
35S promoter
Ω enhancer
Er signal
GCD
Vacuolar signal
Terminator
SmaI
XbaI

Fig. 1a

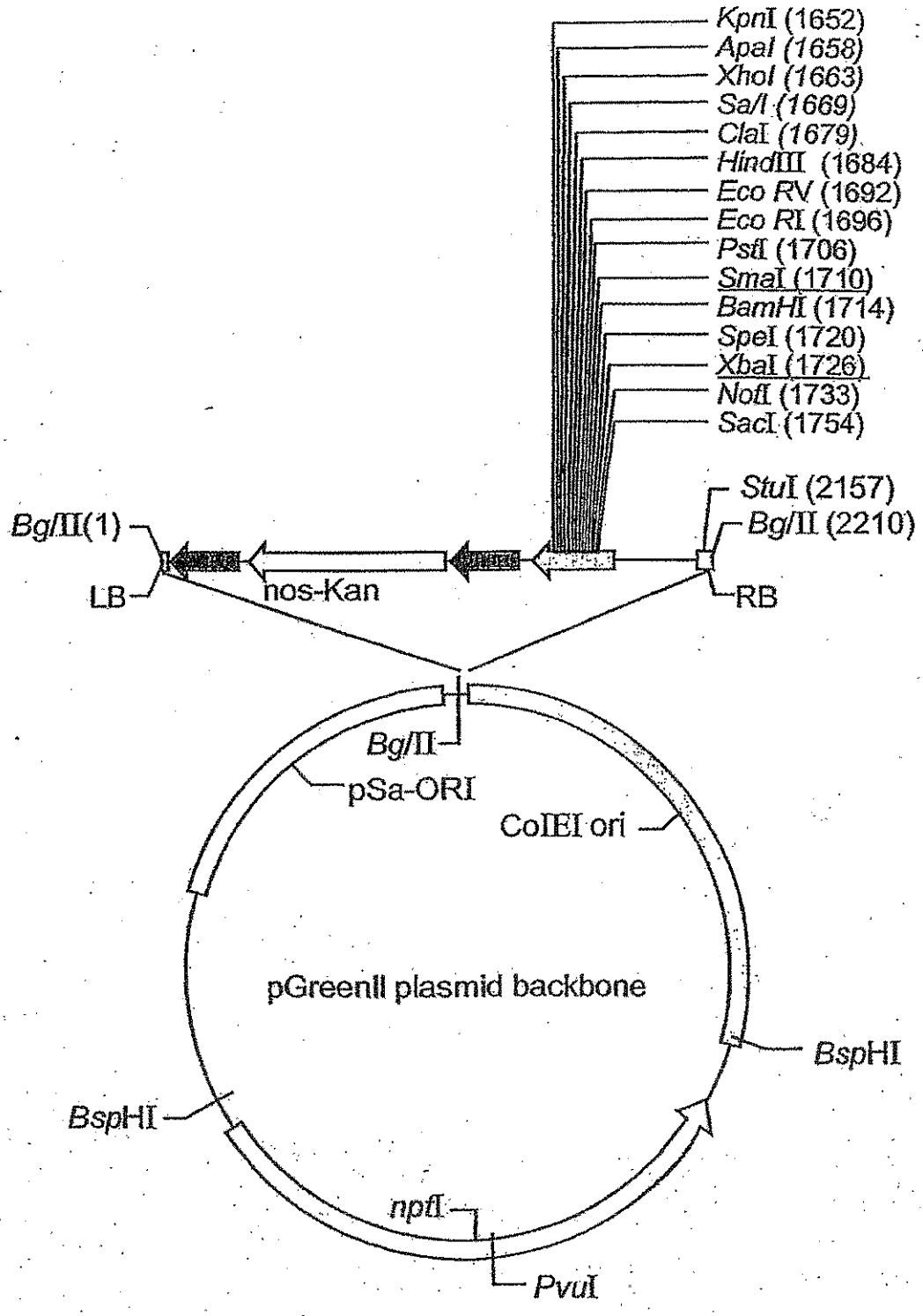

KpnI (1652)
ApaI (1658)
XhoI (1663)
SalI (1669)
ClaI (1679)
HindIII (1684)
Eco RV (1692)
Eco RI (1696)
PstI (1706)
SmaI (1710)
BamHI (1714)
SpeI (1720)
XbaI (1726)
NotI (1733)
SacI (1754)
StuI (2157)
Bg/II (2210)
Bg/II(1)
LB
nos-Kan
RB
Bg/II
pSa-ORI
CoIEI ori
pGreenII plasmid backbone
BspHI
BspHI
nptI
PvuI

Fig. 1b

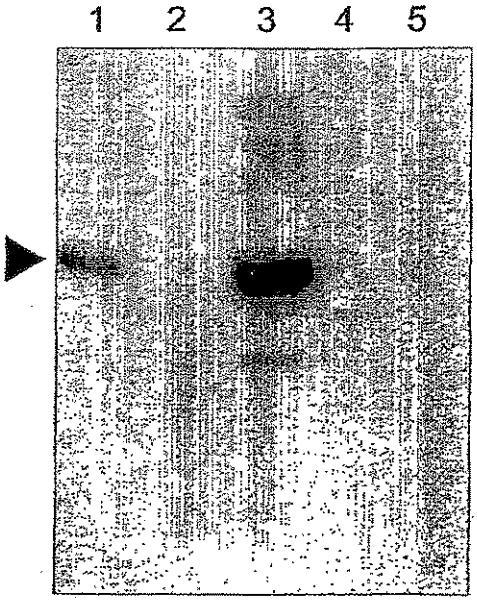


Fig. 2

Transformed cells express rGCD. 1 gram calli tissue was homogenized and 15 microgram of soluble cell extract were run on SDS-PAGE. Expression of rGCD in selected transformed calli was tested by western blot analysis with specific anti hGCD antibodies. 1: standard cerezyme, 2: untransformed callus extract, 3-5: various selected transformed calli extracts .

