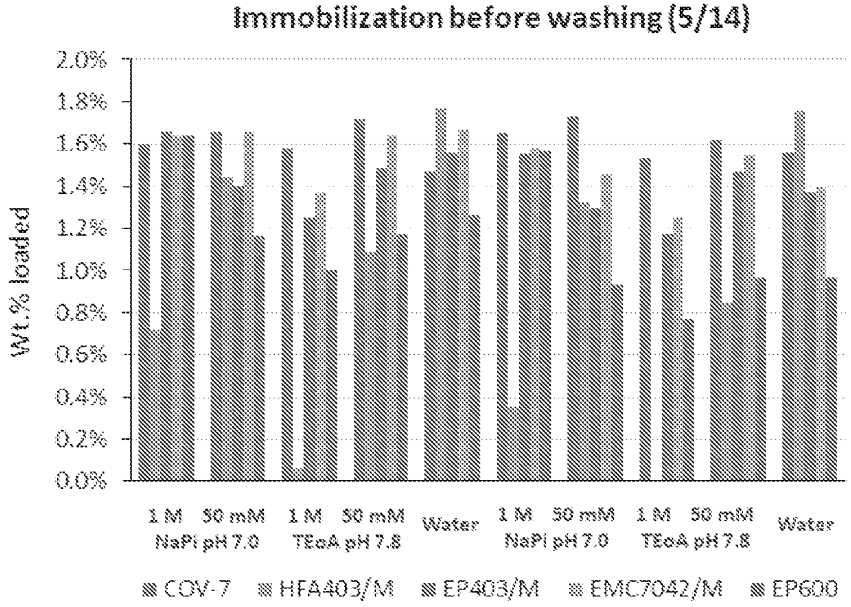

Immobilization before washing (5/14)
Wt.% loaded
2.0%
1.8%
1.6%
1.4%
1.2%
1.0%
0.8%
0.6%
0.4%
0.2%
0.0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 1A

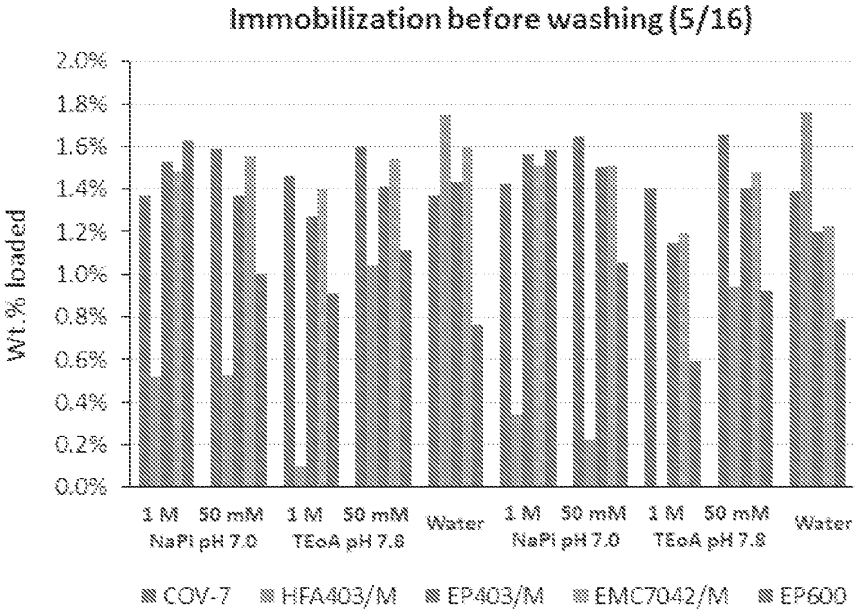

Immobilization before washing (5/16)
Wt.% loaded
2.0%
1.8%
1.6%
1.4%
1.2%
1.0%
0.8%
0.6%
0.4%
0.2%
0.0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 1B

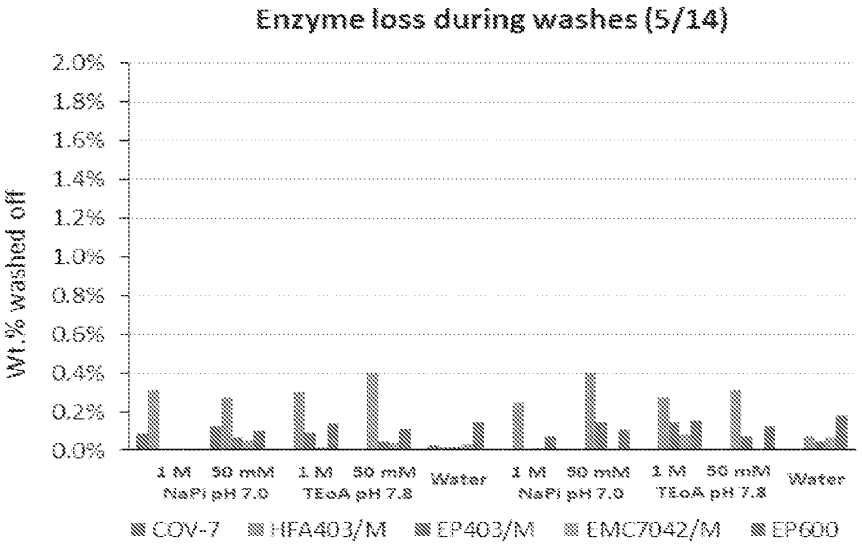

Enzyme loss during washes (5/14)
Wt.% washed off
2.0%
1.8%
1.6%
1.4%
1.2%
1.0%
0.8%
0.6%
0.4%
0.2%
0.0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 2A

