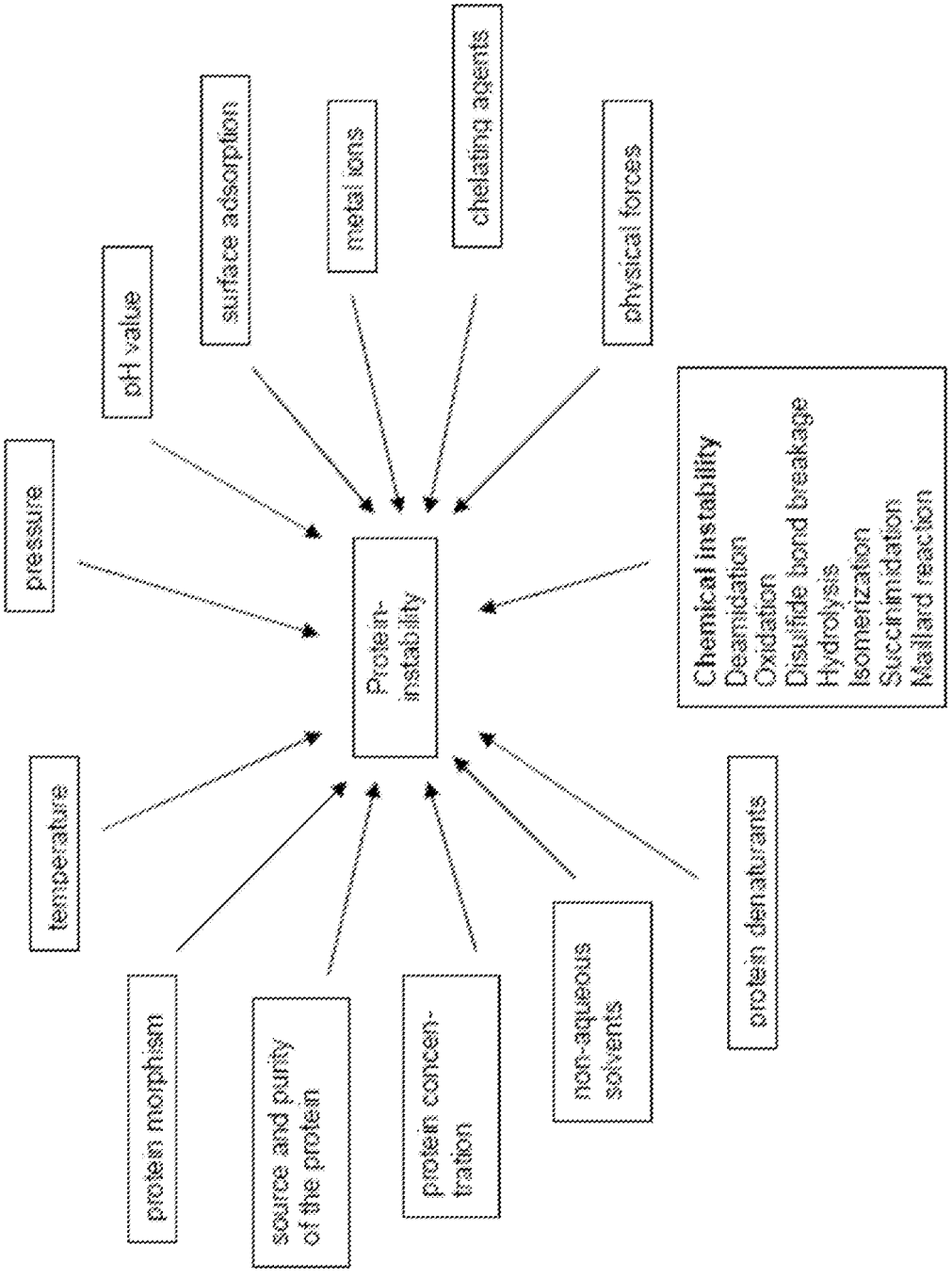

Protein-instability
pressure
pH value
surface adsorption
metal ions
chelating agents
physical forces
Chemical instability
Deamidation
Oxidation
Disulfide bond breakage
Hydrolysis
Isomerization
Succinimidation
Maillard reaction
temperature
protein morphism
source and purity of the protein
protein concen-tration
non-aqueous solvents
protein denaturants

