

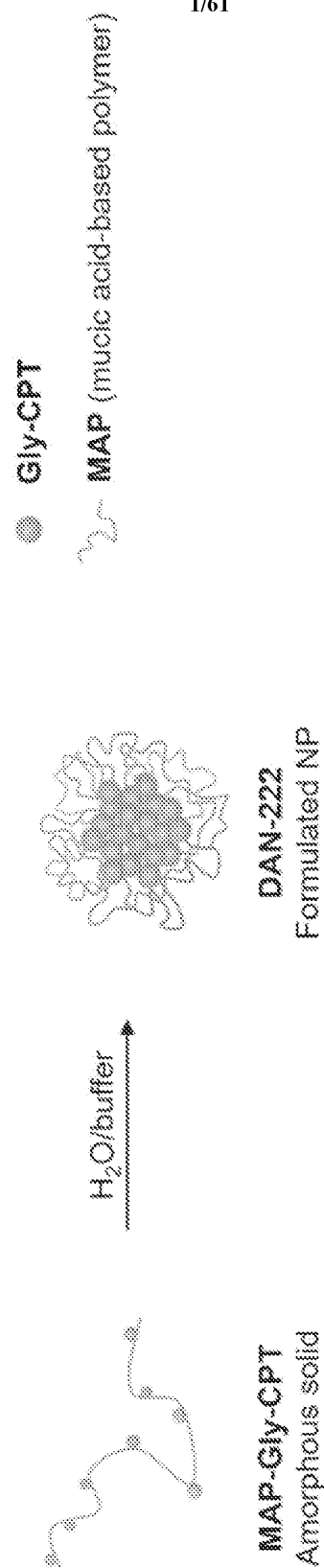
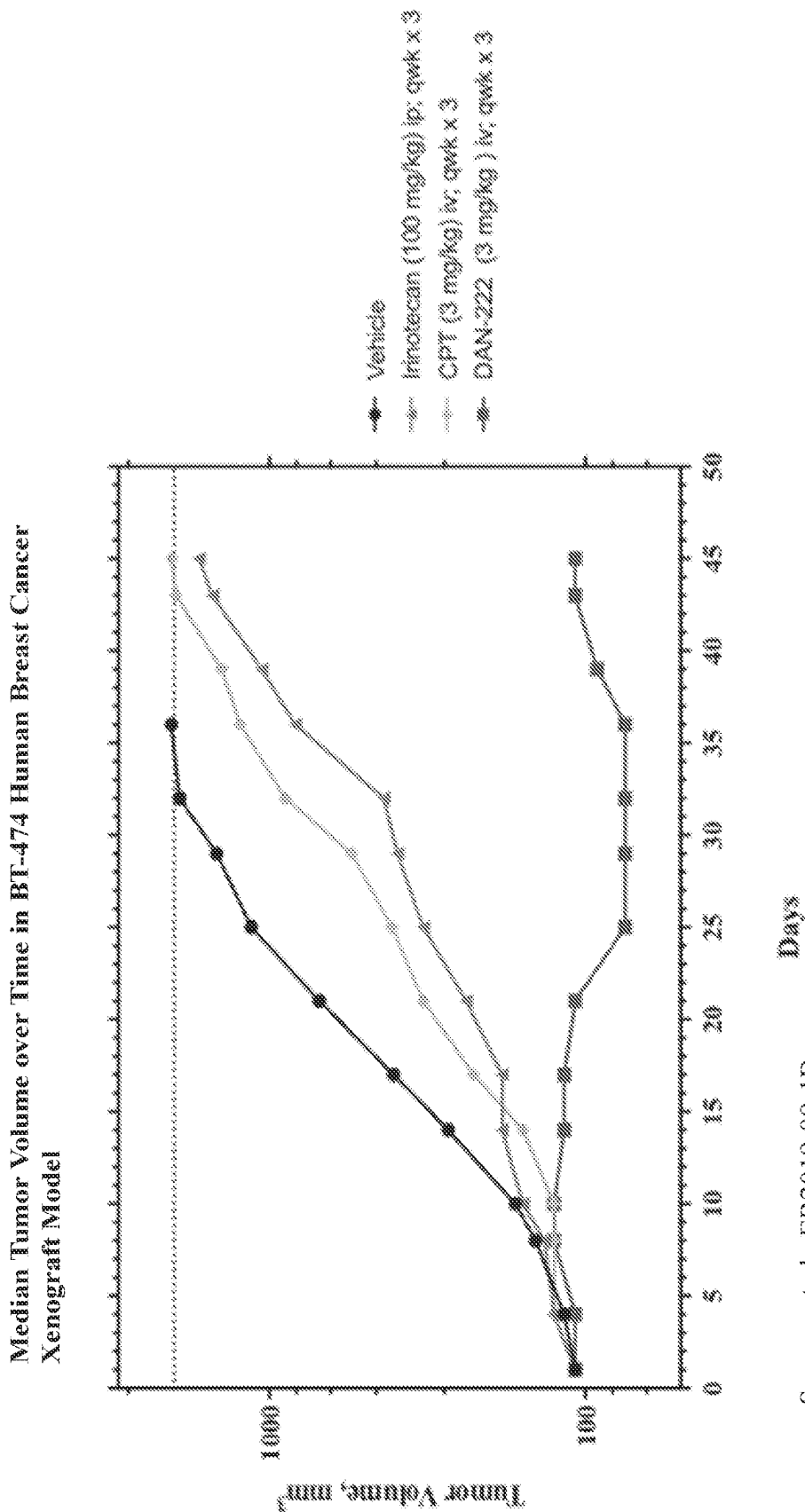
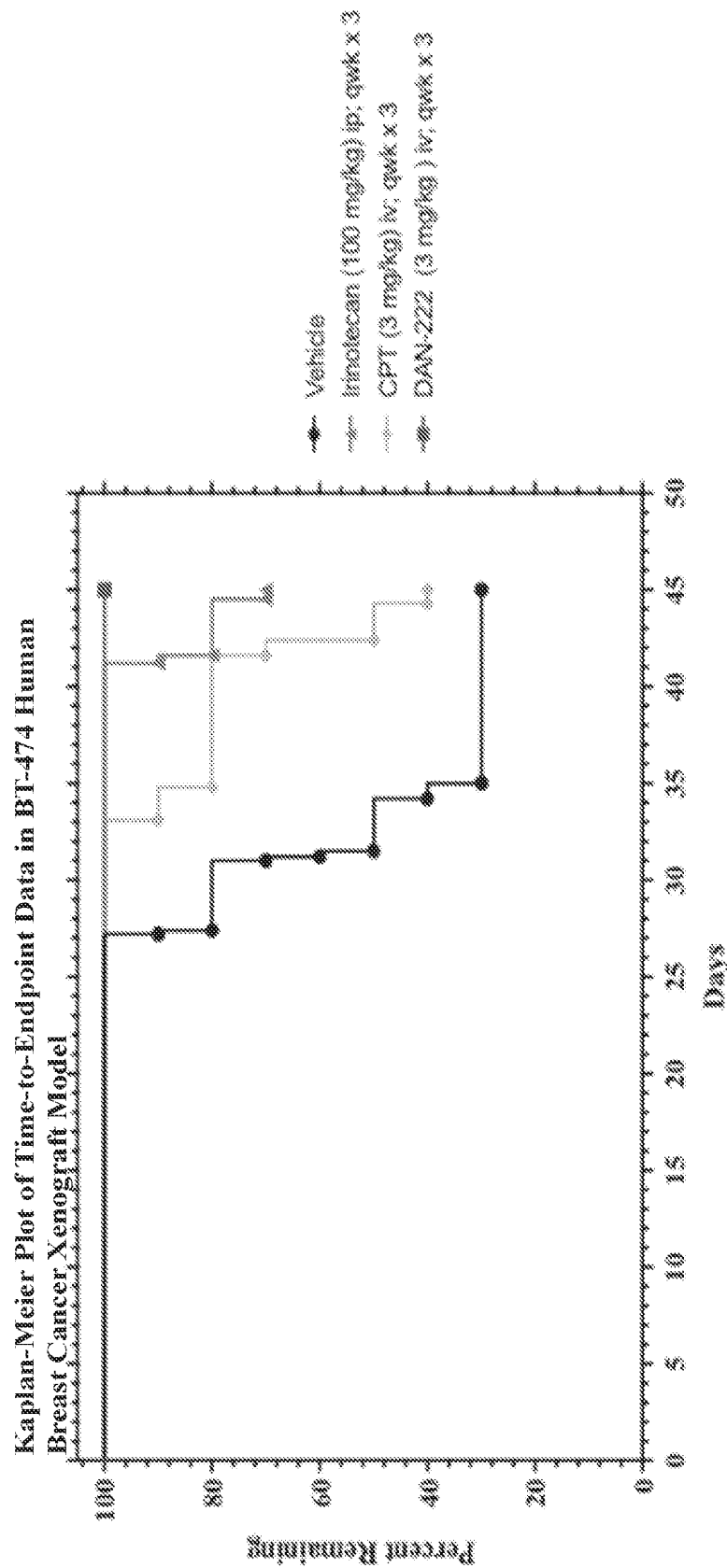


FIG. 1



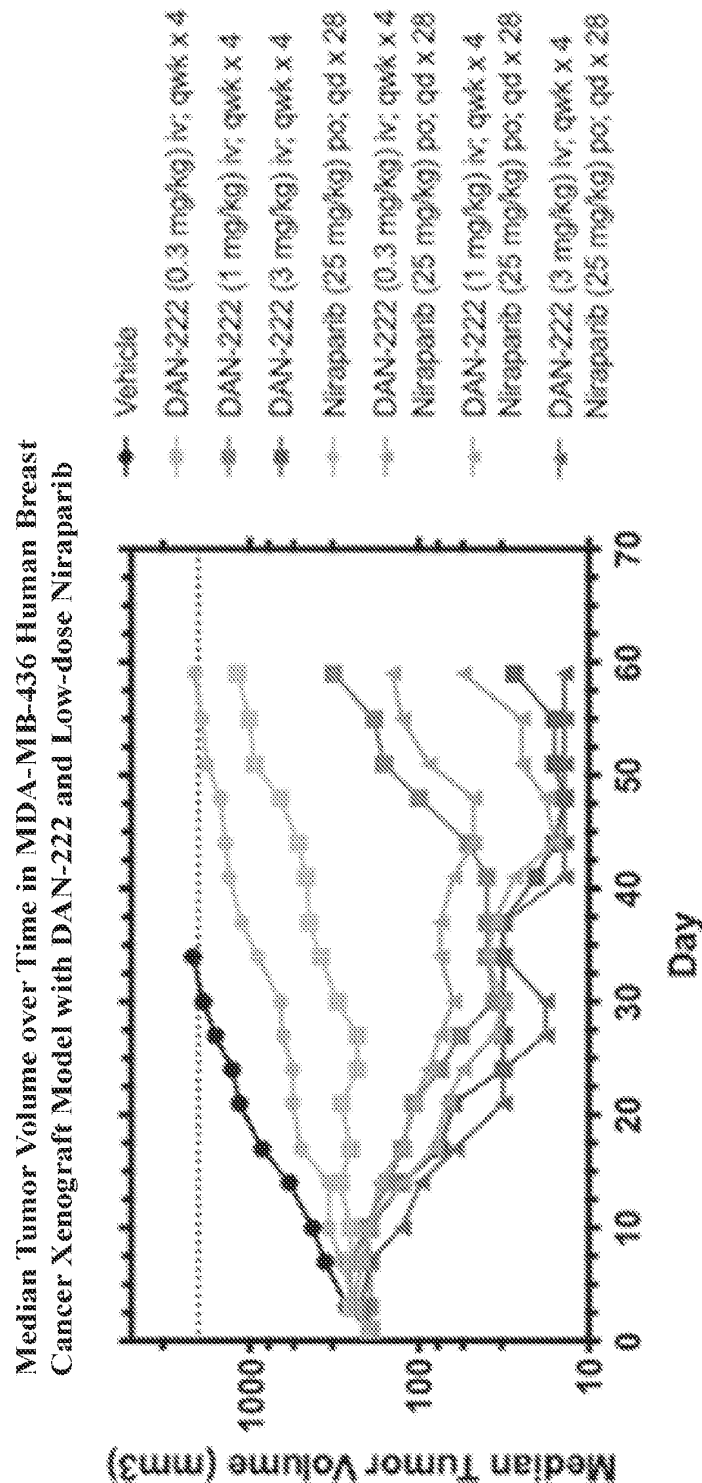
Source: study ER2019-09-1B
CPT = camptothecin; ip=intraperitoneal, iv= intravenously, qwk = once per week
Note: Dashed line indicates tumor volume endpoint for time to endpoint calculations (2000 mm³).

FIG. 2



Source: Study ER2019-09-1B
CPT = camptothecin; ip=intraperitoneal; iv=intravenously, qwk = once per week

FIG. 3



Source: Study ER2020-07-1
iv = intravenous; po = oral administration; qd = once per day; qwk = once per week.
Note: Dashed line indicates tumor volume endpoint for time to endpoint calculations (2000 mm³).

FIG. 4

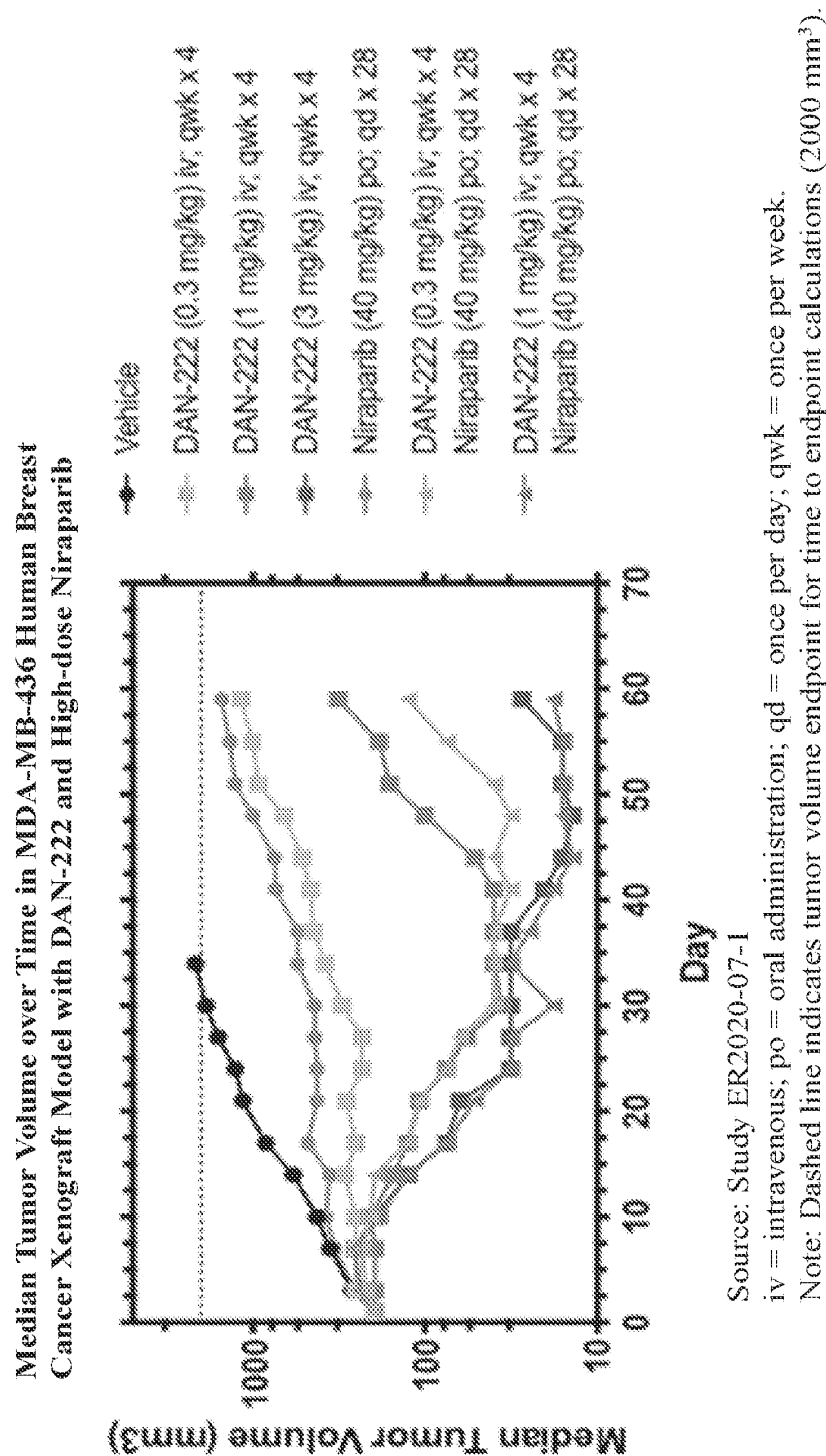


FIG. 5

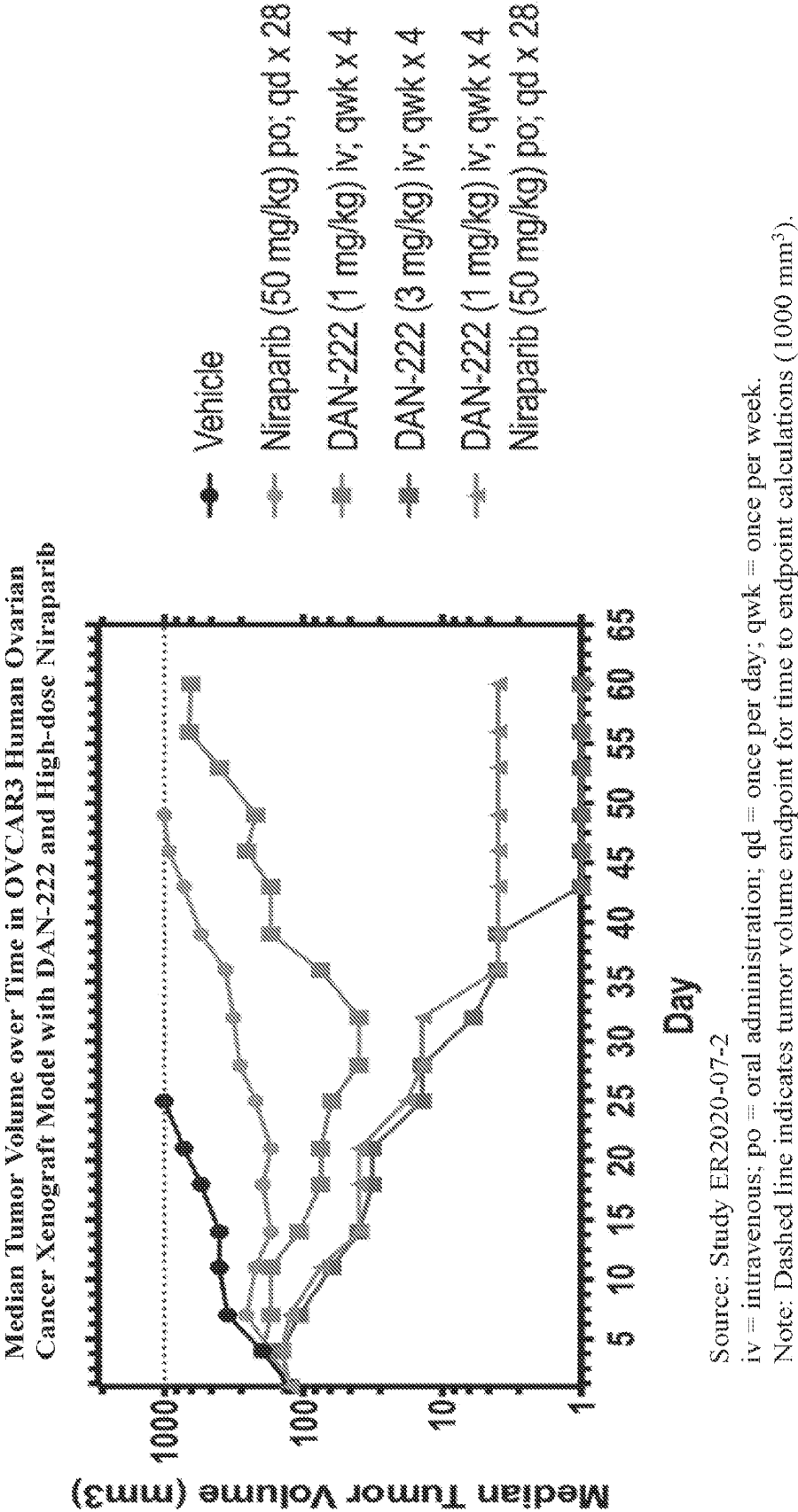


FIG. 6

Concentrations of Camptothecin in Plasma of Male Rats after Intravenous Dosing of DAN-222 on Days 1 and 22

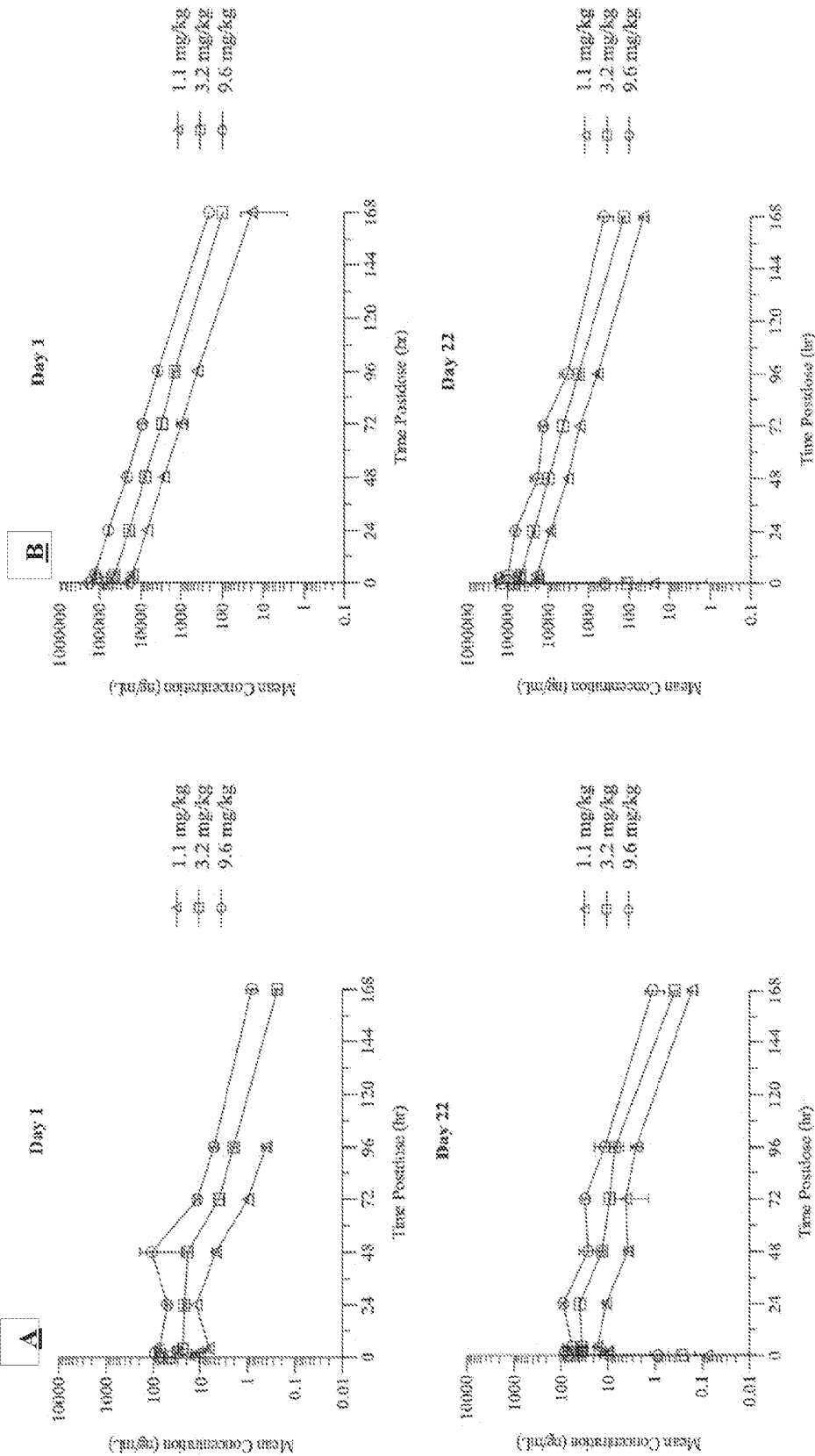
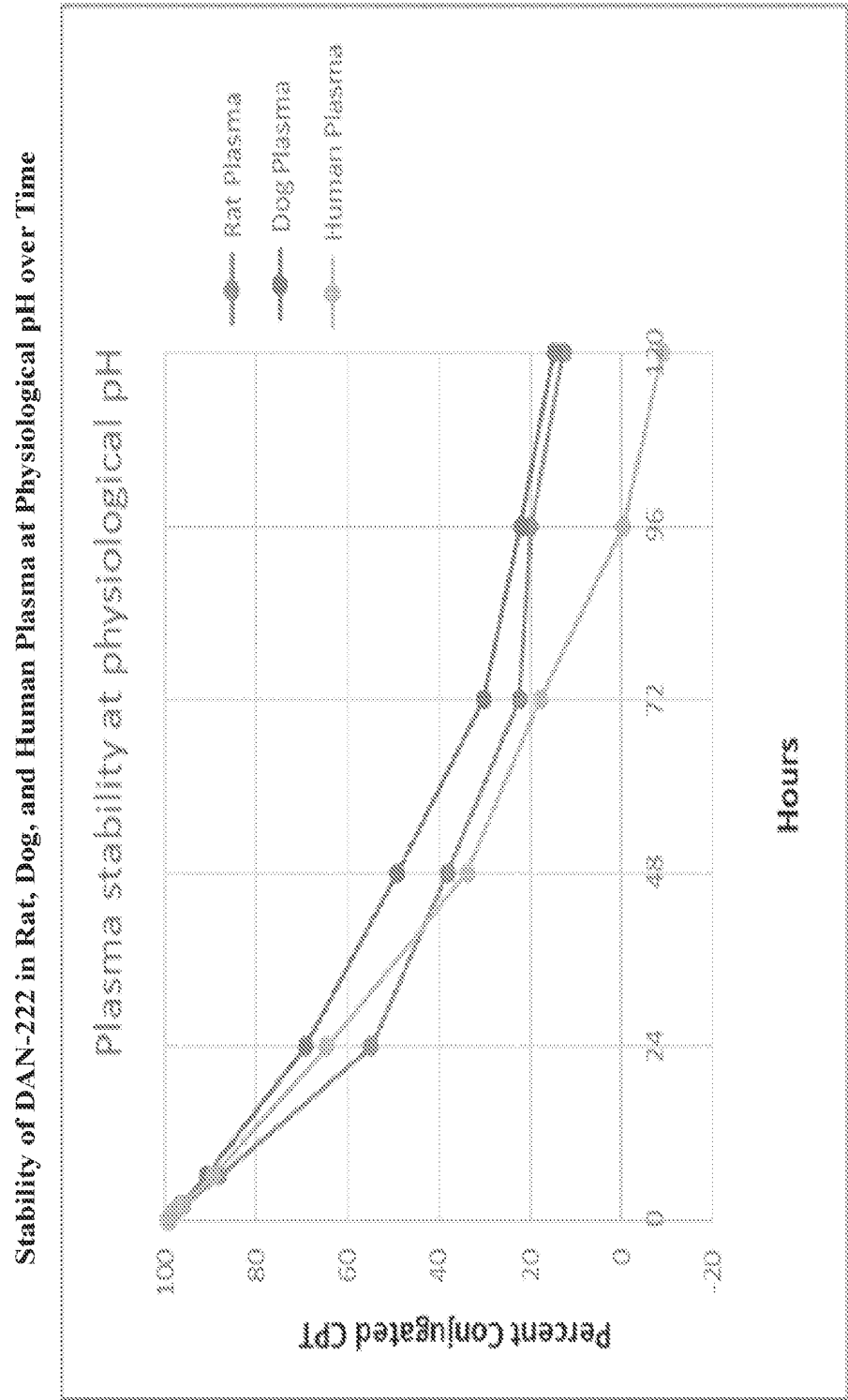


