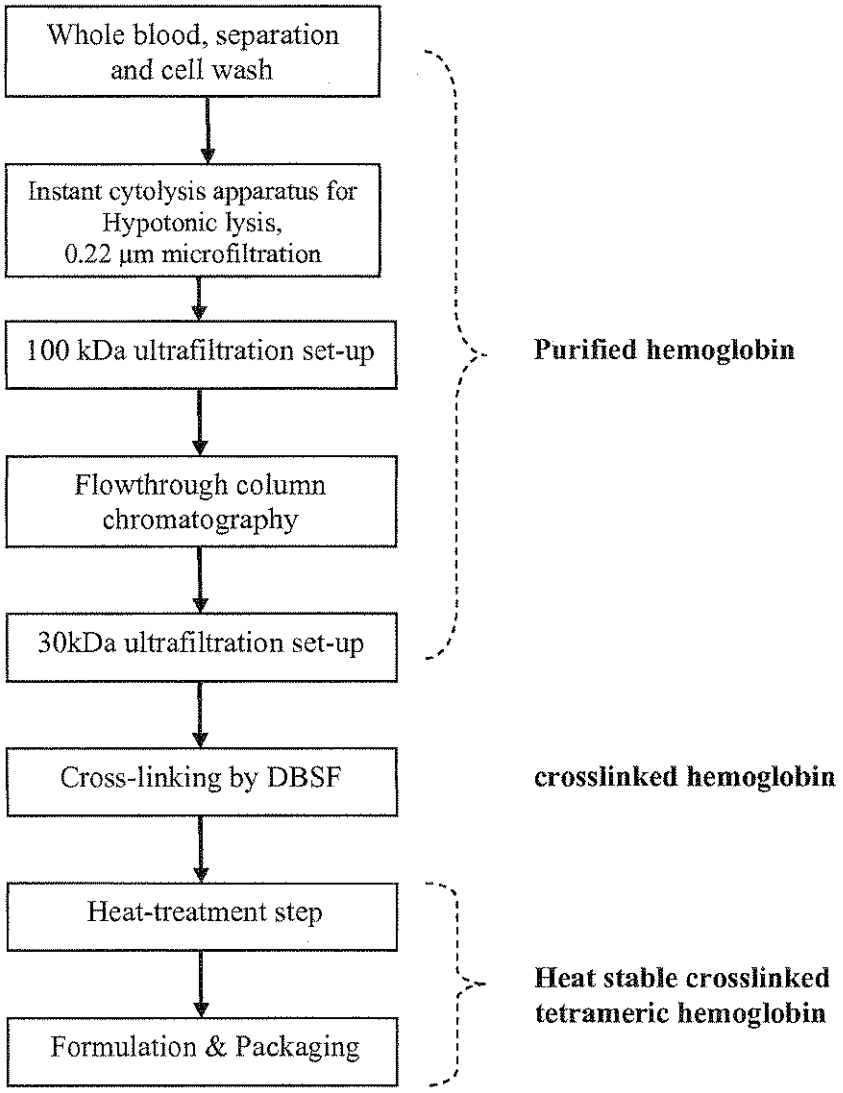

Whole blood, separation and cell wash
Instant cytolysis apparatus for Hypotonic lysis, 0.22 µm microfiltration
100 kDa ultrafiltration set-up
Flowthrough column chromatography
30kDa ultrafiltration set-up
Cross-linking by DBSF
Heat-treatment step
Formulation & Packaging
Purified hemoglobin
crosslinked hemoglobin
Heat stable crosslinked tetrameric hemoglobin

