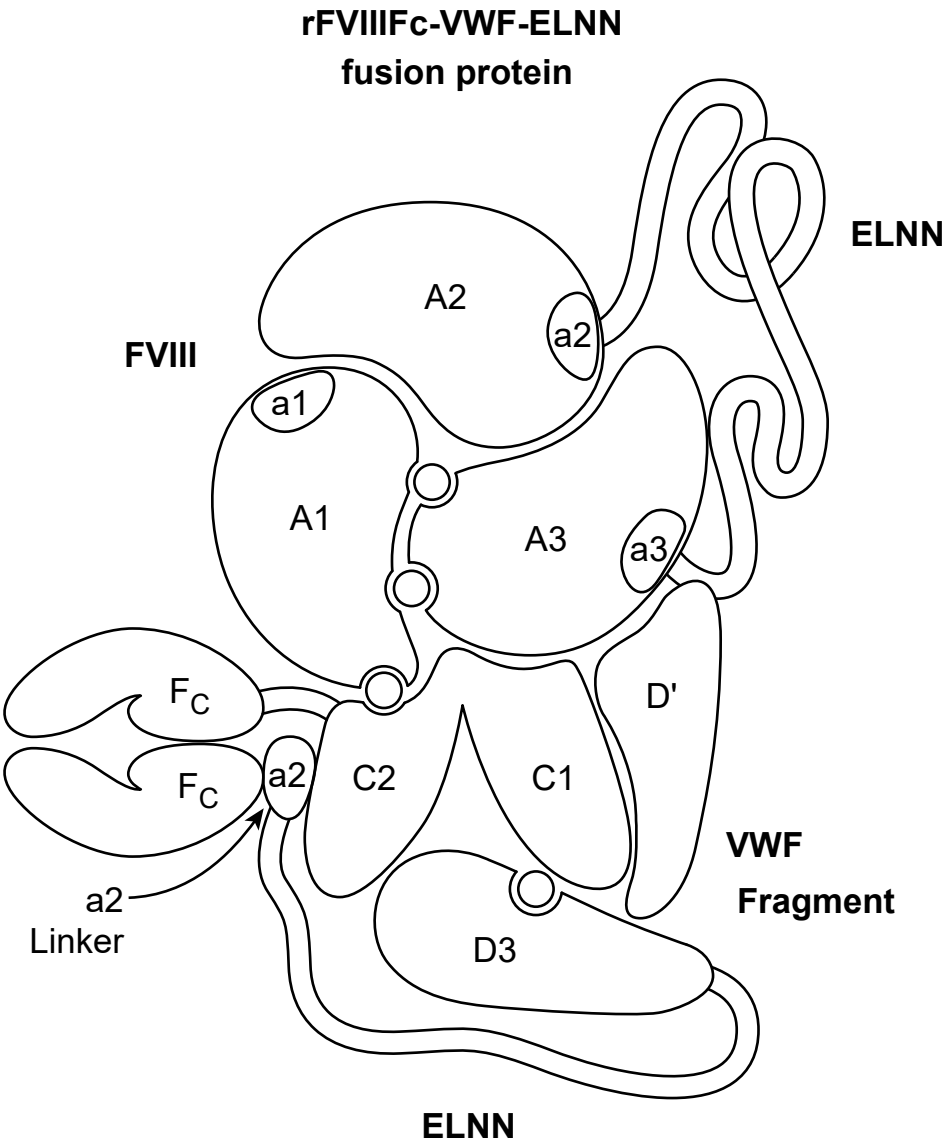

rFVIIIFc-VWF-ELNN
fusion protein
ELNN
FVIII
A2
a2
a1
A1
A3
a3
D'
F_C
F_C
a2
C2
C1
VWF
Fragment
a2
Linker
D3
ELNN

FIG. 1

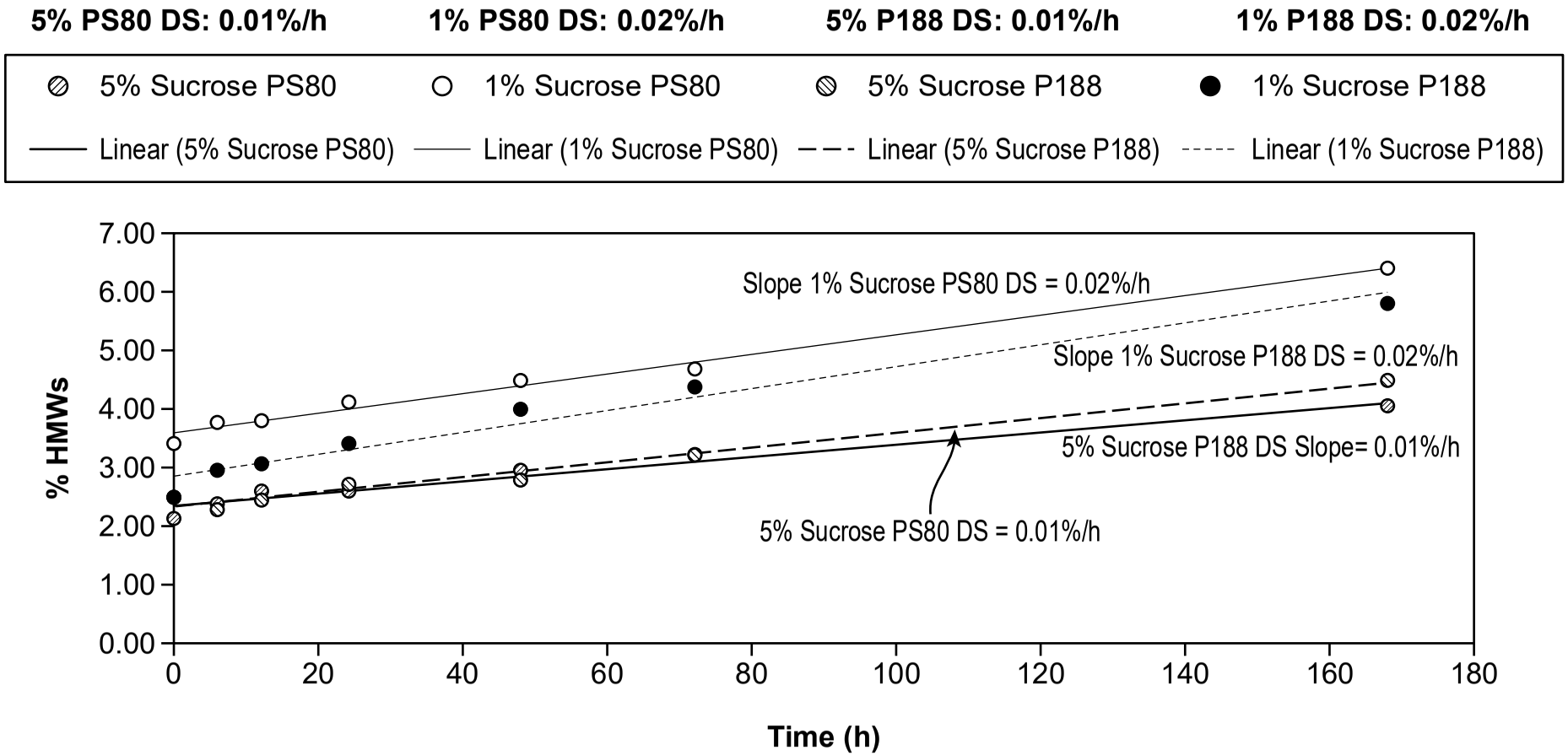

5% PS80 DS: 0.01%/h
1% PS80 DS: 0.02%/h
5% P188 DS: 0.01%/h
1% P188 DS: 0.02%/h
5% Sucrose PS80
1% Sucrose PS80
5% Sucrose P188
1% Sucrose P188
Linear (5% Sucrose PS80)
Linear (1% Sucrose PS80)
Linear (5% Sucrose P188)
Linear (1% Sucrose P188)
Slope 1% Sucrose PS80 DS = 0.02%/h
Slope 1% Sucrose P188 DS = 0.02%/h
5% Sucrose P188 DS Slope= 0.01%/h
5% Sucrose PS80 DS = 0.01%/h
% HMWs
Time (h)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
20
40
60
80
100
120
140
160
180

FIG. 2

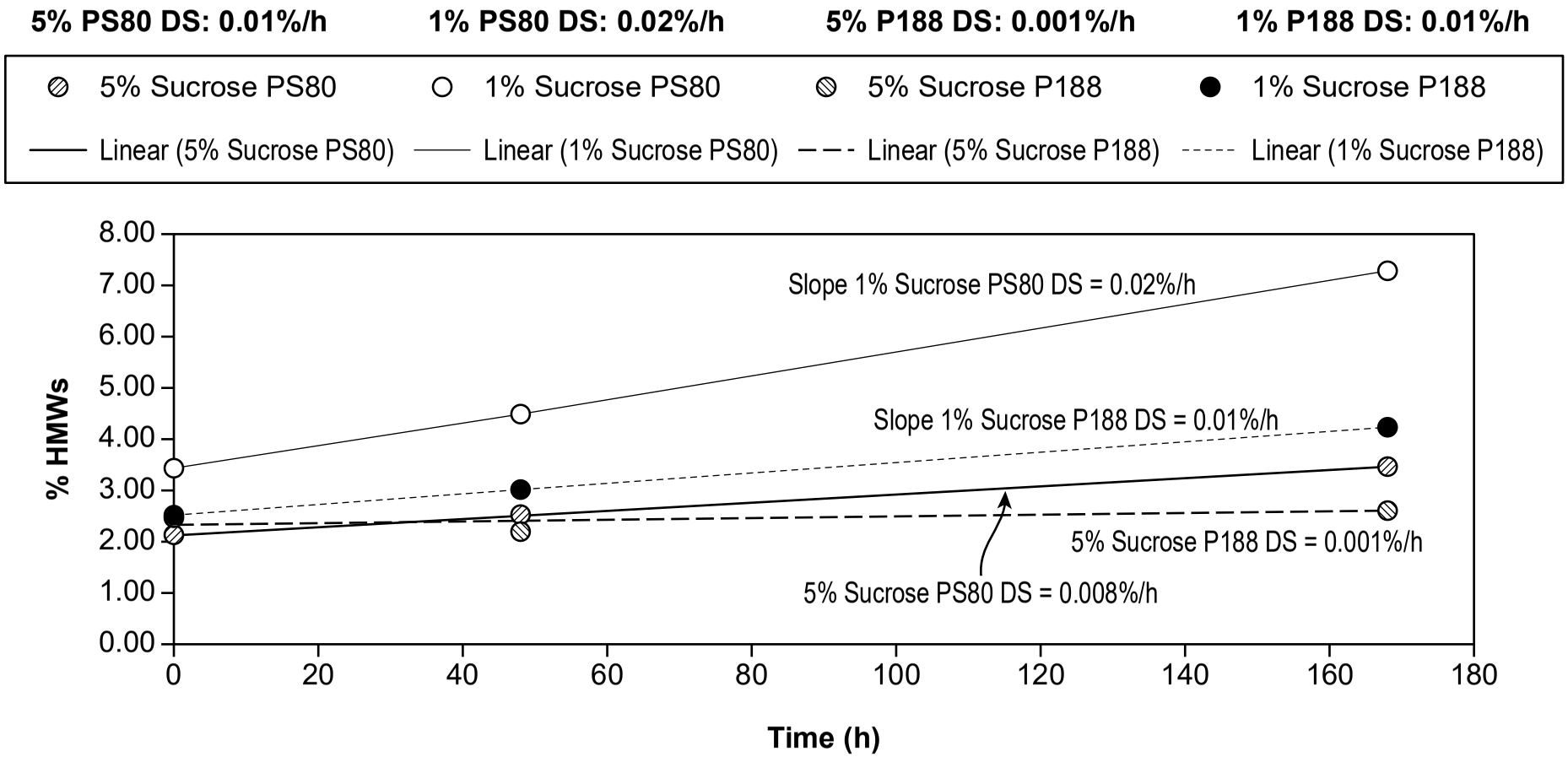

5% PS80 DS: 0.01%/h
1% PS80 DS: 0.02%/h
5% P188 DS: 0.001%/h
1% P188 DS: 0.01%/h
5% Sucrose PS80
1% Sucrose PS80
5% Sucrose P188
1% Sucrose P188
Linear (5% Sucrose PS80)
Linear (1% Sucrose PS80)
Linear (5% Sucrose P188)
Linear (1% Sucrose P188)
% HMWs
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
20
40
60
80
100
120
140
160
180
Time (h)
Slope 1% Sucrose PS80 DS = 0.02%/h
Slope 1% Sucrose P188 DS = 0.01%/h
5% Sucrose P188 DS = 0.001%/h
5% Sucrose PS80 DS = 0.008%/h