FIG. 7

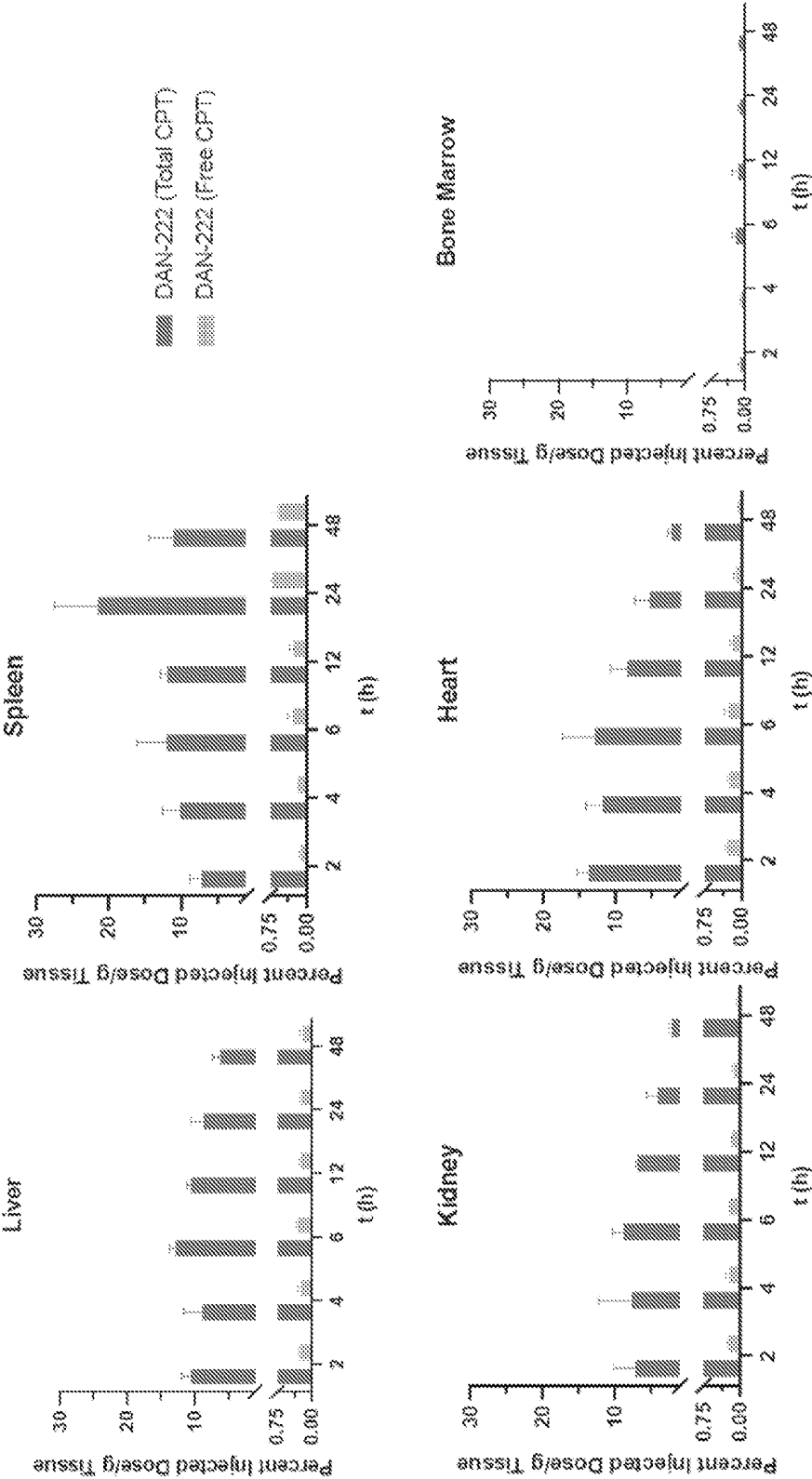
Source: Study No. NC2020-08-3.
Note: Mean ($\pm$ standard deviation) of free (A) and total (B) camptothecin in the plasma of male rats over time



Source: Study PEAA-0002-DB-AC
CPT = camptothecin

FIG. 8

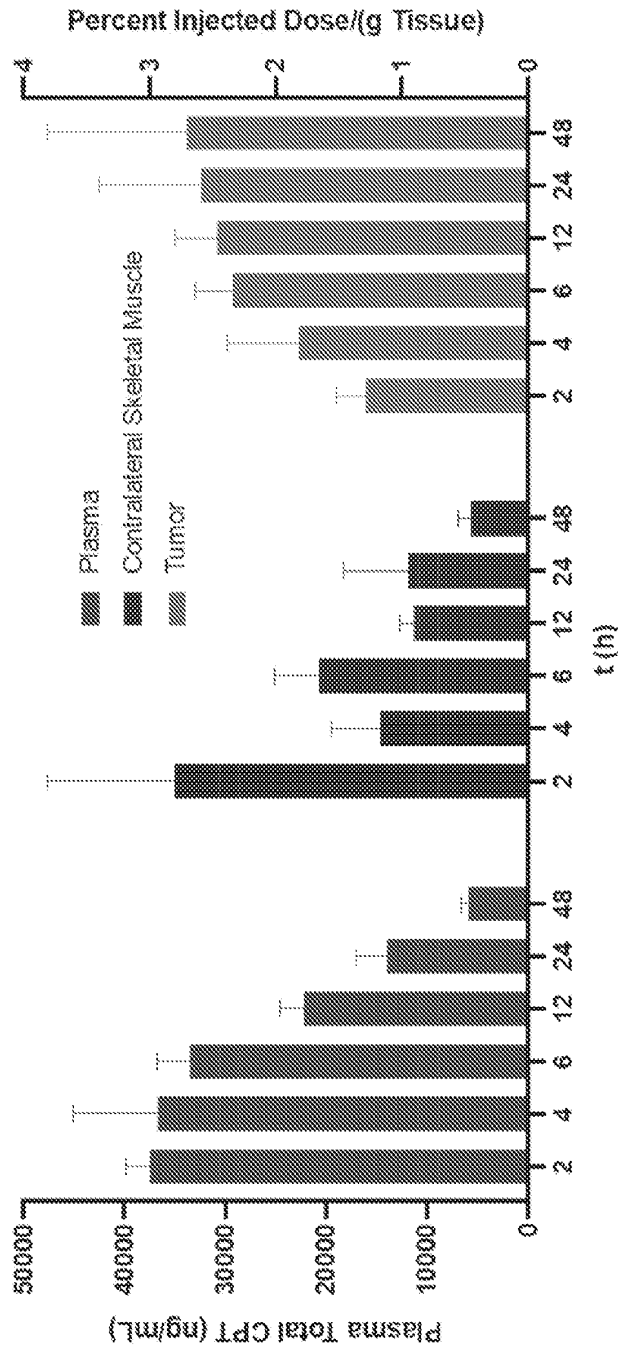
Biodistribution of DAN-222 in JIMT-1 Xenograft Mice



Source(s): Study No. BR2021-01-1 (JIMT-1-e293)
CPT = camptothecin
Note: Total CPT in plasma (ng/mL, left y-axis) and in skeletal muscle and tumor (%ID/g, right y-axis).

FIG. 9A

Biodistribution of DAN-222 in JIMT-1 Xenograft Mice



Source(s): Study No. BR2021-01-1 (JIMT-1-e293)
CPT = camptothecin
Note: Total CPT in plasma (ng/mL, left y-axis) and in skeletal muscle and tumor (%ID/g, right y-axis).

FIG. 9B

Study Reference	Species/Test System	Test Drug/Dose	Major Findings
BR2020-07-1	Mouse/CD-1	DAN-222: 3 mg/kg, IV, single-dose Irinotecan: 60 mg/kg, IV, single-dose	Concentrations of free and total CPT were detectable in the liver, heart, spleen, and kidney for up to 48 hours after dosing. Mean concentrations of free CPT were below the limit of quantitation (< 1.00 ng/mL) in the bone marrow at all time points tested. Free CPT released from DAN-222 was < 0.1% of injected dose per gram of tissue in all organs. Irinotecan was cleared from tissue within 24 hours. DAN-222 and free CPT exposure in bone marrow was greatly reduced as compared to irinotecan.
BR2020-08-1	Mouse/ Athymic nude (Ncr nu/nu)	DAN-222: 3 mg/kg, IV, single-dose Irinotecan: 60 mg/kg, IV, single-dose	Concentrations of free and total CPT were detectable in the liver, heart, spleen, kidney, and tumor tissue for up to 12 hours after dosing. Free CPT released from DAN-222 was < 0.1% of injected dose per gram of tissue in all organs. In tumor tissues, accumulation and retention of total and free CPT was observed over 12 hours after DAN-222 dosing, while irinotecan levels decreased over the same period. In bone marrow, total and free CPT exposure after DAN-222 administration was greatly reduced when compared with irinotecan exposure. Concentrations of free CPT after DAN-222 dosing were below the limit of quantitation (< 1.00 ng/mL) in the bone marrow at all time points tested.
BR2021-01-1	Mouse/CB.17 SCID	DAN-222: 3 mg/kg, IV, single-dose	DAN-222 (total CPT) cleared from plasma and healthy tissue over the course of the study. Levels of free CPT released from DAN-222 were found to be < 1% of injected dose per gram of tissue in all organs tested. Prolonged retention of total CPT was not observed in plasma or normal tissue (i.e., skeletal muscle from the contralateral flank) over 48 hours after dosing; whereas, in tumor tissues, accumulation and retention of total CPT was observed over the course of the study with measured levels doubling from 2 to 48 hours.

CPT = camptothecin; IV = intravenous; SCID = severe combined immunodeficient.

FIG. 10

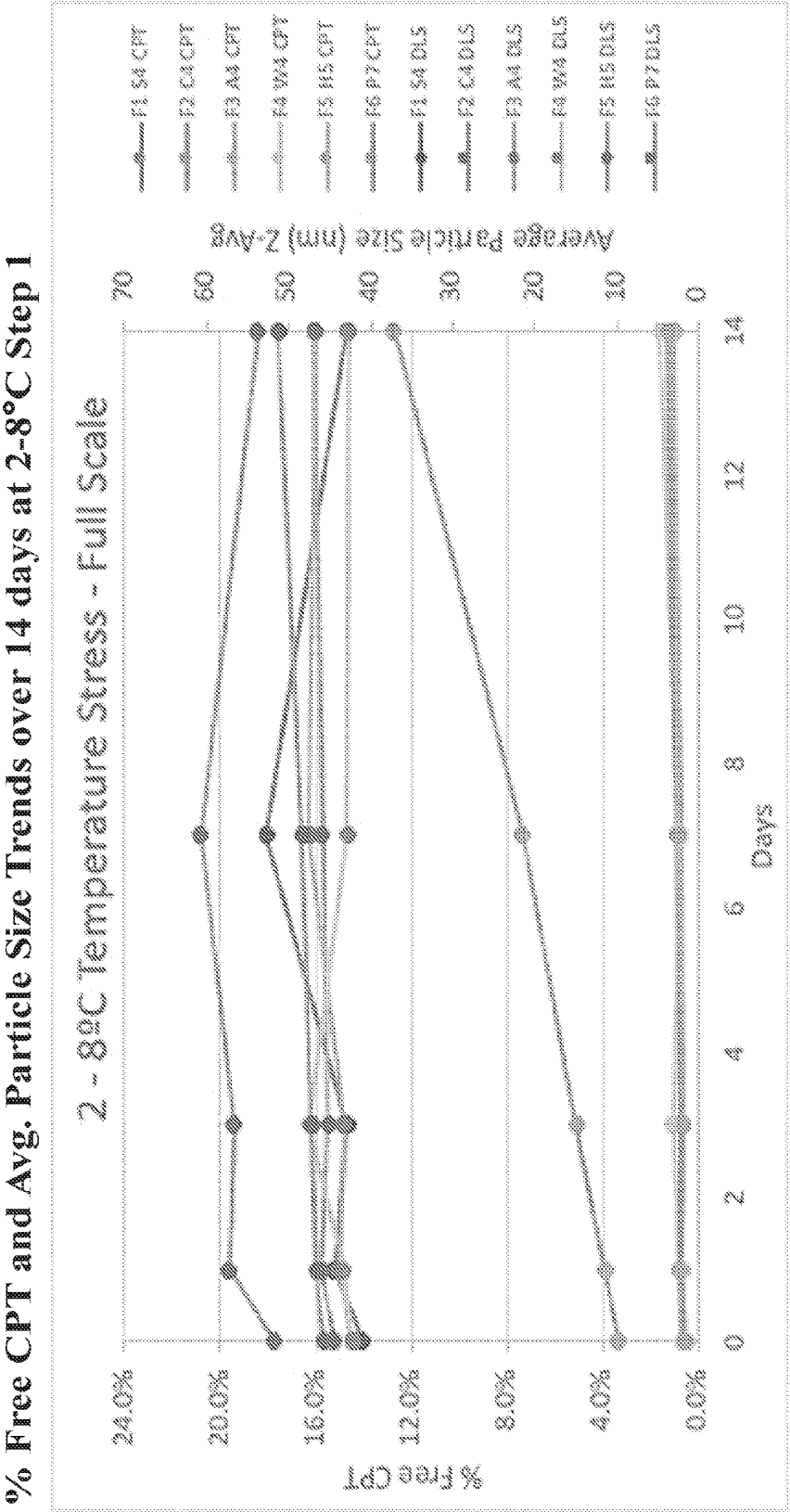


FIG. 11

**% Free CPT and Avg. Particle Size Trends
over 14 days at 20-25°C Step 1**

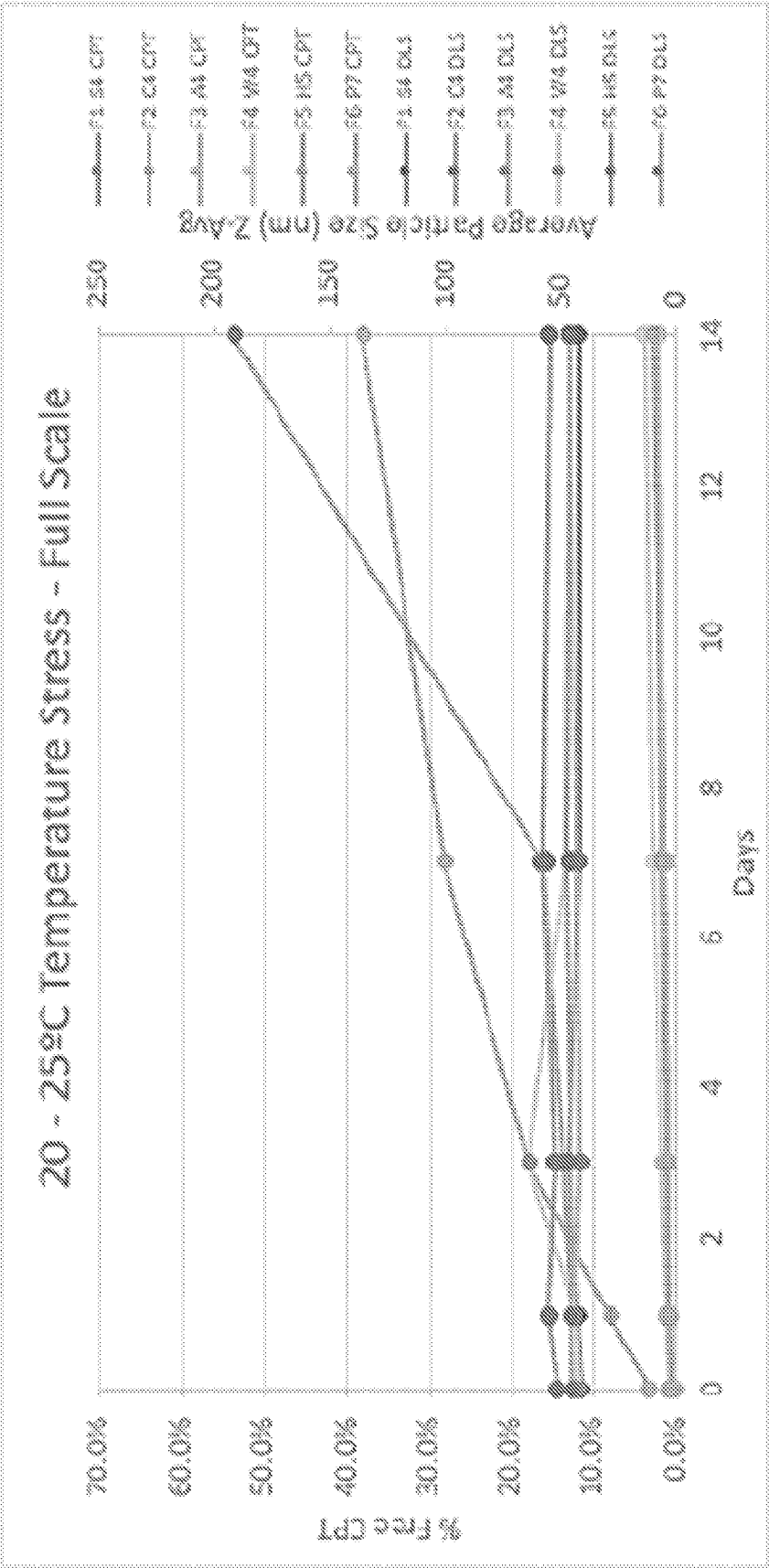


FIG. 12

**% Free CPT and Avg. Particle Size Trends
over 14 days at 40°C Step 1**

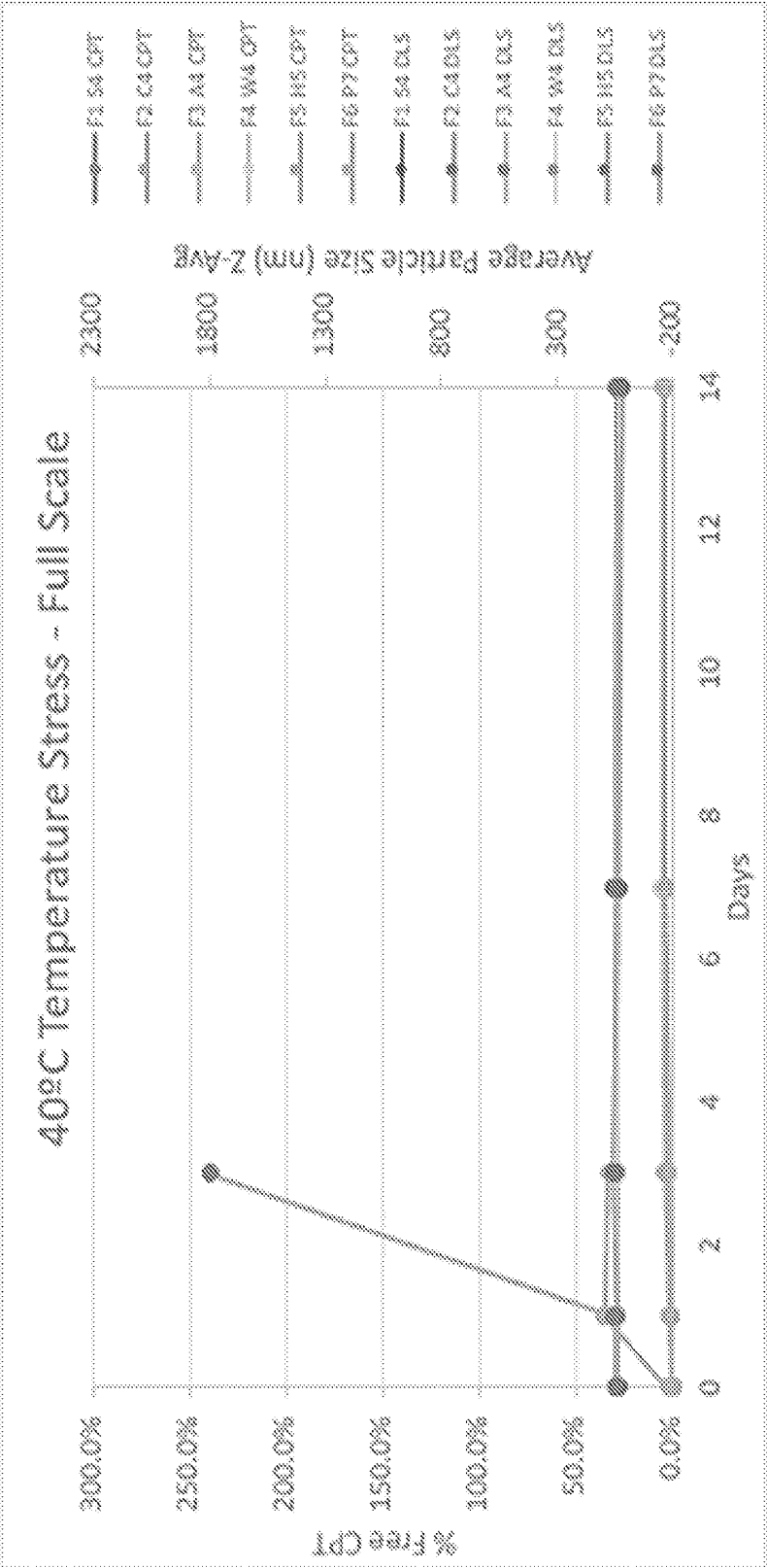


FIG. 13

% Free CPT and Avg. Particle Size Trends over 24 hours of Agitation Step 1

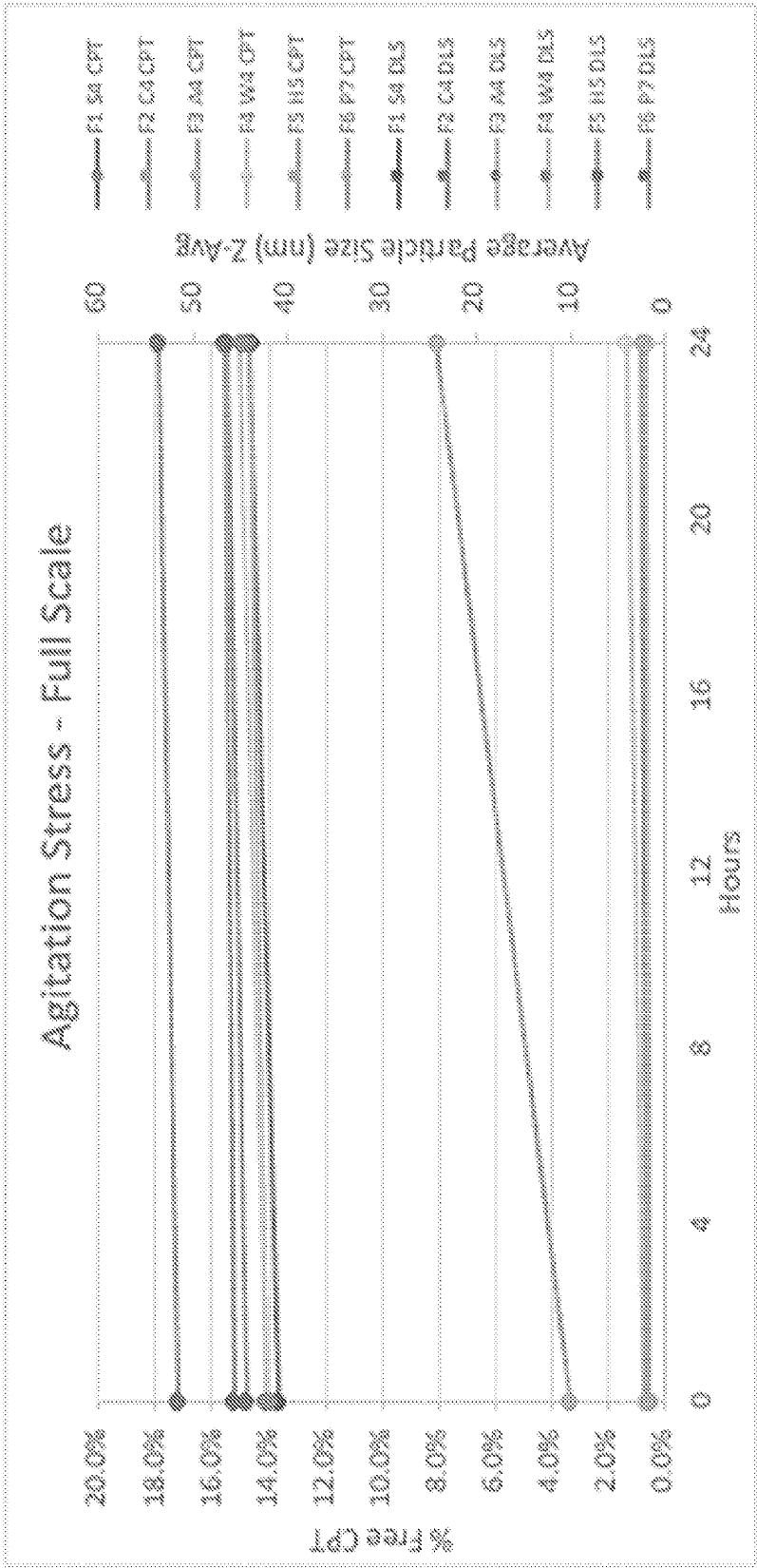


FIG. 14

%Free CPT and Avg. Particle Size Trends over 6 Freeze/Thaw Cycles Step 1

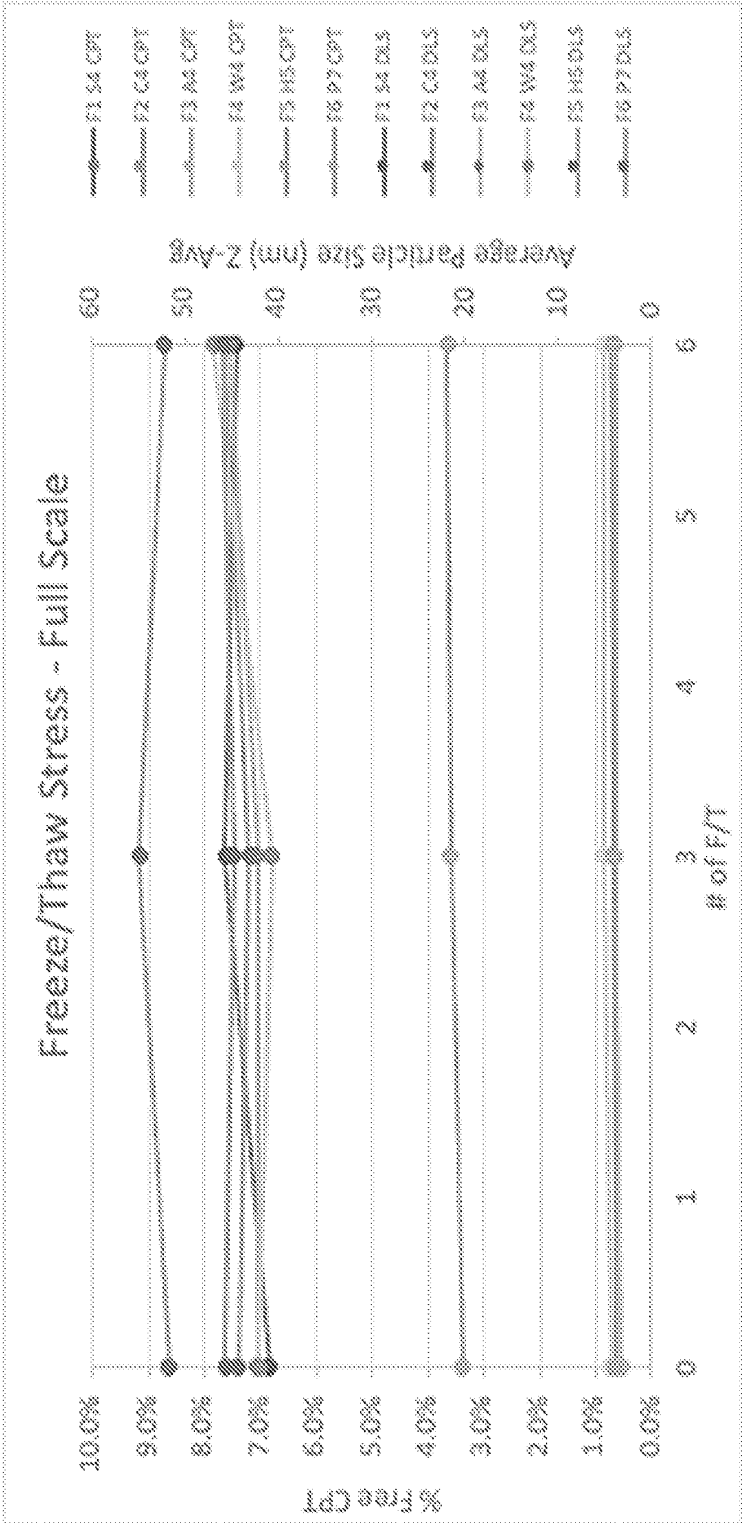


FIG. 15

% Free CPT and Avg. Particle Size Trends over 24 hours of 0.02% H₂O₂ Stress
Step 1