FIG. 1

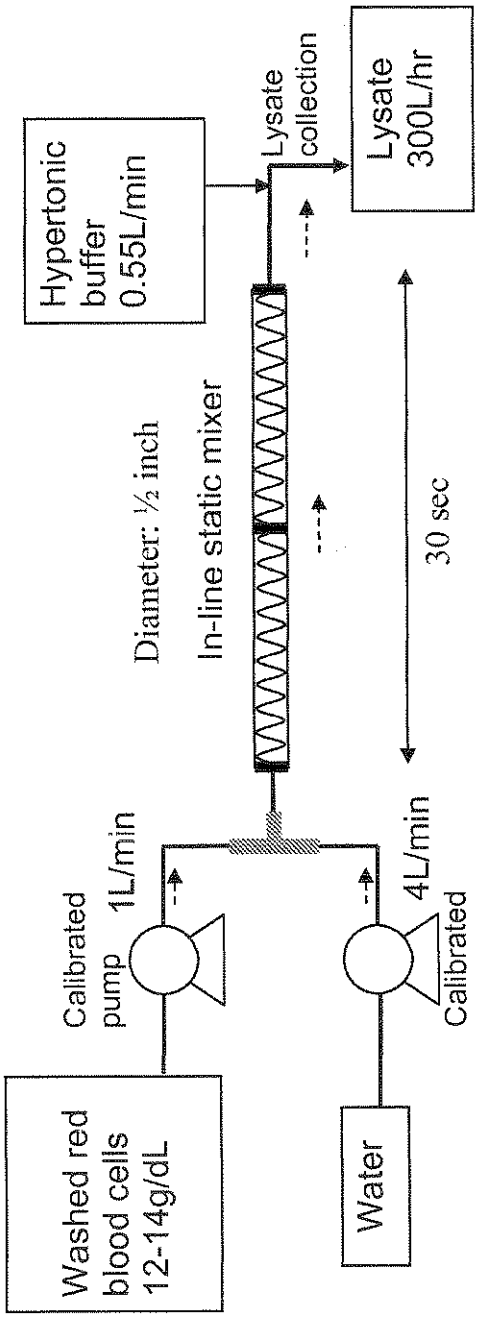
