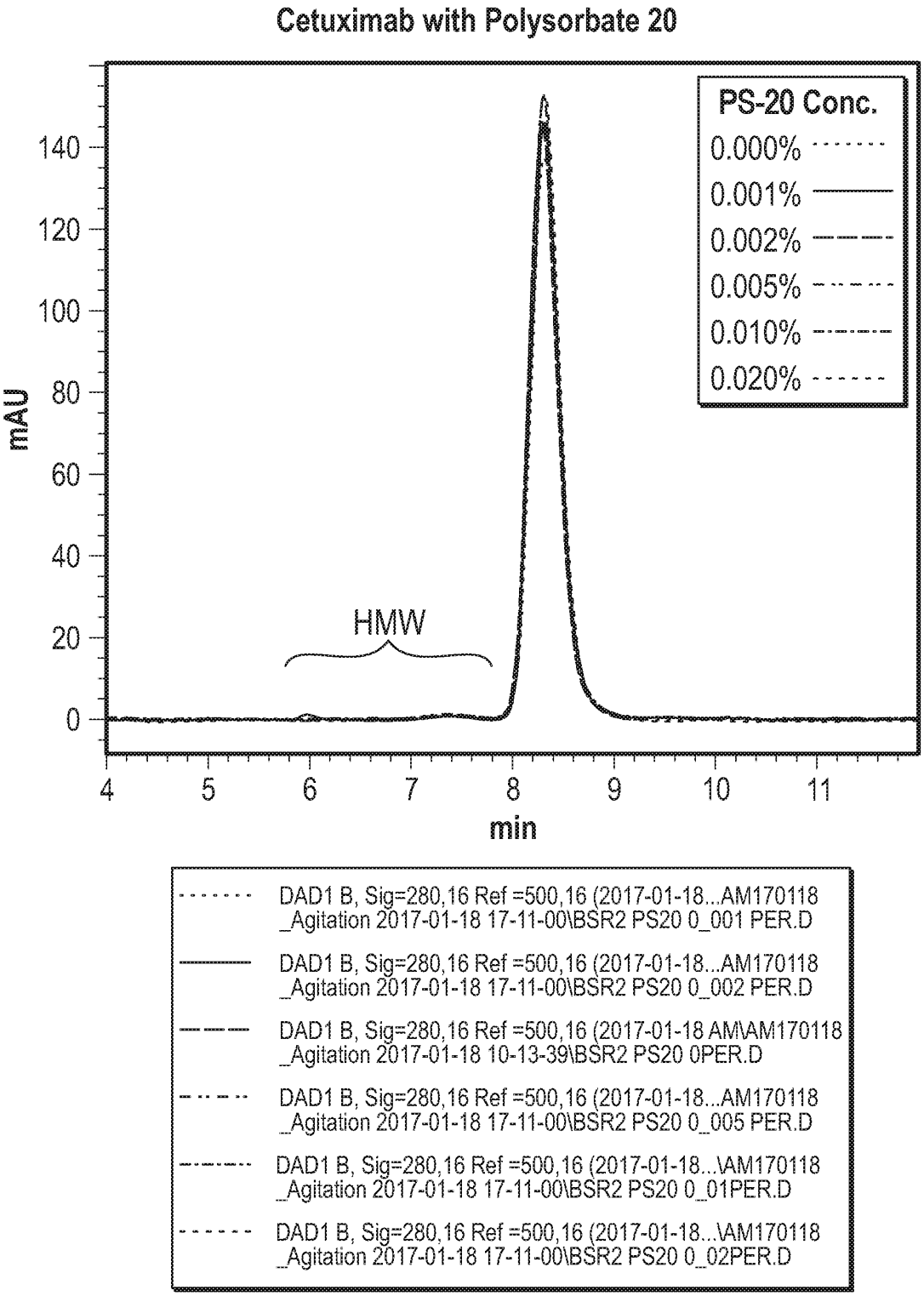

Cetuximab with Polysorbate 20
PS-20 Conc.
0.000%
0.001%
0.002%
0.005%
0.010%
0.020%
HMW
mAU
min
0
20
40
60
80
100
120
140
4
5
6
7
8
9
10
11
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...AM170118 _Agitation 2017-01-18 17-11-00\BSR2 PS20 0_001 PER.D
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...AM170118 _Agitation 2017-01-18 17-11-00\BSR2 PS20 0_002 PER.D
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118 _Agitation 2017-01-18 10-13-39\BSR2 PS20 0PER.D
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...AM170118 _Agitation 2017-01-18 17-11-00\BSR2 PS20 0_005 PER.D
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...\AM170118 _Agitation 2017-01-18 17-11-00\BSR2 PS20 0_01PER.D
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...\AM170118 _Agitation 2017-01-18 17-11-00\BSR2 PS20 0_02PER.D

FIG. 1A

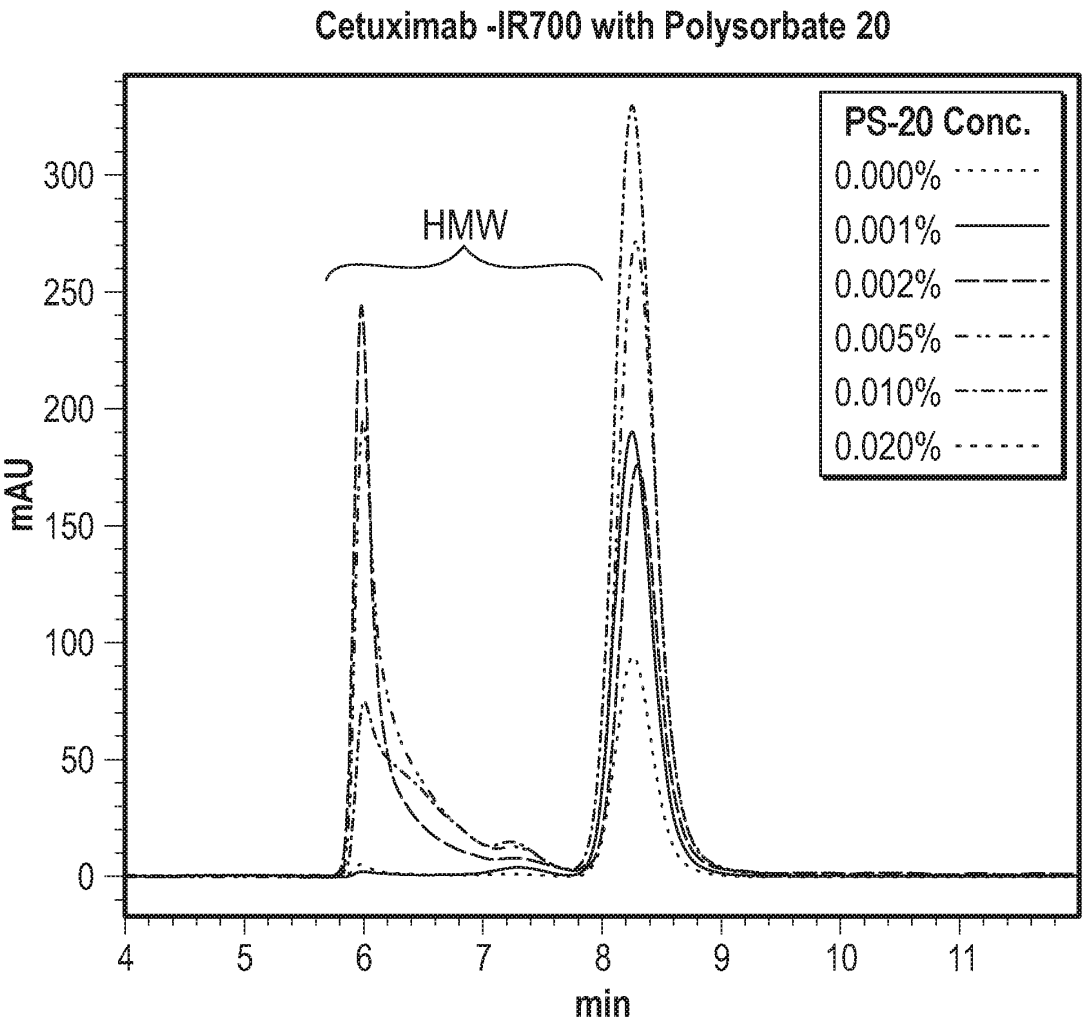

Cetuximab -IR700 with Polysorbate 20
PS-20 Conc.
0.000%
0.001%
0.002%
0.005%
0.010%
0.020%
HMW
mAU
300
250
200
150
100
50
0
4
5
6
7
8
9
10
11
min
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\054-1701.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\053-1601.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\055-1801.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\056-1901.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\057-2001.D)

FIG. 1B

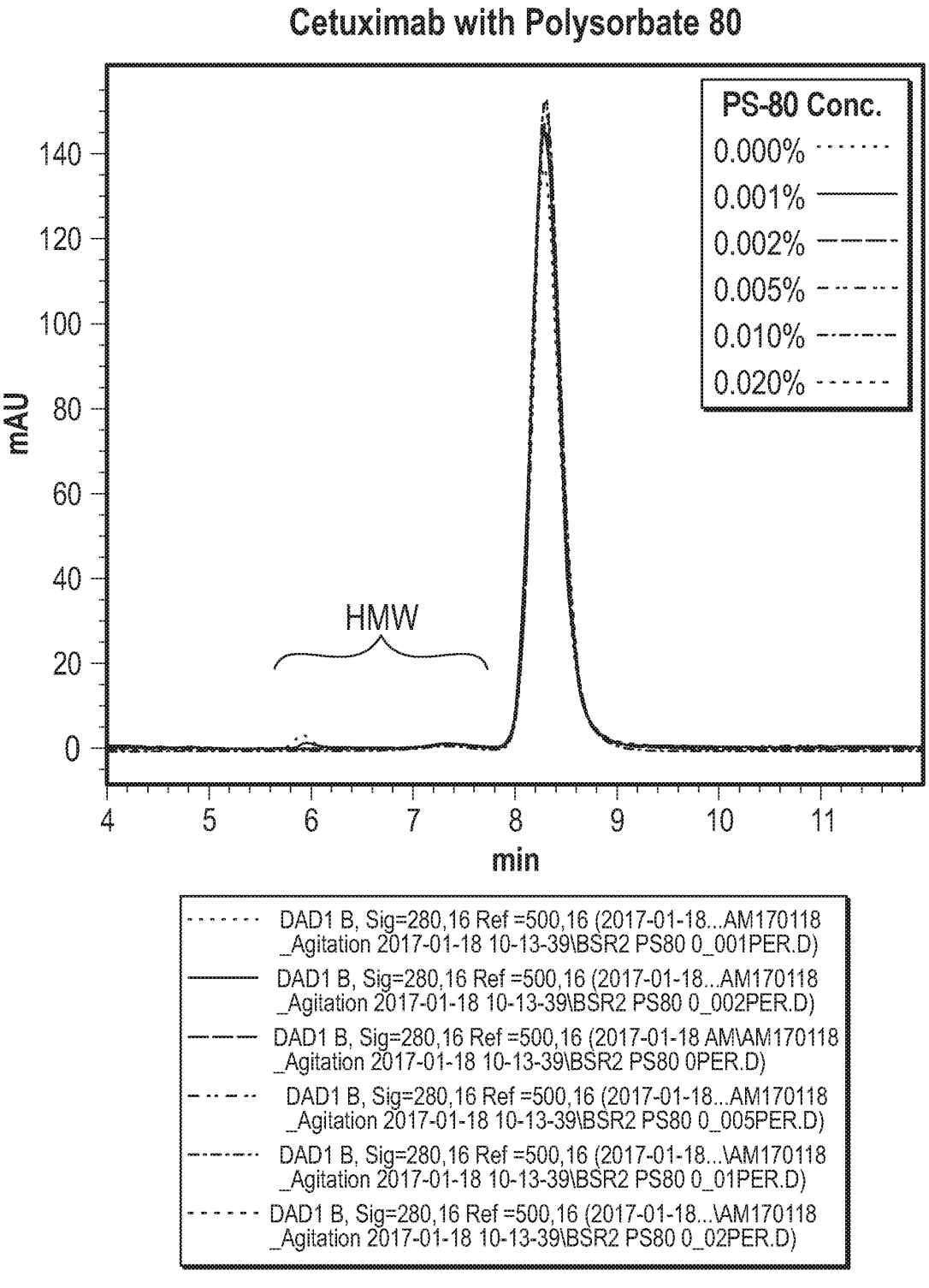

Cetuximab with Polysorbate 80
PS-80 Conc.
0.000%
0.001%
0.002%
0.005%
0.010%
0.020%
HMW
mAU
min
0
20
40
60
80
100
120
140
4
5
6
7
8
9
10
11
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...AM170118 _Agitation 2017-01-18 10-13-39\BSR2 PS80 0_001PER.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...AM170118 _Agitation 2017-01-18 10-13-39\BSR2 PS80 0_002PER.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118 _Agitation 2017-01-18 10-13-39\BSR2 PS80 0PER.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...AM170118 _Agitation 2017-01-18 10-13-39\BSR2 PS80 0_005PER.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...\AM170118 _Agitation 2017-01-18 10-13-39\BSR2 PS80 0_01PER.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18...\AM170118 _Agitation 2017-01-18 10-13-39\BSR2 PS80 0_02PER.D)

