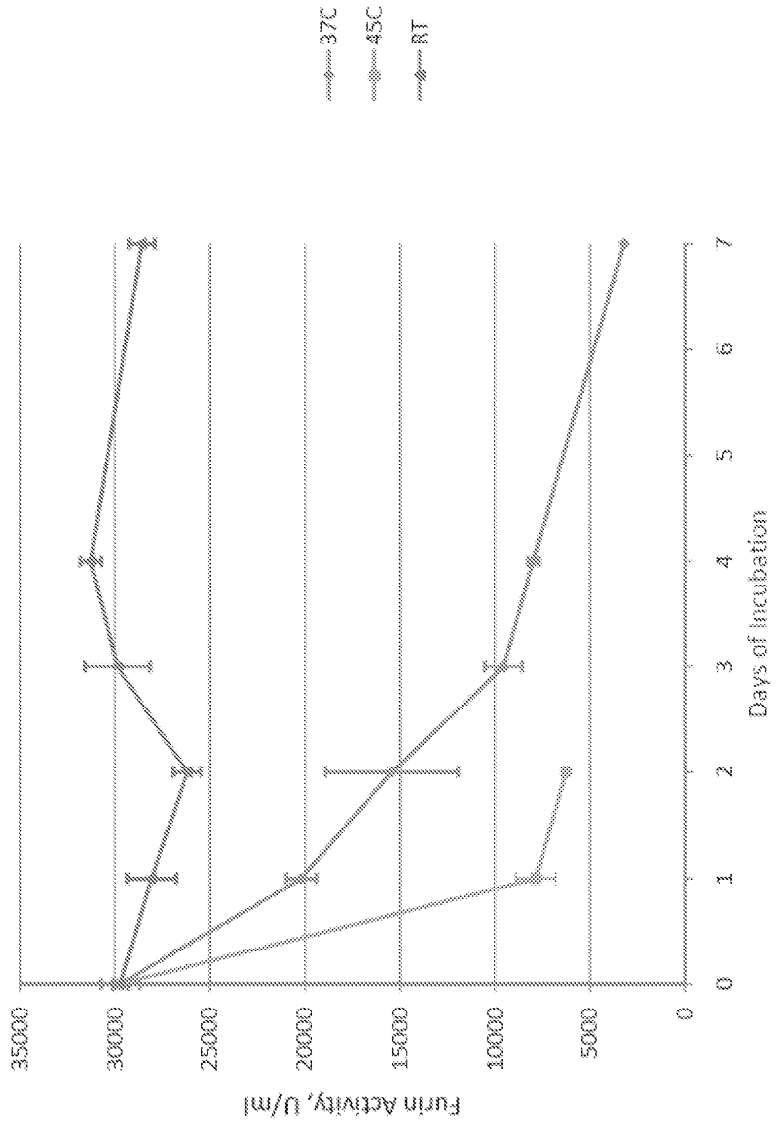

37C
45C
RT
Furin Activity, U/ml
35000
30000
25000
20000
15000
10000
5000
0
0
1
2
3
4
5
6
7
Days of Incubation

FIG. 1

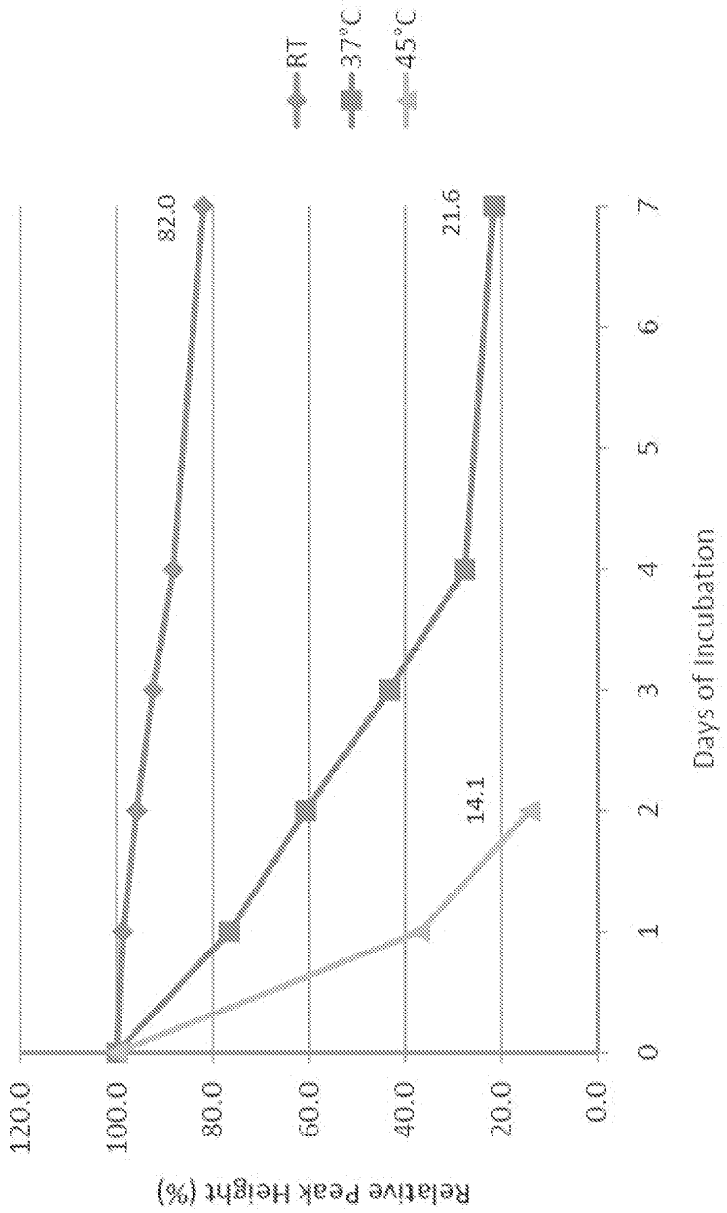

RT
37°C
45°C
82.0
21.6
14.1
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0
1
2
3
4
5
6
7
Days of Incubation
Relative Peak Height (%)

FIG. 2

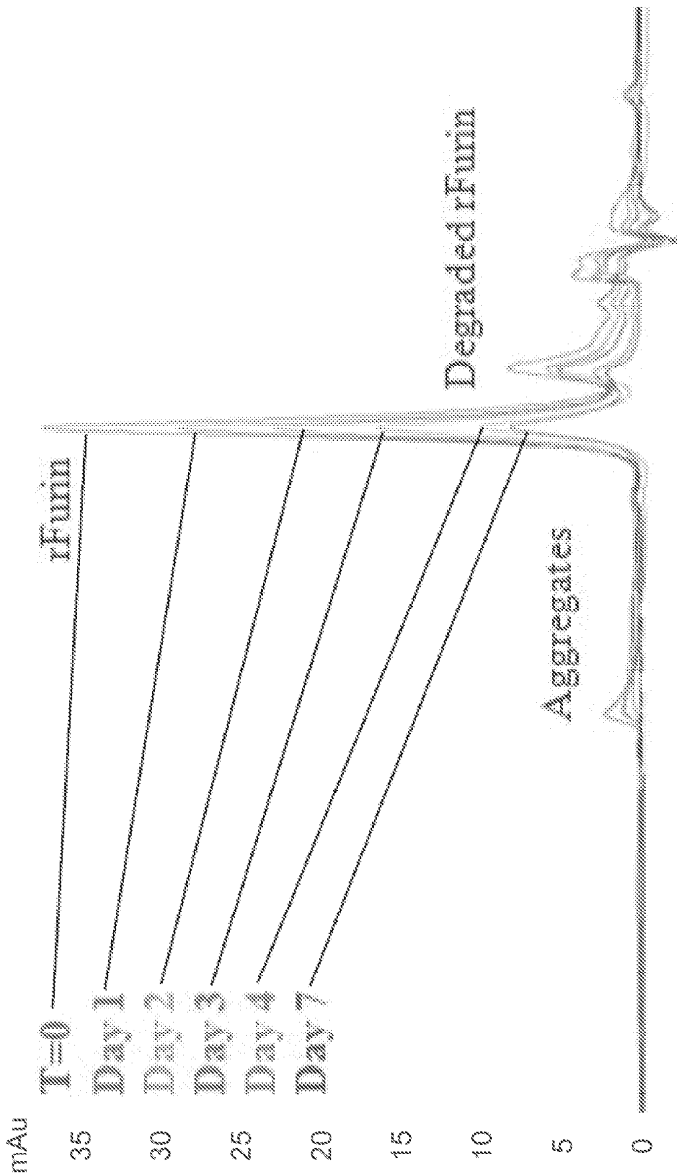

mAu
35
30
25
20
15
10
5
0
T=0
Day 1
Day 2
Day 3
Day 4
Day 7
rFurin
Degraded rFurin
Aggregates

FIG. 3

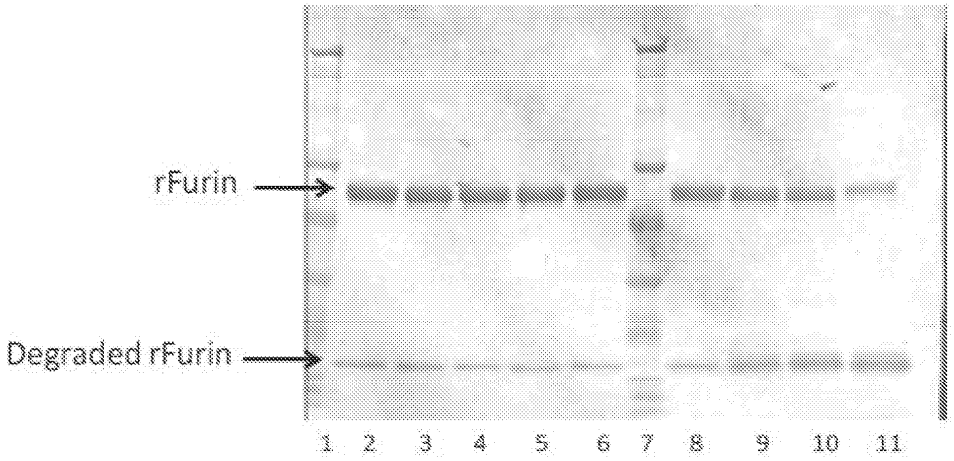


FIG. 4

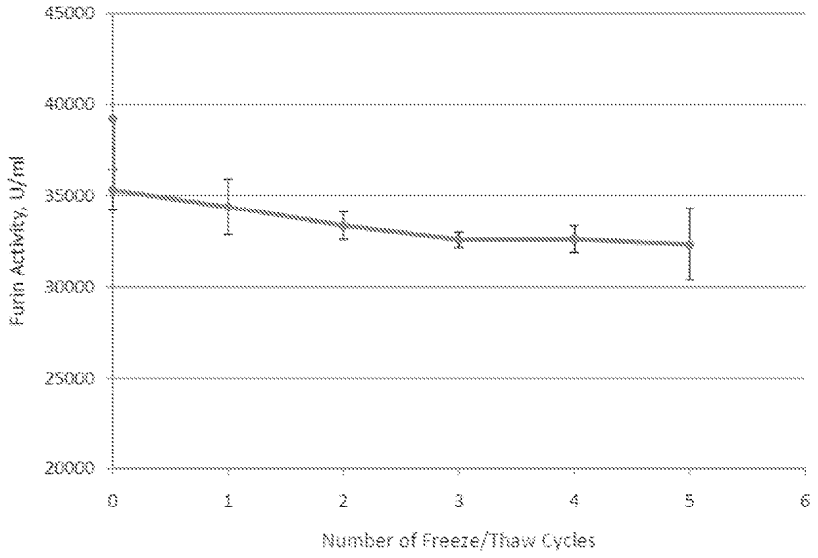

45000
40000
35000
30000
25000
20000
Furin Activity, U/ml
0
1
2
3
4
5
6
Number of Freeze/Thaw Cycles

FIG. 5

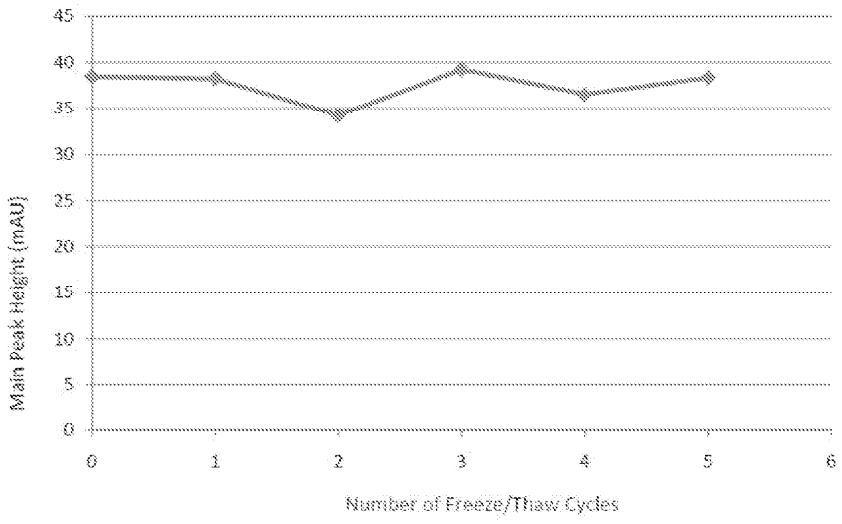

45
40
35
30
25
20
15
10
5
0
Main Peak Height (mAU)
0
1
2
3
4
5
6
Number of Freeze/Thaw Cycles

FIG. 6

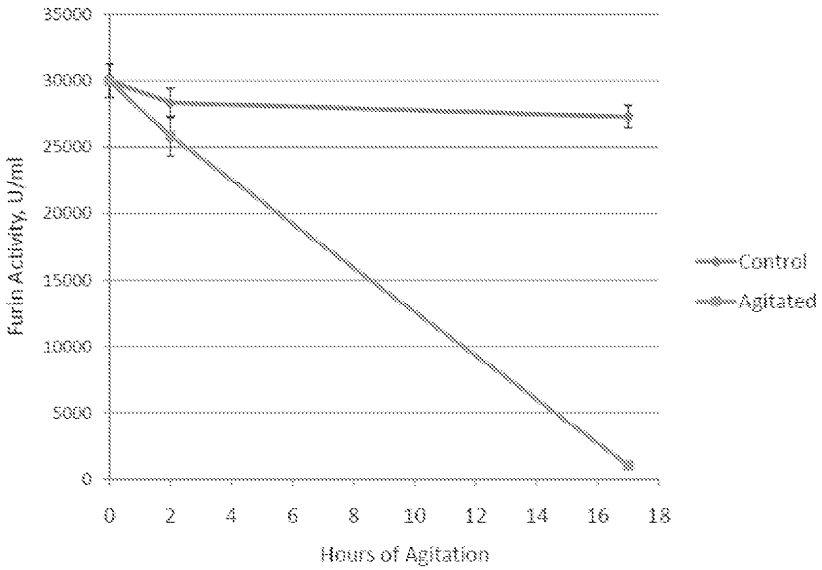

35000
30000
25000
20000
15000
10000
5000
0
Furin Activity, U/ml
0
2
4
6
8
10
12
14
16
18
Hours of Agitation
Control
Agitated

FIG. 7

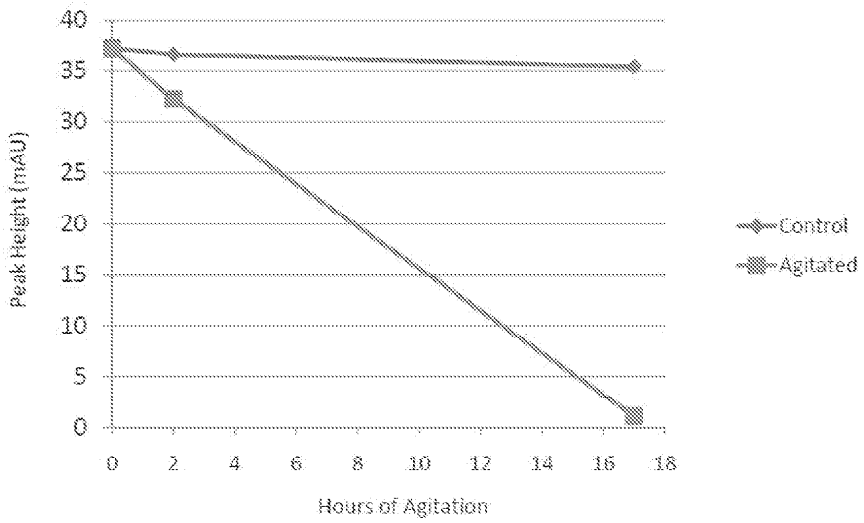

40
35
30
25
20
15
10
5
0
Peak Height (mAU)
0
2
4
6
8
10
12
14
16
18
Hours of Agitation
Control
Agitated