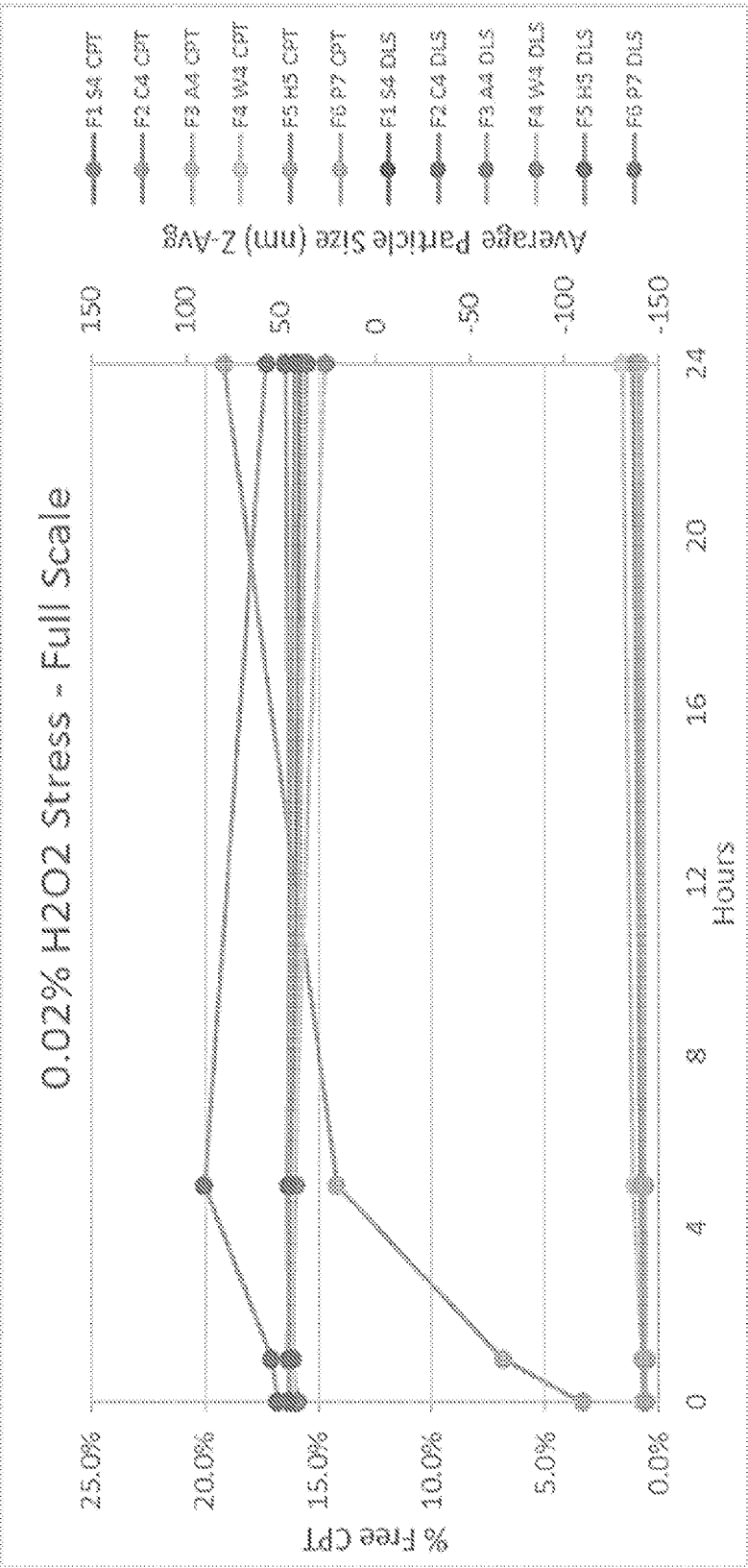


FIG. 16

**% Free CPT and Avg. Particle Size Trends
over 24 hours of 0.05% H₂O₂ Stress**

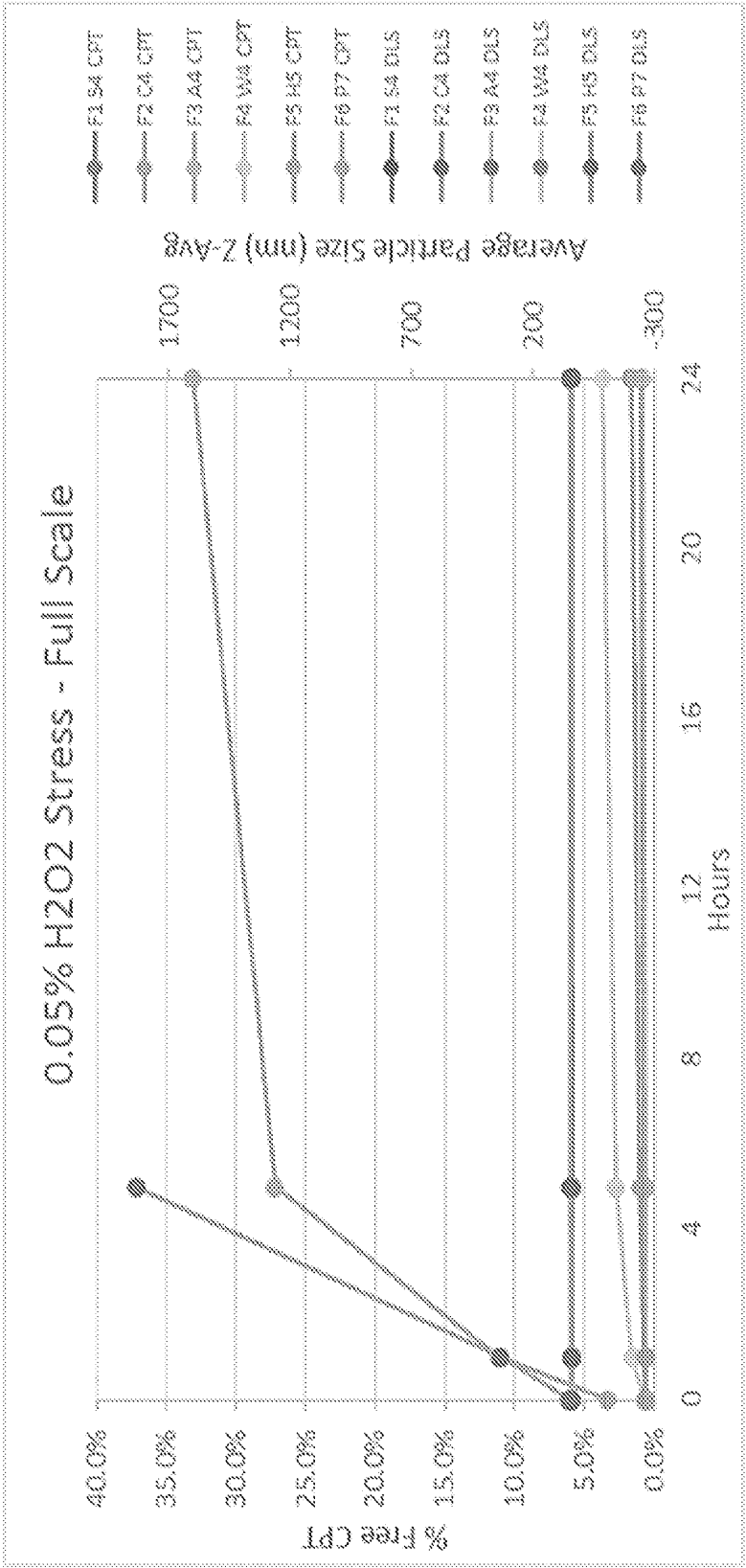


FIG. 17

%Free CPT and Avg. Particle Size Trends over 1 month at 2-8°C Step 2

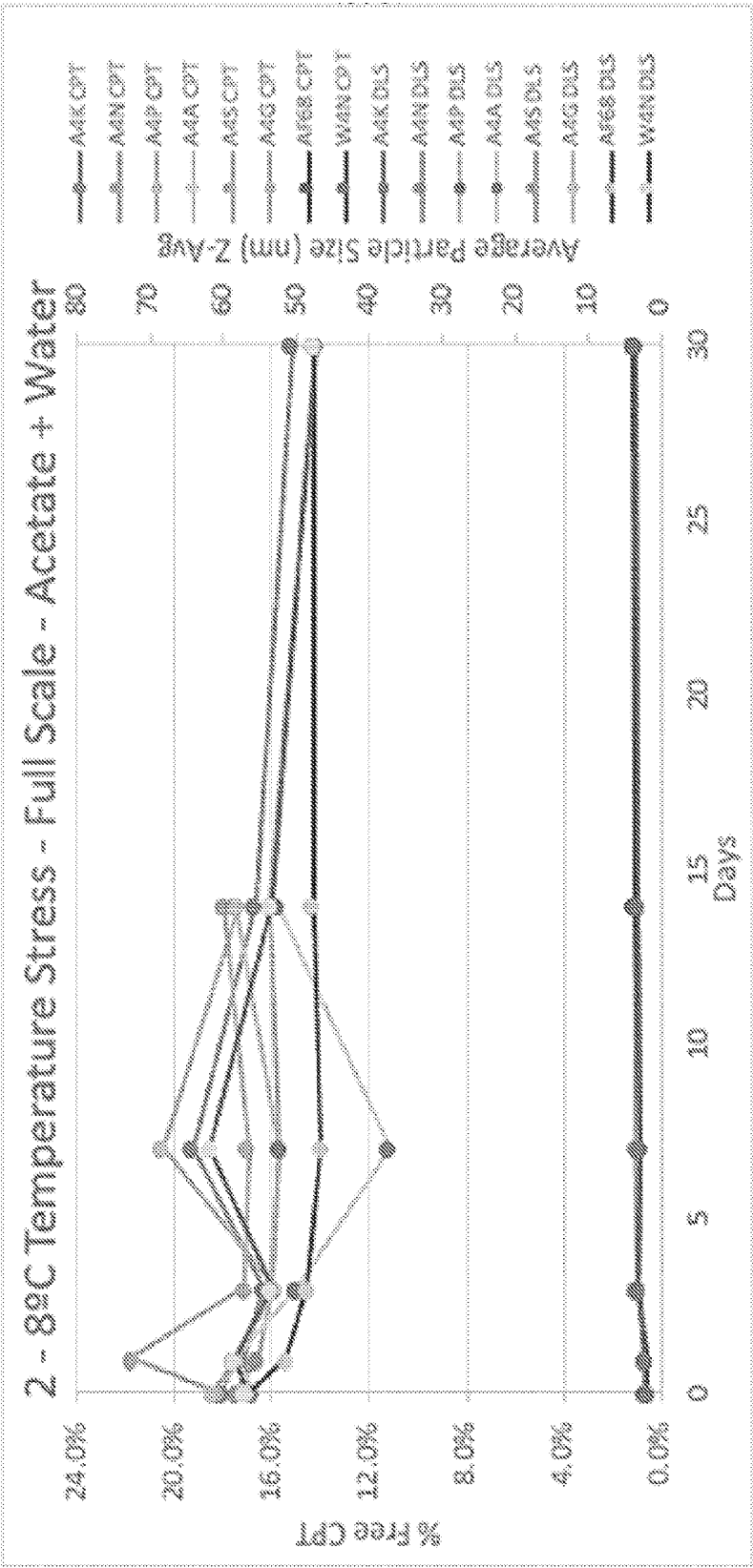


FIG. 18A

%Free CPT and Avg. Particle Size Trends over 1 month at 2-8°C Step 2

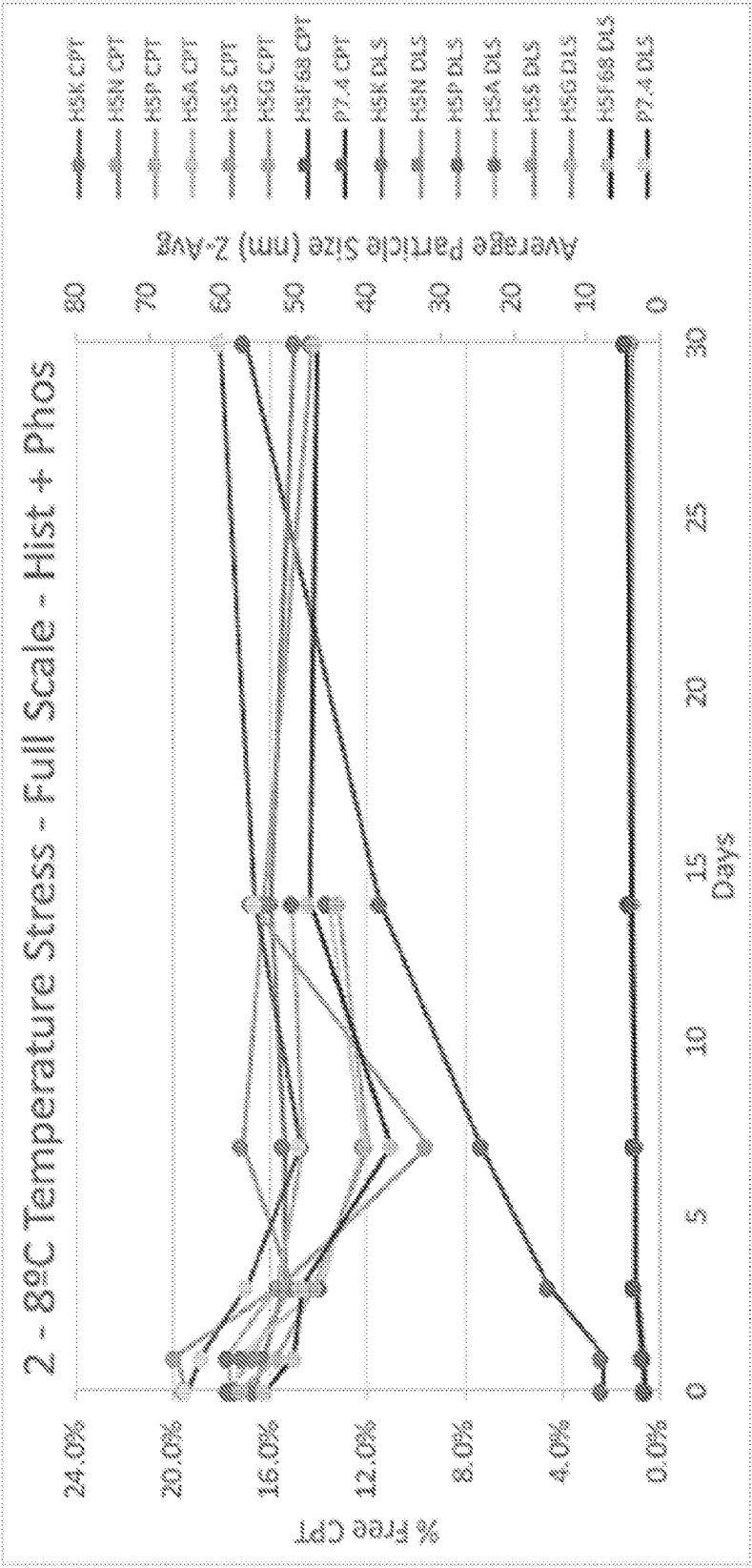


FIG. 18B

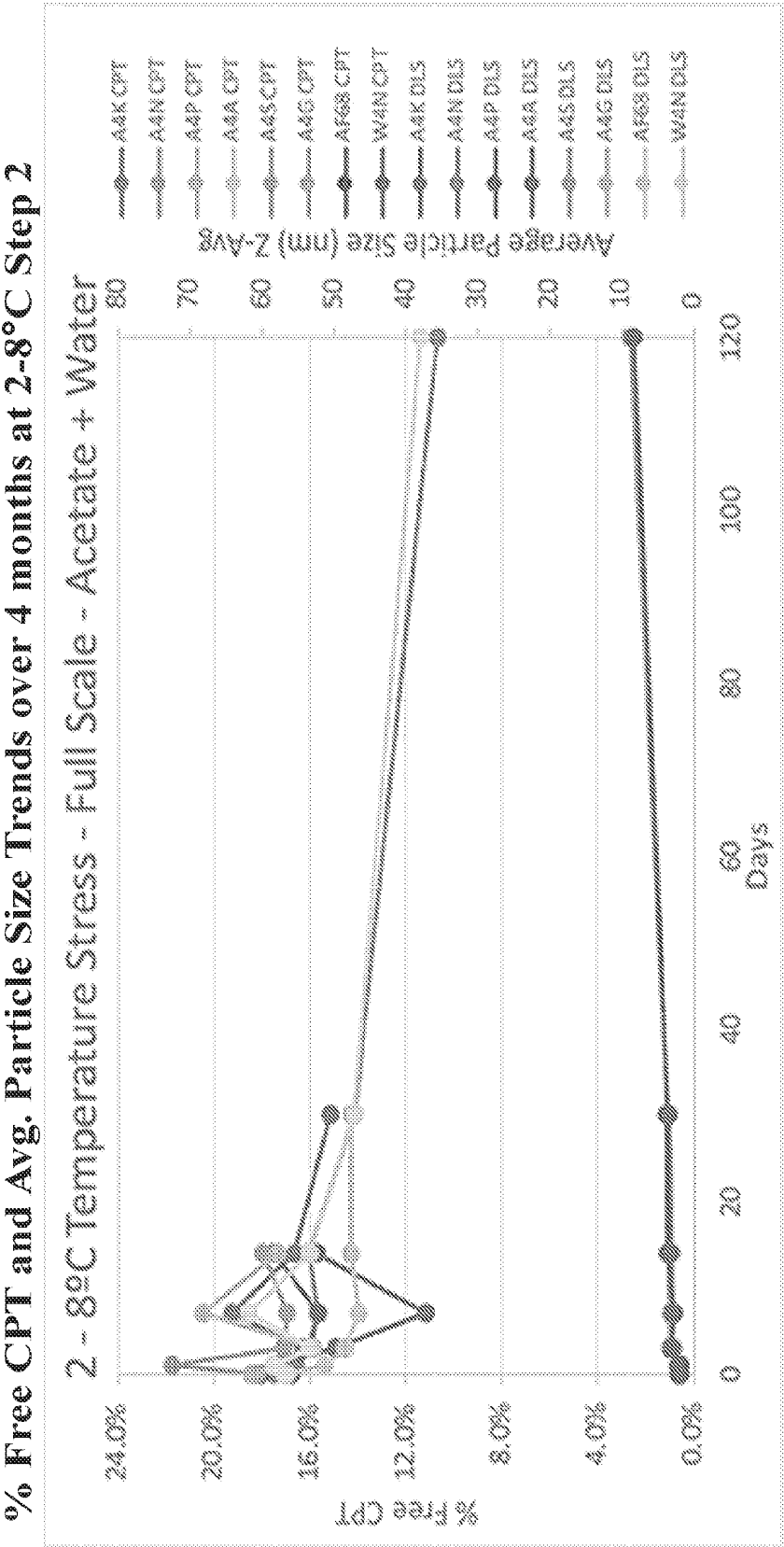


FIG. 19

% Free CPT and Avg. Particle Size Trends over 1 month at 20-25°C Step 2

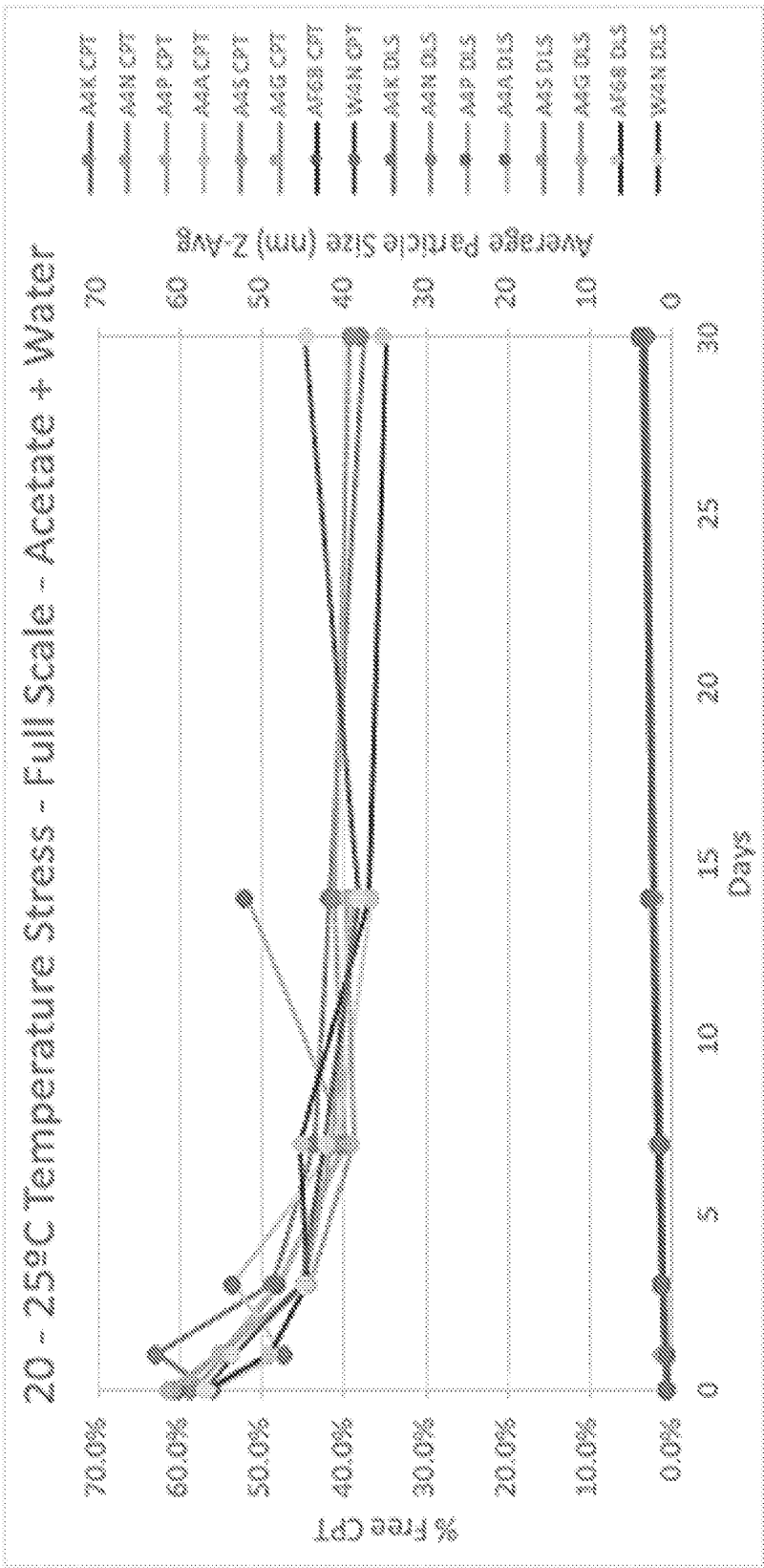


FIG. 20A

% Free CPT and Avg. Particle Size Trends over 1 month at 20-25°C Step 2

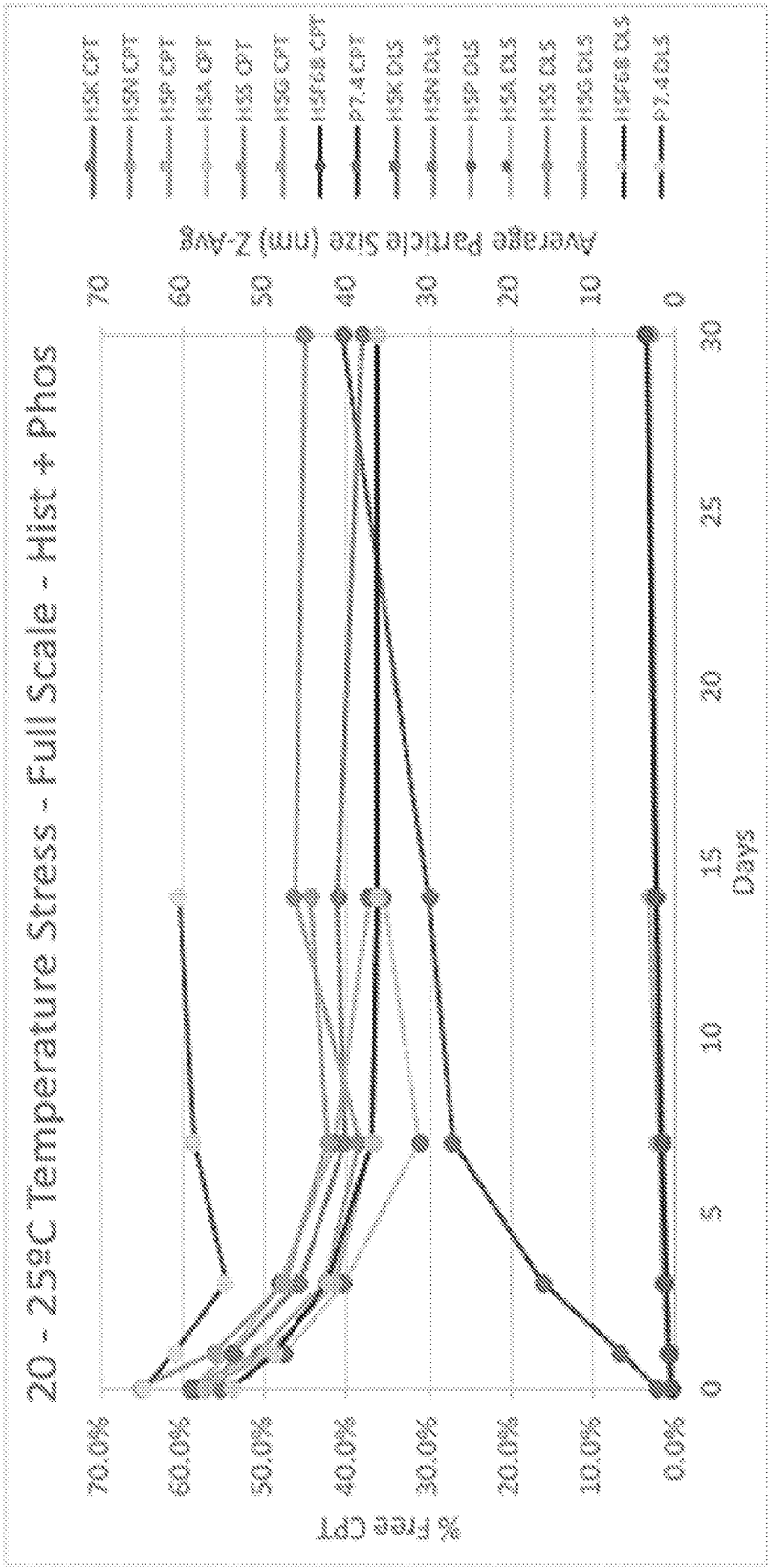


FIG. 20B

% Free CPT and Avg. Particle Size Trends over 7 days at 40°C Step 2

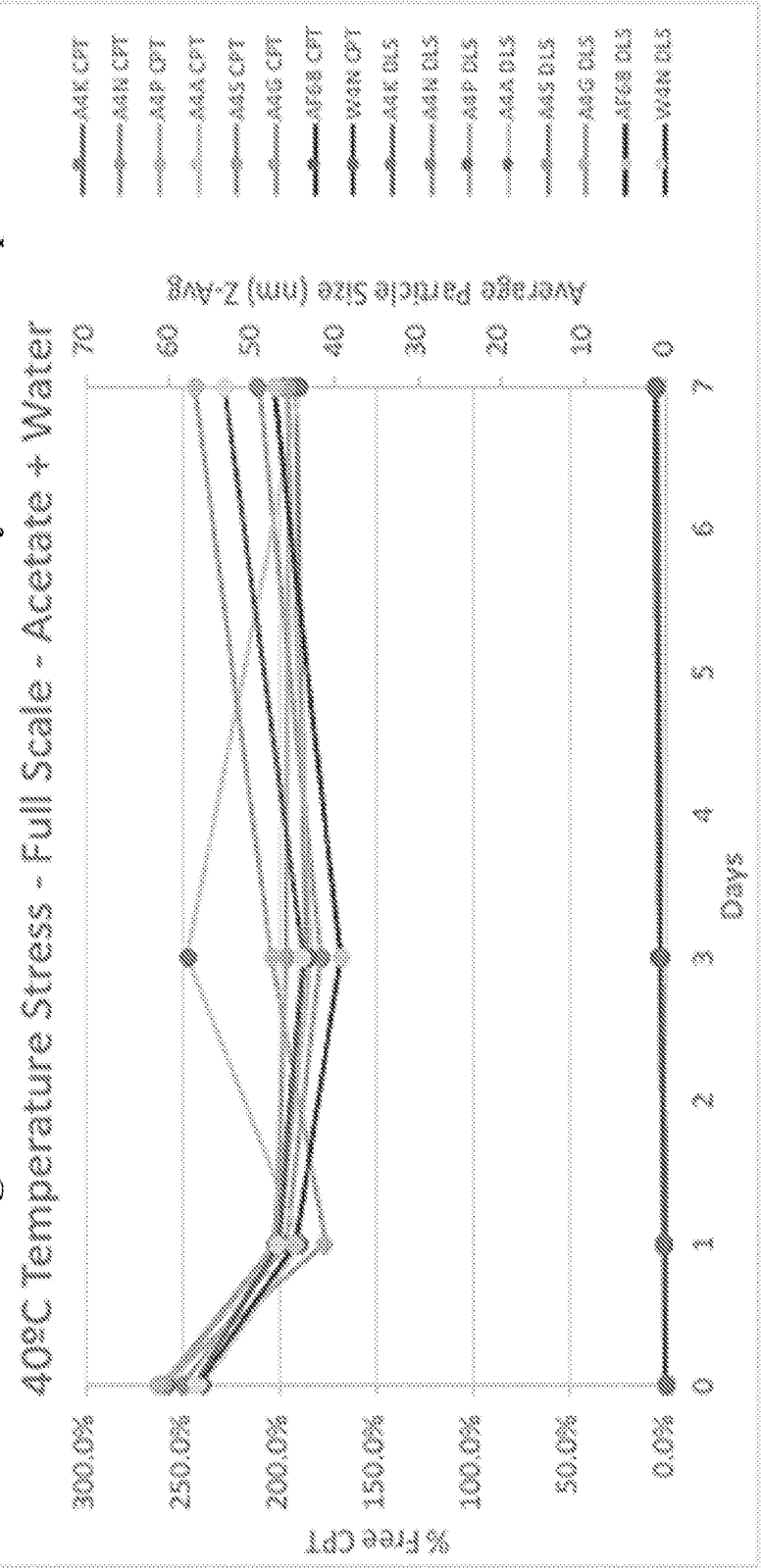


FIG. 21A

% Free CPT and Avg. Particle Size Trends over 7 days at 40°C Step 2

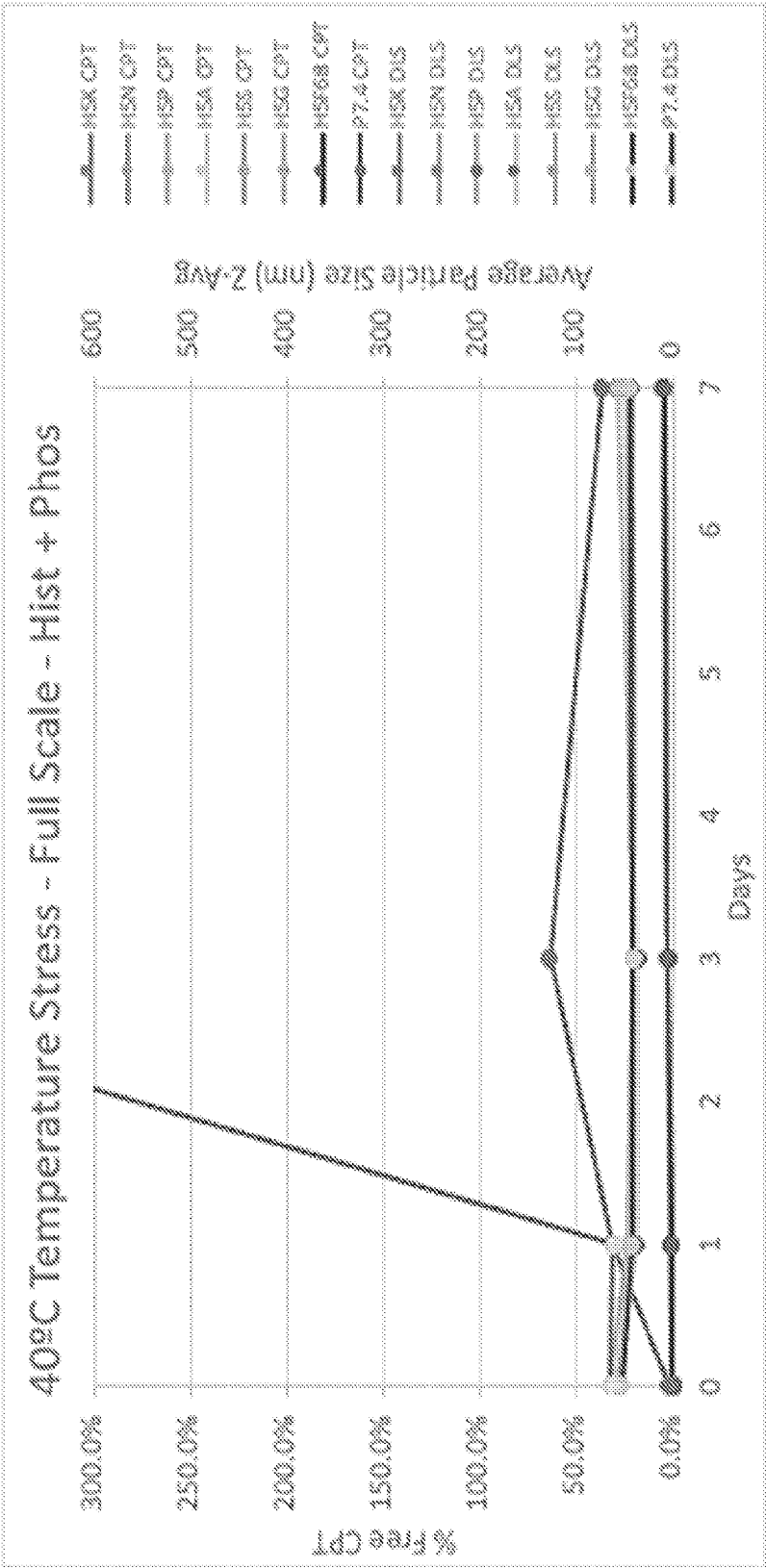


FIG. 21B

