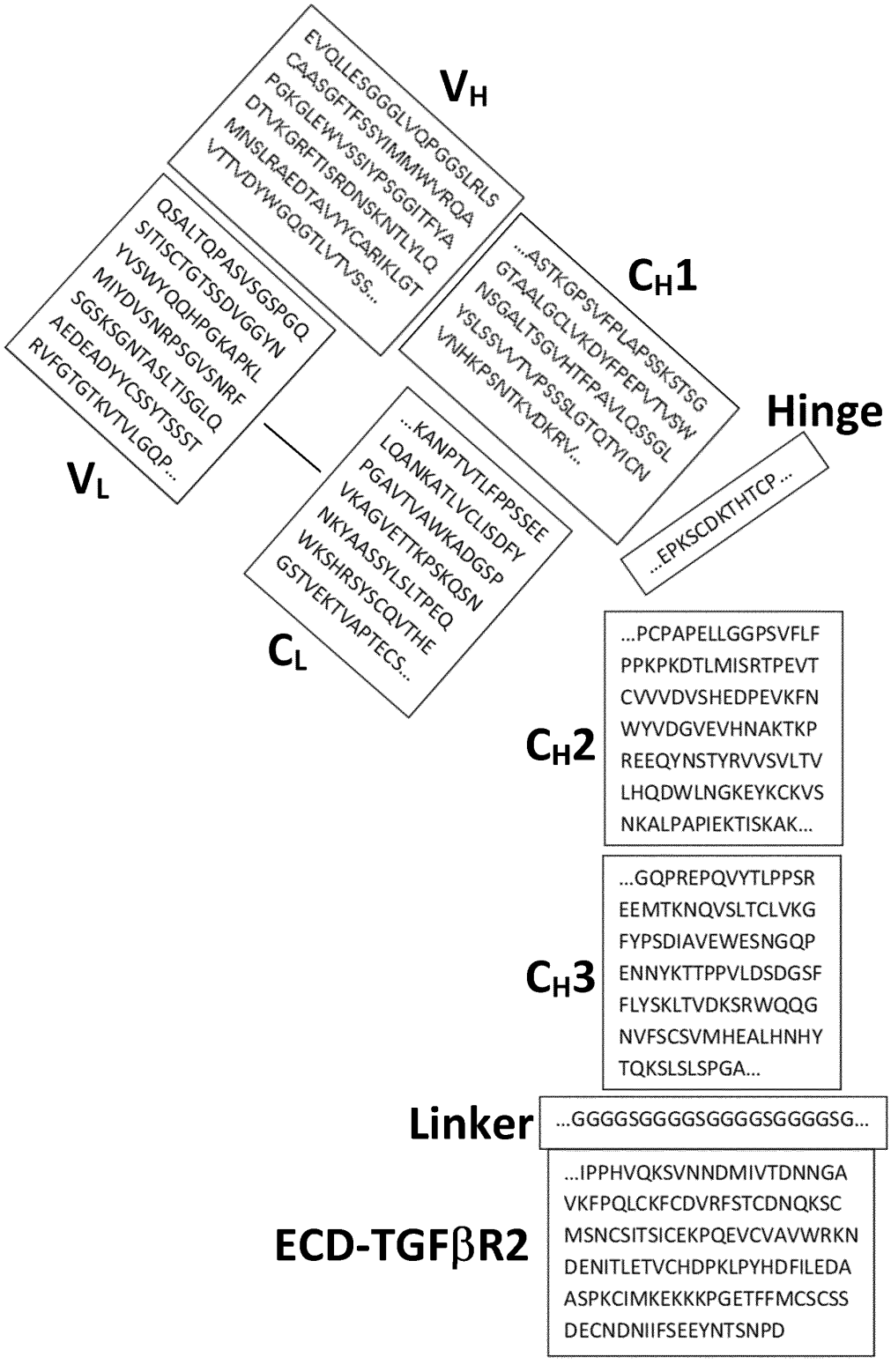

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CAASGFTFSSYIMMWVRQA
PGKGLEWVSSIYPSGGITFYA
DTVKGRFTISRDNSKNTLYLQ
MNSLRAEDTAVYYCARIKLGT
VTTVDYWGQGTLVTVSS...
VH
QSALTQPASVSGSPGQ
SITISCTGTSSDVGGYN
YVSWYQQHPGKAPKL
MIYDVSNRPSGVSNRF
SGSKSGNTASLTISGLQ
AEDEADYYCSSYTSST
RVFGTGTKVTVLGQP...
VL
...ASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKRV...
CH1
Hinge
...EPKSCDKTHTCP...
...KANPTVTLFPPSSEE
LQANKATLVCLISDFY
PGAVTVAWKADGSP
VKAGVETTKPSKQSN
NKYAASSYLSLTPEQ
WKSHRSYSCQVTHE
GSTVEKTVAPTECS...
CL
...PCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVS
NKALPAPIEKTISKAK...
CH2
...GQPREPQVYTLPPSR
EEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHY
TQKSLSLSPGA...
CH3
Linker
...GGGGSGGGGSGGGGSGGGGSG...
...IPPHVQKSVNNDMIVTDNNGA
VKFPQLCKFCDVRFSTCDNQKSC
MSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDA
ASPKCIMKEKKKPGETFFMCSCSS
DECNDNIIFSEEYNTSNPD
ECD-TGFβR2

Figure 1A

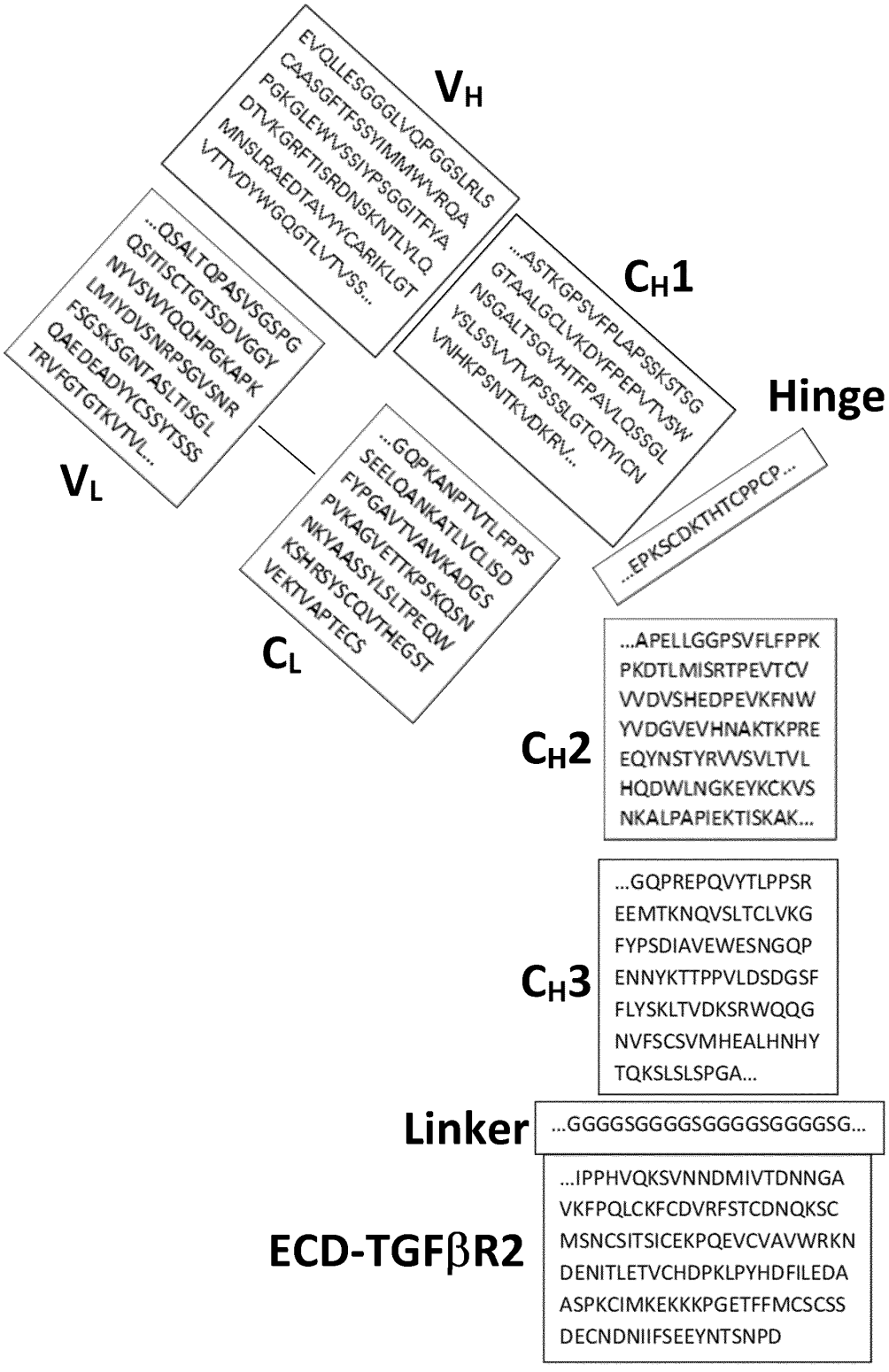

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CAASGFTFSSYIMMWVRQA
PGKGLEWVSSIYPSGGITFYA
DTVKGRFTISRDNSKNTLYLQ
MNSLRAEDTAVYYCARIKLGT
VTTVDYWGQGTLVTVSS...
VH
...QSALTQPASVSGSPG
QSITISCTGTSSDVGGV
NYVSWYQQHPGKAPK
LMIYDVSNRPSGVSNR
FSGSKSGNTASLTISGL
QAEDEADYYCSSYTSSS
TRVFGTGTKVTVL...
VL
...ASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKRV...
CH1
Hinge
...EPKSCDKTHTCPPCP...
...GQPKANPTVTLFPPS
SEELQANKATLVCLISD
FYPGAVTVAWKADGS
PVKAGVETTKPSKQSN
NKYAASSYLSLTPEQW
KSHRSYSCQVTHEGST
VEKTVAPTECS
CL
...APELLGGPSVFLFPPK
PKDTLMISRTPEVTCV
VVDVSHEDPEVKFNW
YVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVS
NKALPAPIEKTISKAK...
CH2
...GQPREPQVYTLPPSR
EEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHY
TQKSLSLSPGA...
CH3
Linker
...GGGGSGGGGSGGGGSGGGGSG...
ECD-TGFβR2
...IPPHVQKSVNNDMIVTDNNGA
VKFPQLCKFCDVRFSTCDNQKSC
MSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDA
ASPKCIMKEKKKPGETFFMCSCSS
DECNDNIIFSEEYNTSNPD

Figure 1B

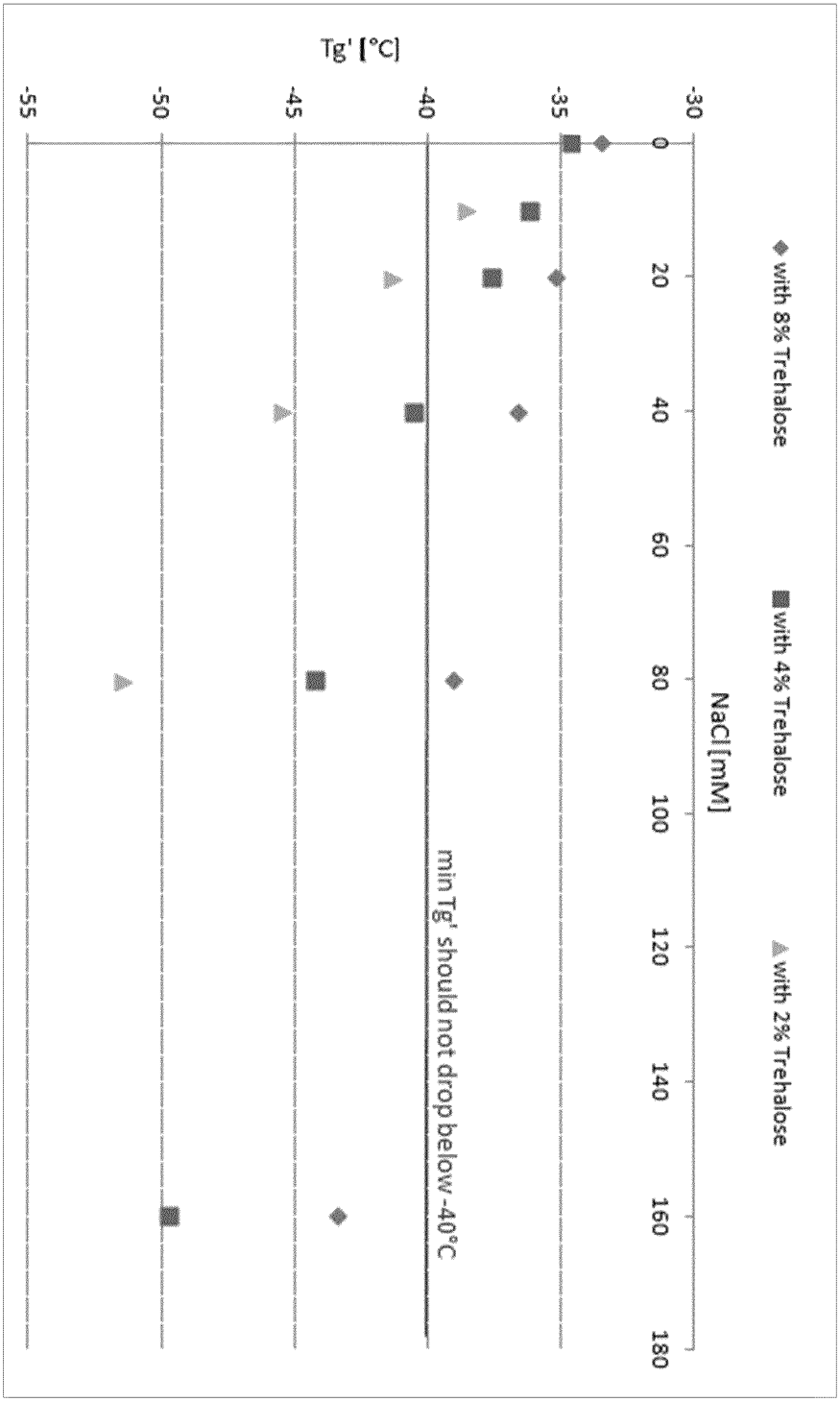

Tg' [°C]
-30
-35
-40
-45
-50
-55
0
20
40
60
80
100
120
140
160
180
NaCl [mM]
with 8% Trehalose
with 4% Trehalose
with 2% Trehalose
min Tg' should not drop below -40°C