FIG. 8

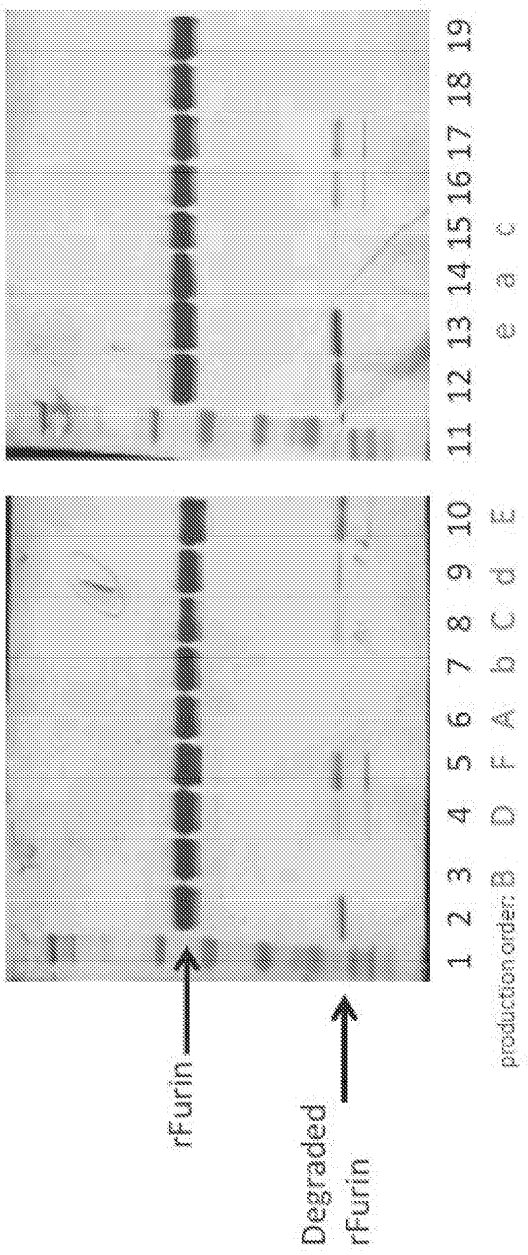

rFurin
Degraded rFurin
production order: B D F A b C d E
1 2 3 4 5 6 7 8 9 10
11 12 13 14 15 16 17 18 19
e a c

FIG. 9

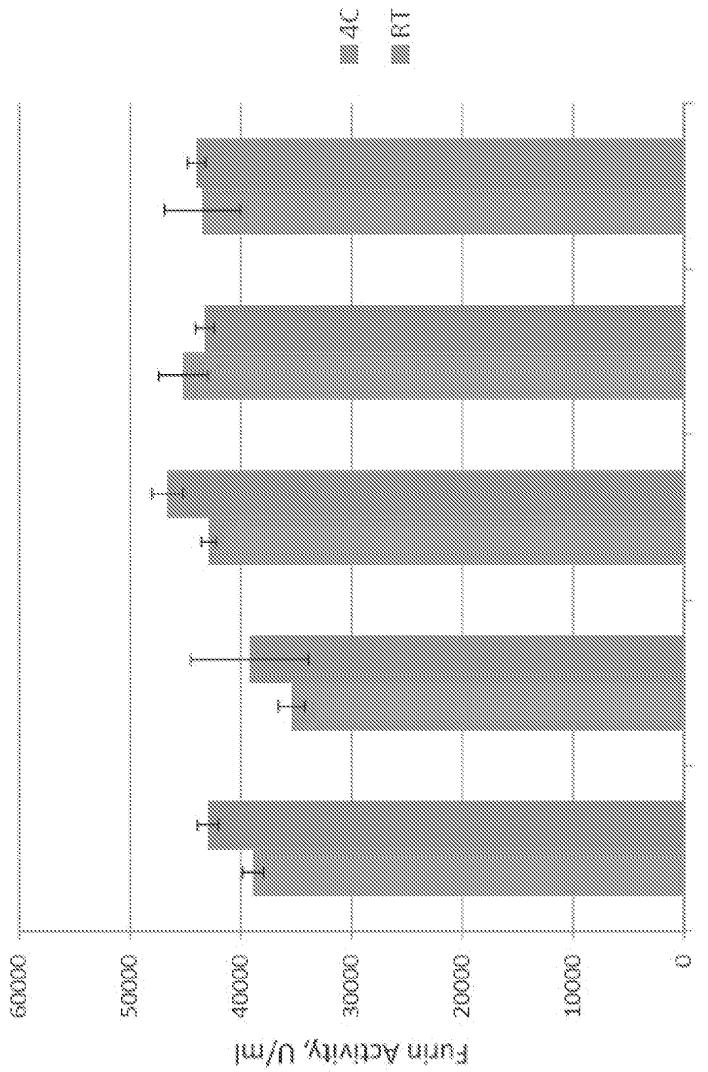

4C
RT
Furin Activity, U/ml
60000
50000
40000
30000
20000
10000
0
3
4
5
8
9
TR4 Cycle Number

FIG. 10

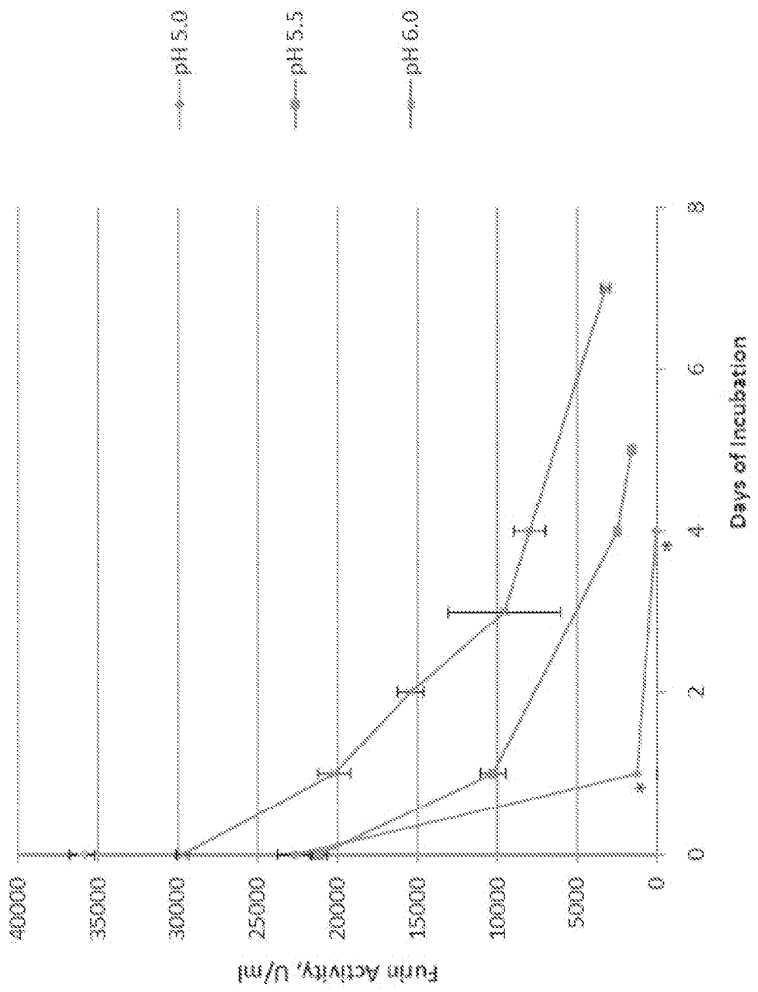

pH 5.0
pH 5.5
pH 6.0
Furin Activity, U/ml
40000
35000
30000
25000
20000
15000
10000
5000
0
0
2
4
6
8
Days of Incubation

FIG. 11

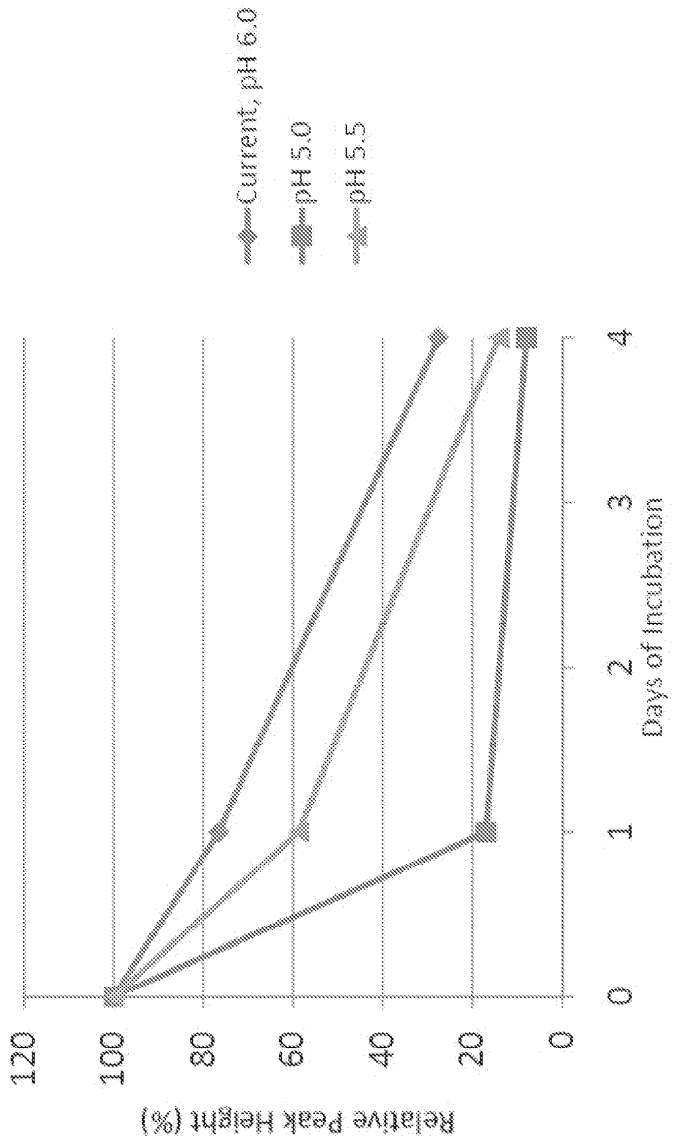

Current, pH 6.0
pH 5.0
pH 5.5
Relative Peak Height (%)
120
100
80
60
40
20
0
0
1
2
3
4
Days of Incubation