% Free CPT and Avg. Particle Size Trends over 4 months at -20°C Step 2

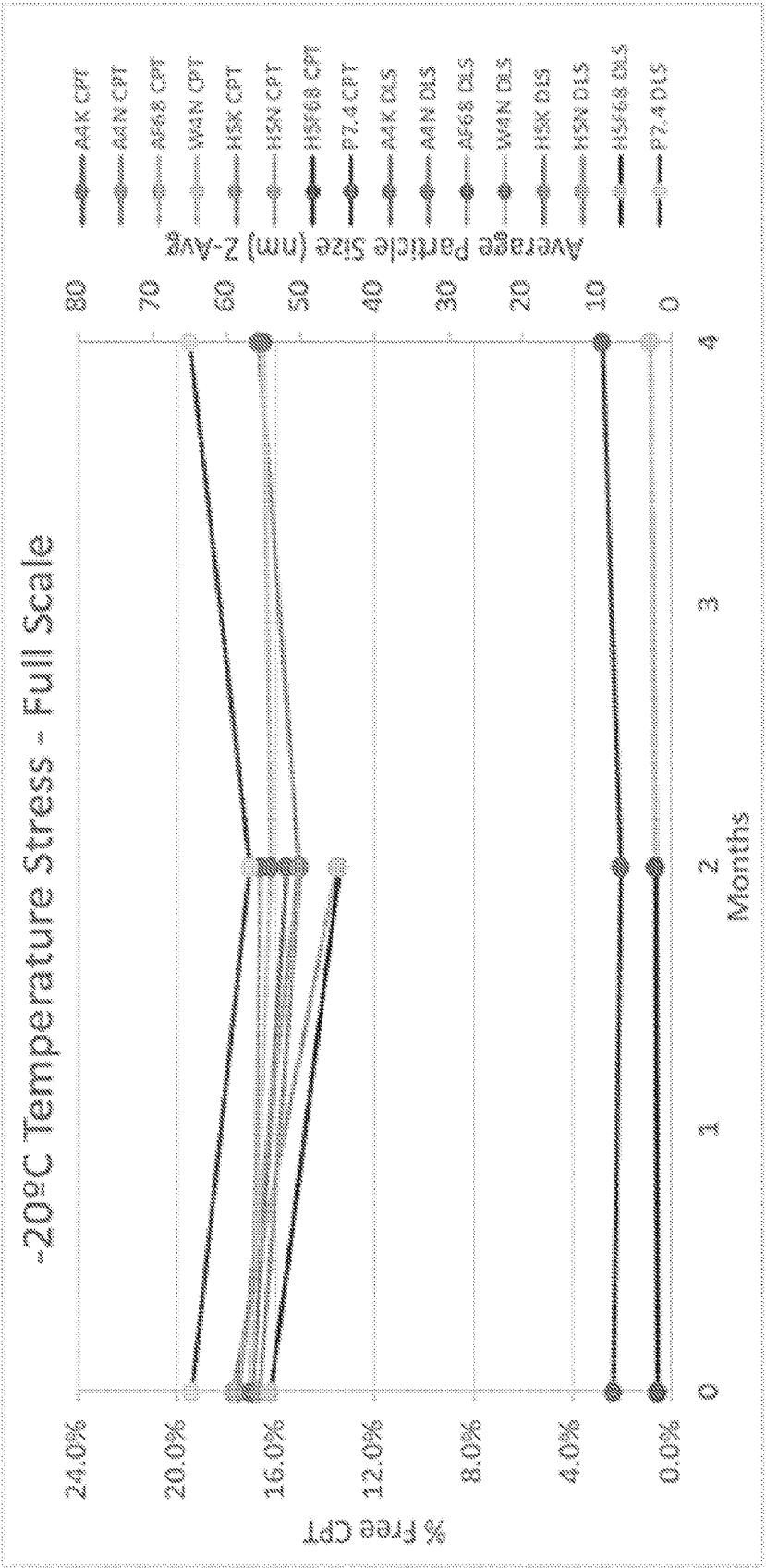


FIG. 22

% Free CPT and Avg. Particle Size Trends over 4 months at -80°C Step 2

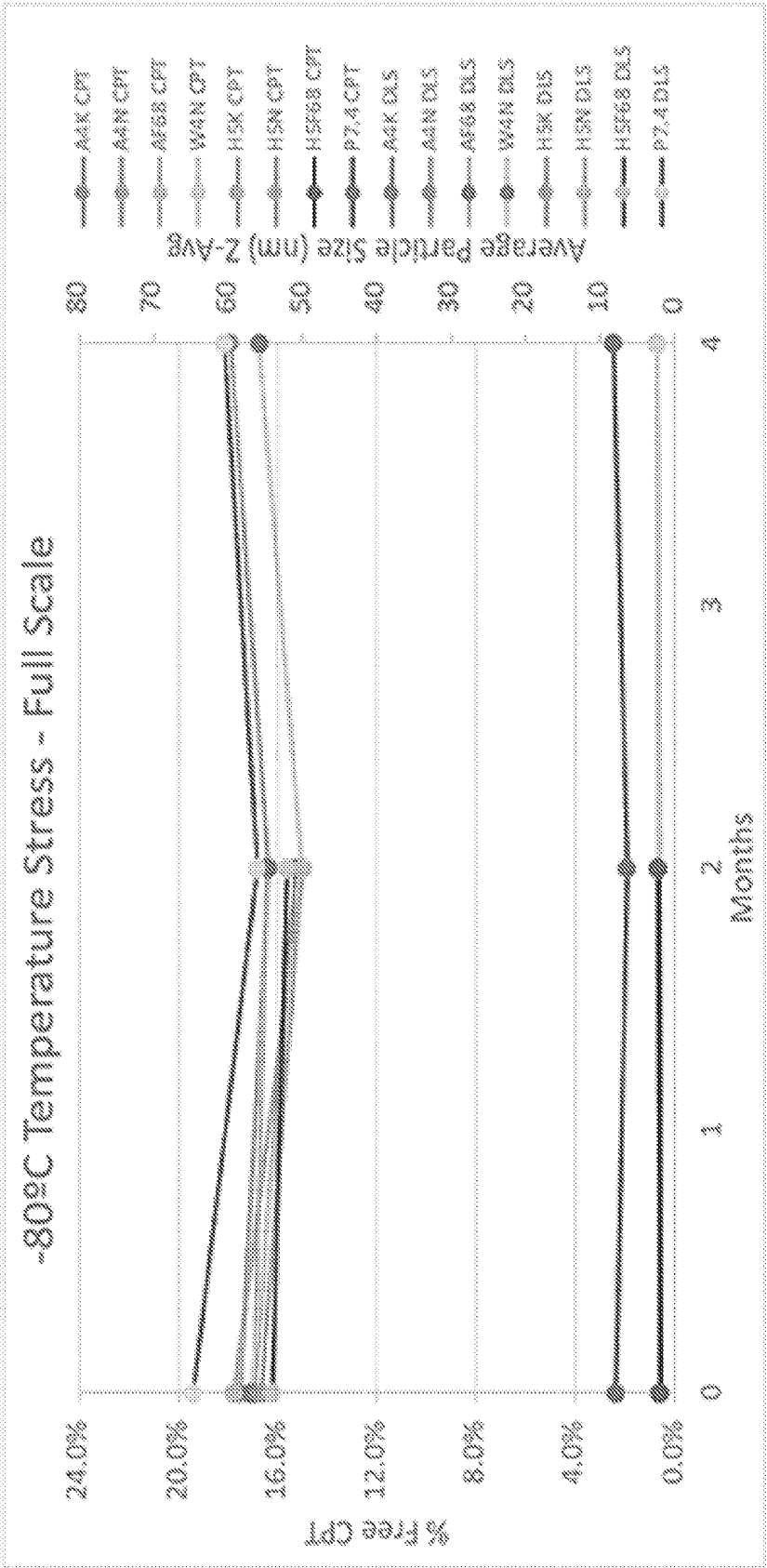


FIG. 23

% Free CPT and Avg. Particle Size Trends over 24 hours of Agitation Step 2

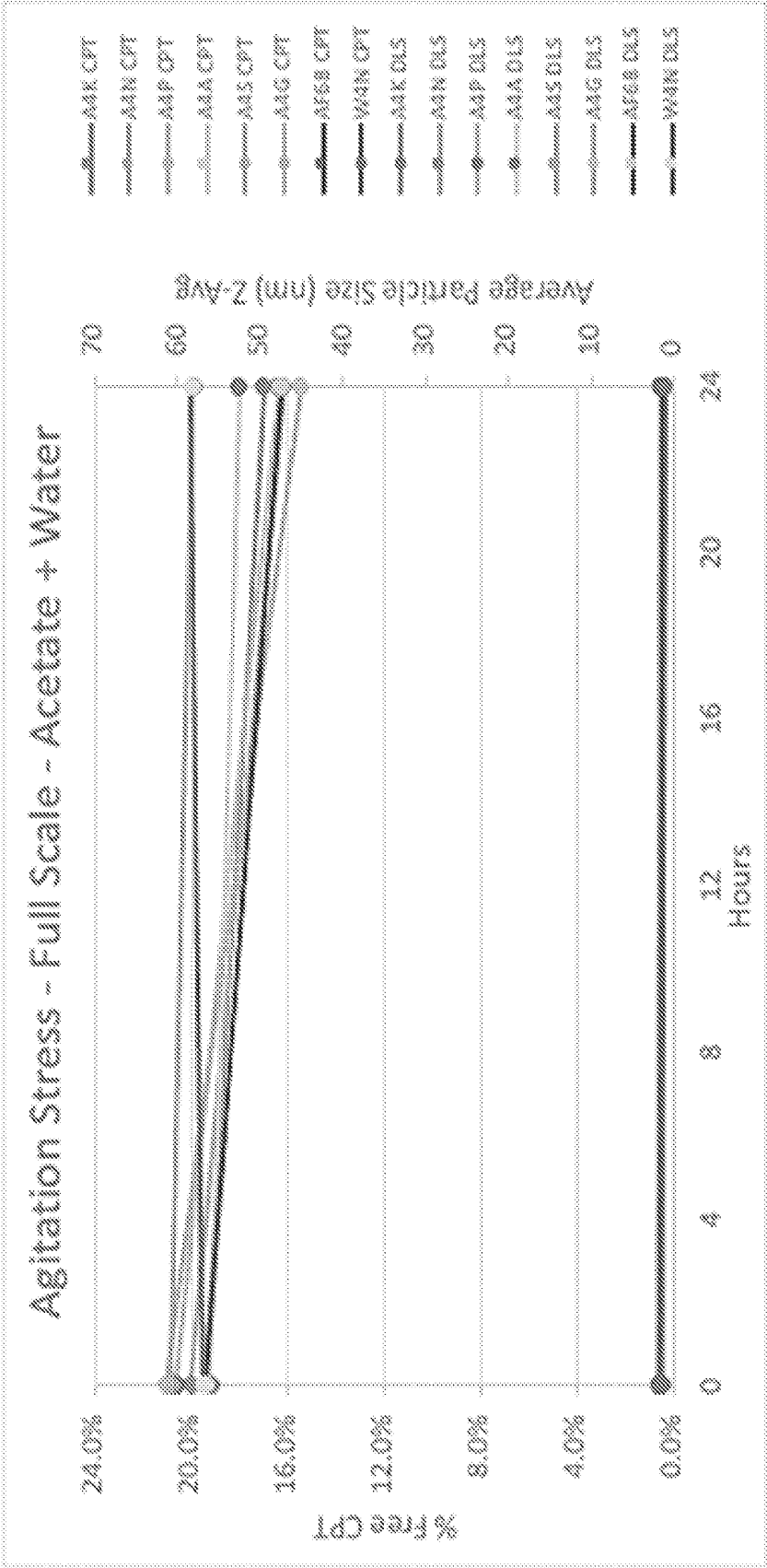


FIG. 24A

% Free CPT and Avg. Particle Size Trends over 24 hours of Agitation Step 2

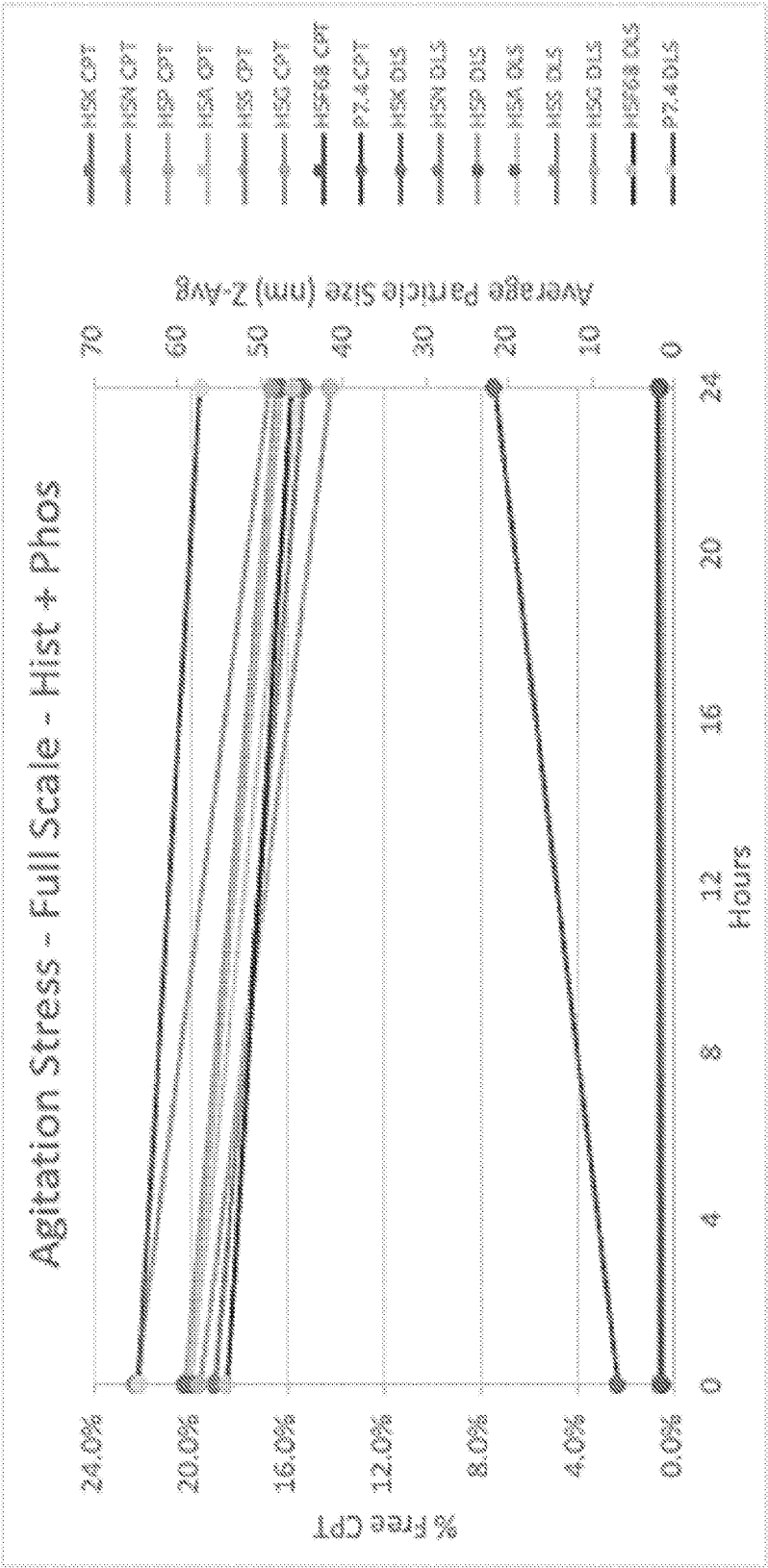


FIG. 24B

% Free CPT and Avg. Particle Size Trends over 6 Freeze/Thaw Cycles Step 2

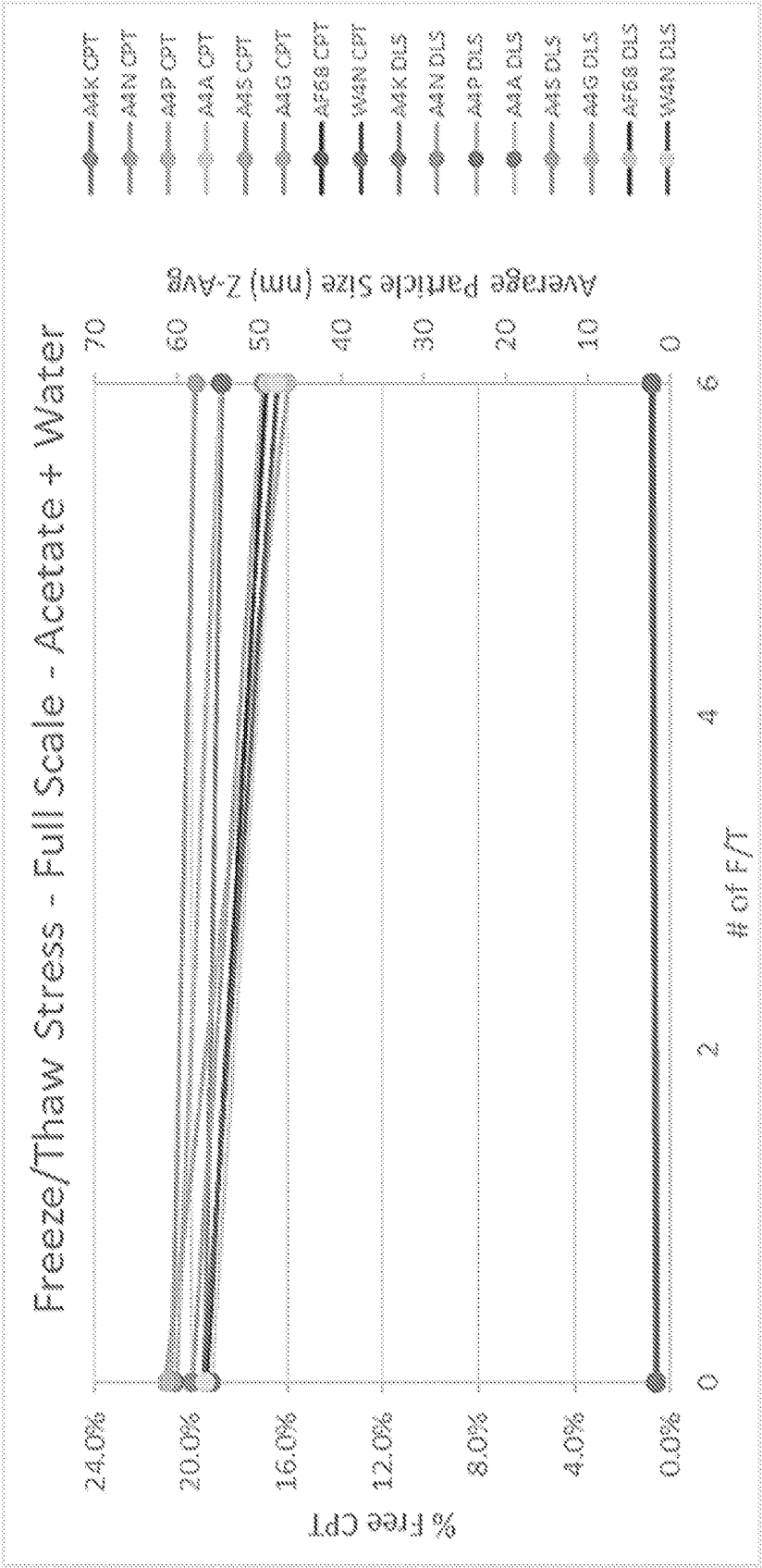


FIG. 25A

% Free CPT and Avg. Particle Size Trends over 6 Freeze/Thaw Cycles Step 2

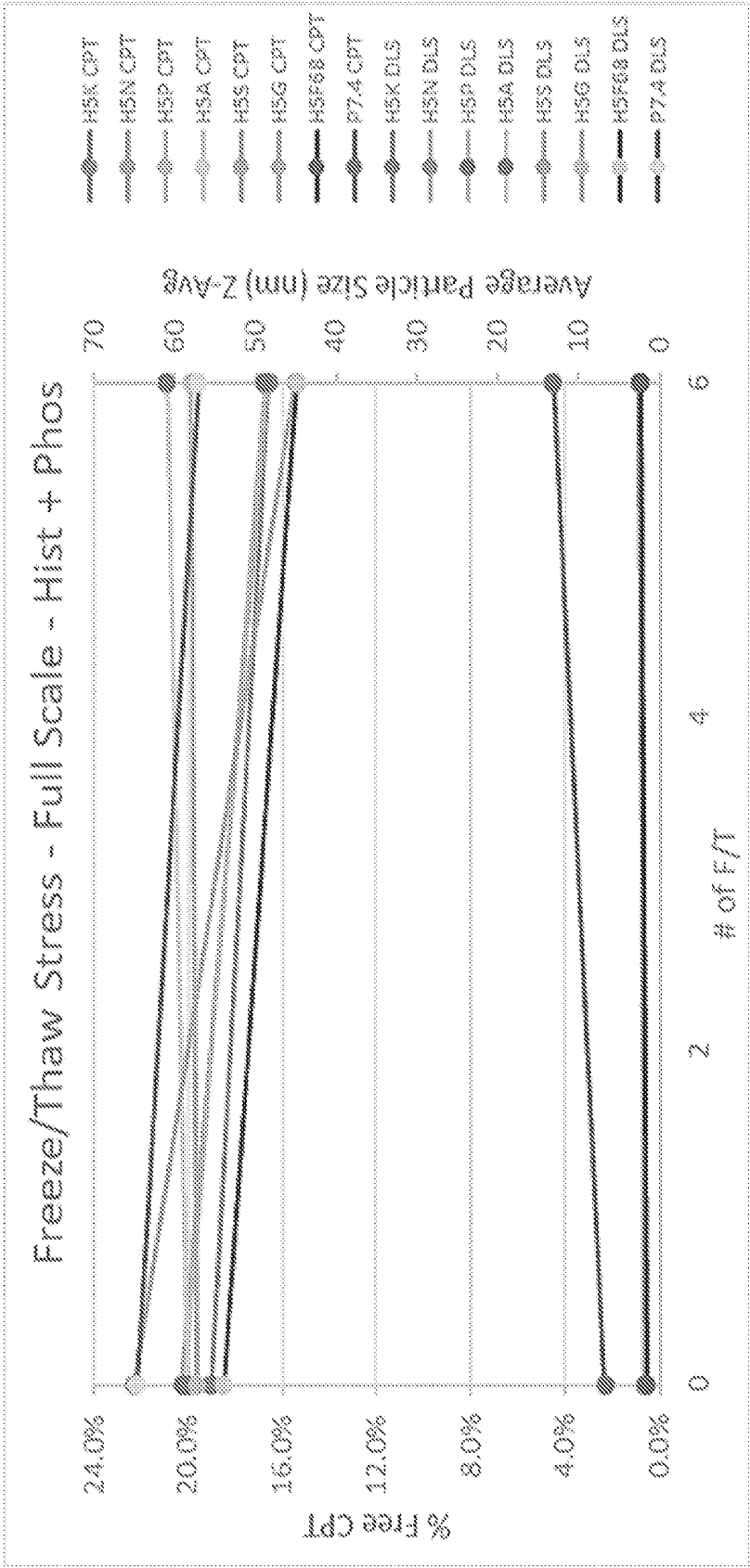


FIG. 25B

% Free CPT and Avg. Particle Size Trends over 24 hours of 0.05% H₂O₂ Stress
Step 2

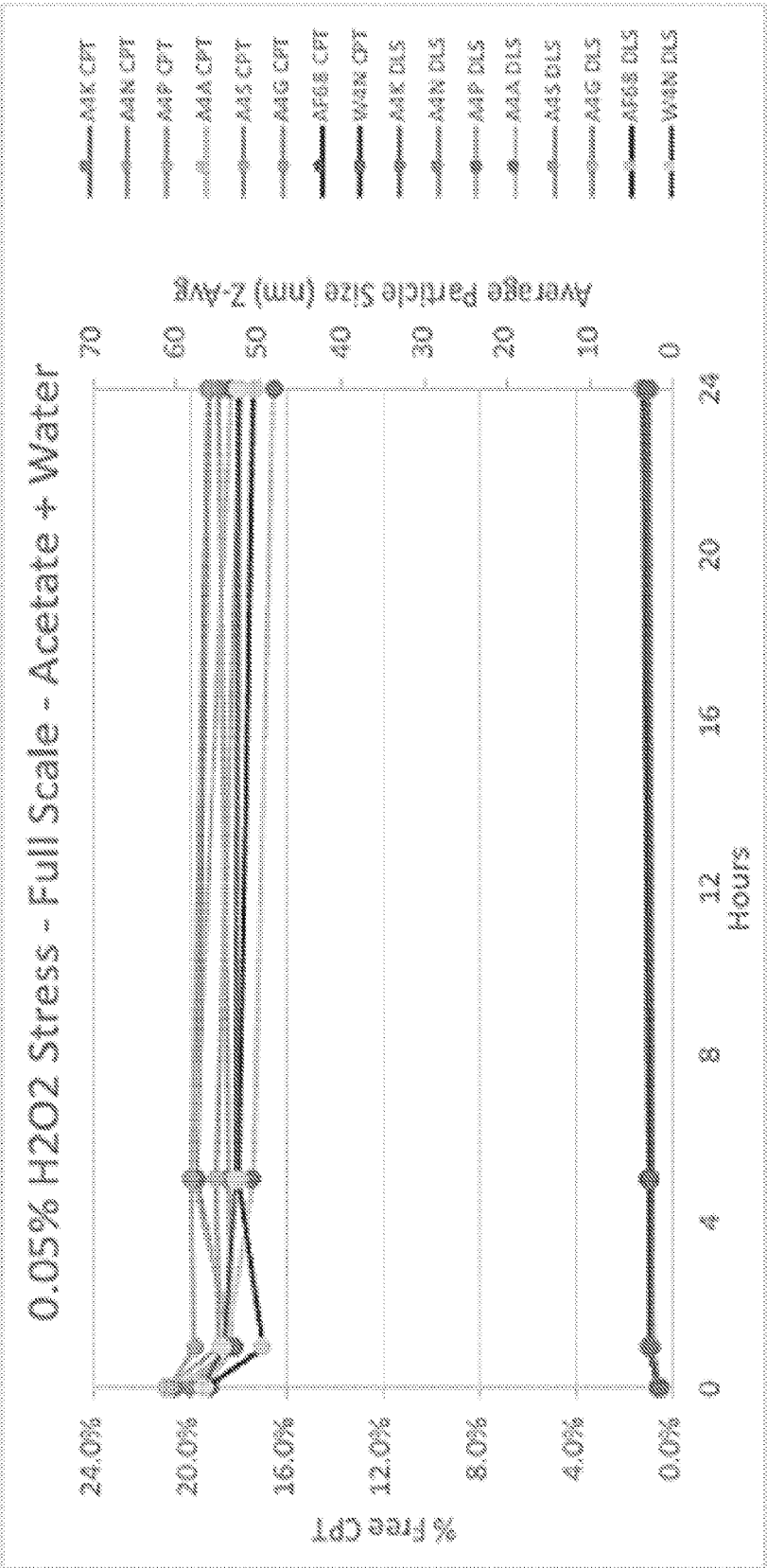


FIG. 26A

% Free CPT and Avg. Particle Size Trends over 24 hours of 0.05% H₂O₂ Stress
Step 2

