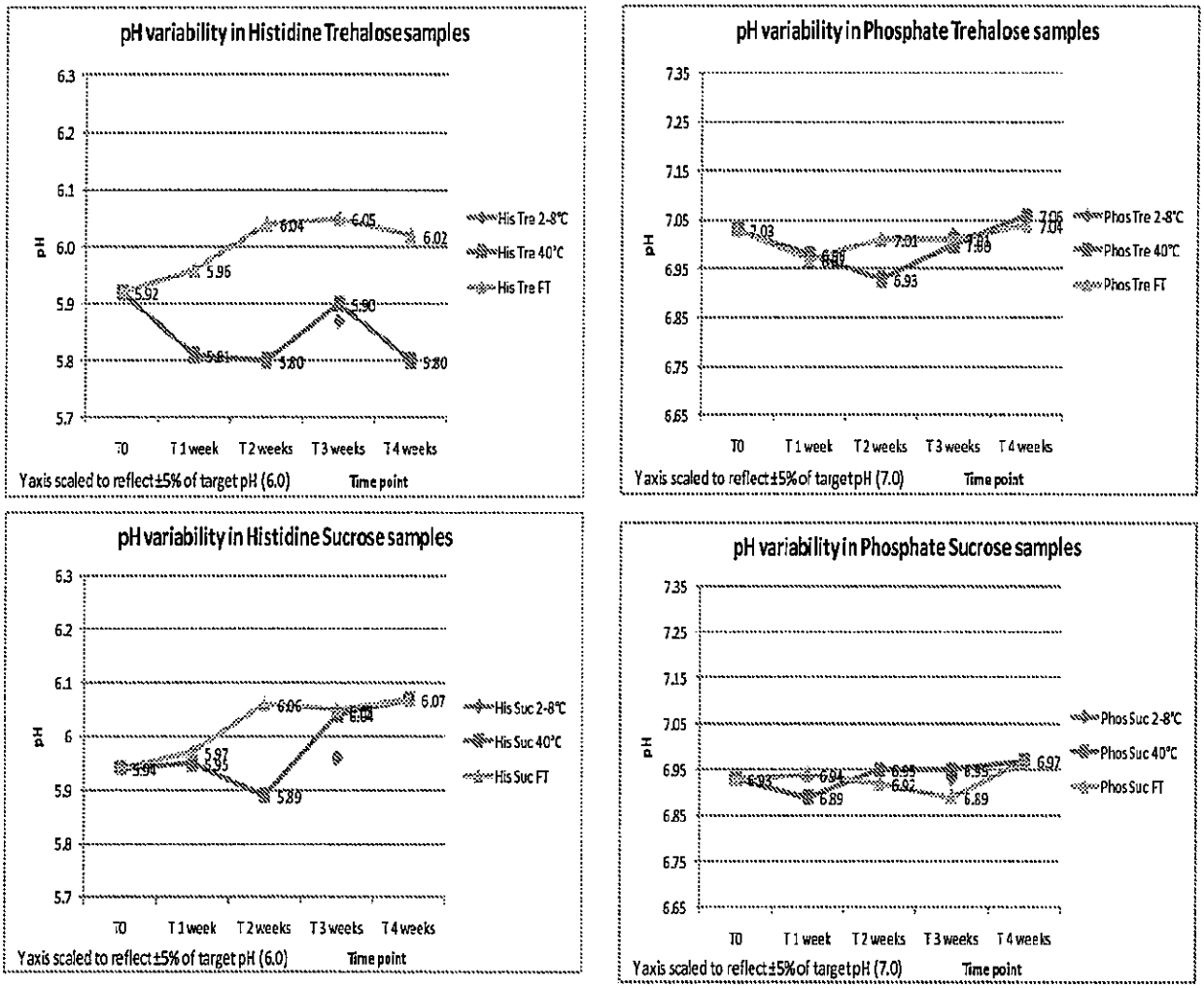
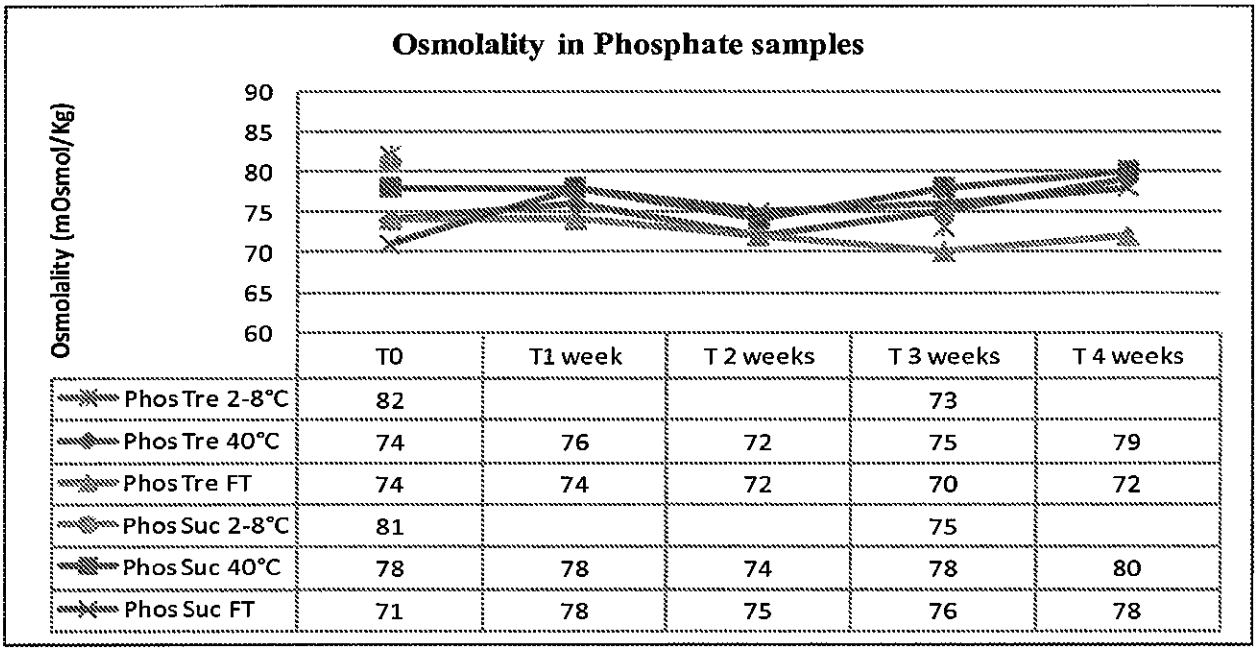
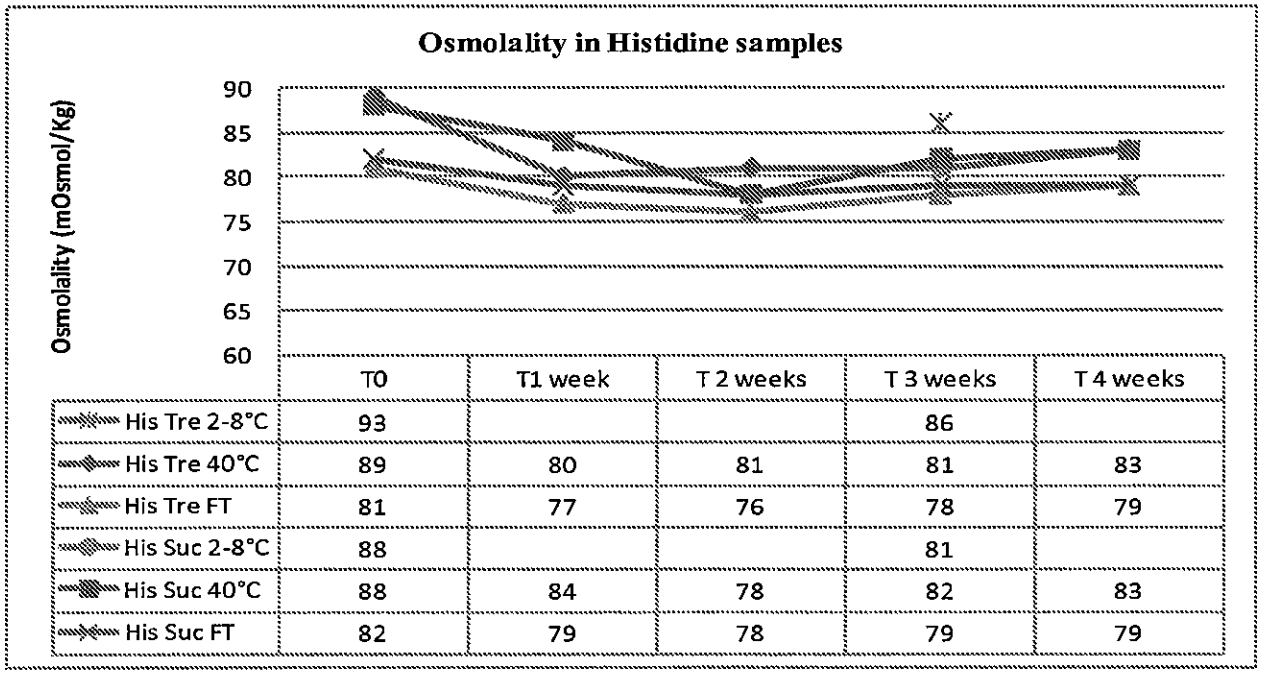

pH variability in Histidine Trehalose samples
pH
His Tre 2-8°C
His Tre 40°C
His Tre FT
T0
T1 week
T2 weeks
T3 weeks
T4 weeks
Yaxis scaled to reflect ±5% of target pH (6.0)
Time point
pH variability in Phosphate Trehalose samples
Phos Tre 2-8°C
Phos Tre 40°C
Phos Tre FT
Yaxis scaled to reflect ±5% of target pH (7.0)
pH variability in Histidine Sucrose samples
His Suc 2-8°C
His Suc 40°C
His Suc FT
pH variability in Phosphate Sucrose samples
Phos Suc 2-8°C
Phos Suc 40°C
Phos Suc FT

Figure1



	T0	T1 week	T 2 weeks	T 3 weeks	T 4 weeks
Phos Tre 2-8°C	82			73	
Phos Tre 40°C	74	76	72	75	79
Phos Tre FT	74	74	72	70	72
Phos Suc 2-8°C	81			75	
Phos Suc 40°C	78	78	74	78	80
Phos Suc FT	71	78	75	76	78



	T0	T1 week	T 2 weeks	T 3 weeks	T 4 weeks
His Tre 2-8°C	93			86	
His Tre 40°C	89	80	81	81	83
His Tre FT	81	77	76	78	79
His Suc 2-8°C	88			81	
His Suc 40°C	88	84	78	82	83
His Suc FT	82	79	78	79	79

