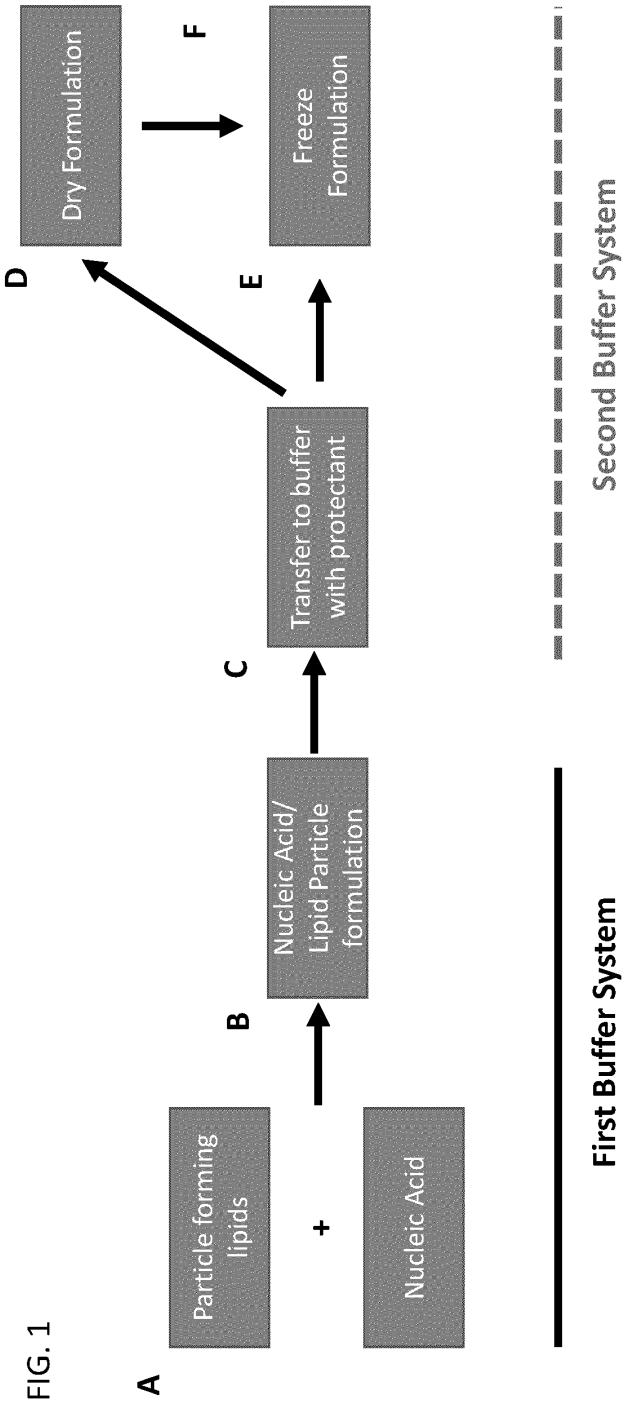

Particle forming lipids
+
Nucleic Acid
A
B
Nucleic Acid/ Lipid Particle formulation
C
Transfer to buffer with protectant
D
Dry Formulation
E
F
Freeze Formulation
First Buffer System
Second Buffer System

FIG. 1

FIG. 2A

#	Cryo/LyoP	Buffer	Salt (Y/N)^	Anneal (Y/N)
F1	10% Sucrose	10 mM Tris	No	No – Cycle 3 (NA) Yes – Cycle 4 (A)
F2	10% Trehalose			
F3	5% Sucrose - 5% Trehalose			
F4	10% Sucrose	10 mM Tris	Yes	No – Cycle 1 (NA) Yes – Cycle 2 (A)
F5	10% Trehalose			
F6	5% Sucrose - 5% Trehalose			
F7	10% Sucrose	10 mM His	No	Yes – Cycle 4 (A)
F8	10% Trehalose			
F9	5% Sucrose - 5% Trehalose			
F10	10% Sucrose	10 mM HEPES	No	Yes – Cycle 4 (A)
F11	10% Trehalose			
F12	5% Sucrose - 5% Trehalose			
F13	10% Sucrose	NaCl free PBS	No	Yes – Cycle 4 (A)
F15	10% Sucrose**	10 mM Tris	No	Yes – Cycle 4 (A)
F16	10% Sucrose**	10 mM Tris	No	
F17	10% Sucrose	NaCl PBS	Yes	No – Cycle 1 (NA) Yes – Cycle 2 (A)
F18	10% Sucrose		Yes	

