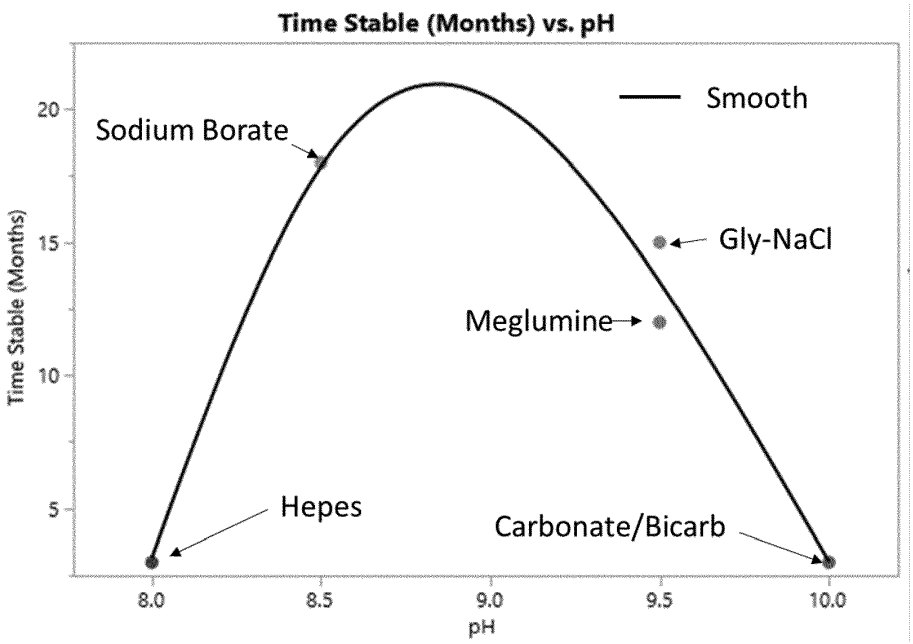
Figure 1

A Stored at 2-8°C



B Accelerated Conditions 25°C

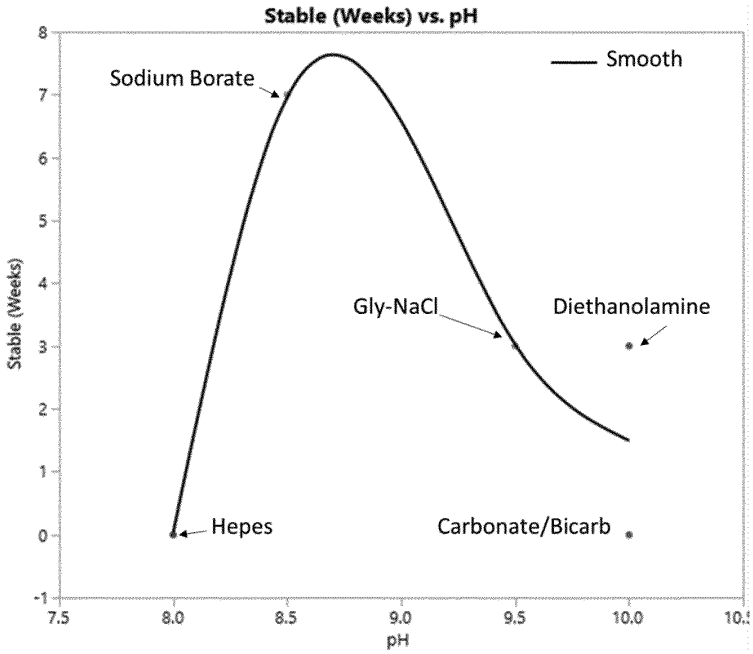


Figure 2 Accelerated Design of Experiments (DOE) Showing 15-20% Glycerol is Beneficial

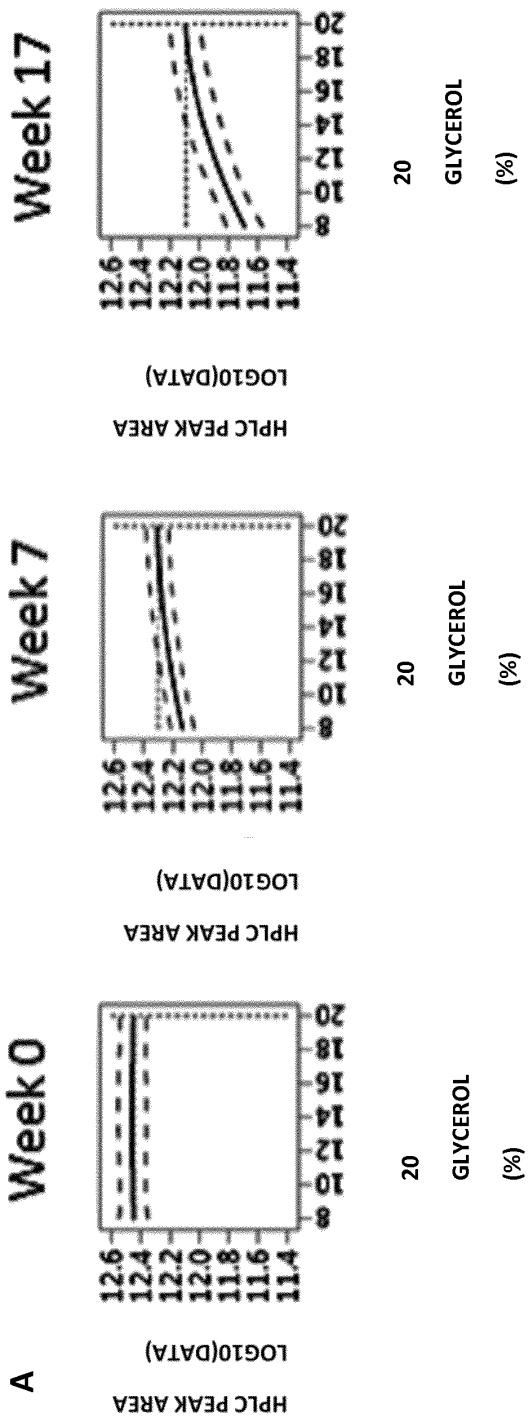
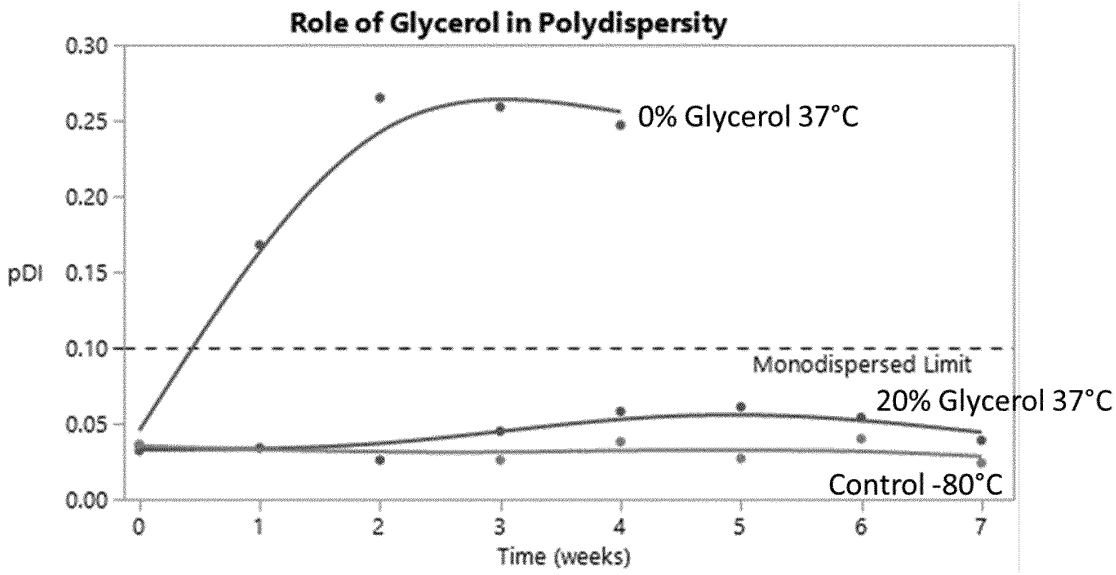