Figure 2

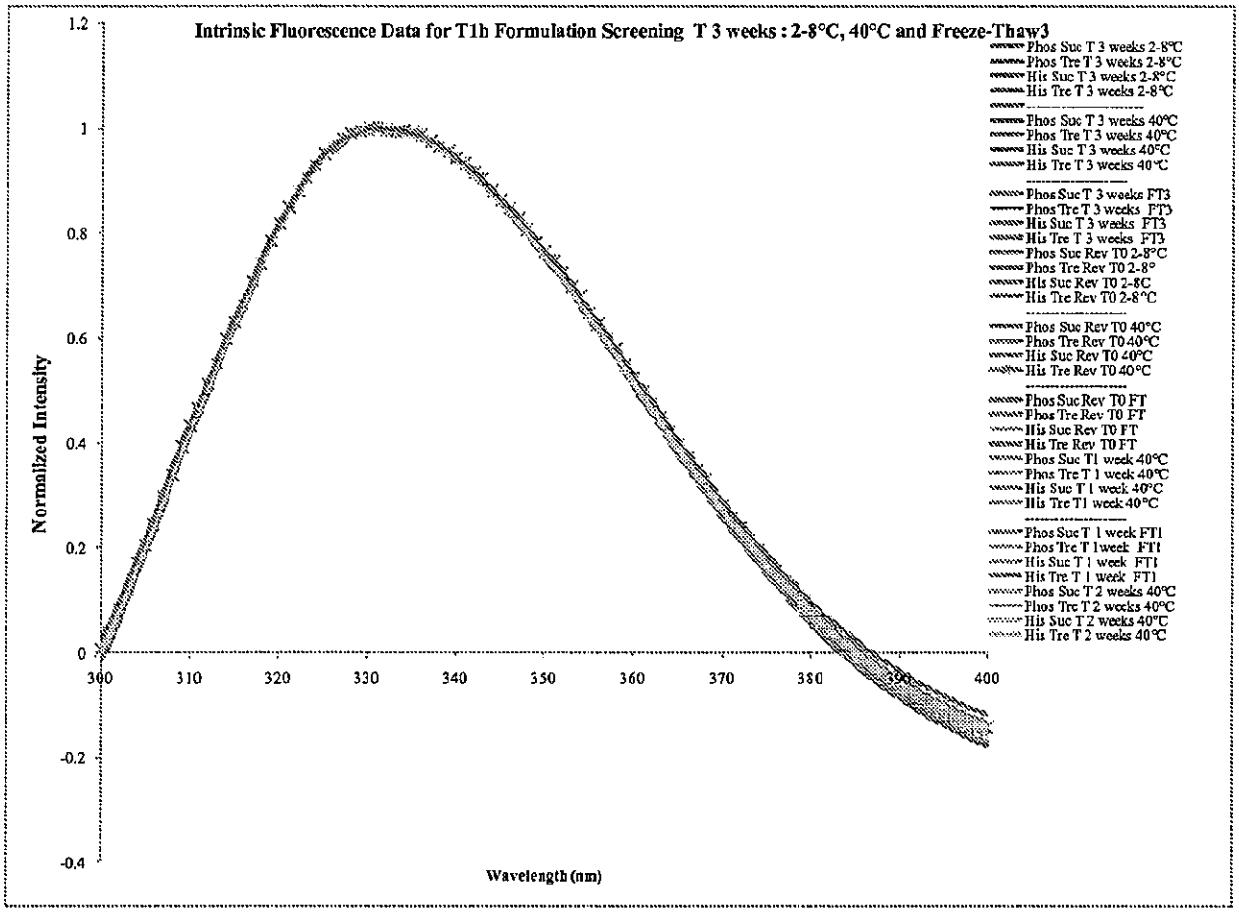
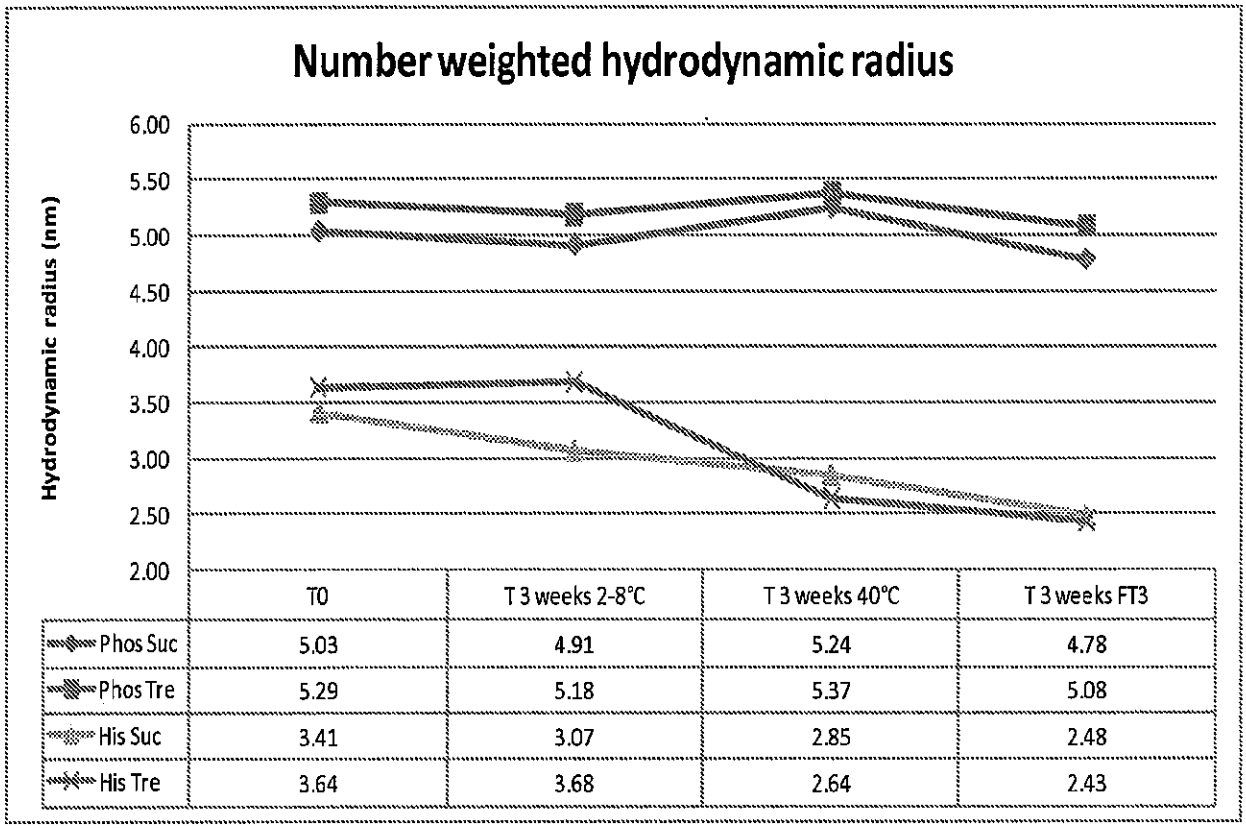

Intrinsic Fluorescence Data for T1h Formulation Screening T 3 weeks : 2-8°C, 40°C and Freeze-Thaw3
Normalized Intensity
Wavelength (nm)
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
300
310
320
330
340
350
360
370
380
400
Phos Suc T 3 weeks 2-8°C
Phos Tre T 3 weeks 2-8°C
His Suc T 3 weeks 2-8°C
His Tre T 3 weeks 2-8°C
Phos Suc T 3 weeks 40°C
Phos Tre T 3 weeks 40°C
His Suc T 3 weeks 40°C
His Tre T 3 weeks 40°C
Phos Suc T 3 weeks FT3
Phos Tre T 3 weeks FT3
His Suc T 3 weeks FT3
His Tre T 3 weeks FT3
Phos Suc Rev T0 2-8°C
Phos Tre Rev T0 2-8°
His Suc Rev T0 2-8C
His Tre Rev T0 2-8°C
Phos Suc Rev T0 40°C
Phos Tre Rev T0 40°C
His Suc Rev T0 40°C
His Tre Rev T0 40°C
Phos Suc Rev T0 FT
Phos Tre Rev T0 FT
His Suc Rev T0 FT
His Tre Rev T0 FT
Phos Suc T1 week 40°C
Phos Tre T 1 week 40°C
His Suc T 1 week 40°C
His Tre T1 week 40°C
Phos Suc T 1 week FT1
Phos Tre T 1week FT1
His Suc T 1 week FT1
His Tre T 1 week FT1
Phos Suc T 2 weeks 40°C
Phos Tre T 2 weeks 40°C
His Suc T 2 weeks 40°C
His Tre T 2 weeks 40°C

Figure 3



	T0	T 3 weeks 2-8°C	T 3 weeks 40°C	T 3 weeks FT3
Phos Suc	5.03	4.91	5.24	4.78
Phos Tre	5.29	5.18	5.37	5.08
His Suc	3.41	3.07	2.85	2.48
His Tre	3.64	3.68	2.64	2.43

Figure 4

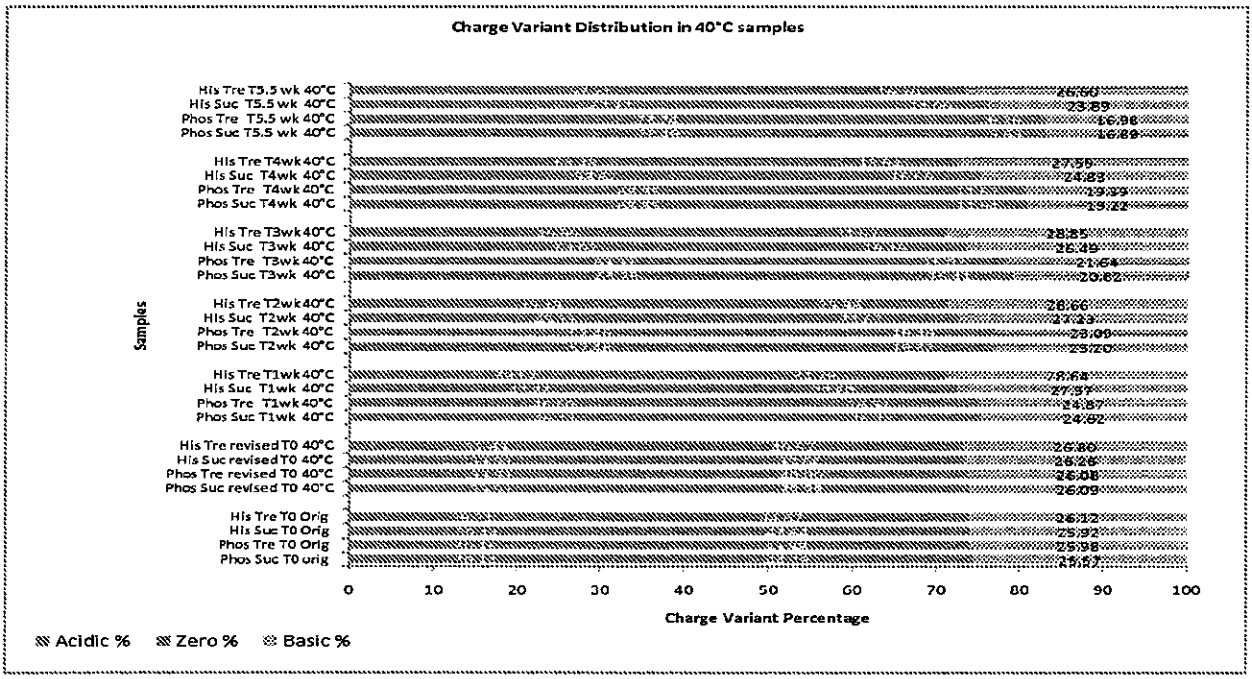
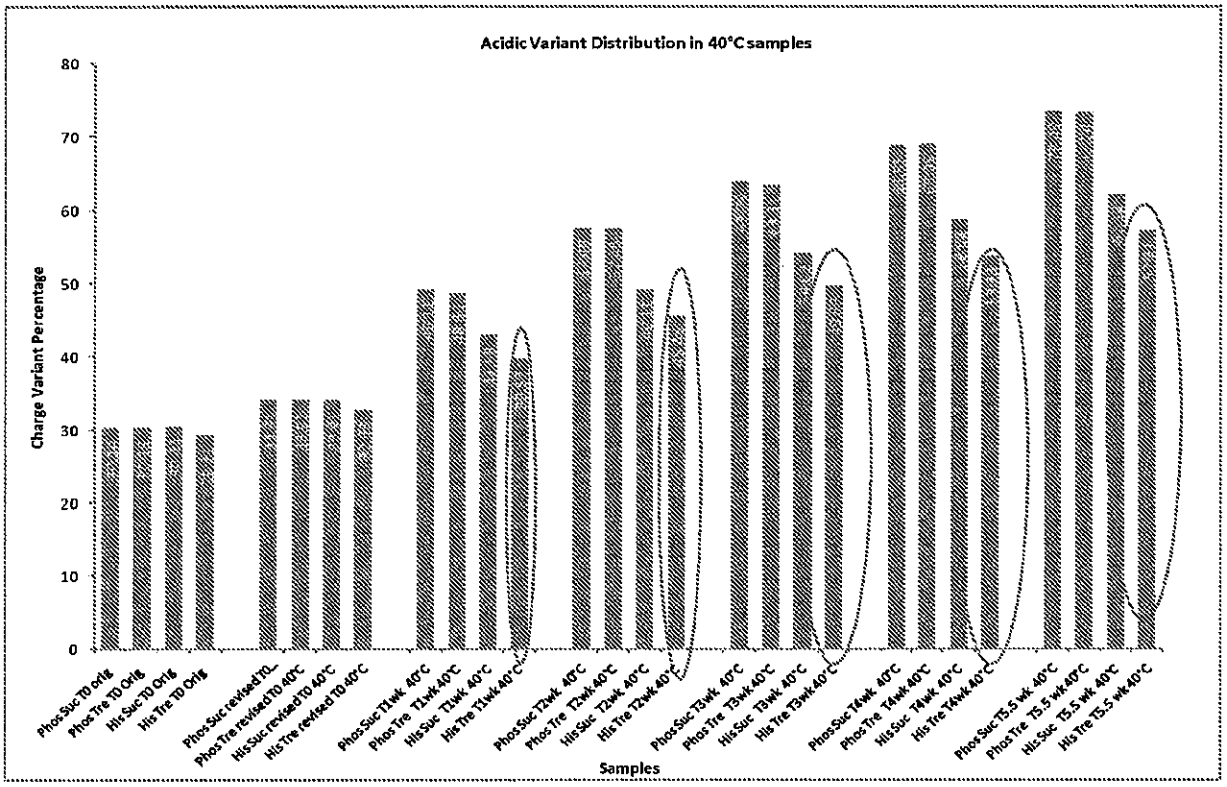

