Figure 1

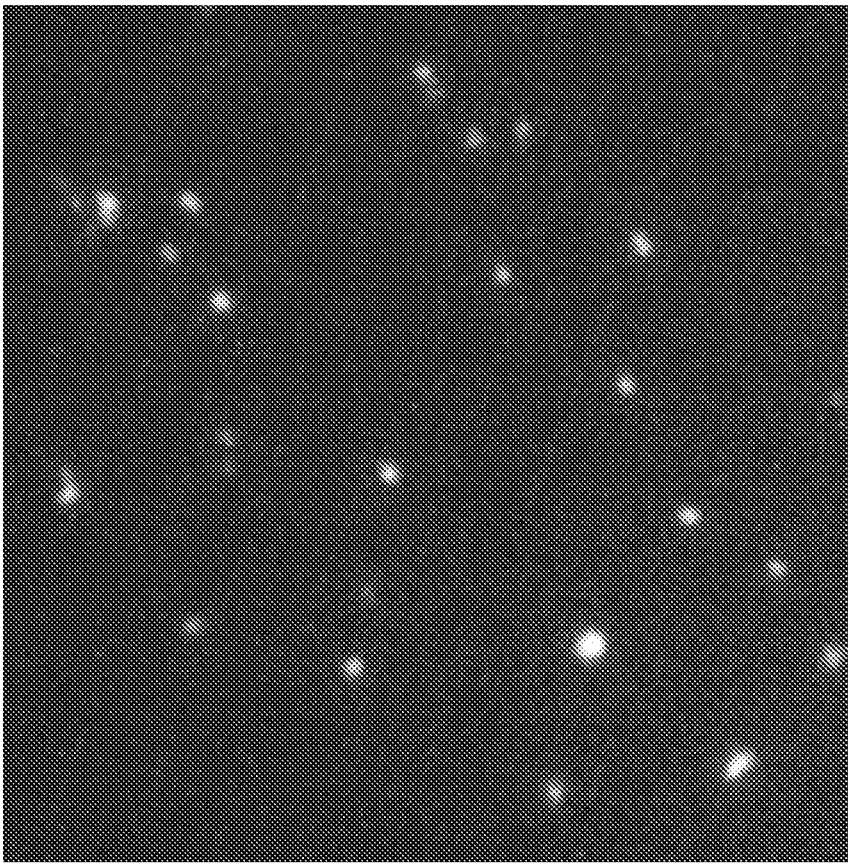


Figure 2

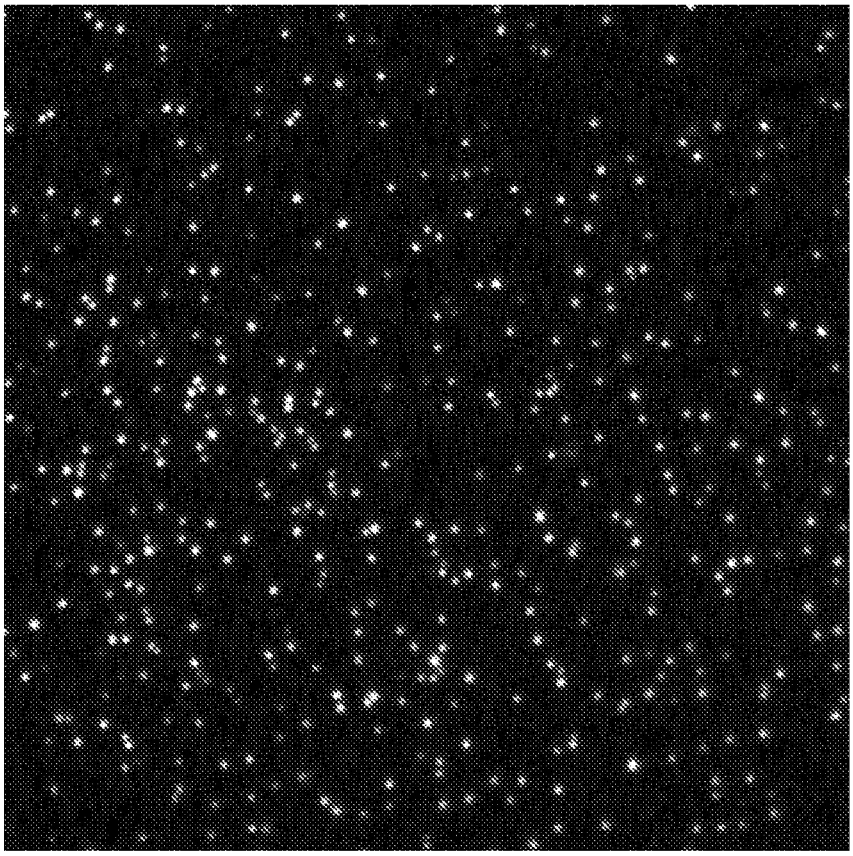


Figure 3

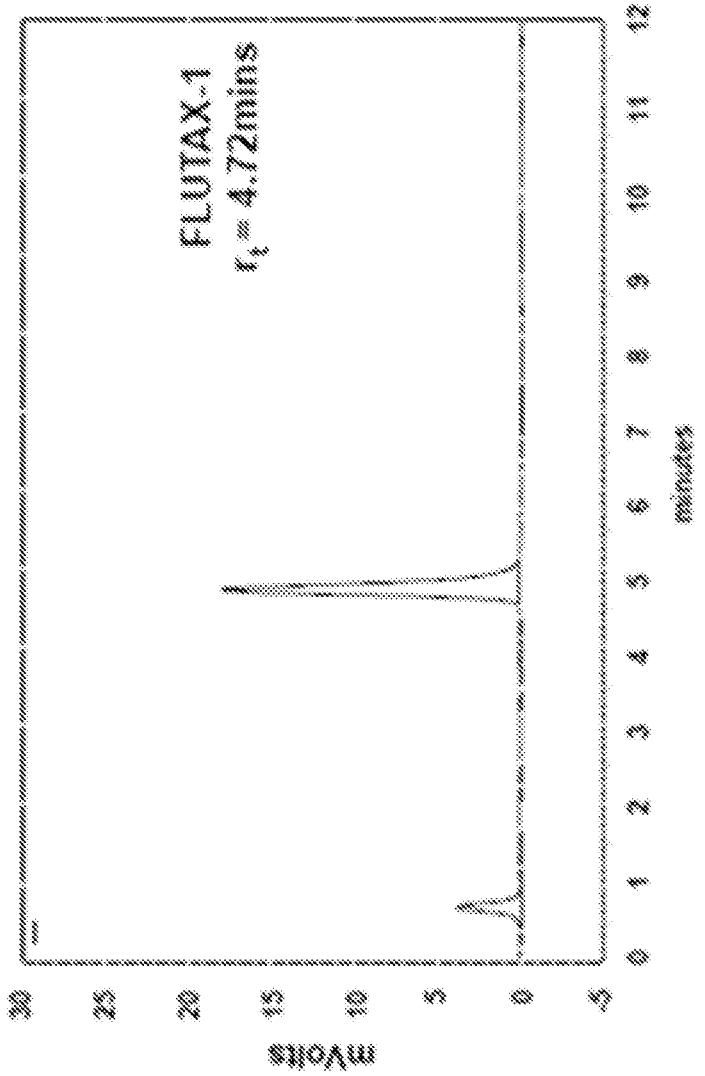


Figure 4

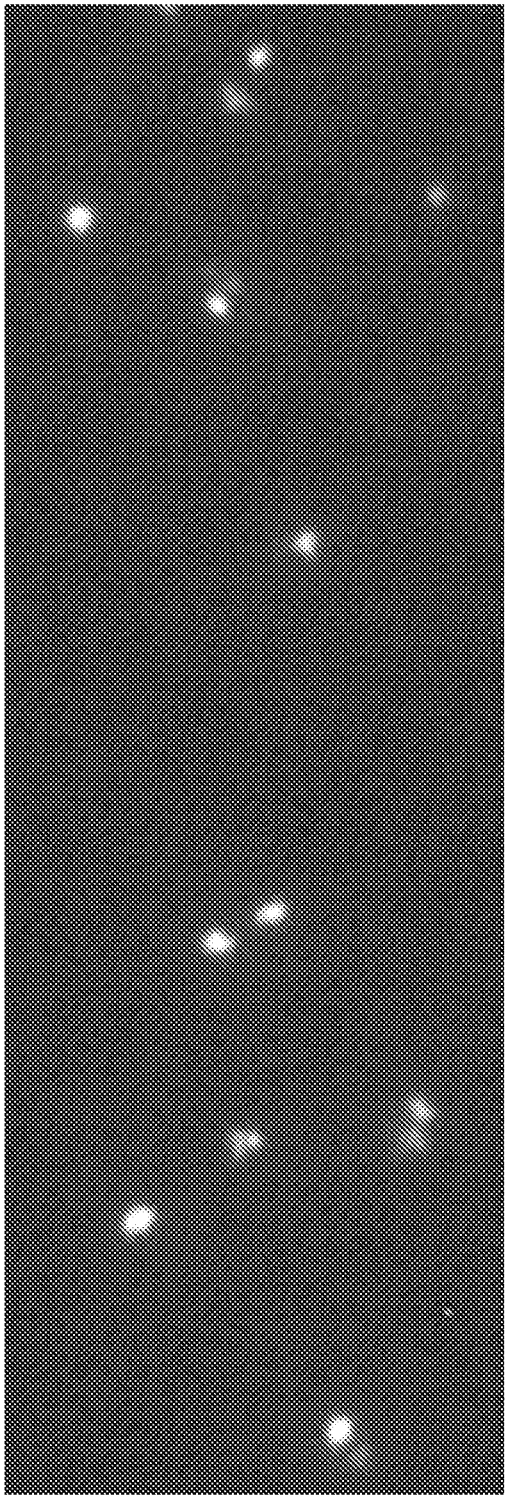


Figure 5



