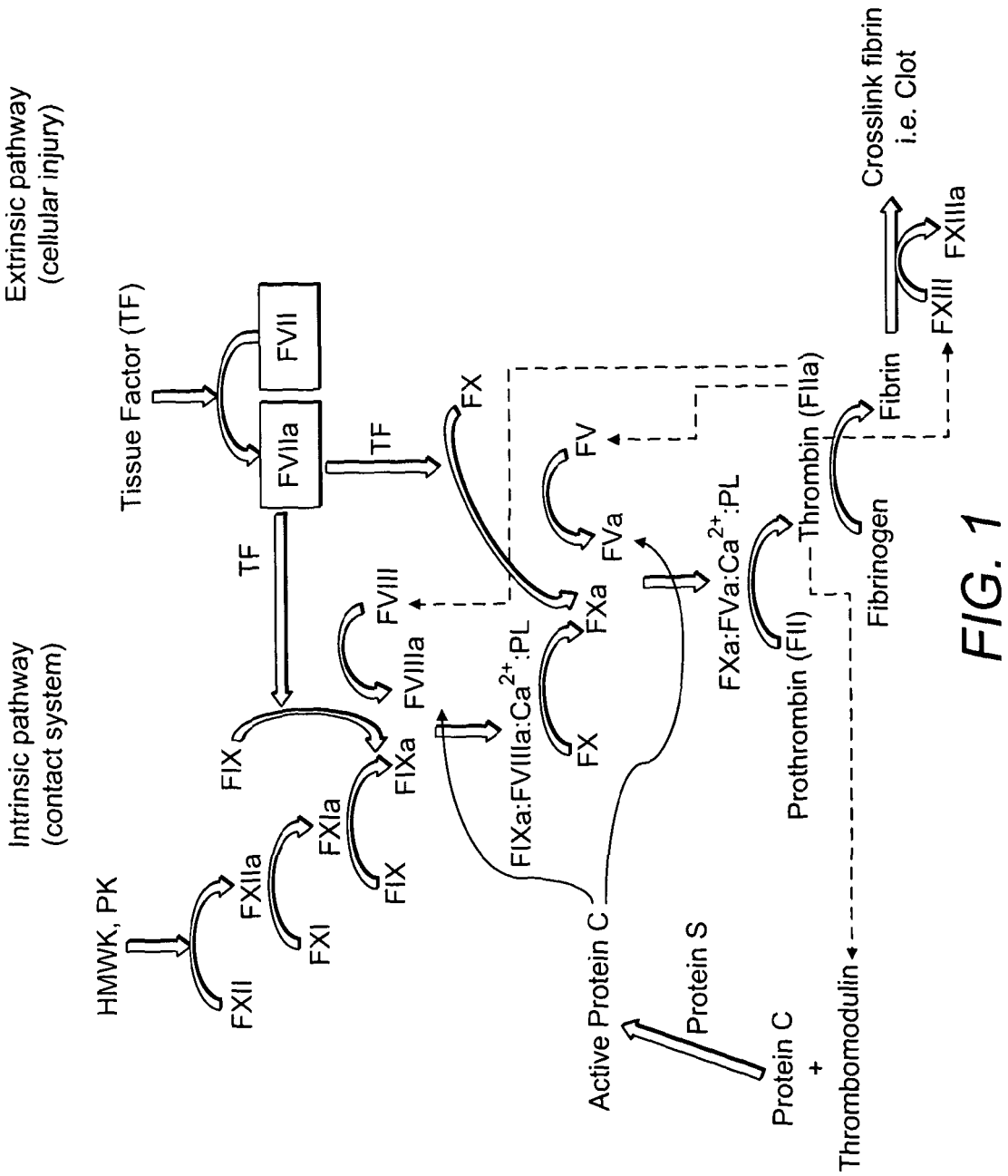

Extrinsic pathway
(cellular injury)
Intrinsic pathway
(contact system)
Tissue Factor (TF)
FVII
FVIIa
TF
TF
FX
FV
FVa
FVIII
FVIIIa
FIX
FIXa
FIXa:FVIIIa:Ca2+:PL
FX
FXa
FXa:FVa:Ca2+:PL
Thrombin (FIIa)
Prothrombin (FII)
Fibrin
Fibrinogen
FXIII
FXIIIa
Crosslink fibrin
i.e. Clot
HMWK, PK
FXII
FXIIa
FXI
FXIa
FIX
Active Protein C
Protein S
Protein C
+
Thrombomodulin

FIG. 1

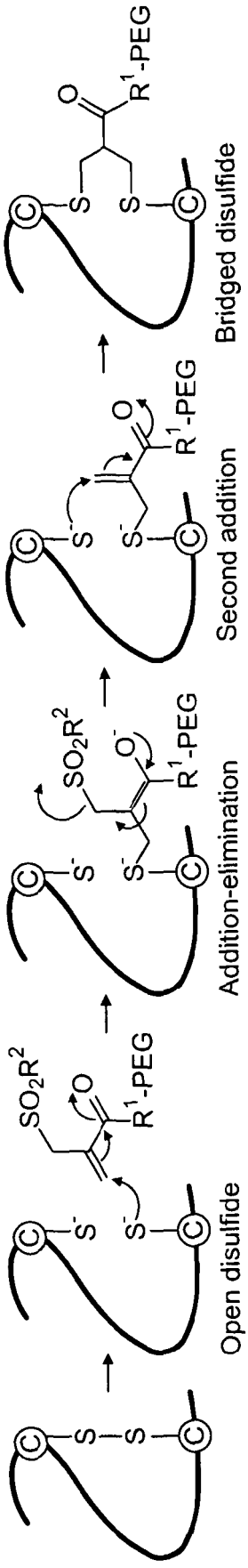

Open disulfide
Addition-elimination
Second addition
Bridged disulfide
SO2R2
R1-PEG

FIG. 2

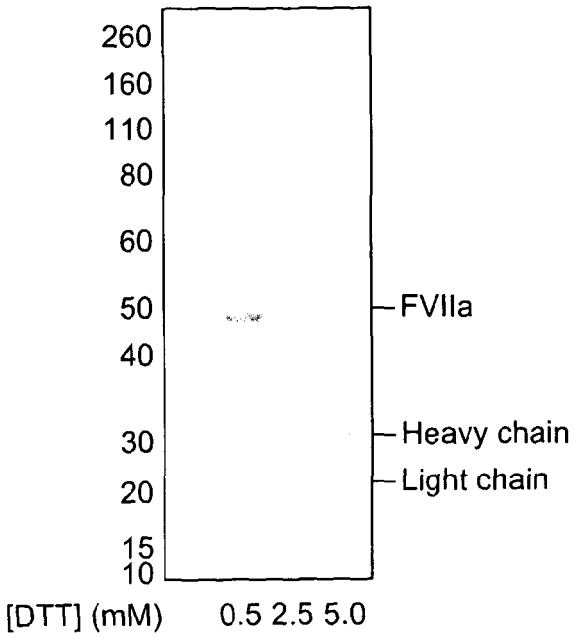

260
160
110
80
60
50
40
30
20
15
10
FVIIa
Heavy chain
Light chain
[DTT] (mM) 0.5 2.5 5.0