Figure 2

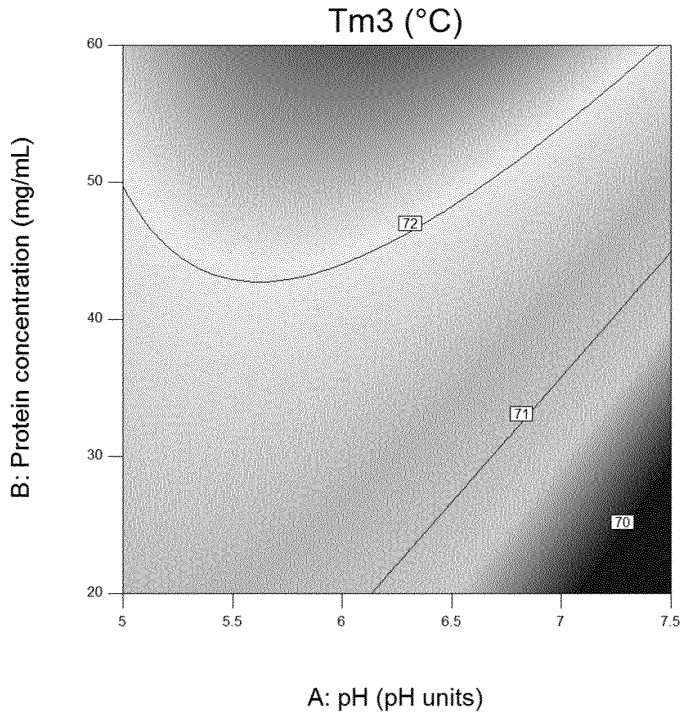


Figure 3

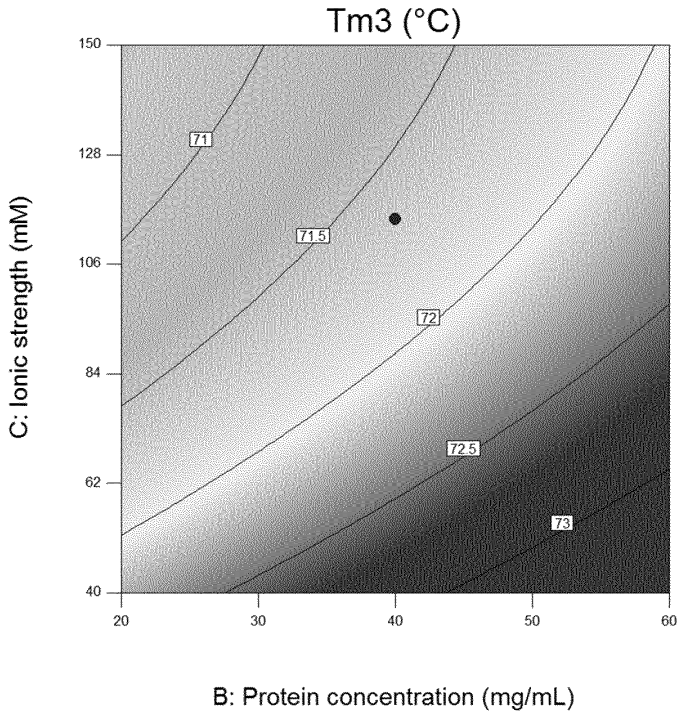


Figure 4

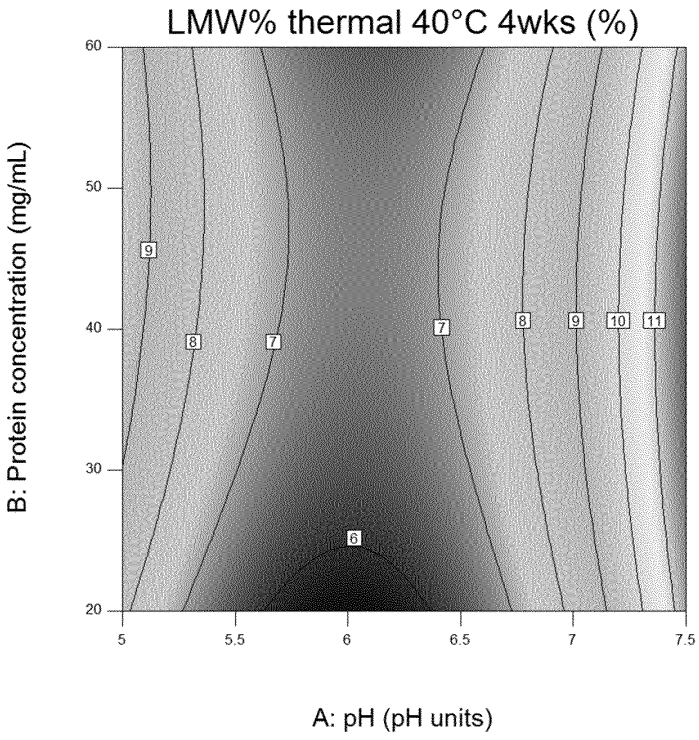


Figure 5

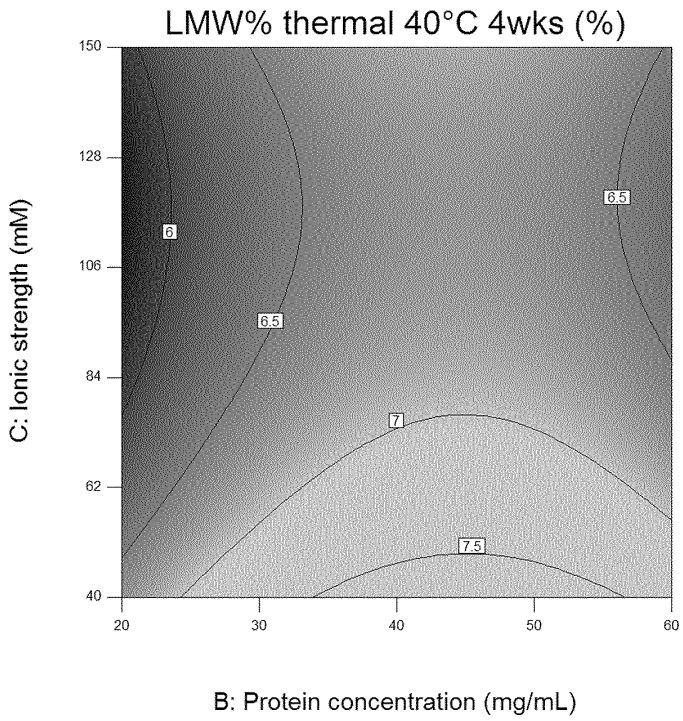


Figure 6

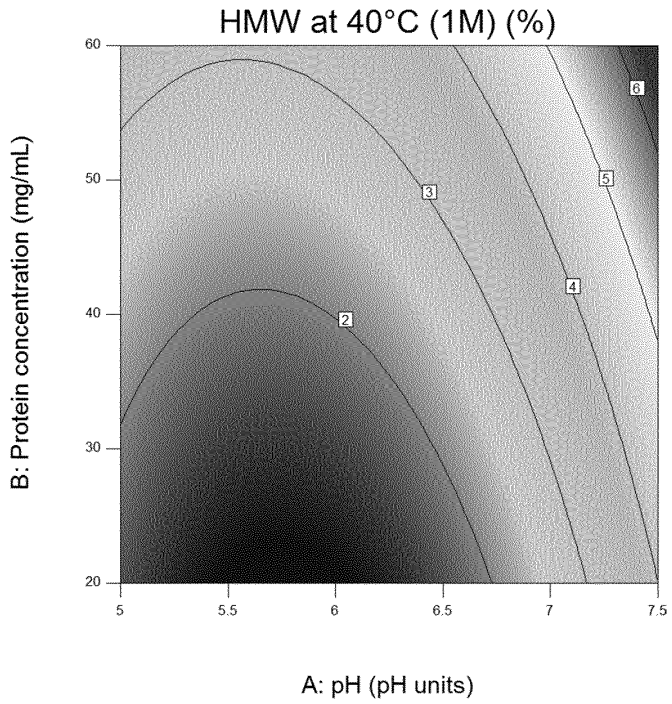


Figure 7

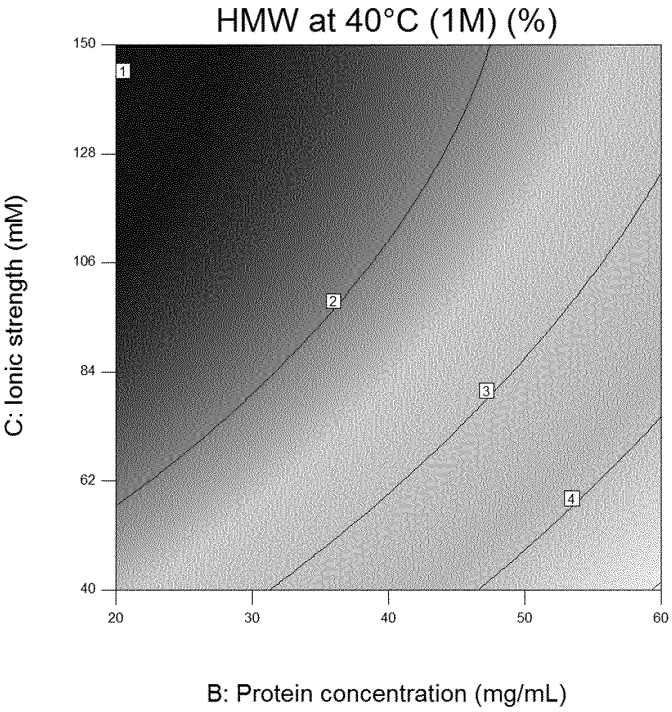


Figure 8

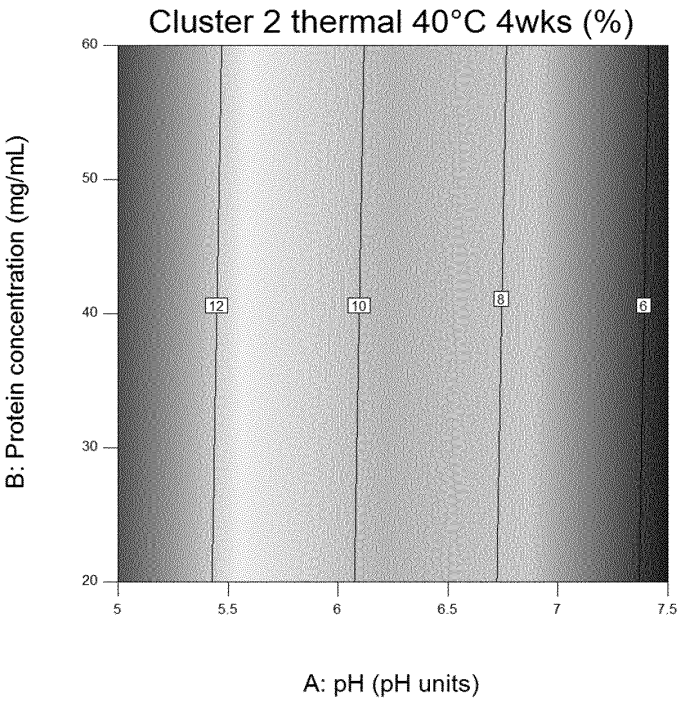


Figure 9

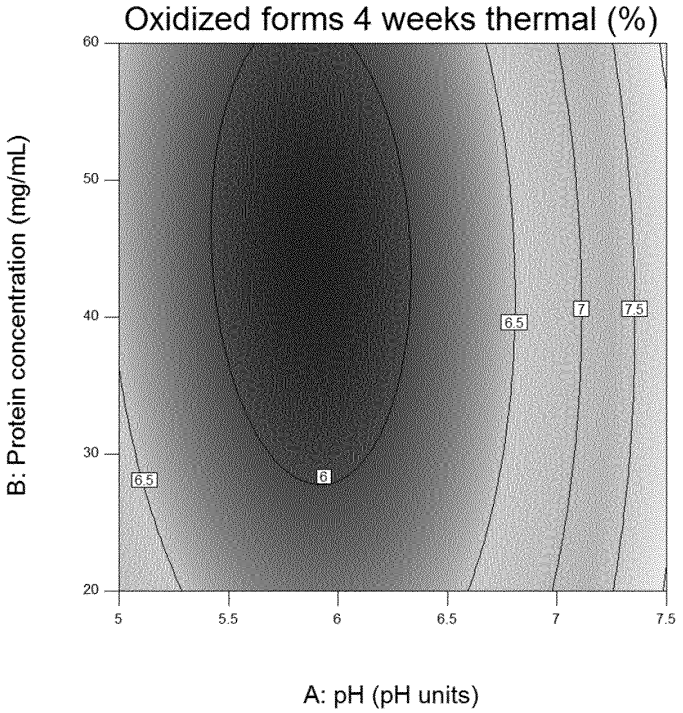


Figure 10

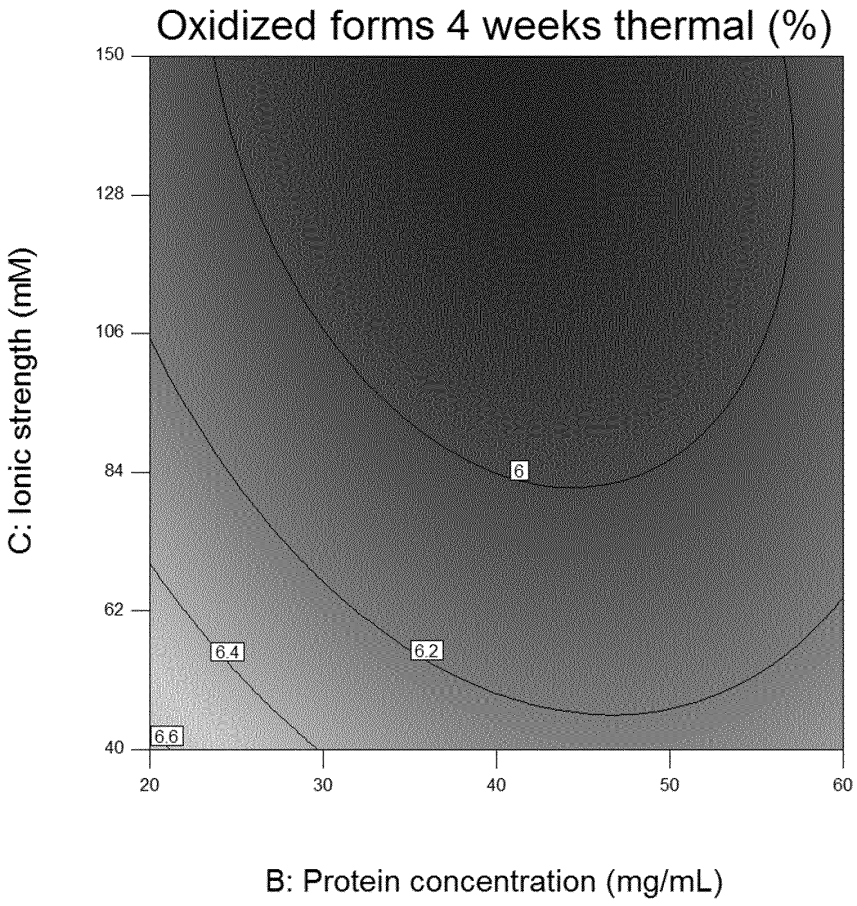


Figure 11

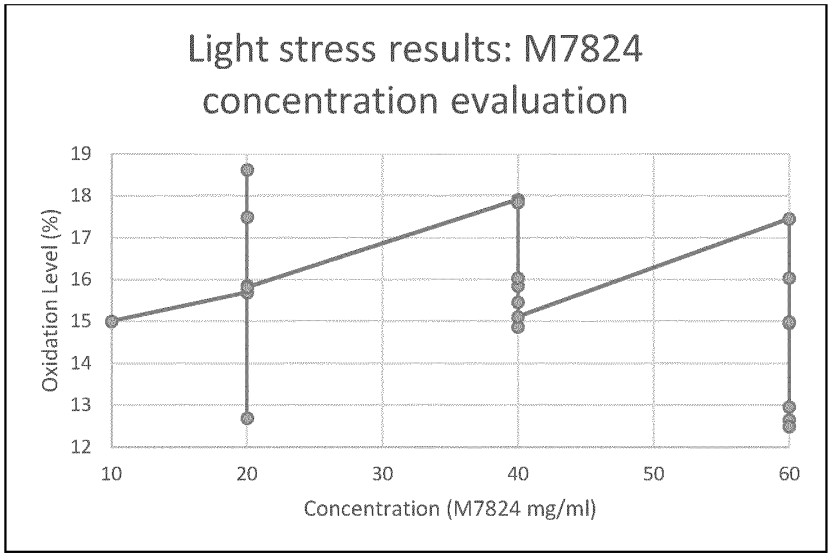


Figure 12

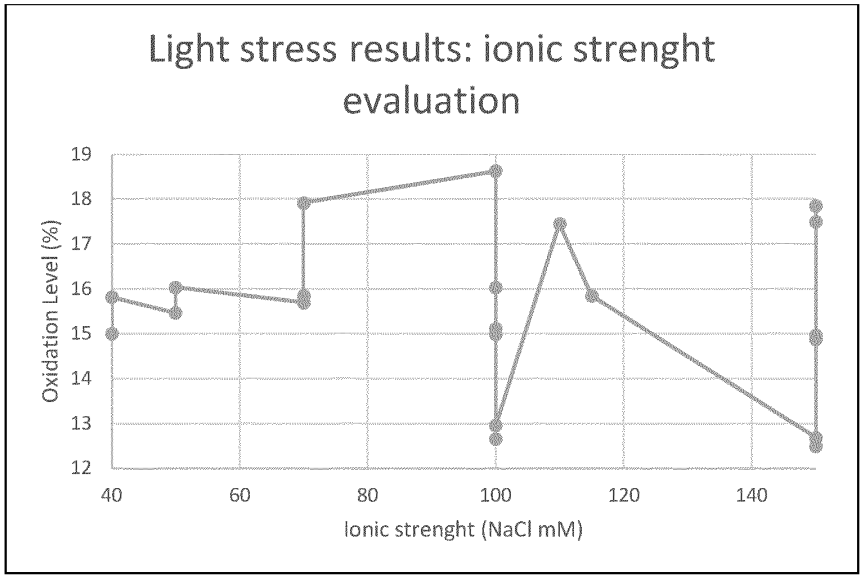


Figure 13

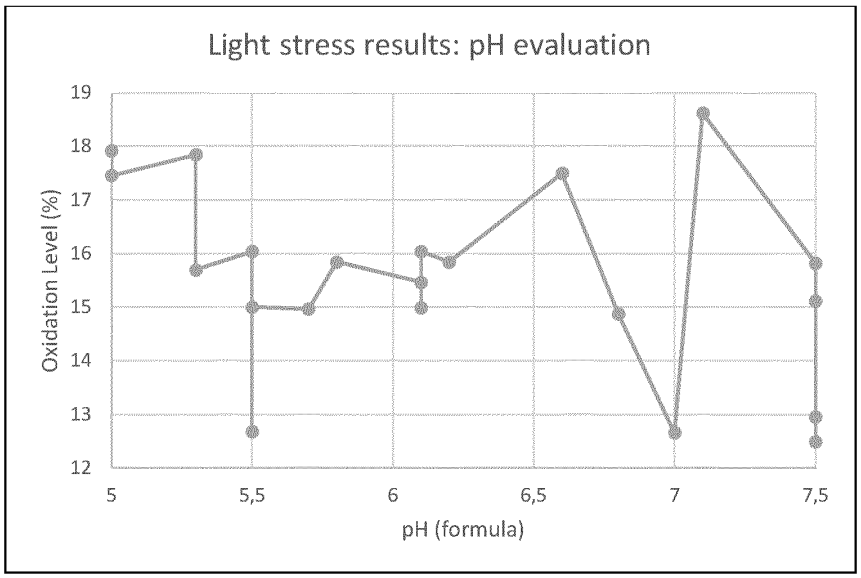


Figure 14

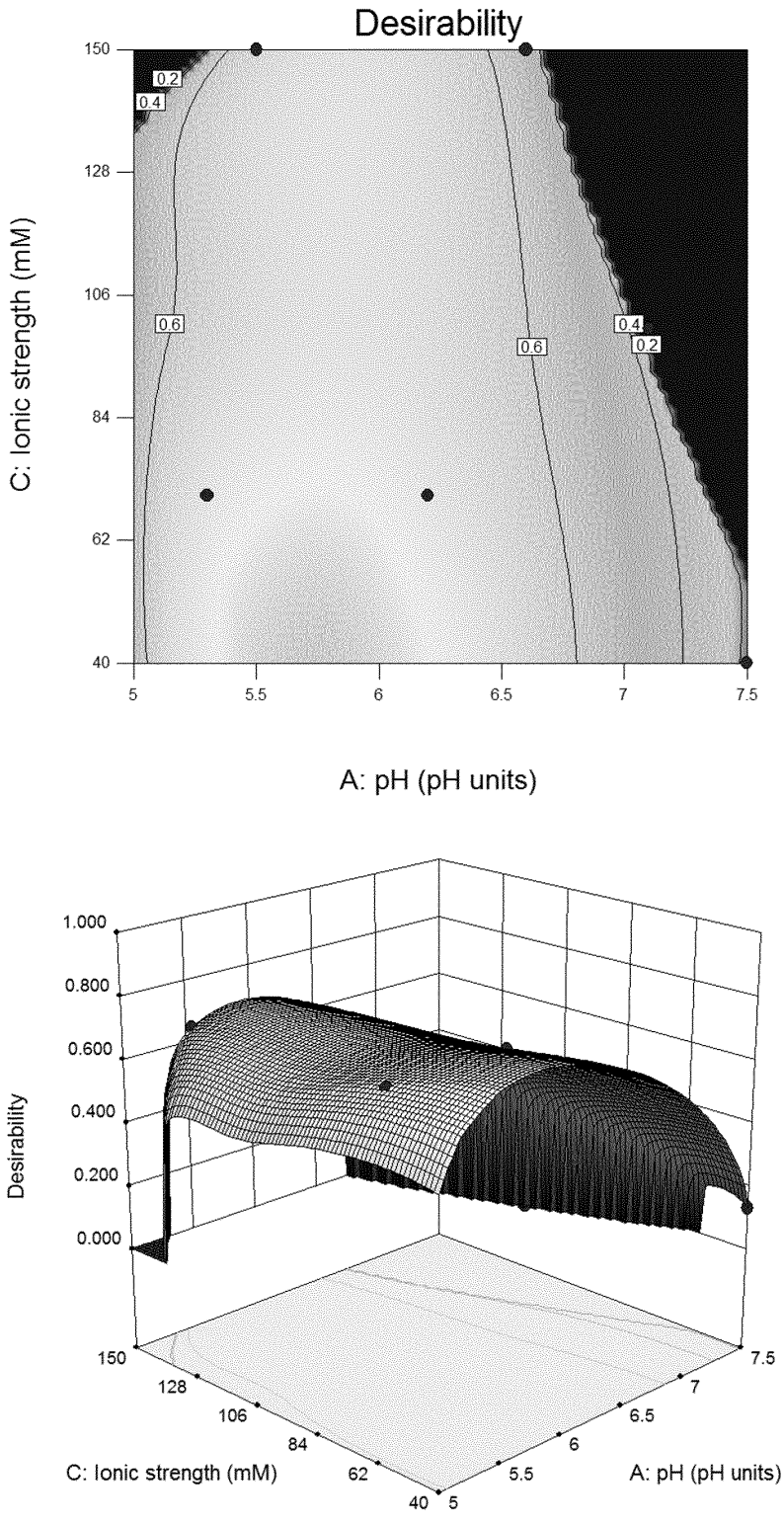

Desirability
150
128
106
84
62
40
C: Ionic strength (mM)
0.2
0.4
0.6
0.6
0.4
0.2
5
5.5
6
6.5
7
7.5
A: pH (pH units)
1.000
0.800
0.600
0.400
0.200
0.000
Desirability
150
128
106
84
62
40
C: Ionic strength (mM)
5
5.5
6
6.5
7
7.5
A: pH (pH units)

Figure 15

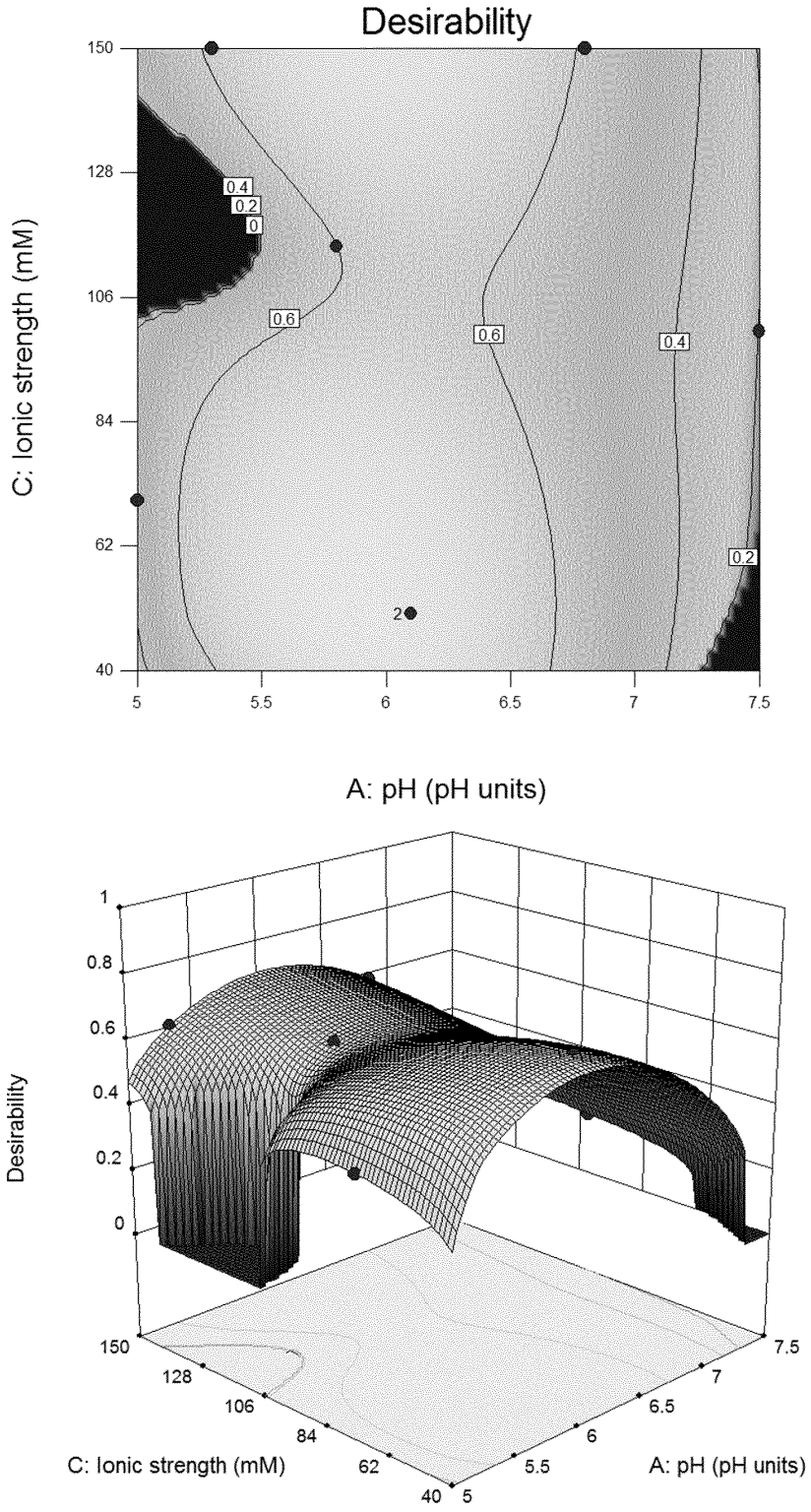

Desirability
C: Ionic strength (mM)
150
128
106
84
62
40
0.4
0.2
0
0.6
0.6
0.4
0.2
2
5
5.5
6
6.5
7
7.5
A: pH (pH units)
Desirability
1
0.8
0.6
0.4
0.2
0
C: Ionic strength (mM)
150
128
106
84
62
40
A: pH (pH units)
5
5.5
6
6.5
7
7.5

Figure 16

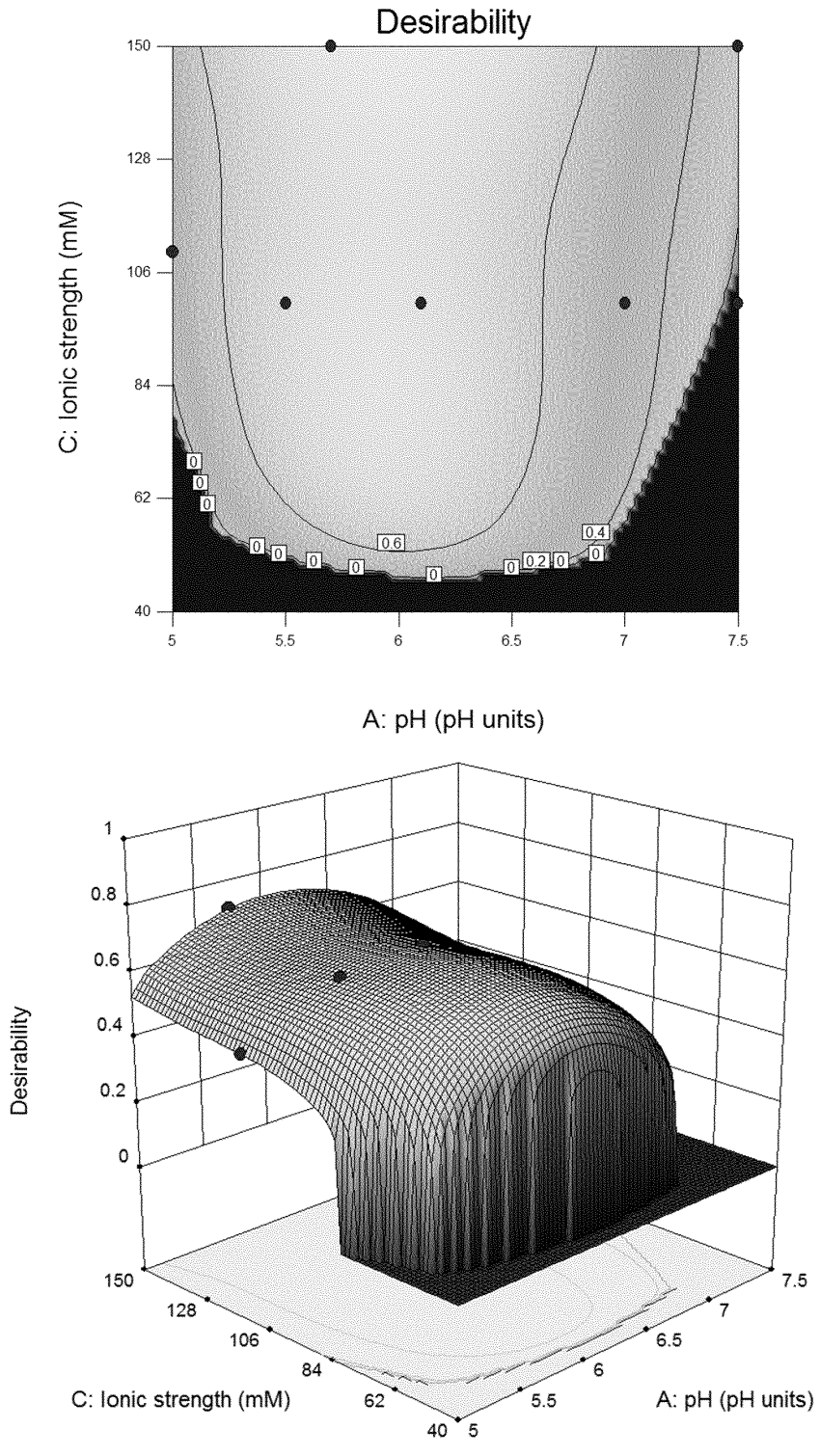

Desirability
C: Ionic strength (mM)
150
128
106
84
62
40
5
5.5
6
6.5
7
7.5
A: pH (pH units)
0
0.2
0.4
0.6
Desirability
1
0.8
0.6
0.4
0.2
0
C: Ionic strength (mM)
A: pH (pH units)