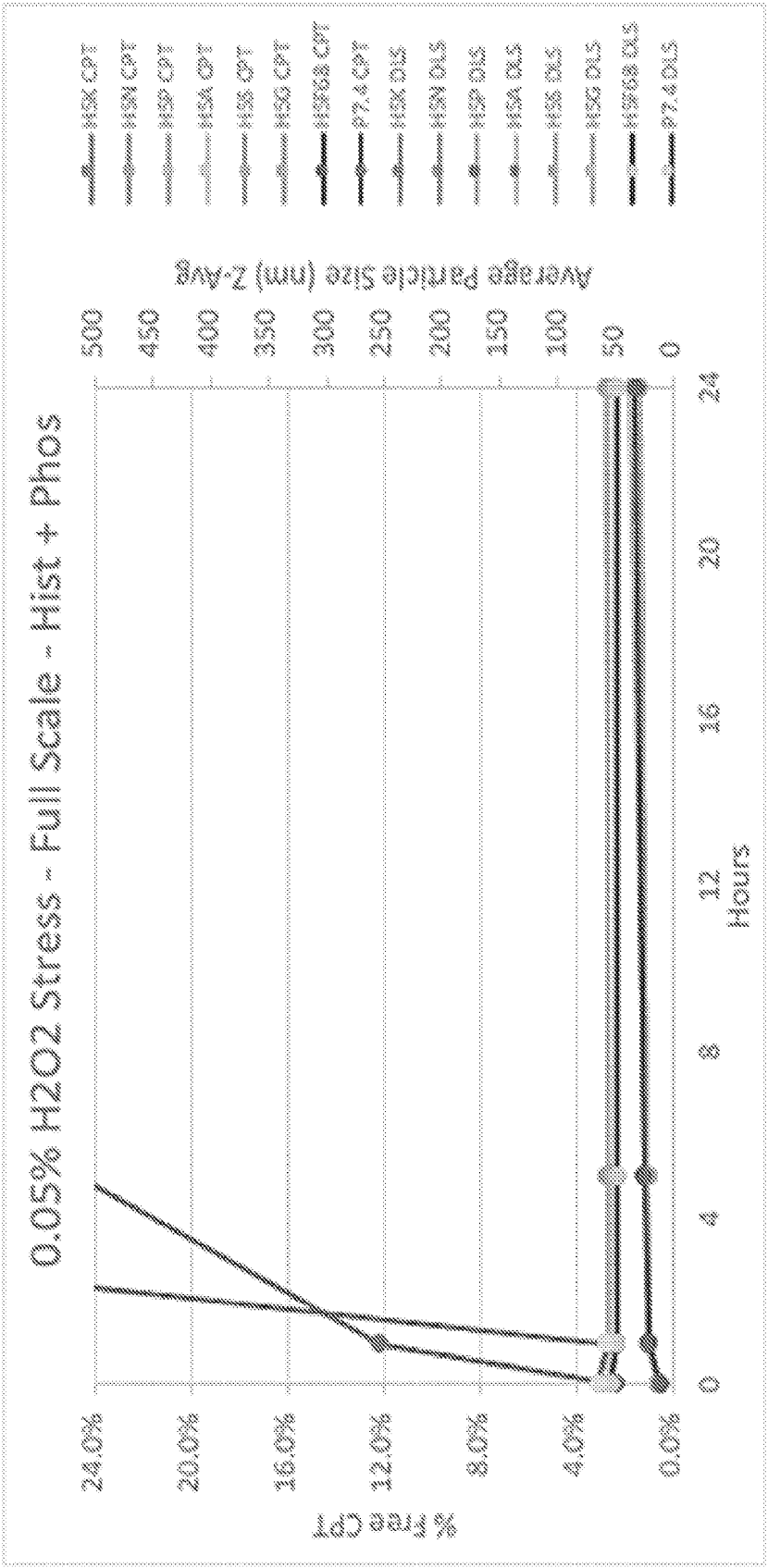


FIG. 26B

% Free CPT and Avg. Particle Size Trends over 1 month at 2-8°C Step 3

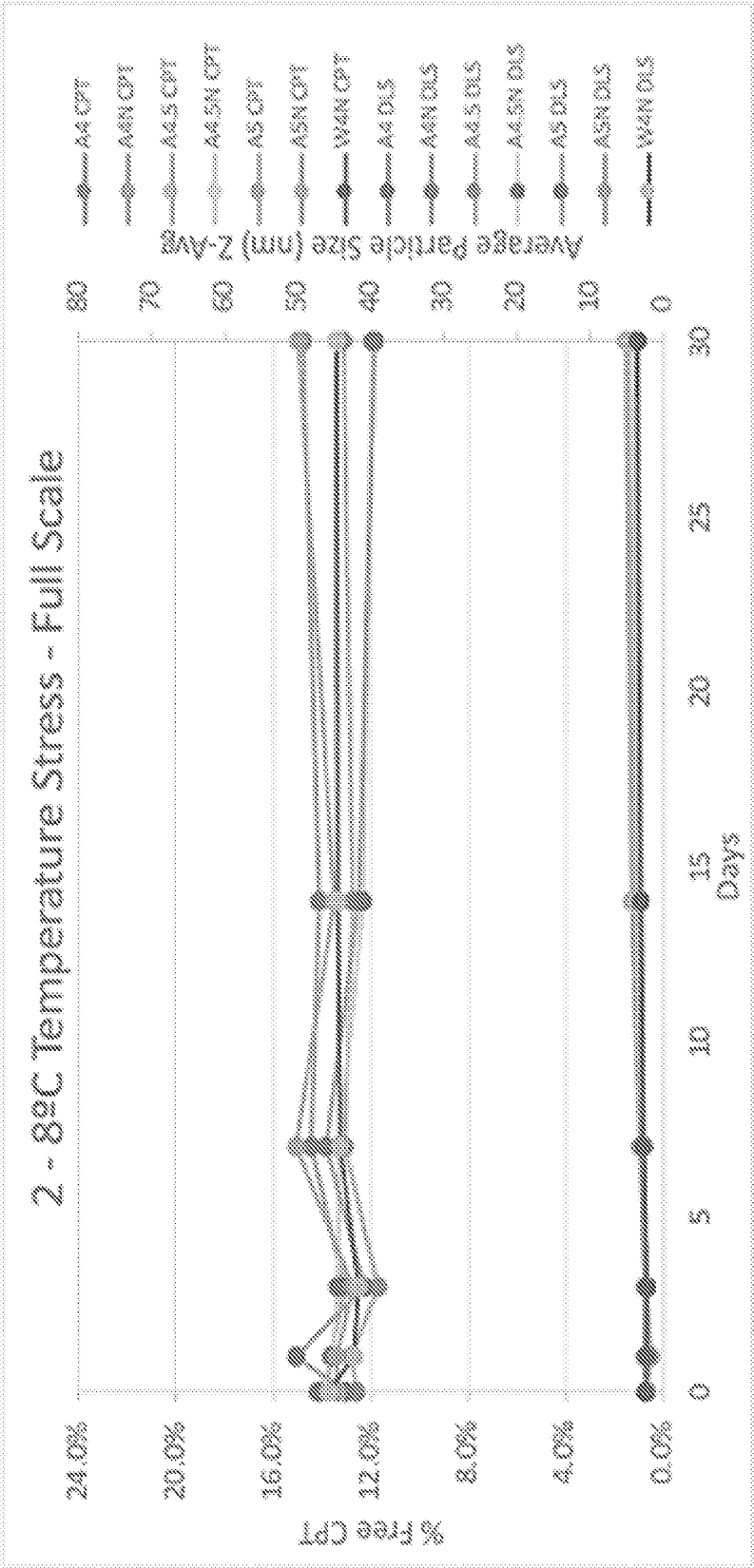


FIG. 27

% Free CPT and Avg. Particle Size Trends over 3 months at 2-8°C Step 3

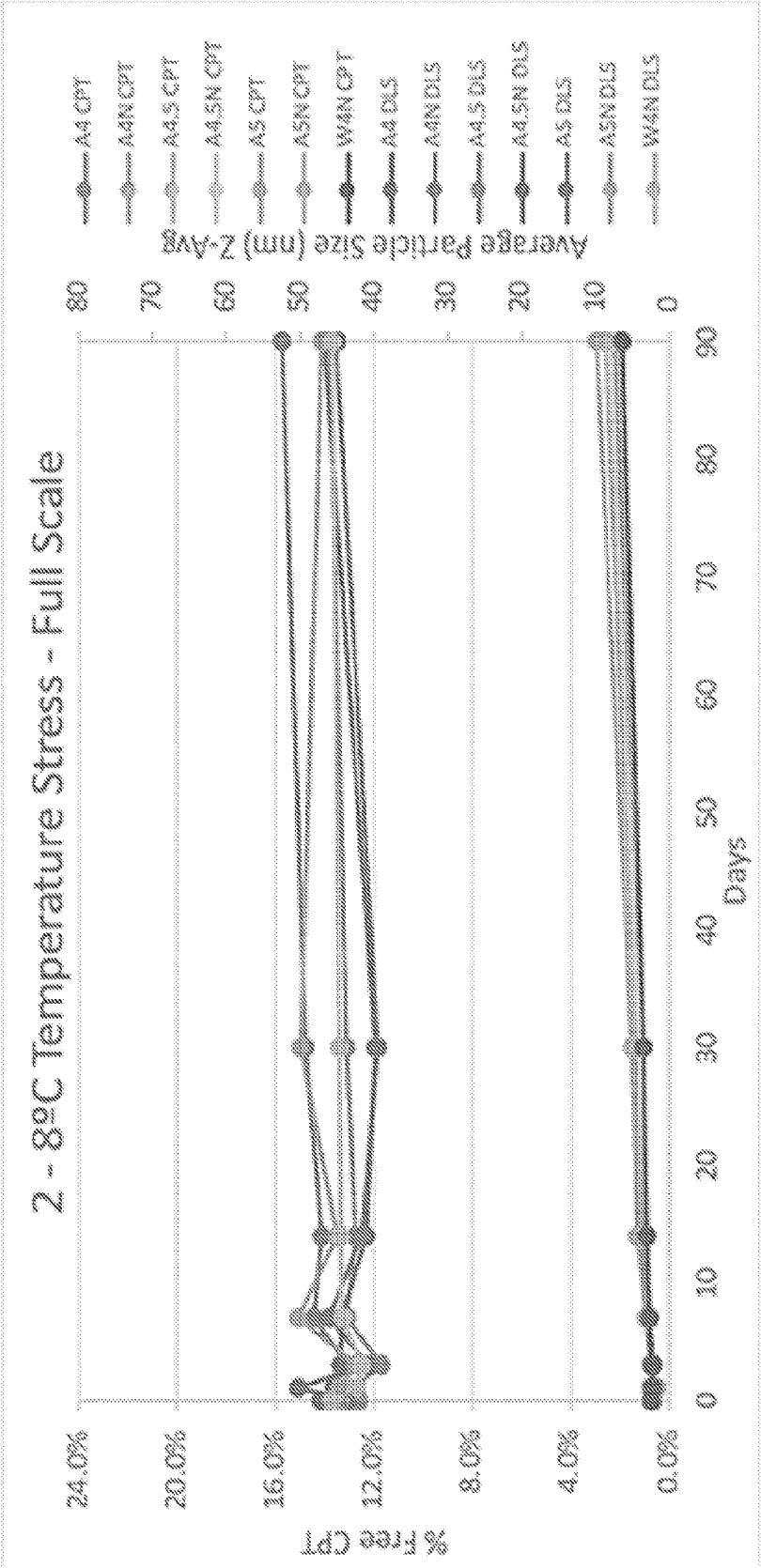


FIG. 28

% Free CPT and Avg. Particle Size Trends over 7 days at 20-25°C Step 3



FIG. 29

% Free CPT and Avg. Particle Size Trends over 7 days at 40°C Step 3

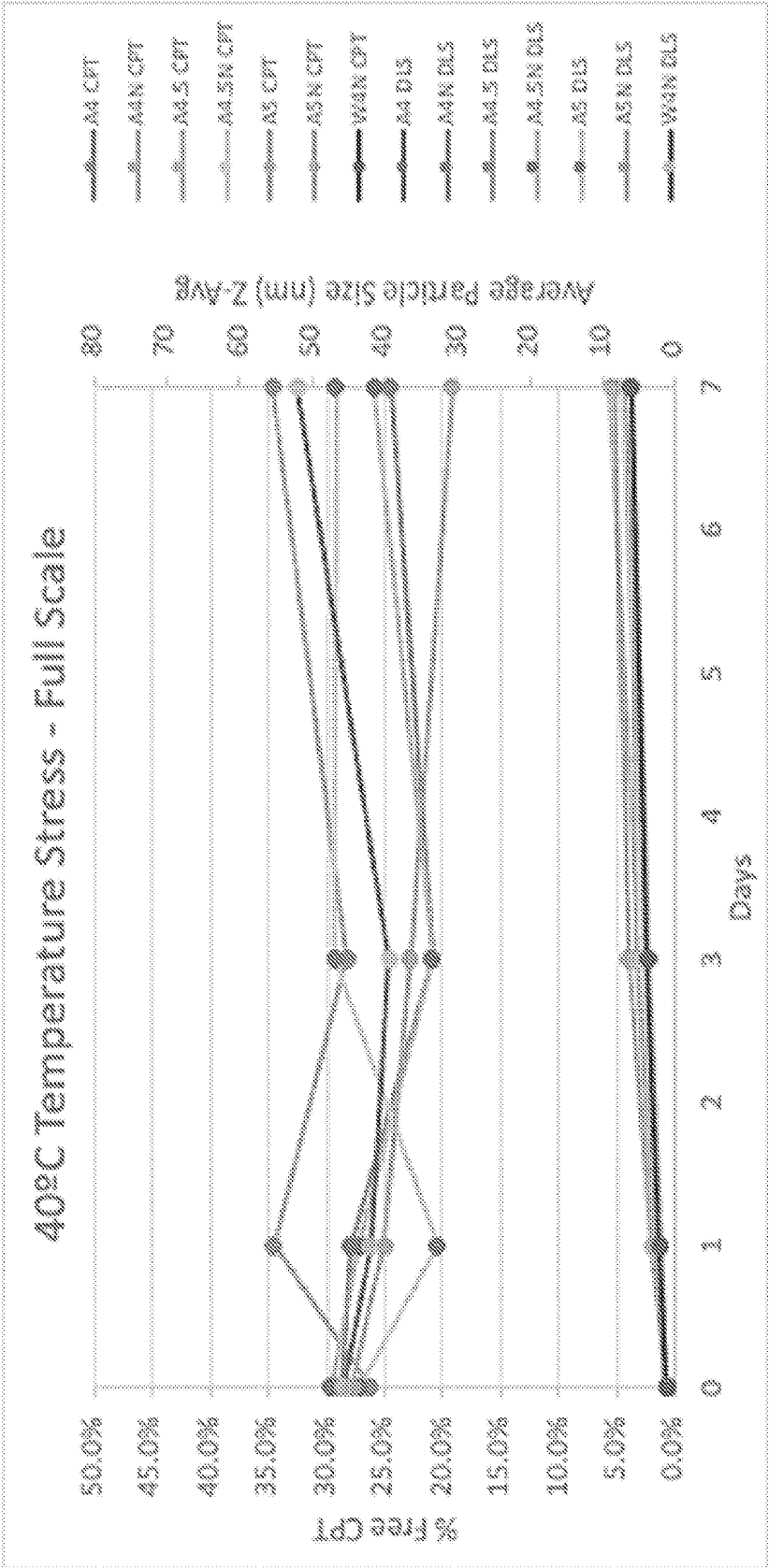


FIG. 30

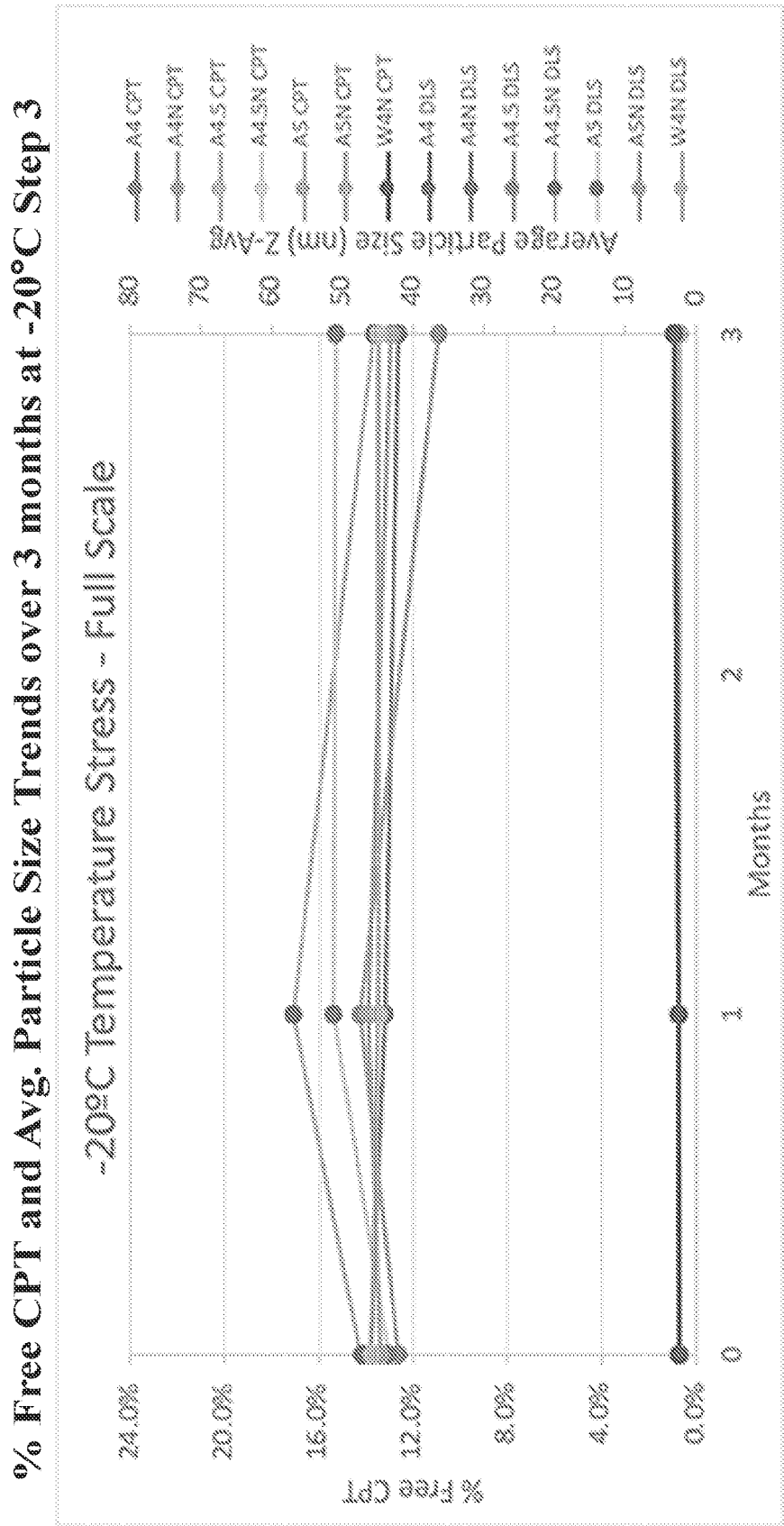


FIG. 31

% Free CPT and Avg. Particle Size Trends over 3 months at -80°C Step 3

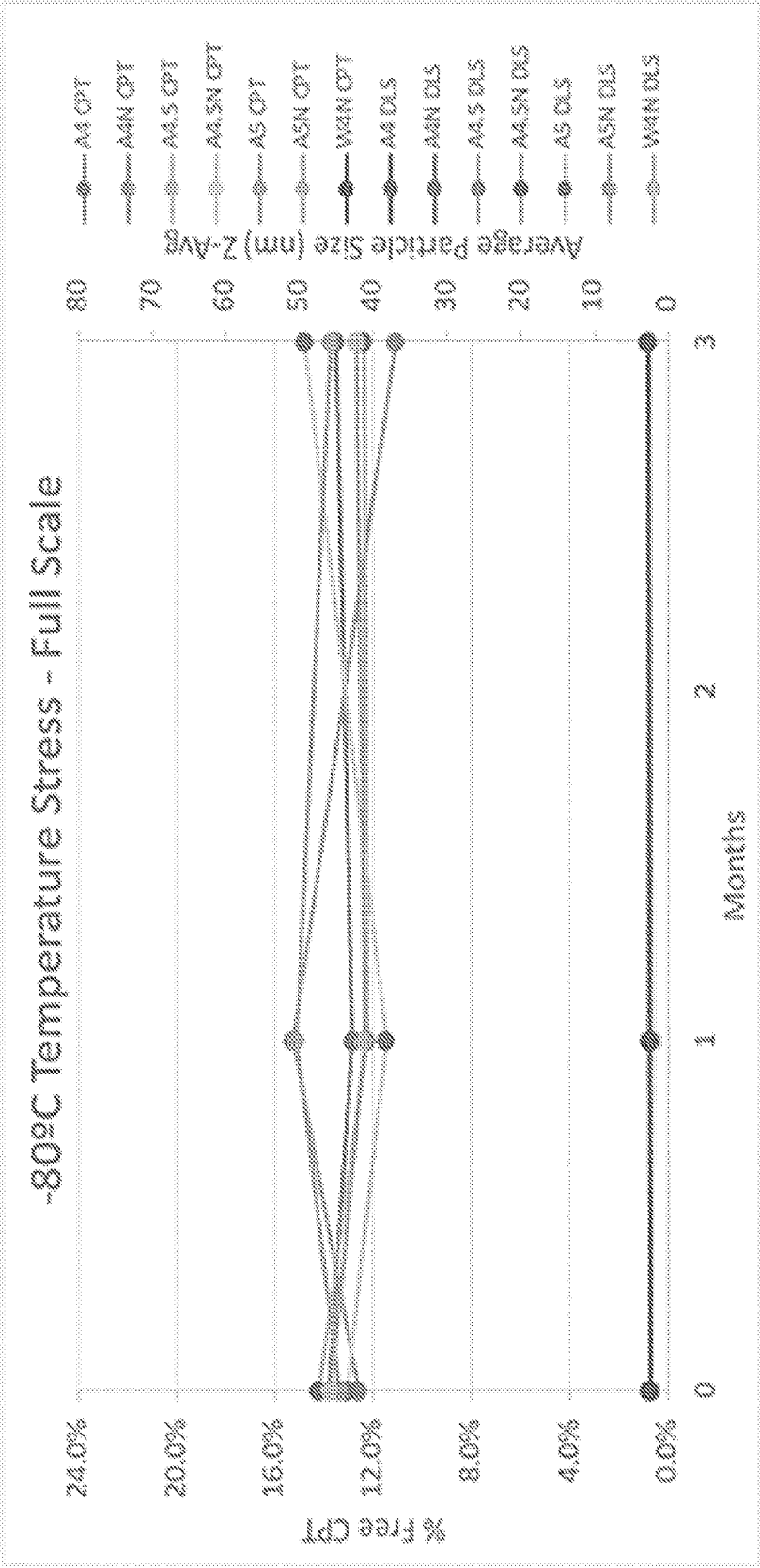


