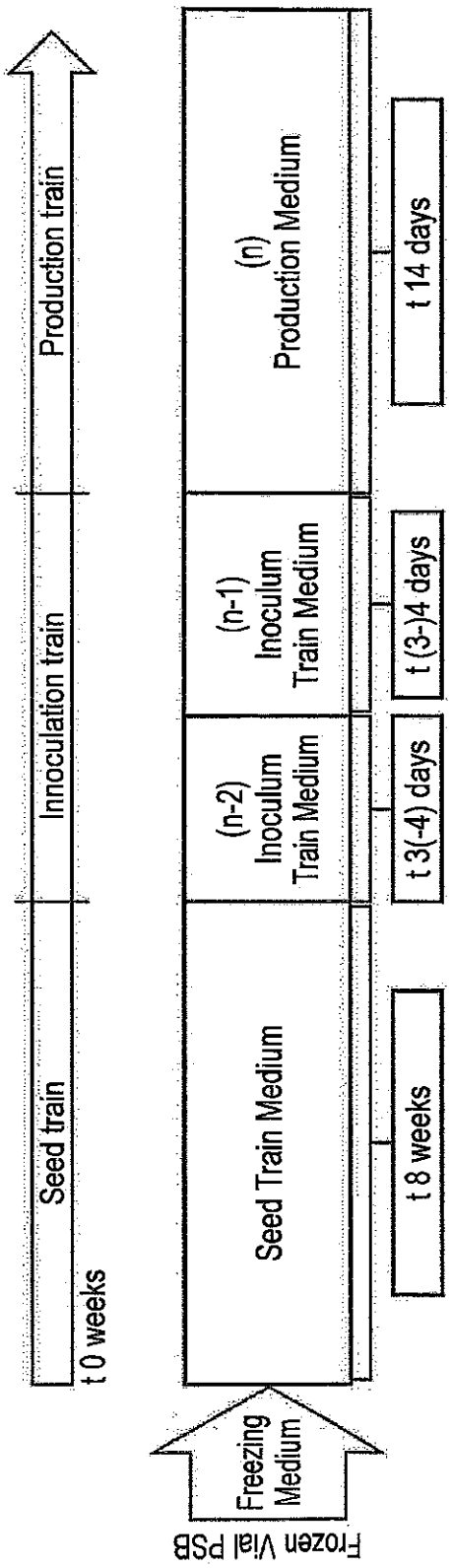

Frozen Vial PSB
Freezing Medium
t 0 weeks
Seed train
Innoculation train
Production train
Seed Train Medium
t 8 weeks
(n-2) Inoculum Train Medium
t 3(-4) days
(n-1) Inoculum Train Medium
t (3-)4 days
(n) Production Medium
t 14 days

FIG. 1

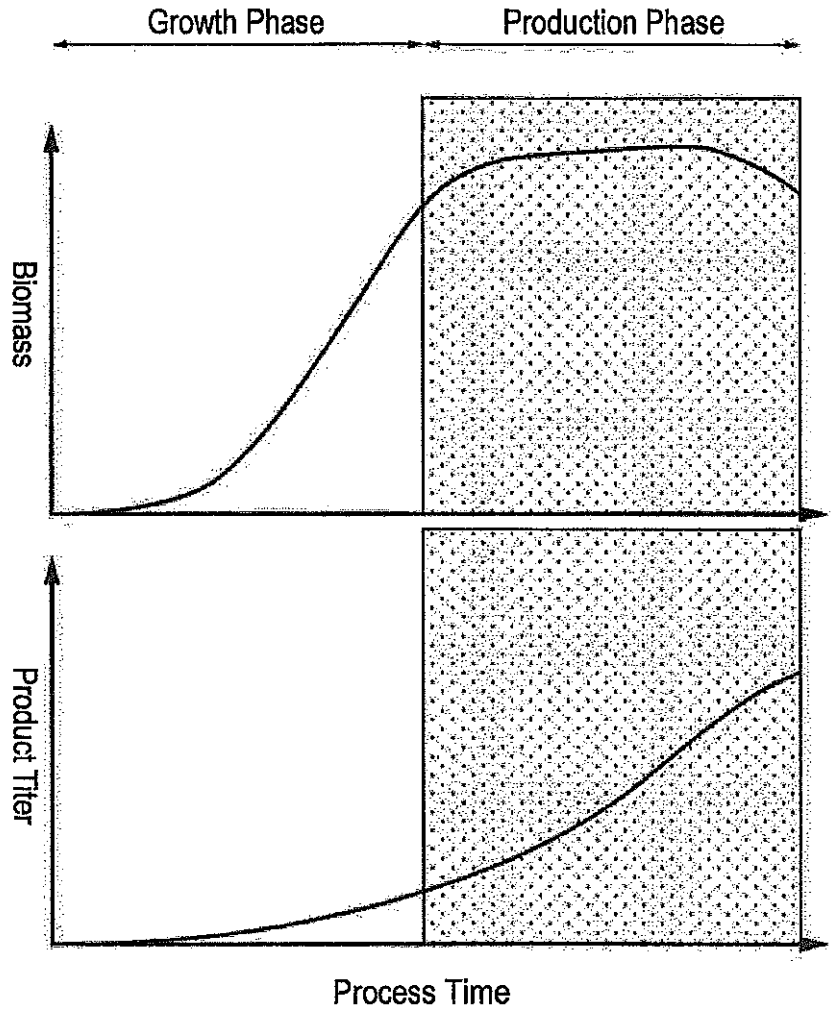

Growth Phase
Production Phase
Biomass
Product Titer
Process Time

FIG. 2

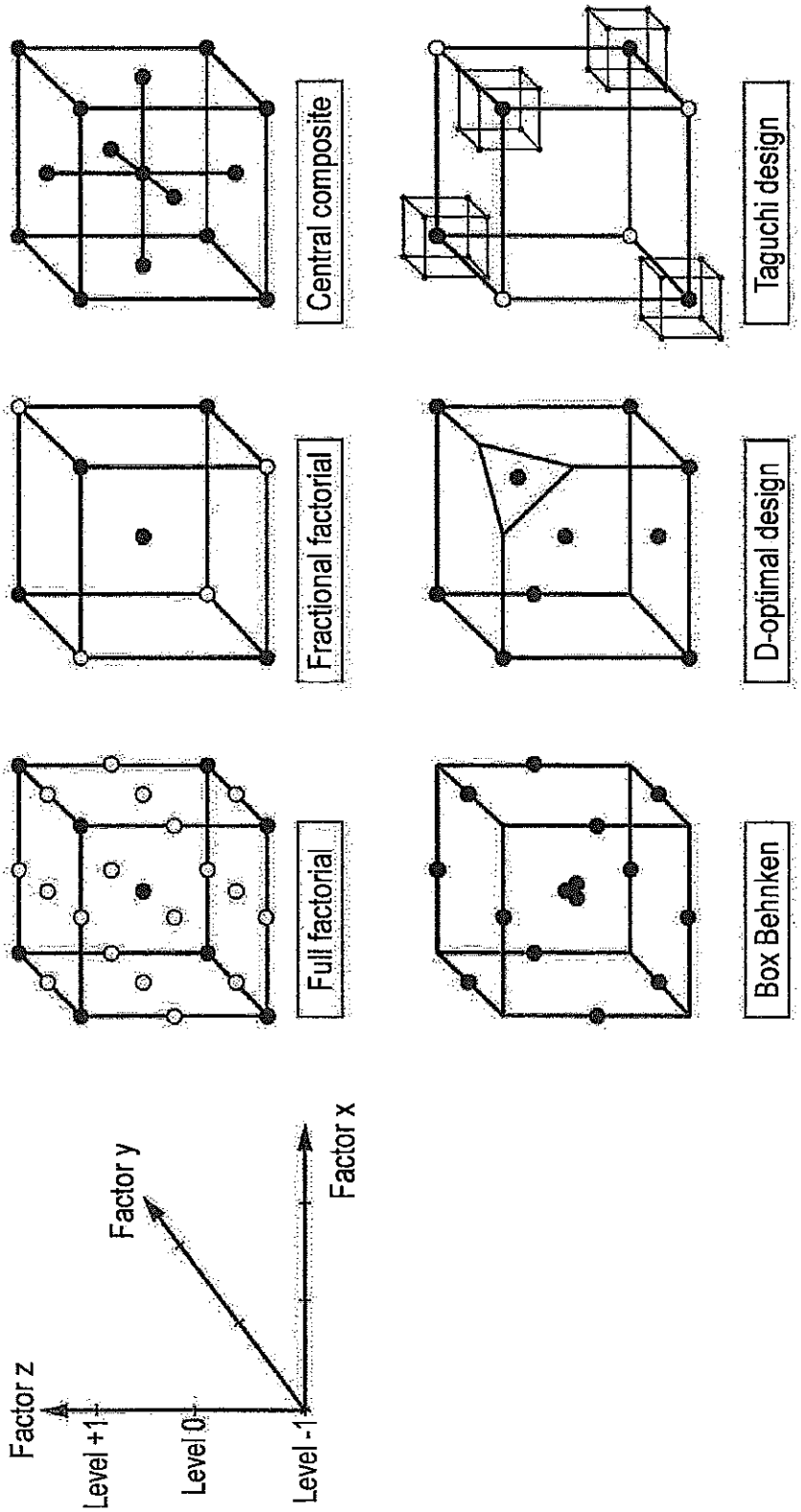

Central composite
Taguchi design
Fractional factorial
D-optimal design
Full factorial
Box Behnken
Factor z
Factor y
Factor x
Level +1
Level 0
Level -1

FIG. 3

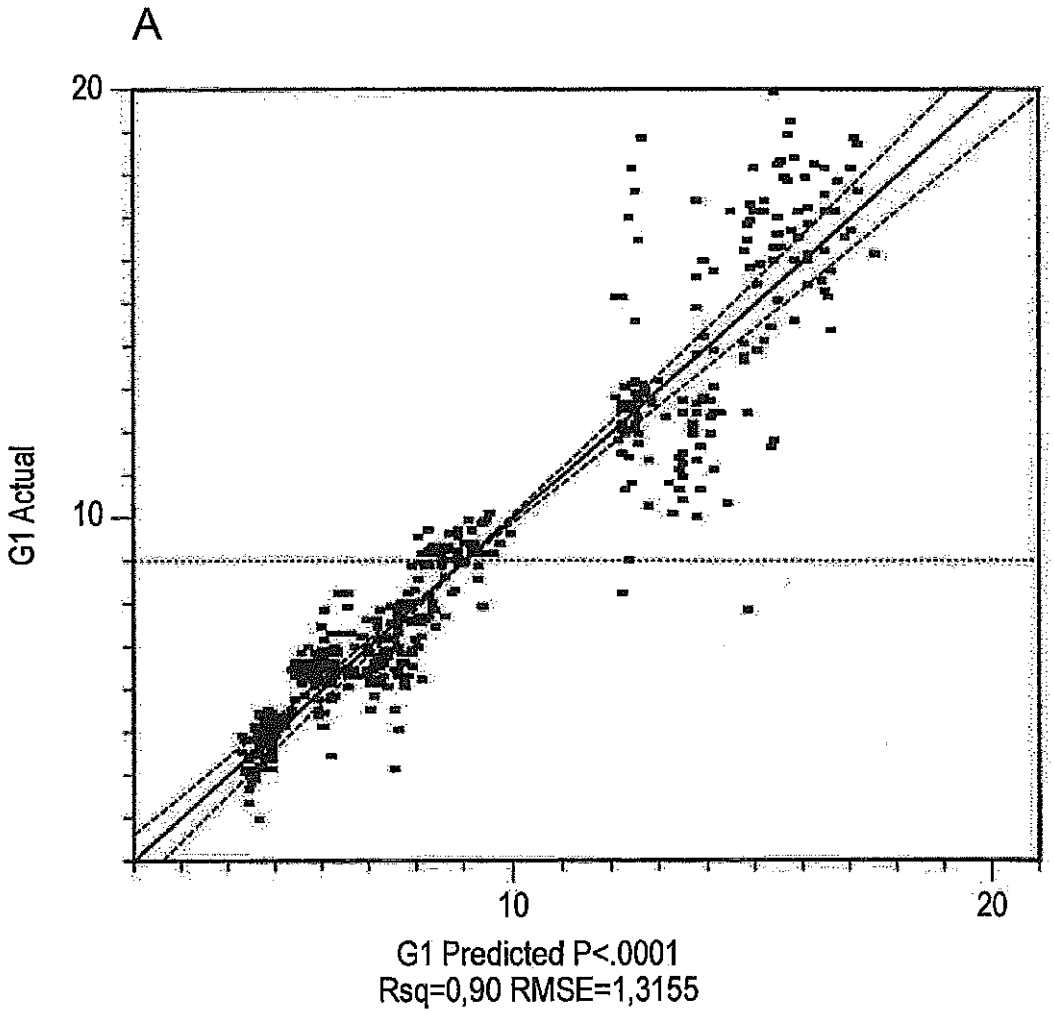


FIG. 4

B

Scaled Estimates

Nominal factors expanded to all levels
Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	7,5468579	0,192689	39,17	<,0001*
Plate[1]	-0,645483	0,144044	-4,48	<,0001*
Plate[2]	-0,594215	0,145802	-4,08	<,0001*
Plate[3]	-0,522473	0,148566	-3,52	0,0005*
Plate[4]	-0,291317	0,144044	-2,02	0,0437*
Plate[5]	1,0248815	0,145743	7,03	<,0001*
Plate[6]	0,6463246	0,146707	4,41	<,0001*
Plate[7]	0,3822824	0,150661	2,54	0,0115*
Clone[1]	0	0	0,00	1,0000
Clone[3]	0	0	0,00	1,0000
Day	-2,955056	0,159919	-18,48	<,0001*
Aluminium	-0,089947	0,067986	-1,32	0,1865
Molybdenum	0,1175024	0,067359	1,74	0,0817
Barium	0,085942	0,067347	1,28	0,2026
Chromium	0,0518704	0,067606	0,77	0,4433
Bromine	-0,013796	0,66447	-0,21	0,8356
Iodine	0,1141258	0,06765	1,69	0,0923
Copper	-0,187321	0,067638	-2,77	0,0058*
Manganese	1,3656831	0,067168	20,33	<,0001*
Rubidium	0,1045994	0,068002	1,54	0,1247
Silver	-0,061647	0,066229	-0,93	0,3524
Zinc	0,0951202	0,066402	1,43	0,1527
Tin	0,0541508	0,067361	0,80	0,4219
Zirconium	-0,060032	0,067283	-0,89	0,3727
(Day-10,7361)*(Day-10,7361)	1,9508852	0,242891	8,03	<,0001*

FIG. 4 (continued)

C

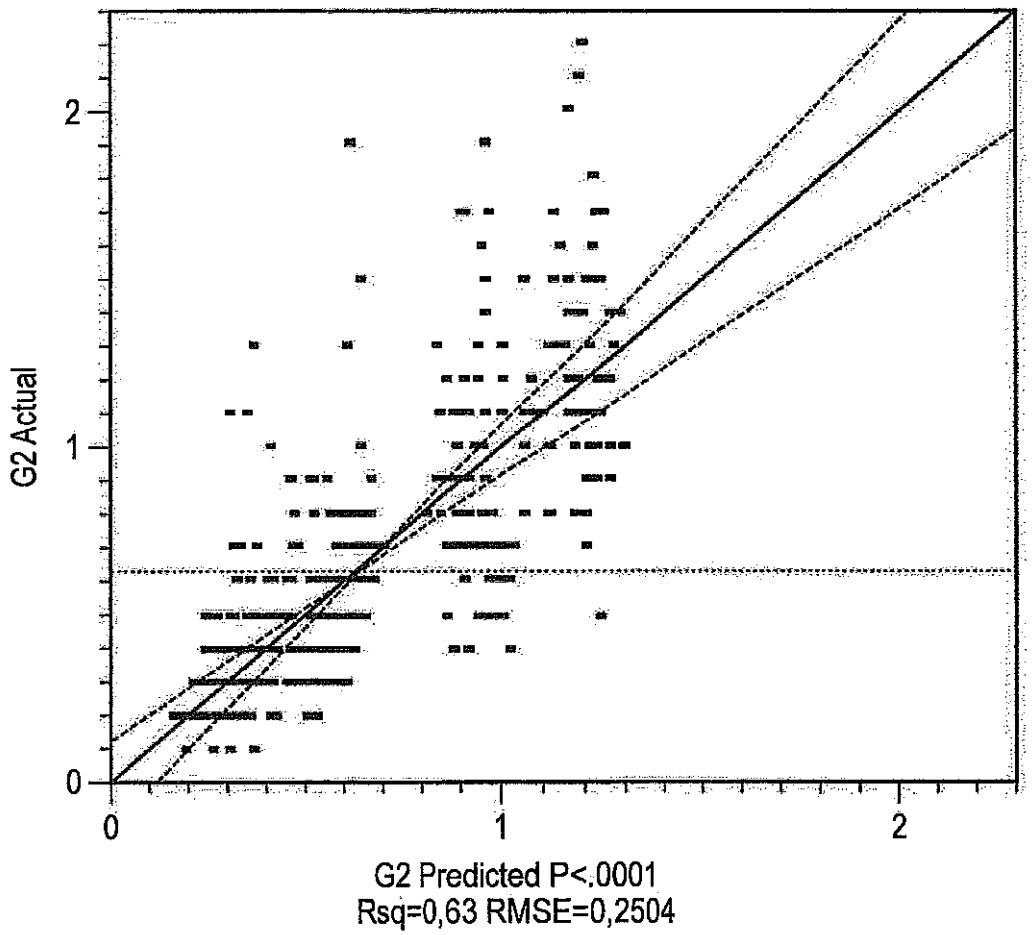


FIG. 4 (continued)