^ Buffers with salt comprise 6 mg/ml NaCl; buffers without salt have no added NaCl

** Sucrose added in first buffer system

Process steps	Process parameters								Time, (hrs)
	Cycle 1 (resistance from Case 1, Table 2 was used), target temperature -40ºC	Time,(hrs)	Cycle 2 (resistance from Case 2, Table 2 was used), target temperature -40ºC	Time,(hrs)	Cycle 3 (resistance from Case 1, Table 2 was used), target temperature -36ºC	Time,(hrs)	Cycle 4 (resistance from Case 2, Table 2 was used), target temperature -36ºC	
Freezing	R to -45ºC in 130 min	2.17	R to -45ºC in 130 min	2.17	R to -45ºC in 130 min	2.17	R to -45ºC in 130 min	2.17
	H at -45ºC for 60 min	3.17	H at -45ºC for 60 min	3.17	H at -45ºC for 60 min	3.17	H at -45ºC for 60 min	3.17
			R to -10ºC in 70 min	4.33			R to -10ºC in 70 min	4.33
			H at -10ºC for 180 min	7.33			H at -10ºC for 180 min	7.33
			R to -45ºC in 70 min	8.50			R to -45ºC in 70 min	8.50
			H at -45ºC for 60 min	9.50			H at -45ºC for 60 min	9.50
Chill condenser and pull vacuum	Tcond<-50ºC; Pch=30 mT	4.17	Tcond<-50ºC; Pch=40 mT	10.50	Tcond<-50ºC; Pch=40 mT	4.17	Tcond<-50ºC; Pch=50 mT	10.50
Primary drying	R to -38ºC in 14 min	4.40	R to -36ºC in 18 min	10.80	R to -32ºC in 26 min	4.60	R to -29ºC in 32 min	11.03
	H at -38ºC for 3600 min	64.40	H at -36ºC for 2700 min	55.80	H at -32ºC for 2400 min	44.60	H at -36ºC for 1800 min	41.03
Secondary drying	R to 30ºC in 340 min	70.07	R to 30ºC in 330 min	61.30	R to 30ºC in 310 min	49.77	R to 30ºC in 295 min	45.95
	H at 30º for 480 min	78.07	H at 30º for 480 min	69.30	H at 30º for 480 min	57.77	H at 30º for 480 min	53.95

R=ramp; H=hold

Note that cycle parameters were calculated assuming edge effect of 1.5. Also, the chamber pressure was chosen based on the performance of 40 sq.m commercial freeze-dryer when it is fully loaded (175770 vials). Pressure set point was at least 9 mT higher than minimum controllable pressure (with resistance scale up factor of 1) for each cycle proposed. The calculations of Pmin for Lyostar III were also made to ensure that pressure can be controlled when 560 vials (one full tray) is loaded in Lyostar III.