FIG. 3(a)

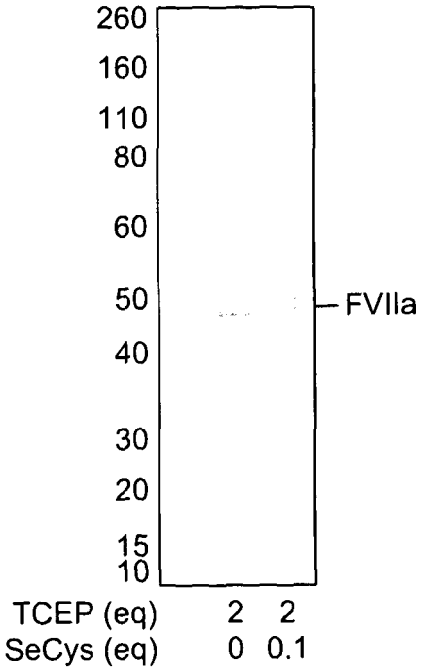

260
160
110
80
60
50
40
30
20
15
10
FVIIa
TCEP (eq) 2 2
SeCys (eq) 0 0.1

FIG. 3(b)

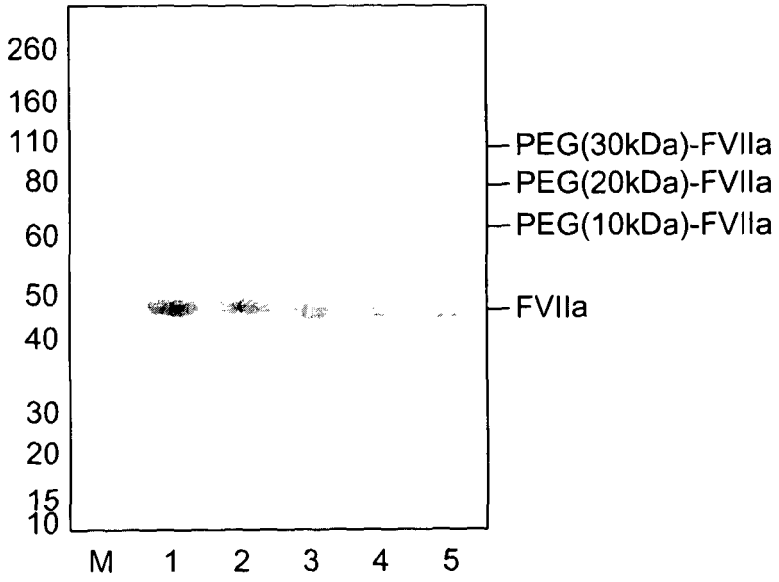

260
160
110
80
60
50
40
30
20
15
10
M
1
2
3
4
5
PEG(30kDa)-FVIIa
PEG(20kDa)-FVIIa
PEG(10kDa)-FVIIa
FVIIa

FIG. 4

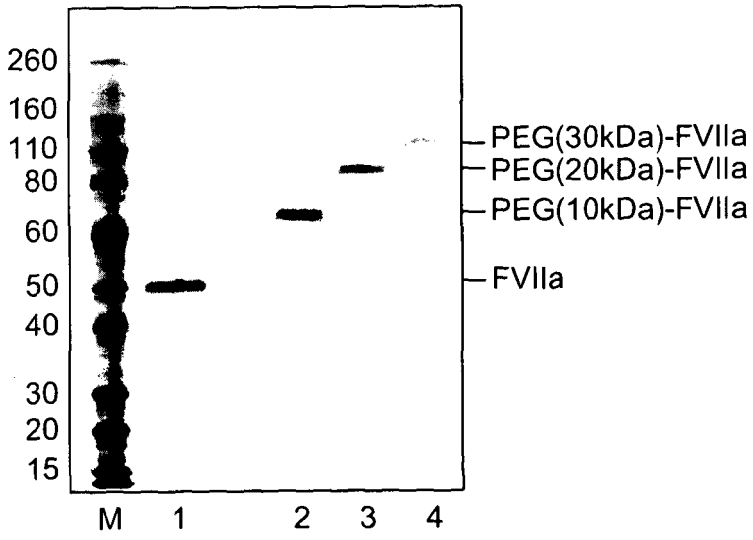

260
160
110
80
60
50
40
30
20
15
M
1
2
3
4
PEG(30kDa)-FVIIa
PEG(20kDa)-FVIIa
PEG(10kDa)-FVIIa
FVIIa