Figure 2 cont.

B 10Mm HEPES, with Glycerol/Accelerated Conditions



C 10Mm HEPES with Glycerol Stored at 25°C

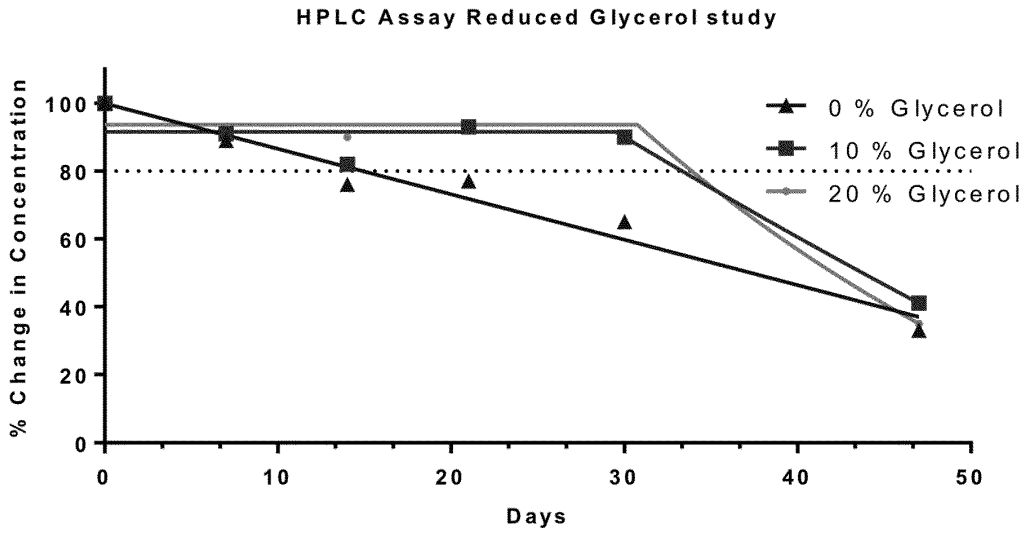
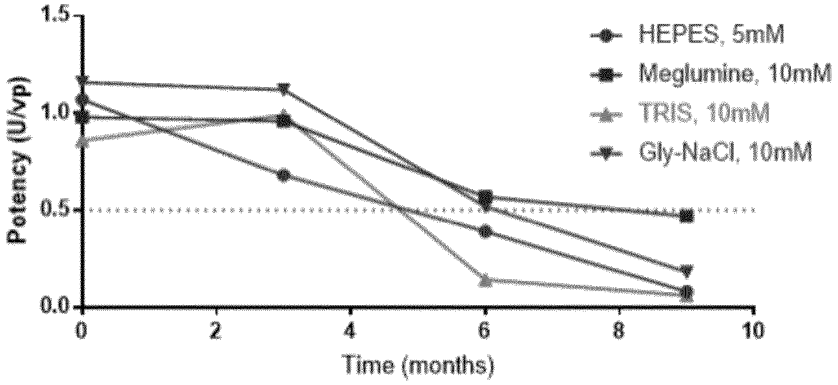
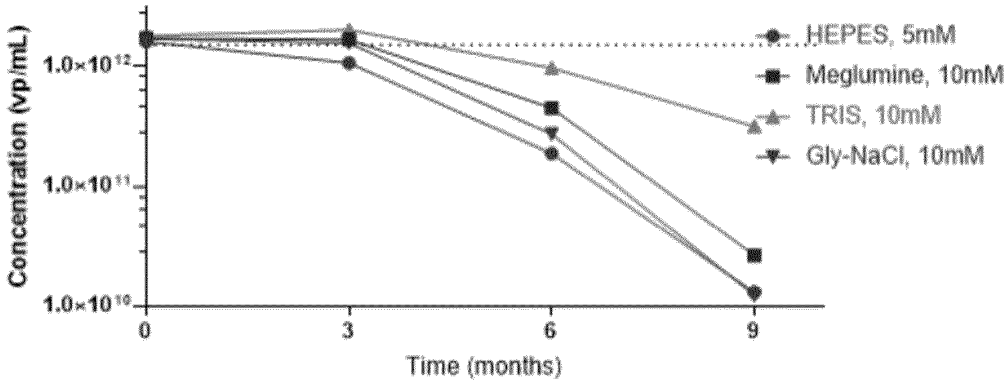