Figure 17

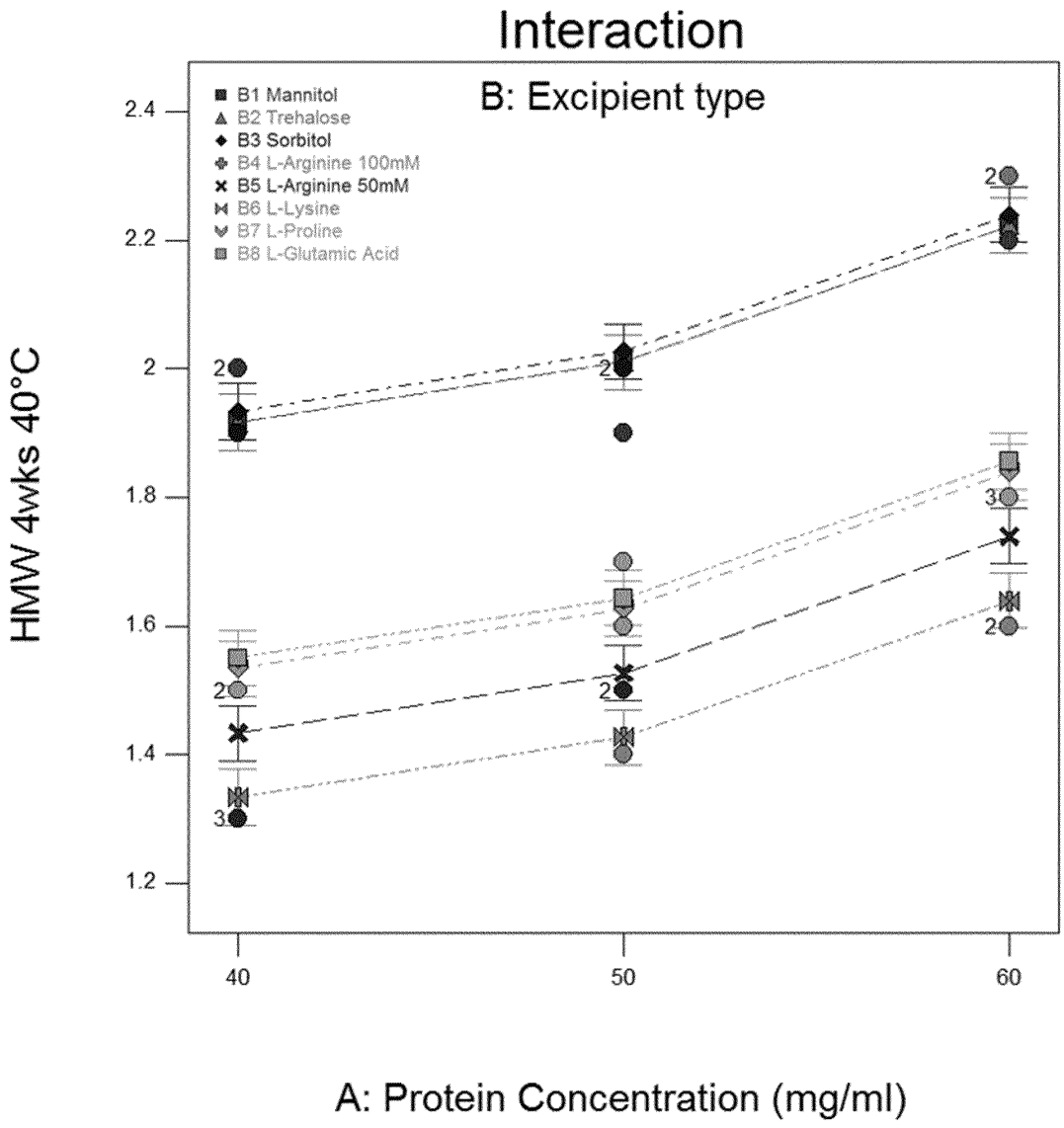

Interaction
B: Excipient type
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid
HMW 4wks 40°C
2.4
2.2
2
1.8
1.6
1.4
1.2
40
50
60
A: Protein Concentration (mg/ml)

Figure 18

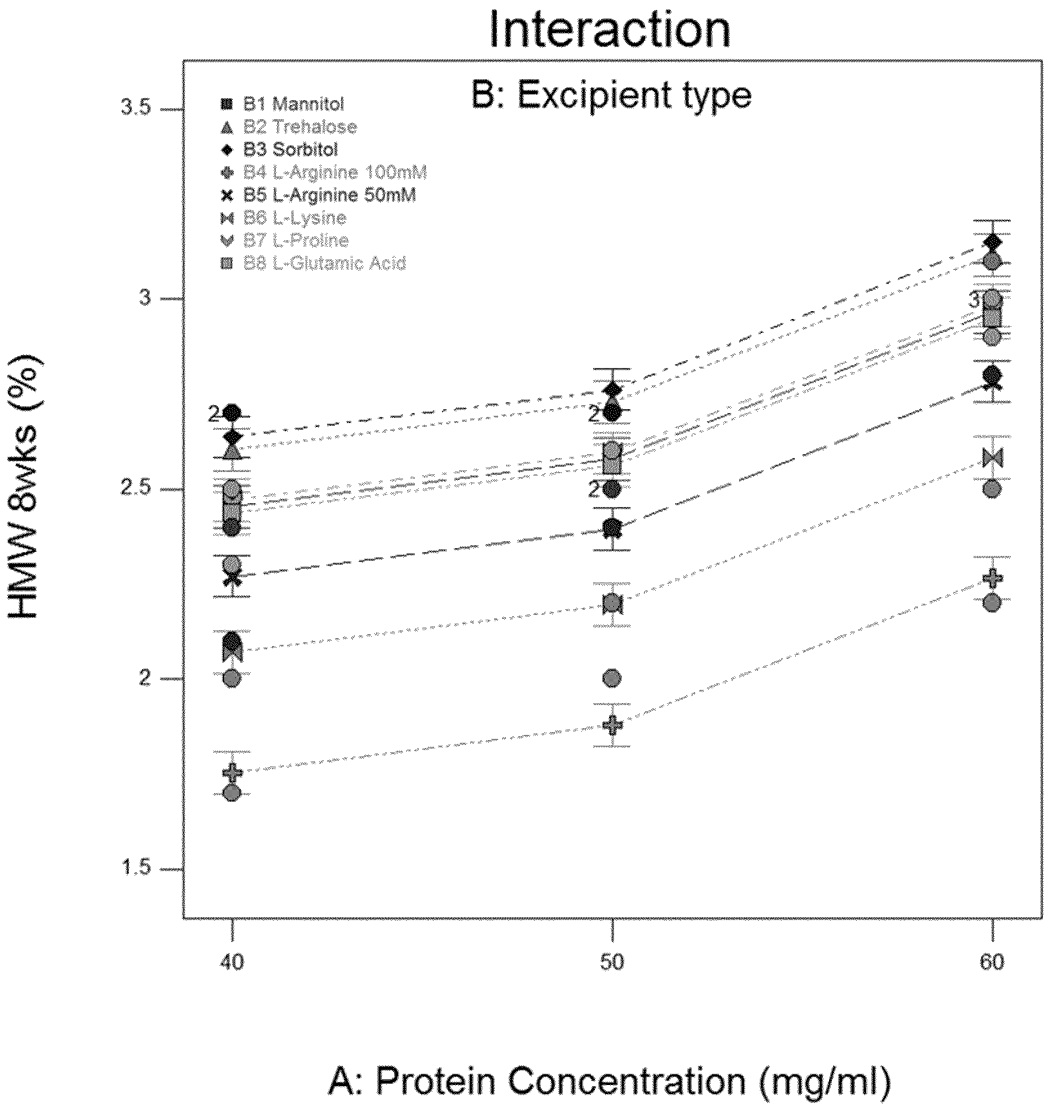

Interaction
B: Excipient type
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid
HMW 8wks (%)
3.5
3
2.5
2
1.5
40
50
60
A: Protein Concentration (mg/ml)

Figure 19

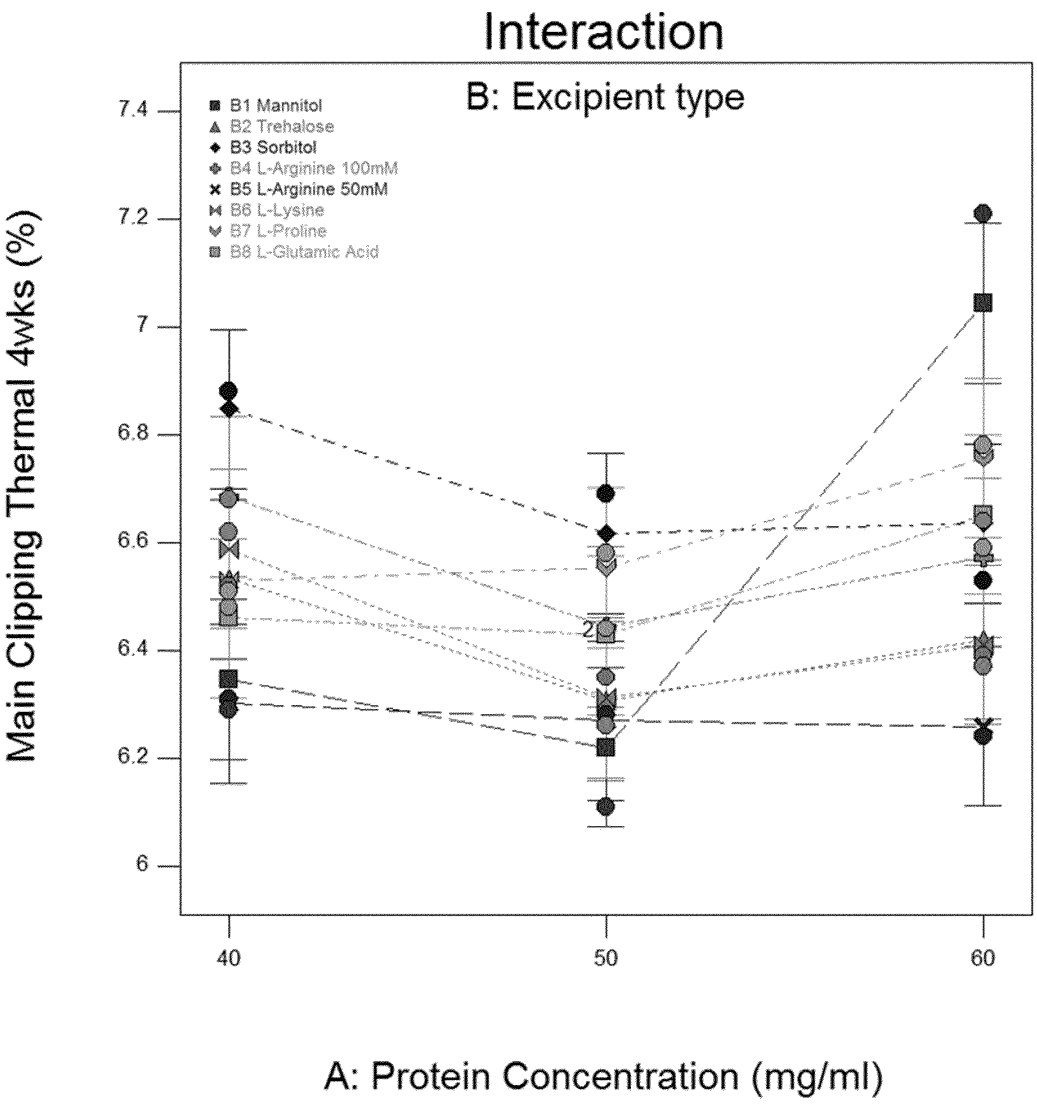

Interaction
B: Excipient type
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid
Main Clipping Thermal 4wks (%)
7.4
7.2
7
6.8
6.6
6.4
6.2
6
40
50
60
A: Protein Concentration (mg/ml)

Figure 20

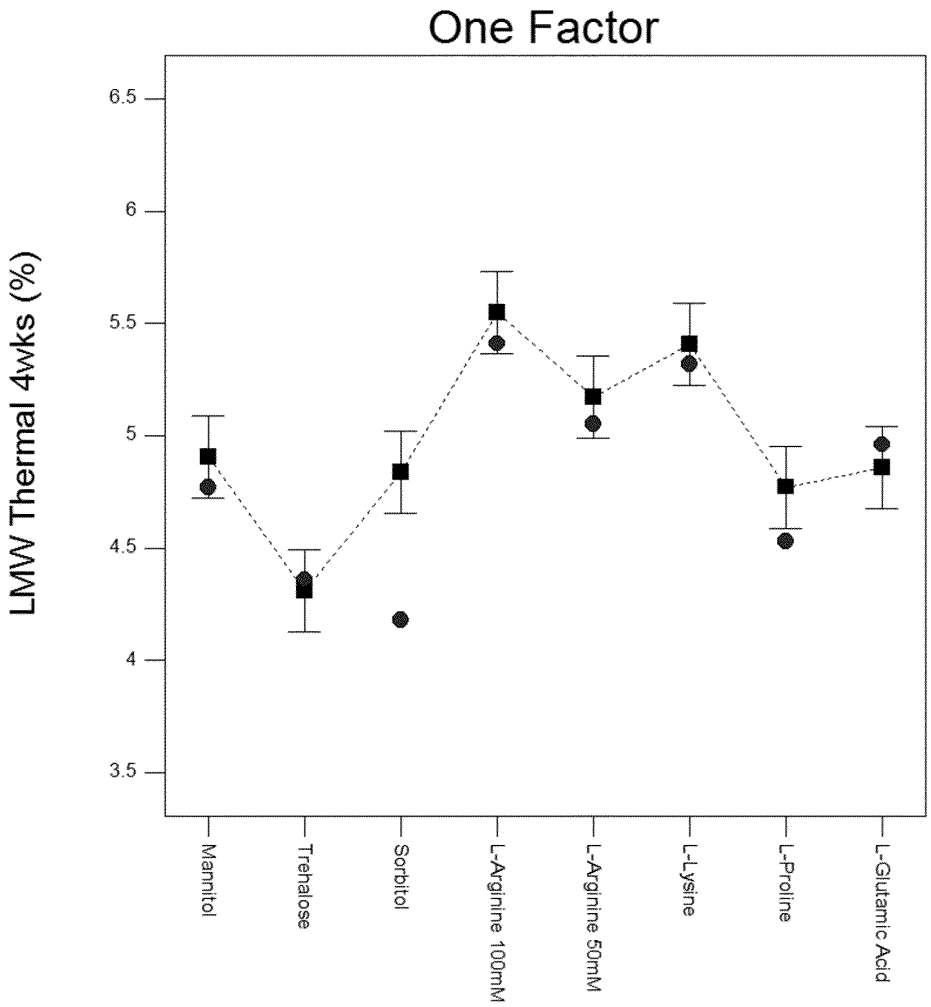

One Factor
LMW Thermal 4wks (%)
6.5
6
5.5
5
4.5
4
3.5
Mannitol
Trehalose
Sorbitol
L-Arginine 100mM
L-Arginine 50mM
L-Lysine
L-Proline
L-Glutamic Acid

Figure 21

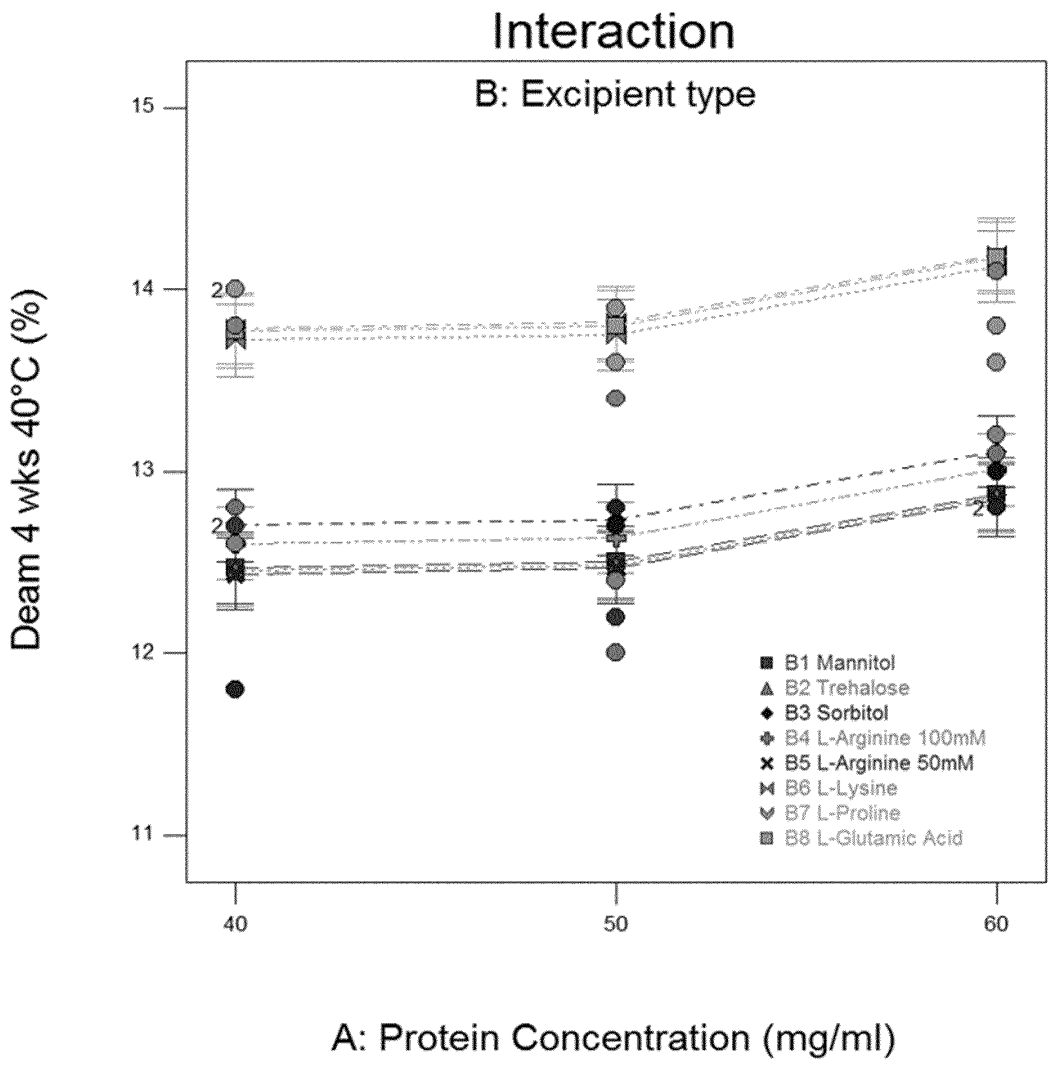

Interaction
B: Excipient type
Deam 4 wks 40°C (%)
15
14
13
12
11
40
50
60
A: Protein Concentration (mg/ml)
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid

Figure 22

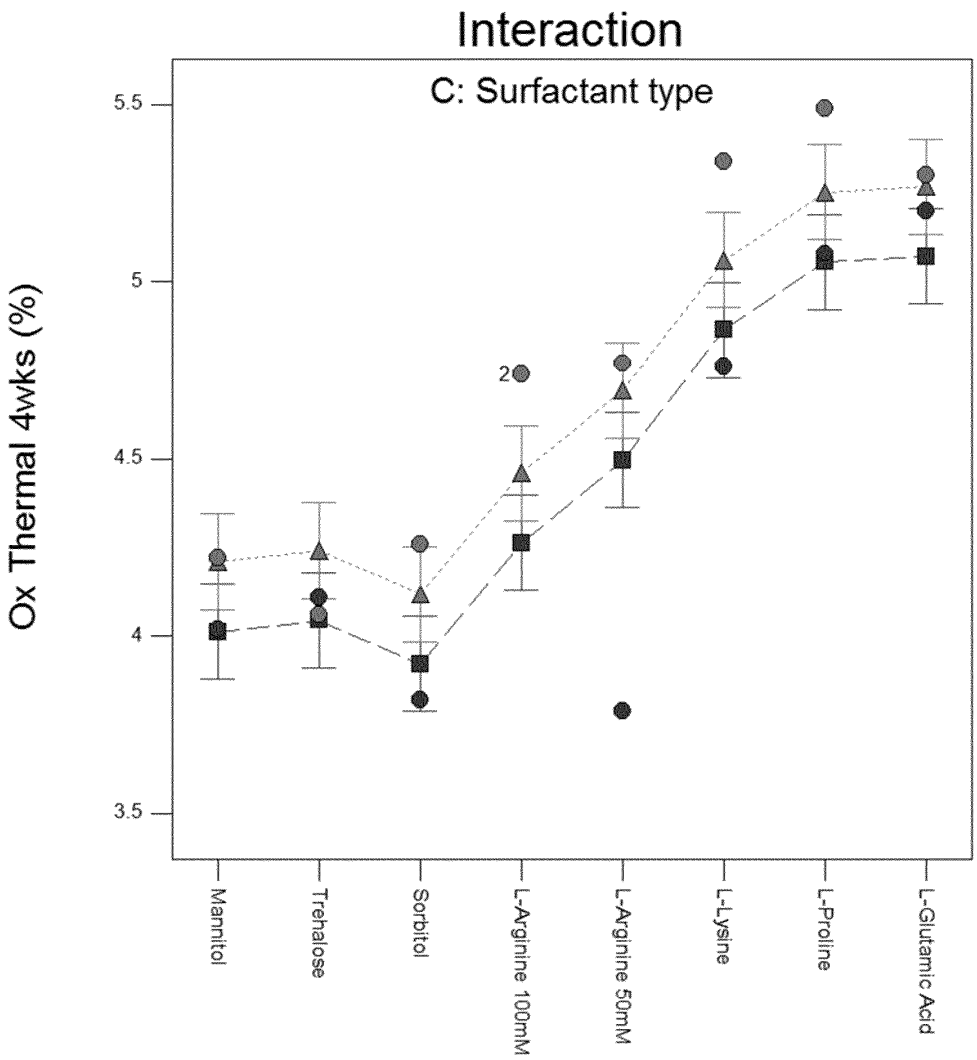

Interaction
C: Surfactant type
Ox Thermal 4wks (%)
5.5
5
4.5
4
3.5
2
Mannitol
Trehalose
Sorbitol
L-Arginine 100mM
L-Arginine 50mM
L-Lysine
L-Proline
L-Glutamic Acid