Figure 1

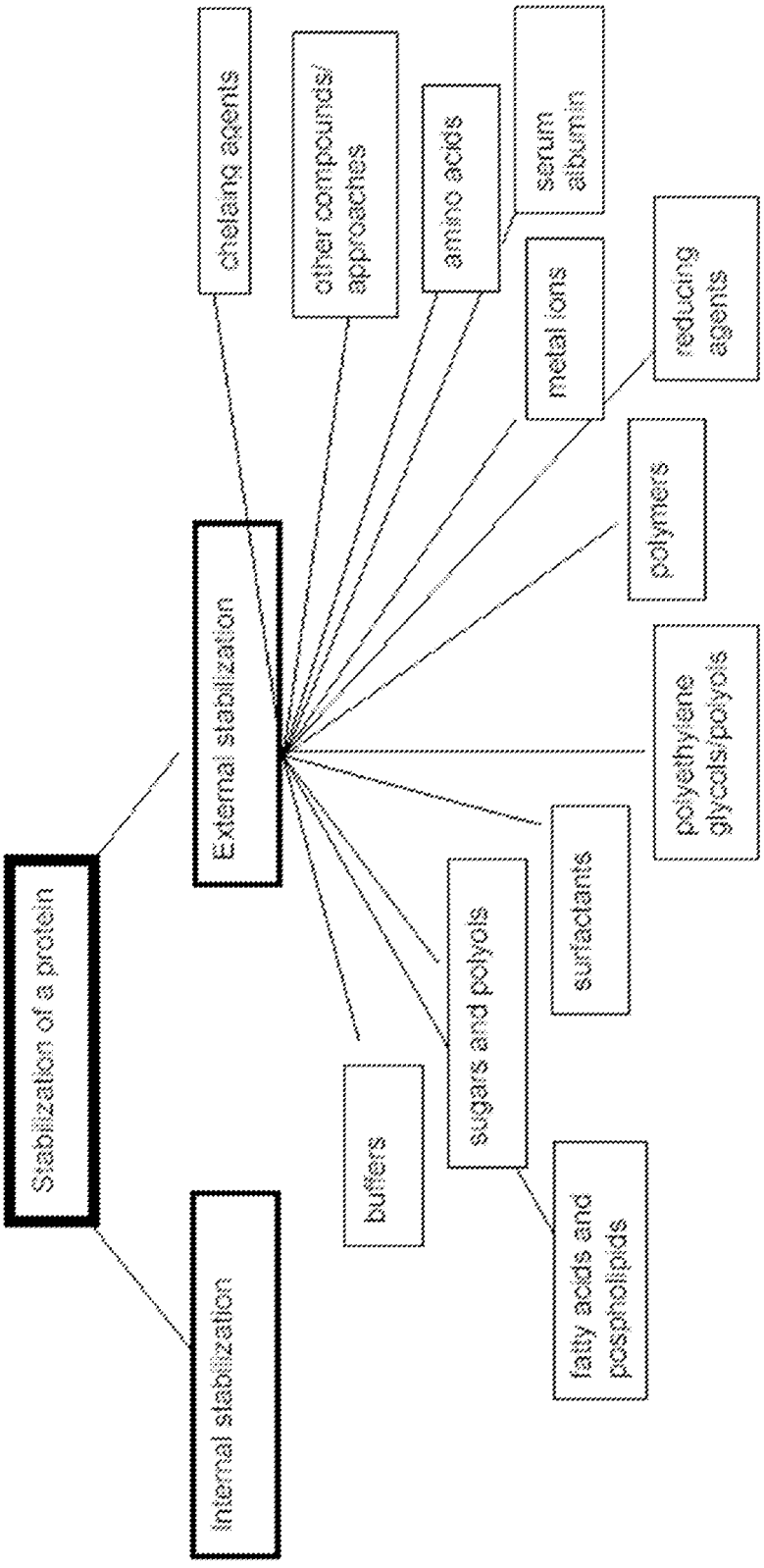

Stabilization of a protein
External stabilization
Internal stabilization
chelating agents
other compounds/ approaches
amino acids
serum albumin
metal ions
reducing agents
polymers
polyethylene glycols/polyols
surfactants
sugars and polyols
buffers
fatty acids and posphonlipids