FIG. 3

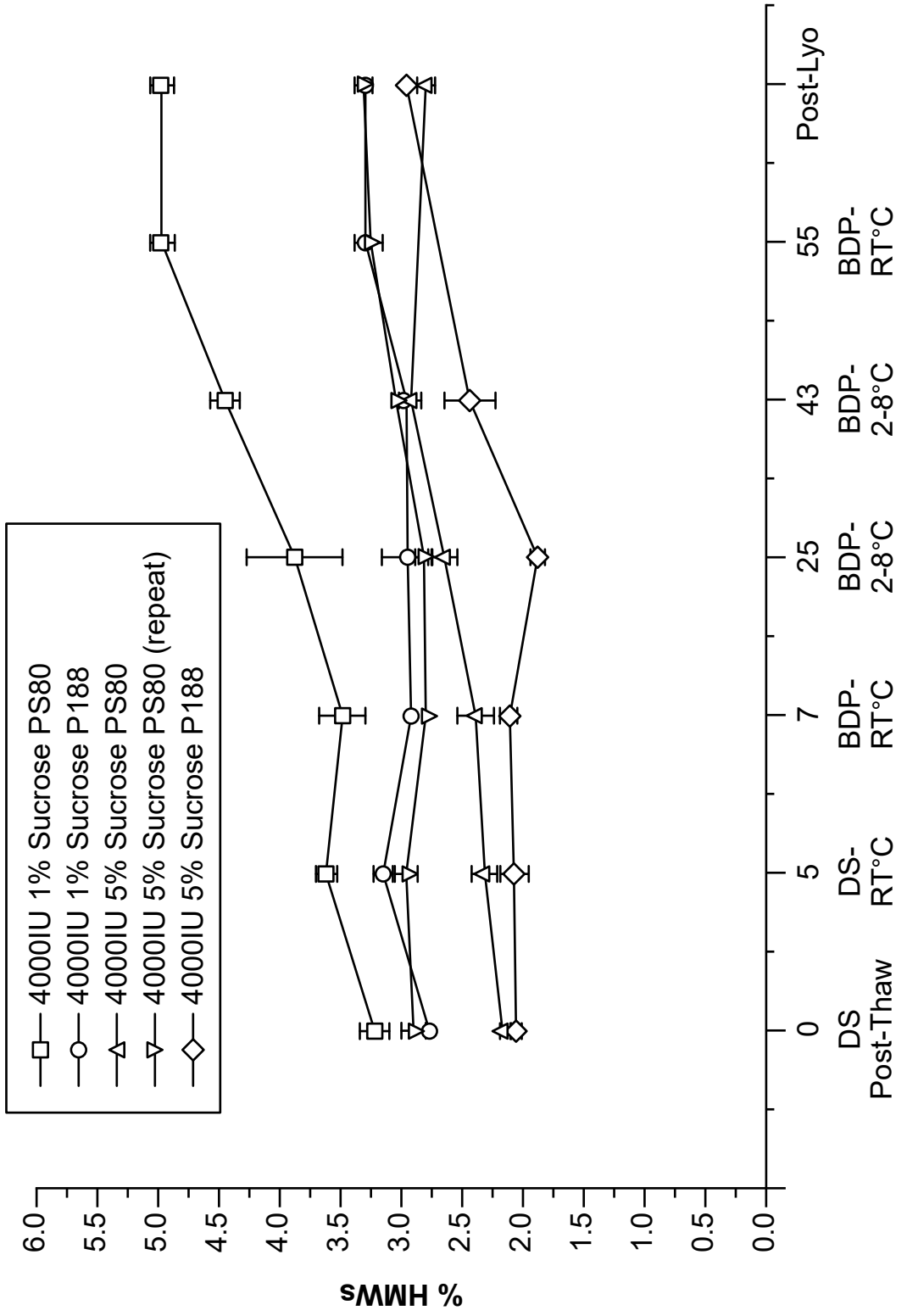

4000IU 1% Sucrose PS80
4000IU 1% Sucrose P188
4000IU 5% Sucrose PS80
4000IU 5% Sucrose PS80 (repeat)
4000IU 5% Sucrose P188
% HMWS
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
DS Post-Thaw
5
DS- RT°C
7
BDP- RT°C
25
BDP- 2-8°C
43
BDP- 2-8°C
55
BDP- RT°C
Post-Lyo
Time

FIG. 4

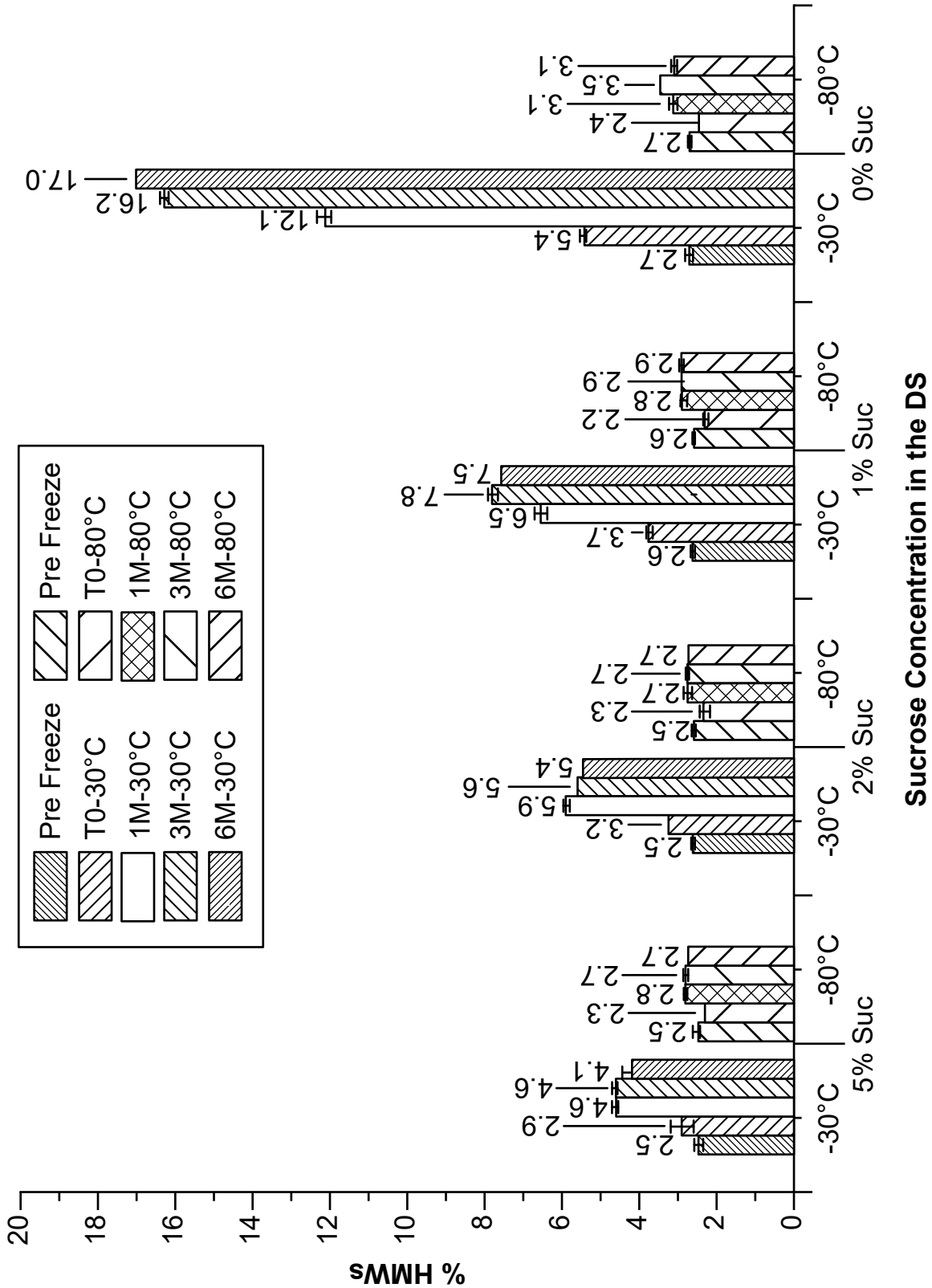

% HMWs
Sucrose Concentration in the DS
Pre Freeze
T0-30°C
1M-30°C
3M-30°C
6M-30°C
Pre Freeze
T0-80°C
1M-80°C
3M-80°C
6M-80°C
5% Suc
1% Suc
2% Suc
0% Suc
-30°C
-80°C

FIG. 5

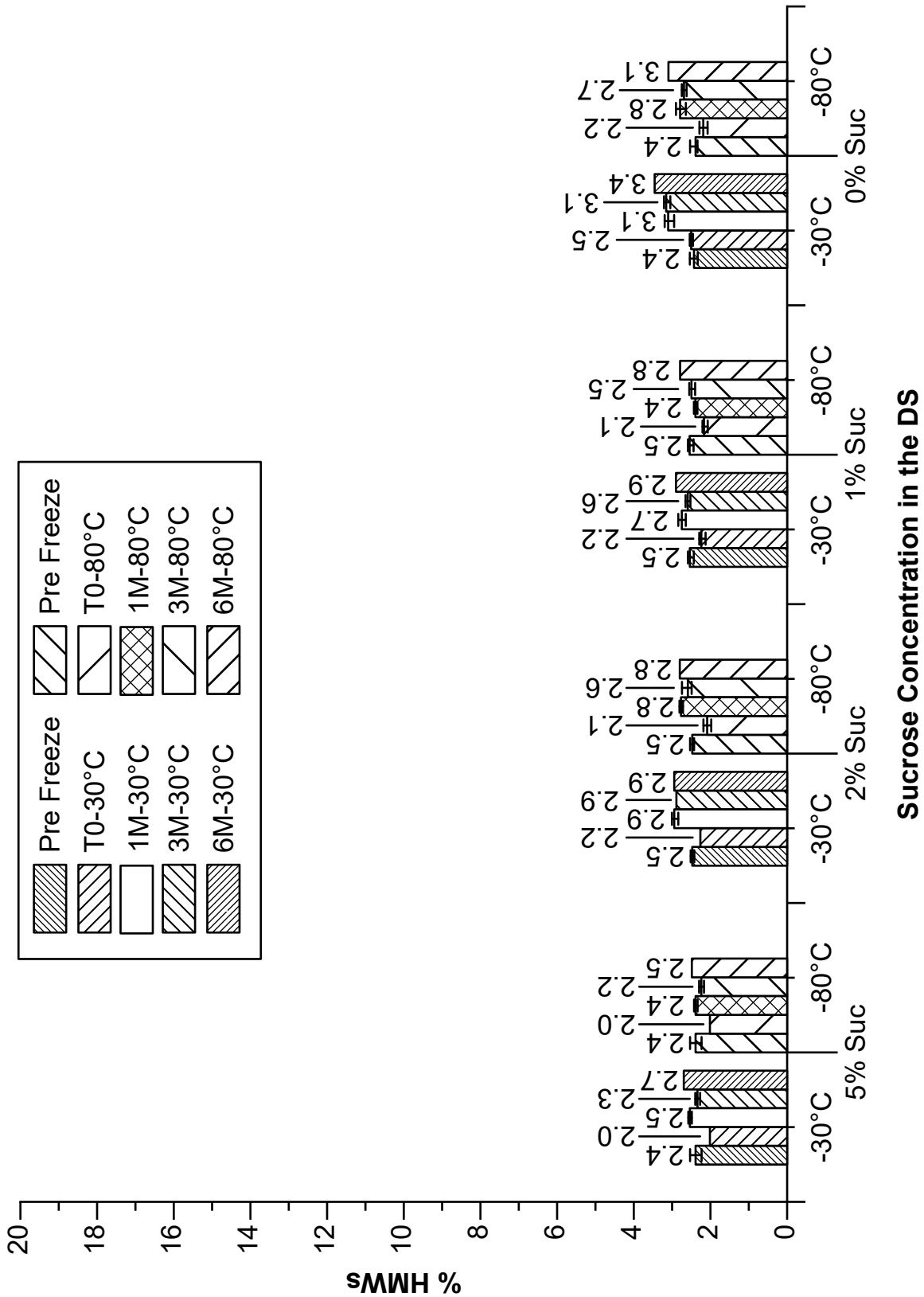

% HMWs
Sucrose Concentration in the DS
Pre Freeze
T0-30°C
1M-30°C
3M-30°C
6M-30°C
T0-80°C
1M-80°C
3M-80°C
6M-80°C
5% Suc
2% Suc
1% Suc
0% Suc
-30°C
-80°C

FIG. 6

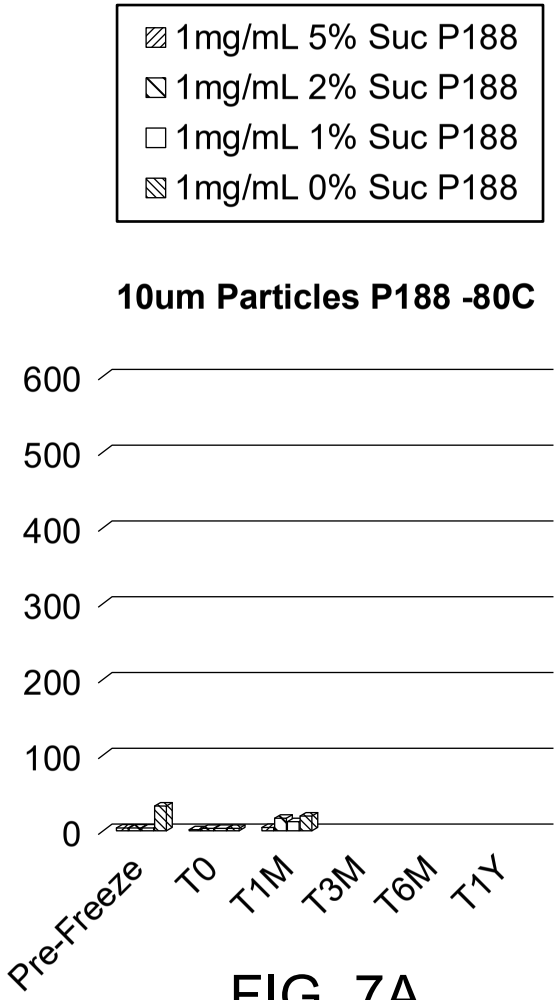

1mg/mL 5% Suc P188
1mg/mL 2% Suc P188
1mg/mL 1% Suc P188
1mg/mL 0% Suc P188
10um Particles P188 -80C
600
500
400
300
200
100
0
Pre-Freeze
T0
T1M
T3M
T6M
T1Y

FIG. 7A

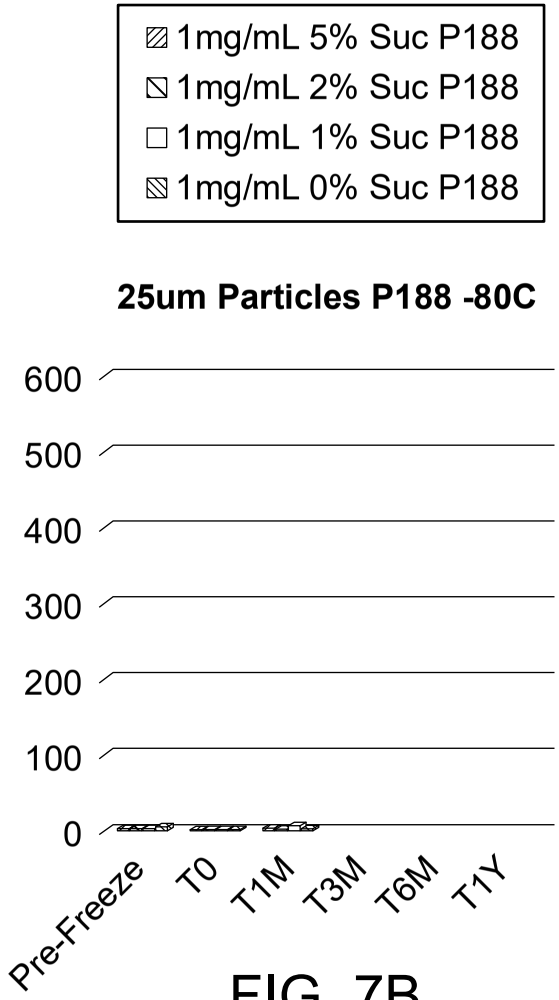

1mg/mL 5% Suc P188
1mg/mL 2% Suc P188
1mg/mL 1% Suc P188
1mg/mL 0% Suc P188
25um Particles P188 -80C
600
500
400
300
200
100
0
Pre-Freeze
T0
T1M
T3M
T6M
T1Y