FIG. 12

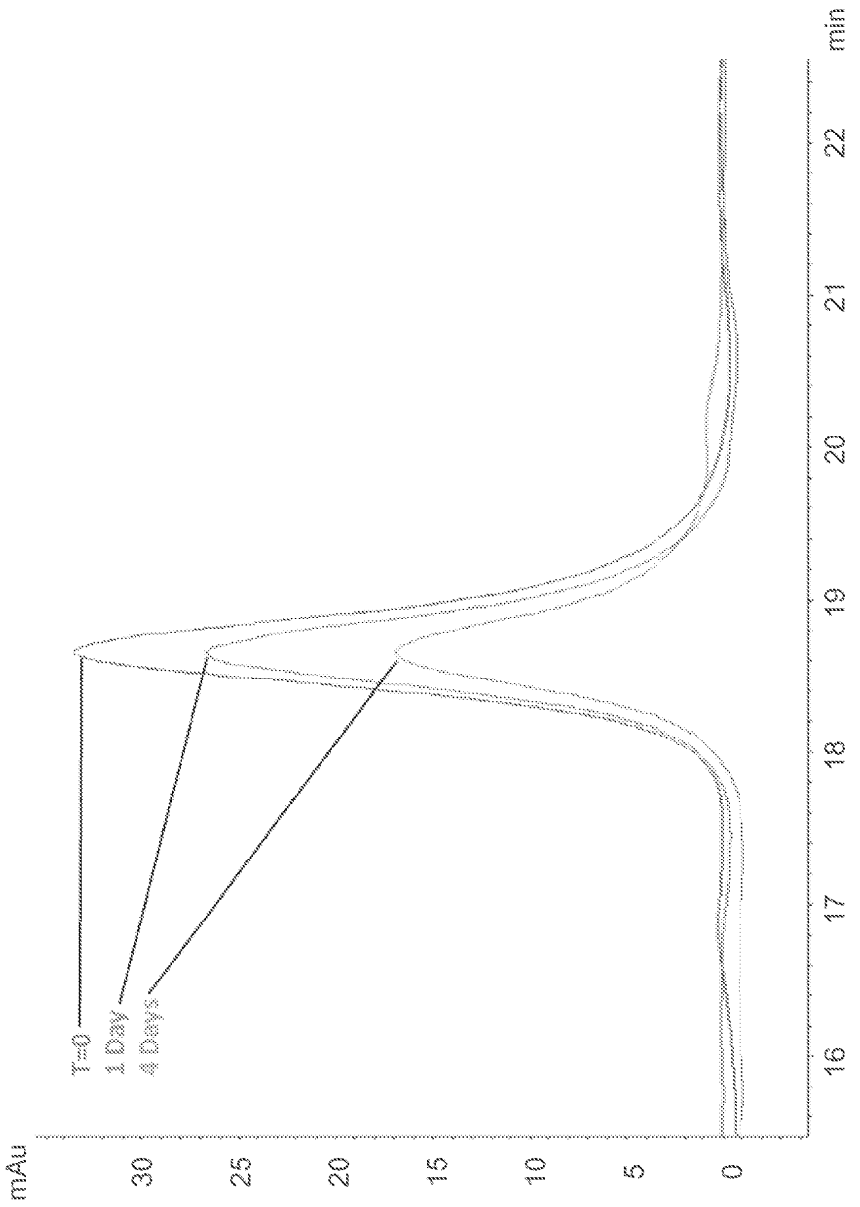


FIG. 13

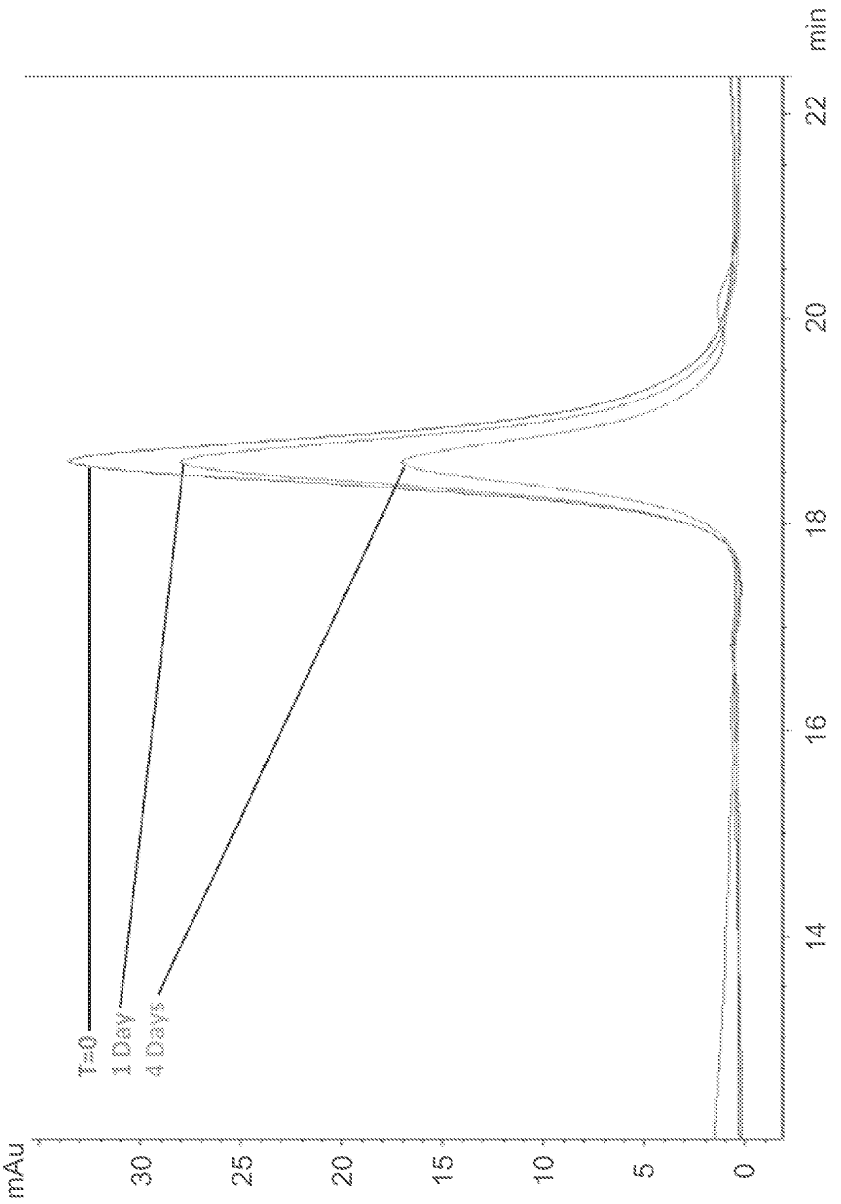


FIG. 14

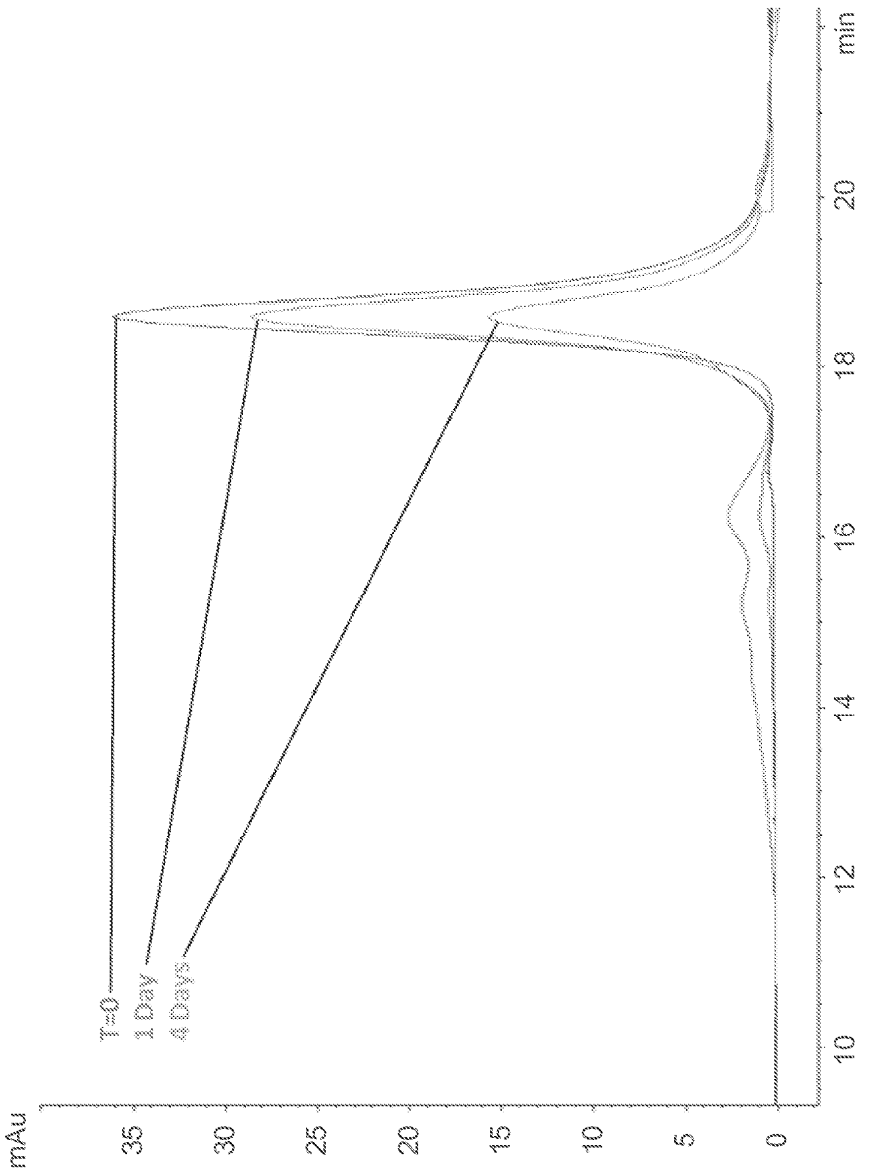


FIG. 15

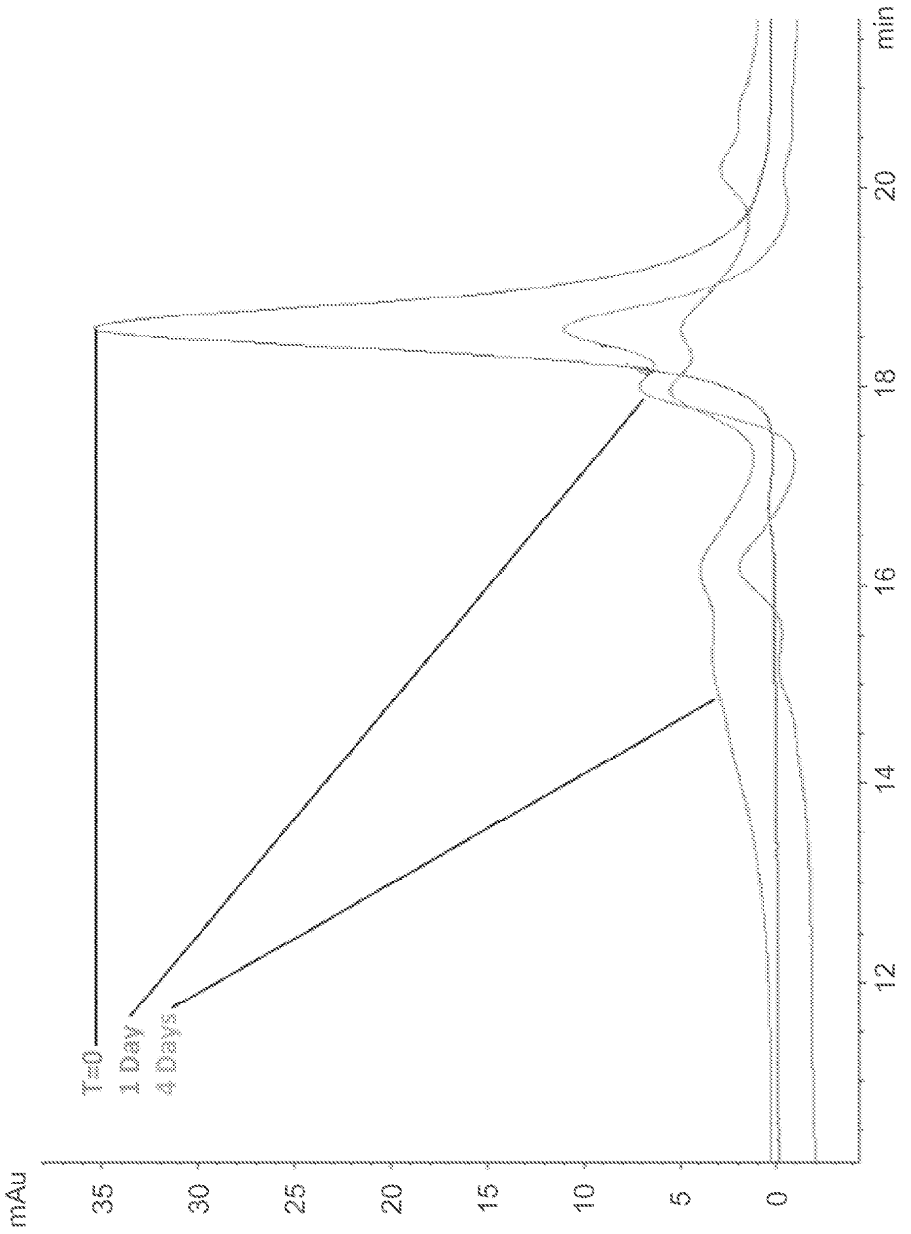


FIG. 16

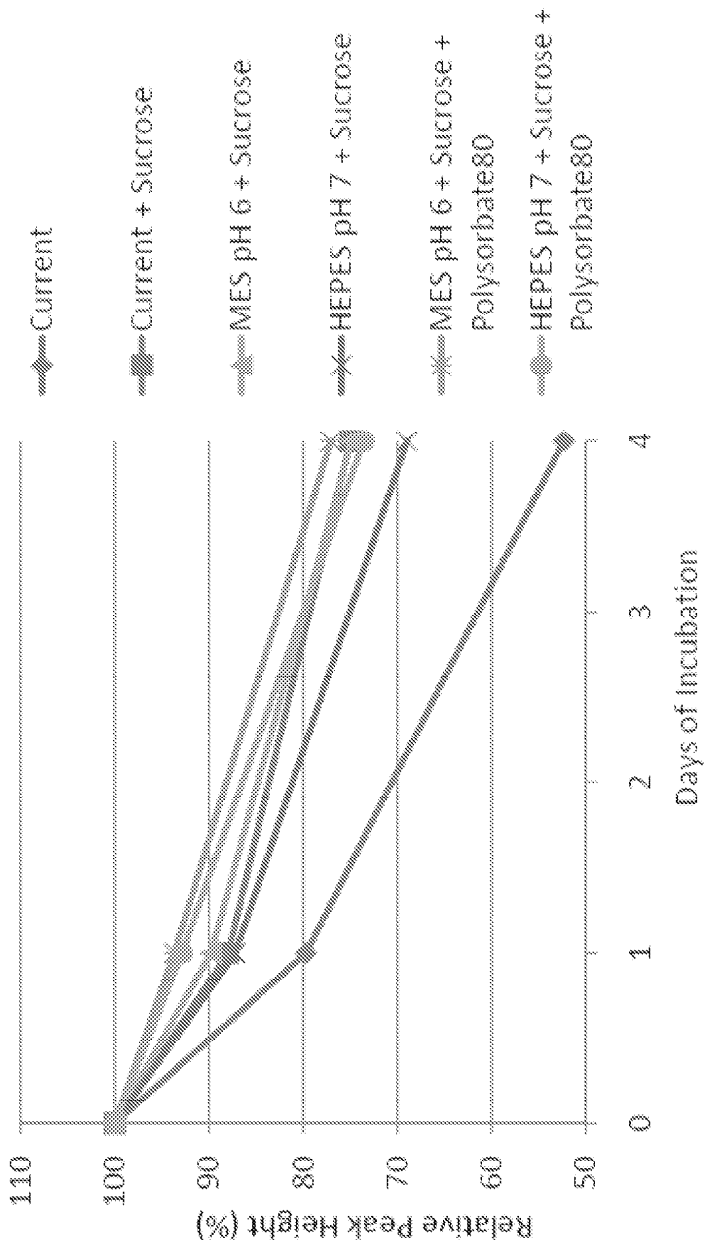

Current
Current + Sucrose
MES pH 6 + Sucrose
HEPES pH 7 + Sucrose
MES pH 6 + Sucrose + Polysorbate80
HEPES pH 7 + Sucrose + Polysorbate80
Relative Peak Height (%)
110
100
90
80
70
60
50
0
1
2
3
4
Days of Incubation

FIG. 17

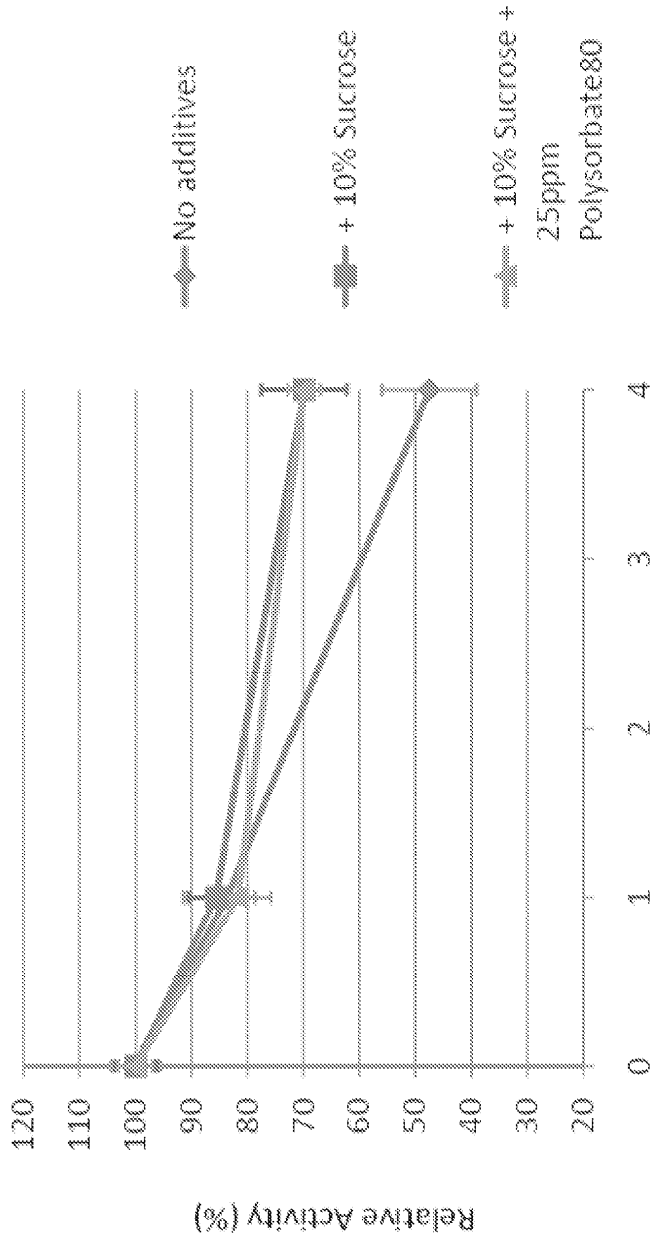

No additives
+ 10% Sucrose
+ 10% Sucrose + 25ppm Polysorbate80
Relative Activity (%)
120
110
100
90
80
70
60
50
40
30
20
0
1
2
3
4
Days of Incubation

FIG. 18

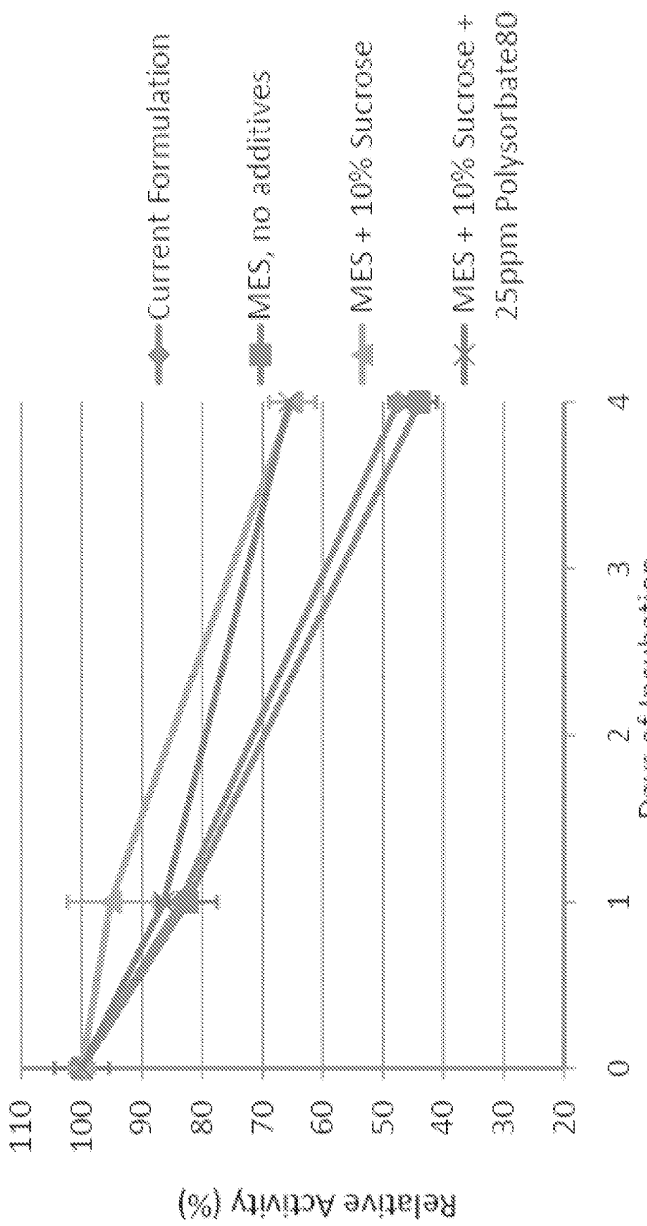

Current Formulation
MES, no additives
MES + 10% Sucrose
MES + 10% Sucrose + 25ppm Polysorbate80
Relative Activity (%)
110
100
90
80
70
60
50
40
30
20
0
1
2
3
4
Days of Incubation