FIG. 2B

Colloidal Stability

FIG. 3A

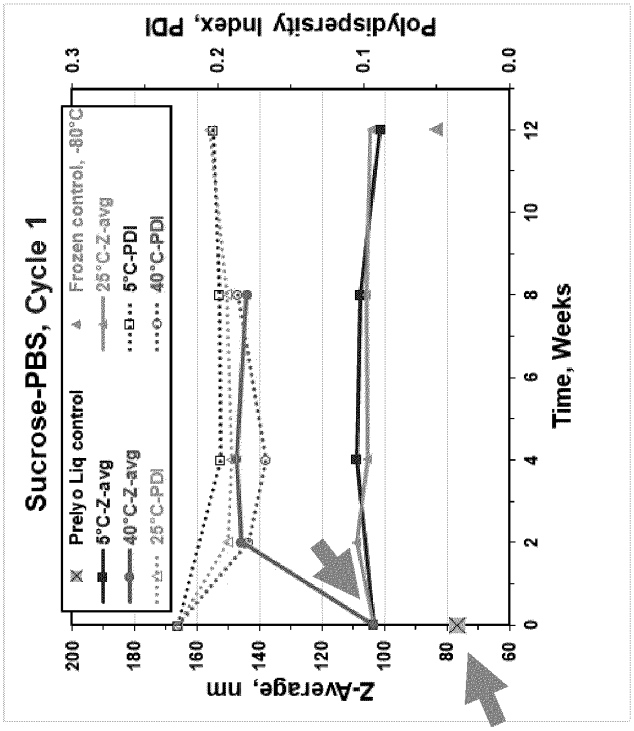