FIG. 7B

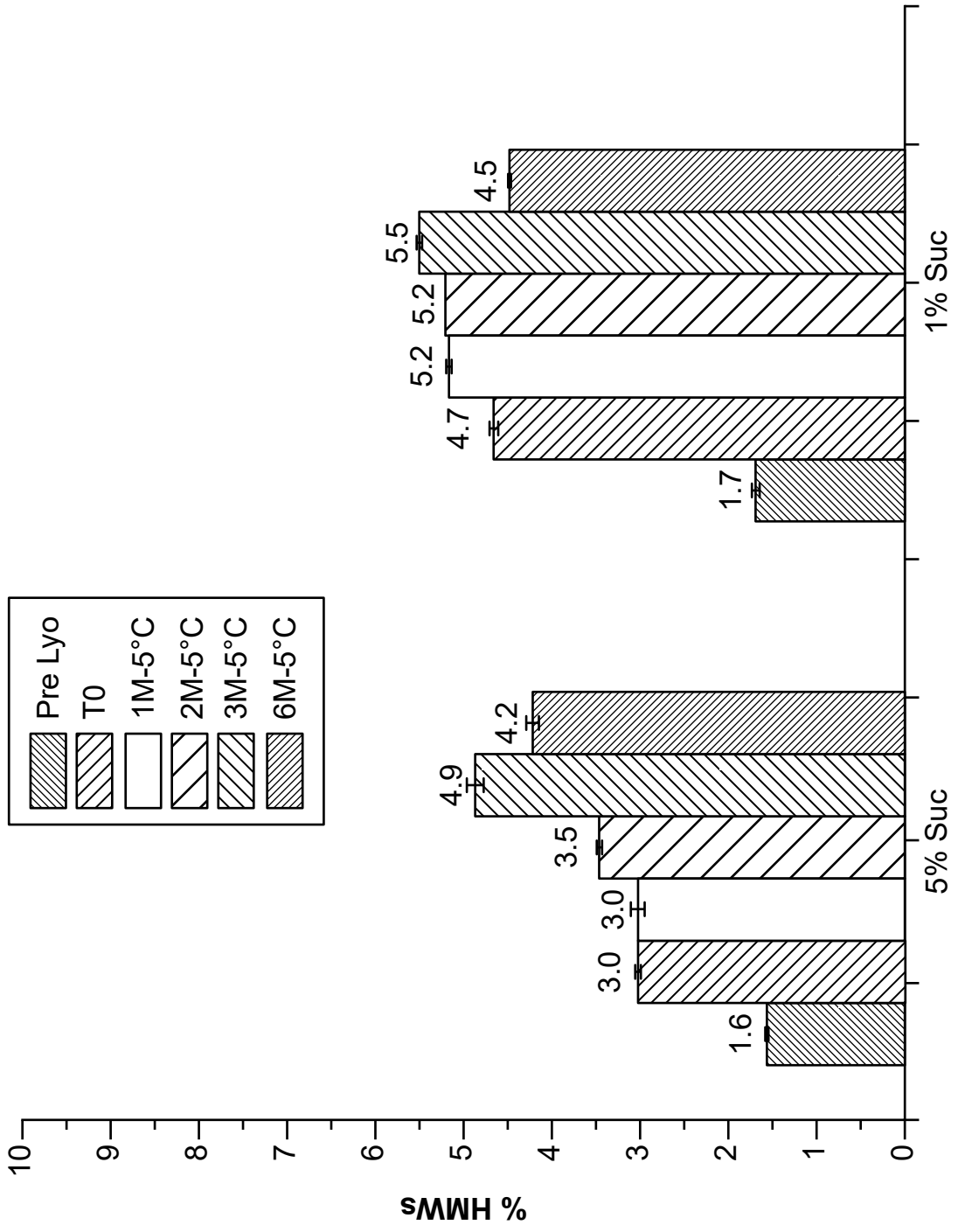

% HMWS
0
1
2
3
4
5
6
7
8
9
10
Pre Lyo
T0
1M-5°C
2M-5°C
3M-5°C
6M-5°C
1.6
3.0
3.0
3.5
4.9
4.2
5% Suc
1.7
4.7
5.2
5.2
5.5
4.5
1% Suc

FIG. 8A

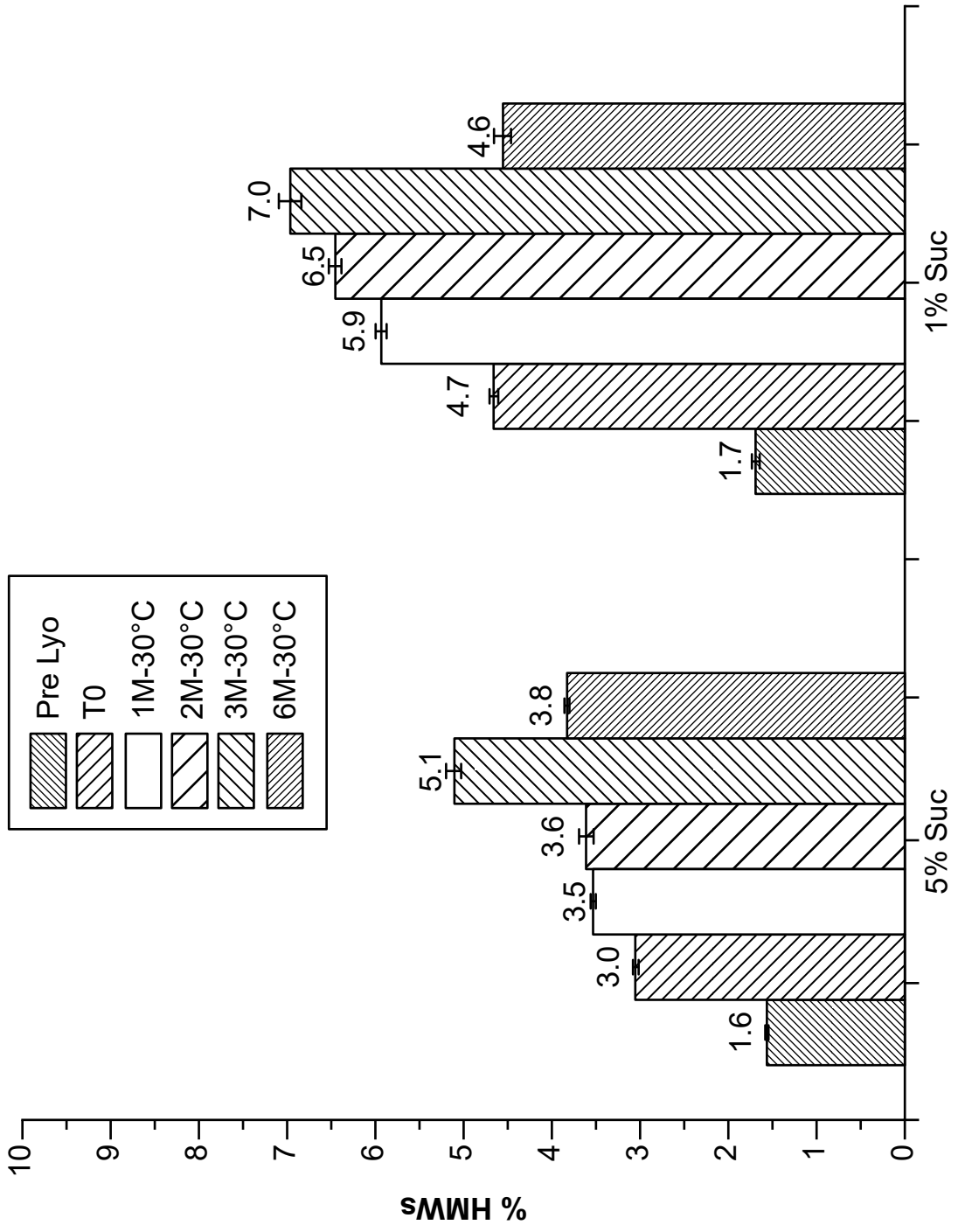

% HMWS
0
1
2
3
4
5
6
7
8
9
10
Pre Lyo
T0
1M-30°C
2M-30°C
3M-30°C
6M-30°C
1.6
3.0
3.5
3.6
5.1
3.8
5% Suc
1.7
4.7
5.9
6.5
7.0
4.6
1% Suc

FIG. 8B

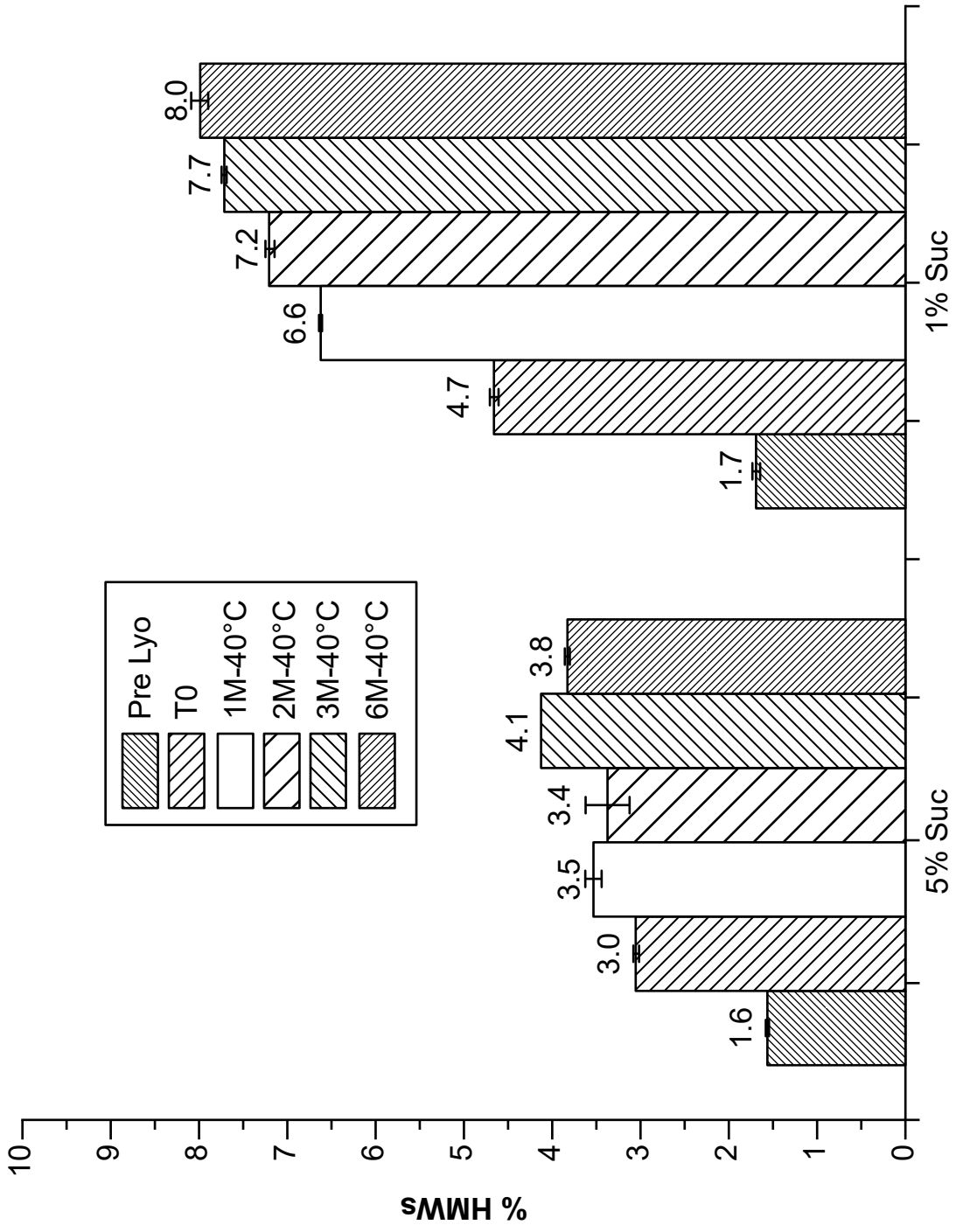

% HMWS
0
1
2
3
4
5
6
7
8
9
10
Pre Lyo
T0
1M-40°C
2M-40°C
3M-40°C
6M-40°C
1.6
3.0
3.5
3.4
4.1
3.8
5% Suc
1.7
4.7
6.6
7.2
7.7
8.0
1% Suc

FIG. 8C

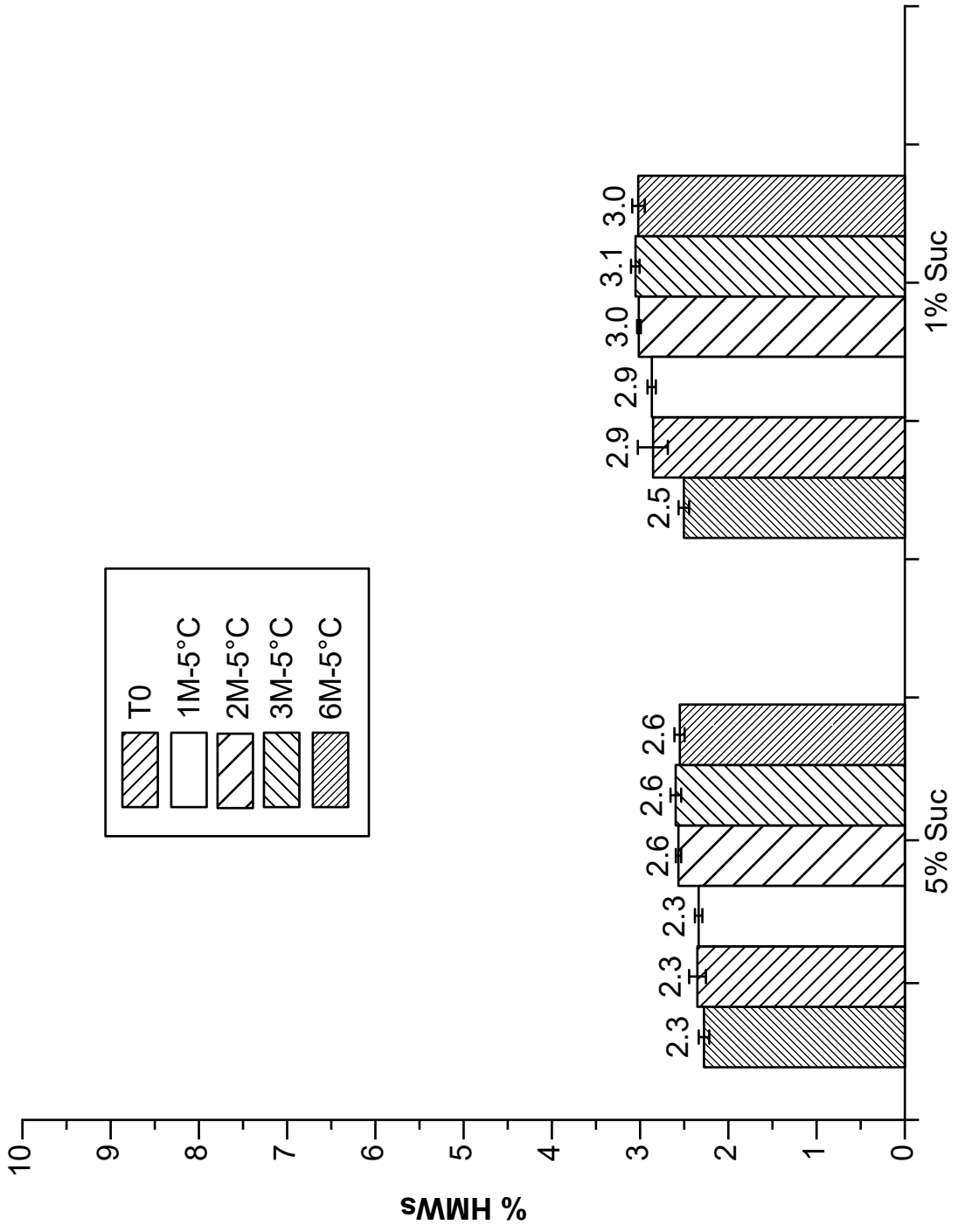

% HMWS
0
1
2
3
4
5
6
7
8
9
10
T0
1M-5°C
2M-5°C
3M-5°C
6M-5°C
2.3
2.3
2.3
2.6
2.6
2.6
2.5
2.9
2.9
3.0
3.1
3.0
5% Suc
1% Suc