FIG. 1C

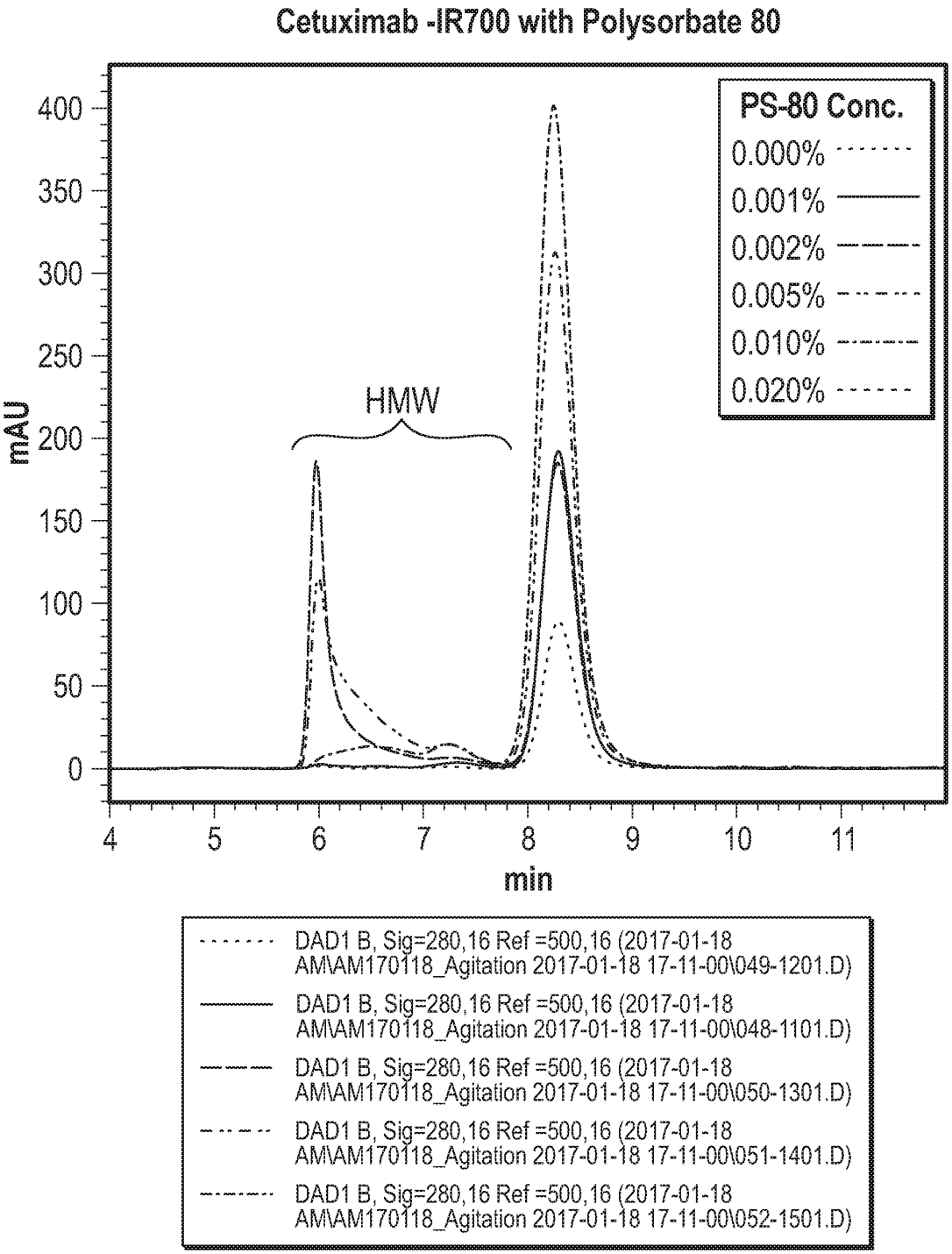

Cetuximab -IR700 with Polysorbate 80
PS-80 Conc.
0.000%
0.001%
0.002%
0.005%
0.010%
0.020%
HMW
mAU
400
350
300
250
200
150
100
50
0
4
5
6
7
8
9
10
11
min
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\049-1201.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\048-1101.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\050-1301.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\051-1401.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18 AM\AM170118_Agitation 2017-01-18 17-11-00\052-1501.D)

FIG. 1D

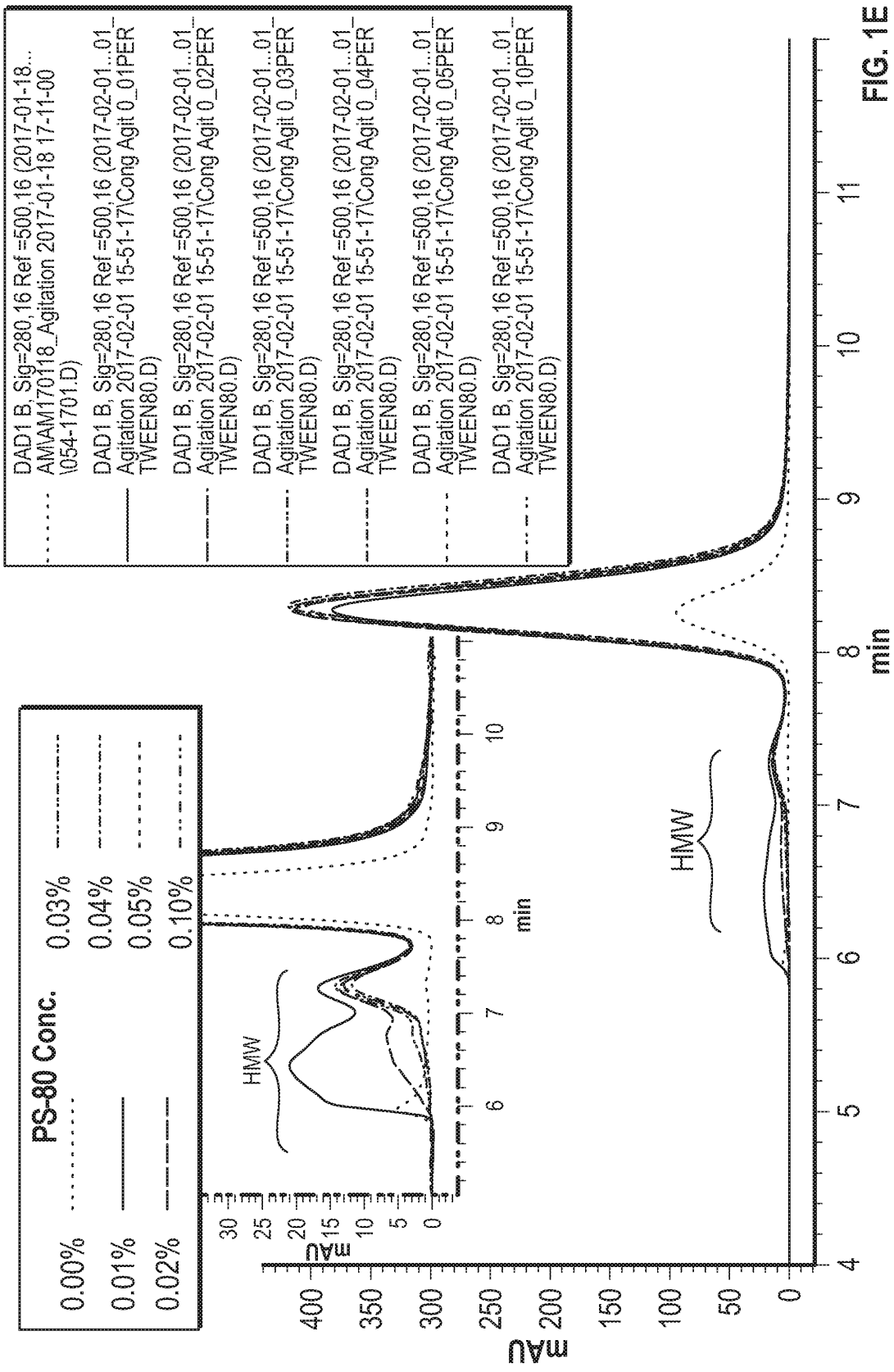

PS-80 Conc.
0.00%
0.01%
0.02%
0.03%
0.04%
0.05%
0.10%
DAD1 B, Sig=280,16 Ref =500,16 (2017-01-18... AM\AM170118_Agitation 2017-01-18 17-11-00 \054-1701.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-02-01...01_Agitation 2017-02-01 15-51-17\Cong Agit 0_01PER TWEEN80.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-02-01...01_Agitation 2017-02-01 15-51-17\Cong Agit 0_02PER TWEEN80.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-02-01...01_Agitation 2017-02-01 15-51-17\Cong Agit 0_03PER TWEEN80.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-02-01...01_Agitation 2017-02-01 15-51-17\Cong Agit 0_04PER TWEEN80.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-02-01...01_Agitation 2017-02-01 15-51-17\Cong Agit 0_05PER TWEEN80.D)
DAD1 B, Sig=280,16 Ref =500,16 (2017-02-01...01_Agitation 2017-02-01 15-51-17\Cong Agit 0_10PER TWEEN80.D)
mAU
min
HMW
HMW

FIG. 1E

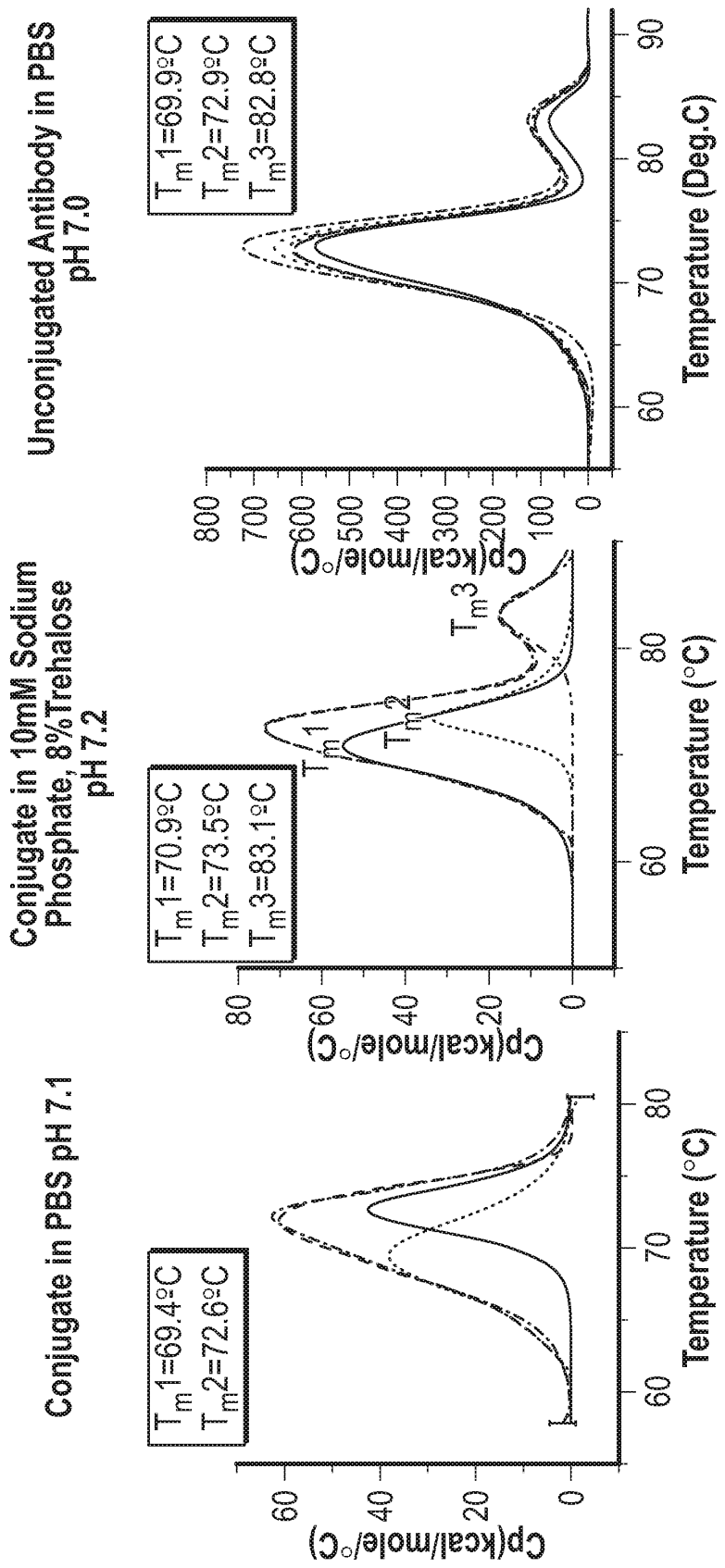

Conjugate in PBS pH 7.1
Tm1=69.4°C
Tm2=72.6°C
Cp(kcal/mole/°C)
Temperature (°C)
Conjugate in 10mM Sodium Phosphate, 8%Trehalose pH 7.2
Tm1=70.9°C
Tm2=73.5°C
Tm3=83.1°C
Tm1
Tm2
Tm3
Cp(kcal/mole/°C)
Temperature (°C)
Unconjugated Antibody in PBS pH 7.0
Tm1=69.9°C
Tm2=72.9°C
Tm3=82.8°C
Cp(kcal/mole/°C)
Temperature (Deg.C)