FIG. 19

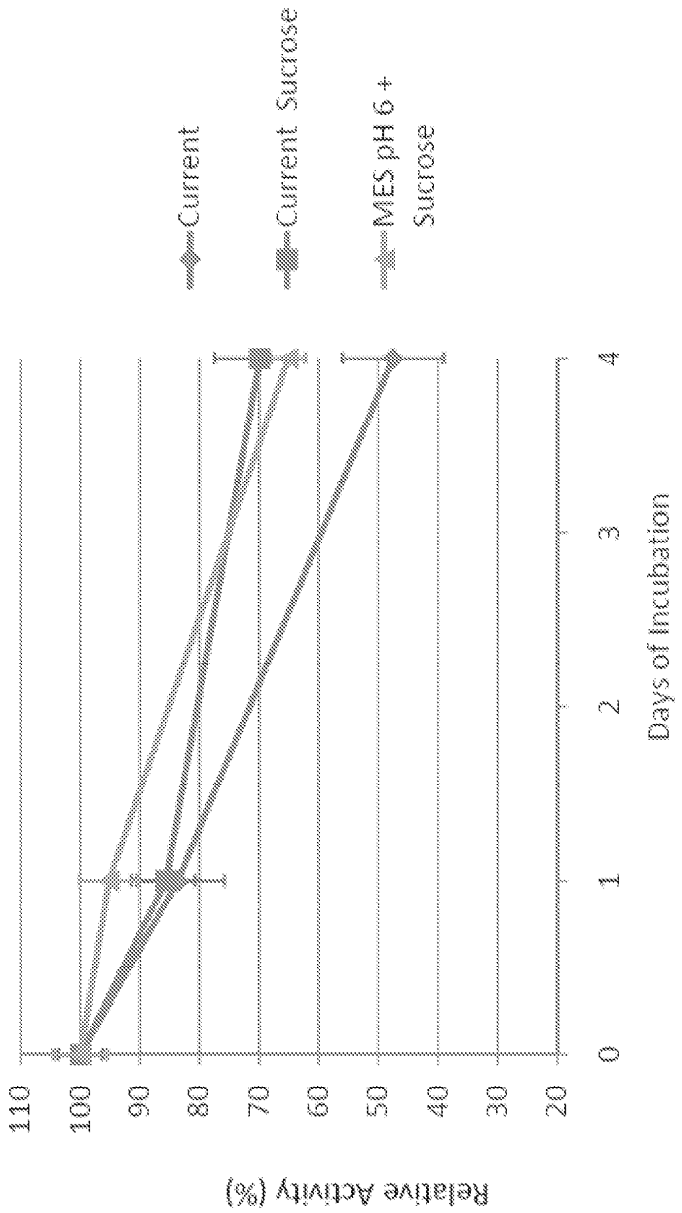

Current
Current Sucrose
MES pH 6 + Sucrose
Relative Activity (%)
110
100
90
80
70
60
50
40
30
20
0
1
2
3
4
Days of Incubation

FIG. 20

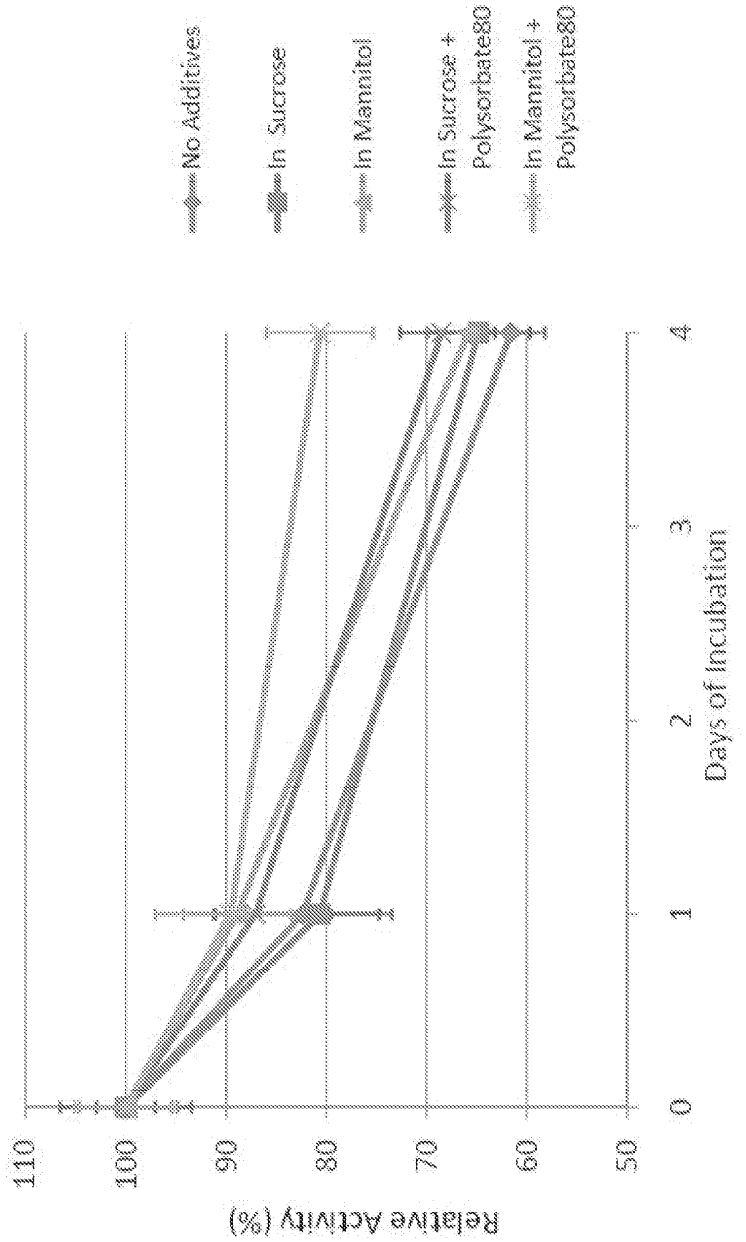

No Additives
In Sucrose
In Mannitol
In Sucrose + Polysorbate80
In Mannitol + Polysorbate80
Relative Activity (%)
110
100
90
80
70
60
50
0
1
2
3
4
Days of Incubation

FIG. 21

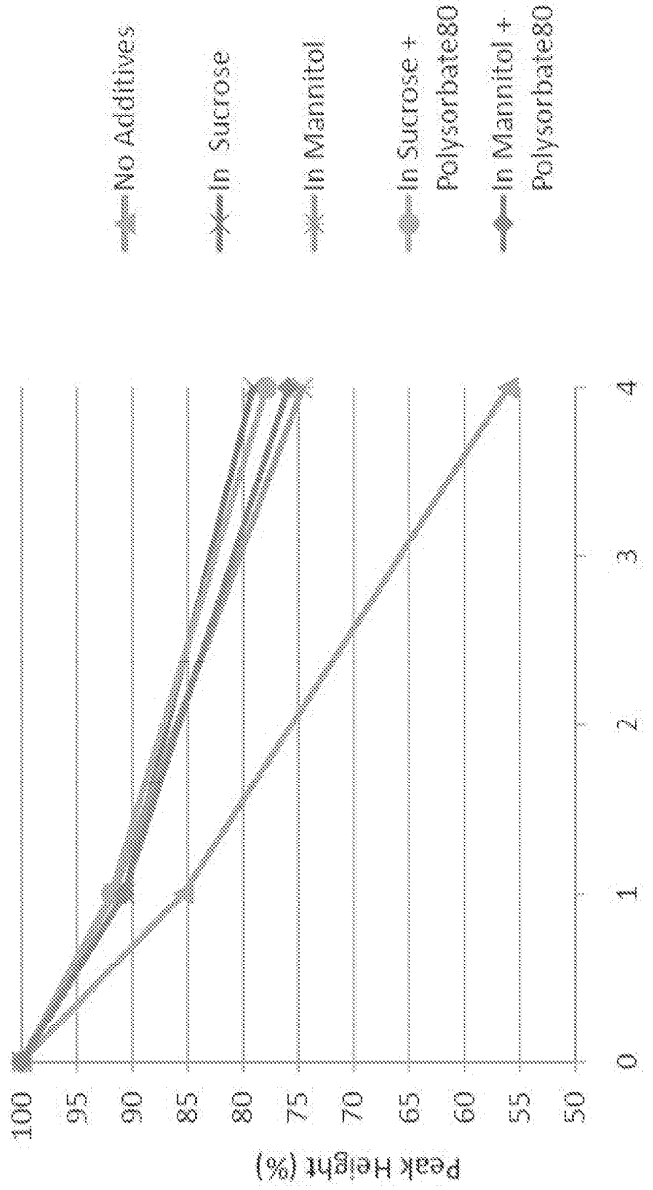

No Additives
In Sucrose
In Mannitol
In Sucrose + Polysorbate80
In Mannitol + Polysorbate80
Peak Height (%)
100
95
90
85
80
75
70
65
60
55
50
0
1
2
3
4
Days of Incubation

FIG. 22

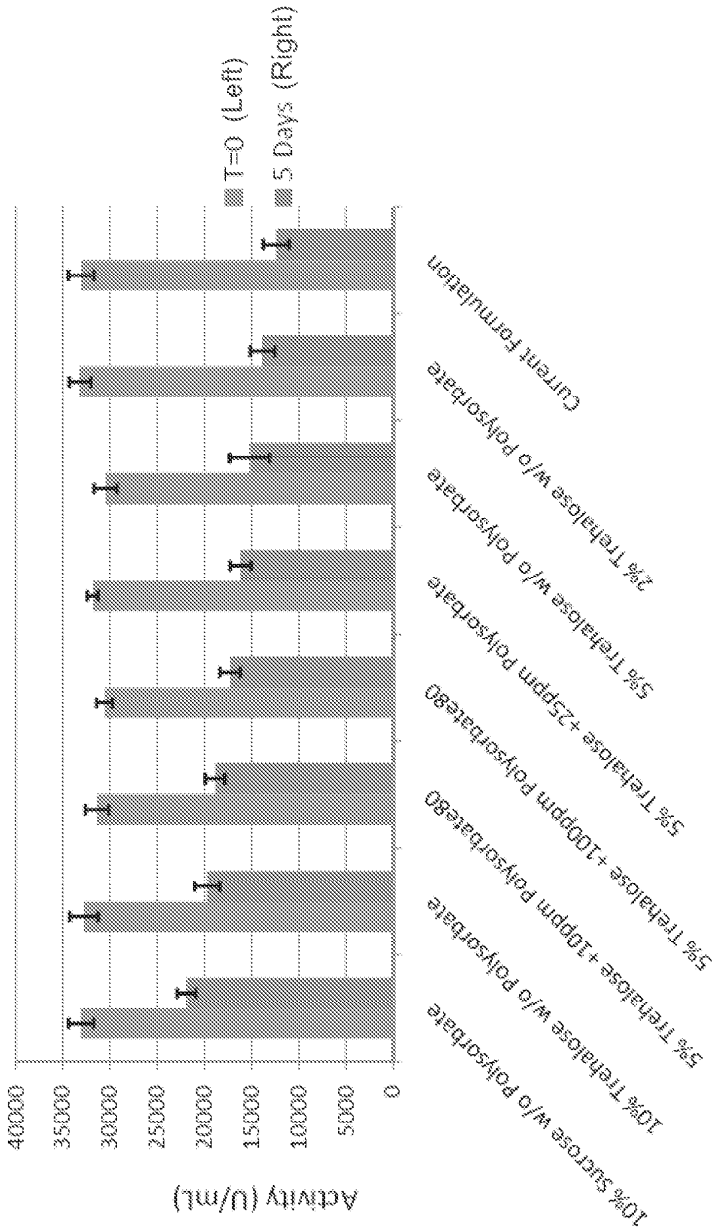

T=0 (Left)
5 Days (Right)
Activity (U/mL)
40000
35000
30000
25000
20000
15000
10000
5000
0
10% Sucrose w/o Polysorbate
10% Trehalose w/o Polysorbate
5% Trehalose +100ppm Polysorbate80
5% Trehalose +100ppm Polysorbate80
5% Trehalose +25ppm Polysorbate
5% Trehalose w/o Polysorbate
3% Trehalose w/o Polysorbate
Current formulation

FIG. 23

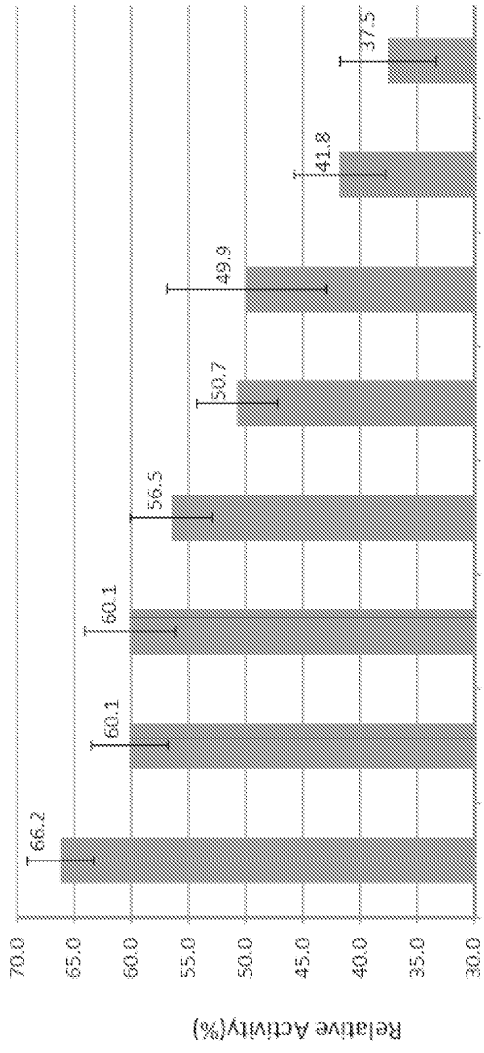

Relative Activity(%)
70.0
65.0
60.0
55.0
50.0
45.0
40.0
35.0
30.0
66.2
60.1
60.1
56.5
50.7
49.9
41.8
37.5
10% Sucrose w/o Polysorbate
5% Trehalose +100ppm Polysorbate80
10% Trehalose w/o Polysorbate
5% Trehalose +100ppm Polysorbate80
5% Trehalose +25ppm Polysorbate80
5% Trehalose w/o Polysorbate
2% Trehalose w/o Polysorbate
Current Formulation

FIG. 24

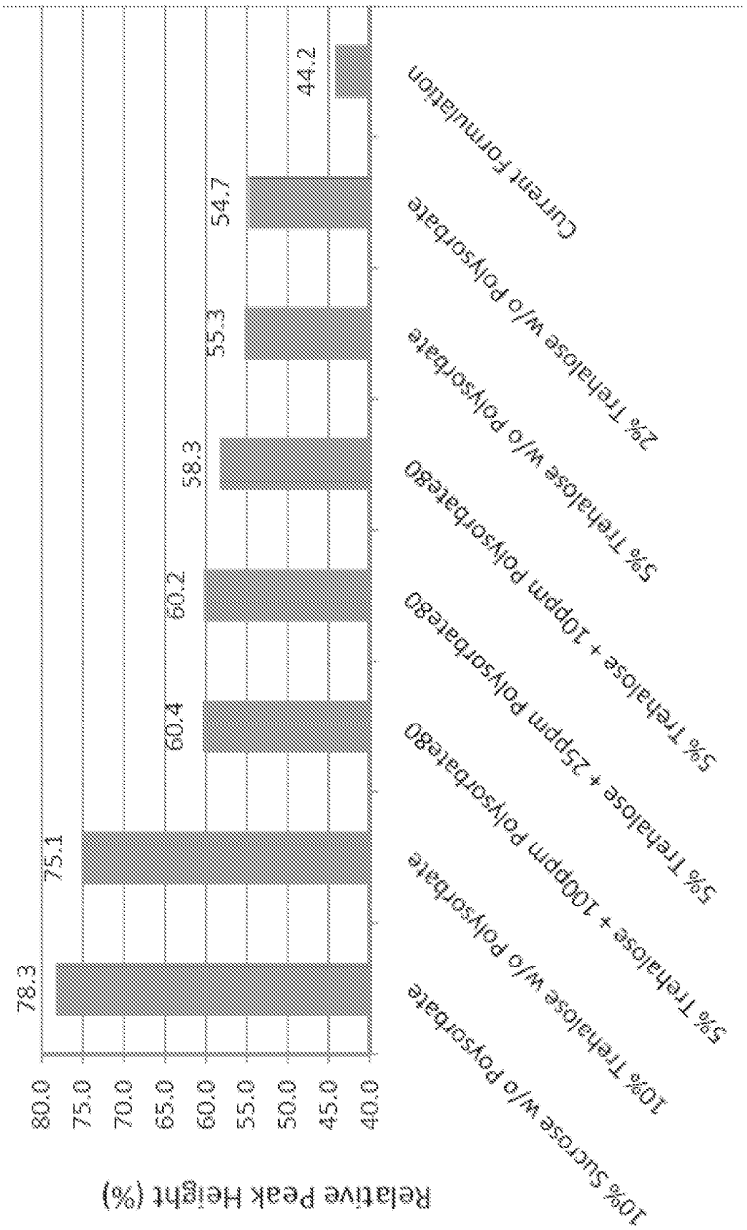

Relative Peak Height (%)
80.0
75.0
70.0
65.0
60.0
55.0
50.0
45.0
40.0
78.3
75.1
60.4
60.2
58.3
55.3
54.7
44.2
10% Sucrose w/o Polysorbate
10% Trehalose w/o Polysorbate
5% Trehalose + 100ppm Polysorbate80
5% Trehalose + 25ppm Polysorbate80
5% Trehalose + 10ppm Polysorbate80
5% Trehalose w/o Polysorbate
2% Trehalose w/o Polysorbate
Current formulation