Figure 3

A 20% Glycerol and buffer at pH9 with HEPES control and stored at 4°C (no ethanol)



B 20% Glycerol and buffer at pH9 and 4°C (no ethanol)



C 20% Glycerol, Meglumine or Gly-NaCl, Ethanol, Methionine, Polysobrate at pH9 and 4°C, HPLC analysis of conc. vp/ml

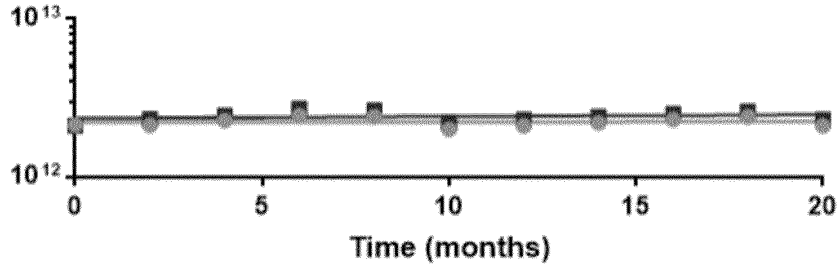


Figure 4

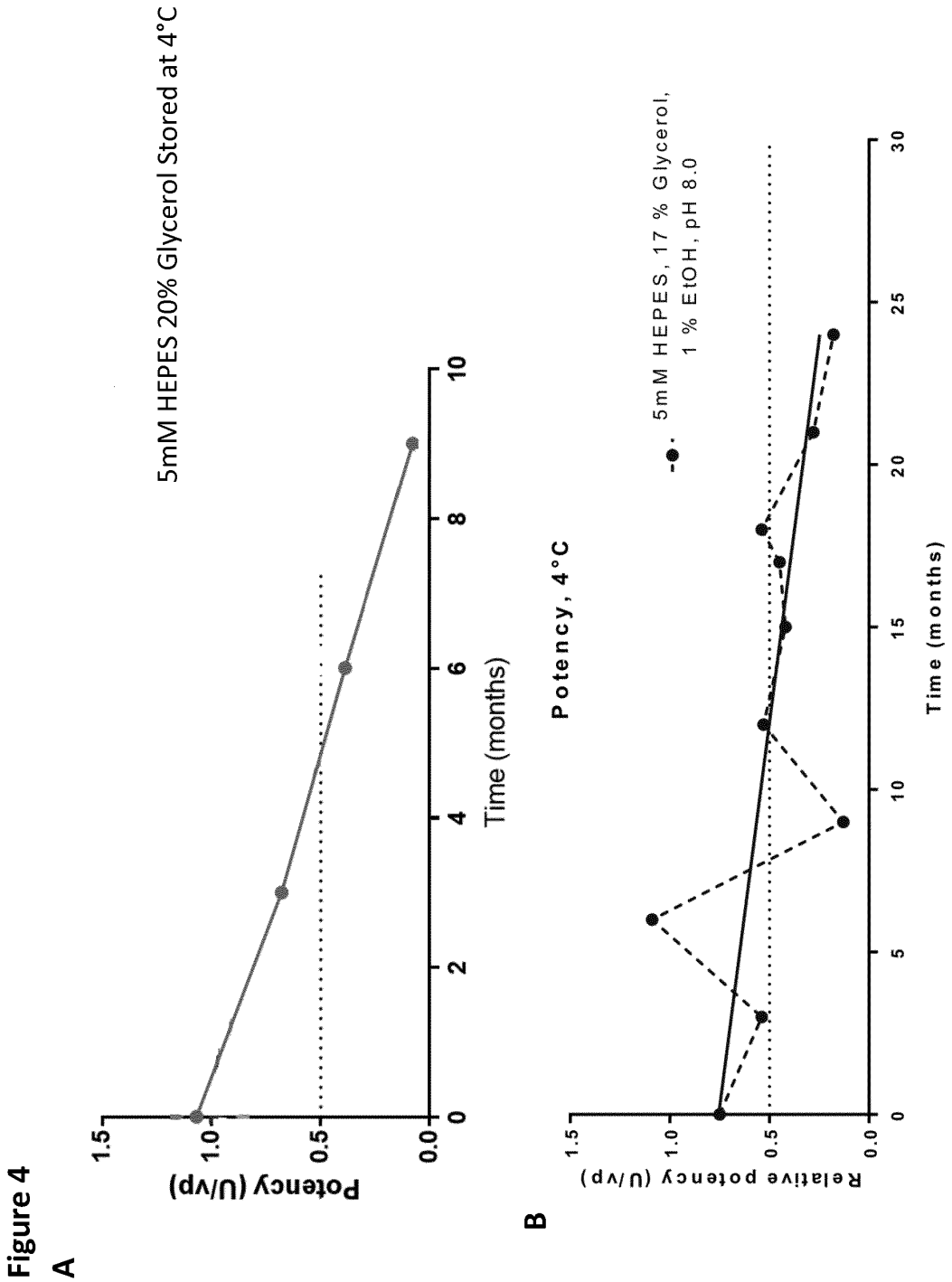

A
5mM HEPES 20% Glycerol Stored at 4°C
Potency (U/vp)
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Time (months)
B
Potency, 4°C
5mM HEPES, 17 % Glycerol,
1 % EtOH, pH 8.0
Relative potency (U/vp)
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
Time (months)

Figure 4 cont.