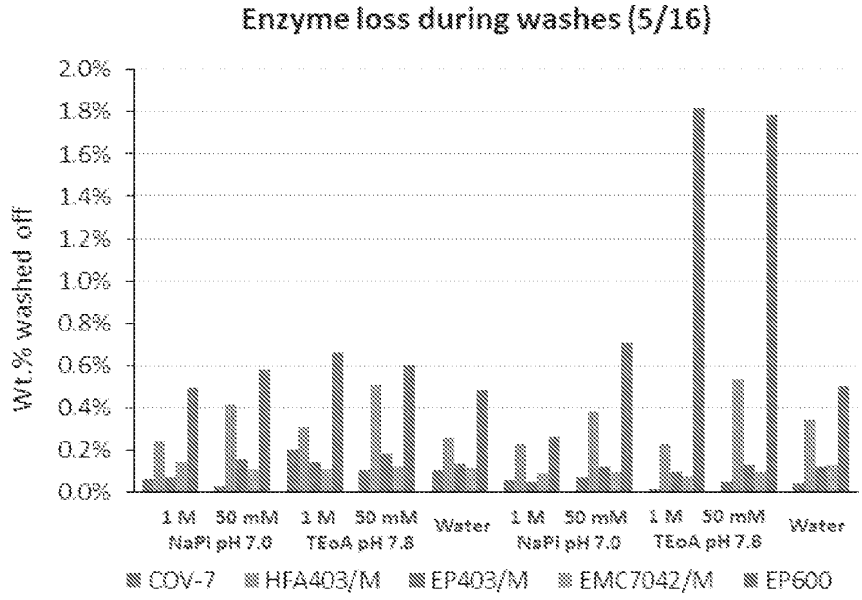

Enzyme loss during washes (5/16)
Wt.% washed off
2.0%
1.8%
1.6%
1.4%
1.2%
1.0%
0.8%
0.6%
0.4%
0.2%
0.0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 2B

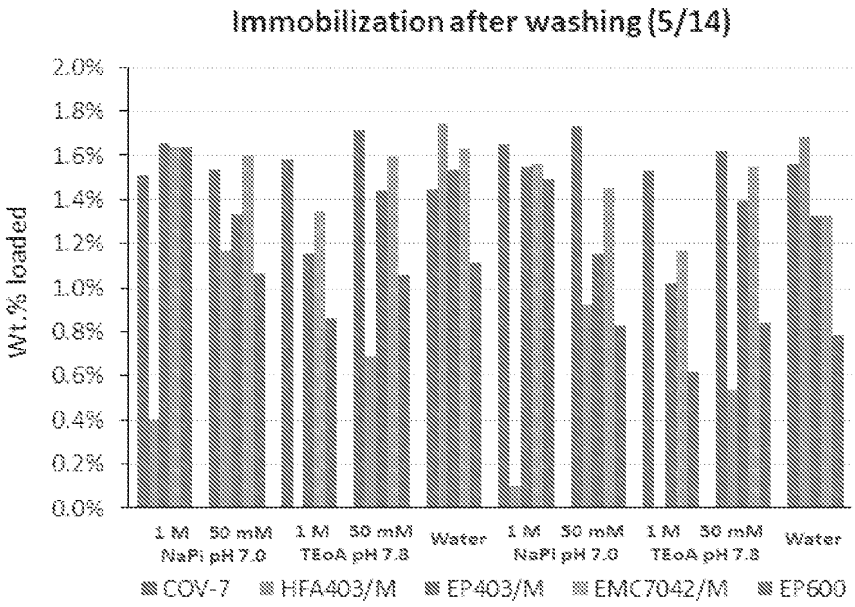

Immobilization after washing (5/14)
Wt.% loaded
2.0%
1.8%
1.6%
1.4%
1.2%
1.0%
0.8%
0.6%
0.4%
0.2%
0.0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 3A