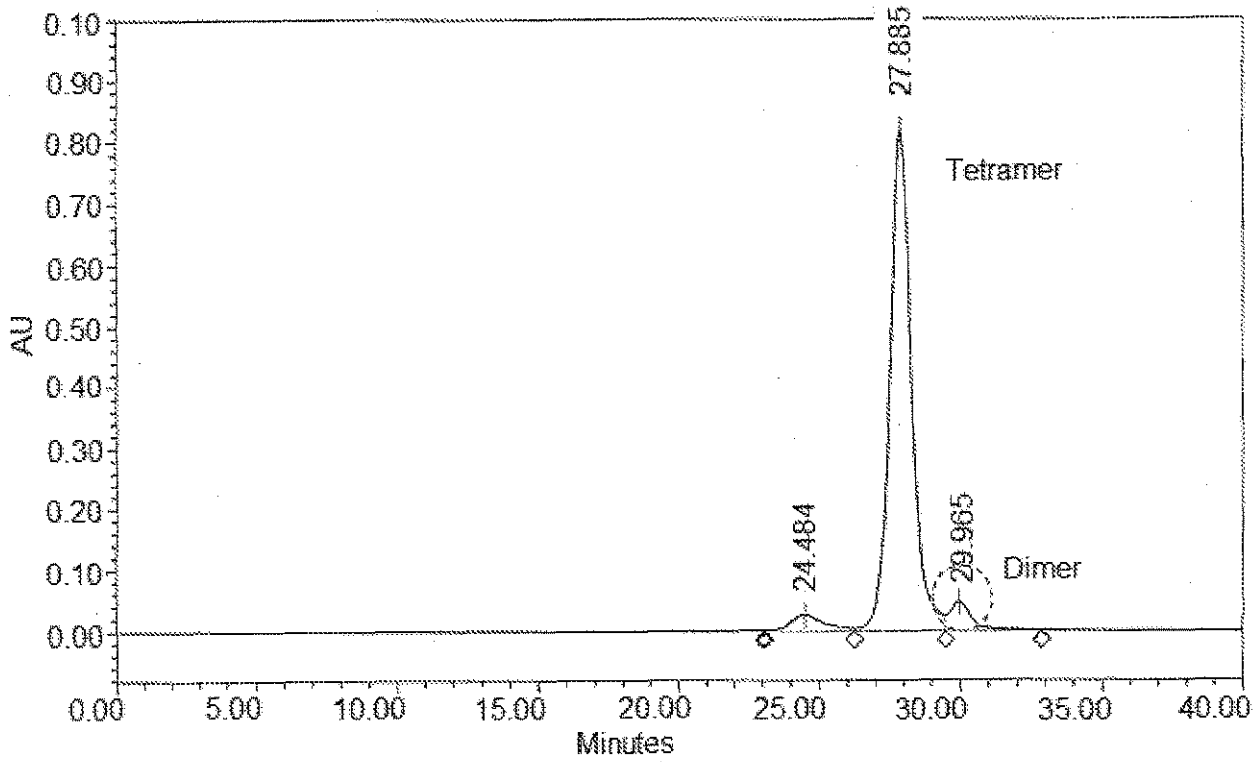
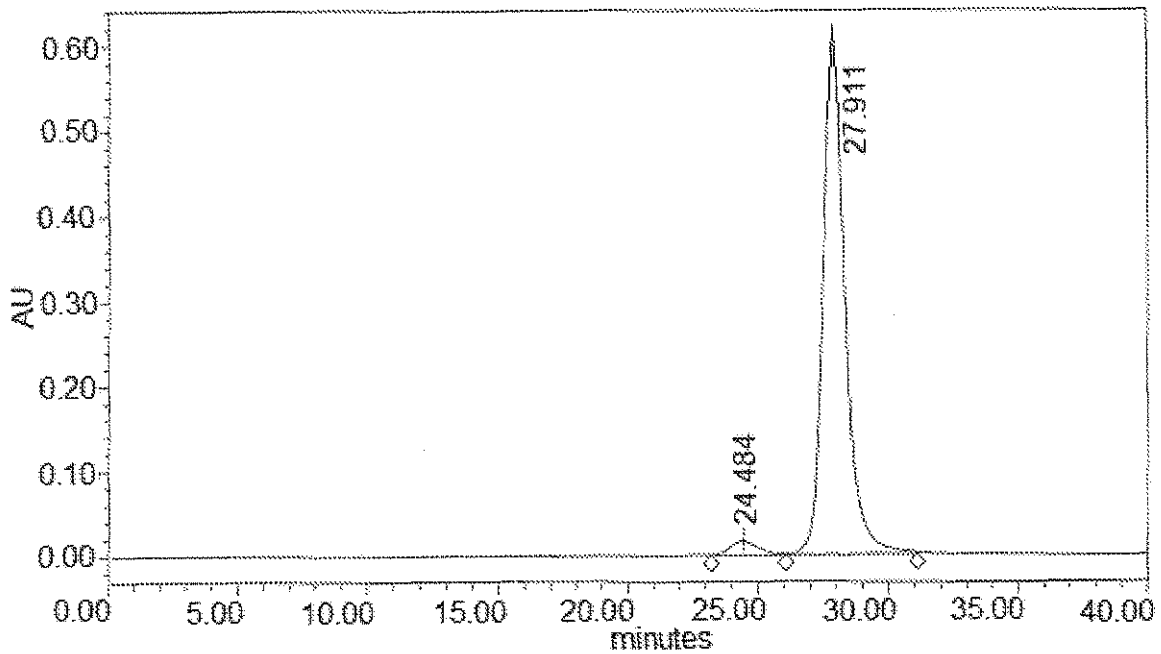