FIG. 9A

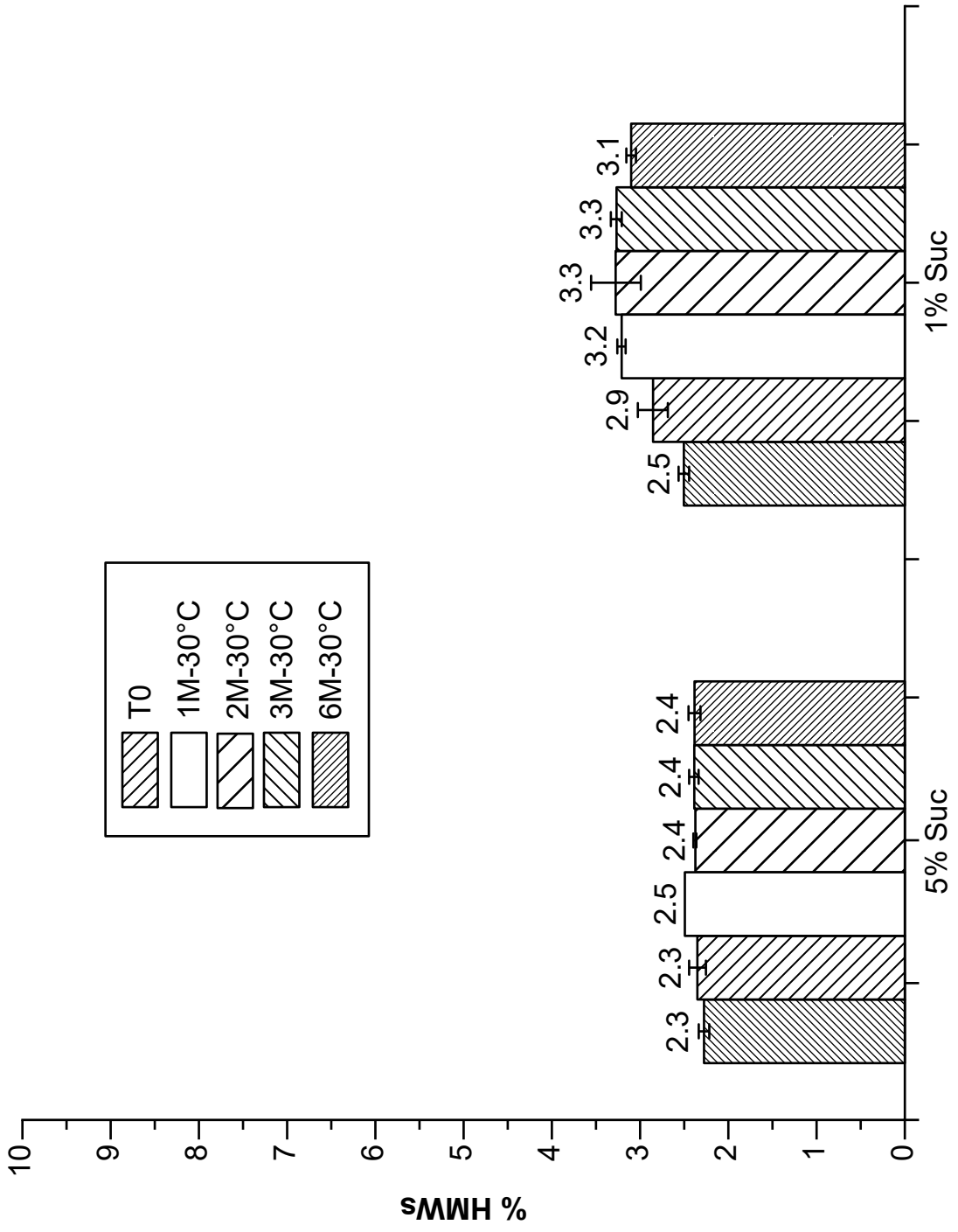

% HMWS
0
1
2
3
4
5
6
7
8
9
10
T0
1M-30°C
2M-30°C
3M-30°C
6M-30°C
2.3
2.3
2.5
2.4
2.4
2.4
5% Suc
2.5
2.9
3.2
3.3
3.3
3.3
3.1
1% Suc

FIG. 9B

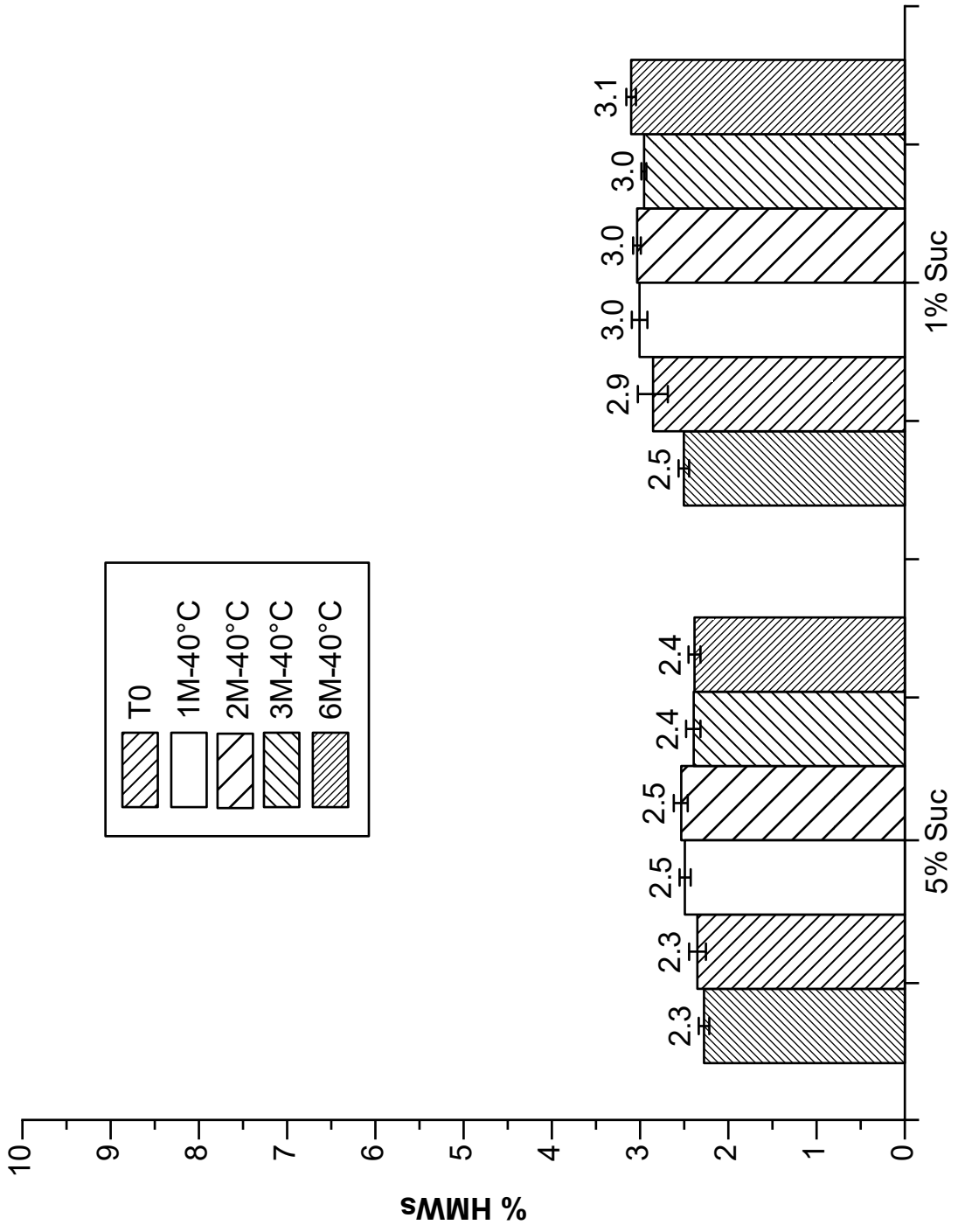

% HMWS
0
1
2
3
4
5
6
7
8
9
10
T0
1M-40°C
2M-40°C
3M-40°C
6M-40°C
2.3
2.3
2.5
2.5
2.4
2.4
2.5
5% Suc
2.9
3.0
3.0
3.0
3.1
1% Suc

FIG. 9C

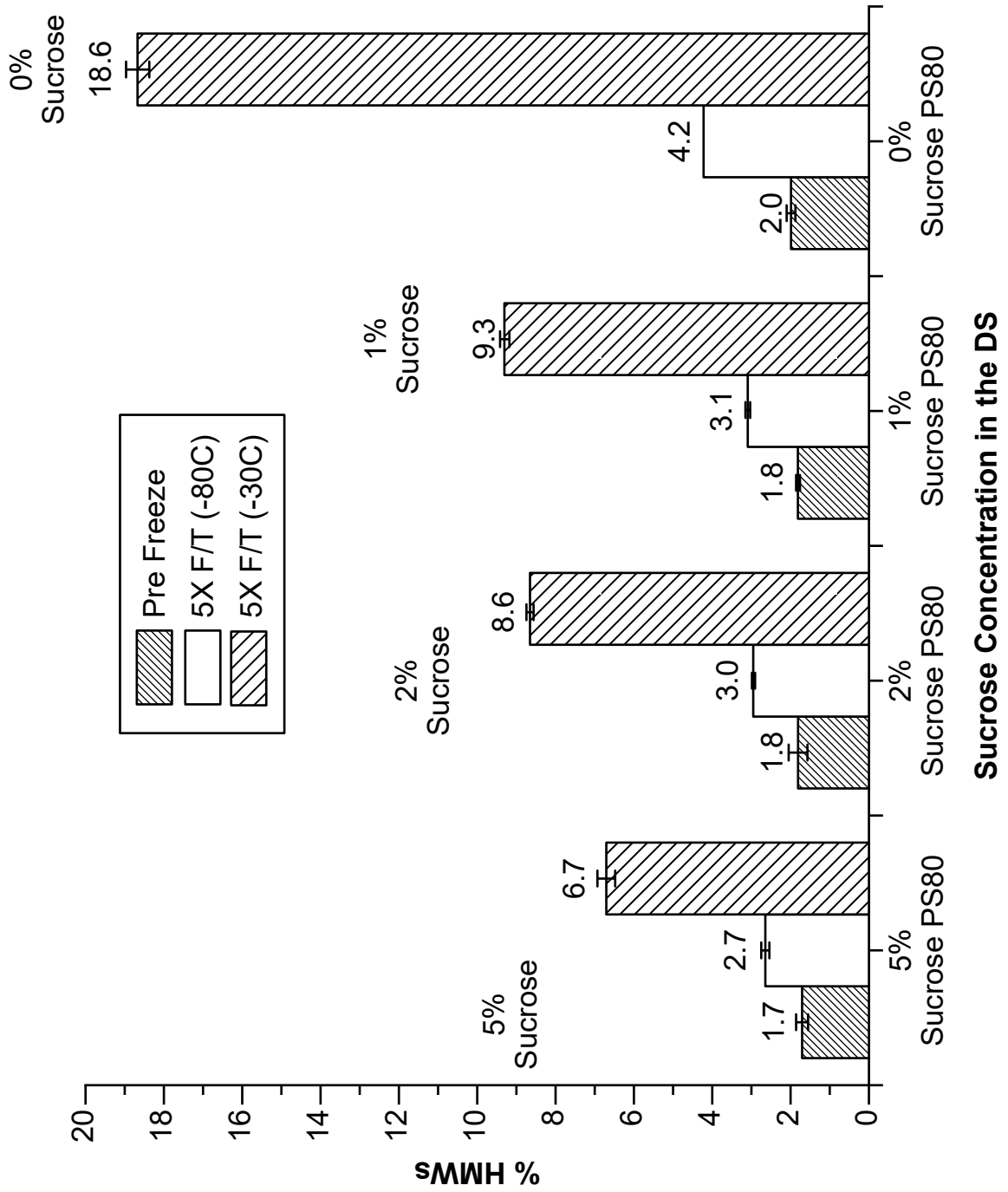

% HMWs
Sucrose Concentration in the DS
Pre Freeze
5X F/T (-80C)
5X F/T (-30C)
0% Sucrose
1% Sucrose
2% Sucrose
5% Sucrose
Sucrose PS80
18.6
4.2
2.0
9.3
3.1
1.8
8.6
3.0
1.8
6.7
2.7
1.7
0
2
4
6
8
10
12
14
16
18
20