Charge Variant Distribution in 40°C samples
His Tre T5.5 wk 40°C
His Suc T5.5 wk 40°C
Phos Tre T5.5 wk 40°C
Phos Suc T5.5 wk 40°C
His Tre T4wk 40°C
His Suc T4wk 40°C
Phos Tre T4wk 40°C
Phos Suc T4wk 40°C
His Tre T3wk 40°C
His Suc T3wk 40°C
Phos Tre T3wk 40°C
Phos Suc T3wk 40°C
His Tre T2wk 40°C
His Suc T2wk 40°C
Phos Tre T2wk 40°C
Phos Suc T2wk 40°C
His Tre T1wk 40°C
His Suc T1wk 40°C
Phos Tre T1wk 40°C
Phos Suc T1wk 40°C
His Tre revised T0 40°C
His Suc revised T0 40°C
Phos Tre revised T0 40°C
Phos Suc revised T0 40°C
His Tre T0 Orig
His Suc T0 Orig
Phos Tre T0 Orig
Phos Suc T0 orig
Samples
0
10
20
30
40
50
60
70
80
90
100
Charge Variant Percentage
Acidic %
Zero %
Basic %

Figure 5


Acidic Variant Distribution in 40°C samples
Charge Variant Percentage
80
70
60
50
40
30
20
10
0
PhosSuc T0 orig
PhosTre T0 Orig
HisSuc T0 Orig
HisTre T0 Orig
PhosSuc revised T0
PhosTre revised T0 40°C
HisSuc revised T0 40°C
HisTre revised T0 40°C
PhosSuc T1wk 40°C
PhosTre T1wk 40°C
HisSuc T1wk 40°C
HisTre T1wk 40°C
PhosSuc T2wk 40°C
PhosTre T2wk 40°C
HisSuc T2wk 40°C
HisTre T2wk 40°C
PhosSuc T3wk 40°C
PhosTre T3wk 40°C
HisSuc T3wk 40°C
HisTre T3wk 40°C
PhosSuc T4wk 40°C
PhosTre T4wk 40°C
HisSuc T4wk 40°C
HisTre T4wk 40°C
PhosSuc T5.5 wk 40°C
PhosTre T5.5 wk 40°C
HisSuc T5.5 wk 40°C
HisTre T5.5 wk 40°C
Samples

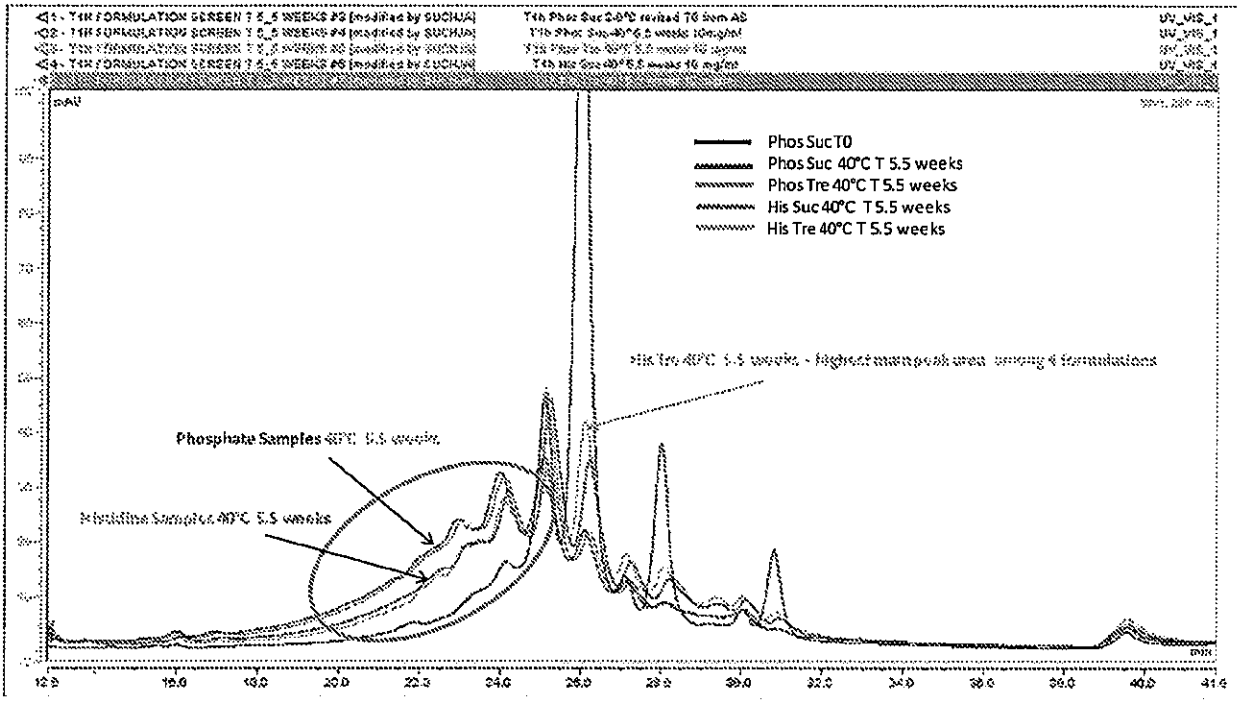
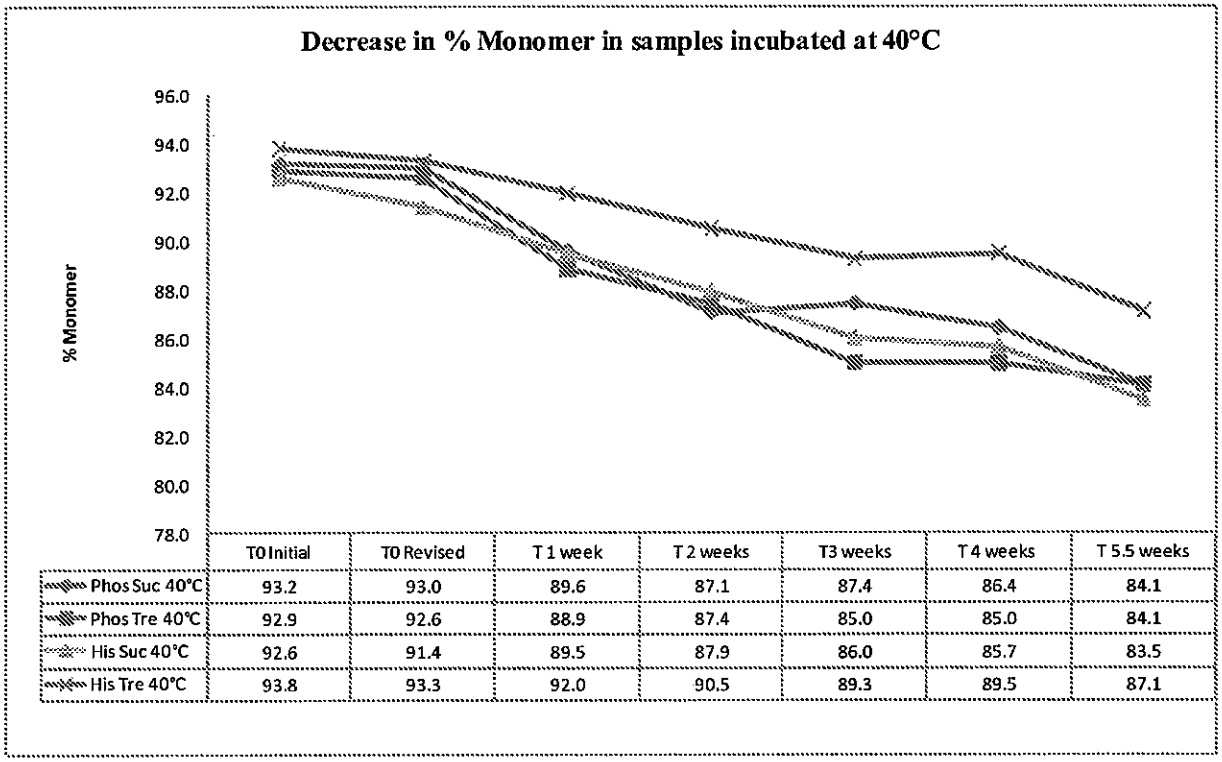
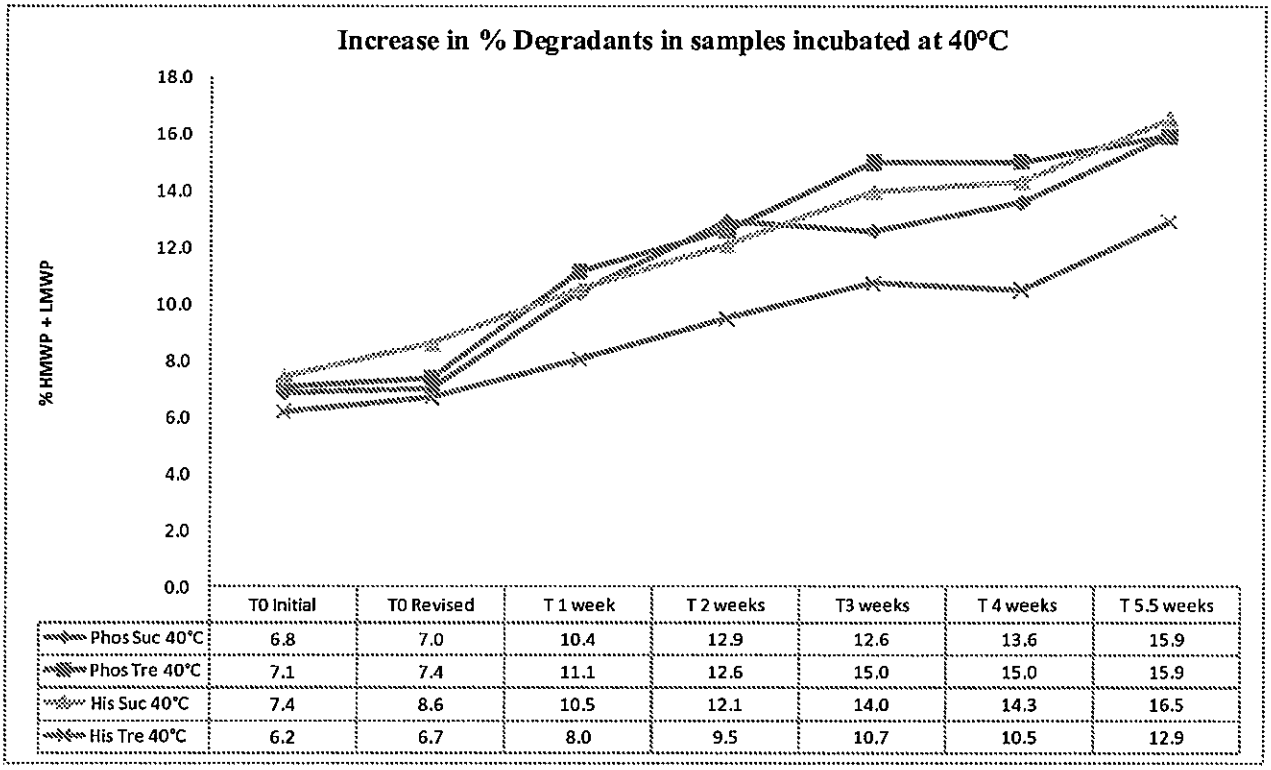

Phos Suc T0
Phos Suc 40°C T 5.5 weeks
Phos Tre 40°C T 5.5 weeks
His Suc 40°C T 5.5 weeks
His Tre 40°C T 5.5 weeks
Phosphate Samples
Histidine Samples 40°C 5.5 weeks