D

Scaled Estimates

Nominal factors expanded to all levels
Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0,4188584	0,036673	11,42	<,0001*
Plate[1]	-0,005623	0,027415	-0,21	0,8376
Plate[2]	-0,052605	0,02775	-1,90	0,0586
Plate[3]	-0,058561	0,028276	-2,07	0,0389*
Plate[4]	0,0096544	0,027415	0,35	0,7249
Plate[5]	0,0565108	0,027738	2,04	0,0422*
Plate[6]	0,0533356	0,027922	1,91	0,0567
Plate[7]	-0,002712	0,028674	-0,09	0,9247
Clone[1]	0	0	0,00	1,0000
Clone[3]	0	0	0,00	1,0000
Day	-0,157669	0,030436	-5,18	<,0001*
Aluminium	-0,01299	0,012939	-1,00	0,3159
Molybdenum	0,0007346	0,01282	0,06	0,9543
Barium	0,0071756	0,012818	0,56	0,5759
Chromium	0,0014852	0,012867	0,12	0,9082
Bromine	0,0036169	0,012646	0,29	0,7750
Iodine	0,0087184	0,012875	0,68	0,4987
Copper	-0,011456	0,012873	-0,89	0,3740
Manganese	0,1315323	0,012784	10,29	<,0001*
Rubidium	0,0067806	0,012942	0,52	0,6006
Silver	-0,000594	0,012605	-0,05	0,9624
Zinc	0,0136294	0,012638	1,08	0,2814
Tin	-0,015355	0,01282	-1,20	0,2317
Zirconium	-0,016528	0,012806	-1,29	0,1975
(Day-10,7361)*(Day-10,7361)	0,2807794	0,046228	6,07	<,0001*

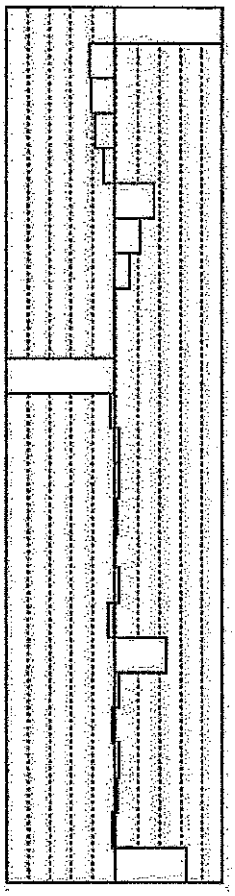


FIG. 4 (continued)

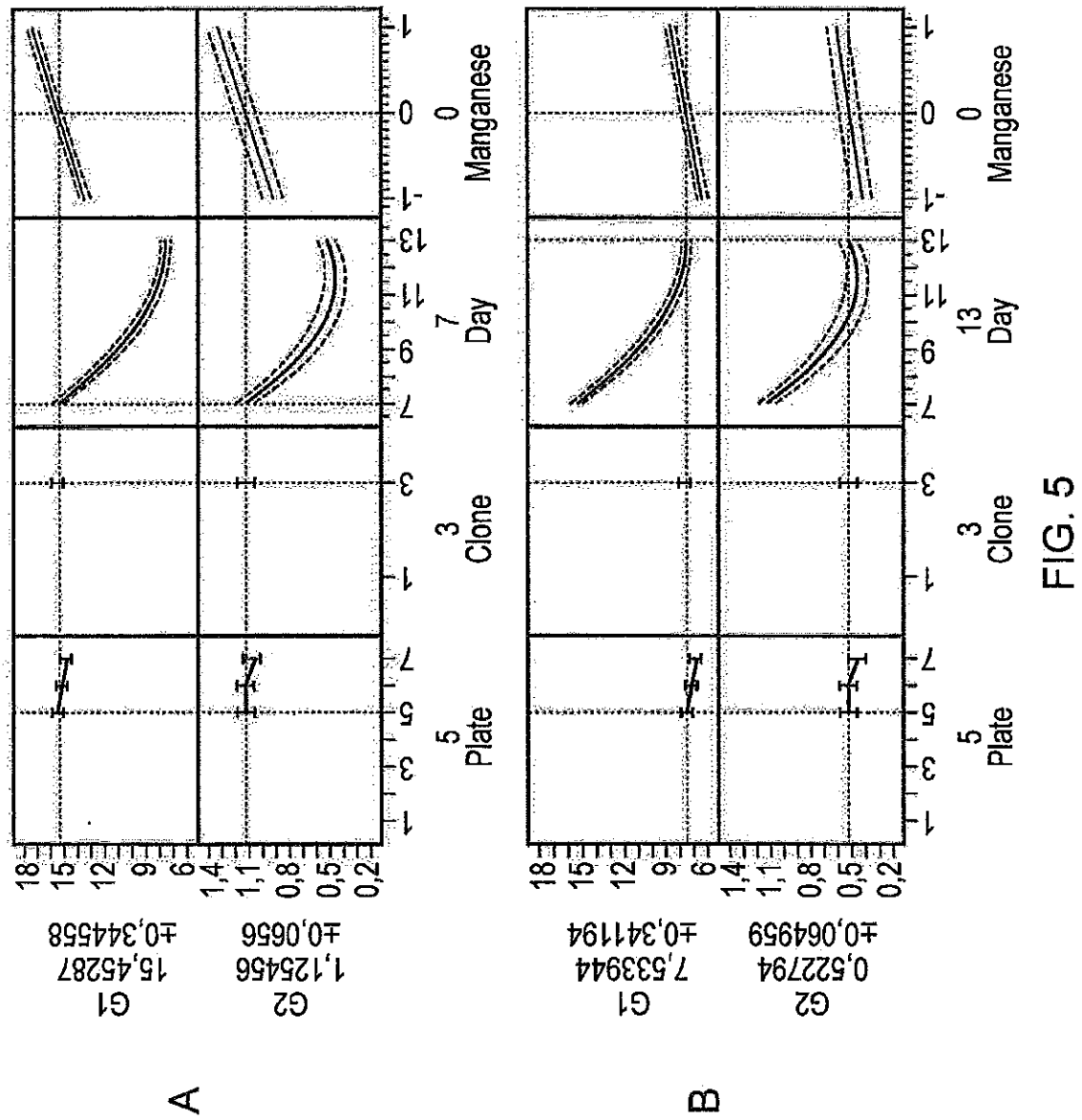

A
G1
15,45287
±0,34558
G2
1,12456
±0,0656
B
G1
7,53394
±0,34194
G2
0,52279
±0,06495
Plate
Clone
Day
Manganese

FIG. 5

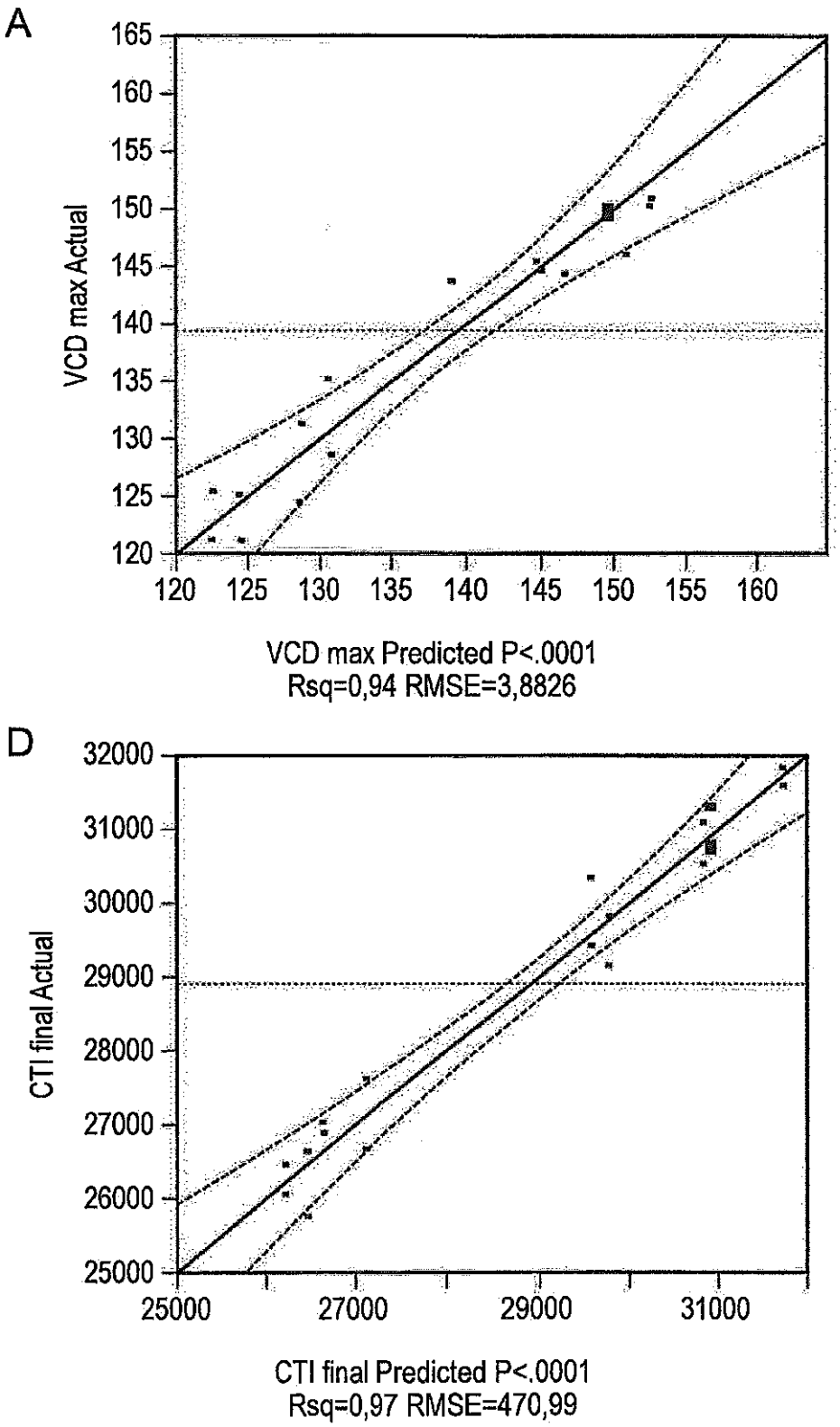
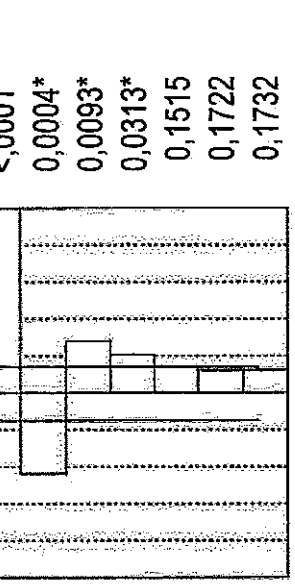

A
VCD max Actual
165
160
155
150
145
140
135
130
125
120
120 125 130 135 140 145 150 155 160
VCD max Predicted P<.0001
Rsq=0,94 RMSE=3,8826
D
CTI final Actual
32000
31000
30000
29000
28000
27000
26000
25000
25000 27000 29000 31000
CTI final Predicted P<.0001
Rsq=0,97 RMSE=470,99

FIG. 6

B

Term	Estimate	Std Error	t Ratio	Prob>|t|
Iron	11,12	0,970661	11,46	<,0001*
Iron*Iron	-12,07417	2,442777	-4,94	0,0004*
Manganese	3,05625	0,970661	3,15	0,0093*
Zinc*Iron	2,395	0,970661	2,47	0,0313*
Iron*Copper	1,49625	0,970661	1,54	0,1515
Zinc	1,4175	0,970661	1,46	0,1722
Copper	1,41375	0,970661	1,46	0,1732



C

FIG. 6 (continued)

E

Term	Estimate	Std Error	t Ratio	Prob>|t|
Iron	1928,1563	117,7482	16,38	<,0001*
Iron*Iron	-2378,123	296,3265	-8,03	<,0001*
Zinc	425,515	117,7482	3,61	0,0036*
Zinc*Iron	367,86625	117,7482	3,12	0,0088*
Manganese	271,09875	117,7482	2,30	0,0400*
Zinc*Manganese	174,75875	117,7482	1,48	0,1635

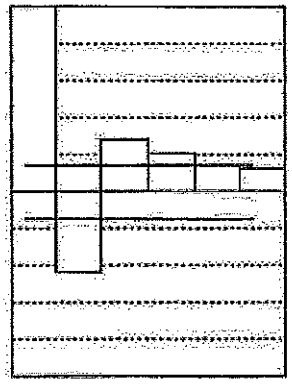
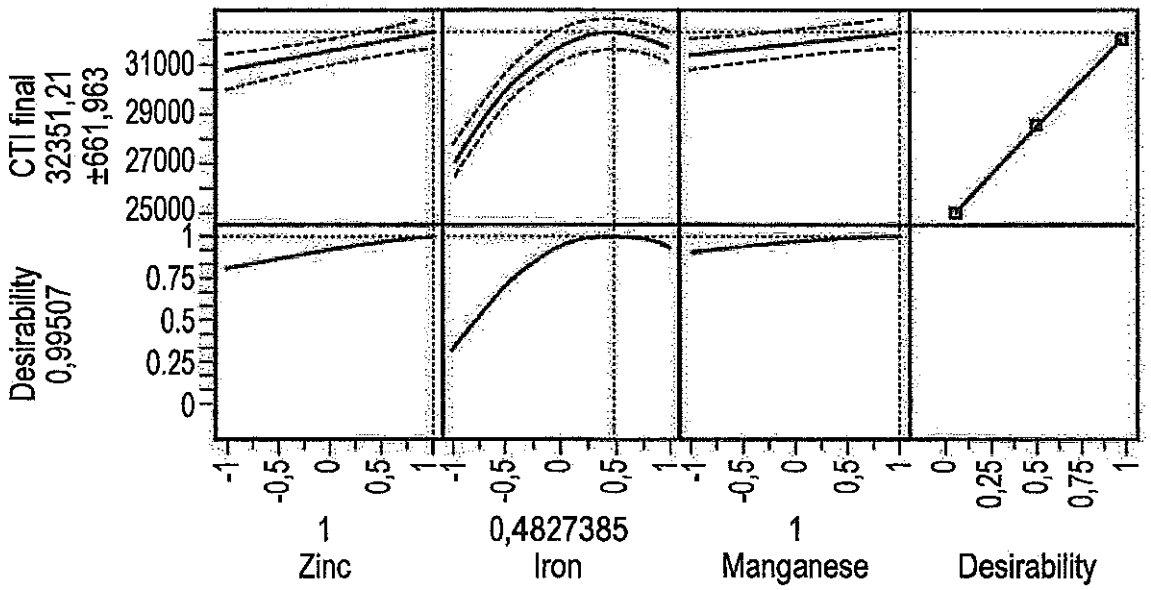


FIG. 6 (continued)

F



G

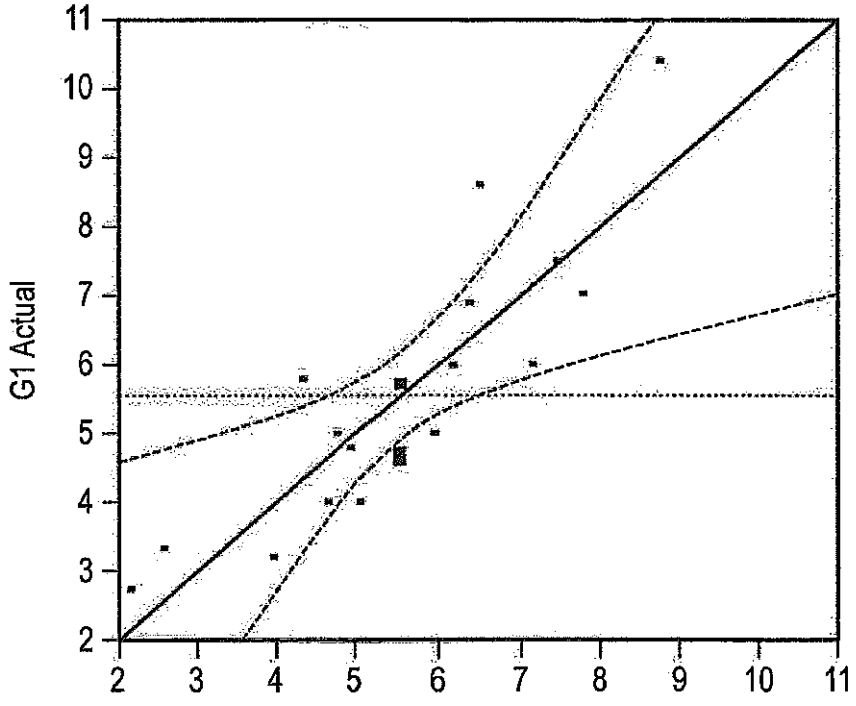


FIG. 6 (continued)

H

Term	Estimate	Std Error	t Ratio	Prob>|t|
Zinc*Copper	-1,05	0,29895	-3,51	0,0043*
Manganese	1,0375	0,29895	3,47	0,0046*
Iron	0,875	0,29895	2,93	0,0127*
Zinc	0,35	0,29895	1,17	0,2644
Iron*Manganese	-0,225	0,29895	-0,75	0,4662
Copper	-0,1375	0,29895	-0,46	0,6538