Figure 23

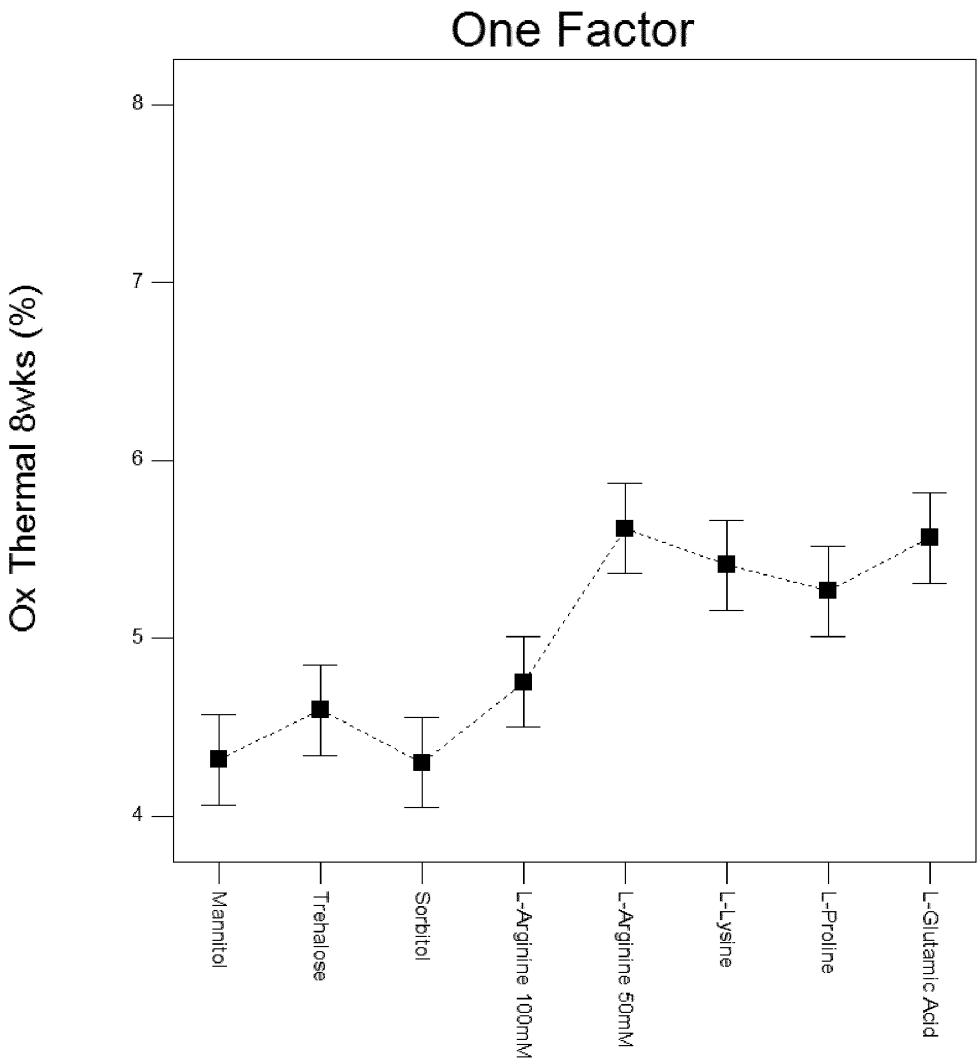

One Factor
Ox Thermal 8wks (%)
8
7
6
5
4
Mannitol
Trehalose
Sorbitol
L-Arginine 100mM
L-Arginine 50mM
L-Lysine
L-Proline
L-Glutamic Acid

Figure 24

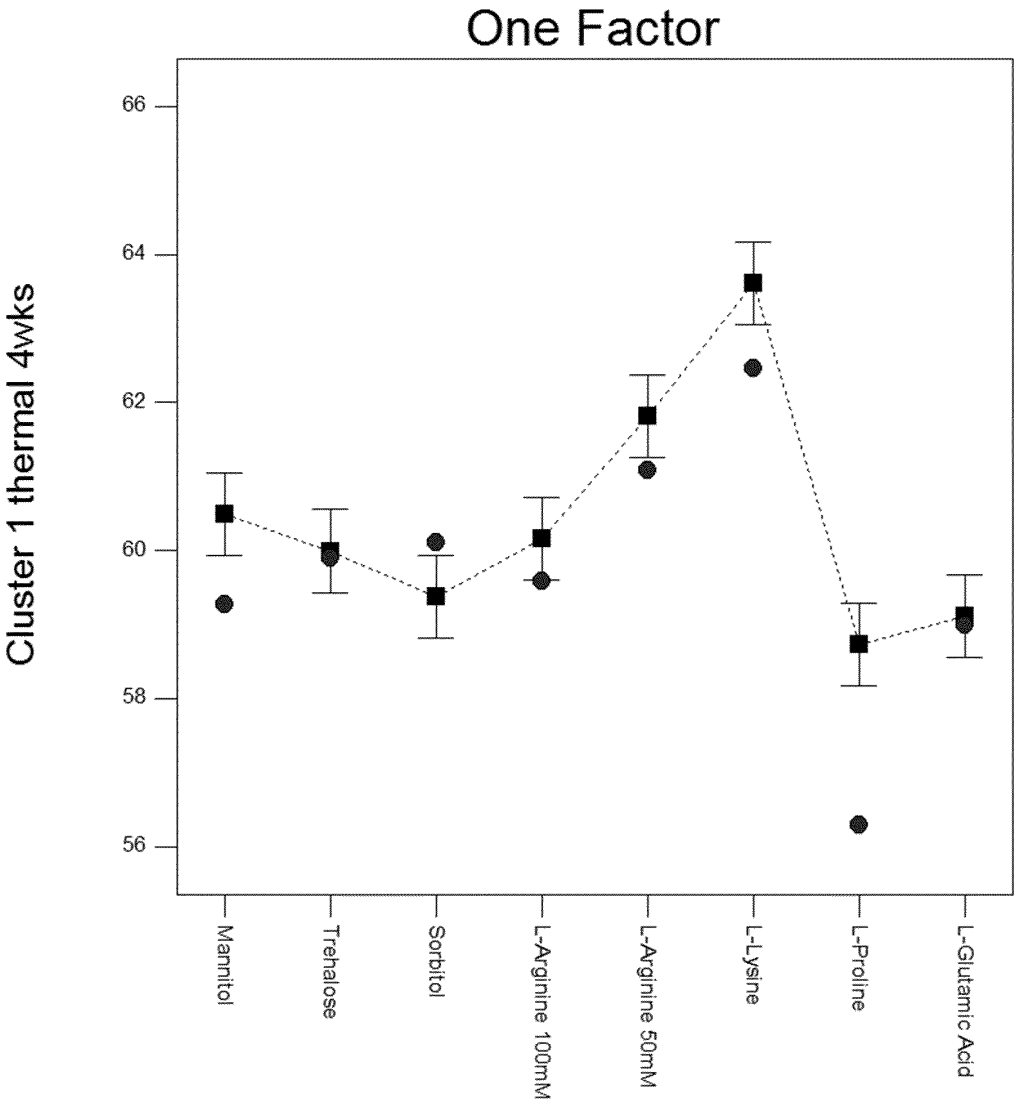

One Factor
Cluster 1 thermal 4wks
66
64
62
60
58
56
Mannitol
Trehalose
Sorbitol
L-Arginine 100mM
L-Arginine 50mM
L-Lysine
L-Proline
L-Glutamic Acid

Figure 25

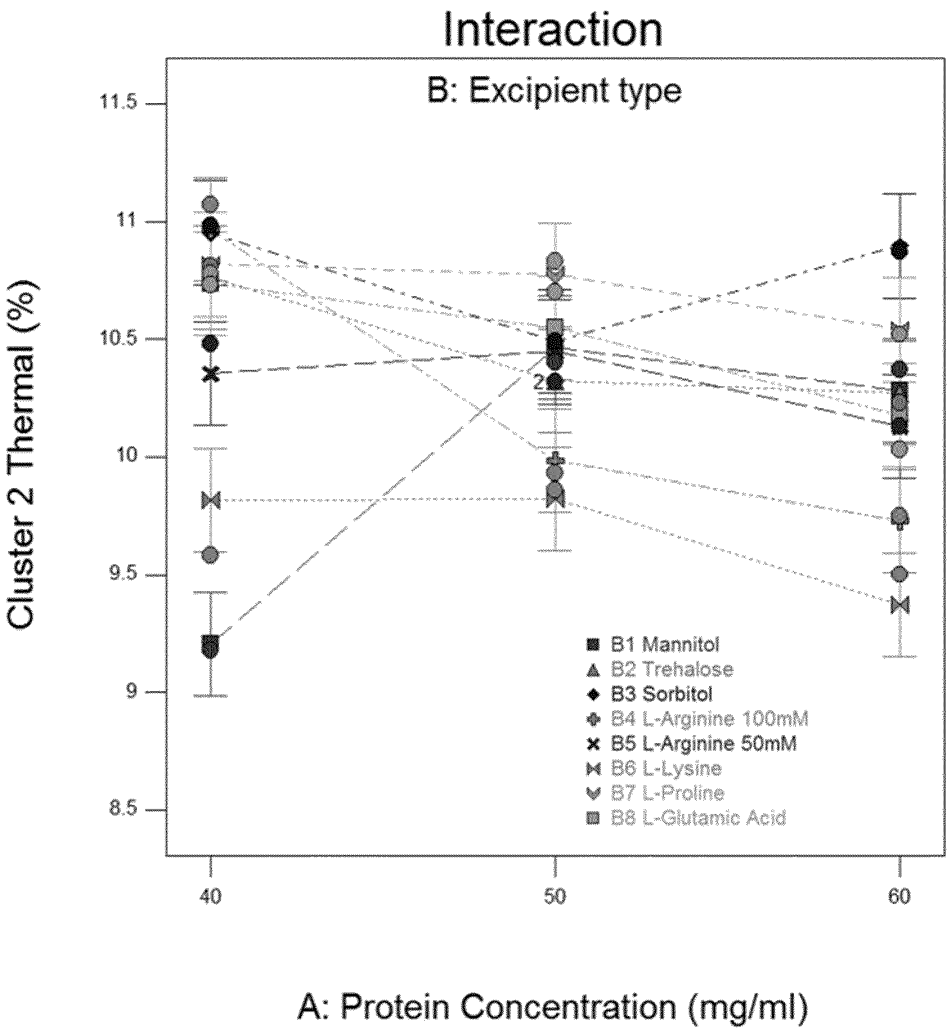

Interaction
B: Excipient type
Cluster 2 Thermal (%)
11.5
11
10.5
10
9.5
9
8.5
40
50
60
A: Protein Concentration (mg/ml)
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid

Figure 26

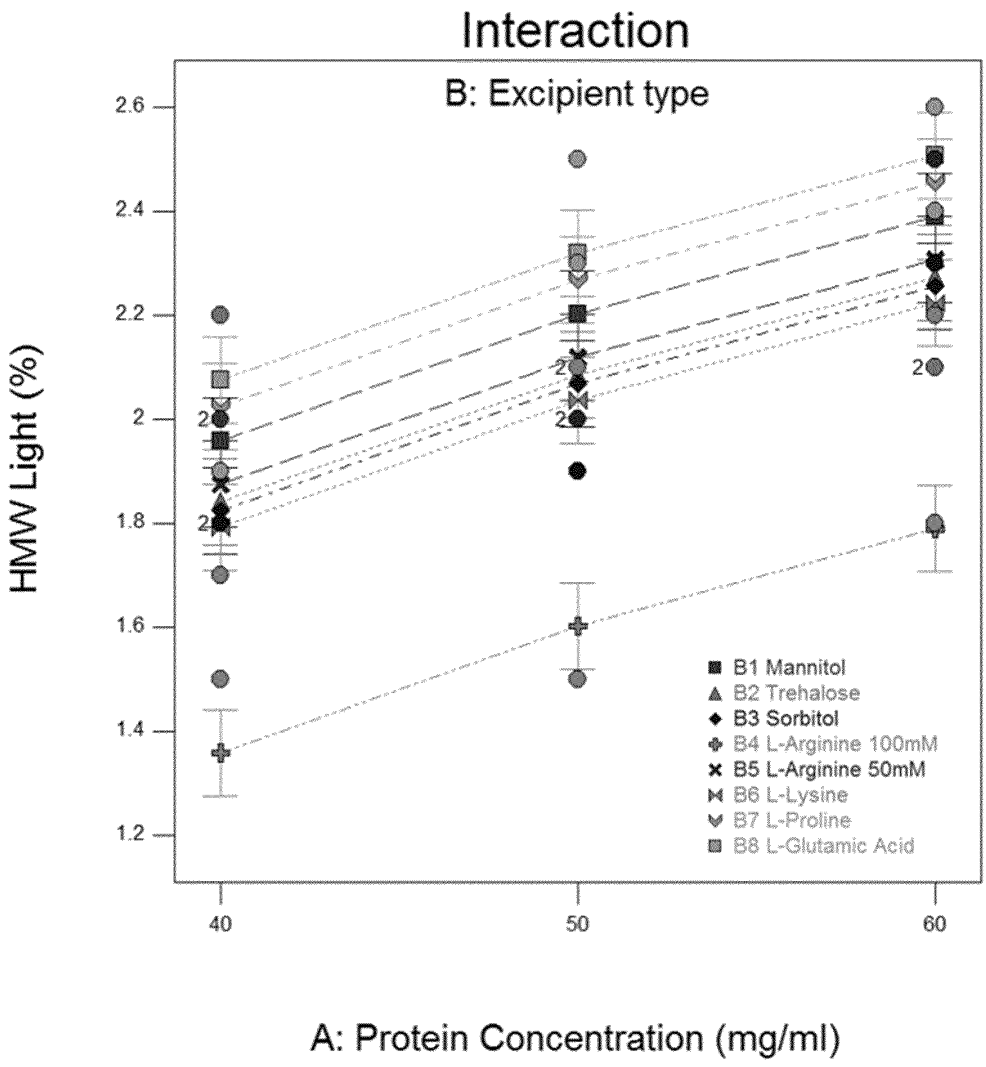

Interaction
B: Excipient type
HMW Light (%)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
40
50
60
A: Protein Concentration (mg/ml)
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid

Figure 27

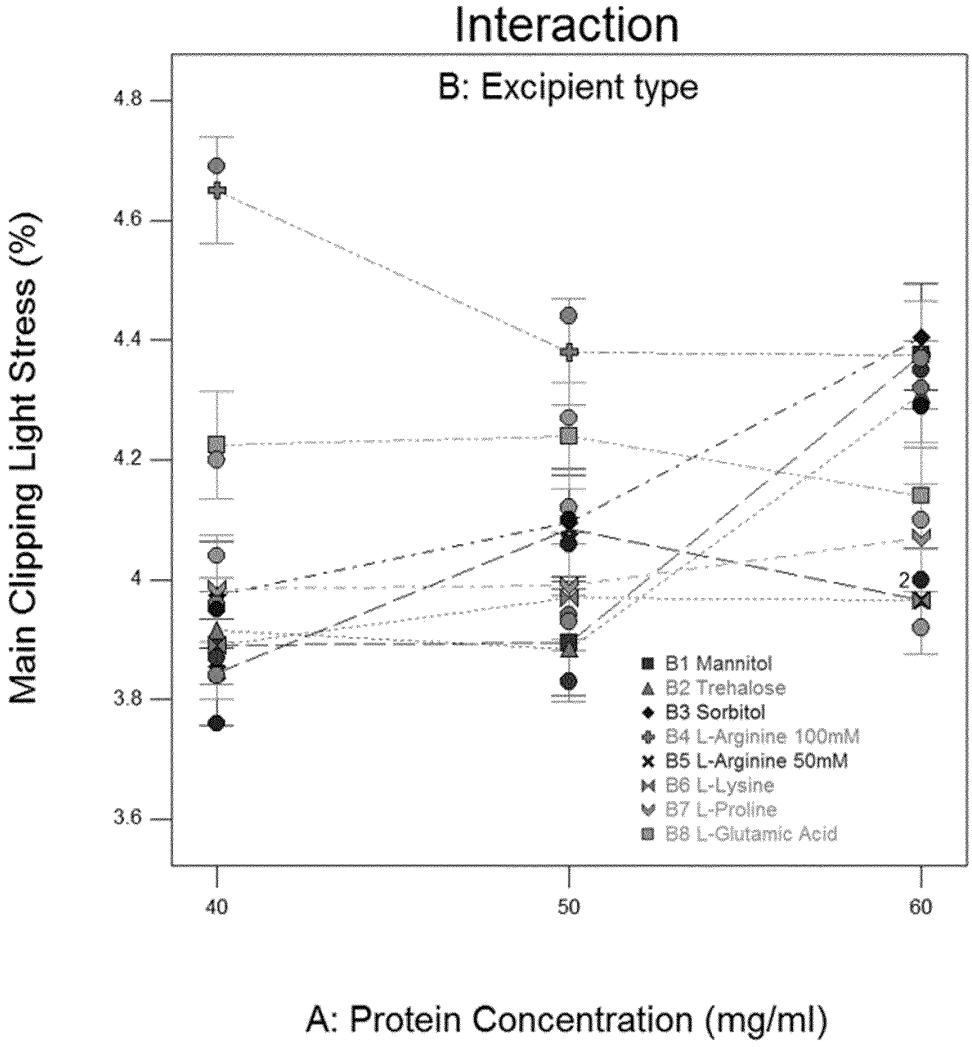

Interaction
B: Excipient type
Main Clipping Light Stress (%)
4.8
4.6
4.4
4.2
4
3.8
3.6
40
50
60
A: Protein Concentration (mg/ml)
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid

Figure 28

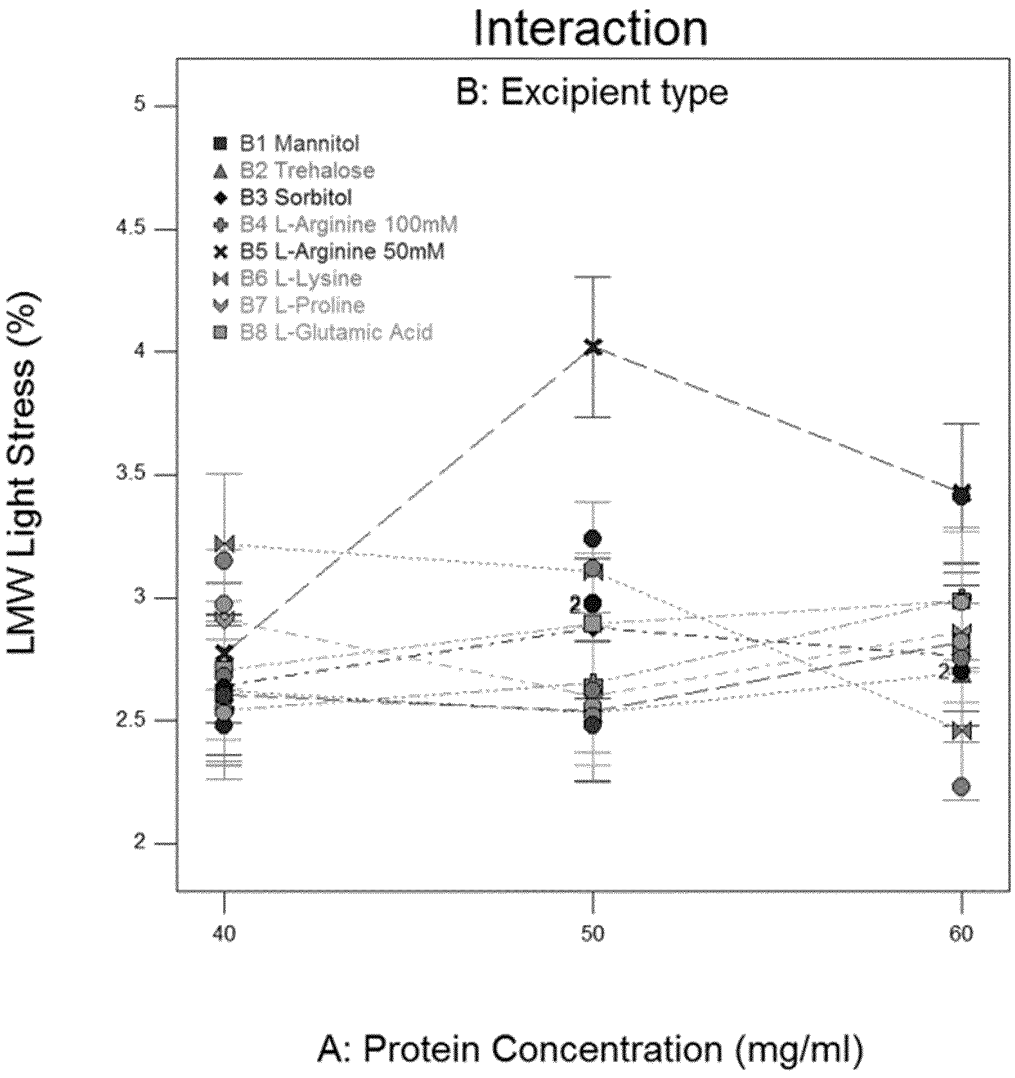

Interaction
B: Excipient type
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid
LMW Light Stress (%)
5
4.5
4
3.5
3
2.5
2
40
50
60
A: Protein Concentration (mg/ml)