Figure 7



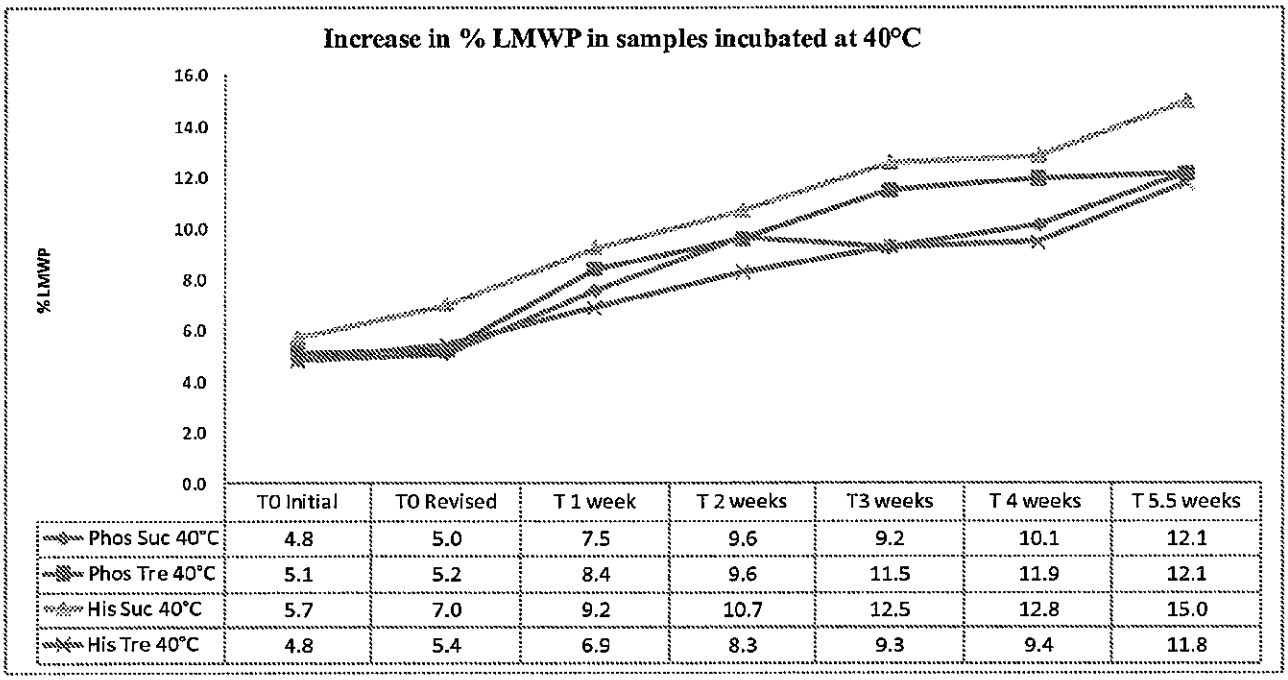
	T0 Initial	T0 Revised	T 1 week	T 2 weeks	T3 weeks	T 4 weeks	T 5.5 weeks
Phos Suc 40°C	93.2	93.0	89.6	87.1	87.4	86.4	84.1
Phos Tre 40°C	92.9	92.6	88.9	87.4	85.0	85.0	84.1
His Suc 40°C	92.6	91.4	89.5	87.9	86.0	85.7	83.5
His Tre 40°C	93.8	93.3	92.0	90.5	89.3	89.5	87.1

Figure 8



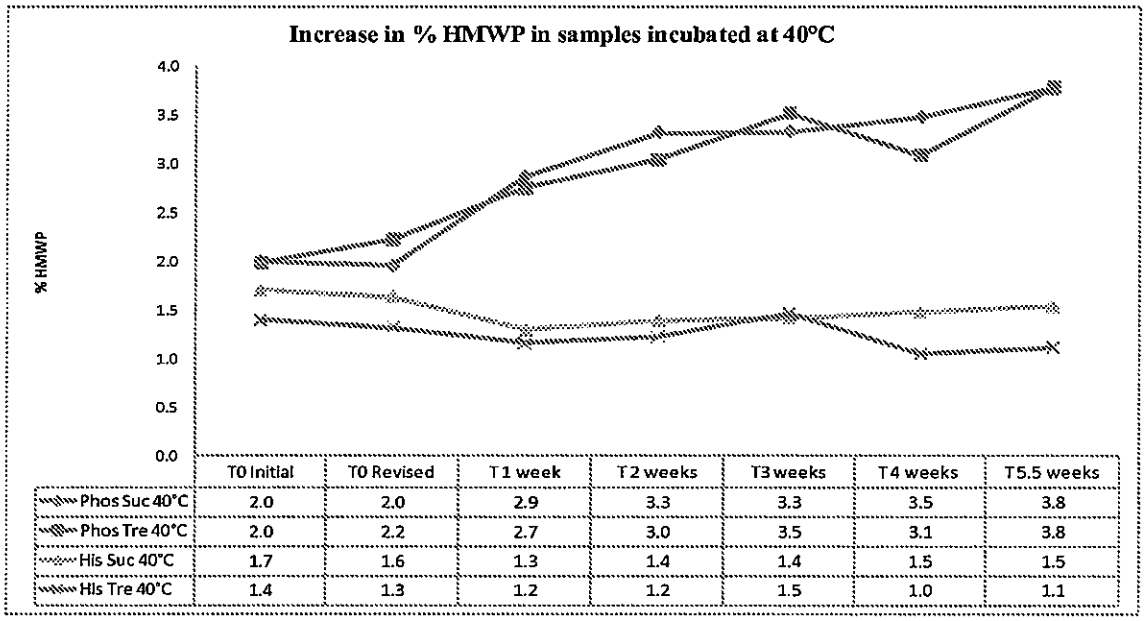
	T0 Initial	T0 Revised	T 1 week	T 2 weeks	T3 weeks	T 4 weeks	T 5.5 weeks
Phos Suc 40°C	6.8	7.0	10.4	12.9	12.6	13.6	15.9
Phos Tre 40°C	7.1	7.4	11.1	12.6	15.0	15.0	15.9
His Suc 40°C	7.4	8.6	10.5	12.1	14.0	14.3	16.5
His Tre 40°C	6.2	6.7	8.0	9.5	10.7	10.5	12.9

Figure 9



	T0 Initial	T0 Revised	T 1 week	T 2 weeks	T3 weeks	T 4 weeks	T 5.5 weeks
Phos Suc 40°C	4.8	5.0	7.5	9.6	9.2	10.1	12.1
Phos Tre 40°C	5.1	5.2	8.4	9.6	11.5	11.9	12.1
His Suc 40°C	5.7	7.0	9.2	10.7	12.5	12.8	15.0
His Tre 40°C	4.8	5.4	6.9	8.3	9.3	9.4	11.8

Figure 10



	T0 Initial	T0 Revised	T1 week	T2 weeks	T3 weeks	T4 weeks	T5.5 weeks
Phos Suc 40°C	2.0	2.0	2.9	3.3	3.3	3.5	3.8
Phos Tre 40°C	2.0	2.2	2.7	3.0	3.5	3.1	3.8
His Suc 40°C	1.7	1.6	1.3	1.4	1.4	1.5	1.5
His Tre 40°C	1.4	1.3	1.2	1.2	1.5	1.0	1.1

Figure 11

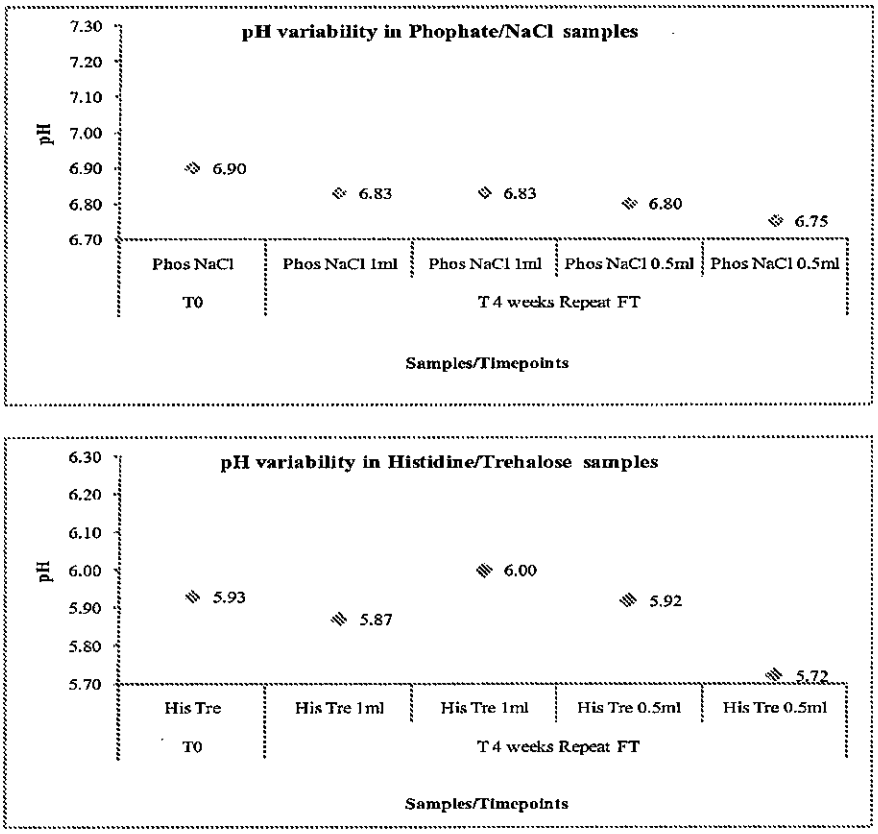

pH variability in Phophate/NaCl samples
pH
7.30
7.20
7.10
7.00
6.90
6.80
6.70
6.90
6.83
6.83
6.80
6.75
Phos NaCl
Phos NaCl 1ml
Phos NaCl 1ml
Phos NaCl 0.5ml
Phos NaCl 0.5ml
T0
T 4 weeks Repeat FT
Samples/Timepoints
pH variability in Histidine/Trehalose samples
pH
6.30
6.20
6.10
6.00
5.90
5.80
5.70
5.93
5.87
6.00
5.92
5.72
His Tre
His Tre 1ml
His Tre 1ml
His Tre 0.5ml
His Tre 0.5ml
T0
T 4 weeks Repeat FT
Samples/Timepoints

Figure 12

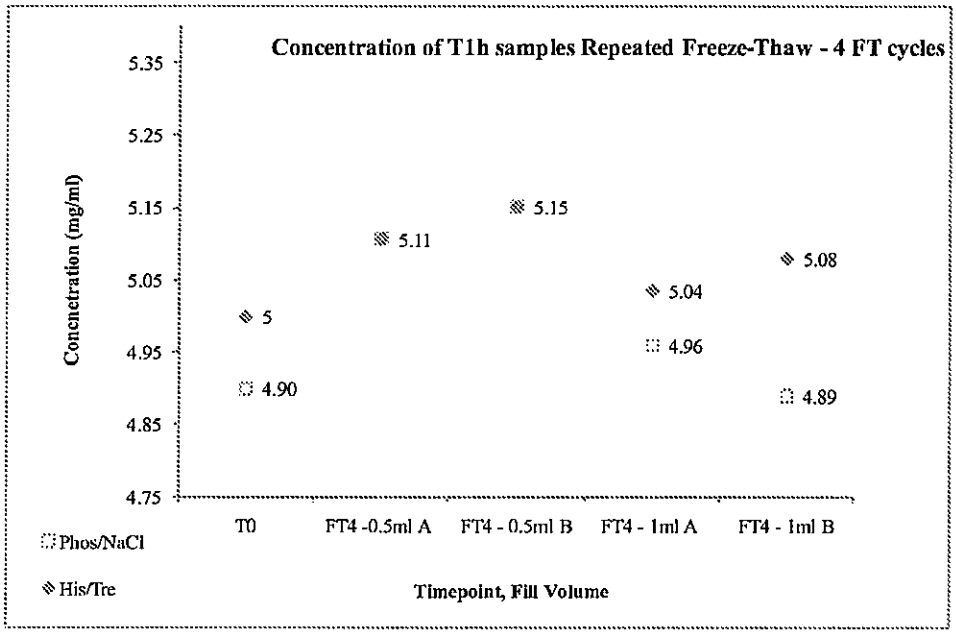

Concentration of T1h samples Repeated Freeze-Thaw - 4 FT cycles
Concentration (mg/ml)
5.35
5.25
5.15
5.05
4.95
4.85
4.75
5
5.11
5.15
5.04
5.08
4.90
4.96
4.89
T0
FT4 -0.5ml A
FT4 - 0.5ml B
FT4 - 1ml A
FT4 - 1ml B
Phos/NaCl
His/Tre
Timepoint, Fill Volume

