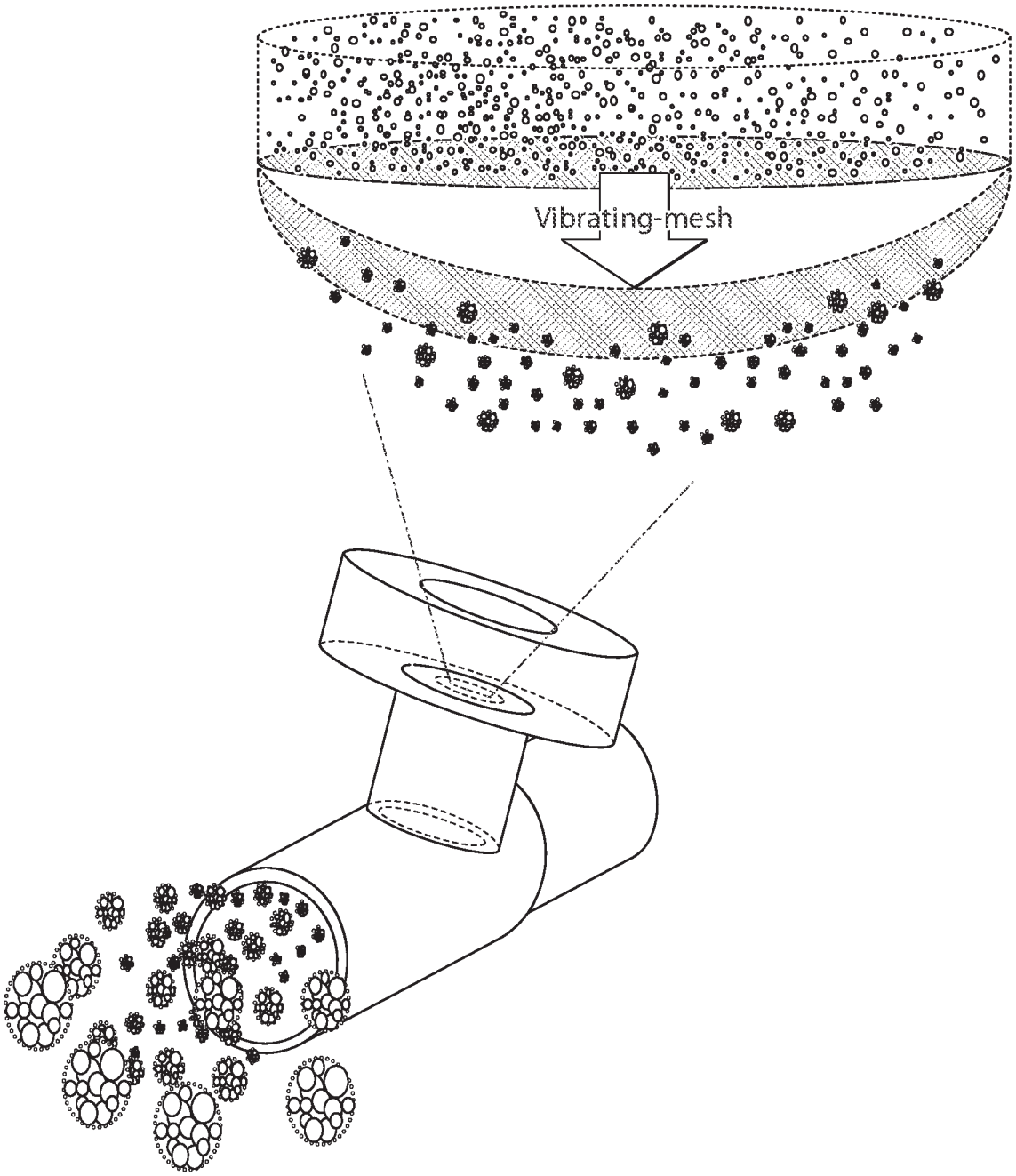


Fig. 1A

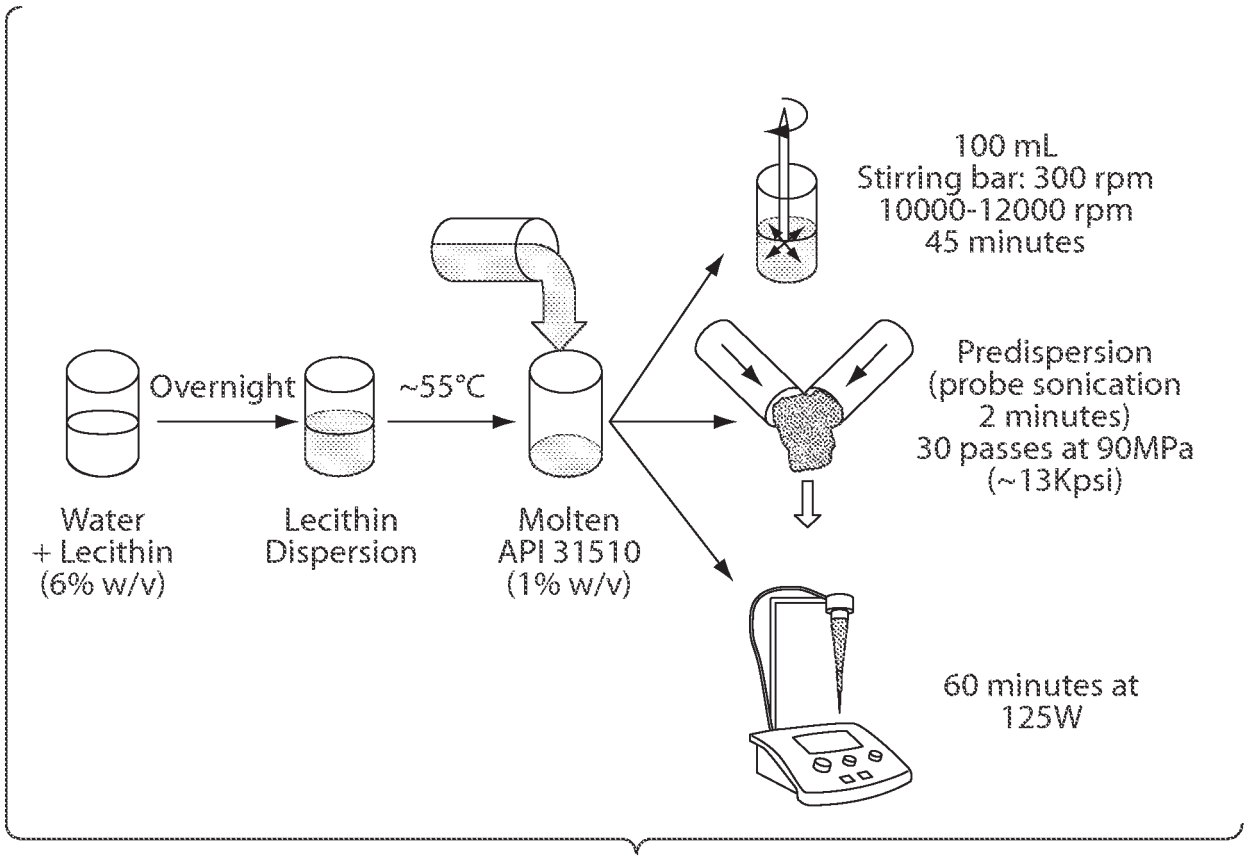

100 mL
Stirring bar: 300 rpm
10000-12000 rpm
45 minutes
Predispersion
(probe sonication
2 minutes)
30 passes at 90MPa
(~13Kpsi)
60 minutes at
125W
Overnight
~55°C
Water
+ Lecithin
(6% w/v)
Lecithin
Dispersion
Molten
API 31510
(1% w/v)

Fig. 1B

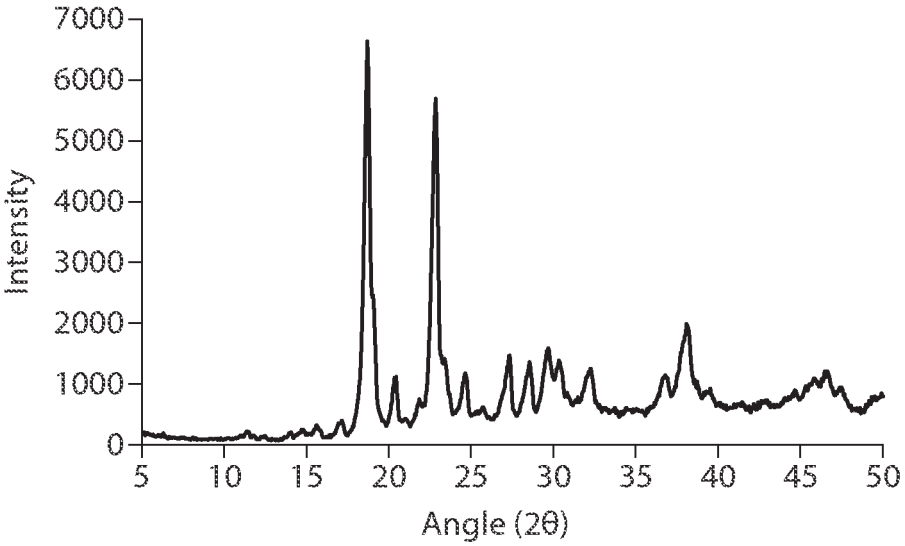

7000
6000
5000
4000
3000
2000
1000
0
Intensity
5
10
15
20
25
30
35
40
45
50
Angle (2θ)

Fig. 2

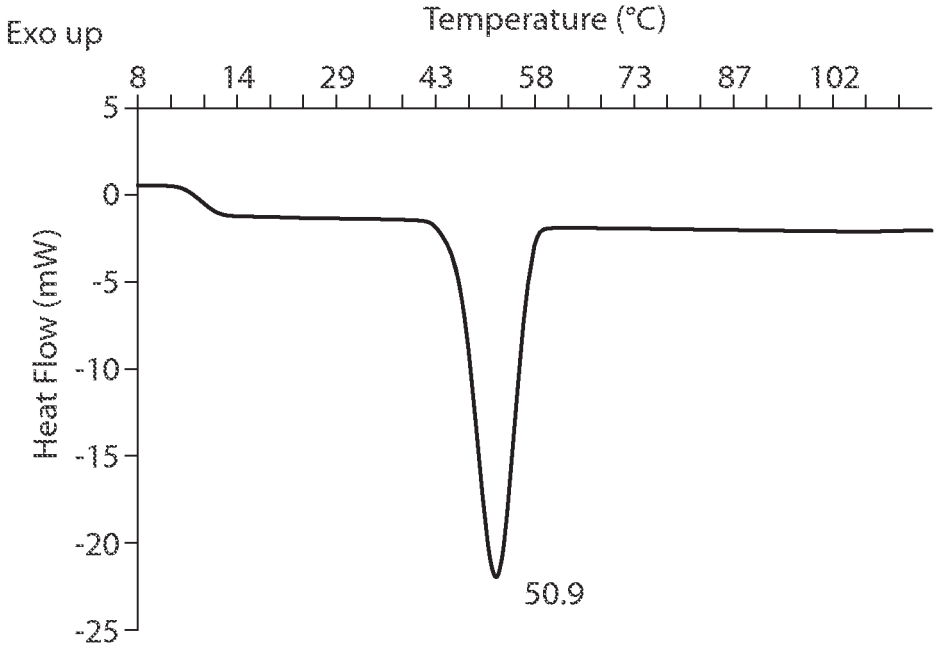

Exo up
Temperature (°C)
8
14
29
43
58
73
87
102
5
0
-5
-10
-15
-20
-25
Heat Flow (mW)
50.9

Fig. 3

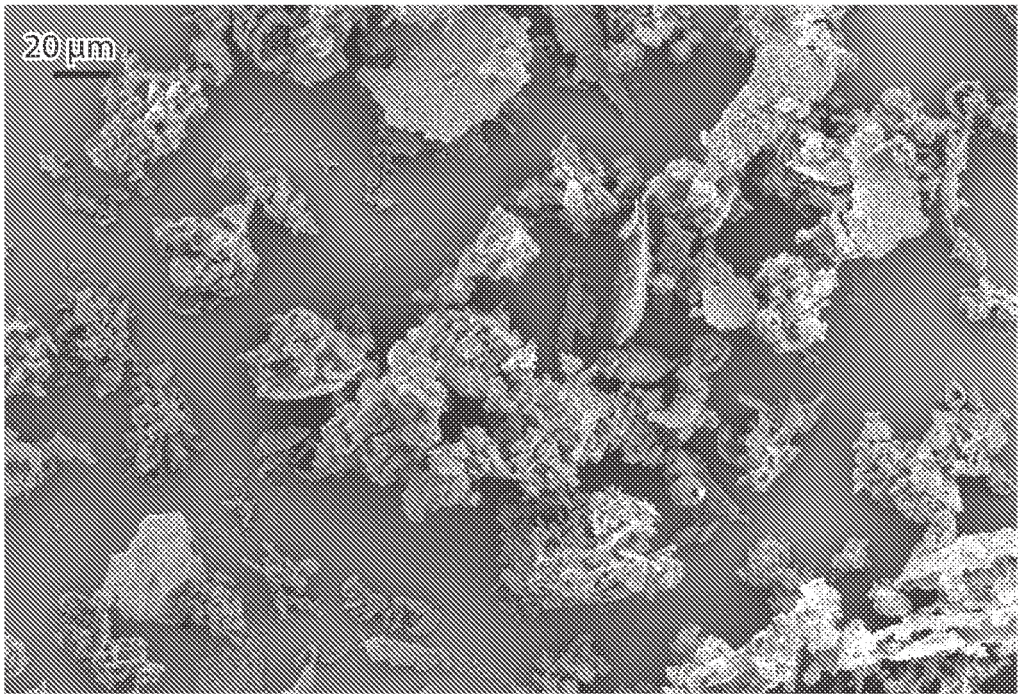

20 µm

Fig. 4

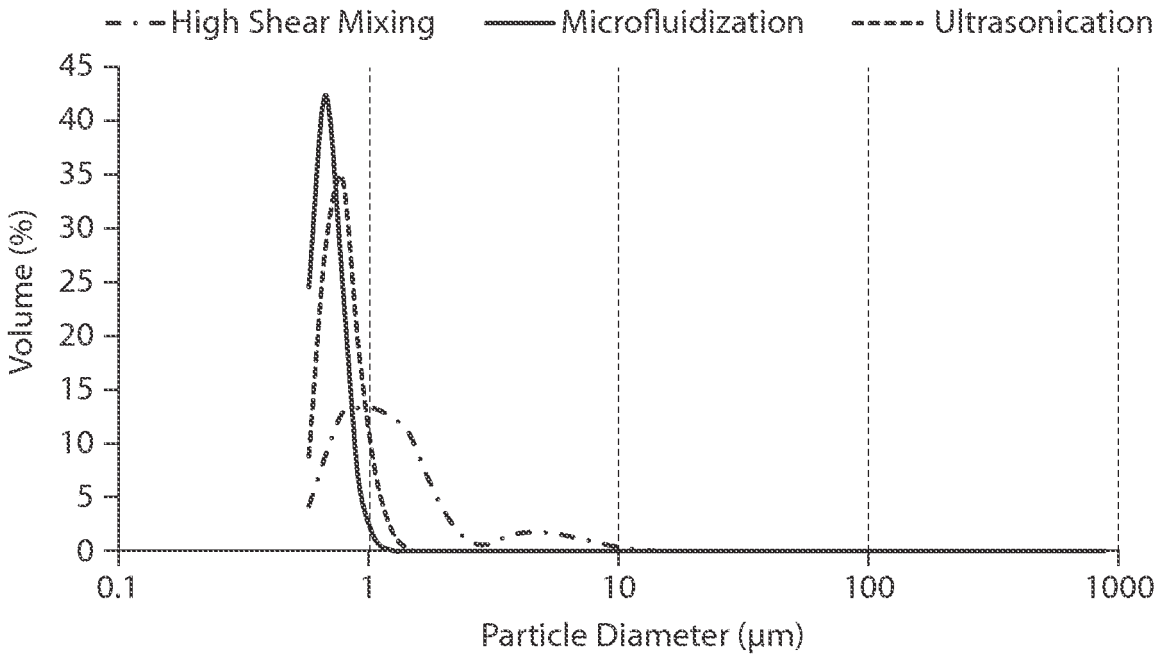

High Shear Mixing
Microfluidization
Ultrasonication
Volume (%)
45
40
35
30
25
20
15
10
5
0
0.1
1
10
100
1000
Particle Diameter (µm)

Fig. 5

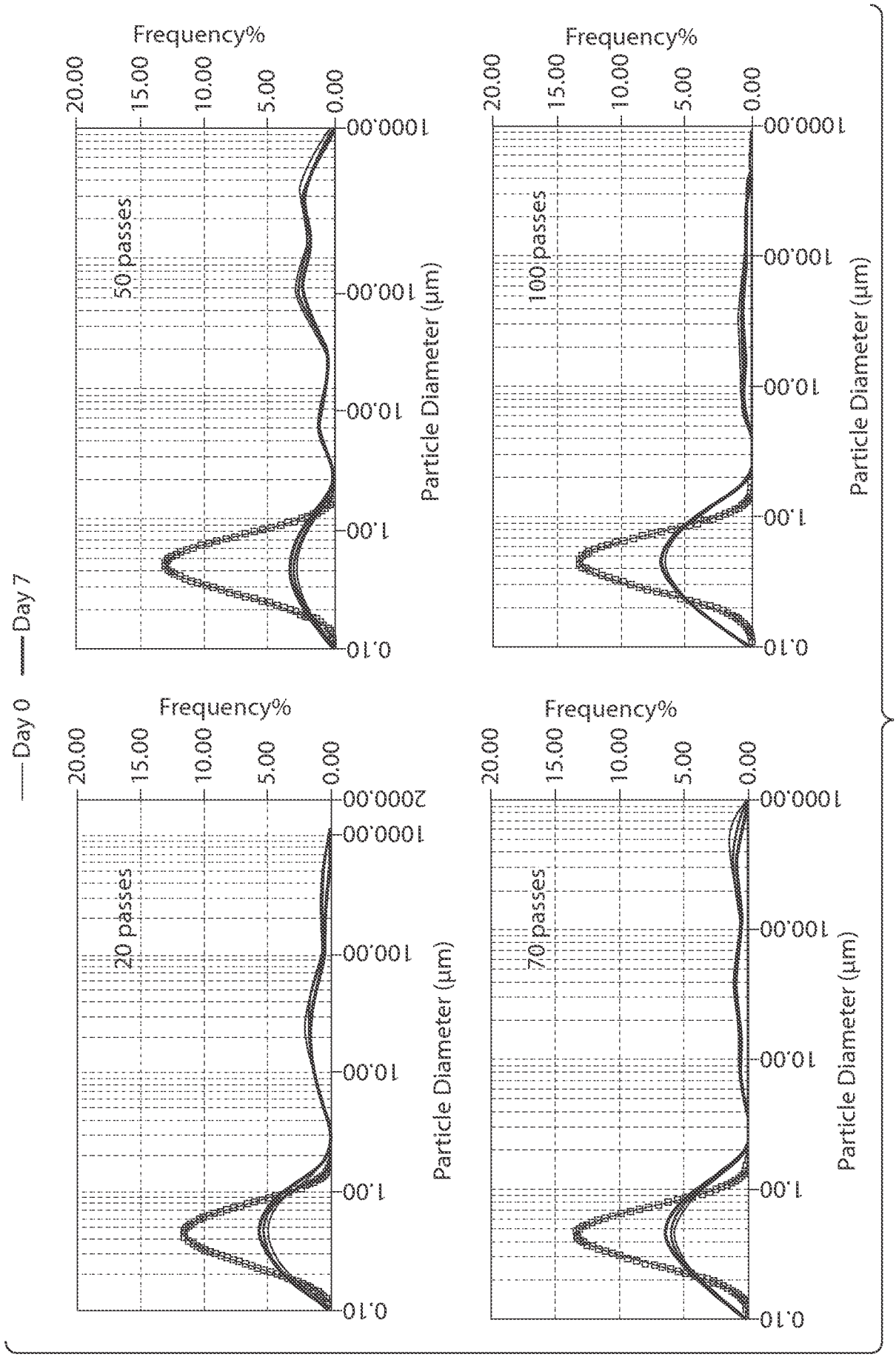

Day 0
Day 7
Frequency%
20.00
15.00
10.00
5.00
0.00
50 passes
100 passes
20 passes
70 passes
Particle Diameter (µm)
2000.00
1000.00
100.00
10.00
1.00
0.10