Figure 29

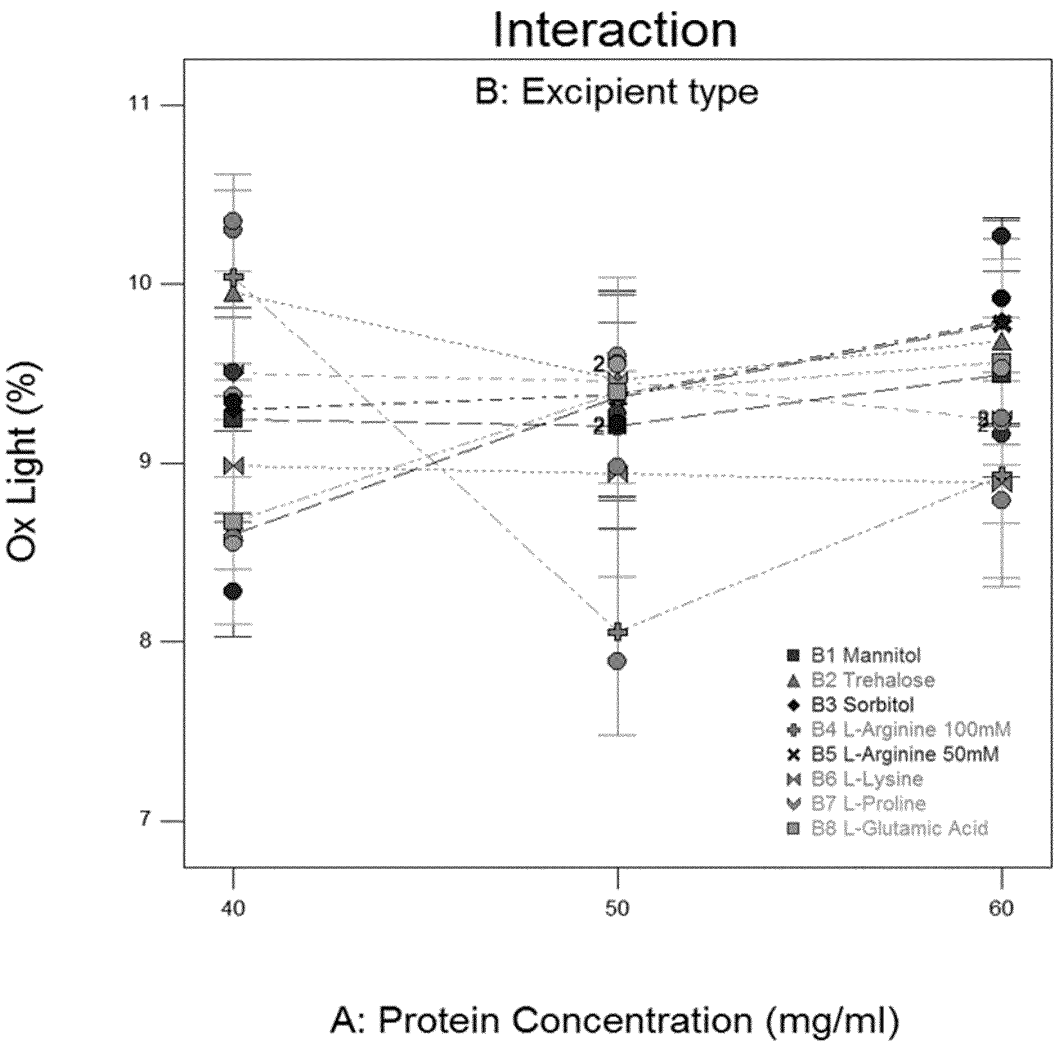

Interaction
B: Excipient type
Ox Light (%)
11
10
9
8
7
40
50
60
A: Protein Concentration (mg/ml)
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid

Figure 30

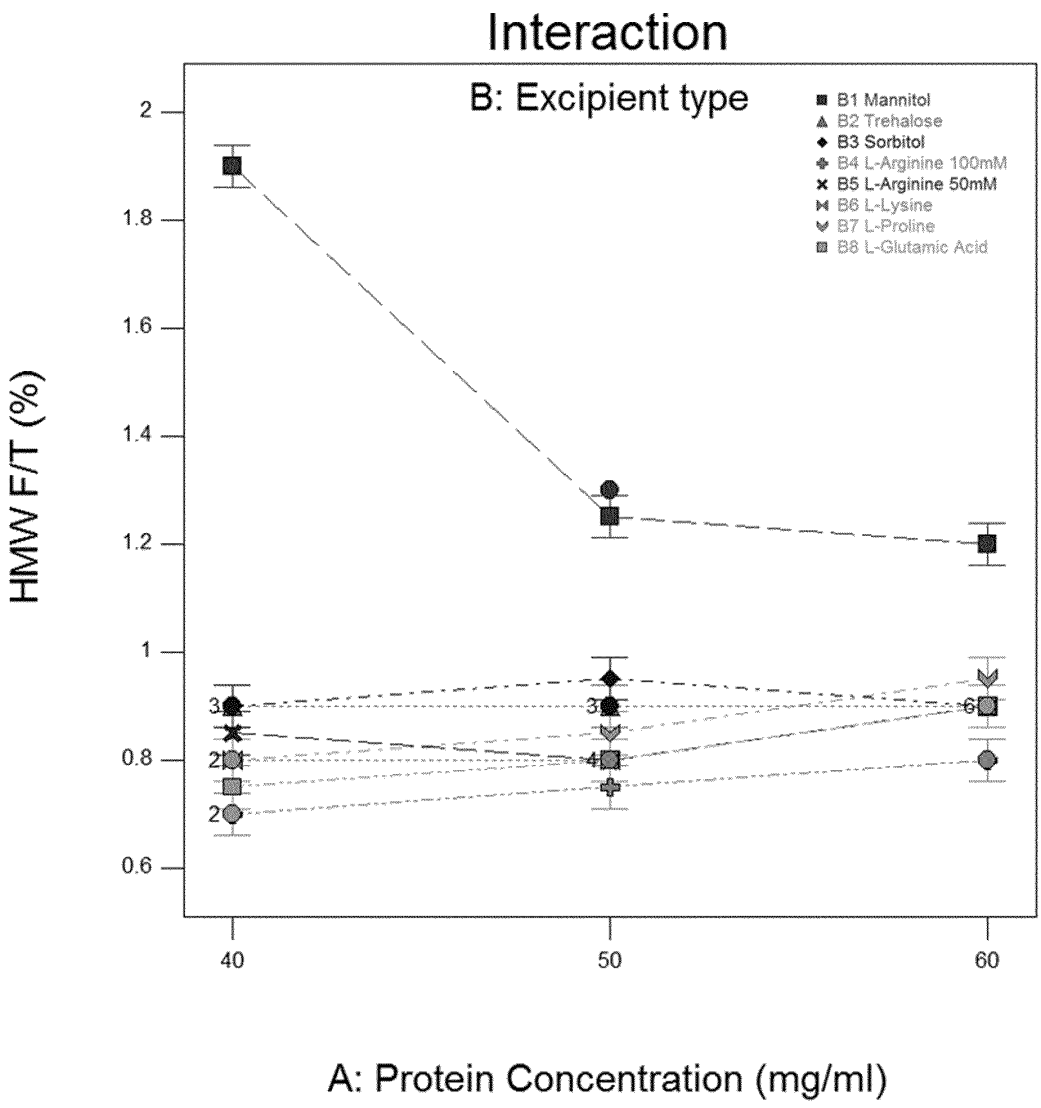

Interaction
B: Excipient type
B1 Mannitol
B2 Trehalose
B3 Sorbitol
B4 L-Arginine 100mM
B5 L-Arginine 50mM
B6 L-Lysine
B7 L-Proline
B8 L-Glutamic Acid
HMW F/T (%)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
40
50
60
A: Protein Concentration (mg/ml)

Figure 31

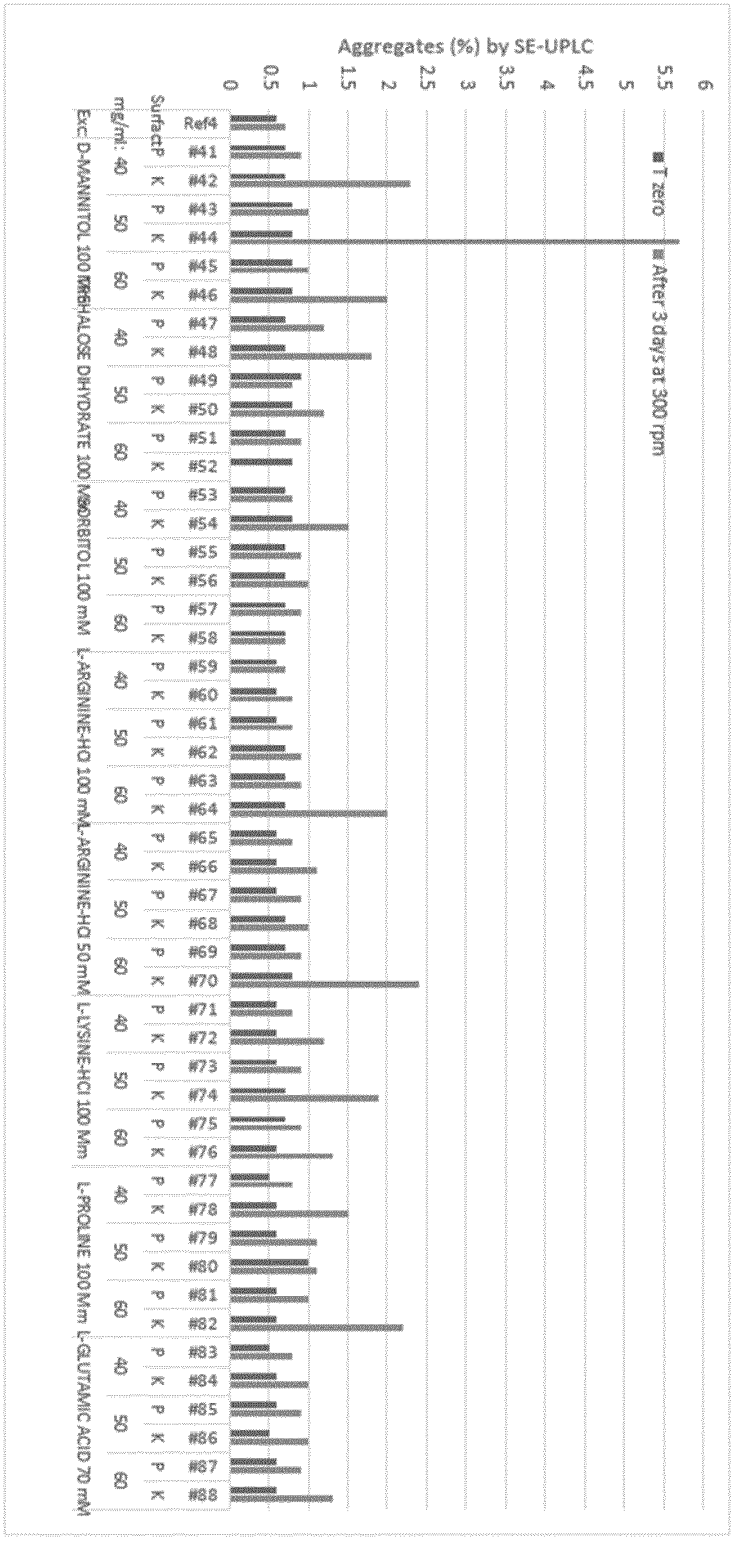

Aggregates (%) by SE-UPLC
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
T zero
After 3 days at 300 rpm
Excipient
mg/ml:
Surfactant
Ref4
#41
#42
#43
#44
#45
#46
#47
#48
#49
#50
#51
#52
#53
#54
#55
#56
#57
#58
#59
#60
#61
#62
#63
#64
#65
#66
#67
#68
#69
#70
#71
#72
#73
#74
#75
#76
#77
#78
#79
#80
#81
#82
#83
#84
#85
#86
#87
#88
D-MANNITOL 100 mM
TREHALOSE DIHYDRATE 100 mM
SORBITOL 100 mM
L-ARGININE-HCl 100 mM
L-ARGININE-HCl 50 mM
L-LYSINE-HCl 100 Mm
L-PROLINE 100 Mm
L-GLUTAMIC ACID 70 mM

Figure 32

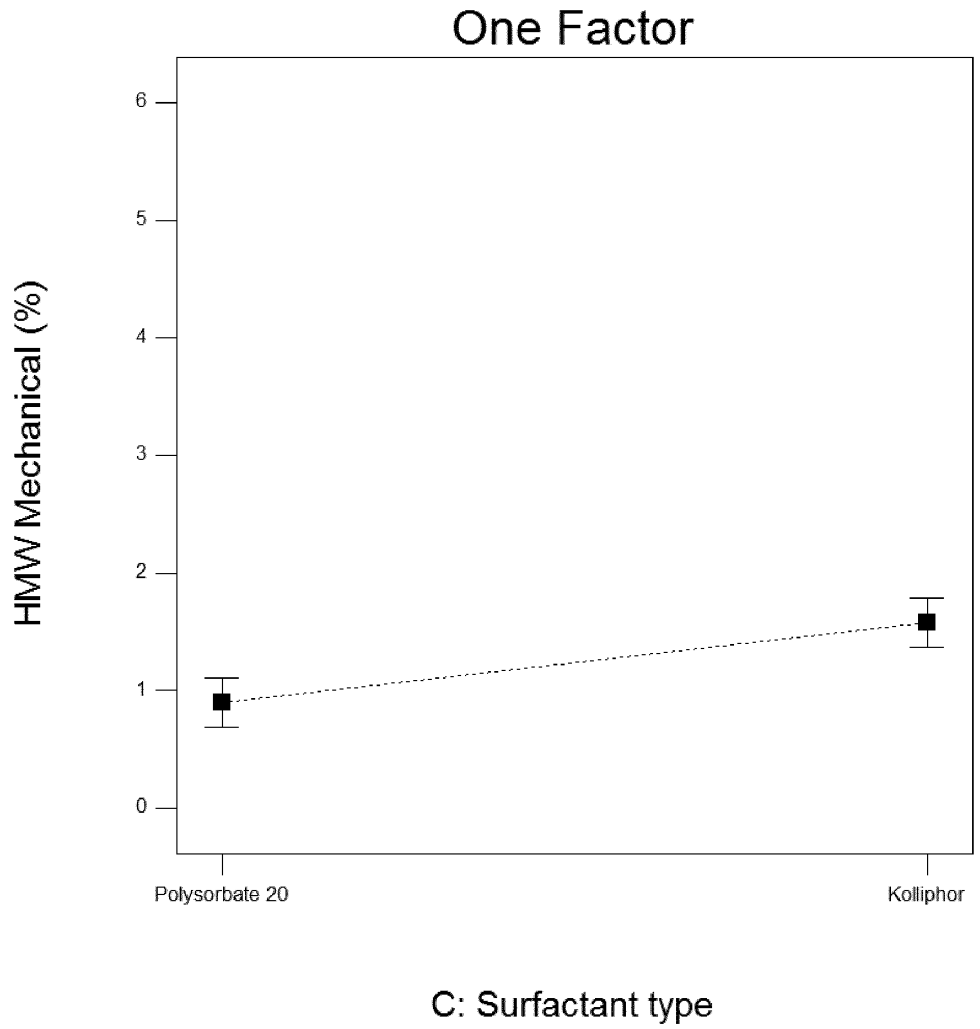

One Factor
HMW Mechanical (%)
6
5
4
3
2
1
0
Polysorbate 20
Kolliphor
C: Surfactant type

Figure 33

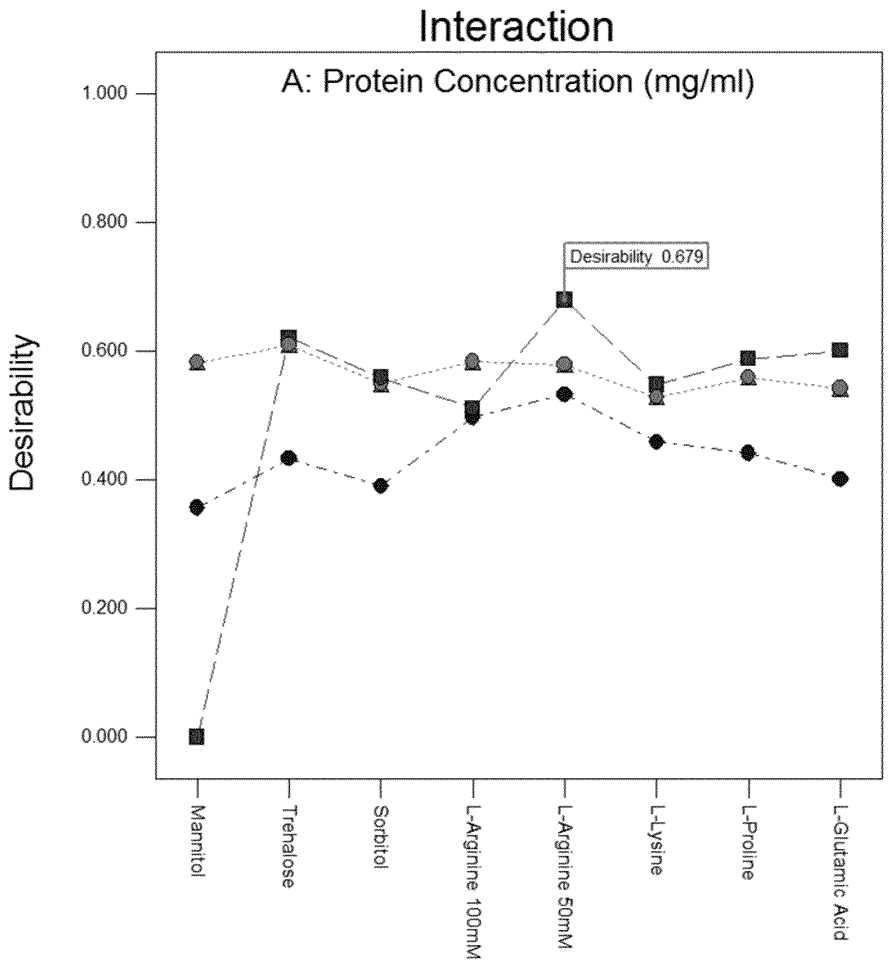

Interaction
A: Protein Concentration (mg/ml)
Desirability
1.000
0.800
0.600
0.400
0.200
0.000
Desirability 0.679
Mannitol
Trehalose
Sorbitol
L-Arginine 100mM
L-Arginine 50mM
L-Lysine
L-Proline
L-Glutamic Acid

Figure 34

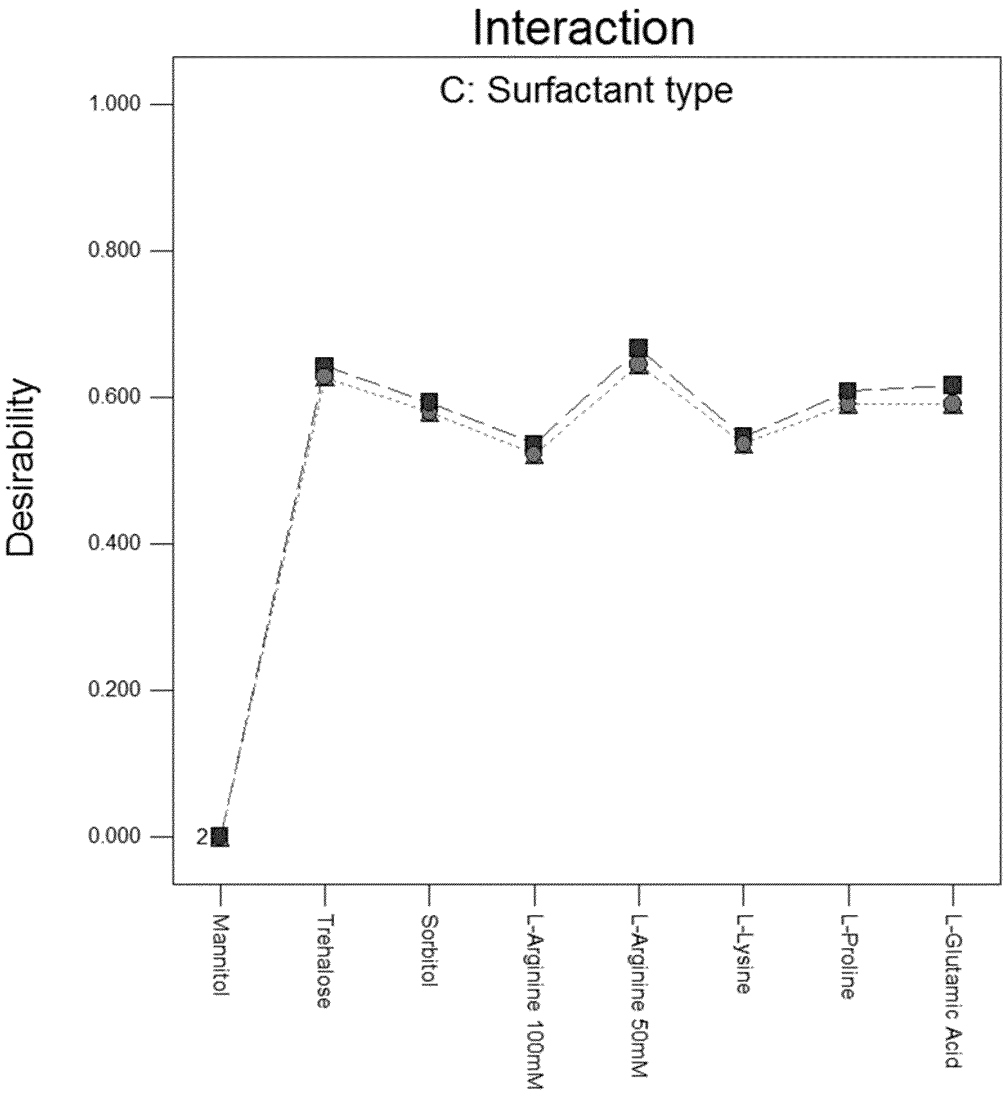

Interaction
C: Surfactant type
Desirability
1.000
0.800
0.600
0.400
0.200
0.000
2
Mannitol
Trehalose
Sorbitol
L-Arginine 100mM
L-Arginine 50mM
L-Lysine
L-Proline
L-Glutamic Acid