Figure 13

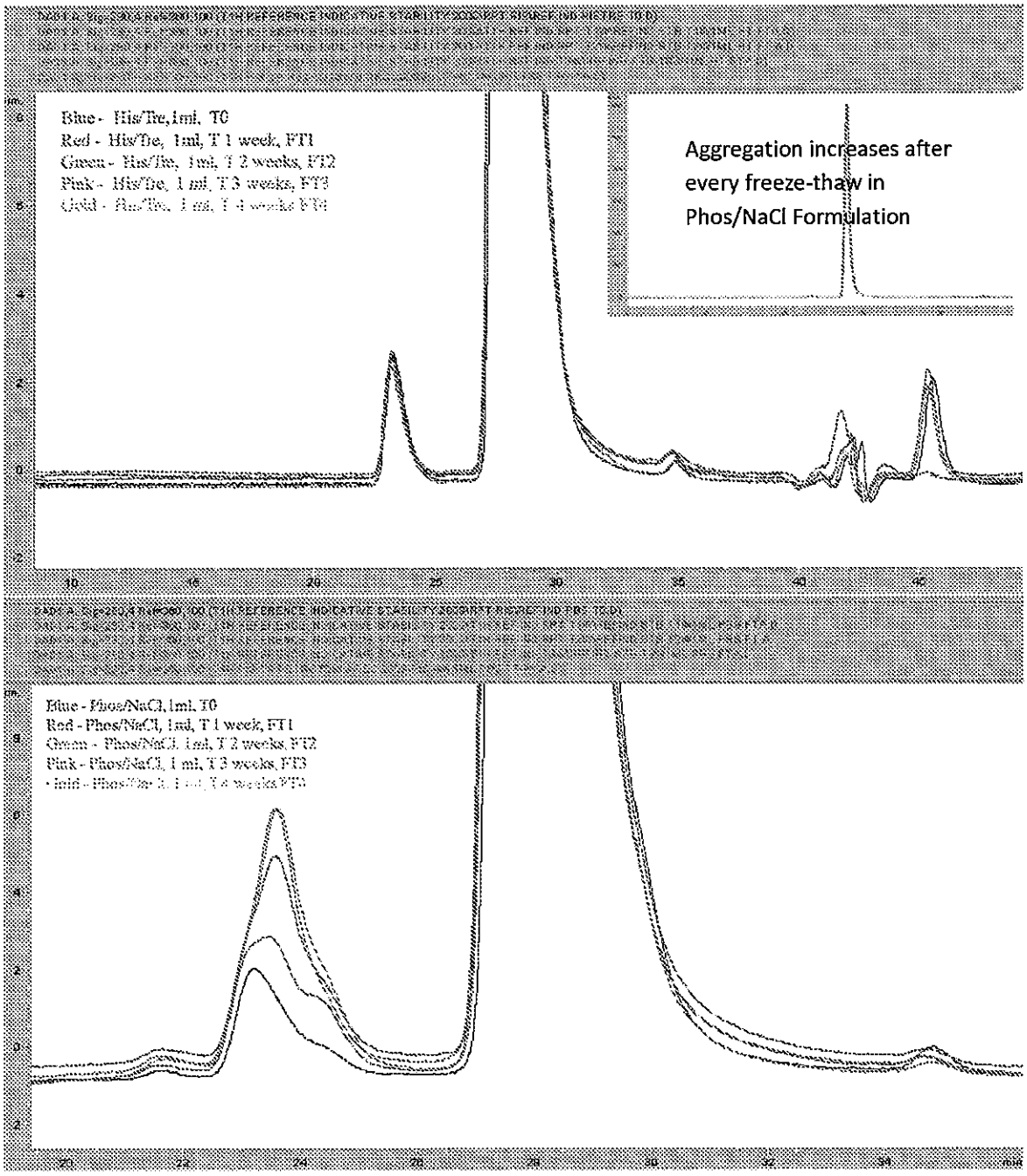
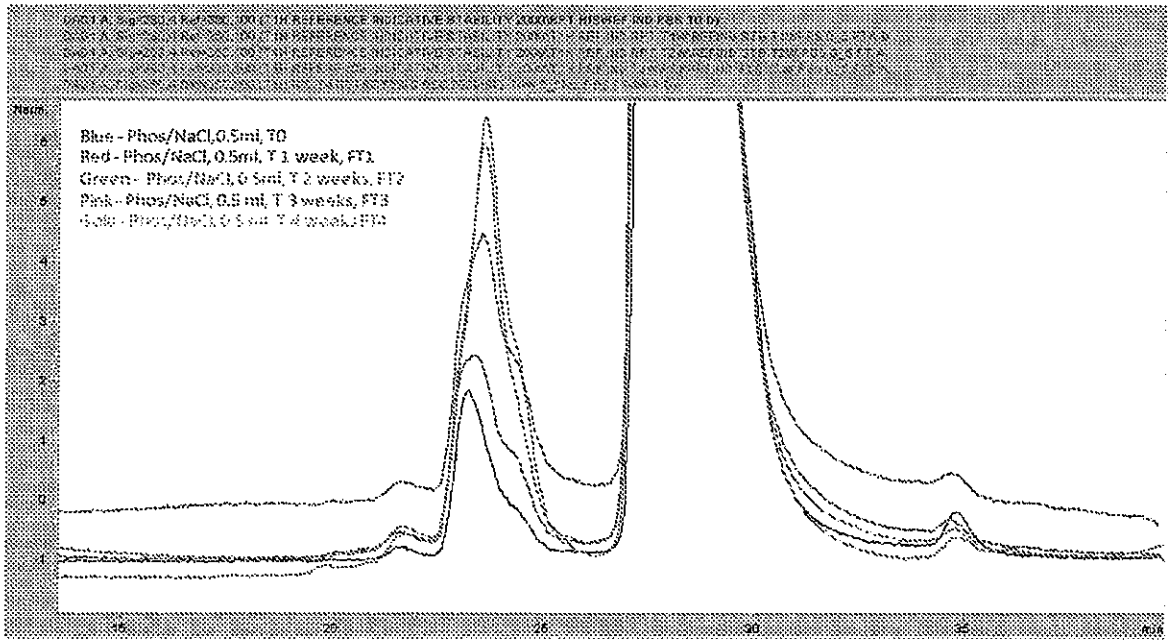

Blue - His/Tre, 1ml, T0
Red - His/Tre, 1ml, T 1 week, FT1
Green - His/Tre, 1ml, T 2 weeks, FT2
Pink - His/Tre, 1 ml, T 3 weeks, FT3
Aggregation increases after every freeze-thaw in Phos/NaCl Formulation
Blue - Phos/NaCl, 1ml, T0
Red - Phos/NaCl, 1ml, T 1 week, FT1
Green - Phos/NaCl, 1ml, T 2 weeks, FT2
Pink - Phos/NaCl, 1 ml, T 3 weeks, FT3

Figure 14


Blue - Phos/NaCl,0.5ml, T0
Red - Phos/NaCl, 0.5ml, T 1 week, FT1
Green - Phos/NaCl, 0.5ml, T 2 weeks, FT2
Pink - Phos/NaCl, 0.5 ml, T 3 weeks, FT3

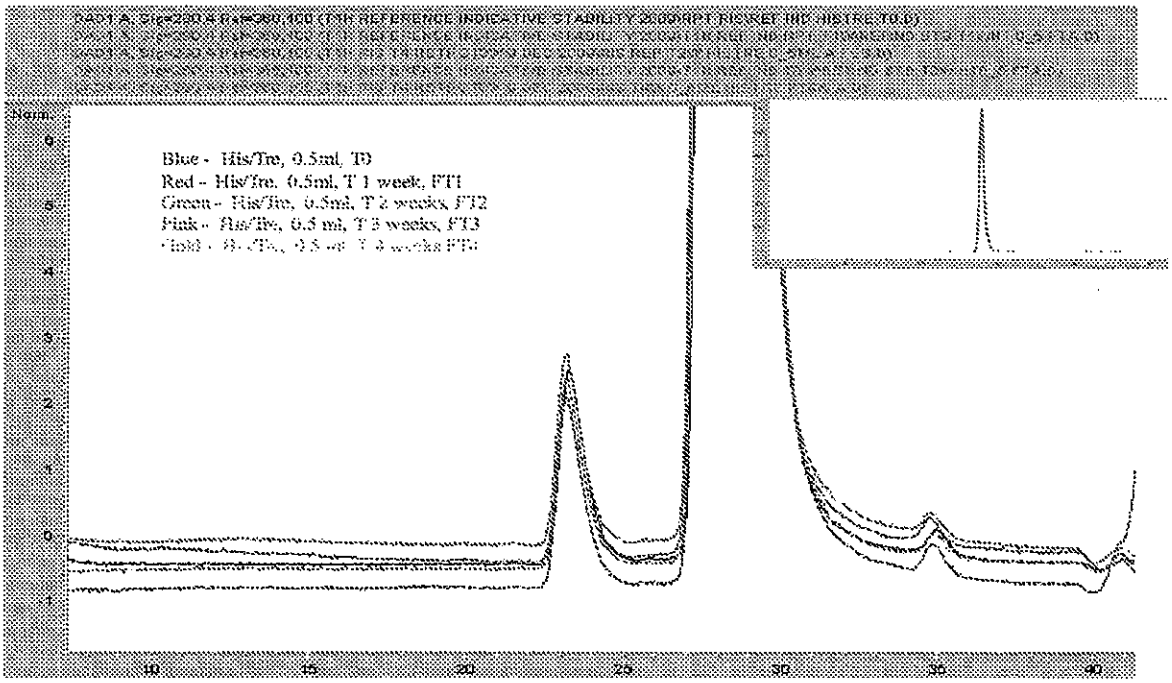

Blue - His/Tre, 0.5ml, T0
Red - His/Tre, 0.5ml, T 1 week, FT1
Green - His/Tre, 0.5ml, T 2 weeks, FT2
Pink - His/Tre, 0.5 ml, T 3 weeks, FT3