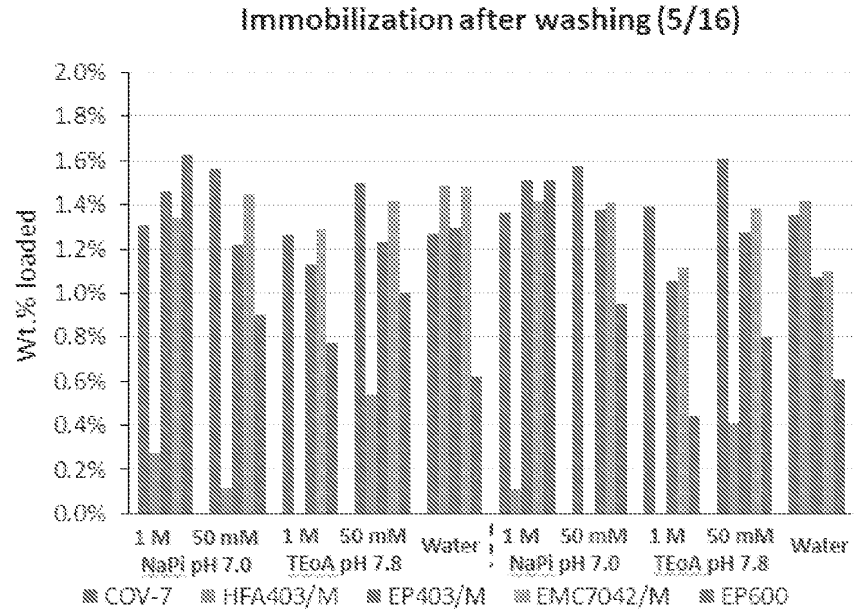

Immobilization after washing (5/16)
Wt.% loaded
2.0%
1.8%
1.6%
1.4%
1.2%
1.0%
0.8%
0.6%
0.4%
0.2%
0.0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 3B

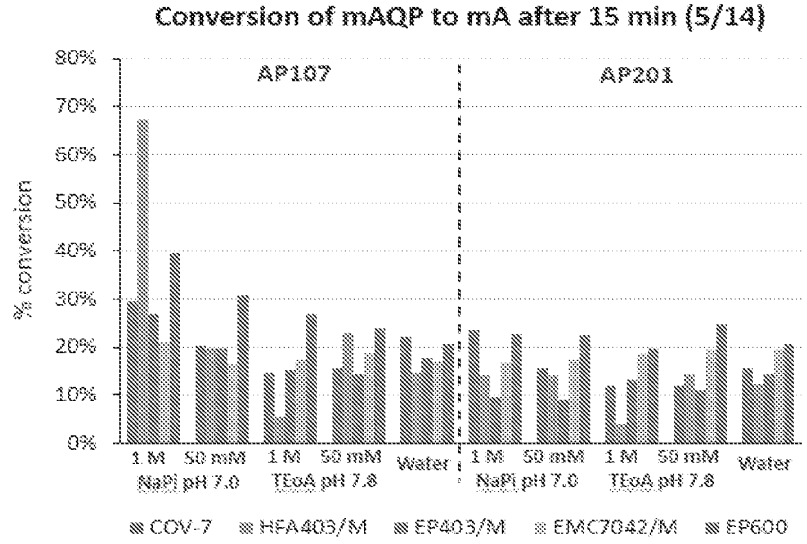

Conversion of mAQP to mA after 15 min (5/14)
AP107
AP201
% conversion
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 4A

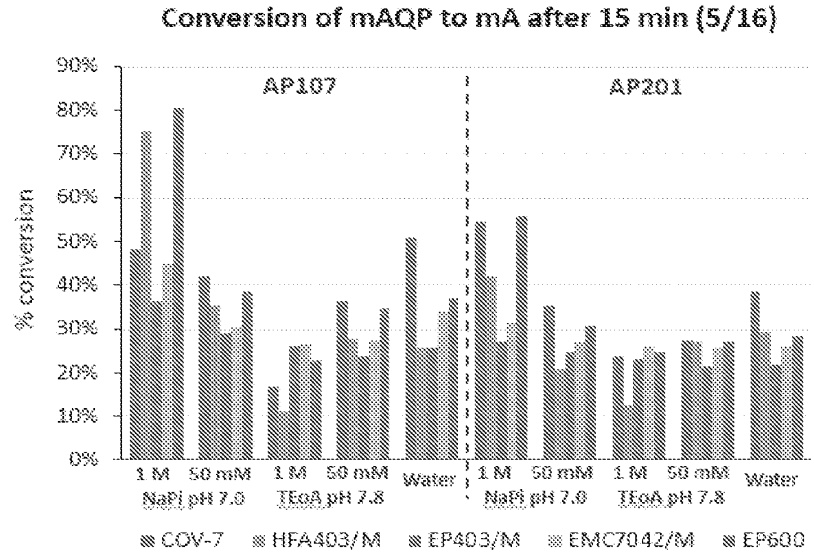

Conversion of mAQP to mA after 15 min (5/16)
AP107
AP201
% conversion
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 4B