FIG. 5

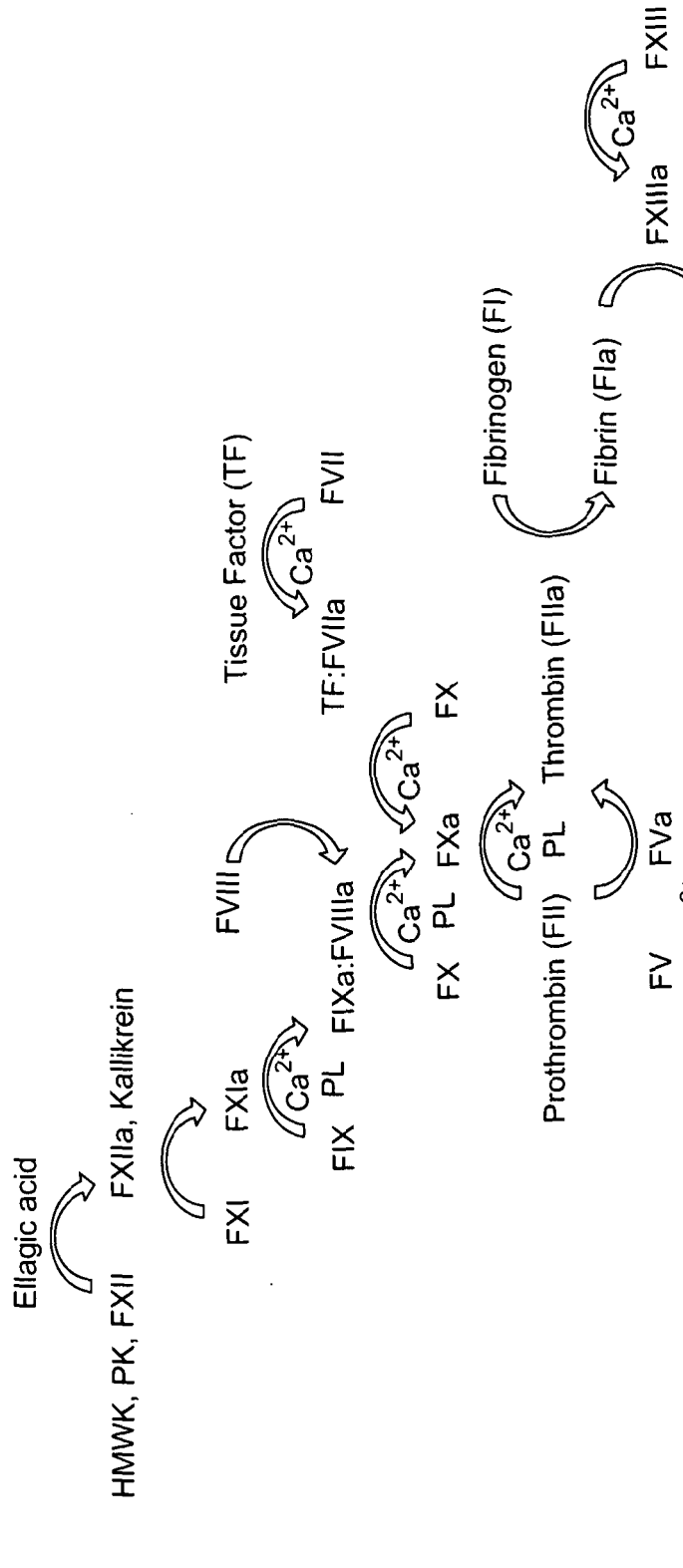

Ellagic acid
HMWK, PK, FXII
FXIIa, Kallikrein
FXI
FXIa
Ca2+
FIX PL FIXa:FVIIIa
FVIII
Ca2+
FX PL FXa
Ca2+
FX
Tissue Factor (TF)
Ca2+
TF:FVIIa
FVII
Ca2+
Prothrombin (FII) PL Thrombin (FIIa)
Fibrinogen (FI)
Fibrin (Fla)
FV
FVa
Ca2+
PL
Ca2+
FXIIIa
FXIII
Cross-linked fibrin
HMWK: High MW kininogen
PK: Prekallikrein
PL: Phospholipid

FIG. 6

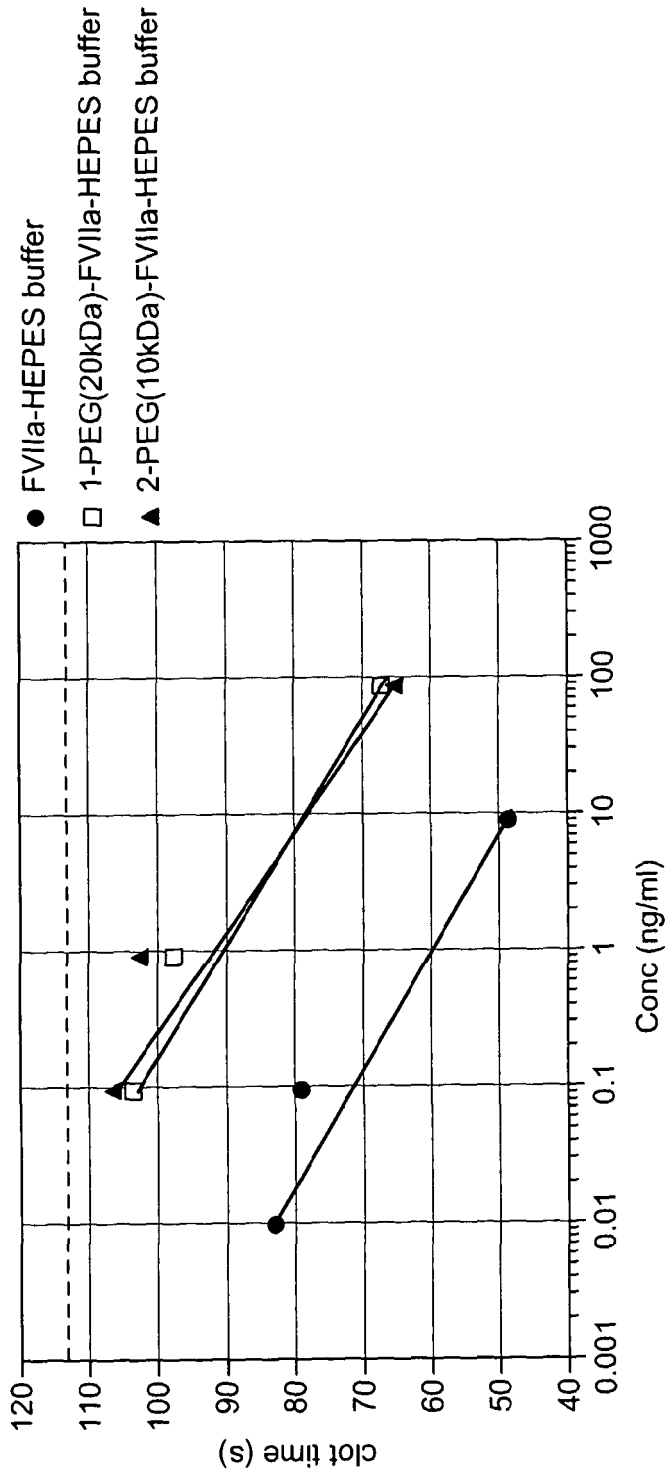

FVIIa-HEPES buffer
1-PEG(20kDa)-FVIIa-HEPES buffer
2-PEG(10kDa)-FVIIa-HEPES buffer
clot time (s)
120
110
100
90
80
70
60
50
40
0.001
0.01
0.1
1
10
100
1000
Conc (ng/ml)