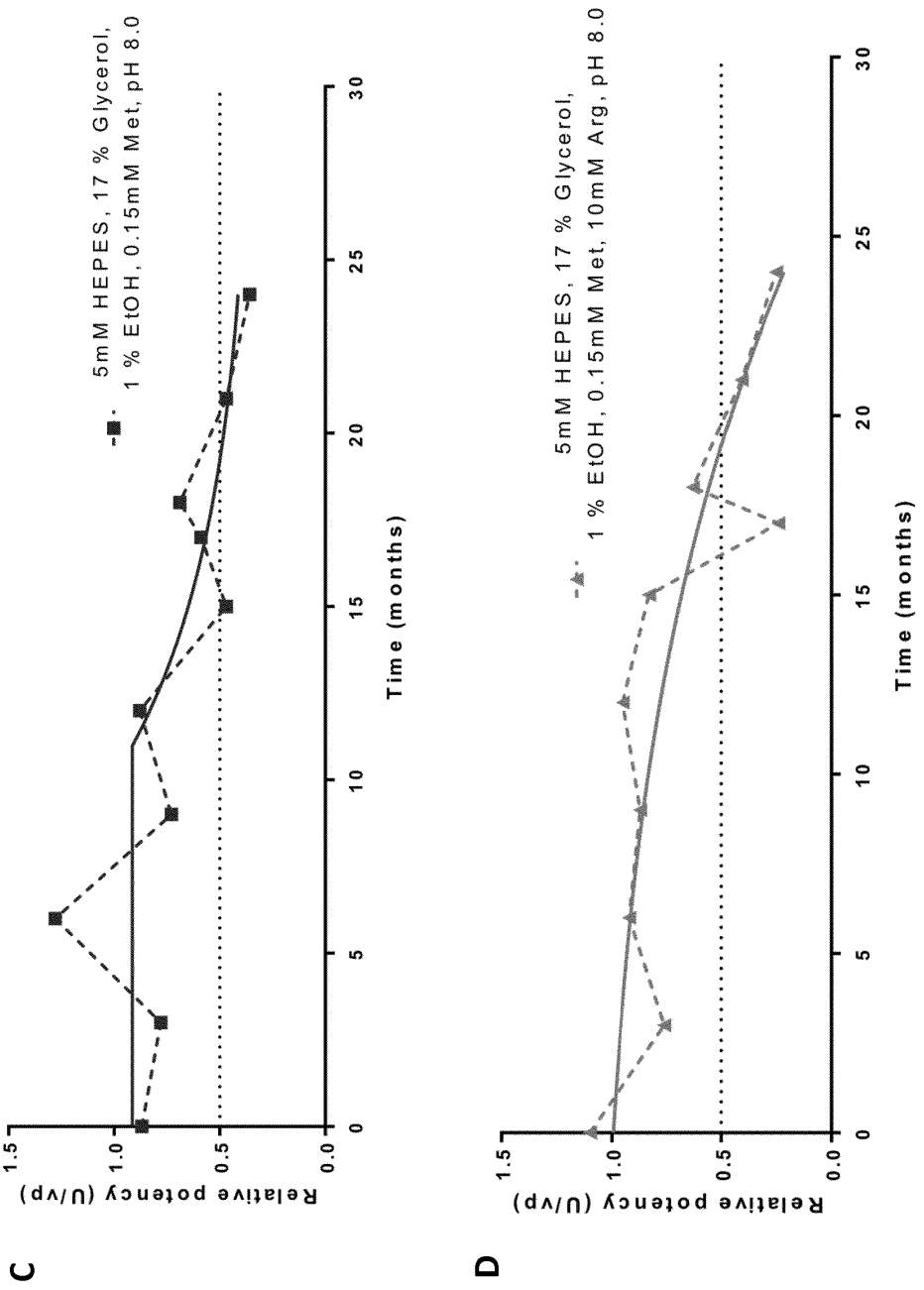


Figure 4 cont.