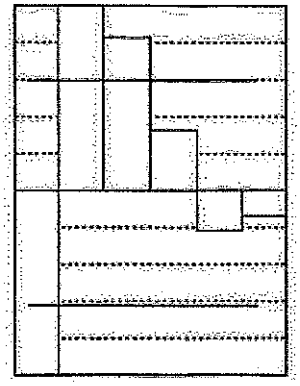
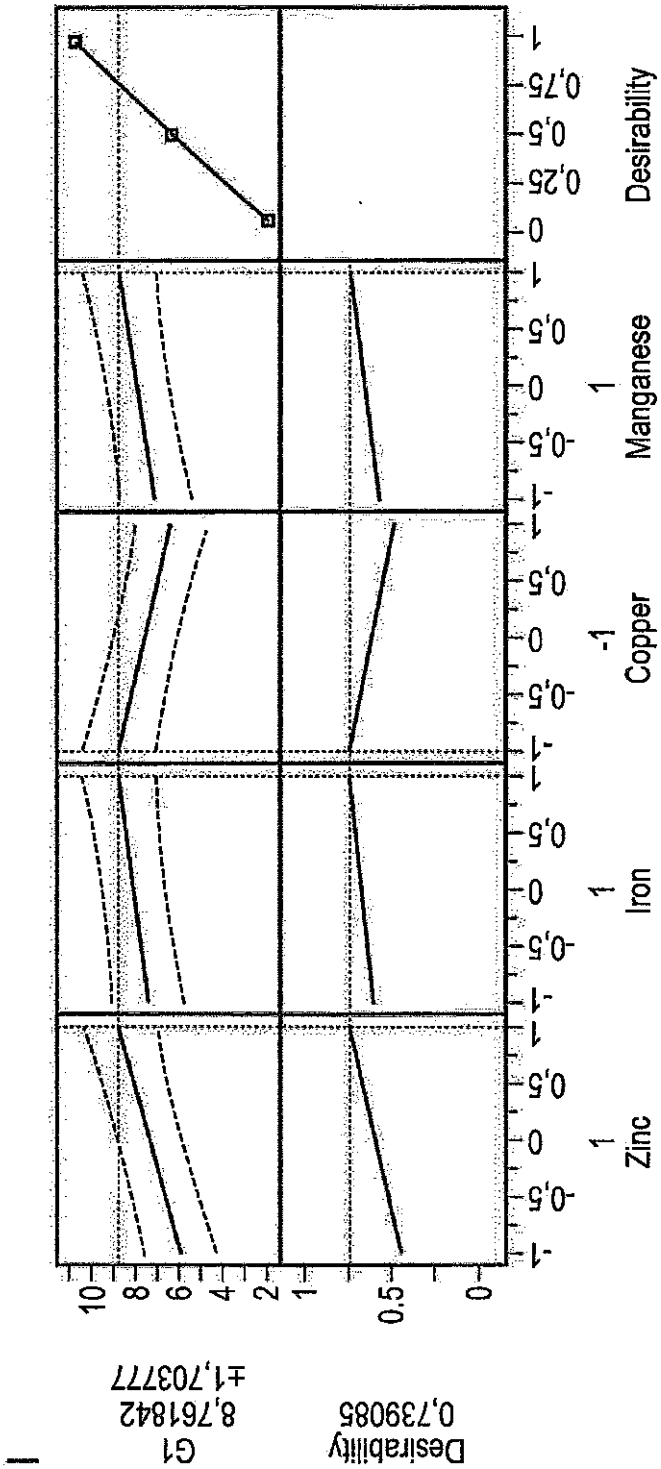


FIG. 6 (continued)



K

Term	Estimate	Std Error	t Ratio	Prob>|t|
Manganese	-1,80625	0,486821	-3,71	0,0023*
Zinc*Copper	1,59375	0,486821	3,27	0,0055*
Zinc	-0,95625	0,486821	-1,96	0,0697
Copper	0,31875	0,486821	0,65	0,5232

FIG. 6 (continued)

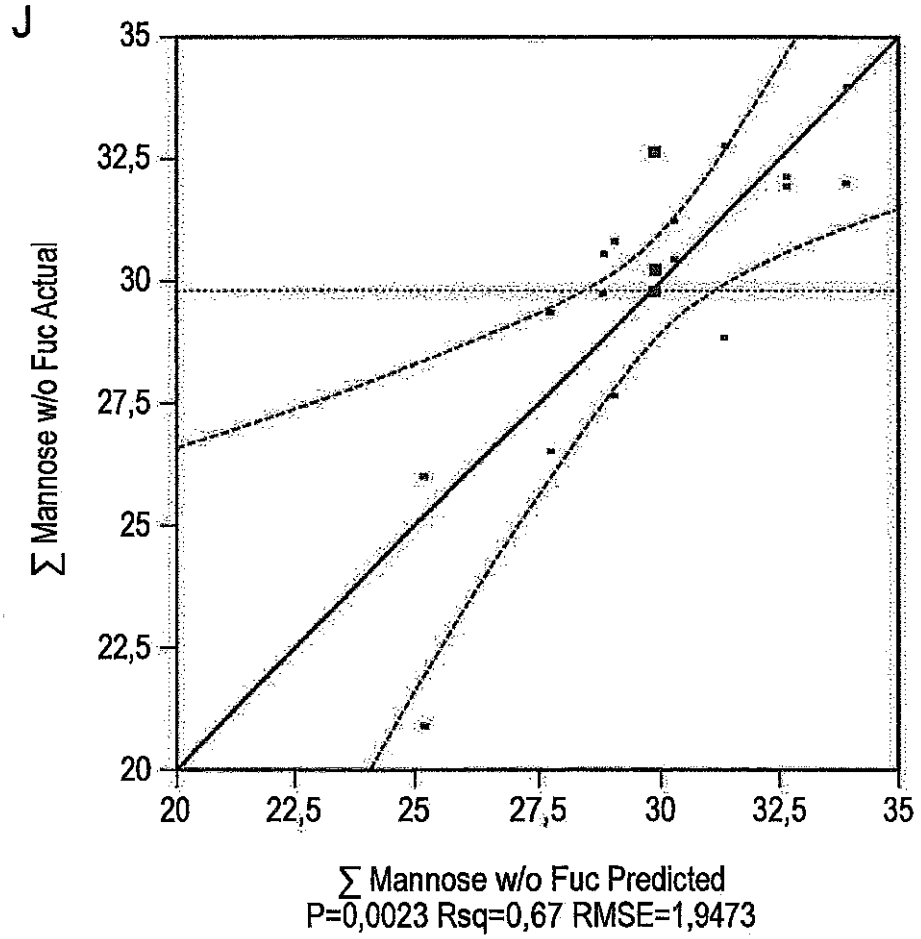


FIG. 6 (continued)

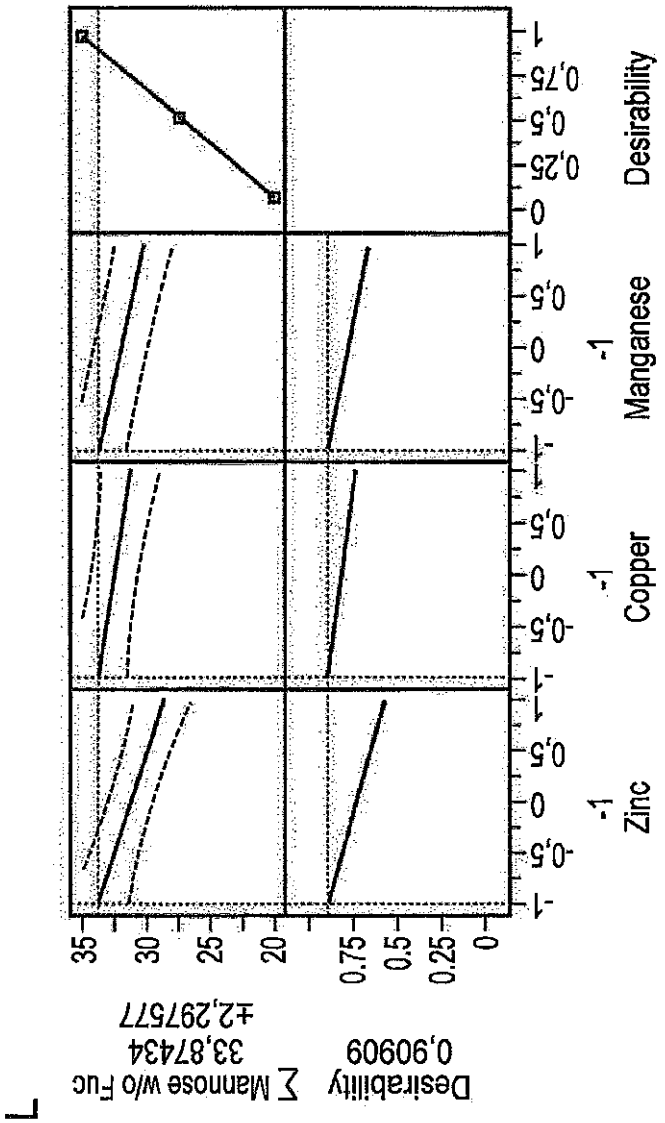


FIG. 6 (continued)

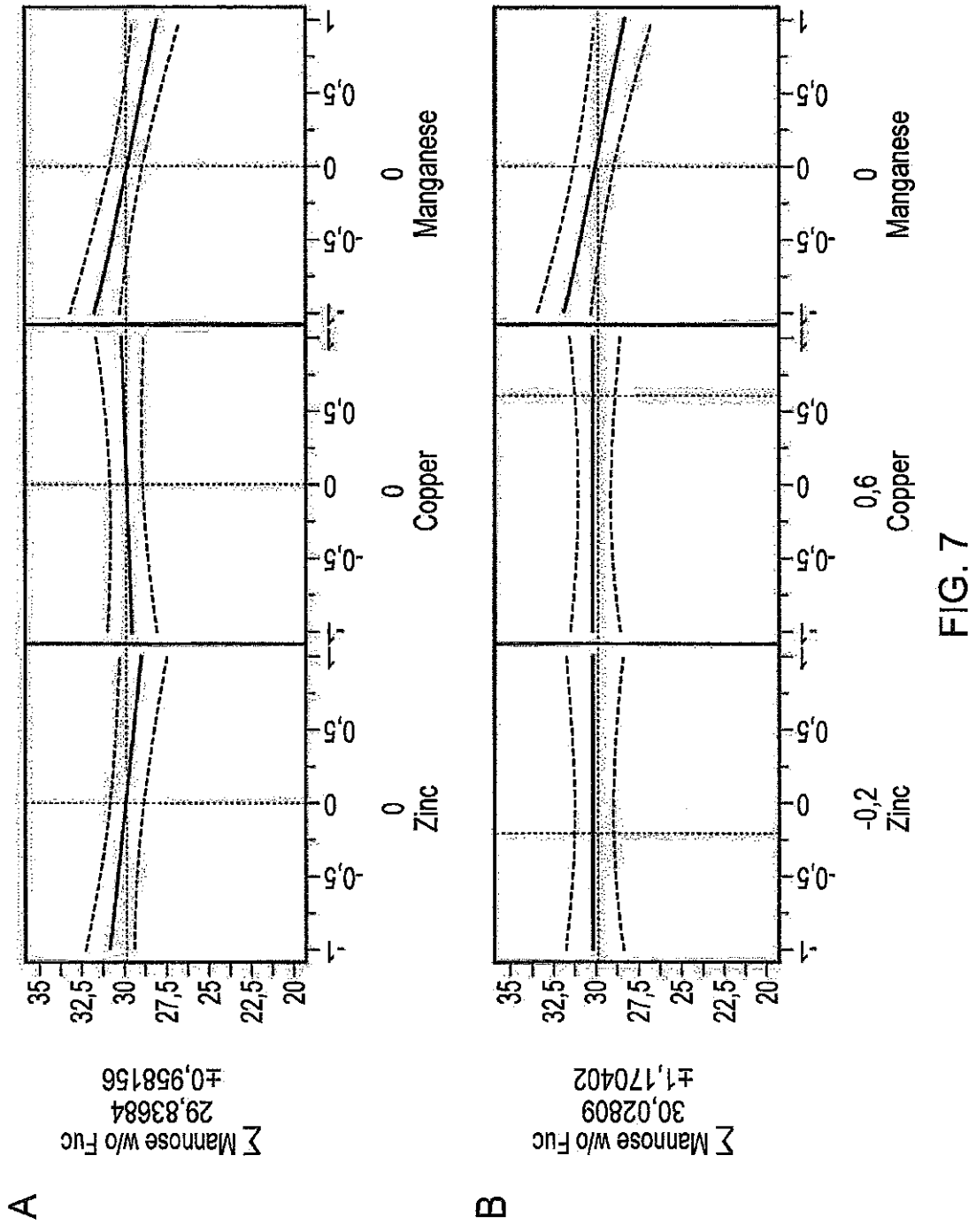

A
Σ Mannose w/o Fuc
29,83684
±0,958156
35
32,5
30
27,5
25
22,5
20
-1
-0,5
0
0,5
1
Zinc
0
Copper
0
Manganese
0
B
Σ Mannose w/o Fuc
30,02809
±1,170402
-0,2
Zinc
0,6
Copper
0
Manganese

FIG. 7

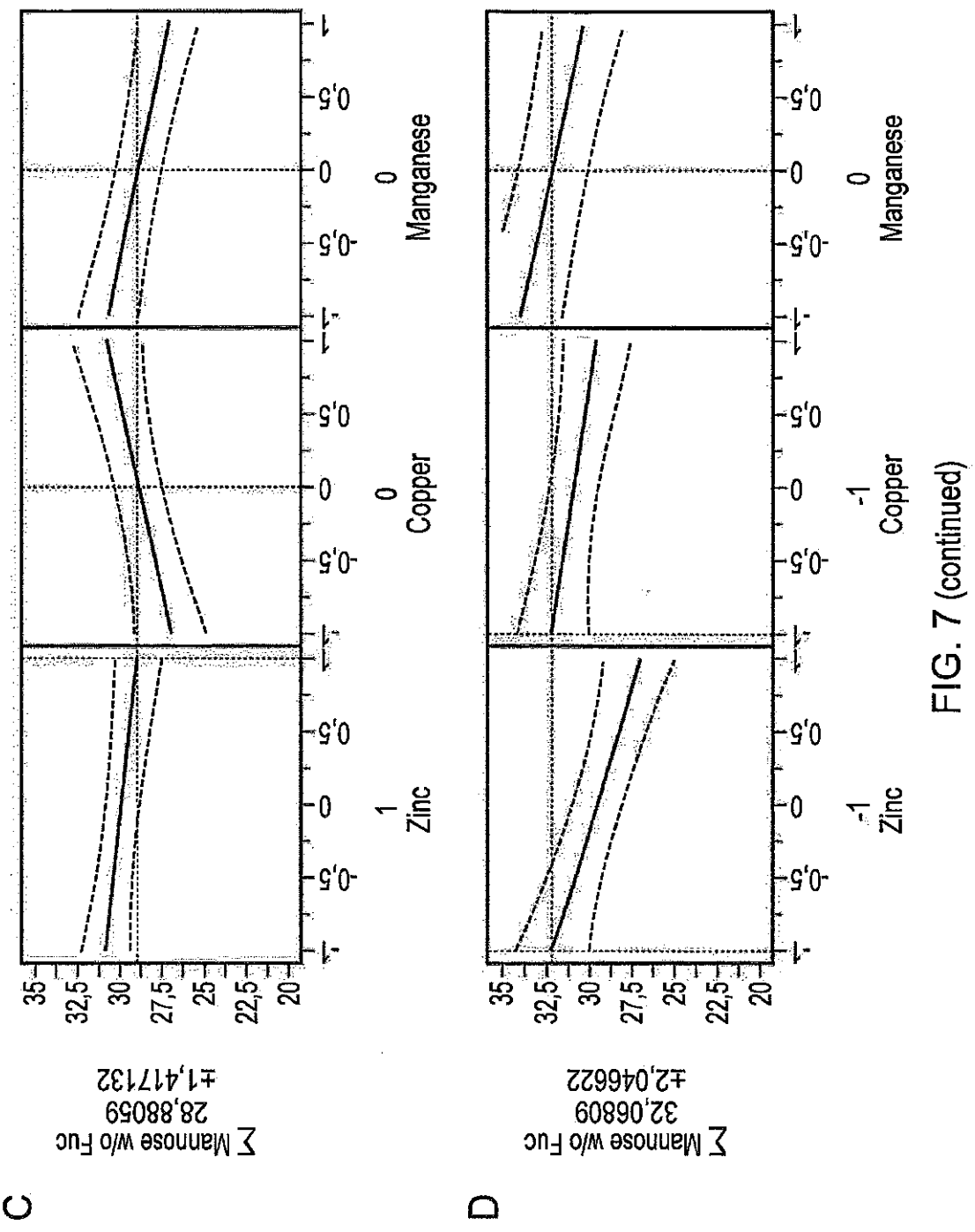


FIG. 7 (continued)

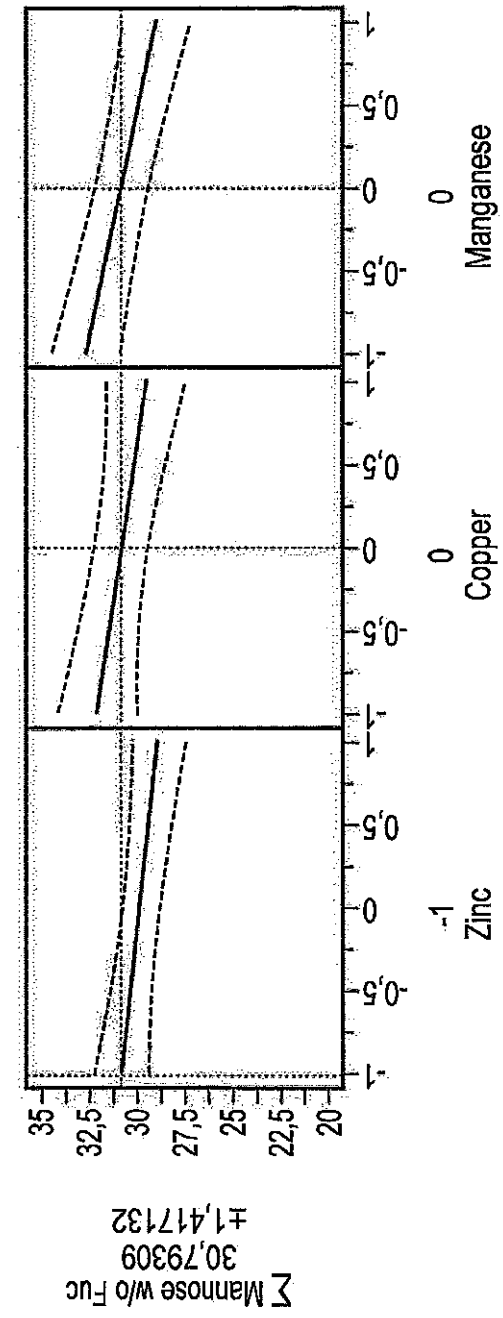
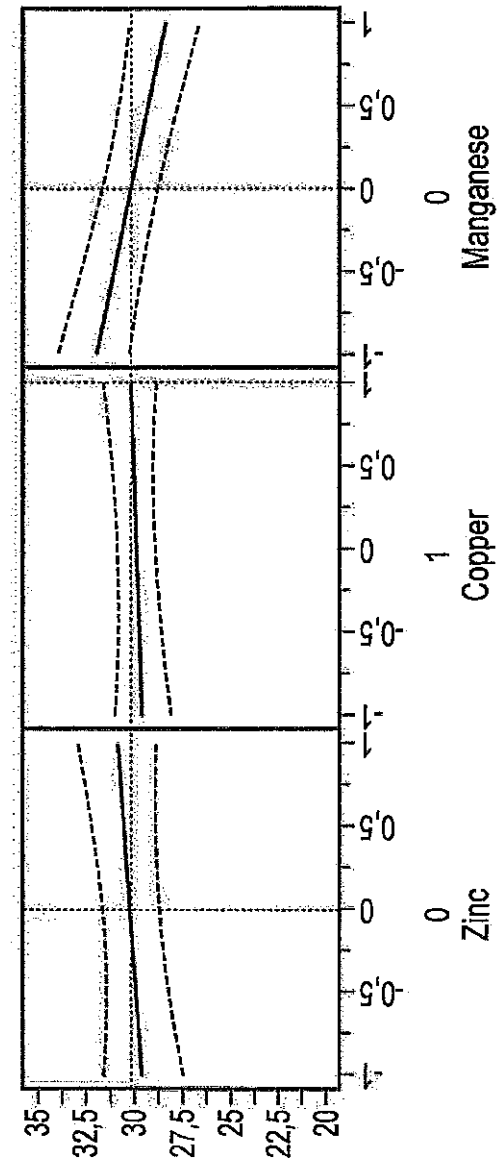