FIG. 2

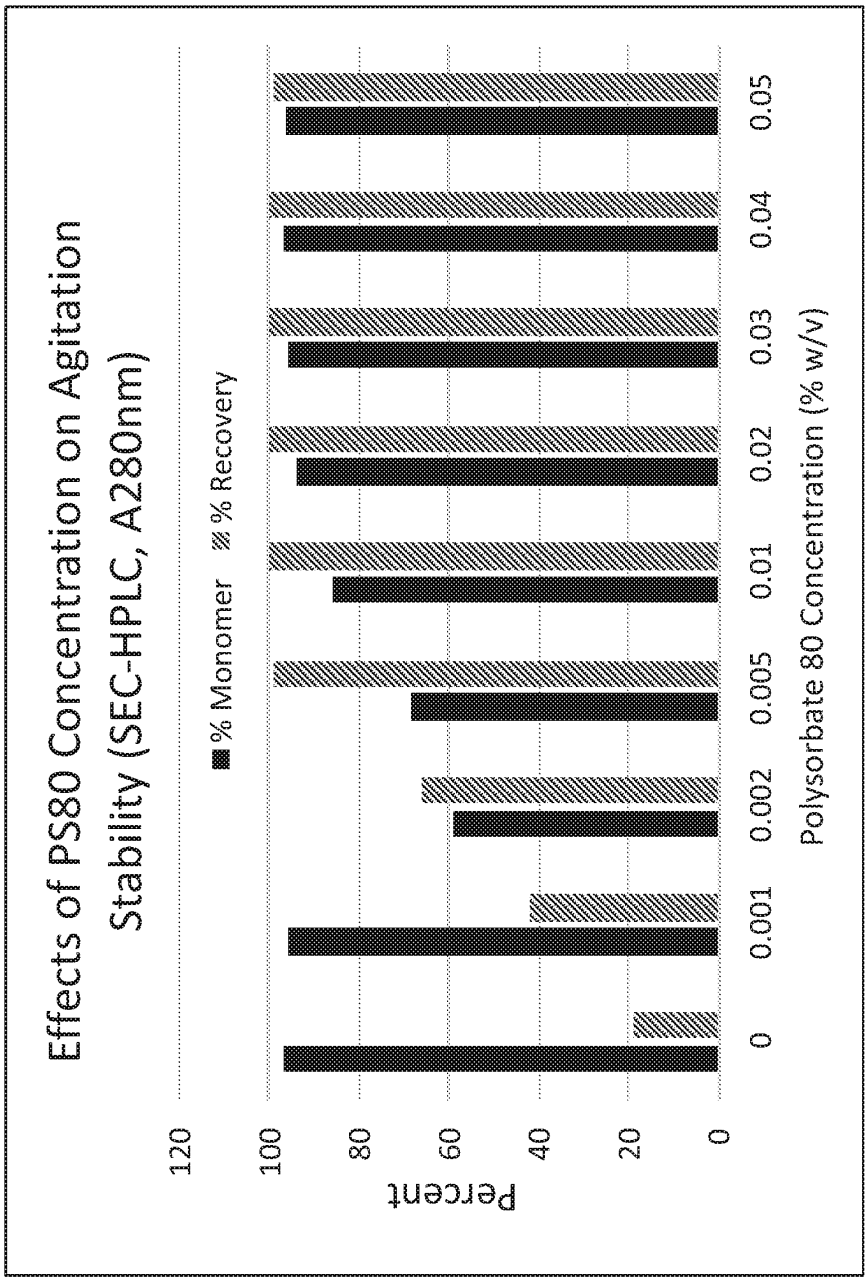
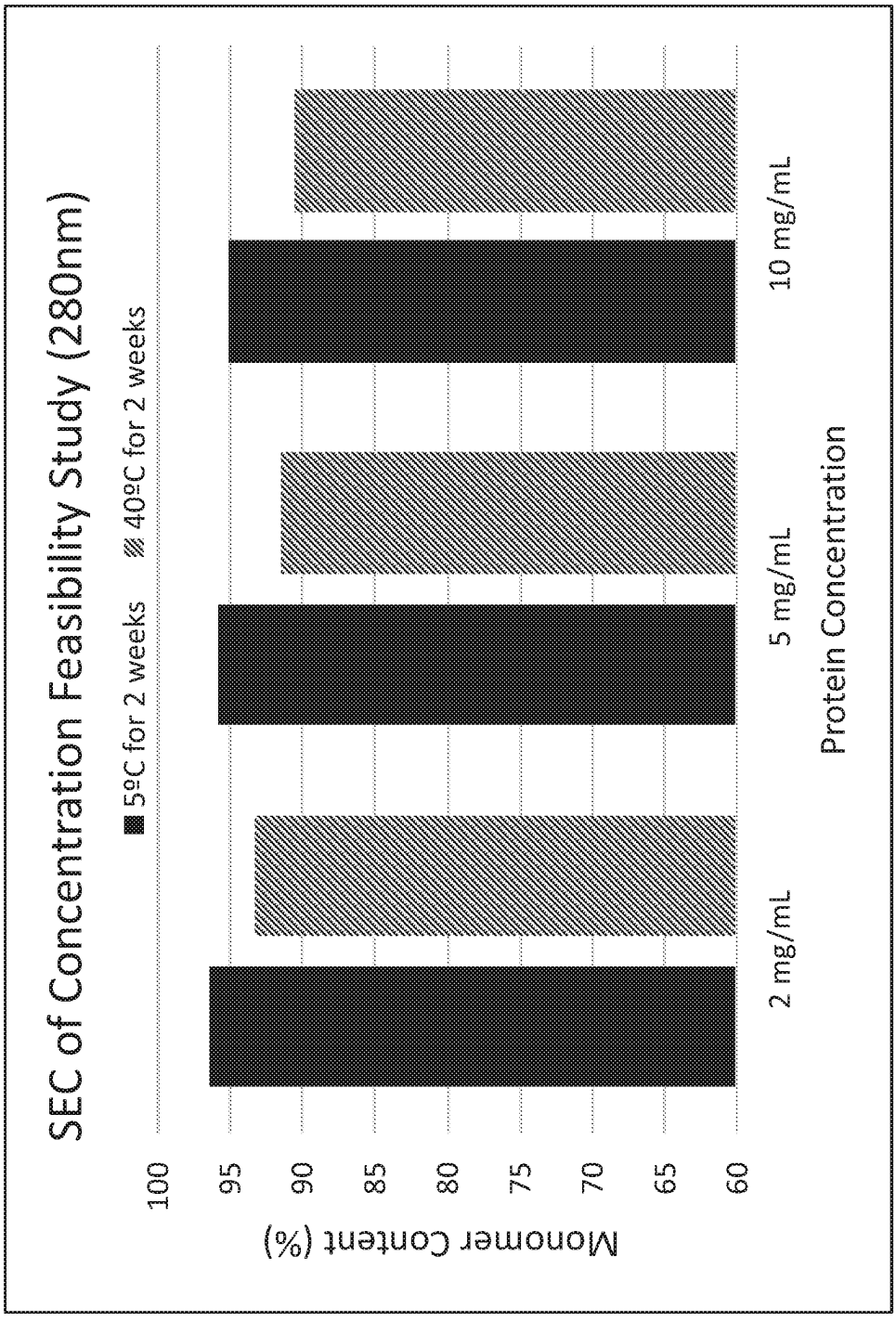

Effects of PS80 Concentration on Agitation Stability (SEC-HPLC, A280nm)
% Monomer
% Recovery
Percent
120
100
80
60
40
20
0
0
0.001
0.002
0.005
0.01
0.02
0.03
0.04
0.05
Polysorbate 80 Concentration (% w/v)

FIG. 3

FIG. 4


SEC of Concentration Feasibility Study (280nm)
5ºC for 2 weeks
40ºC for 2 weeks
Monomer Content (%)
100
95
90
85
80
75
70
65
60
2 mg/mL
5 mg/mL
10 mg/mL
Protein Concentration

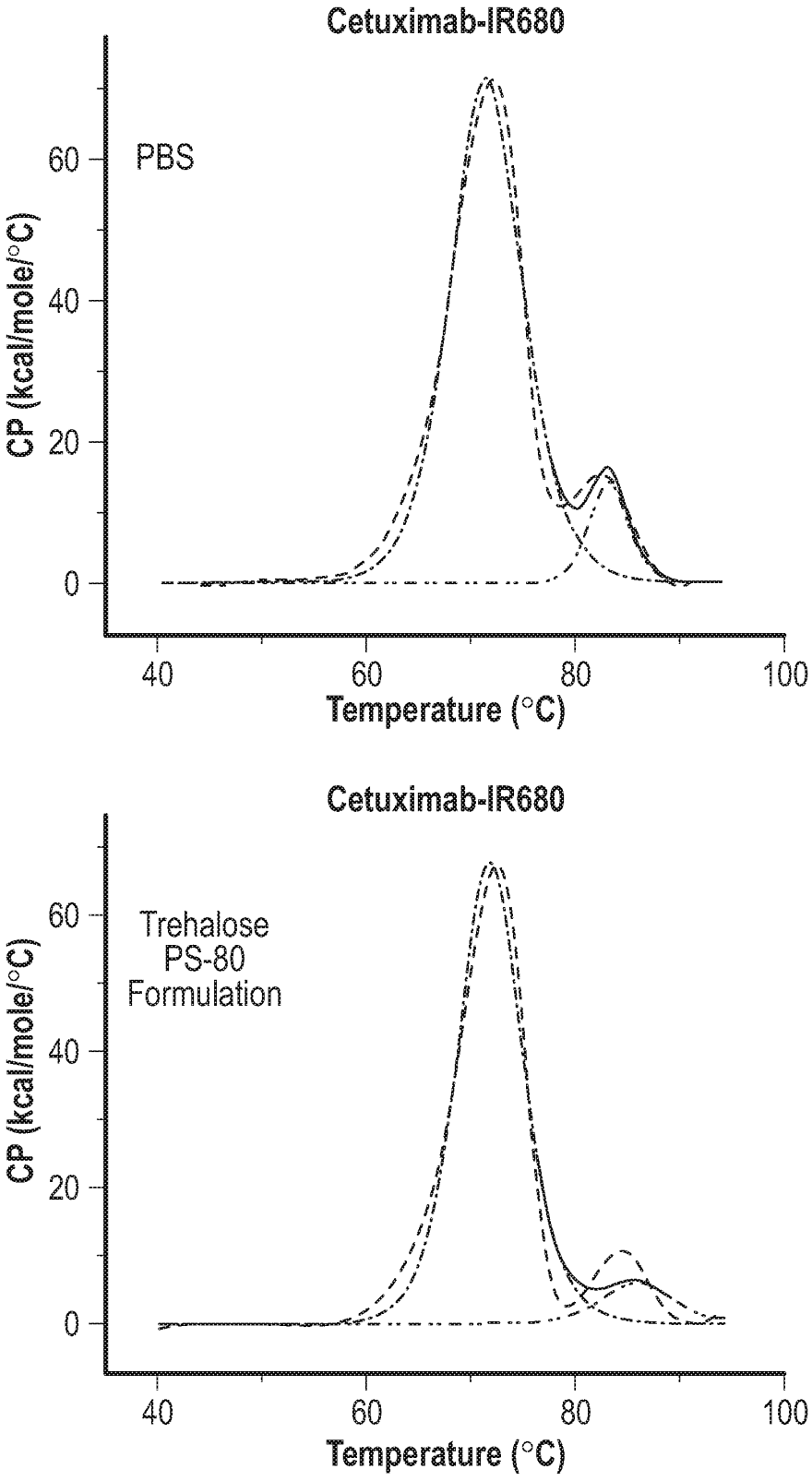

Cetuximab-IR680
PBS
CP (kcal/mole/°C)
0
20
40
60
40
60
80
100
Temperature (°C)
Cetuximab-IR680
Trehalose
PS-80
Formulation
CP (kcal/mole/°C)
0
20
40
60
40
60
80
100
Temperature (°C)

