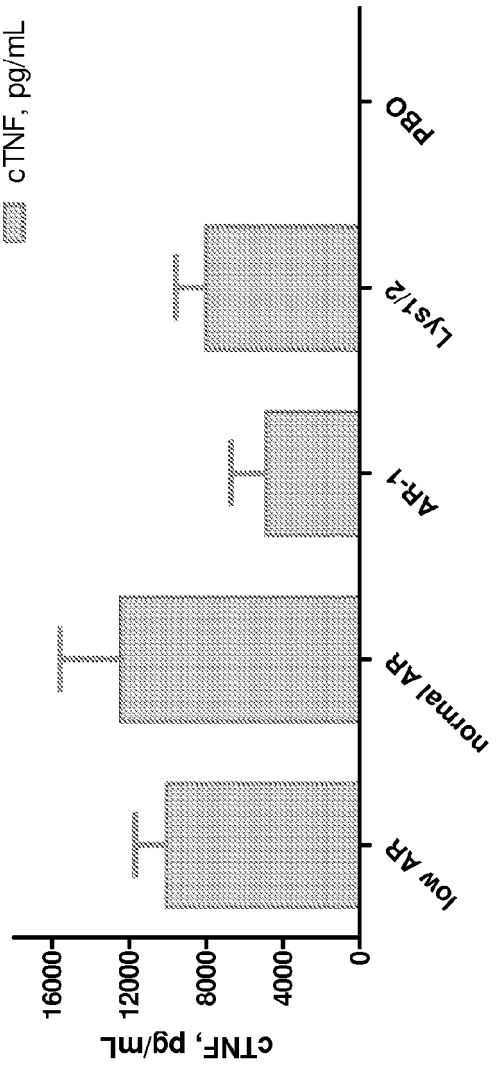

cTNF, pg/mL
16000
12000
8000
4000
0
low AR
normal AR
AR-1
Lys1/2
PBO
cTNF, pg/mL

Figure 198

Figure 199

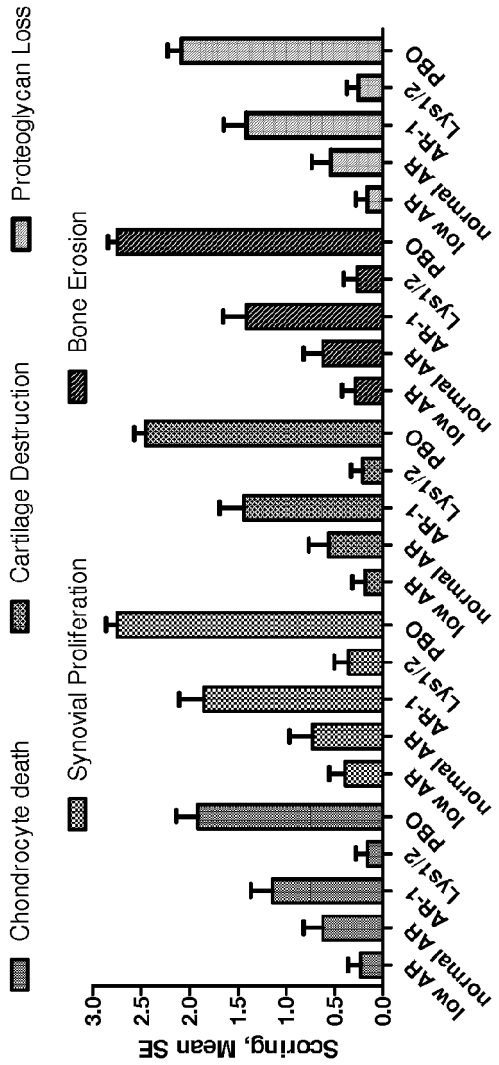

Chondrocyte death
Cartilage Destruction
Proteoglycan Loss
Synovial Proliferation
Bone Erosion
Scoring, Mean SE
3.0
2.5
2.0
1.5
1.0
0.5
0.0
low AR
normal AR
AR-1
Lys1/2
PBO