Figure 35

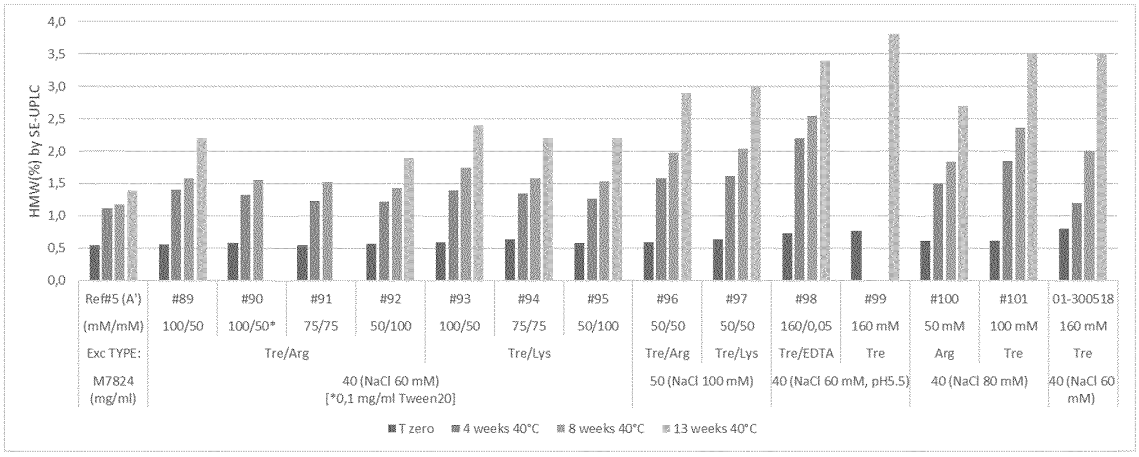


Figure 36

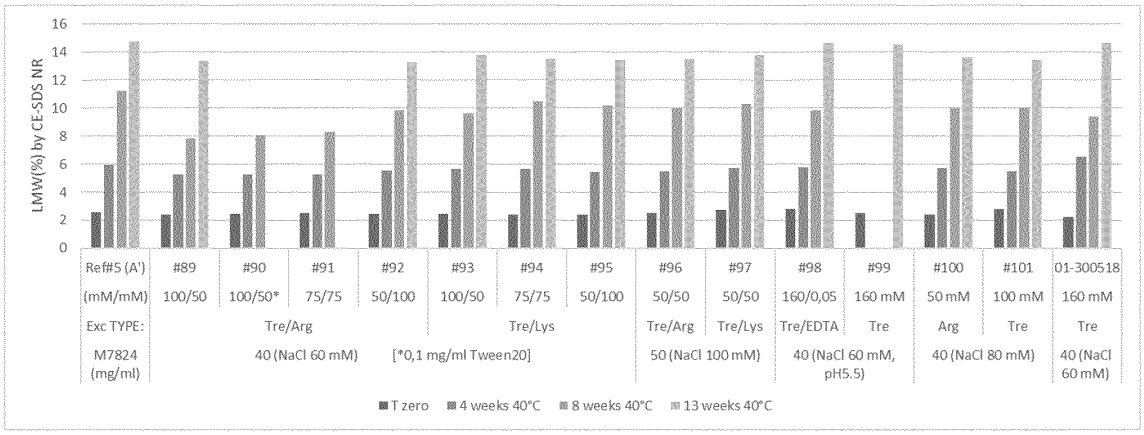


Figure 37

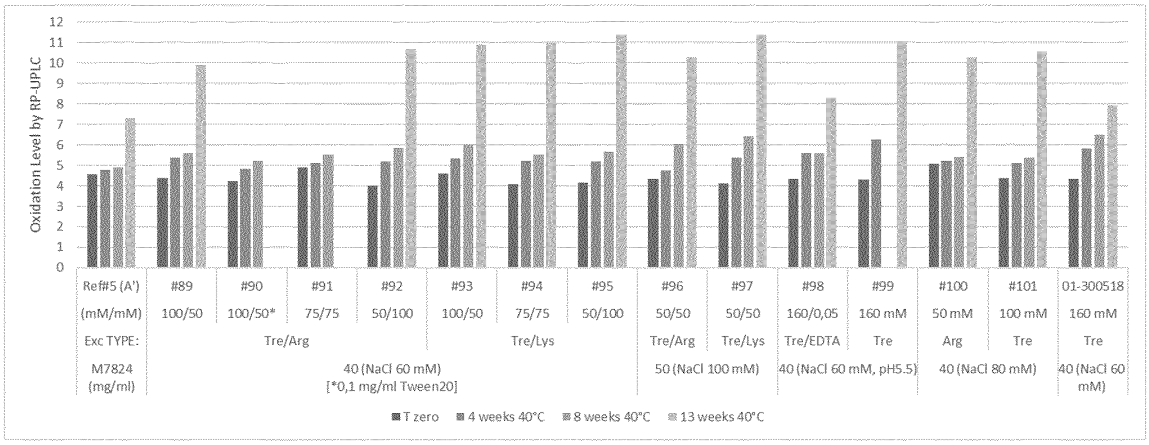


Figure 38

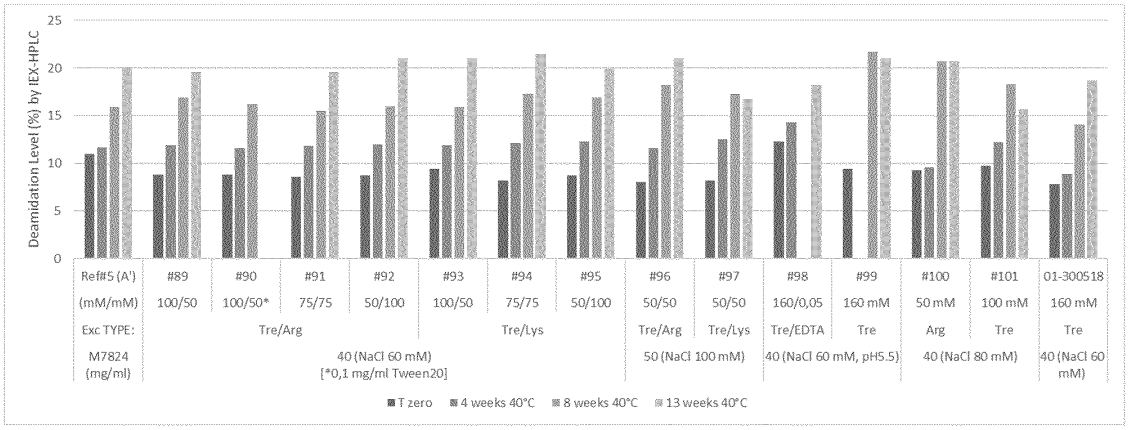


Figure 39

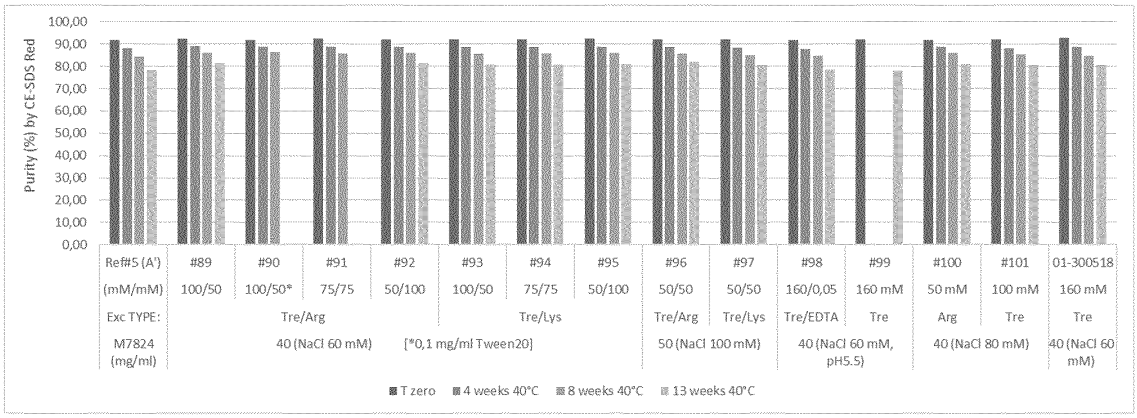


Figure 40

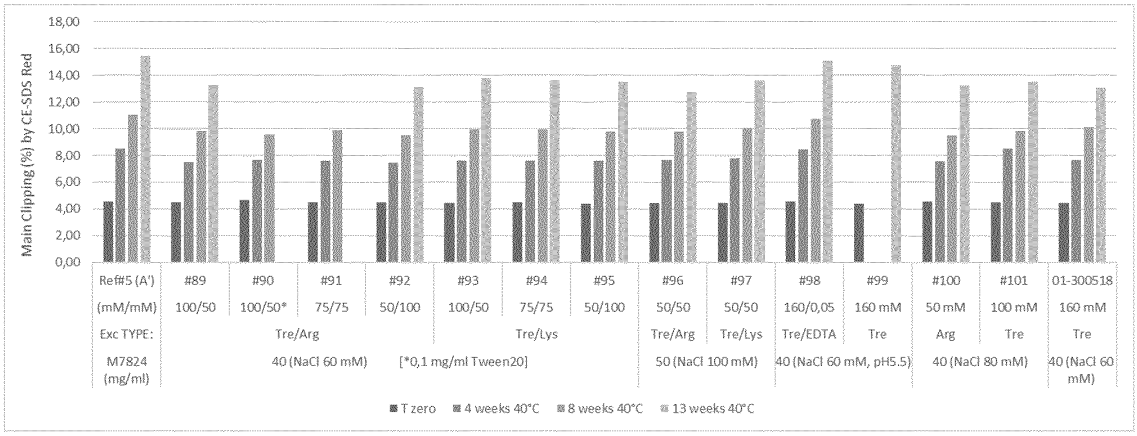


Figure 41

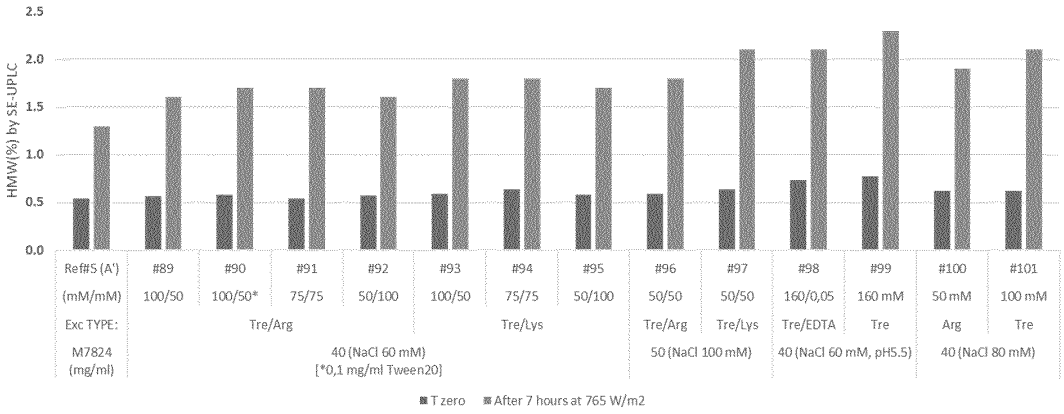


Figure 42

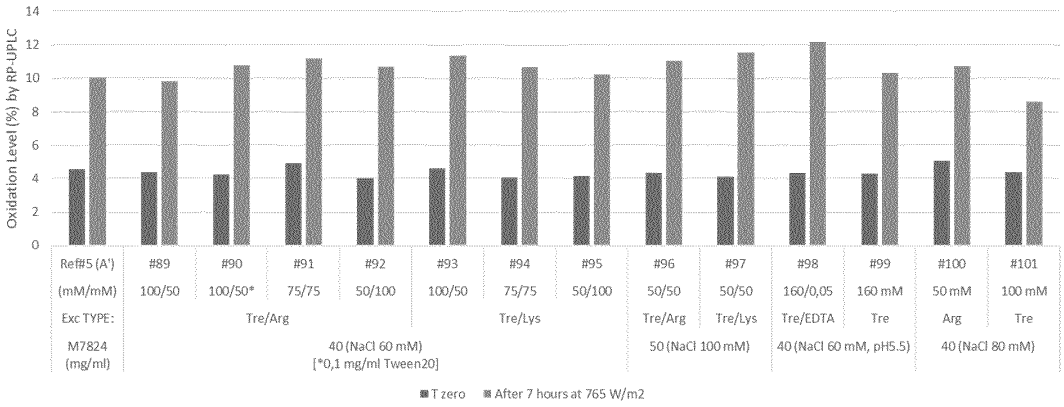


Figure 43

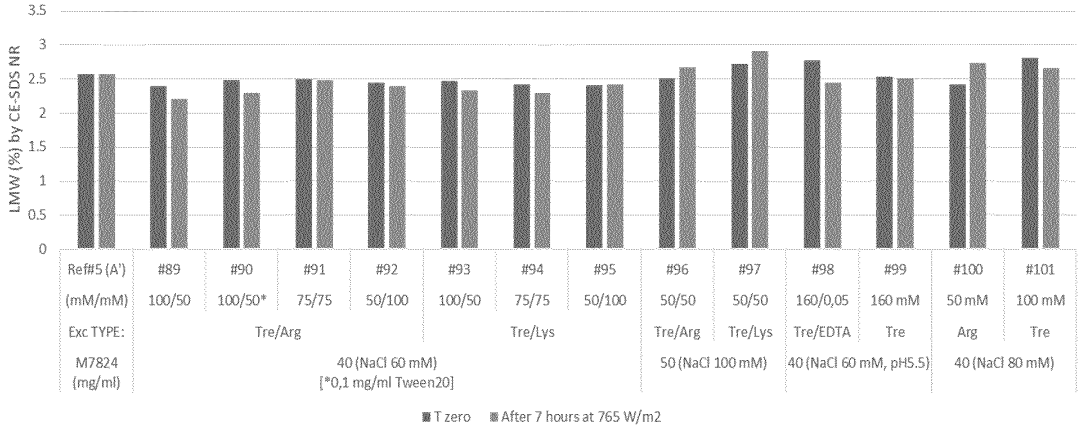


Figure 44

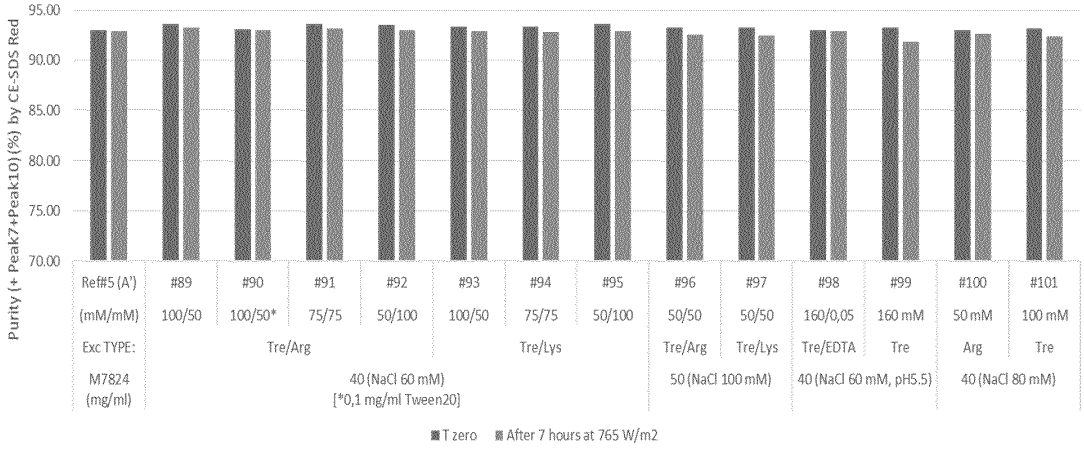


Figure 45

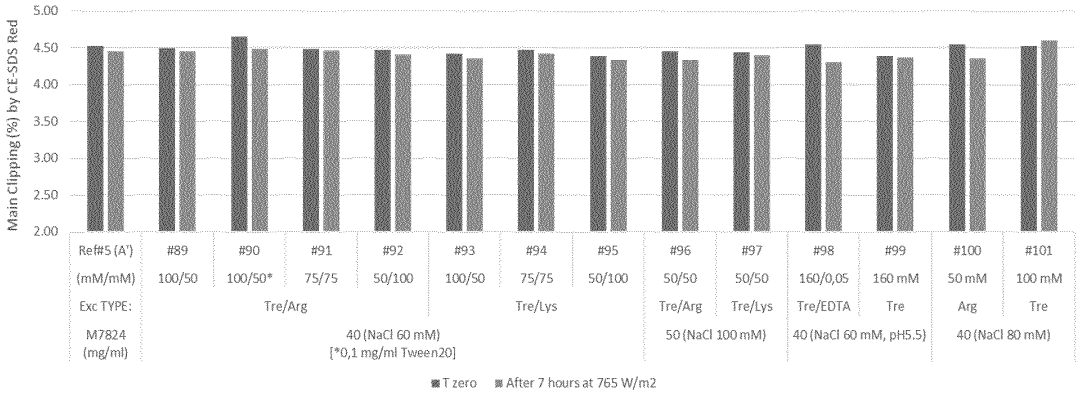


Figure 46

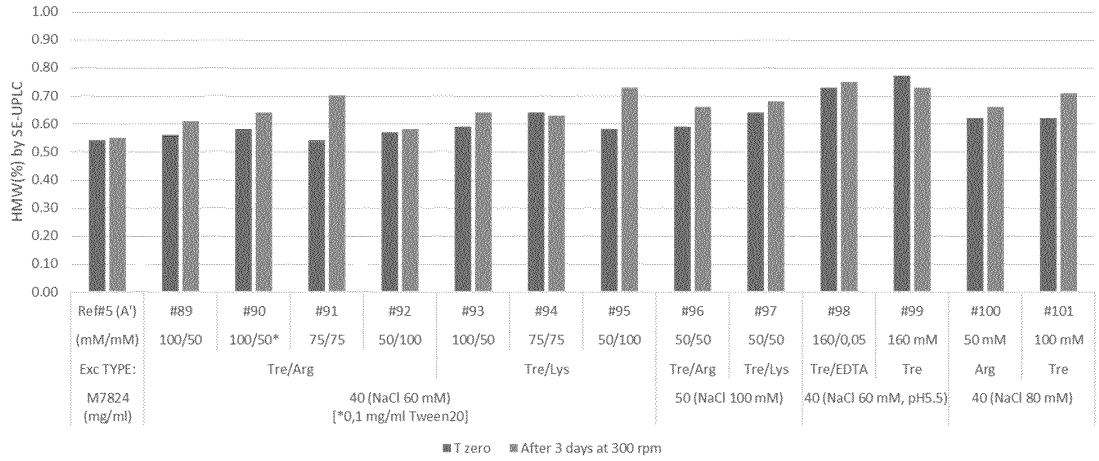


Figure 47

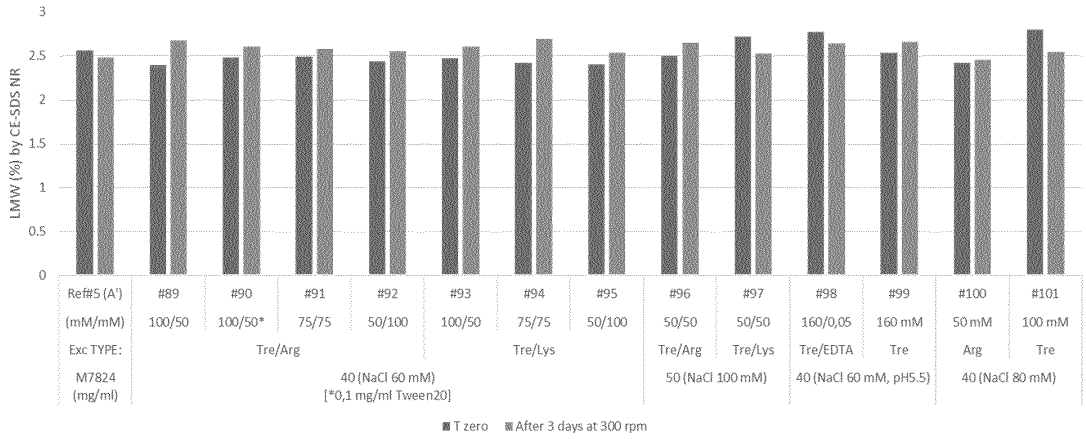


Figure 48