Fig. 6

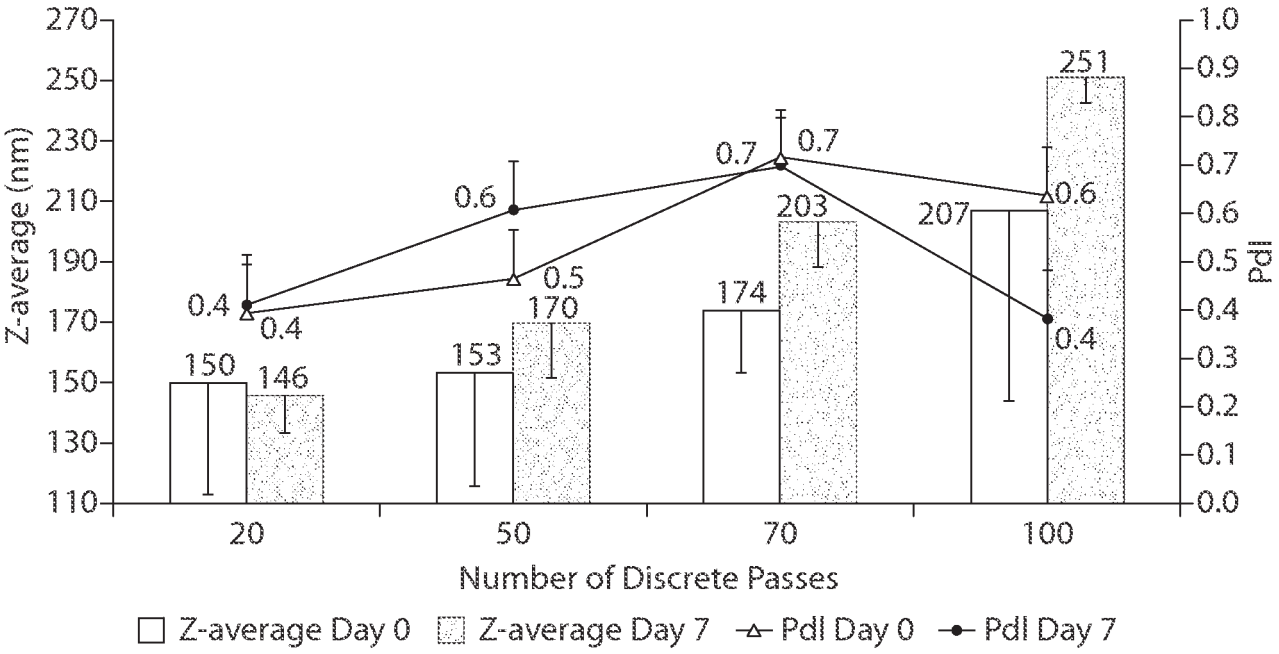

Z-average (nm)
270
250
230
210
190
170
150
130
110
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
PdI
150
146
153
170
174
203
207
251
0.4
0.4
0.6
0.5
0.7
0.7
0.6
0.4
20
50
70
100
Number of Discrete Passes
Z-average Day 0
Z-average Day 7
PdI Day 0
PdI Day 7

Fig. 7

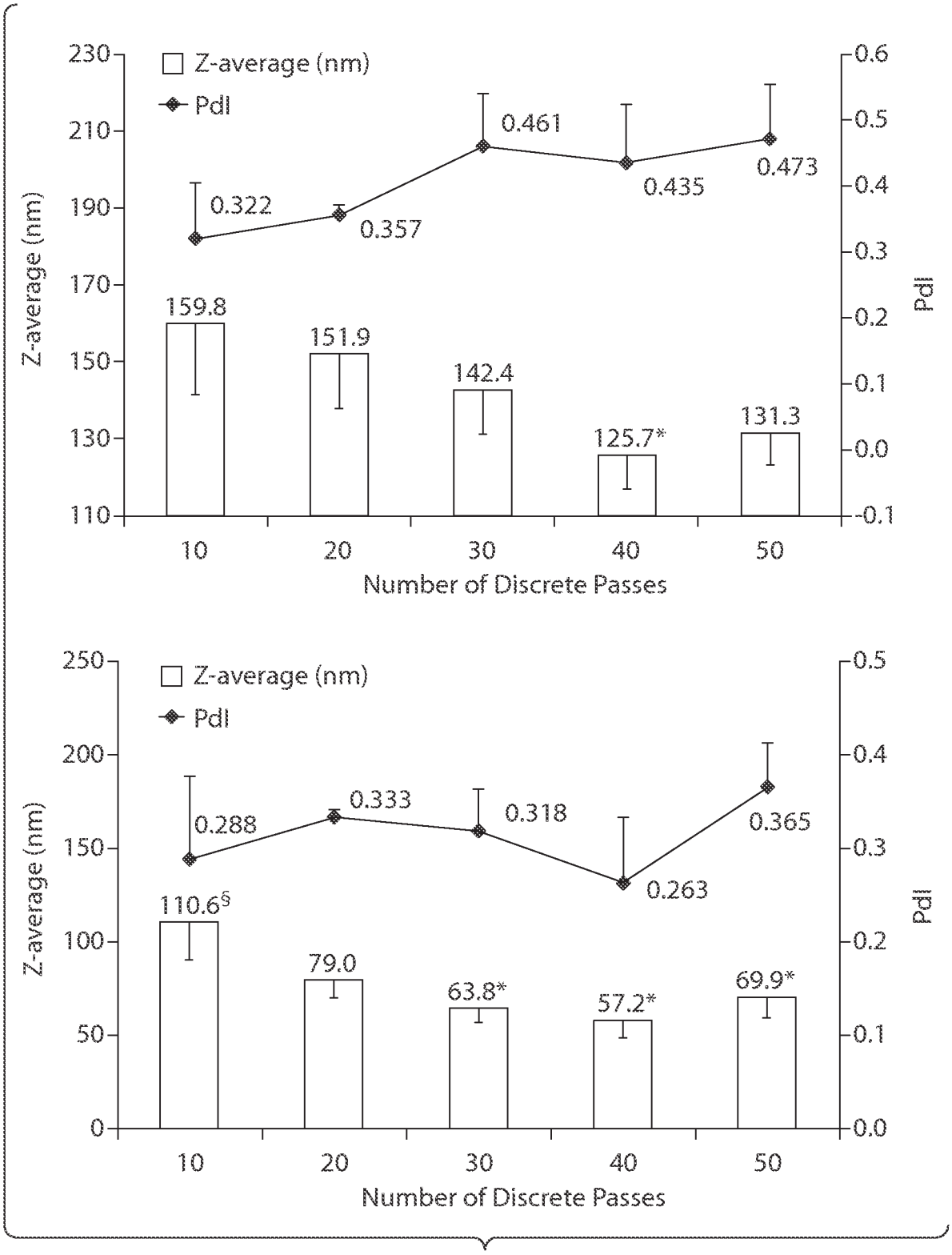

Z-average (nm)
PdI
230
210
190
170
150
130
110
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0.322
0.357
0.461
0.435
0.473
159.8
151.9
142.4
125.7*
131.3
10
20
30
40
50
Number of Discrete Passes
Z-average (nm)
PdI
250
200
150
100
50
0
0.5
0.4
0.3
0.2
0.1
0.0
0.288
0.333
0.318
0.263
0.365
110.6§
79.0
63.8*
57.2*
69.9*
10
20
30
40
50
Number of Discrete Passes

Fig. 8

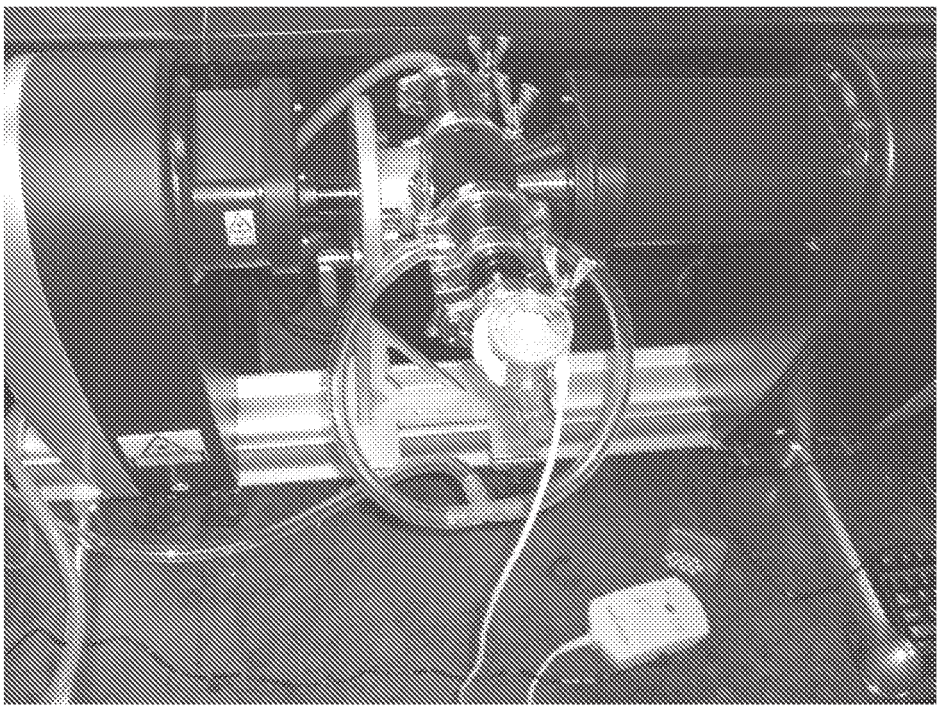


Fig. 9A

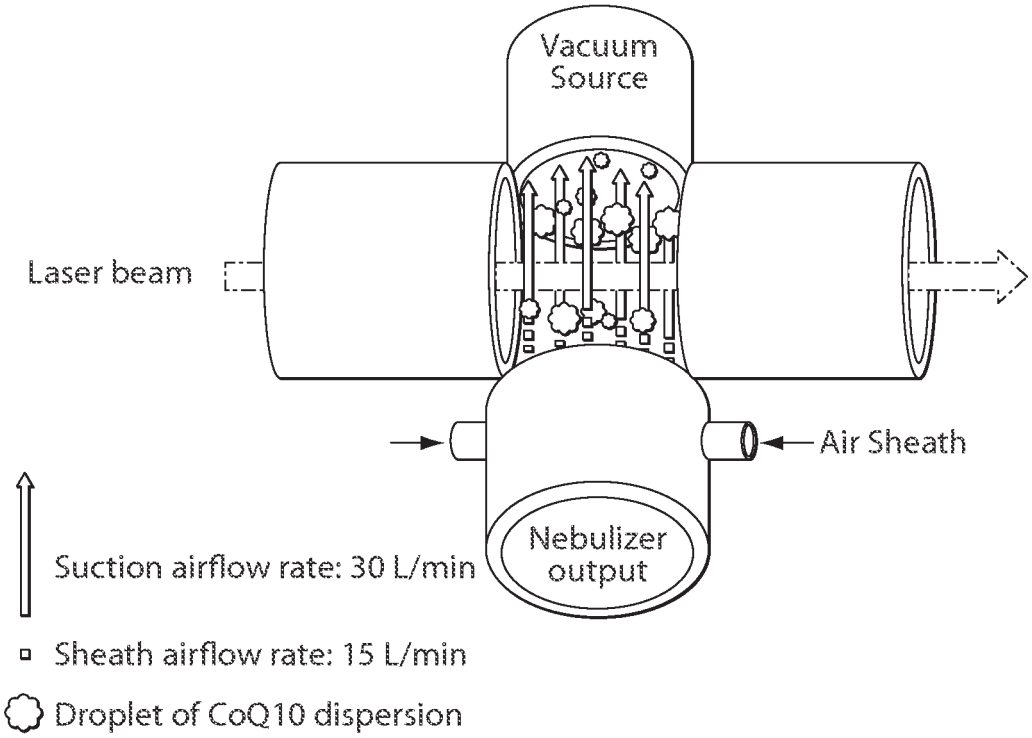

Vacuum Source
Laser beam
Air Sheath
Nebulizer output
Suction airflow rate: 30 L/min
Sheath airflow rate: 15 L/min
Droplet of CoQ10 dispersion

Fig. 9B

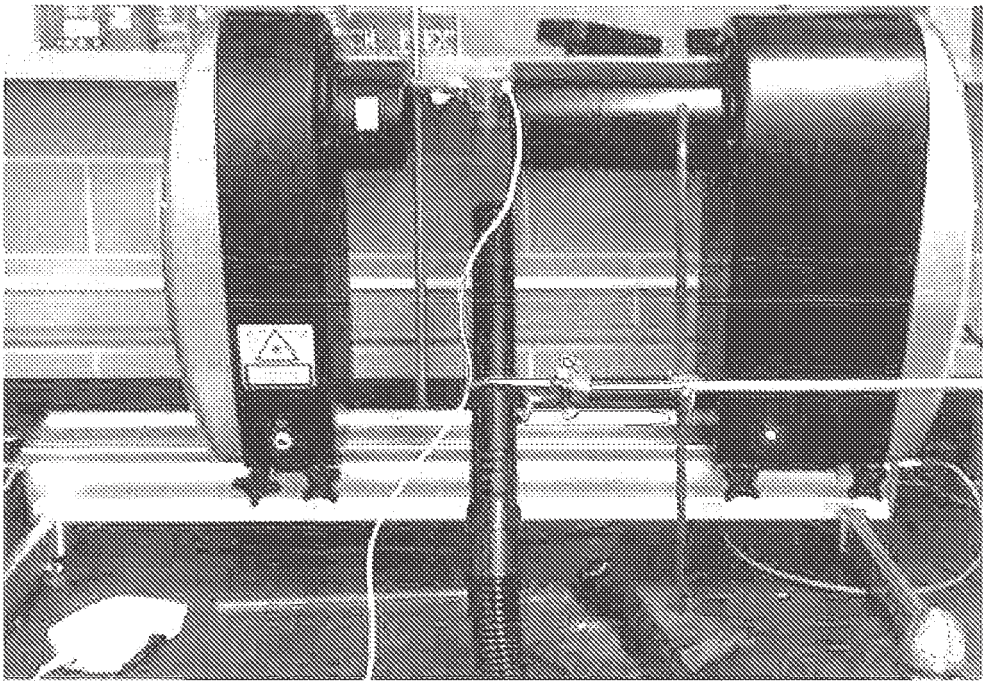


Fig. 9C

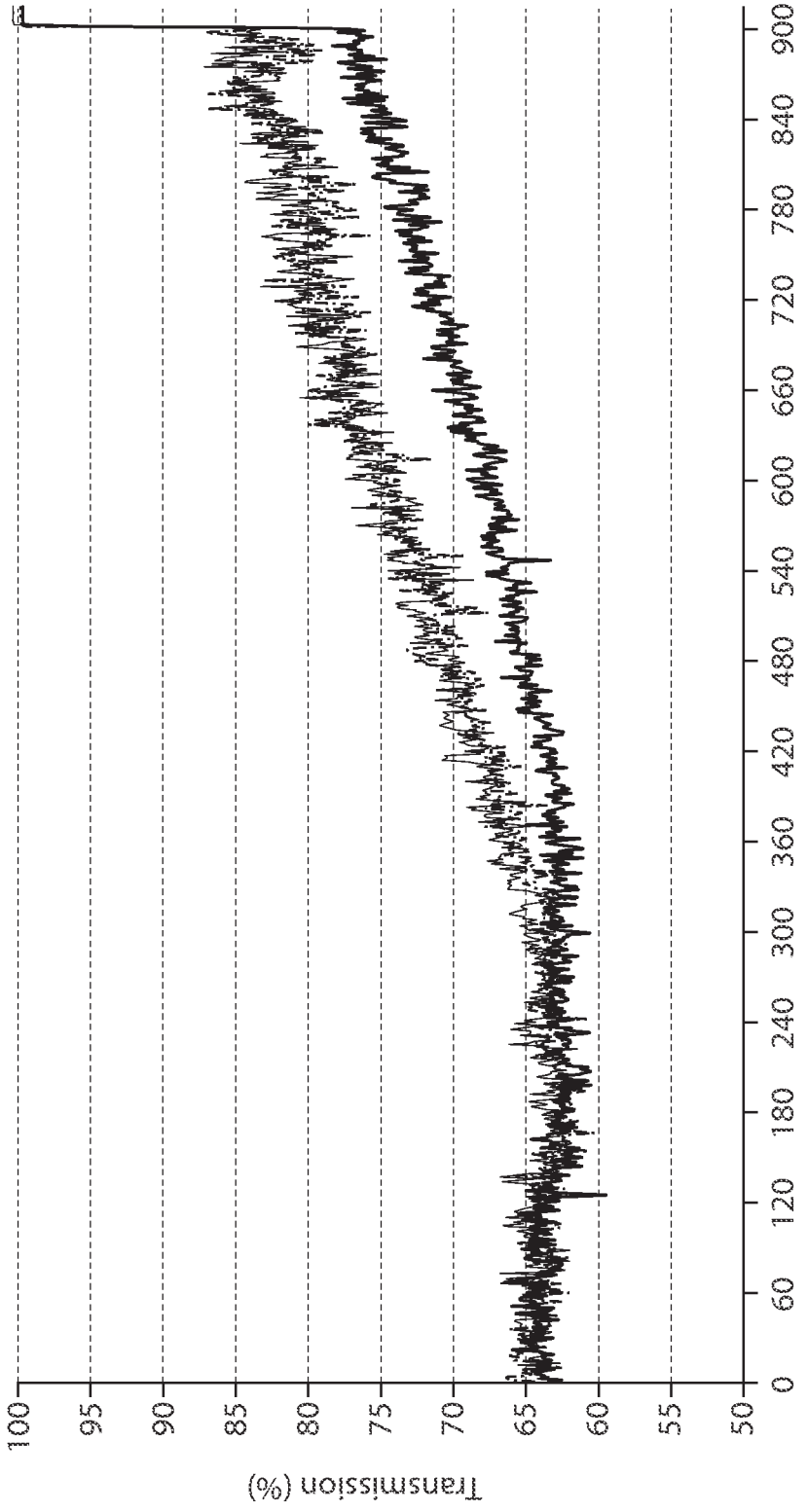

Transmission (%)
100
95
90
85
80
75
70
65
60
55
50
0
60
120
180
240
300
360
420
480
540
600
660
720
780
840
900
10 passes
30 passes
50 passes