FIG. 10

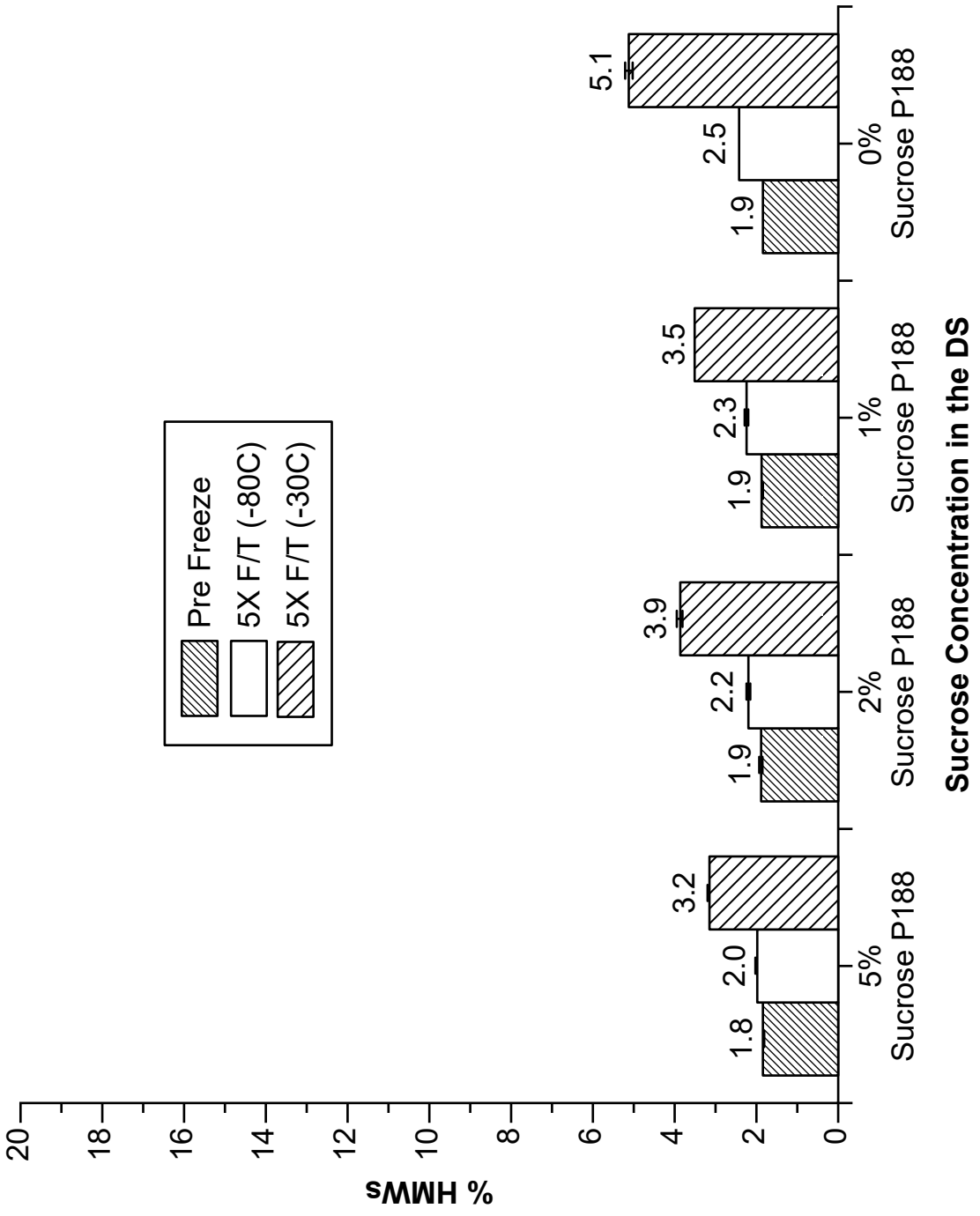

Pre Freeze
5X F/T (-80C)
5X F/T (-30C)
% HMWs
0
2
4
6
8
10
12
14
16
18
20
1.8
2.0
3.2
1.9
2.2
3.9
1.9
2.3
3.5
1.9
2.5
5.1
5%
Sucrose P188
2%
Sucrose P188
1%
Sucrose P188
0%
Sucrose P188
Sucrose Concentration in the DS

FIG. 11

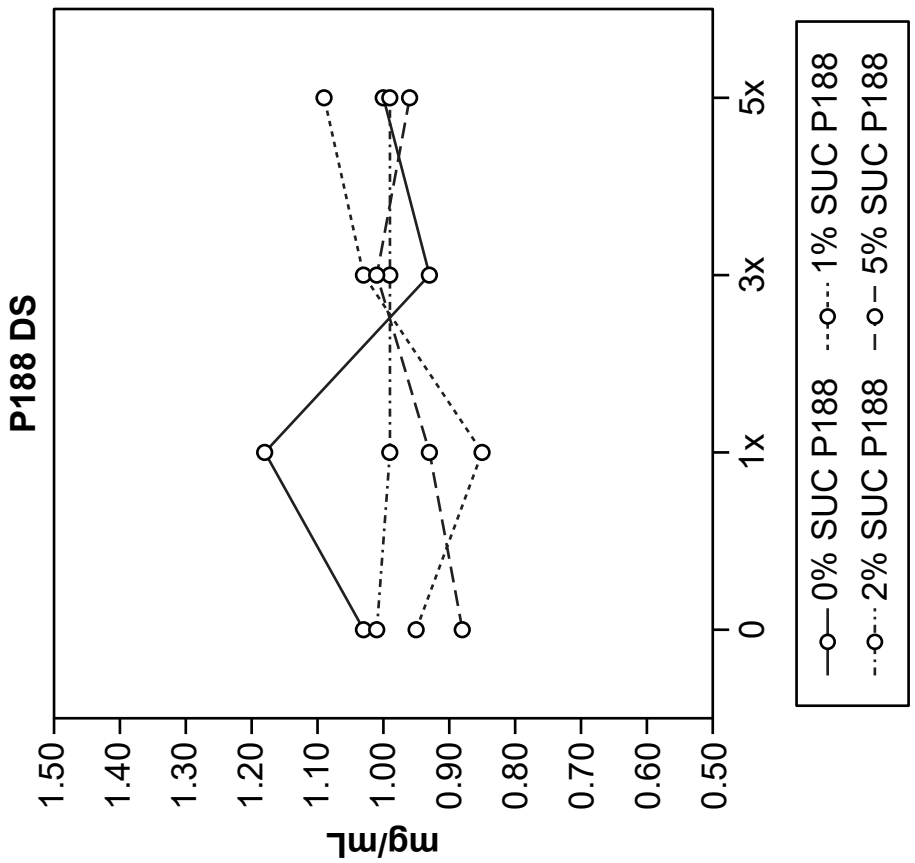


FIG. 12B

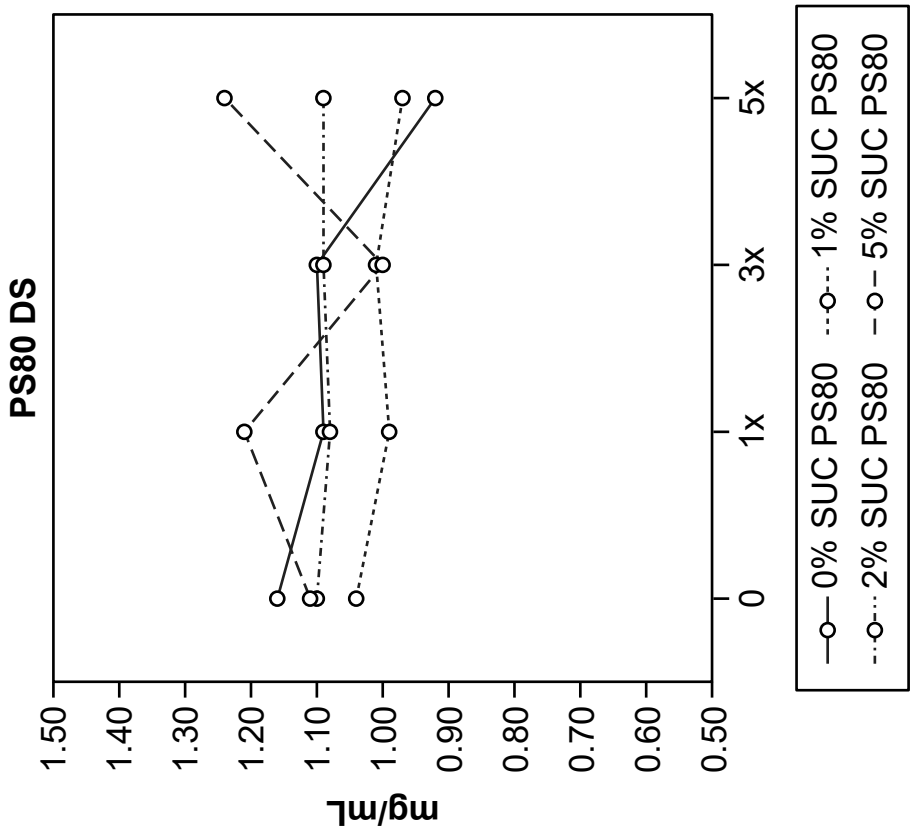


FIG. 12A

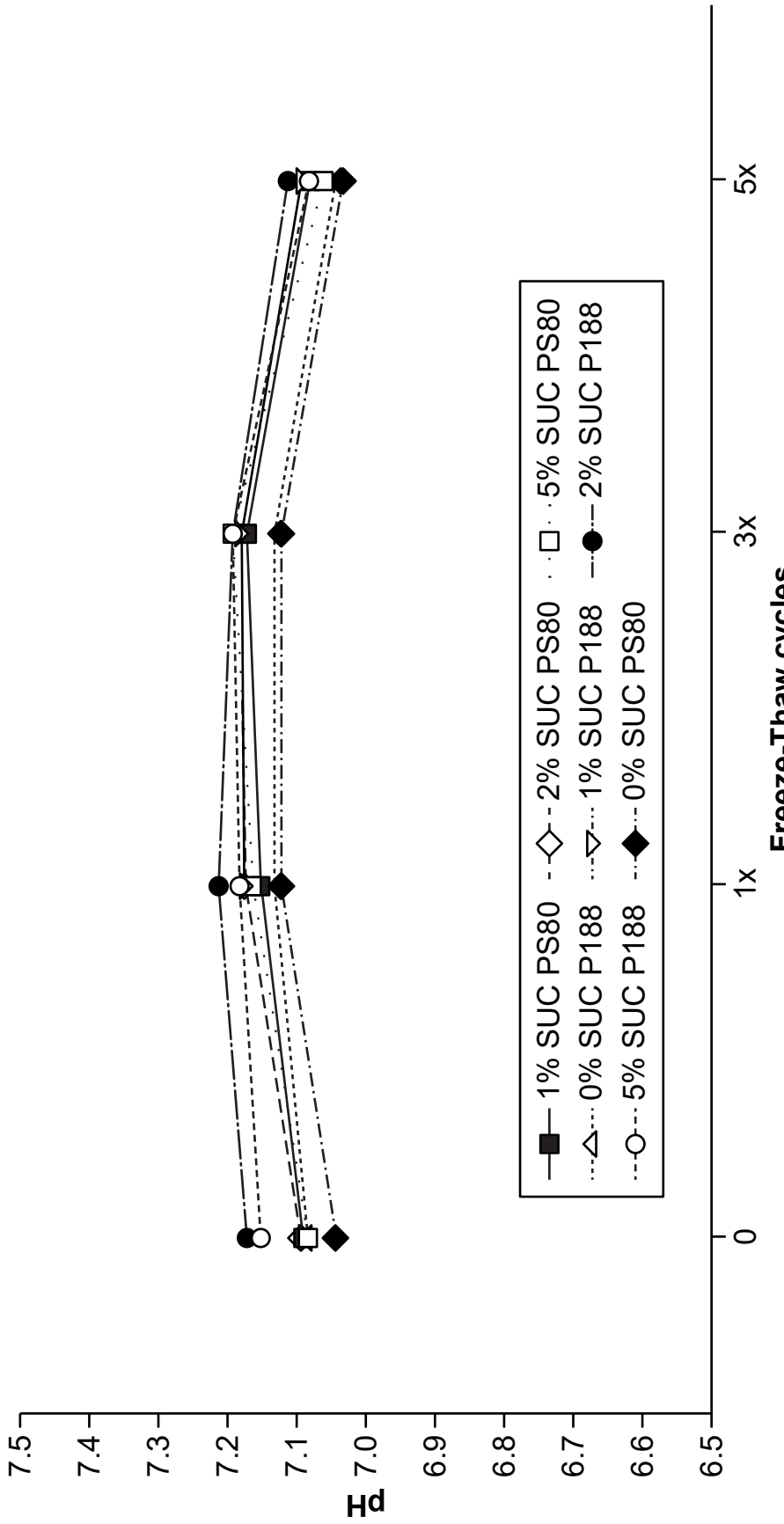

pH
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
0
1x
3x
5x
Freeze-Thaw cycles
1% SUC PS80
2% SUC PS80
5% SUC PS80
0% SUC P188
1% SUC P188
2% SUC P188
5% SUC P188
0% SUC PS80

