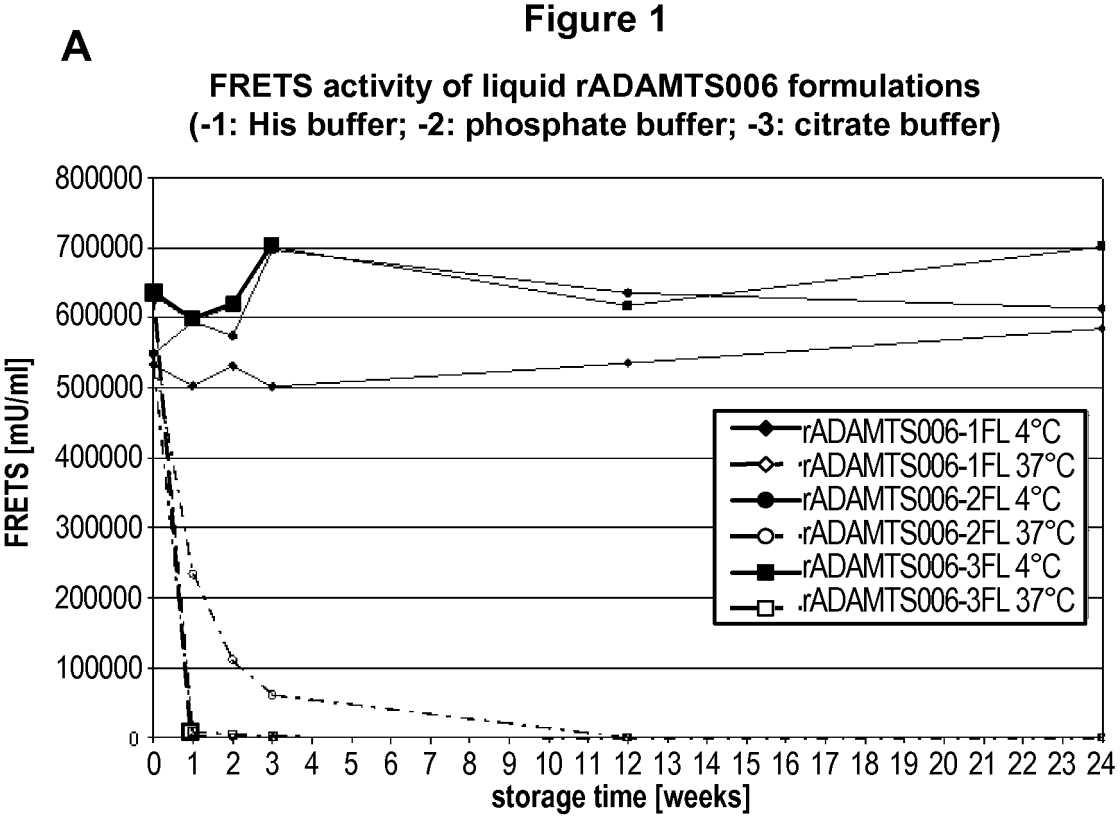
Figure 1

A


FRETS activity of liquid rADAMTS006 formulations
(-1: His buffer; -2: phosphate buffer; -3: citrate buffer)
FRETS [mU/ml]
800000
700000
600000
500000
400000
300000
200000
100000
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
storage time [weeks]
rADAMTS006-1FL 4°C
rADAMTS006-1FL 37°C
rADAMTS006-2FL 4°C
rADAMTS006-2FL 37°C
rADAMTS006-3FL 4°C
rADAMTS006-3FL 37°C

B

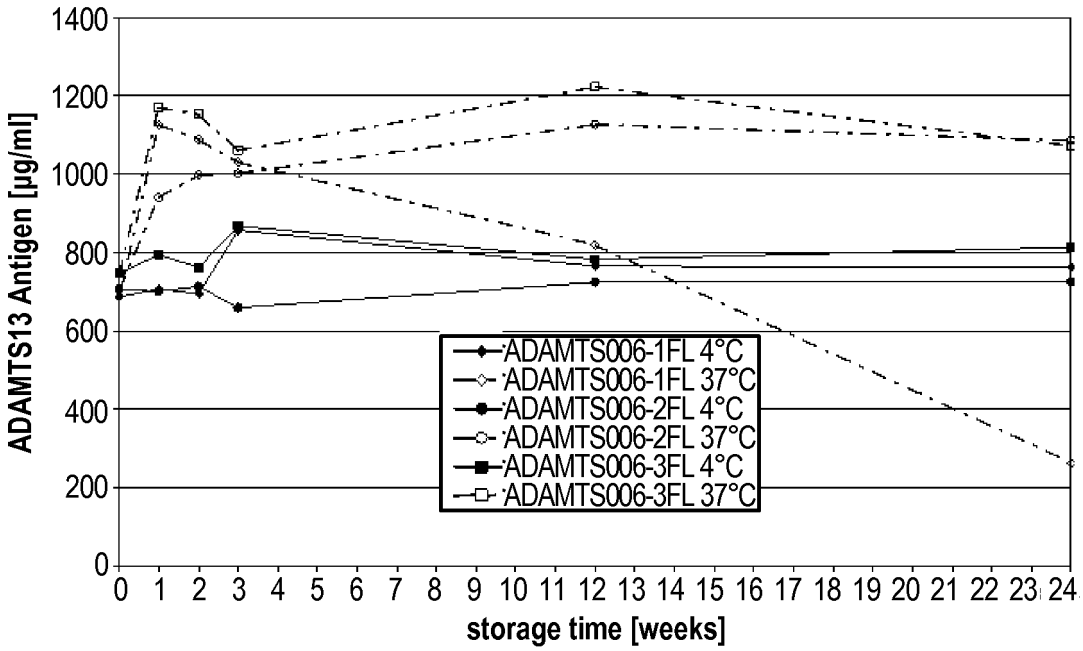
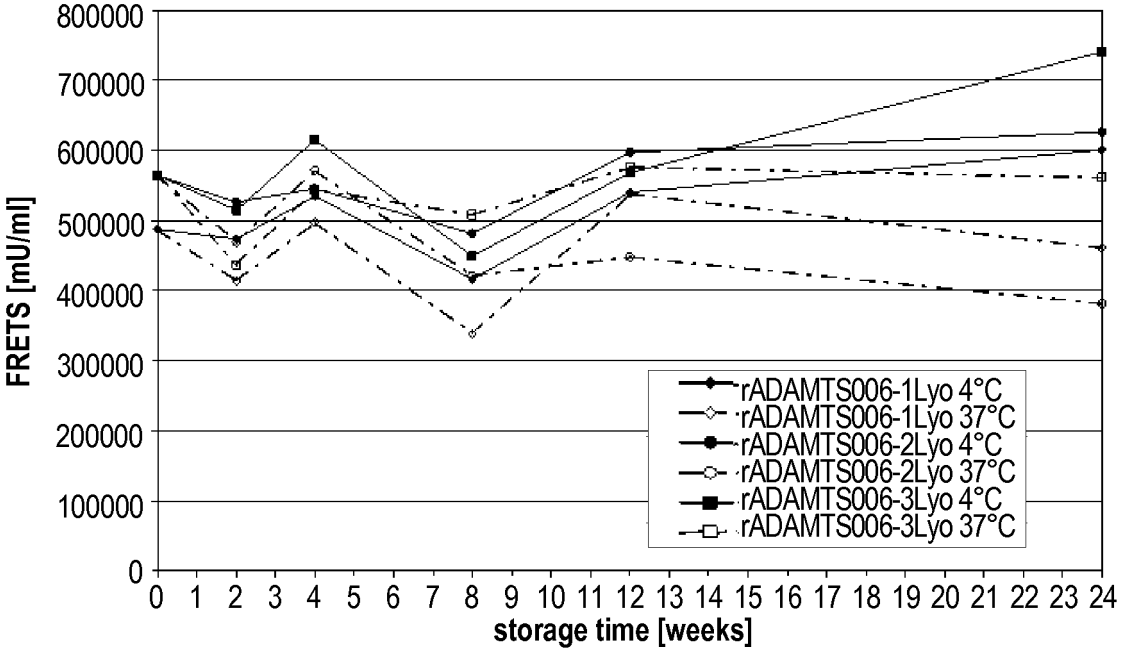

ADAMTS13 antigen of liquid rADAMTS006 formulations
(-1: His buffer; -2: phosphate buffer; -3: citrate buffer)
ADAMTS13 Antigen [µg/ml]
1400
1200
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
storage time [weeks]
ADAMTS006-1FL 4°C
ADAMTS006-1FL 37°C
ADAMTS006-2FL 4°C
ADAMTS006-2FL 37°C
ADAMTS006-3FL 4°C
ADAMTS006-3FL 37°C

Figure 2

A


FRETS activity of lyophilized rADAMTS006 formulations
(-1: His buffer; -2: phosphate buffer; -3: citrate buffer)
FRETS [mU/ml]
800000
700000
600000
500000
400000
300000
200000
100000
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
storage time [weeks]
rADAMTS006-1Lyo 4°C
rADAMTS006-1Lyo 37°C
rADAMTS006-2Lyo 4°C
rADAMTS006-2Lyo 37°C
rADAMTS006-3Lyo 4°C
rADAMTS006-3Lyo 37°C

B

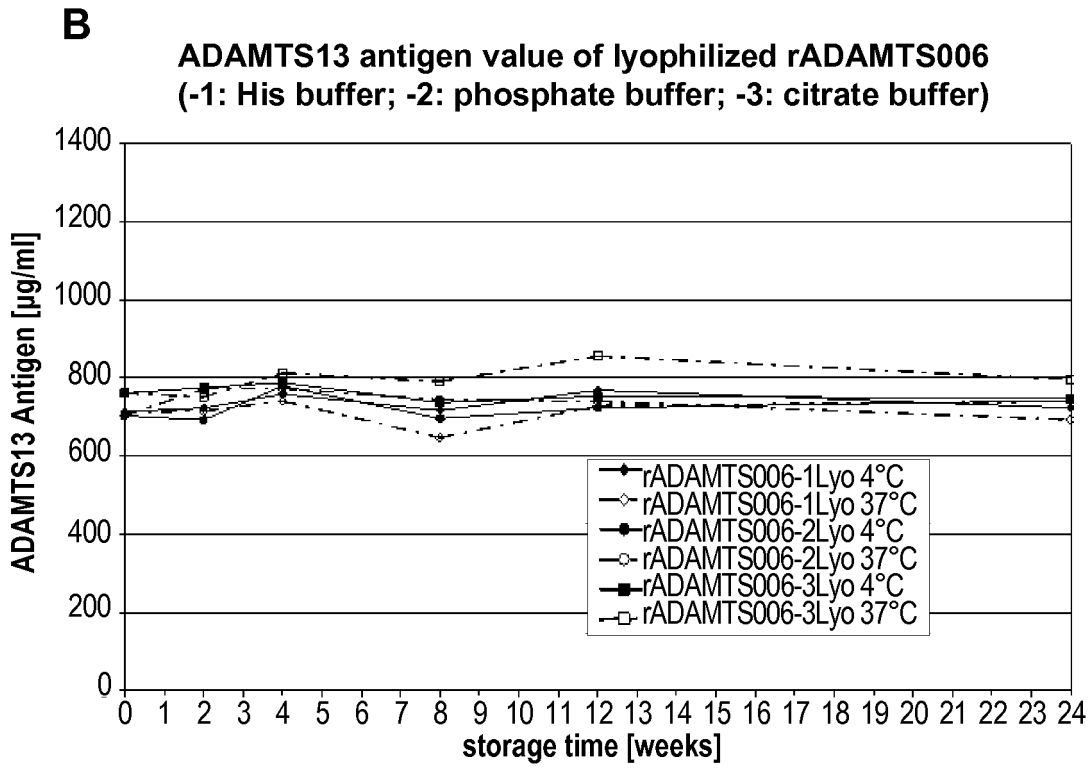

ADAMTS13 antigen value of lyophilized rADAMTS006
(-1: His buffer; -2: phosphate buffer; -3: citrate buffer)
ADAMTS13 Antigen [µg/ml]
1400
1200
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
storage time [weeks]
rADAMTS006-1Lyo 4°C
rADAMTS006-1Lyo 37°C
rADAMTS006-2Lyo 4°C
rADAMTS006-2Lyo 37°C
rADAMTS006-3Lyo 4°C
rADAMTS006-3Lyo 37°C

Figure 3

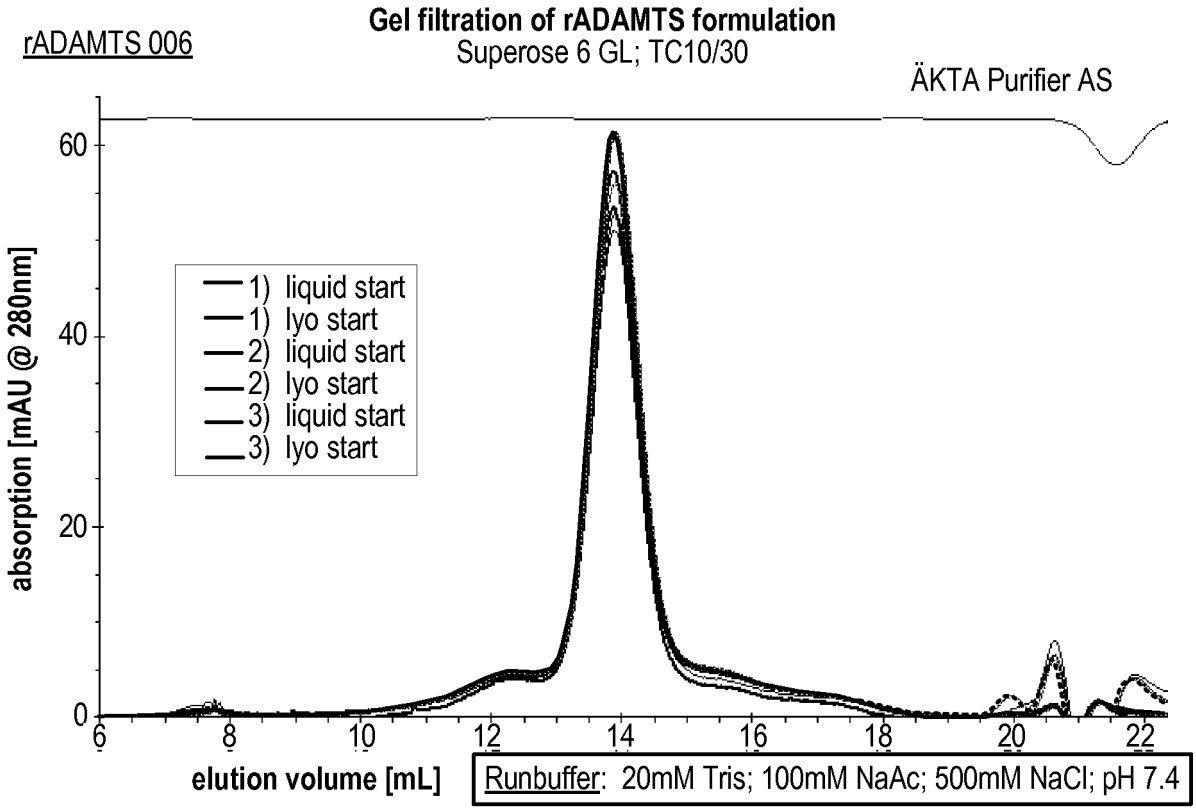
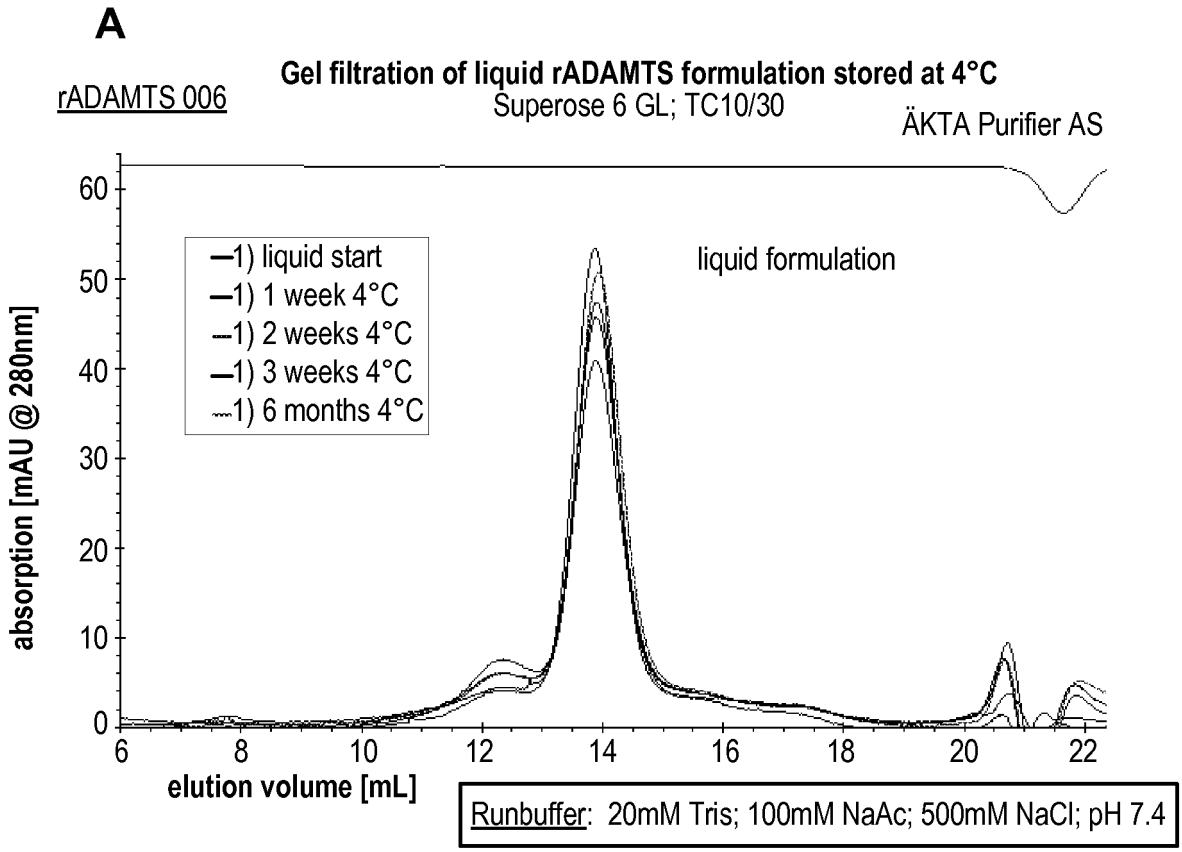

rADAMTS 006
Gel filtration of rADAMTS formulation
Superose 6 GL; TC10/30
ÄKTA Purifier AS
absorption [mAU @ 280nm]
elution volume [mL]
1) liquid start
1) lyo start
2) liquid start
2) lyo start
3) liquid start
3) lyo start
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

Figure 4


A
Gel filtration of liquid rADAMTS formulation stored at 4°C
rADAMTS 006
Superose 6 GL; TC10/30
ÄKTA Purifier AS
liquid formulation
1) liquid start
1) 1 week 4°C
1) 2 weeks 4°C
1) 3 weeks 4°C
1) 6 months 4°C
absorption [mAU @ 280nm]
elution volume [mL]
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