Fig. 10

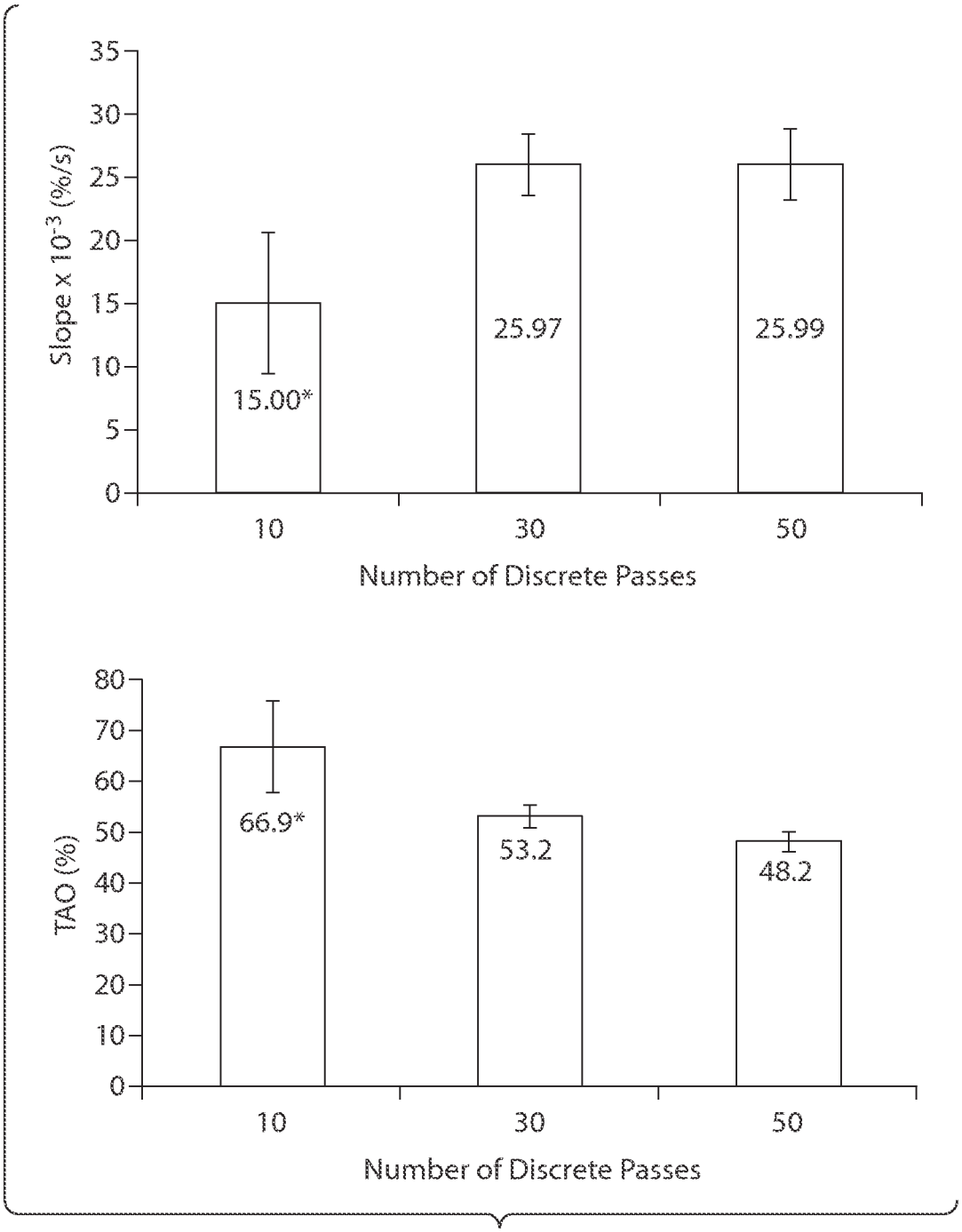

Slope x 10^{-3} (%/s)
35
30
25
20
15
10
5
0
15.00*
25.97
25.99
10
30
50
Number of Discrete Passes
TAO (%)
80
70
60
50
40
30
20
10
0
66.9*
53.2
48.2
10
30
50
Number of Discrete Passes

Fig. 11

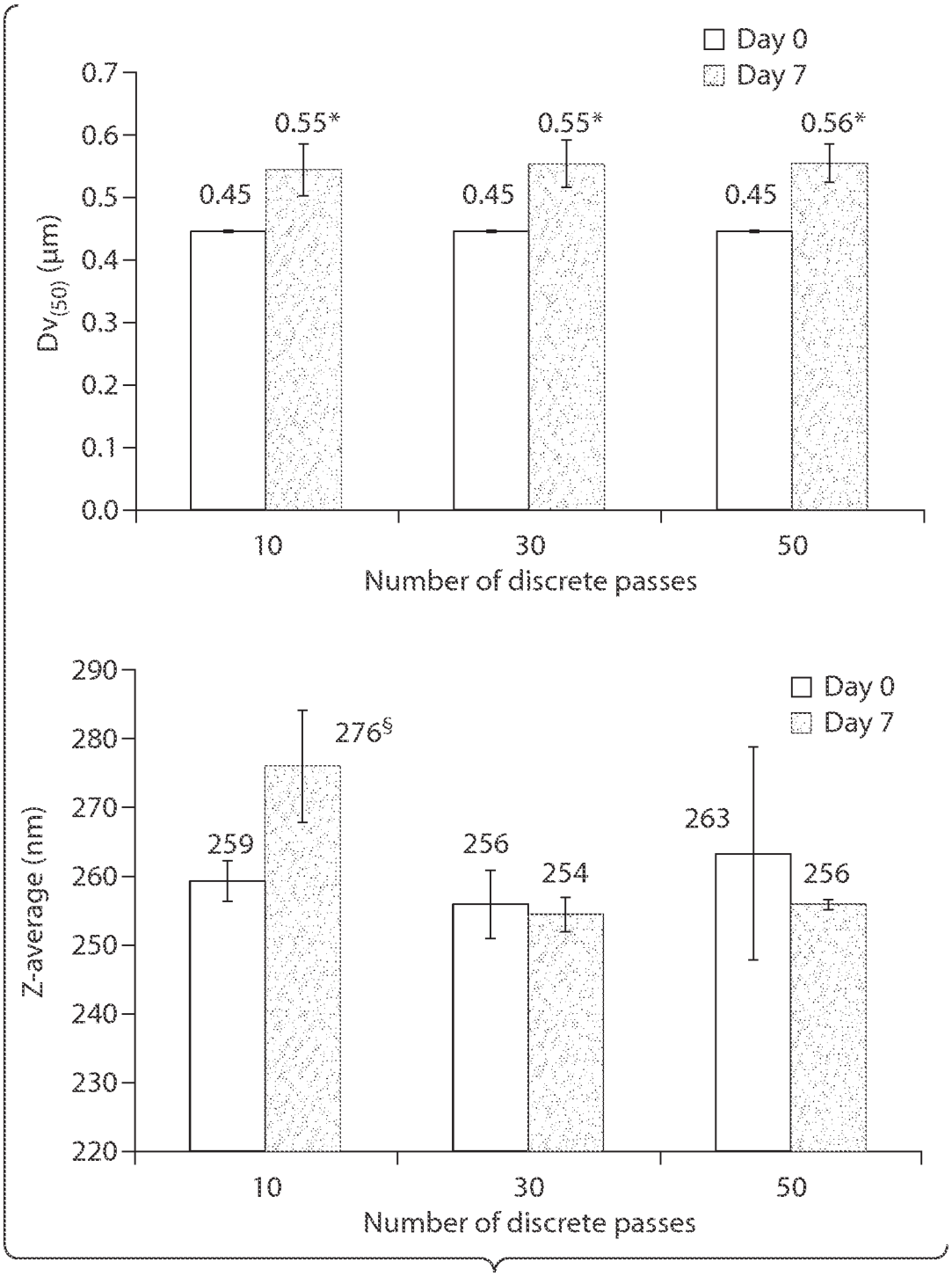

Day 0
Day 7
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
$Dv_{(50)}$ (µm)
0.45
0.55*
0.45
0.55*
0.45
0.56*
10
30
50
Number of discrete passes
290
280
270
260
250
240
230
220
Z-average (nm)
259
276§
256
254
263
256
Day 0
Day 7
10
30
50
Number of discrete passes

Fig. 12

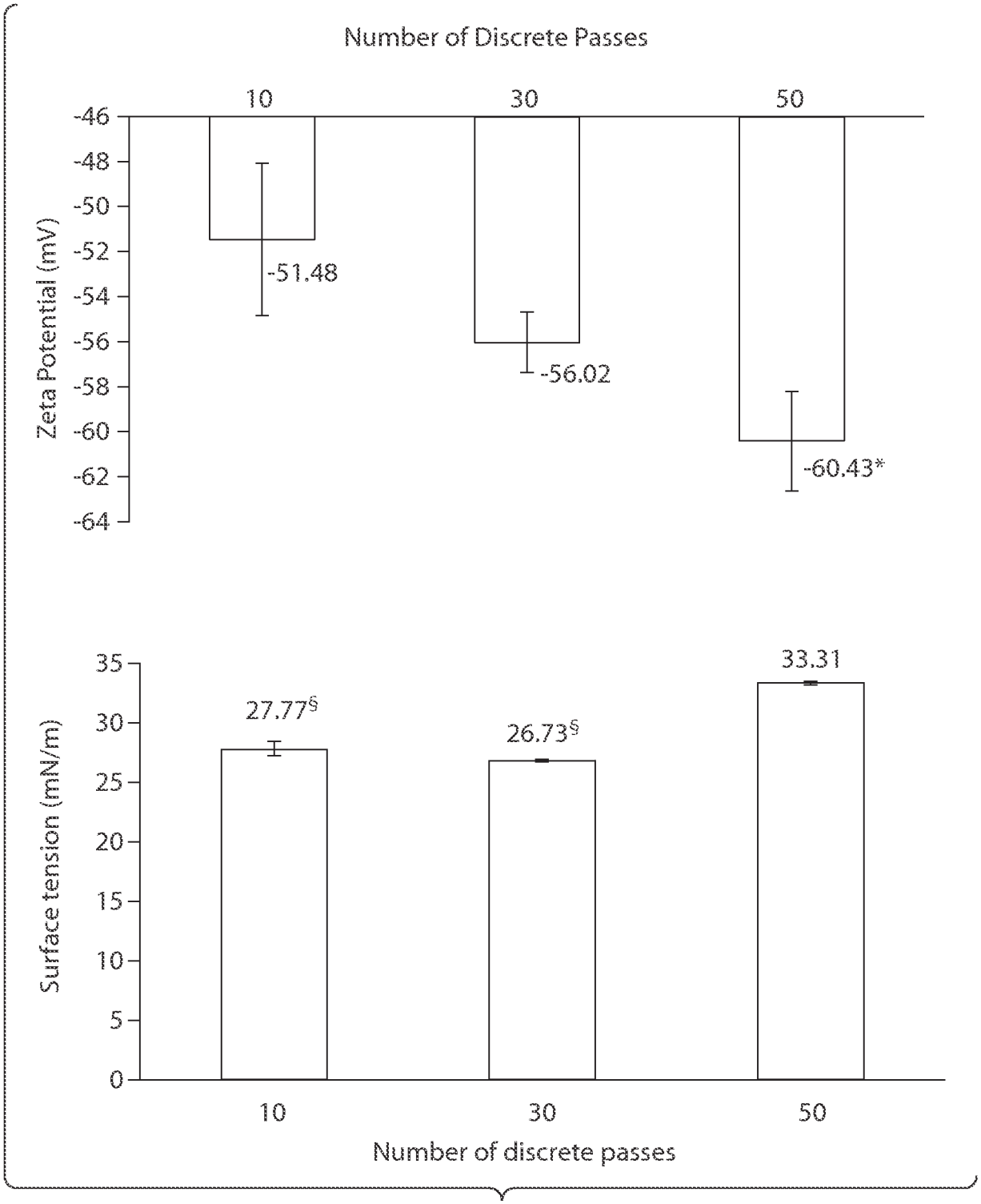

Number of Discrete Passes
10
30
50
-46
-48
-50
-52
-54
-56
-58
-60
-62
-64
Zeta Potential (mV)
-51.48
-56.02
-60.43*
35
30
25
20
15
10
5
0
Surface tension (mN/m)
27.77§
26.73§
33.31
10
30
50
Number of discrete passes

Fig. 13

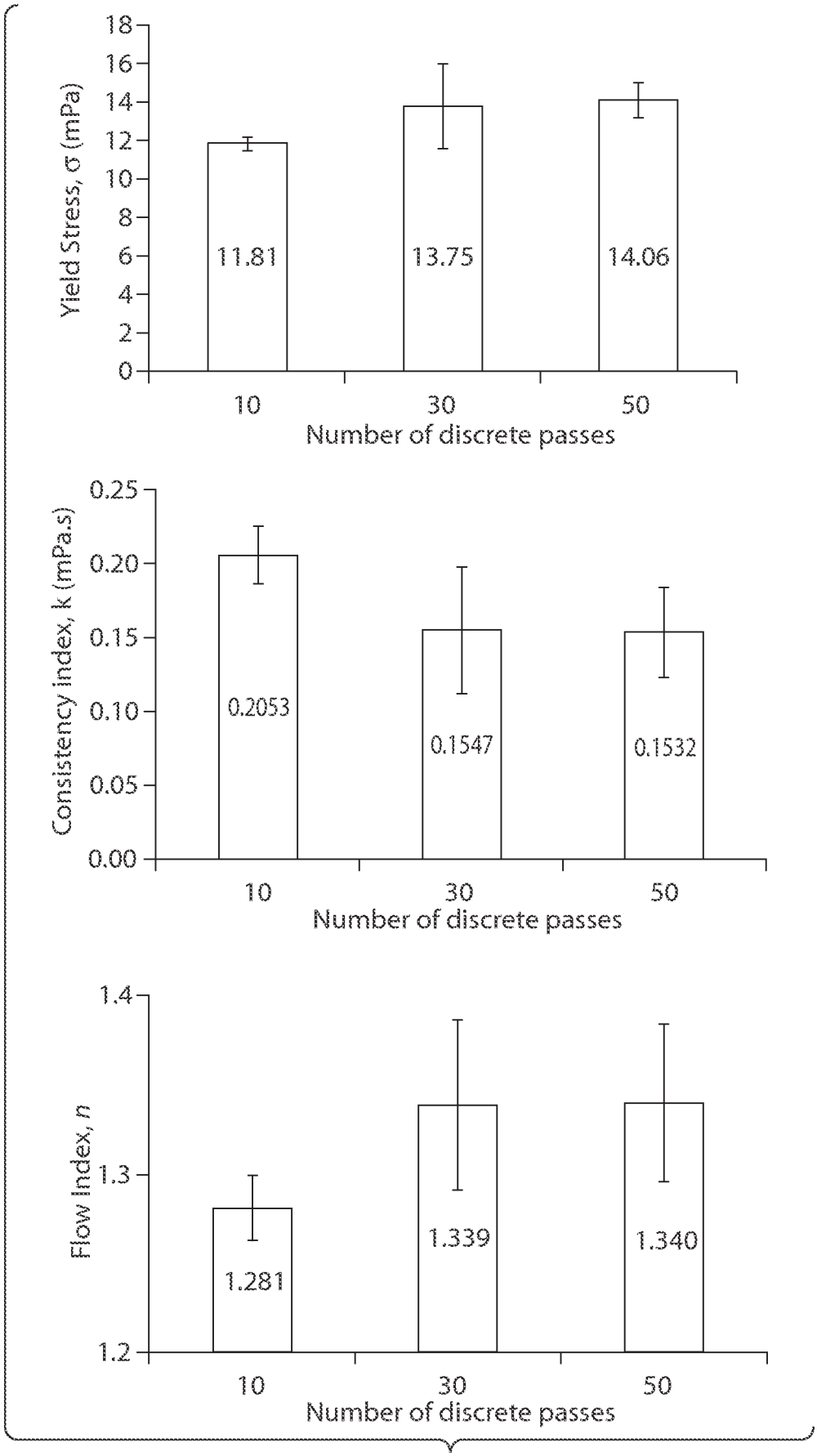

Yield Stress, σ (mPa)
18
16
14
12
10
8
6
4
2
0
11.81
13.75
14.06
10
30
50
Number of discrete passes
Consistency index, k (mPa.s)
0.25
0.20
0.15
0.10
0.05
0.00
0.2053
0.1547
0.1532
10
30
50
Number of discrete passes
Flow Index, n
1.4
1.3
1.2
1.281
1.339
1.340
10
30
50
Number of discrete passes

Fig. 14A

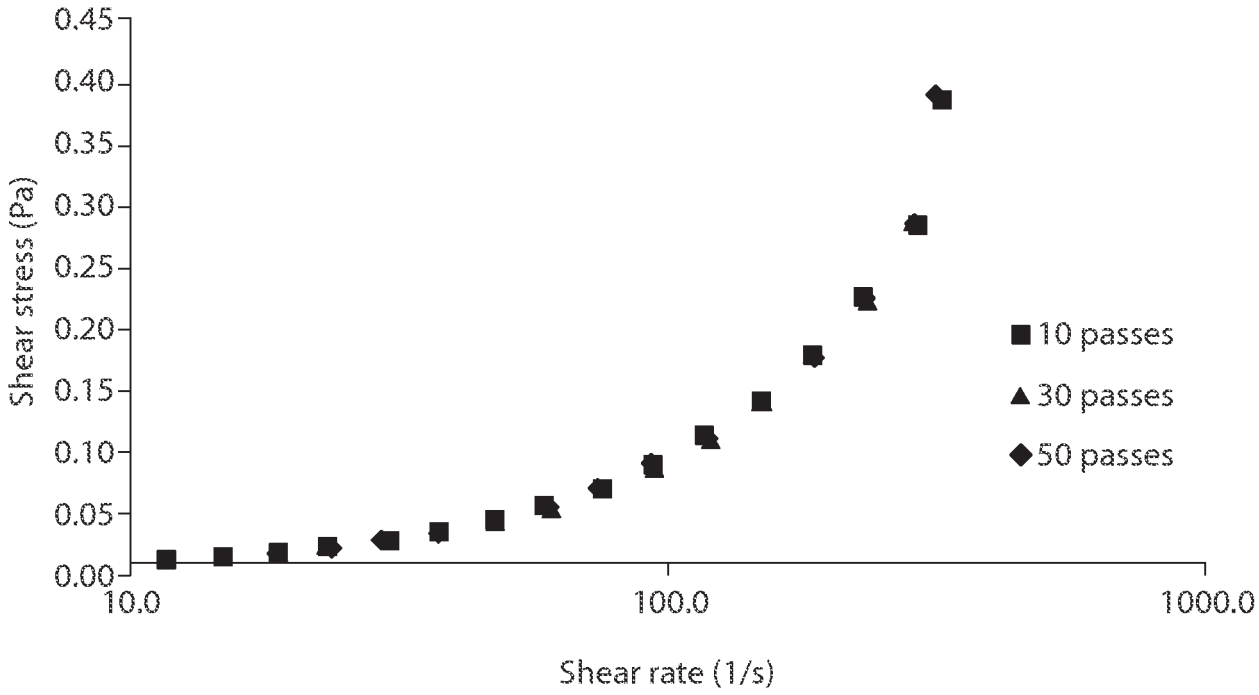

0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Shear stress (Pa)
10.0
100.0
1000.0
Shear rate (1/s)
10 passes
30 passes
50 passes

Fig. 14B

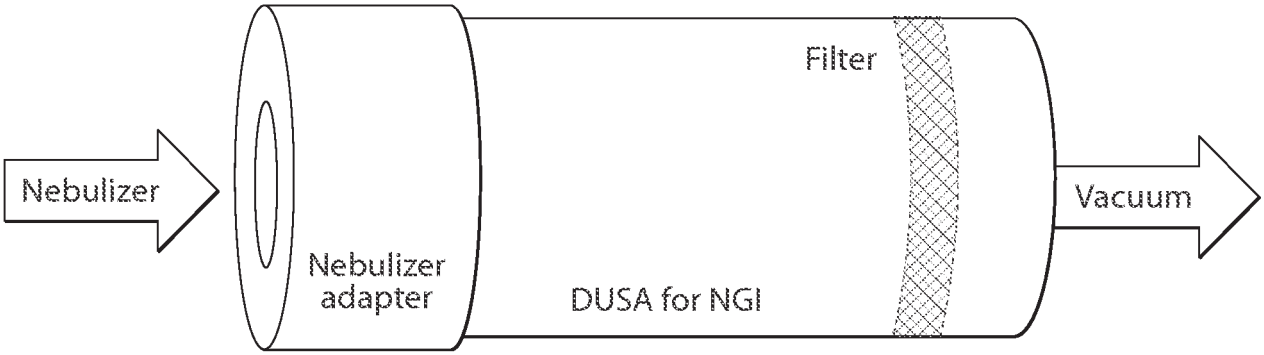


Fig. 15

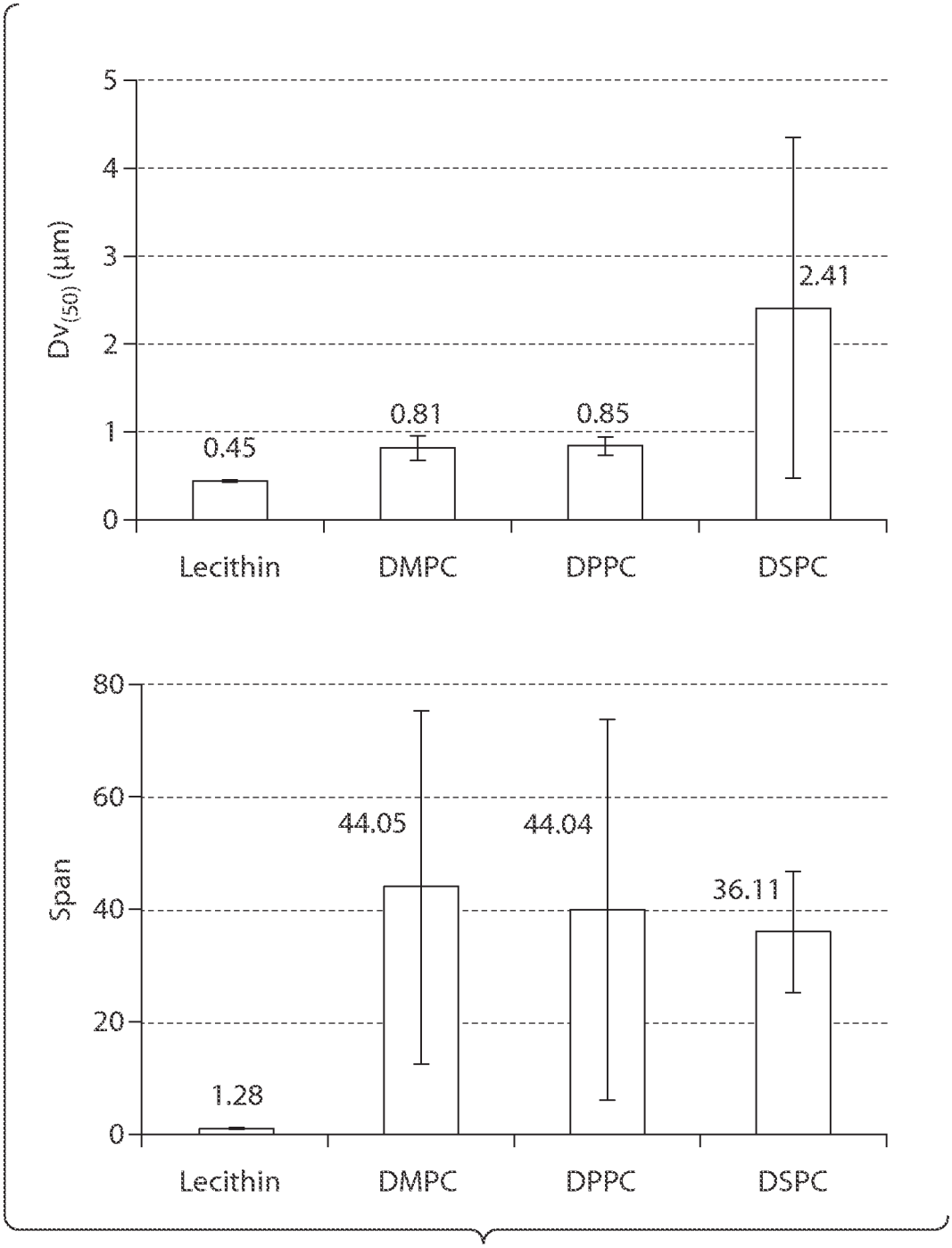

5
4
3
2
1
0
$Dv_{(50)}$ (µm)
0.45
0.81
0.85
2.41
Lecithin
DMPC
DPPC
DSPC
80
60
40
20
0
Span
1.28
44.05
44.04
36.11
Lecithin
DMPC
DPPC
DSPC