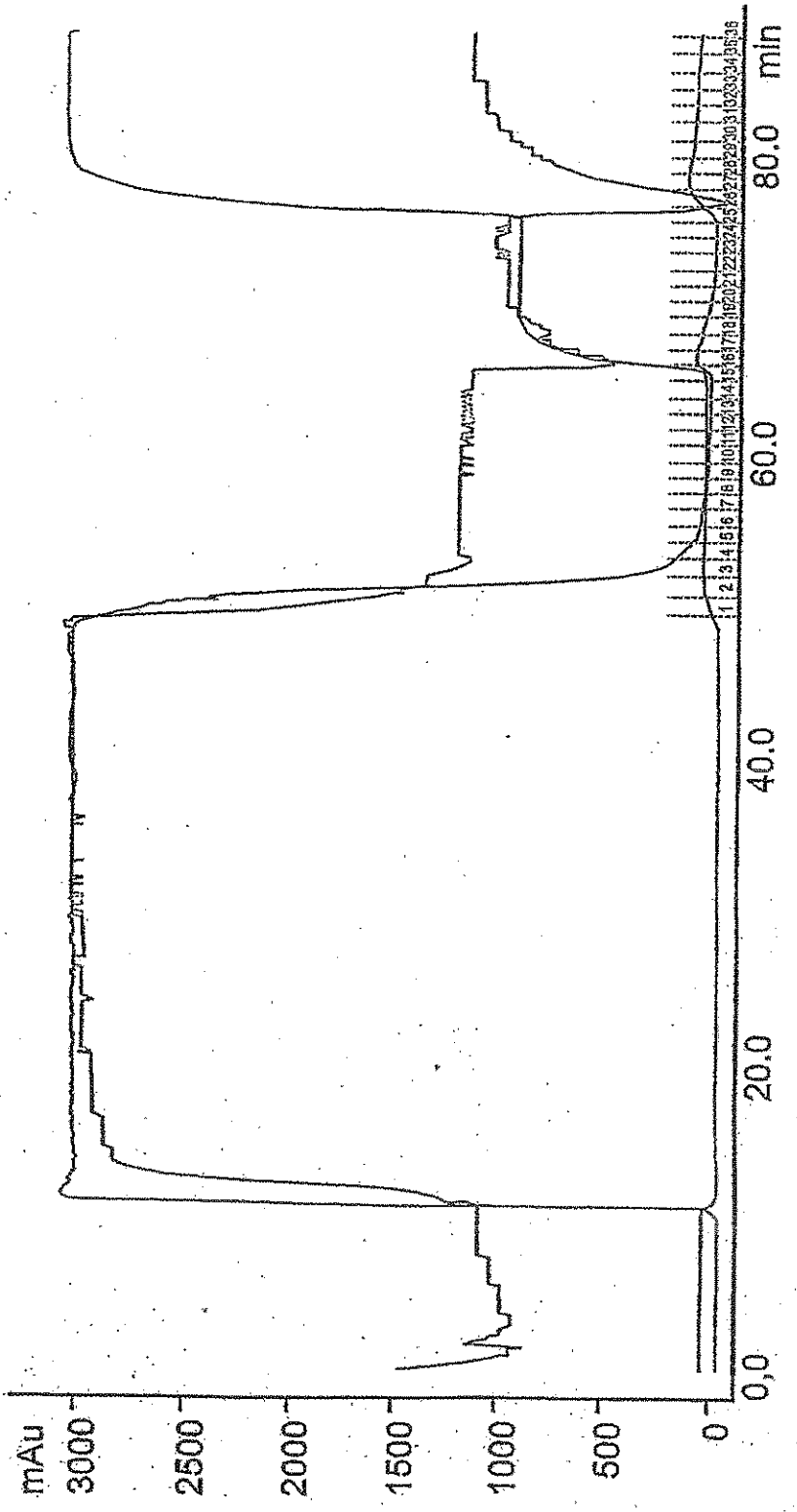

UV
Cond
pH
Fractions
mAu
3000
2500
2000
1500
1000
500
0
0,0
20.0
40.0
60.0
80.0
min