Figure 200A-D

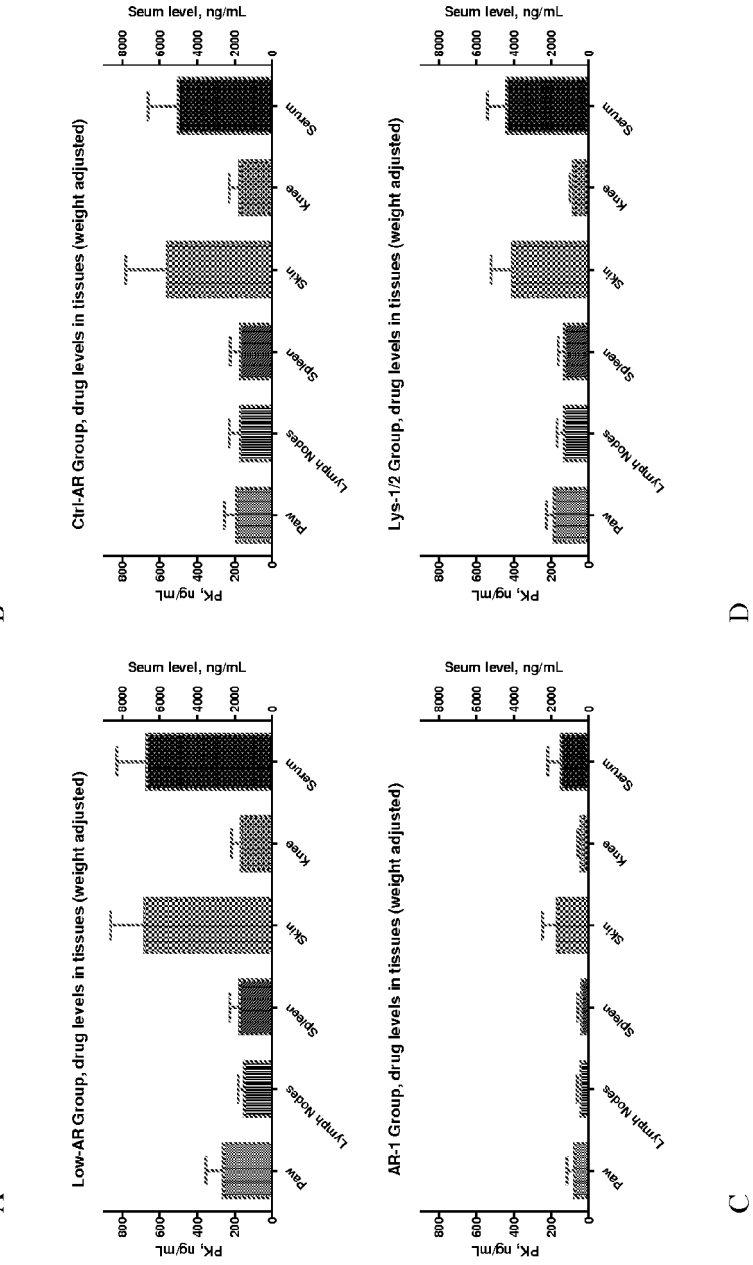


Figure 201A-D