FIG. 7 (continued)



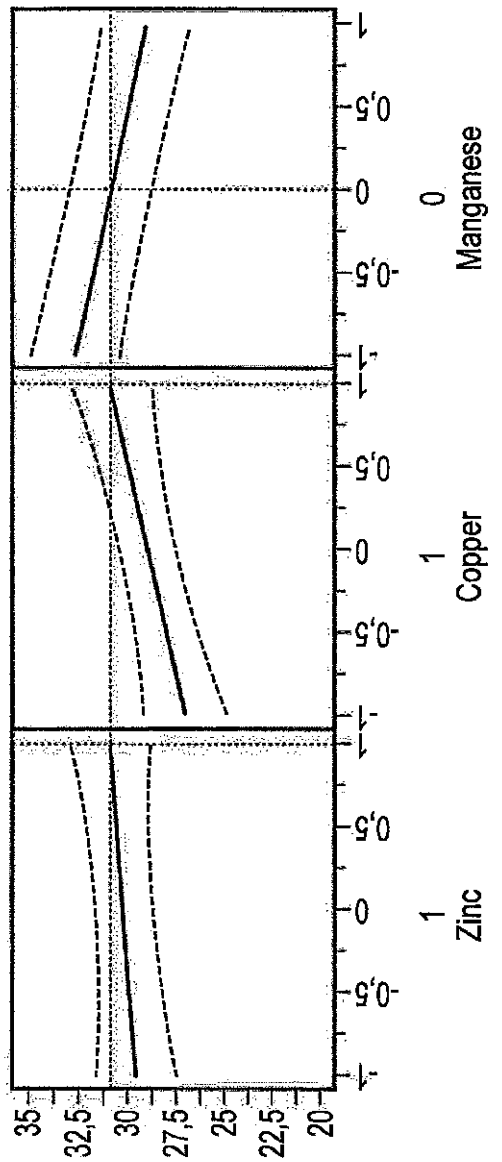


FIG. 7 (continued)

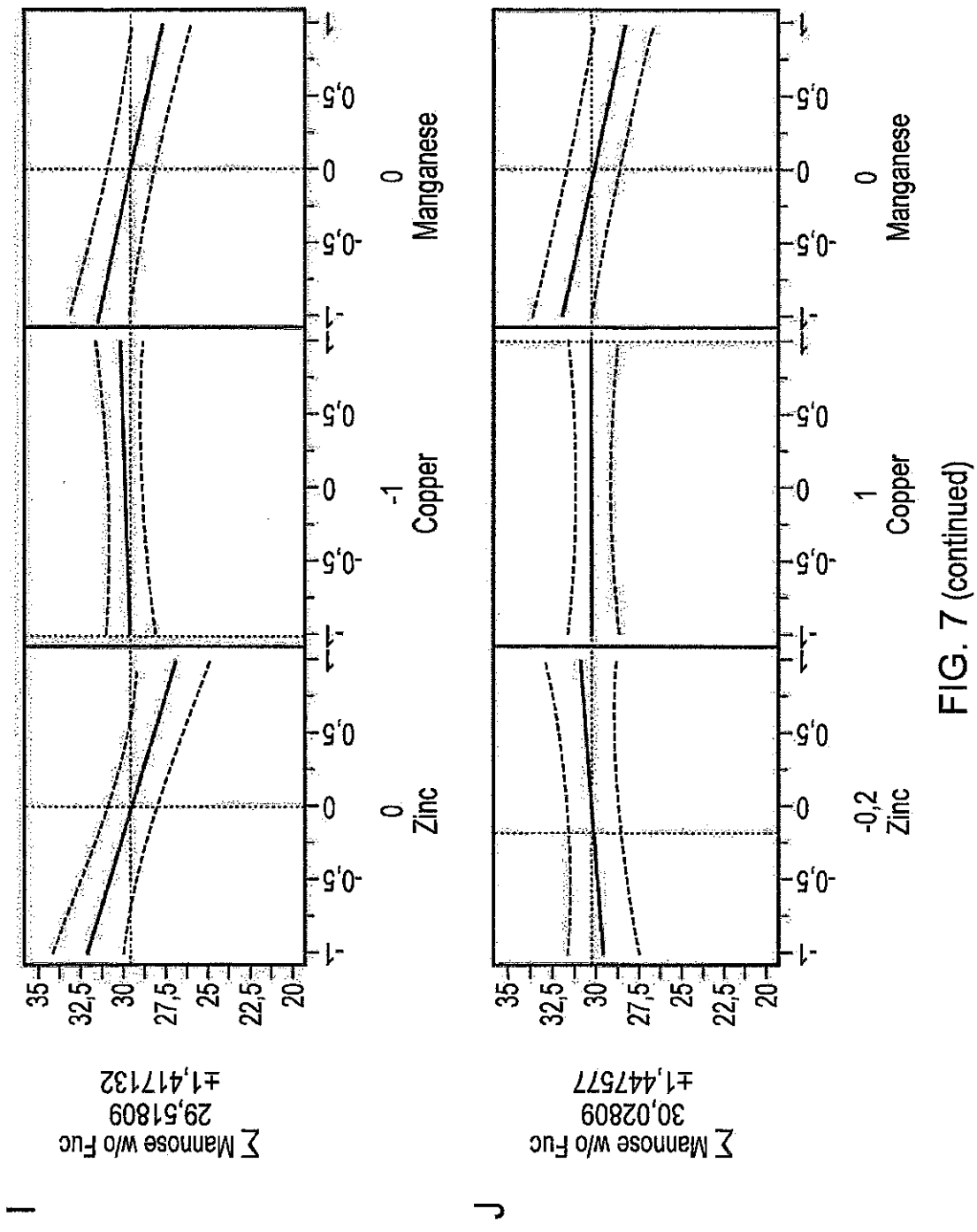


FIG. 7 (continued)

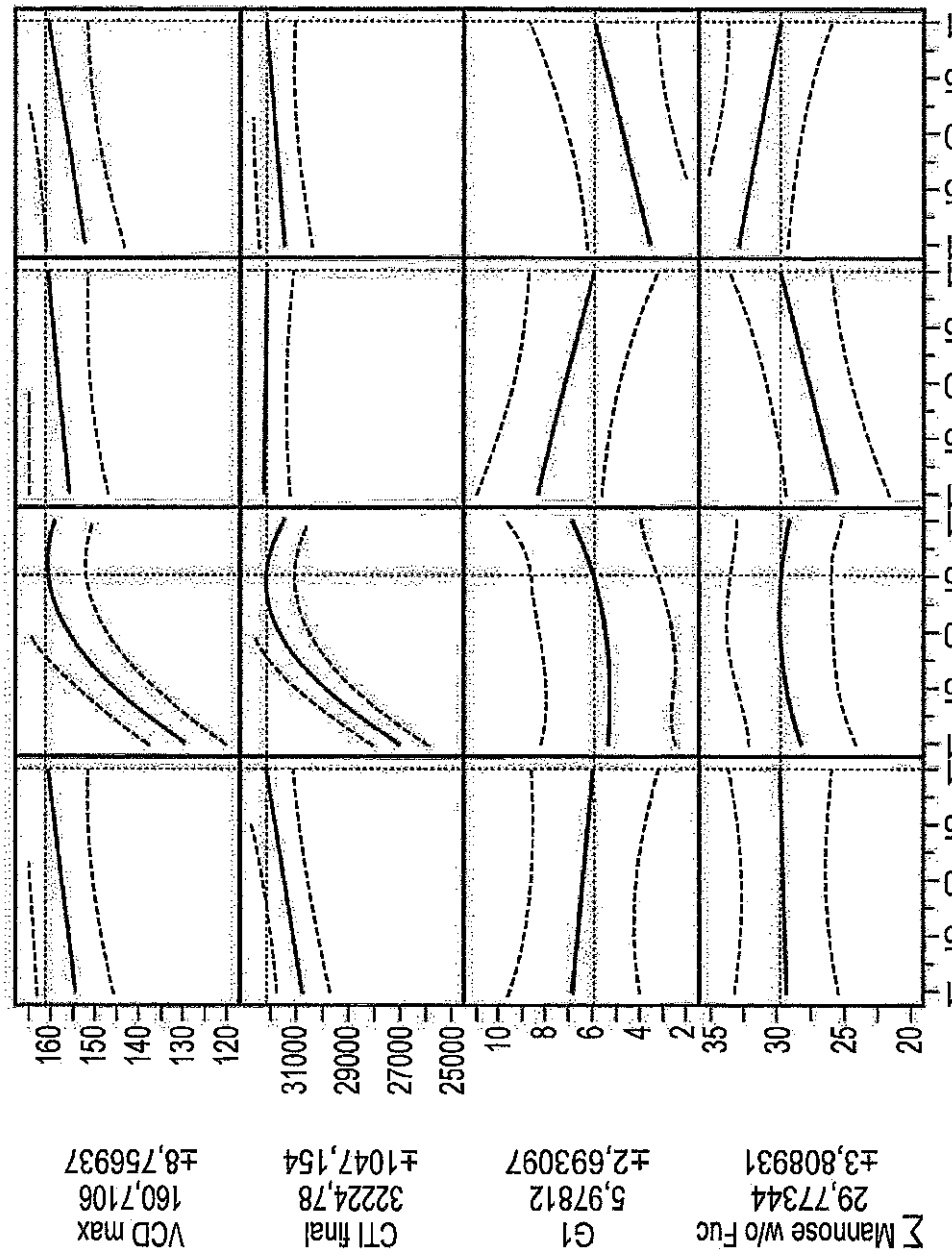

A
Σ Mannose w/o Fuc
29,77344
±3,808931
G1
5,97812
±2,693097
CTI final
3224,78
±1047,154
VCD max
160,7106
±8,756937
Zinc
Iron
Copper
Manganese

FIG. 8

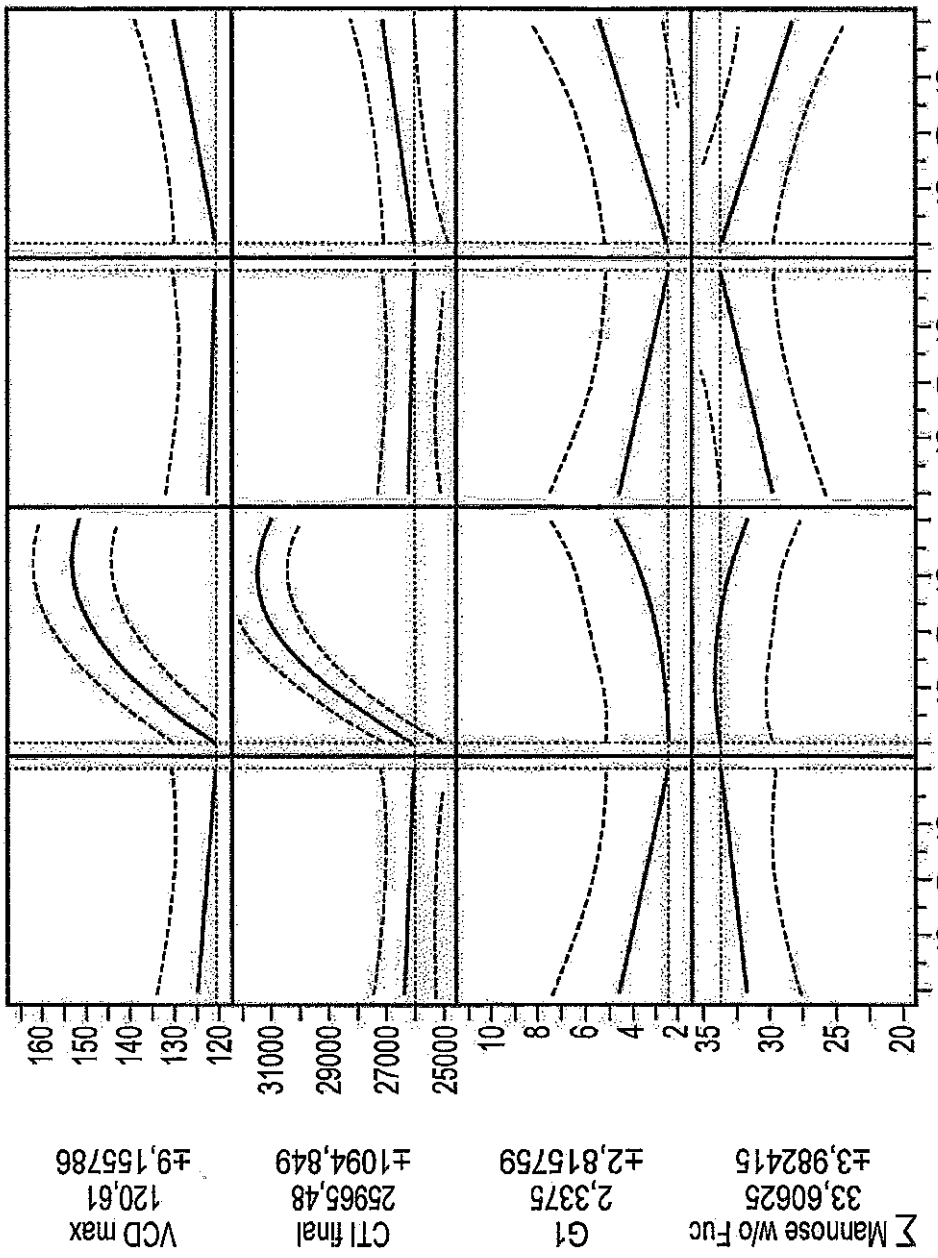


FIG. 8 (continued)

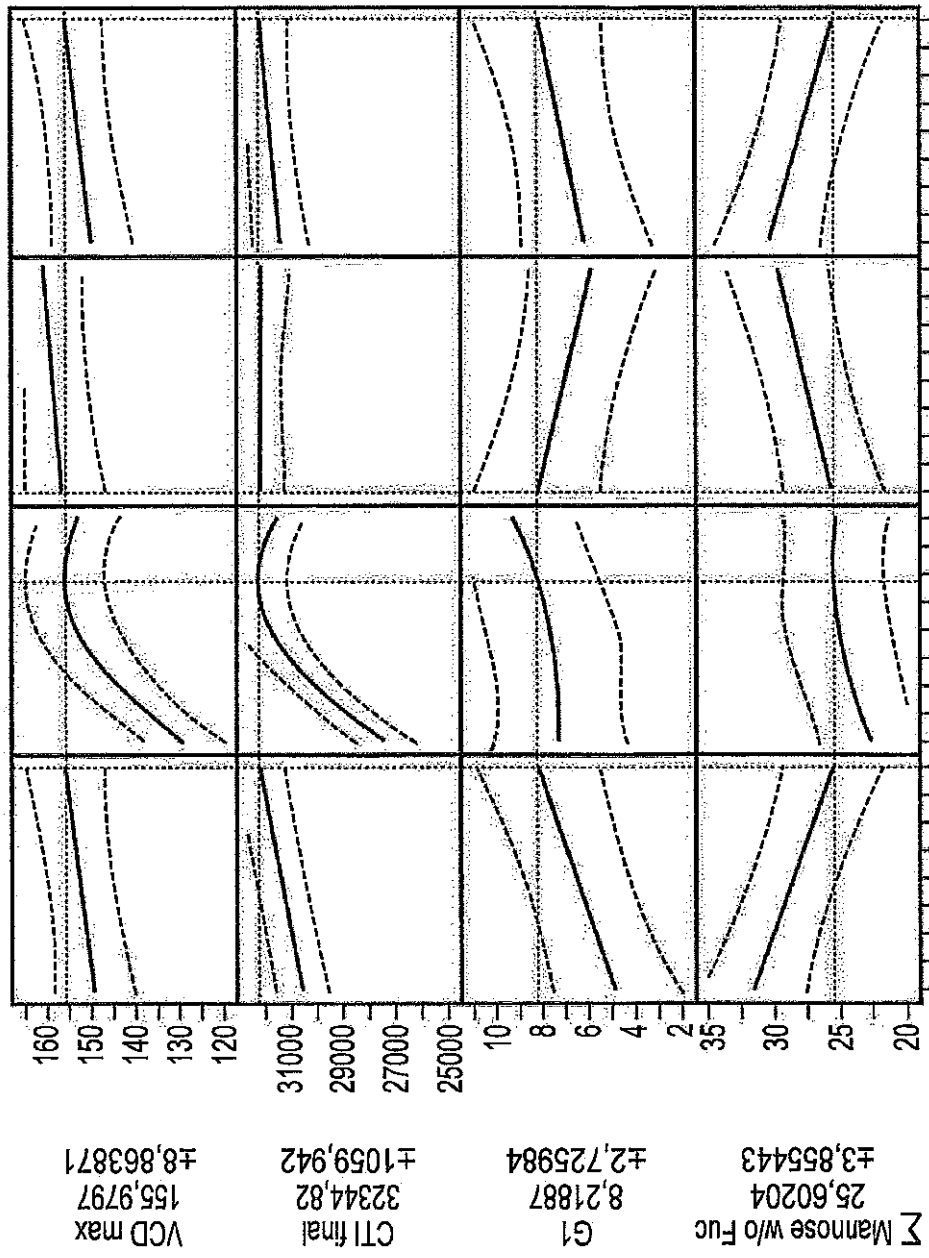


FIG. 8 (continued)