FIG. 32

% Free CPT and Avg. Particle Size Trends over 24 Hour Agitation at 450 rpm
Step 3

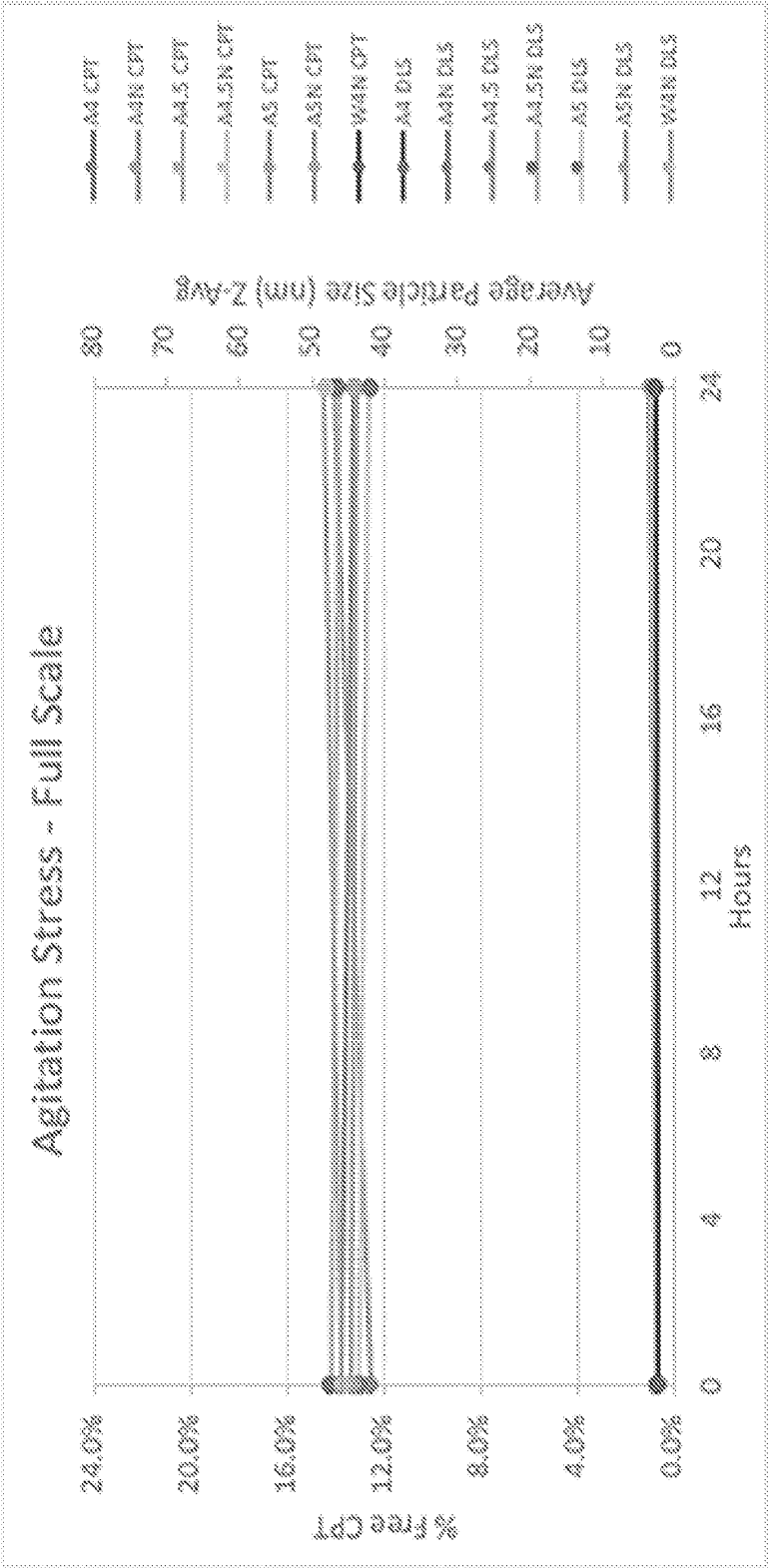


FIG. 33

% Free CPT and Avg. Particle Size Trends over 6x Freeze/Thaw Step 3

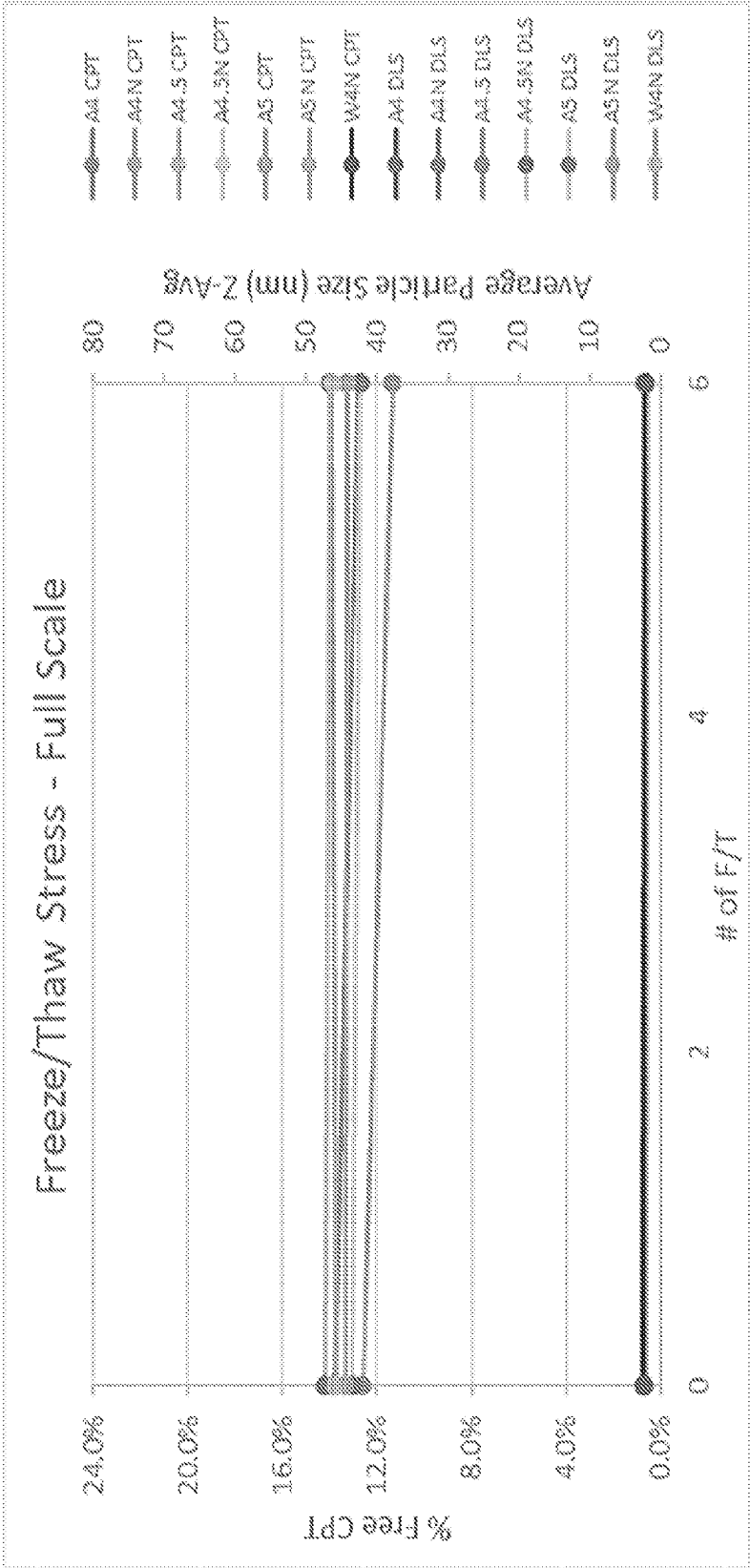
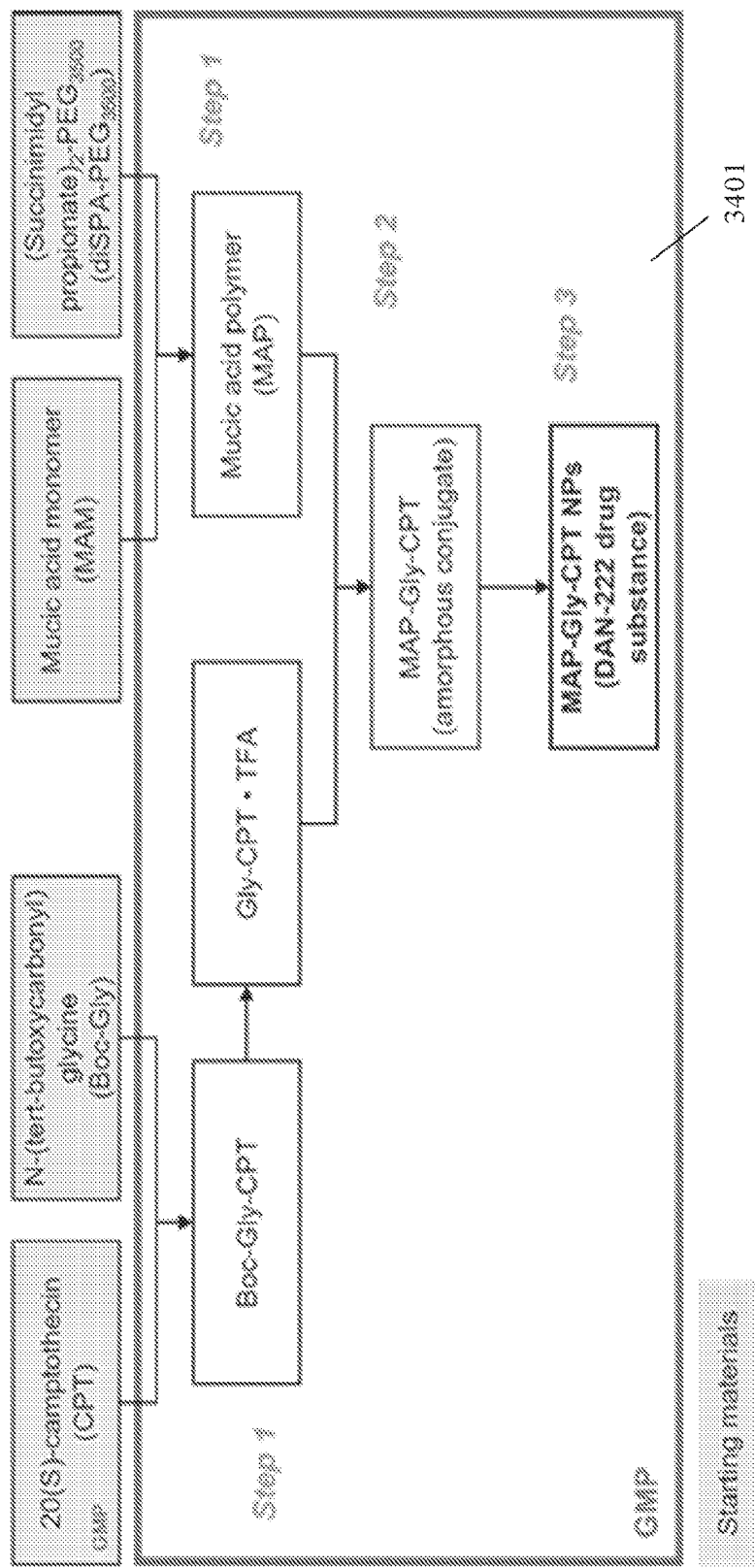


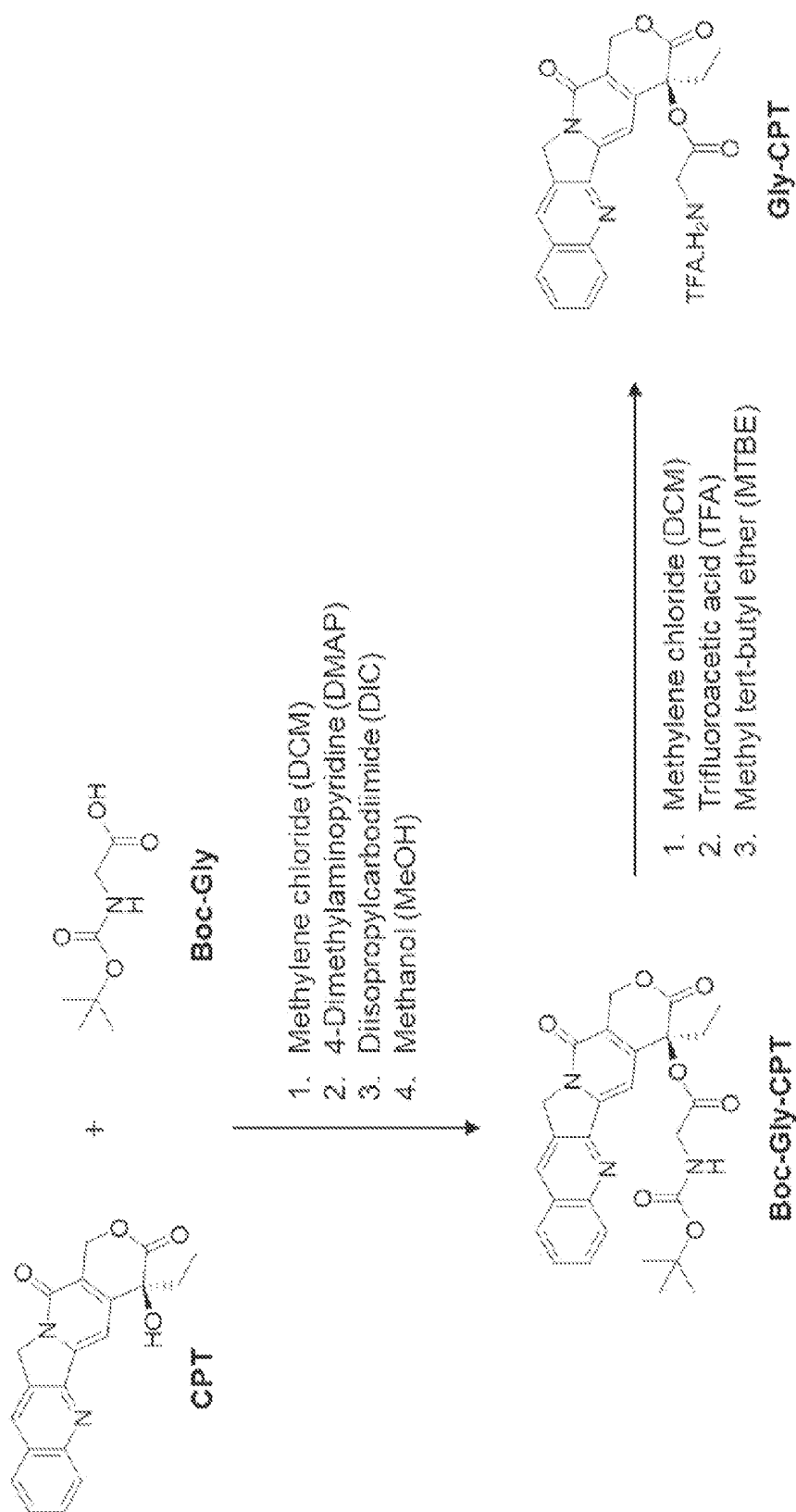
FIG. 34



Boc = tert-butoxycarbonyl; Gly = glycine; GMP = current good manufacturing practice; NP = nanoparticle; PEG = polyethylene glycol; TFA = trifluoroacetic acid

FIG. 35

Synthetic scheme to prepare Gly-CPT



Boc = tert-butoxycarbonyl; CPT = 20(S)-camptothecin; Gly = glycine; Gly-CPT = glycylicamptothecin (TFA salt)