Figure 2

Figure 3

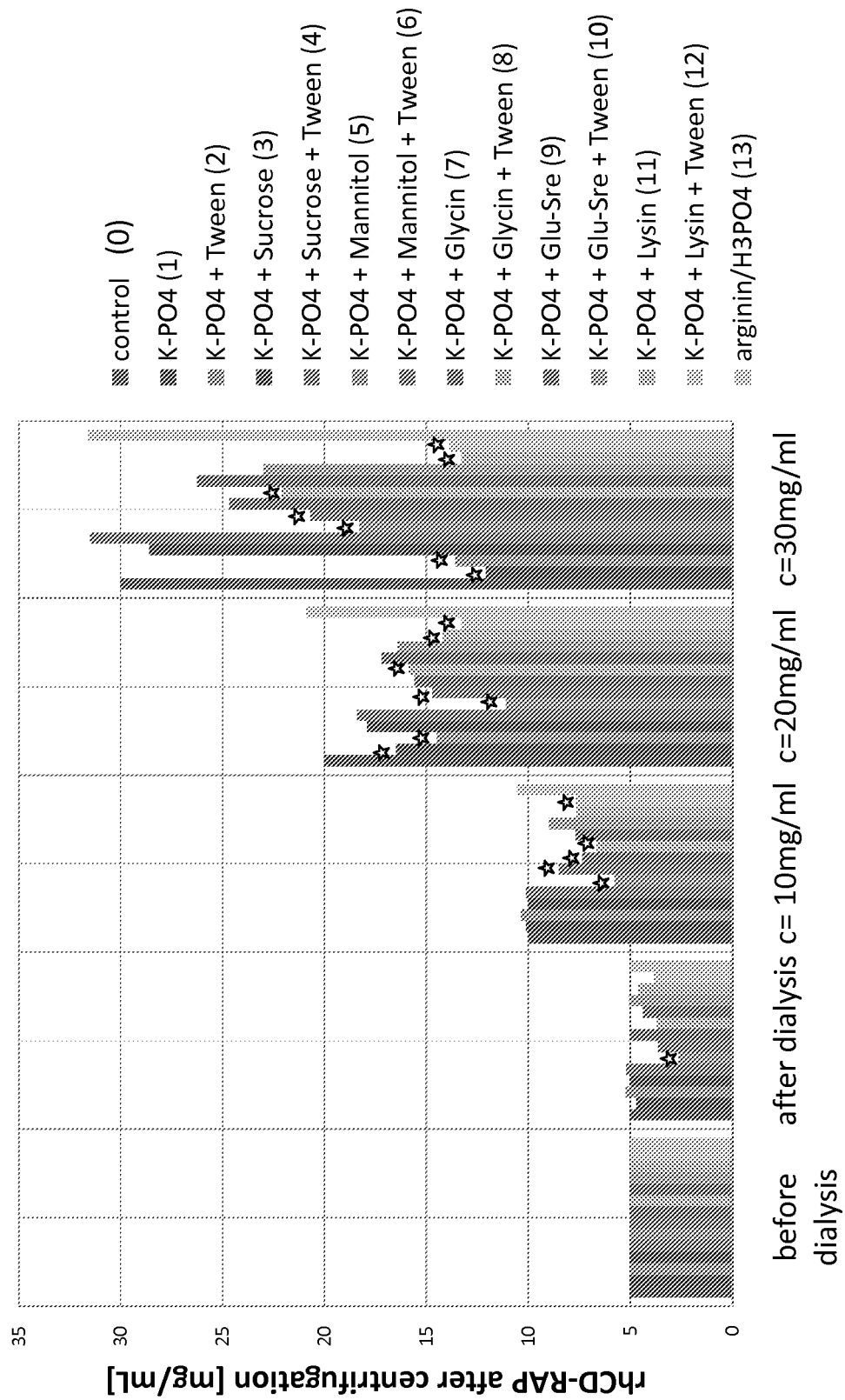

concentration of rhCD-RAP in K-PO4-buffer systems
rhCD-RAP after centrifugation [mg/mL]
0
5
10
15
20
25
30
35
control (0)
K-PO4 (1)
K-PO4 + Tween (2)
K-PO4 + Sucrose (3)
K-PO4 + Sucrose + Tween (4)
K-PO4 + Mannitol (5)
K-PO4 + Mannitol + Tween (6)
K-PO4 + Glycin (7)
K-PO4 + Glycin + Tween (8)
K-PO4 + Glu-Sre (9)
K-PO4 + Glu-Sre + Tween (10)
K-PO4 + Lysin (11)
K-PO4 + Lysin + Tween (12)
arginin/H3PO4 (13)
before dialysis
after dialysis c= 10mg/ml c=20mg/ml c=30mg/ml
☆ = pellet after centrifugation

Figure 4

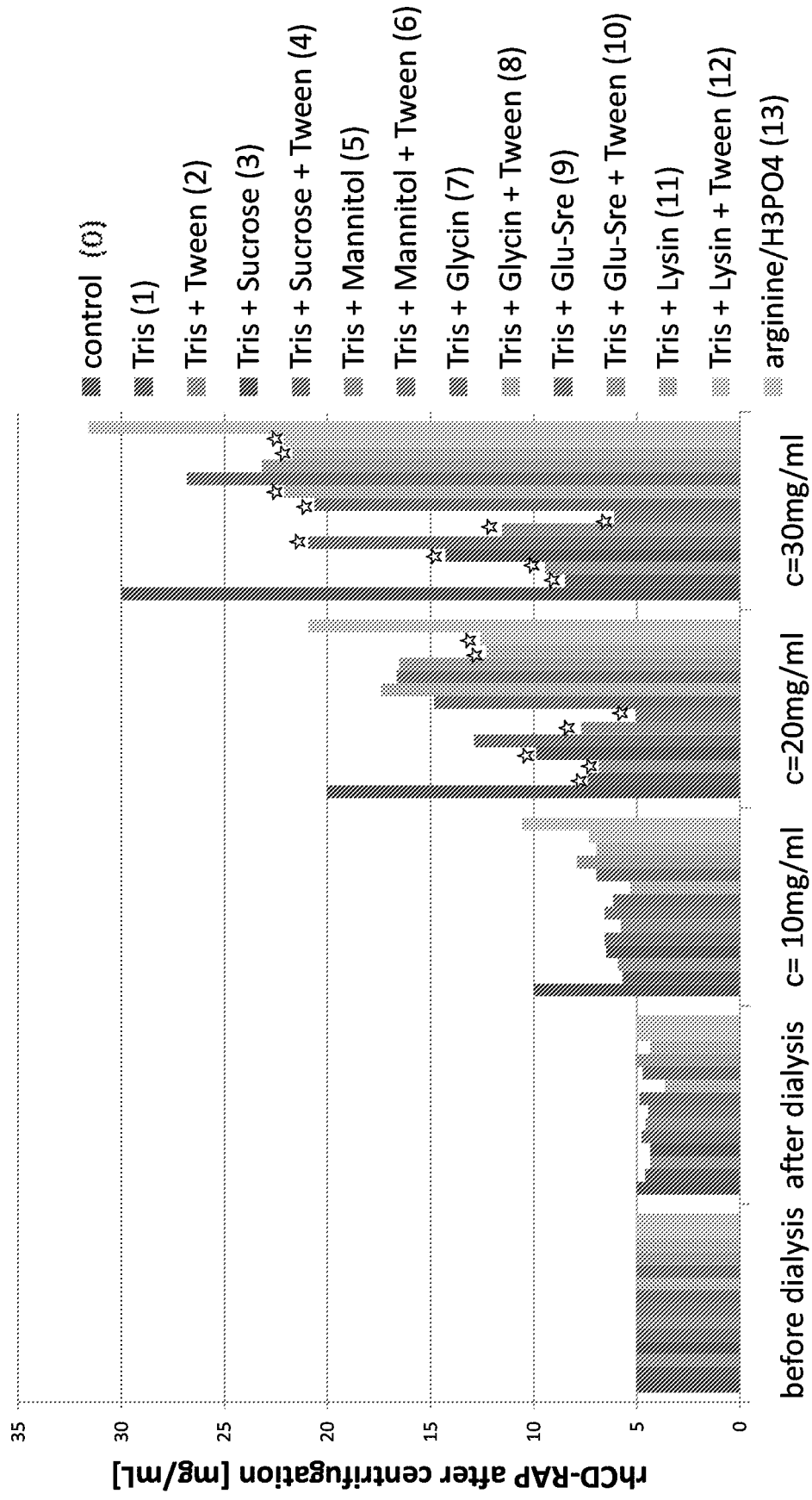
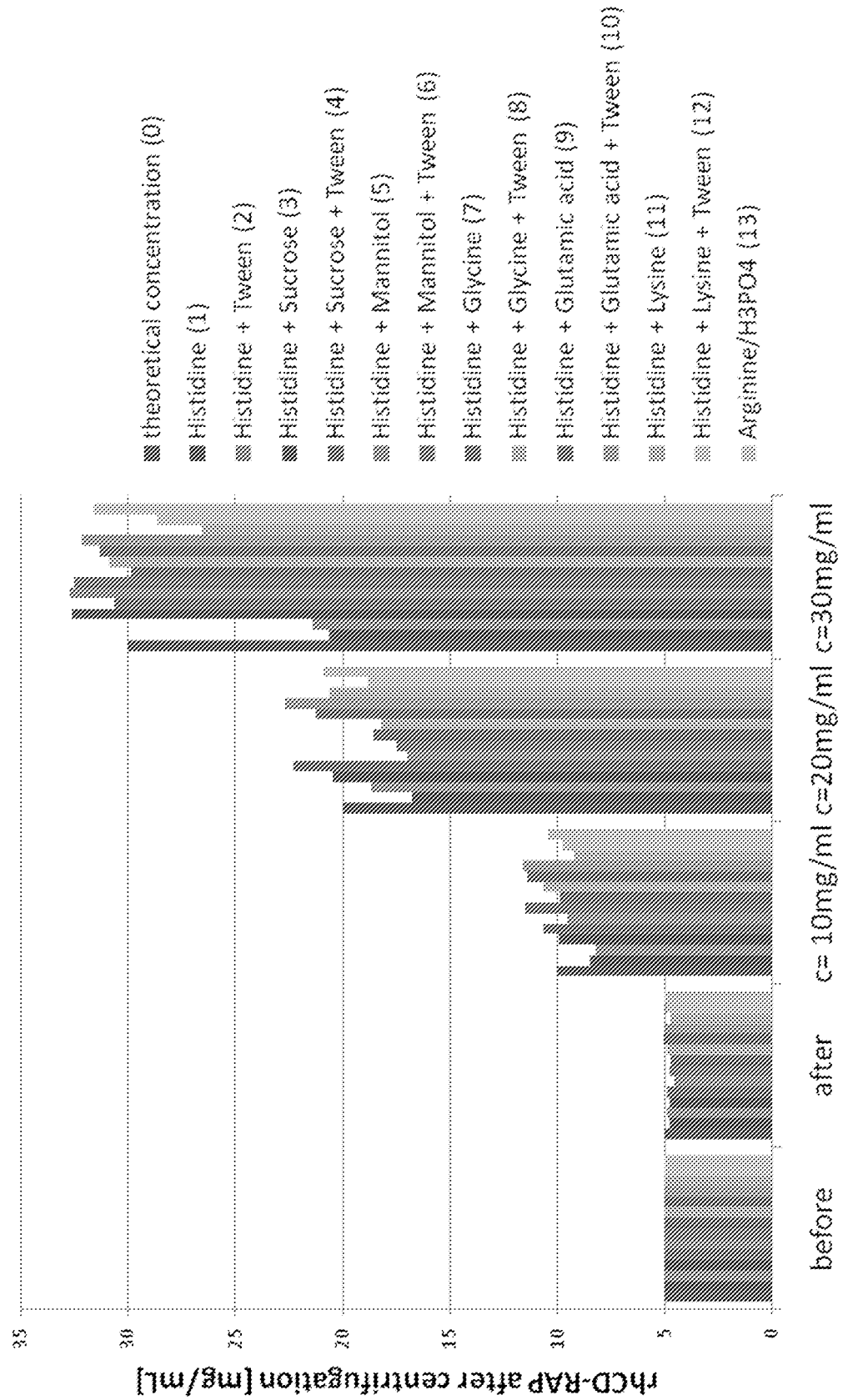