Figure 6

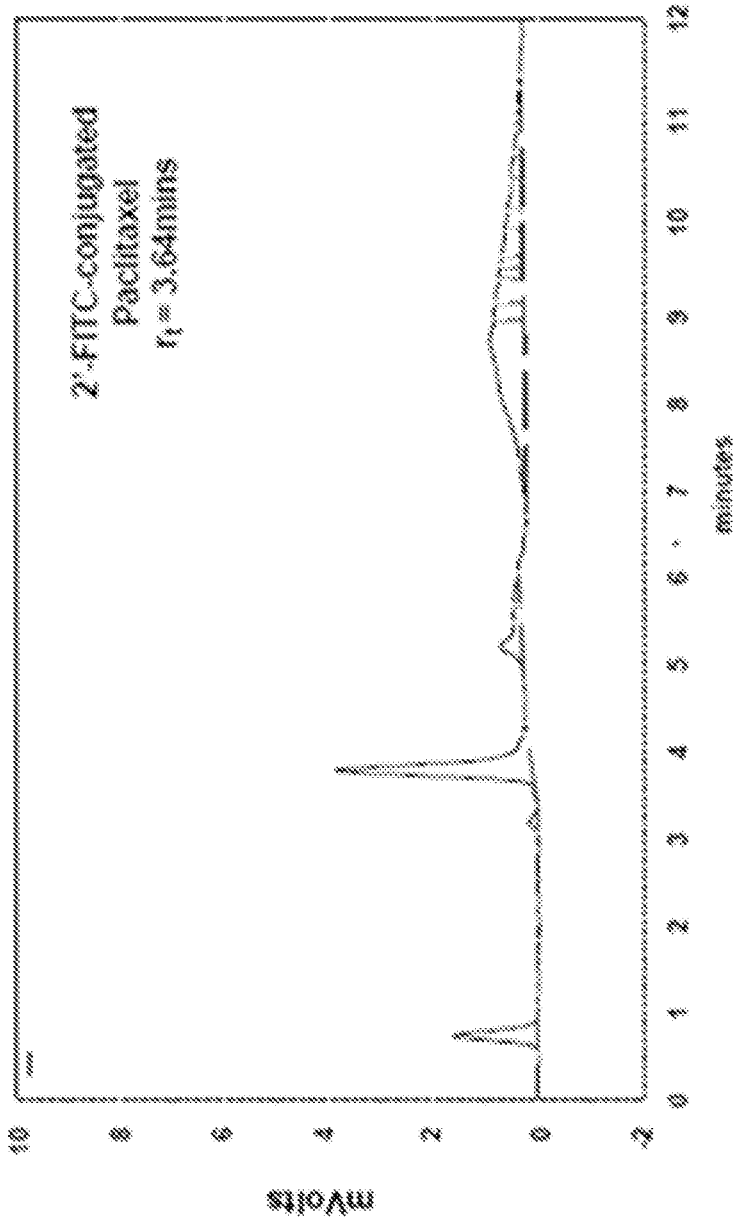


Figure 7

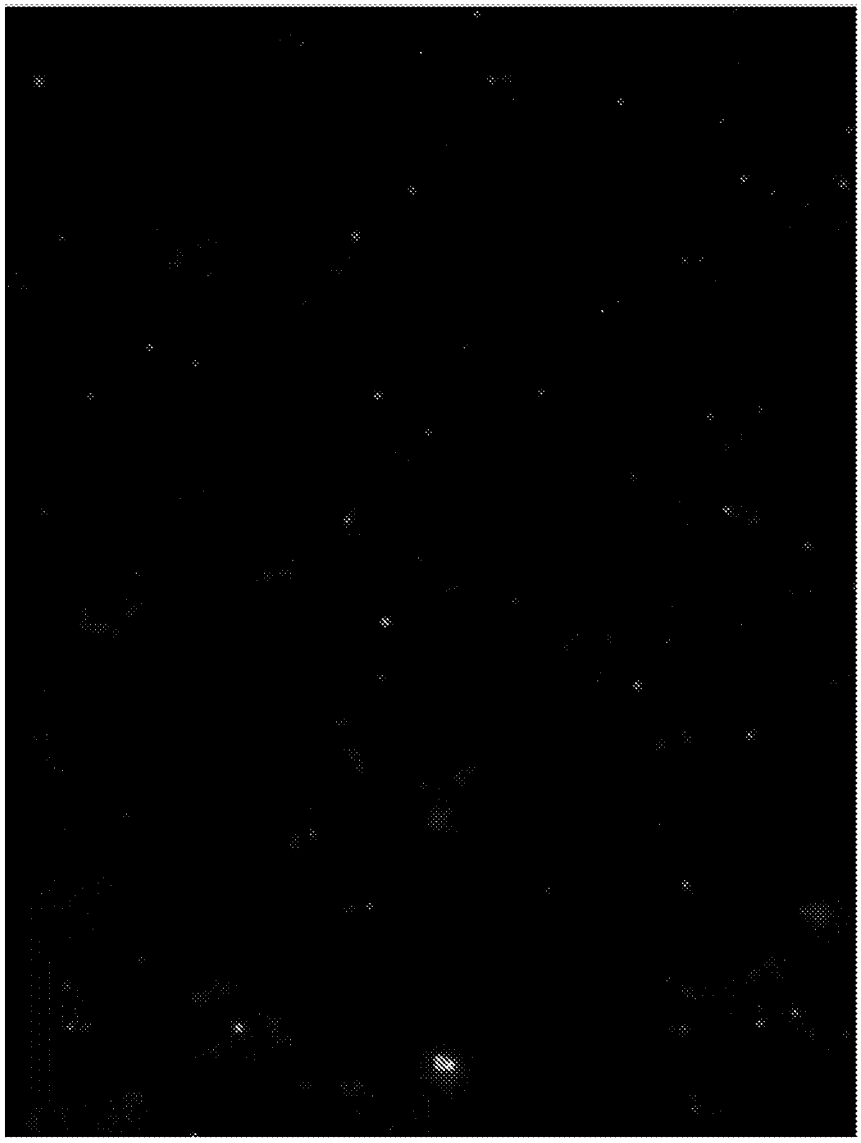


Figure 8

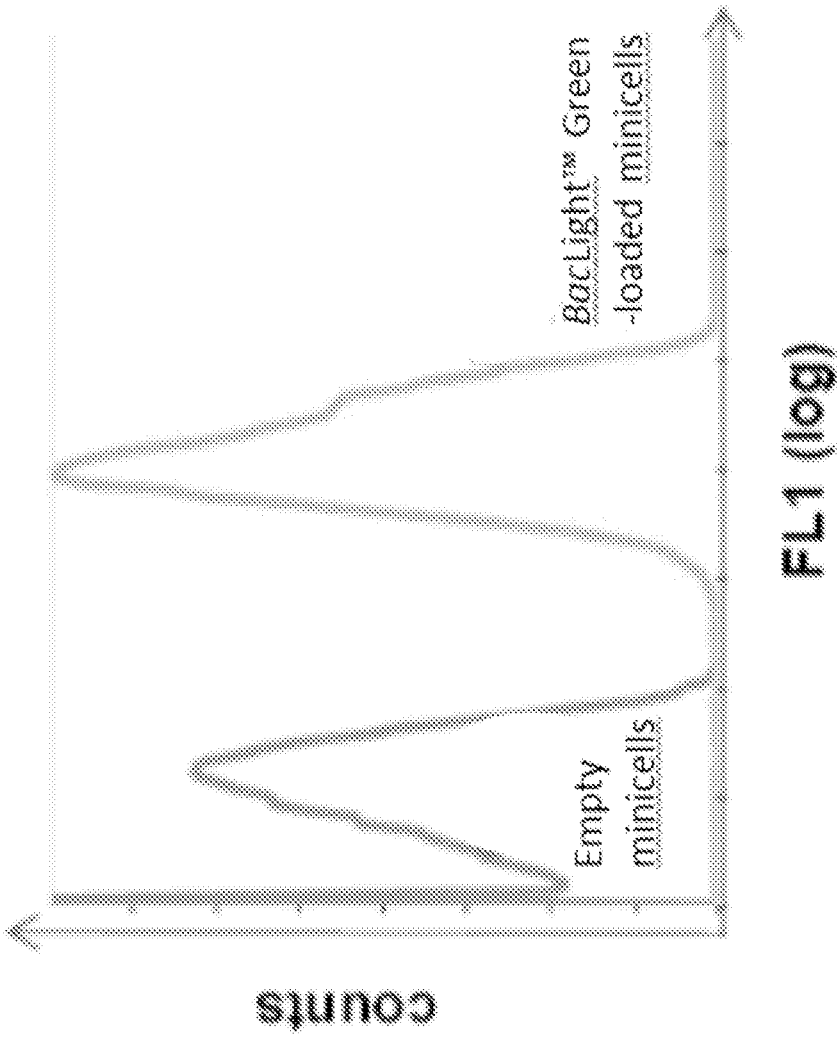

counts
Empty minicells
BacLight™ Green -loaded minicells
FL1 (log)