FIG. 7

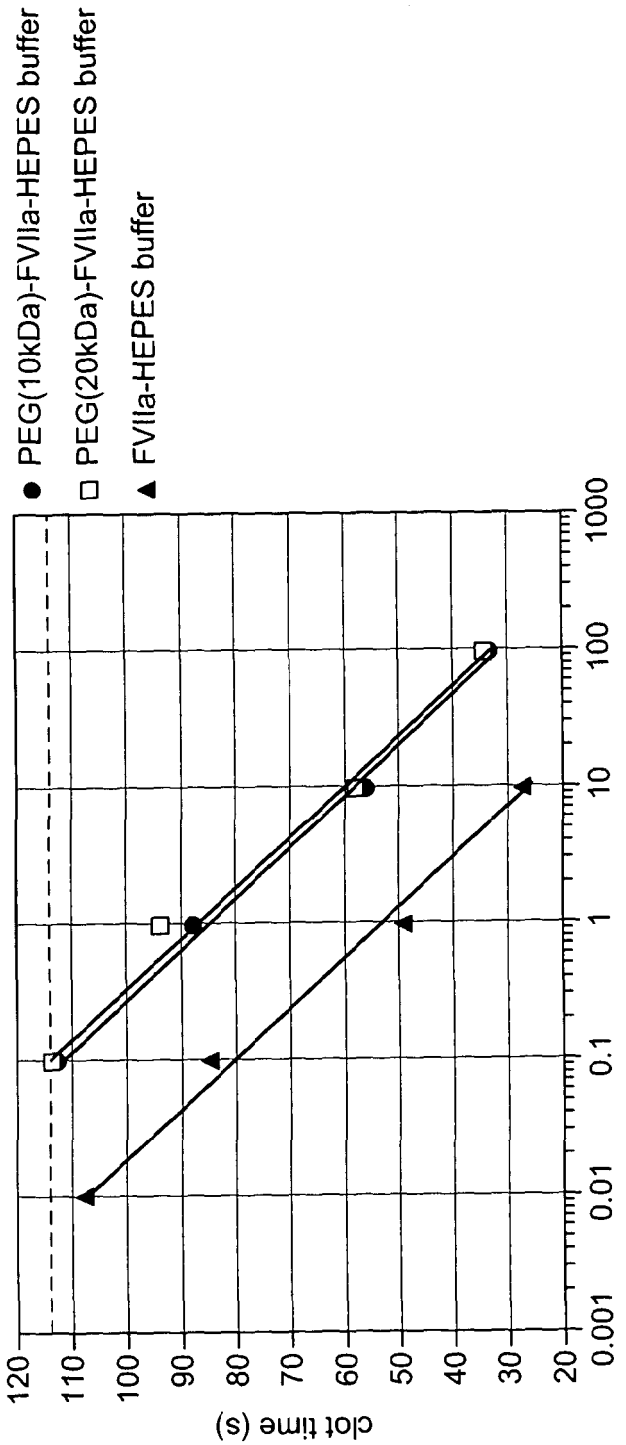

● PEG(10kDa)-FVIIa-HEPES buffer
□ PEG(20kDa)-FVIIa-HEPES buffer
▲ FVIIa-HEPES buffer
clot time (s)
120
110
100
90
80
70
60
50
40
30
20
0.001
0.01
0.1
1
10
100
1000
Conc (ng/ml)

FIG. 8

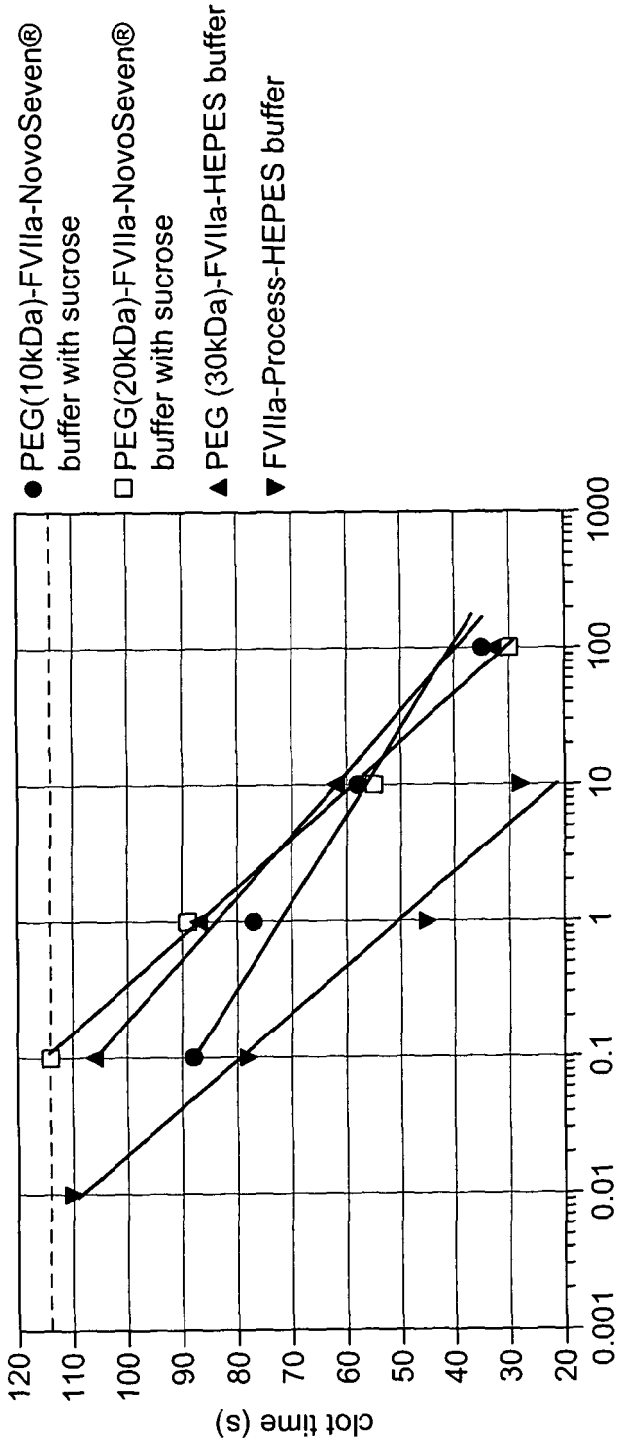

● PEG(10kDa)-FVIIa-NovoSeven® buffer with sucrose
□ PEG(20kDa)-FVIIa-NovoSeven® buffer with sucrose
▲ PEG (30kDa)-FVIIa-HEPES buffer
▼ FVIIa-Process-HEPES buffer
clot time (s)
120
110
100
90
80
70
60
50
40
30
20
0.001
0.01
0.1
1
10
100
1000
Conc (ng/ml)