FIG. 5A

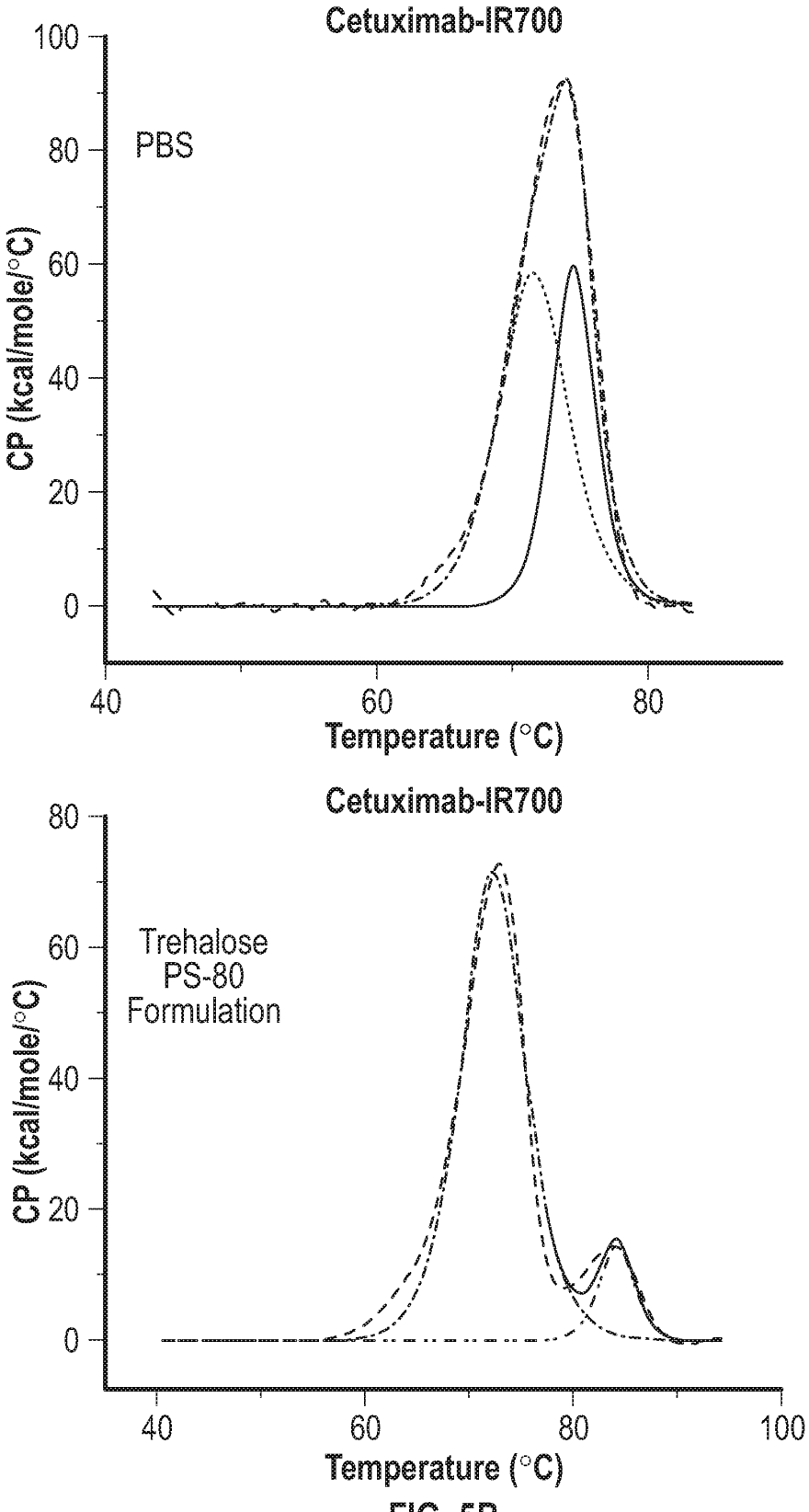
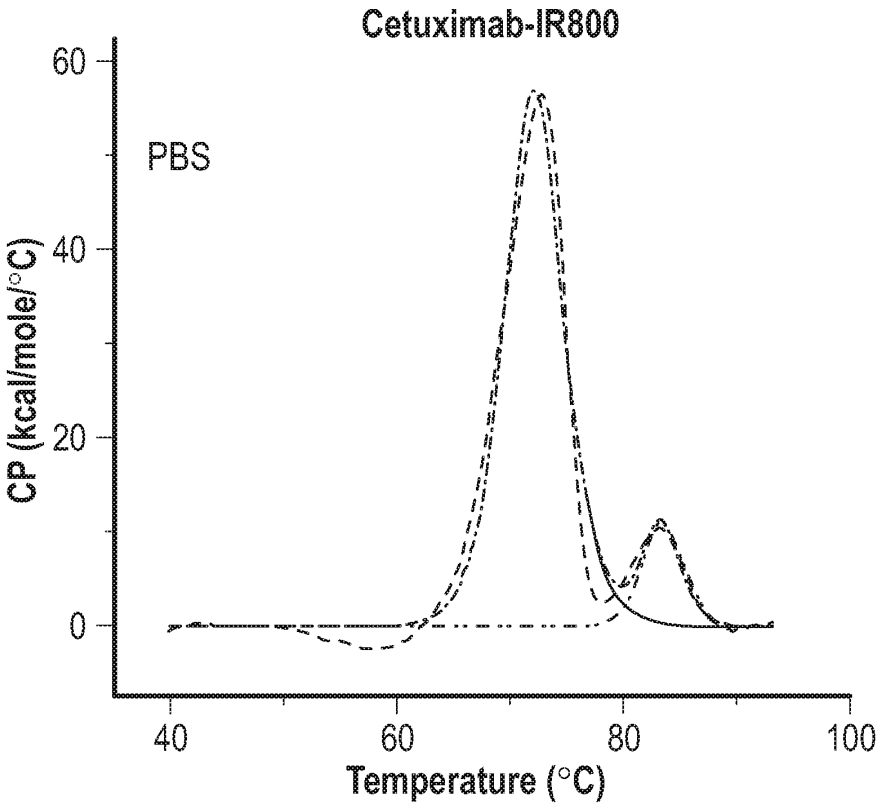

Cetuximab-IR700
PBS
CP (kcal/mole/°C)
Temperature (°C)
Cetuximab-IR700
Trehalose
PS-80
Formulation
CP (kcal/mole/°C)
Temperature (°C)

FIG. 5B


Cetuximab-IR800
PBS
CP (kcal/mole/°C)
60
40
20
0
40
60
80
100
Temperature (°C)

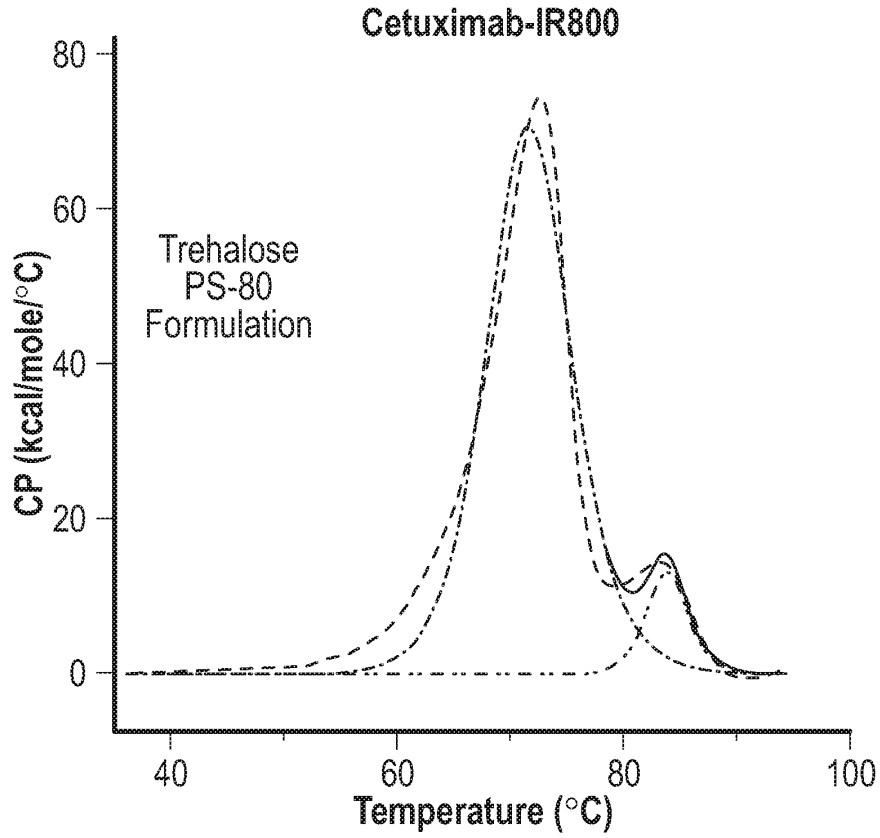

Cetuximab-IR800
Trehalose
PS-80
Formulation
CP (kcal/mole/°C)
80
60
40
20
0
40
60
80
100
Temperature (°C)

FIG. 5C

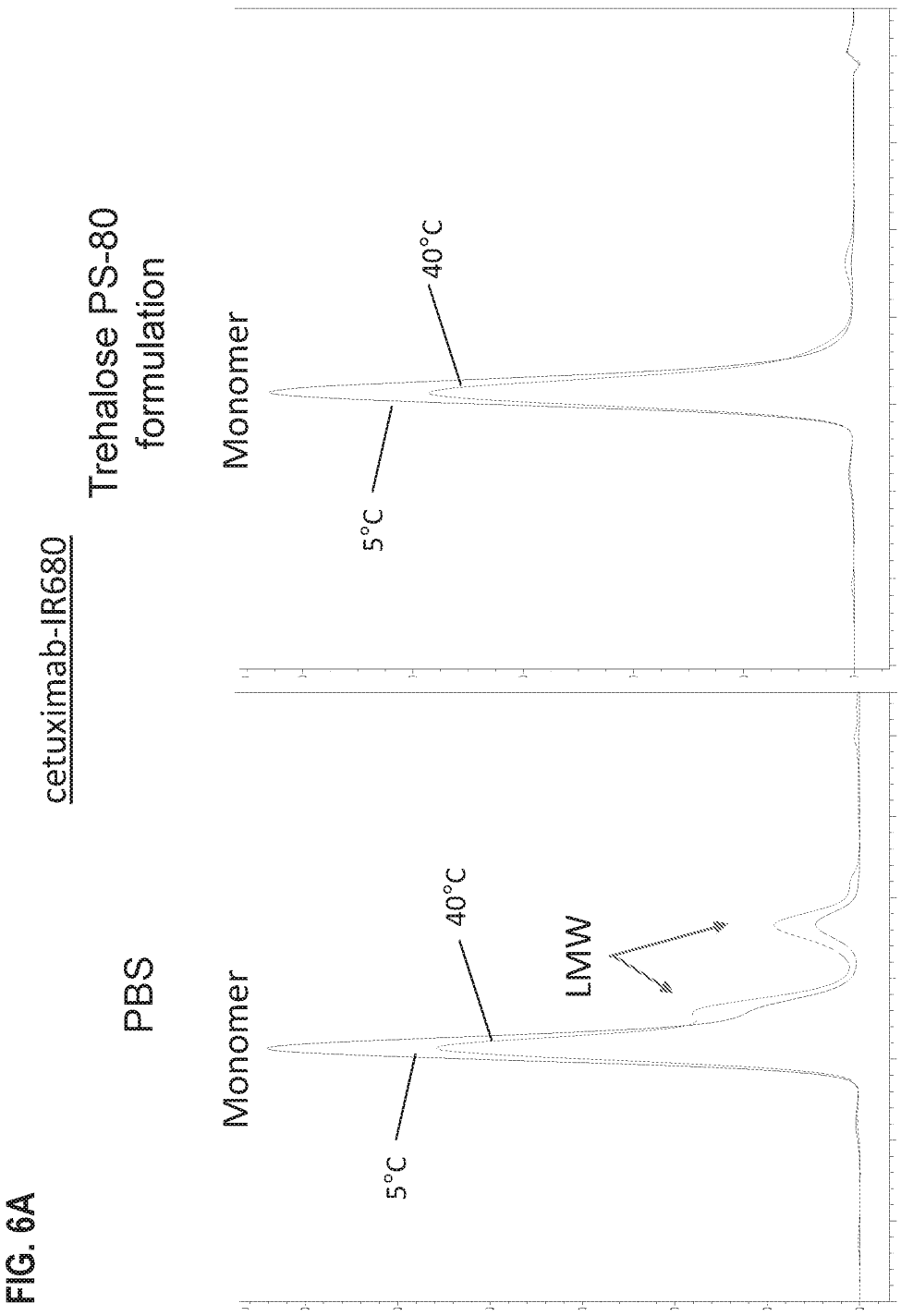
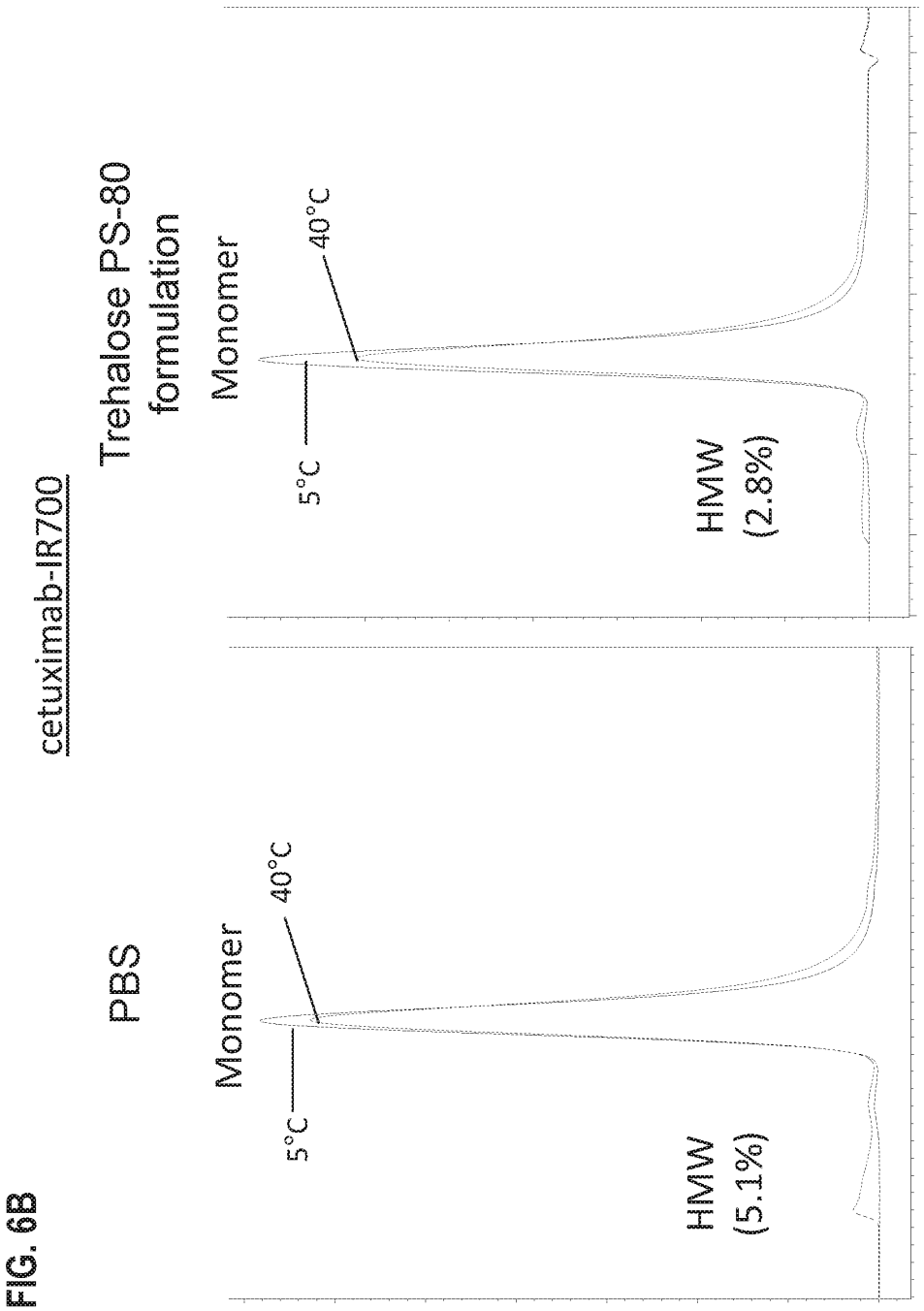