Fig. 3a

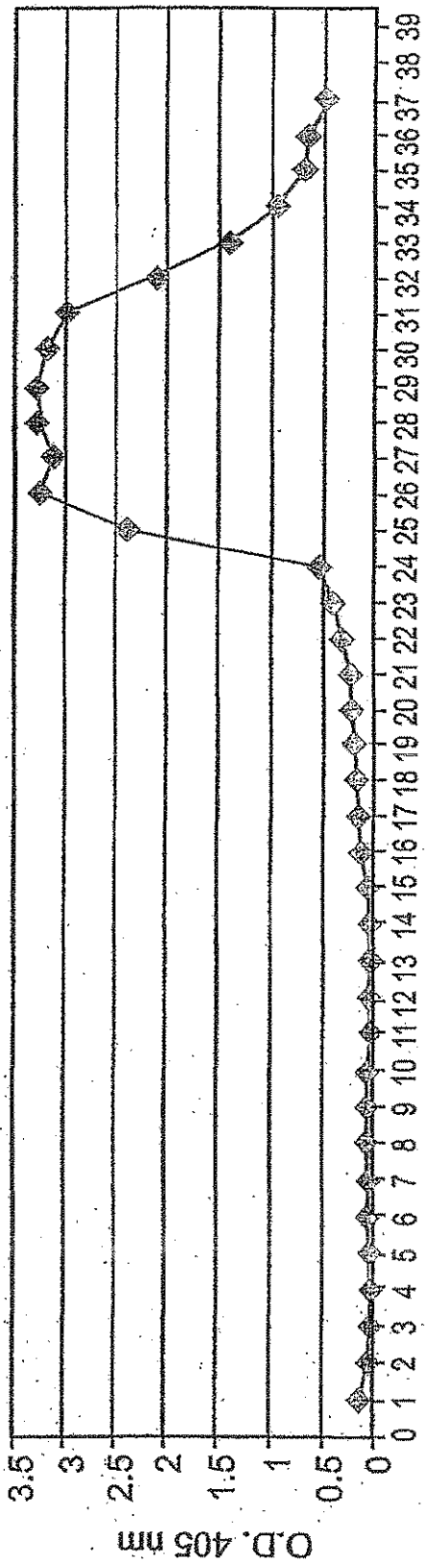

O.D. 405 nm
3.5
3
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
Fraction

Fig. 3b

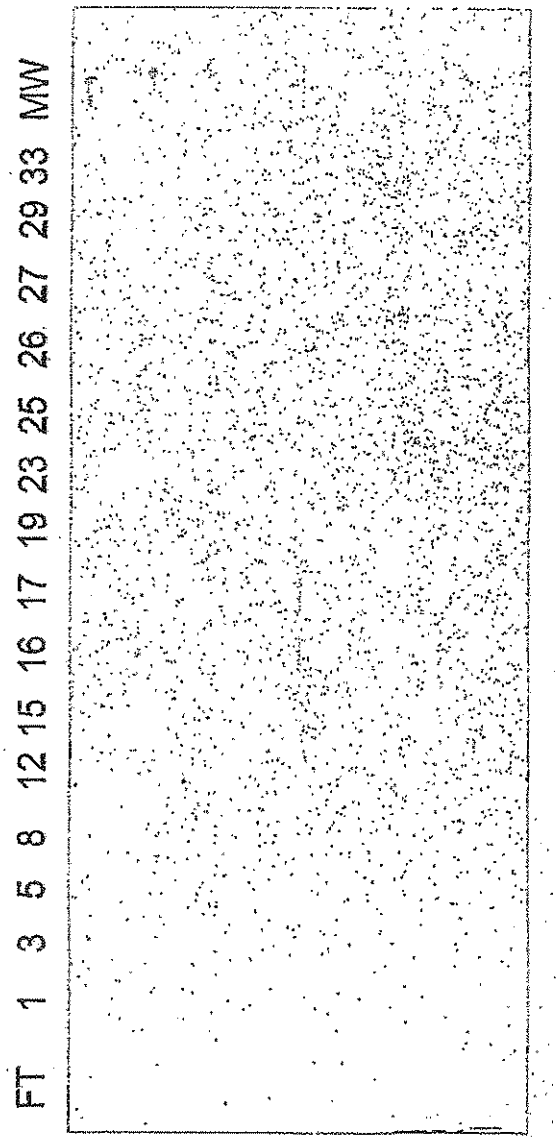

FT 1 3 5 8 12 15 16 17 19 23 25 26 27 29 33 MW

Fig. 3c

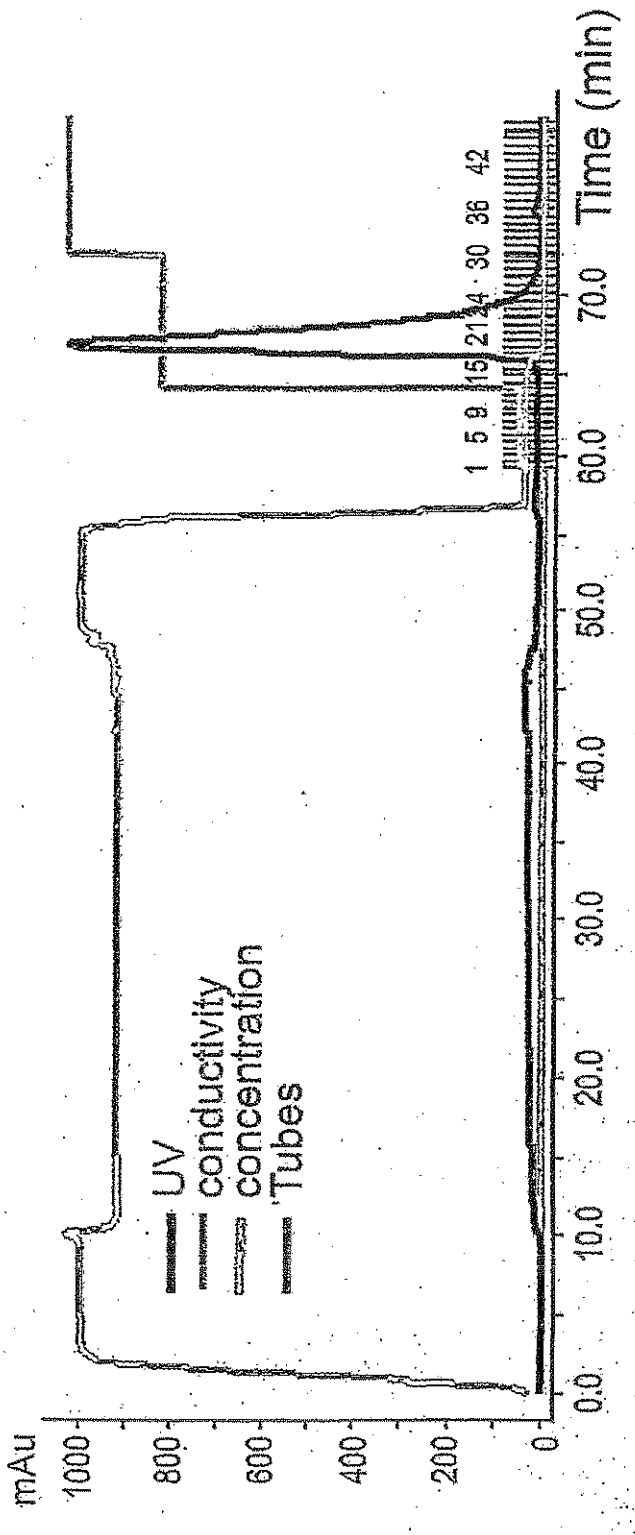

mAu
1000
800
600
400
200
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Time (min)
UV
conductivity
concentration
Tubes
1 5 9 15 21 24 30 36 42

Fig. 3d

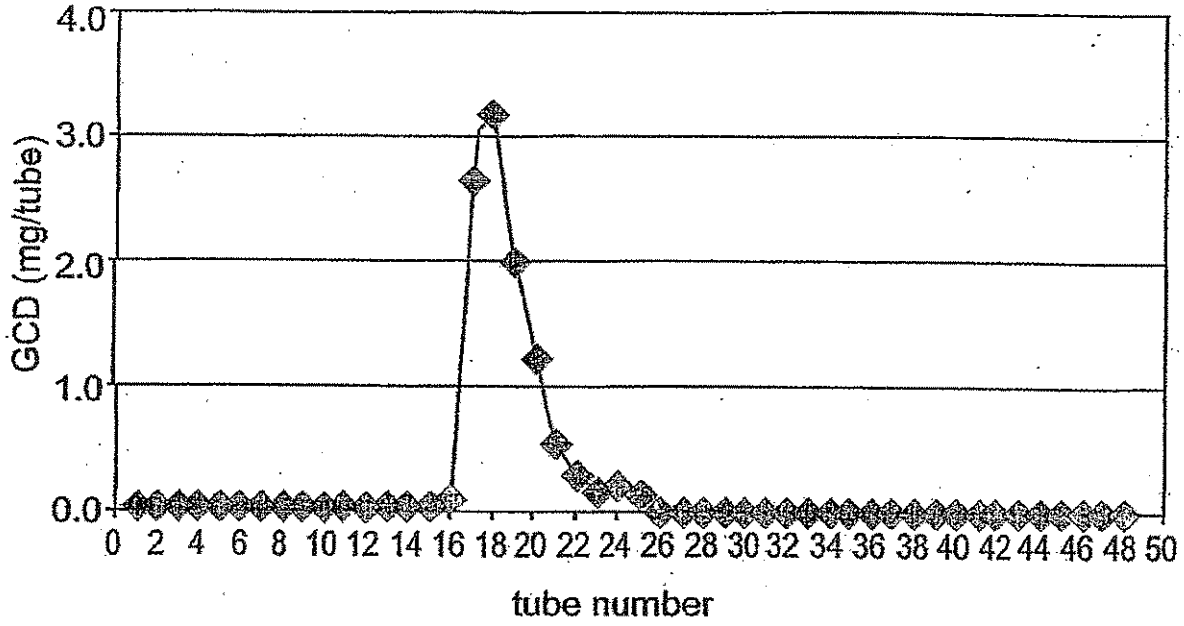

4.0
3.0
2.0
1.0
0.0
GCD (mg/tube)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
tube number