Figure 9

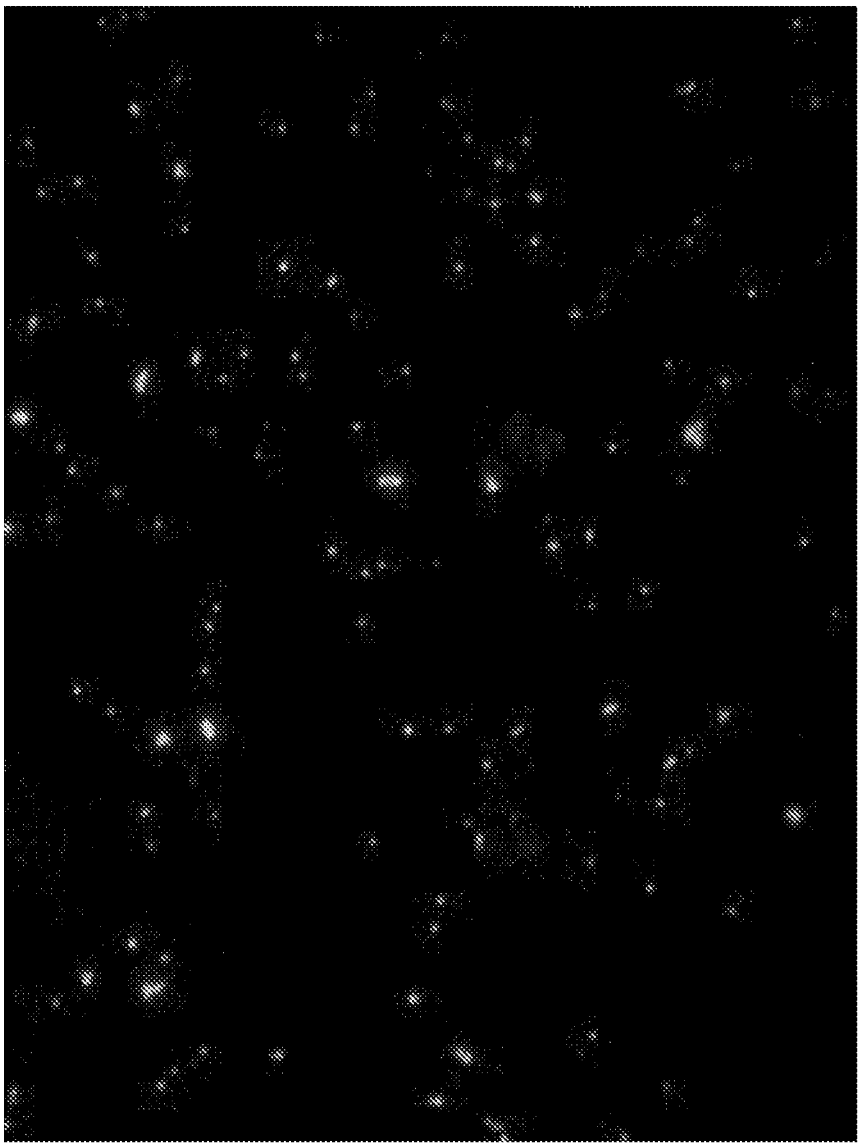


Figure 10

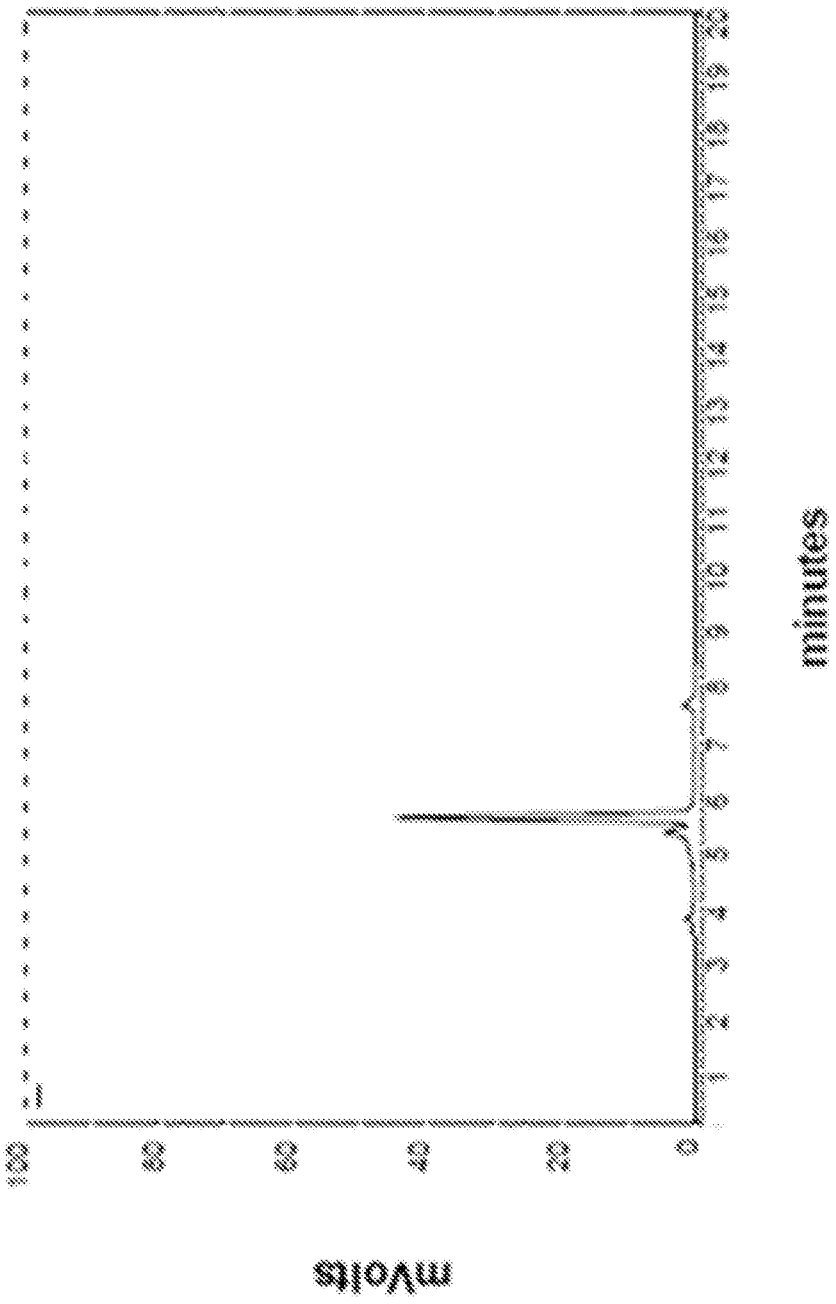


Figure 11

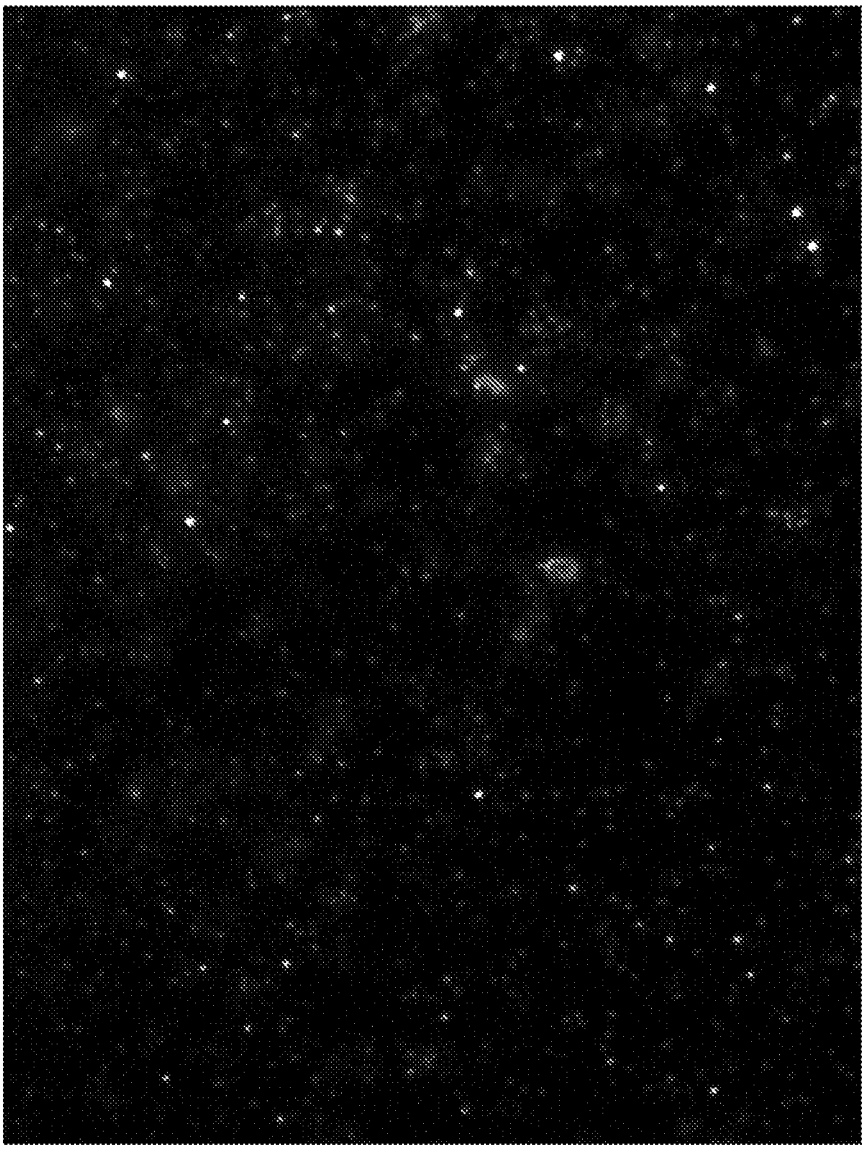


Figure 12