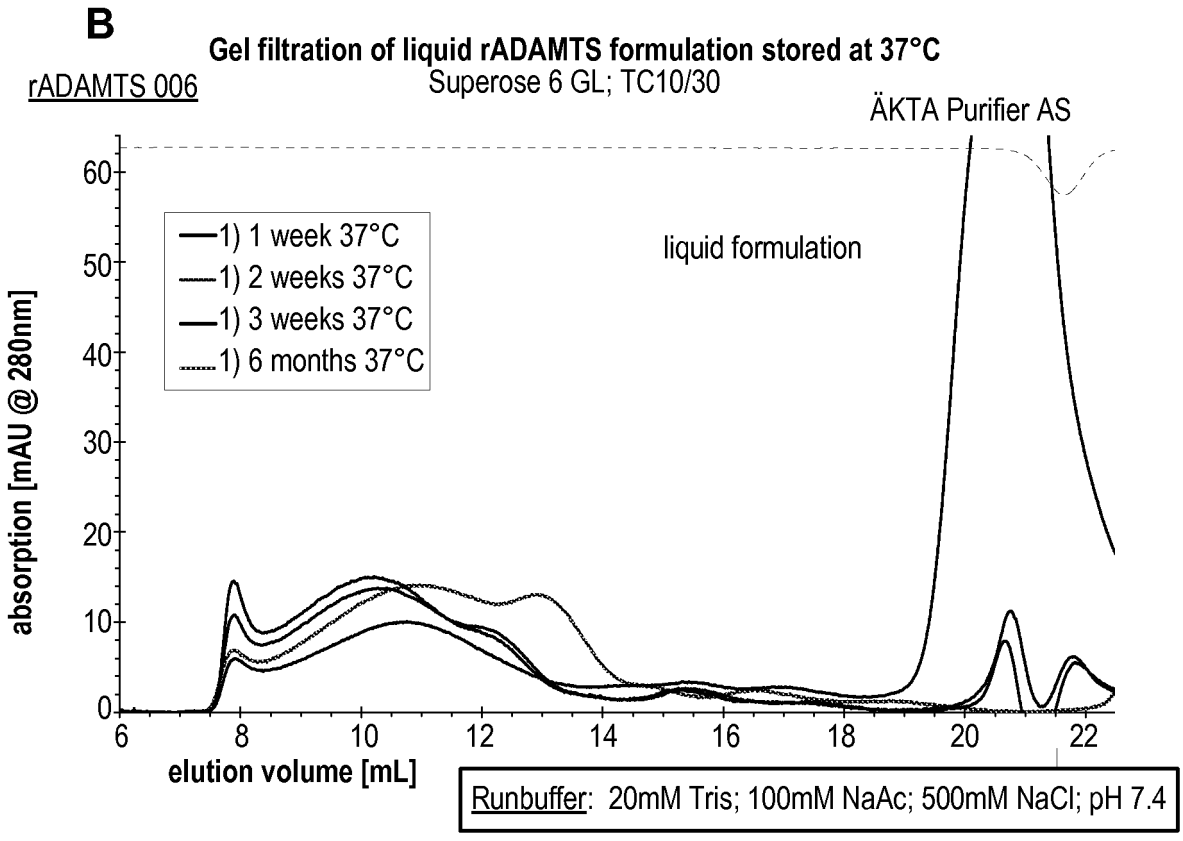
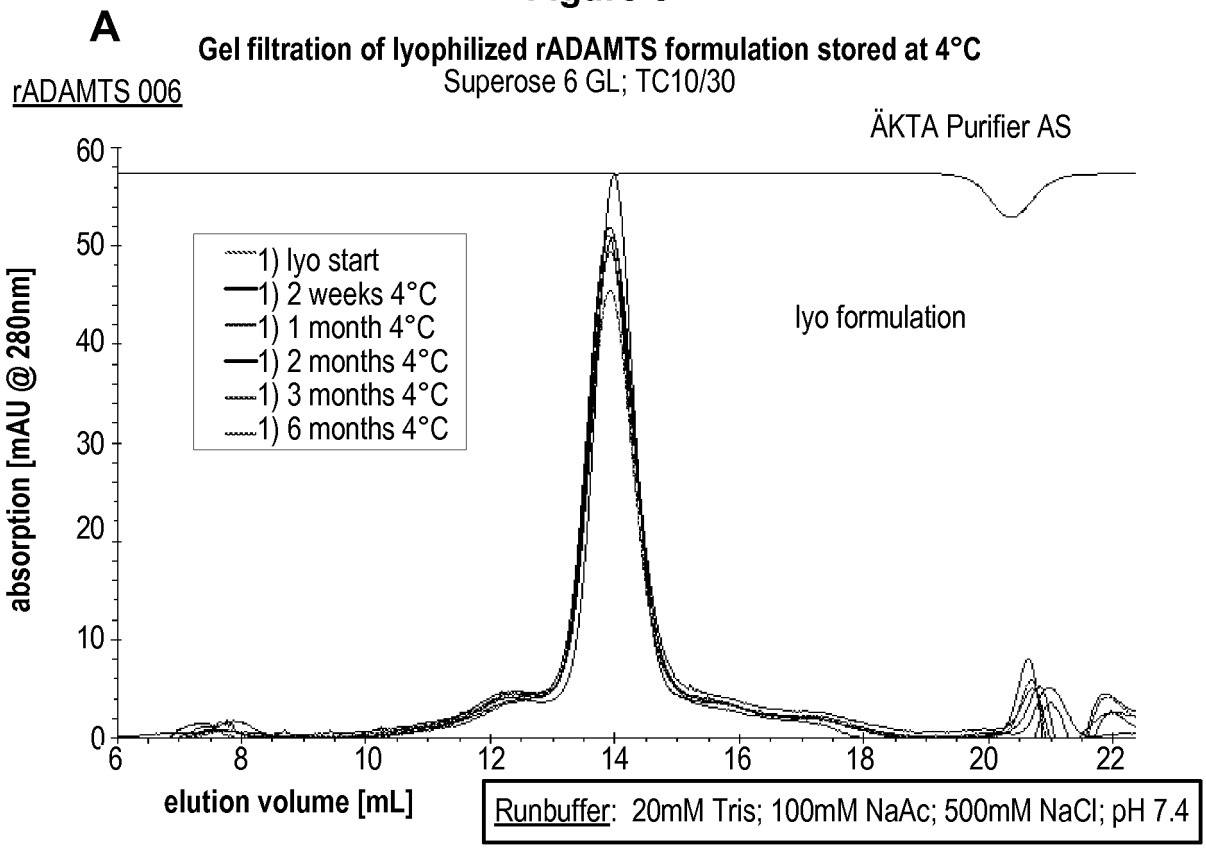

B
Gel filtration of liquid rADAMTS formulation stored at 37°C
rADAMTS 006
Superose 6 GL; TC10/30
ÄKTA Purifier AS
liquid formulation
1) 1 week 37°C
1) 2 weeks 37°C
1) 3 weeks 37°C
1) 6 months 37°C
absorption [mAU @ 280nm]
elution volume [mL]
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

Figure 5


A
Gel filtration of lyophilized rADAMTS formulation stored at 4°C
Superose 6 GL; TC10/30
rADAMTS 006
ÄKTA Purifier AS
1) lyo start
1) 2 weeks 4°C
1) 1 month 4°C
1) 2 months 4°C
1) 3 months 4°C
1) 6 months 4°C
lyo formulation
absorption [mAU @ 280nm]
elution volume [mL]
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

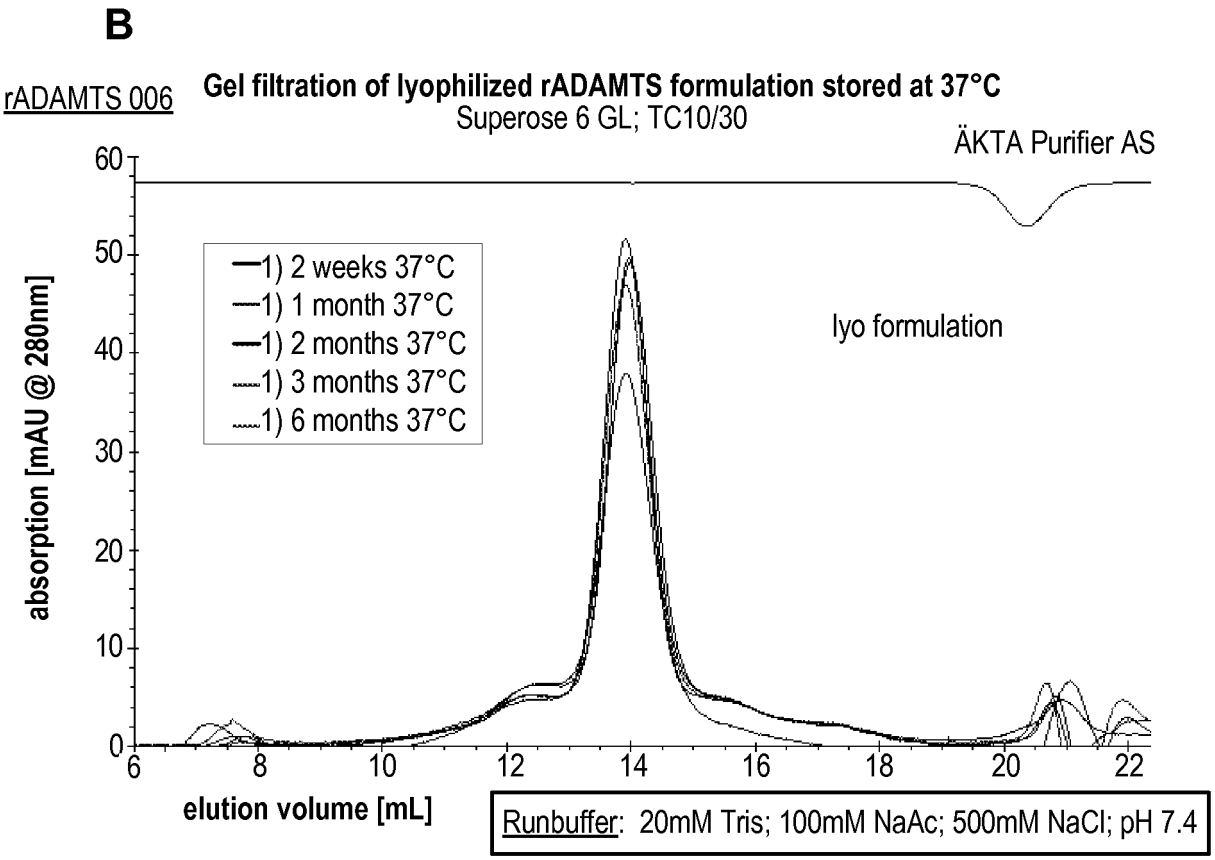

B
rADAMTS 006
Gel filtration of lyophilized rADAMTS formulation stored at 37°C
Superose 6 GL; TC10/30
ÄKTA Purifier AS
1) 2 weeks 37°C
1) 1 month 37°C
1) 2 months 37°C
1) 3 months 37°C
1) 6 months 37°C
lyo formulation
absorption [mAU @ 280nm]
elution volume [mL]
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

Figure 6

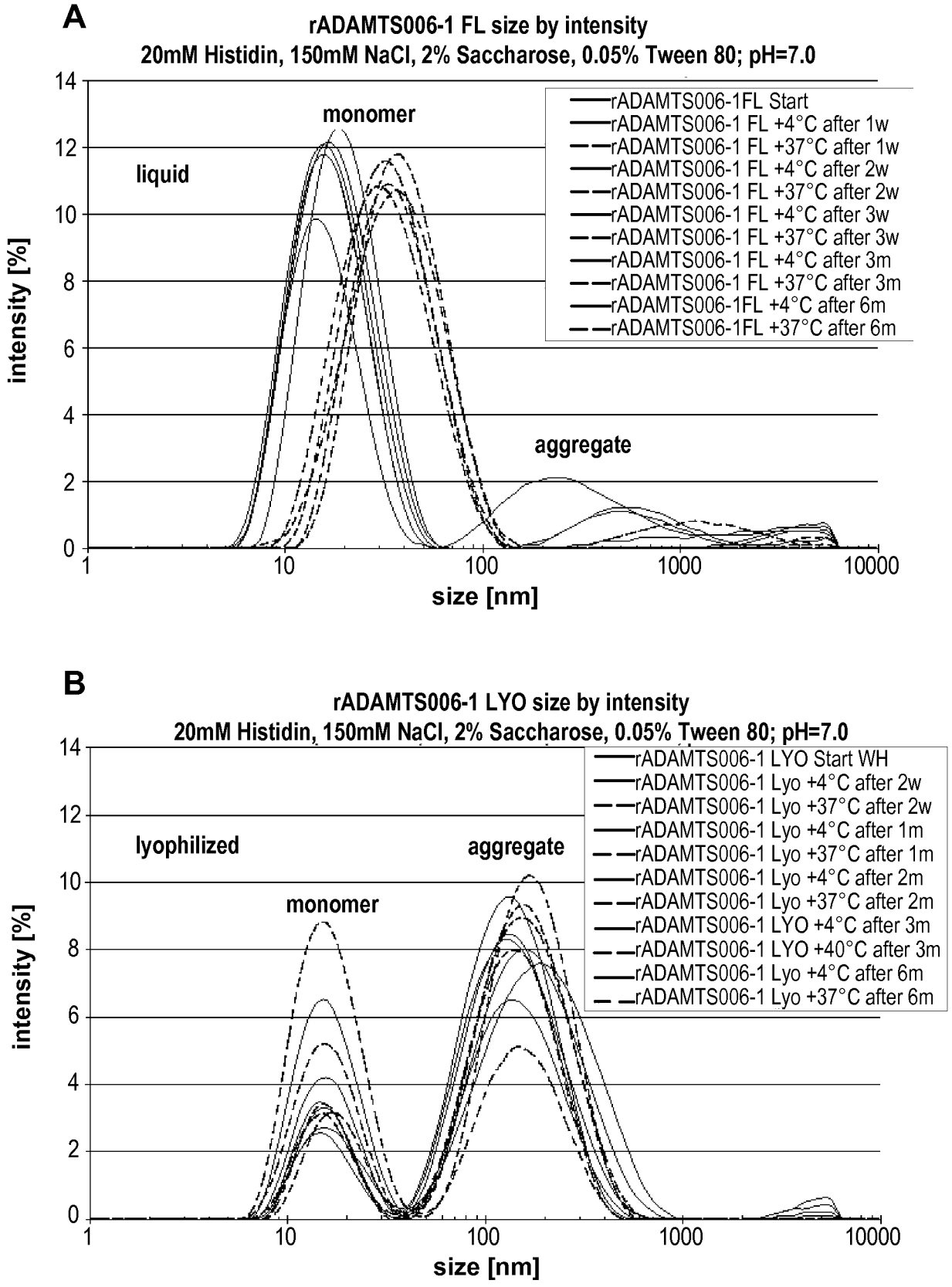

A
rADAMTS006-1 FL size by intensity
20mM Histidin, 150mM NaCl, 2% Saccharose, 0.05% Tween 80; pH=7.0
monomer
liquid
aggregate
rADAMTS006-1FL Start
rADAMTS006-1 FL +4°C after 1w
rADAMTS006-1 FL +37°C after 1w
rADAMTS006-1 FL +4°C after 2w
rADAMTS006-1 FL +37°C after 2w
rADAMTS006-1 FL +4°C after 3w
rADAMTS006-1 FL +37°C after 3w
rADAMTS006-1 FL +4°C after 3m
rADAMTS006-1 FL +37°C after 3m
rADAMTS006-1FL +4°C after 6m
rADAMTS006-1FL +37°C after 6m
intensity [%]
size [nm]
B
rADAMTS006-1 LYO size by intensity
20mM Histidin, 150mM NaCl, 2% Saccharose, 0.05% Tween 80; pH=7.0
lyophilized
aggregate
monomer
rADAMTS006-1 LYO Start WH
rADAMTS006-1 Lyo +4°C after 2w
rADAMTS006-1 Lyo +37°C after 2w
rADAMTS006-1 Lyo +4°C after 1m
rADAMTS006-1 Lyo +37°C after 1m
rADAMTS006-1 Lyo +4°C after 2m
rADAMTS006-1 Lyo +37°C after 2m
rADAMTS006-1 LYO +4°C after 3m
rADAMTS006-1 LYO +40°C after 3m
rADAMTS006-1 Lyo +4°C after 6m
rADAMTS006-1 Lyo +37°C after 6m
intensity [%]
size [nm]

Figure 7

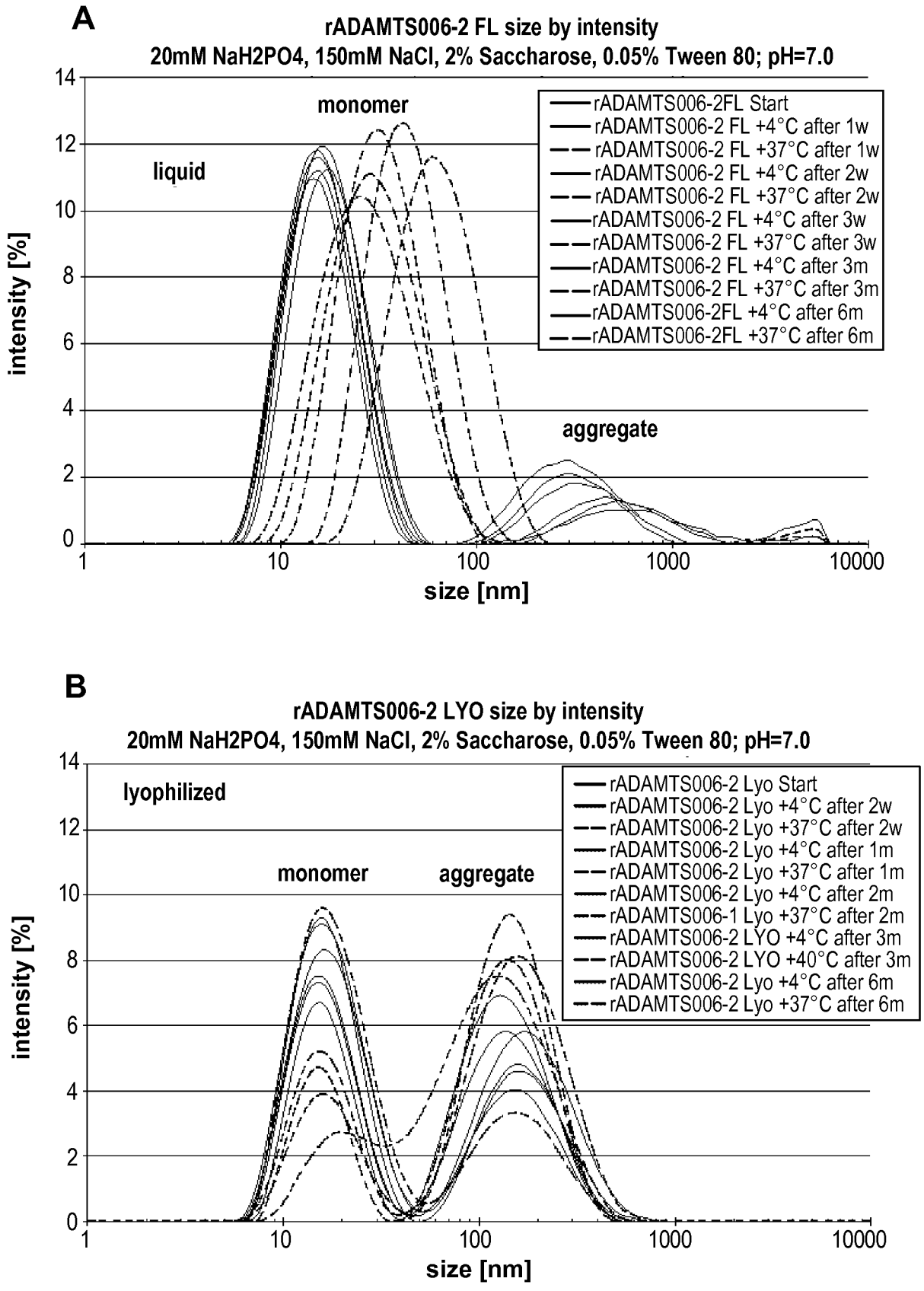

A
rADAMTS006-2 FL size by intensity
20mM NaH2PO4, 150mM NaCl, 2% Saccharose, 0.05% Tween 80; pH=7.0
monomer
liquid
aggregate
rADAMTS006-2FL Start
rADAMTS006-2 FL +4°C after 1w
rADAMTS006-2 FL +37°C after 1w
rADAMTS006-2 FL +4°C after 2w
rADAMTS006-2 FL +37°C after 2w
rADAMTS006-2 FL +4°C after 3w
rADAMTS006-2 FL +37°C after 3w
rADAMTS006-2 FL +4°C after 3m
rADAMTS006-2 FL +37°C after 3m
rADAMTS006-2FL +4°C after 6m
rADAMTS006-2FL +37°C after 6m
intensity [%]
size [nm]
B
rADAMTS006-2 LYO size by intensity
20mM NaH2PO4, 150mM NaCl, 2% Saccharose, 0.05% Tween 80; pH=7.0
lyophilized
monomer
aggregate
rADAMTS006-2 Lyo Start
rADAMTS006-2 Lyo +4°C after 2w
rADAMTS006-2 Lyo +37°C after 2w
rADAMTS006-2 Lyo +4°C after 1m
rADAMTS006-2 Lyo +37°C after 1m
rADAMTS006-2 Lyo +4°C after 2m
rADAMTS006-1 Lyo +37°C after 2m
rADAMTS006-2 LYO +4°C after 3m
rADAMTS006-2 LYO +40°C after 3m
rADAMTS006-2 Lyo +4°C after 6m
rADAMTS006-2 Lyo +37°C after 6m
intensity [%]
size [nm]