Hypertonic buffer 0.55L/min
Lysate collection
Lysate 300L/hr
Diameter: ½ inch
In-line static mixer
30 sec
Calibrated pump
1L/min
4L/min
Calibrated pump
Washed red blood cells 12-14g/dL
Water

FIG. 2



(a) No Heat Treatment



(b) Heat-treated (90°C for 45 sec - 2 min or 80°C for 30 min), stabilized crosslinked tetrameric hemoglobin

FIG. 3

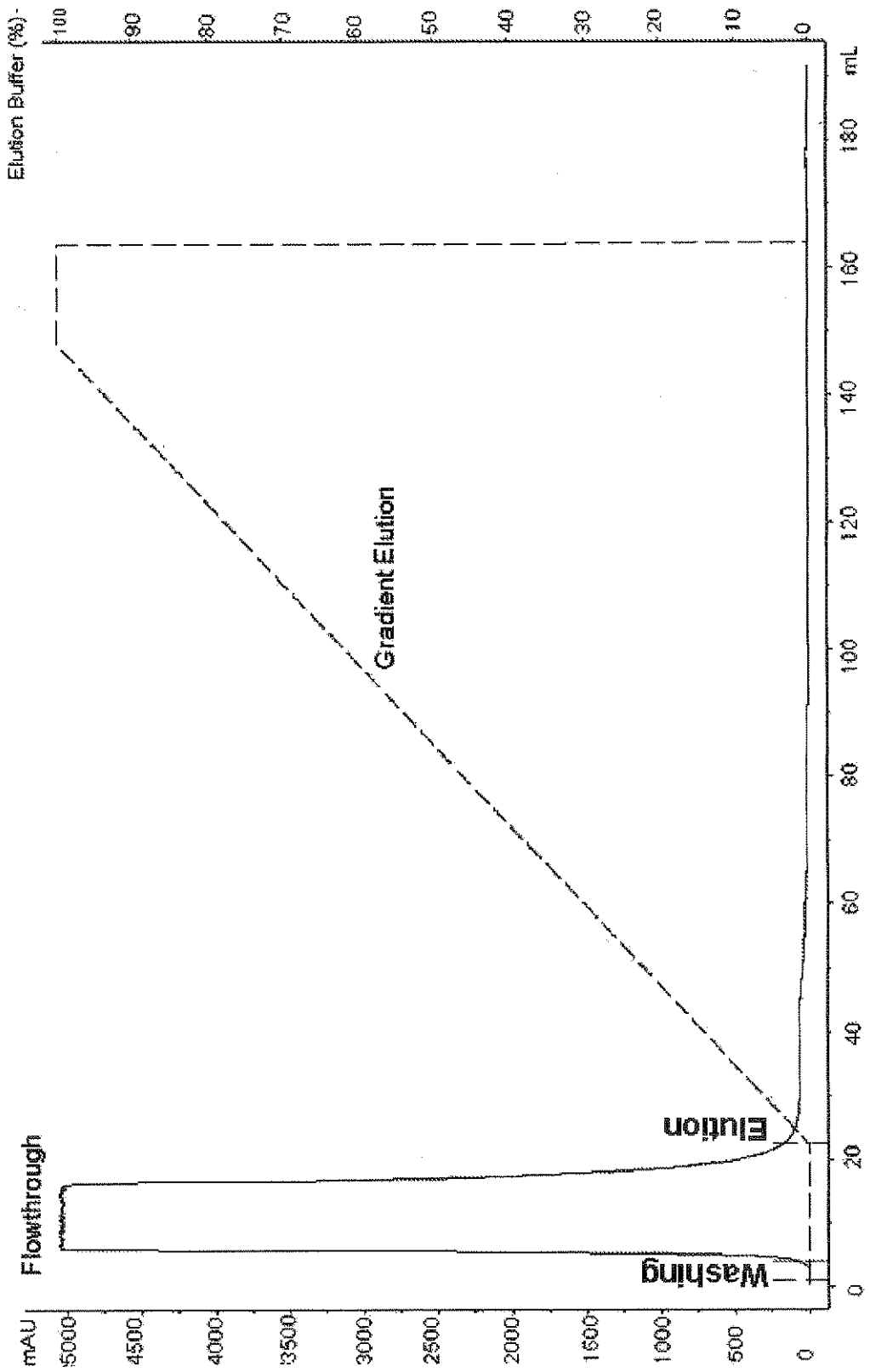

Elution Buffer (%)
100
90
80
70
60
50
40
30
20
10
0
mL
180
160
140
120
100
80
60
40
20
0
mAU
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Flowthrough
Washing
Elution
Gradient Elution

FIG. 4

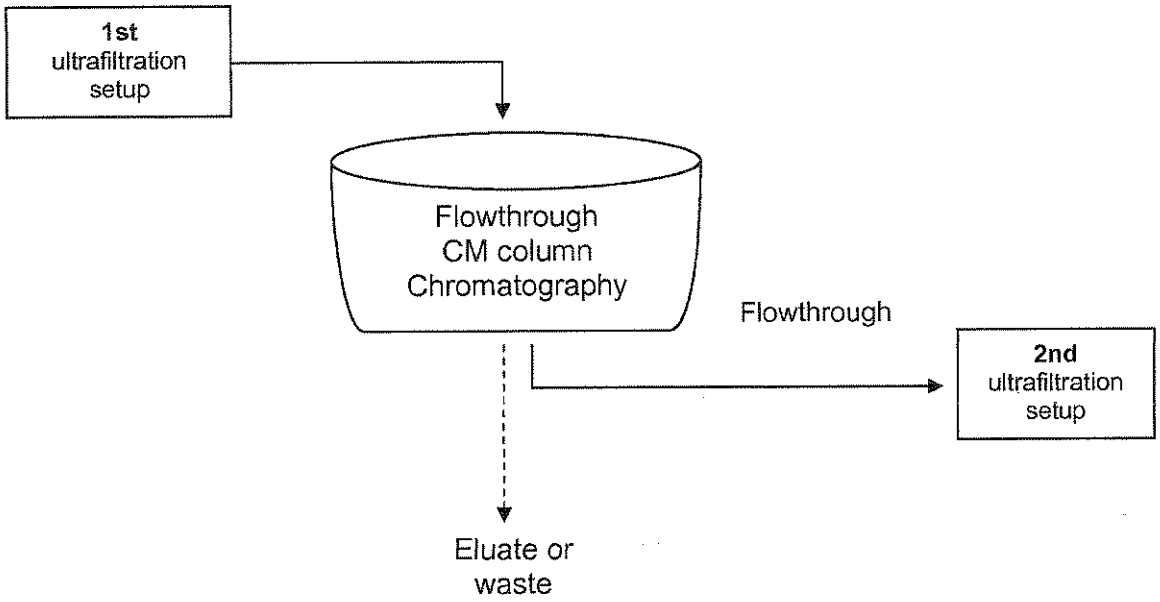

1st
ultrafiltration
setup
Flowthrough
CM column
Chromatography
Flowthrough
2nd
ultrafiltration
setup
Eluate or
waste