cetuximab-IR680
PBS
Trehalose PS-80 formulation
Monomer
5°C
40°C
LMW
Monomer
5°C
40°C

FIG. 6A

FIG. 6B


cetuximab-IR700
PBS
Monomer
40°C
5°C
HMW
(5.1%)
Trehalose PS-80
formulation
Monomer
40°C
5°C
HMW
(2.8%)

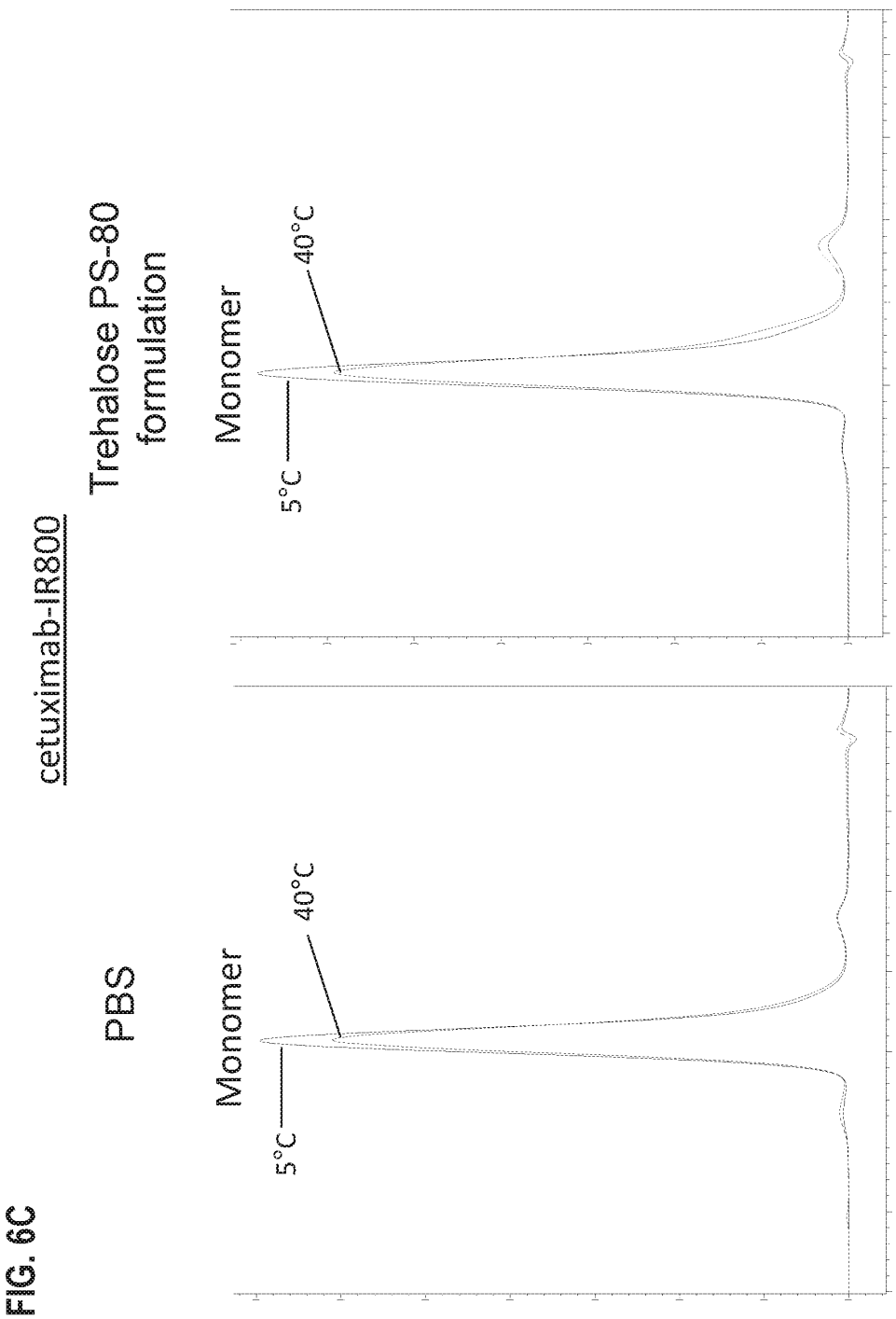

cetuximab-IR800
PBS
Monomer
5°C
40°C
Trehalose PS-80
formulation
Monomer
5°C
40°C

FIG. 6C

FIG. 7A

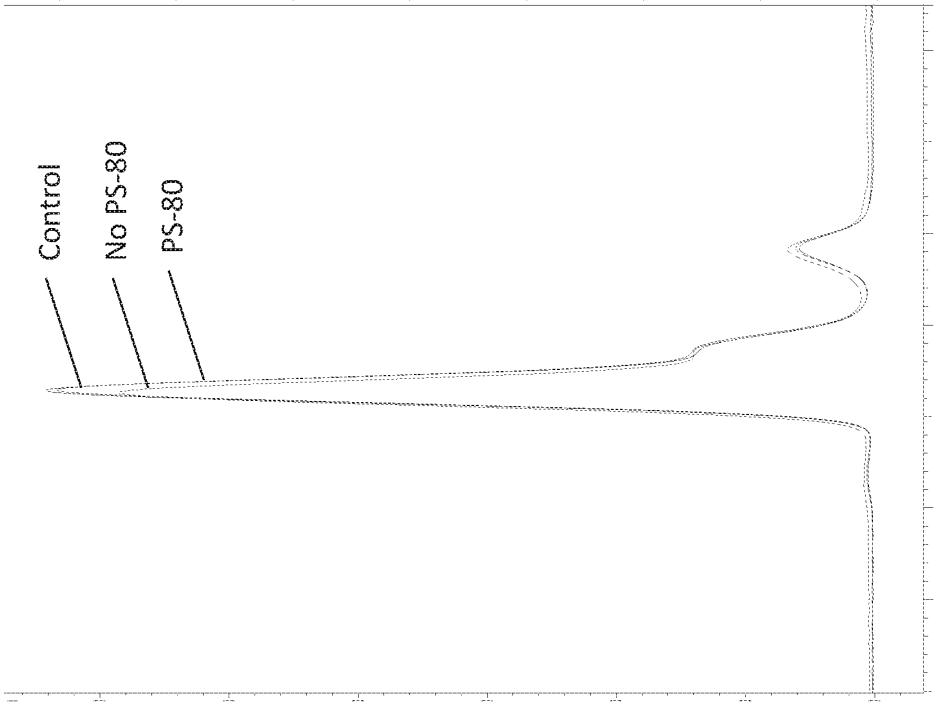

cetuximab-IR680
Control
No PS-80
PS-80

FIG. 7B

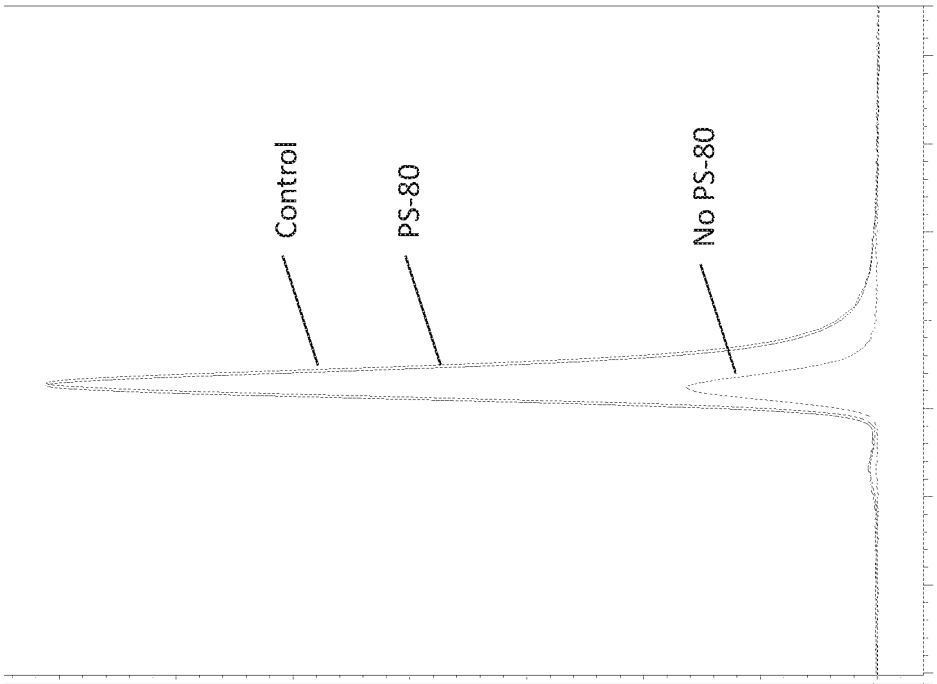
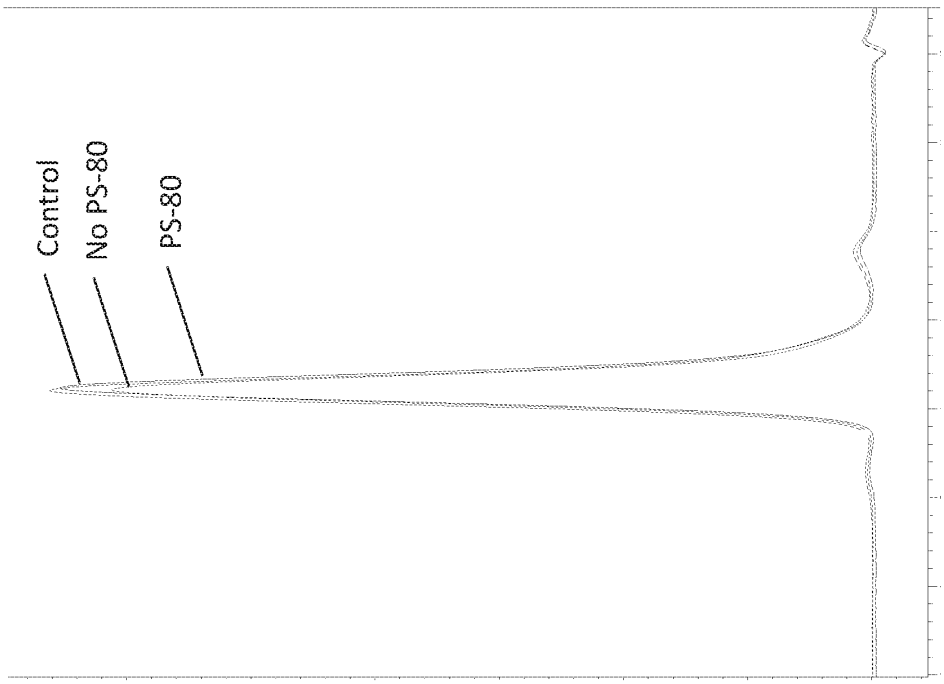