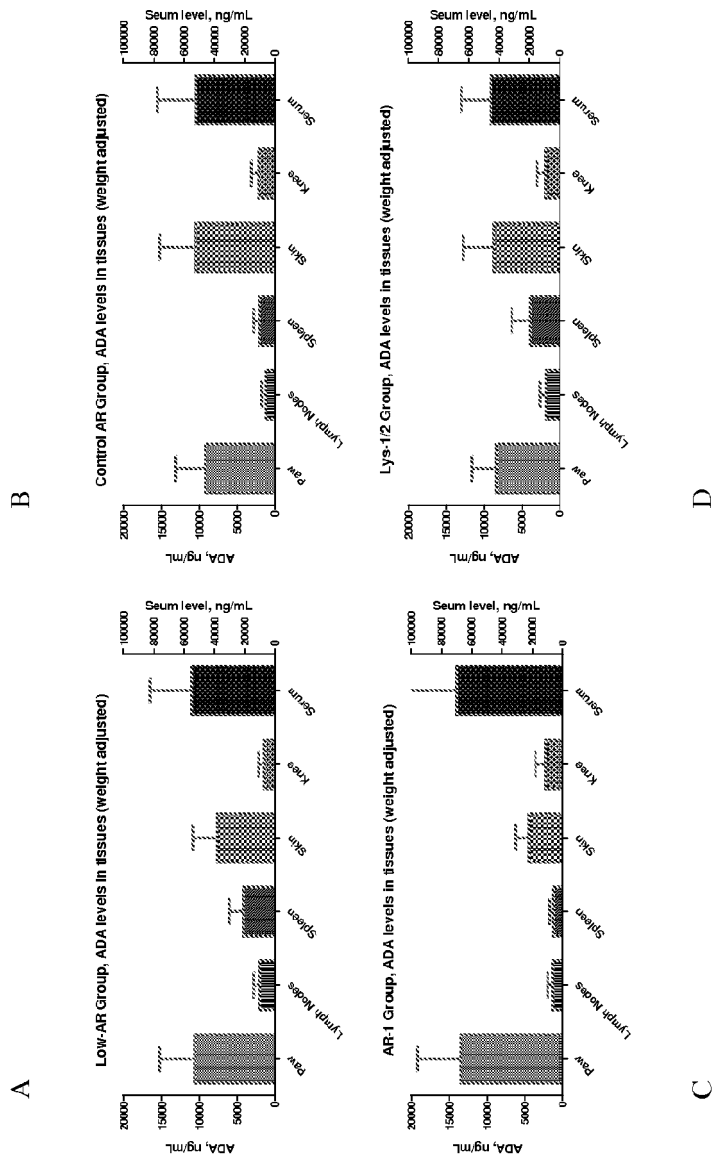


Figure 202A-D