FIG. 5

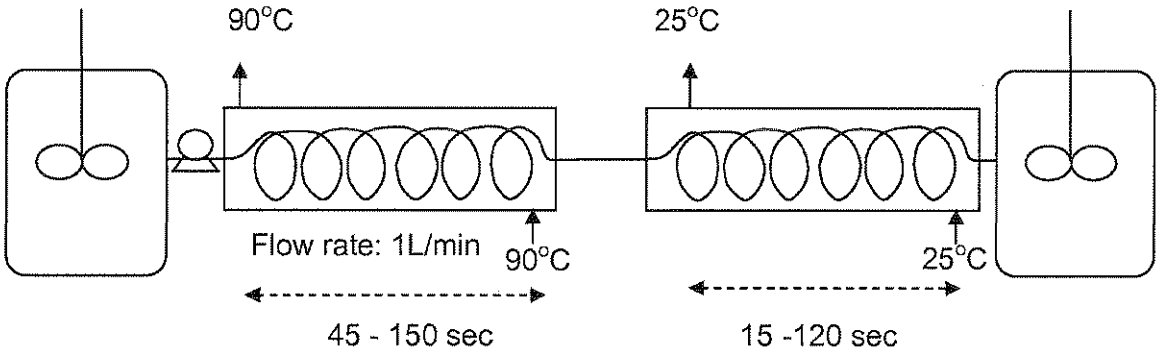


FIG. 6

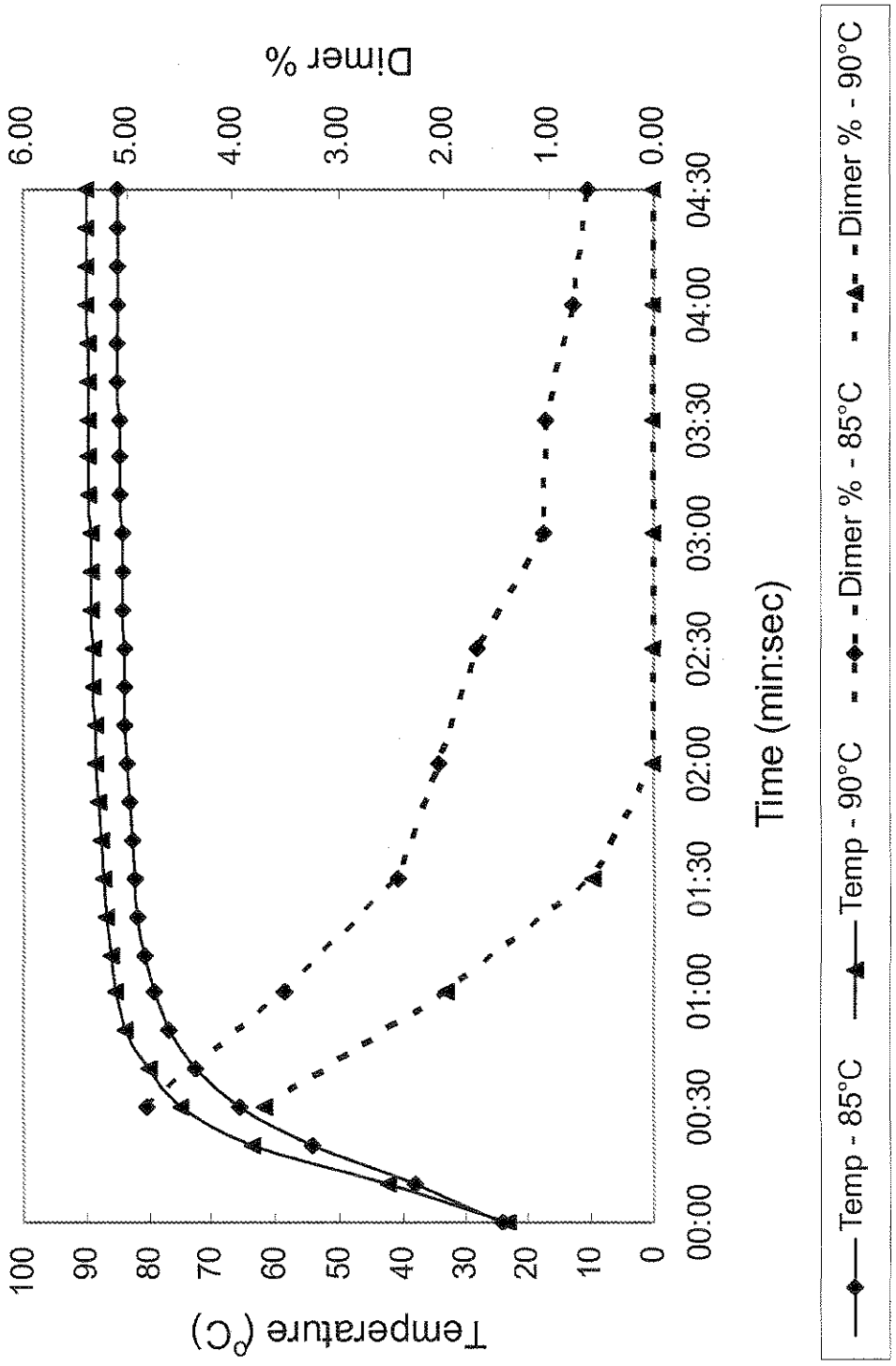

Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
Dimer %
6.00
5.00
4.00
3.00
2.00
1.00
0.00
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
Time (min:sec)
Temp - 85°C
Temp - 90°C
Dimer % - 85°C
Dimer % - 90°C