Fig. 16

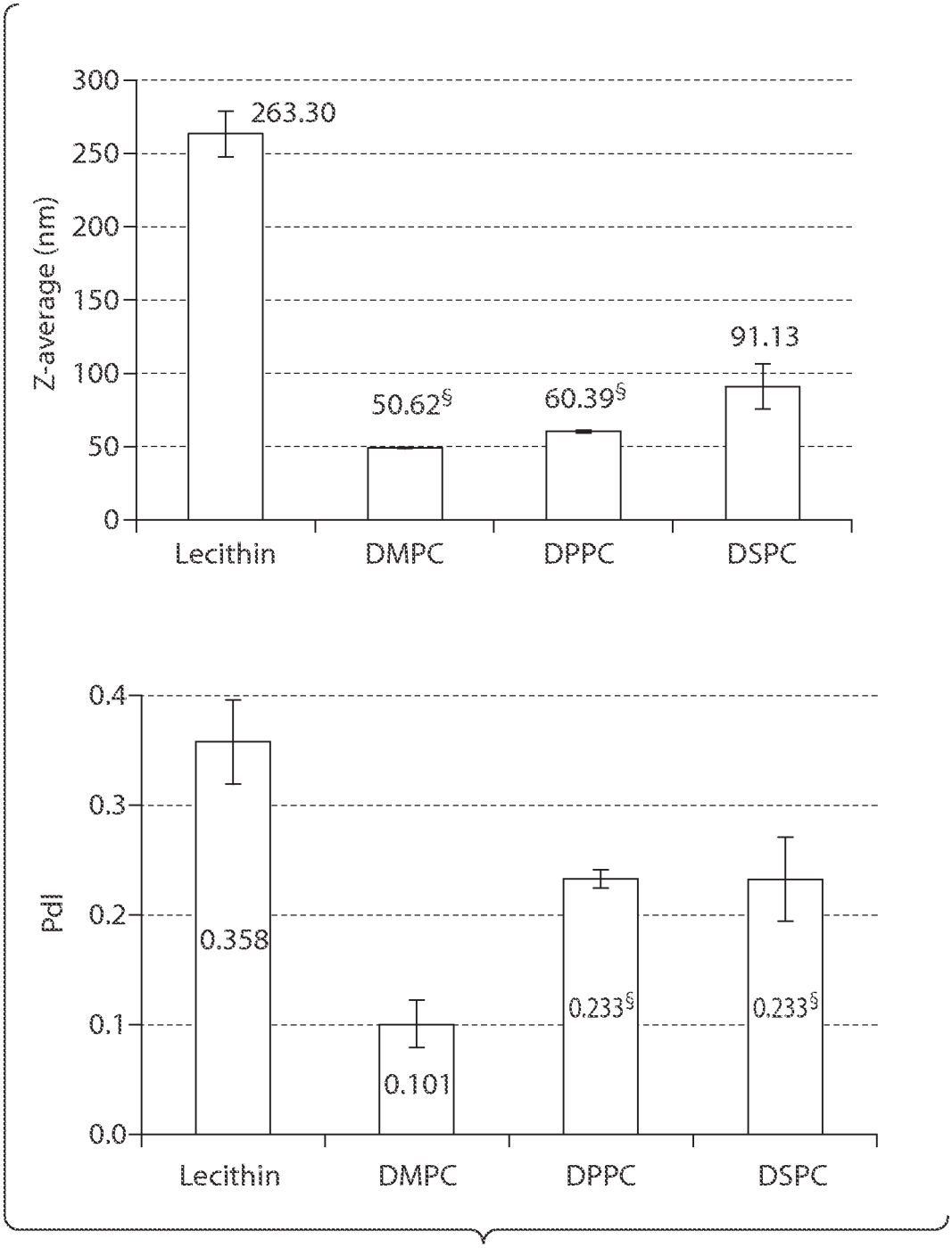

Z-average (nm)
300
250
200
150
100
50
0
263.30
50.62§
60.39§
91.13
Lecithin
DMPC
DPPC
DSPC
PdI
0.4
0.3
0.2
0.1
0.0
0.358
0.101
0.233§
0.233§
Lecithin
DMPC
DPPC
DSPC

Fig. 17

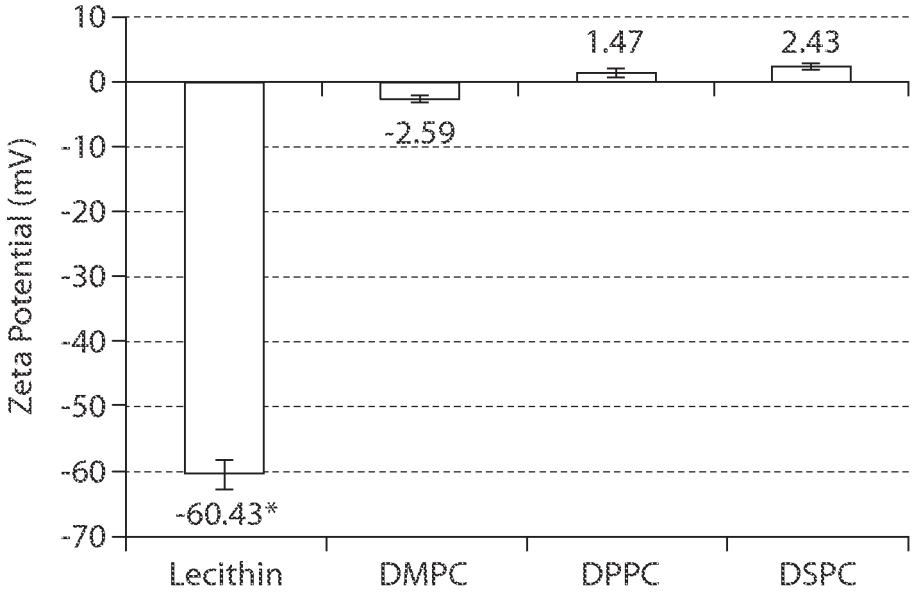

10
0
-10
-20
-30
-40
-50
-60
-70
Zeta Potential (mV)
1.47
2.43
-2.59
-60.43*
Lecithin
DMPC
DPPC
DSPC

Fig. 18

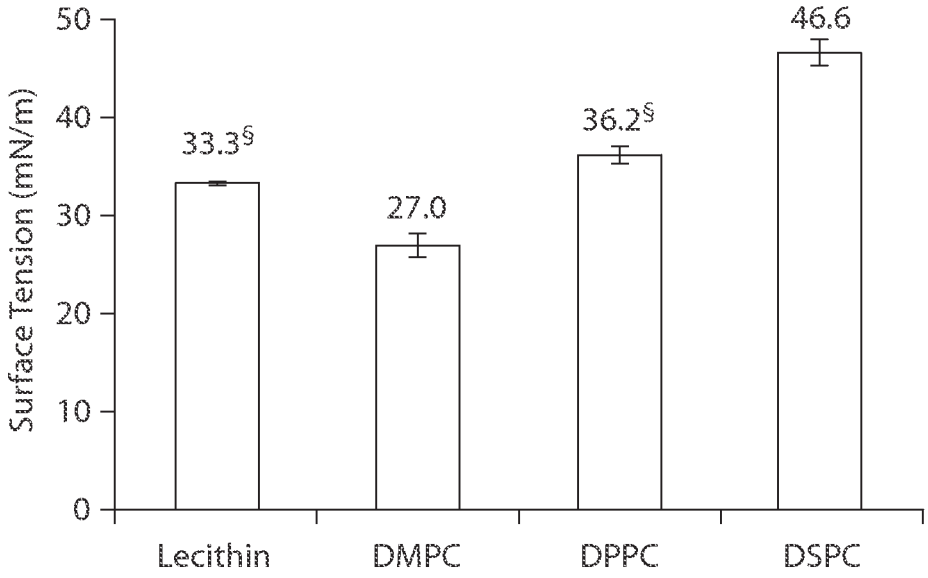

50
40
30
20
10
0
Surface Tension (mN/m)
46.6
36.2§
33.3§
27.0
Lecithin
DMPC
DPPC
DSPC

Fig. 19

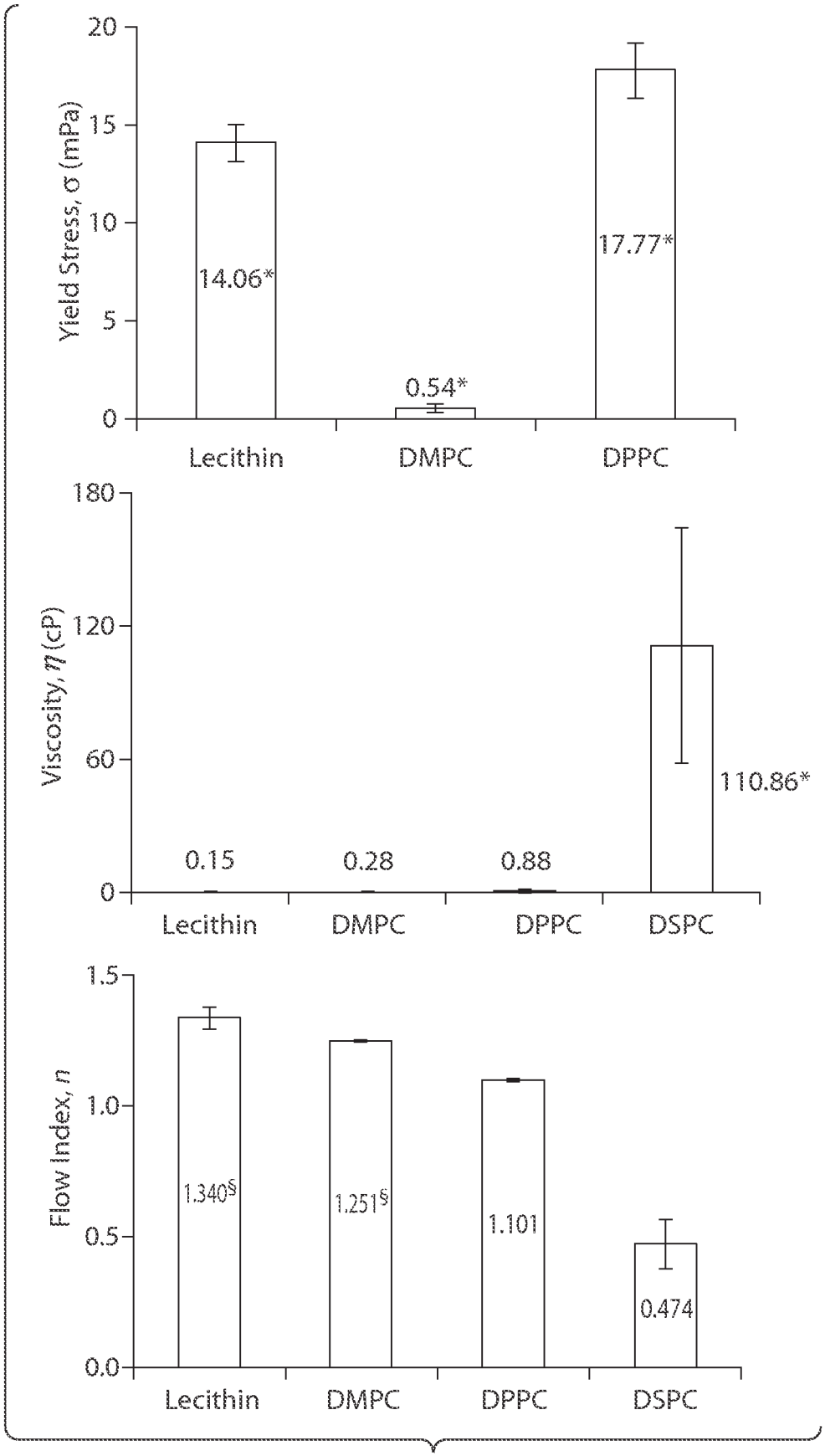

Yield Stress, σ (mPa)
20
15
10
5
0
14.06*
0.54*
17.77*
Lecithin
DMPC
DPPC
Viscosity, η (cP)
180
120
60
0
0.15
0.28
0.88
110.86*
Lecithin
DMPC
DPPC
DSPC
Flow Index, n
1.5
1.0
0.5
0.0
1.340§
1.251§
1.101
0.474
Lecithin
DMPC
DPPC
DSPC

Fig. 20

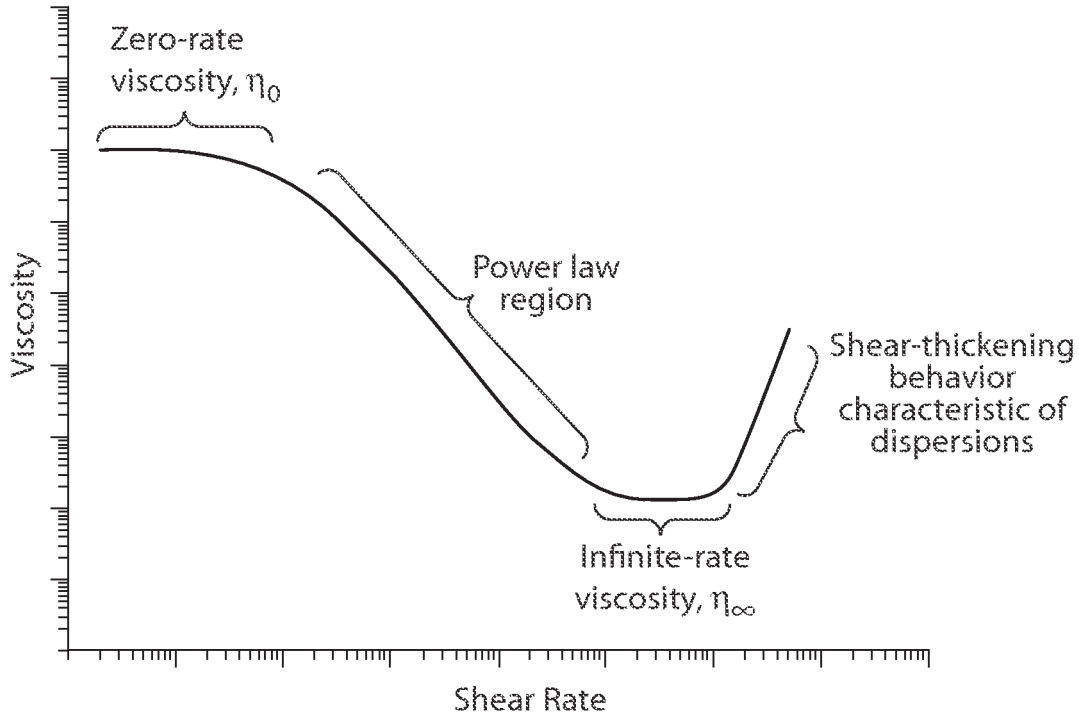

Zero-rate
viscosity, η_0
Power law
region
Shear-thickening
behavior
characteristic of
dispersions
Infinite-rate
viscosity, η_∞
Viscosity
Shear Rate

Fig. 21

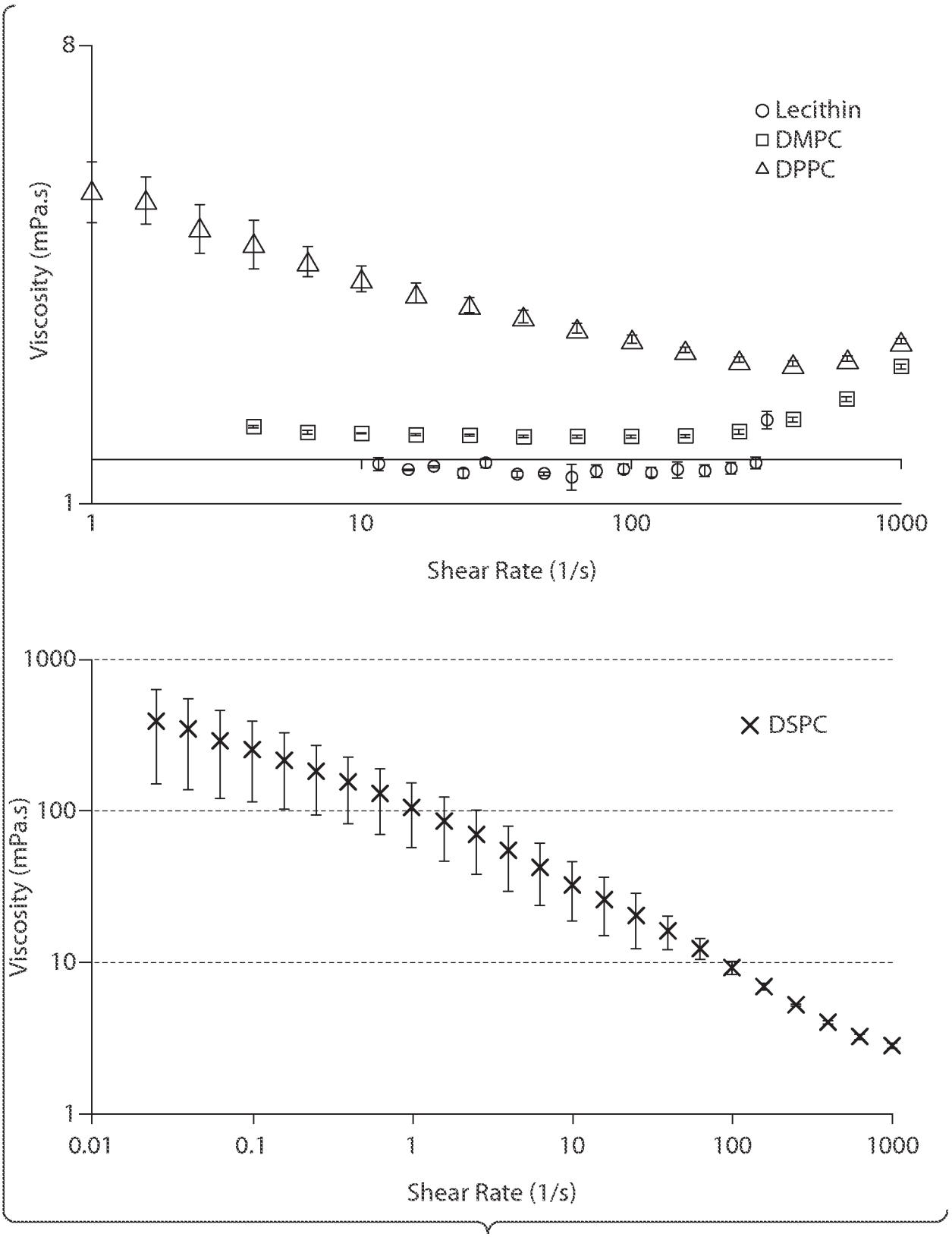

8
1
Viscosity (mPa.s)
Lecithin
DMPC
DPPC
1
10
100
1000
Shear Rate (1/s)
1000
100
10
1
Viscosity (mPa.s)
DSPC
0.01
0.1
1
10
100
1000
Shear Rate (1/s)