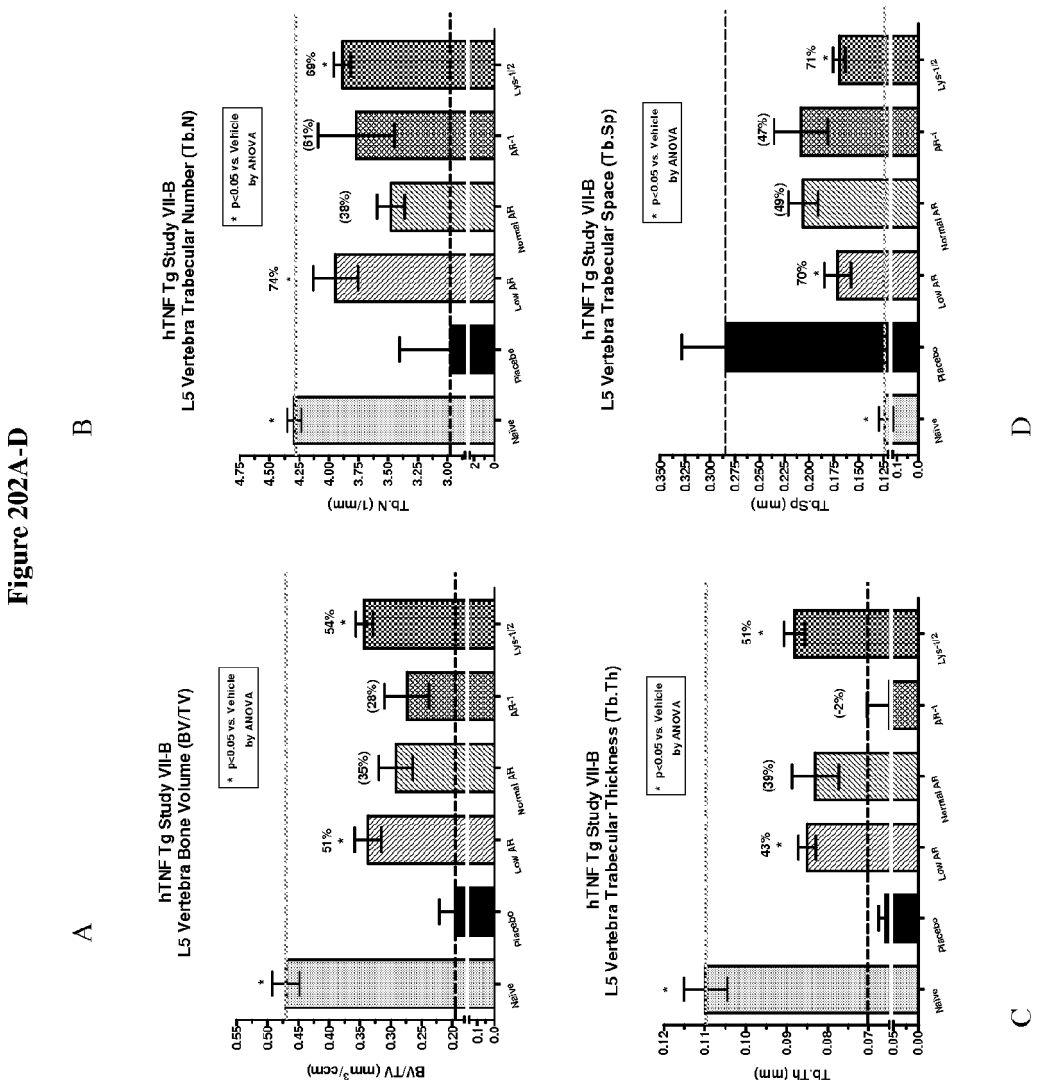


Figure 203A-D