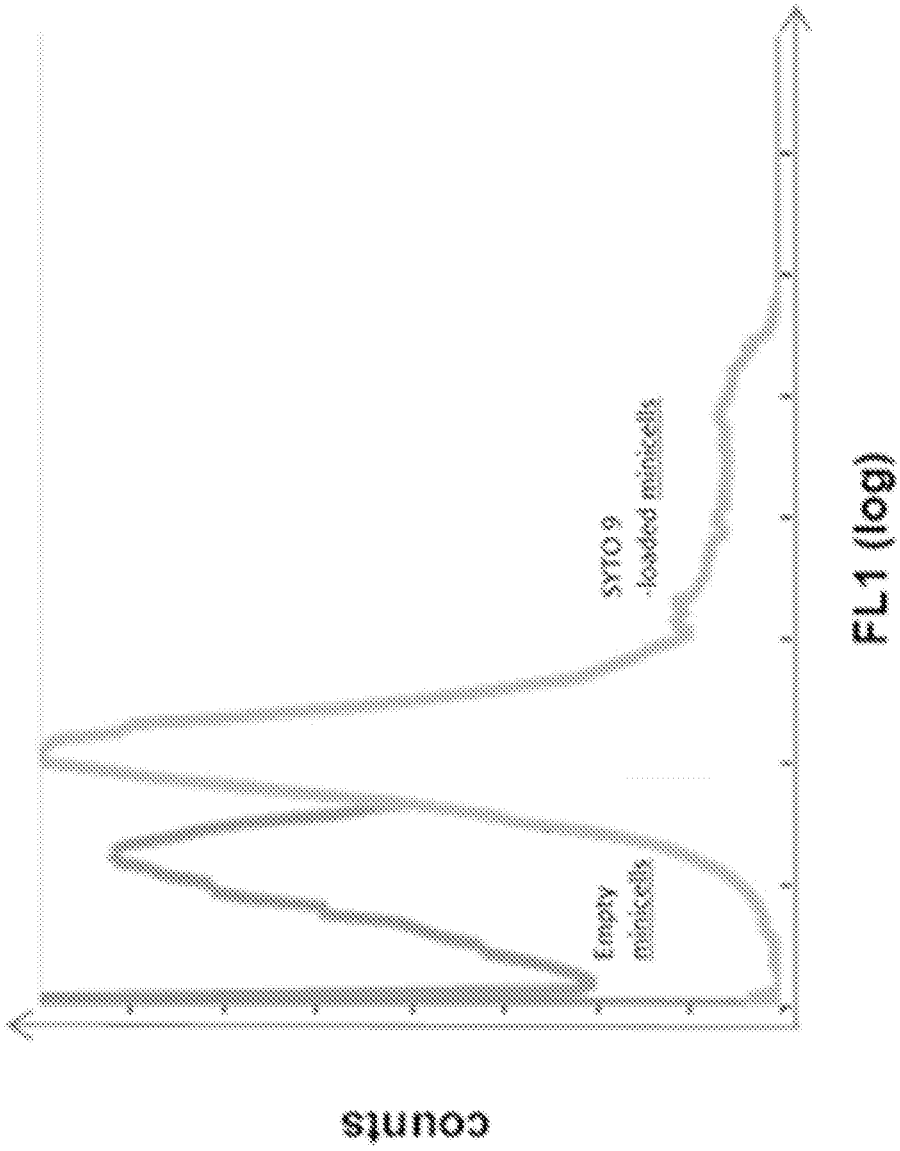


Figure 13

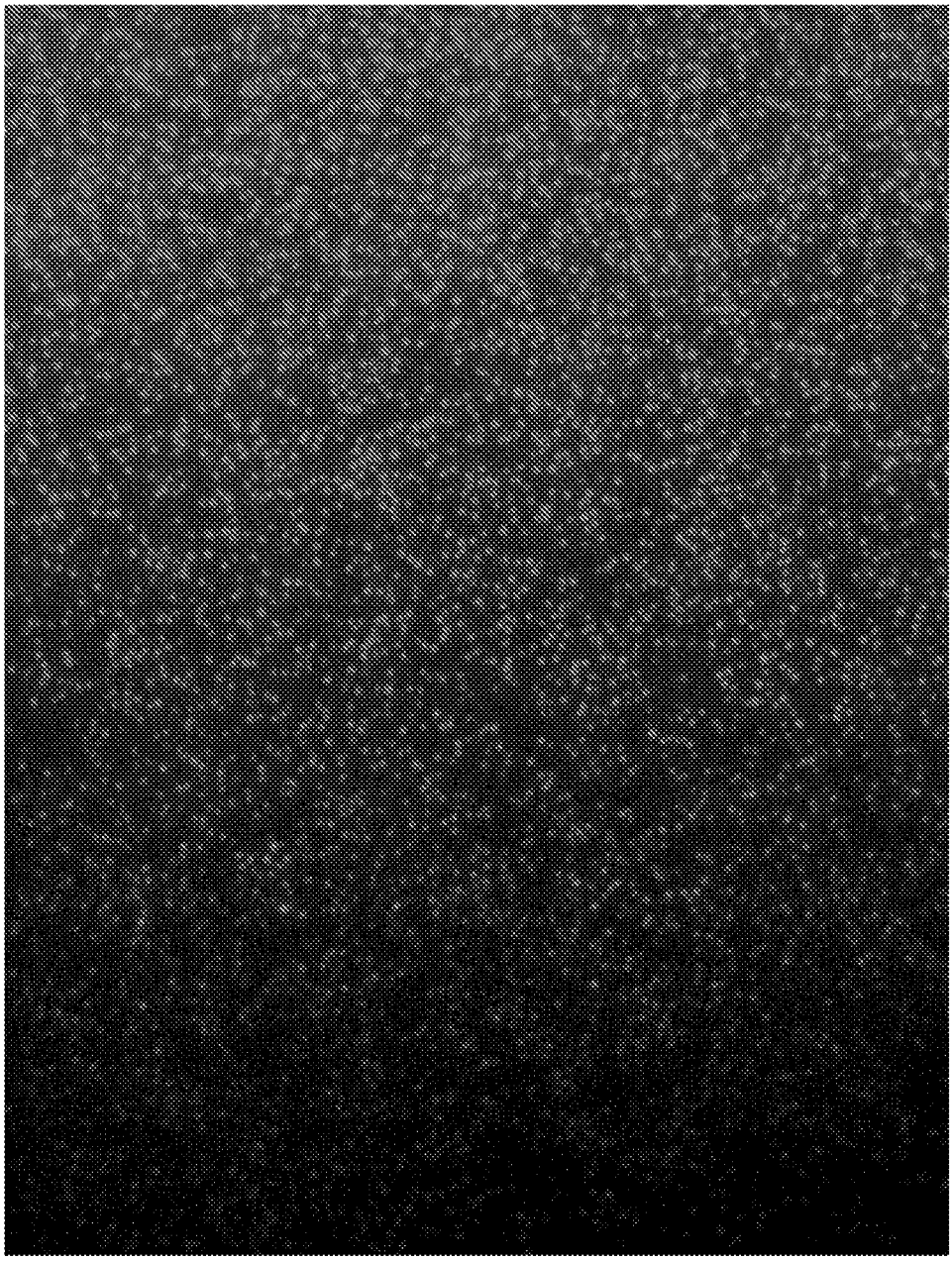


Figure 14

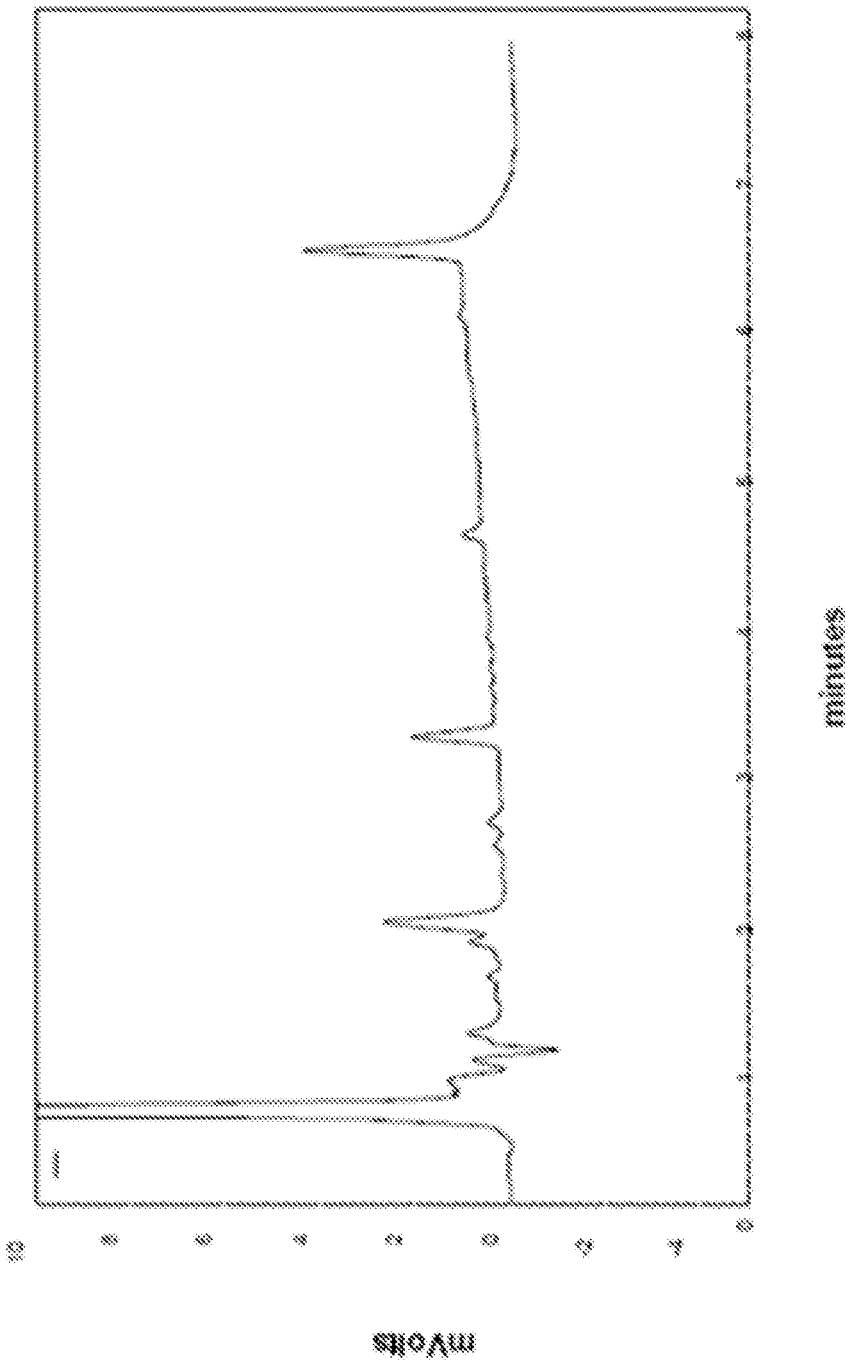


Figure 15

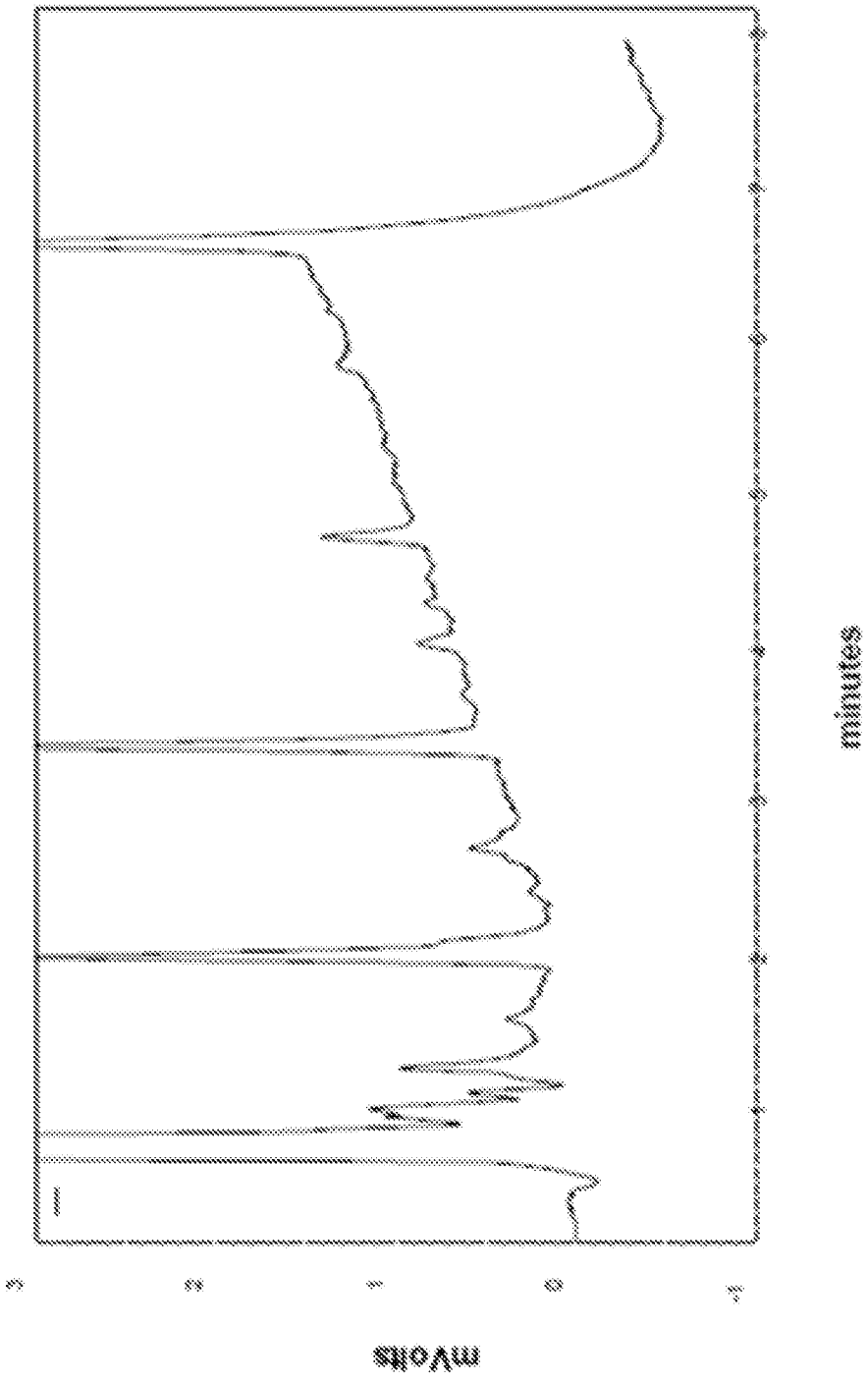