FIG. 25

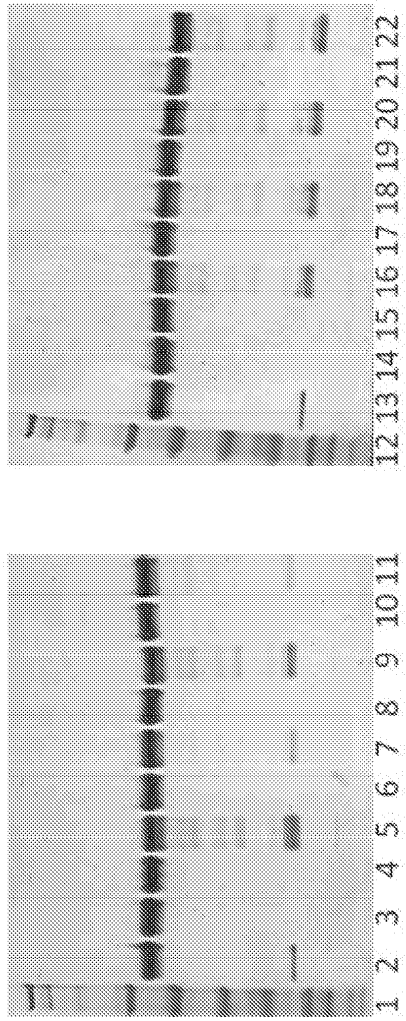


FIG. 26

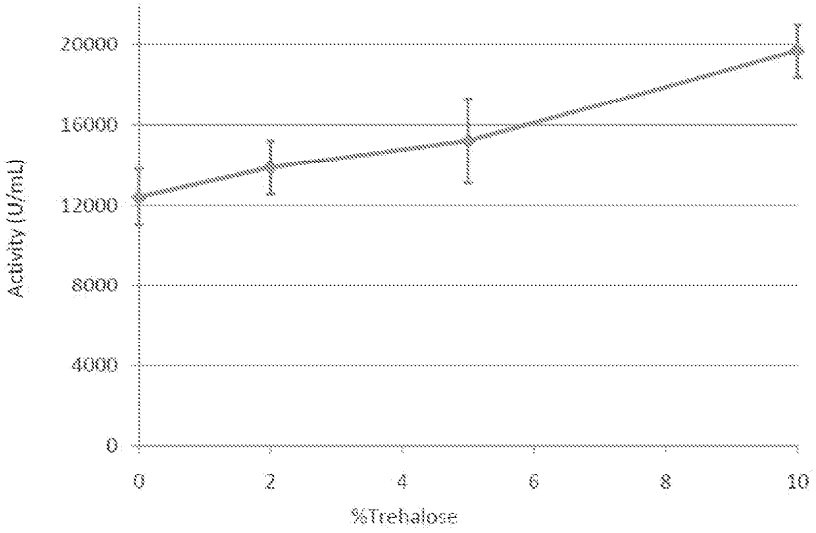

20000
16000
12000
8000
4000
0
Activity (U/mL)
0
2
4
6
8
10
%Trehalose

FIG. 27

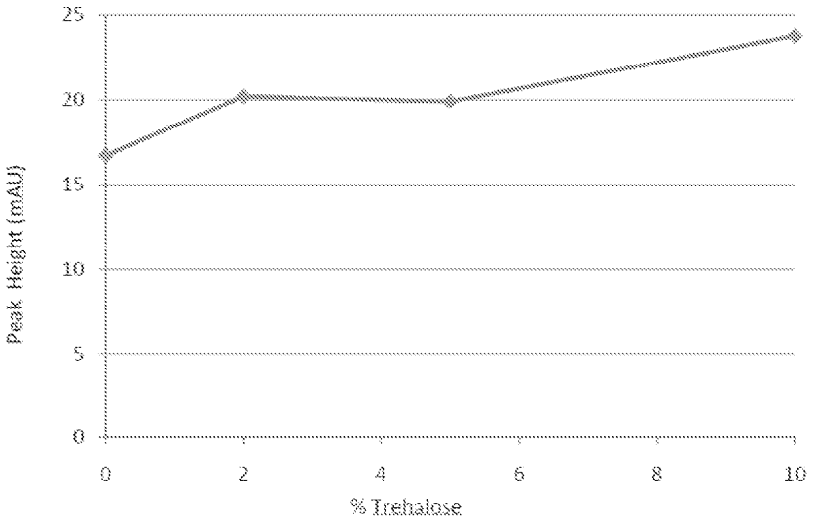

25
20
15
10
5
0
Peak Height (mAU)
0
2
4
6
8
10
% Trehalose

FIG. 28

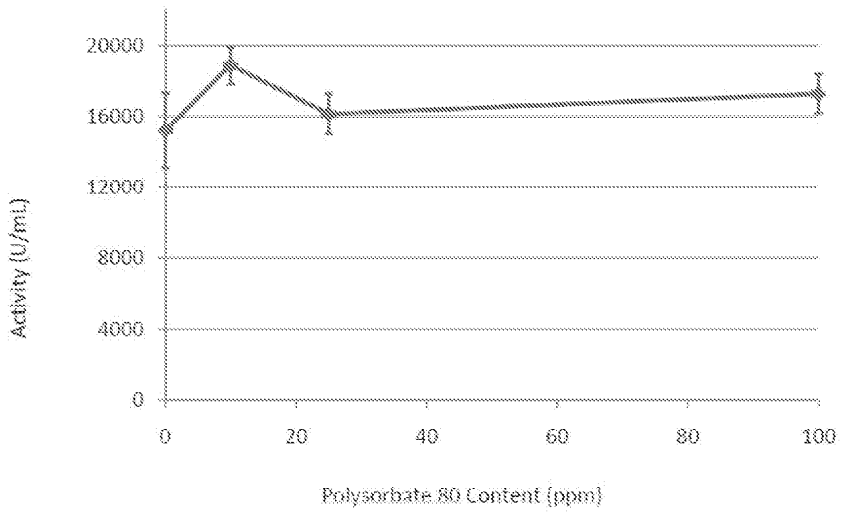

20000
16000
12000
8000
4000
0
Activity (U/mL)
0
20
40
60
80
100
Polysorbate 80 Content (ppm)

FIG. 29

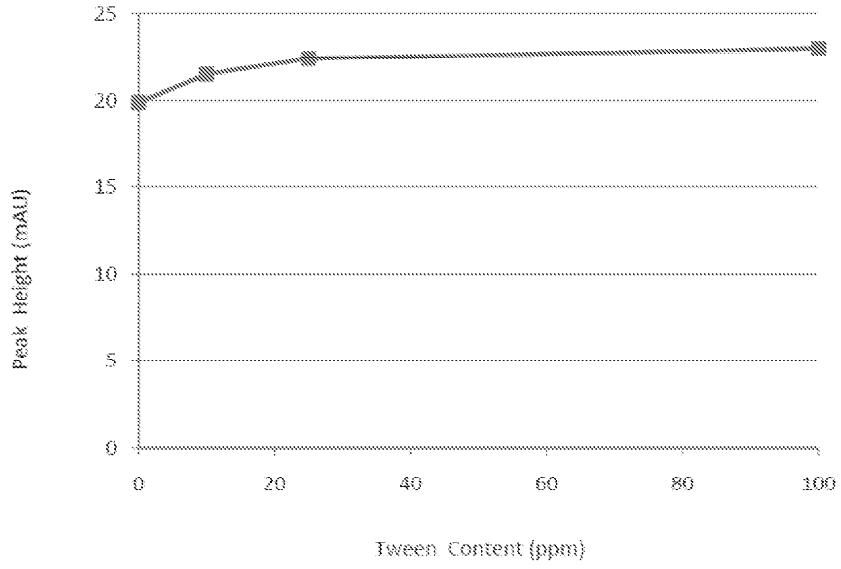

25
20
15
10
5
0
Peak Height (mAU)
0
20
40
60
80
100
Tween Content (ppm)

FIG. 30

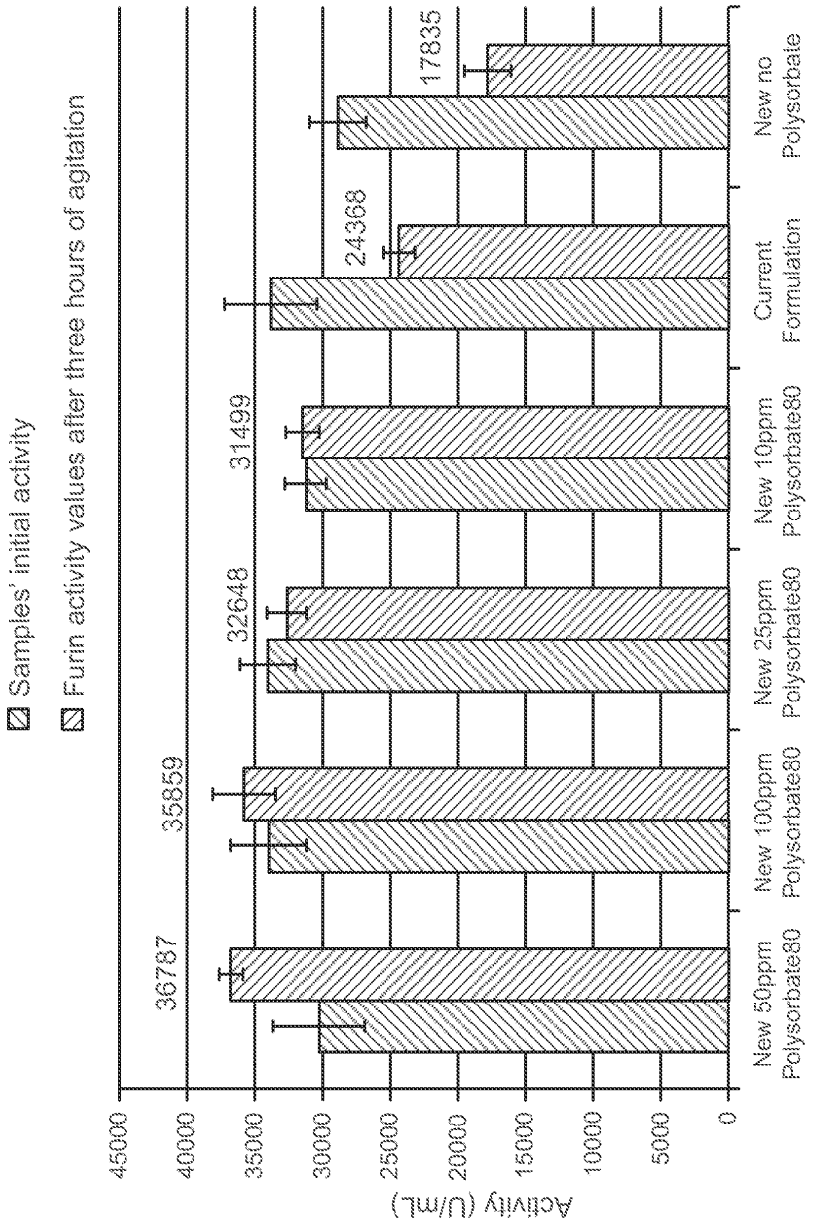

Samples' initial activity
Furin activity values after three hours of agitation
Activity (U/mL)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
36787
35859
32648
31499
24368
17835
New 50ppm Polysorbate80
New 100ppm Polysorbate80
New 25ppm Polysorbate80
New 10ppm Polysorbate80
Current Formulation
New no Polysorbate

FIG. 31

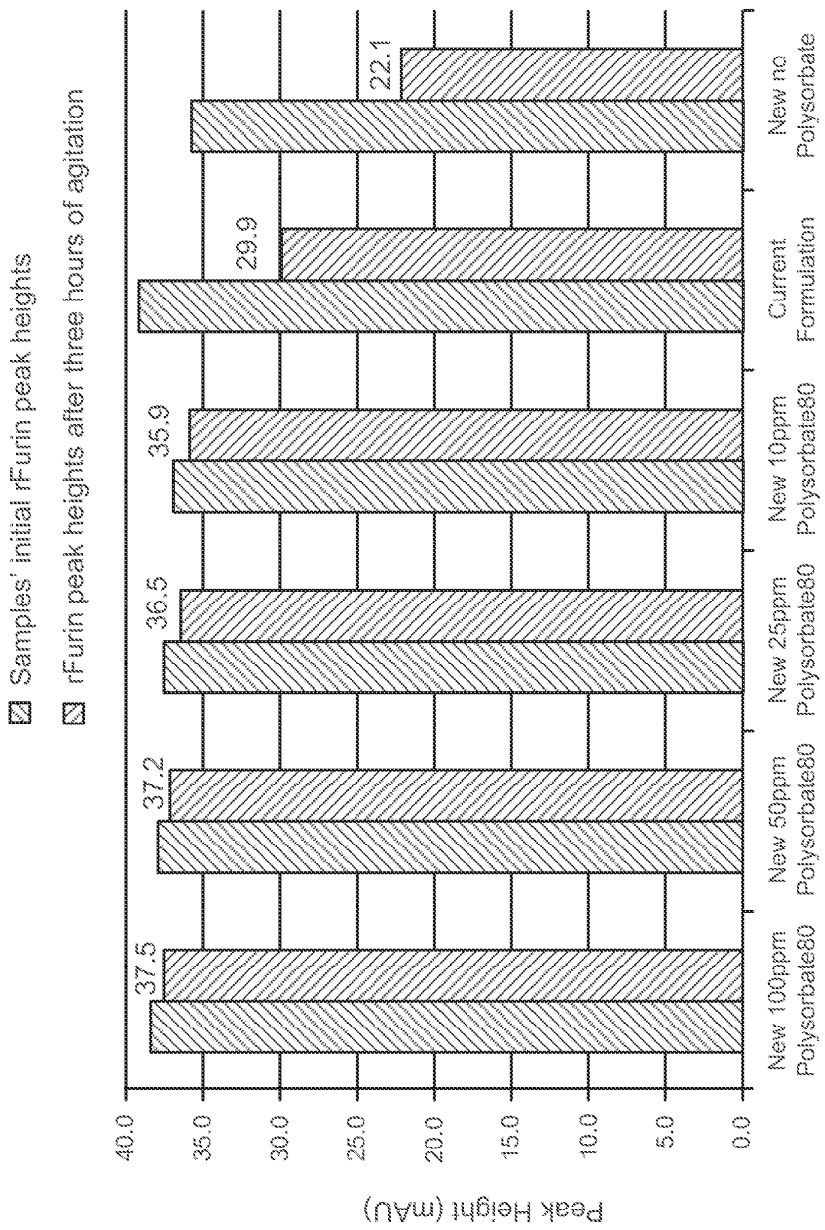

Samples' initial rFurin peak heights
rFurin peak heights after three hours of agitation
Peak Height (mAU)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
37.5
37.2
36.5
35.9
29.9
22.1
New 100ppm Polysorbate80
New 50ppm Polysorbate80
New 25ppm Polysorbate80
New 10ppm Polysorbate80
Current Formulation
New no Polysorbate

FIG. 32

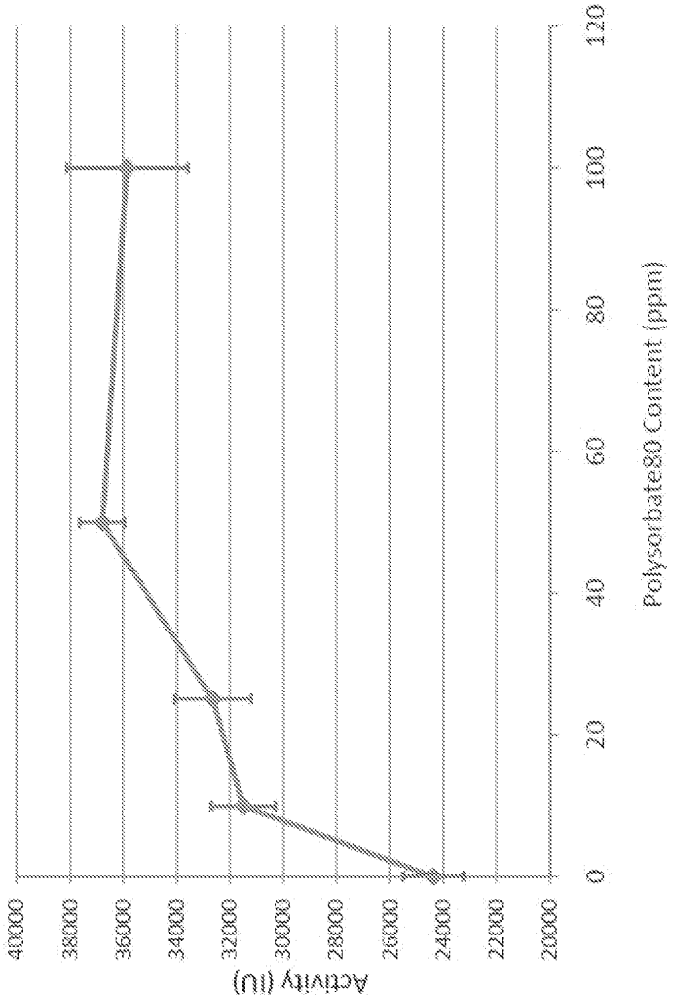

Activity (IU)
40000
38000
36000
34000
32000
30000
28000
26000
24000
22000
20000
0
20
40
60
80
100
120
Polysorbate80 Content (ppm)