Figure 15

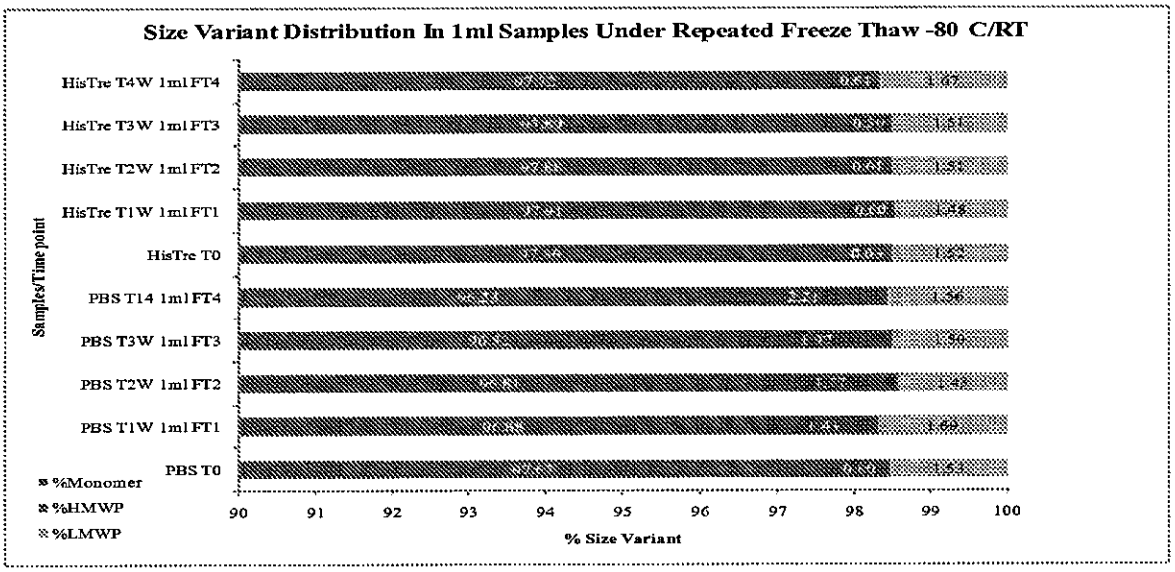


Figure 16

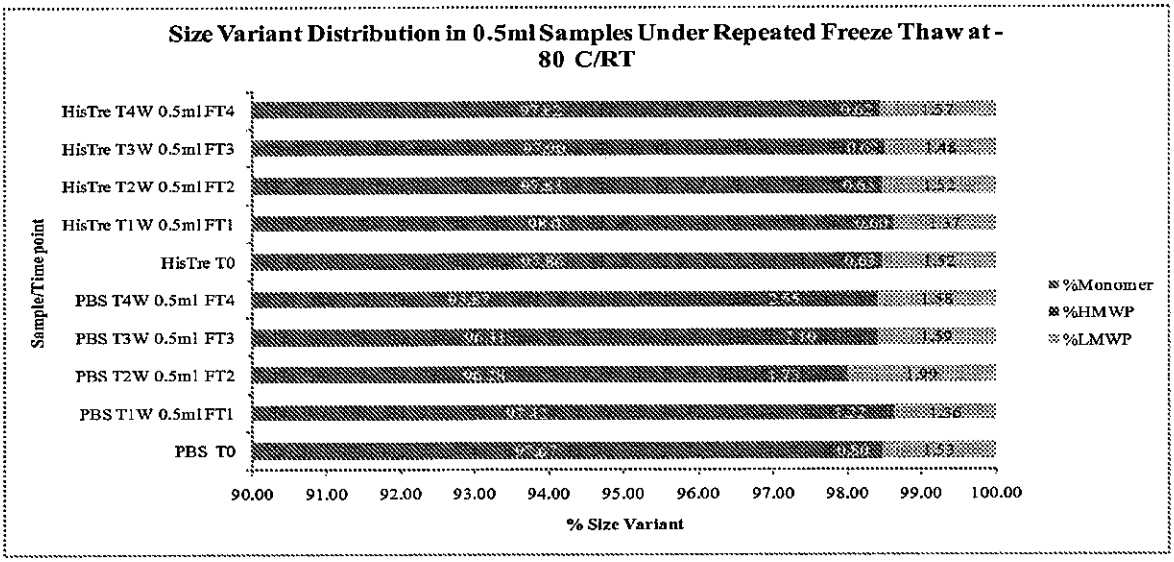


Figure 17

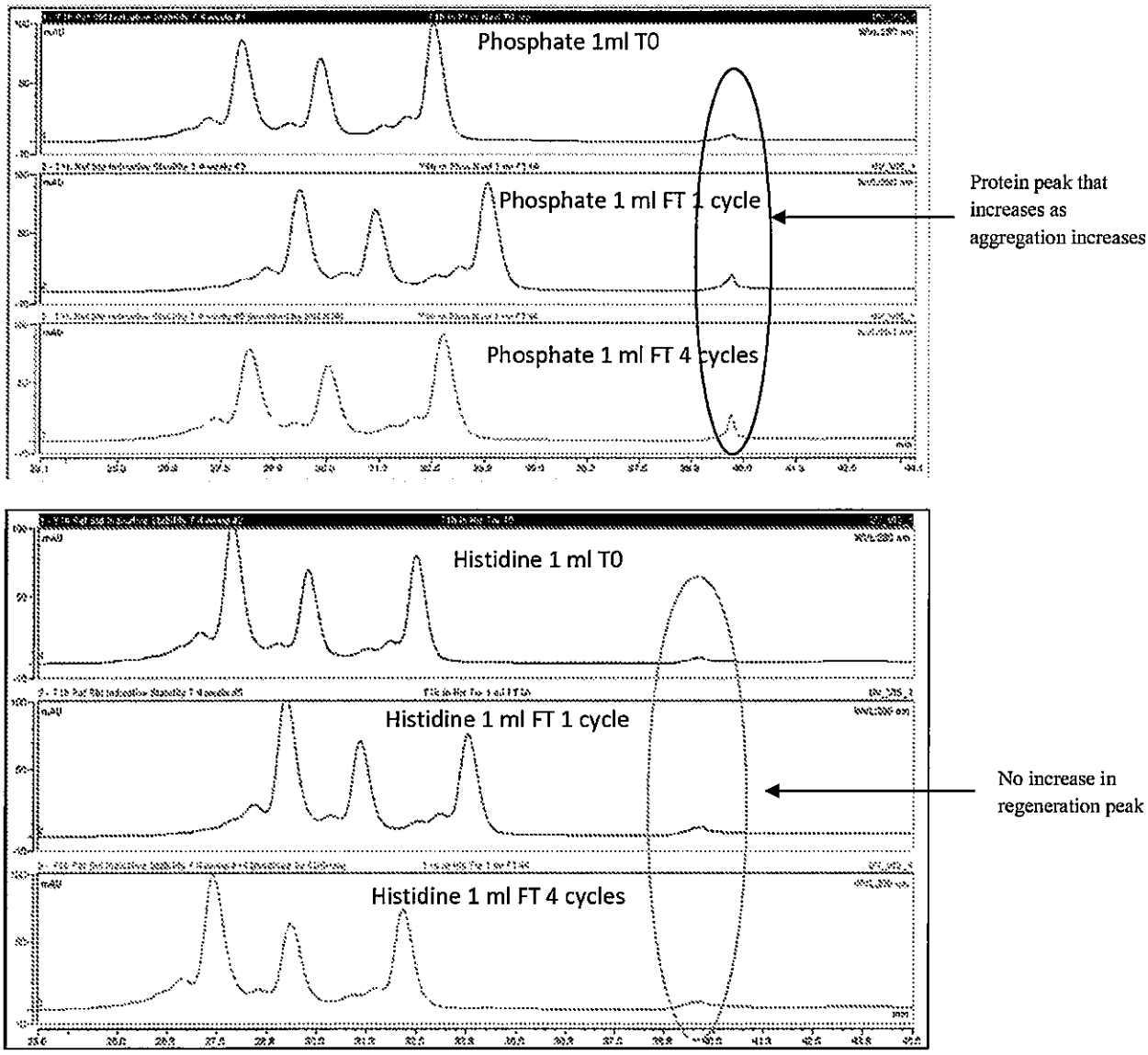

Phosphate 1ml T0
Phosphate 1 ml FT 1 cycle
Phosphate 1 ml FT 4 cycles
Protein peak that increases as aggregation increases
Histidine 1 ml T0
Histidine 1 ml FT 1 cycle
Histidine 1 ml FT 4 cycles
No increase in regeneration peak

Figure 18

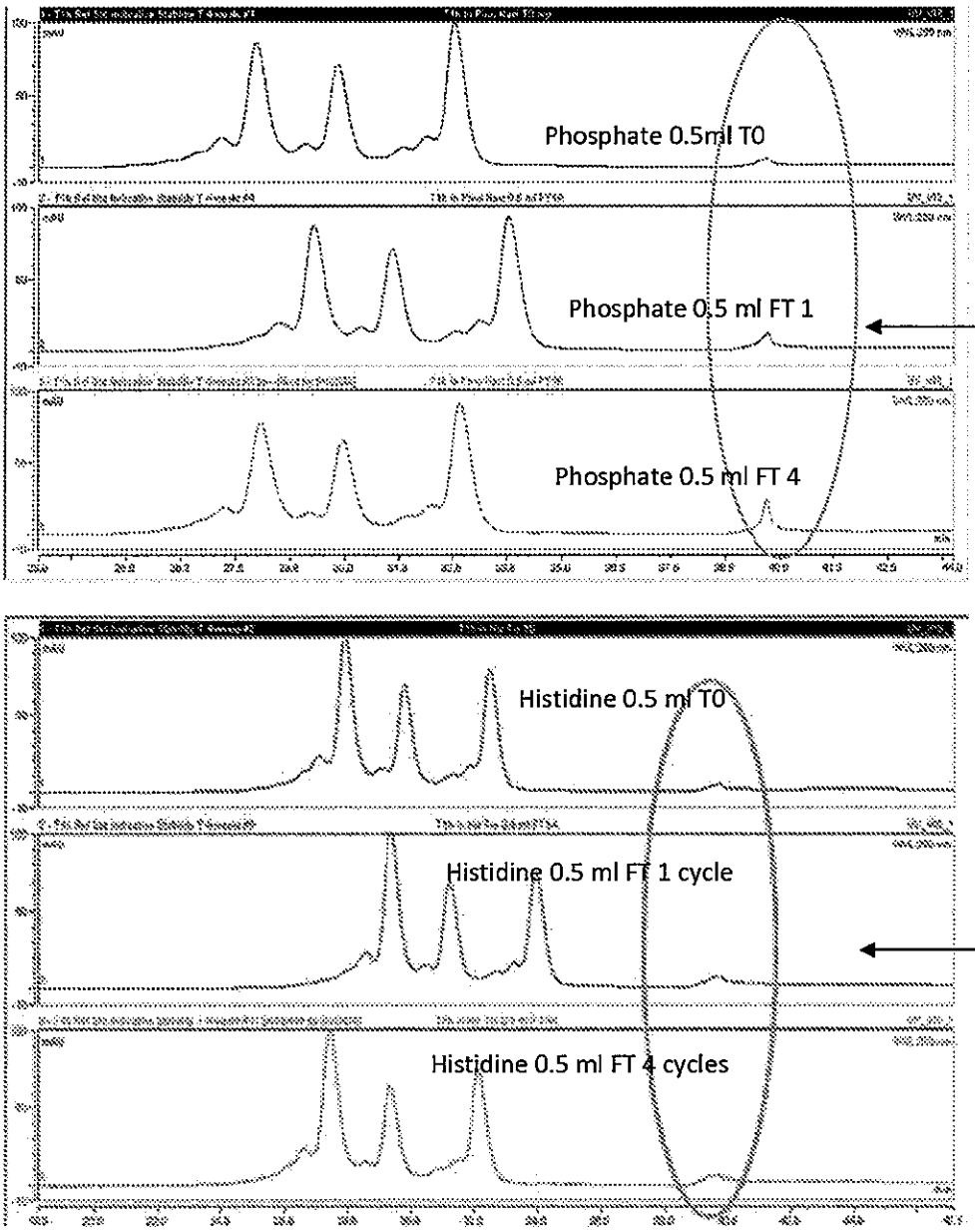

Phosphate 0.5ml T0
Phosphate 0.5 ml FT 1
Phosphate 0.5 ml FT 4
Protein peak that increases as aggregation increases
Histidine 0.5 ml T0
Histidine 0.5 ml FT 1 cycle
Histidine 0.5 ml FT 4 cycles
No increase in regeneration peak