Fig. 3e

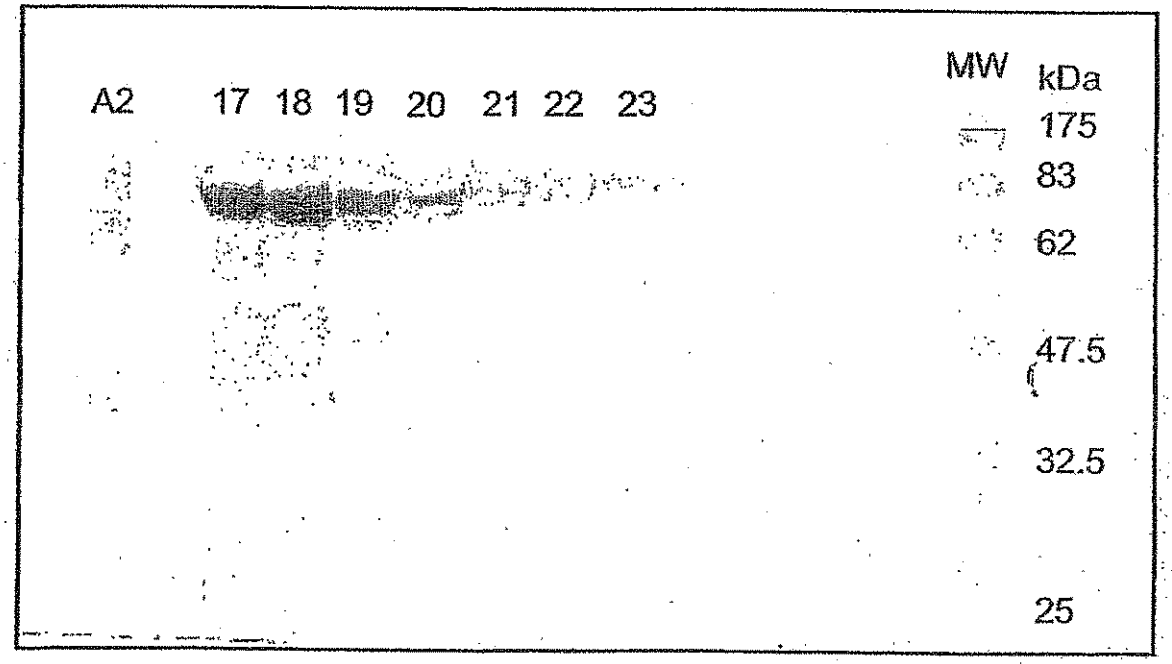

A2
17 18 19 20 21 22 23
MW kDa
175
83
62
47.5
32.5
25

Fig. 3f

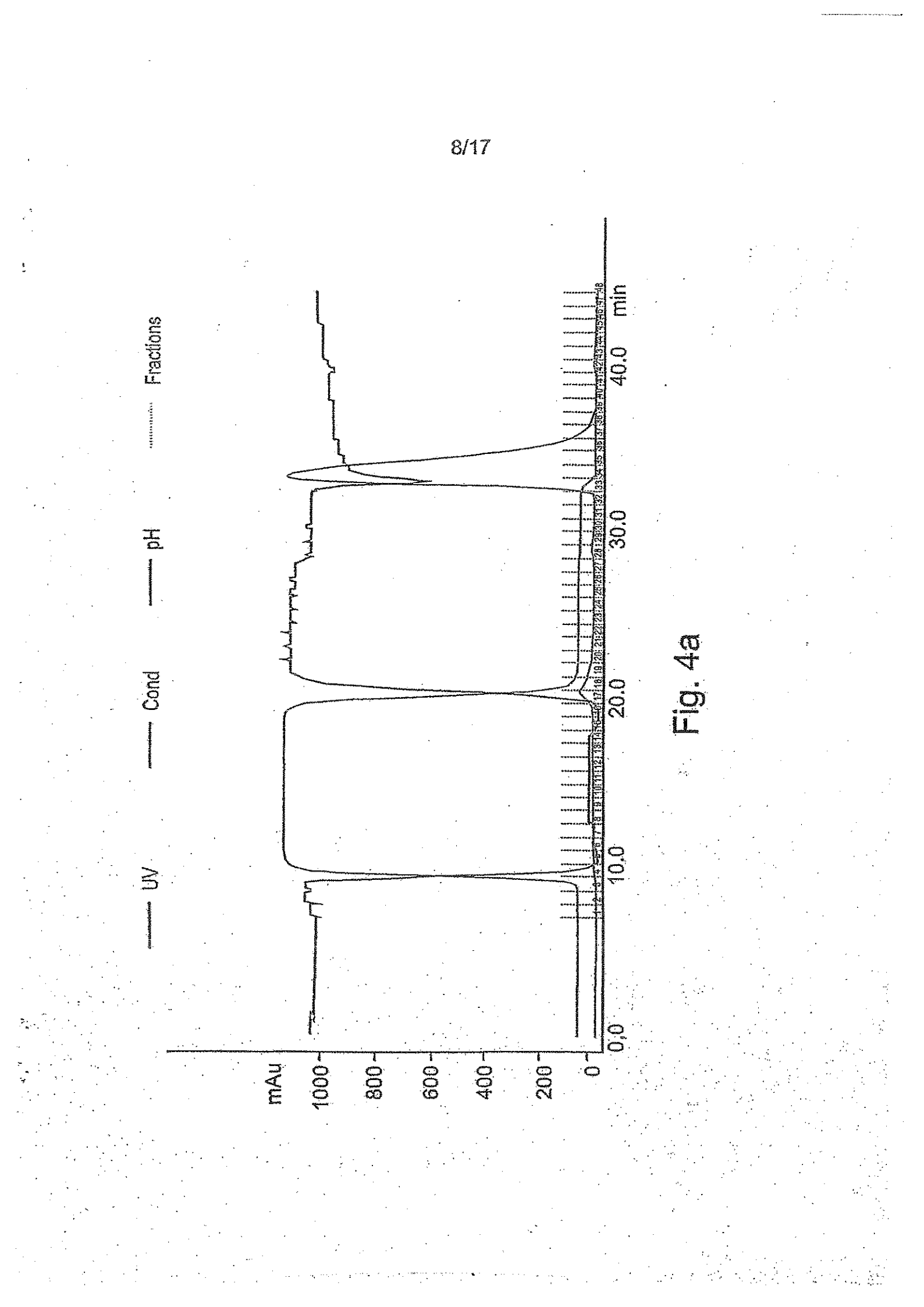

UV
Cond
pH
Fractions
mAu
1000
800
600
400
200
0
0,0
10,0
20,0
30,0
40,0
min

Fig. 4a

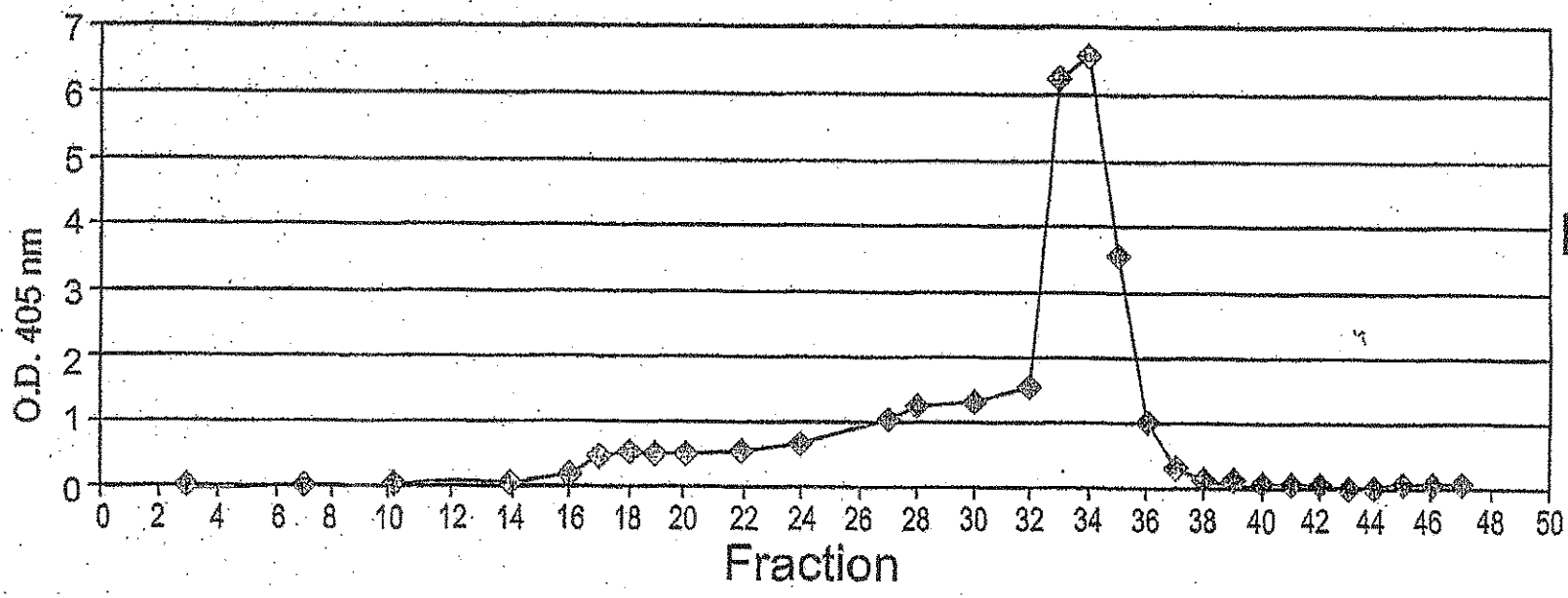

O.D. 405 nm
0
1
2
3
4
5
6
7