Figure 8

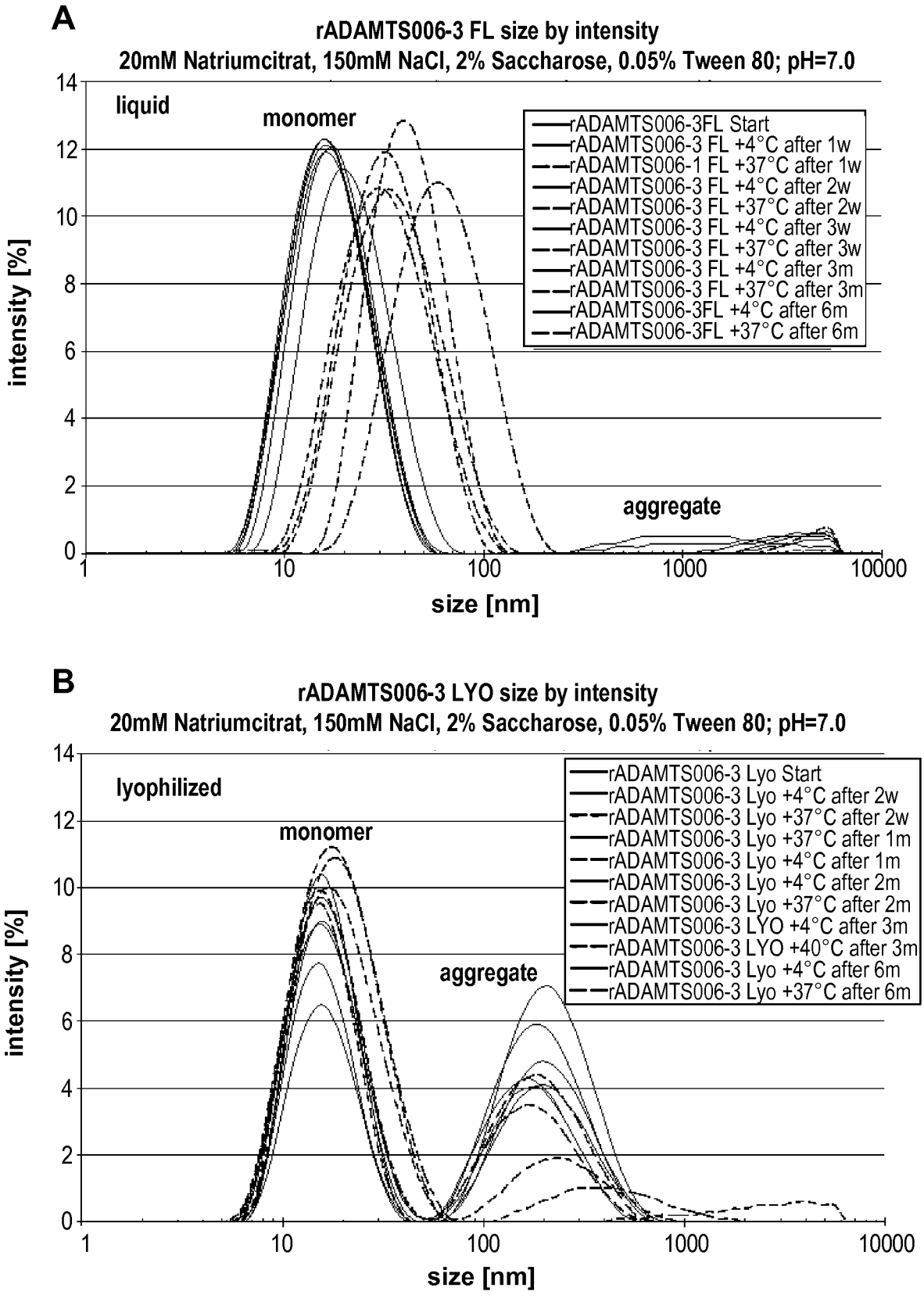

A
rADAMTS006-3 FL size by intensity
20mM Natriumcitrat, 150mM NaCl, 2% Saccharose, 0.05% Tween 80; pH=7.0
liquid
monomer
aggregate
rADAMTS006-3FL Start
rADAMTS006-3 FL +4°C after 1w
rADAMTS006-1 FL +37°C after 1w
rADAMTS006-3 FL +4°C after 2w
rADAMTS006-3 FL +37°C after 2w
rADAMTS006-3 FL +4°C after 3w
rADAMTS006-3 FL +37°C after 3w
rADAMTS006-3 FL +4°C after 3m
rADAMTS006-3 FL +37°C after 3m
rADAMTS006-3FL +4°C after 6m
rADAMTS006-3FL +37°C after 6m
intensity [%]
size [nm]
B
rADAMTS006-3 LYO size by intensity
20mM Natriumcitrat, 150mM NaCl, 2% Saccharose, 0.05% Tween 80; pH=7.0
lyophilized
monomer
aggregate
rADAMTS006-3 Lyo Start
rADAMTS006-3 Lyo +4°C after 2w
rADAMTS006-3 Lyo +37°C after 2w
rADAMTS006-3 Lyo +37°C after 1m
rADAMTS006-3 Lyo +4°C after 1m
rADAMTS006-3 Lyo +4°C after 2m
rADAMTS006-3 Lyo +37°C after 2m
rADAMTS006-3 LYO +4°C after 3m
rADAMTS006-3 LYO +40°C after 3m
rADAMTS006-3 Lyo +4°C after 6m
rADAMTS006-3 Lyo +37°C after 6m
intensity [%]
size [nm]

Figure 9

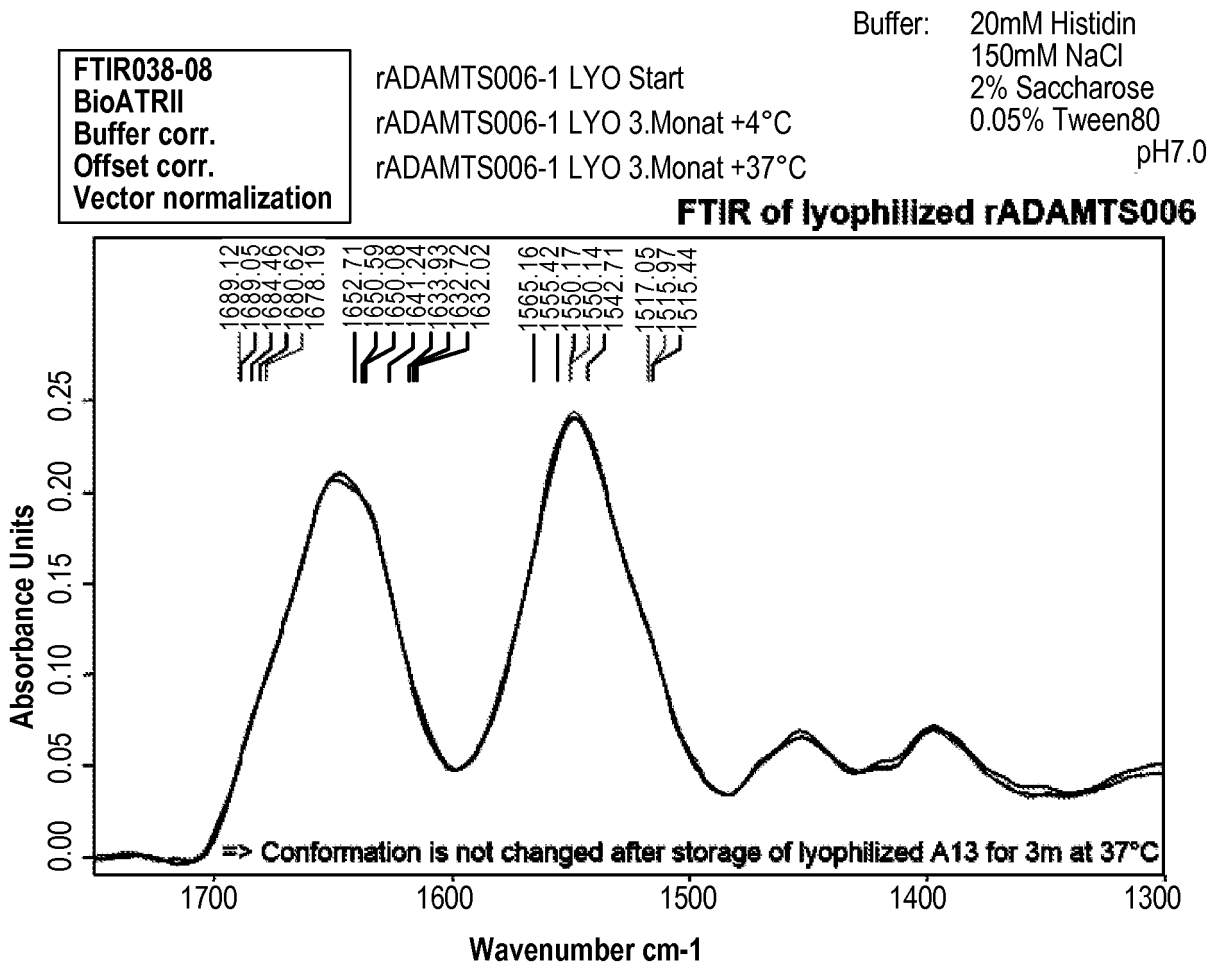

Buffer: 20mM Histidin
150mM NaCl
2% Saccharose
0.05% Tween80
pH7.0
FTIR038-08
BioATRII
Buffer corr.
Offset corr.
Vector normalization
rADAMTS006-1 LYO Start
rADAMTS006-1 LYO 3.Monat +4°C
rADAMTS006-1 LYO 3.Monat +37°C
FTIR of lyophilized rADAMTS006
1689.12
1689.05
1684.46
1680.62
1678.19
1652.71
1650.59
1650.08
1641.24
1633.93
1632.72
1632.02
1565.16
1555.42
1550.17
1550.14
1542.71
1517.05
1515.97
1515.44
Absorbance Units
0.00
0.05
0.10
0.15
0.20
0.25
=> Conformation is not changed after storage of lyophilized A13 for 3m at 37°C
1700
1600
1500
1400
1300
Wavenumber cm-1

Figure 10

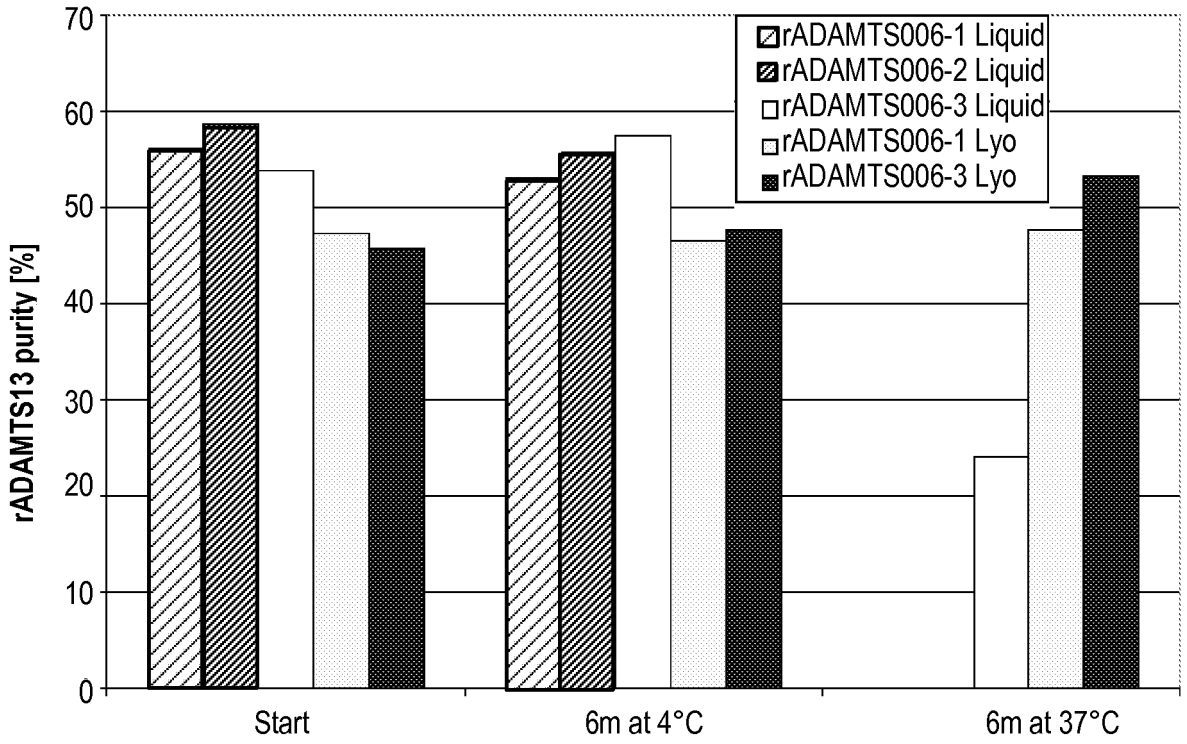

rADAMTS13 purity [%]
70
60
50
40
30
20
10
0
Start
6m at 4°C
6m at 37°C
rADAMTS006-1 Liquid
rADAMTS006-2 Liquid
rADAMTS006-3 Liquid
rADAMTS006-1 Lyo
rADAMTS006-3 Lyo

Figure 11

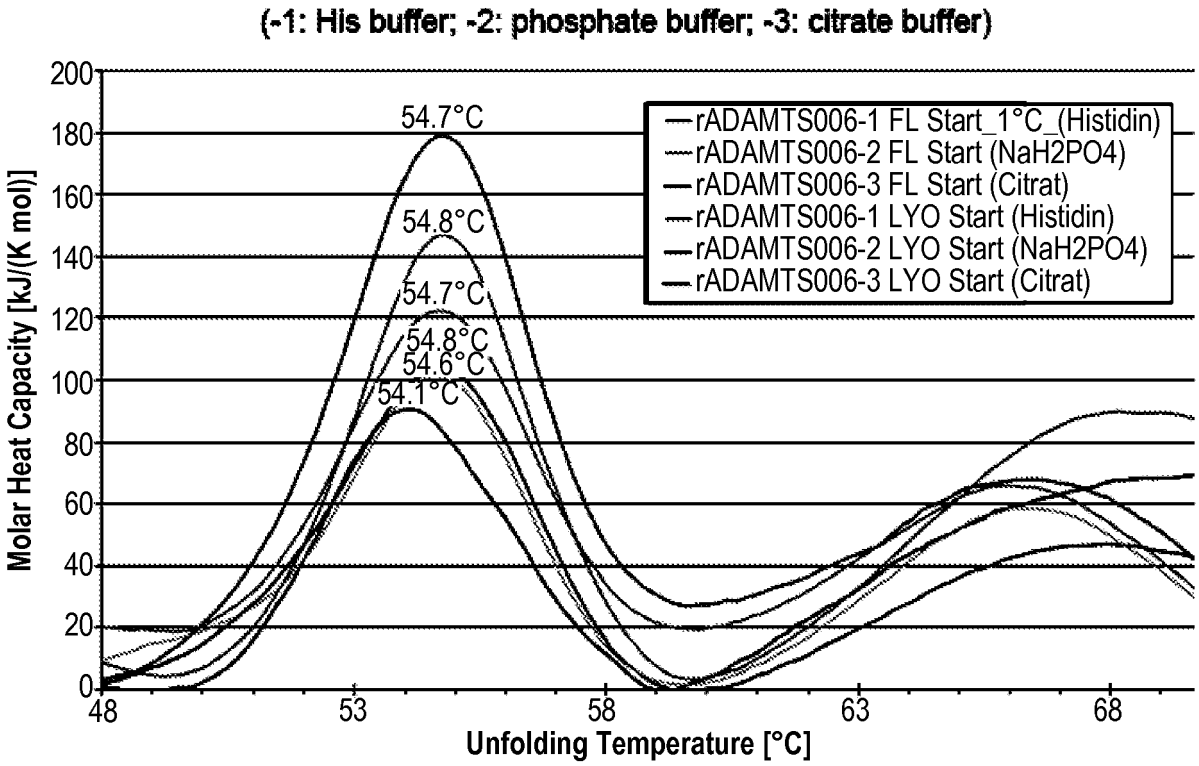

(-1: His buffer; -2: phosphate buffer; -3: citrate buffer)
Molar Heat Capacity [kJ/(K mol)]
Unfolding Temperature [°C]
54.7°C
54.8°C
54.7°C
54.8°C
54.6°C
54.1°C
rADAMTS006-1 FL Start_1°C_(Histidin)
rADAMTS006-2 FL Start (NaH2PO4)
rADAMTS006-3 FL Start (Citrat)
rADAMTS006-1 LYO Start (Histidin)
rADAMTS006-2 LYO Start (NaH2PO4)
rADAMTS006-3 LYO Start (Citrat)

Figure 12

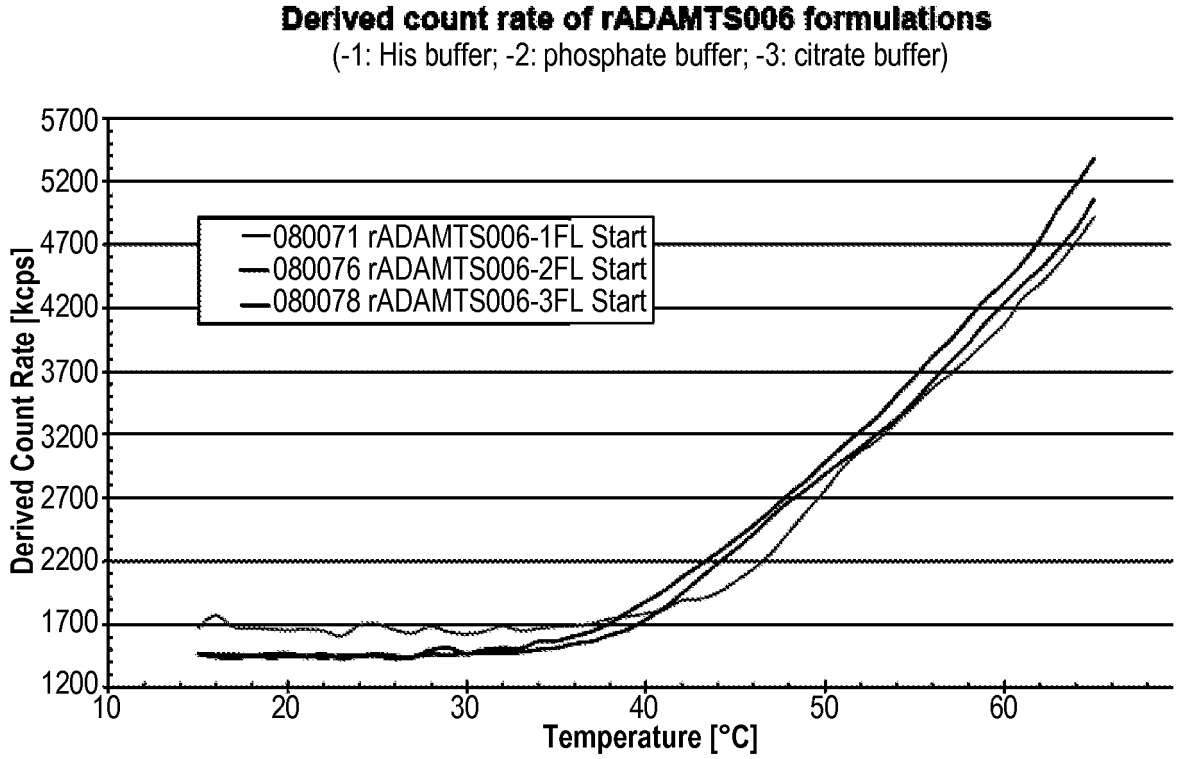
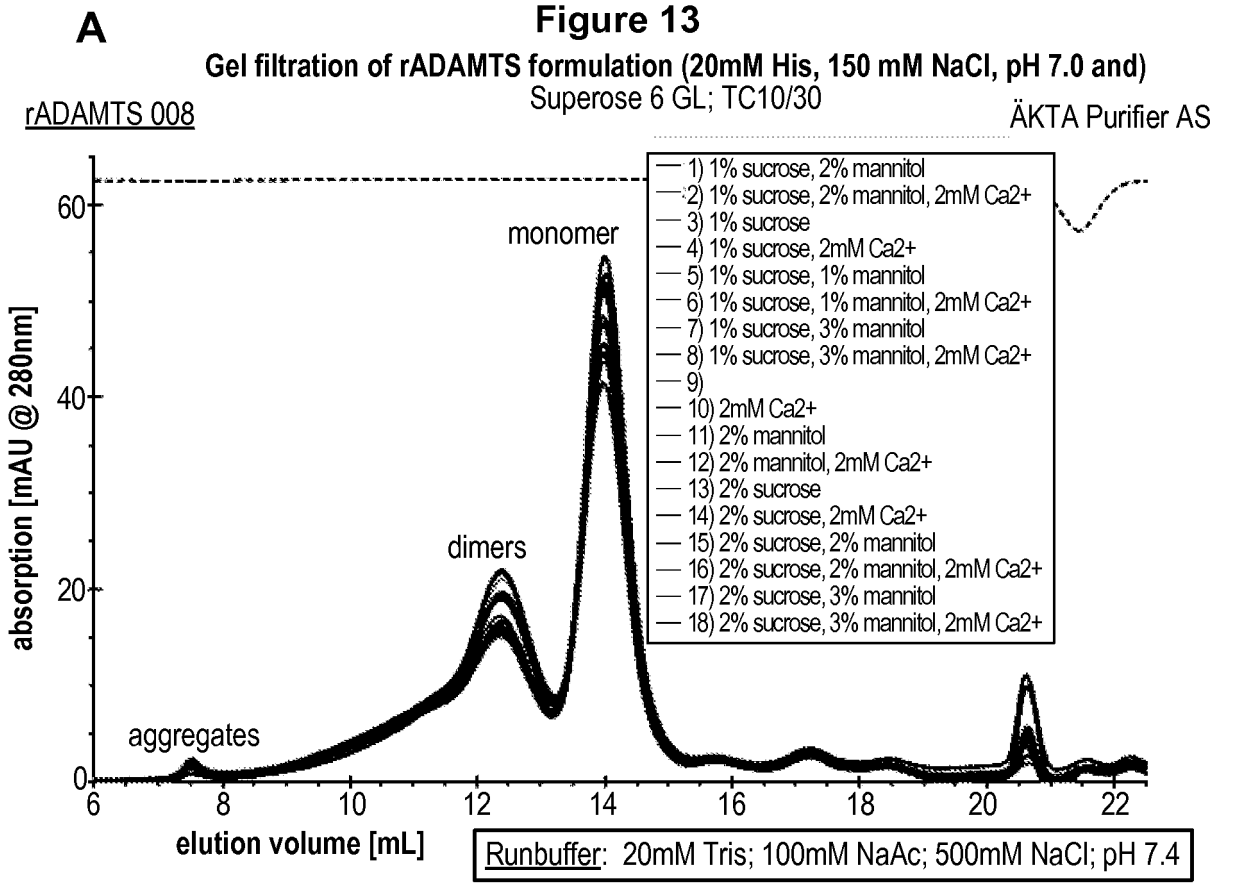

Derived count rate of rADAMTS006 formulations
(-1: His buffer; -2: phosphate buffer; -3: citrate buffer)
Derived Count Rate [kcps]
5700
5200
4700
4200
3700
3200
2700
2200
1700
1200
10
20
30
40
50
60
Temperature [°C]
080071 rADAMTS006-1FL Start
080076 rADAMTS006-2FL Start
080078 rADAMTS006-3FL Start

Figure 13


A
Gel filtration of rADAMTS formulation (20mM His, 150 mM NaCl, pH 7.0 and)
Superose 6 GL; TC10/30
rADAMTS 008
ÄKTA Purifier AS
1) 1% sucrose, 2% mannitol
2) 1% sucrose, 2% mannitol, 2mM Ca2+
3) 1% sucrose
4) 1% sucrose, 2mM Ca2+
5) 1% sucrose, 1% mannitol
6) 1% sucrose, 1% mannitol, 2mM Ca2+
7) 1% sucrose, 3% mannitol
8) 1% sucrose, 3% mannitol, 2mM Ca2+
9)
10) 2mM Ca2+
11) 2% mannitol
12) 2% mannitol, 2mM Ca2+
13) 2% sucrose
14) 2% sucrose, 2mM Ca2+
15) 2% sucrose, 2% mannitol
16) 2% sucrose, 2% mannitol, 2mM Ca2+
17) 2% sucrose, 3% mannitol
18) 2% sucrose, 3% mannitol, 2mM Ca2+
monomer
dimers
aggregates
absorption [mAU @ 280nm]
elution volume [mL]
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