Figure 16

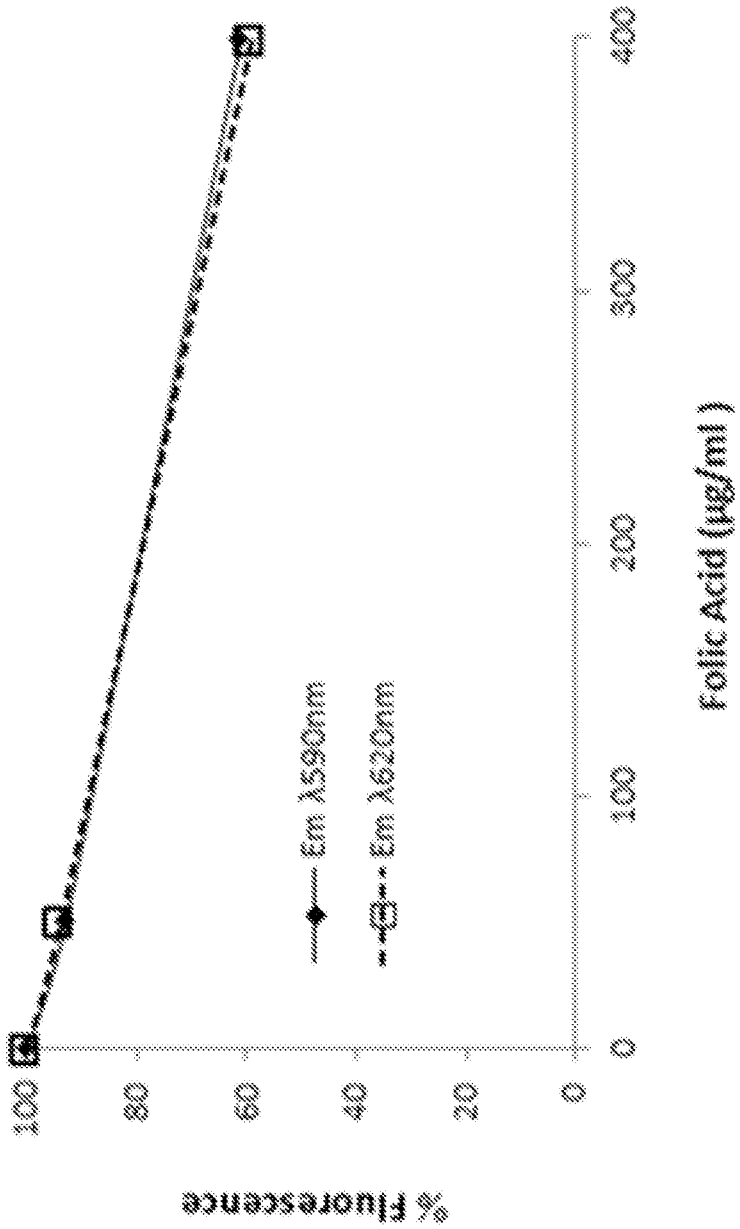

Quenching of Doxorubicin Fluorescence by Folic Acid
% fluorescence
100
80
60
40
20
0
0
100
200
300
400
Folic Acid (µg/ml)
Em λ590nm
Em λ620nm