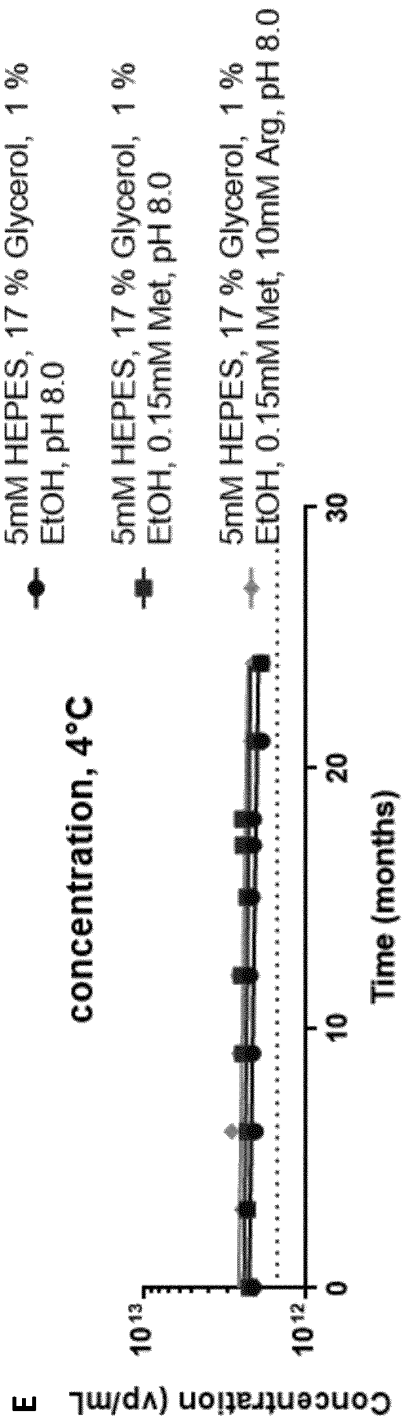


Figure 5

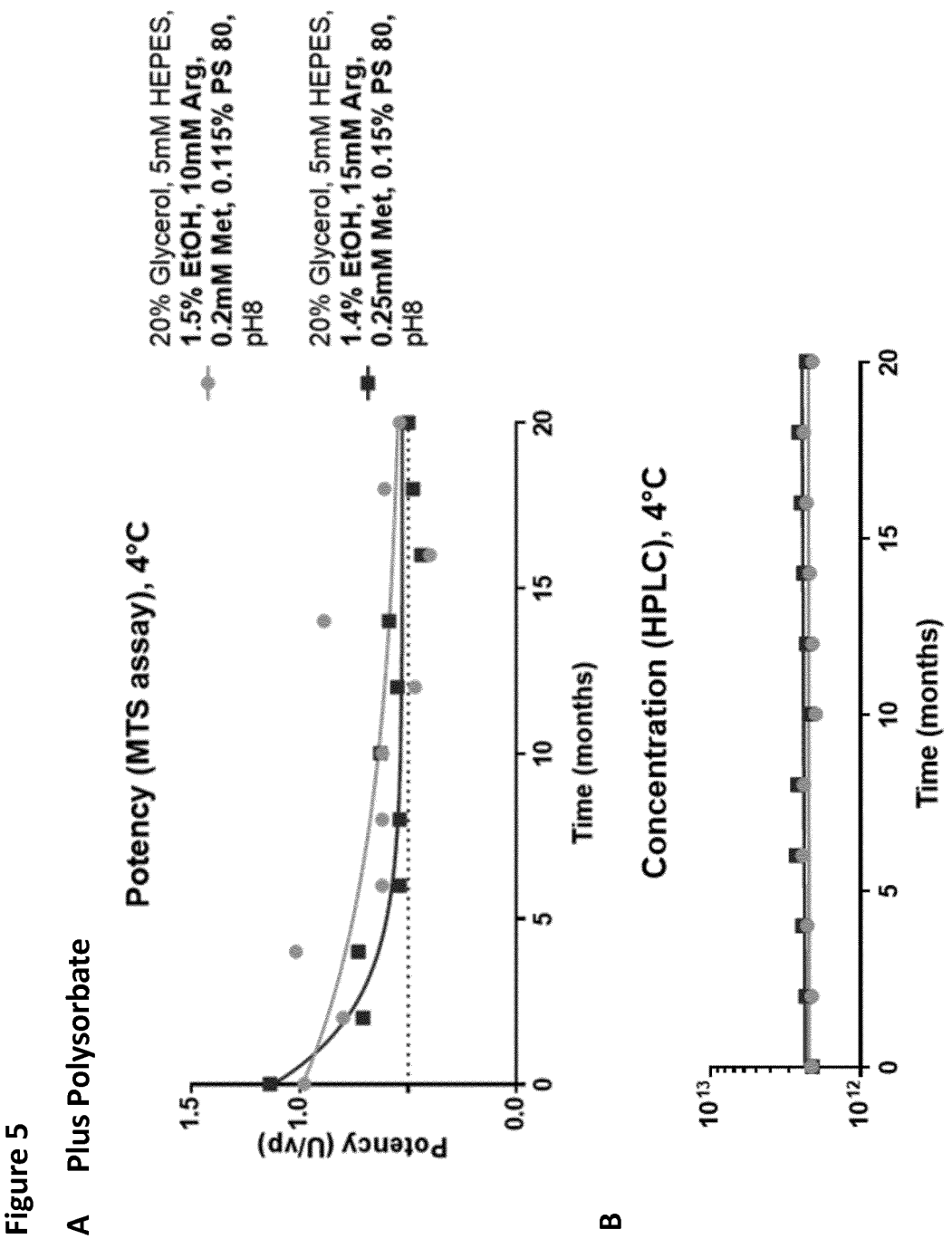

A Plus Polysorbate
Potency (MTS assay), 4°C
20% Glycerol, 5mM HEPES, 1.5% EtOH, 10mM Arg, 0.2mM Met, 0.115% PS 80, pH8
20% Glycerol, 5mM HEPES, 1.4% EtOH, 15mM Arg, 0.25mM Met, 0.15% PS 80, pH8
Potency (U/vp)
1.5
1.0
0.0
0
5
10
15
20
Time (months)
B
Concentration (HPLC), 4°C
10^{13}
10^{12}
0
5
10
15
20
Time (months)