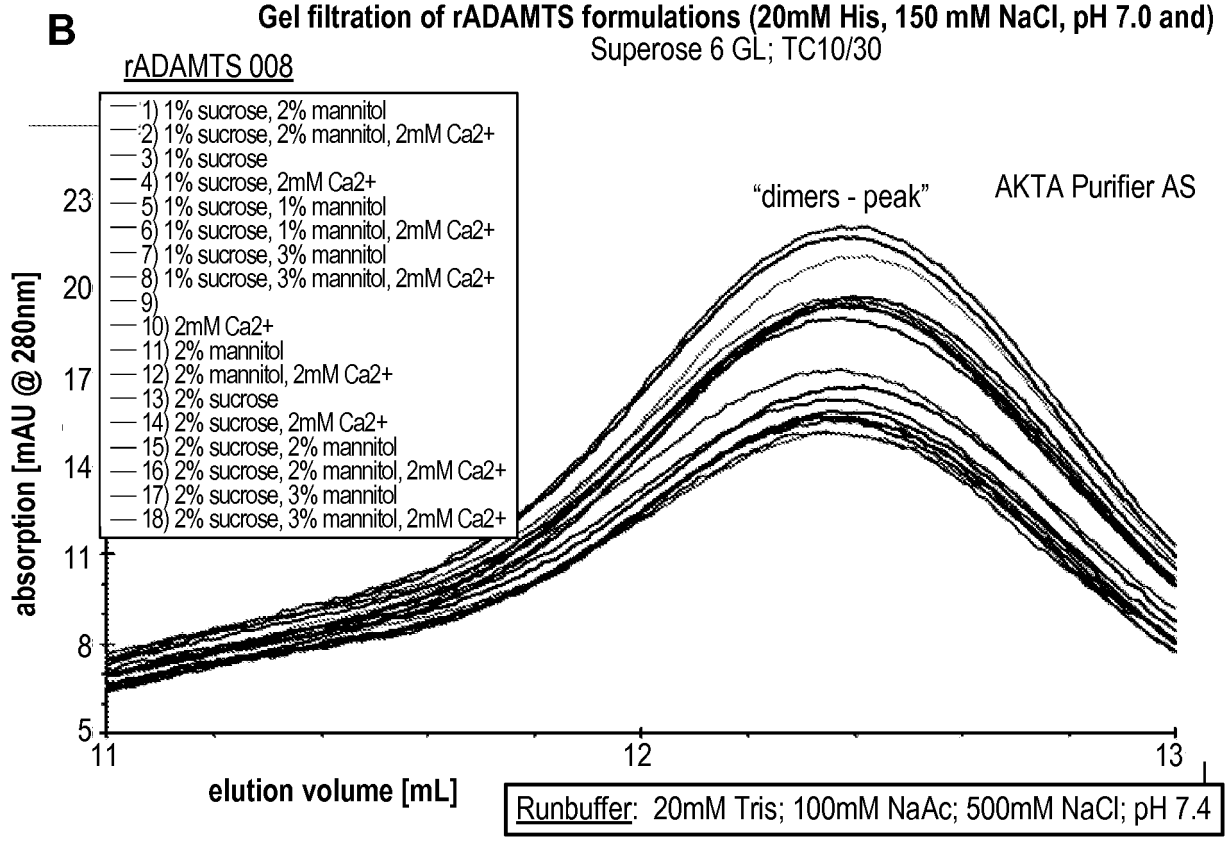

B
Gel filtration of rADAMTS formulations (20mM His, 150 mM NaCl, pH 7.0 and)
Superose 6 GL; TC10/30
rADAMTS 008
1) 1% sucrose, 2% mannitol
2) 1% sucrose, 2% mannitol, 2mM Ca2+
3) 1% sucrose
4) 1% sucrose, 2mM Ca2+
5) 1% sucrose, 1% mannitol
6) 1% sucrose, 1% mannitol, 2mM Ca2+
7) 1% sucrose, 3% mannitol
8) 1% sucrose, 3% mannitol, 2mM Ca2+
9)
10) 2mM Ca2+
11) 2% mannitol
12) 2% mannitol, 2mM Ca2+
13) 2% sucrose
14) 2% sucrose, 2mM Ca2+
15) 2% sucrose, 2% mannitol
16) 2% sucrose, 2% mannitol, 2mM Ca2+
17) 2% sucrose, 3% mannitol
18) 2% sucrose, 3% mannitol, 2mM Ca2+
"dimers - peak"
AKTA Purifier AS
absorption [mAU @ 280nm]
elution volume [mL]
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

Figure 14

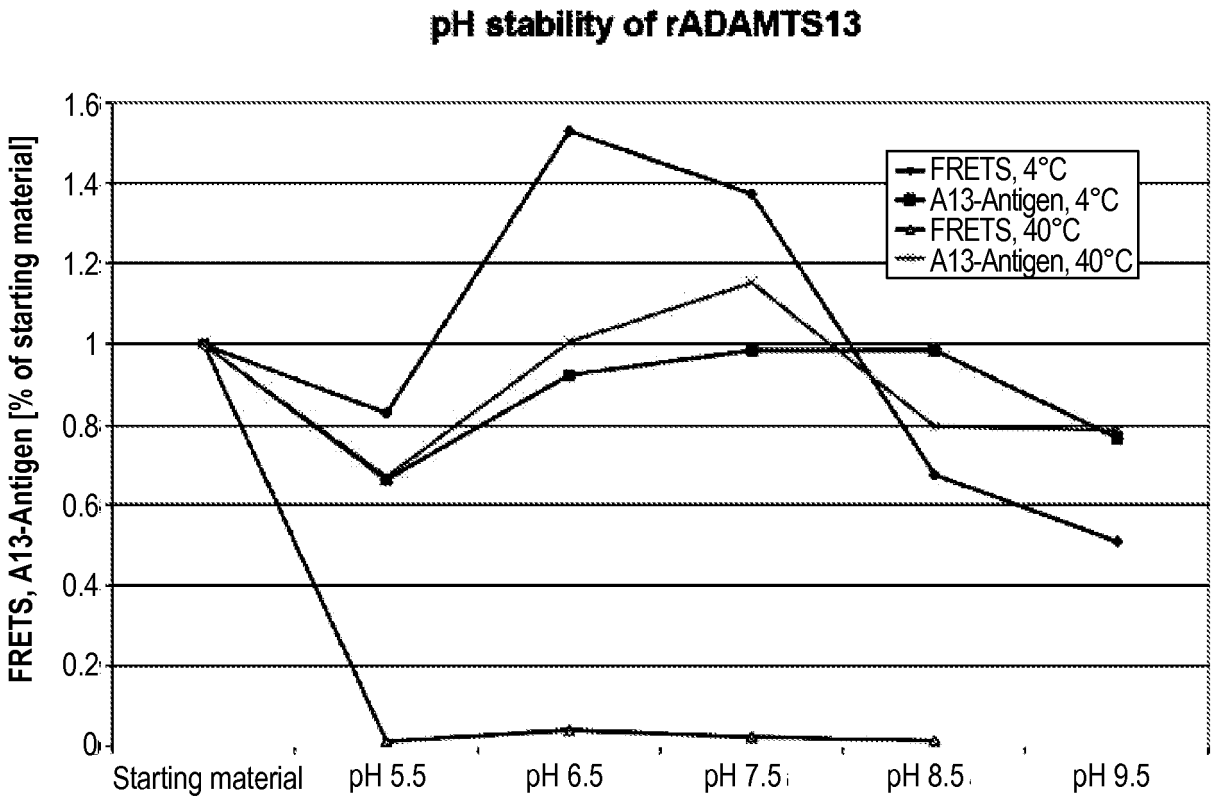

pH stability of rADAMTS13
FRETS, A13-Antigen [% of starting material]
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
FRETS, 4°C
A13-Antigen, 4°C
FRETS, 40°C
A13-Antigen, 40°C
Starting material
pH 5.5
pH 6.5
pH 7.5
pH 8.5
pH 9.5

Figure 15

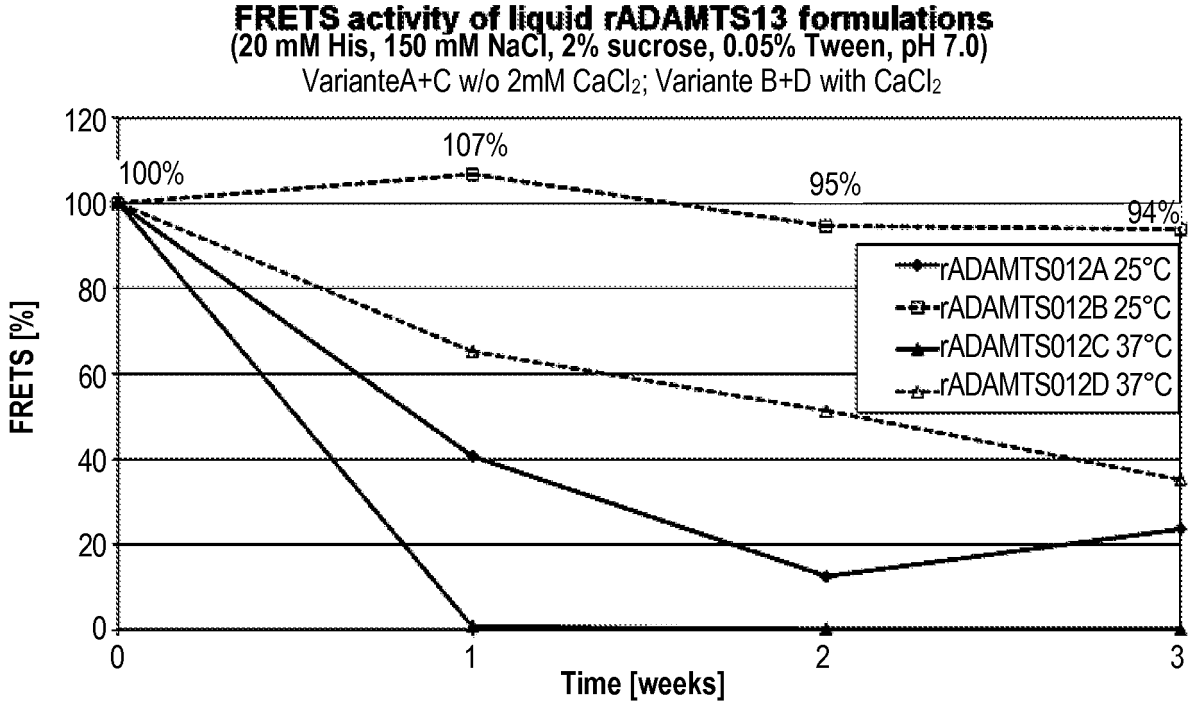
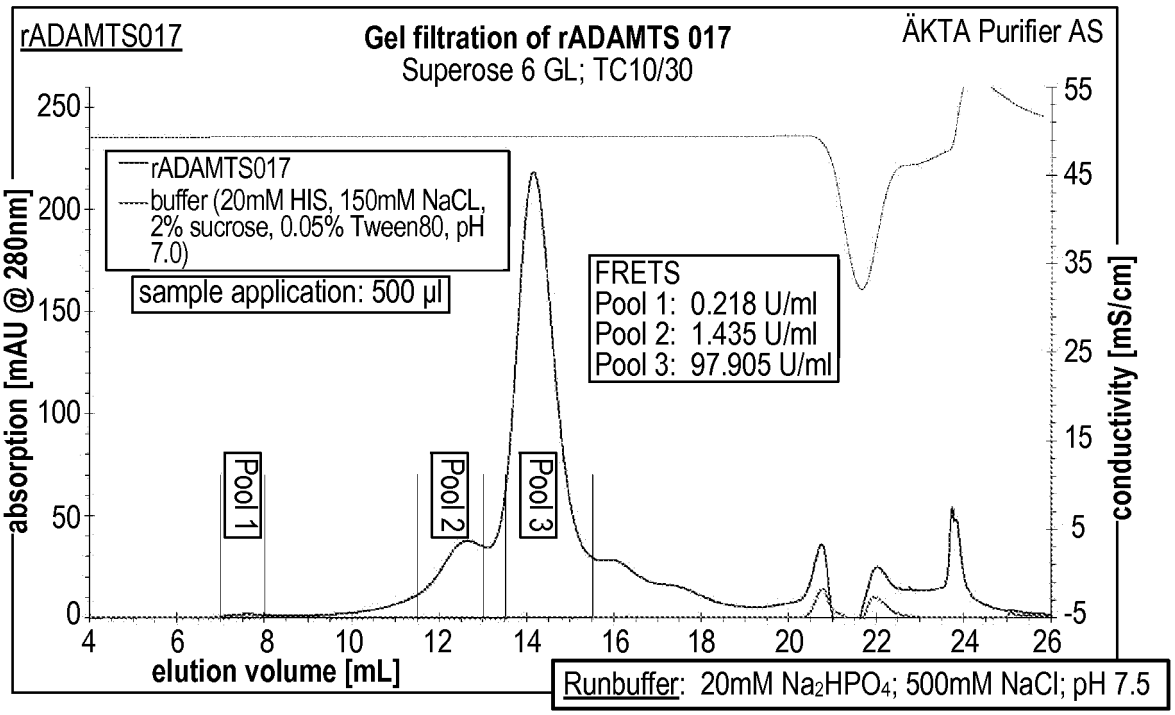

FRETS activity of liquid rADAMTS13 formulations
(20 mM His, 150 mM NaCl, 2% sucrose, 0.05% Tween, pH 7.0)
VarianteA+C w/o 2mM CaCl2; Variante B+D with CaCl2
FRETS [%]
Time [weeks]
100%
107%
95%
94%
rADAMTS012A 25°C
rADAMTS012B 25°C
rADAMTS012C 37°C
rADAMTS012D 37°C

Figure 16

A


rADAMTS017
Gel filtration of rADAMTS 017
Superose 6 GL; TC10/30
ÄKTA Purifier AS
rADAMTS017
buffer (20mM HIS, 150mM NaCL, 2% sucrose, 0.05% Tween80, pH 7.0)
sample application: 500 µl
FRETS
Pool 1: 0.218 U/ml
Pool 2: 1.435 U/ml
Pool 3: 97.905 U/ml
Pool 1
Pool 2
Pool 3
absorption [mAU @ 280nm]
conductivity [mS/cm]
elution volume [mL]
Runbuffer: 20mM Na2HPO4; 500mM NaCl; pH 7.5

B

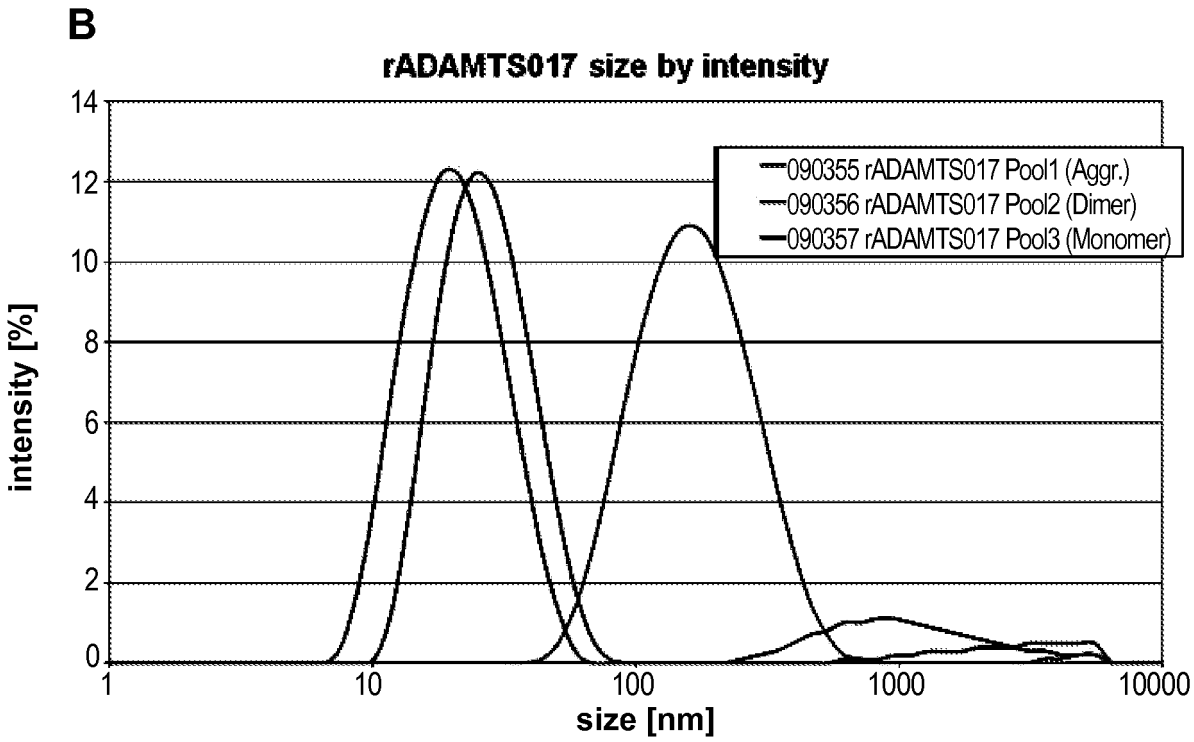

rADAMTS017 size by intensity
090355 rADAMTS017 Pool1 (Aggr.)
090356 rADAMTS017 Pool2 (Dimer)
090357 rADAMTS017 Pool3 (Monomer)
intensity [%]
size [nm]