Figure 17

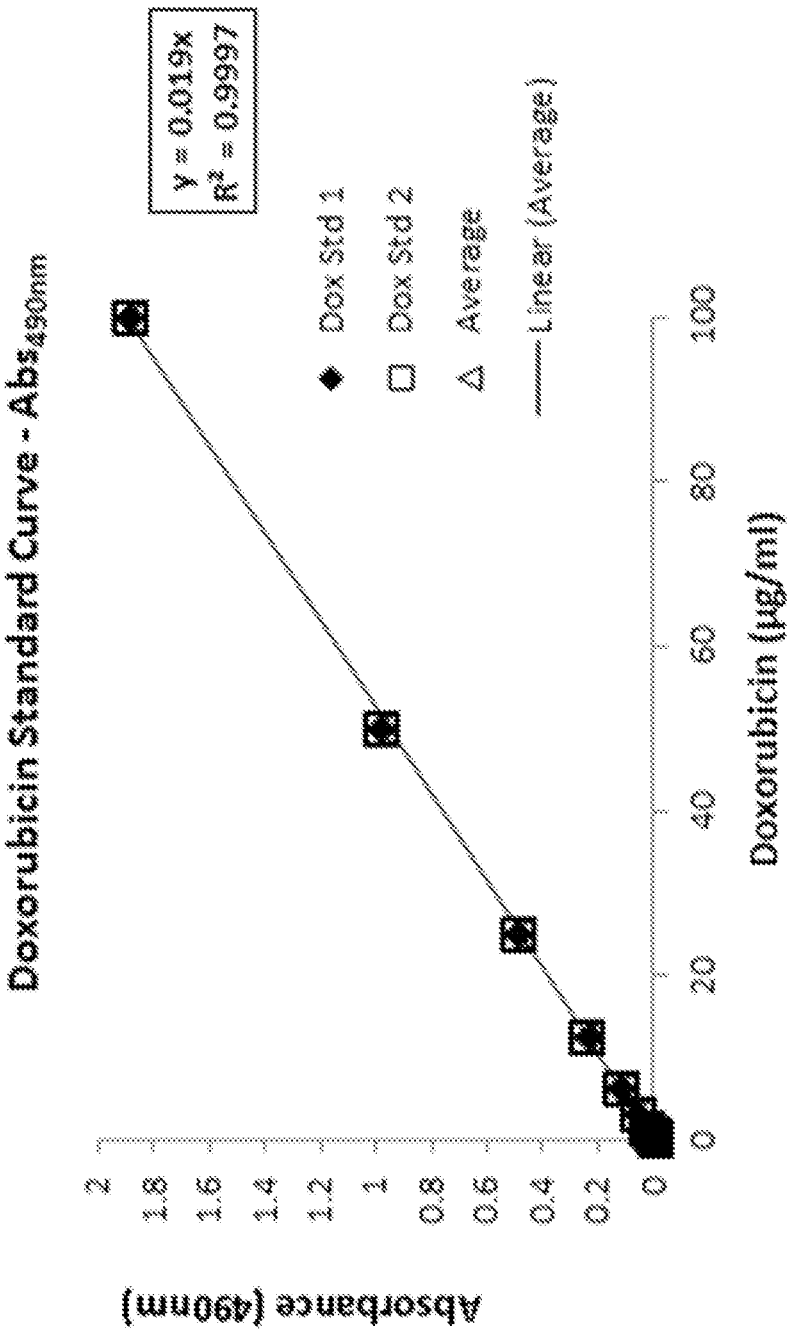

Doxorubicin Standard Curve - Abs490nm
y = 0.019x
R² = 0.9997
Dox Std 1
Dox Std 2
Average
Linear (Average)
Absorbance (490nm)
Doxorubicin (µg/ml)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
20
40
60
80
100

Figure 18

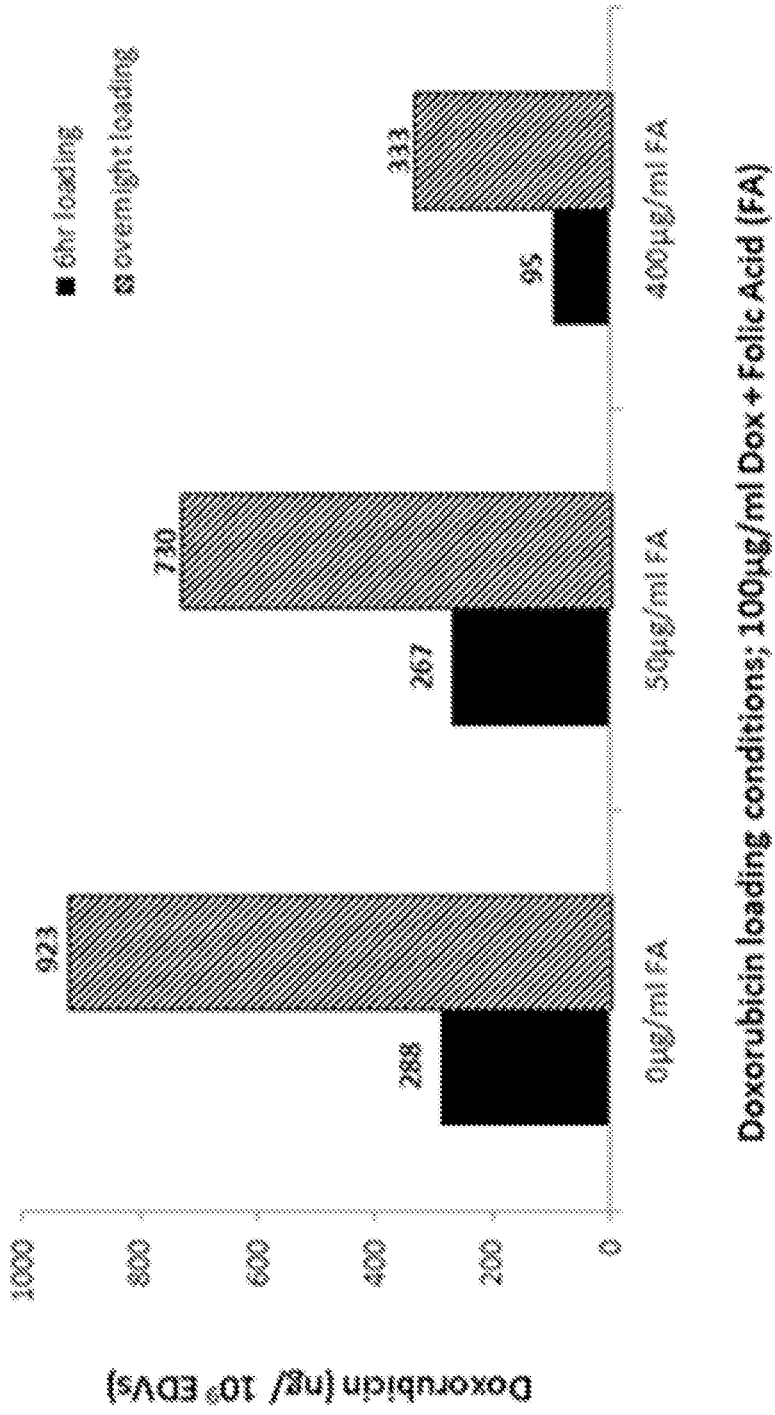

Effect of the fluorescence quencher Folic Acid on Doxorubicin loading
6hr loading
overnight loading
Doxorubicin (ng/ 10^9 EDVs)
1000
800
600
400
200
0
923
288
730
267
333
95
0µg/ml FA
50µg/ml FA
400µg/ml FA
Doxorubicin loading conditions; 100µg/ml Dox + Folic Acid (FA)