FIG. 9

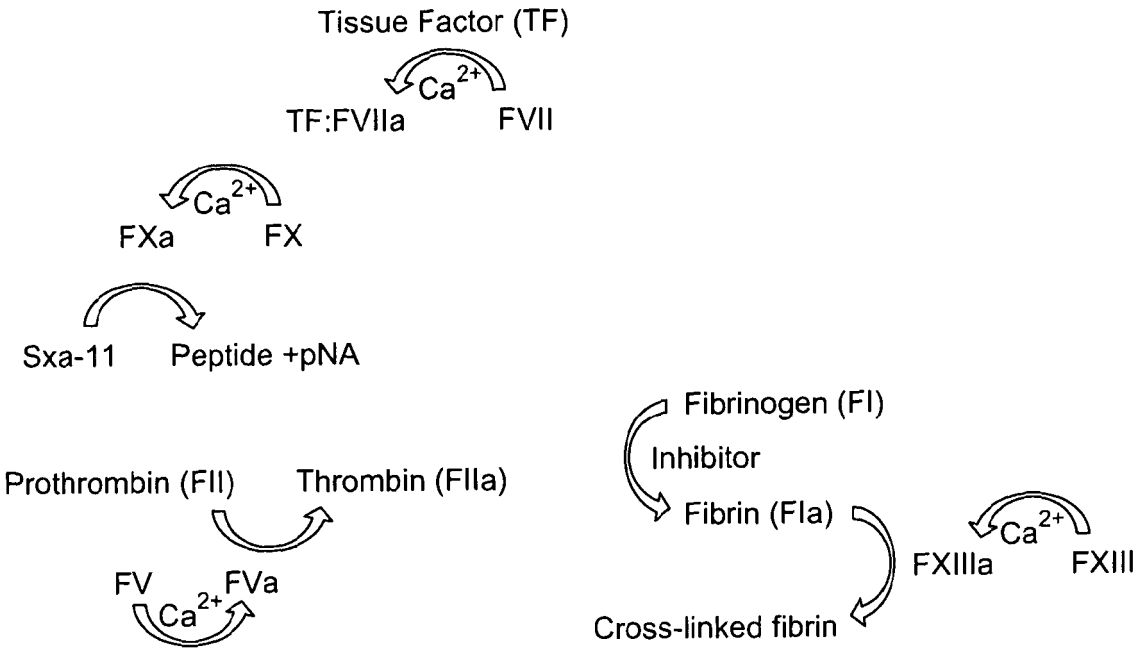

Tissue Factor (TF)
Ca2+
TF:FVIIa
FVII
Ca2+
FXa
FX
Sxa-11
Peptide +pNA
Prothrombin (FII)
Thrombin (FIIa)
FV
FVa
Ca2+
Fibrinogen (FI)
Inhibitor
Fibrin (FIa)
Ca2+
FXIIIa
FXIII
Cross-linked fibrin

FIG. 10

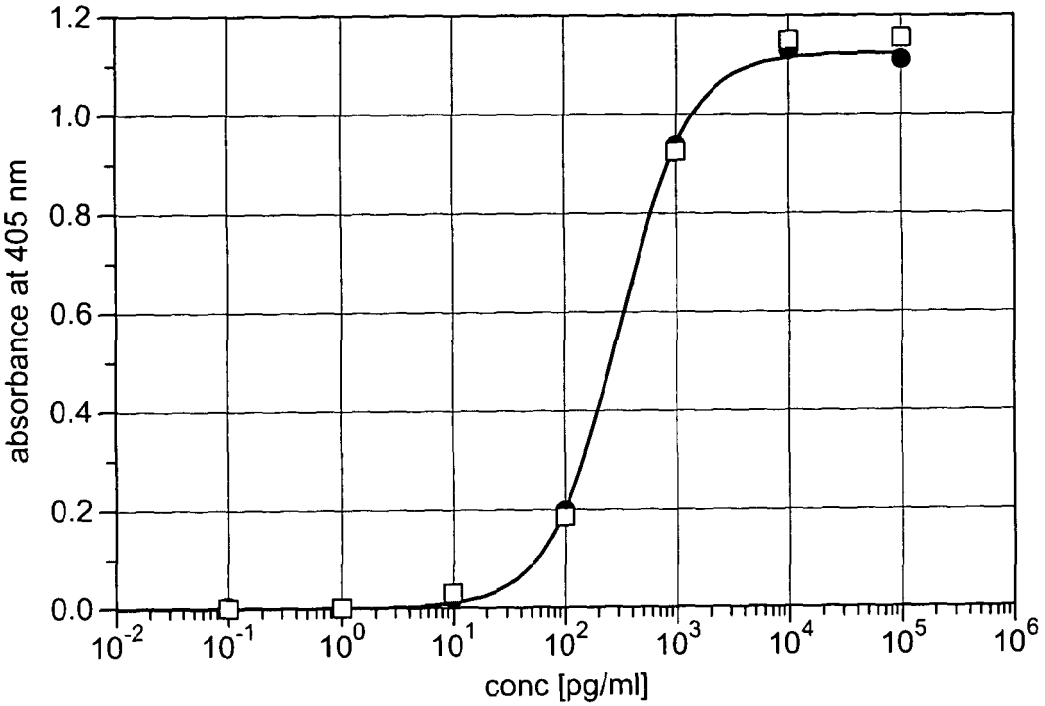

absorbance at 405 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
conc [pg/ml]

FIG. 11

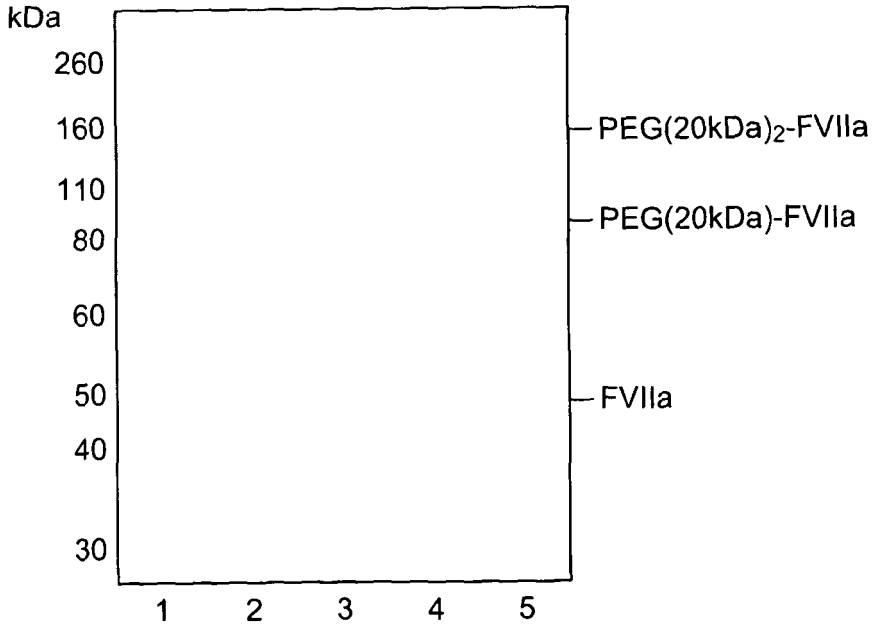

kDa
260
160
110
80
60
50
40
30
1
2
3
4
5
PEG(20kDa)$_2$-FVIIa
PEG(20kDa)-FVIIa
FVIIa