Figure 19

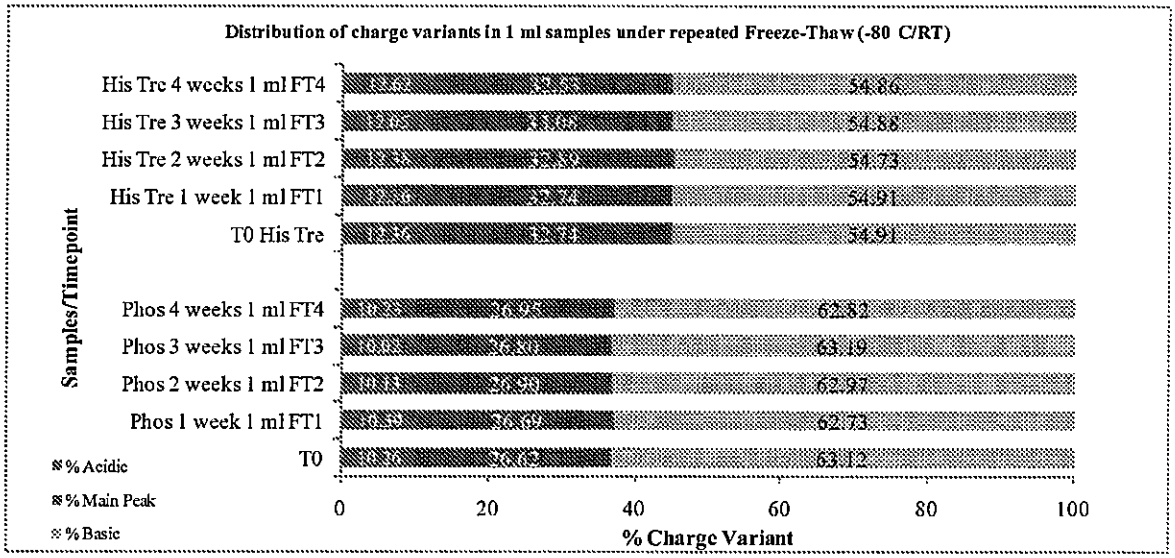


Figure 1

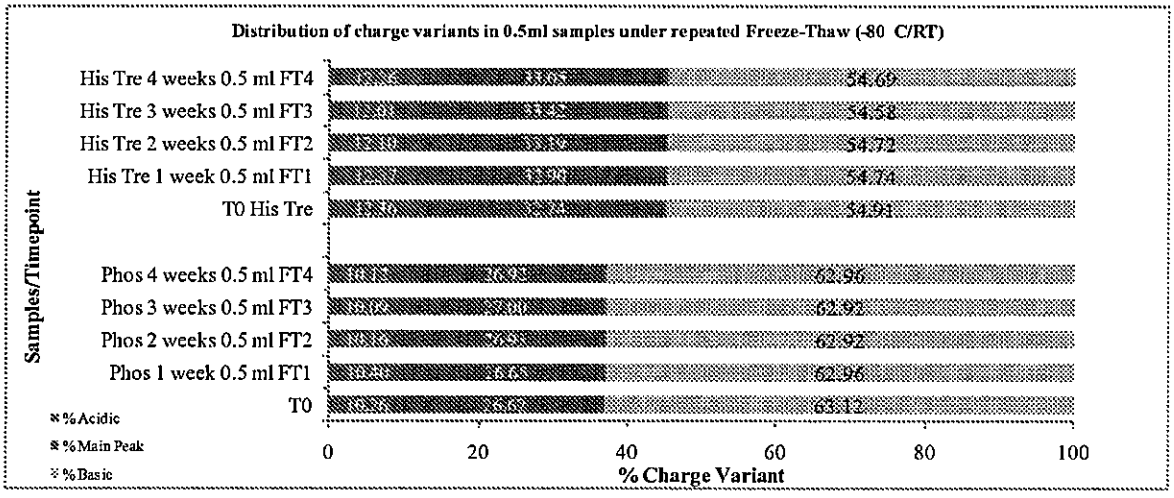


Figure 2

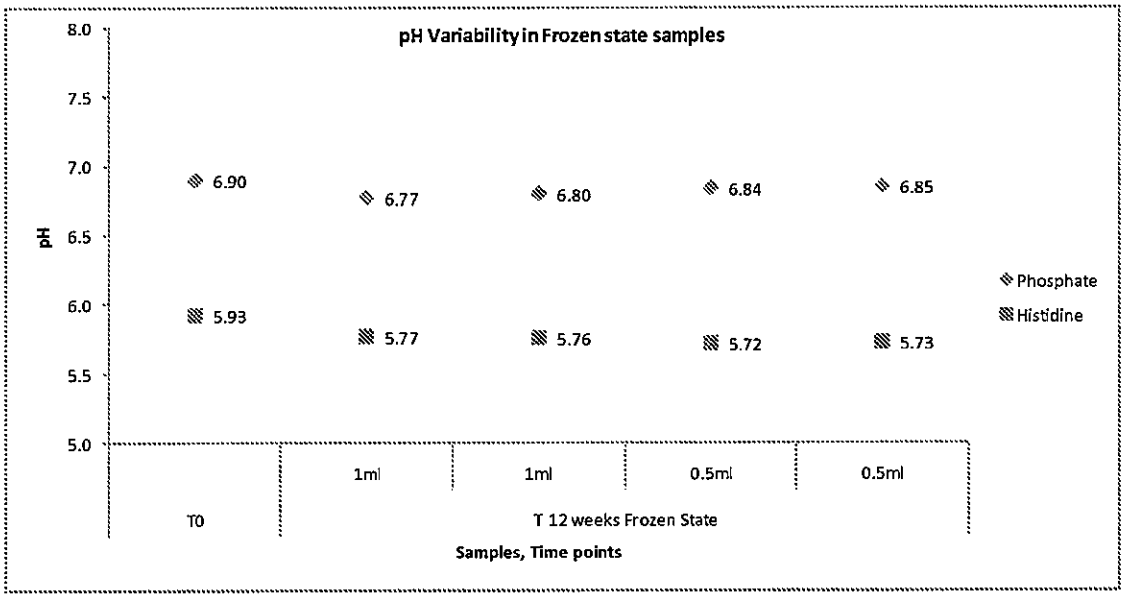

pH Variability in Frozen state samples
8.0
7.5
7.0
6.5
6.0
5.5
5.0
pH
6.90
6.77
6.80
6.84
6.85
5.93
5.77
5.76
5.72
5.73
Phosphate
Histidine
T0
1ml
1ml
0.5ml
0.5ml
T 12 weeks Frozen State
Samples, Time points

Figure 22

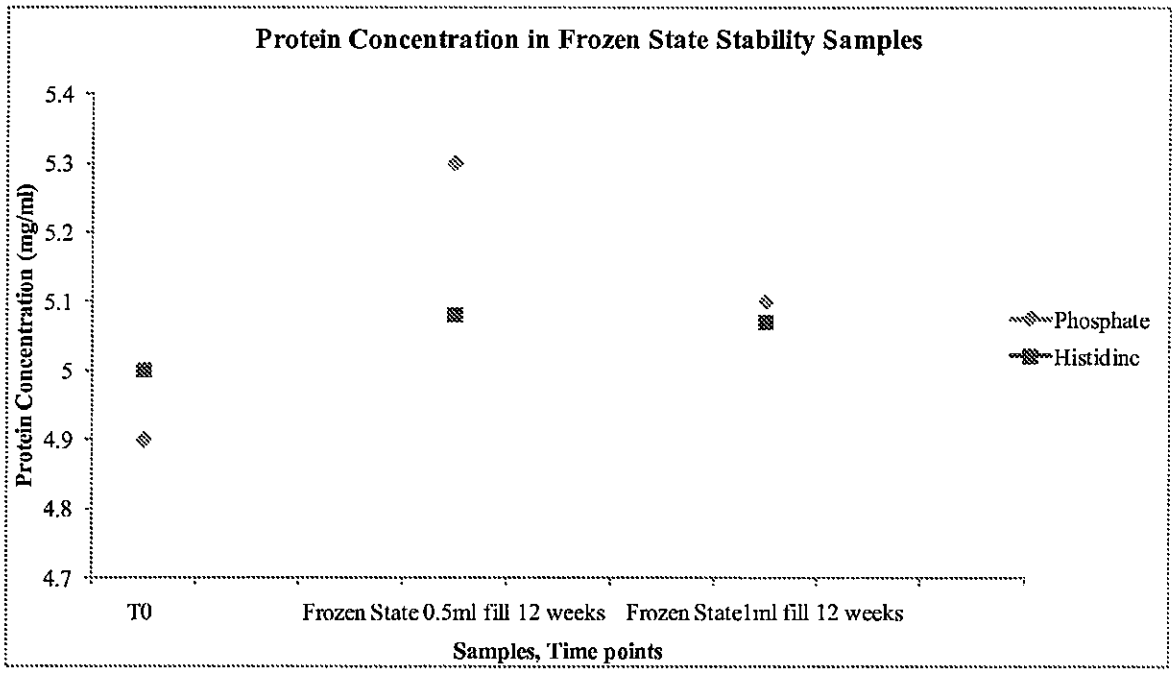

Protein Concentration in Frozen State Stability Samples
Protein Concentration (mg/ml)
5.4
5.3
5.2
5.1
5
4.9
4.8
4.7
T0
Frozen State 0.5ml fill 12 weeks
Frozen State1ml fill 12 weeks
Samples, Time points
Phosphate
Histidine

Figure 23

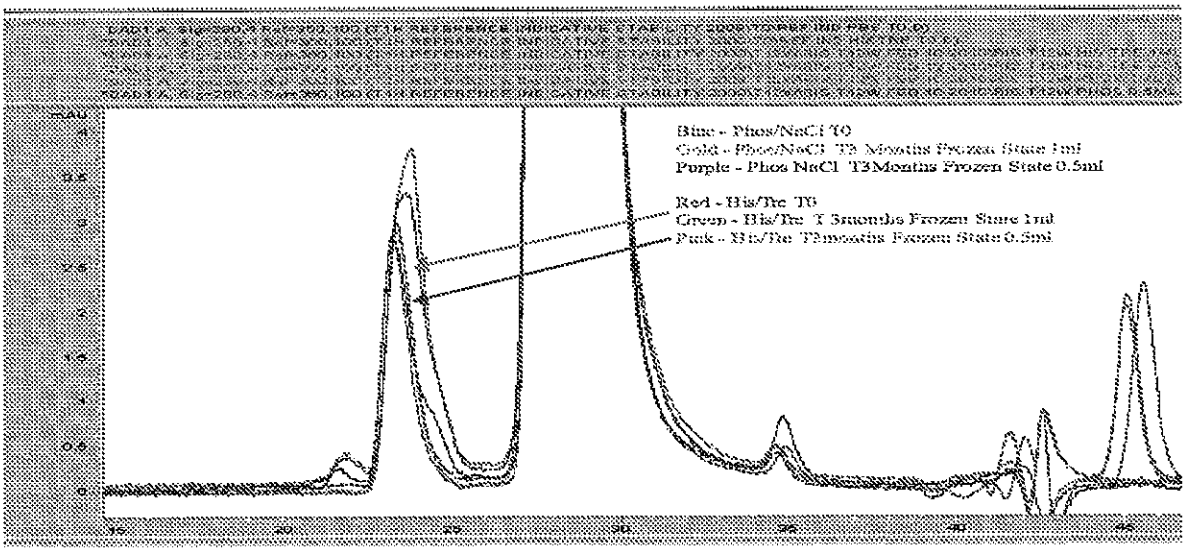
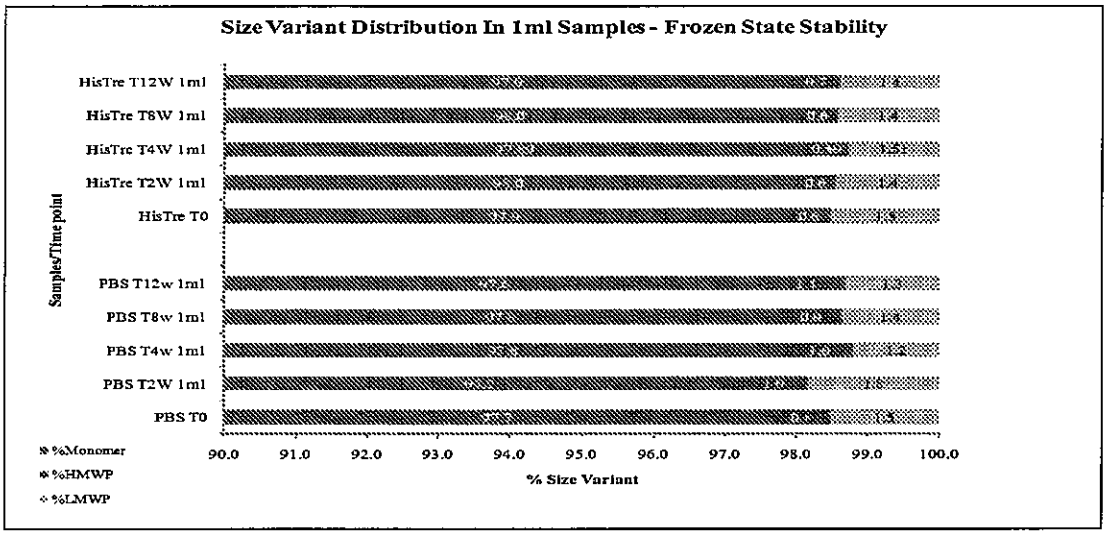

mAU
Blue - Phos/NaCl T0
Gold - Phos/NaCl T3 Months Frozen State 1ml
Purple - Phos NaCl T3Months Frozen State 0.5ml
Red - His/Tre T0
Green - His/Tre T 3months Frozen State 1ml
Pink - His/Tre T3months Frozen State 0.5ml