Figure 19

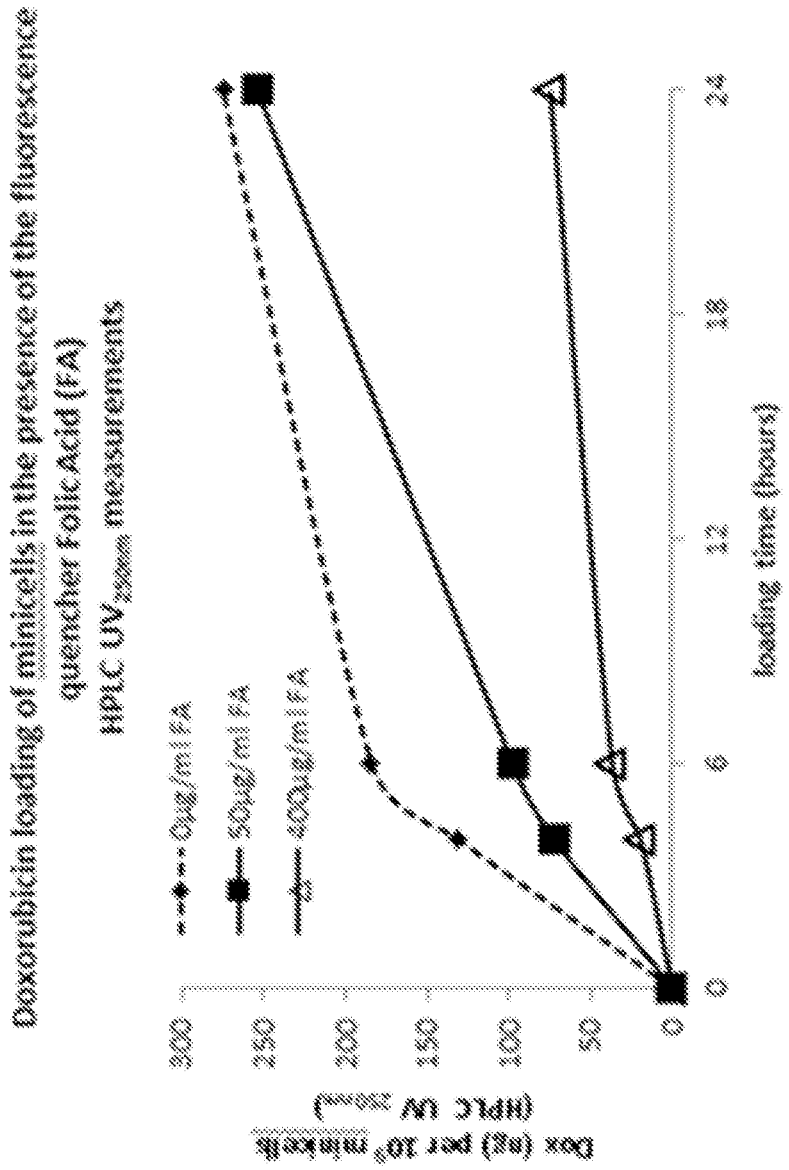

Doxorubicin loading of minicells in the presence of the fluorescence quencher Folic Acid (FA)
HPLC UV495nm measurements
0µg/ml FA
50µg/ml FA
400µg/ml FA
Dox (ng) per 10^9 minicells (HPLC UV 495nm)
300
250
200
150
100
50
0
0
6
12
18
24
loading time (hours)

Figure 20

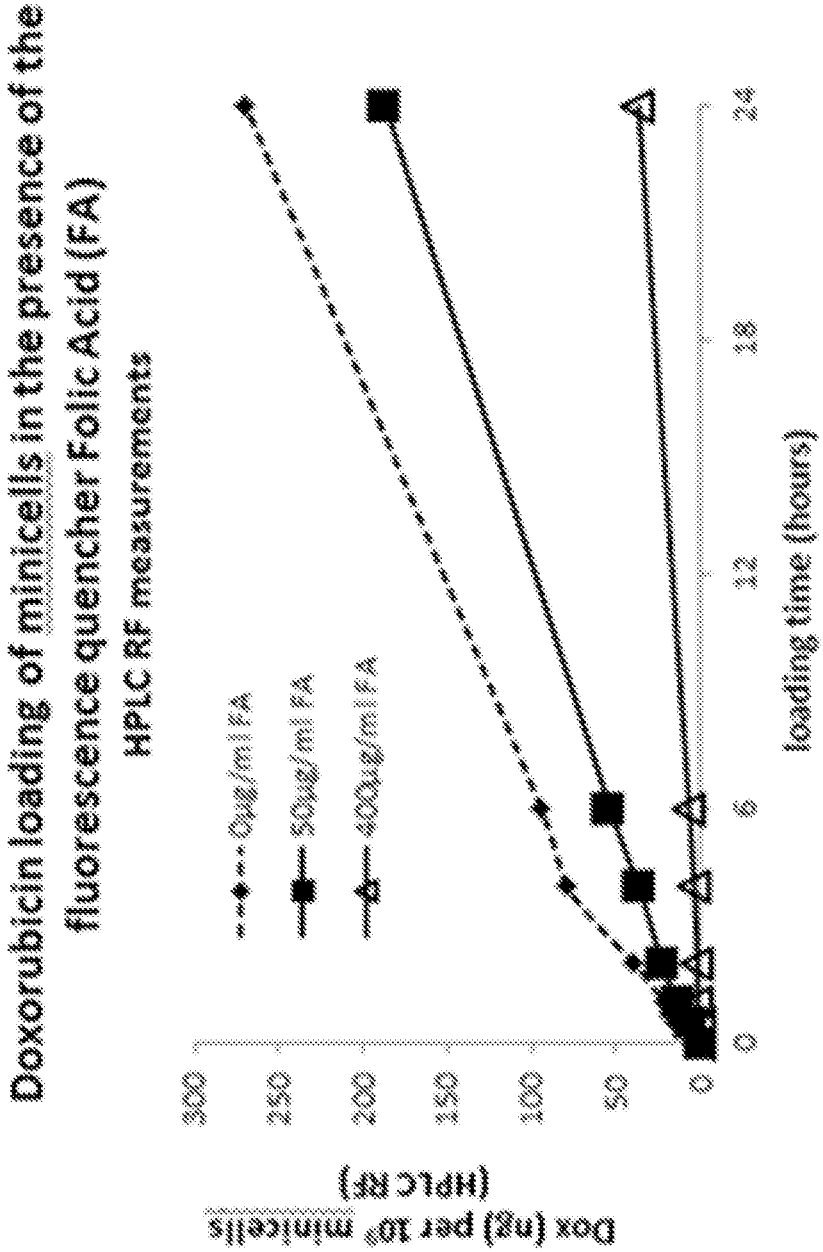

Doxorubicin loading of minicells in the presence of the fluorescence quencher Folic Acid (FA)
HPLC RF measurements
0μg/ml FA
50μg/ml FA
400μg/ml FA
Dox (ng) per 10^9 minicells (HPLC RF)
300
250
200
150
100
50
0
0
6
12
18
24
loading time (hours)

Figure 21

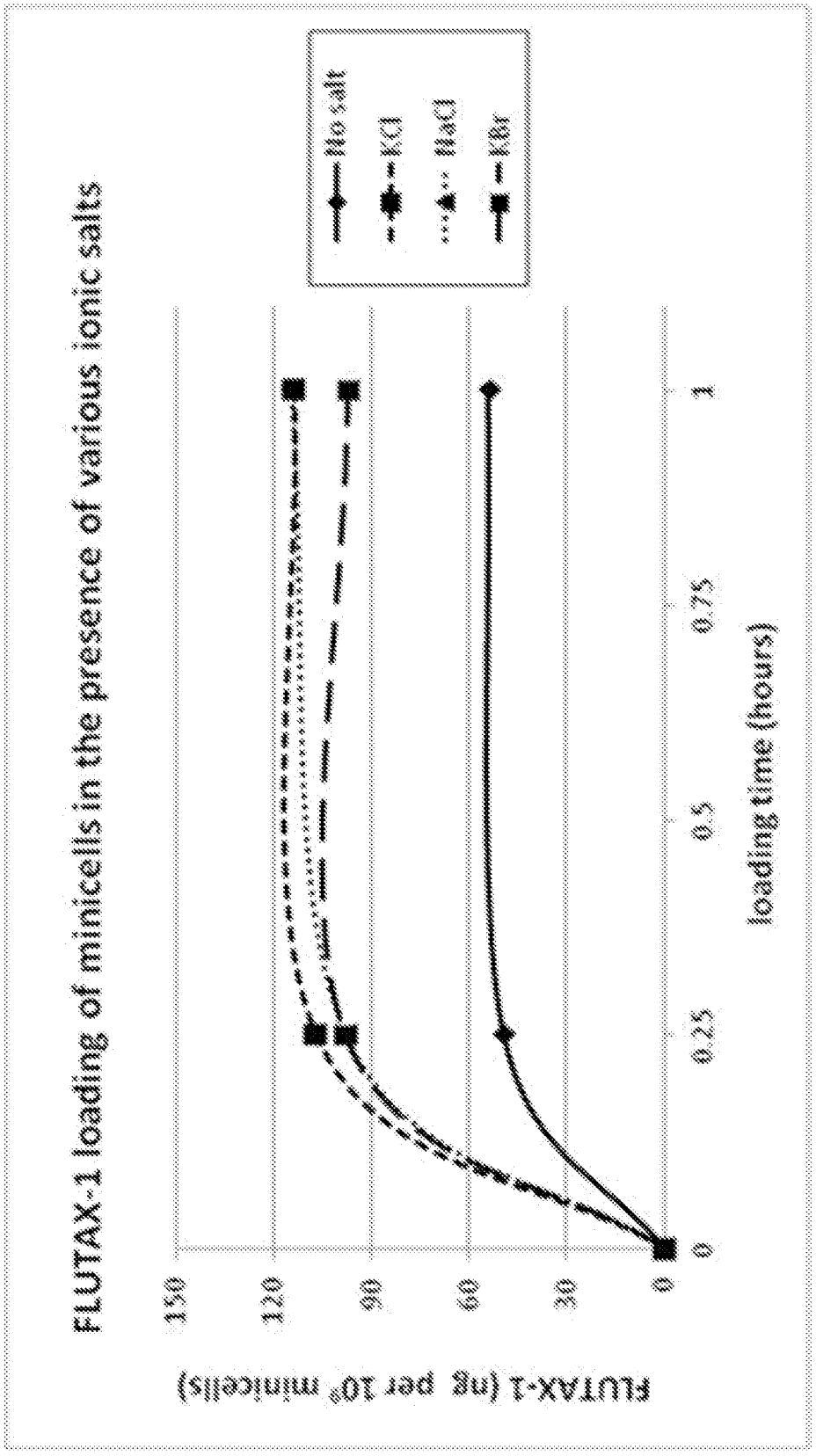

FLUTAX-1 loading of minicells in the presence of various ionic salts
No salt
KCl
NaCl
KBr
FLUTAX-1 (ng per 10⁹ minicells)
150
120
90
60
30
0
0
0.25
0.5
0.75
1
loading time (hours)