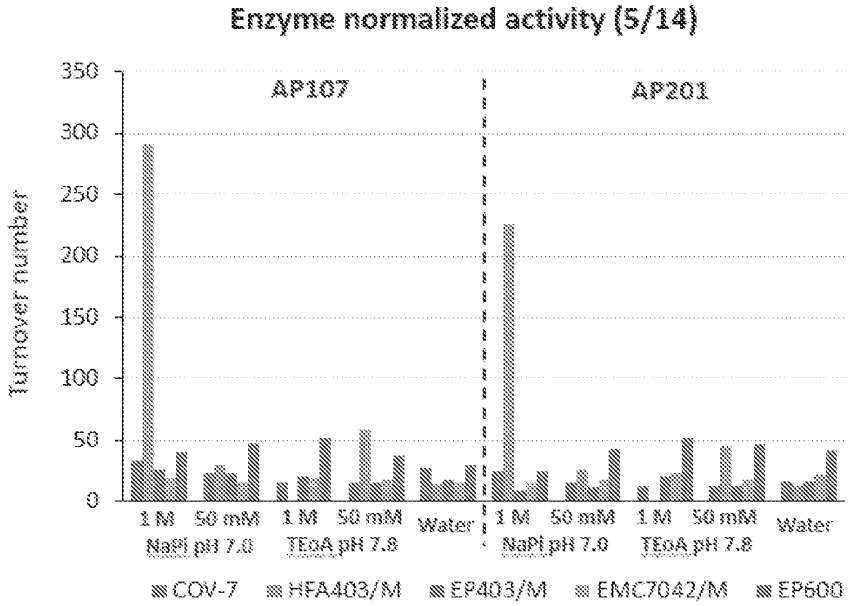

Enzyme normalized activity (5/14)
AP107
AP201
Turnover number
350
300
250
200
150
100
50
0
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 5A

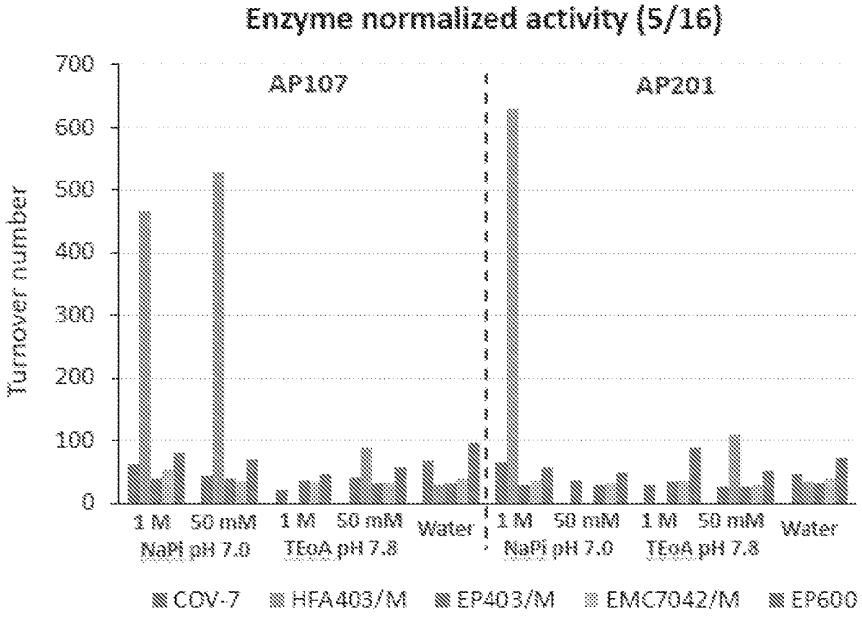

Enzyme normalized activity (5/16)
AP107
AP201
Turnover number
700
600
500
400
300
200
100
0
1 M NaPi pH 7.0
50 mM NaPi pH 7.0
1 M TEoA pH 7.8
50 mM TEoA pH 7.8
Water
COV-7
HFA403/M
EP403/M
EMC7042/M
EP600

Fig. 5B

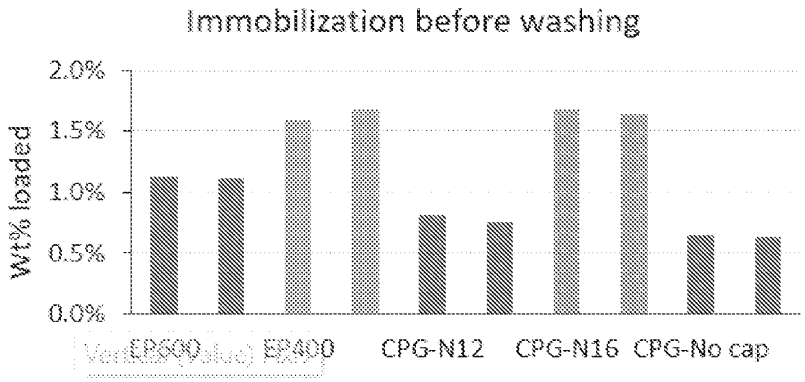

Immobilization before washing
Wt% loaded
2.0%
1.5%
1.0%
0.5%
0.0%
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap

Fig. 6A

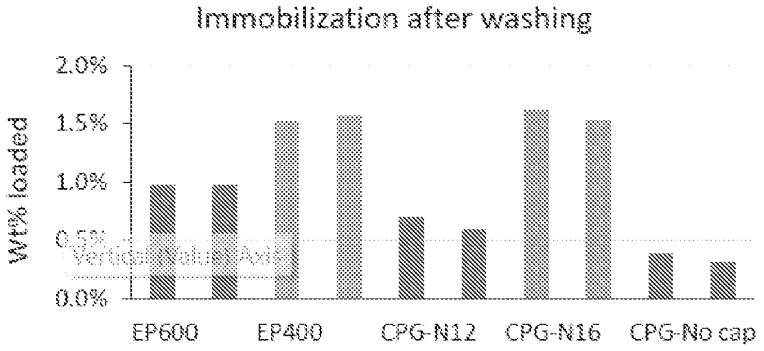

Immobilization after washing
Wt% loaded
2.0%
1.5%
1.0%
0.5%
0.0%
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap

Fig. 6B

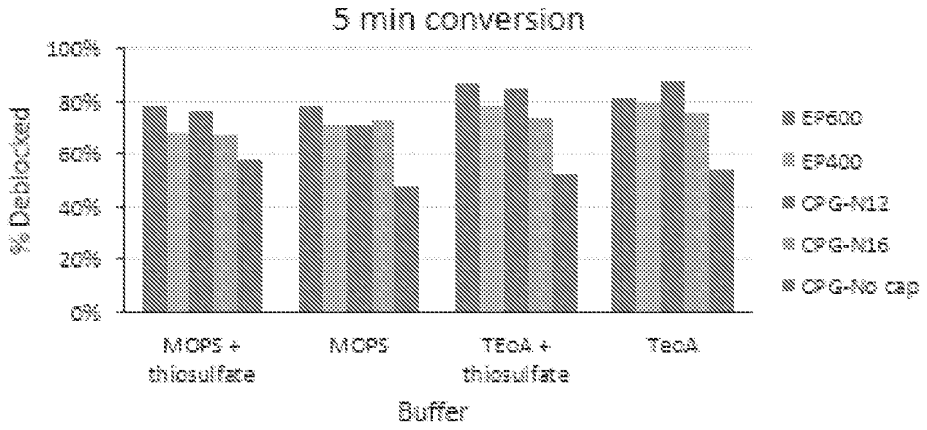

5 min conversion
100%
80%
60%
40%
20%
0%
% Deblocked
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap
MOPS + thiosulfate
MOPS
TEoA + thiosulfate
TeoA
Buffer

Fig. 7A

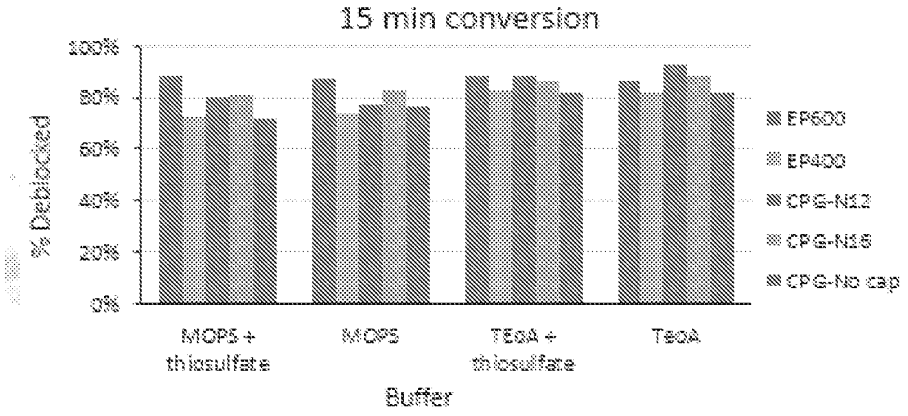

15 min conversion
100%
80%
60%
40%
20%
0%
% Deblocked
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap
MOPS + thiosulfate
MOPS
TEoA + thiosulfate
TeoA
Buffer

Fig. 7B

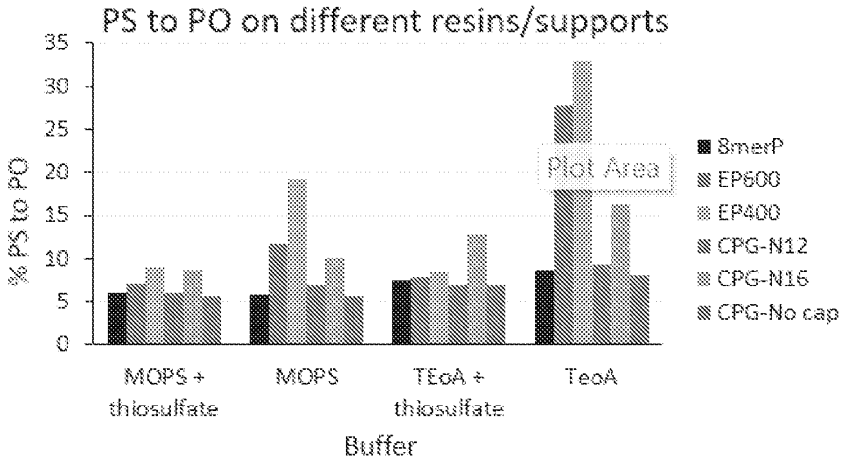

PS to PO on different resins/supports
% PS to PO
35
30
25
20
15
10
5
0
Plot Area
8merP
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap
MOPS + thiosulfate
MOPS
TEoA + thiosulfate
TeoA
Buffer