FIG. 13

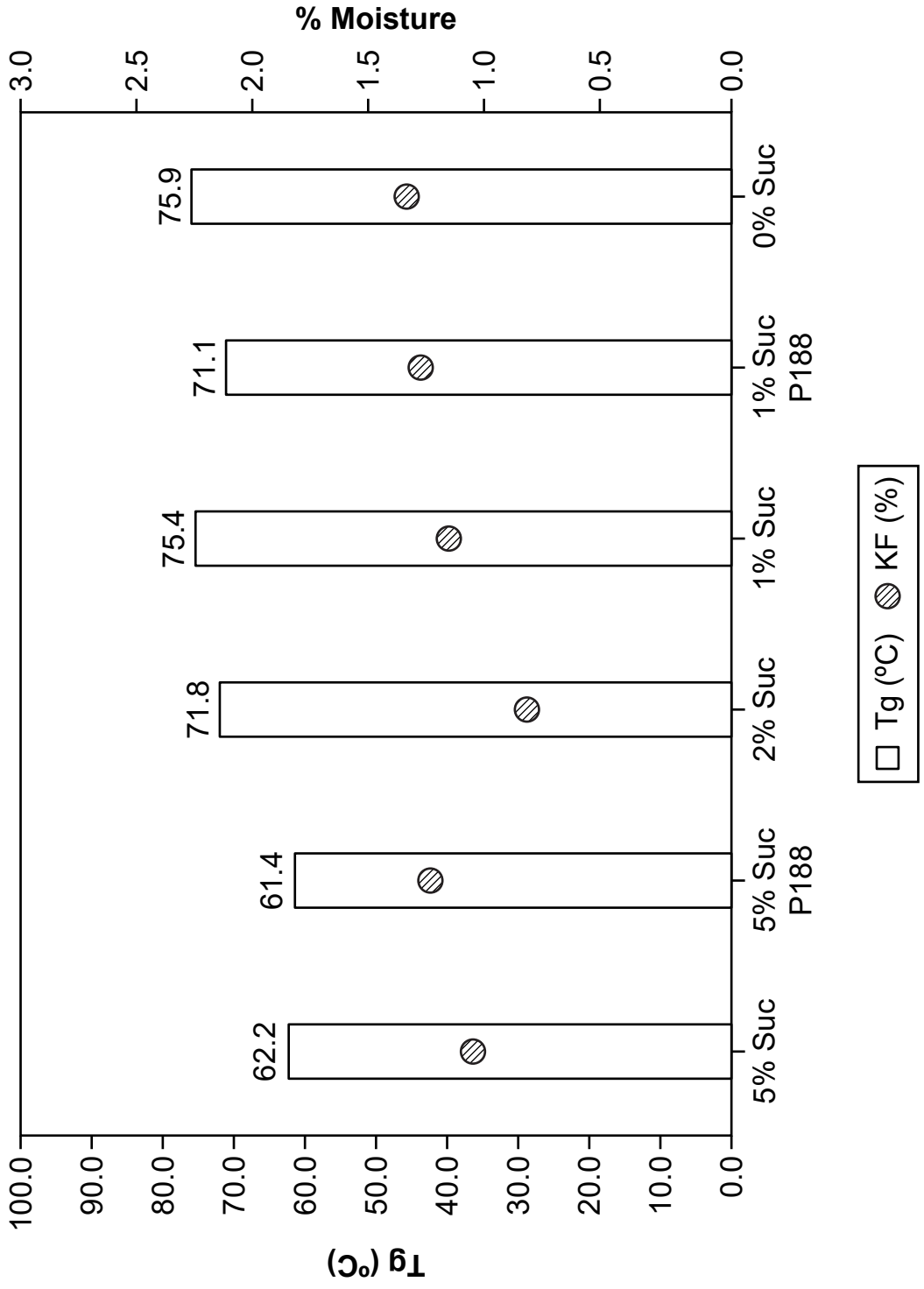

% Moisture
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Tg (°C)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
62.2
61.4
71.8
75.4
71.1
75.9
5% Suc
5% Suc P188
2% Suc
1% Suc
1% Suc P188
0% Suc
Tg (°C)
KF (%)

FIG. 14

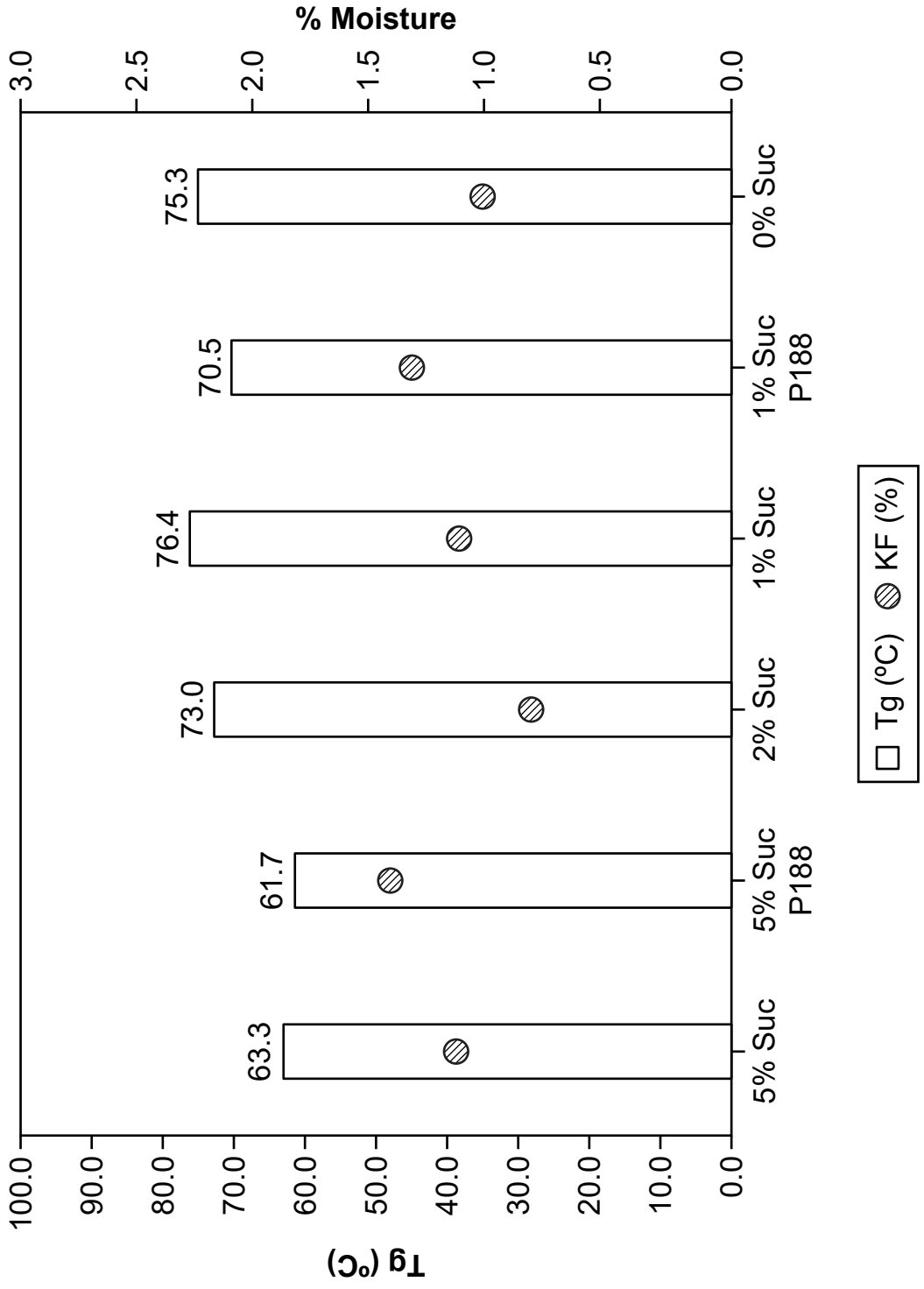

% Moisture
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Tg (°C)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
63.3
61.7
73.0
76.4
70.5
75.3
5% Suc
5% Suc P188
2% Suc
1% Suc
1% Suc P188
0% Suc
Tg (°C)
KF (%)

FIG. 15

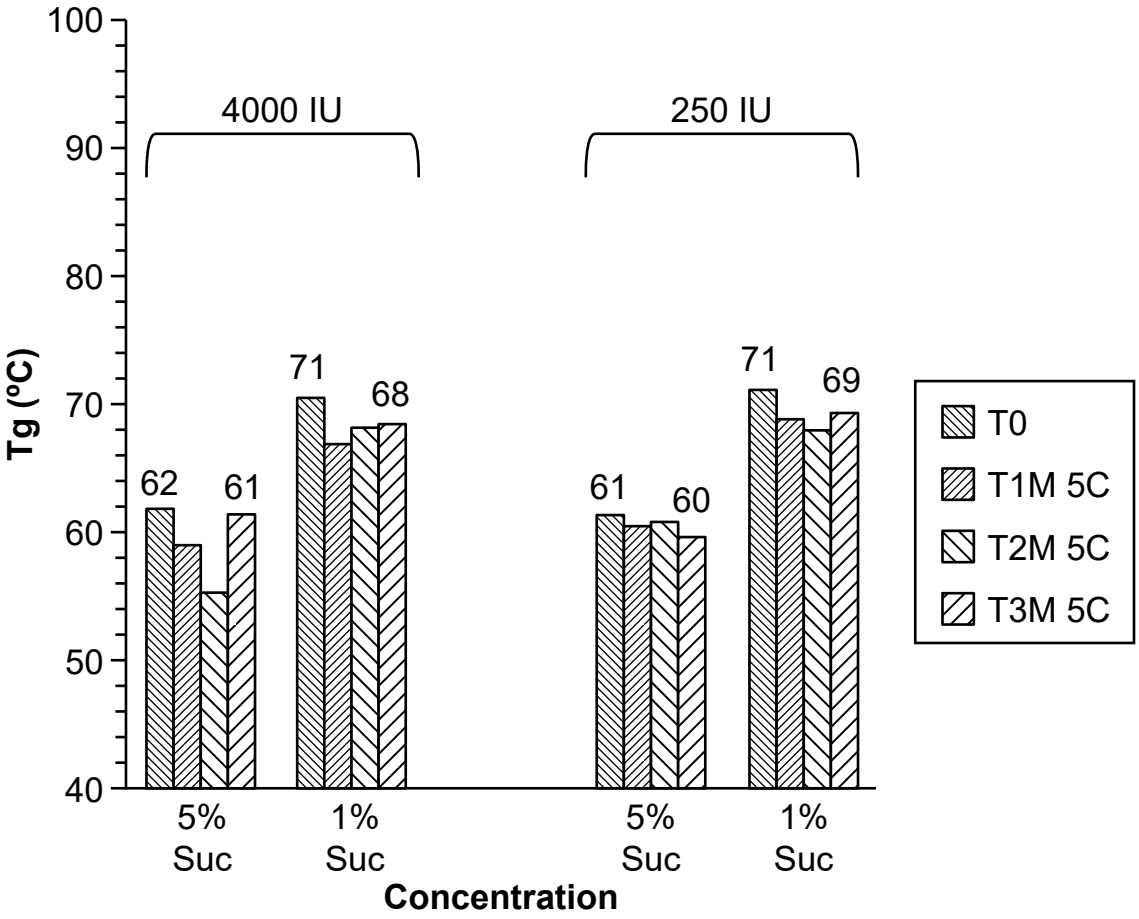

100
90
80
70
60
50
40
Tg (°C)
4000 IU
250 IU
62
61
71
68
61
60
71
69
5%
Suc
1%
Suc
5%
Suc
1%
Suc
Concentration
T0
T1M 5C
T2M 5C
T3M 5C

FIG. 16A

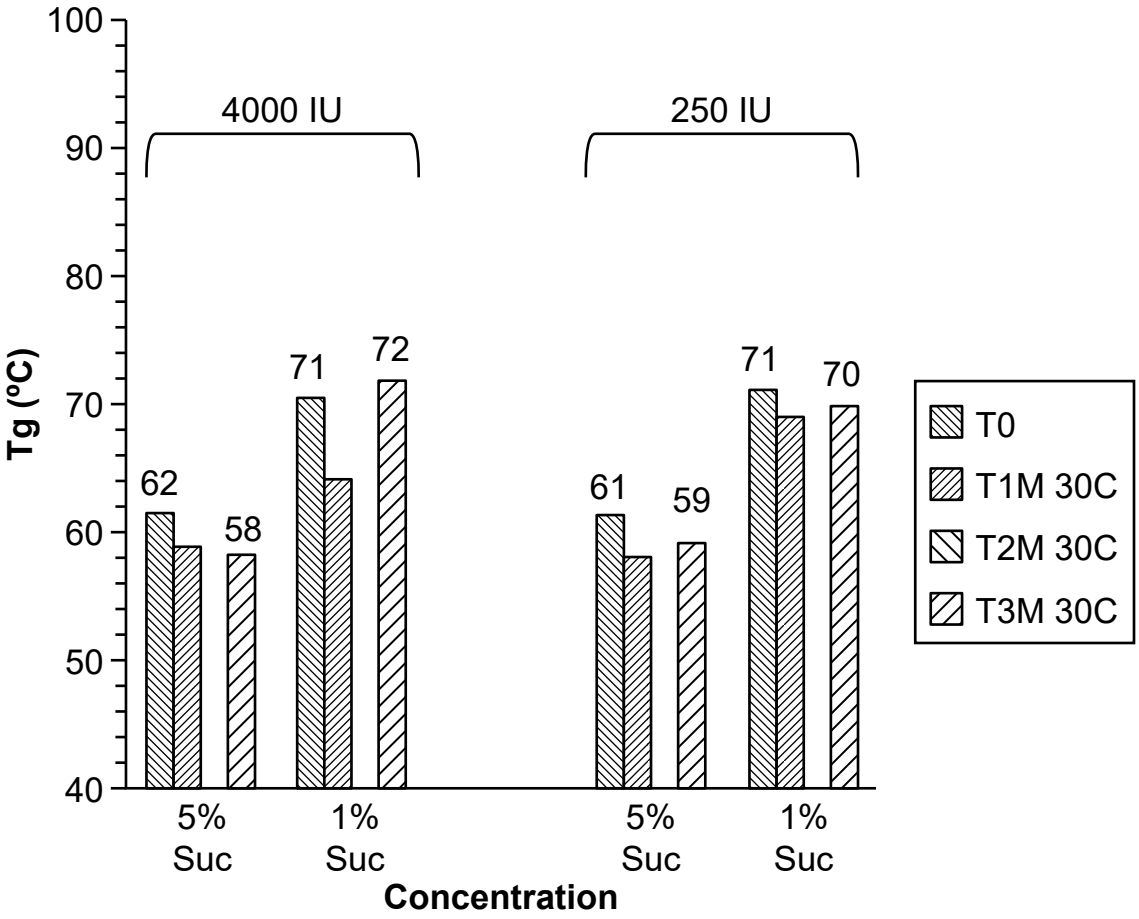

100
90
80
70
60
50
40
Tg (ºC)
4000 IU
250 IU
62
58
71
72
61
59
71
70
5%
Suc
1%
Suc
5%
Suc
1%
Suc
Concentration
T0
T1M 30C
T2M 30C
T3M 30C