concentration of rhCD-RAP in Tris-Cl buffer systems
rhCD-RAP after centrifugation [mg/mL]
0
5
10
15
20
25
30
35
control (0)
Tris (1)
Tris + Tween (2)
Tris + Sucrose (3)
Tris + Sucrose + Tween (4)
Tris + Mannitol (5)
Tris + Mannitol + Tween (6)
Tris + Glycin (7)
Tris + Glycin + Tween (8)
Tris + Glu-Sre (9)
Tris + Glu-Sre + Tween (10)
Tris + Lysin (11)
Tris + Lysin + Tween (12)
arginine/H3PO4 (13)
before dialysis
after dialysis c= 10mg/ml
c=20mg/ml
c=30mg/ml

Figure 5


concentration of rhCR-RAP in Histidin-Cl buffer systems
rhCD-RAP after centrifugation [mg/mL]
0
5
10
15
20
25
30
35
before dialysis
after dialysis
c= 10mg/ml c=20mg/ml c=30mg/ml
theoretical concentration (0)
Histidine (1)
Histidine + Tween (2)
Histidine + Sucrose (3)
Histidine + Sucrose + Tween (4)
Histidine + Mannitol (5)
Histidine + Mannitol + Tween (6)
Histidine + Glycine (7)
Histidine + Glycine + Tween (8)
Histidine + Glutamic acid (9)
Histidine + Glutamic acid + Tween (10)
Histidine + Lysine (11)
Histidine + Lysine + Tween (12)
Arginine/H3PO4 (13)