Fig. 8A

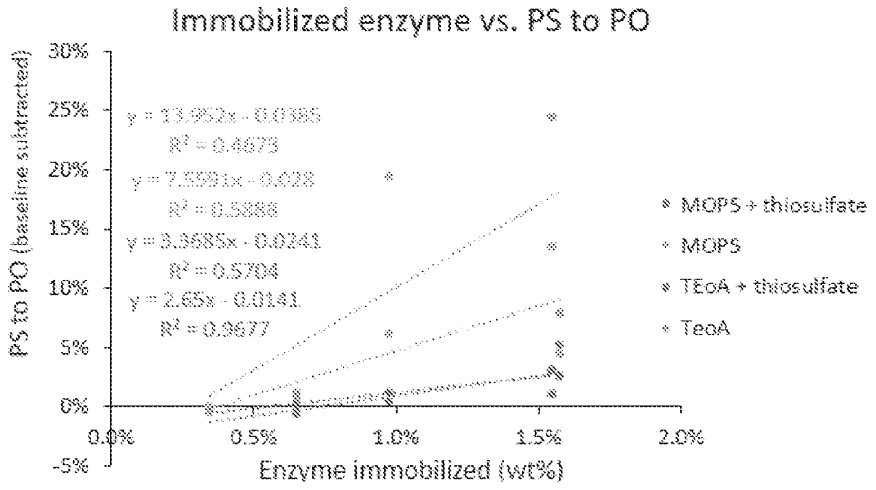

Immobilized enzyme vs. PS to PO
PS to PO (baseline subtracted)
30%
25%
20%
15%
10%
5%
0%
-5%
y = 13.952x - 0.0385
R² = 0.4673
y = 7.5591x - 0.028
R² = 0.5888
y = 3.3685x - 0.0241
R² = 0.5704
y = 2.65x - 0.0141
R² = 0.9677
0.0%
0.5%
1.0%
1.5%
2.0%
Enzyme immobilized (wt%)
MOPS + thiosulfate
MOPS
TEoA + thiosulfate
TeoA

Fig. 8B

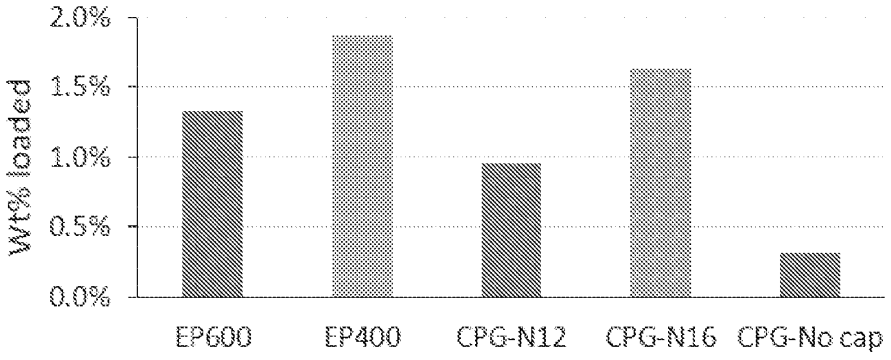

2.0%
1.5%
1.0%
0.5%
0.0%
Wt% loaded
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap

Fig. 9A

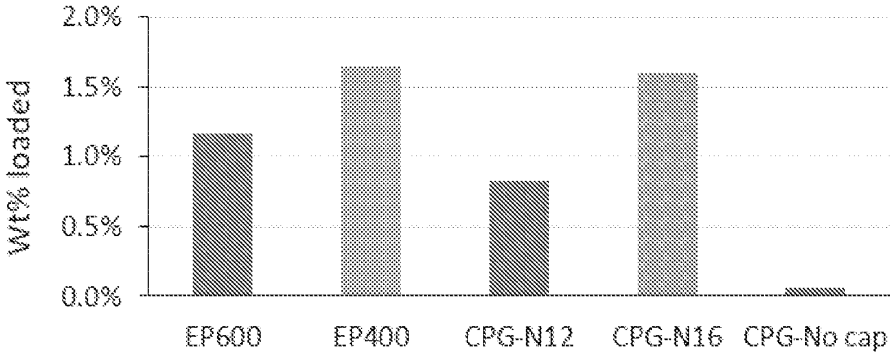

2.0%
1.5%
1.0%
0.5%
0.0%
Wt% loaded
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap

Fig. 9B

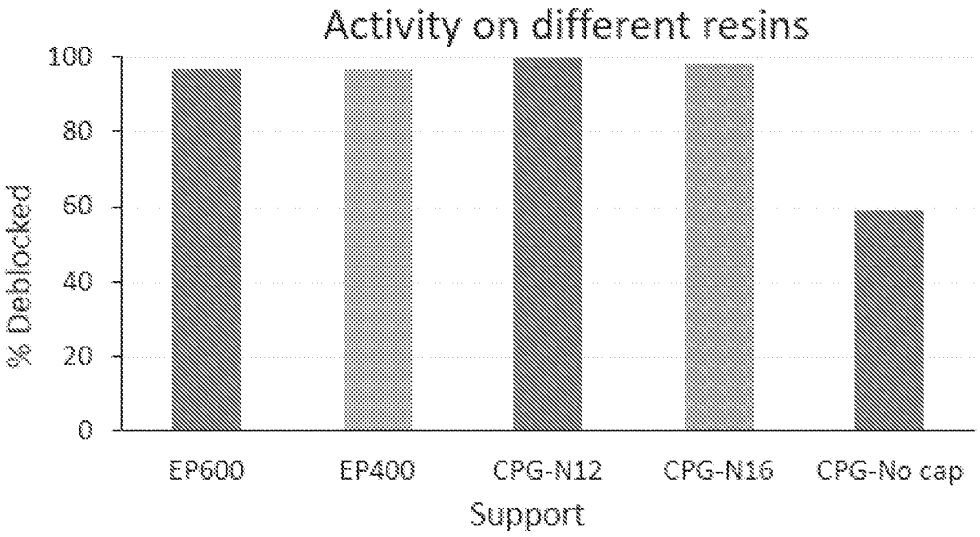


Fig. 10A

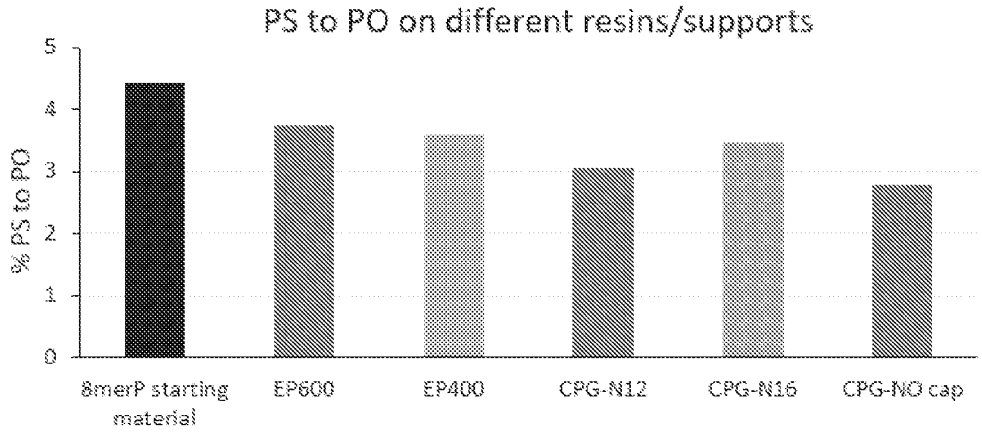


Fig. 10B

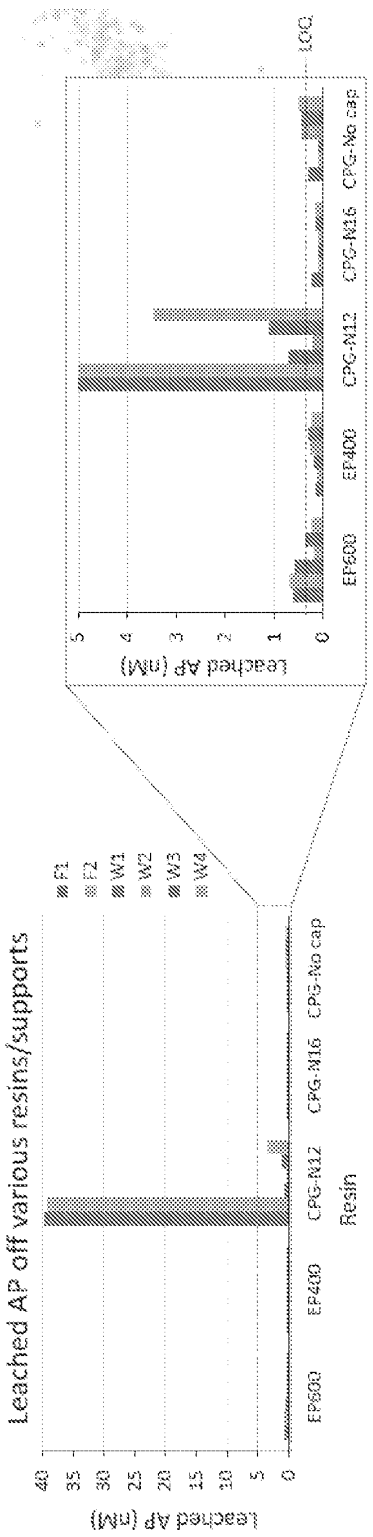

Leached AP off various resins/supports
Leached AP (nM)
0
5
10
15
20
25
30
35
40
F1
F2
W1
W2
W3
W4
EP600
EP400
CPG-N12
CPG-N16
CPG-No cap
Resin
LOQ
Leached AP (nM)
0
1
2
3
4
5