cetuximab-IR700
Control
PS-80
No PS-80

FIG. 7C



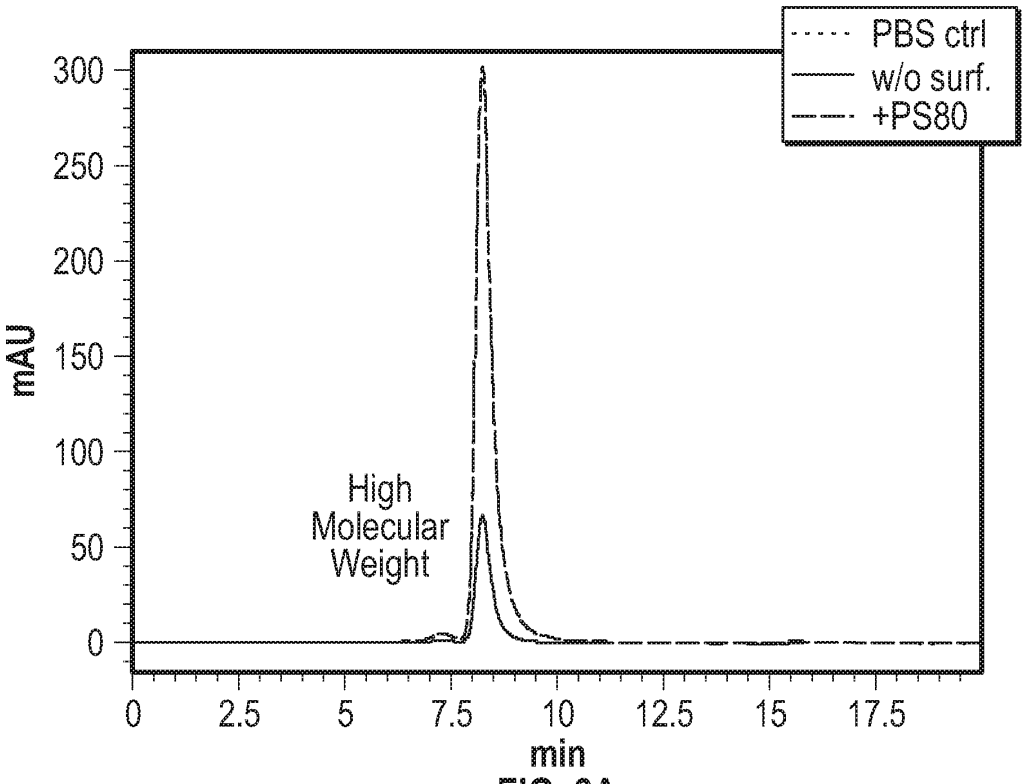

PBS ctrl
w/o surf.
+PS80
300
250
200
150
100
50
0
mAU
High
Molecular
Weight
0
2.5
5
7.5
10
12.5
15
17.5
min

FIG. 8A

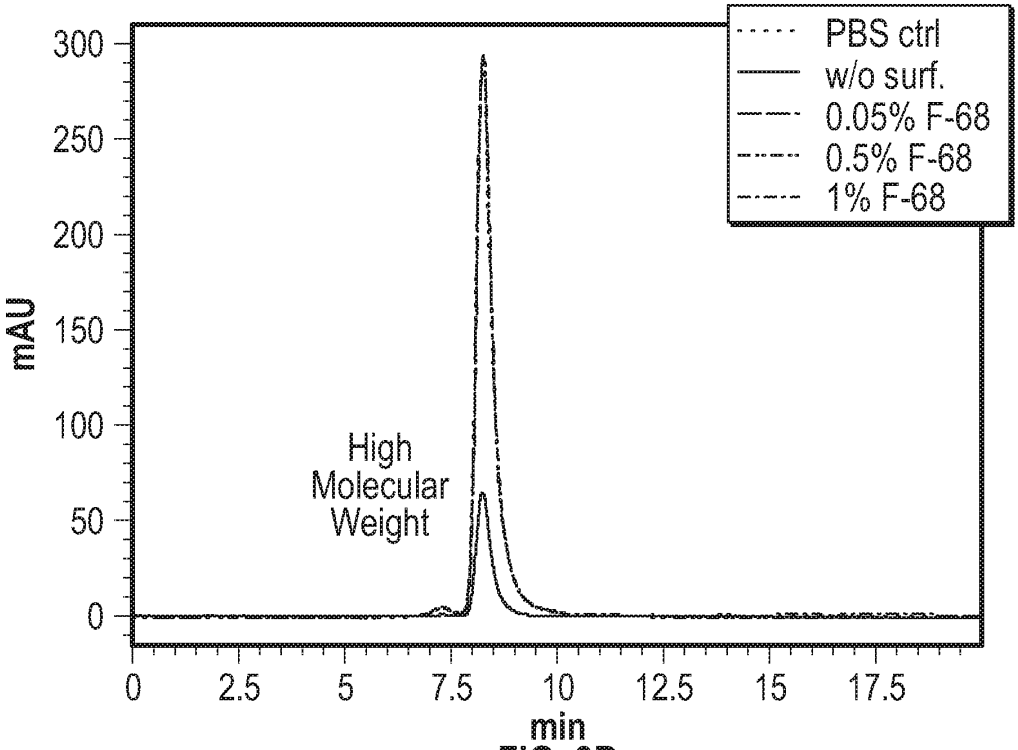

PBS ctrl
w/o surf.
0.05% F-68
0.5% F-68
1% F-68
mAU
300
250
200
150
100
50
0
High
Molecular
Weight
0
2.5
5
7.5
10
12.5
15
17.5
min

FIG. 8B

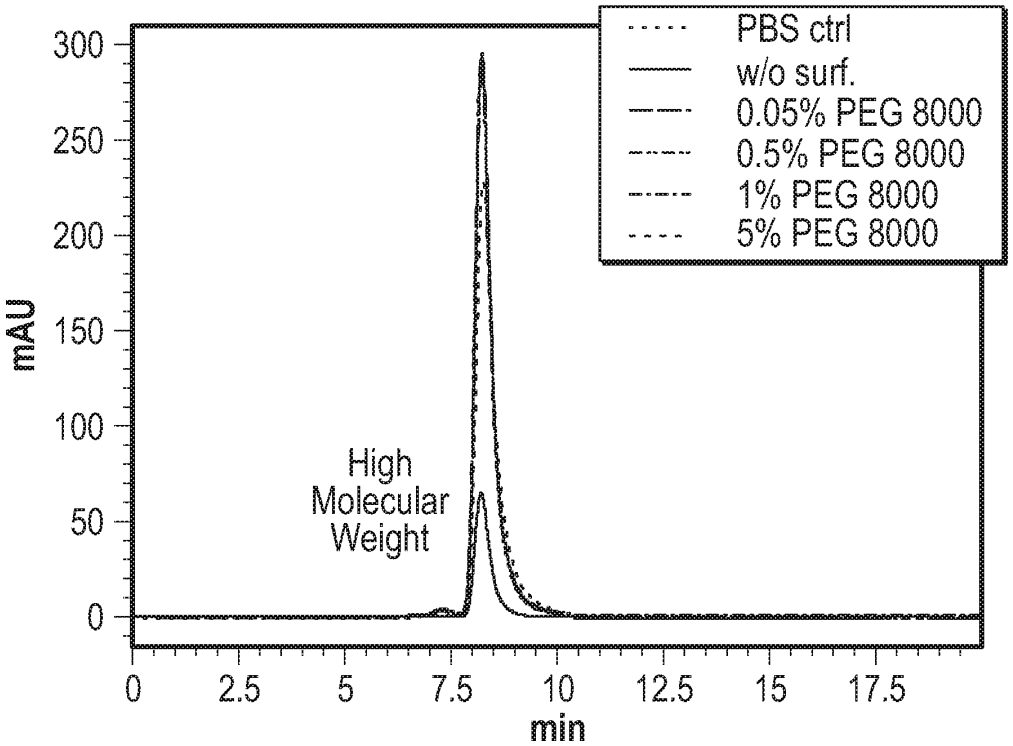

PBS ctrl
w/o surf.
0.05% PEG 8000
0.5% PEG 8000
1% PEG 8000
5% PEG 8000
mAU
300
250
200
150
100
50
0
High
Molecular
Weight
0
2.5
5
7.5
10
12.5
15
17.5
min

FIG. 8C

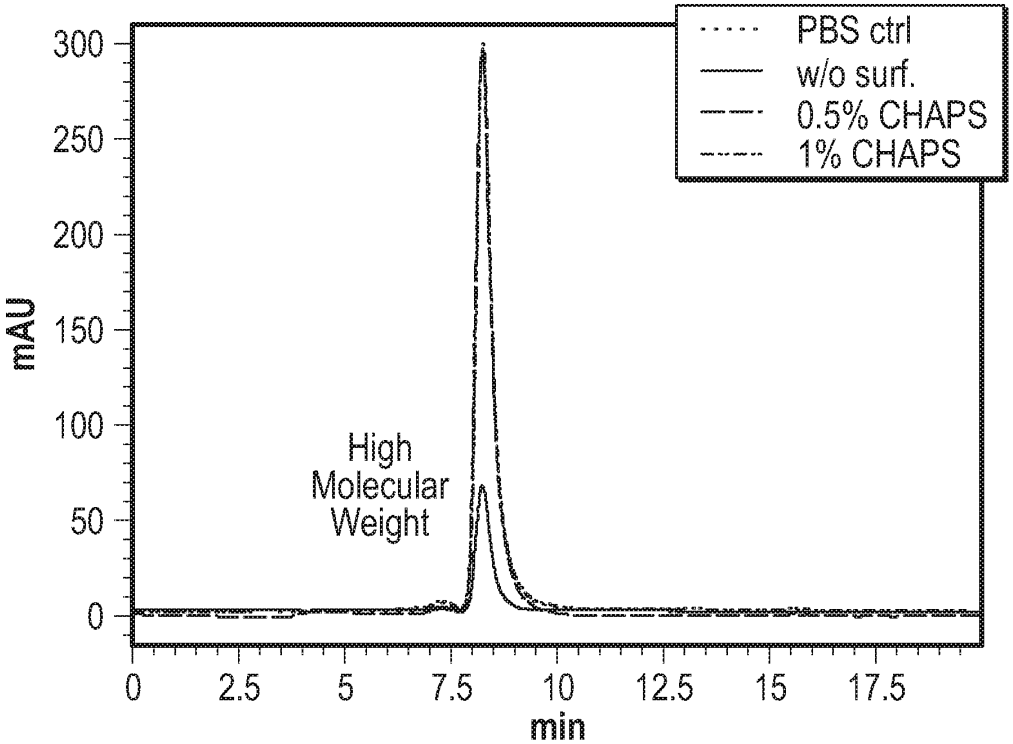

PBS ctrl
w/o surf.
0.5% CHAPS
1% CHAPS
mAU
300
250
200
150
100
50
0
High
Molecular
Weight
0
2.5
5
7.5
10
12.5
15
17.5
min