FIG. 7

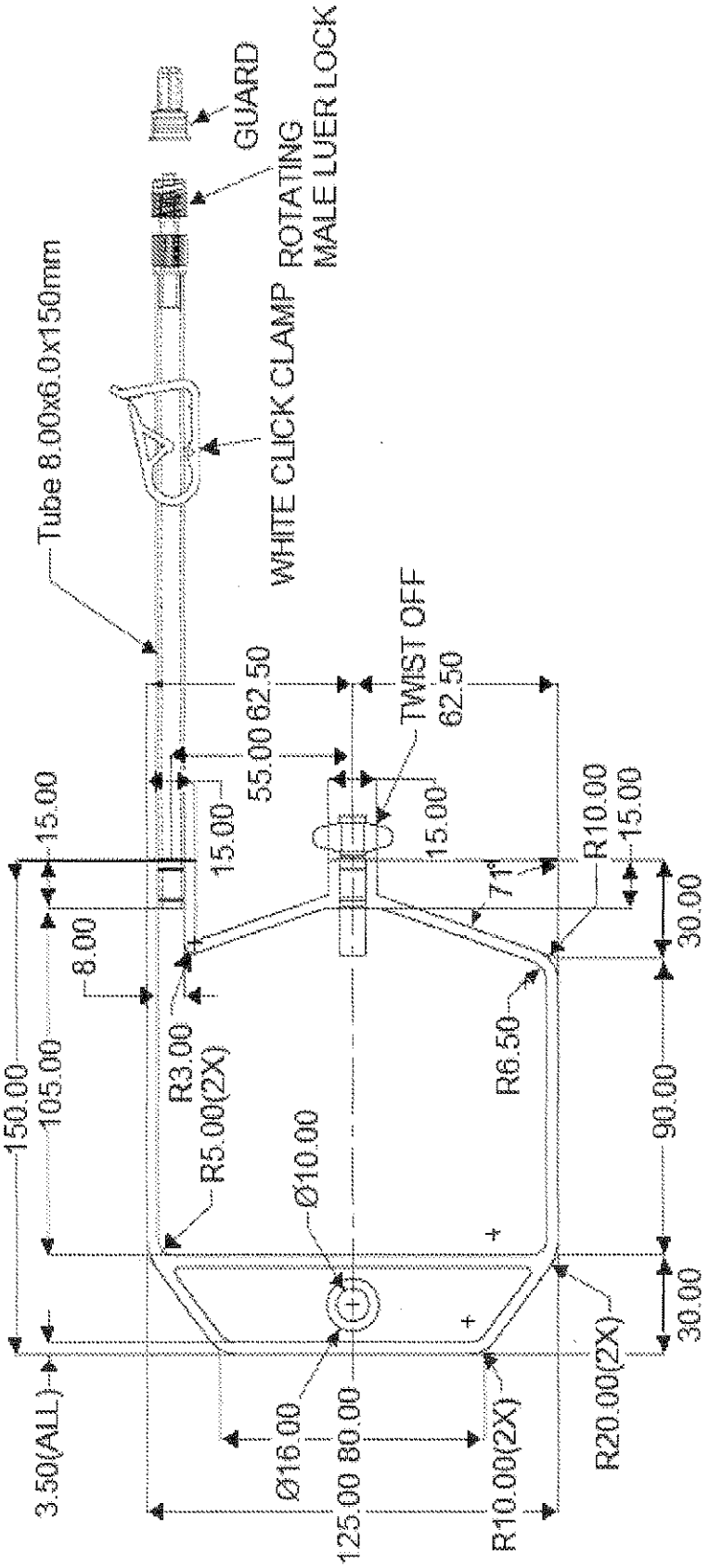

GUARD
ROTATING MALE LUER LOCK
WHITE CLICK CLAMP
Tube 8.00x6.0x150mm
TWIST OFF
62.50
55.00 62.50
15.00
15.00
15.00
15.00
R10.00
71°
30.00
8.00
R3.00
R5.00(2X)
R6.50
150.00
105.00
Ø10.00
90.00
30.00
3.50(ALL)
Ø16.00
80.00
125.00
R10.00(2X)
R20.00(2X)