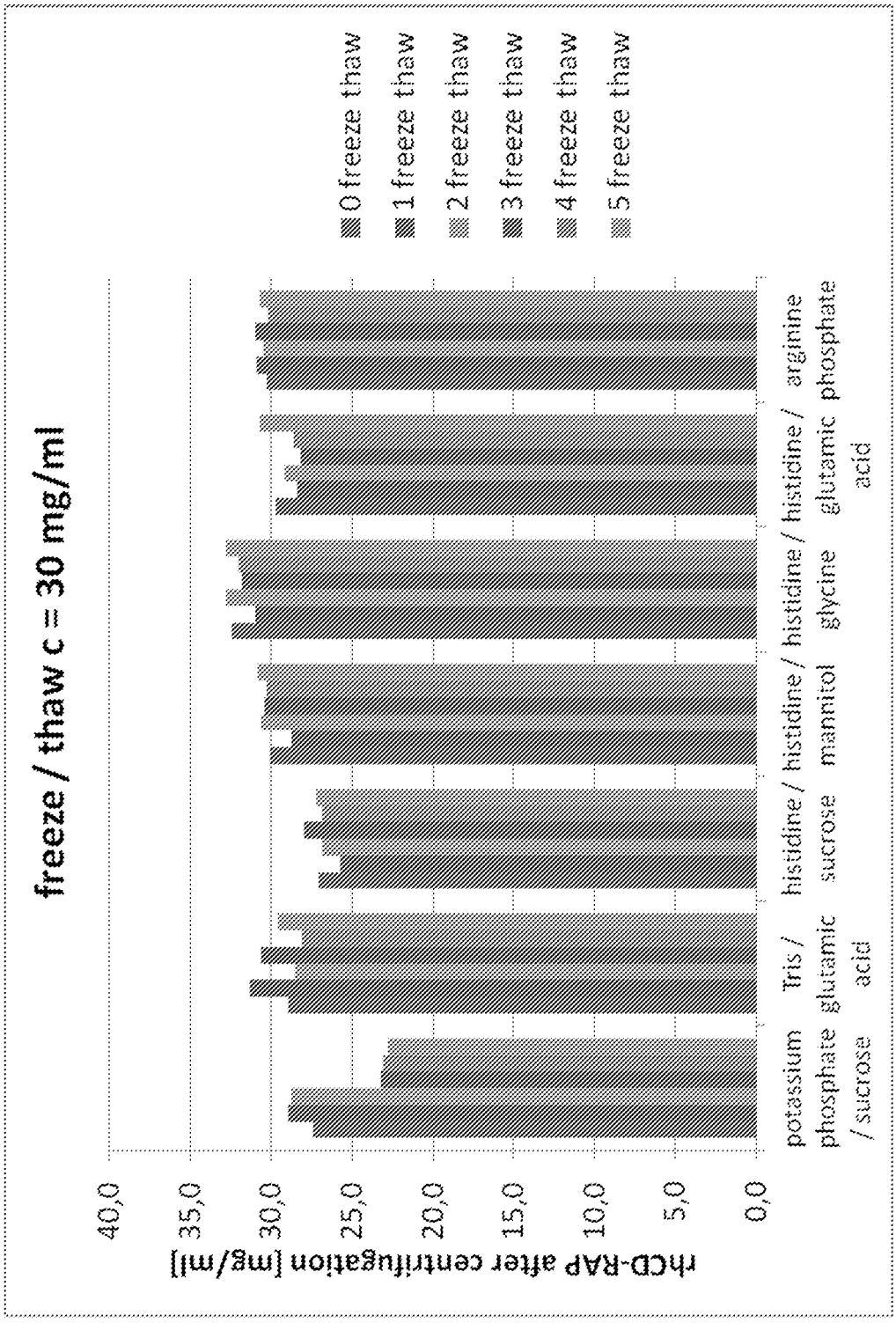

freeze / thaw c = 30 mg/ml
rhCD-RAP after centrifugation [mg/ml]
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0 freeze thaw
1 freeze thaw
2 freeze thaw
3 freeze thaw
4 freeze thaw
5 freeze thaw
potassium phosphate / sucrose
Tris / glutamic acid
histidine / sucrose
histidine / mannitol
histidine / glycine
histidine / glutamic acid
arginine phosphate