FIG. 16B

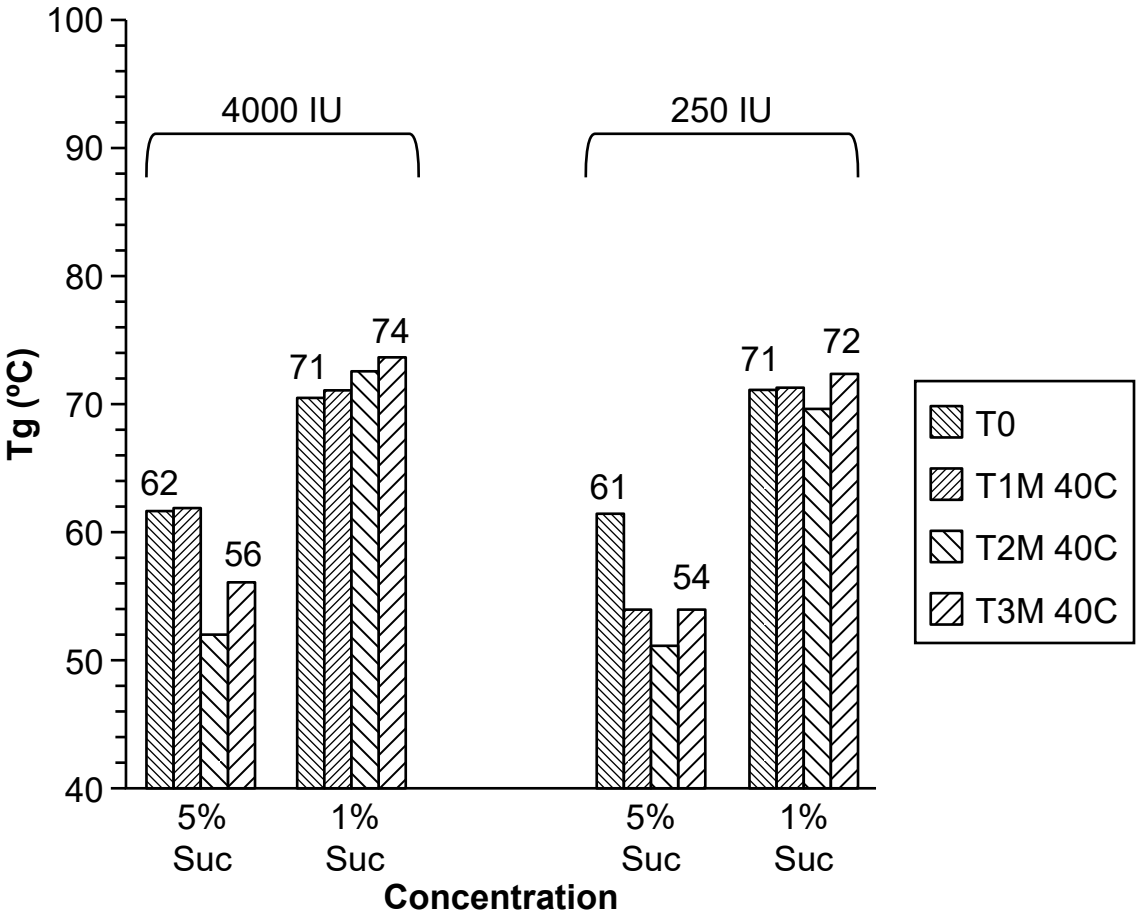

100
90
80
70
60
50
40
Tg (ºC)
4000 IU
250 IU
62
56
71
74
61
54
71
72
5%
Suc
1%
Suc
5%
Suc
1%
Suc
Concentration
T0
T1M 40C
T2M 40C
T3M 40C

FIG. 16C

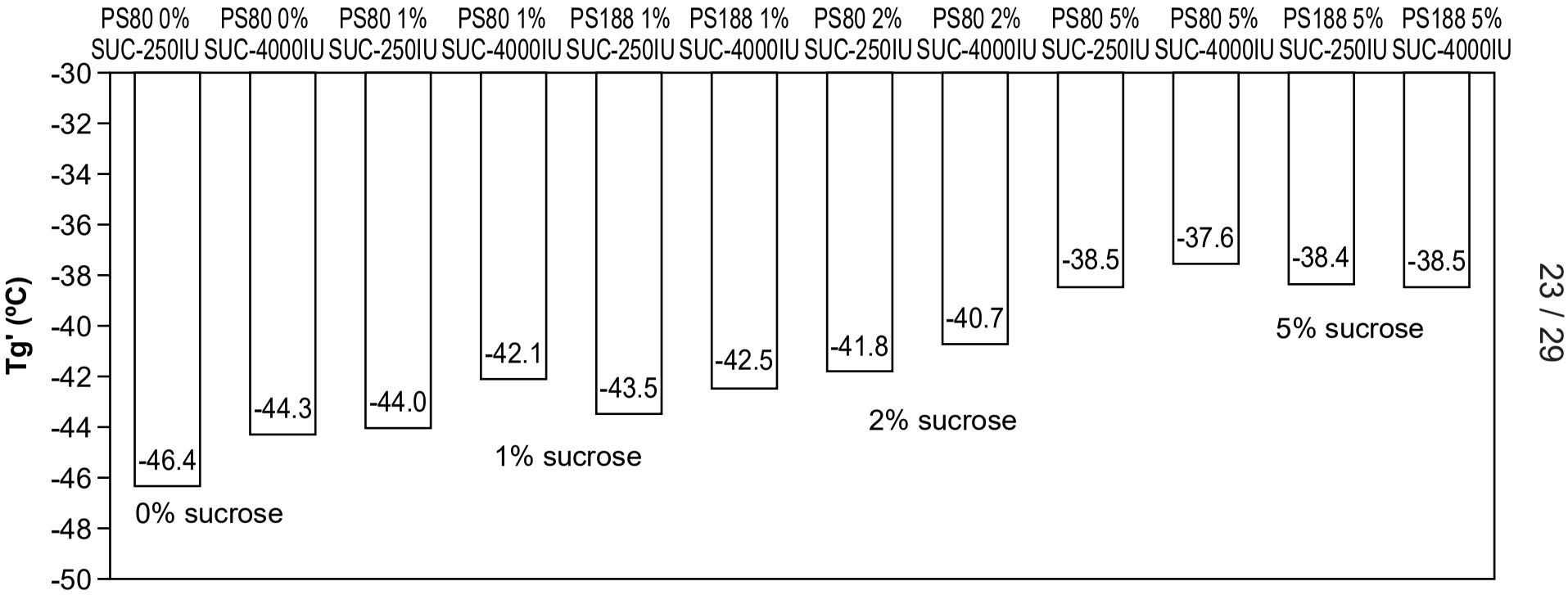

PS80 0% SUC-250IU
PS80 0% SUC-4000IU
PS80 1% SUC-250IU
PS80 1% SUC-4000IU
PS188 1% SUC-250IU
PS188 1% SUC-4000IU
PS80 2% SUC-250IU
PS80 2% SUC-4000IU
PS80 5% SUC-250IU
PS80 5% SUC-4000IU
PS188 5% SUC-250IU
PS188 5% SUC-4000IU
Tg' (°C)
-30
-32
-34
-36
-38
-40
-42
-44
-46
-48
-50
-46.4
-44.3
-44.0
-42.1
-43.5
-42.5
-41.8
-40.7
-38.5
-37.6
-38.4
-38.5
0% sucrose
1% sucrose
2% sucrose
5% sucrose

FIG. 17

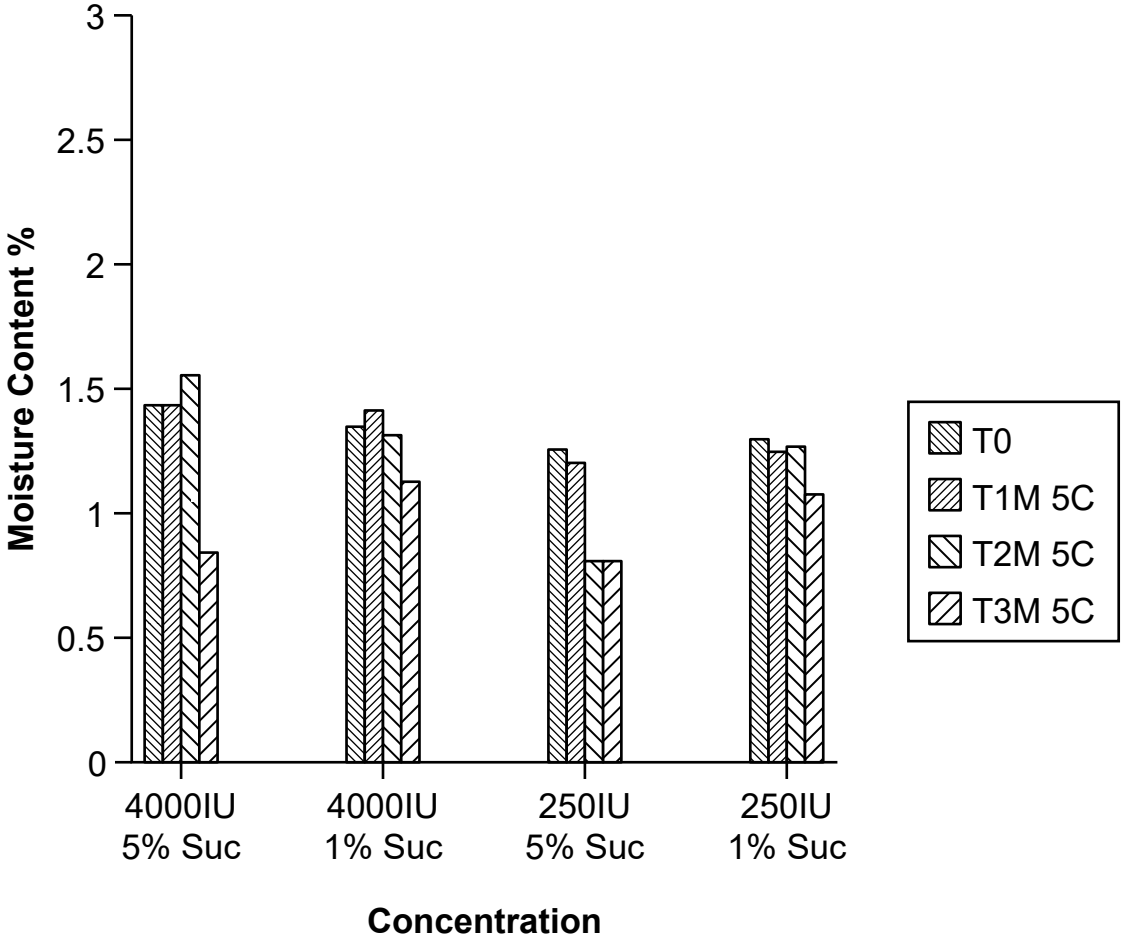

Moisture Content %
3
2.5
2
1.5
1
0.5
0
4000IU
5% Suc
4000IU
1% Suc
250IU
5% Suc
250IU
1% Suc
Concentration
T0
T1M 5C
T2M 5C
T3M 5C

FIG. 18A

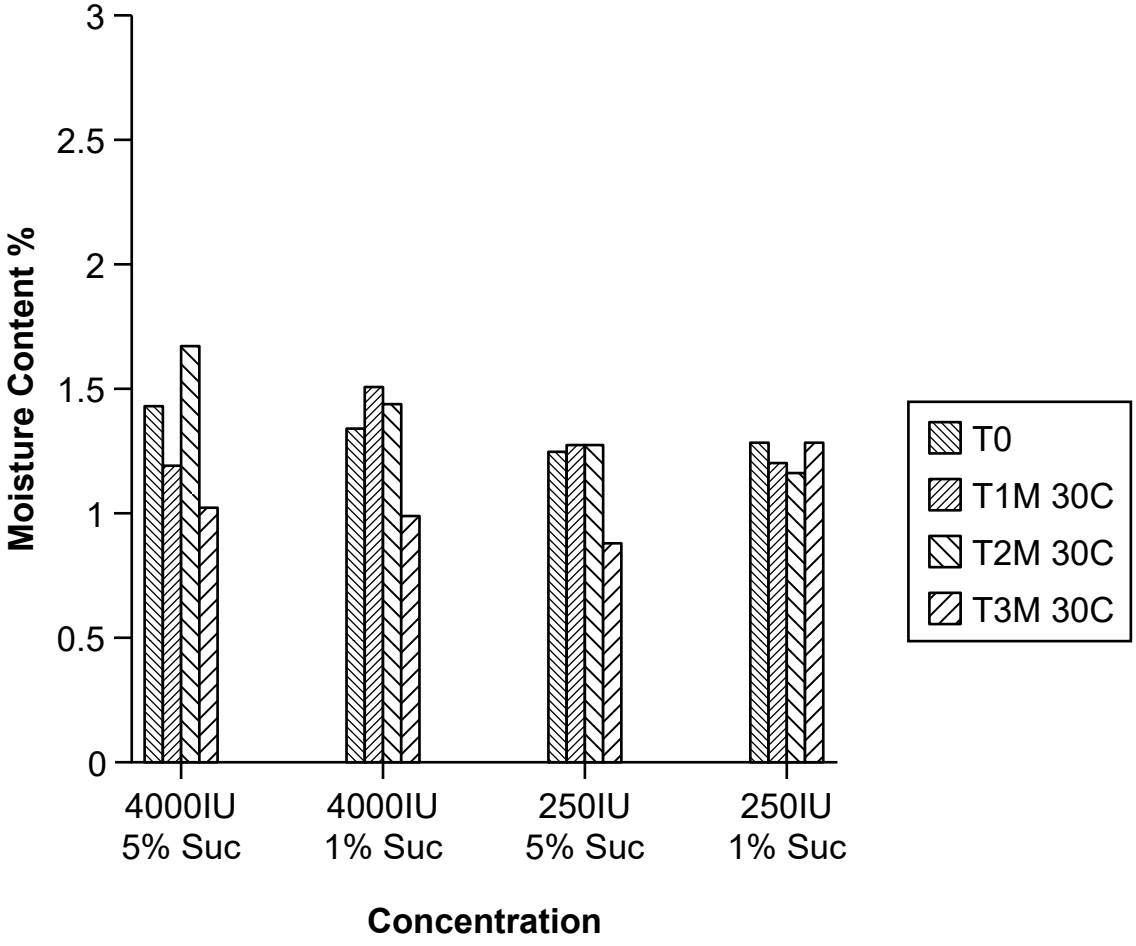

Moisture Content %
3
2.5
2
1.5
1
0.5
0
4000IU
5% Suc
4000IU
1% Suc
250IU
5% Suc
250IU
1% Suc
Concentration
T0
T1M 30C
T2M 30C
T3M 30C