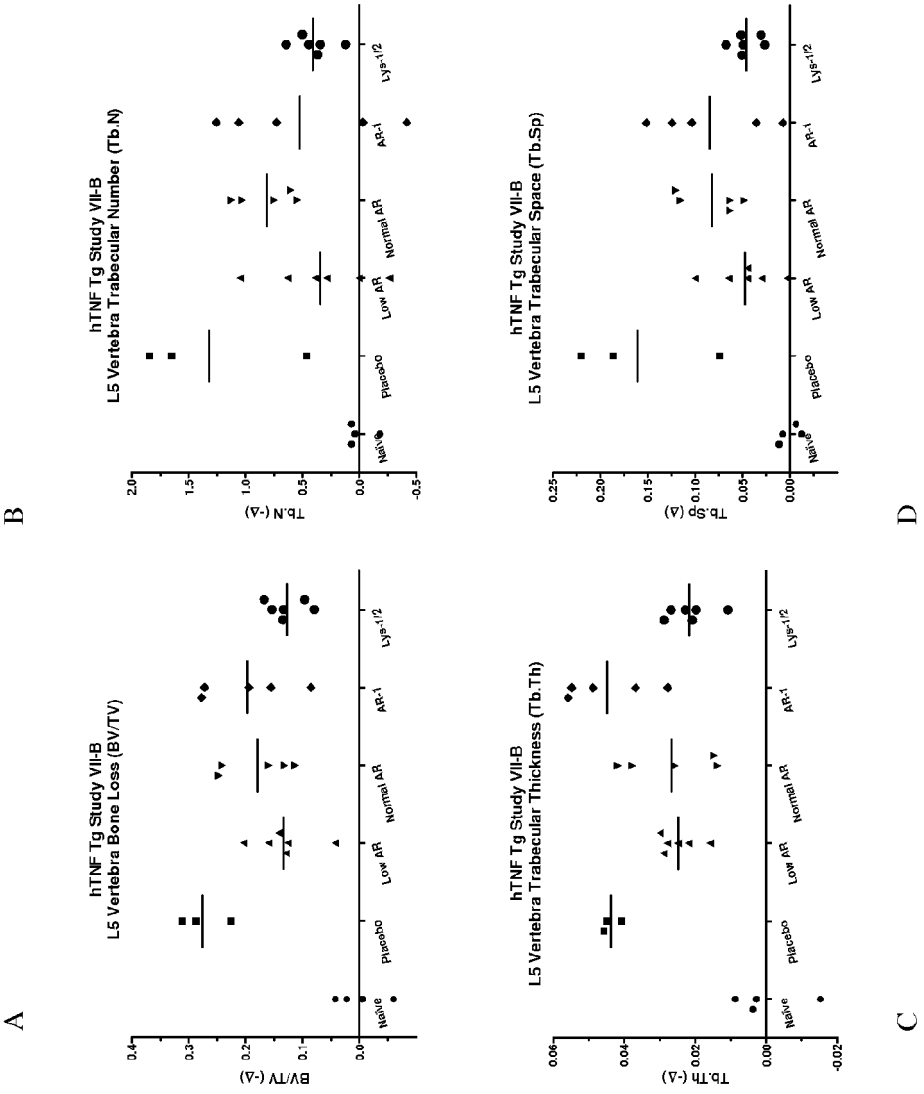
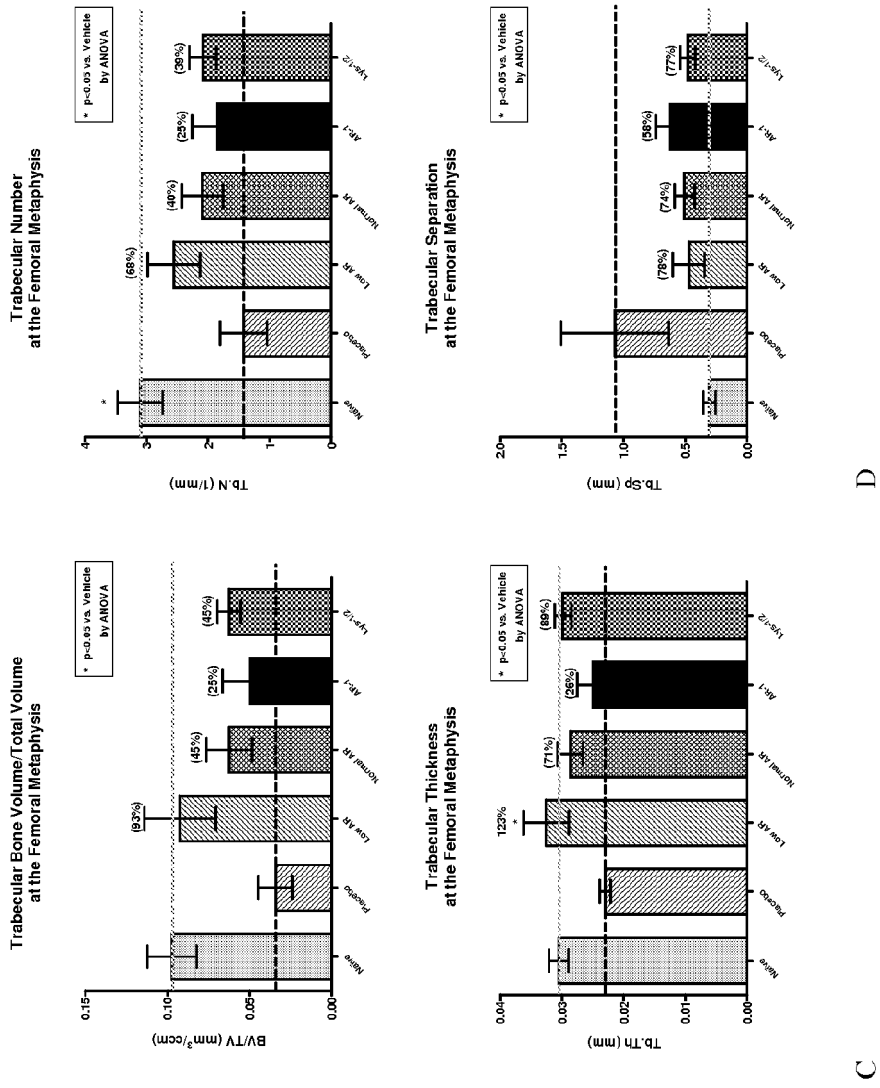