Fig. 22

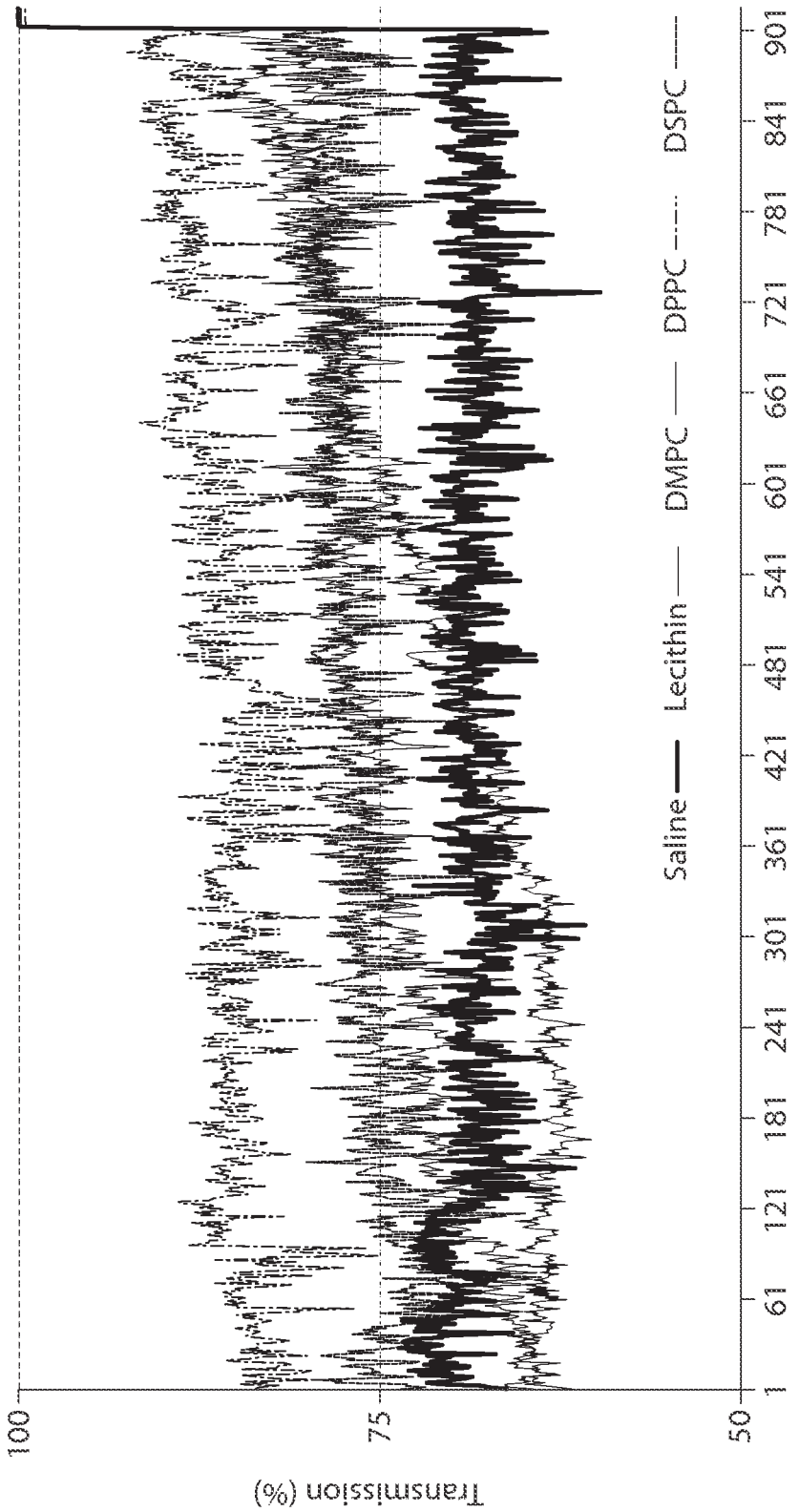

Transmission (%)
100
75
50
1
61
121
181
241
301
361
421
481
541
601
661
721
781
841
901
Relative Time (seconds)
Saline
Lecithin
DMPC
DPPC
DSPC

Fig. 23

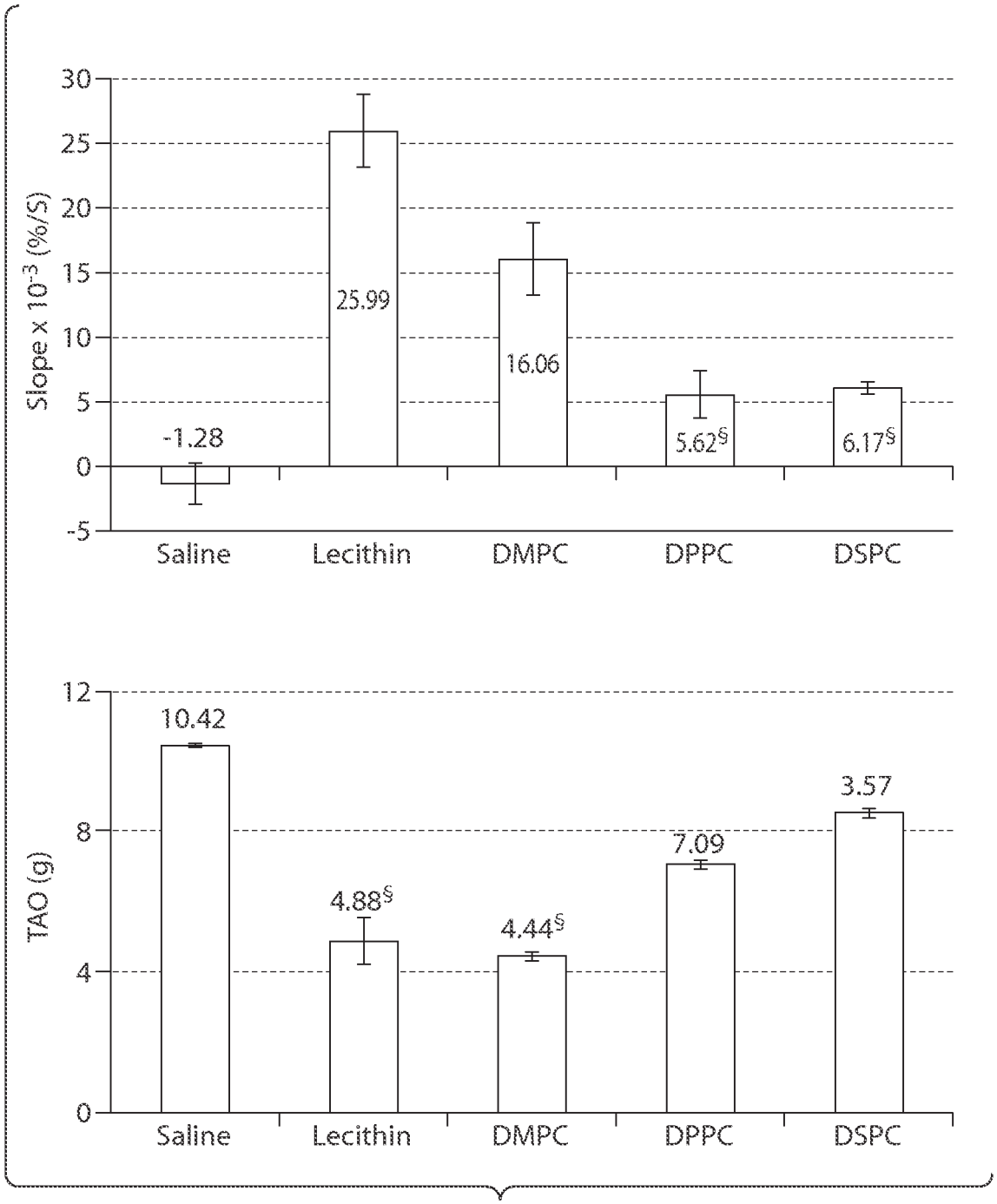

Slope x 10-3 (%/S)
30
25
20
15
10
5
0
-5
-1.28
25.99
16.06
5.62§
6.17§
Saline
Lecithin
DMPC
DPPC
DSPC
TAO (g)
12
8
4
0
10.42
4.88§
4.44§
7.09
3.57
Saline
Lecithin
DMPC
DPPC
DSPC

Fig. 24

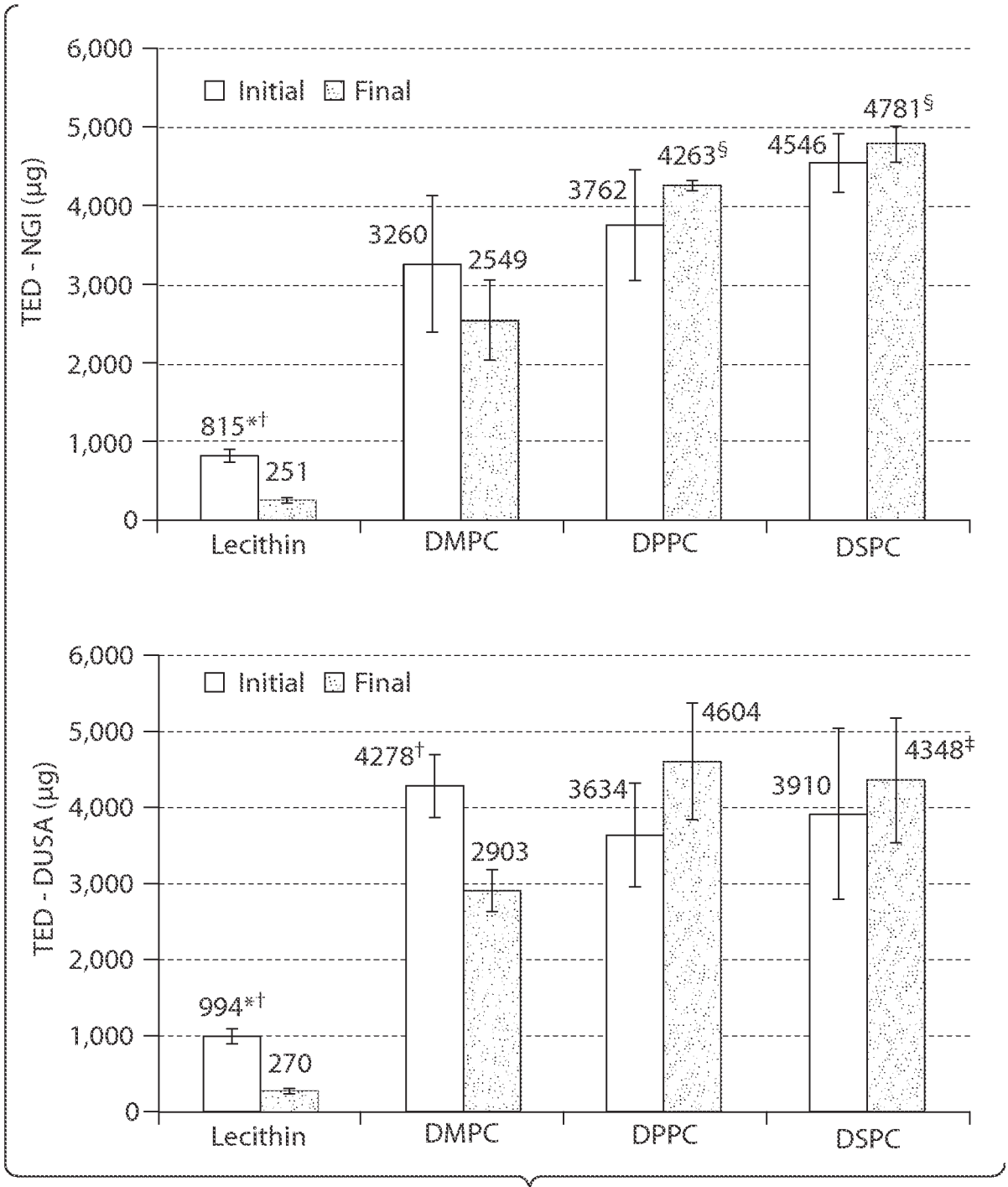

TED - NGI (µg)
6,000
5,000
4,000
3,000
2,000
1,000
0
Initial
Final
815*†
251
3260
2549
3762
4263§
4546
4781§
Lecithin
DMPC
DPPC
DSPC
TED - DUSA (µg)
6,000
5,000
4,000
3,000
2,000
1,000
0
Initial
Final
994*†
270
4278†
2903
3634
4604
3910
4348‡
Lecithin
DMPC
DPPC
DSPC

Fig. 25

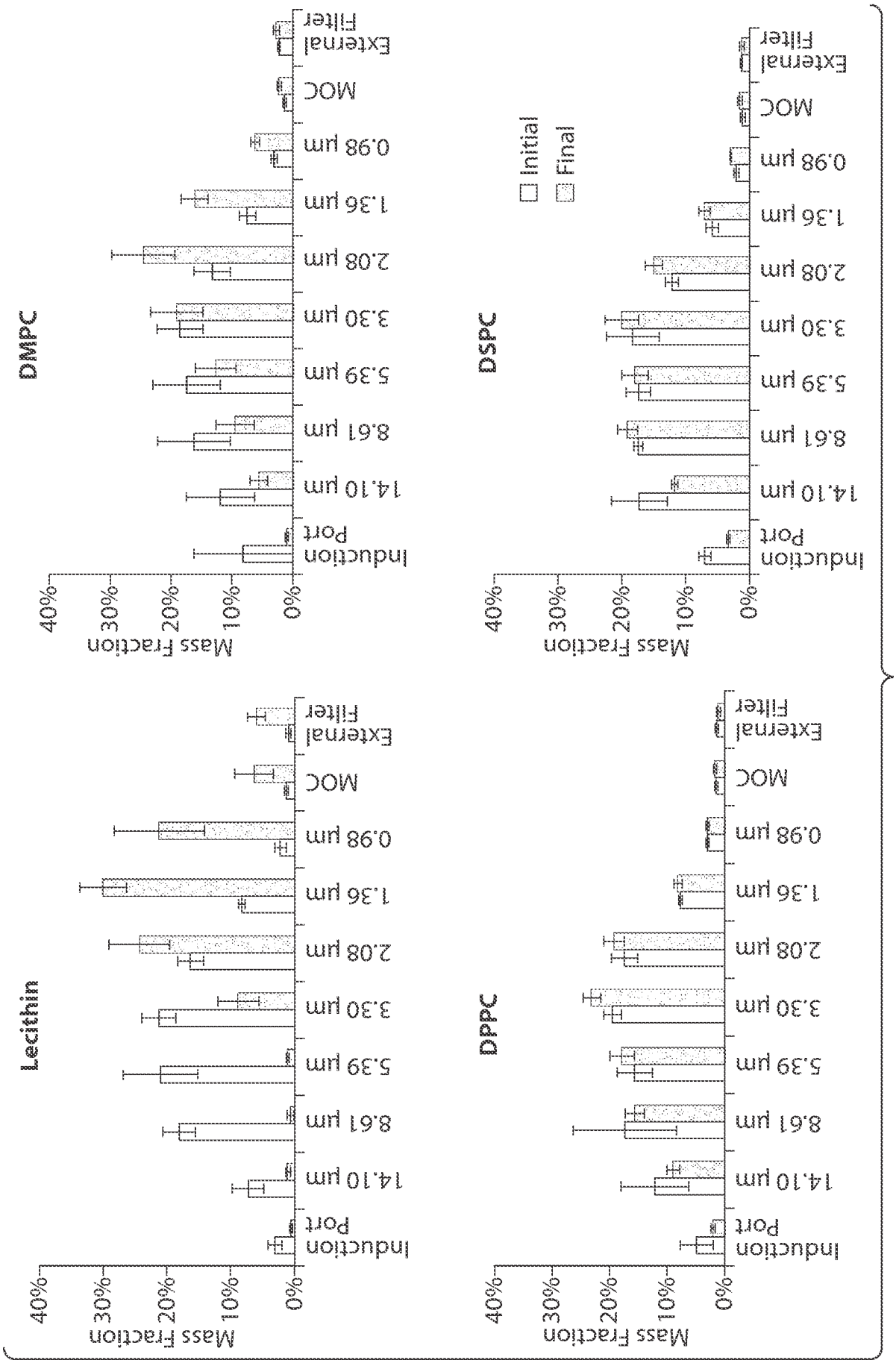

Lecithin
DMPC
DSPC
DPPC
Initial
Final
Mass Fraction
40%
30%
20%
10%
0%
Induction Port
14.10 µm
8.61 µm
5.39 µm
3.30 µm
2.08 µm
1.36 µm
0.98 µm
MOC
External Filter

Fig. 26

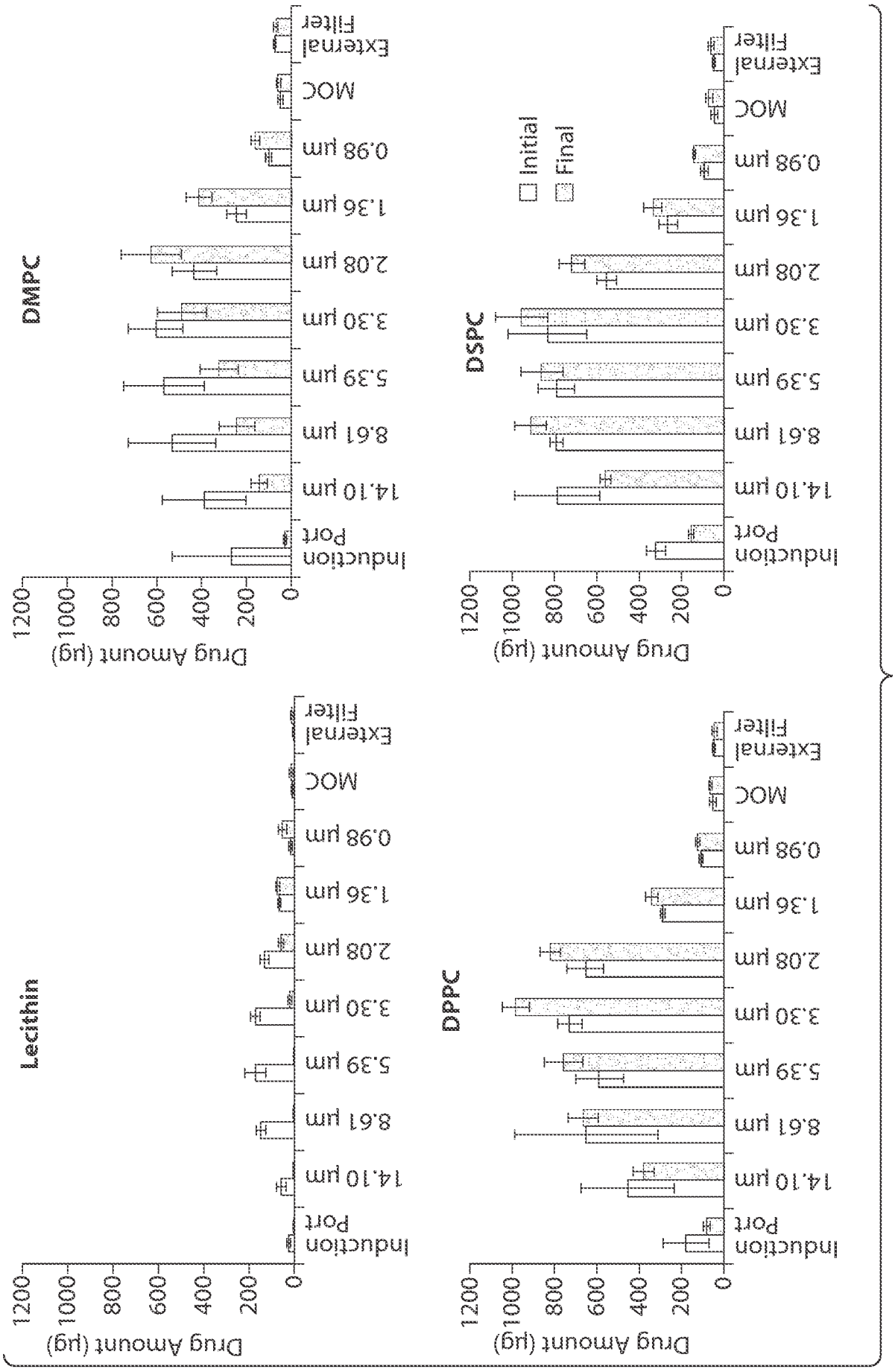

Drug Amount (µg)
1200
1000
800
600
400
200
0
Induction Port
14.10 µm
8.61 µm
5.39 µm
3.30 µm
2.08 µm
1.36 µm
0.98 µm
MOC
External Filter
Initial
Final
DMPC
DSPC
DPPC
Lecithin