FIG. 8D

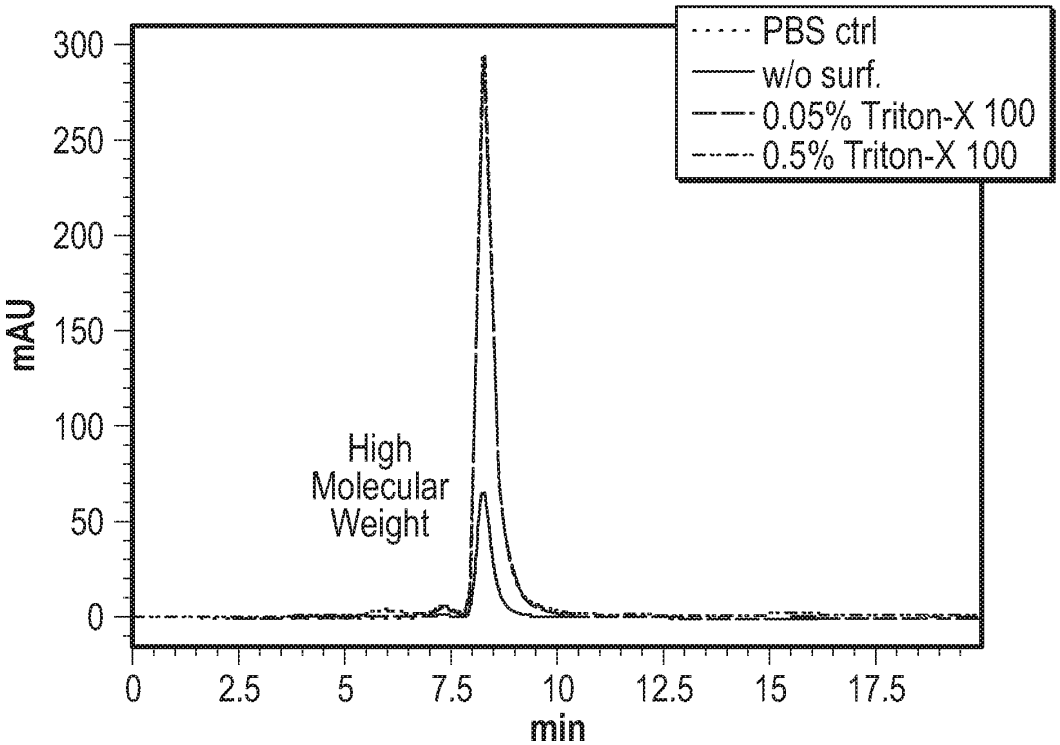

PBS ctrl
w/o surf.
0.05% Triton-X 100
0.5% Triton-X 100
mAU
300
250
200
150
100
50
0
High
Molecular
Weight
0
2.5
5
7.5
10
12.5
15
17.5
min

FIG. 8E

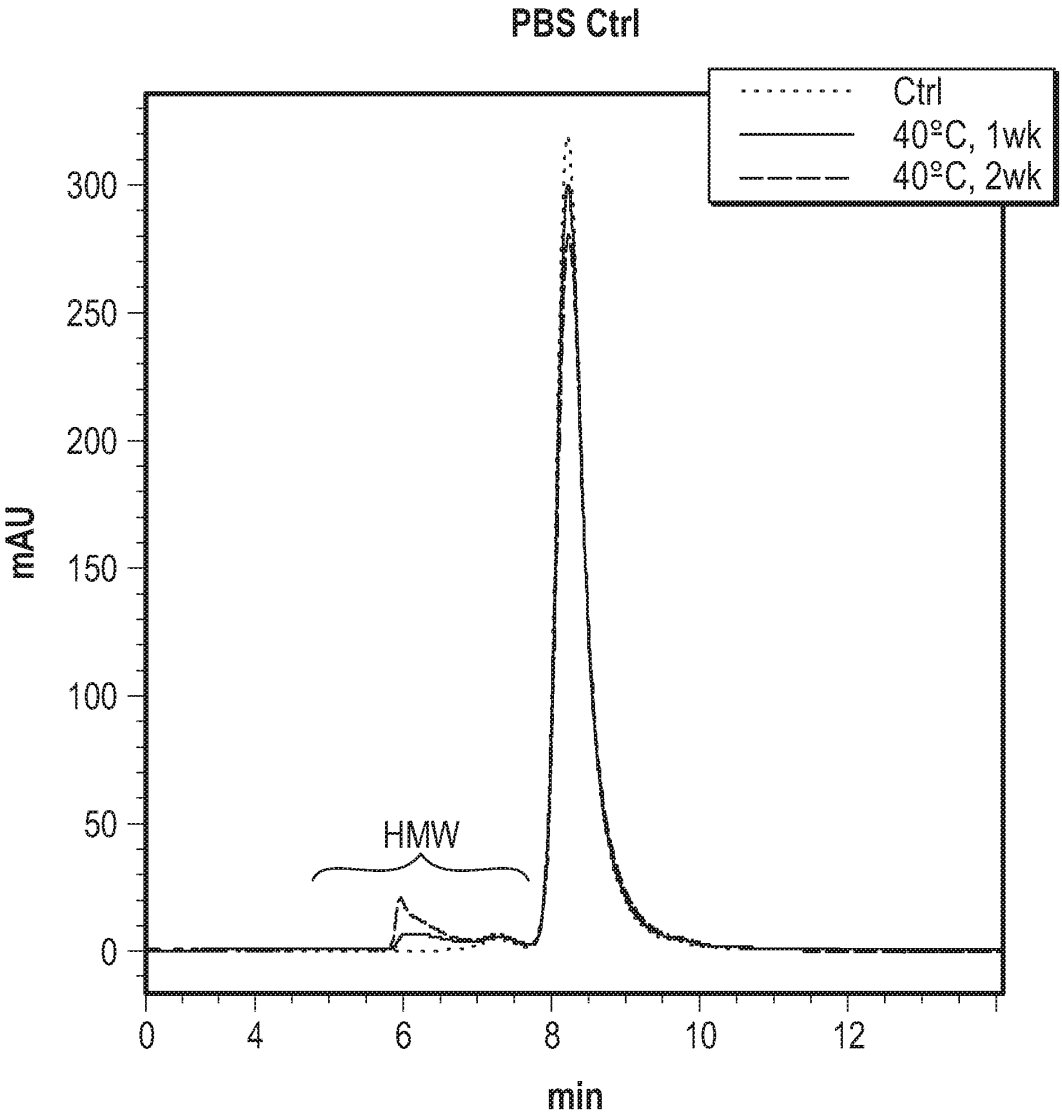

PBS Ctrl
Ctrl
40ºC, 1wk
40ºC, 2wk
mAU
300
250
200
150
100
50
0
HMW
0
4
6
8
10
12
min

FIG. 9A

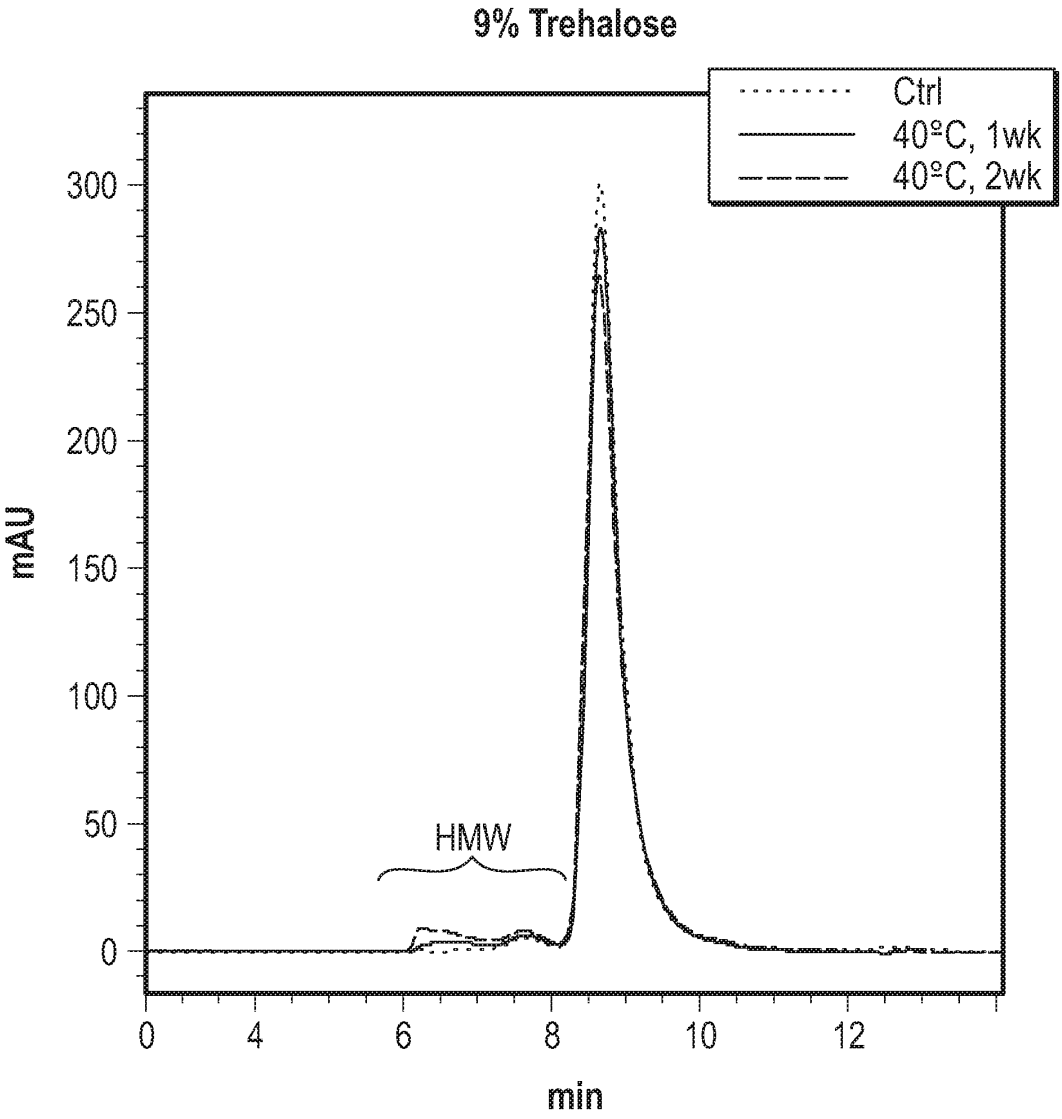

9% Trehalose
Ctrl
40ºC, 1wk
40ºC, 2wk
mAU
300
250
200
150
100
50
0
HMW
0
4
6
8
10
12
min

FIG. 9B

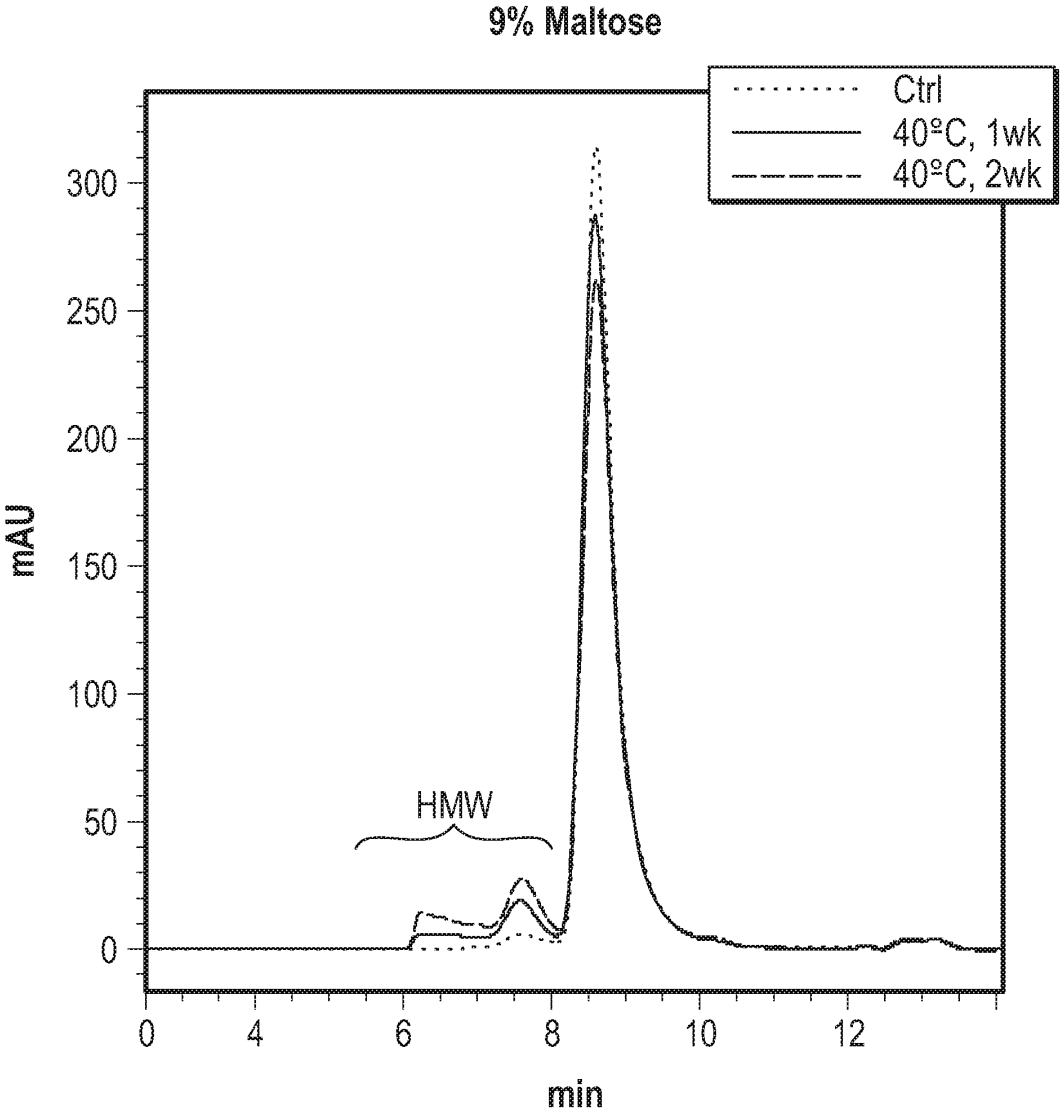

9% Maltose
Ctrl
40ºC, 1wk
40ºC, 2wk
mAU
300
250
200
150
100
50
0
HMW
0
4
6
8
10
12
min