Figure 204A-D



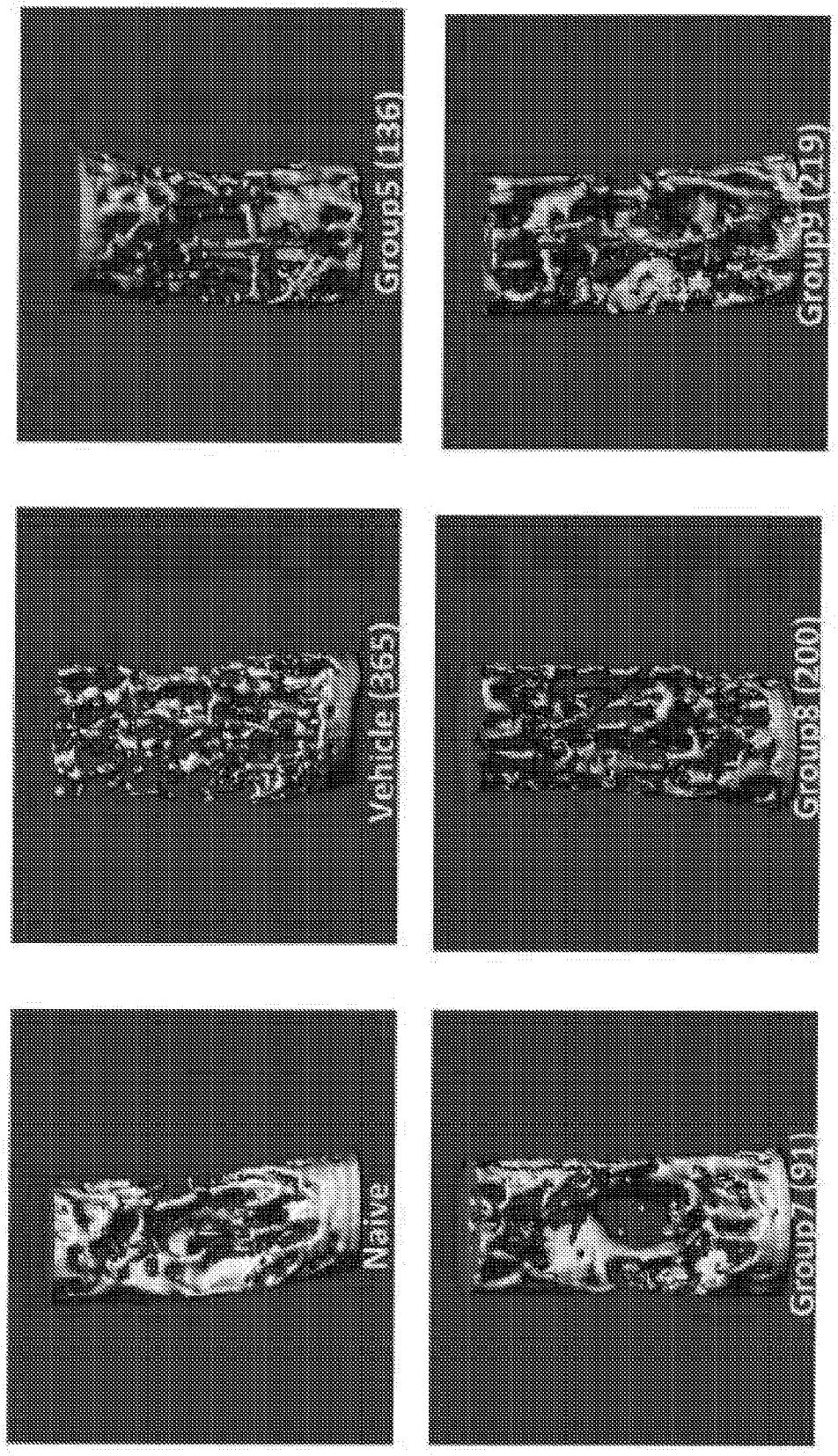

Group5 (136)
Group1 (219)
Vehicle (365)
Group3 (200)
Naïve
Group7 (191)