Fig. 27

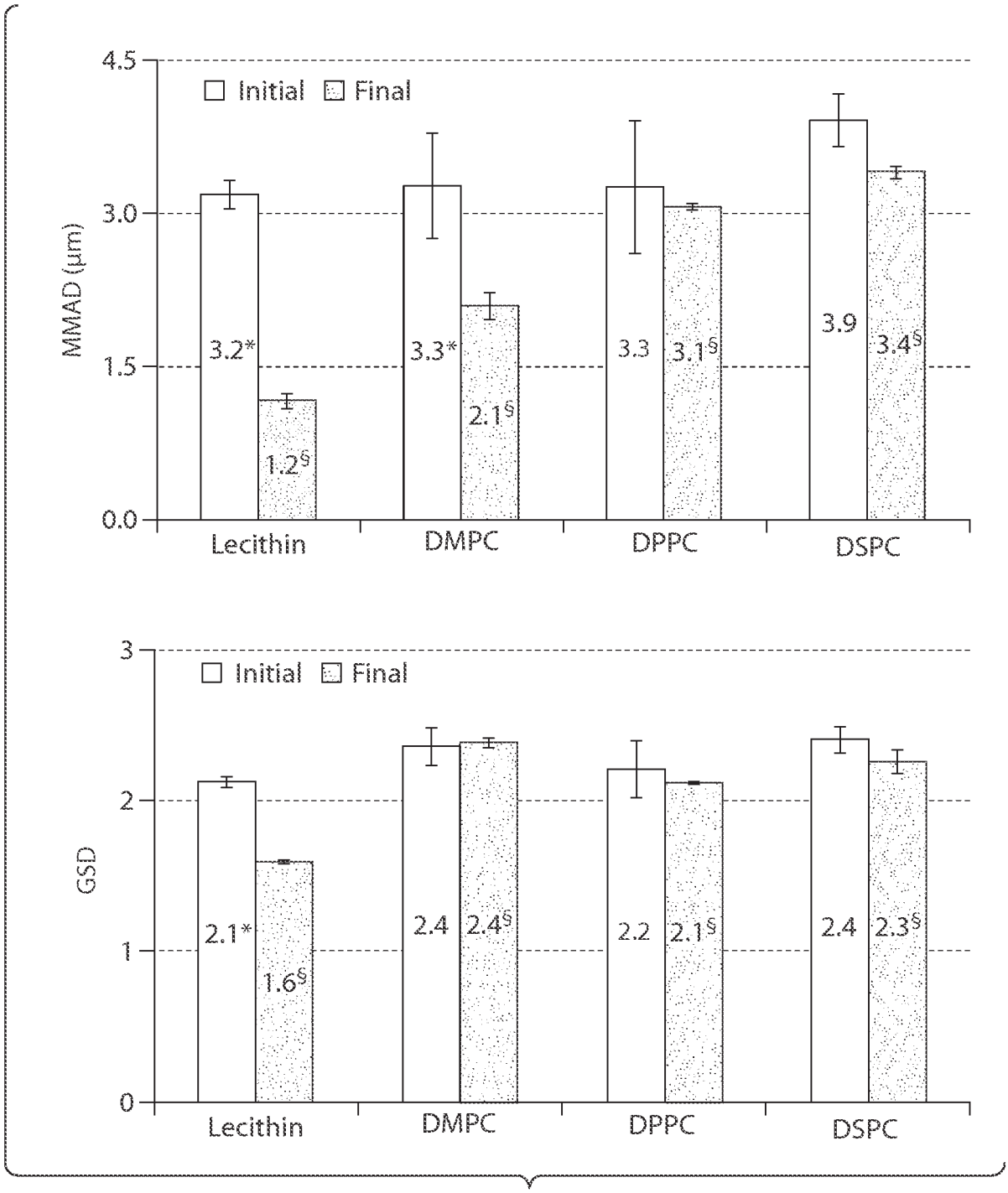

4.5
3.0
1.5
0.0
MMAD (μm)
Initial
Final
3.2*
1.2§
3.3*
2.1§
3.3
3.1§
3.9
3.4§
Lecithin
DMPC
DPPC
DSPC
3
2
1
0
GSD
Initial
Final
2.1*
1.6§
2.4
2.4§
2.2
2.1§
2.4
2.3§
Lecithin
DMPC
DPPC
DSPC

Fig. 28

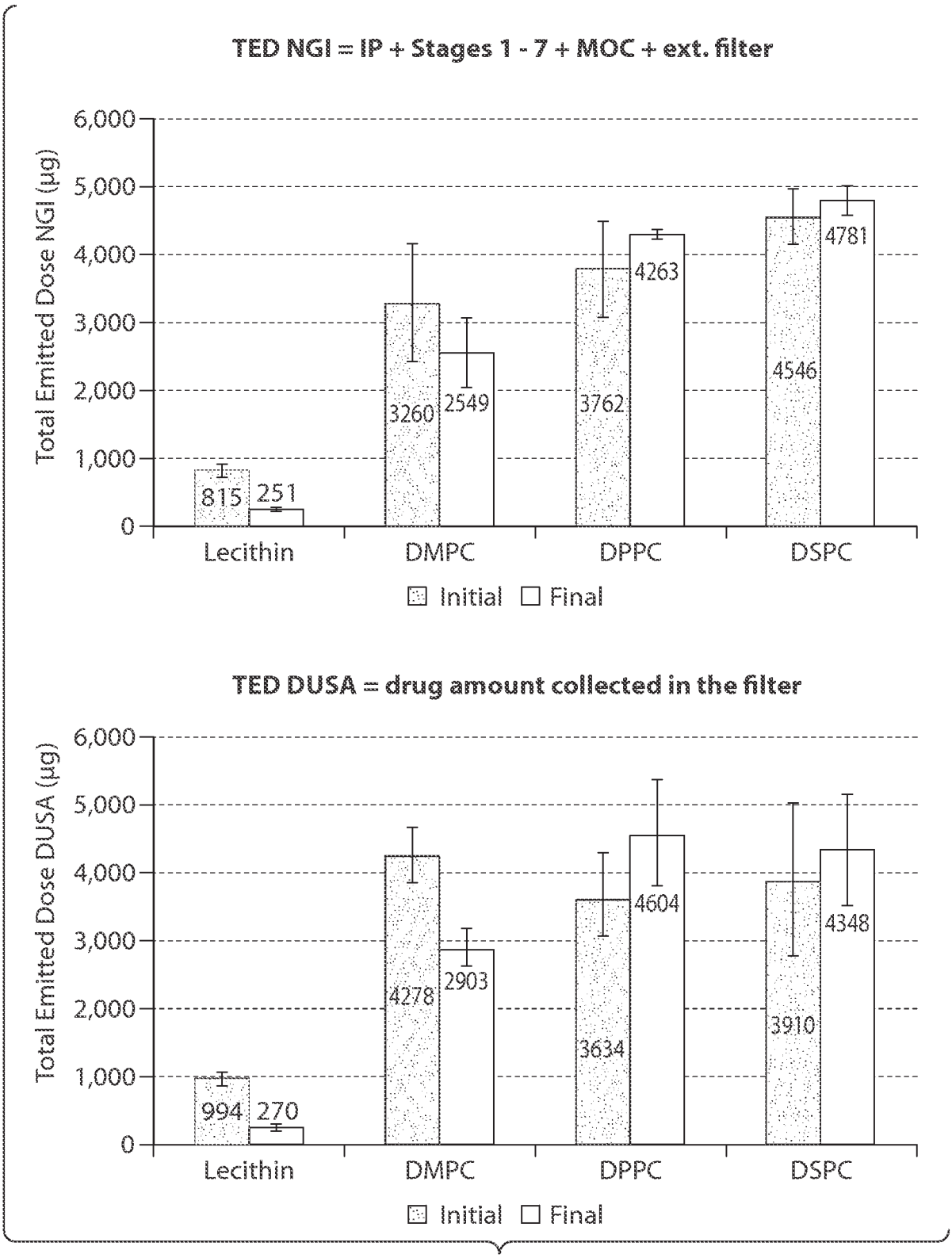

TED NGI = IP + Stages 1 - 7 + MOC + ext. filter
Total Emitted Dose NGI (µg)
6,000
5,000
4,000
3,000
2,000
1,000
0
815
251
3260
2549
3762
4263
4546
4781
Lecithin
DMPC
DPPC
DSPC
Initial
Final
TED DUSA = drug amount collected in the filter
Total Emitted Dose DUSA (µg)
6,000
5,000
4,000
3,000
2,000
1,000
0
994
270
4278
2903
3634
4604
3910
4348
Lecithin
DMPC
DPPC
DSPC
Initial
Final

Fig. 29A

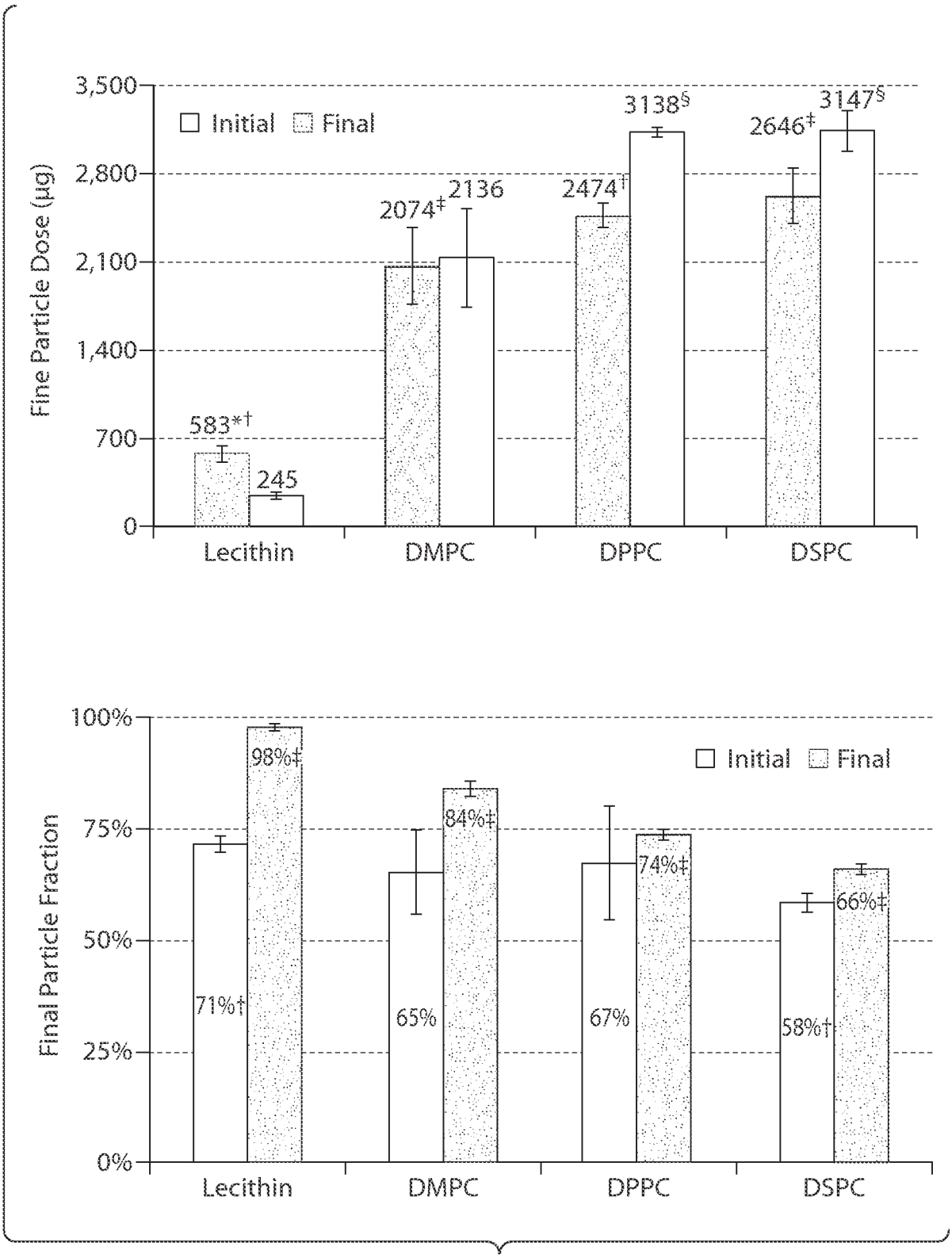

Fine Particle Dose (µg)
3,500
2,800
2,100
1,400
700
0
Initial
Final
583*†
245
2074‡
2136
2474†
3138§
2646‡
3147§
Lecithin
DMPC
DPPC
DSPC
Final Particle Fraction
100%
75%
50%
25%
0%
98%‡
71%†
84%‡
65%
74%‡
67%
66%‡
58%†
Lecithin
DMPC
DPPC
DSPC

Fig. 29B

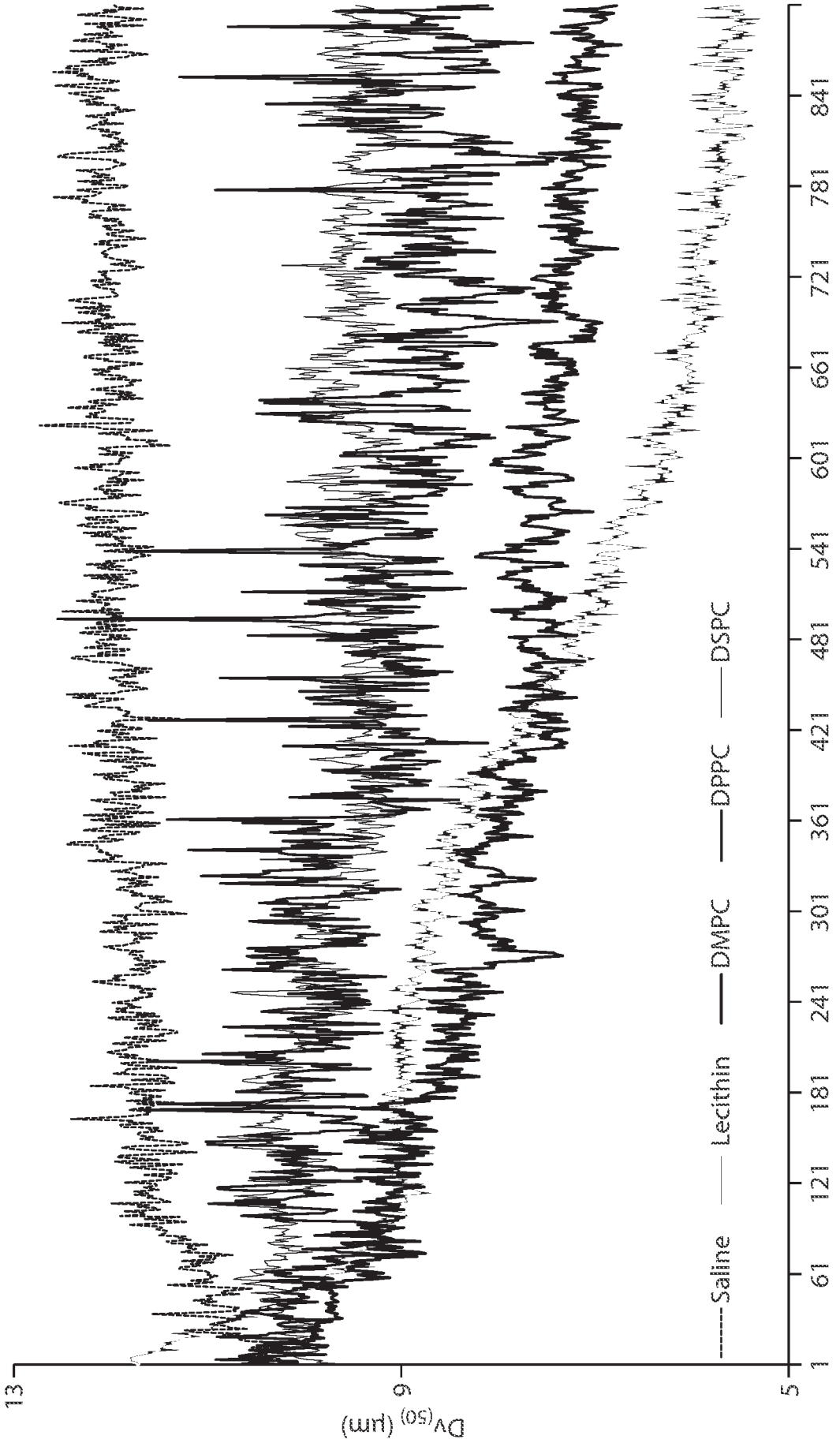

13
9
5
$Dv_{(50)}$ (µm)
1
61
121
181
241
301
361
421
481
541
601
661
721
781
841
Saline
Lecithin
DMPC
DPPC
DSPC