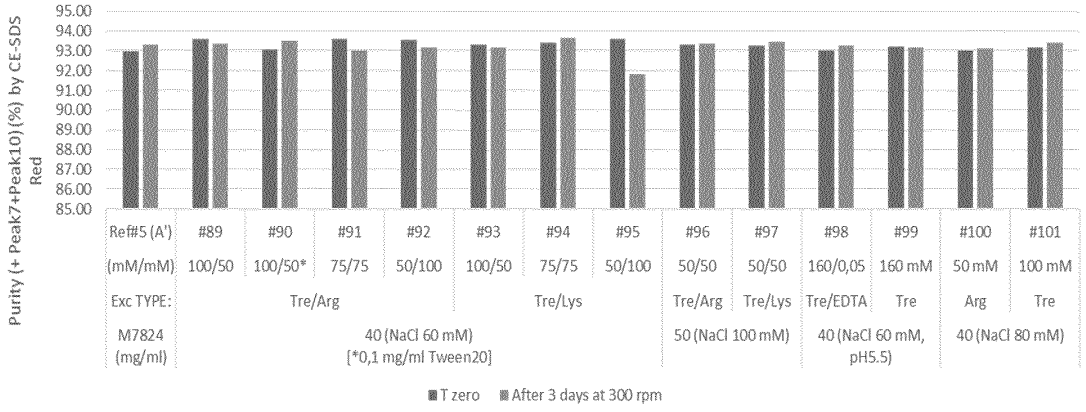


Figure 49

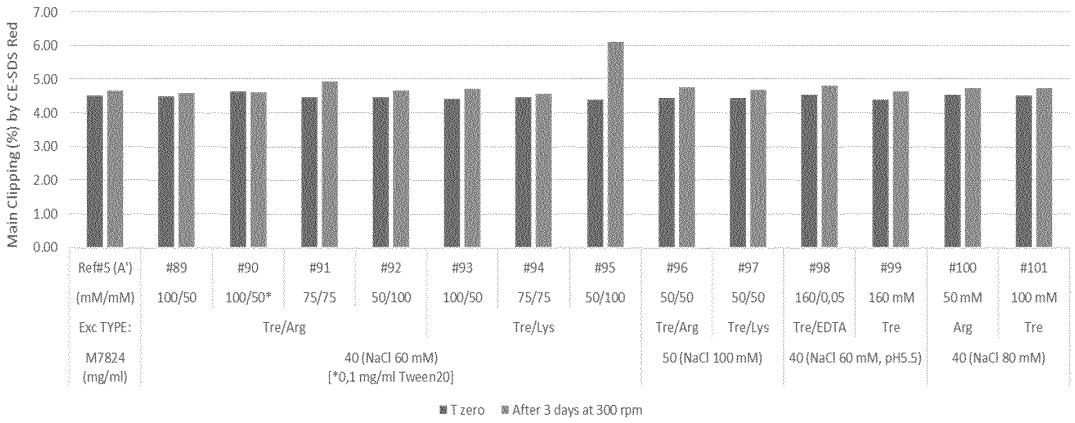


Figure 50

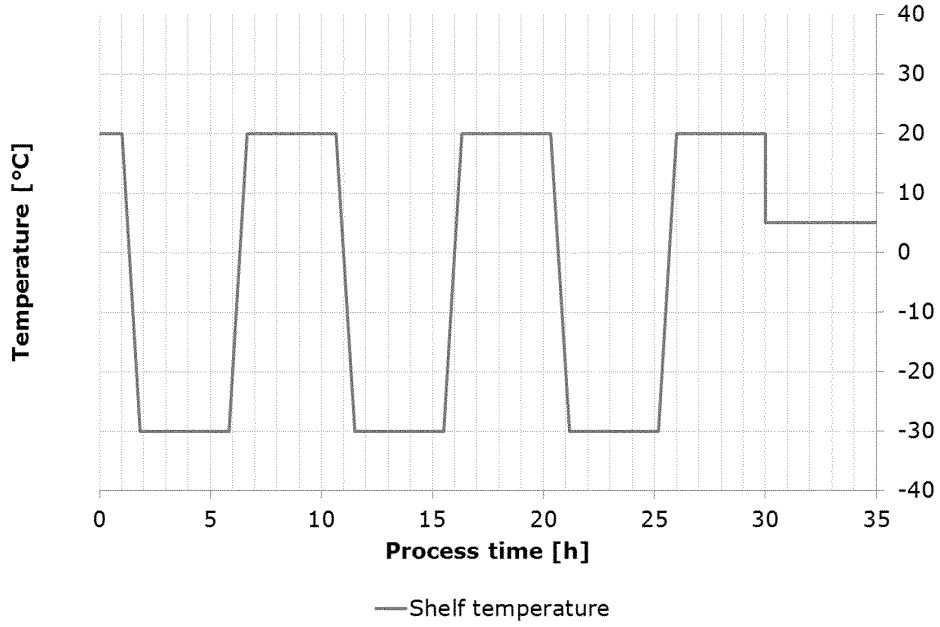


Figure 51

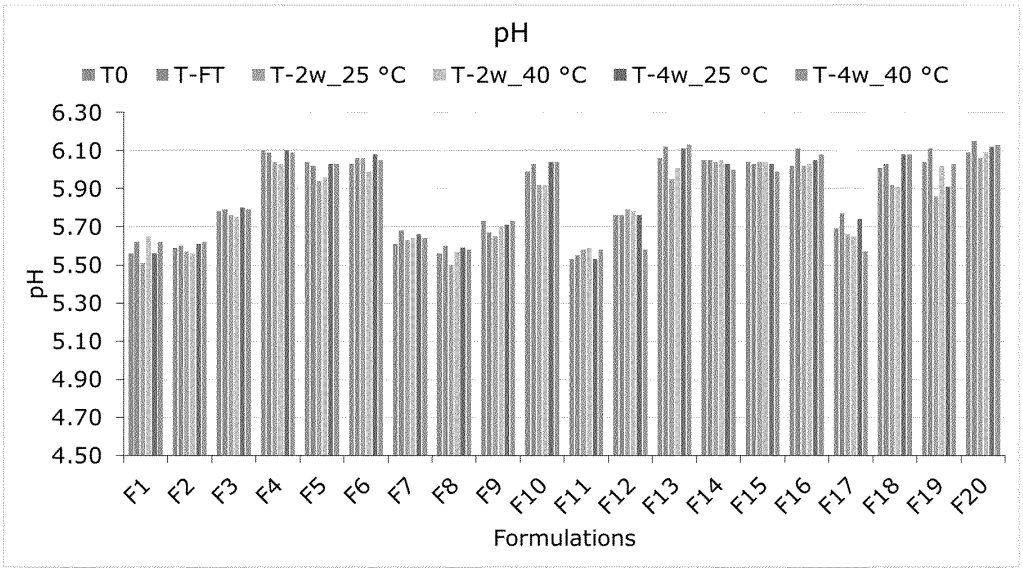


Figure 52

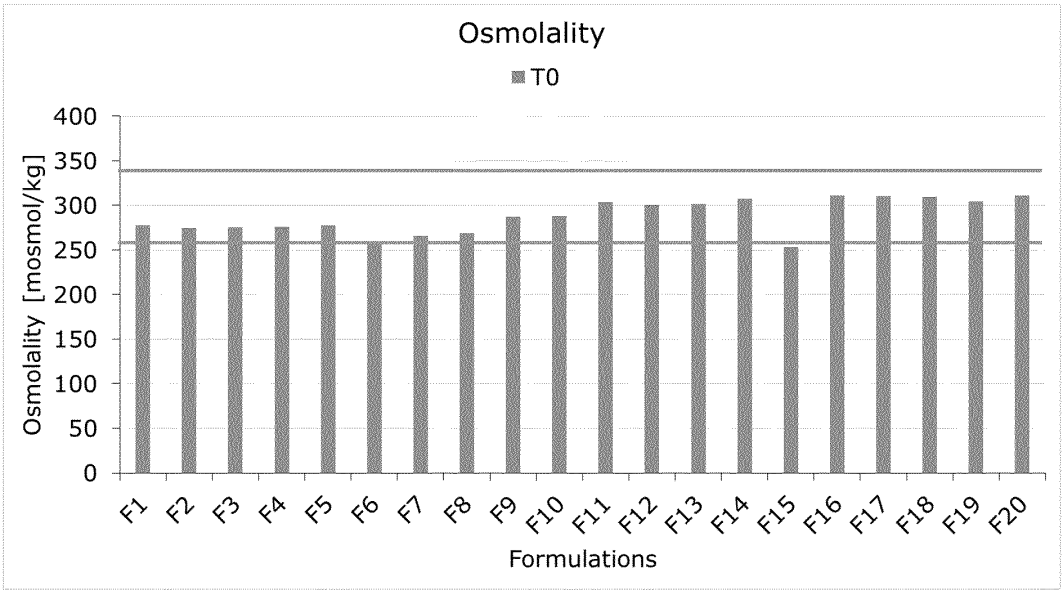


Figure 53

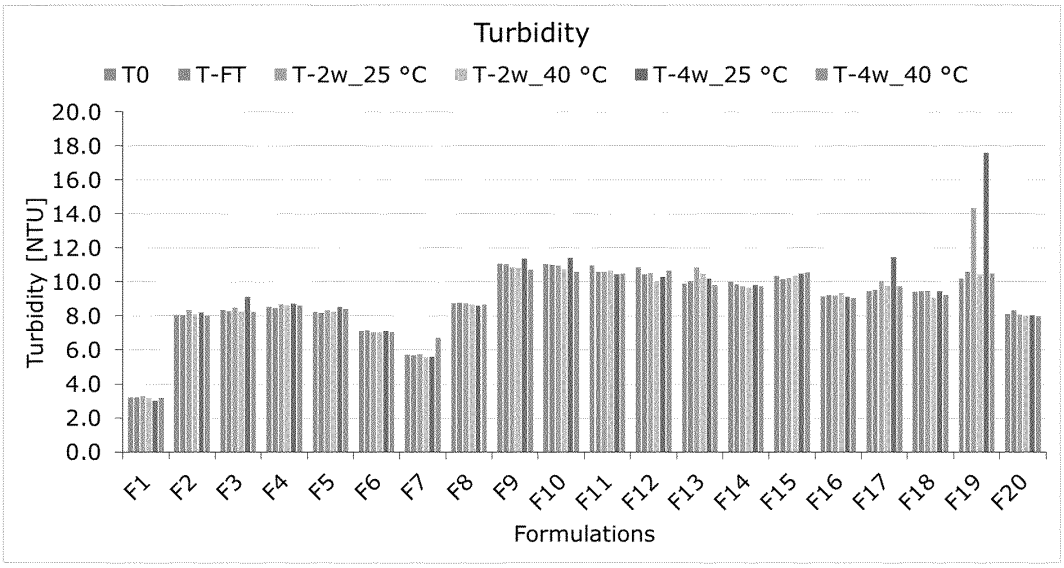


Figure 54

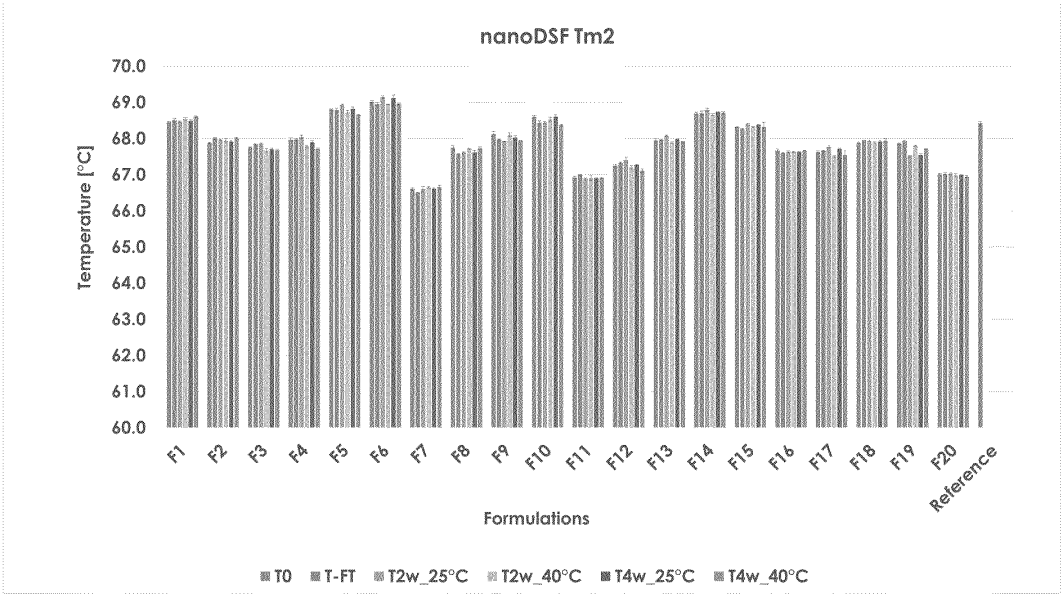


Figure 55

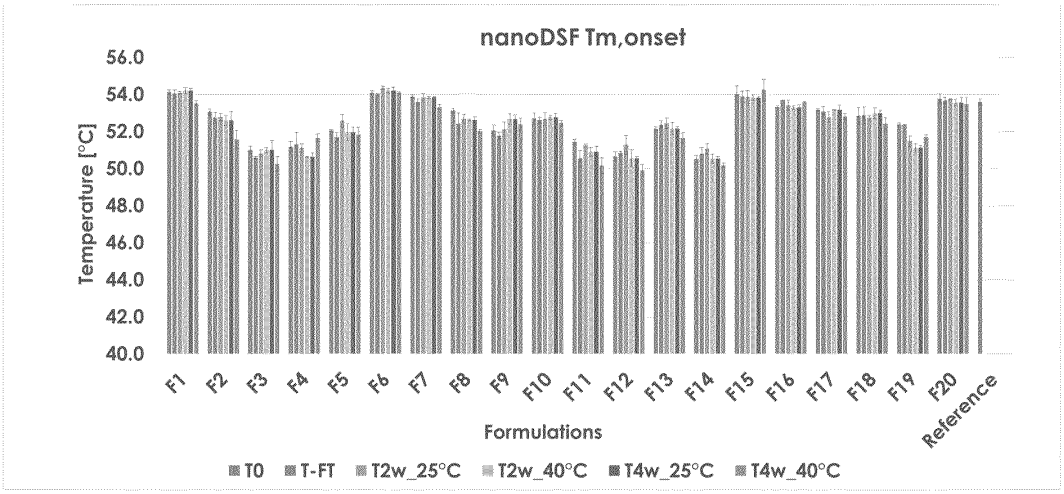


Figure 56

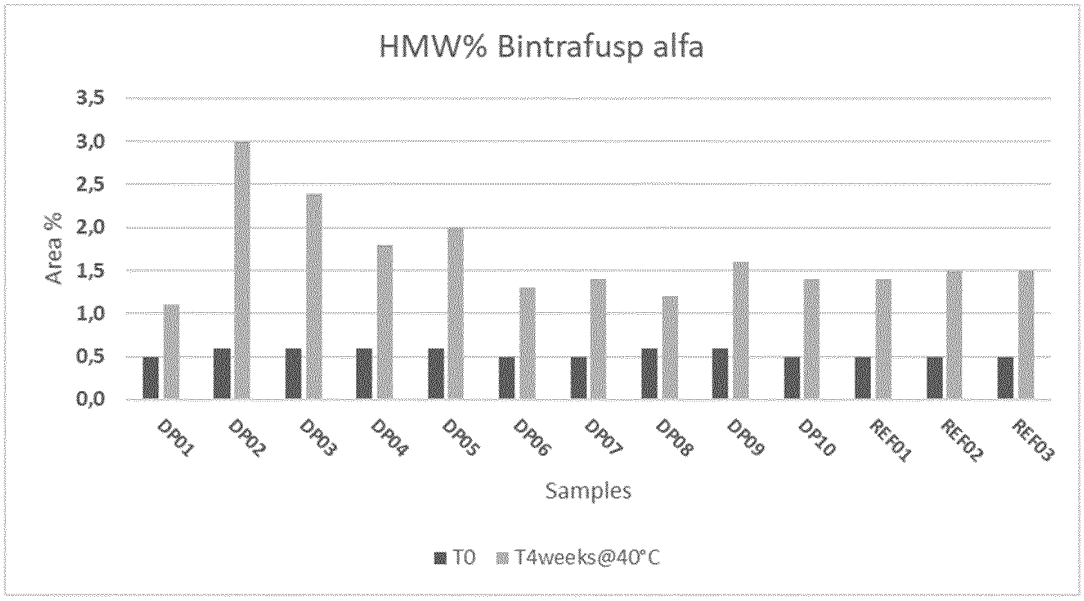


Figure 57 (A)

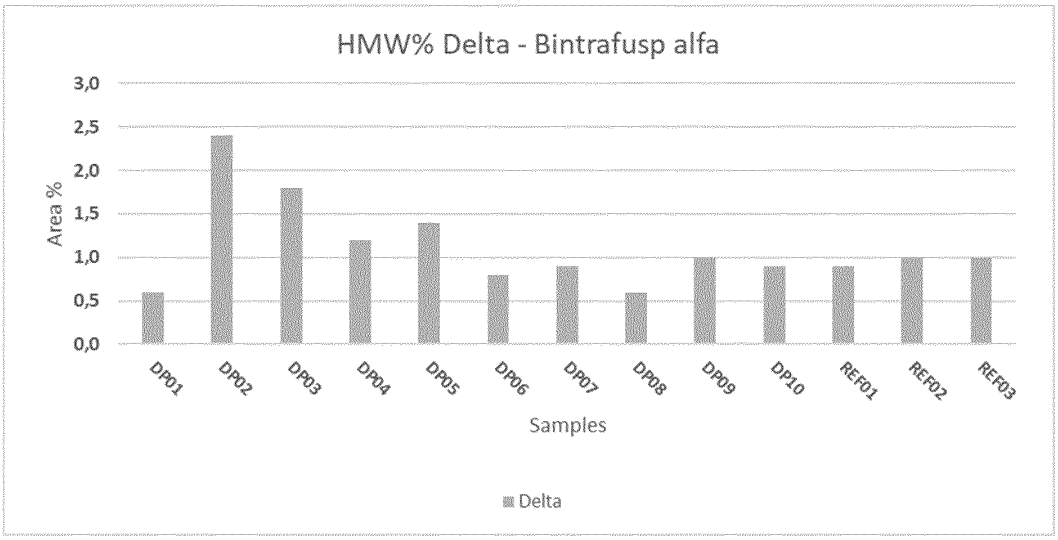


Figure 57 (B)

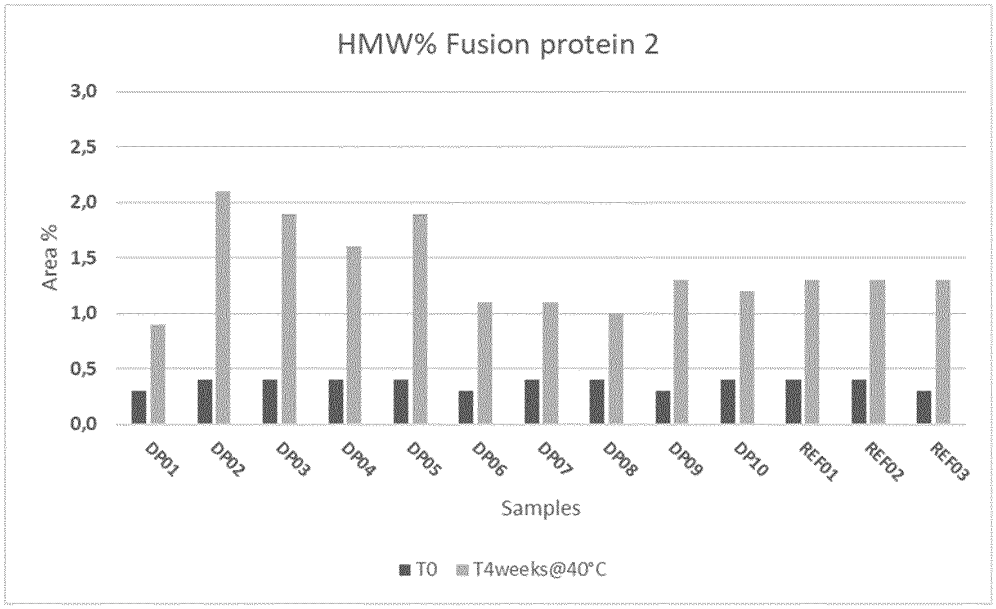


Figure 57 (C)

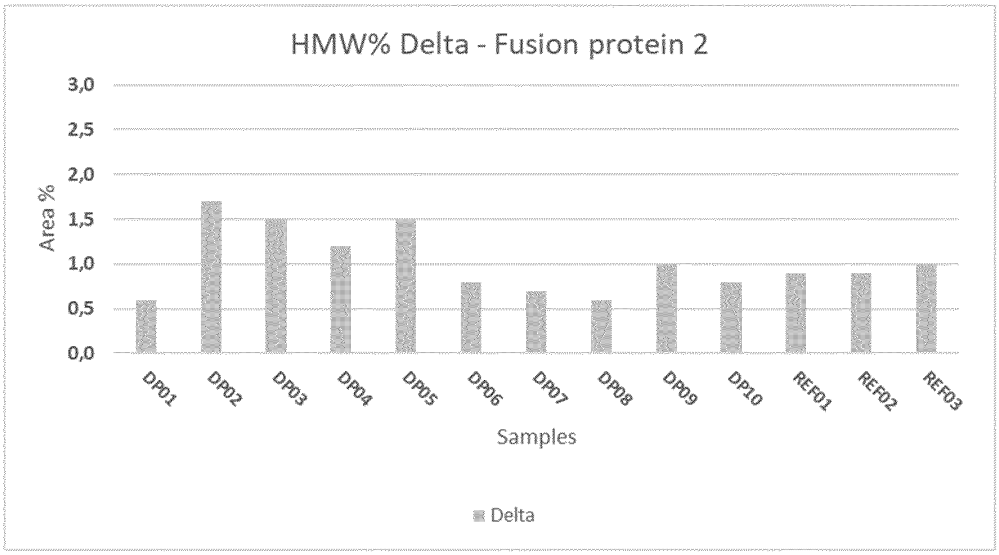


Figure 57 (D)

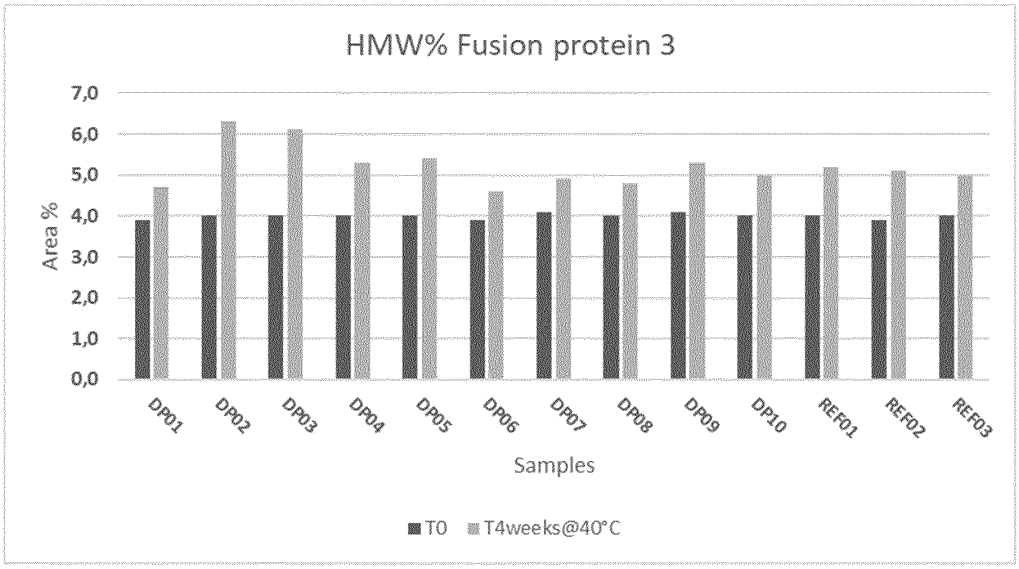


Figure 57 (E)