Figure 17

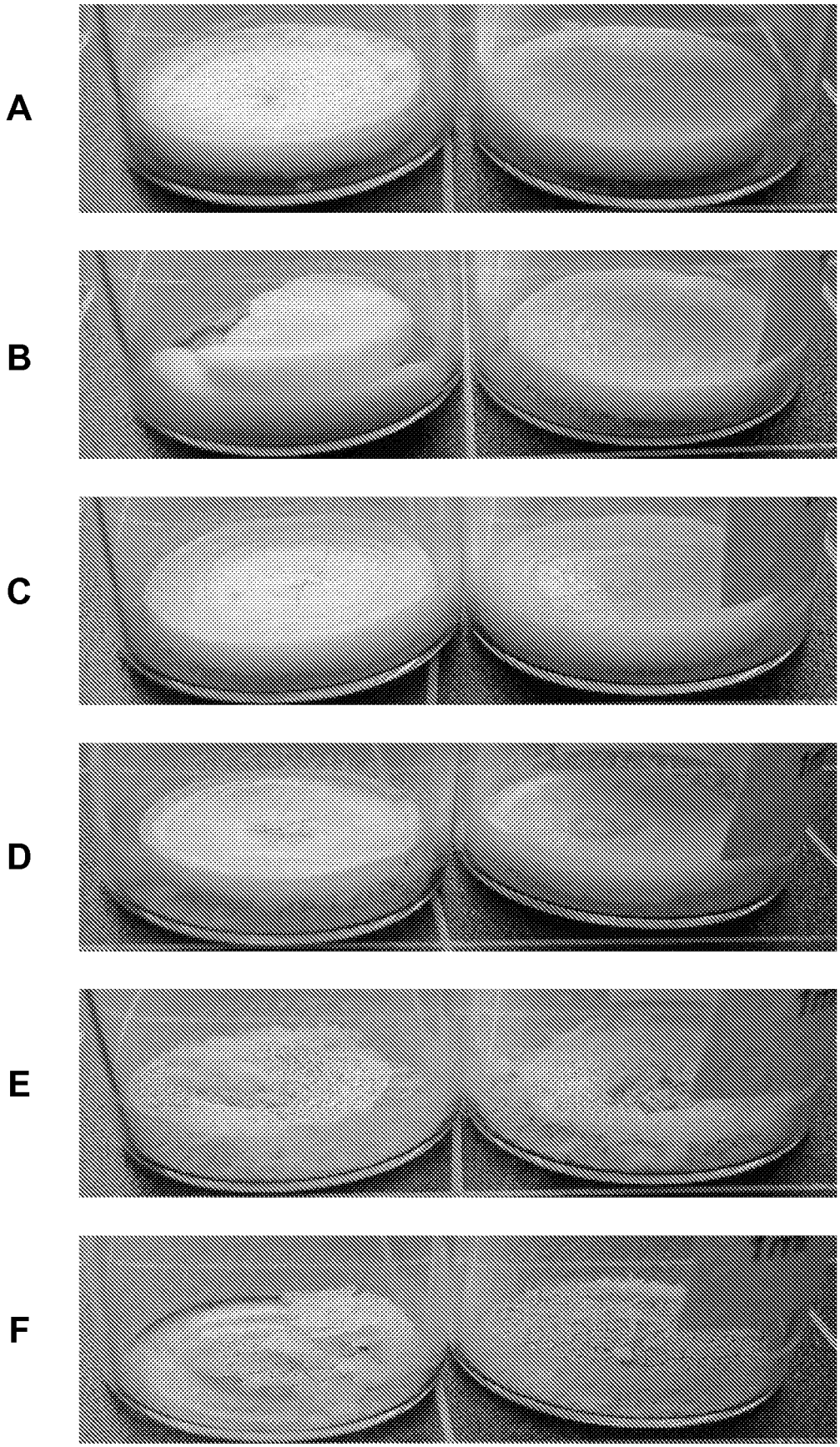


Figure 18

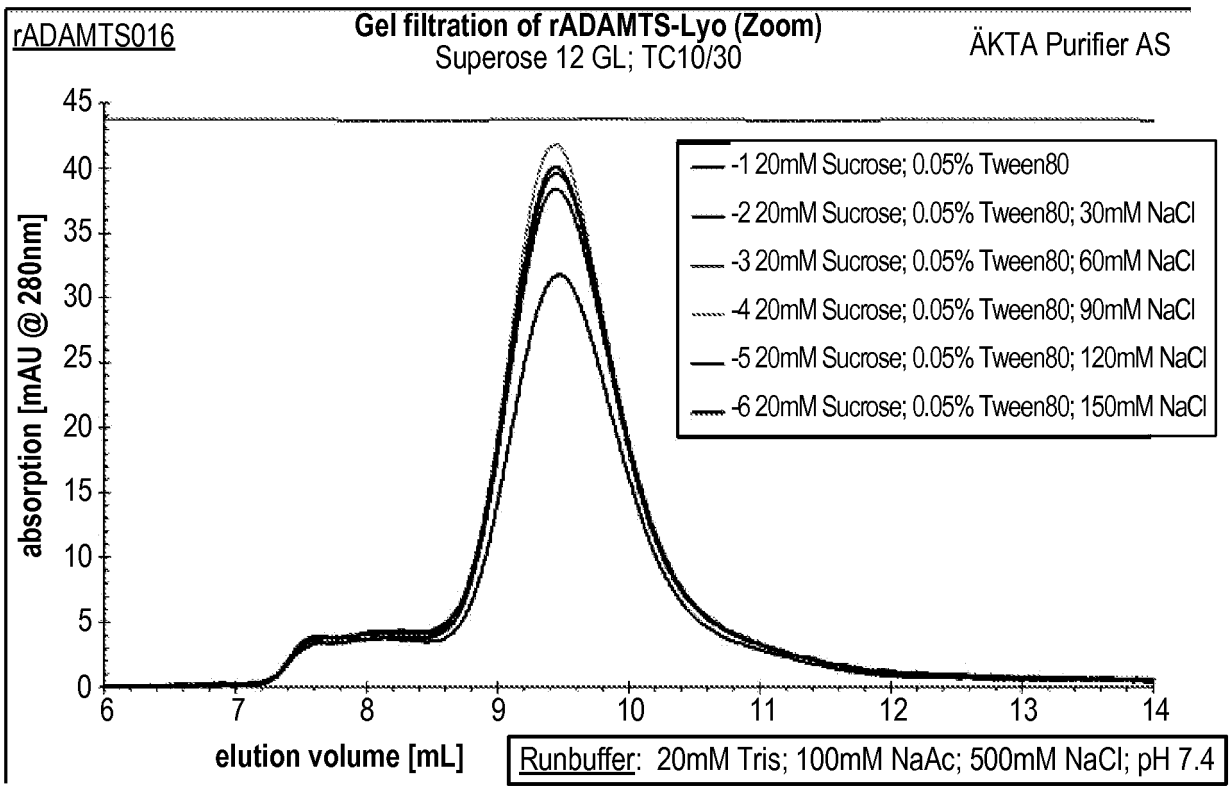

rADAMTS016
Gel filtration of rADAMTS-Lyo (Zoom)
Superose 12 GL; TC10/30
ÄKTA Purifier AS
absorption [mAU @ 280nm]
elution volume [mL]
-1 20mM Sucrose; 0.05% Tween80
-2 20mM Sucrose; 0.05% Tween80; 30mM NaCl
-3 20mM Sucrose; 0.05% Tween80; 60mM NaCl
-4 20mM Sucrose; 0.05% Tween80; 90mM NaCl
-5 20mM Sucrose; 0.05% Tween80; 120mM NaCl
-6 20mM Sucrose; 0.05% Tween80; 150mM NaCl
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

Figure 19

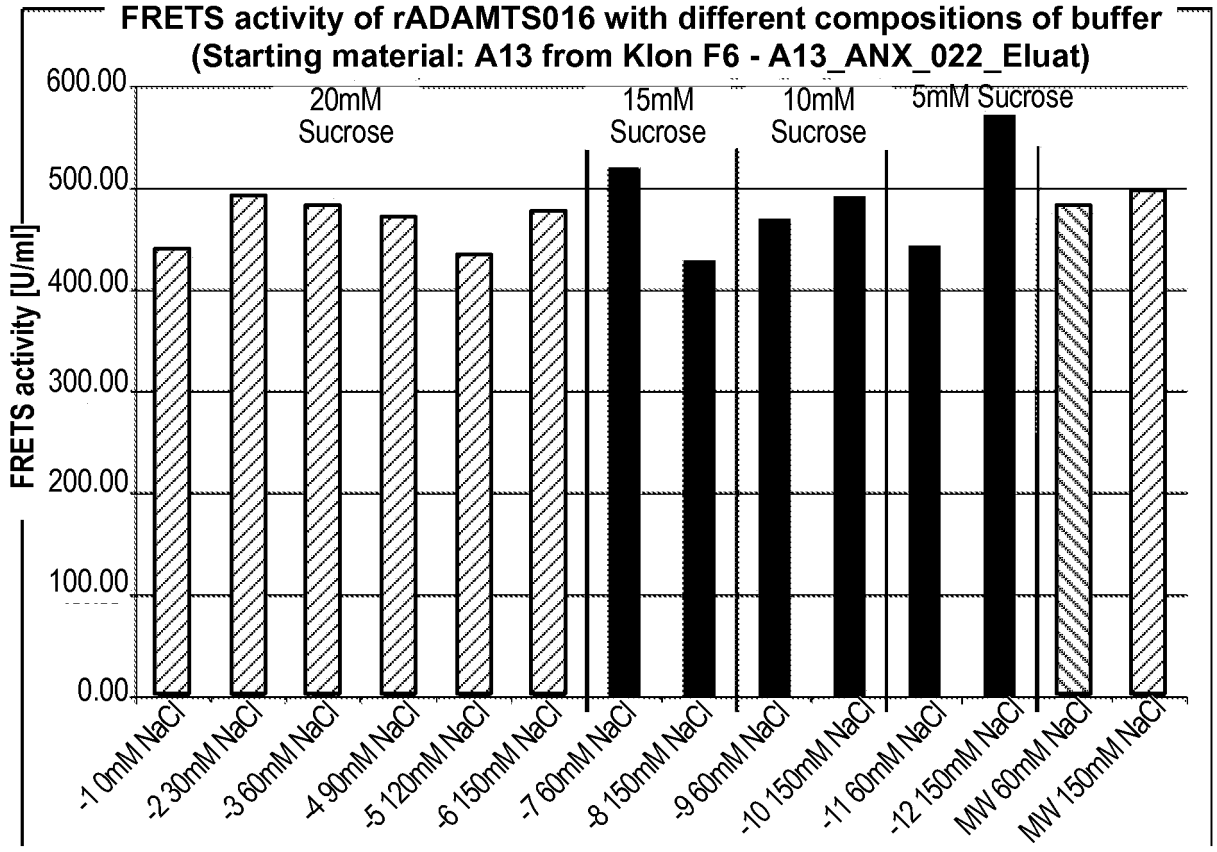
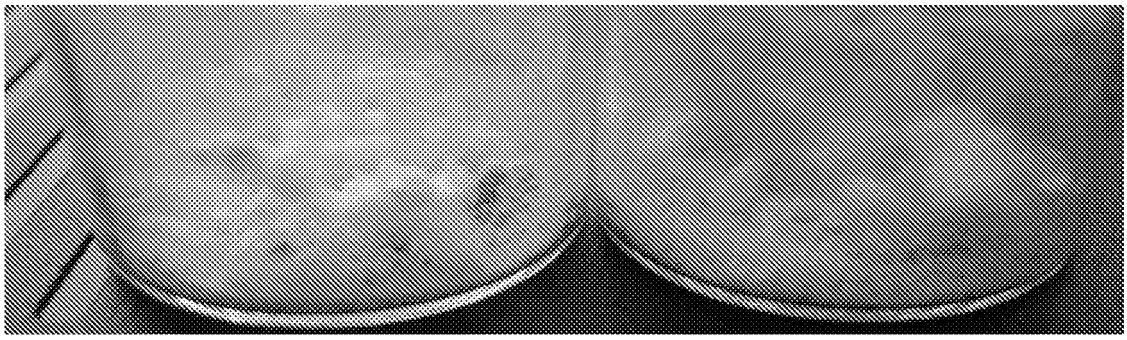

FRETS activity of rADAMTS016 with different compositions of buffer
(Starting material: A13 from Klon F6 - A13_ANX_022_Eluat)
FRETS activity [U/ml]
600.00
500.00
400.00
300.00
200.00
100.00
0.00
20mM Sucrose
15mM Sucrose
10mM Sucrose
5mM Sucrose
-1 0mM NaCl
-2 30mM NaCl
-3 60mM NaCl
-4 90mM NaCl
-5 120mM NaCl
-6 150mM NaCl
-7 60mM NaCl
-8 150mM NaCl
-9 60mM NaCl
-10 150mM NaCl
-11 60mM NaCl
-12 150mM NaCl
MW 60mM NaCl
MW 150mM NaCl

Figure 20

A



B

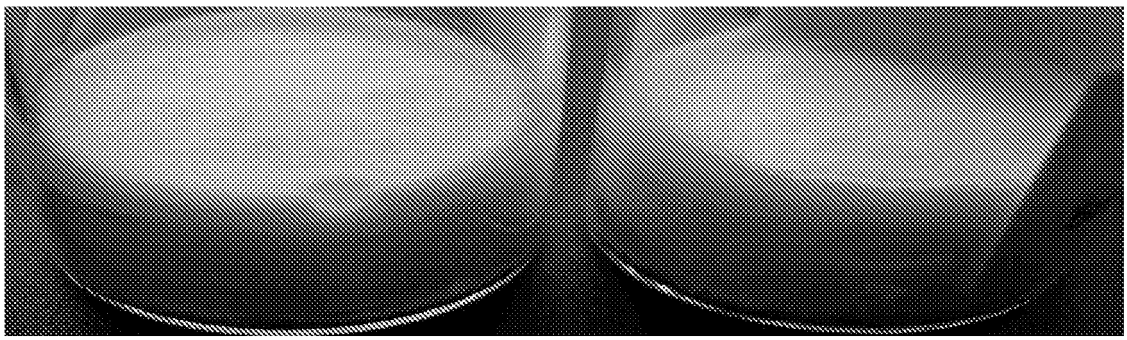
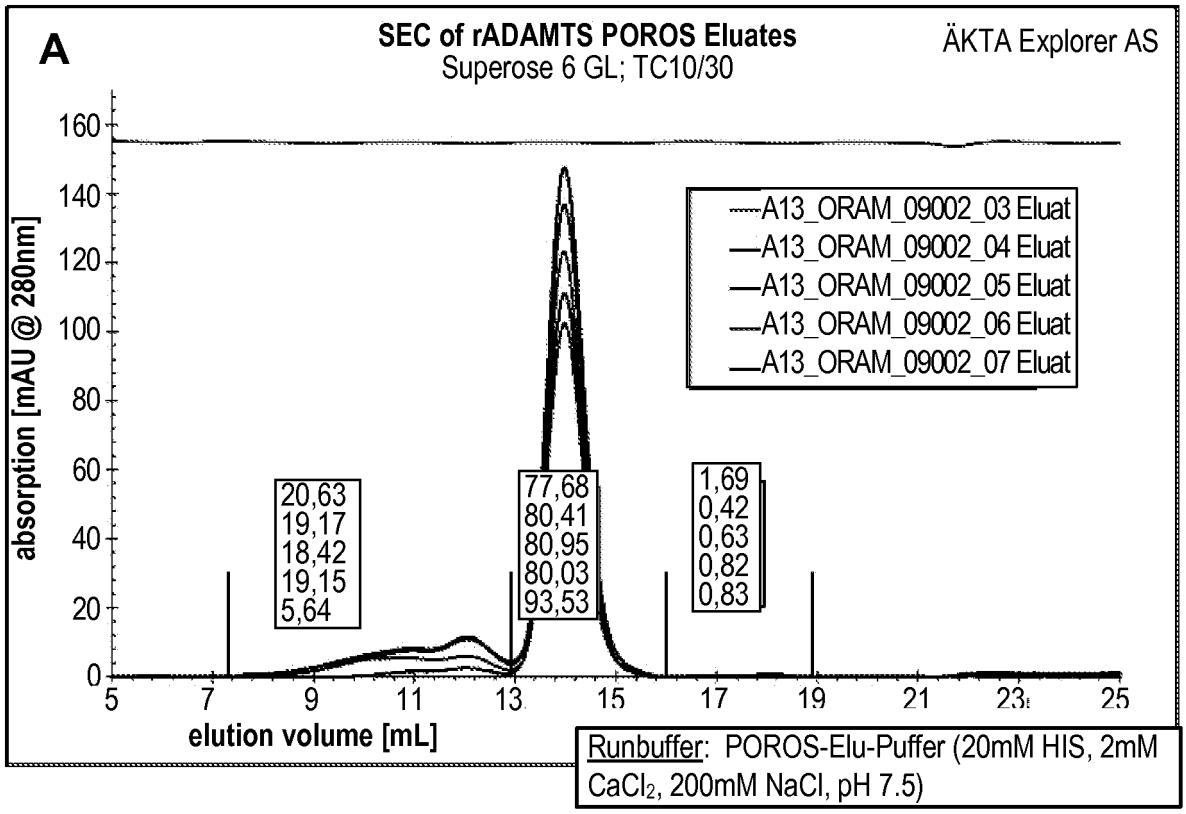


Figure 21


A
SEC of rADAMTS POROS Eluates
Superose 6 GL; TC10/30
ÄKTA Explorer AS
absorption [mAU @ 280nm]
elution volume [mL]
A13_ORAM_09002_03 Eluat
A13_ORAM_09002_04 Eluat
A13_ORAM_09002_05 Eluat
A13_ORAM_09002_06 Eluat
A13_ORAM_09002_07 Eluat
20,63
19,17
18,42
19,15
5,64
77,68
80,41
80,95
80,03
93,53
1,69
0,42
0,63
0,82
0,83
Runbuffer: POROS-Elu-Puffer (20mM HIS, 2mM CaCl2, 200mM NaCl, pH 7.5)

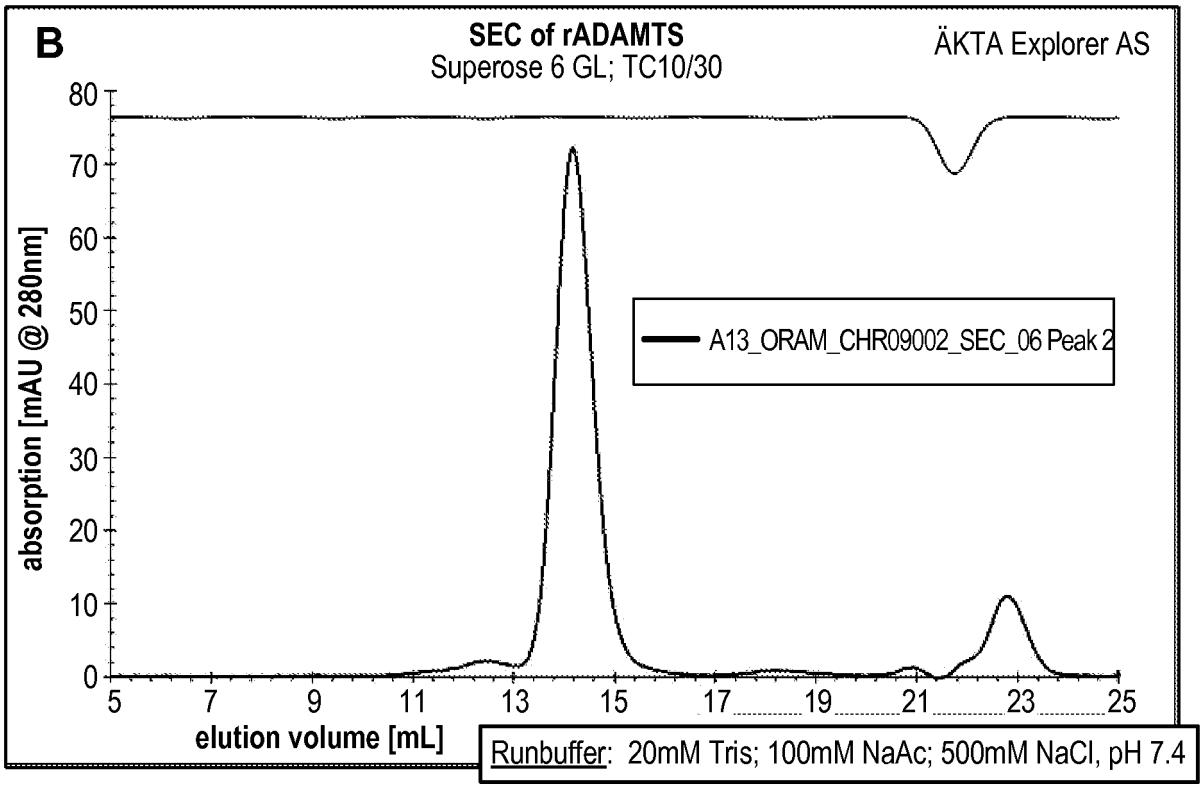

B
SEC of rADAMTS
Superose 6 GL; TC10/30
ÄKTA Explorer AS
absorption [mAU @ 280nm]
elution volume [mL]
A13_ORAM_CHR09002_SEC_06 Peak 2
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl, pH 7.4

Figure 22A

A

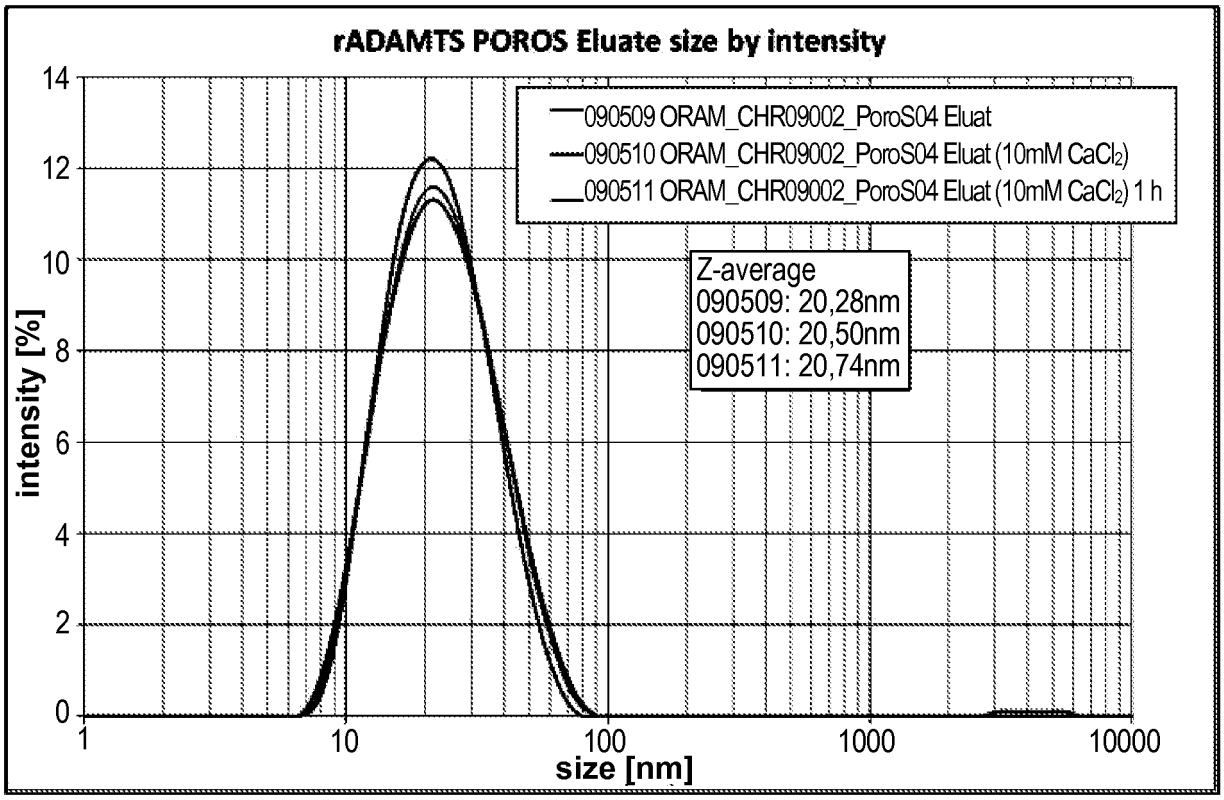

rADAMTS POROS Eluate size by intensity
090509 ORAM_CHR09002_PoroS04 Eluat
090510 ORAM_CHR09002_PoroS04 Eluat (10mM CaCl2)
090511 ORAM_CHR09002_PoroS04 Eluat (10mM CaCl2) 1 h
Z-average
090509: 20,28nm
090510: 20,50nm
090511: 20,74nm
intensity [%]
size [nm]
0
2
4
6
8
10
12
14
1
10
100
1000
10000