Fig. 10C

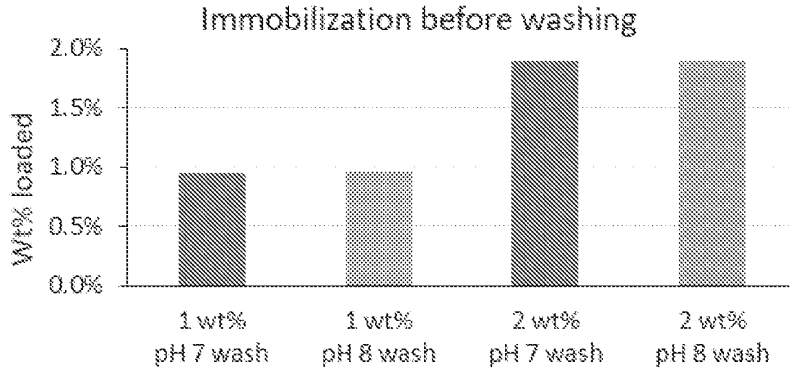


Fig. 11A

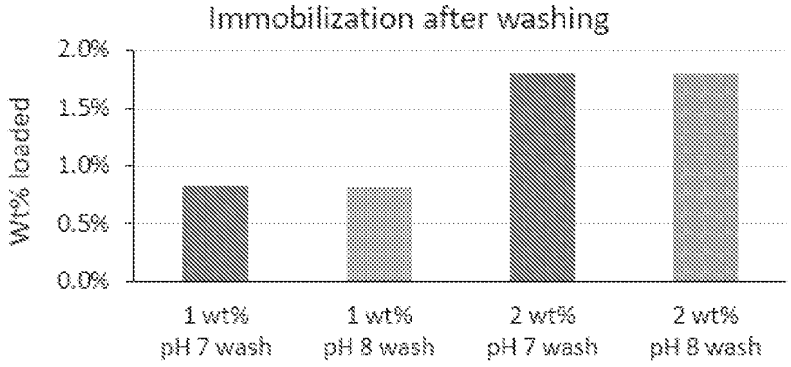


Fig. 11B

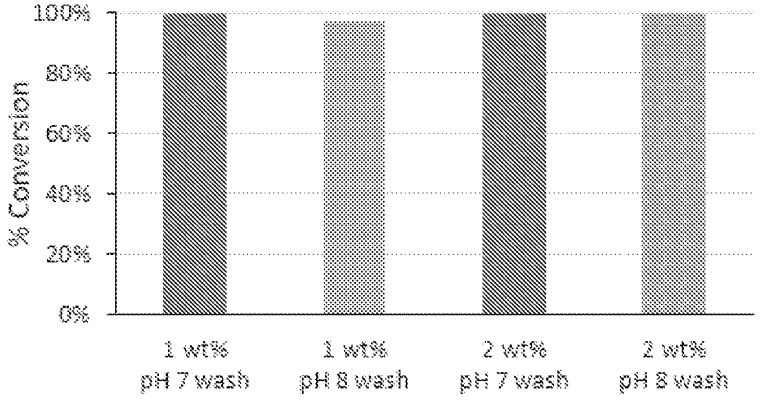


Fig. 11C

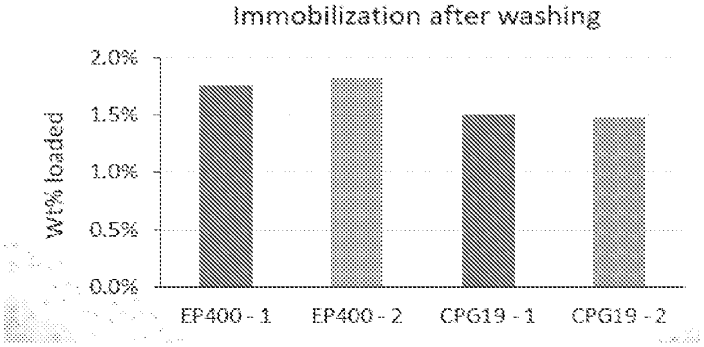


Fig. 12A

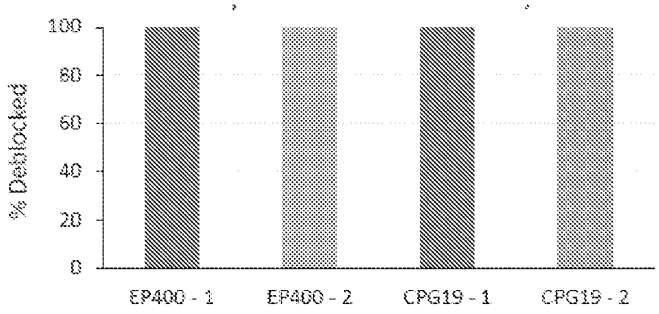


Fig. 12B

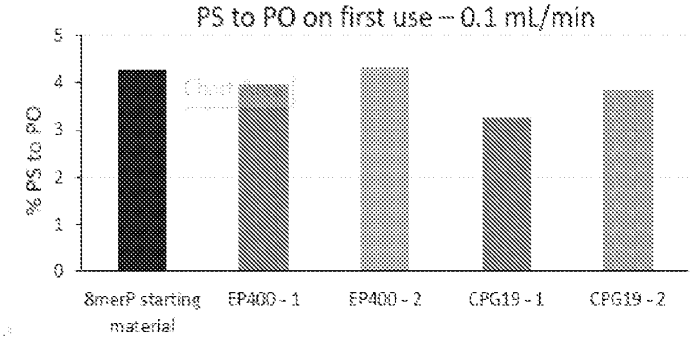


Fig. 12C

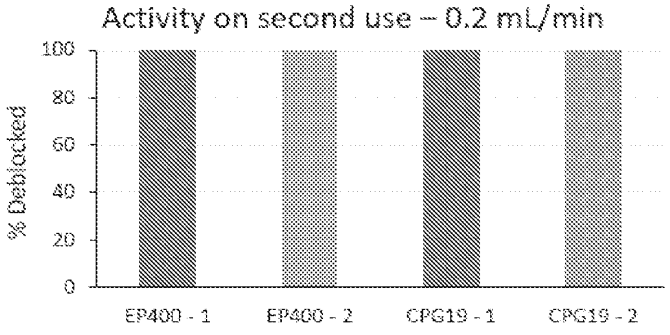


Fig. 12D