FIG. 8

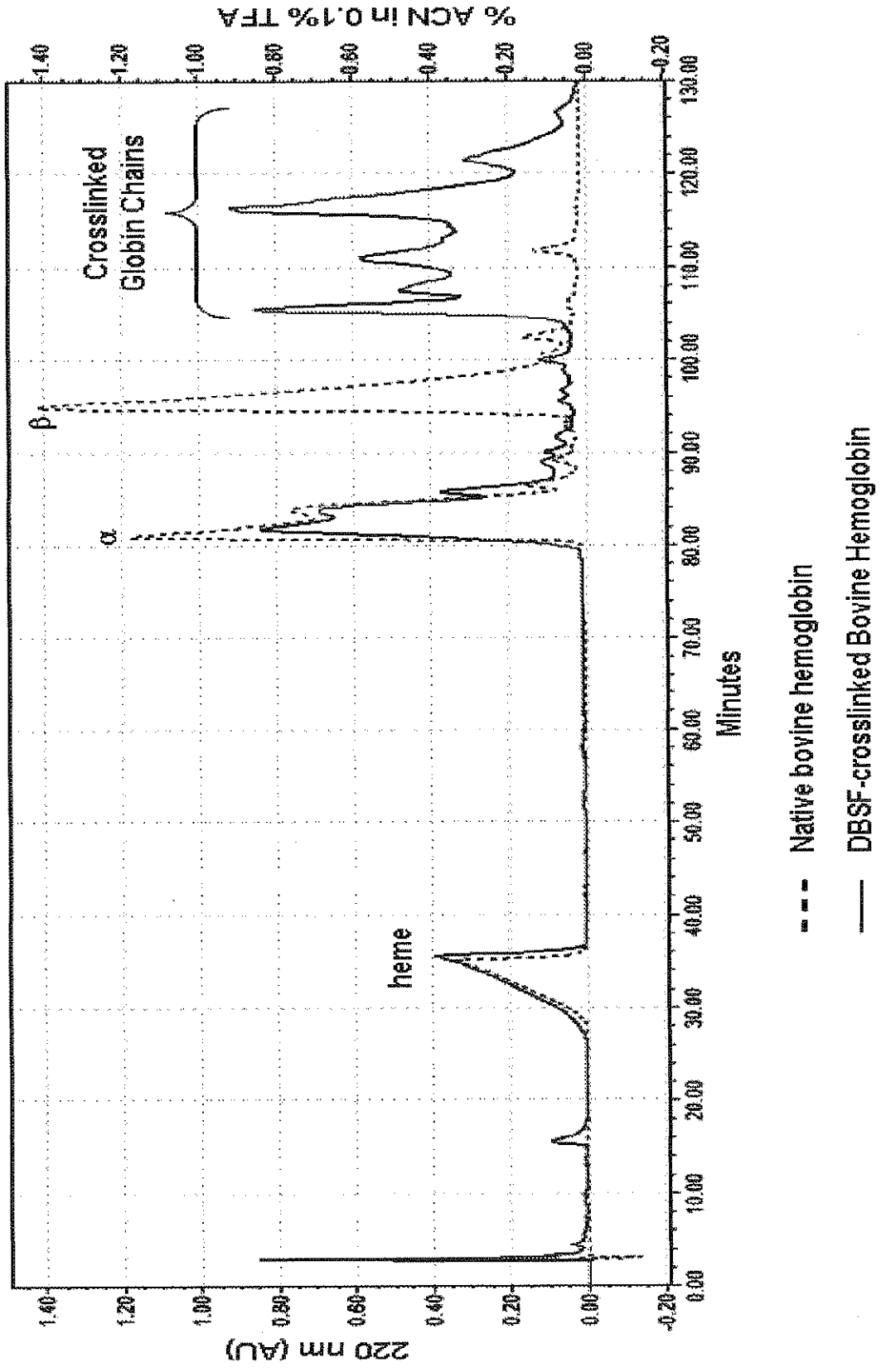
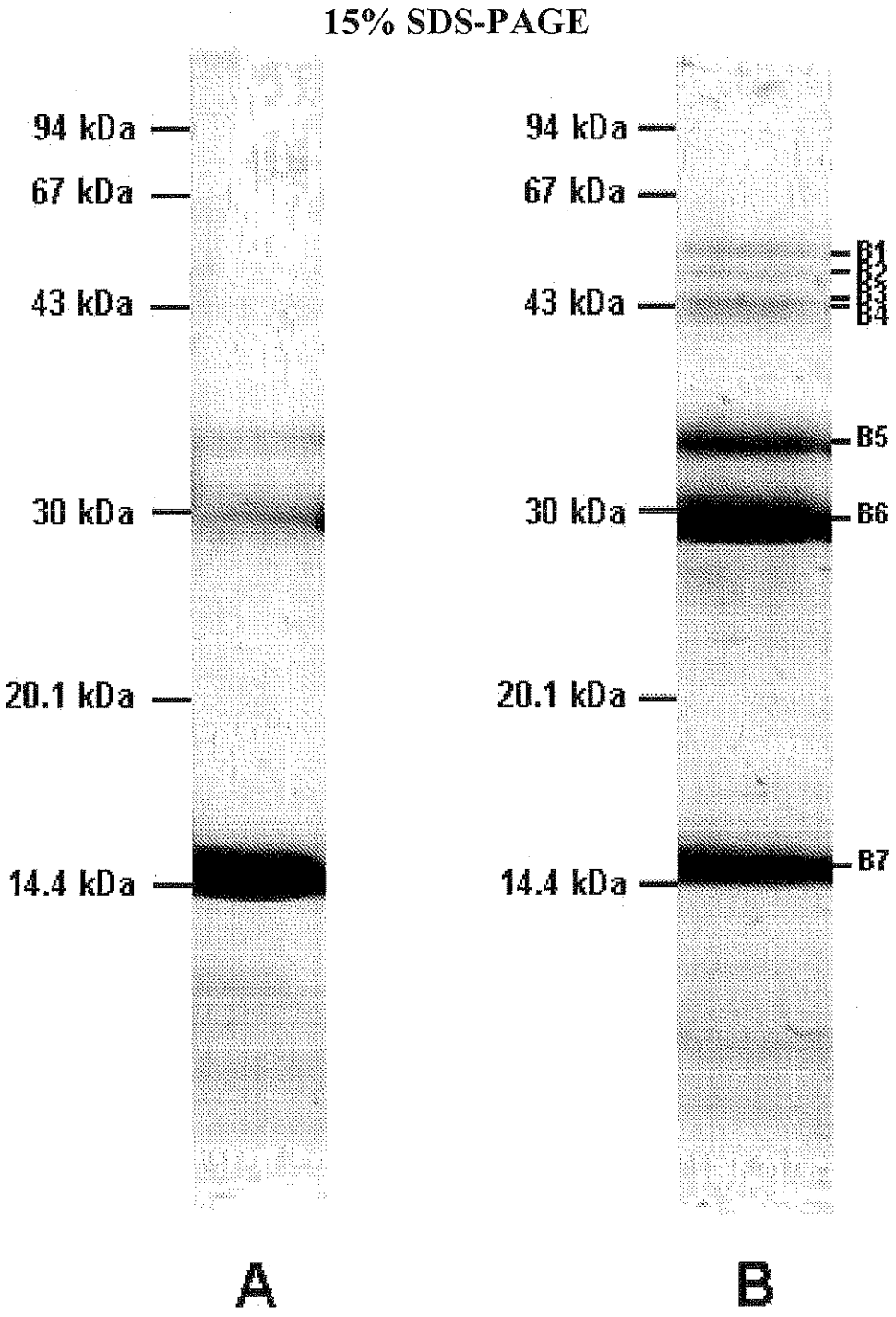