FIG. 36

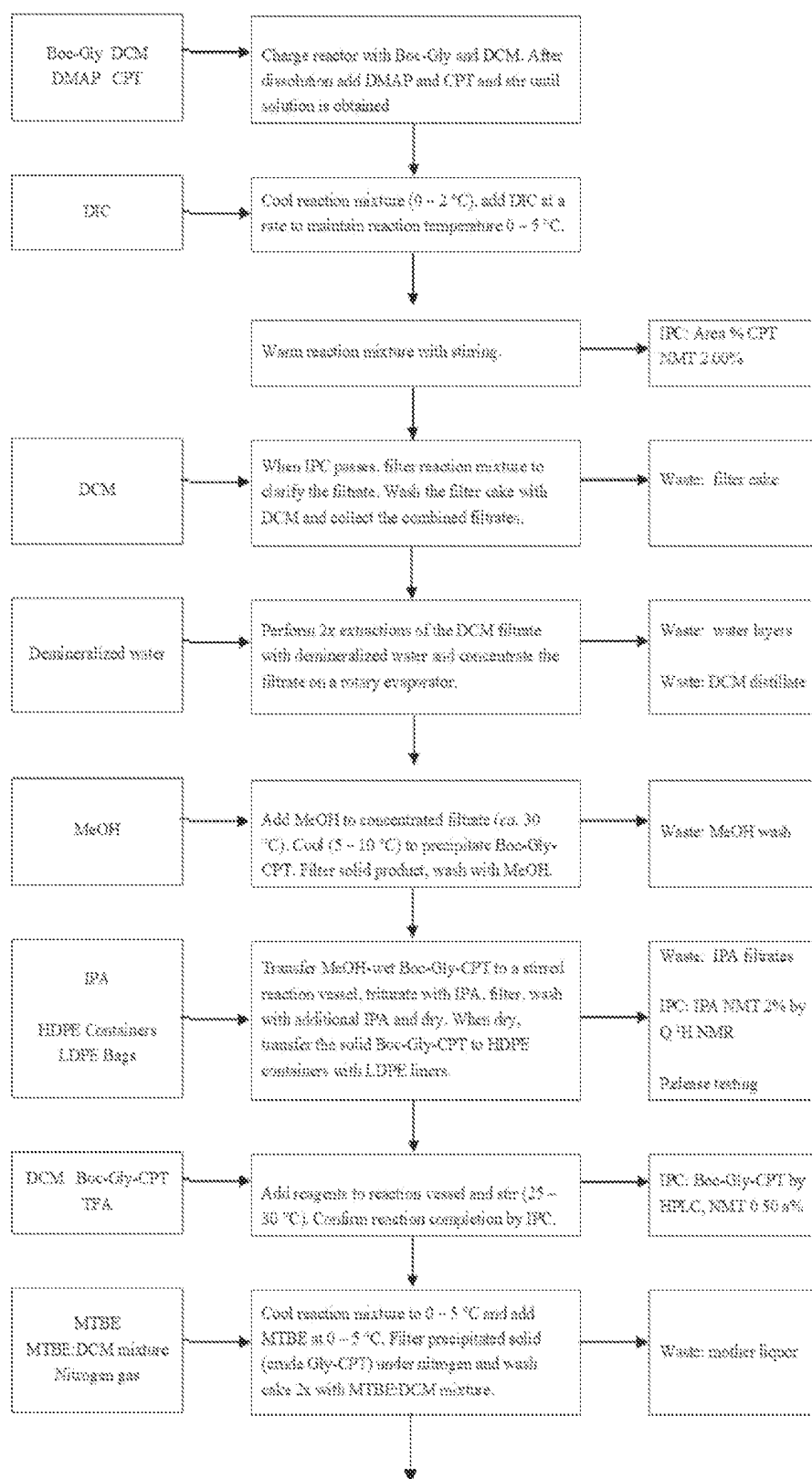


FIG. 37A

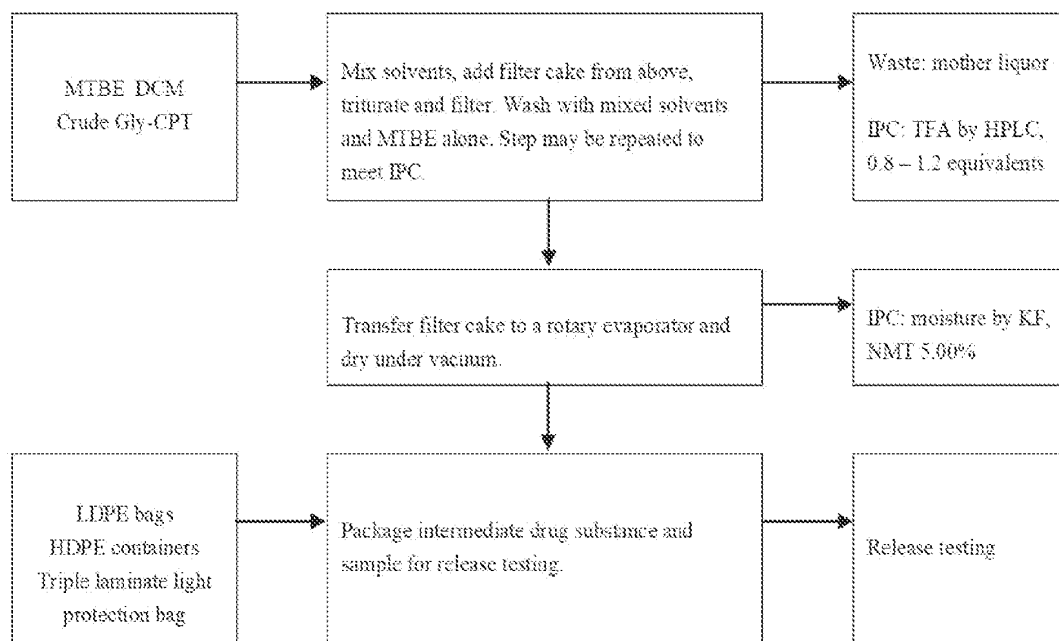
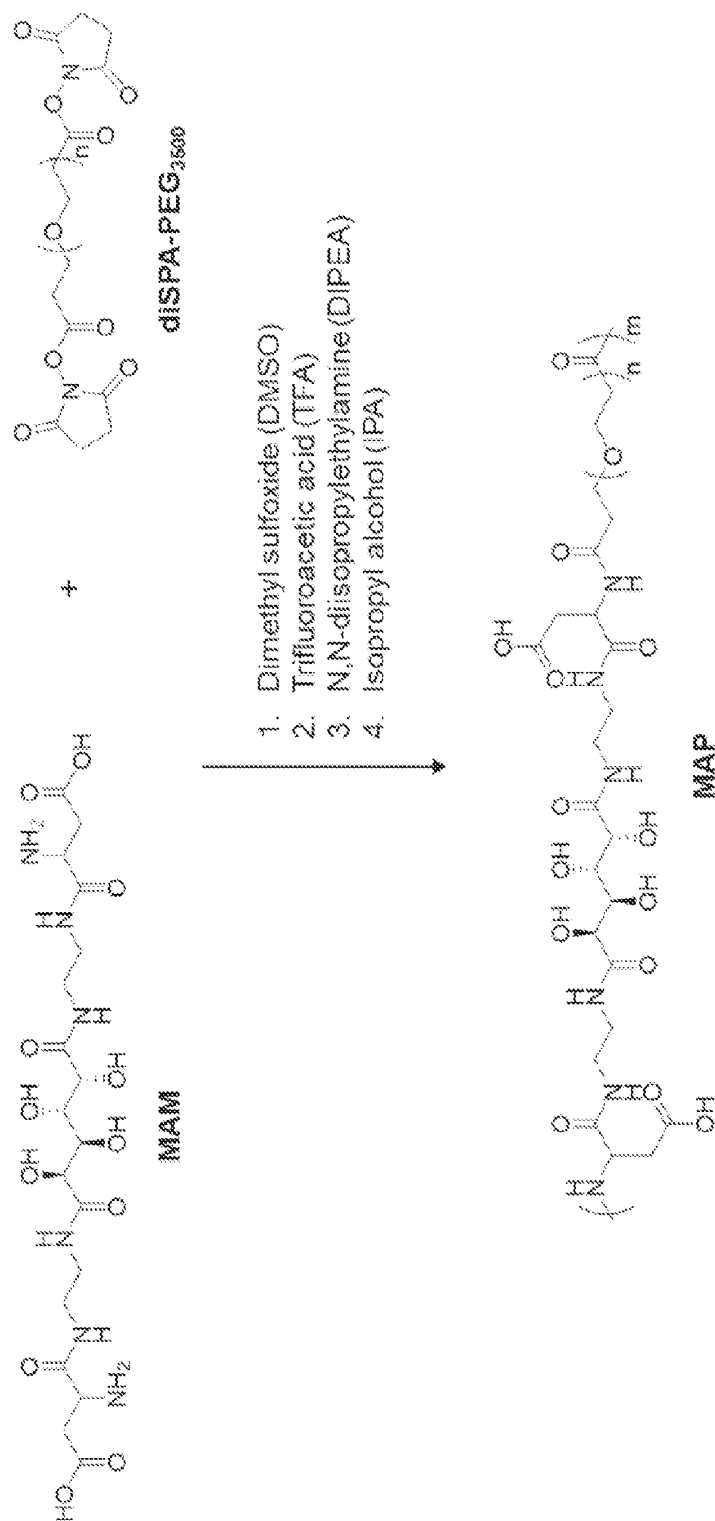


FIG. 37B

Synthetic scheme to prepare mucic acid polymer (MAP)



diSPA-PEG₃₅₀₀ = (succinimidyl propionate)₂-PEG₃₅₀₀; MAM = mucic acid monomer; MAP = mucic acid polymer; PEG = polyethylene glycol

FIG. 38

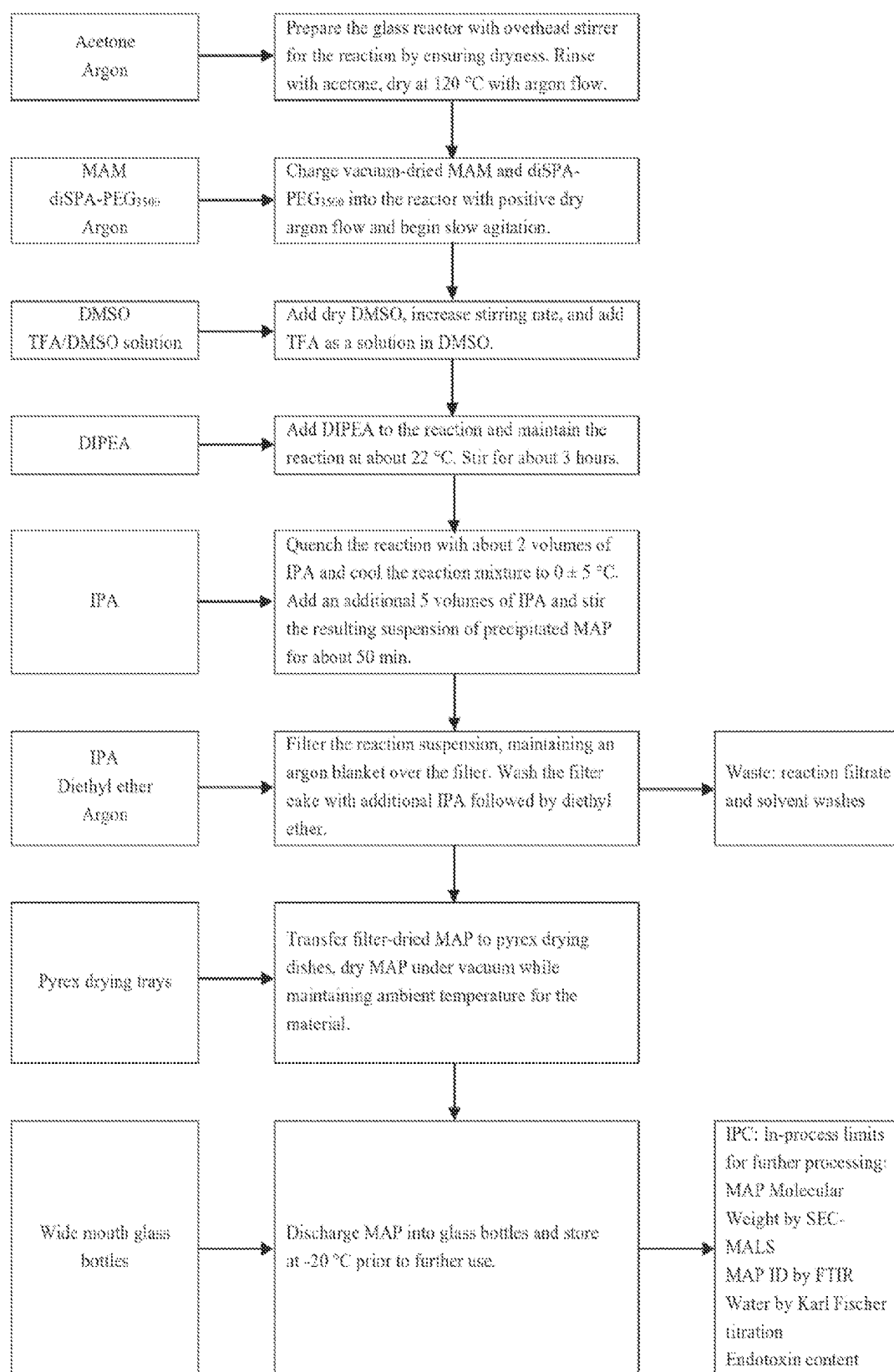
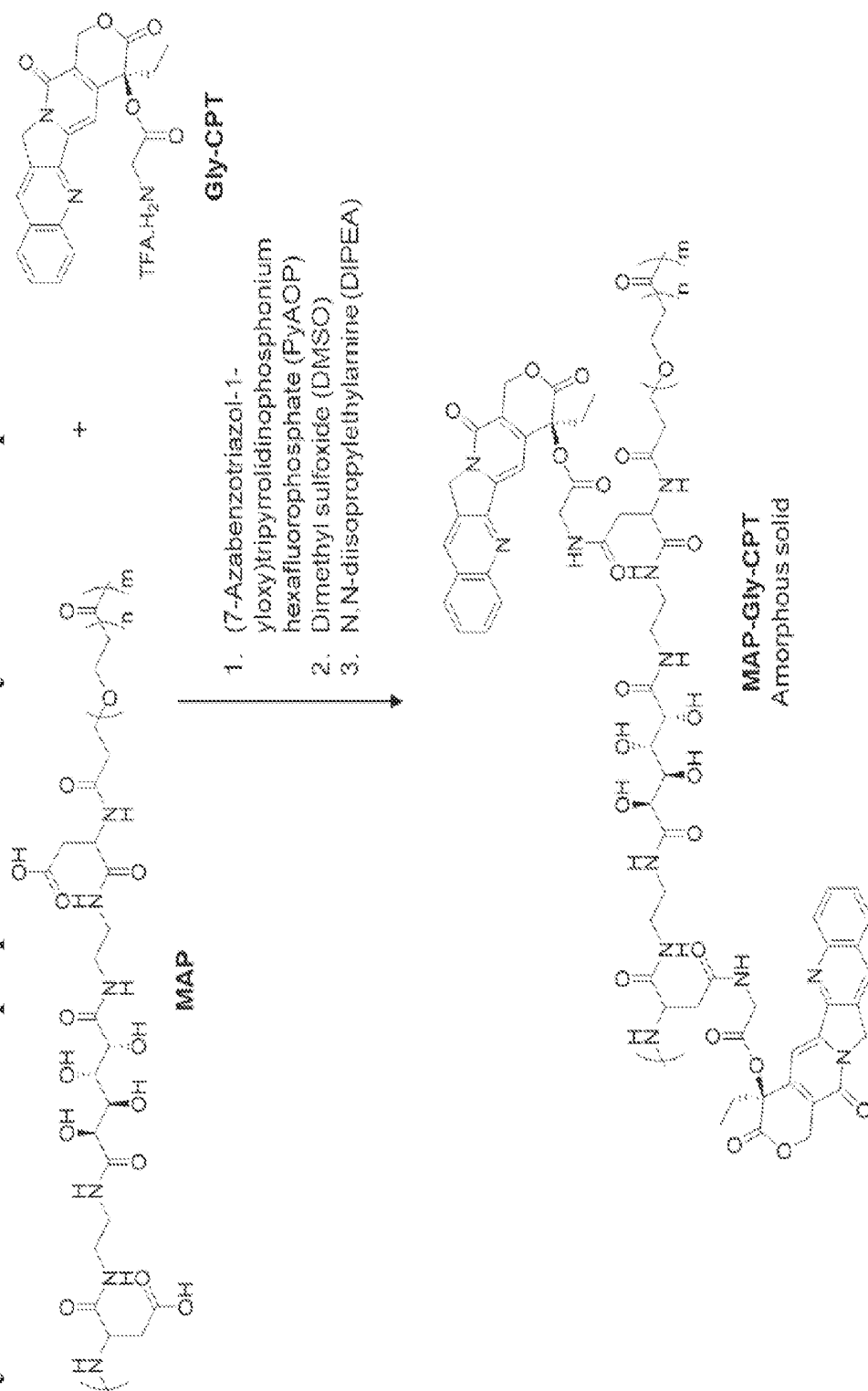


FIG. 39

Synthetic scheme to prepare MAP-Gly-CPT amorphous



Gly-CPT = glycyl-camptothecin (TFA salt); MAP = mucic acid polymer

FIG. 40

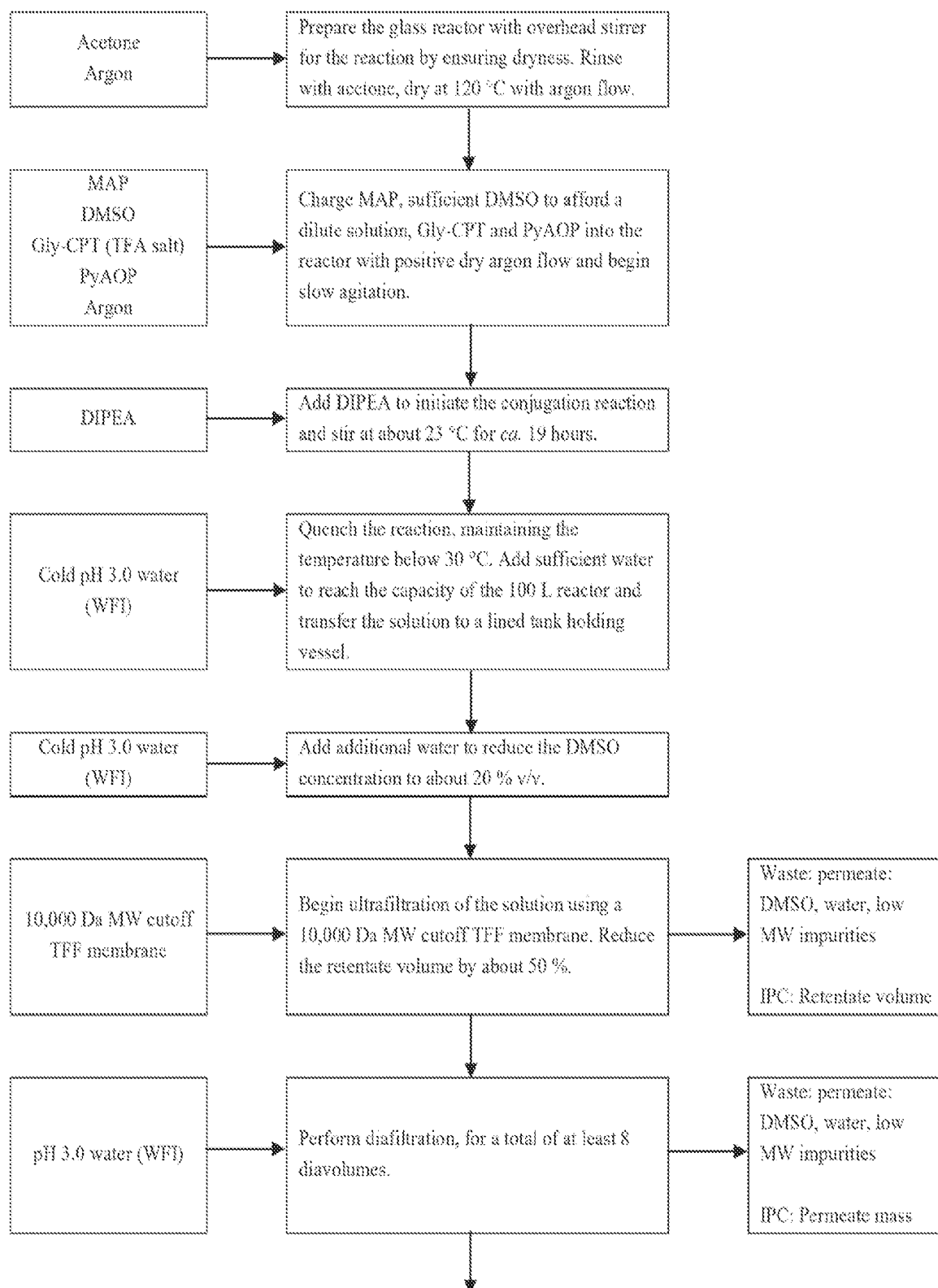


FIG. 41A

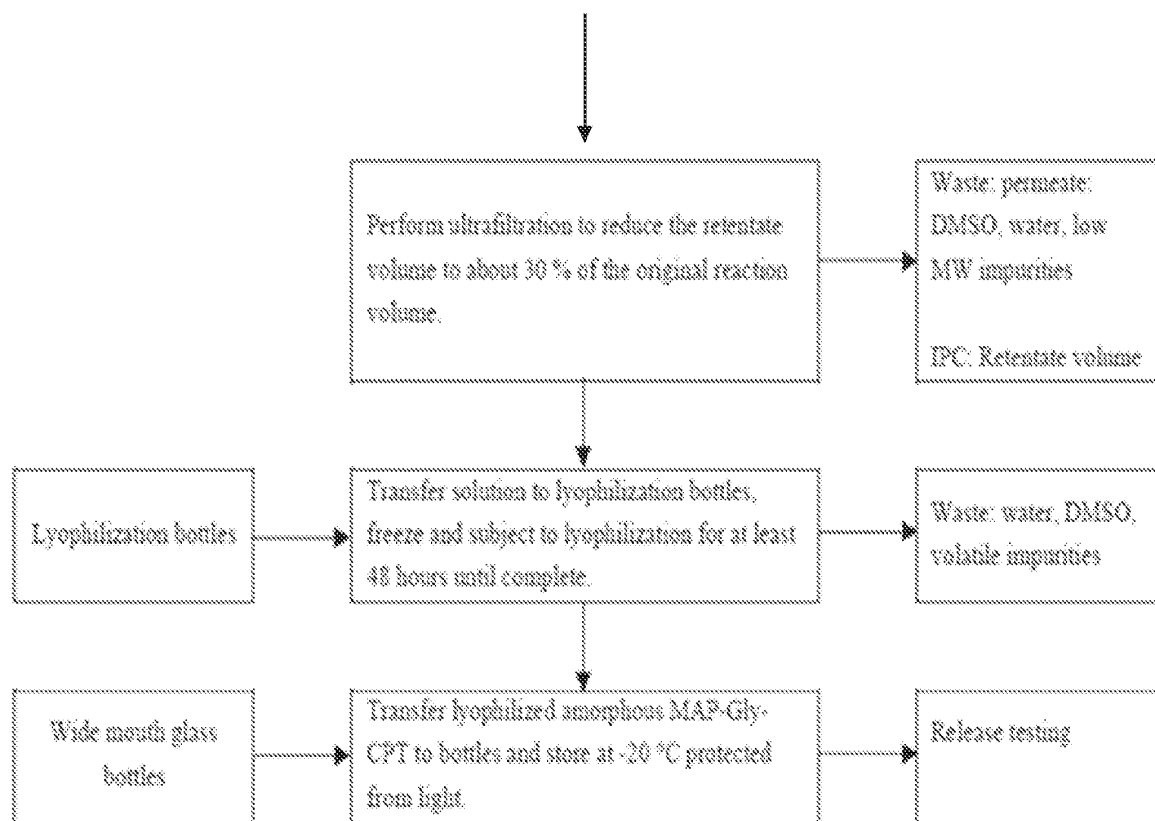


FIG. 41B

Scheme to prepare MAP-Gly-CPT NP solution (DAN-222 FBDS)