FIG. 33

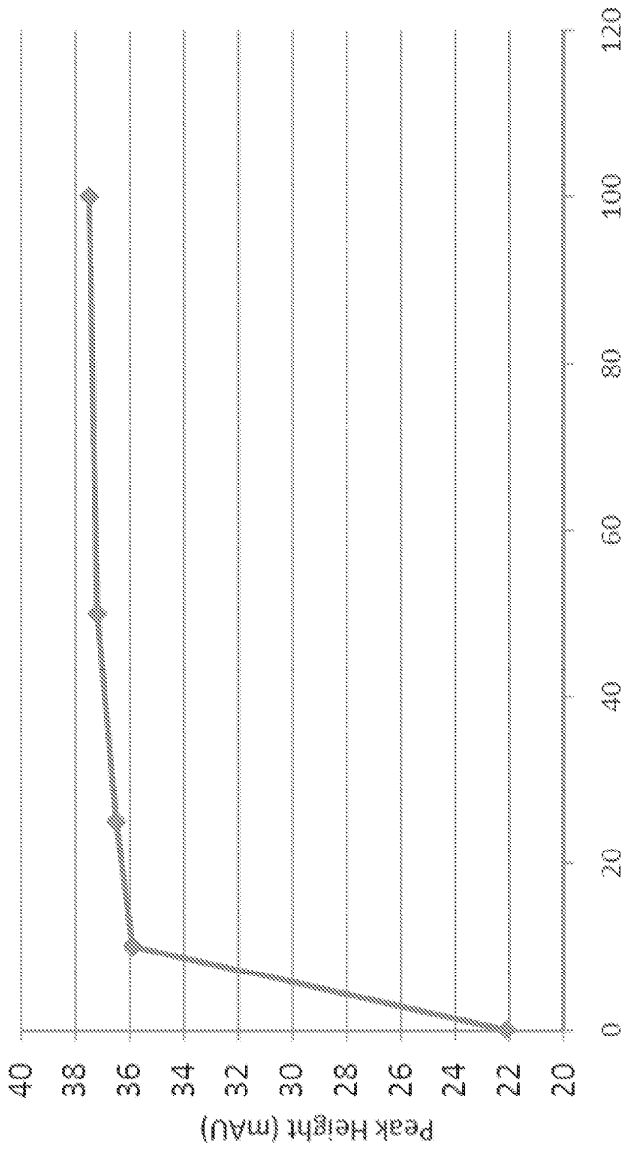

Peak Height (mAU)
40
38
36
34
32
30
28
26
24
22
20
0
20
40
60
80
100
120
Polysorbate80 Content (ppm)

FIG. 34

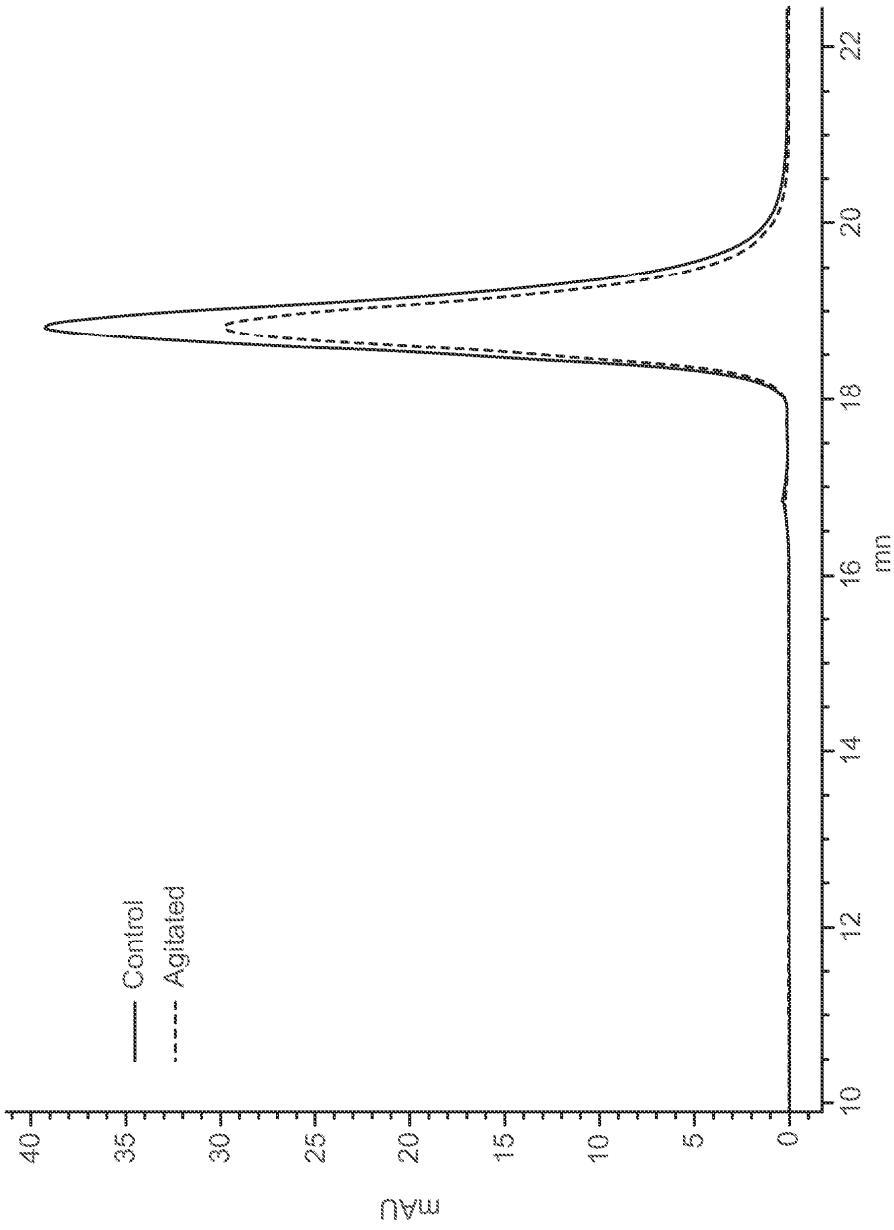

Control
Agitated
mAU
40
35
30
25
20
15
10
5
0
10
12
14
16
18
20
22
min

FIG. 35

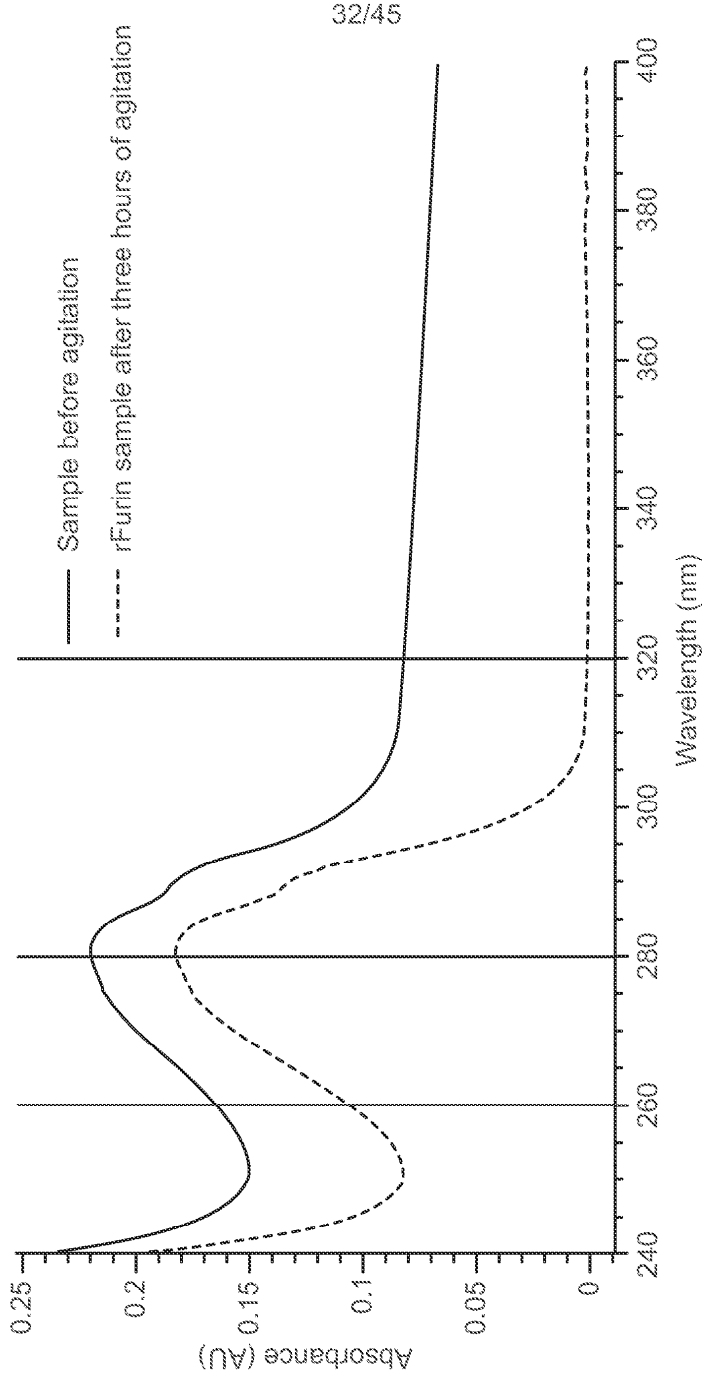

Sample before agitation
rFurin sample after three hours of agitation
Absorbance (AU)
0.25
0.2
0.15
0.1
0.05
0
240
260
280
300
320
340
360
380
400
Wavelength (nm)

FIG. 36

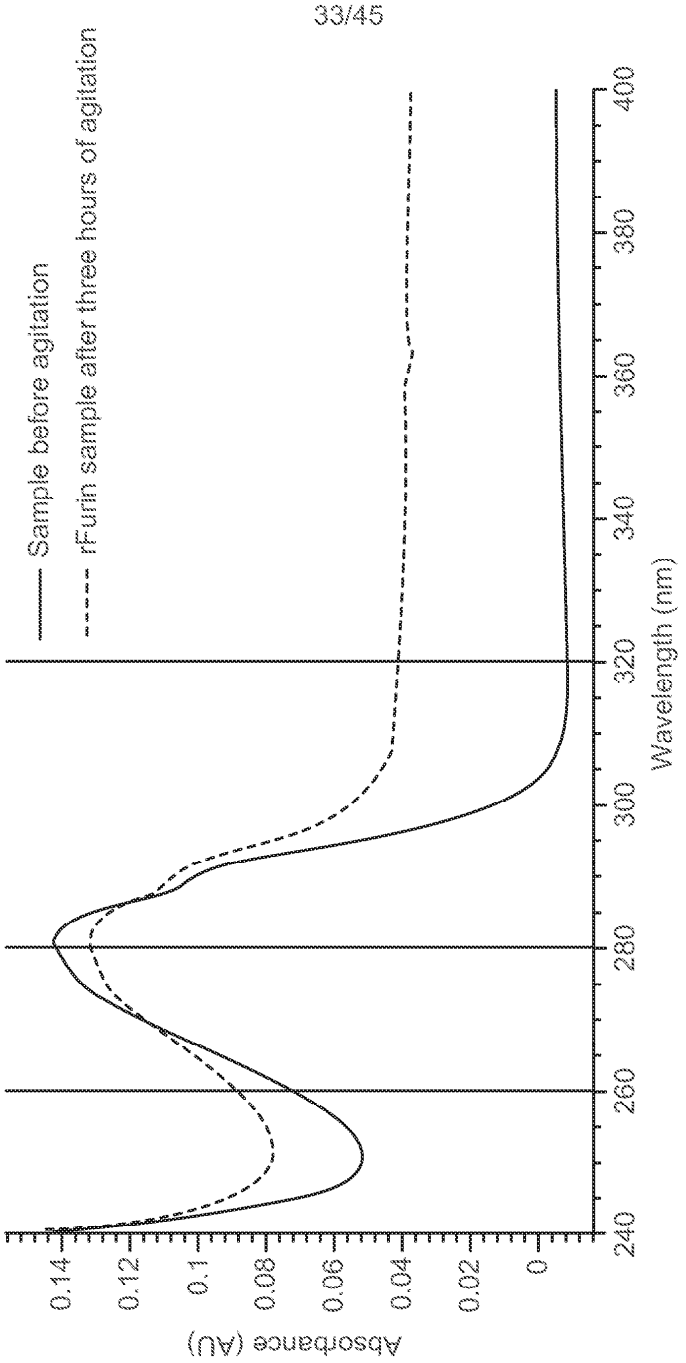

Sample before agitation
rFurin sample after three hours of agitation
Absorbance (AU)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
240
260
280
300
320
340
360
380
400
Wavelength (nm)

FIG. 37

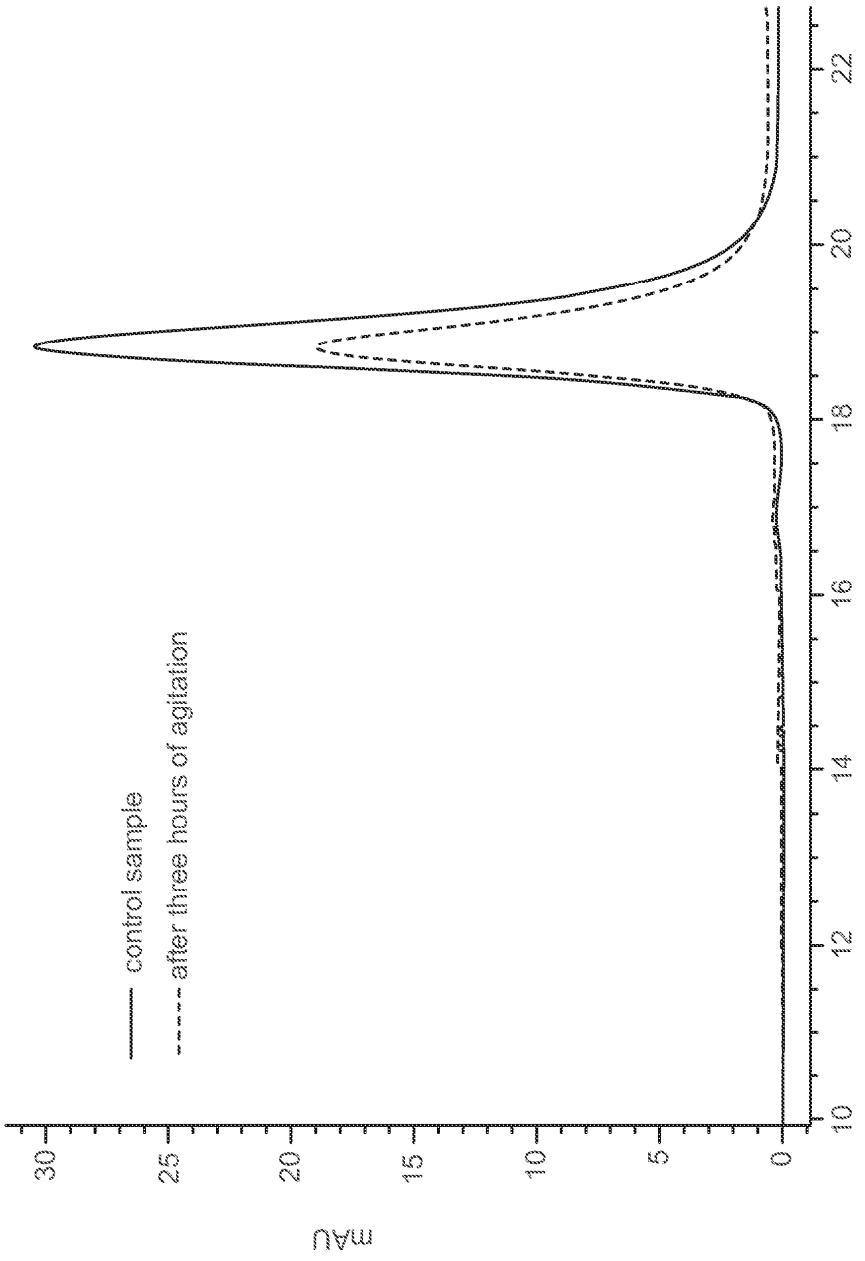

mAU
30
25
20
15
10
5
0
10
12
14
16
18
20
22
nm
control sample
after three hours of agitation