FIG. 12

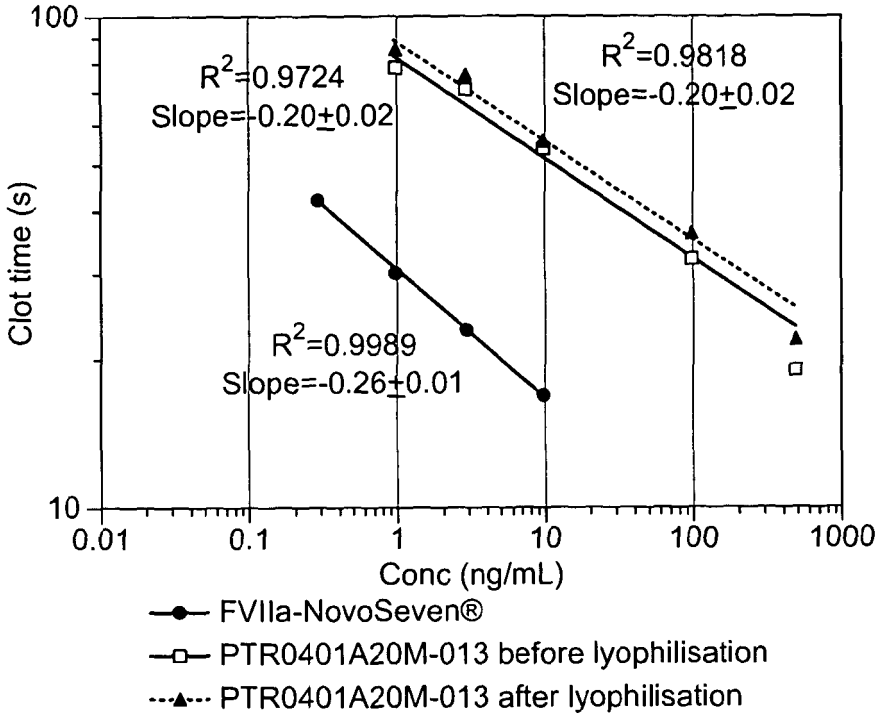


FIG. 13(a)

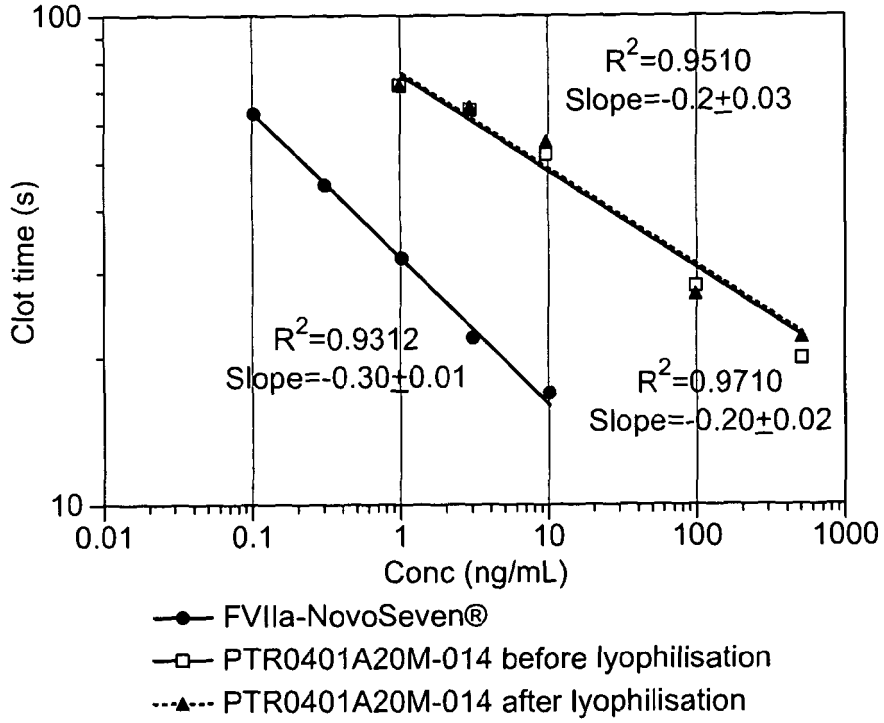


FIG. 13(b)

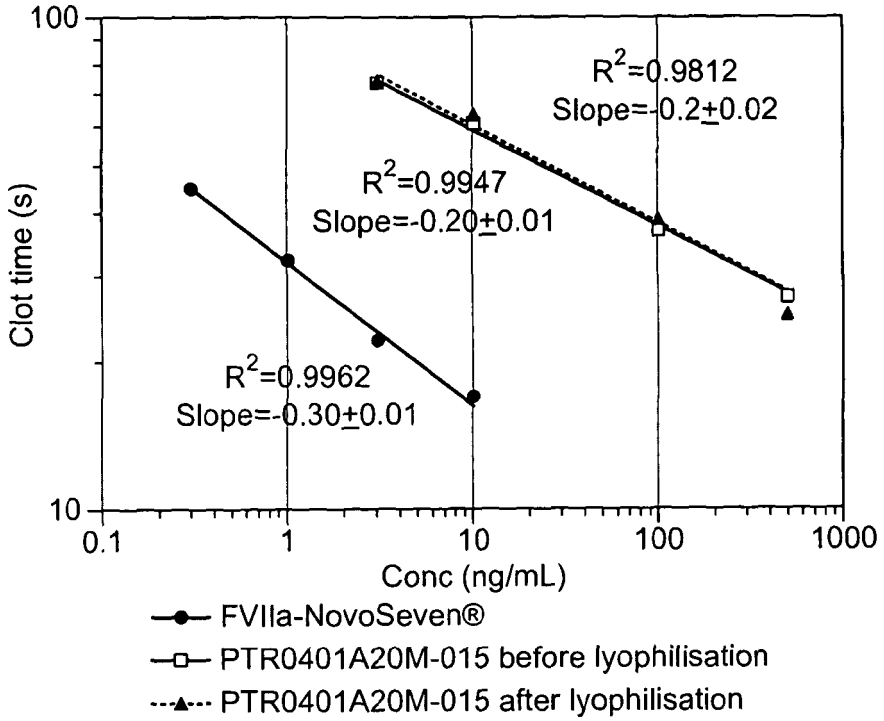


FIG. 13(c)