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Fraction

Fig. 4b

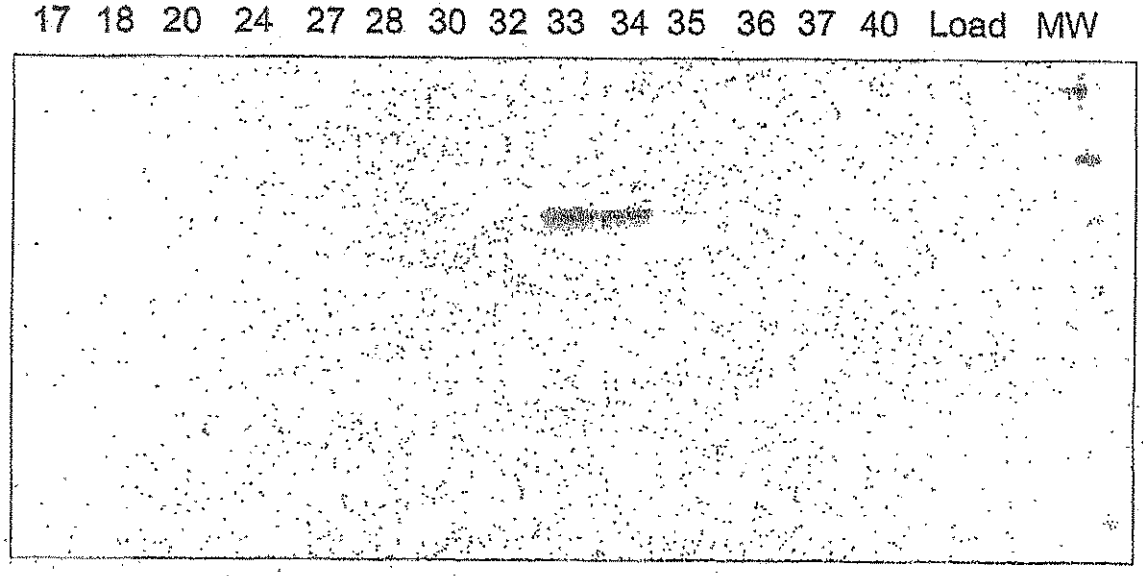

17 18 20 24 27 28 30 32 33 34 35 36 37 40 Load MW

Fig. 4c

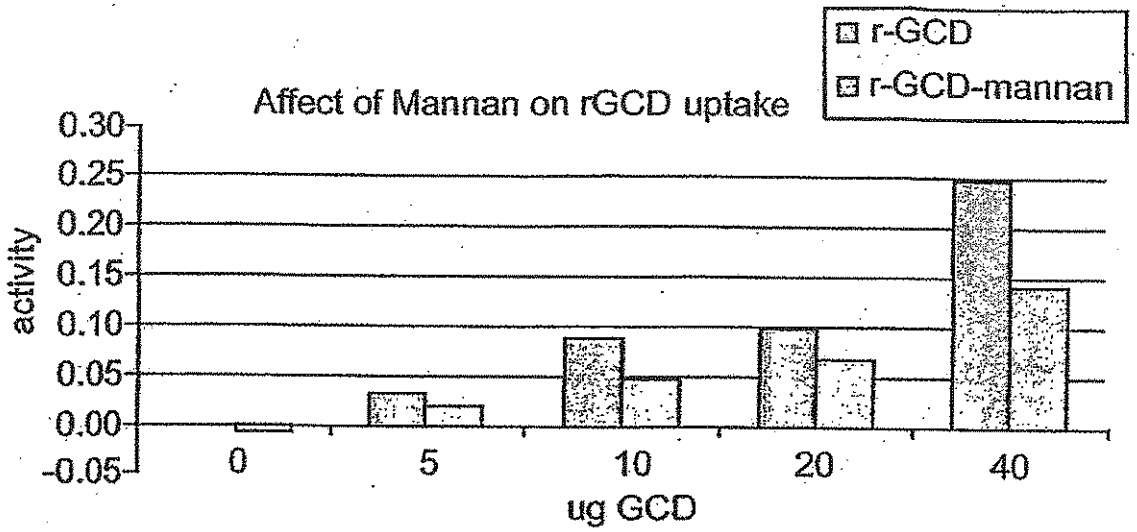

r-GCD
r-GCD-mannan
Affect of Mannan on rGCD uptake
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
activity
0
5
10
20
40
ug GCD