Figure 6

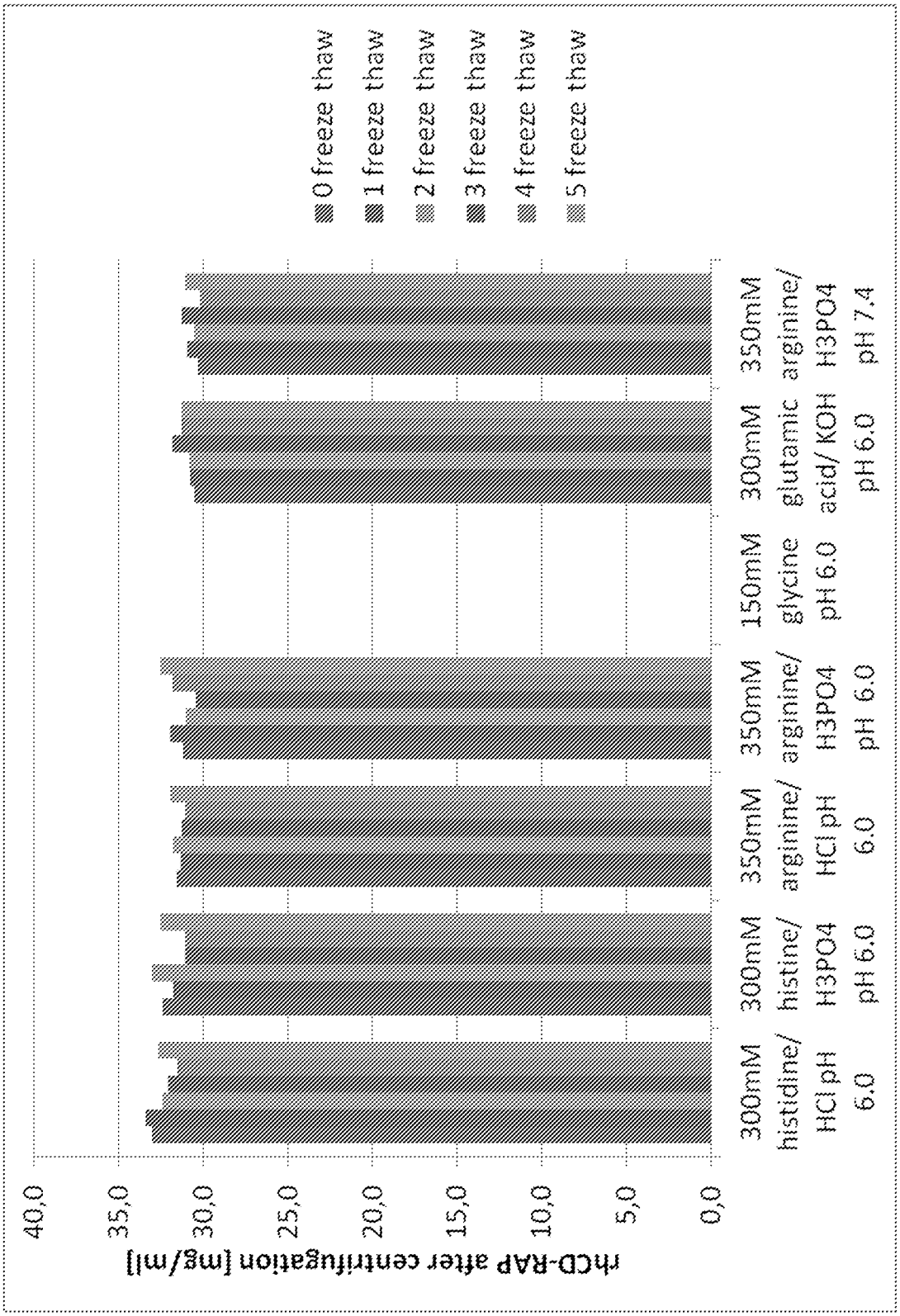

rhCD-RAP after centrifugation [mg/ml]
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0 freeze thaw
1 freeze thaw
2 freeze thaw
3 freeze thaw
4 freeze thaw
5 freeze thaw
300mM histidine/ HCl pH 6.0
300mM histine/ H3PO4 pH 6.0
350mM arginine/ HCl pH 6.0
350mM arginine/ H3PO4 pH 6.0
150mM glycine pH 6.0
300mM glutamic acid/ KOH pH 6.0
350mM arginine/ H3PO4 pH 7.4

Figure 7

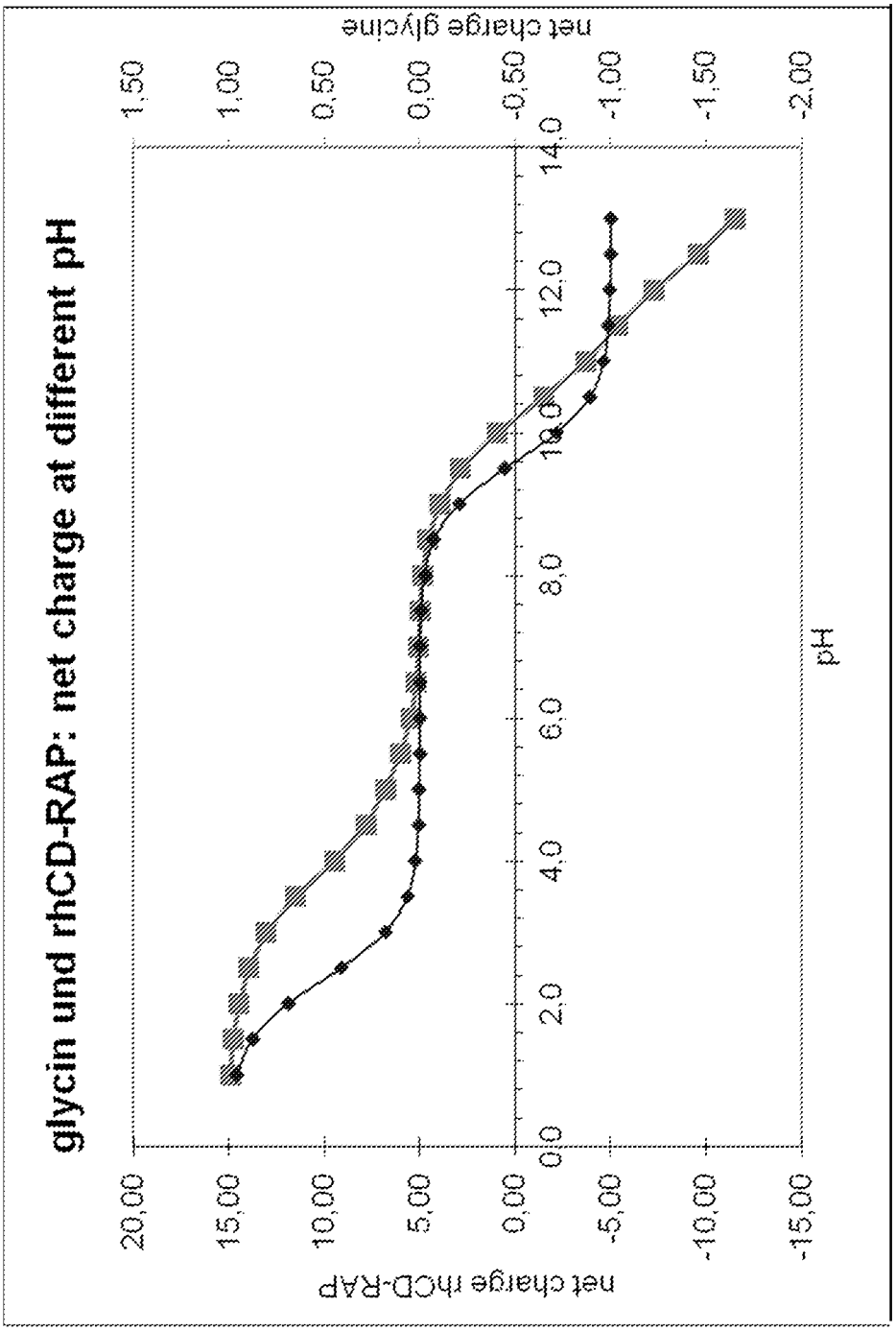

glycin und rhCD-RAP: net charge at different pH
net charge rhCD-RAP
20,00
15,00
10,00
5,00
0,00
-5,00
-10,00
-15,00
net charge glycine
1,50
1,00
0,50
0,00
-0,50
-1,00
-1,50
-2,00
0,0
2,0
4,0
6,0
8,0
10,0
12,0
14,0
pH