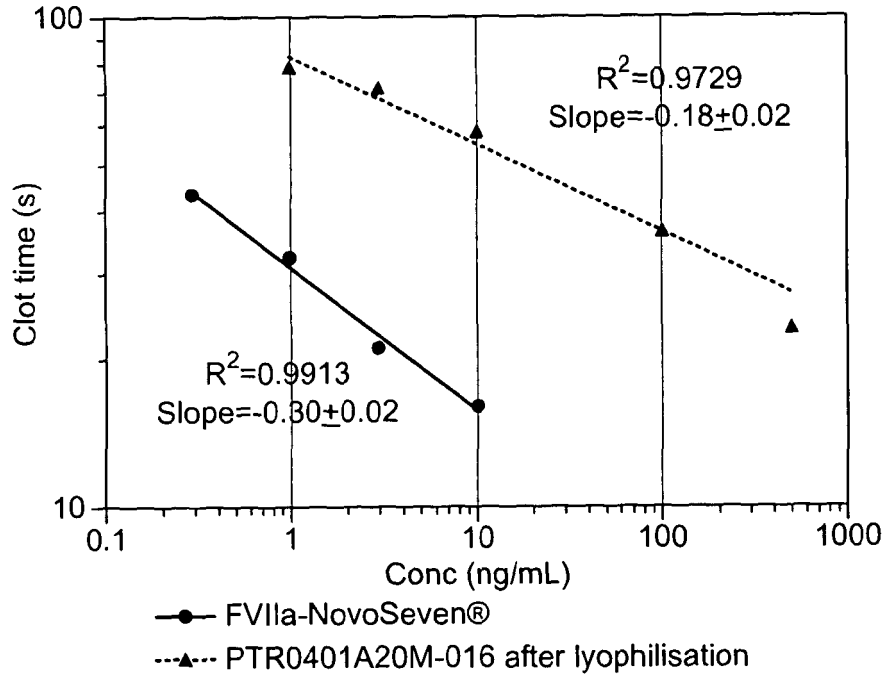


FIG. 13(d)

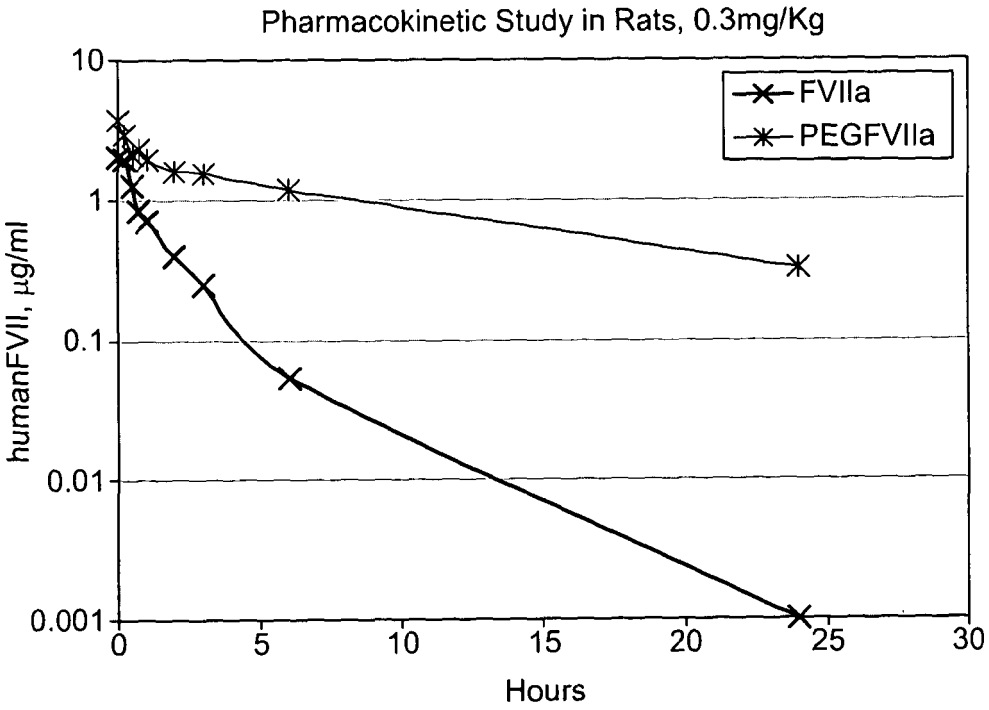

Pharmacokinetic Study in Rats, 0.3mg/Kg
FVIIa
PEGFVIIa
humanFVII, µg/ml
10
1
0.1
0.01
0.001
0
5
10
15
20
25
30
Hours