FIG. 18B

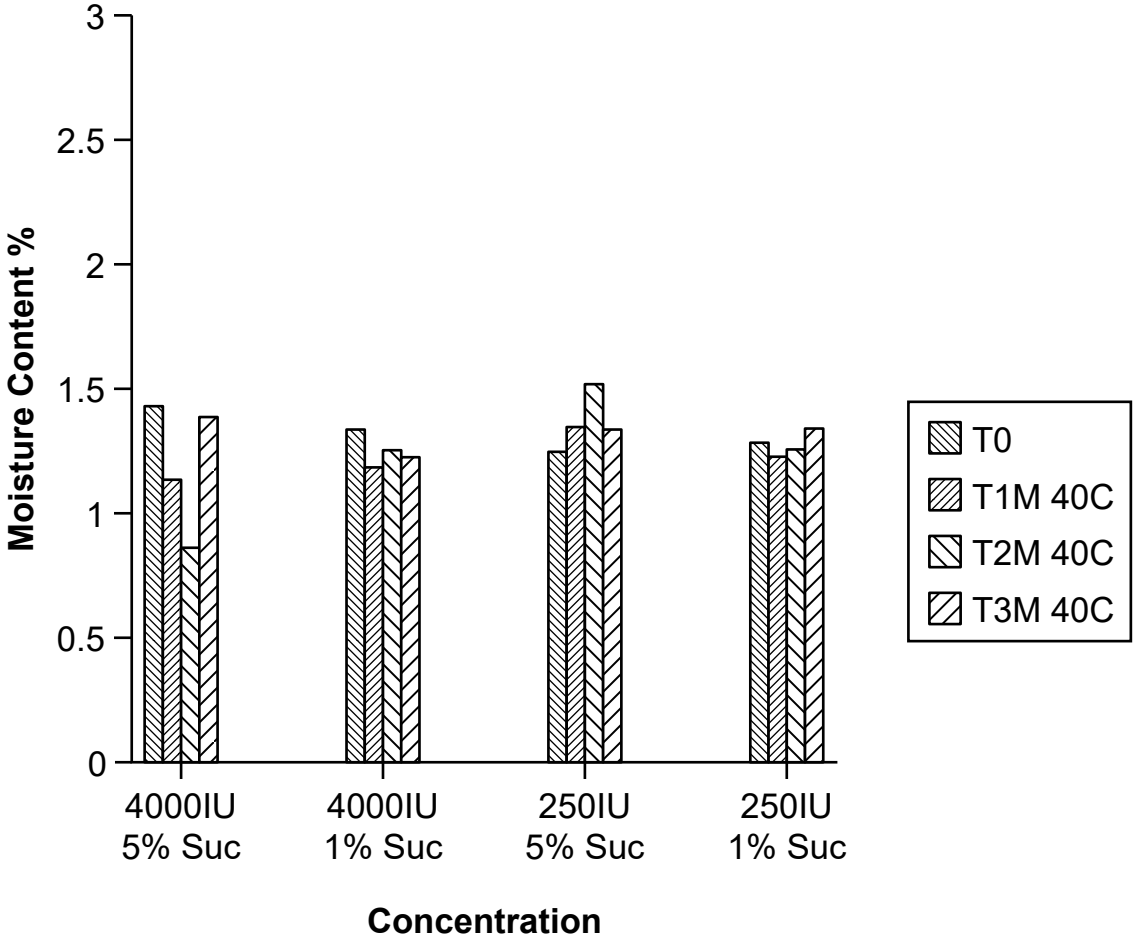

Moisture Content %
3
2.5
2
1.5
1
0.5
0
4000IU
5% Suc
4000IU
1% Suc
250IU
5% Suc
250IU
1% Suc
Concentration
T0
T1M 40C
T2M 40C
T3M 40C

FIG. 18C

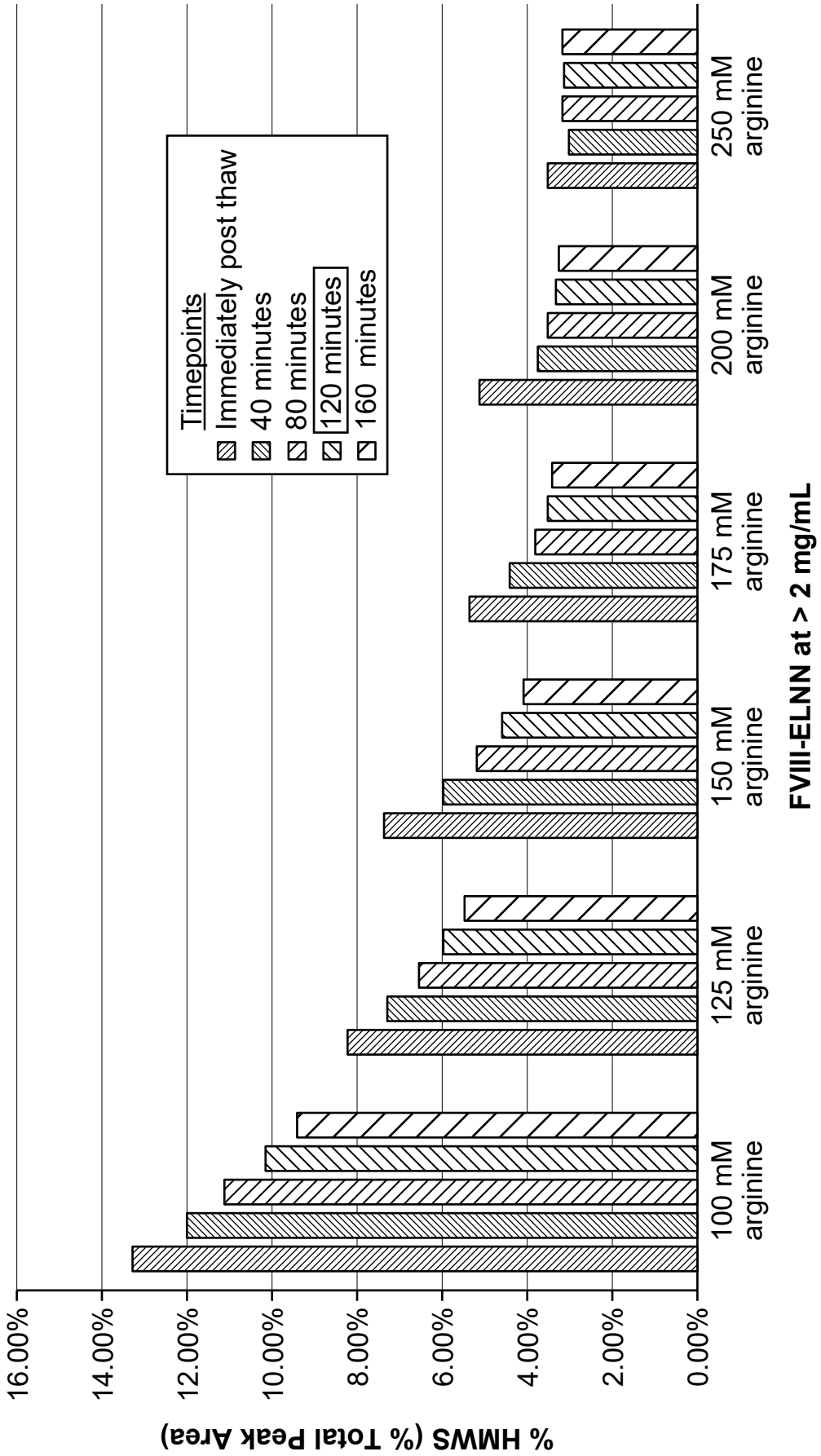

% HMWS (% Total Peak Area)
16.00%
14.00%
12.00%
10.00%
8.00%
6.00%
4.00%
2.00%
0.00%
Timepoints
Immediately post thaw
40 minutes
80 minutes
120 minutes
160 minutes
100 mM arginine
125 mM arginine
150 mM arginine
175 mM arginine
200 mM arginine
250 mM arginine
FVIII-ELNN at > 2 mg/mL

FIG. 19

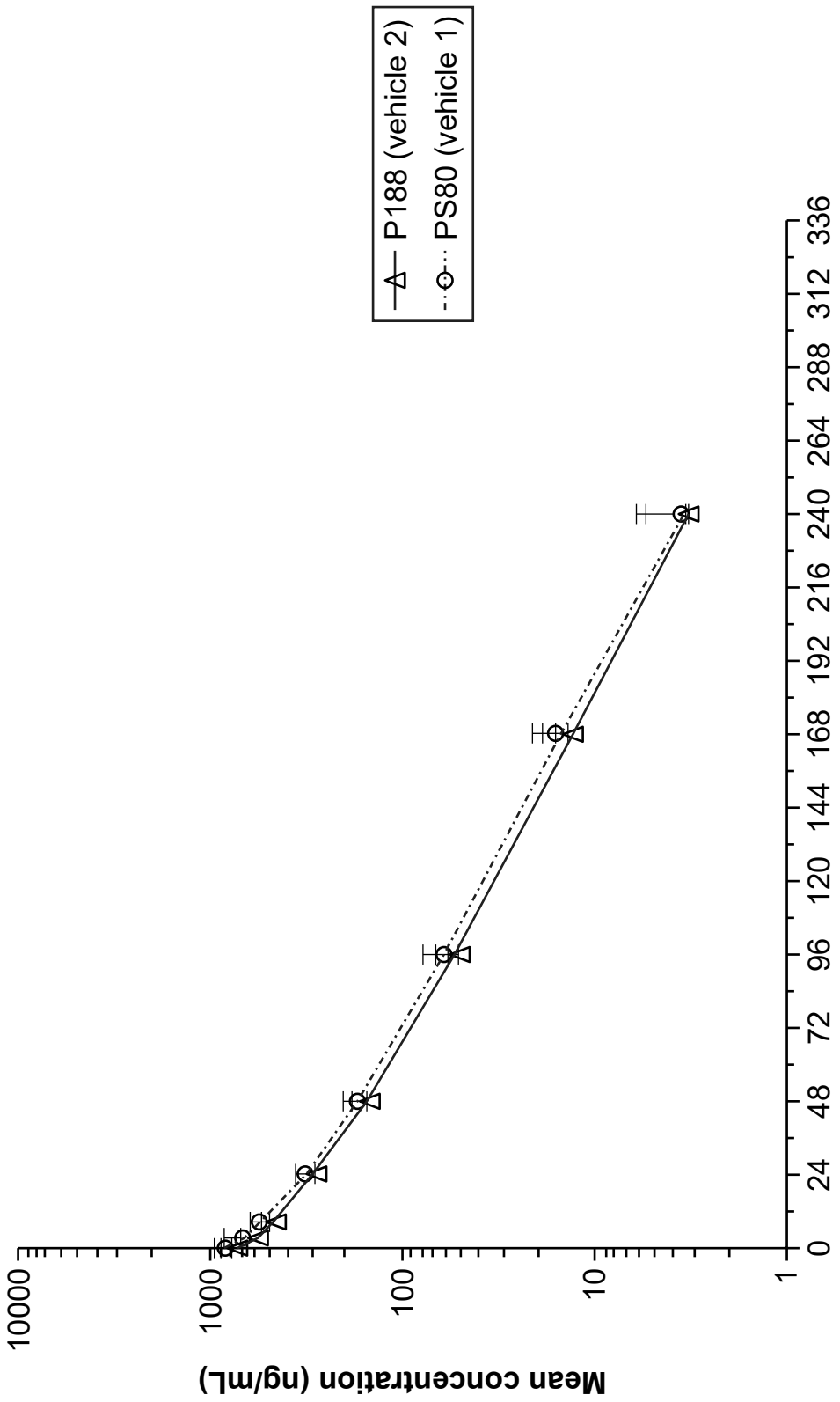

P188 (vehicle 2)
PS80 (vehicle 1)
Mean concentration (ng/mL)
10000
1000
100
10
1
Time (hr)
0
24
48
72
96
120
144
168
192
216
240
264
288
312
336

FIG. 20

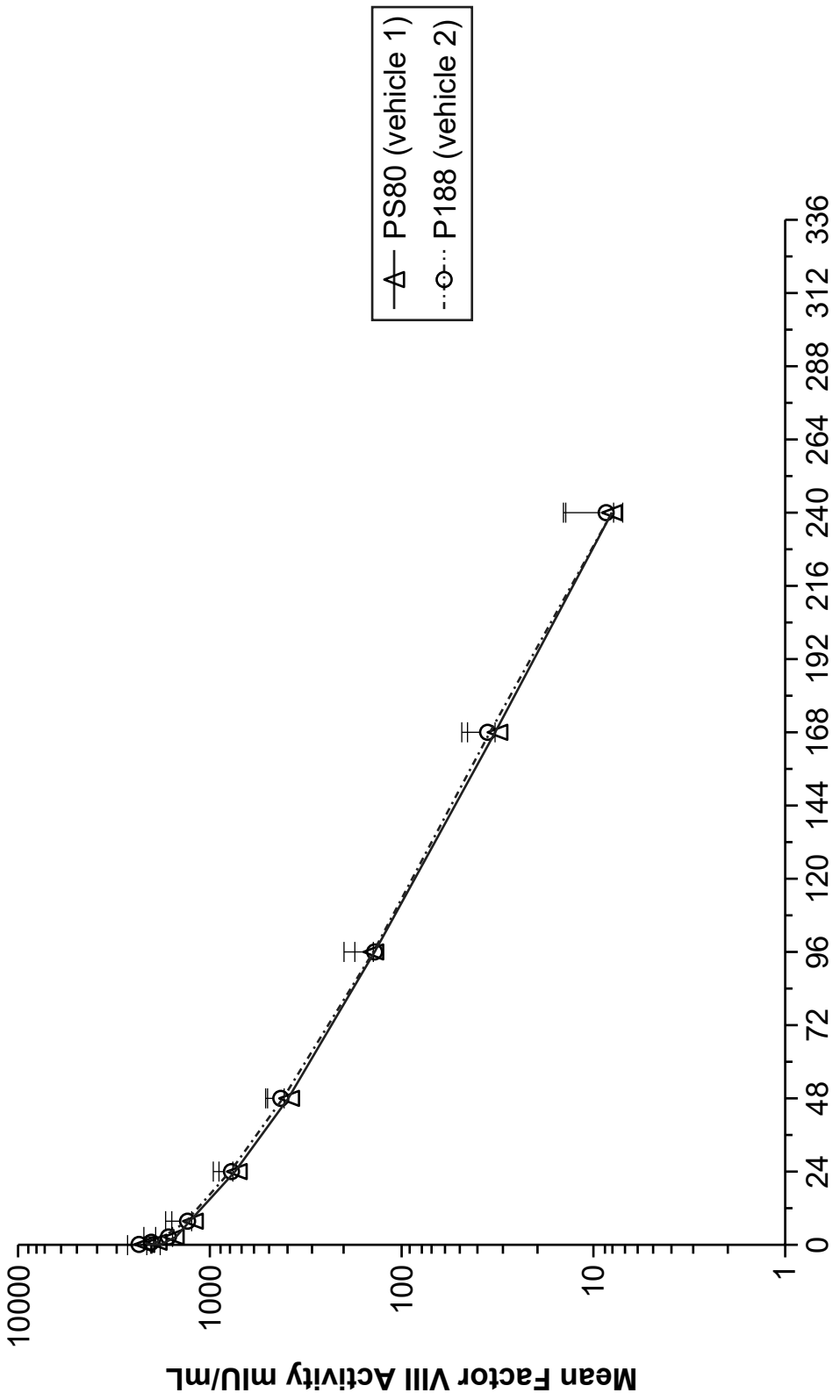

PS80 (vehicle 1)
P188 (vehicle 2)
Mean Factor VIII Activity mIU/mL
10000
1000
100
10
1
Time (hr)
0
24
48
72
96
120
144
168
192
216
240
264
288
312
336

FIG. 21