Figure 22

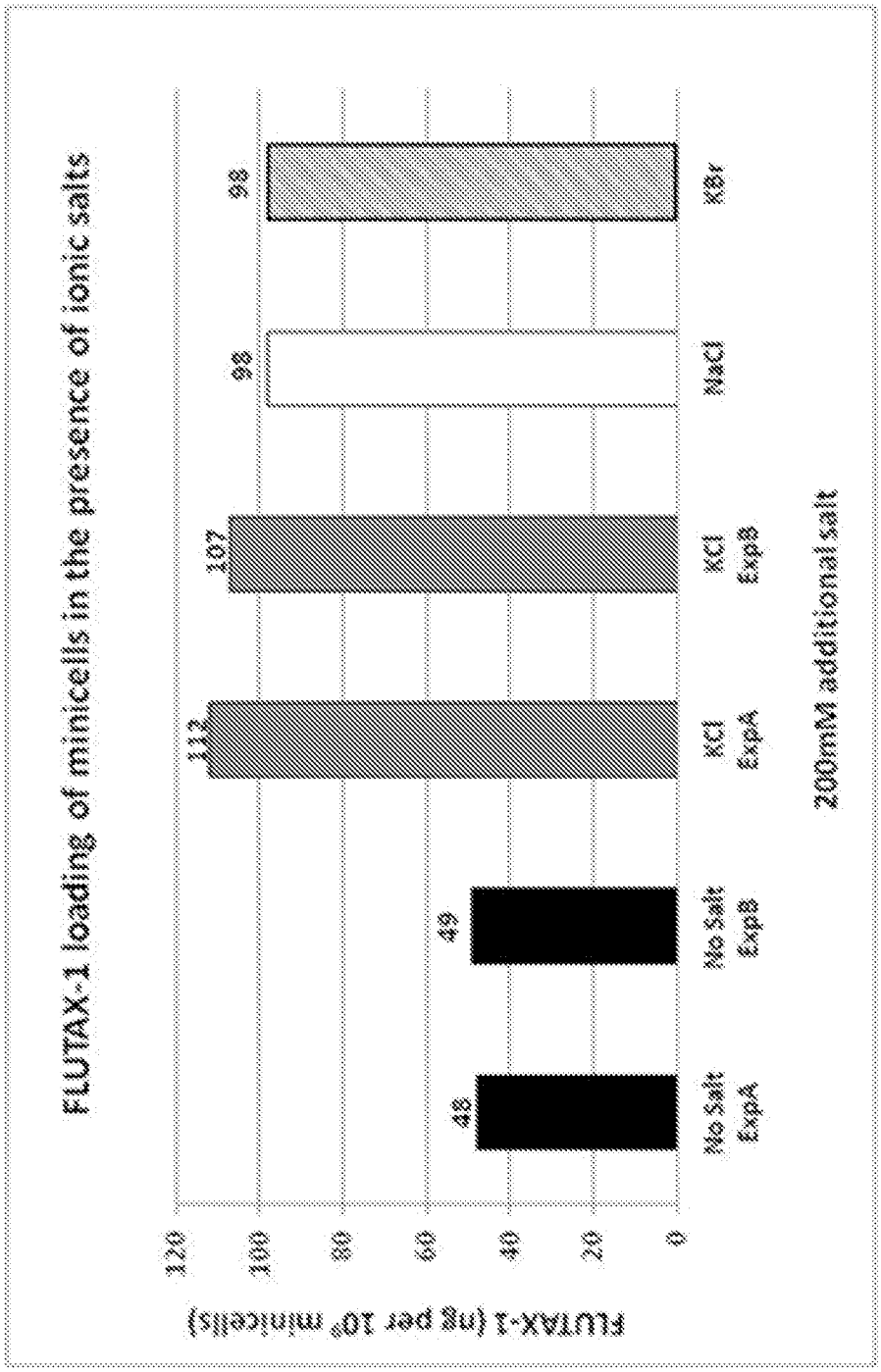

FLUTAX-1 loading of minicells in the presence of ionic salts
FLUTAX-1 (ng per 10⁸ minicells)
120
100
80
60
40
20
0
48
49
111
107
98
98
No Salt ExpA
No Salt ExpB
KCl ExpA
KCl ExpB
NaCl
KBr
200mM additional salt

Figure 23

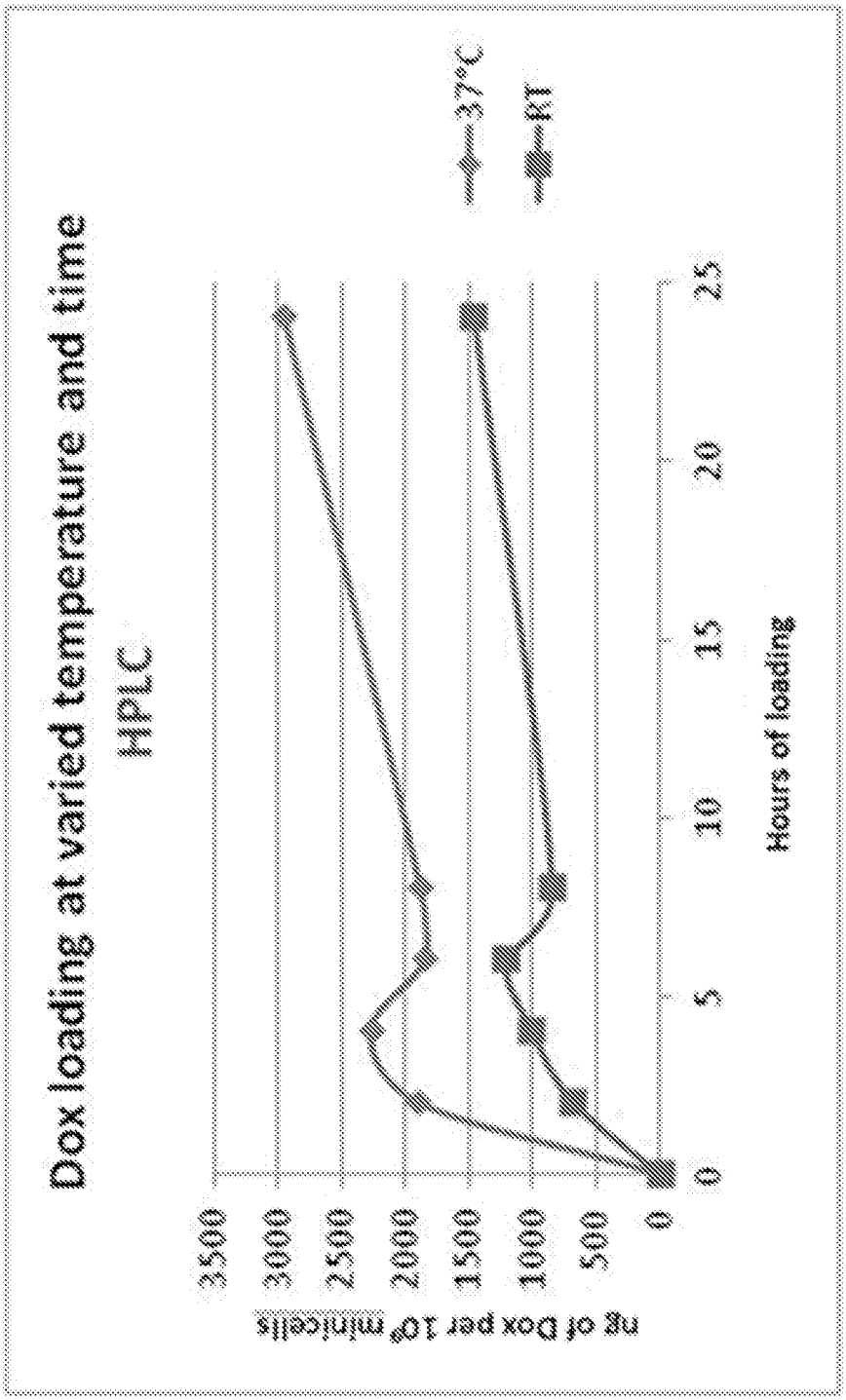

Dox loading at varied temperature and time
HPLC
ng of Dox per 10⁶ mlnlcells
3500
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
Hours of loading
37°C
RT

Figure 24