Fig. 30

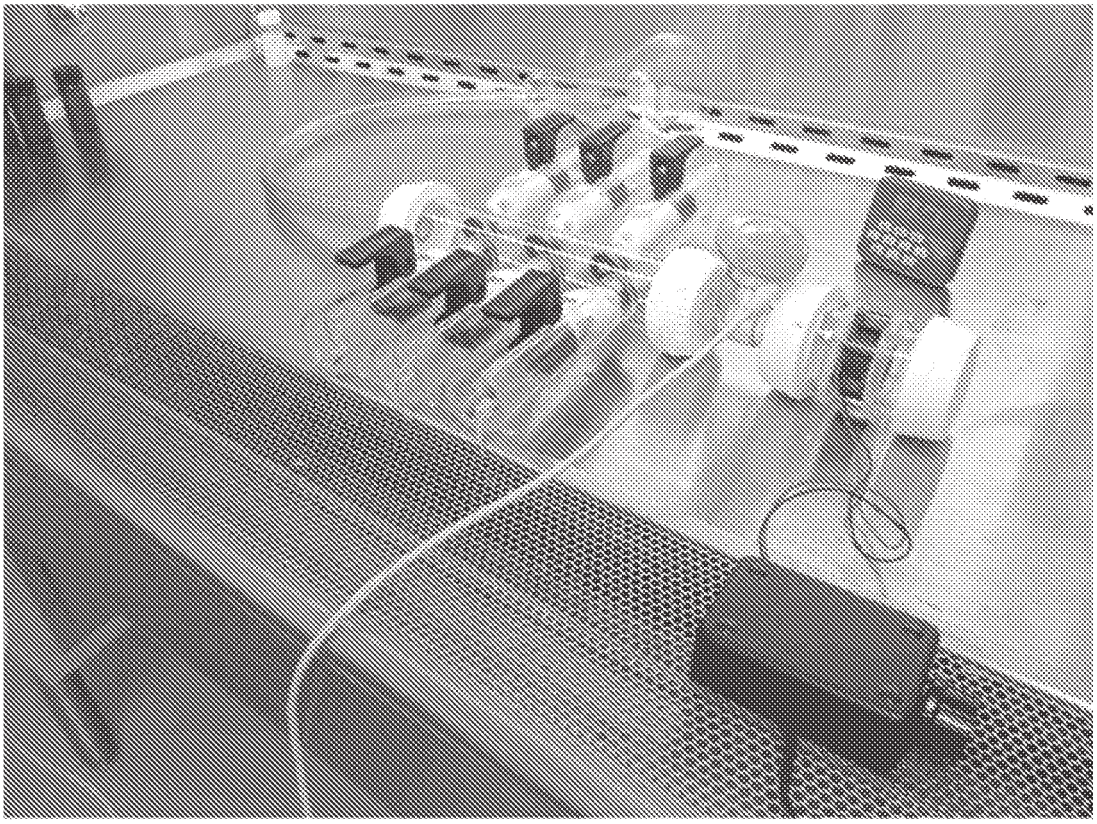


Fig. 31

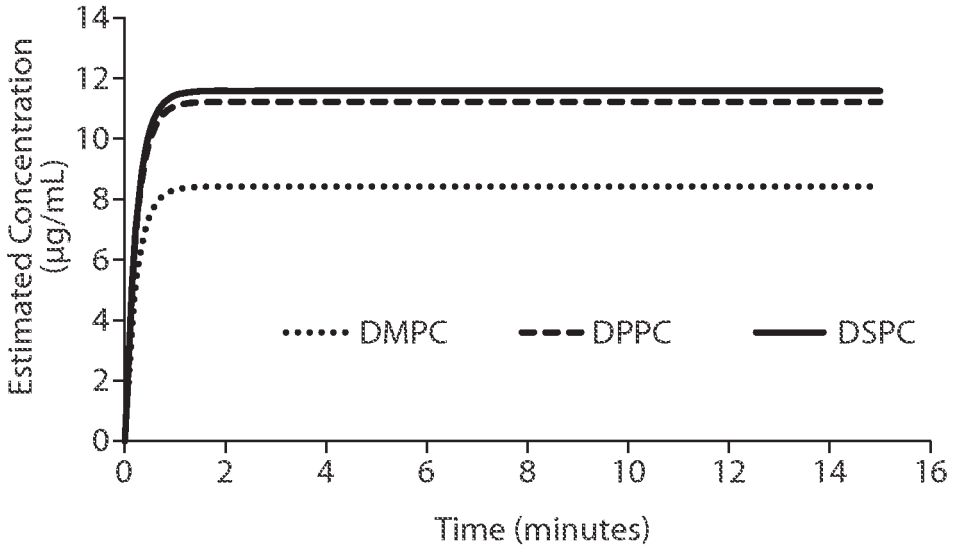

Estimated Concentration (µg/mL)
14
12
10
8
6
4
2
0
DMPC
DPPC
DSPC
0
2
4
6
8
10
12
14
16
Time (minutes)

Fig. 32

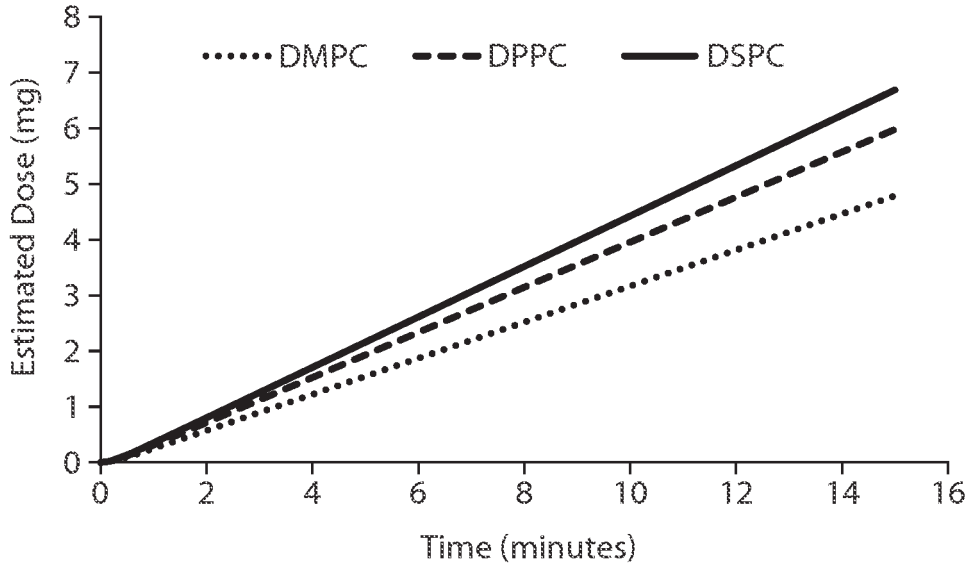

Estimated Dose (mg)
8
7
6
5
4
3
2
1
0
DMPC
DPPC
DSPC
0
2
4
6
8
10
12
14
16
Time (minutes)

Fig. 33

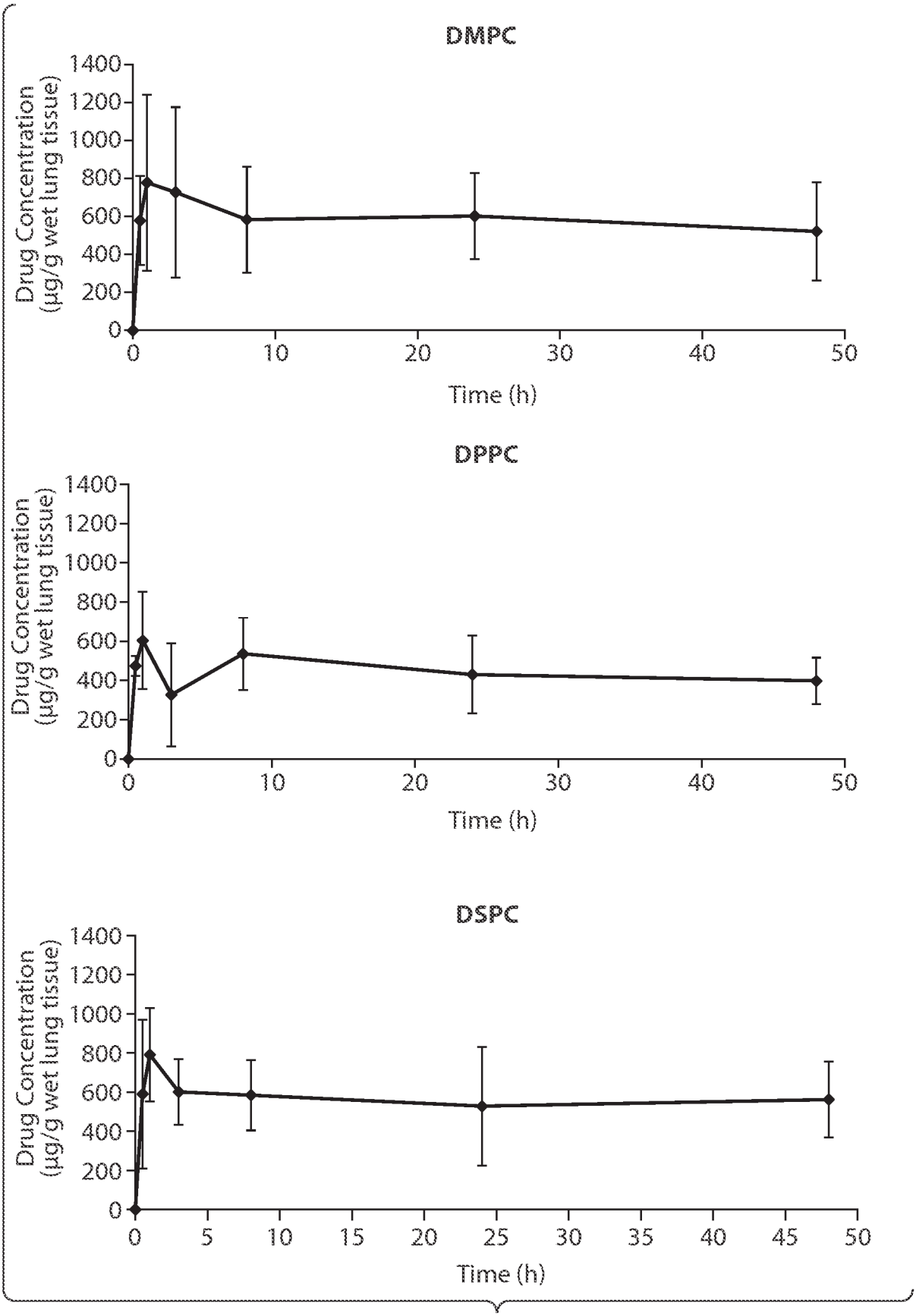

DMPC
Drug Concentration (µg/g wet lung tissue)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time (h)
DPPC
Drug Concentration (µg/g wet lung tissue)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time (h)
DSPC
Drug Concentration (µg/g wet lung tissue)
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
50
Time (h)

Fig. 34

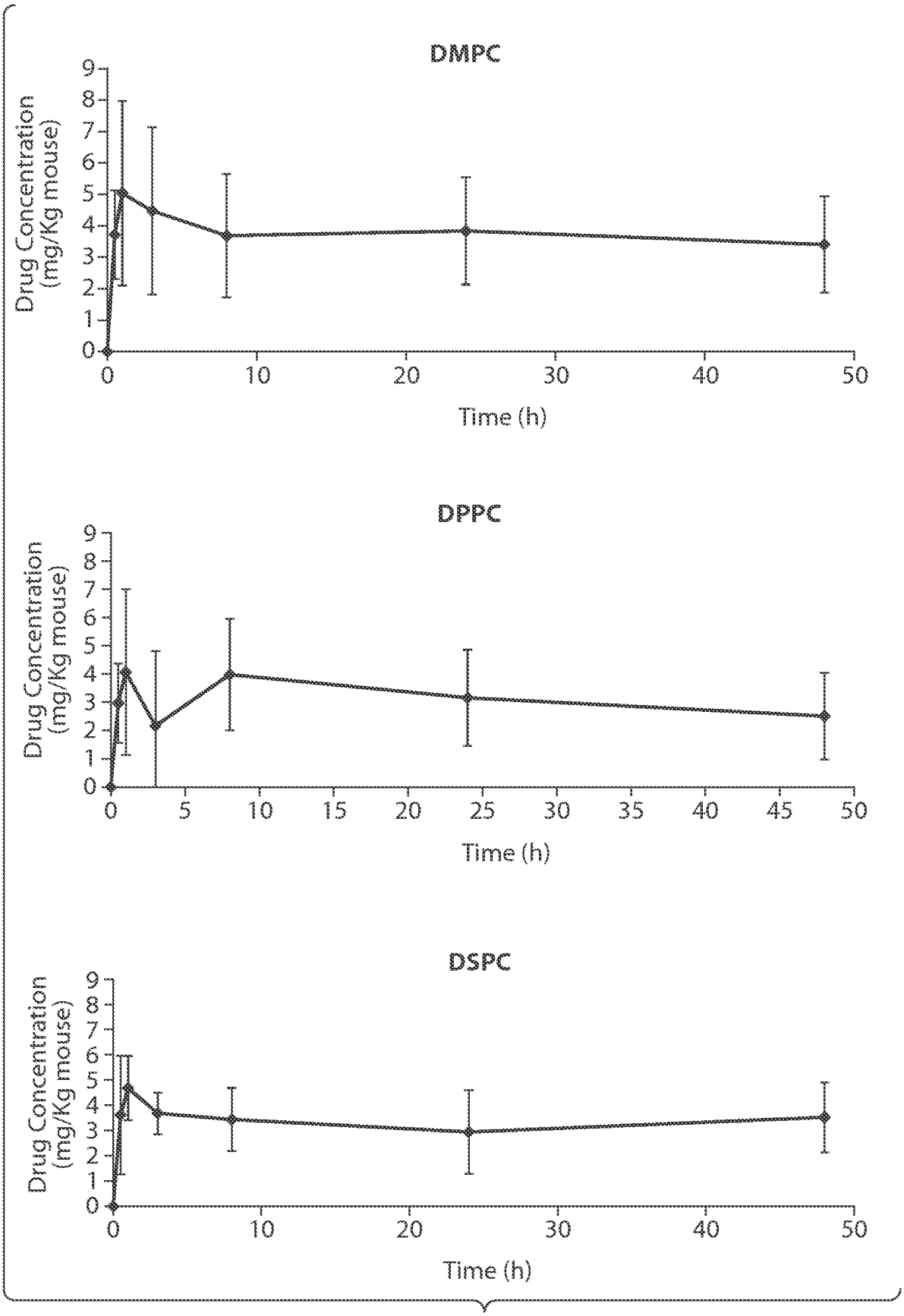

DMPC
Drug Concentration (mg/Kg mouse)
0 1 2 3 4 5 6 7 8 9
0 10 20 30 40 50
Time (h)
DPPC
Drug Concentration (mg/Kg mouse)
0 1 2 3 4 5 6 7 8 9
0 5 10 15 20 25 30 35 40 45 50
Time (h)
DSPC
Drug Concentration (mg/Kg mouse)
0 1 2 3 4 5 6 7 8 9
0 10 20 30 40 50
Time (h)

Fig. 35

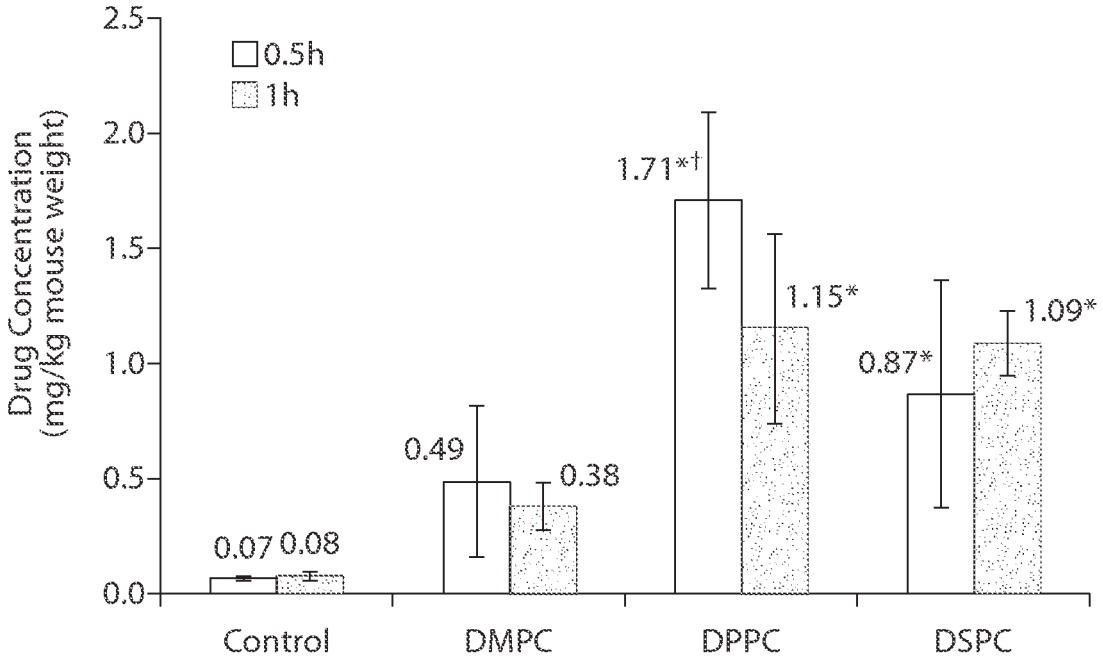

Drug Concentration (mg/kg mouse weight)
2.5
2.0
1.5
1.0
0.5
0.0
0.5h
1h
0.07
0.08
0.49
0.38
1.71*†
1.15*
0.87*
1.09*
Control
DMPC
DPPC
DSPC

Fig. 36

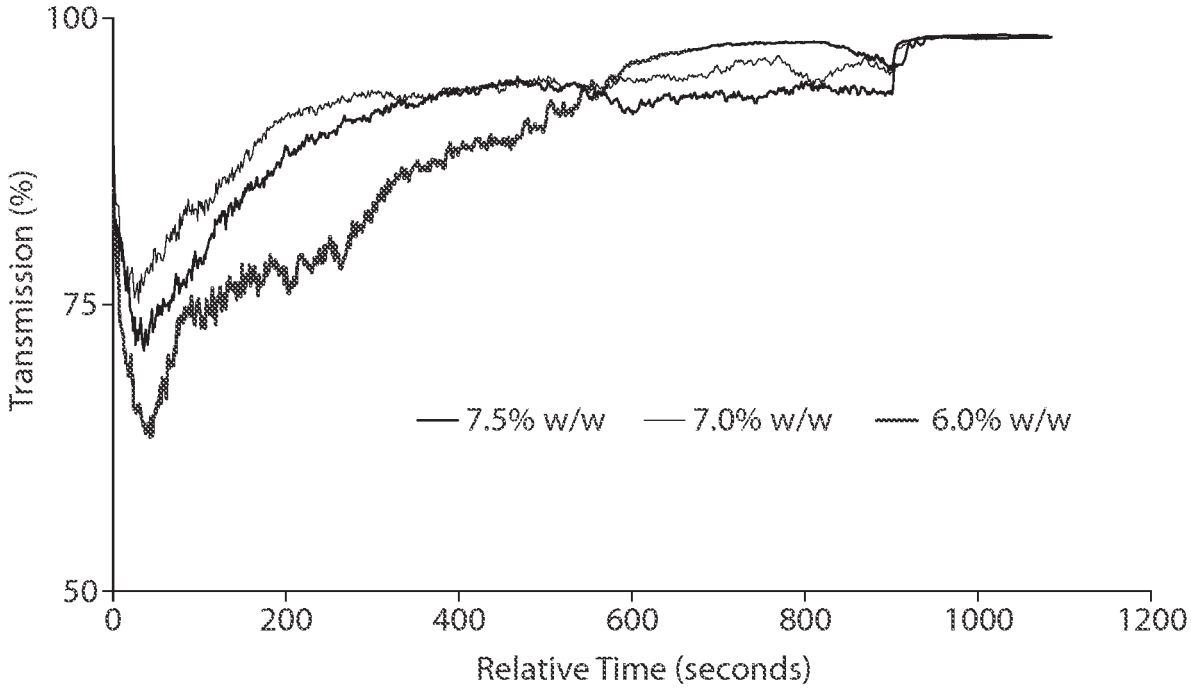

100
75
50
Transmission (%)
0
200
400
600
800
1000
1200
Relative Time (seconds)
7.5% w/w
7.0% w/w
6.0% w/w

Fig. 37

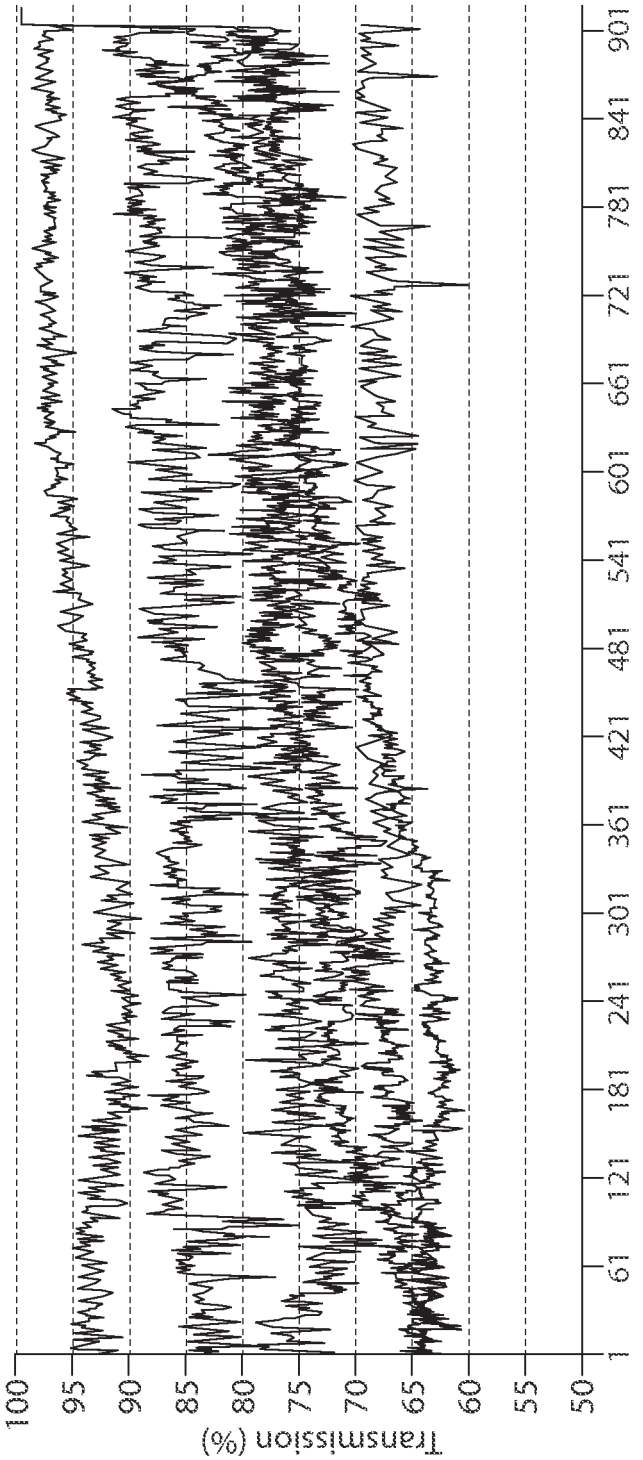

Transmission (%)
100
95
90
85
80
75
70
65
60
55
50
1
61
121
181
241
301
361
421
481
541
601
661
721
781
841
901
Relative Time (seconds)
Saline
Lecithin (50 passes)
CO,DPPC06
CO,DSPC01
CO,DMPC05
CO,DMPC06
IV Cyto tech