Figure 6A

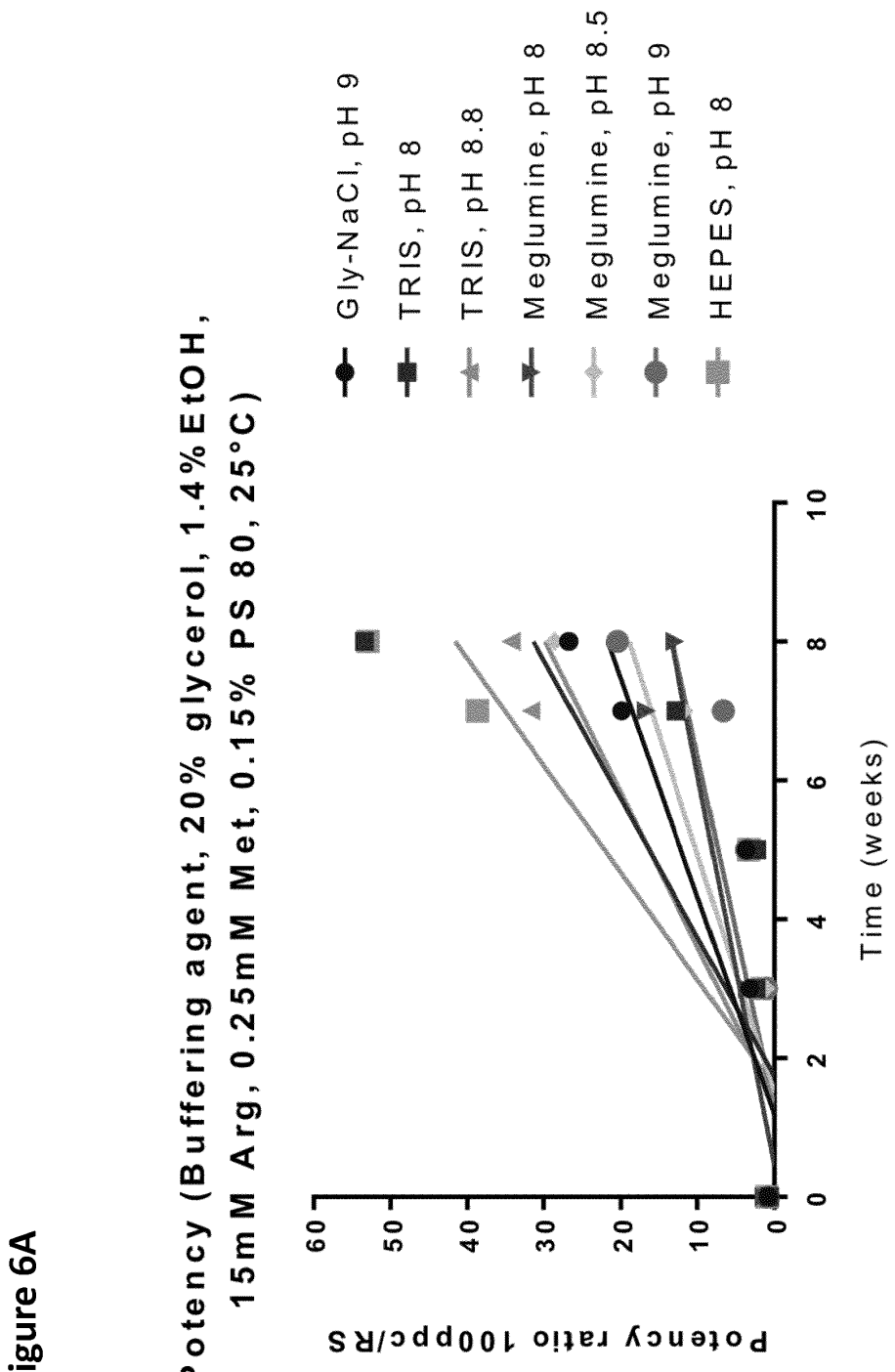
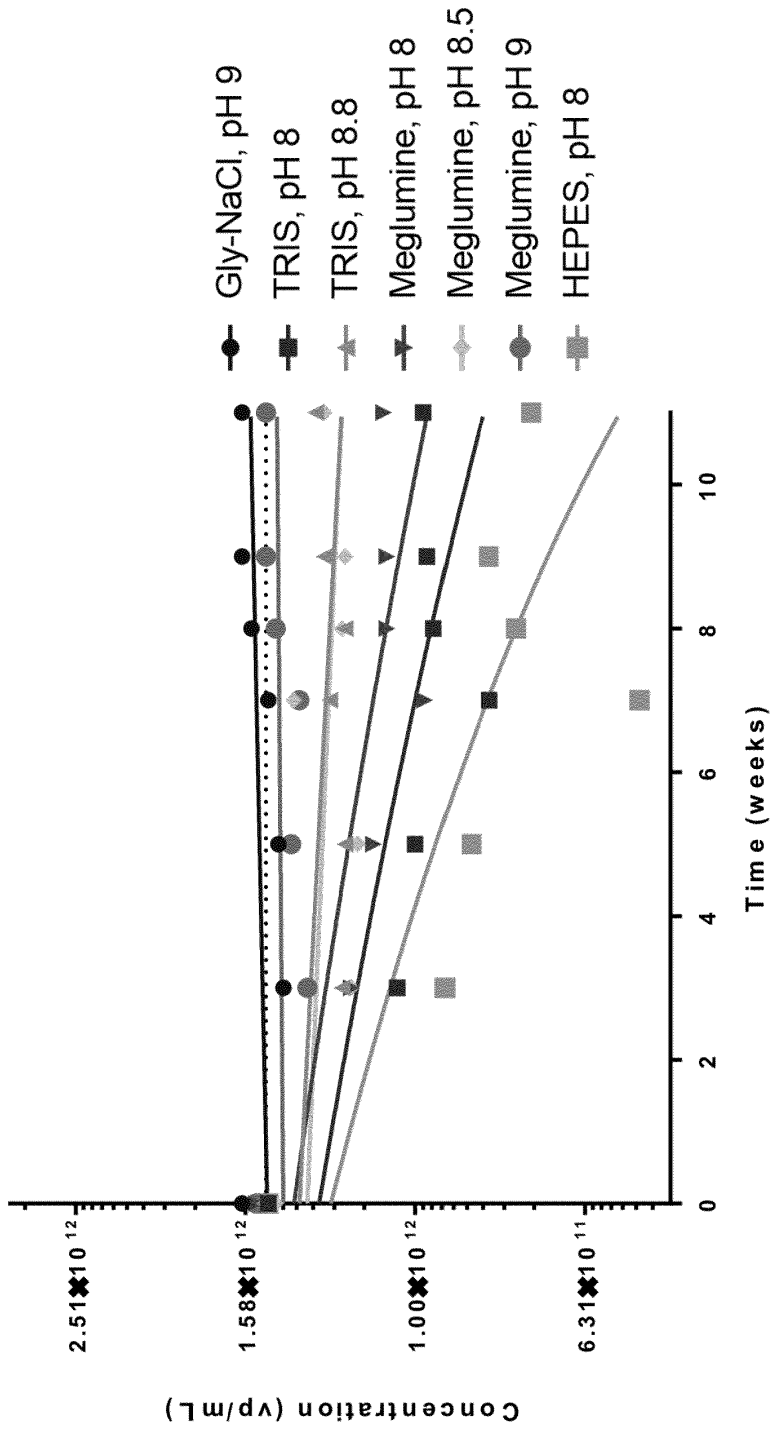

Potency (Buffering agent, 20% glycerol, 1.4%EtOH, 15mM Arg, 0.25mM Met, 0.15% PS 80, 25°C)
Potency ratio 100ppc/RS
Time (weeks)
Gly-NaCl, pH 9
TRIS, pH 8
TRIS, pH 8.8
Meglumine, pH 8
Meglumine, pH 8.5
Meglumine, pH 9
HEPES, pH 8
0
10
20
30
40
50
60
0
2
4
6
8
10

Figure 6B


Concentration (Buffering agent, 20% glycerol, 1.4% EtOH, 15mM Arg, 0.25mM Met, 0.15% PS 80, 25°C)
Gly-NaCl, pH 9
TRIS, pH 8
TRIS, pH 8.8
Meglumine, pH 8
Meglumine, pH 8.5
Meglumine, pH 9
HEPES, pH 8
Concentration (vp/mL)
2.51✖10 12
1.58✖10 12
1.00✖10 12
6.31✖10 11
0
2
4
6
8
10
Time (weeks)