FIG. 38

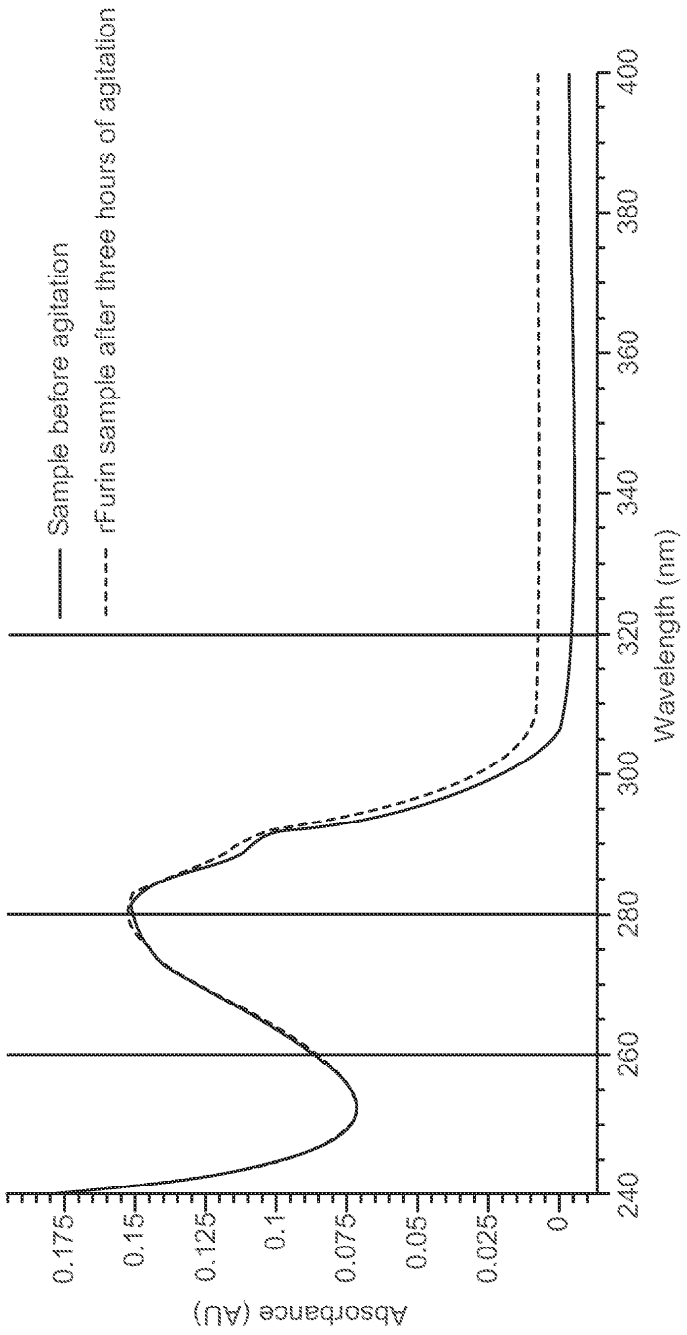

Sample before agitation
rFurin sample after three hours of agitation
Absorbance (AU)
0.175
0.15
0.125
0.1
0.075
0.05
0.025
0
240
260
280
300
320
340
360
380
400
Wavelength (nm)

FIG. 39

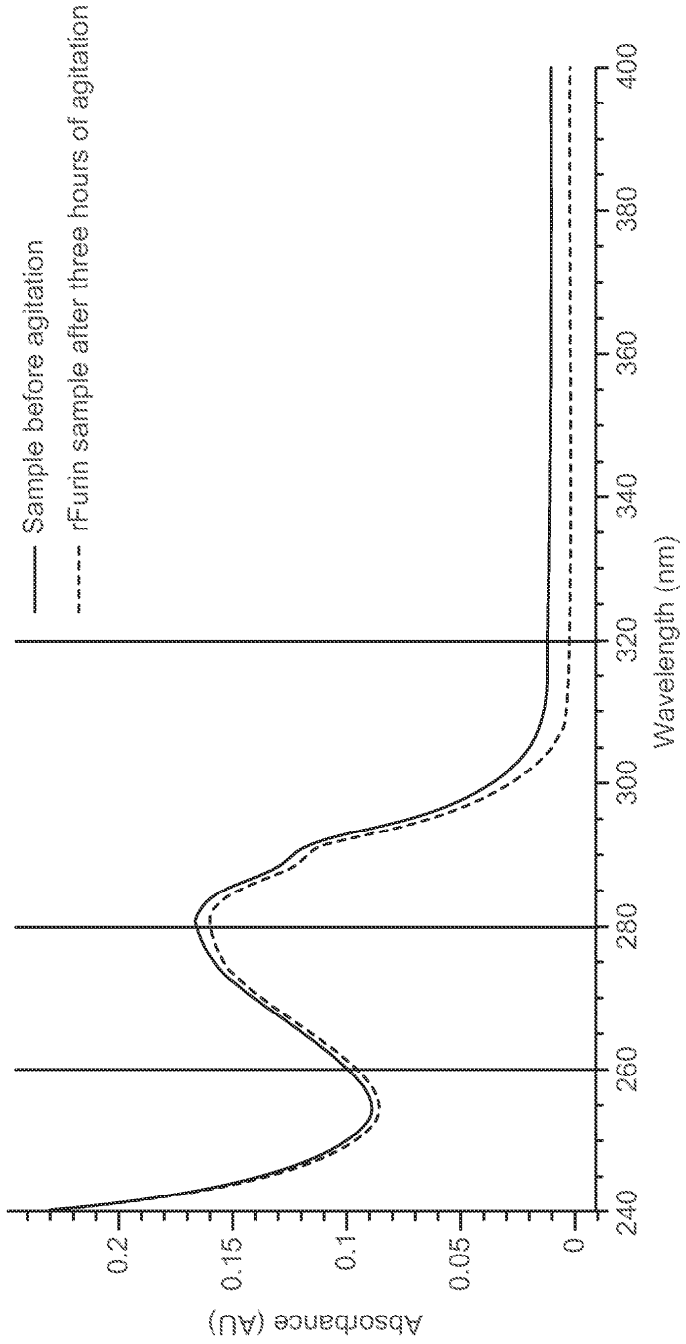

Sample before agitation
rFurin sample after three hours of agitation
Absorbance (AU)
0.2
0.15
0.1
0.05
0
240
260
280
300
320
340
360
380
400
Wavelength (nm)

FIG. 40

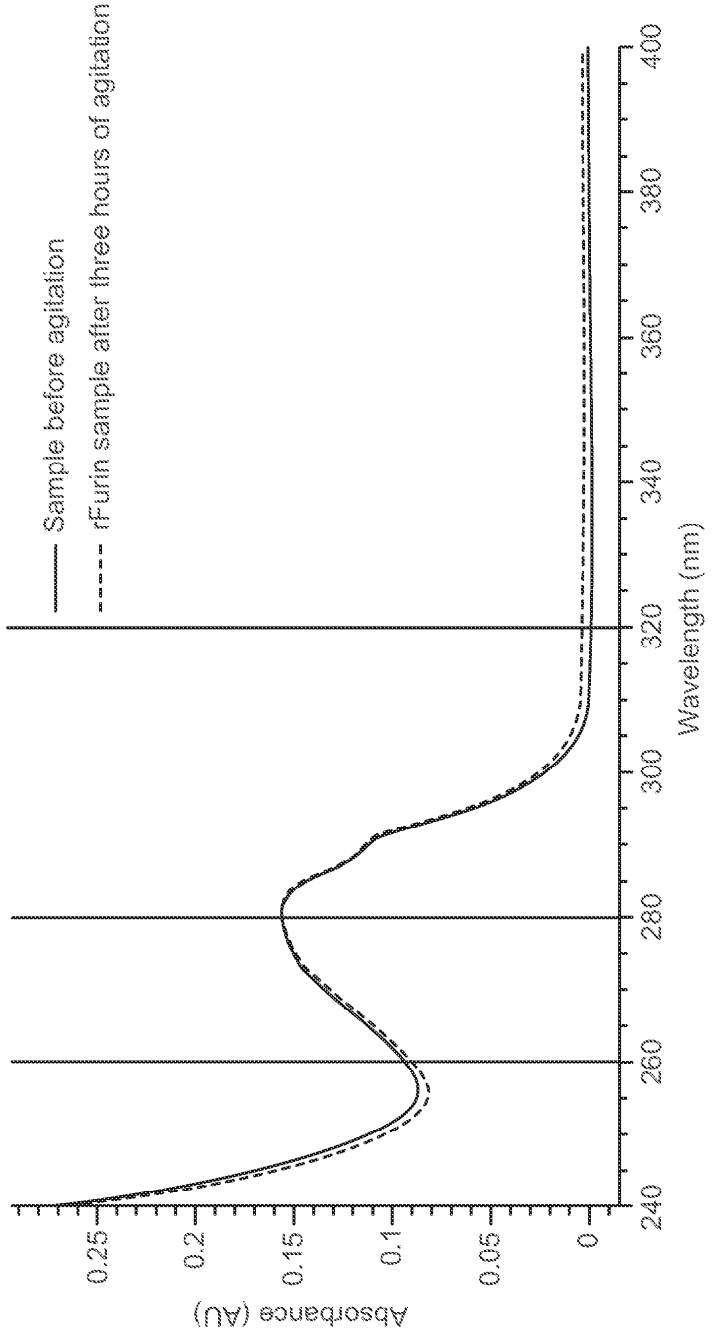

Sample before agitation
rFurin sample after three hours of agitation
Absorbance (AU)
0.25
0.2
0.15
0.1
0.05
0
240
260
280
300
320
340
360
380
400
Wavelength (nm)

FIG. 41

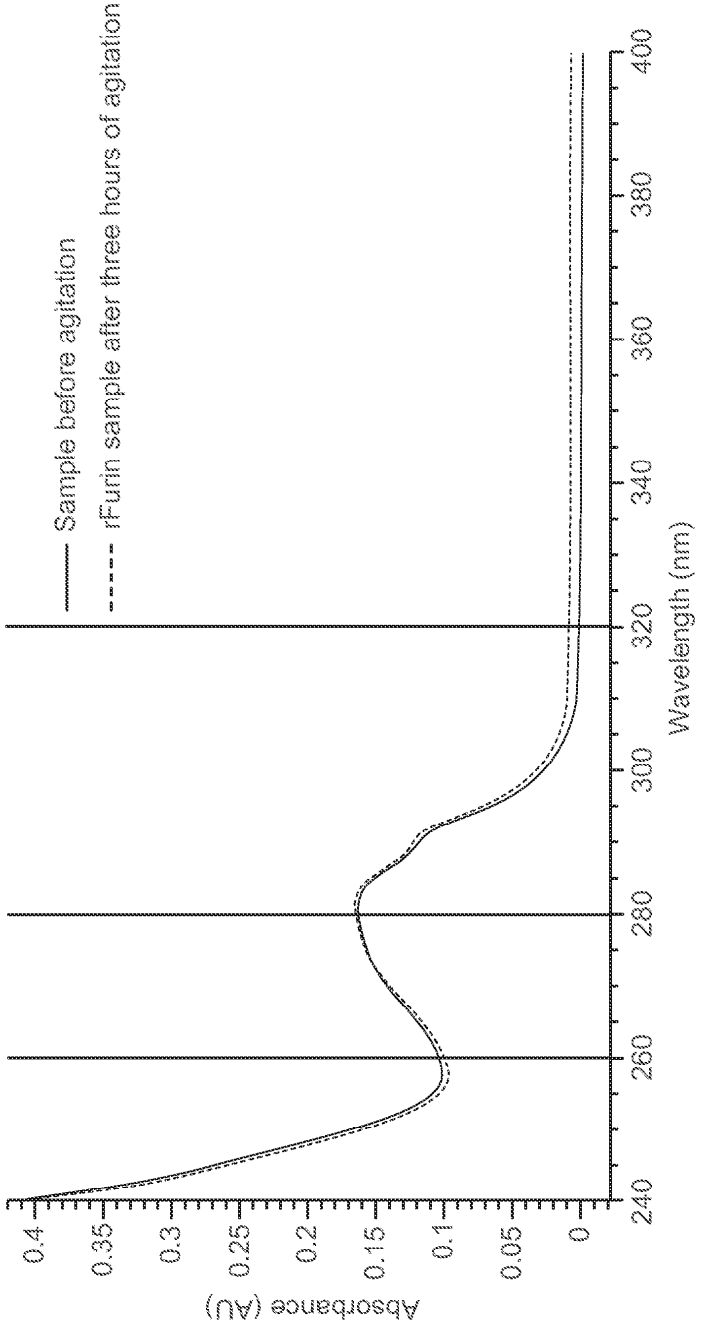

Sample before agitation
rFurin sample after three hours of agitation
Absorbance (AU)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
240
260
280
300
320
340
360
380
400
Wavelength (nm)

FIG. 42

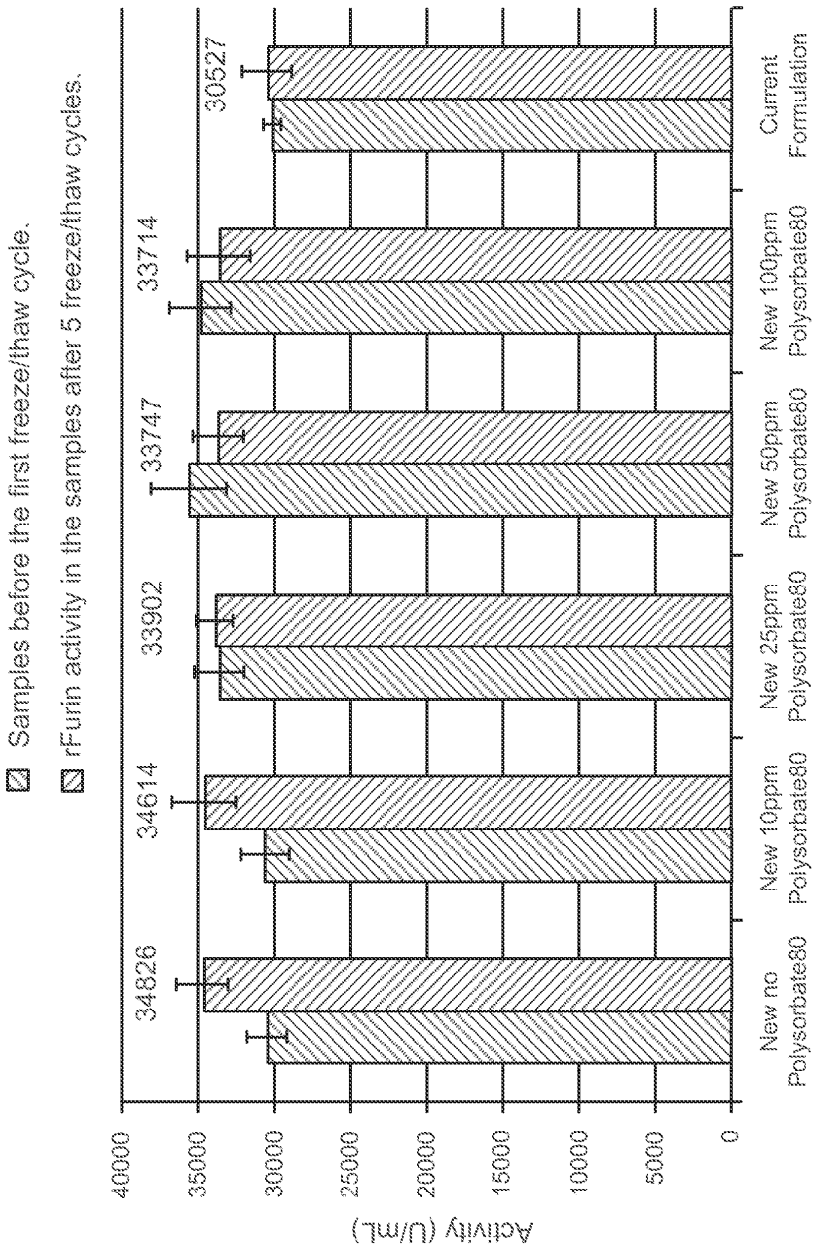

Samples before the first freeze/thaw cycle.
rFurin activity in the samples after 5 freeze/thaw cycles.
Activity (U/mL)
40000
35000
30000
25000
20000
15000
10000
5000
0
34826
34614
33902
33747
33714
30527
New no Polysorbate80
New 10ppm Polysorbate80
New 25ppm Polysorbate80
New 50ppm Polysorbate80
New 100ppm Polysorbate80
Current Formulation