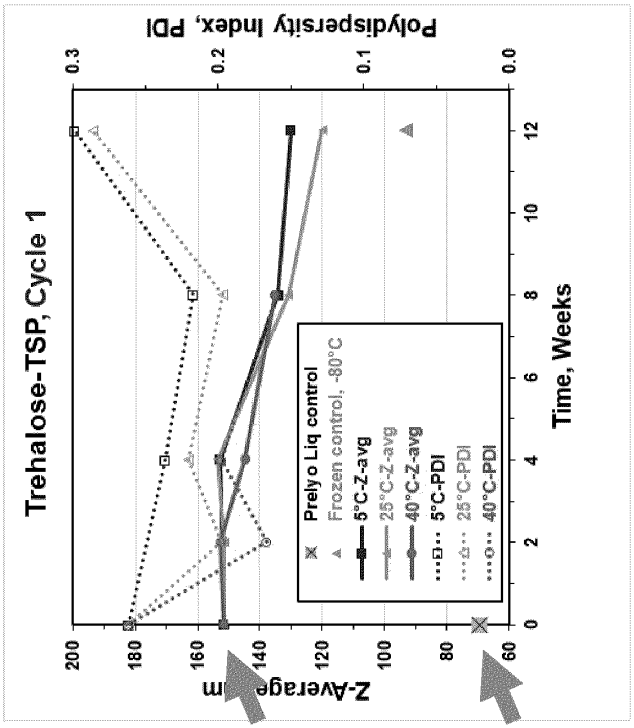
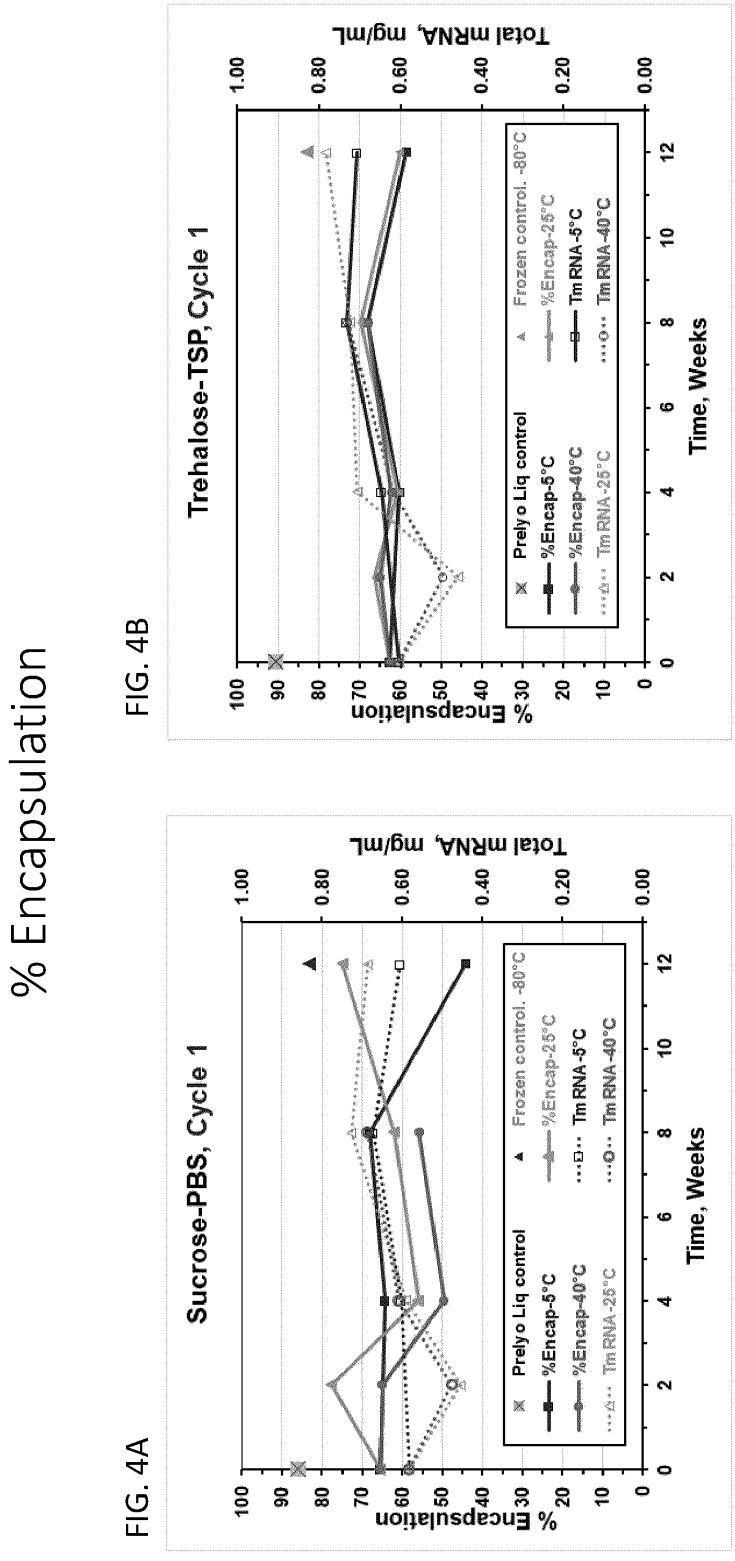