220 nm (AU)
% ACN in 0.1% TFA
Minutes
Crosslinked Globin Chains
β
α
heme
Native bovine hemoglobin
DBSF-crosslinked Bovine Hemoglobin

FIG. 9



A) Native Bovine Hemoglobin
B) Hemoglobin crosslinked with DBSF under deoxygenated condition

FIG.10

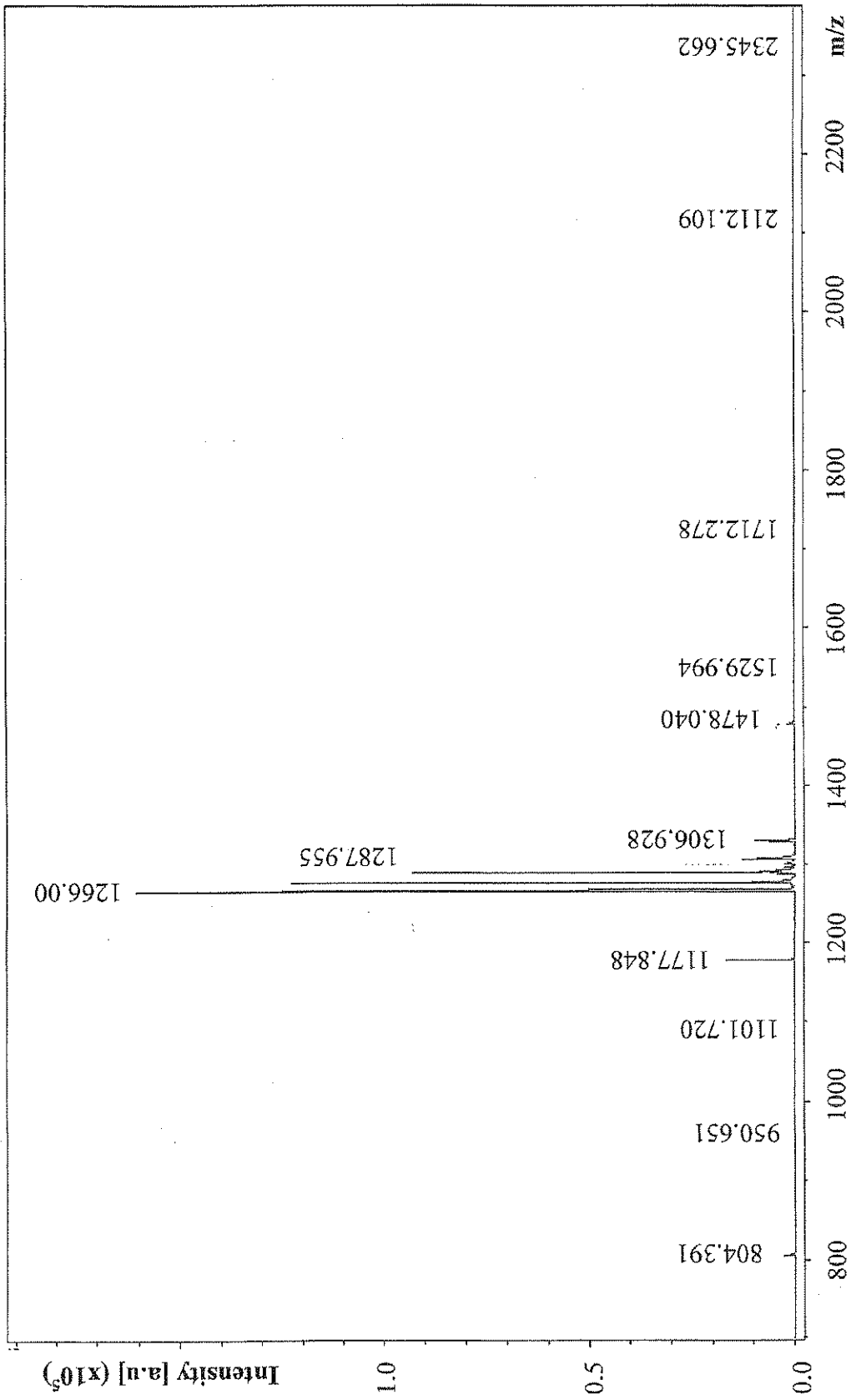

Intensity [a.u] (x10⁵)
0.0
0.5
1.0
m/z
800
1000
1200
1400
1600
1800
2000
2200
804.391
950.651
1101.720
1177.848
1266.00
1287.955
1306.928
1478.040
1529.994
1712.278
2112.109
2345.662

FIG.11