Figure 8

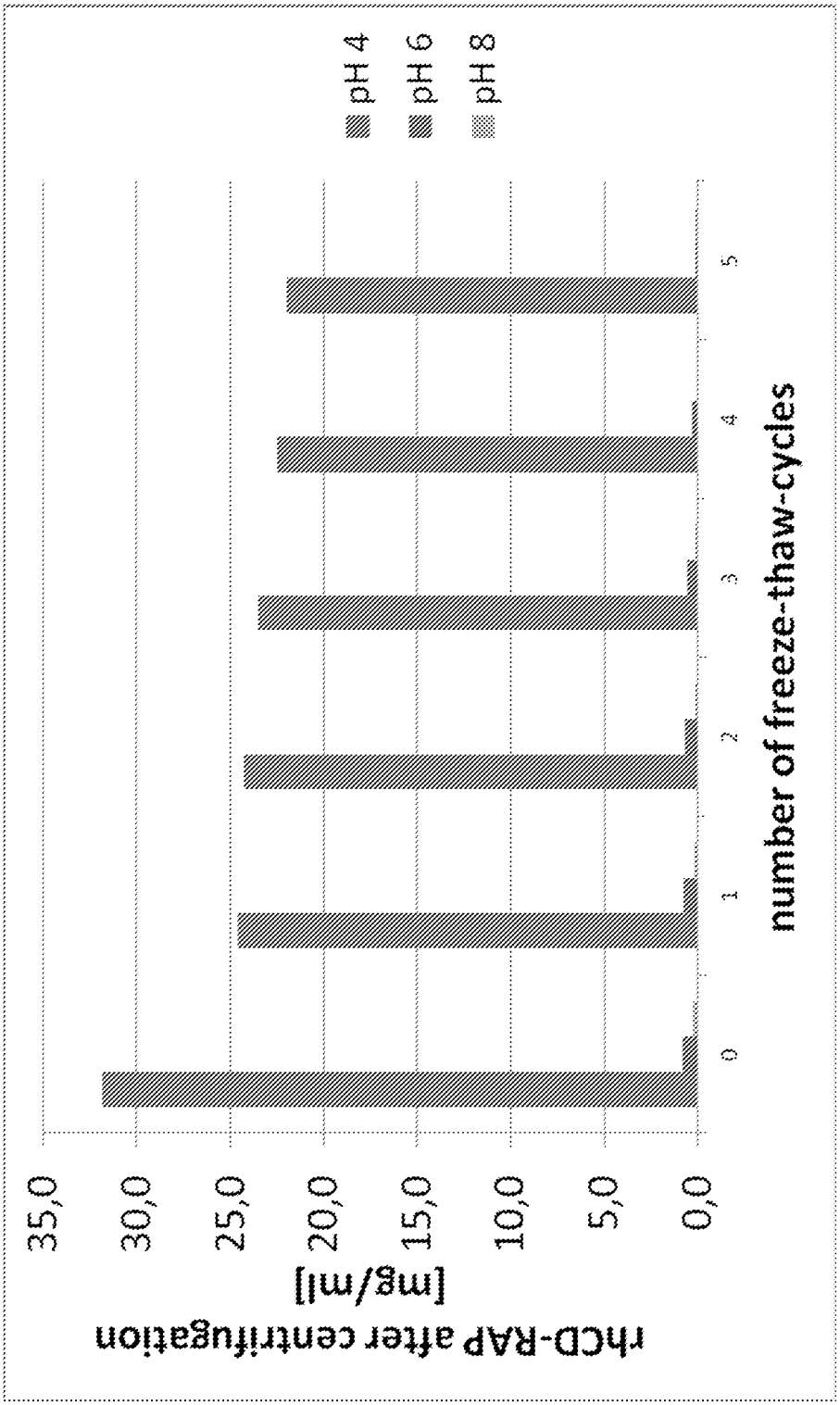

rhCD-RAP after centrifugation
[mg/ml]
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0
1
2
3
4
5
number of freeze-thaw-cycles
pH 4
pH 6
pH 8