Figure 22B

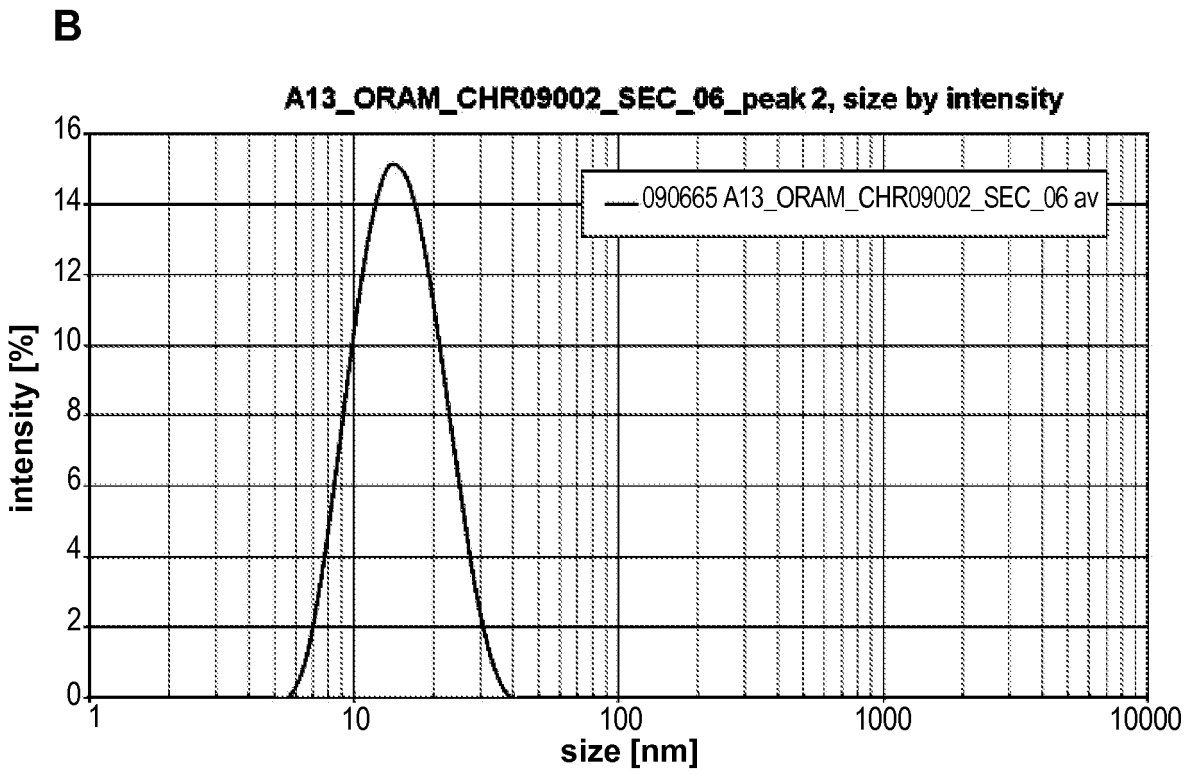
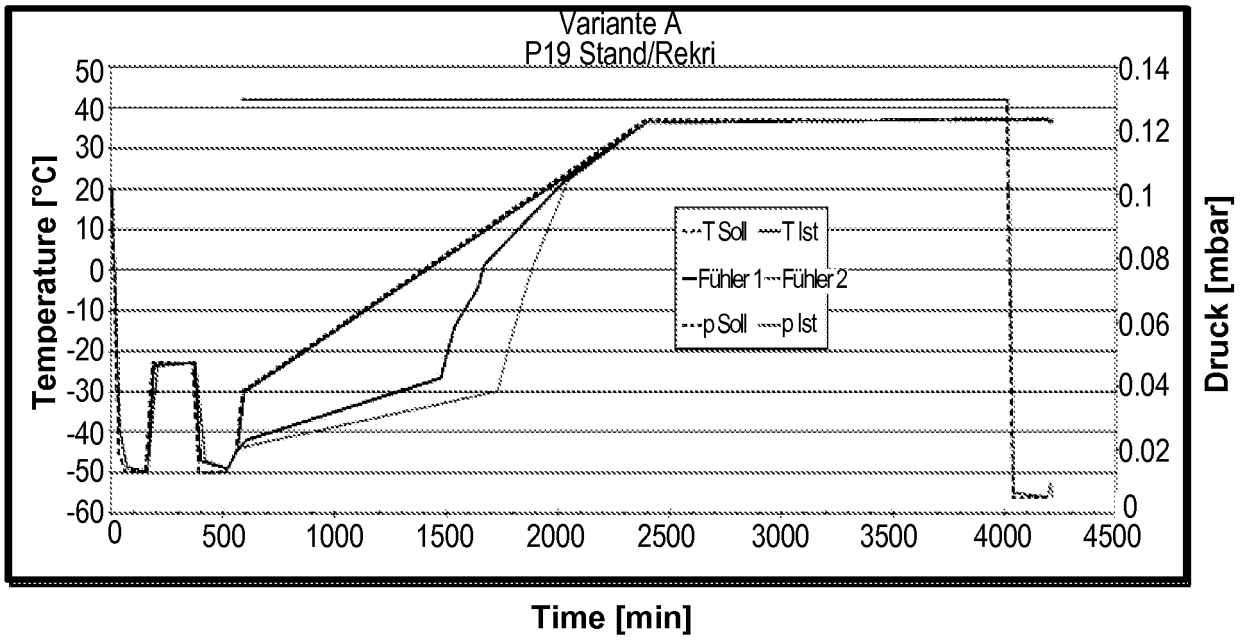

B
A13_ORAM_CHR09002_SEC_06_peak 2, size by intensity
090665 A13_ORAM_CHR09002_SEC_06 av
intensity [%]
size [nm]
0
2
4
6
8
10
12
14
16
1
10
100
1000
10000

Figure 23

A


Variante A
P19 Stand/Rekri
Temperature [°C]
Druck [mbar]
Time [min]
T Soll
T Ist
Fühler 1
Fühler 2
p Soll
p Ist
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
500
1000
1500
2000
2500
3000
3500
4000
4500

B

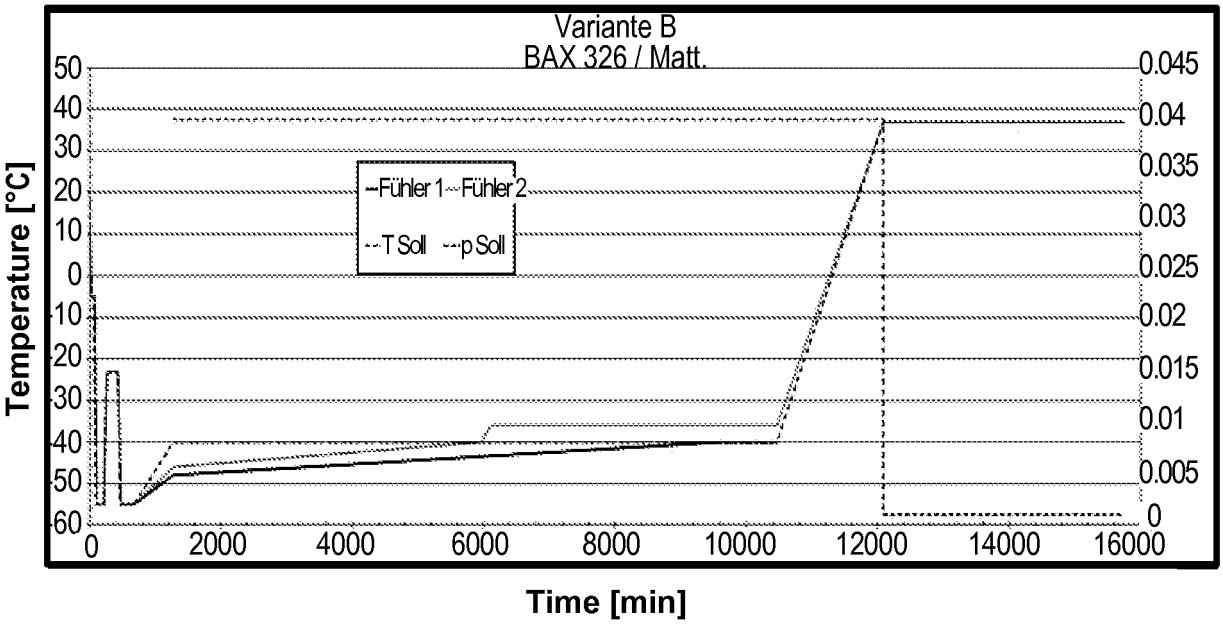

Variante B
BAX 326 / Matt.
Temperature [°C]
Time [min]
Fühler 1
Fühler 2
T Soll
p Soll
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
2000
4000
6000
8000
10000
12000
14000
16000

Figure 24A

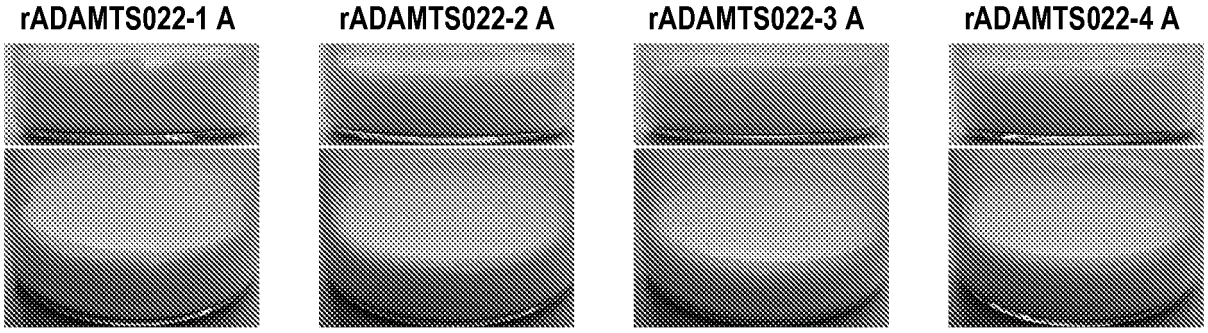


Figure 24B

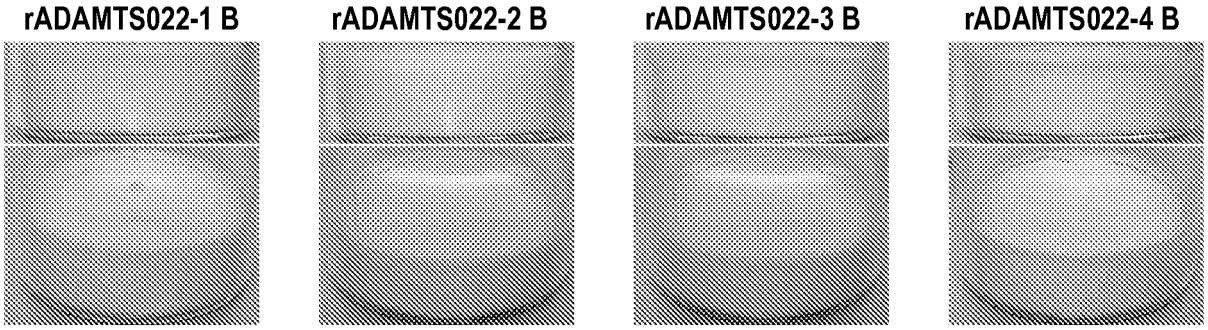


Figure 24C

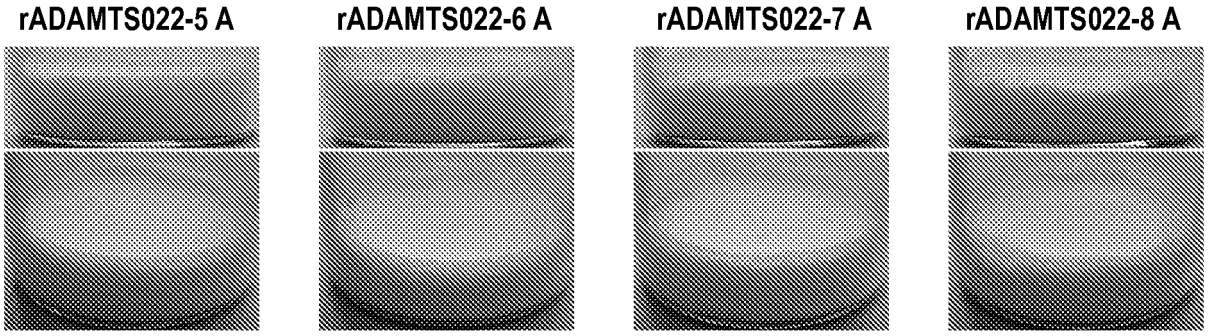


Figure 24D

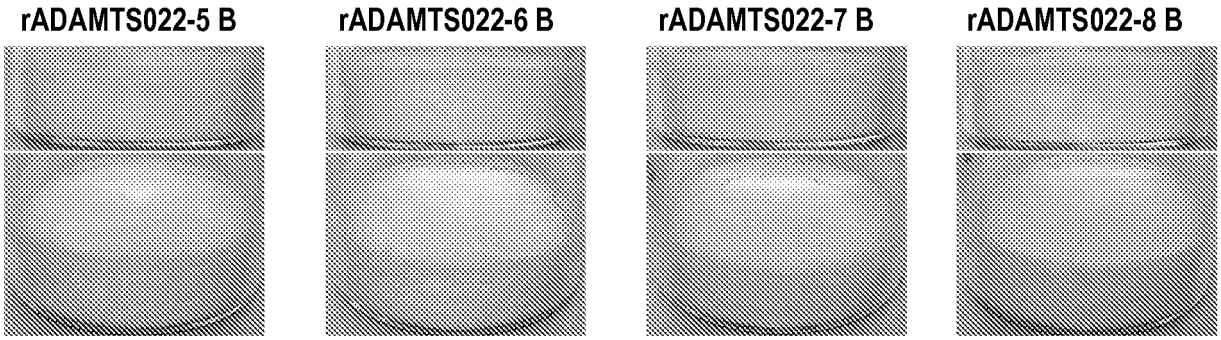
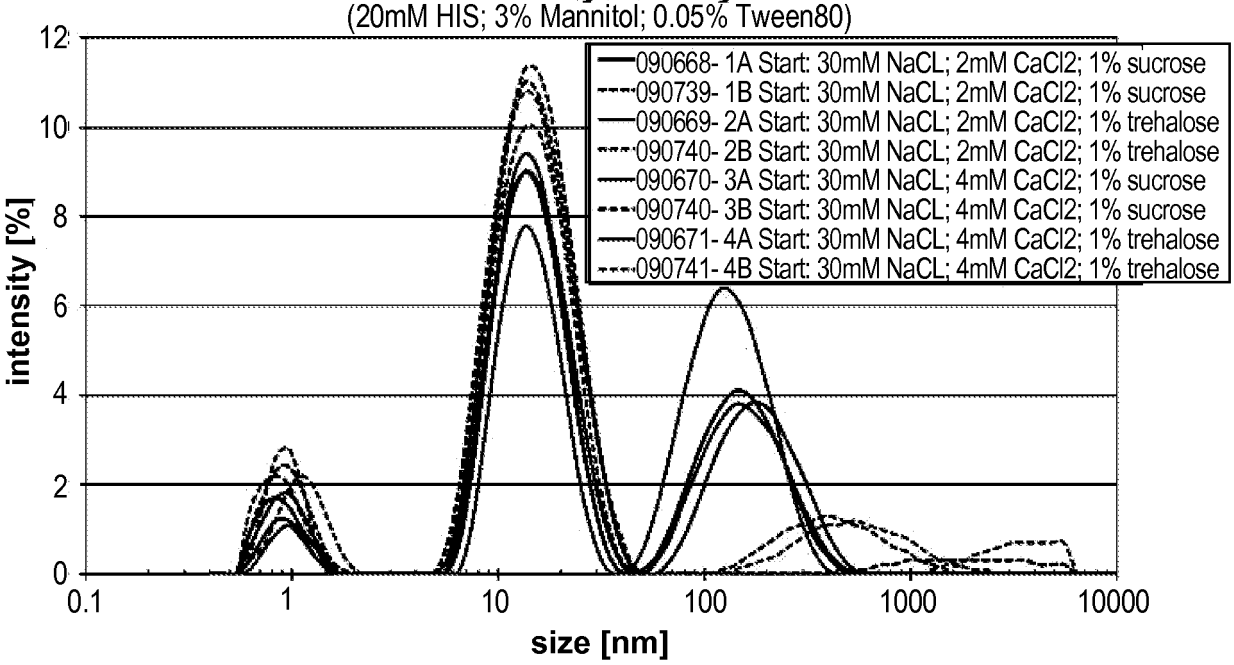


Figure 25

A


rADAMTS022 A LYO start and rADAMTS022 B Lyo start:
size by intensity
(20mM HIS; 3% Mannitol; 0.05% Tween80)
090668- 1A Start: 30mM NaCL; 2mM CaCl2; 1% sucrose
090739- 1B Start: 30mM NaCL; 2mM CaCl2; 1% sucrose
090669- 2A Start: 30mM NaCL; 2mM CaCl2; 1% trehalose
090740- 2B Start: 30mM NaCL; 2mM CaCl2; 1% trehalose
090670- 3A Start: 30mM NaCL; 4mM CaCl2; 1% sucrose
090740- 3B Start: 30mM NaCL; 4mM CaCl2; 1% sucrose
090671- 4A Start: 30mM NaCL; 4mM CaCl2; 1% trehalose
090741- 4B Start: 30mM NaCL; 4mM CaCl2; 1% trehalose
intensity [%]
size [nm]
0
2
4
6
8
10
12
0.1
1
10
100
1000
10000

B

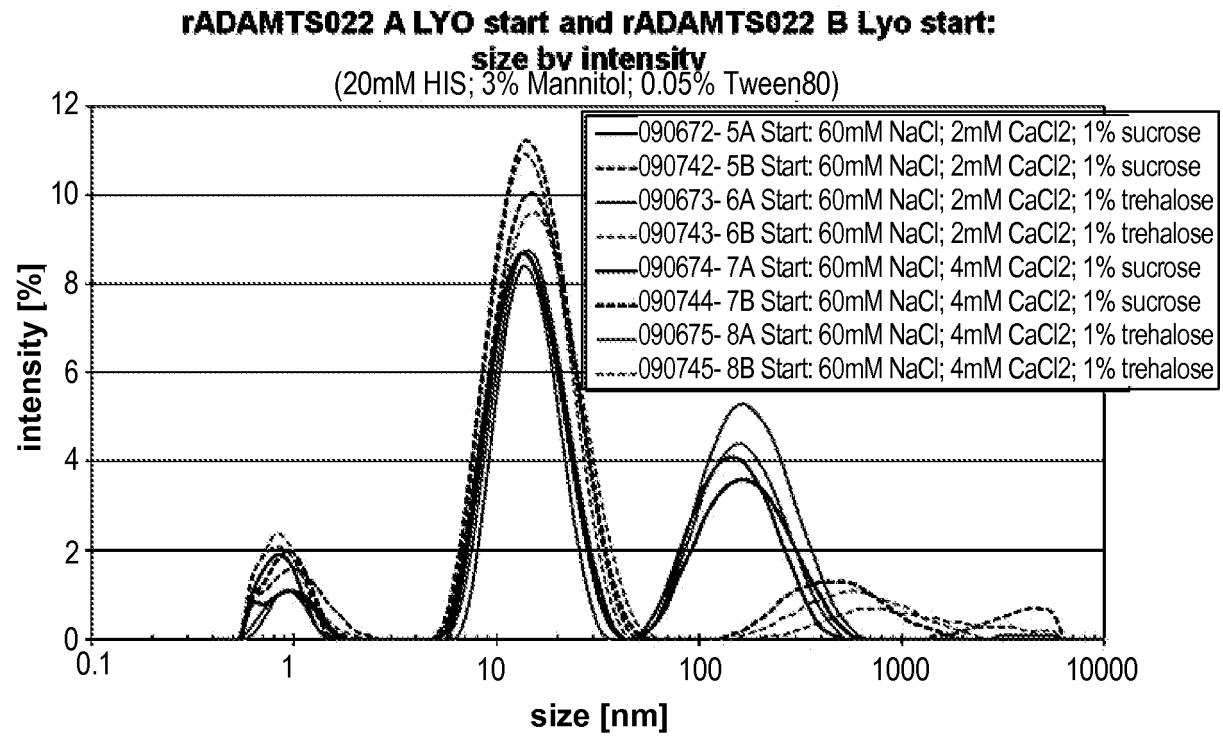

rADAMTS022 A LYO start and rADAMTS022 B Lyo start:
size by intensity
(20mM HIS; 3% Mannitol; 0.05% Tween80)
090672- 5A Start: 60mM NaCl; 2mM CaCl2; 1% sucrose
090742- 5B Start: 60mM NaCl; 2mM CaCl2; 1% sucrose
090673- 6A Start: 60mM NaCl; 2mM CaCl2; 1% trehalose
090743- 6B Start: 60mM NaCl; 2mM CaCl2; 1% trehalose
090674- 7A Start: 60mM NaCl; 4mM CaCl2; 1% sucrose
090744- 7B Start: 60mM NaCl; 4mM CaCl2; 1% sucrose
090675- 8A Start: 60mM NaCl; 4mM CaCl2; 1% trehalose
090745- 8B Start: 60mM NaCl; 4mM CaCl2; 1% trehalose
intensity [%]
size [nm]
0
2
4
6
8
10
12
0.1
1
10
100
1000
10000

Figure 26

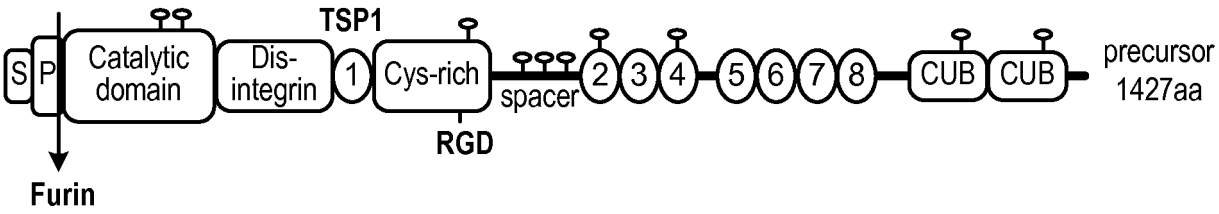

S
P
Catalytic domain
Dis-integrin
TSP1
1
Cys-rich
RGD
spacer
2
3
4
5
6
7
8
CUB
CUB
precursor 1427aa
Furin