Fig. 5a

Uptake of GCD in peritoneal macrophages by mannose receptors
GCD (CB-mix1 = rGCD of the present invention) Vs. Cerezyme®

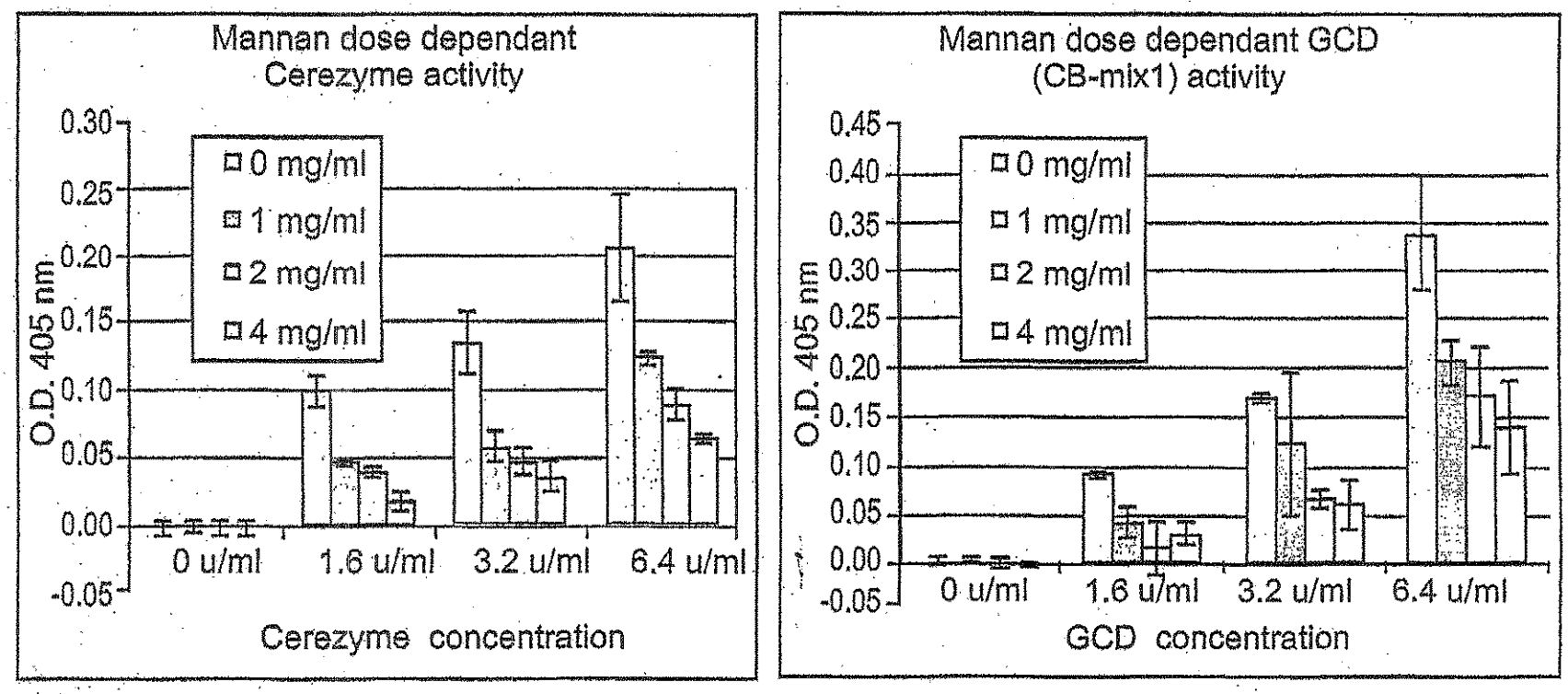
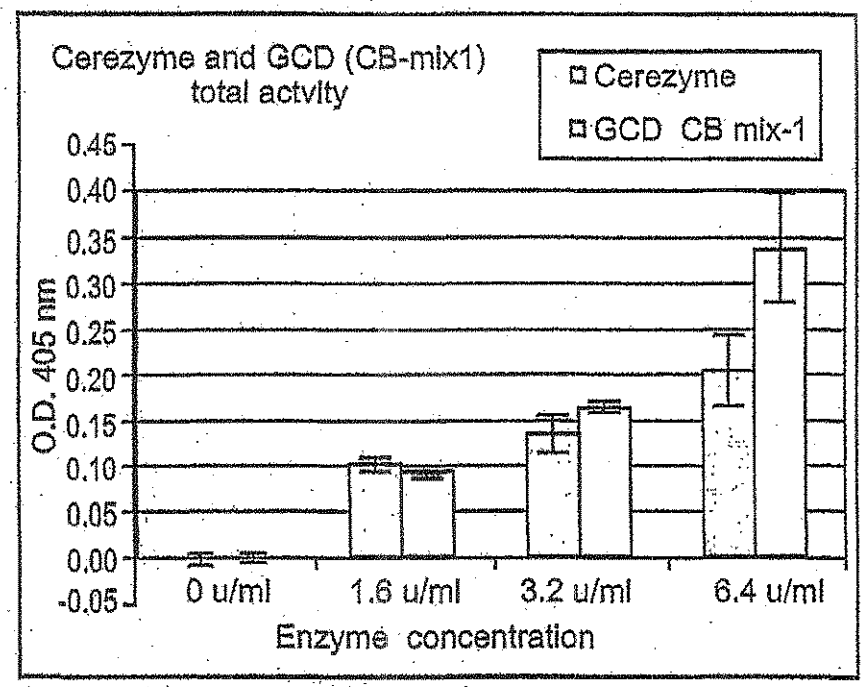


Fig. 5b

Uptake of GCD in peritoneal macrophages by mannose receptors
GCD (CB-mix1 – rGCD of the present invention) Vs. Cerezyme®