Figure 205

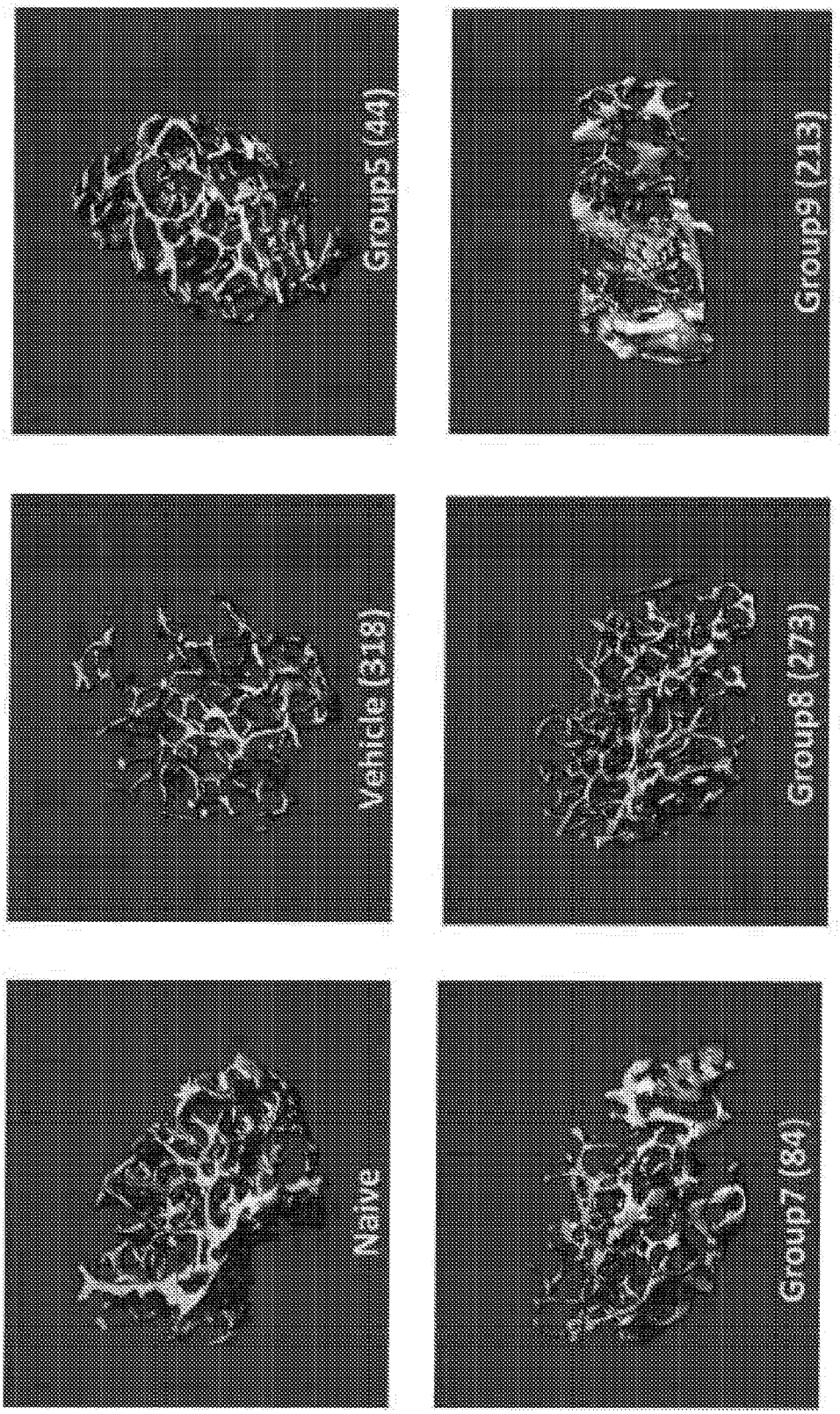


Figure 206