Figure 27

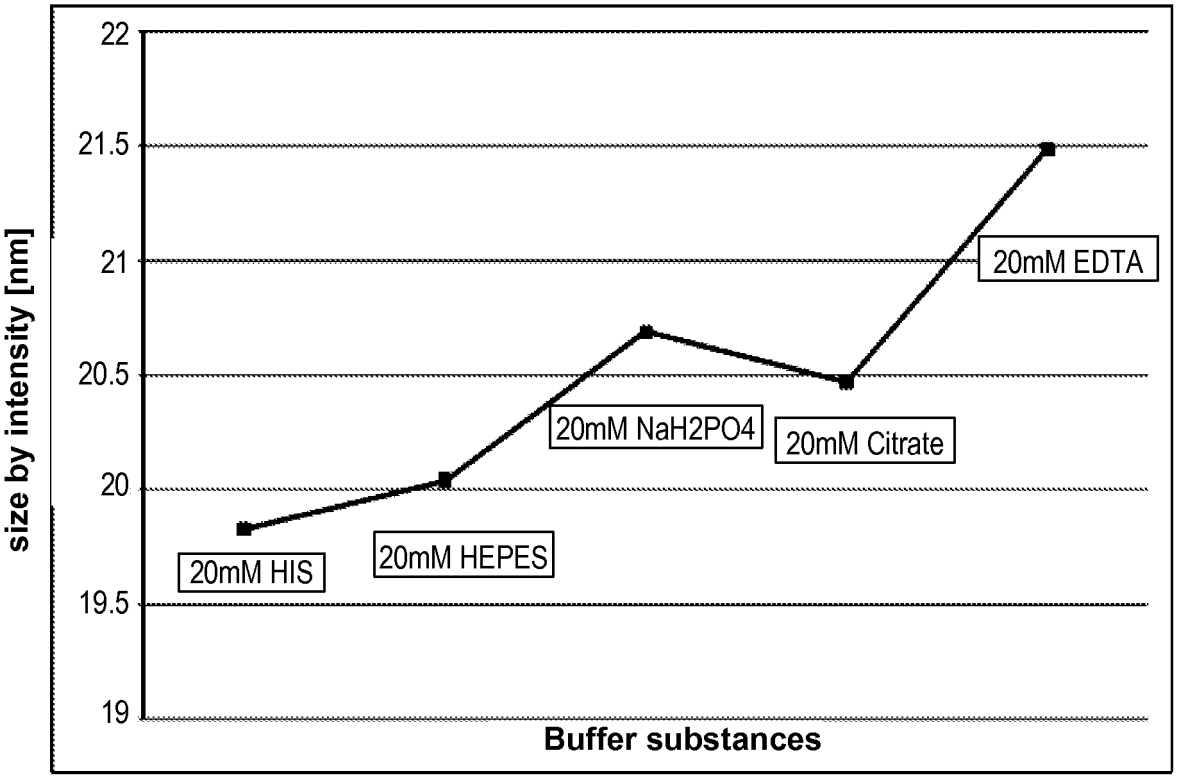

22
21.5
21
20.5
20
19.5
19
size by intensity [nm]
20mM HIS
20mM HEPES
20mM NaH2PO4
20mM Citrate
20mM EDTA
Buffer substances

Figure 28

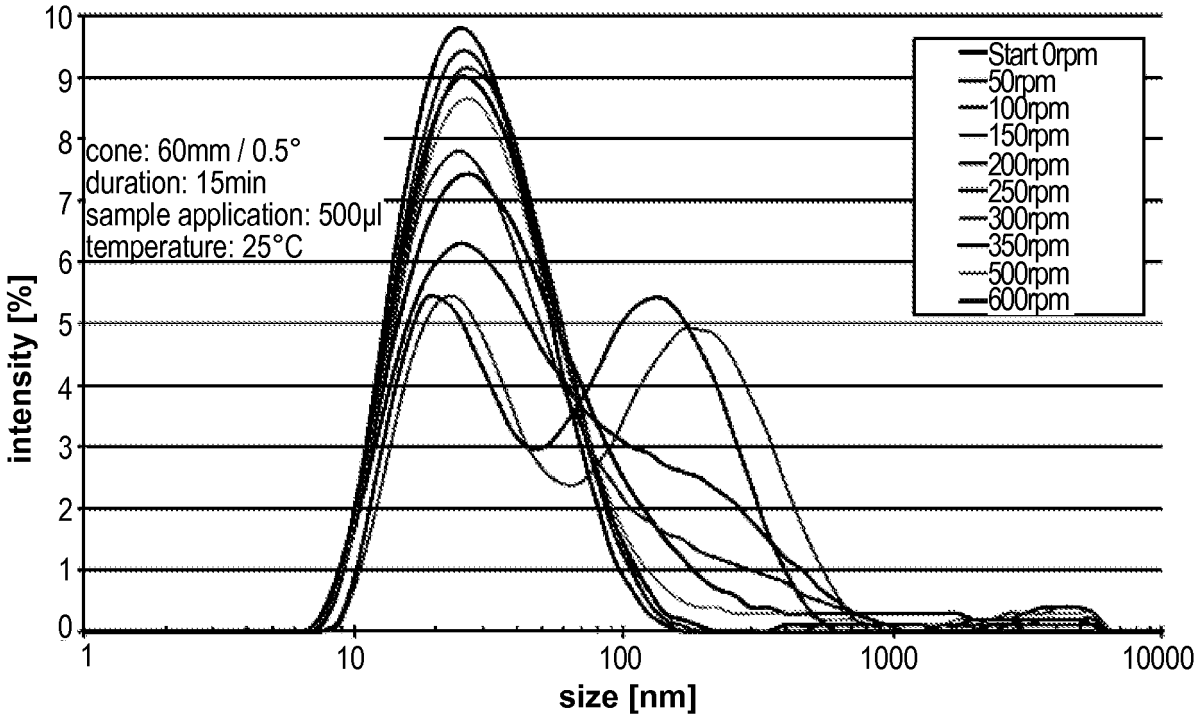

intensity [%]
size [nm]
cone: 60mm / 0.5°
duration: 15min
sample application: 500µl
temperature: 25°C
Start 0rpm
50rpm
100rpm
150rpm
200rpm
250rpm
300rpm
350rpm
500rpm
600rpm

Figure 29

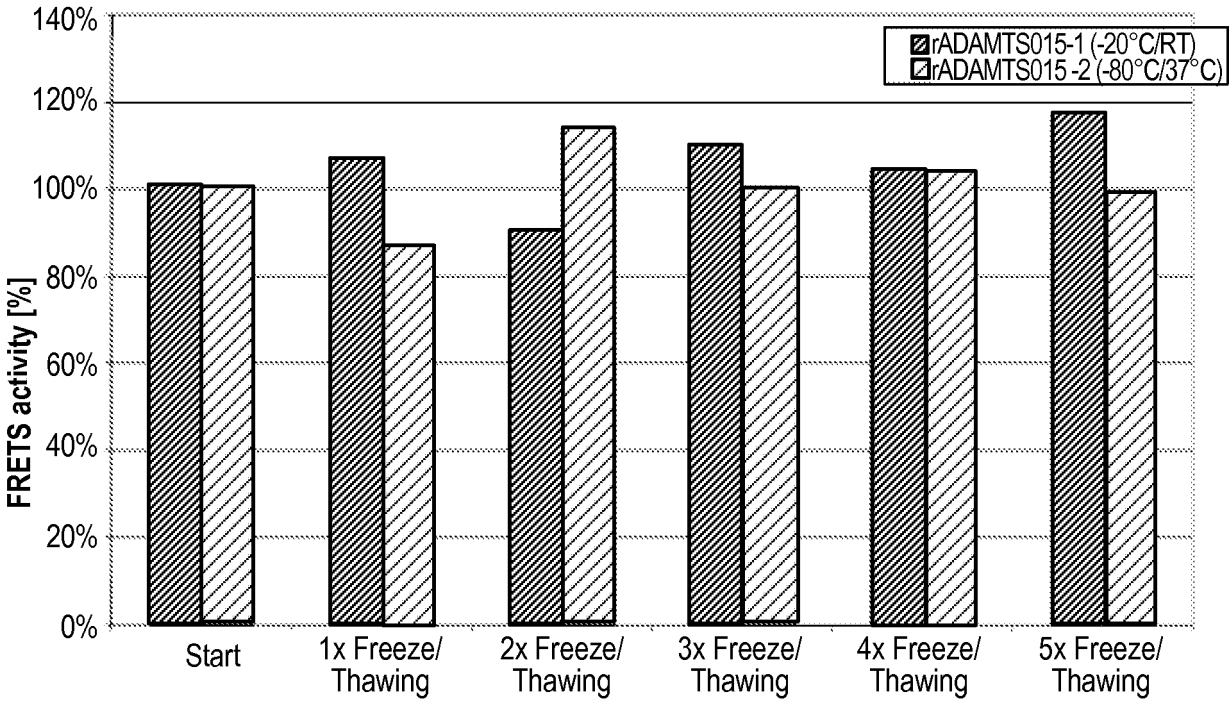

140%
120%
100%
80%
60%
40%
20%
0%
FRETS activity [%]
rADAMTS015-1 (-20°C/RT)
rADAMTS015 -2 (-80°C/37°C)
Start
1x Freeze/ Thawing
2x Freeze/ Thawing
3x Freeze/ Thawing
4x Freeze/ Thawing
5x Freeze/ Thawing

Figure 30

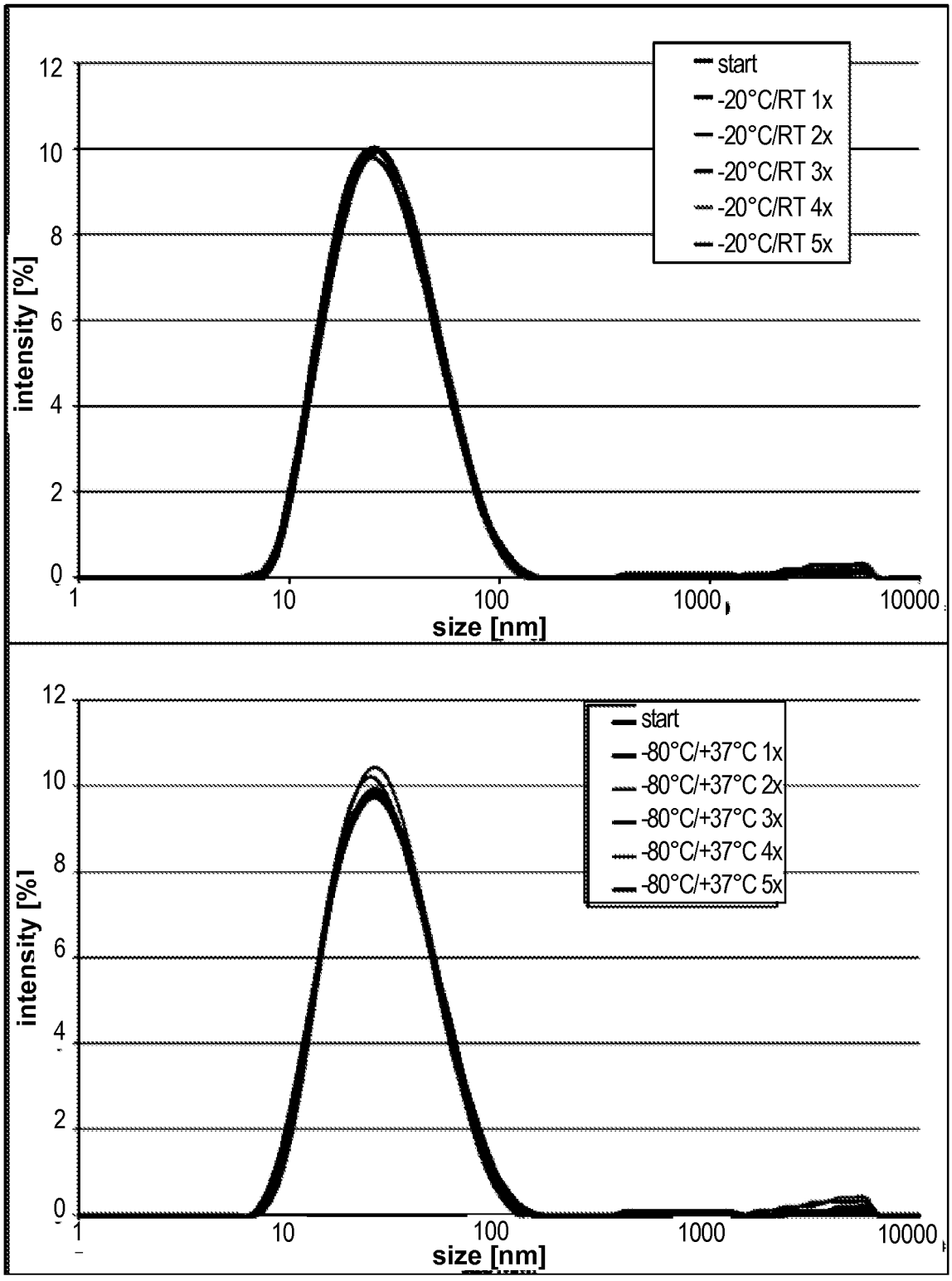

start
-20°C/RT 1x
-20°C/RT 2x
-20°C/RT 3x
-20°C/RT 4x
-20°C/RT 5x
intensity [%]
size [nm]
start
-80°C/+37°C 1x
-80°C/+37°C 2x
-80°C/+37°C 3x
-80°C/+37°C 4x
-80°C/+37°C 5x
intensity [%]
size [nm]

Figure 31

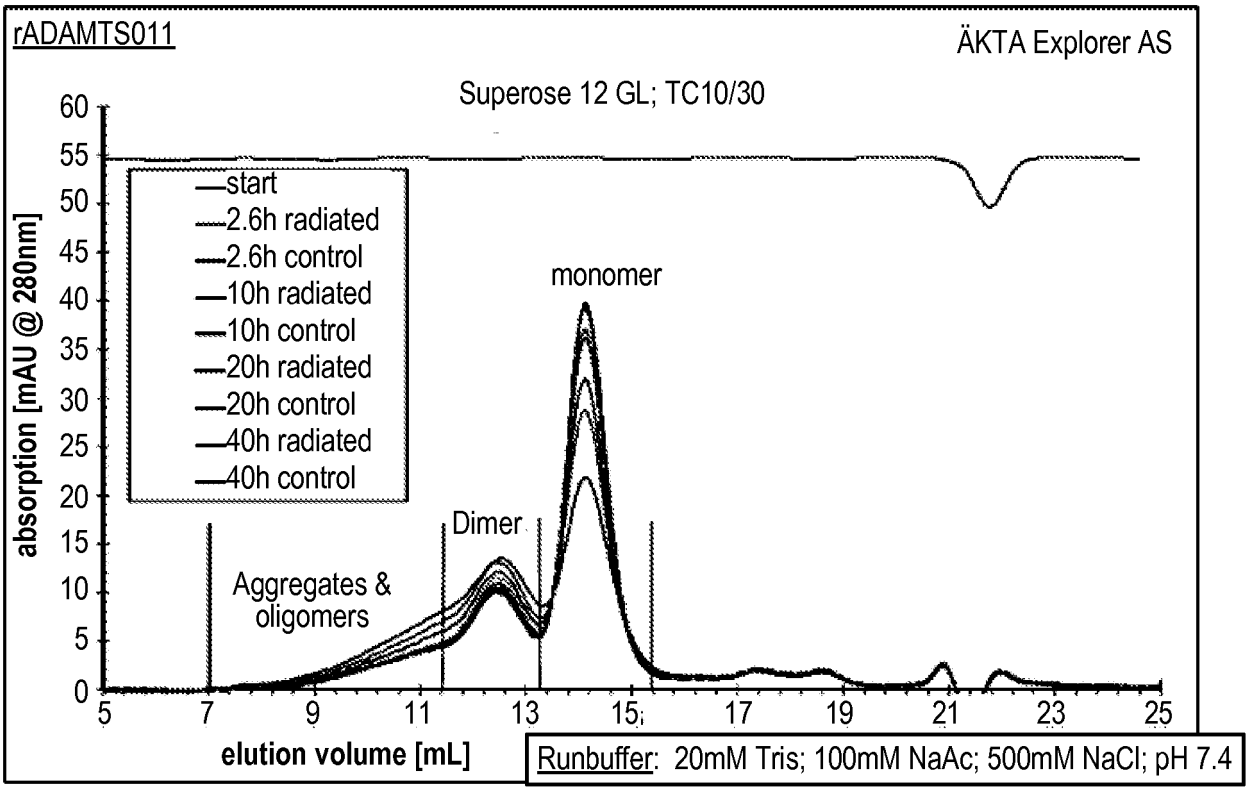

rADAMTS011
ÄKTA Explorer AS
Superose 12 GL; TC10/30
absorption [mAU @ 280nm]
elution volume [mL]
start
2.6h radiated
2.6h control
10h radiated
10h control
20h radiated
20h control
40h radiated
40h control
monomer
Dimer
Aggregates & oligomers
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

Figure 32

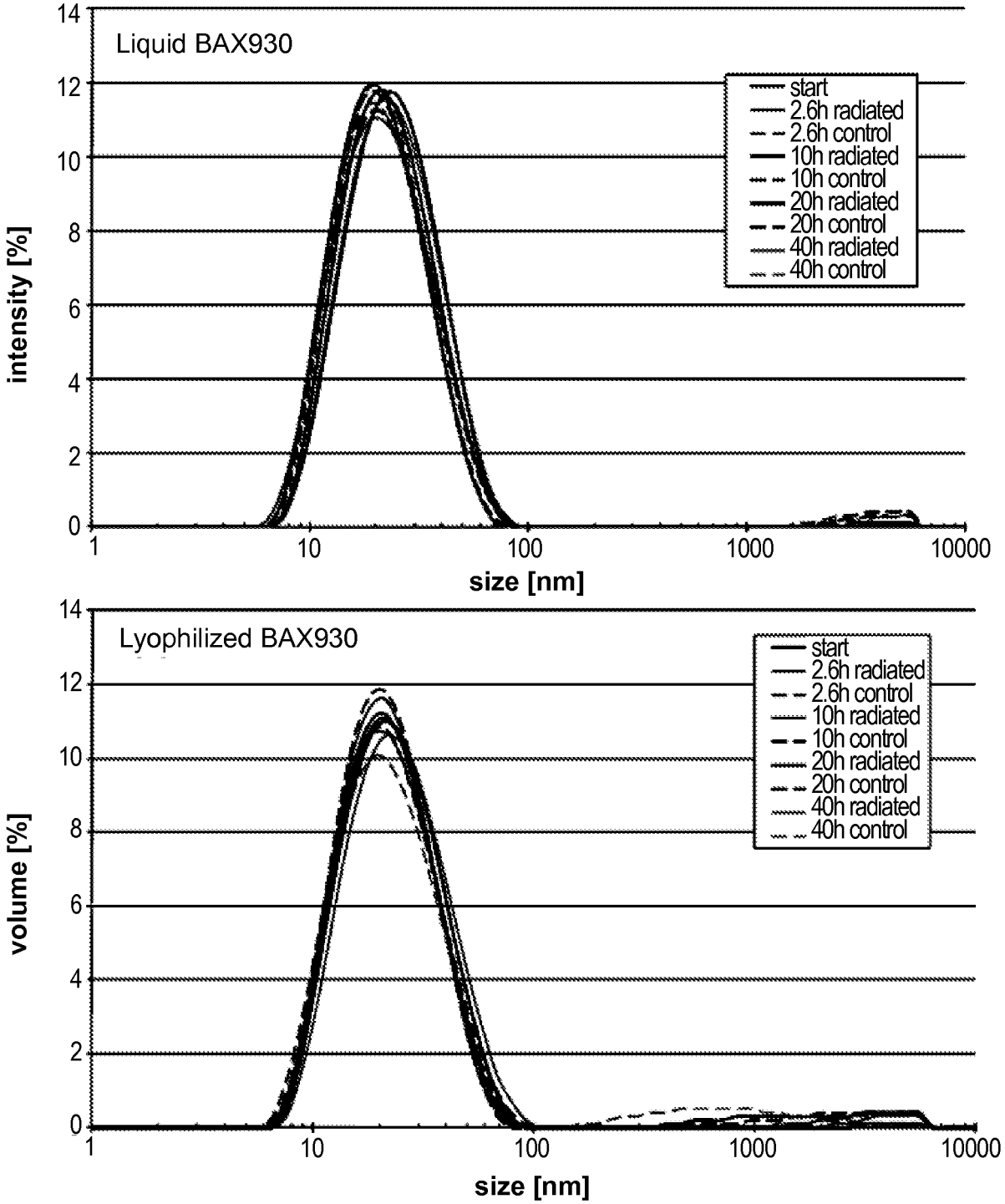

Liquid BAX930
intensity [%]
size [nm]
start
2.6h radiated
2.6h control
10h radiated
10h control
20h radiated
20h control
40h radiated
40h control
Lyophilized BAX930
volume [%]
size [nm]