FIG. 3B




% Encapsulation
FIG. 4A
Sucrose-PBS, Cycle 1
% Encapsulation
Total mRNA, mg/mL
Time, Weeks
Prelyo Liq control
%Encap-5°C
%Encap-40°C
Tm RNA-25°C
Frozen control. -80°C
%Encap-25°C
Tm RNA-5°C
Tm RNA-40°C
FIG. 4B
Trehalose-TSP, Cycle 1
% Encapsulation
Total mRNA, mg/mL
Time, Weeks
Prelyo Liq control
%Encap-5°C
%Encap-40°C
Tm RNA-25°C
Frozen control. -80°C
%Encap-25°C
Tm RNA-5°C
Tm RNA-40°C

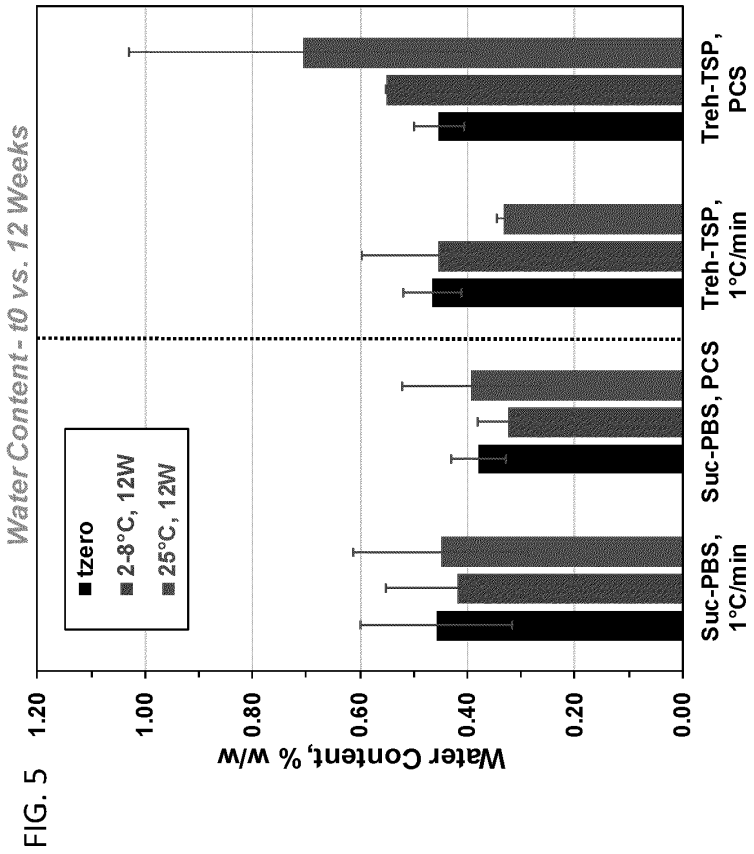

Water Content - t0 vs. 12 Weeks
tzero
2-8°C, 12W
25°C, 12W
Water Content, % w/w
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Suc-PBS, 1°C/min
Suc-PBS, PCS
Treh-TSP, 1°C/min
Treh-TSP, PCS