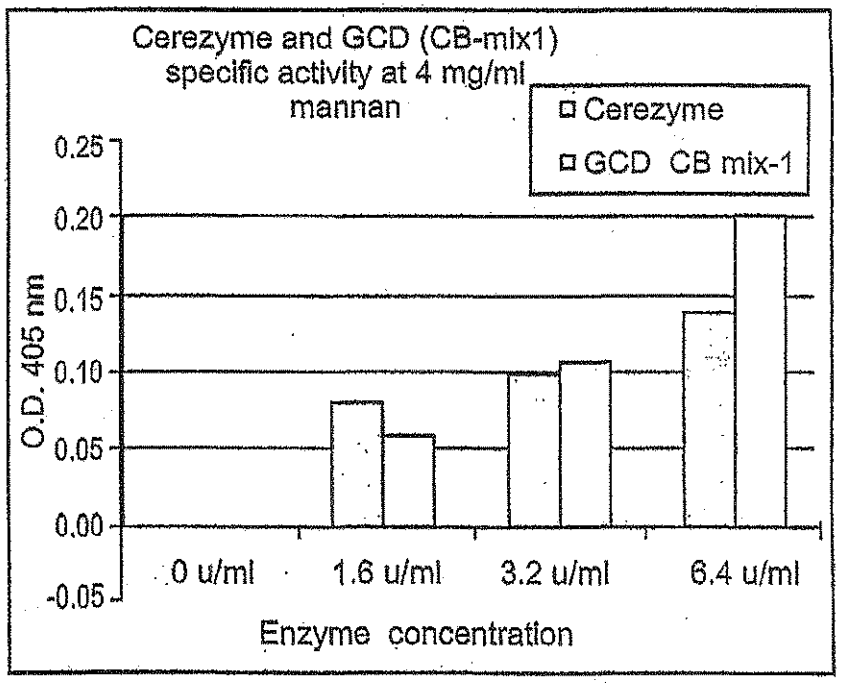


Fig. 5c

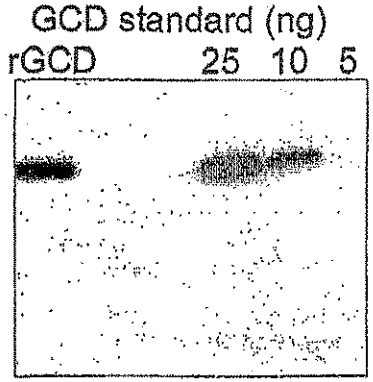
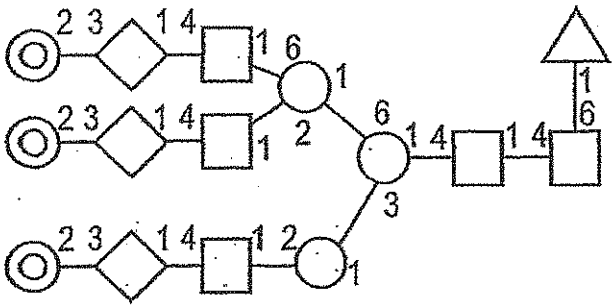
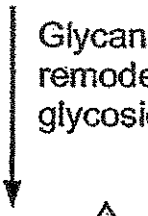


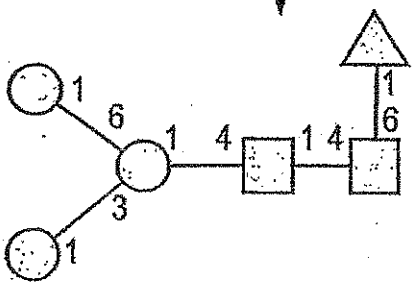
Fig. 5d



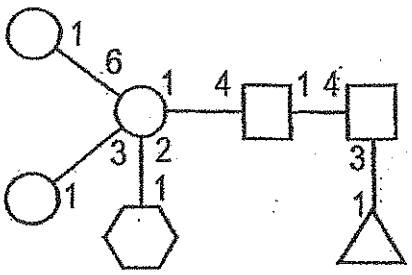
Major glycan structure
from CHO cells



Glycan
remodeling with
glycosidases



Major remodeled glycan
structure on Cerezyme



Major glycan structure
from carrot cells:

Mannose terminal glycan

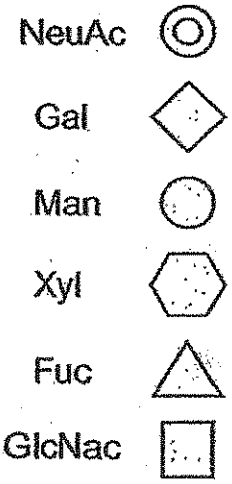


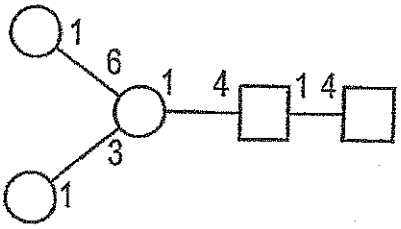
Fig. 6

a) Ma3—Mb4GNb4—GN
Ma6-|
Xb2-+
|
Fa3-+

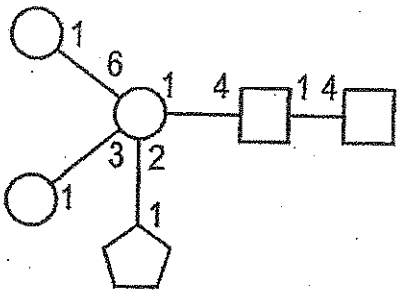
b) Ma3—Mb4GNb4GN
Ma6 -+

c) Ma3—Mb4GNb4—GN
Ma6-+
|
Fa3-+

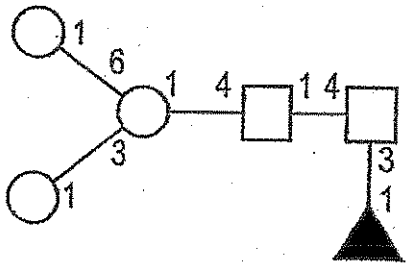
Fig. 7



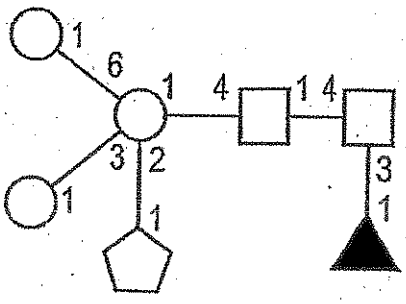
Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1171.5