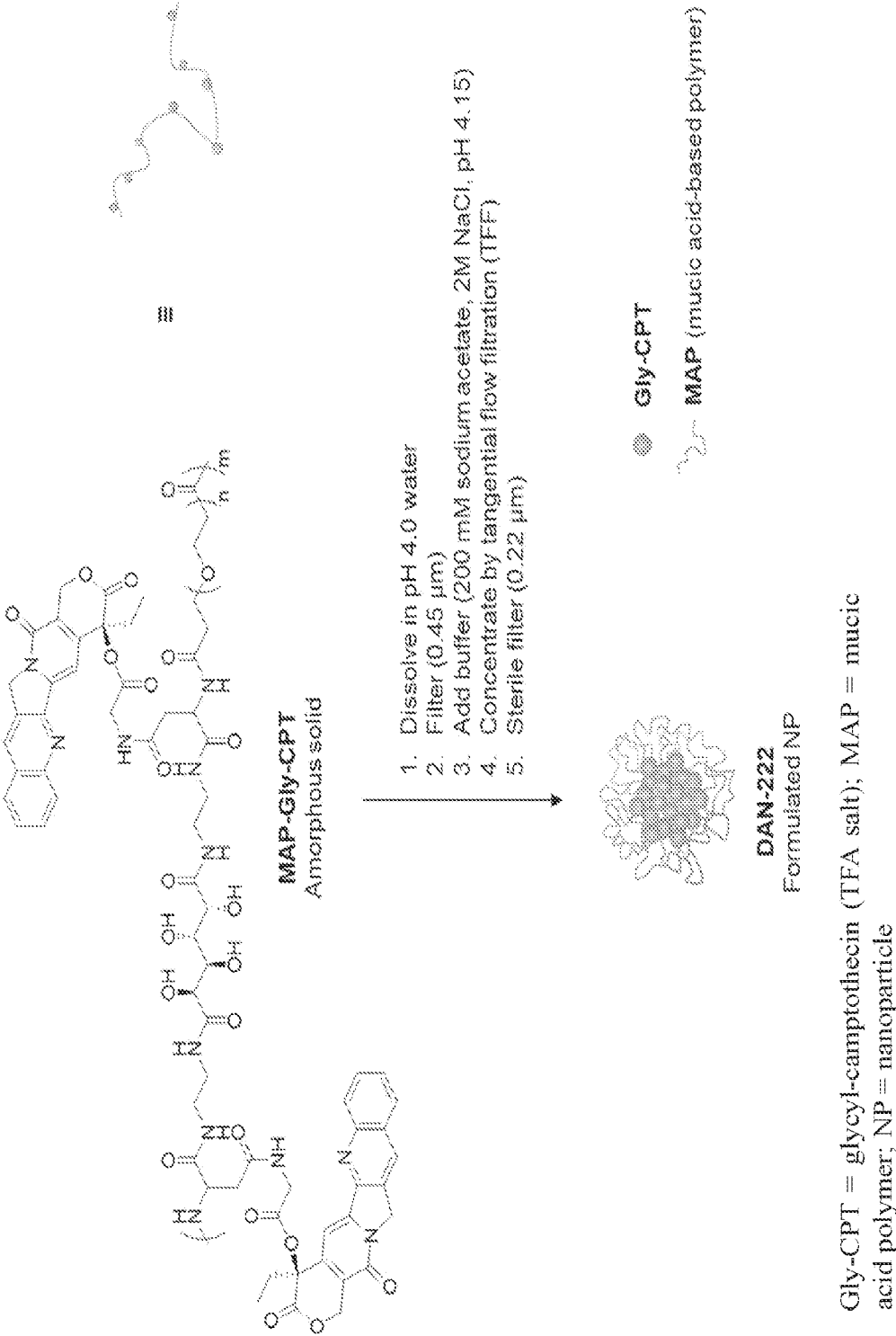


FIG. 42

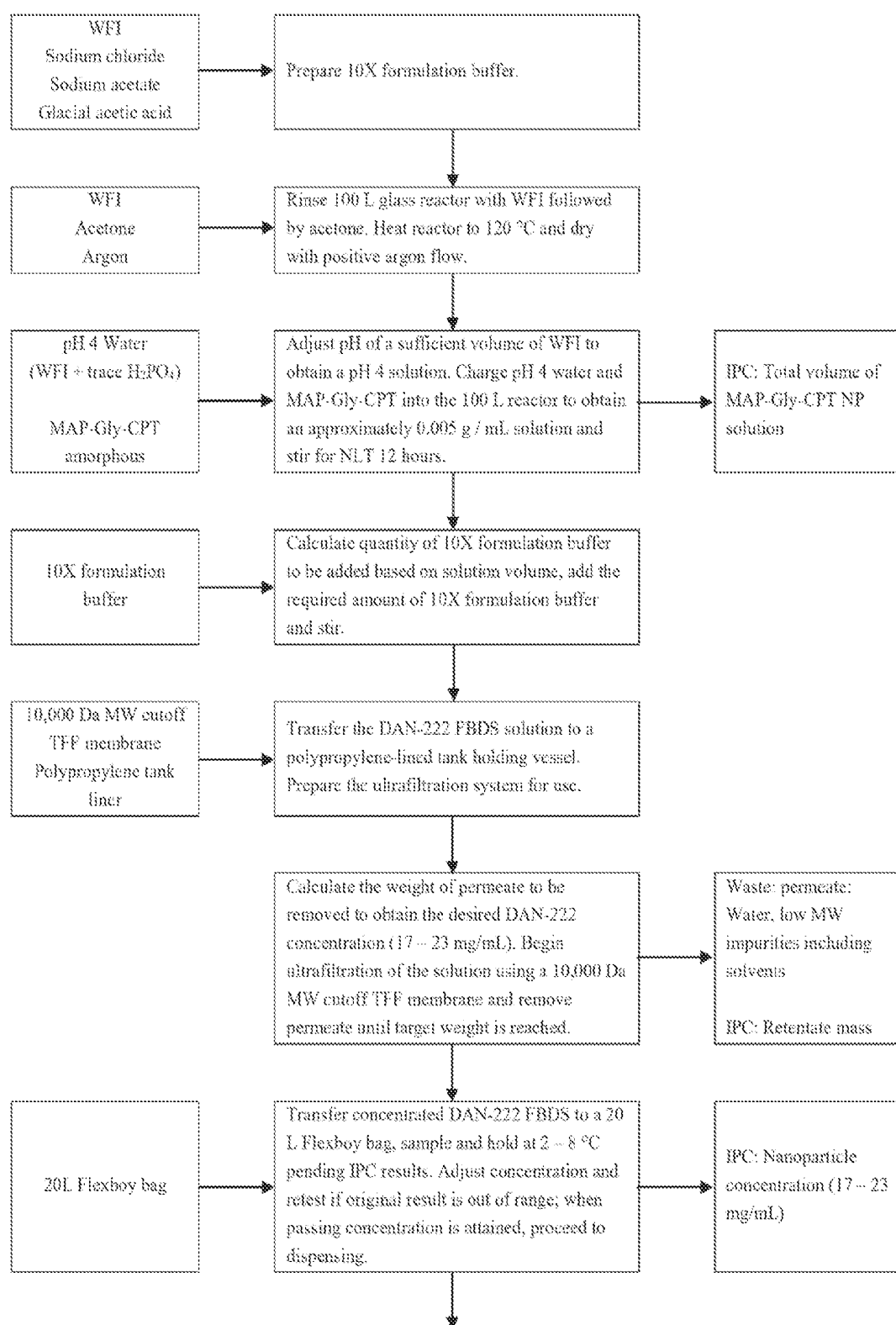


FIG. 43A

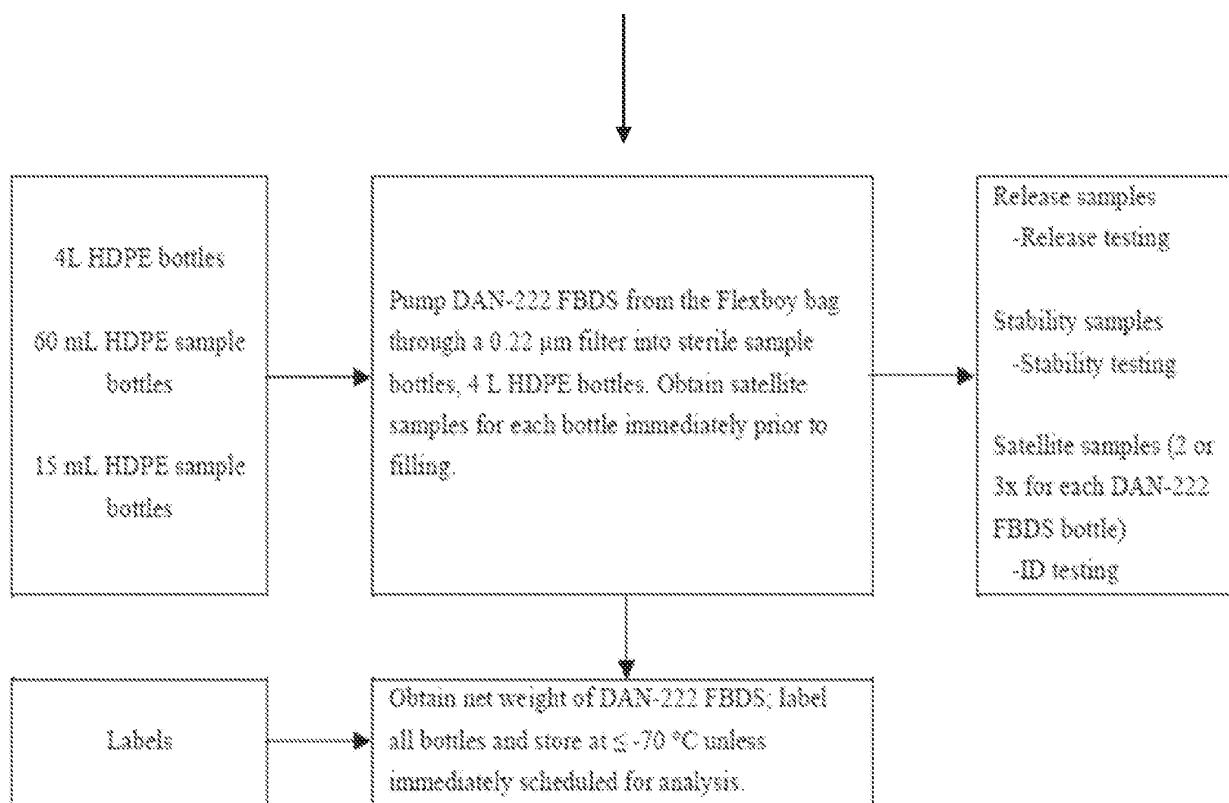


FIG. 43B

MAP MW control by MAM:diSPA-PEG comonomer ratio

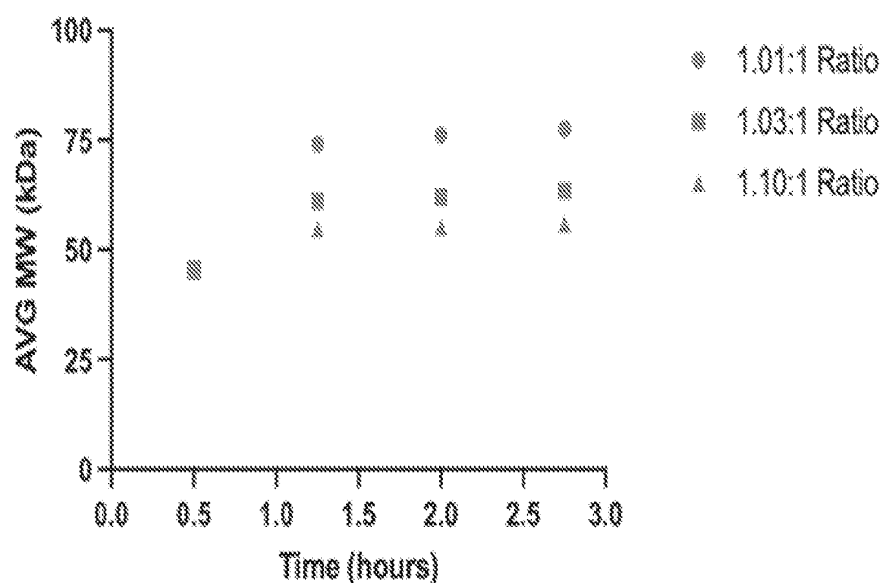


FIG. 44

Chemical structures of MAM diTFA salt and neutral species

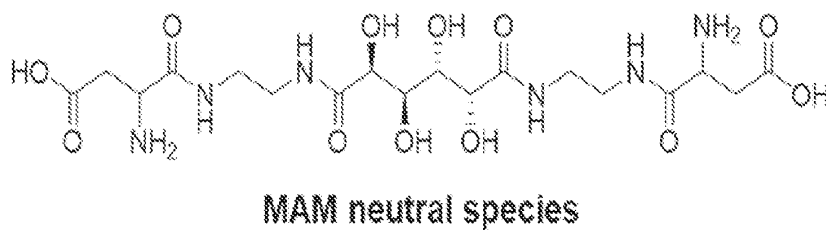
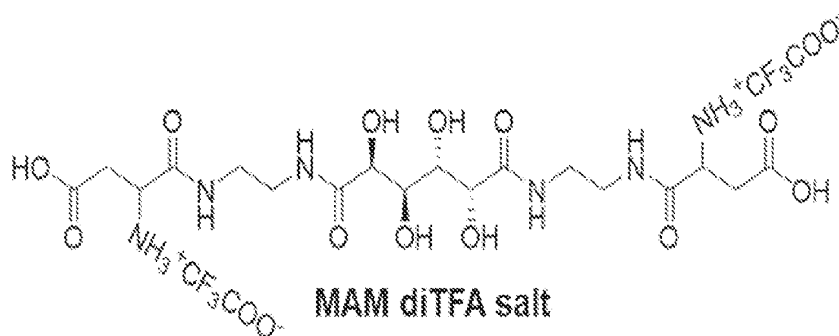
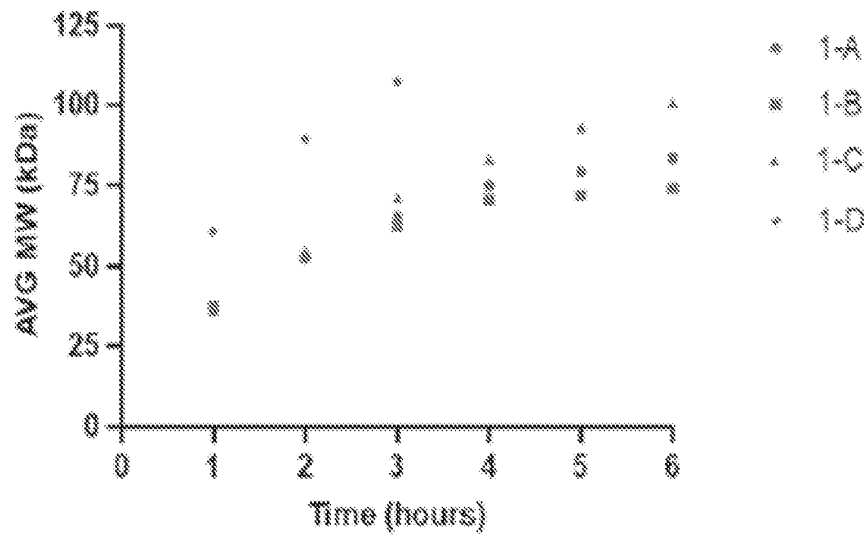


FIG. 45

MAP MW over time for screen 1 reaction conditions

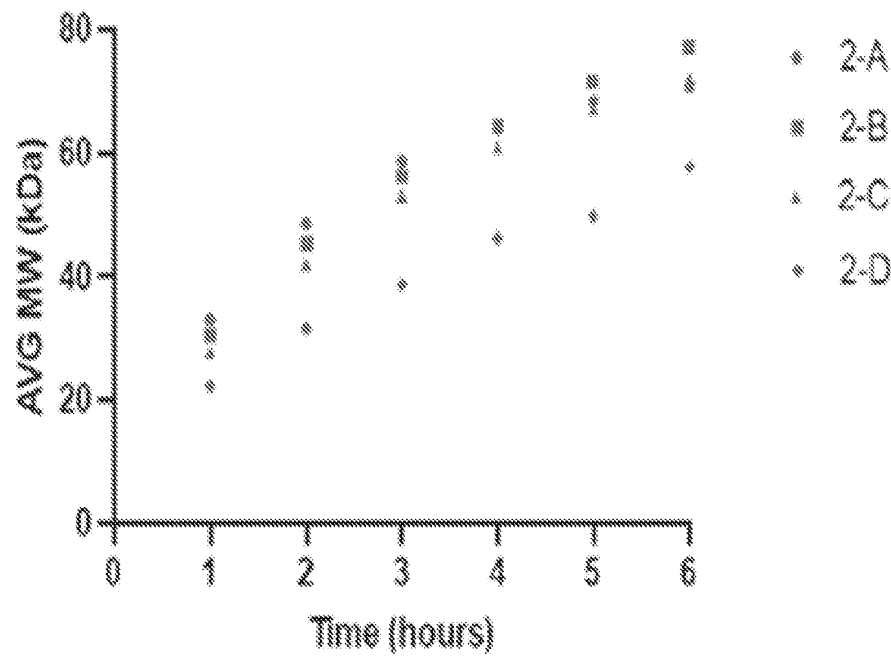


Reaction conditions evaluated in polymerization screen 1

Reaction	1-A	1-B	1-C	1-D
Comonomer ratio (MAM:diSPA-PEG)	1.10 : 1	1.10 : 1	1.05 : 1	1.05 : 1
TFA equivalents (TFA:MAM)	1.4 : 1	1.6 : 1	1.6 : 1	1.6 : 1
DIPEA equivalents (DIPEA:MAM)	2.5 : 1	2.5 : 1	2.5 : 1	4.0 : 1

FIG. 46

MAP MW over time for screen 2 reaction conditions



Reaction conditions evaluated in polymerization screen 2

Reaction	2-A	2-B	2-C	2-D
Comonomer ratio (MAM:diSPA-PEG)	1.10 : 1	1.07 : 1	1.07 : 1	1.07 : 1
TFA equivalents (TFA:MAM)	1.6 : 1	1.6 : 1	1.8 : 1	1.8 : 1
DIPEA equivalents (DIPEA:MAM)	2.5 : 1	2.5 : 1	2.5 : 1	2.2 : 1

FIG. 47

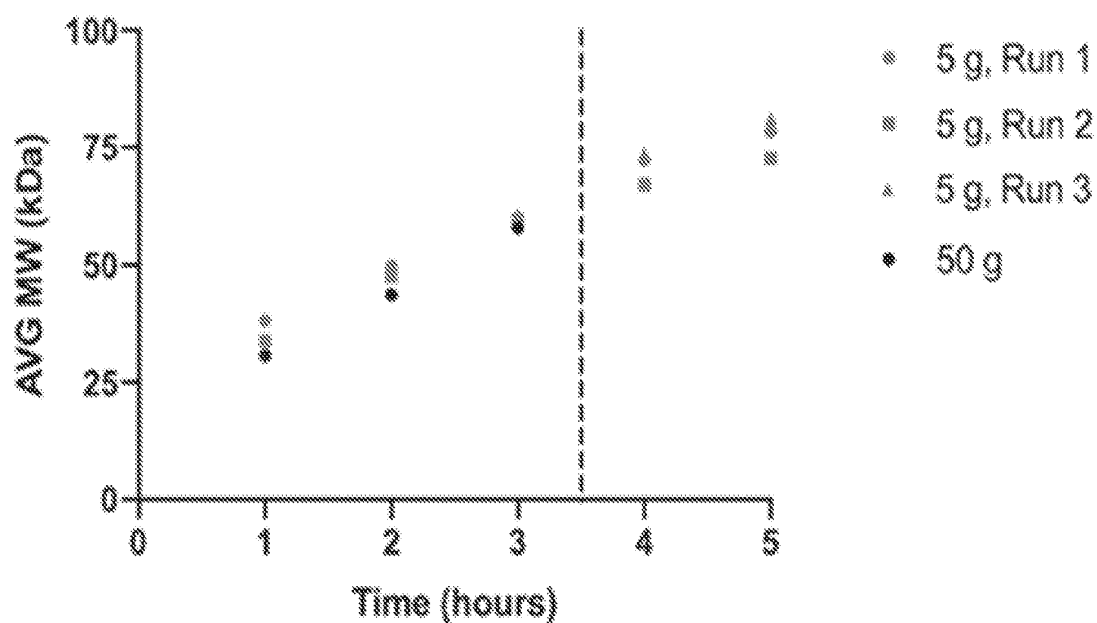
MAP MW over time for scale-up reactions

FIG. 48

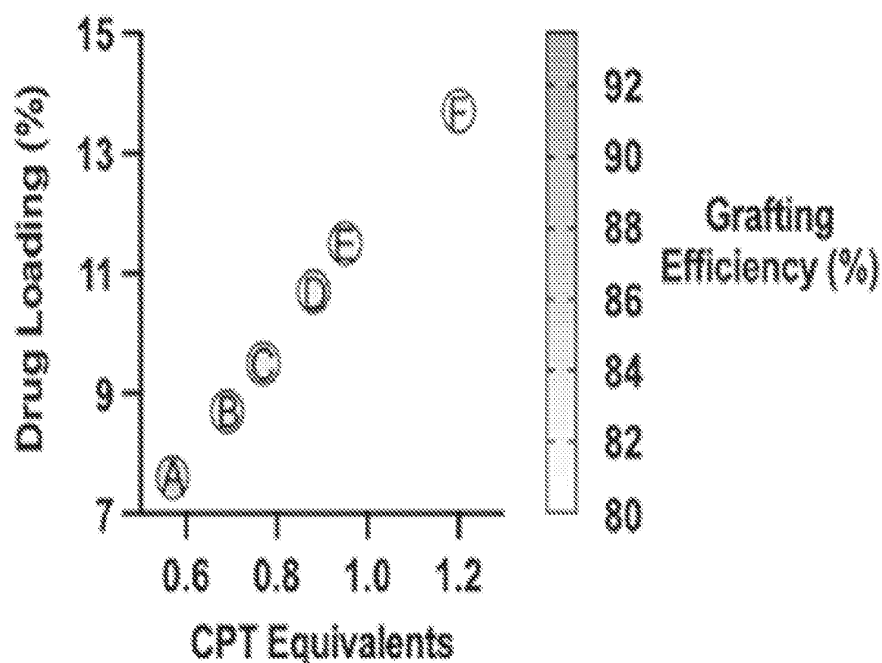
Drug loading percentage as a function of Gly-CPT equivalents and grafting efficiency for reactions with PyAOP

FIG. 49

Comparison of manufacturing processes for DAN-222 Formulated Bulk Drug Substance lots				
Process Step	Parameter	Dose range finding	Purpose/Use	Clinical phase I
Step 1 (Gly-CPT)	Lot #	PS533 ¹	PS533 ¹	K009P03L2001 K009P03L2003
	Lot #	SKT-757-046-5G-ppp SKT-757-044-5G-dia SKT-757-060-5G-dia	SKT-874-012-DRY SKT-874-017-DRY SKT-874-019-DRY SKT-874-026-DRY	21-M002 21-M003 21-M004
Step 2 (MAP)	Monomer	MAM as dTFA salt	MAM as dTFA salt	MAM as neutral species
Step 3 (MAP-Gly-CPT amorphous)	Purification method	Dialysis and precipitation	Precipitation	Precipitation
	Lot #	SKT-757-069-lyo SKT-757-072-1-lyo SKT-757-072-2-lyo SKT-757-075-T1-lyo SKT-757-075-T2-lyo SKT-757-075-T3-lyo	SKT-874-027-A SKT-874-028-B SKT-874-029-C SKT-874-030-D	21-M005 21-M005-1 21-M005-2 21-M006
	Coupling reagent	PyAOP	PyAOP	PyAOP
	Purification method	Dialysis	TFF	TFF
	Lot #	SKT-757-077-NPS	SKT-874-031-NPS	21-M007
Step 4 (MAP-Gly-CPT NPs) (DAN-222 FBDS)	Formulation	0.9 % NaCl, pH 4.0	20 mM sodium acetate, 200 mM NaCl, pH 4.3	20 mM sodium acetate, 200 mM NaCl, pH 4.3
	Concentration method	TFF	TFF	TFF
	Filtration steps	0.22-micron sterile filtration	0.45-micron conditioning filtration and 0.22-micron sterile filtration	0.45-micron conditioning filtration and 0.22-micron sterile filtration
¹ Custom-manufactured batch used for development, ca. 10x Avra Scale				

FIG. 50

Stability and recovery of DAN-222 in low (target 0.022 mg/mL CPT concentration) IV bags

	PVC Bag with 0.9 % (w/v) Saline Diluent	PVC Bag with 5 % (w/v) Dextrose Diluent	PO Bag with 0.9 % (w/v) Saline Diluent	PO Bag with 5 % (w/v) Dextrose Diluent	PE-PP Bag with 0.9 % (w/v) Saline Diluent	PE-PP Bag with 5 % (w/v) Dextrose Diluent
Visual inspection	Free from visible particles	Free from visible particles	Free from visible particles	Free from visible particles	Free from visible particles	Free from visible particles
% free of total CPT	0.82 % (+0.06 % increase from starting DP (0.76 %))	0.79 % (+0.03 % increase from starting DP (0.76 %))	0.85 % (+0.07 % increase from starting DP (0.78 %))	0.89 % (+0.11 % increase from starting DP (0.78 %))	0.83 % (-0.01 % decrease from starting DP (0.84 %))	0.87 % (+0.03 % increase from starting DP (0.84 %))
CPT concentration	0.0208 mg/mL (95.9 % of theoretical mg/mL CPT calculated)	0.0208 mg/mL (95.0 % of theoretical mg/mL CPT calculated)	0.0204 mg/mL (93.6 % of theoretical mg/mL CPT calculated)	0.0200 mg/mL (94.3 % of theoretical mg/mL CPT calculated)	0.0181 mg/mL (94.3 % of theoretical mg/mL CPT calculated)	0.0184 mg/mL (93.4 % of theoretical mg/mL CPT calculated)
Particle size	48.3 nm	30.8 nm	20.9 nm	29.1 nm	23.2 nm	34.3 nm
Polydispersity index	0.683	0.557	0.331	0.643	0.388	0.849
pH	5.03	4.94	4.80	4.51	4.95	4.79
Osmolality	285 mOsm/kg	267 mOsm/kg	289 mOsm/kg	271 mOsm/kg	290 mOsm/kg	271 mOsm/kg
Sub-visible particles (per mL)	≥ 2 µm: 4 ≥ 10 µm: 0.5 ≥ 25 µm: 0	≥ 2 µm: 3 ≥ 10 µm: 0.5 ≥ 25 µm: 0	≥ 2 µm: 3 ≥ 10 µm: 0.5 ≥ 25 µm: 0	≥ 2 µm: 1.5 ≥ 10 µm: 0.5 ≥ 25 µm: 0	≥ 2 µm: 0 ≥ 10 µm: 0 ≥ 25 µm: 0	≥ 2 µm: 2 ≥ 10 µm: 0.5 ≥ 25 µm: 0

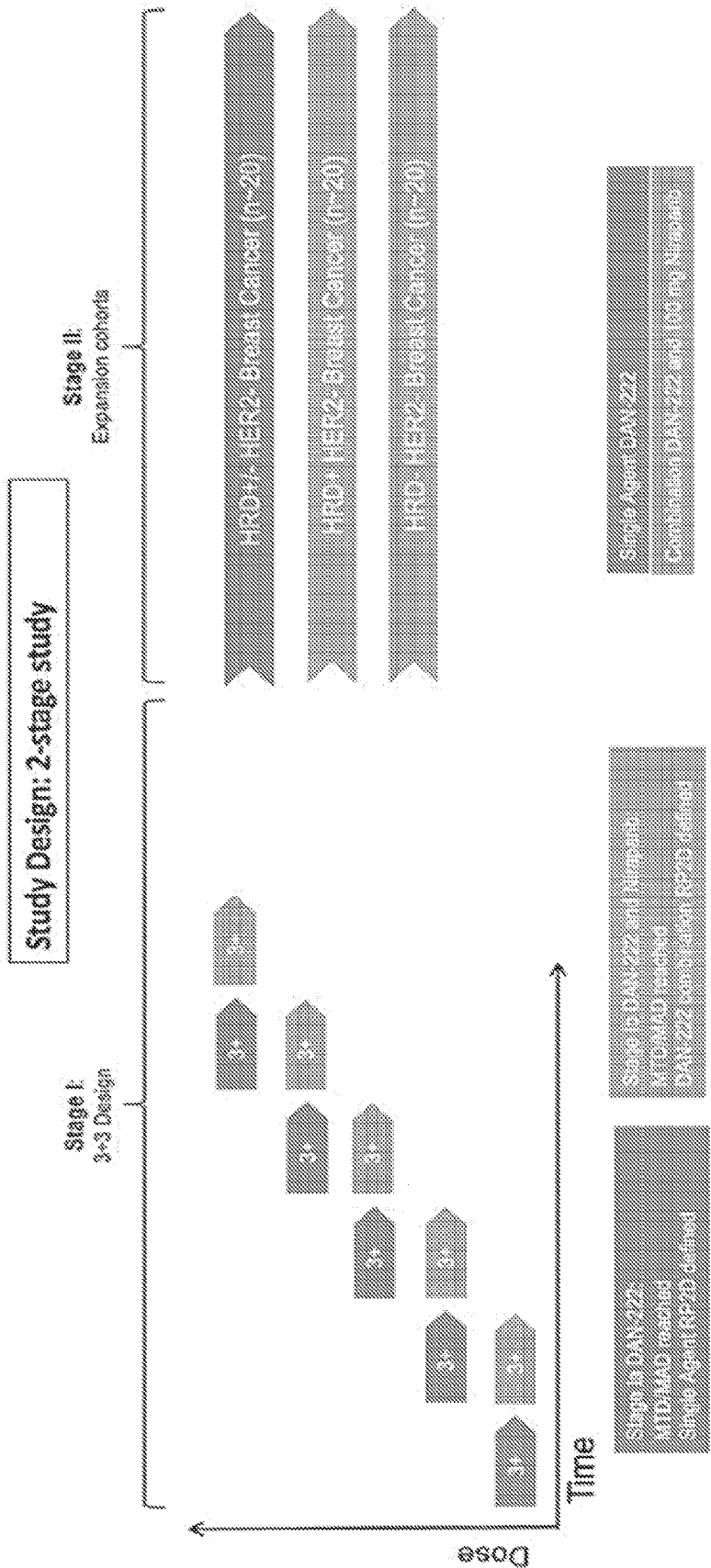
CPT = 20(S)-camptothecin; DP = drug product; mg = milligram; mL = milliliter; mOsm/kg = milliosmole kilogram; nm = nanometer; PVC = polyvinyl chloride; PO = polyolefin; PE-PP = polyethylene-polypropylene; µm = micron

FIG. 51

Stability and recovery of DAN-222 in high dose (target 0.400 mg/mL CPT concentration) IV bags						
	PVC Bag with 0.9 % (w/v) Saline Diluent	PVC Bag with 5 % (w/v) Dextrose Diluent	PO Bag with 0.9 % (w/v) Saline Diluent	PO Bag with 5 % (w/v) Dextrose Diluent	PE-PP Bag with 0.9 % (w/v) Saline Diluent	PE-PP Bag with 5 % (w/v) Dextrose Diluent
Visual inspection	Free from visible particles	Free from visible particles	Free from visible particles	Free from visible particles	Free from visible particles	Free from visible particles
% free of total CPT	0.77 % (~0.01 % increase from starting DP (0.76 %))	0.79 % (~0.03 % increase from starting DP (0.76 %))	0.79 % (~0.01 % increase from starting DP (0.78 %))	0.82 % (~0.04 % increase from starting DP (0.78 %))	0.94 % (~0.09 % decrease from starting DP (0.85 %))	0.92 % (~0.07 % increase from starting DP (0.85 %))
CPT concentration	0.383 mg/mL (100.9 % of theoretical mg/mL CPT calculated)	0.373 mg/mL (99.2 % of theoretical mg/mL CPT calculated)	0.388 mg/mL (103.2 % of theoretical mg/mL CPT calculated)	0.368 mg/mL (99.5 % of theoretical mg/mL CPT calculated)	0.376 mg/mL (97.9 % of theoretical mg/mL CPT calculated)	0.378 mg/mL (97.2 % of theoretical mg/mL CPT calculated)
Particle size	48.3 nm	30.8 nm	20.9 nm	29.1 nm	23.2 nm	34.3 nm
Polydispersity index	0.445	0.451	0.417	0.417	0.403	0.409
pH	4.31	4.33	4.33	4.32	4.35	4.37
Osmolality	303 mOsm/kg	289 mOsm/kg	303 mOsm/kg	291 mOsm/kg	304 mOsm/kg	286 mOsm/kg
Sub-visible particles (per mL)	≥ 2 µm: 5.5 ≥ 10 µm: 2 ≥ 25 µm: 1	≥ 2 µm: 4 ≥ 10 µm: 1 ≥ 25 µm: 10	≥ 2 µm: 3 ≥ 10 µm: 1 ≥ 25 µm: 0	≥ 2 µm: 4 ≥ 10 µm: 1 ≥ 25 µm: 10	≥ 2 µm: 2.5 ≥ 10 µm: 0 ≥ 25 µm: 0	≥ 2 µm: 4.5 ≥ 10 µm: 0.5 ≥ 25 µm: 0

CPT = 20(S)-camptothecin; DP = drug product; mg = milligram; mL = milliliter; mOsm/kg = milliosmole-kilogram; nm = nanometer; PVC = polyvinyl chloride; PO = polyolefin; PE-PP = polyethylene-polypropylene; µm = micrometer

FIG. 52



HER2- = human epidermal growth factor receptor 2 negative; HRD- = homologous recombination repair deficient negative; HRD+ = homologous recombination repair deficient positive; MAD = maximally administered dose; MTD = maximum tolerated dose; RP2D = recommended phase 2 dose.

FIG. 53