FIG. 43

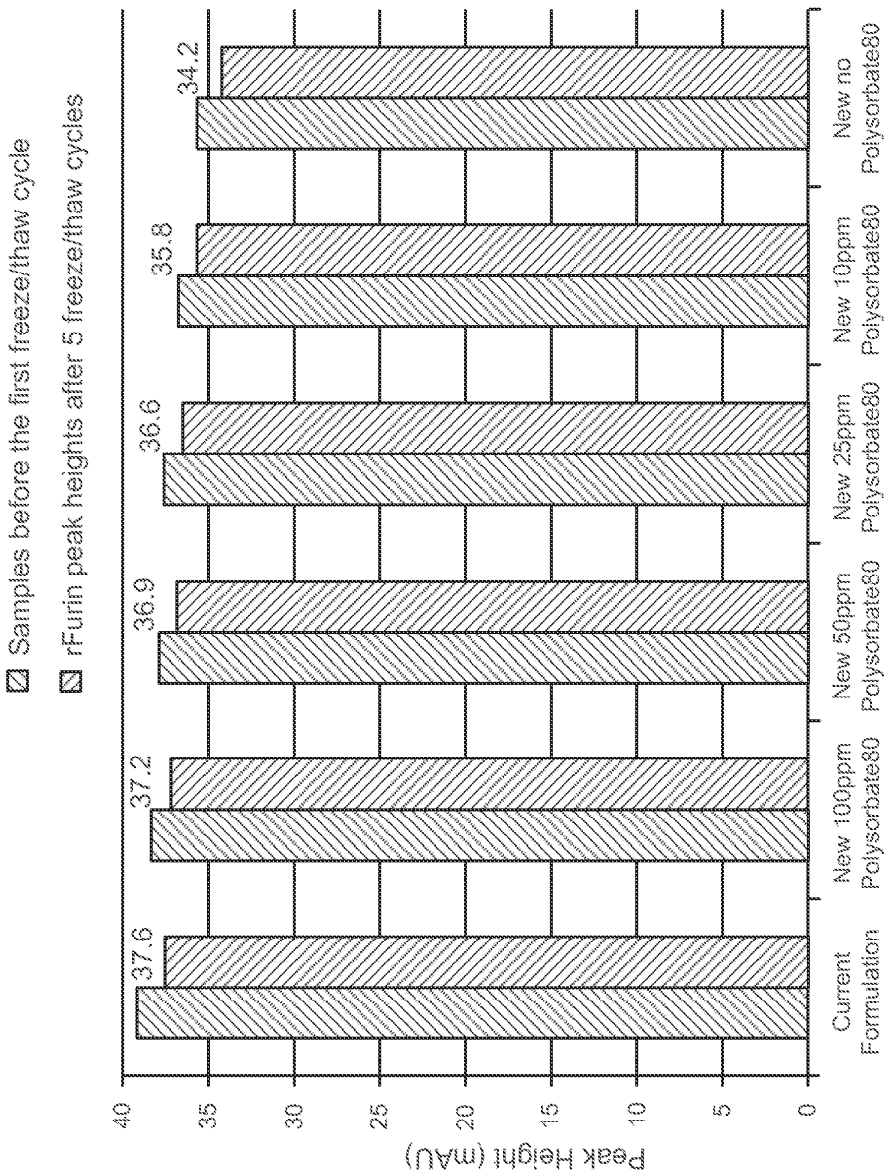

Samples before the first freeze/thaw cycle
rFurin peak heights after 5 freeze/thaw cycles
Peak Height (mAU)
0
5
10
15
20
25
30
35
40
37.6
37.2
36.9
36.6
35.8
34.2
Current Formulation
New 100ppm Polysorbate80
New 50ppm Polysorbate80
New 25ppm Polysorbate80
New 10ppm Polysorbate80
New no Polysorbate80

FIG. 44

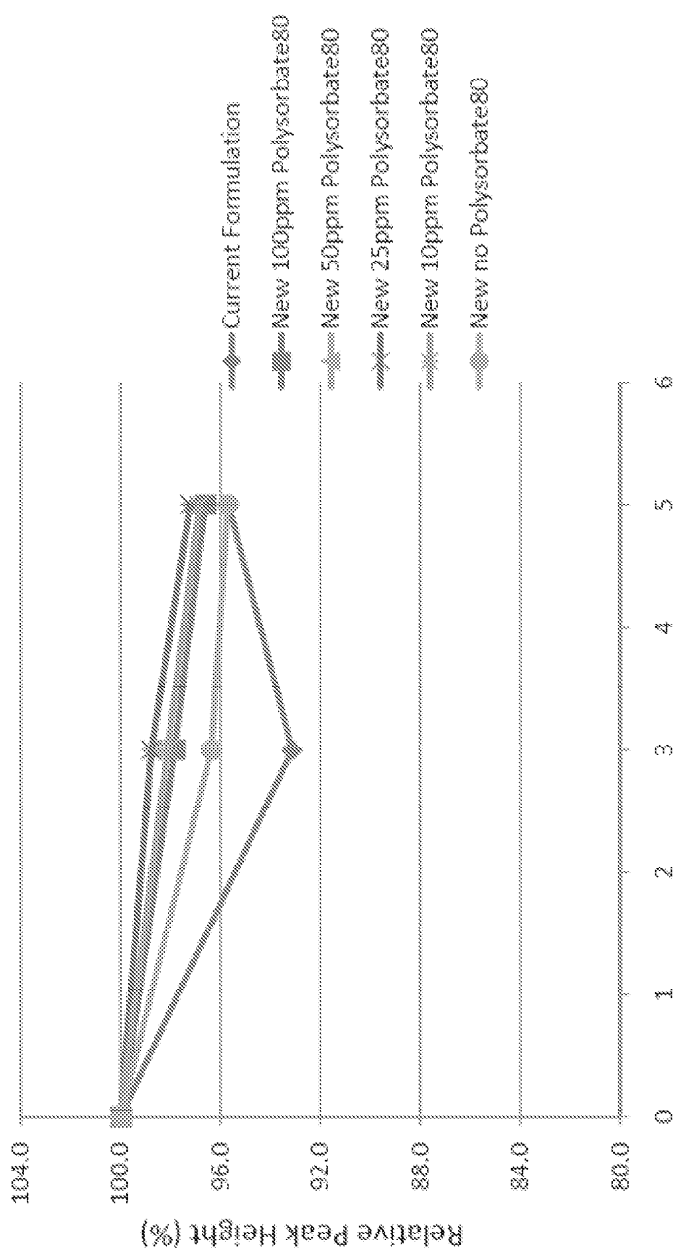

Relative Peak Height (%)
104.0
100.0
96.0
92.0
88.0
84.0
80.0
0
1
2
3
4
5
6
#Freeze/Thaw Cycles
Current Formulation
New 100ppm Polysorbate80
New 50ppm Polysorbate80
New 25ppm Polysorbate80
New 10ppm Polysorbate80
New no Polysorbate80

FIG. 45

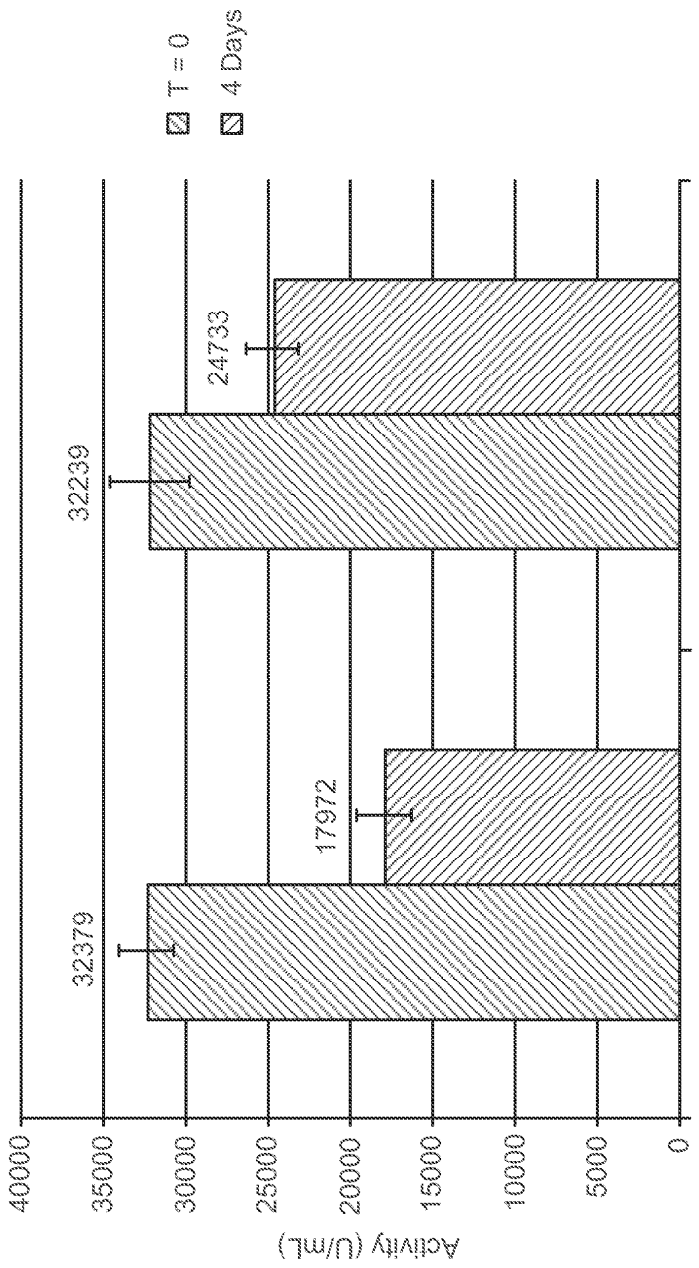

T = 0
4 Days
Activity (U/mL)
40000
35000
30000
25000
20000
15000
10000
5000
0
32379
17972
32239
24733
Current Formulation
New Formulation

FIG. 46

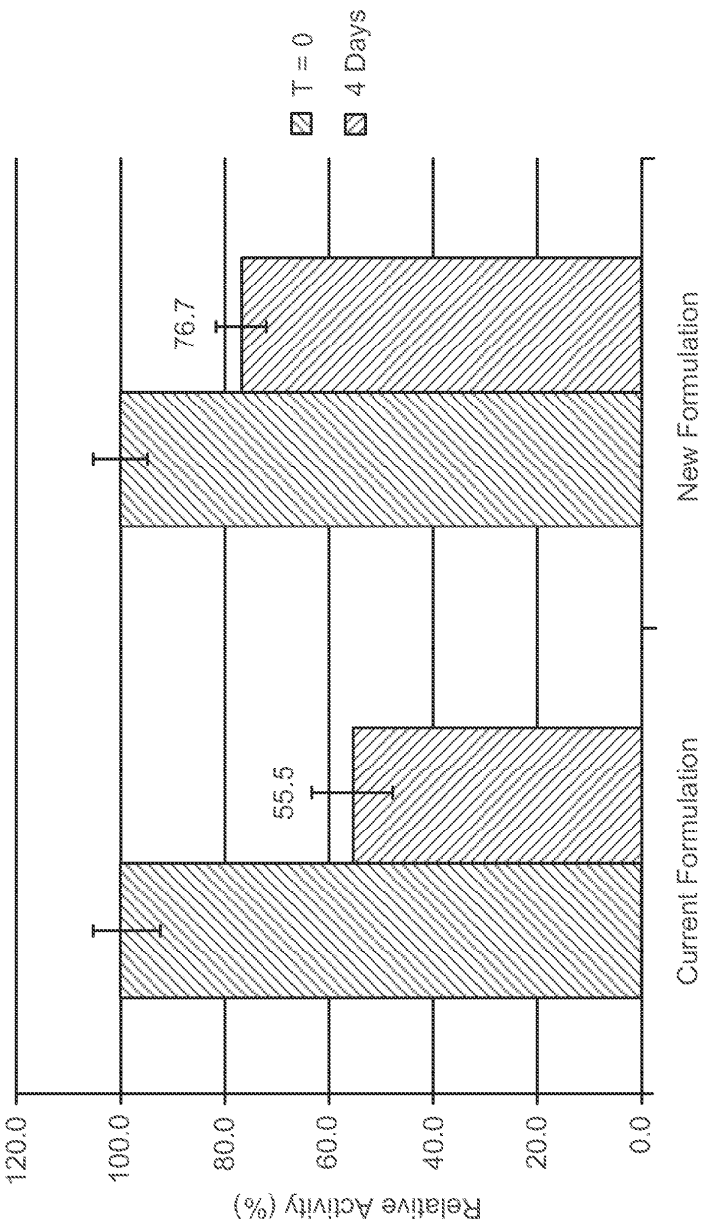

T = 0
4 Days
76.7
55.5
New Formulation
Current Formulation
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Relative Activity (%)

FIG. 47

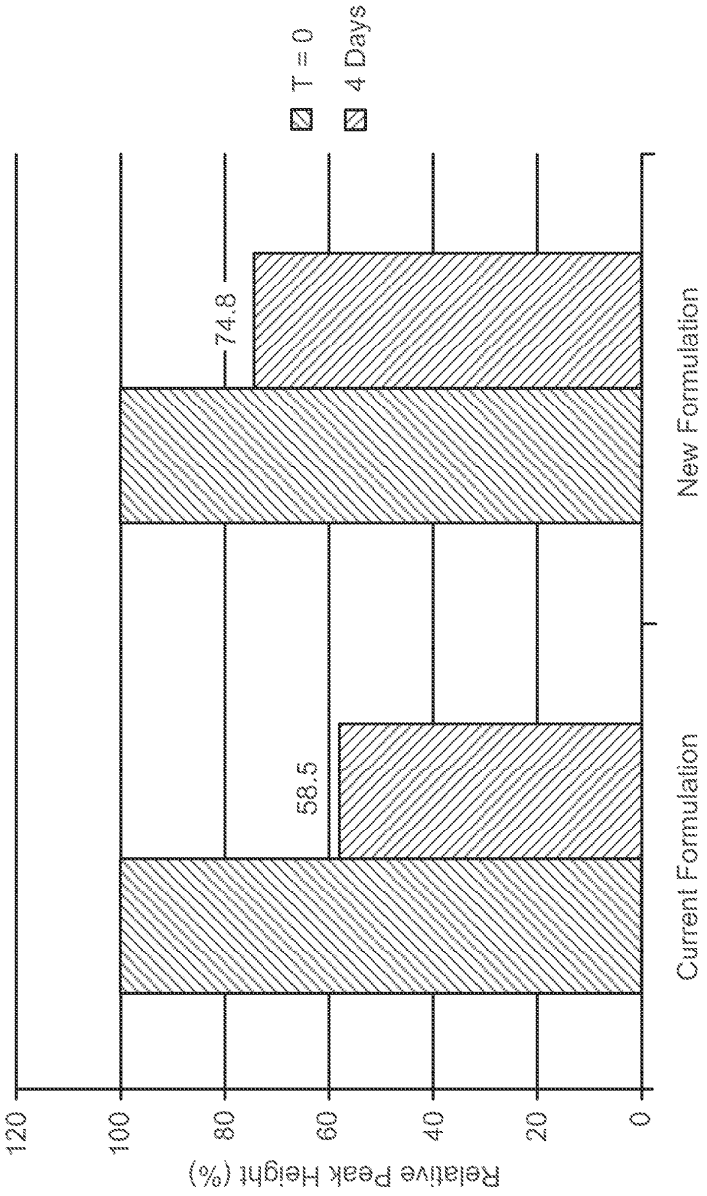

T = 0
4 Days
74.8
58.5
New Formulation
Current Formulation
Relative Peak Height (%)
120
100
80
60
40
20
0

FIG. 48

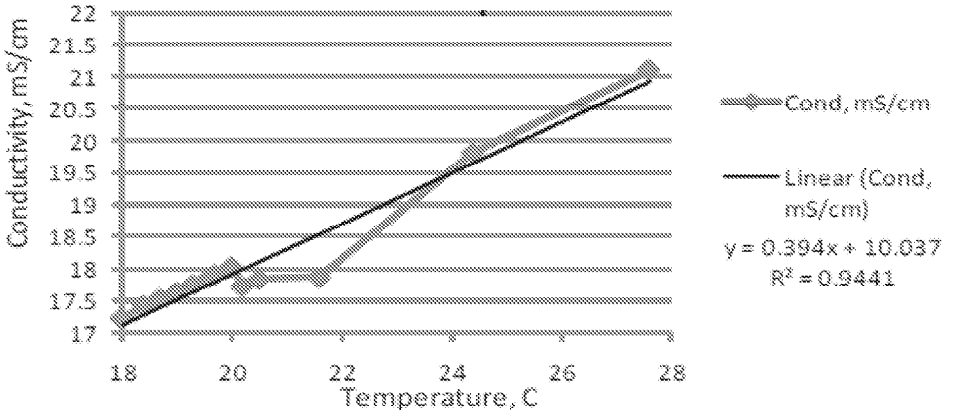


FIG. 49A

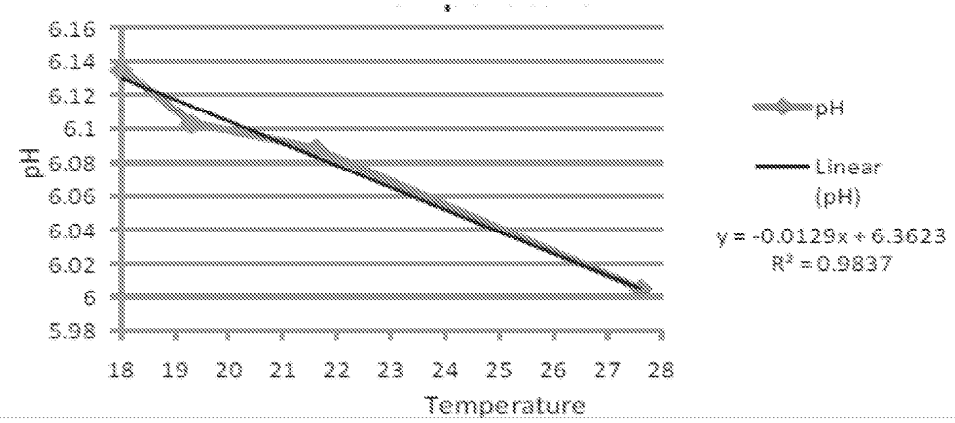


FIG. 49B