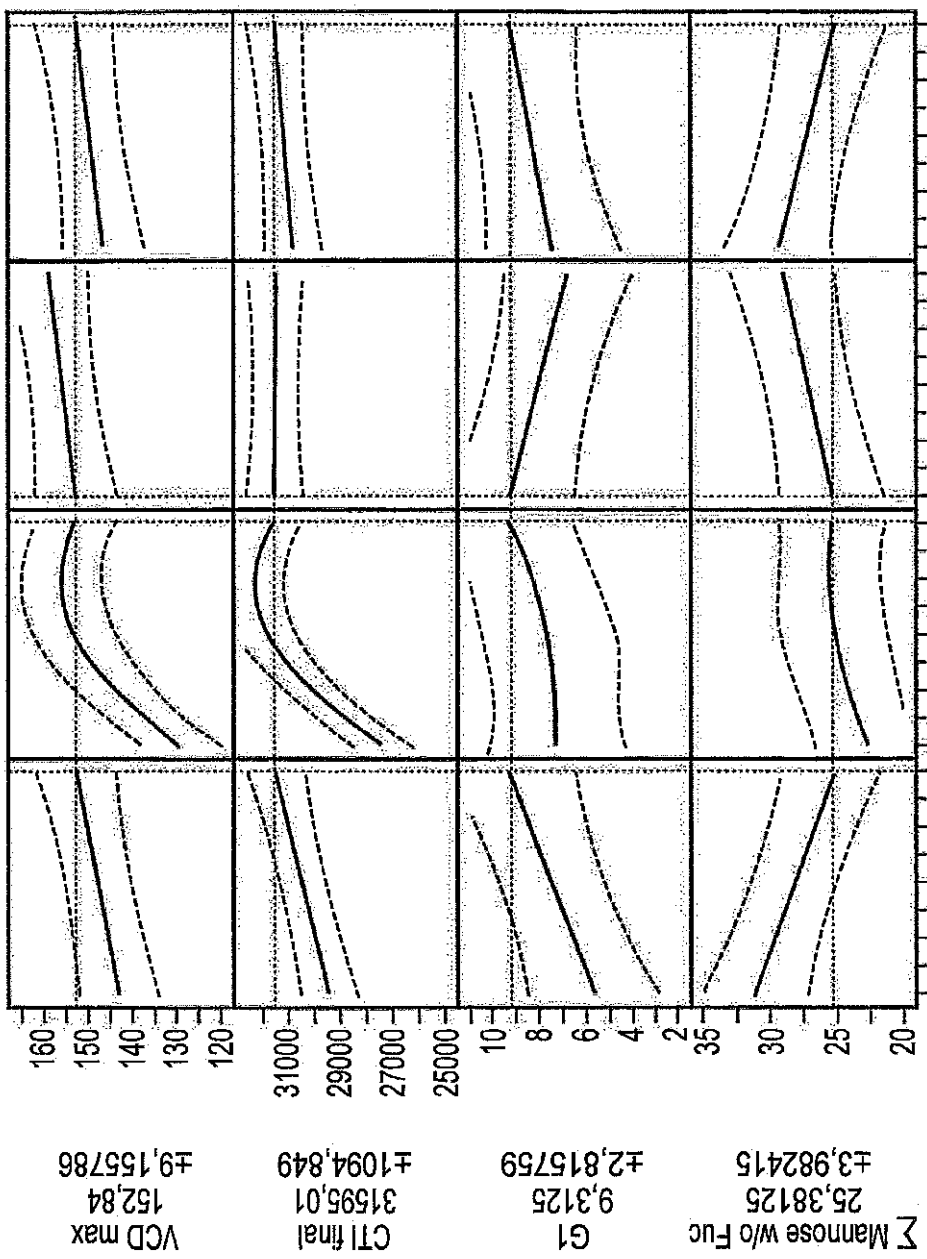


FIG. 8 (continued)

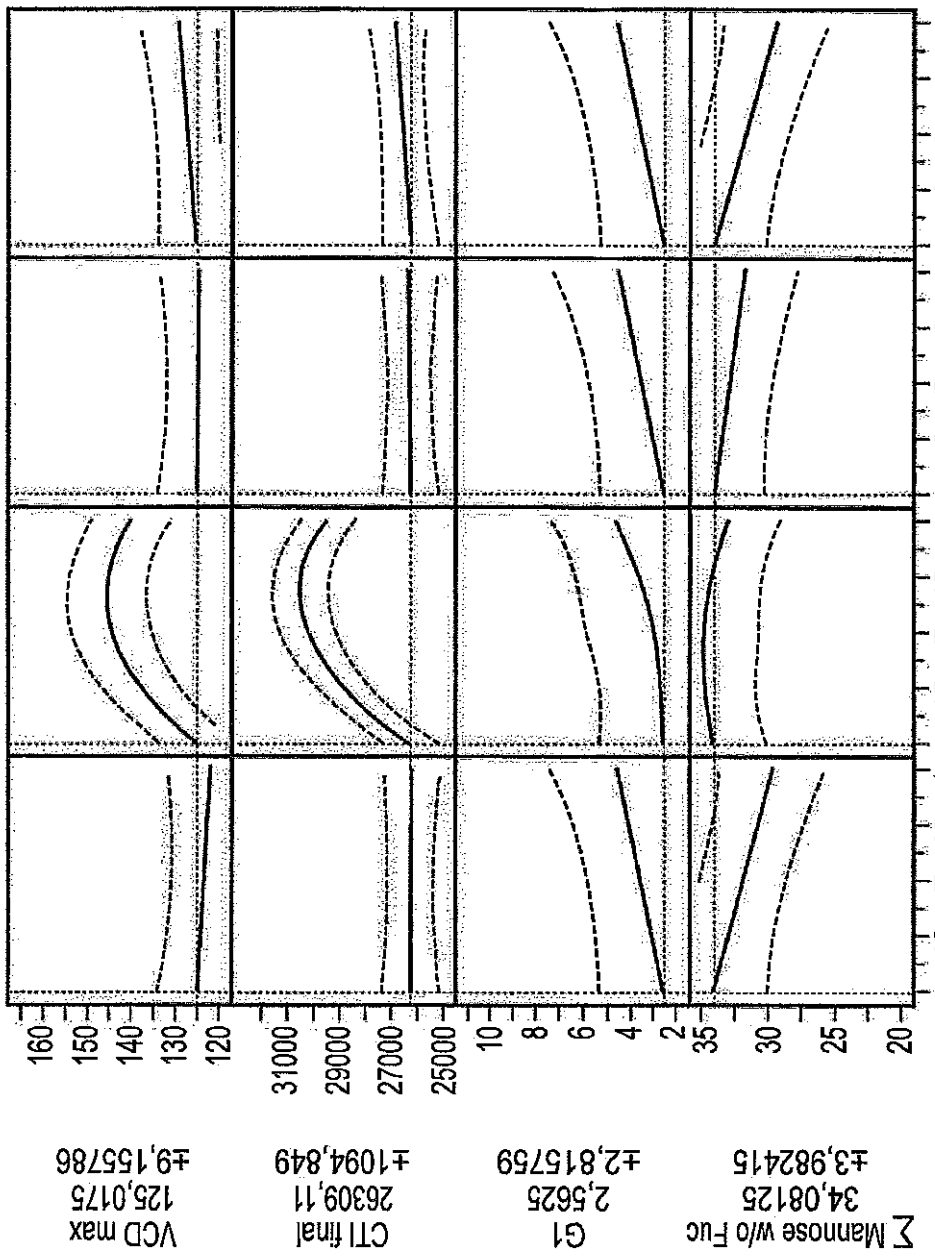


FIG. 8 (continued)

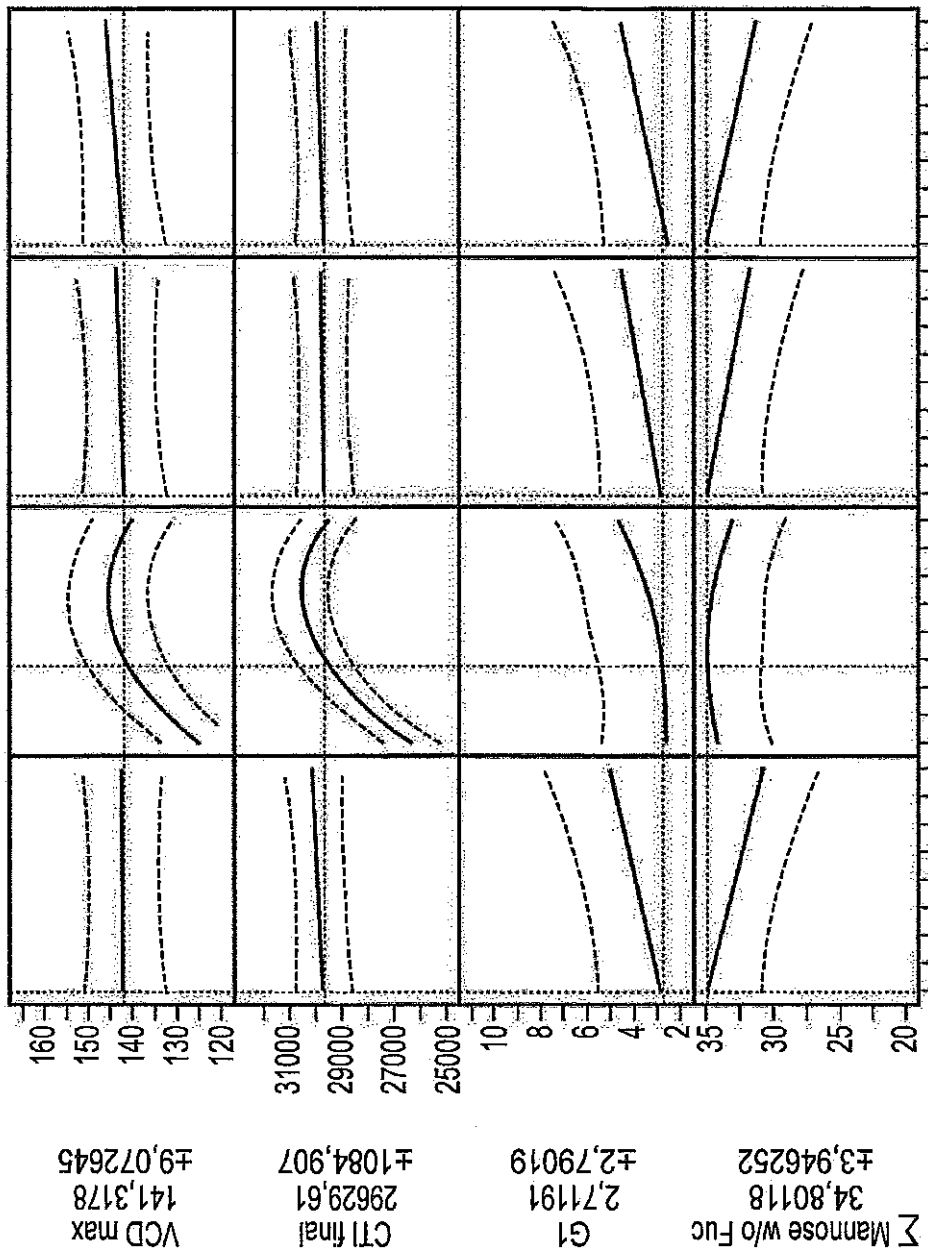


FIG. 8 (continued)

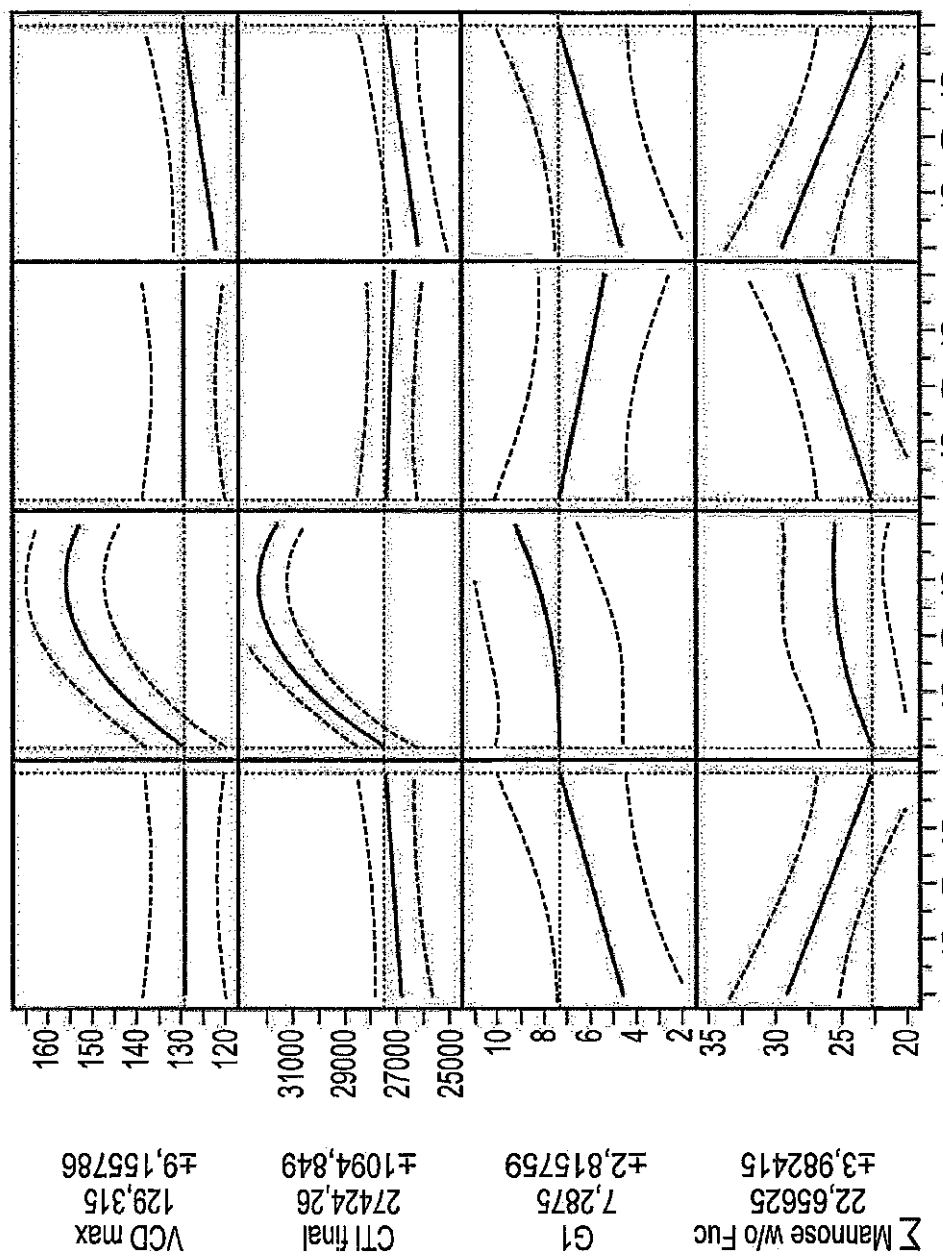


FIG. 8 (continued)

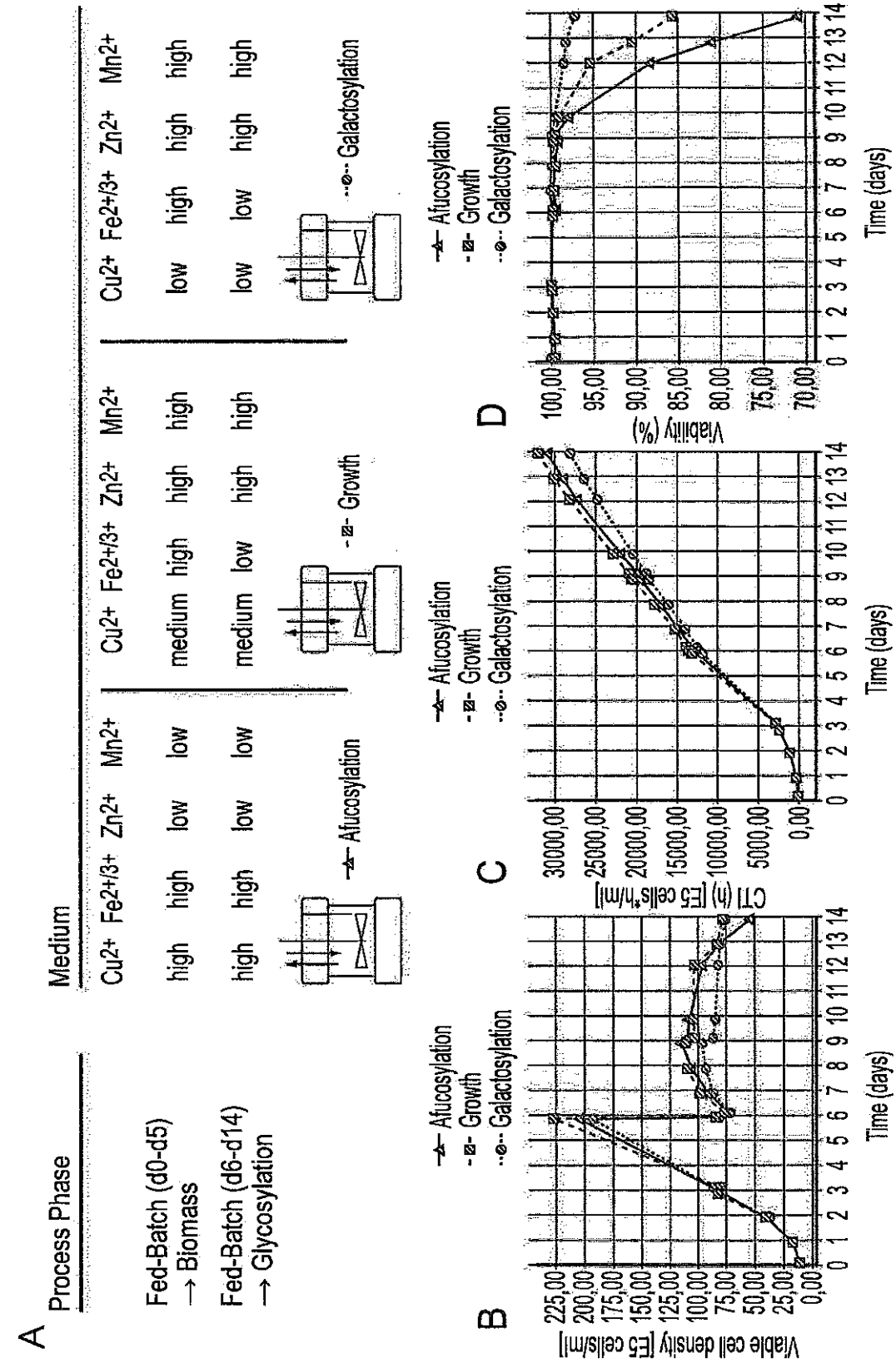

A
Process Phase
Medium
Cu2+ Fe2+/3+ Zn2+ Mn2+
Fed-Batch (d0-d5) → Biomass
high high low low
Fed-Batch (d6-d14) → Glycosylation
high high low low
medium high high high
medium low high high
low high high high
low low high high
Afucosylation
Growth
Galactosylation
B
Viable cell density [E5 cells/ml]
C
CTI (h) [E5 cells*h/ml]
D
Viability (%)
Time (days)