FIG. 9C

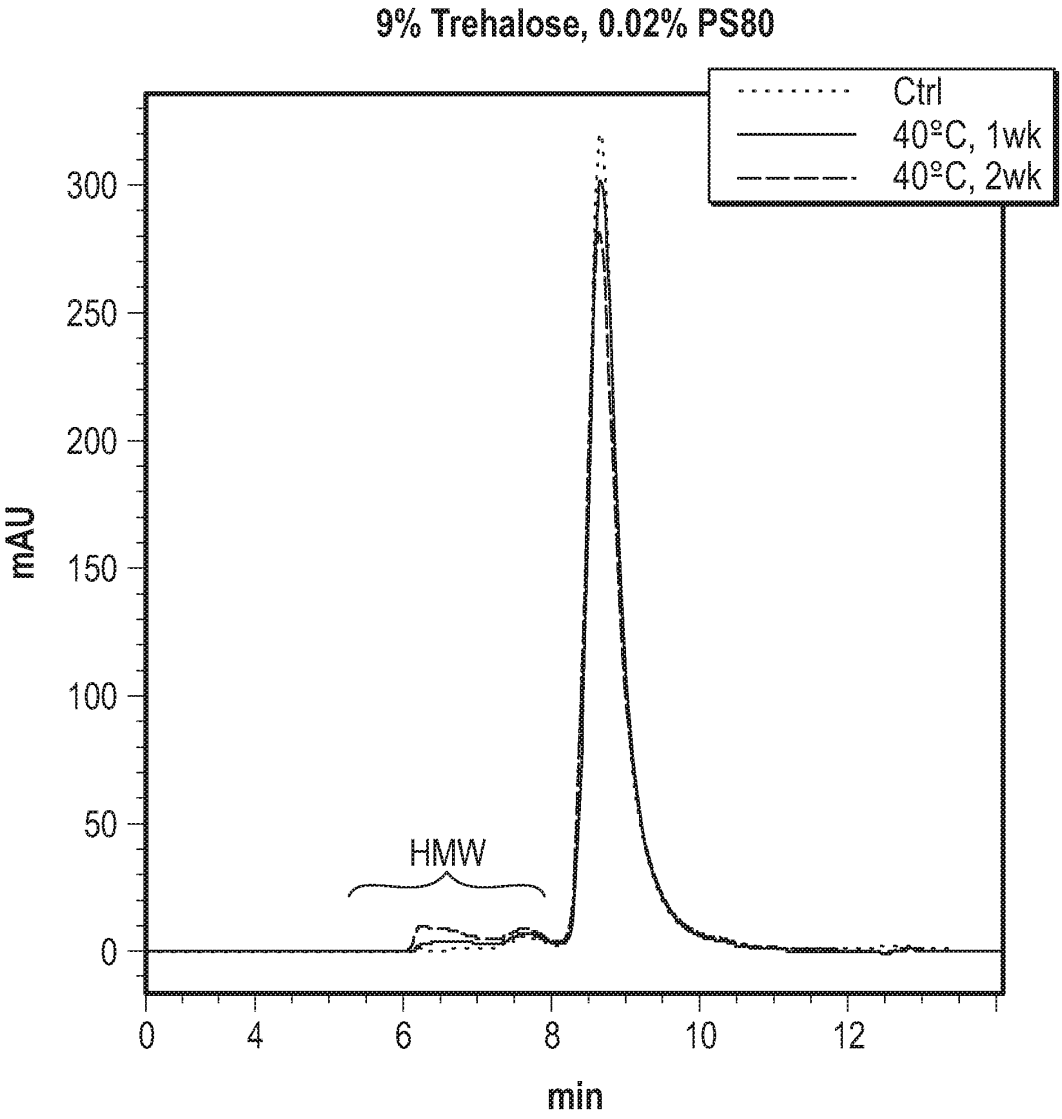

9% Trehalose, 0.02% PS80
Ctrl
40ºC, 1wk
40ºC, 2wk
mAU
300
250
200
150
100
50
0
HMW
0
4
6
8
10
12
min

FIG. 9D

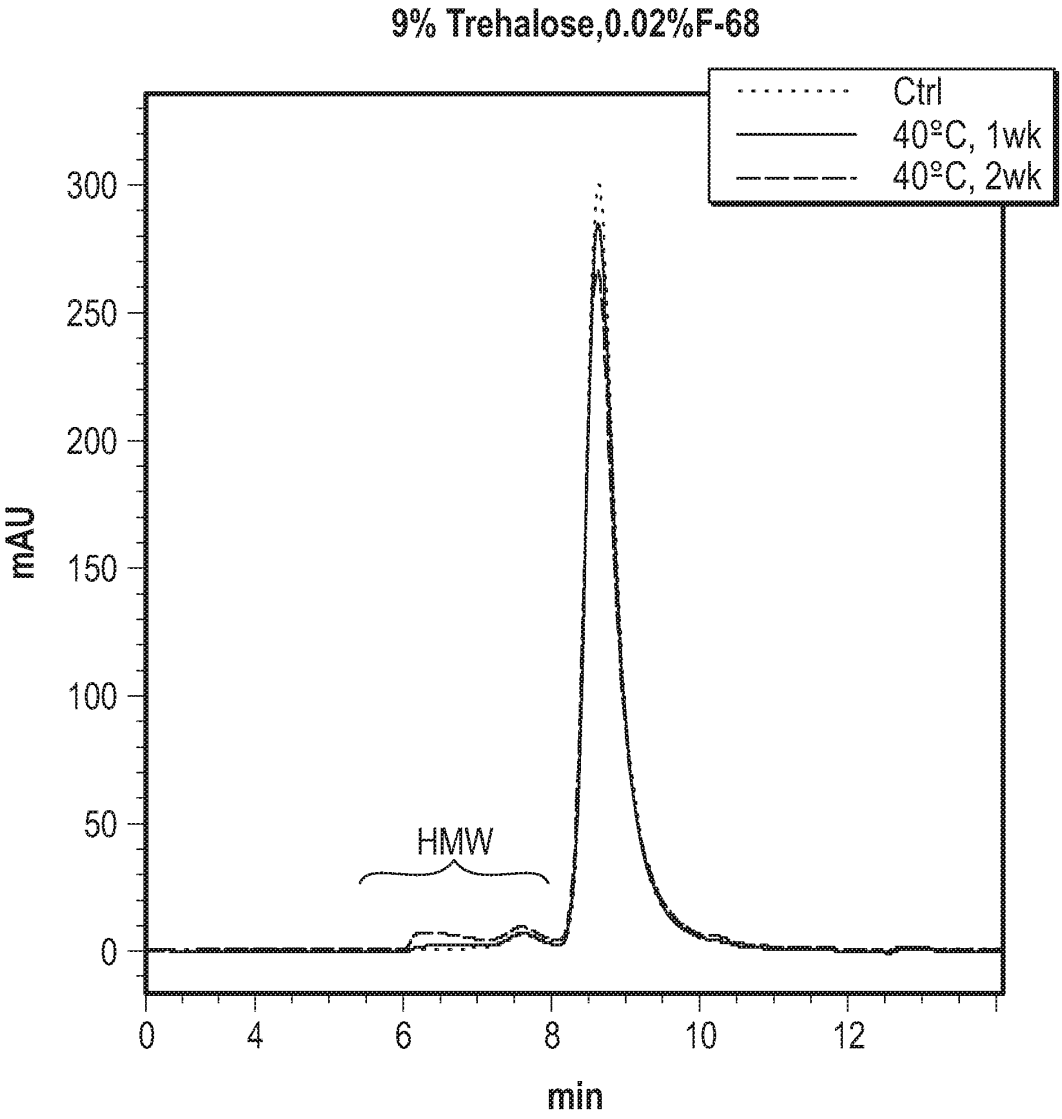

9% Trehalose,0.02%F-68
Ctrl
40ºC, 1wk
40ºC, 2wk
mAU
300
250
200
150
100
50
0
HMW
0
4
6
8
10
12
min

FIG. 9E

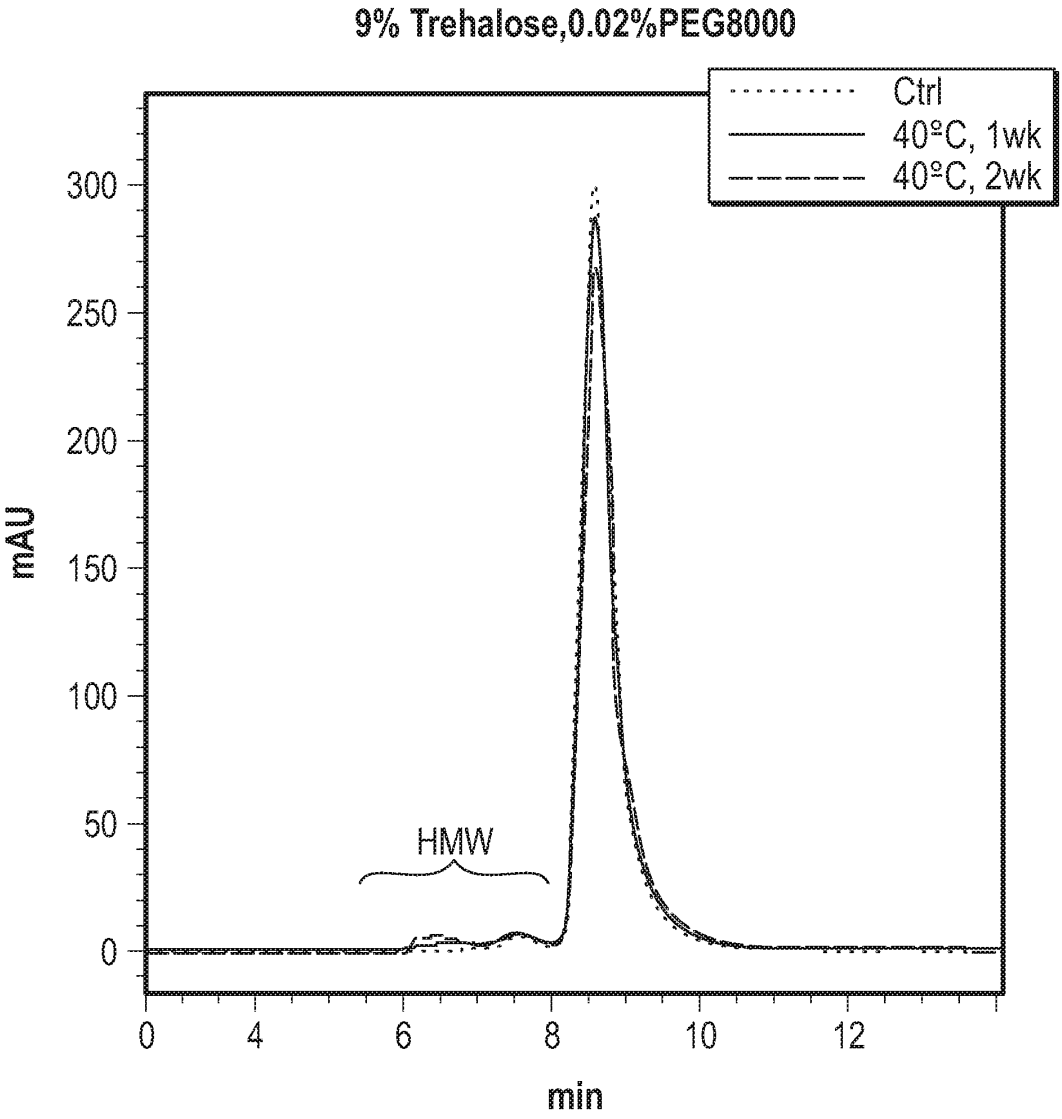

9% Trehalose,0.02%PEG8000
Ctrl
40ºC, 1wk
40ºC, 2wk
mAU
300
250
200
150
100
50
0
HMW
0
4
6
8
10
12
min

FIG. 9F