Fig. 38

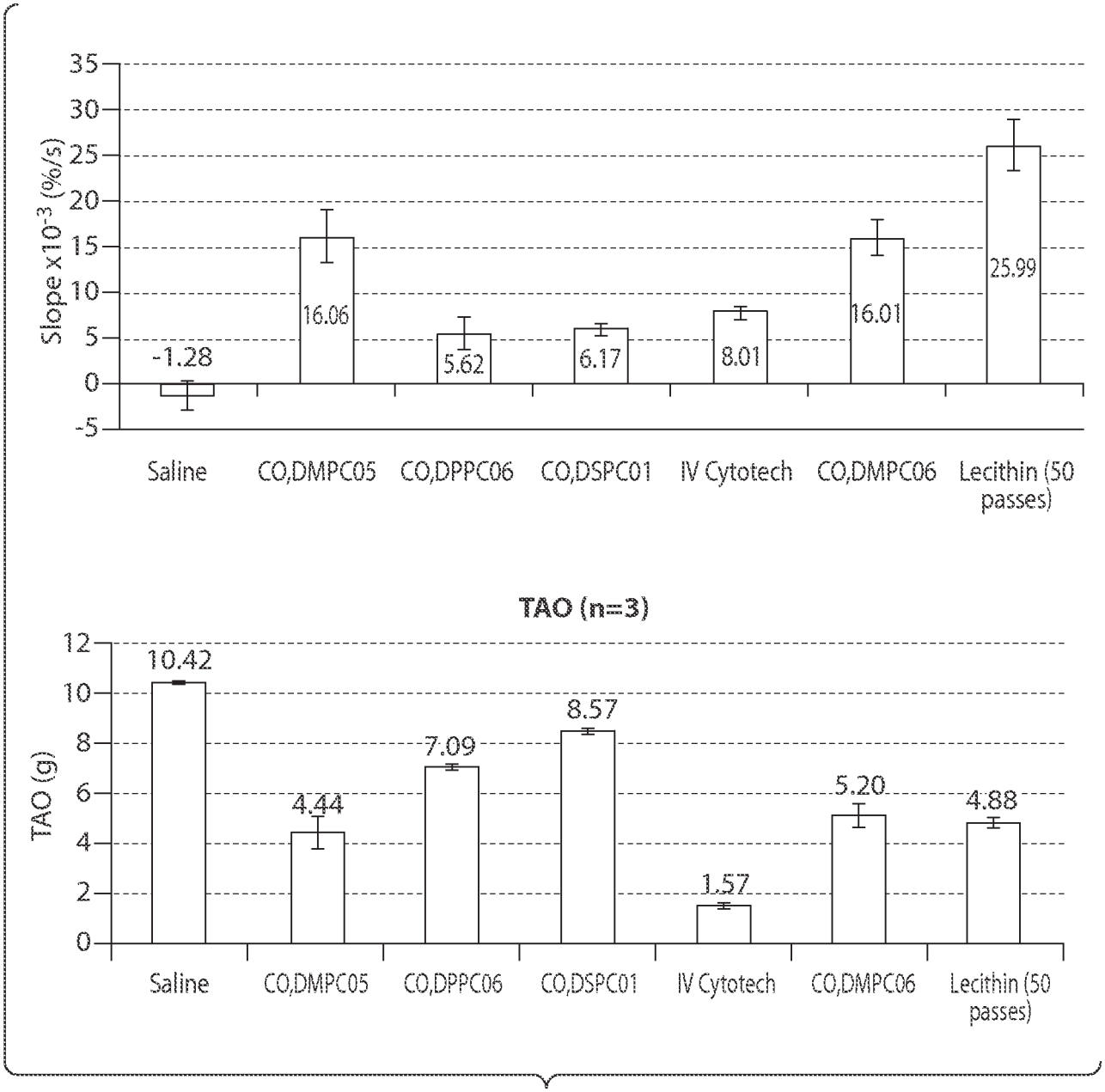

Slope x10-3 (%/s)
35
30
25
20
15
10
5
0
-5
-1.28
16.06
5.62
6.17
8.01
16.01
25.99
Saline
CO,DMPC05
CO,DPPC06
CO,DSPC01
IV Cytotech
CO,DMPC06
Lecithin (50 passes)
TAO (n=3)
TAO (g)
12
10
8
6
4
2
0
10.42
4.44
7.09
8.57
1.57
5.20
4.88
Saline
CO,DMPC05
CO,DPPC06
CO,DSPC01
IV Cytotech
CO,DMPC06
Lecithin (50 passes)

Fig. 39

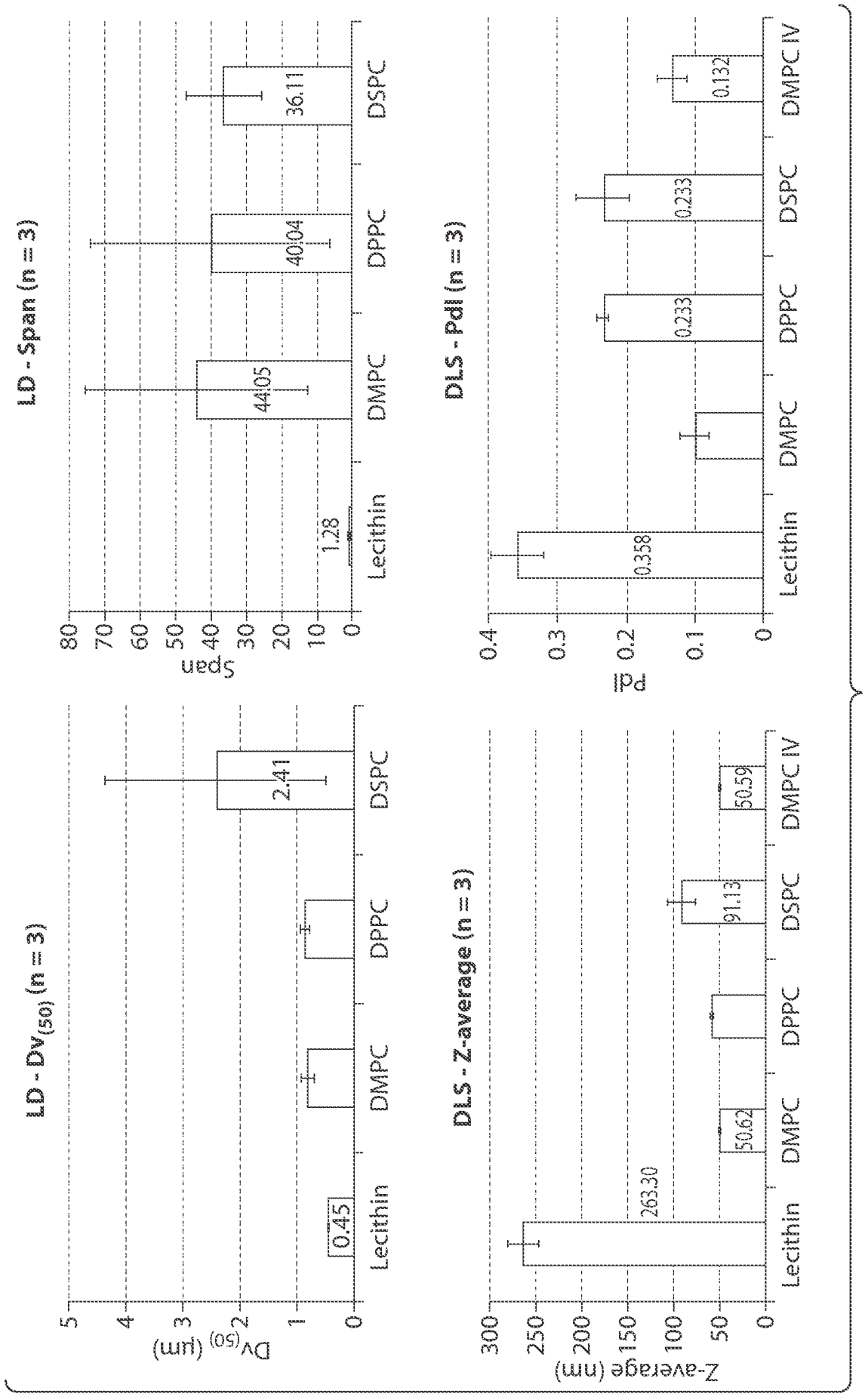

LD - Span (n = 3)
Span
80
70
60
50
40
30
20
10
0
1.28
44.05
40.04
36.11
Lecithin
DMPC
DPPC
DSPC
DLS - PdI (n = 3)
PdI
0.4
0.3
0.2
0.1
0
0.358
0.233
0.233
0.132
Lecithin
DMPC
DPPC
DSPC
DMPC IV
LD - Dv(50) (n = 3)
Dv(50) (µm)
5
4
3
2
1
0
0.45
2.41
Lecithin
DMPC
DPPC
DSPC
DLS - Z-average (n = 3)
Z-average (nm)
300
250
200
150
100
50
0
263.30
50.62
91.13
50.59
Lecithin
DMPC
DPPC
DSPC
DMPC IV

Fig. 40

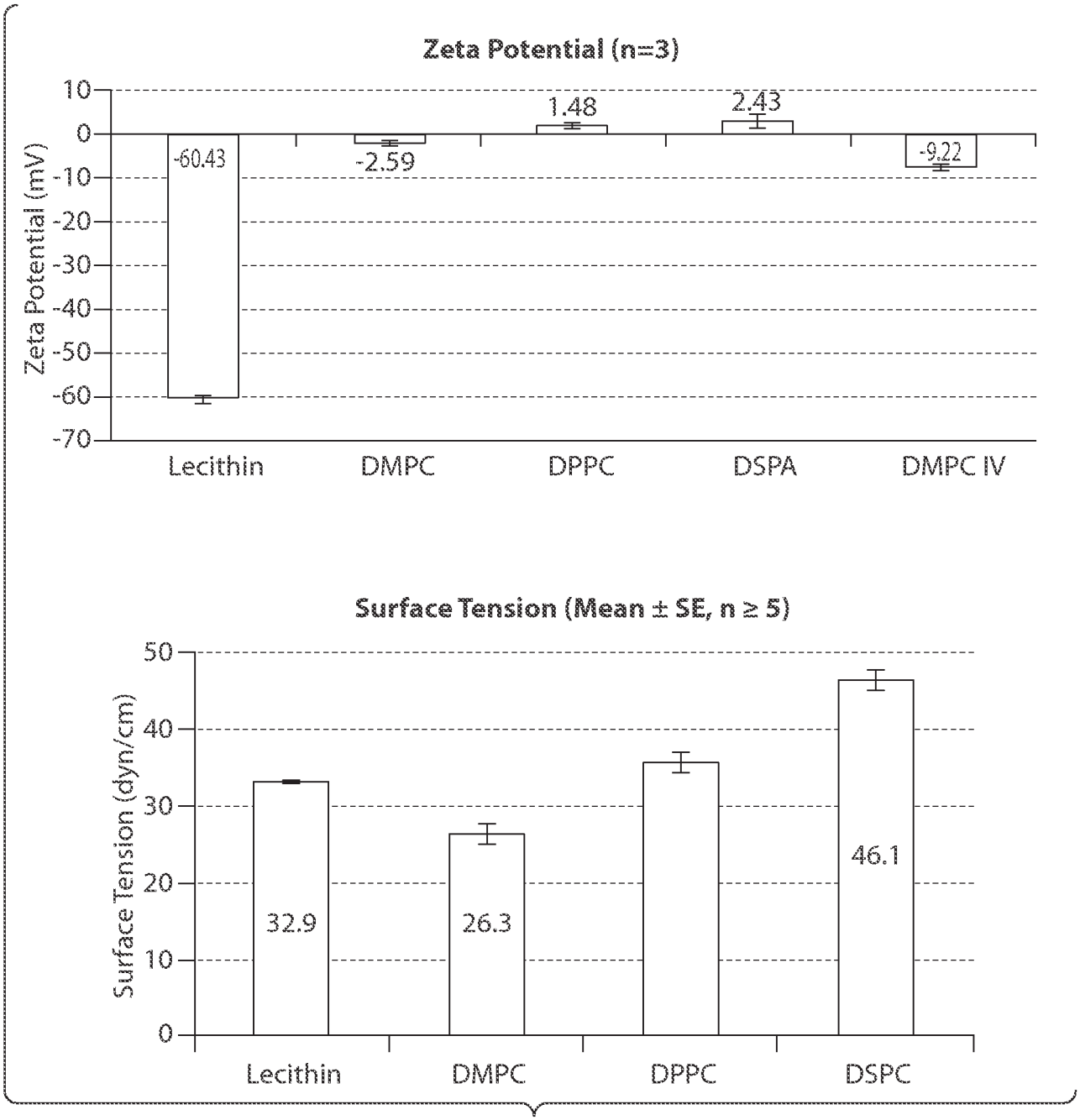

Zeta Potential (n=3)
Zeta Potential (mV)
10
0
-10
-20
-30
-40
-50
-60
-70
-60.43
-2.59
1.48
2.43
-9.22
Lecithin
DMPC
DPPC
DSPA
DMPC IV
Surface Tension (Mean ± SE, n ≥ 5)
Surface Tension (dyn/cm)
50
40
30
20
10
0
32.9
26.3
46.1
Lecithin
DMPC
DPPC
DSPC

Fig. 41

Formulation	Test	Phospholipids		Number of passes	Characterization
		Type(s)	Hydration		
A	Number of passes; stability	Lecithin	~1 hour, no stirring	20, 50, 70 and 100	Particle size distribution
B	Number of passes; type of phospholipids	Lecithin and DPPC	~1 hour, no stirring	10, 20, 30, 40 and 50	Particle size distribution
C*	Nebulization performance	Lecithin	Overnight, stirring	10, 30, and 50	Particle size distribution, surface tension, rheology, zeta potential, and TAO.

*0.9% w/v added to final formulation following processing with microfluidizer.
DPPC: dipalmitoylphosphatidylcholine; TAO: Total Aerosol Output.

Table 1

Manufacturing Process	$Dv_{(50)}$ (μm)	Span Value
Shear Force	1.03	2.076
Microfluidization	0.63	0.367
Ultrasonication	0.71	0.459

Table 2

Formulation	$Dv_{(10)}$ (μm)	$Dv_{(50)}$ (μm)	$Dv_{(90)}$ (μm)
Lecithin	0.24 ± 0.00	0.45 ± 0.00	0.81 ± 0.00
DMPC	0.21 ± 0.01*	0.81 ± 0.14	37.84 ± 27.25
DPPC	0.22 ± 0.01	0.85 ± 0.10	36.30 ± 34.26
DSPC	0.25 ± 0.02*	0.41 ± 1.93	97.24 ± 103.06

Results are expressed as means ± standard deviations (n = 3).
* P>0.05 following Tukey-Kramer test.

Table 3

Model	Equation
Cross	$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{(1 + (K \cdot \dot{\gamma})^m)}$
Sisko	$\eta = \eta_\infty + K \cdot \dot{\gamma}^{n-1}$
Williamson	$\eta = \frac{\eta_0}{(1 + (K \cdot \dot{\gamma})^n)}$

η_0 and η_∞ are the asymptotic values of viscosity at very low (zero-rate viscosity) and very high (infinite-rate viscosity) shear rates; K is the characteristic time (the lower the value, the further to the right the curve lies); m is a dimensionless constant indicating rheological behavior (viscosity as a function of shear rate) in the shear thinning region where $m = 0$ refers to Newtonian behavior and increasing values tending to unity corresponds to increasing shear thinning behavior; n is the power-law index and values below and above unity represent shear-thinning and shear thickening events, respectively; and $\dot{\gamma}$ is shear rate.

Table 4

Formulation	Model	Zero-rate viscosity (mPa.s)[§]	Infinite-rate viscosity (mPa.s)	Characteristic time (s)	Rate index (n) or Cross rate constant (m)	Thixotropy (Pa/s)	Normalized Thixotrpy (s^{-1})
Lecithin	Sisko	N/A	0.95 ± 0.01	$8.60 \times 10^{-4} \pm 14.9 \times 10^{-4}$	23.35 ± 1.90*	85.48 ± 0.79*	$16.92 \times 10^{-4} \pm 0.31 \times 10^{-4}$
DMPC	Cross	1.13 ± 0.01	3.66 ± 3.11	$9.06 \times 10^{-4} \pm 5.38 \times 10^{-4}$	2.59 ± 0.52	Zero	Zero
DMPC	Cross	5.31 ± 0.96	1.61 ± 0.01	0.51 ± 0.03	0.70 ± 0.05	Zero	Zero
DSPC	Williamson	621.17 ± 446.30	N/A	15.19 ± 11.12*	0.54 ± 0.10	Zero	Zero

Results are expressed as means ± standard deviations (n = 3). N/A: not applicable when referred to this rheological model (compare to equations in table 2). [§] Groups are statistically different but *post hoc* test was unable to distinguish differences among groups. * $P < 0.05$ when compared to other formulations.

Table 5

Formulation	FPD_{et} (mg)	FPD_r (µg/min)
Lecithin	24.84	1656
DMPC	126.30	8420
DPPC	168.36	11224
DSPC	173.79	11586

Table 6

Formulation	FPD_r (mg/min)	Mouse weight (g)	RMV (mL/min)	Estimated Dose	
				After 15 minutes of nebulization (mg)	Normalized to body weight (mg/kg)
DMPC	8.420	28.8 ± 1.4	38.50	4.787	166.2
DPPC	11.224	26.1 ± 1.5	36.07	5.980	229.1
DSPC	11.586	29.5 ± 2.0	39.11	6.693	226.9

Results of mouse weight are expressed as mean ± standard deviations (n = 36 per group). FPD_r is the rate of delivery of the Fine Particle Dose (the amount of particles with aerodynamic cutoff diameter below 5.39 μm per minute) and RMV is the Rate Minute Volume calculated based on 10 mL/kg at 120 breaths per minute.[21]

Table 7

Pharmacokinetic Parameter	DMPC	DPPC	DSPC
C_{max} (µg/g wet tissue)	777.7	604.0	791.3
t_{max} (h)	1	1	1
AUC_{0-48} (mg-h/g)	28.228	21.144	26.830

Table 8

Number of passes	Lecithin (test run)		DPPC (n=3)	
	APT (%)	TAO (%)	APT (%)	TAO (%)
10	94.8	11	94.3 ± 1.8	50.0 ± 9.3
20	99.3	0	81.9 ± 1.4	69.6 ± 12.1
30	97.1	10.5	87.7 ± 1.0	70.4 ± 3.0
40	94.2	21	77.8 ± 3.2	76.4 ± 3.4
50	90	30	86.8 ± 1.1	78.0 ± 8.7

Form.	Number of Cycles	Following Preparation		After 7 days	
		APT (%)	TAO (%)	APT (%)	TAO (%)
1	20	97.1 ± 3.4	16.4 ± 15.2	85.4 ± 3.5**	75.1 ± 9.0**
2	50	77.9 ± 12.6	85.6 ± 15.1*	89.1 ± 2.0	66.7 ± 5.3
3	70	85.8 ± 1.5*	72.0 ± 4.4*	91.5 ± 0.5**	56.4 ± 0.4**
4	100	86.8 ± 0.6*	67.8 ± 1.0*	91.7 ± 0.2**	52.7 ± 1.8**

Table 9