FIG. 9

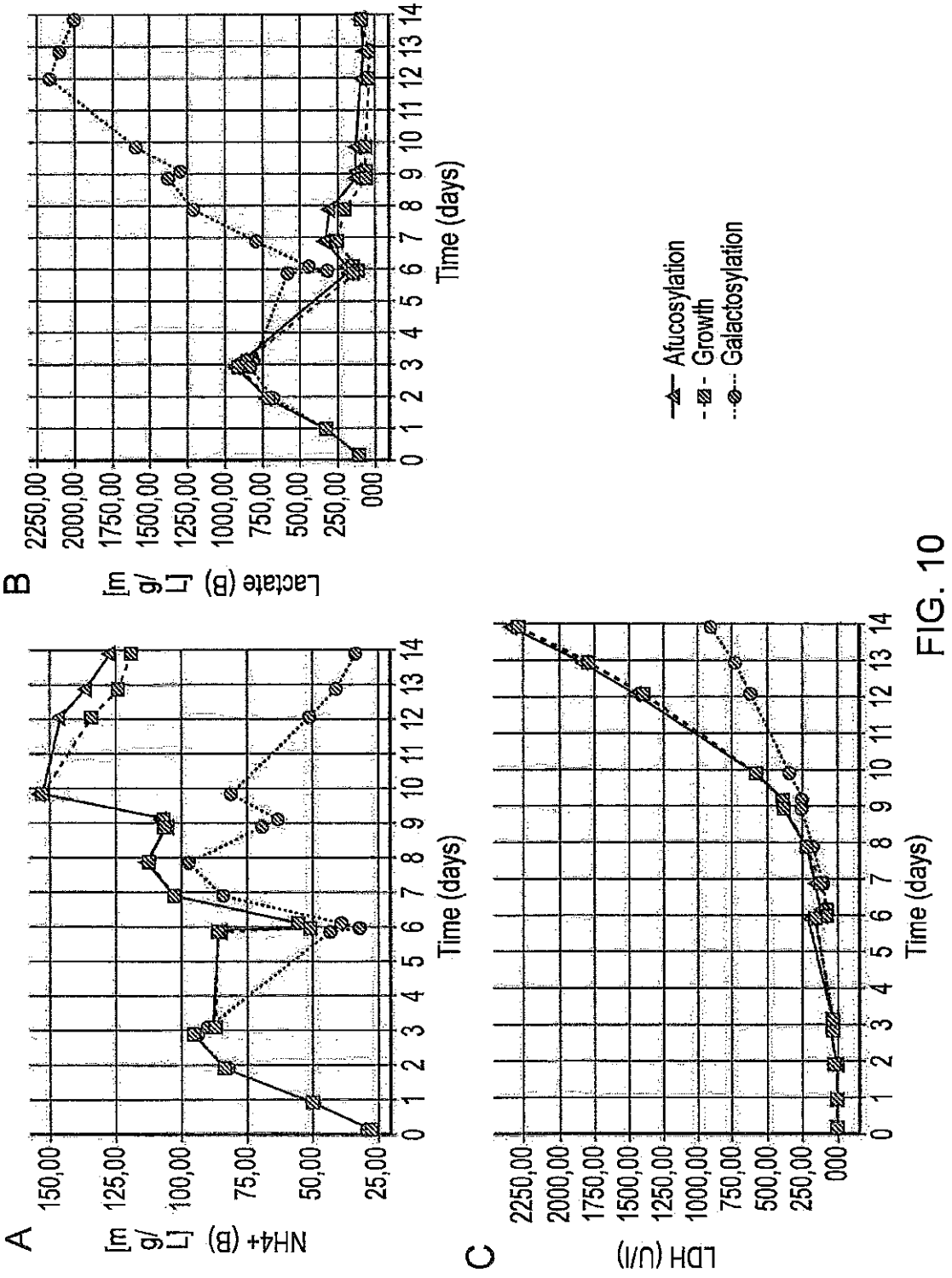

A
NH4+ (B) [g/l]
150,00
125,00
100,00
75,00
50,00
25,00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (days)
B
Lactate (B) [g/l]
2250,00
2000,00
1750,00
1500,00
1250,00
1000,00
750,00
500,00
250,00
000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (days)
Afucosylation
Growth
Galactosylation
C
LDH (U/l)
2250,00
2000,00
1750,00
1500,00
1250,00
1000,00
750,00
500,00
250,00
000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (days)

FIG. 10

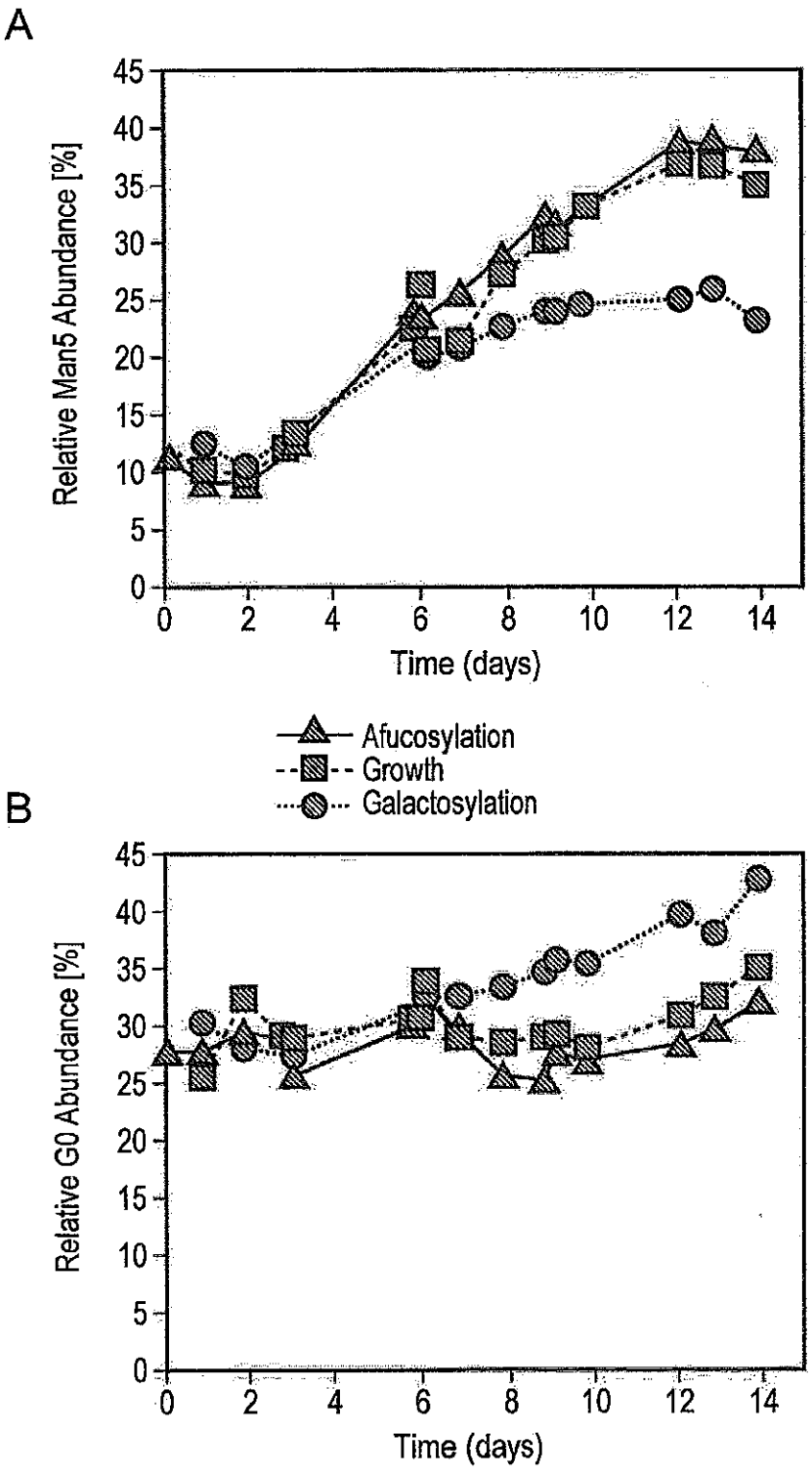
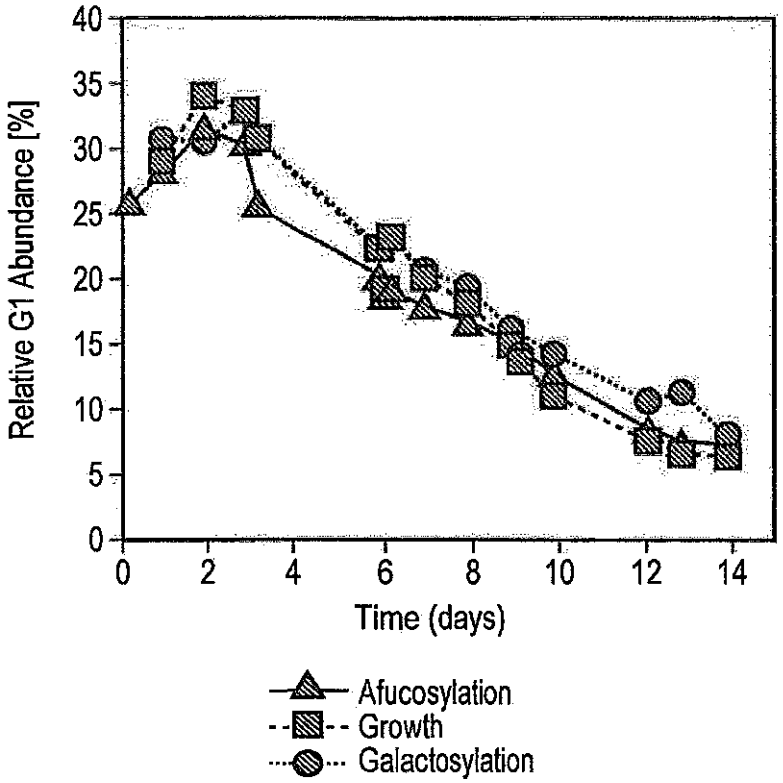

A
Relative Man5 Abundance [%]
Time (days)
Afucosylation
Growth
Galactosylation
B
Relative G0 Abundance [%]
Time (days)

FIG. 11

C



D

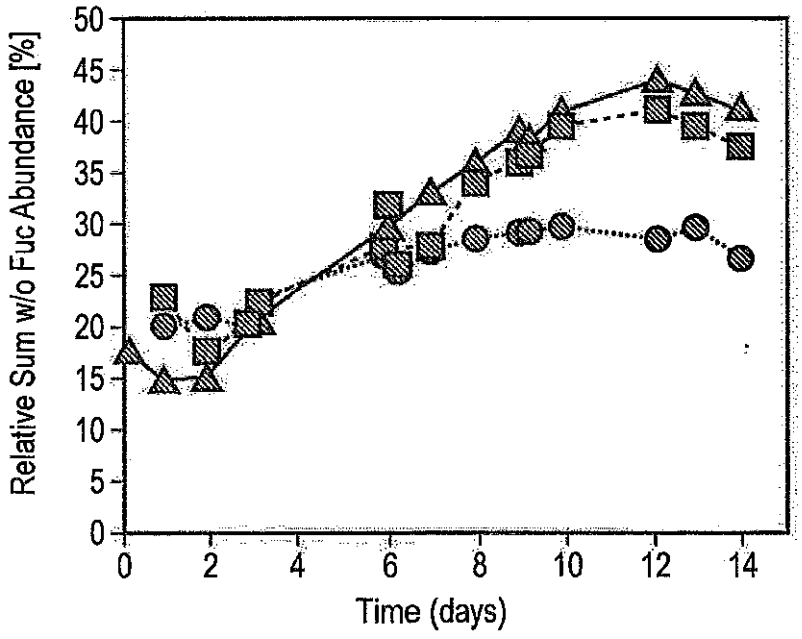


FIG. 11 (continued)

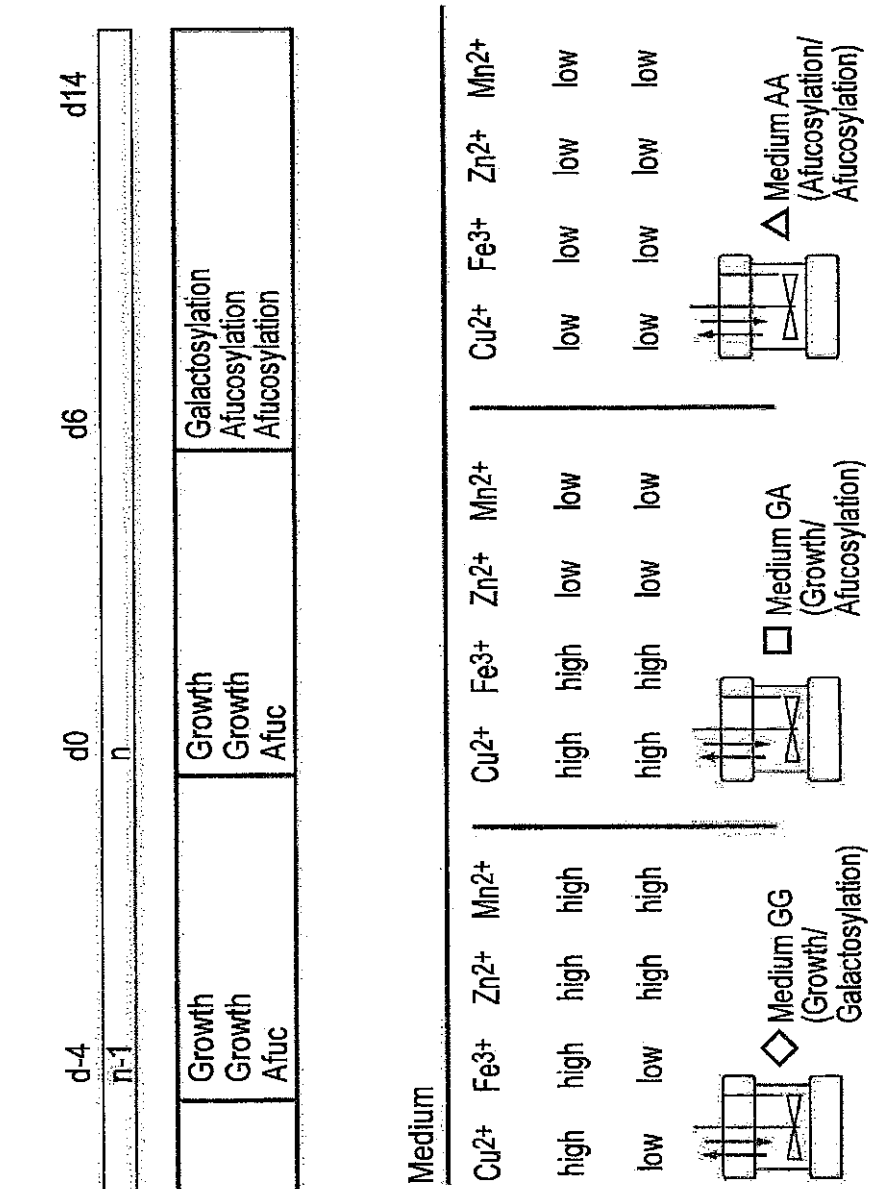

A
Process Time
d-8
d-4
d0
d6
d14
Phases
n-2
n-1
n
Test Cases
1 Growth
2 Growth
3 Afuc
Growth
Growth
Afuc
Growth
Growth
Afuc
Galactosylation
Afucosylation
Afucosylation
B
Process Phase
Medium
Cu2+ Fe3+ Zn2+ Mn2+
Inoculation Train
(phase n-2/n-1)
→ Biomass
high high high high
Fed-Batch
(phase n)
→ Glycosylation
low low high high
◇ Medium GG
(Growth/
Galactosylation)
Cu2+ Fe3+ Zn2+ Mn2+
high high low low
high high low low
□ Medium GA
(Growth/
Afucosylation)
Cu2+ Fe3+ Zn2+ Mn2+
low low low low
low low low low
△ Medium AA
(Afucosylation/
Afucosylation)