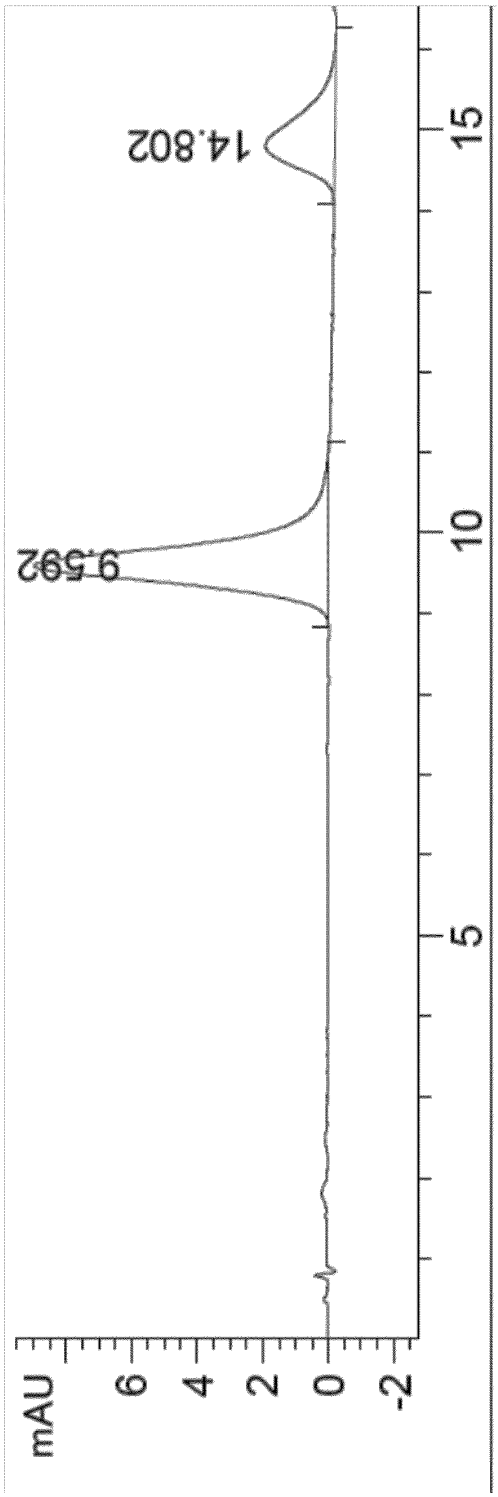


Figure 7 Analytical Separation of Adenovirus 5 (Ad 5) and Adenovirus 11 (Ad 11) by Anion Exchange Chromatography

Figure 8A

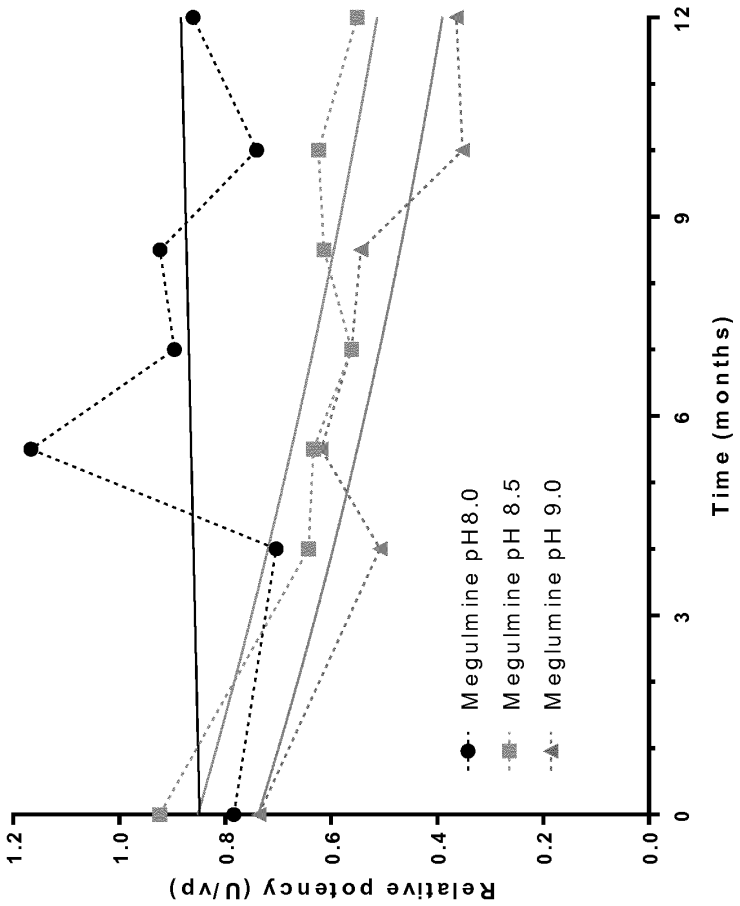

Oncolytic Relative Potency Analysis (Buffering agent, 20% glycerol, 1.4% ethanol, 15 mM arginine, 0.25 mM methionine) 4°C storage
Relative potency (U/vp)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
3
6
9
12
Time (months)
Megulmine pH8.0
Megulmine pH 8.5
Meglumine pH 9.0