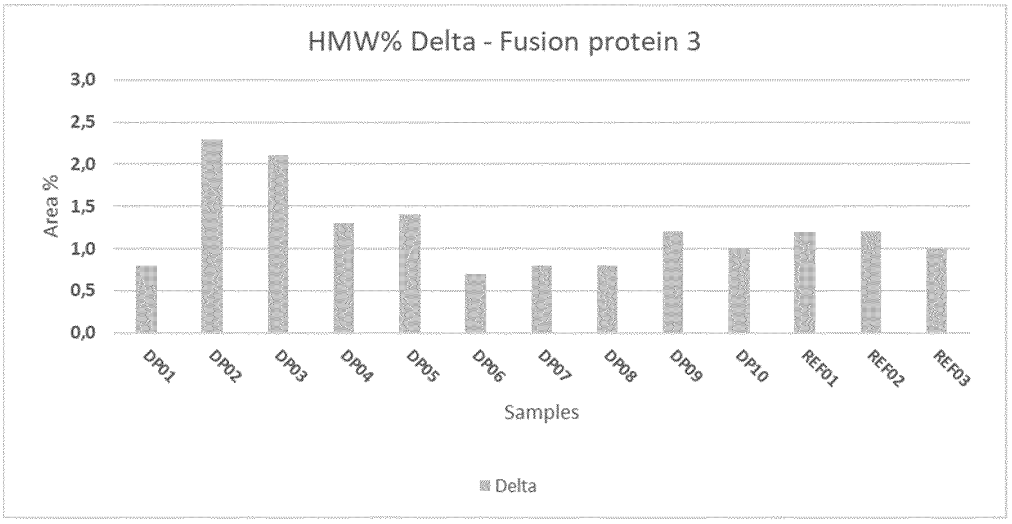


Figure 57 (F)

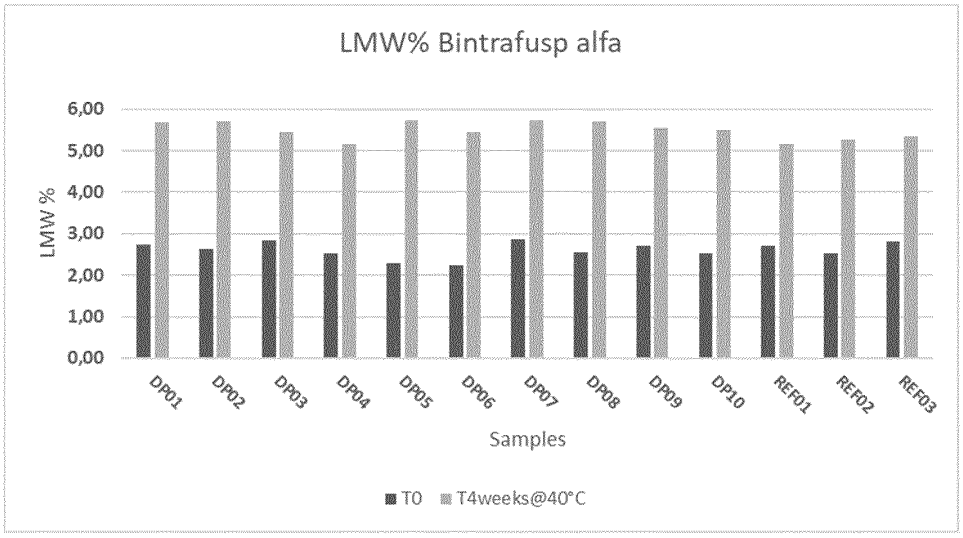


Figure 58 (A)

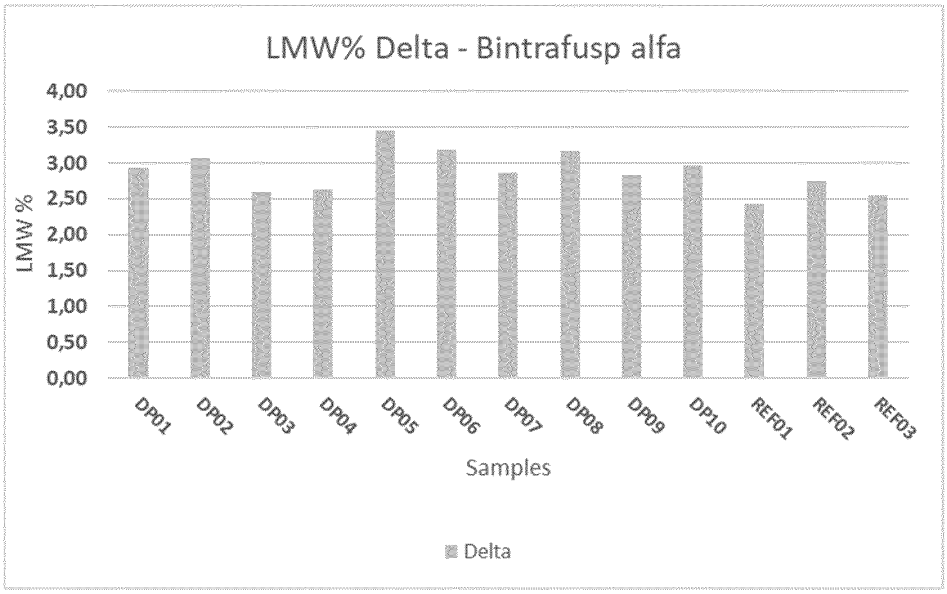


Figure 58 (B)

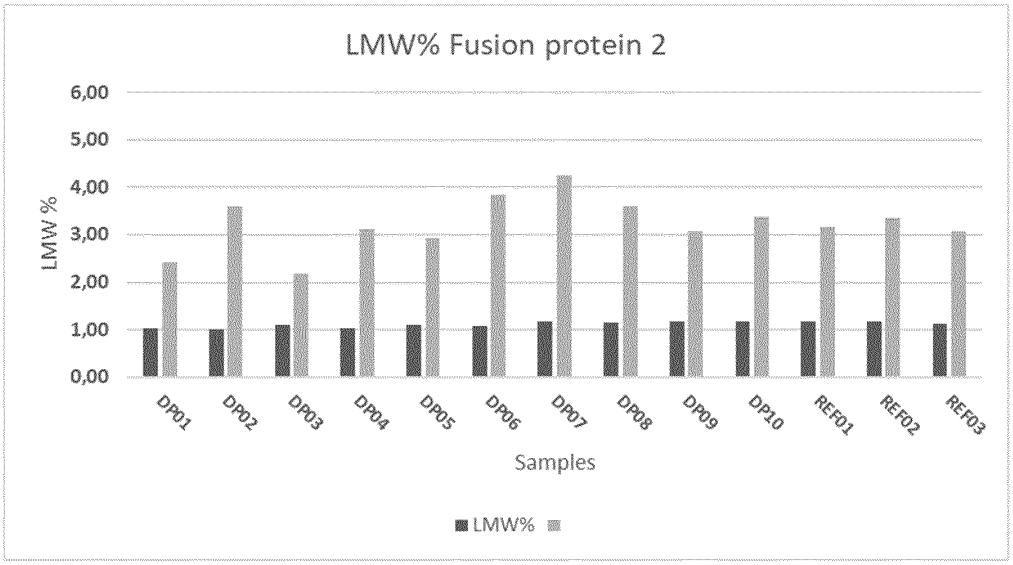


Figure 58 (C)

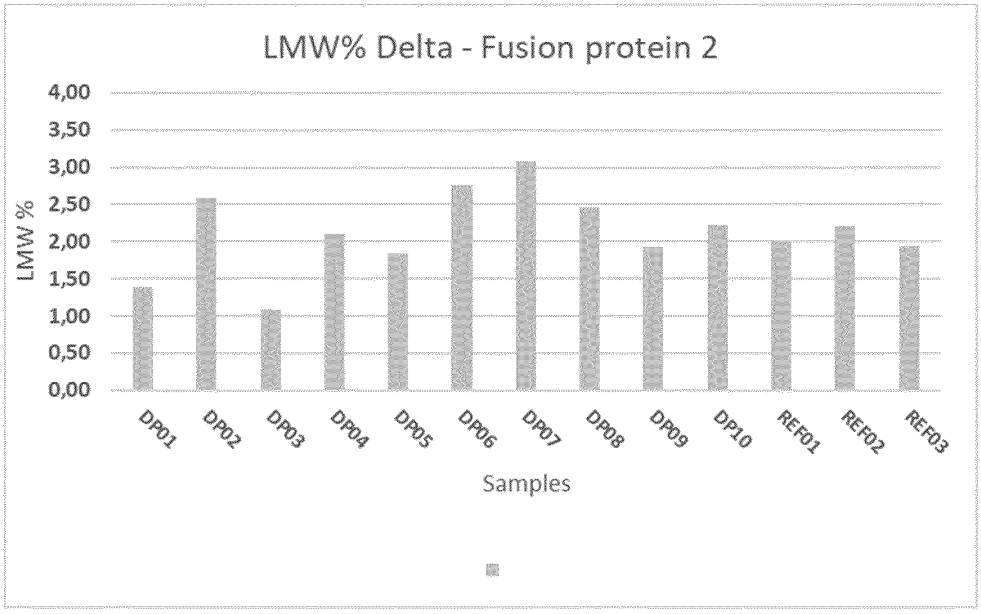


Figure 58 (D)

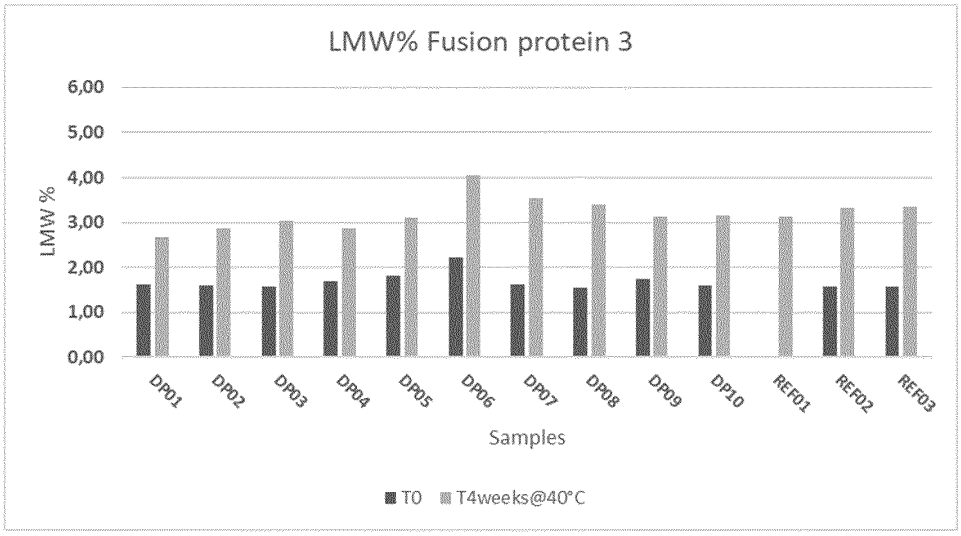


Figure 58 (E)

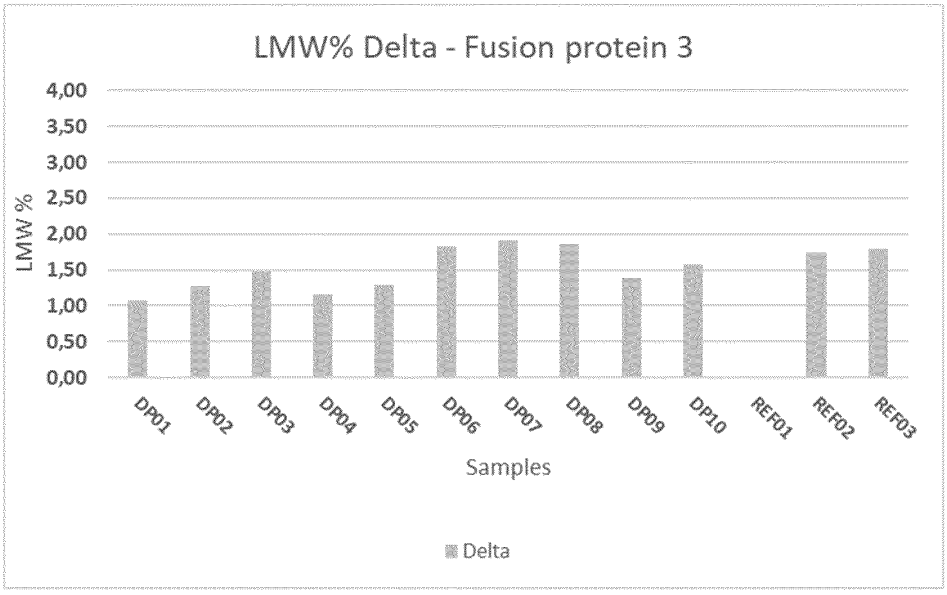
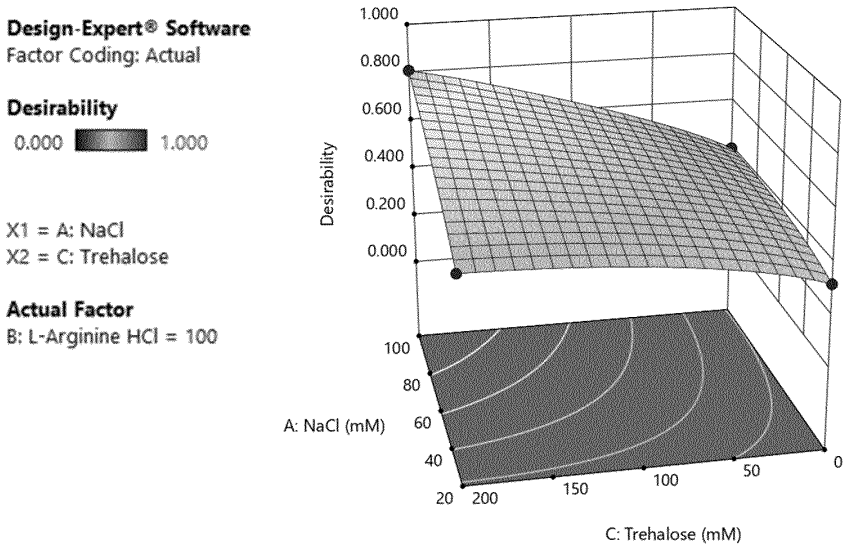
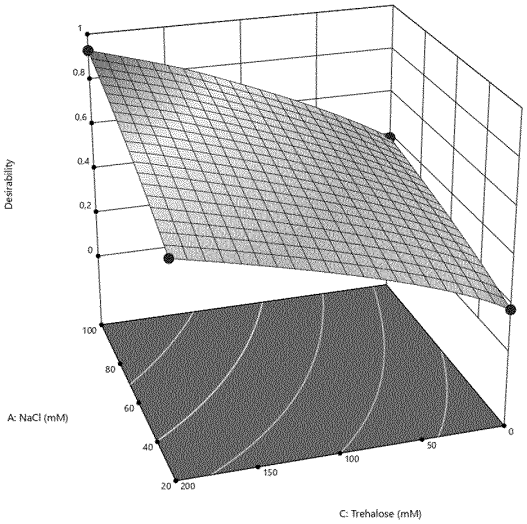


Figure 58 (F)

Bintrafusp alfa



Fusion protein 2



Fusion protein 3

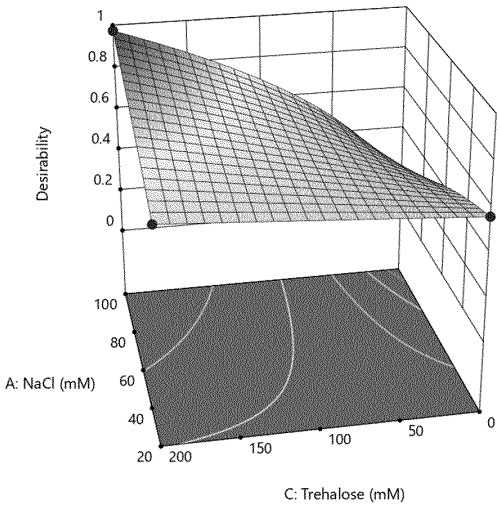
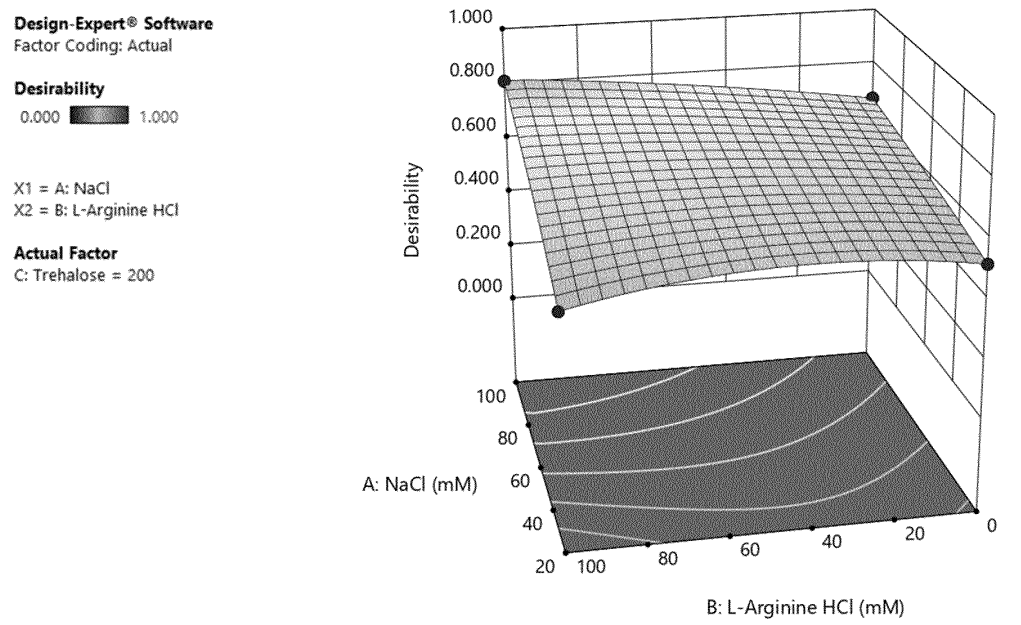
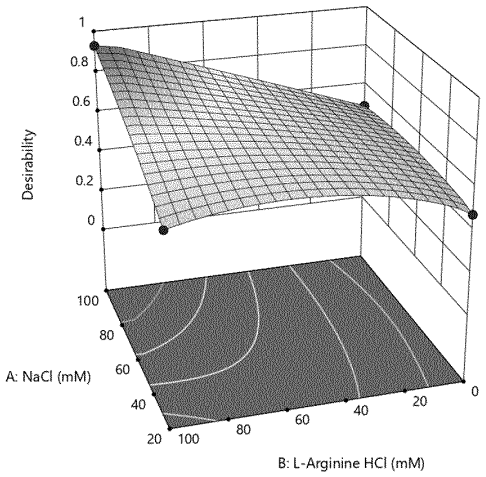


Figure 59

Bintrafusp alfa



Fusion protein 2



Fusion protein 3

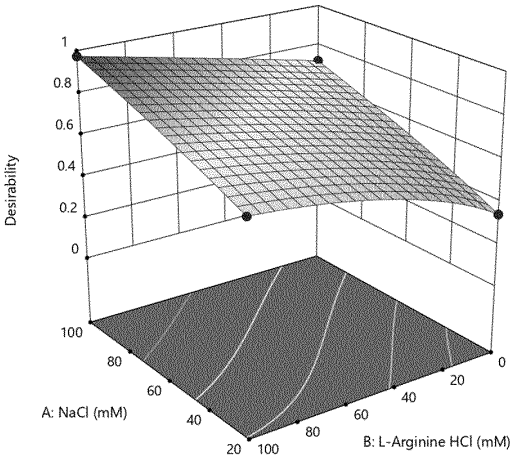
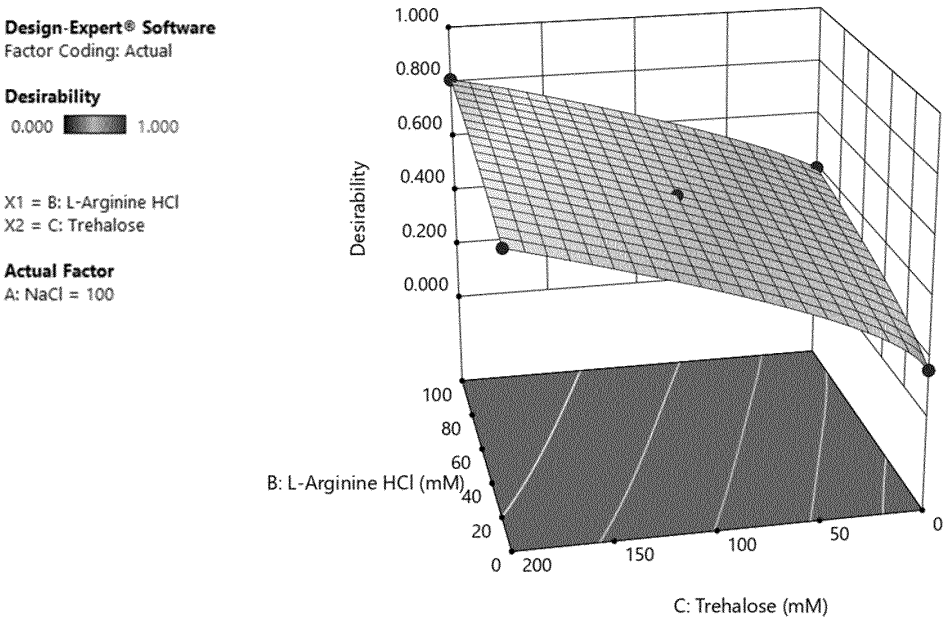
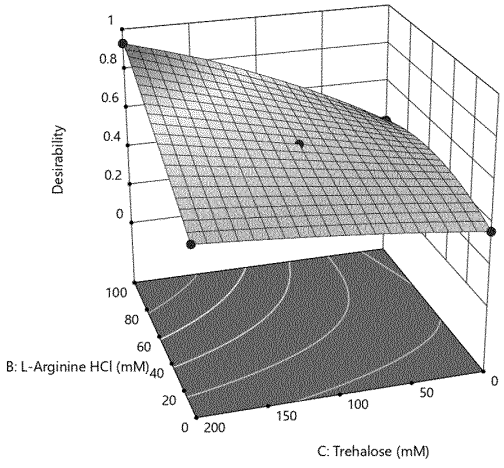


Figure 60



Fusion protein 2



Fusion protein 3

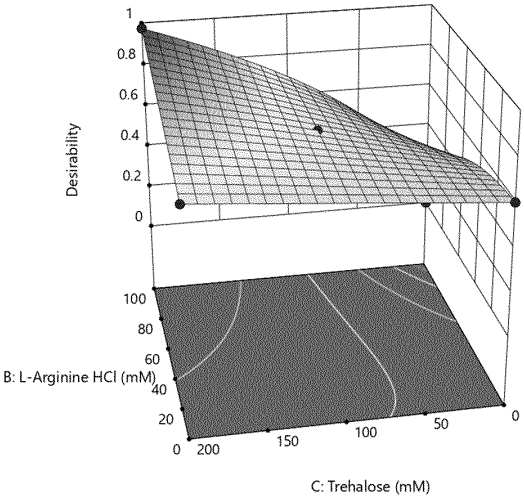


Figure 61