Figure 33

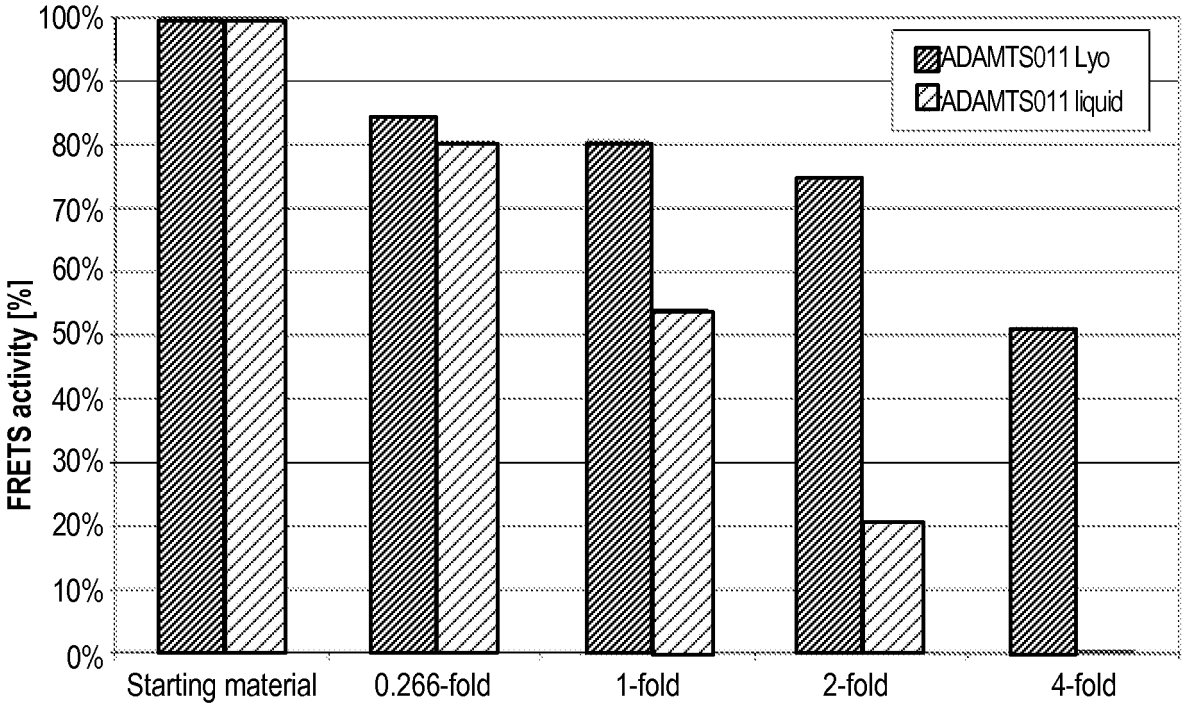

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
FRETS activity [%]
ADAMTS011 Lyo
ADAMTS011 liquid
Starting material
0.266-fold
1-fold
2-fold
4-fold

Figure 34

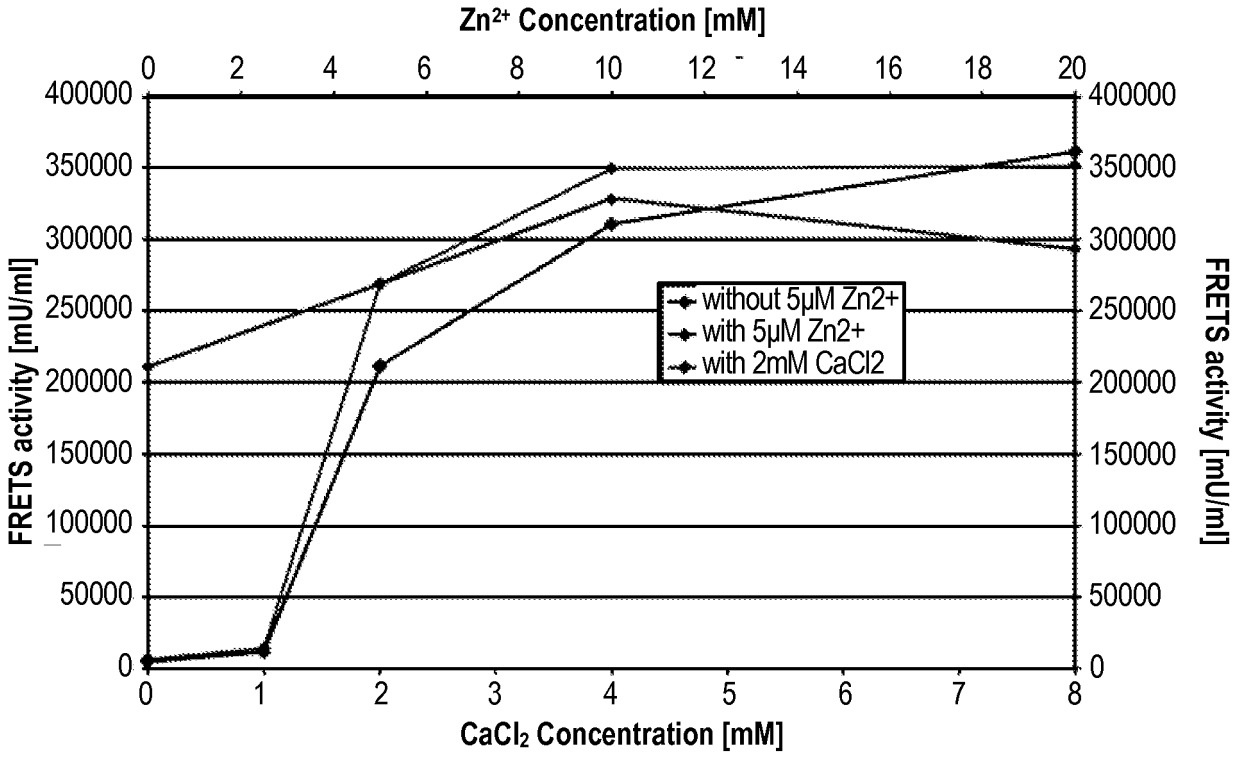

Zn2+ Concentration [mM]
0
2
4
6
8
10
12
14
16
18
20
400000
350000
300000
250000
200000
150000
100000
50000
0
FRETS activity [mU/ml]
without 5µM Zn2+
with 5µM Zn2+
with 2mM CaCl2
0
1
2
3
4
5
6
7
8
CaCl2 Concentration [mM]

Figure 35

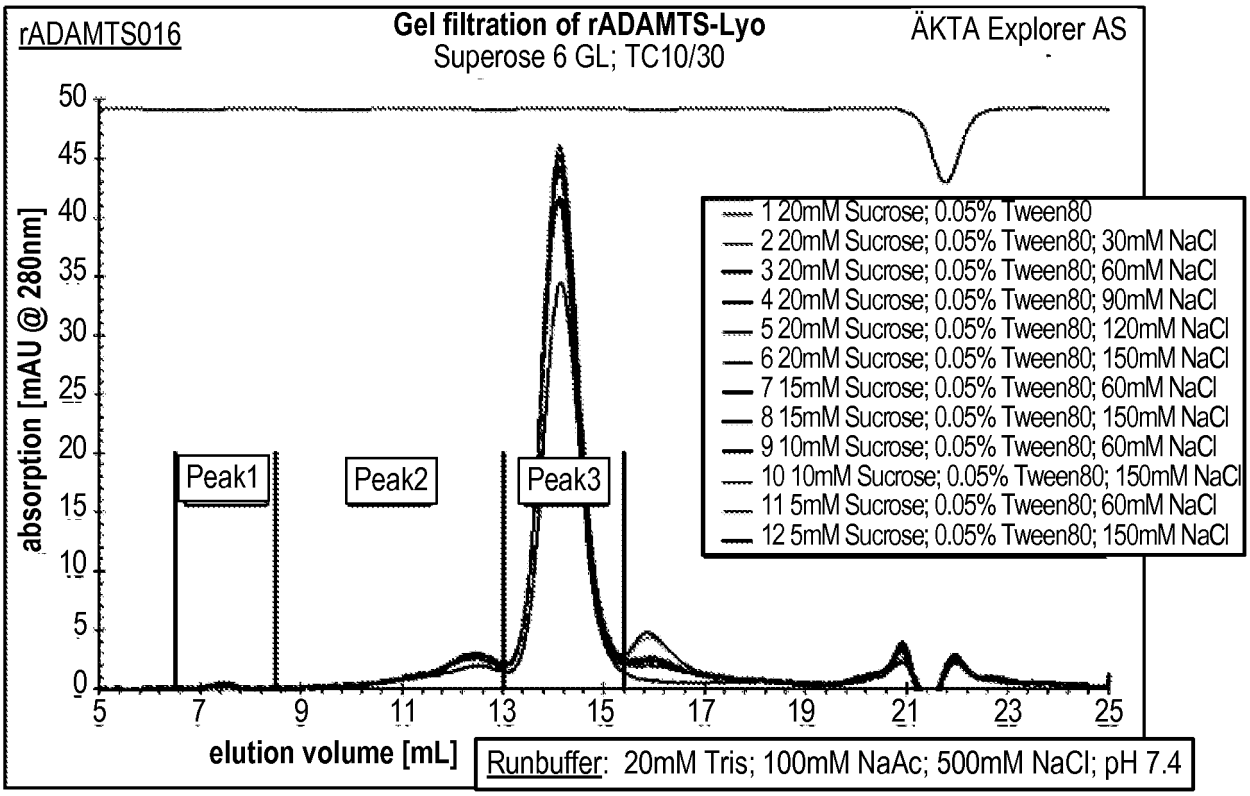

rADAMTS016
Gel filtration of rADAMTS-Lyo
Superose 6 GL; TC10/30
ÄKTA Explorer AS
absorption [mAU @ 280nm]
elution volume [mL]
Peak1
Peak2
Peak3
1 20mM Sucrose; 0.05% Tween80
2 20mM Sucrose; 0.05% Tween80; 30mM NaCl
3 20mM Sucrose; 0.05% Tween80; 60mM NaCl
4 20mM Sucrose; 0.05% Tween80; 90mM NaCl
5 20mM Sucrose; 0.05% Tween80; 120mM NaCl
6 20mM Sucrose; 0.05% Tween80; 150mM NaCl
7 15mM Sucrose; 0.05% Tween80; 60mM NaCl
8 15mM Sucrose; 0.05% Tween80; 150mM NaCl
9 10mM Sucrose; 0.05% Tween80; 60mM NaCl
10 10mM Sucrose; 0.05% Tween80; 150mM NaCl
11 5mM Sucrose; 0.05% Tween80; 60mM NaCl
12 5mM Sucrose; 0.05% Tween80; 150mM NaCl
Runbuffer: 20mM Tris; 100mM NaAc; 500mM NaCl; pH 7.4

Figure 36


rADAMTS016-1
rADAMTS016-2
rADAMTS016-3
rADAMTS016-4
rADAMTS016-5
rADAMTS016-6
rADAMTS016-7
rADAMTS016-8
rADAMTS016-9
rADAMTS016-10
rADAMTS016-11
rADAMTS016-12

Figure 37

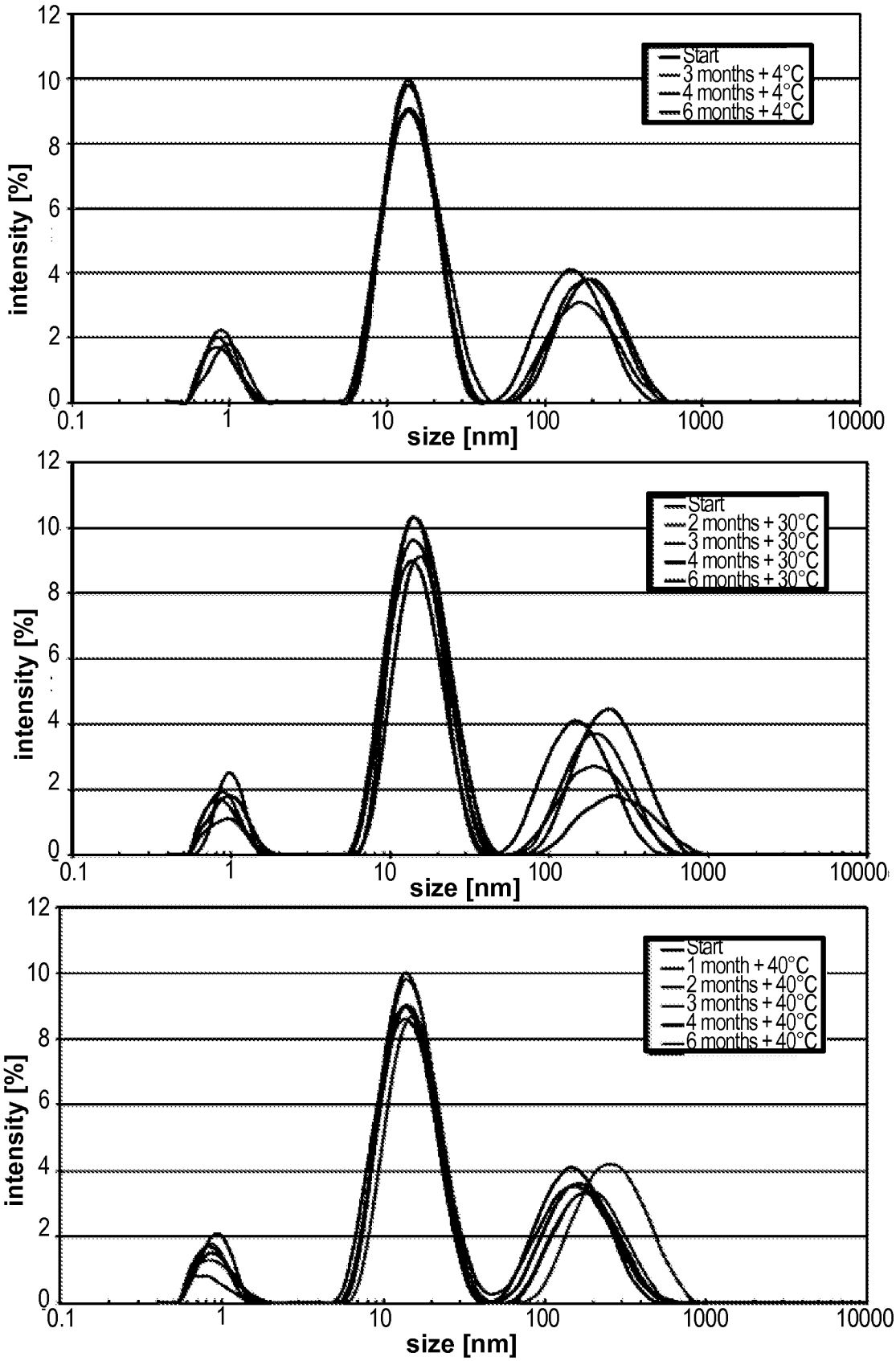

intensity [%]
size [nm]
Start
3 months + 4°C
4 months + 4°C
6 months + 4°C
Start
2 months + 30°C
3 months + 30°C
4 months + 30°C
6 months + 30°C
Start
1 month + 40°C
2 months + 40°C
3 months + 40°C
4 months + 40°C
6 months + 40°C

Figure 38

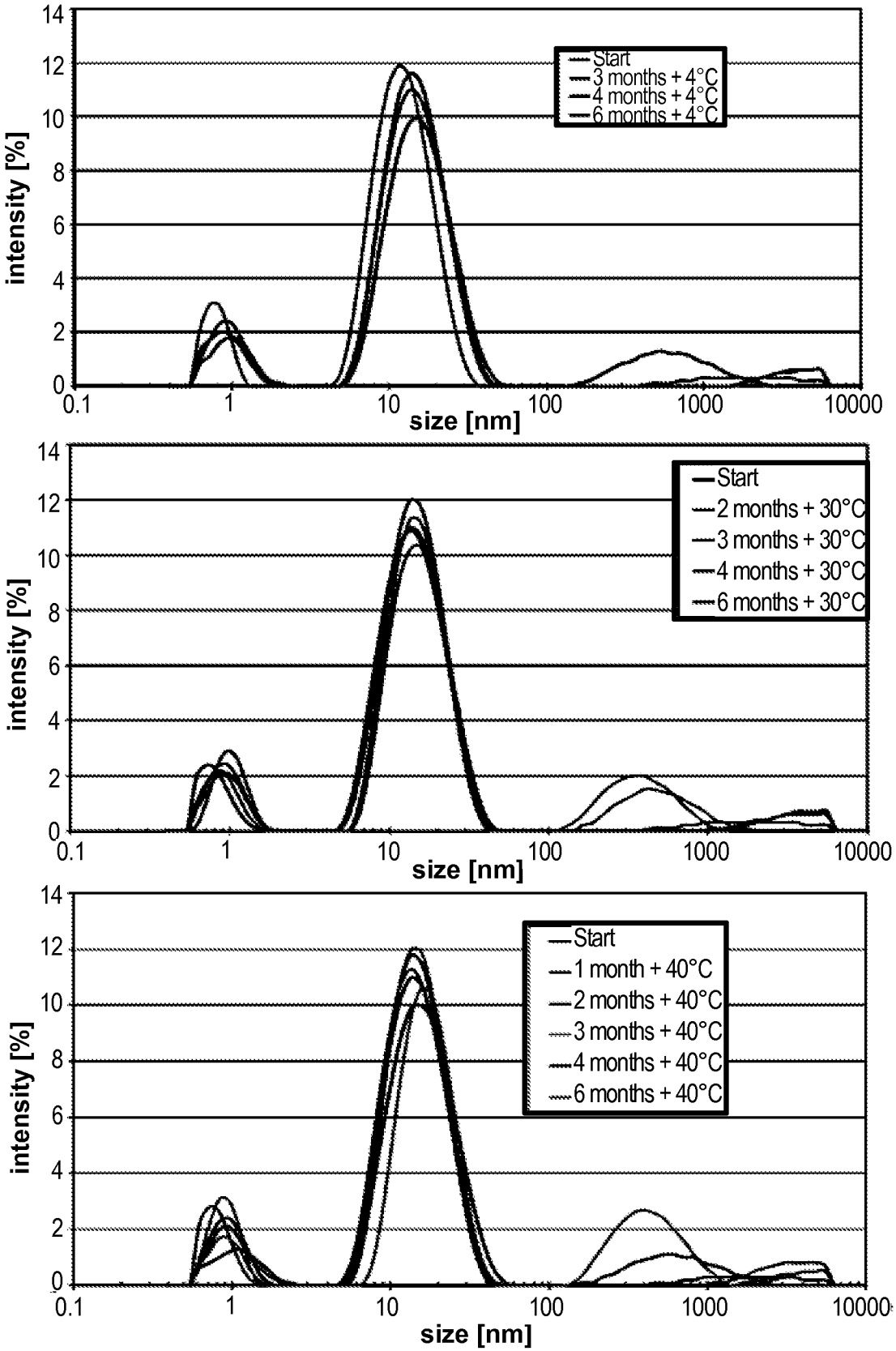
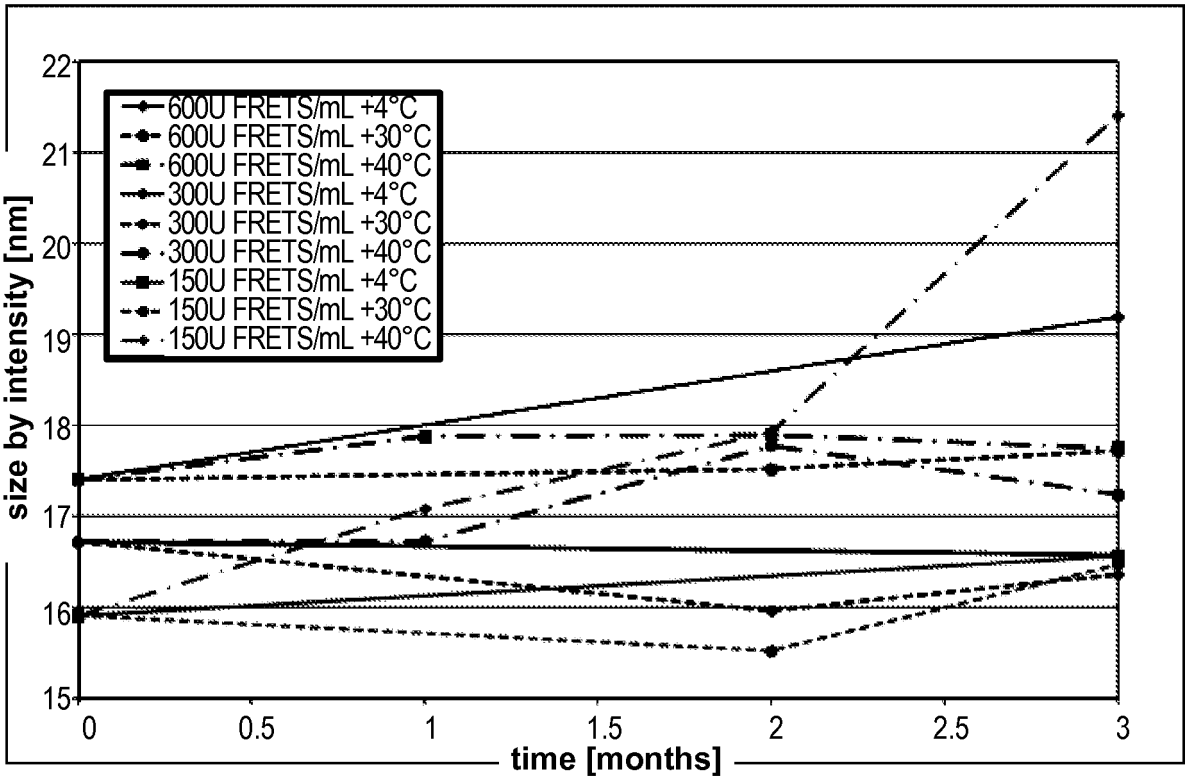

intensity [%]
size [nm]
Start
3 months + 4°C
4 months + 4°C
6 months + 4°C
Start
2 months + 30°C
3 months + 30°C
4 months + 30°C
6 months + 30°C
Start
1 month + 40°C
2 months + 40°C
3 months + 40°C
4 months + 40°C
6 months + 40°C

Figure 39


600U FRETS/mL +4°C
600U FRETS/mL +30°C
600U FRETS/mL +40°C
300U FRETS/mL +4°C
300U FRETS/mL +30°C
300U FRETS/mL +40°C
150U FRETS/mL +4°C
150U FRETS/mL +30°C
150U FRETS/mL +40°C
size by intensity [nm]
22
21
20
19
18
17
16
15
0
0.5
1
1.5
2
2.5
3
time [months]