Figure 9

Figure 10

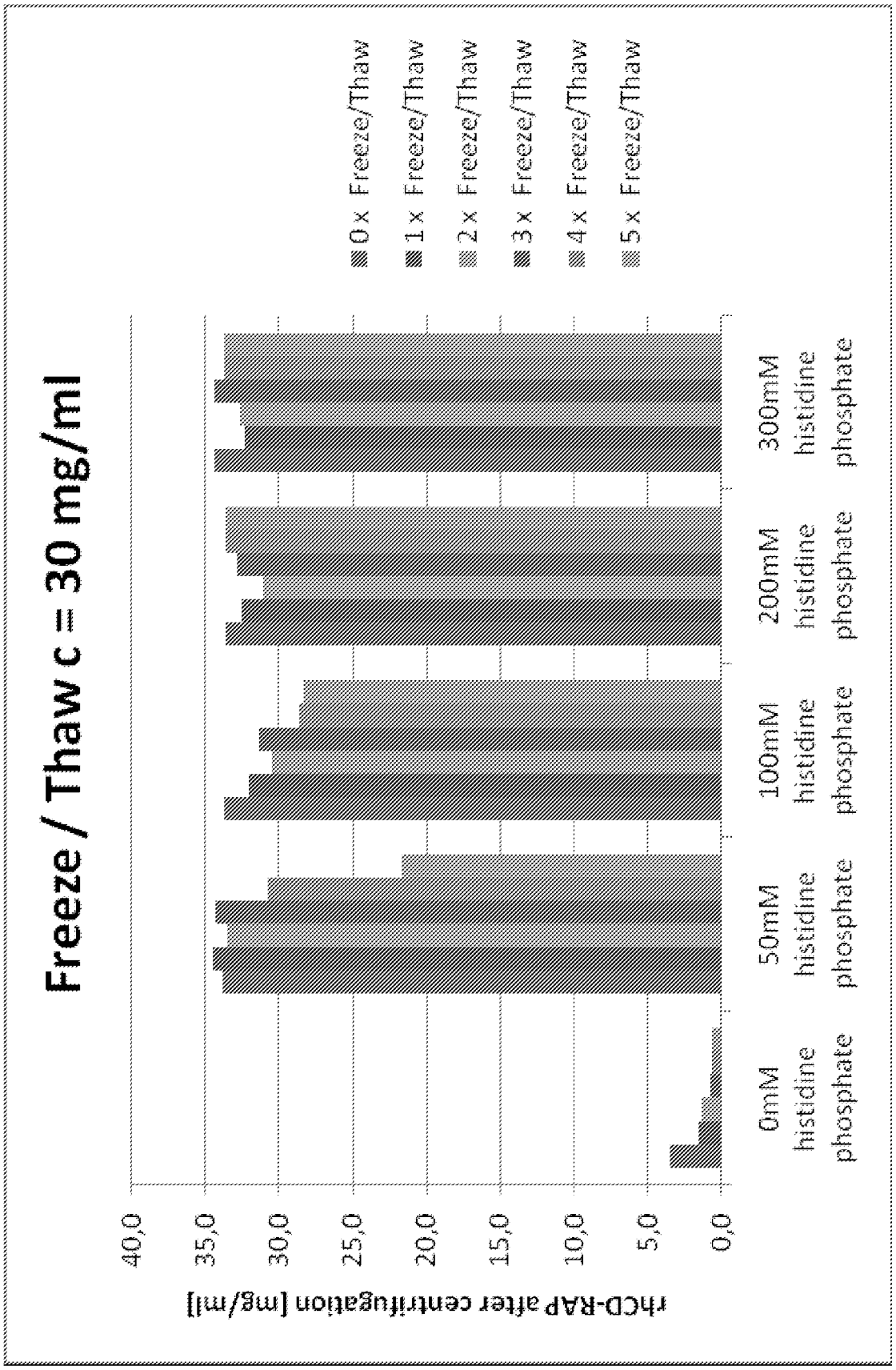
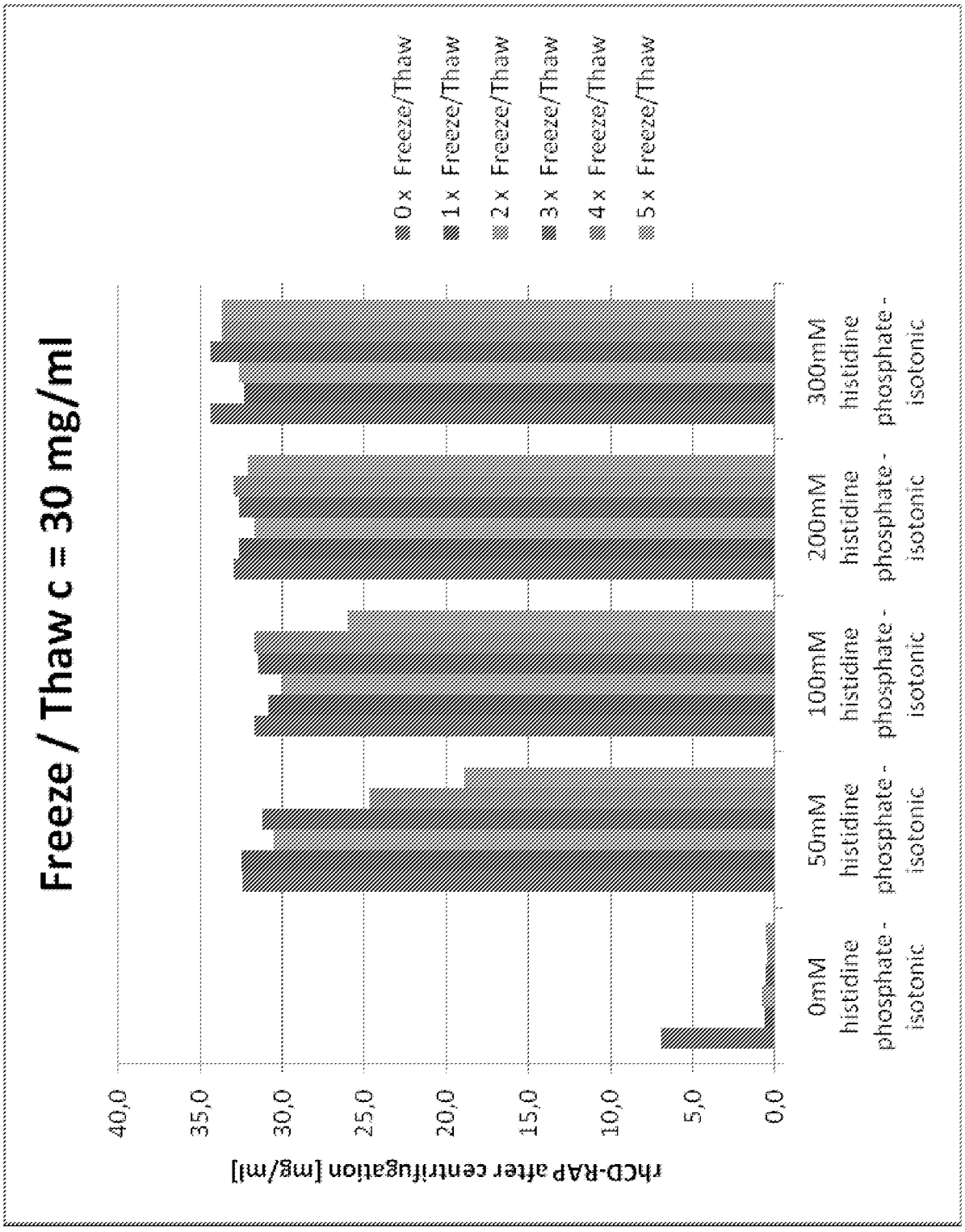

Freeze / Thaw c = 30 mg/ml
rhCD-RAP after centrifugation [mg/ml]
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0x Freeze/Thaw
1x Freeze/Thaw
2x Freeze/Thaw
3x Freeze/Thaw
4x Freeze/Thaw
5x Freeze/Thaw
0mM histidine phosphate
50mM histidine phosphate
100mM histidine phosphate
200mM histidine phosphate
300mM histidine phosphate

Figure 11


Freeze / Thaw c = 30 mg/ml
rhCD-RAP after centrifugation [mg/ml]
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0 x Freeze/Thaw
1 x Freeze/Thaw
2 x Freeze/Thaw
3 x Freeze/Thaw
4 x Freeze/Thaw
5 x Freeze/Thaw
0mM histidine phosphate - isotonic
50mM histidine phosphate - isotonic
100mM histidine phosphate - isotonic
200mM histidine phosphate - isotonic
300mM histidine phosphate - isotonic