Figure 8B

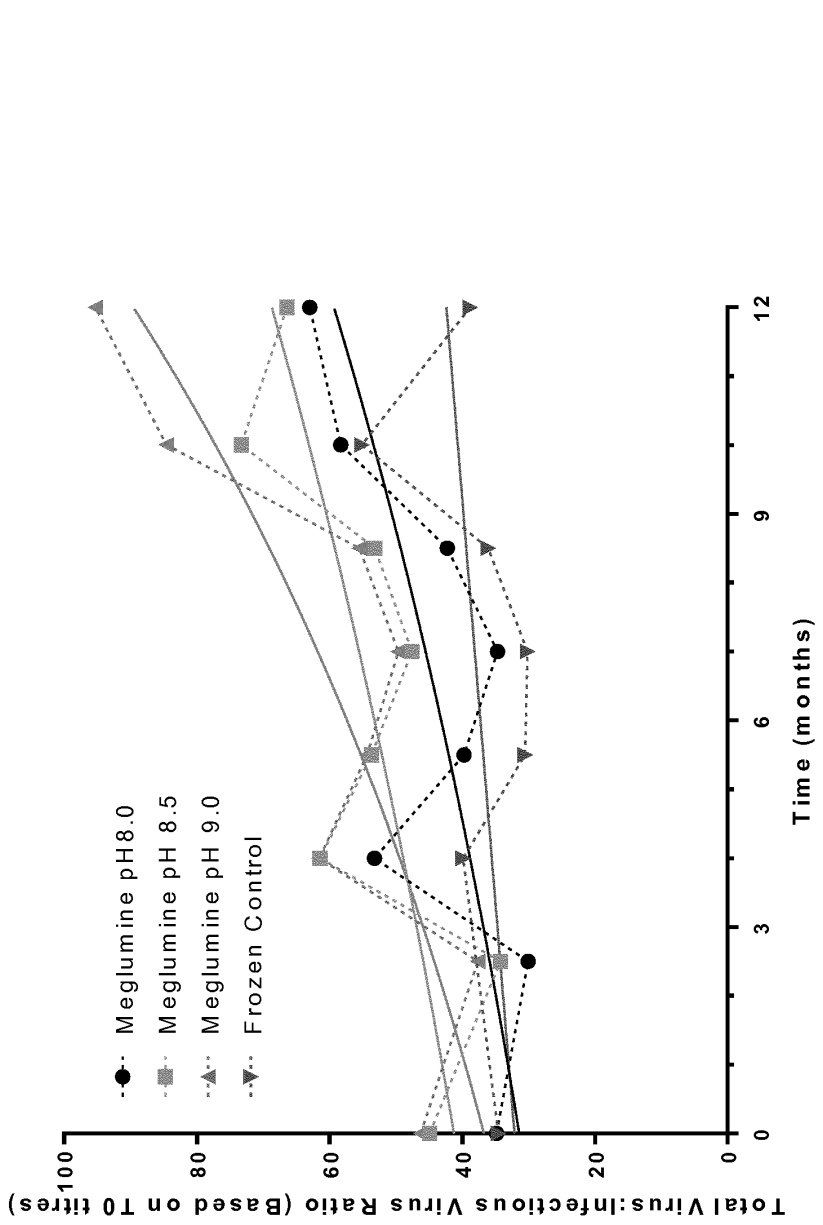

Ratio of Total:Infectious Viral Particles Analysis (Buffering agent, 20% glycerol, 1.4% ethanol, 15 mM arginine, 0.25 mM methionine), 4°C storage
Meglumine pH8.0
Meglumine pH 8.5
Meglumine pH 9.0
Frozen Control
Total Virus:Infectious Virus Ratio (Based on T0 titres)
Time (months)
0
20
40
60
80
100
0
3
6
9
12

Figure 8C Oncolytic Relative Potency Analysis (Buffering Agent, 20% glycerol, 1.4% ethanol, 15 mM arginine, 0.25 mM methionine)

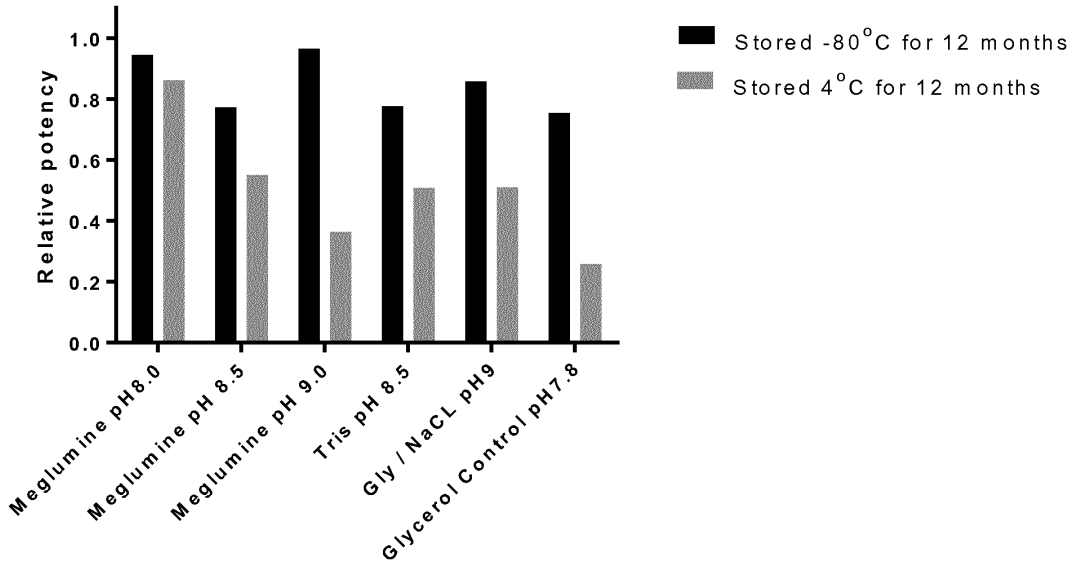


Figure 8D Virus Concentration by AEX-HPLC Analysis (Buffering Agent, 20% glycerol, 1.4% ethanol, 15 mM arginine, 0.25 mM methionine)

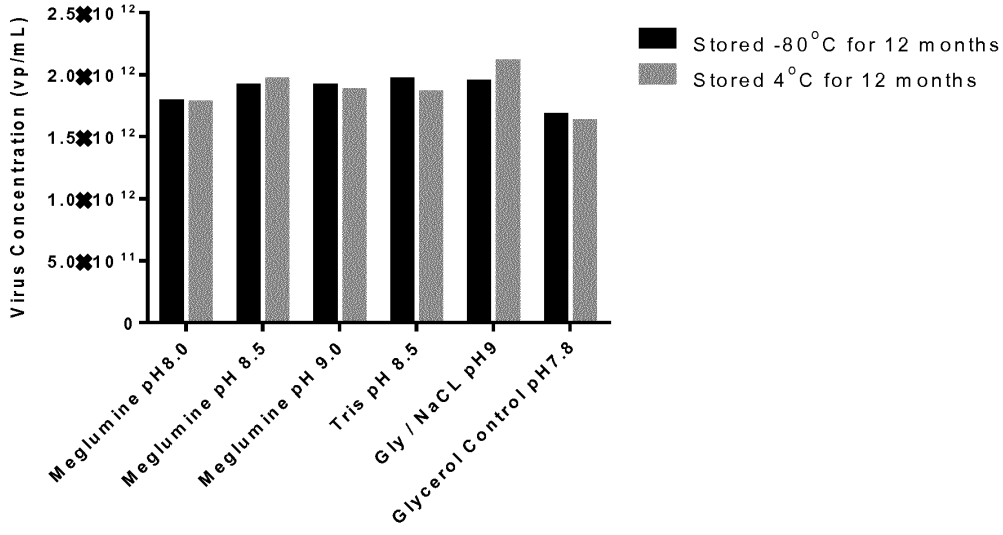


Figure 8E

Ratio of total virus: infectious virus particles (Buffering Agent, 20% glycerol, 1.4% ethanol, 15 mM arginine, 0.25 mM methionine)