FIG. 12

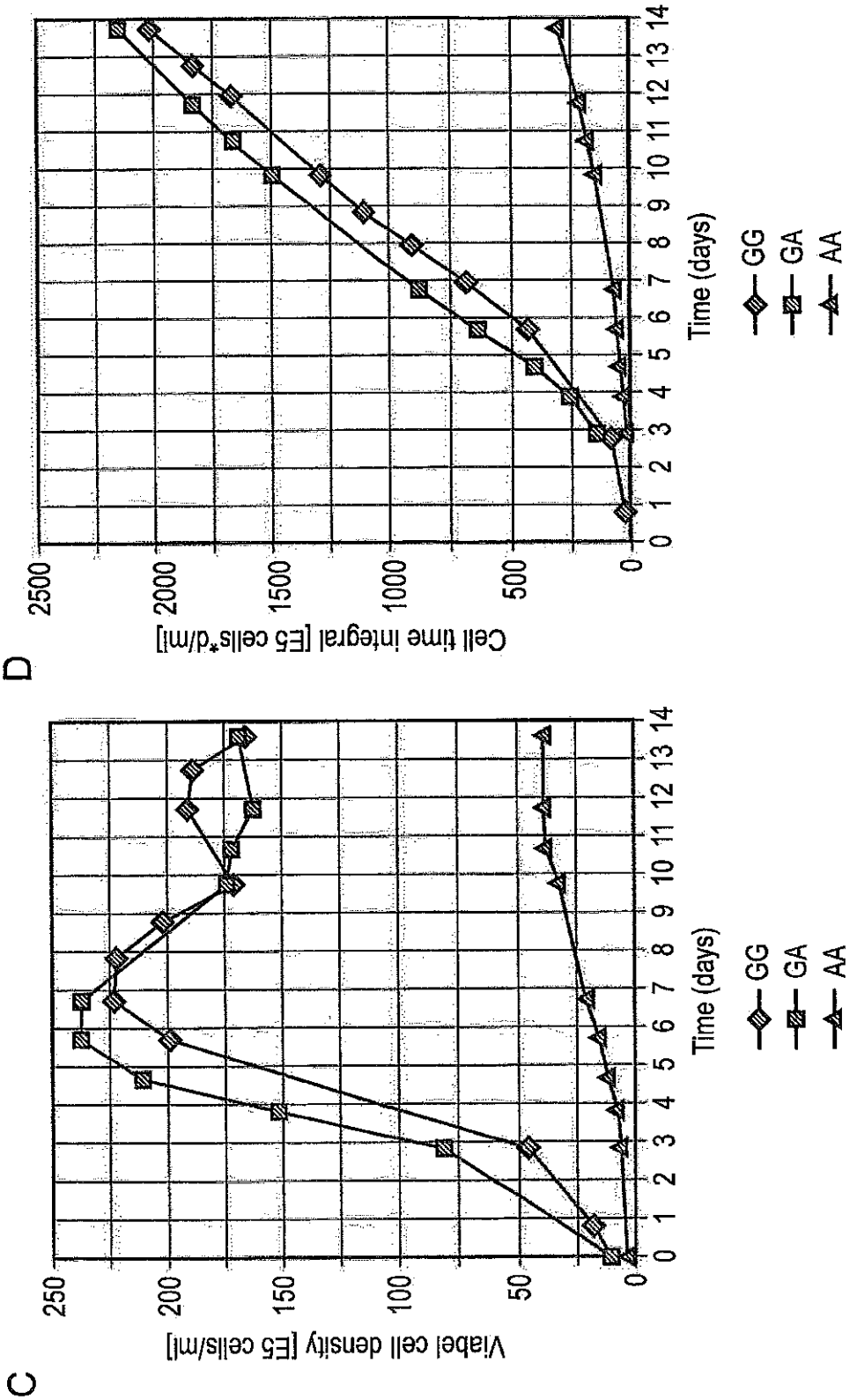
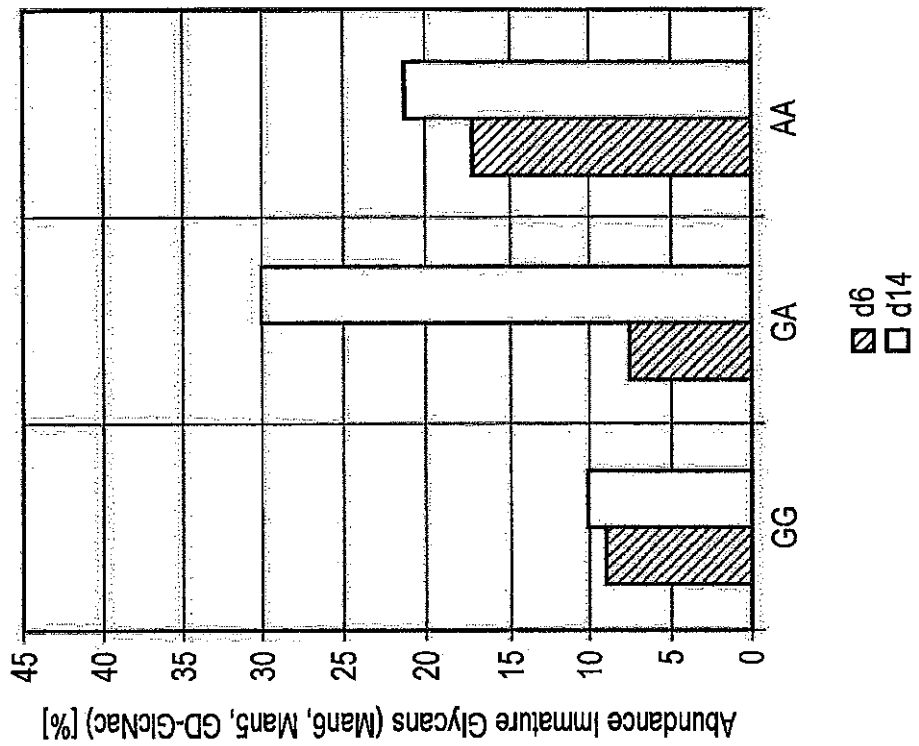


FIG. 12 (continued)

F



E

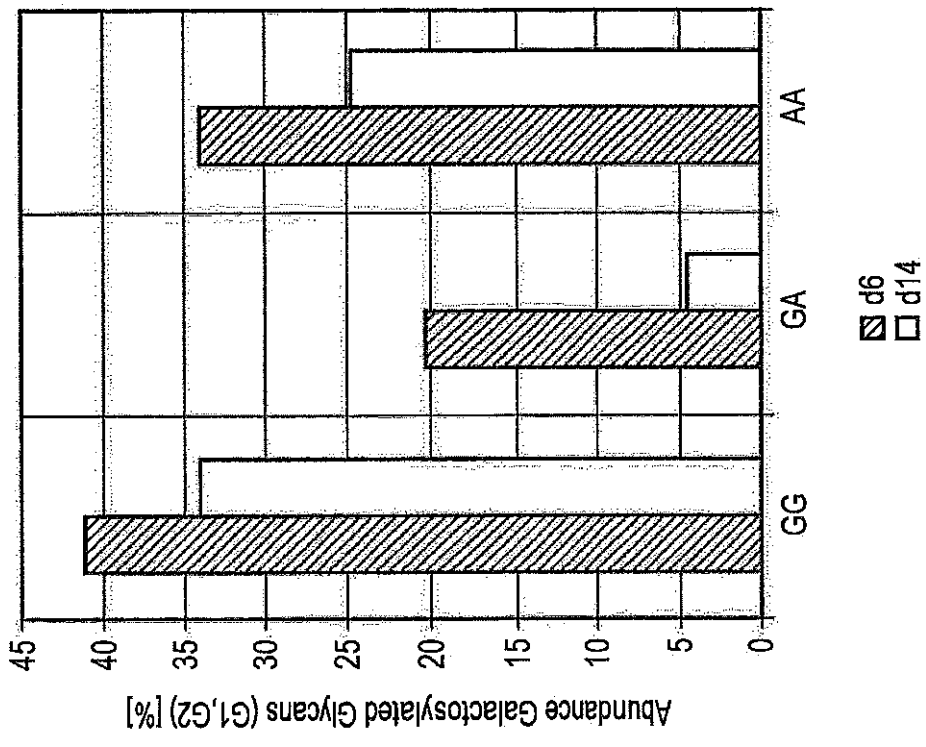
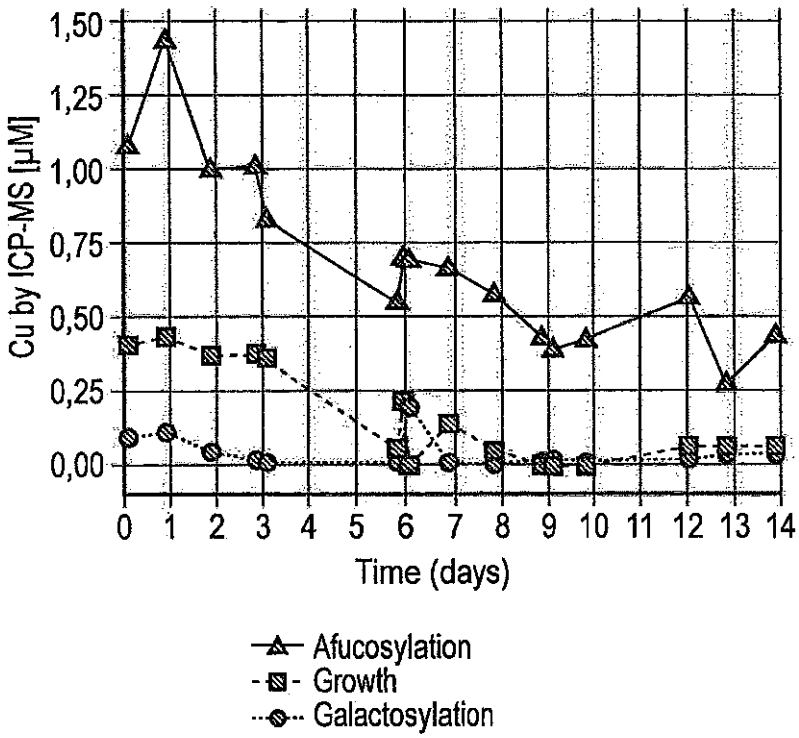


FIG. 12 (continued)

A


Cu by ICP-MS [µM]
1,50
1,25
1,00
0,75
0,50
0,25
0,00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (days)
Afucosylation
Growth
Galactosylation

B

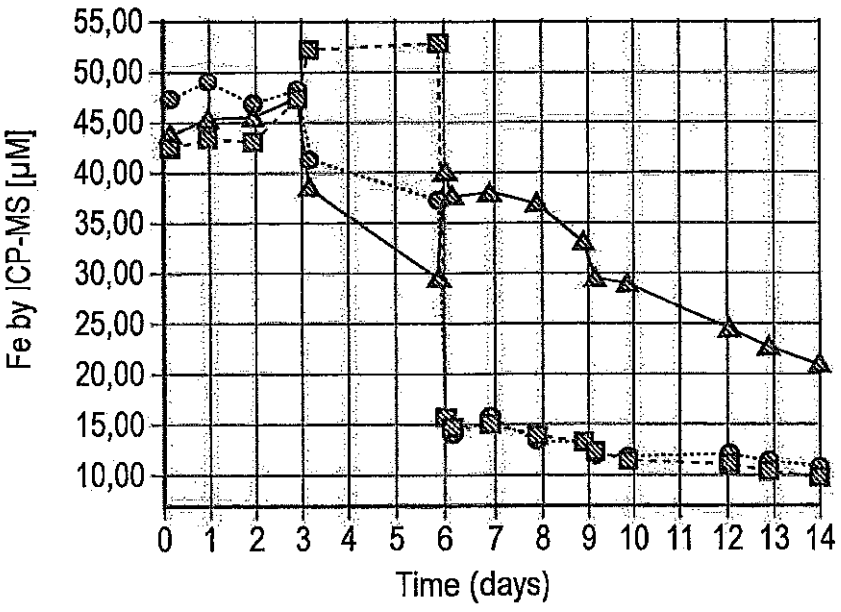
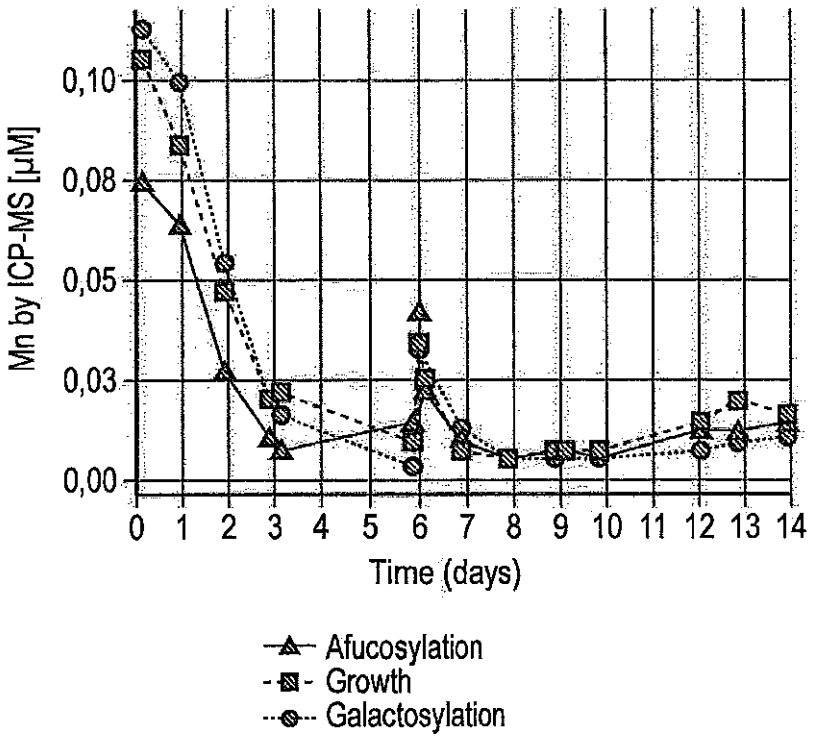

Fe by ICP-MS [µM]
55,00
50,00
45,00
40,00
35,00
30,00
25,00
20,00
15,00
10,00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (days)

FIG. 13

C



D

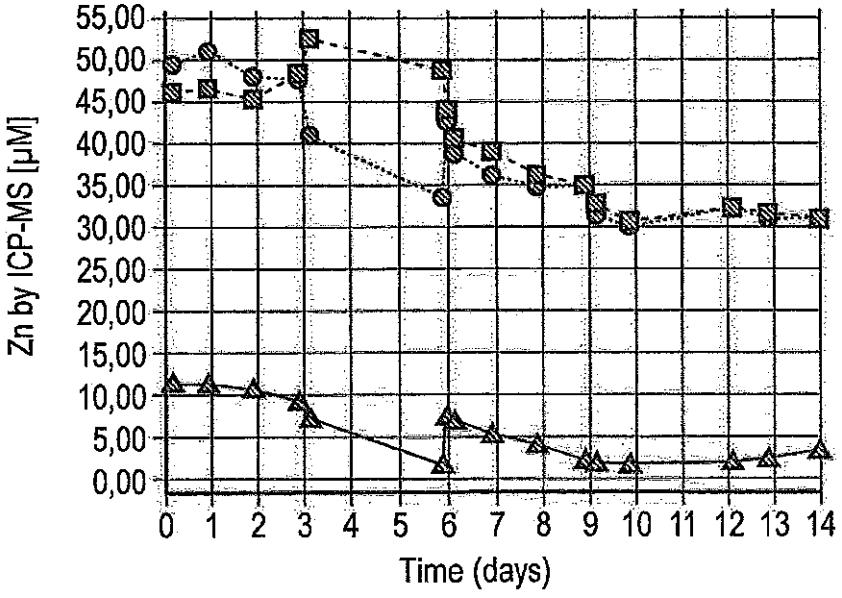


FIG. 13 (continued)