FIG. 5

% Expression

FIG. 6A

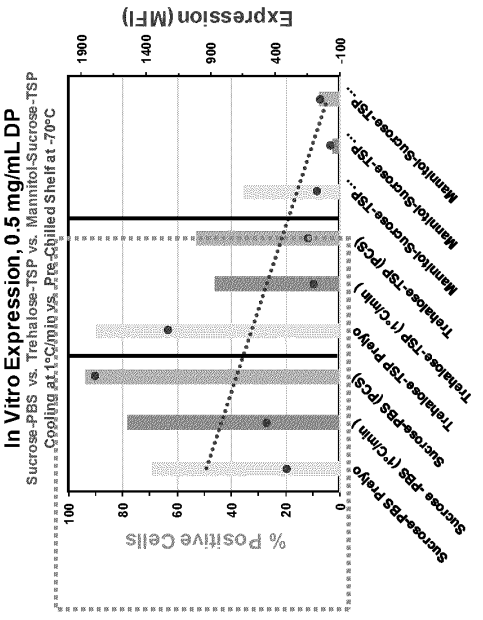


FIG. 6B

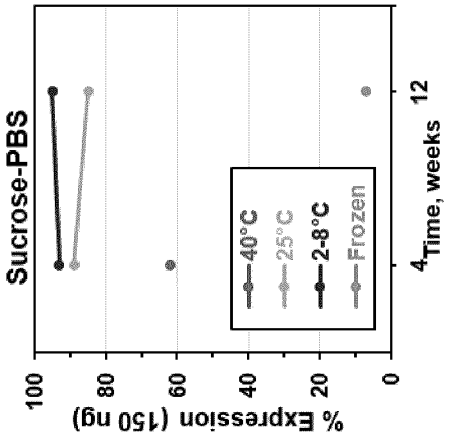


FIG. 6C

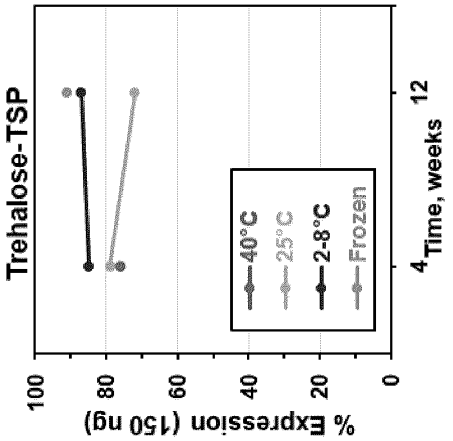


FIG. 7A

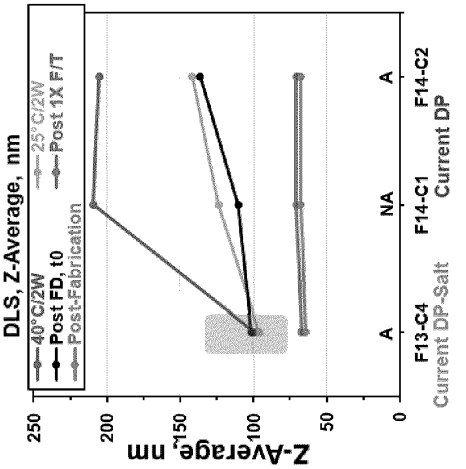


FIG. 7B

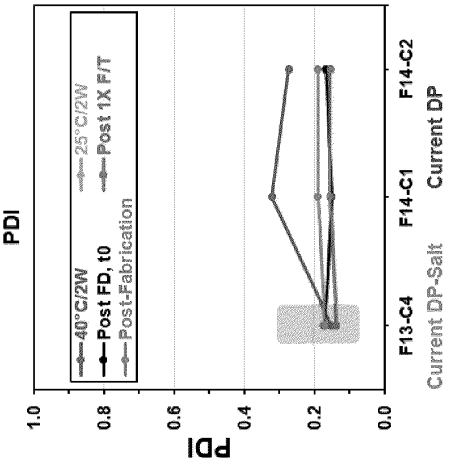


FIG. 7C

FIG. 7D

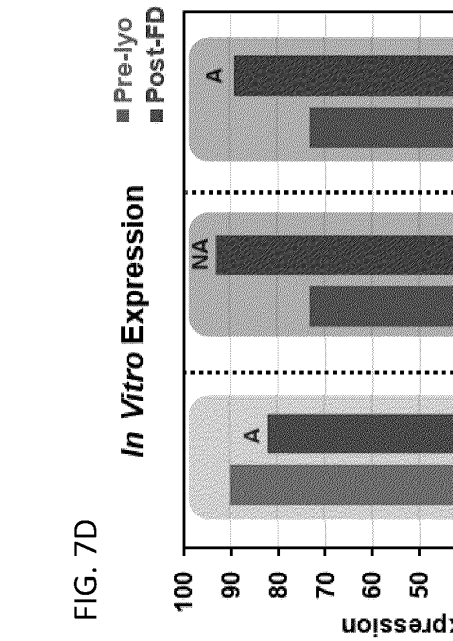
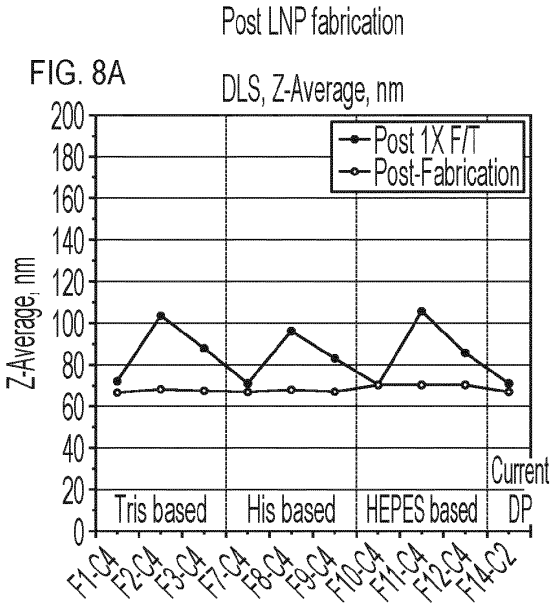
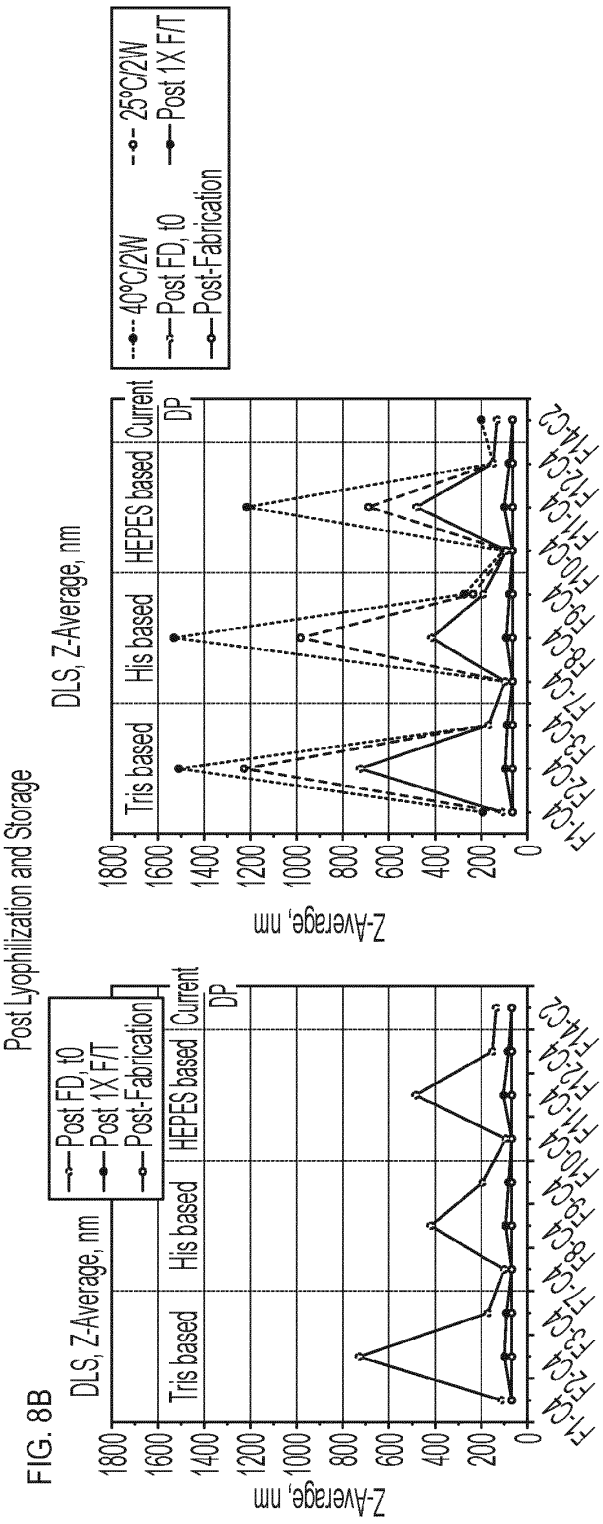


FIG. 8A



- F1, F7, F10 - Sucrose based; salt free; annealed
- F2, F8, F11 - Trehalose based; salt free; annealed
- F3, F9, F12 - Sucrose + Trehalose based; salt free; annealed
- F14 - Current DP; annealed

FIG. 8B


Post Lyophilization and Storage
DLS, Z-Average, nm
Post FD, t0
Post 1X F/T
Post-Fabrication
Tris based
His based
HEPES based
Current DP
Z-Average, nm
1800
1600
1400
1200
1000
800
600
400
200
0
F1-C4
F2-C4
F3-C4
F7-C4
F8-C4
F9-C4
F10-C4
F11-C4
F12-C4
F14-C2
DLS, Z-Average, nm
40°C/2W
25°C/2W
Post FD, t0
Post 1X F/T
Post-Fabrication
Tris based
His based
HEPES based
Current DP
Z-Average, nm
F1-C4
F2-C4
F3-C4
F7-C4
F8-C4
F9-C4
F10-C4
F11-C4
F12-C4
F14-C2