FIG. 14

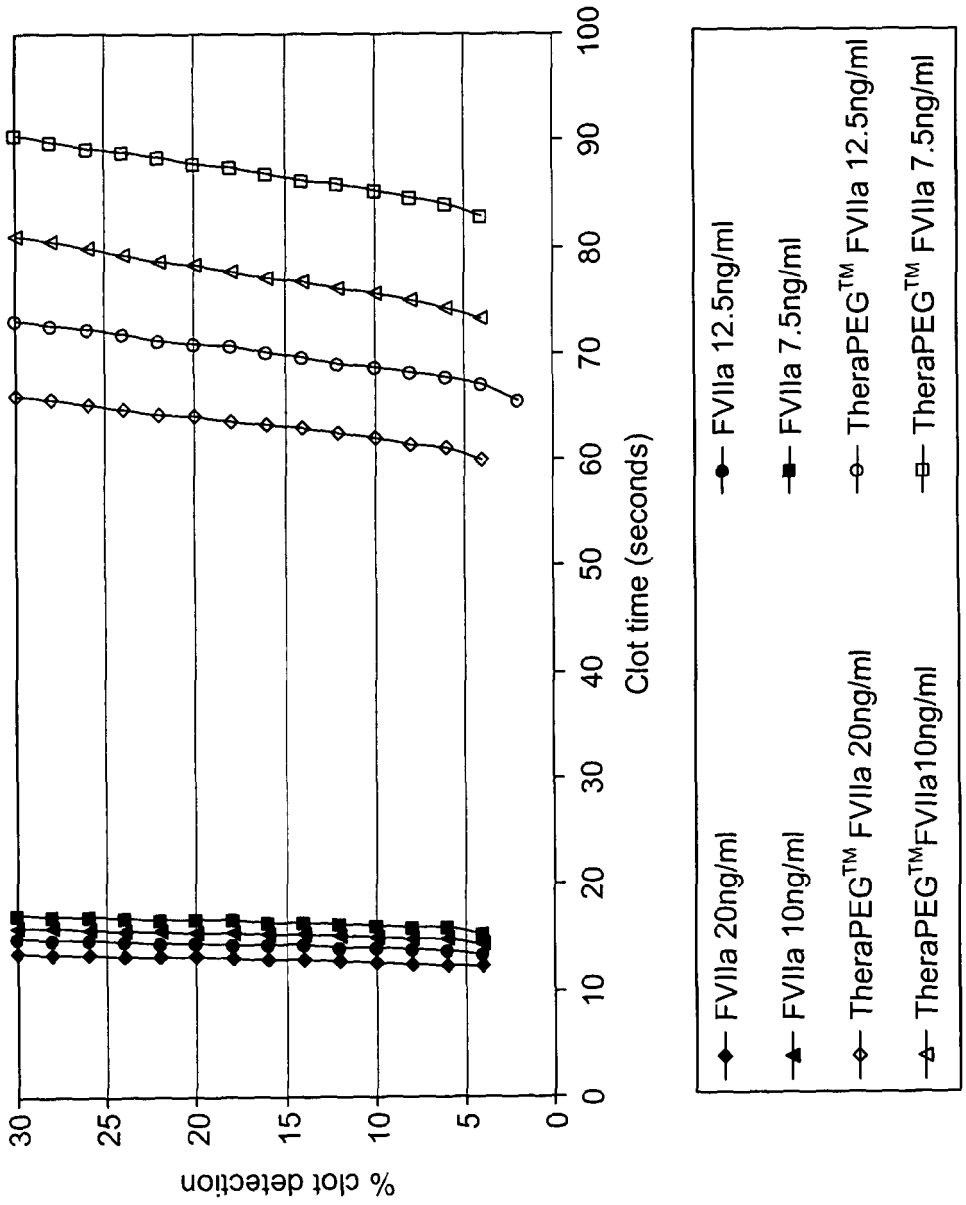

% clot detection
0
5
10
15
20
25
30
Clot time (seconds)
0
10
20
30
40
50
60
70
80
90
100
FVIIa 20ng/ml
FVIIa 10ng/ml
TheraPEG™ FVIIa 20ng/ml
TheraPEG™FVIIa10ng/ml
FVIIa 12.5ng/ml
FVIIa 7.5ng/ml
TheraPEG™ FVIIa 12.5ng/ml
TheraPEG™ FVIIa 7.5ng/ml

FIG. 15

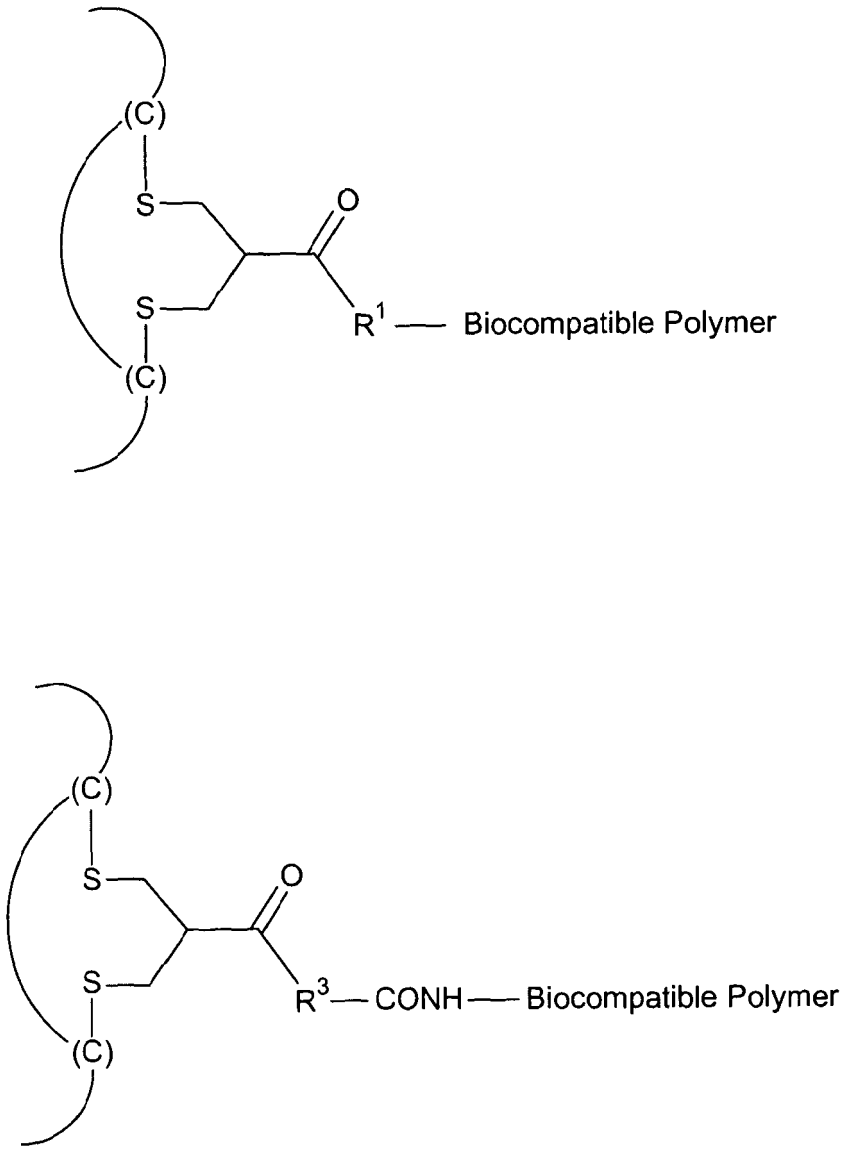

(C)
S
O
S
R1
Biocompatible Polymer
(C)
(C)
S
O
S
R3
CONH
Biocompatible Polymer
(C)

FIG. 16