Figure24


Size Variant Distribution In 1ml Samples - Frozen State Stability
HisTre T12W 1ml
HisTre T8W 1ml
HisTre T4W 1ml
HisTre T2W 1ml
HisTre T0
PBS T12w 1ml
PBS T8w 1ml
PBS T4w 1ml
PBS T2W 1ml
PBS T0
Samples/Time point
%Monomer
%HMWP
%LMWP
90.0 91.0 92.0 93.0 94.0 95.0 96.0 97.0 98.0 99.0 100.0
% Size Variant

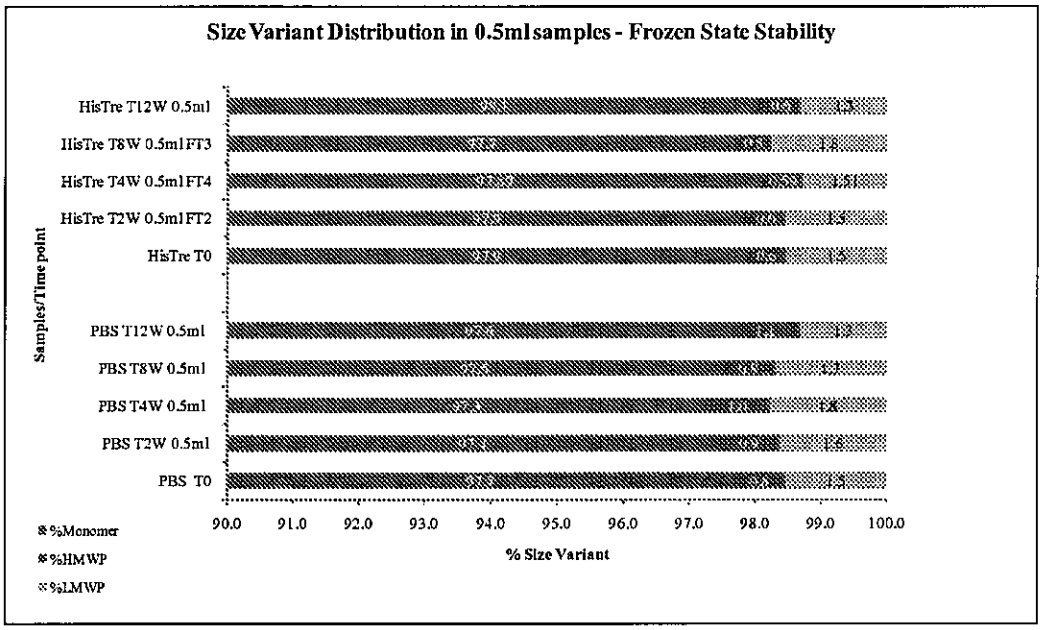

Size Variant Distribution in 0.5ml samples - Frozen State Stability
HisTre T12W 0.5ml
HisTre T8W 0.5ml FT3
HisTre T4W 0.5ml FT4
HisTre T2W 0.5ml FT2
HisTre T0
PBS T12W 0.5ml
PBS T8W 0.5ml
PBS T4W 0.5ml
PBS T2W 0.5ml
PBS T0
Samples/Time point
%Monomer
%HMWP
%LMWP
90.0 91.0 92.0 93.0 94.0 95.0 96.0 97.0 98.0 99.0 100.0
% Size Variant

Figure25

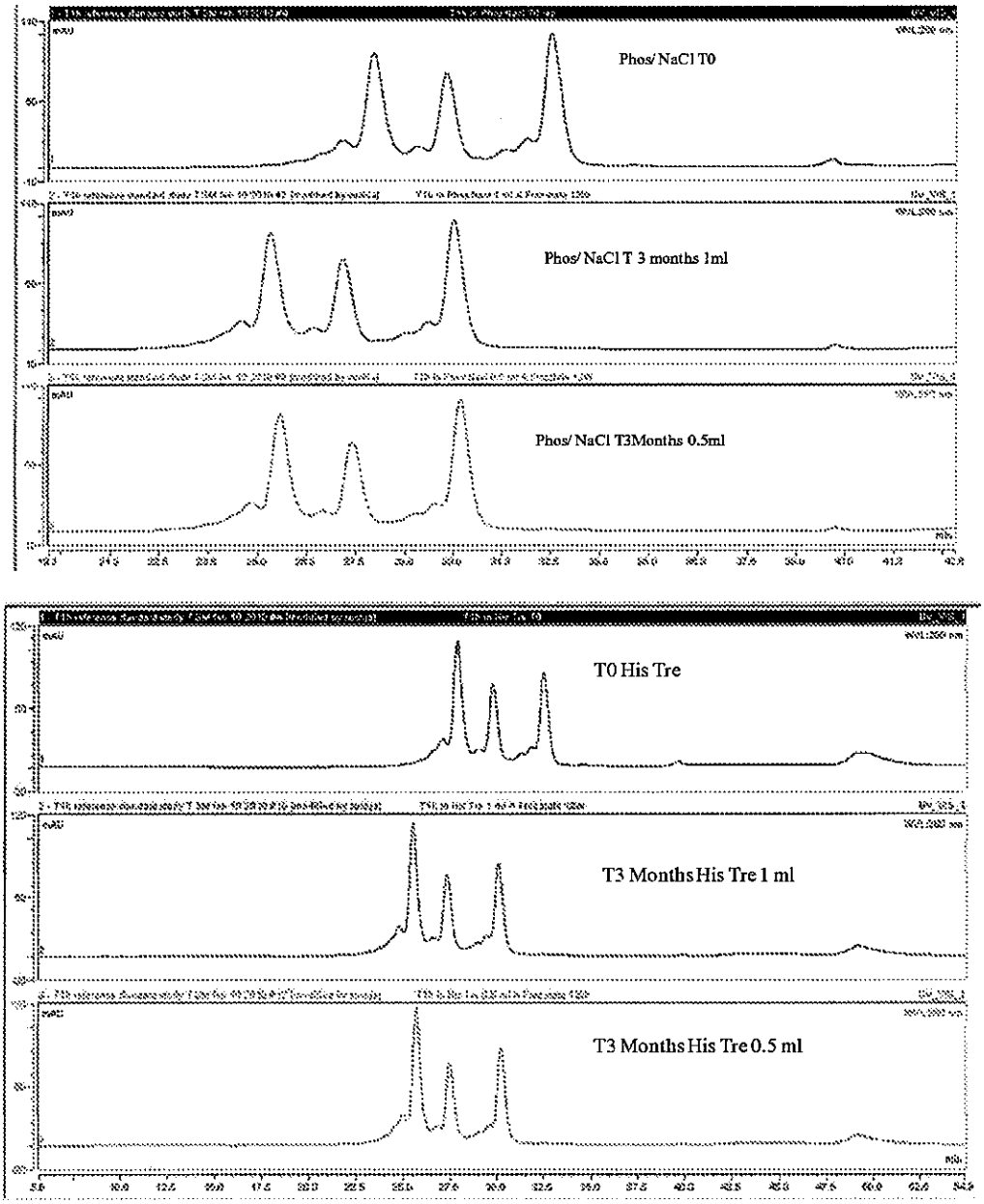
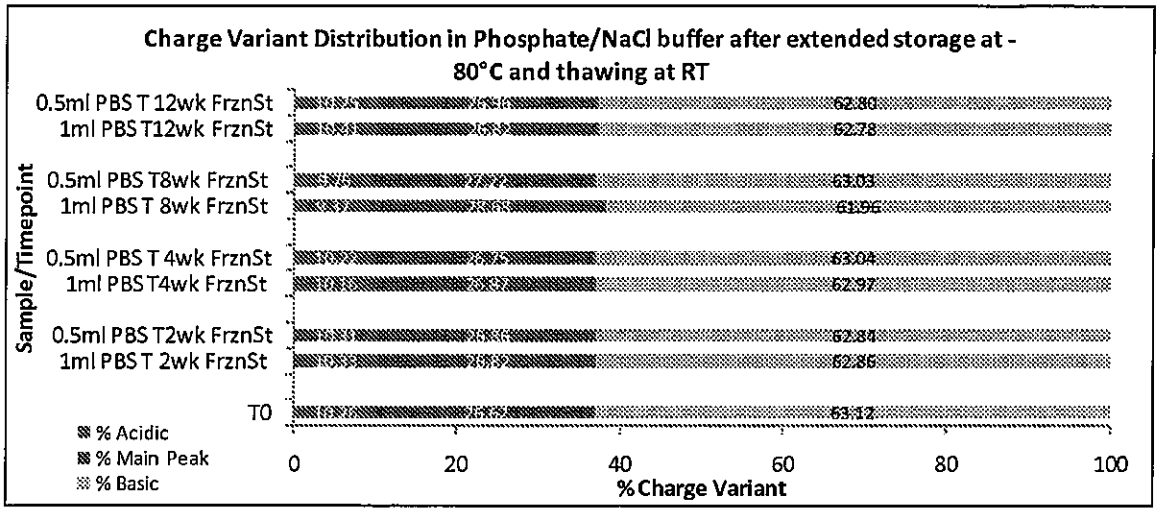

Phos/ NaCl T0
Phos/ NaCl T 3 months 1ml
Phos/ NaCl T3Months 0.5ml
T0 His Tre
T3 Months His Tre 1 ml
T3 Months His Tre 0.5 ml

Figure26


Charge Variant Distribution in Phosphate/NaCl buffer after extended storage at -80°C and thawing at RT
0.5ml PBS T 12wk FrznSt
1ml PBS T12wk FrznSt
0.5ml PBS T8wk FrznSt
1ml PBS T 8wk FrznSt
0.5ml PBS T 4wk FrznSt
1ml PBS T4wk FrznSt
0.5ml PBS T2wk FrznSt
1ml PBS T 2wk FrznSt
T0
62.80
62.78
63.03
61.96
63.04
62.97
62.84
62.86
63.12
Sample/Timepoint
% Acidic
% Main Peak
% Basic
0
20
40
60
80
100
% Charge Variant

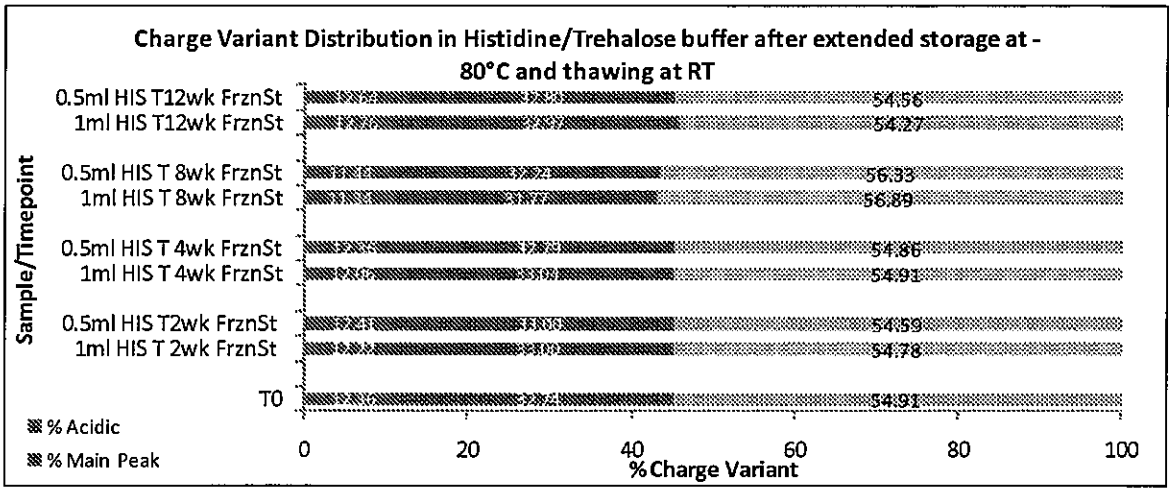

Charge Variant Distribution in Histidine/Trehalose buffer after extended storage at -80°C and thawing at RT
0.5ml HIS T12wk FrznSt
1ml HIS T12wk FrznSt
0.5ml HIS T 8wk FrznSt
1ml HIS T 8wk FrznSt
0.5ml HIS T 4wk FrznSt
1ml HIS T 4wk FrznSt
0.5ml HIS T2wk FrznSt
1ml HIS T 2wk FrznSt
T0
54.56
54.27
56.33
56.89
54.86
54.91
54.59
54.78
54.91
Sample/Timepoint
% Acidic
% Main Peak
0
20
40
60
80
100
% Charge Variant

Figure 27