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1331.6

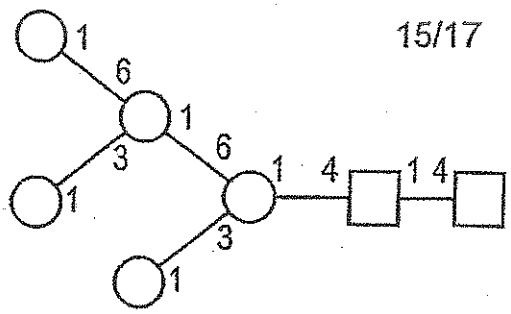


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1345.6

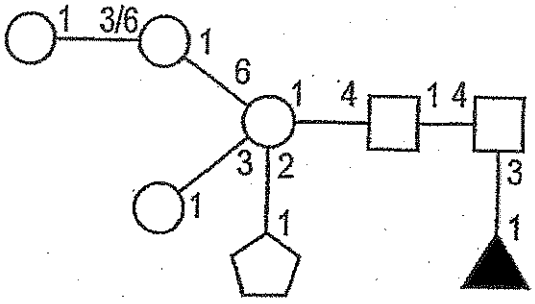


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1505.7

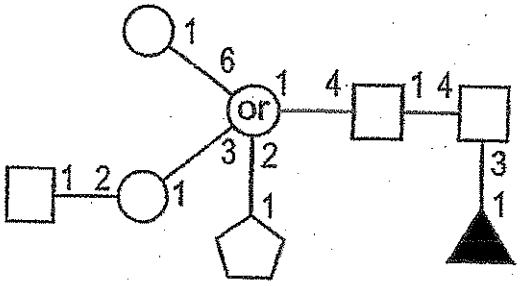
Fig. 8a



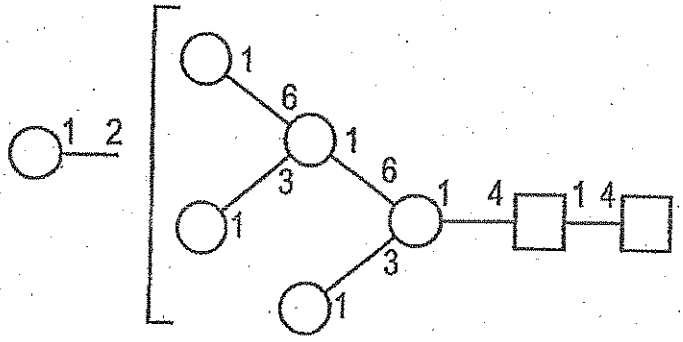
Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1579.8



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1709.7

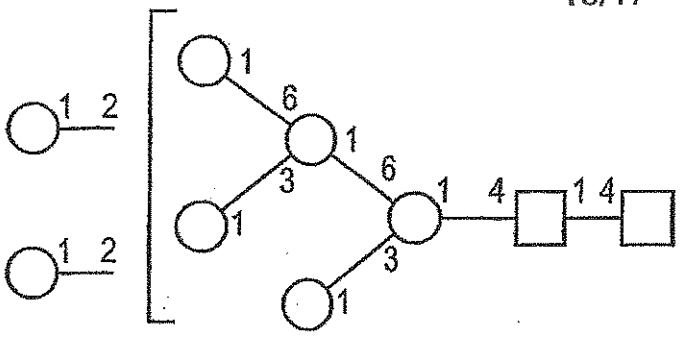


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1750.9

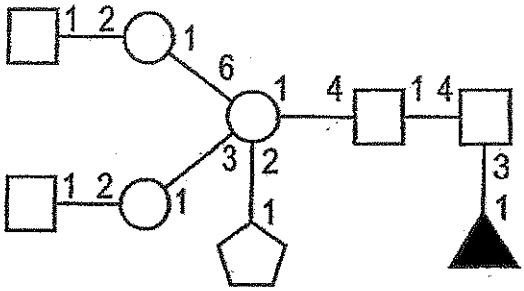


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1783.9

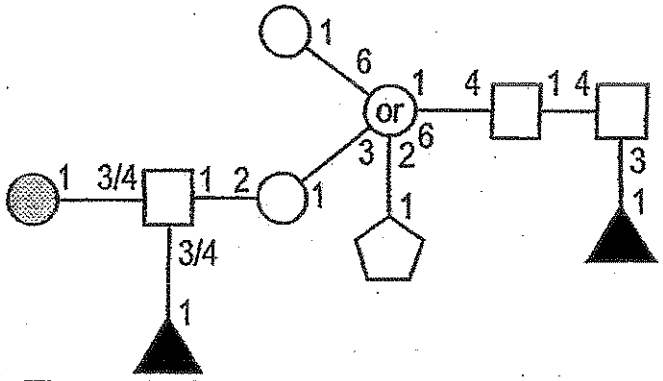
Fig. 8b



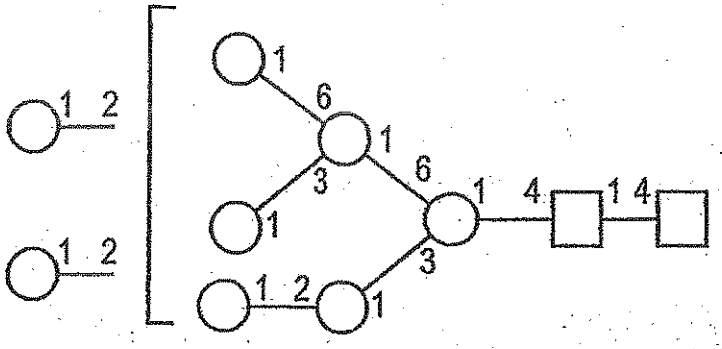
Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1989.0



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 1997.0

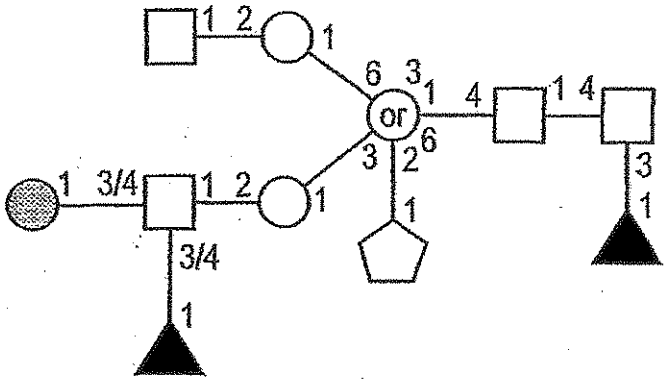


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2130.0

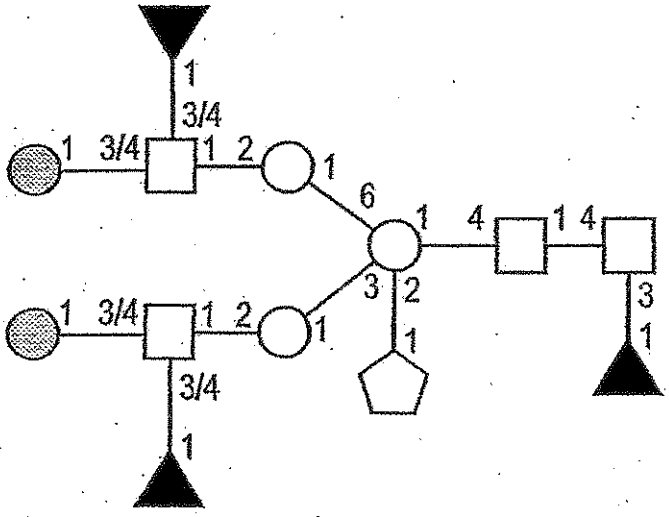


Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2193.1

Fig. 8c



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2375.2



Theoretical monoisotopic mass for $[M+Na]^+$ molecular ion = 2375.2

Key:

- Fucose
- Galactose
- N-Acetylglucosamine
- Mannose
- Xylose

Fig. 8d