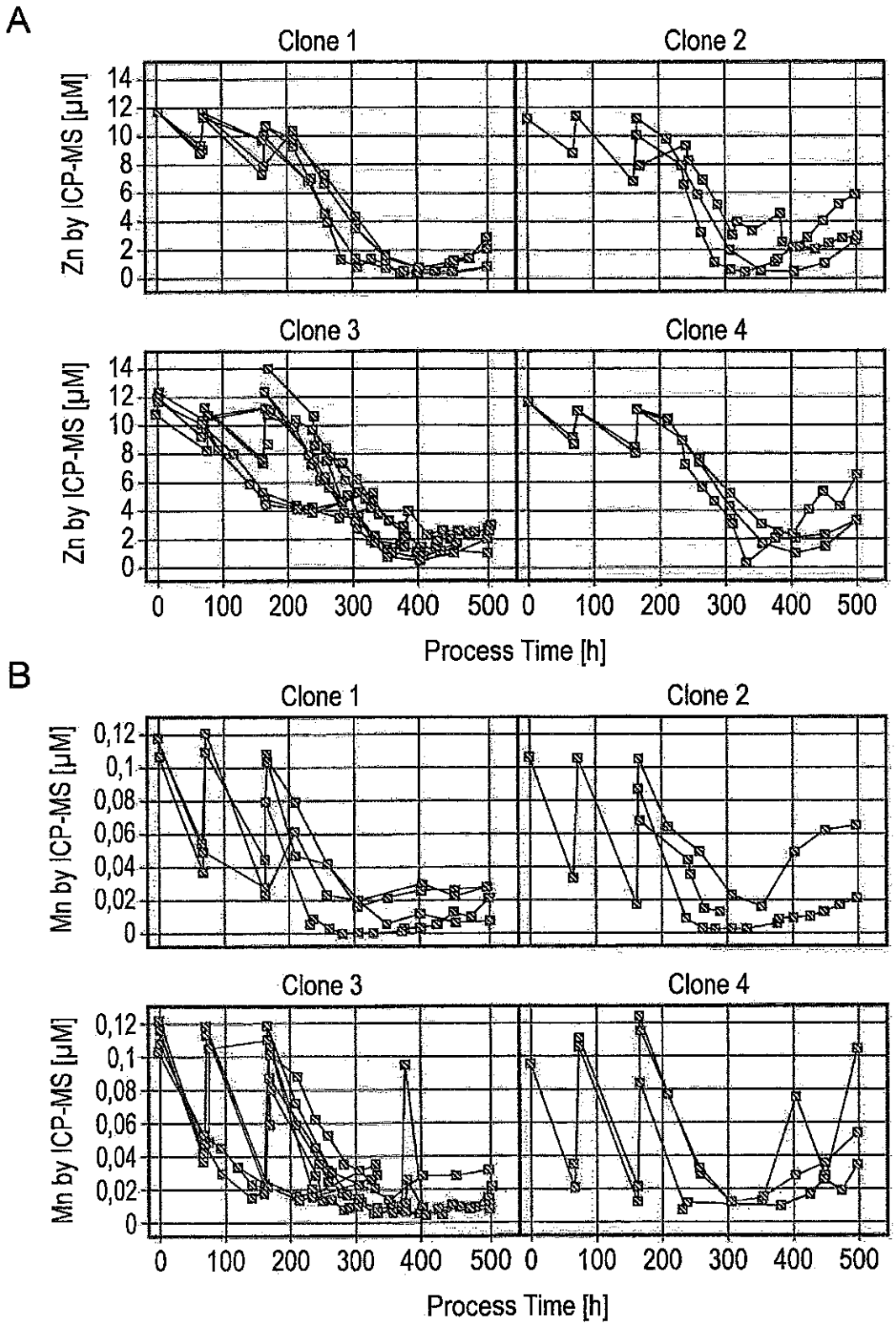
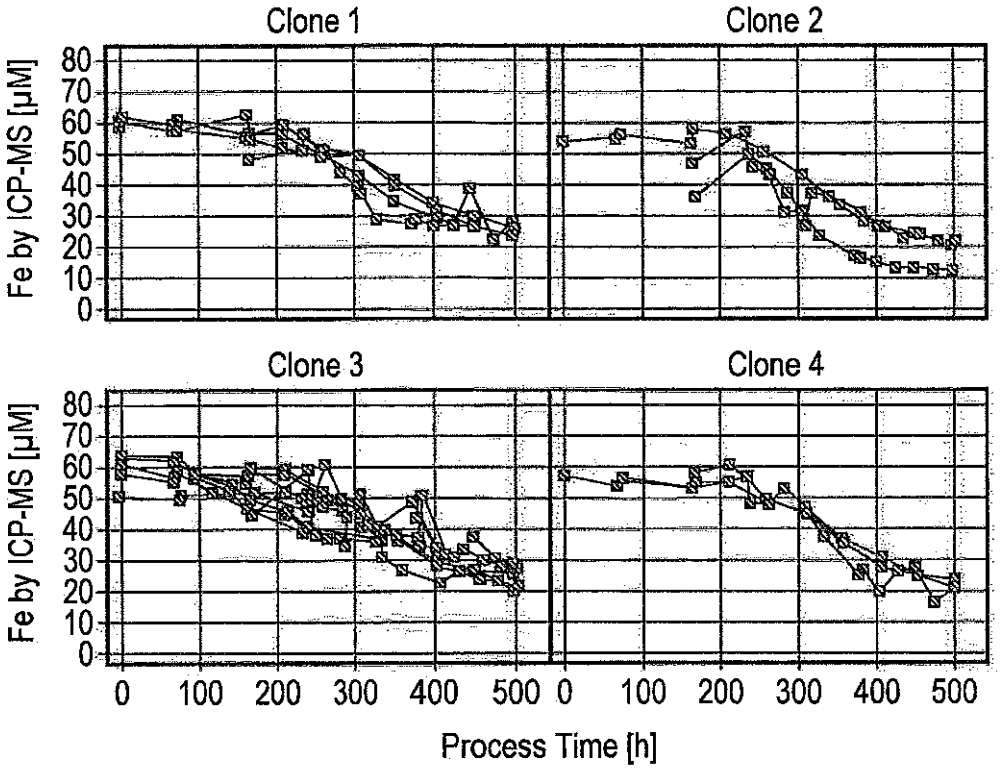

A
Clone 1
Clone 2
Clone 3
Clone 4
Zn by ICP-MS [µM]
0
2
4
6
8
10
12
14
0 100 200 300 400 500
Process Time [h]
B
Clone 1
Clone 2
Clone 3
Clone 4
Mn by ICP-MS [µM]
0
0,02
0,04
0,06
0,08
0,1
0,12
0 100 200 300 400 500
Process Time [h]

FIG. 14

C



D

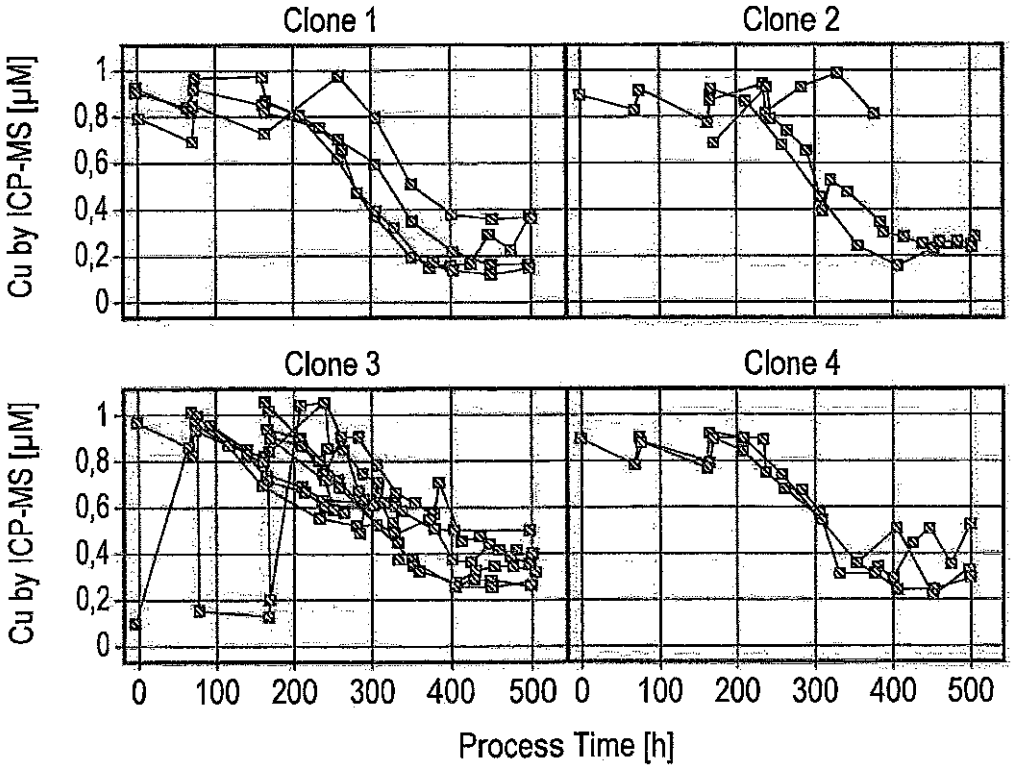


FIG. 14 (continued)

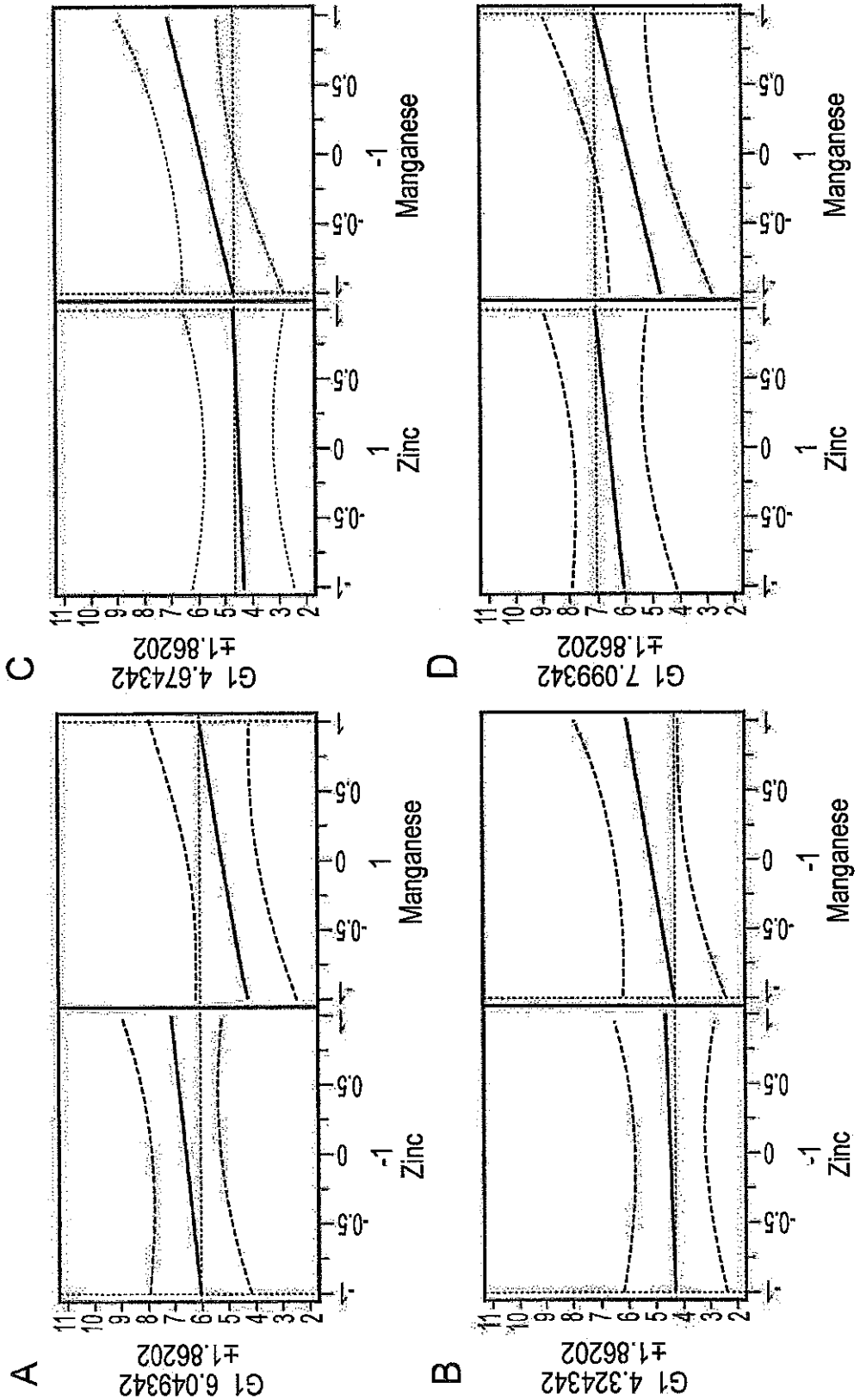

A
G1 6.04934 2
±1.86202
B
G1 4.32434 2
±1.86202
C
G1 4.67434 2
±1.86202
D
G1 7.09934 2
±1.86202
Zinc
Manganese
-1
-0.5
0
0.5
1
2
3
4
5
6
7
8
9
10
11

FIG. 15

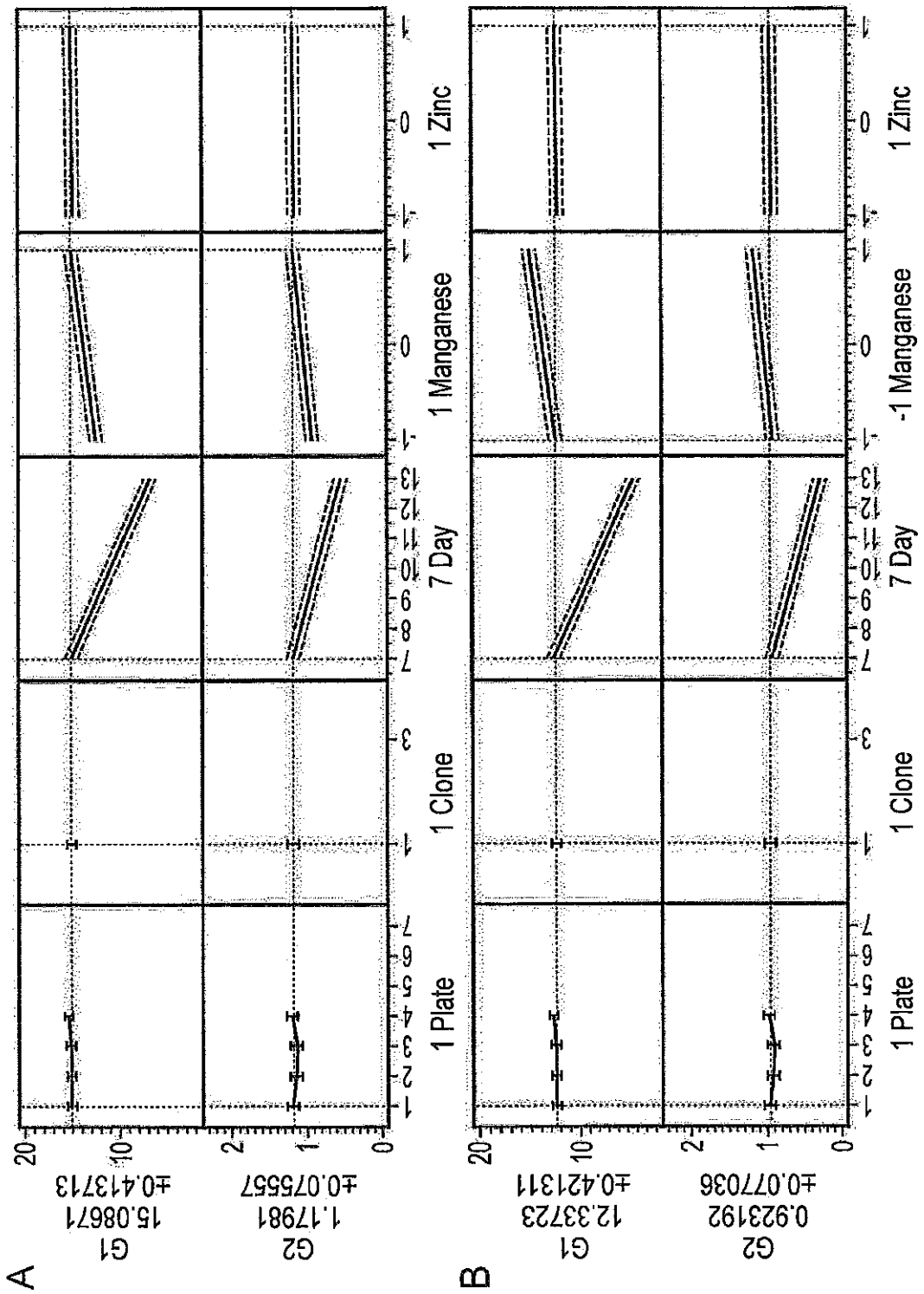

A
G1
15.08671
±0.413713
G2
1.17981
±0.075557
B
G1
12.33723
±0.421311
G2
0.923192
±0.077036
1 Plate
1 Clone
7 Day
1 Manganese
-1 Manganese
1 Zinc

FIG. 16

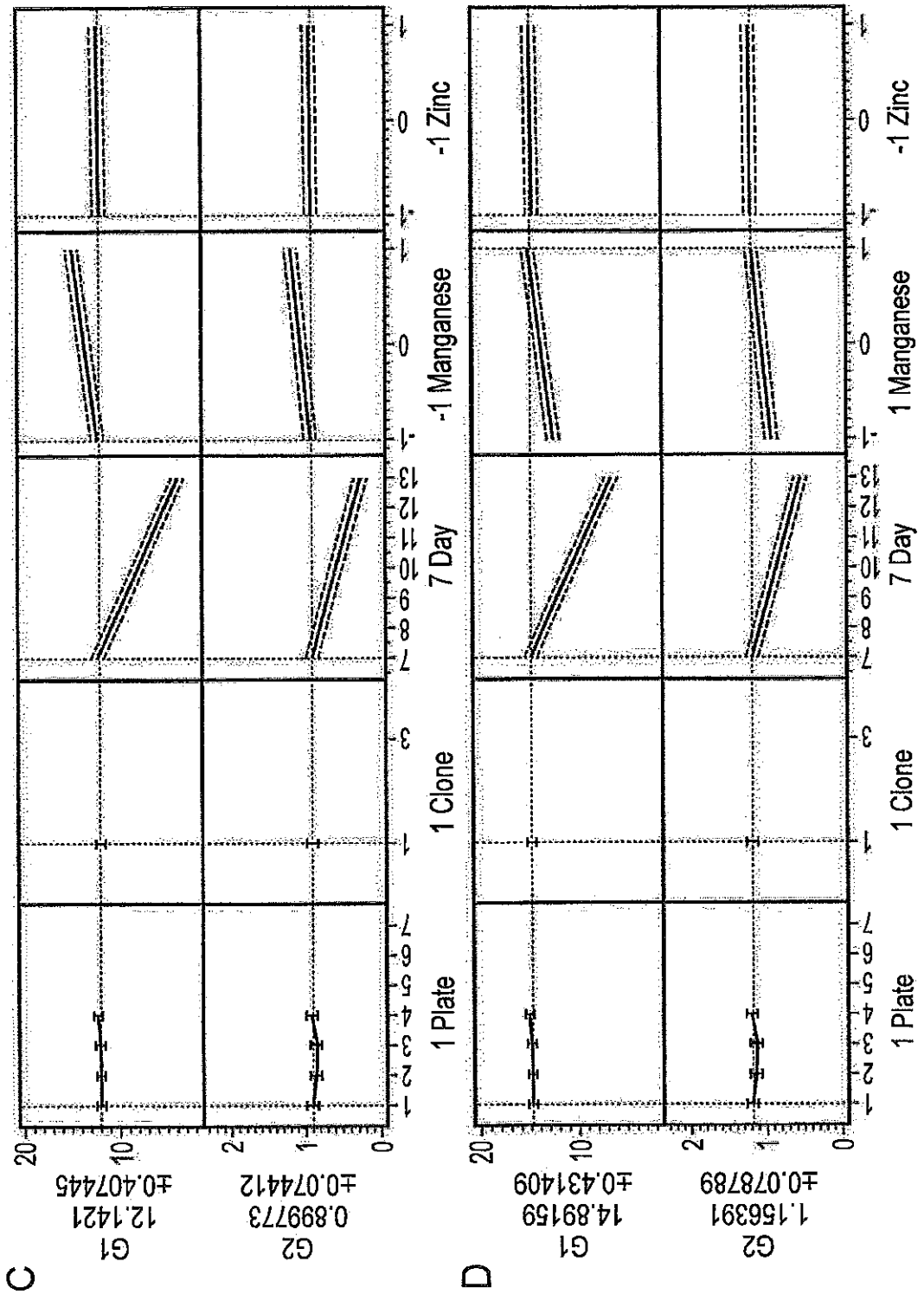


FIG. 16 (continued)